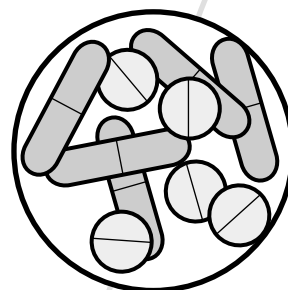
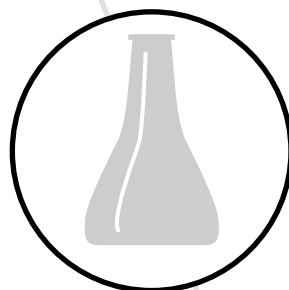
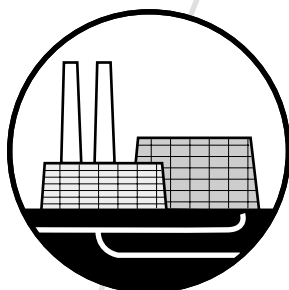


Waters 2410 Differential Refractometer

Operator's Guide



Waters

34 Maple Street
Milford, MA 01757

71500241002, Revision 3

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

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

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



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

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

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

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

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



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

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

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

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

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

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



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

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

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

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



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

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

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

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

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



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

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

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

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

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



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






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

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





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

Precaución: para evitar choques eléctricos, apague el instrumento y desenchufe el cable de alimentación antes de realizar cualquier reparación en el instrumento.

Commonly Used Symbols

| | |
|---|---|
|  | <p>Direct current</p> <p>Courant continu</p> <p>Gleichstrom</p> <p>Corrente continua</p> <p>Corriente continua</p> |
|  | <p>Alternating current</p> <p>Courant alternatif</p> <p>Wechs el s t r om</p> <p>Corrente alternata</p> <p>Corriente alterna</p> |
|  | <p>Protective conductor terminal</p> <p>Borne du conducteur de protection</p> <p>Schutzleiteranschluss</p> <p>Terminale di conduttore con protezione</p> <p>Borne del conductor de tierra</p> |
|  | <p>Frame or chassis terminal</p> <p>Borne du cadre ou du châssis</p> <p>Rahmen- oder Chassisanschluss</p> <p>Terminale di struttura o telaio</p> <p>Borne de la estructura o del chasis</p> |
|  | <p>Caution or refer to manual</p> <p>Attention ou reportez-vous au guide</p> <p>Vorsicht, oder lesen Sie das Handbuch</p> <p>Prestare attenzione o fare riferimento alla guida</p> <p>Actúe con precaución o consulte la guía</p> |

Commonly Used Symbols (Continued)

| | |
|--|--|
|  | <p>Caution, hot surface or high temperature Attention, surface chaude ou température élevée Vorsicht, heiße Oberfläche oder hohe Temperatur Precauzione, superficie calda o elevata temperatura Precaución, superficie caliente o temperatura elevada</p> |
|  | <p>Caution, risk of electric shock (high voltage)•Attention, risque de commotion électrique (haute tension) Vorsicht, Elektroschockgefahr (Hochspannung) Precauzione, rischio di scossa elettrica (alta tensione) Precaución, peligro de descarga eléctrica (alta tensión)</p> |
|  | <p>Caution, risk of needle-stick puncture Attention, risques de perforation de la taille d'une aiguille Vorsicht, Gefahr einer Spritzenpunktierung Precauzione, rischio di puntura con ago Precaución, riesgo de punción con aguja</p> |
|  | <p>Caution, ultraviolet light Attention, rayonnement ultraviolet Vorsicht, Ultraviolettes Licht Precauzione, luce ultravioletta Precaución, emisiones de luz ultravioleta</p> |

Waters 2410 Differential Refractometer Information

Intended Use

The Waters® 2410 Differential Refractometer can be used for in-vitro diagnostic testing to analyze many compounds, including diagnostic indicators and therapeutically monitored compounds. When you develop methods, follow the “Protocol for the Adoption of Analytical Methods in the Clinical Chemistry Laboratory,” American Journal of Medical Technology, 44, 1, pages 30–37 (1978). This protocol covers good operating procedures and techniques necessary to validate system and method performance.

Biological Hazard

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

Calibration

Follow acceptable methods of calibration with pure standards to calibrate methods. Use a minimum of five standards to generate a standard curve. The concentration range should cover the entire range of quality-control samples, typical specimens, and atypical specimens.

Quality Control

Routinely run three quality-control samples. Quality-control samples should represent subnormal, normal, and above-normal levels of a compound. Ensure that quality-control sample results are within an acceptable range, and evaluate precision from day to day and run to run. Data collected when quality-control samples are out of range may not be valid. Do not report this data until you ensure that chromatographic system performance is acceptable.

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How to Use This Guide

Purpose of This Guide

The *Waters 2410 Differential Refractometer Operator's Guide* describes the features and use of the Waters® 2410 Differential Refractometer and provides installation and maintenance procedures.

Audience

This guide is intended for use by anyone interested in installing, using, maintaining, and troubleshooting the 2410 differential refractometer.

Structure of This Guide

The *Waters 2410 Differential Refractometer Operator's Guide* is divided into chapters and appendixes. Each page is marked with a tab and a footer to facilitate access to information within the chapter or appendix.

The table below describes the material covered in each chapter and appendix.

| | |
|---|--|
| <i>Chapter 1, Waters 2410 Theory of Operation</i> | Describes the product and the principles of differential refractometry and 2410 differential refractometer operation. |
| <i>Chapter 2, Installing the 2410 Refractometer</i> | Describes the 2410 differential refractometer installation procedures. |
| <i>Chapter 3, Making Signal Connections</i> | Describes how to connect other components of your chromatography system to the 2410 differential refractometer. |
| <i>Chapter 4, Preparing Solvents</i> | Discusses the importance of filtering and degassing solvents for effective operation. |
| <i>Chapter 5, Using the 2410 Refractometer</i> | Describes how to power on and off and operate the 2410 differential refractometer. |
| <i>Chapter 6, Maintenance Procedures</i> | Describes maintenance and parts replacement procedures for the 2410 differential refractometer. |
| Chapter 7, Troubleshooting | Provides tables describing symptoms, possible causes, and corrective actions for 2410 differential refractometer operational problems. |

| | |
|---|--|
| Appendix A, Specifications | Provides specifications for the 2410 differential refractometer. |
| Appendix B, Spare Parts/Accessories | Lists the recommended spare parts for the 2410 differential refractometer. |
| <i>Appendix C, Warranty Information</i> | Includes warranty and service information for the 2410 differential refractometer. |

Related Documents

The following table lists other documents related to the operation of the Waters 2410 Differential Refractometer.

| | |
|--|---|
| <i>Waters 2690 Separations Module Operator's Guide</i> | Describes the procedures for unpacking, installing, using, maintaining, and troubleshooting the Waters 2690 Separations Module. |
| <i>Waters 600E Multisolvent Delivery System User's Guide</i> | Describes the procedures for installing, using, maintaining, and troubleshooting the Waters 600E Multisolvent Delivery System. |
| <i>Waters Bus SAT/IN Module Installation Guide</i> | Provides the procedures for installing the Waters Bus SAT/IN Module. |
| <i>Millennium Software User's Guide, Vol. I and Vol. II</i> | Describes the Millennium Chromatography Manager software used in both the Millennium 2010 workstation and the Millennium 2020 client/server system. |

Conventions Used in This Guide

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

- **Bold** text indicates user action. For example:

Press **0**, then press **Enter** for the remaining fields.

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

An instrument method tells the software how to acquire data.

- Instructions to click a specific icon include the icon in the left column of the page. For example:



Click the Projects view icon. The Projects view appears with all existing project folders.

Notes, Attentions, and Cautions

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

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

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



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

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



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



Caution: *To avoid the possibility of electrical shock, always power off the detector and unplug the power cord before you perform maintenance procedures.*



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

Chapter 1

Waters 2410 Theory of Operation

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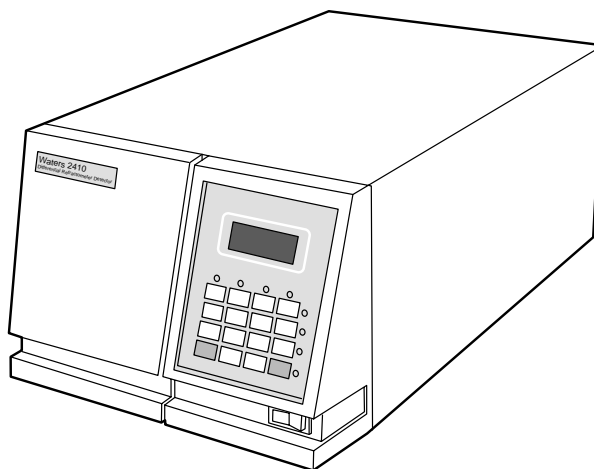
Waters 2410 Theory of Operation

This chapter introduces you to the Waters® 2410 Differential Refractometer. It summarizes the 2410 differential refractometer features and the principles of differential refractometry, and describes the theory and principles of operation.

Refer to Appendix A, Specifications, for system specifications, and to *Chapter 4, Preparing Solvents*, for solvent considerations.

1.1 Overview

The Waters 2410 Differential Refractometer, shown in Figure 1-1, 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 Waters system controller or Waters data system.



TP01531

Figure 1-1 Waters 2410 Differential Refractometer

Range and Sensitivity

The 2410 detector functions with solvents with refractive indices between 1.00 and 1.75. The measurement range of the instrument is 5×10^{-8} to 5×10^{-3} refractive index units full scale (RIUFS).

Features

Features of the 2410 differential 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 external column heater controls
- Battery backup to retain parameter settings when the detector is powered off or during power interruptions
- Long-life LED light source

1.2 Theory of Operation

The Waters 2410 Differential Refractometer uses optical 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 the second medium at an angle that is not perpendicular to the medium's surface, the light is bent (refracted).

The extent to which a medium refracts light is its *refractive index* (RI), calculated as the ratio of the velocity of light in a vacuum 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 that affect RI

- Measuring refraction
- Using changes in RI for sample detection

Factors That Affect RI

The refractive index of a medium is solely dependent on 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.

Wavelength

The refractive index of a medium has a specific value that changes with the wavelength of the incident light beam. Since the 2410 differential refractometer uses monochromatic light at a fixed wavelength, the effect of different wavelengths of light on RI is not discussed in this guide.

Density

The density of the medium also affects its RI. At a fixed wavelength, the relationship between the density of a medium and its RI is generally, but not necessarily, linear. The most important of the factors that affect the density of a medium are:

- Composition
- Temperature
- Pressure

Figure 1-2 illustrates the effect of density on the RI of two solutions. The refractive index of a sucrose solution changes linearly with concentration over this range of compositions, but a methanol solution exhibits a nonlinear region between concentrations of 45 and 55 percent.

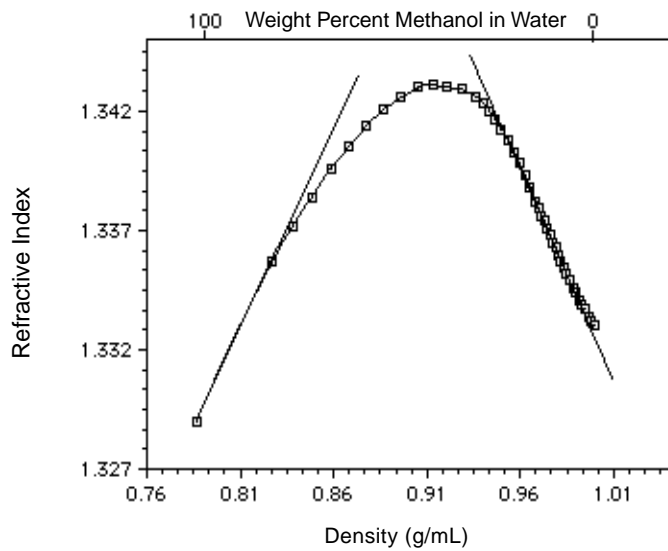
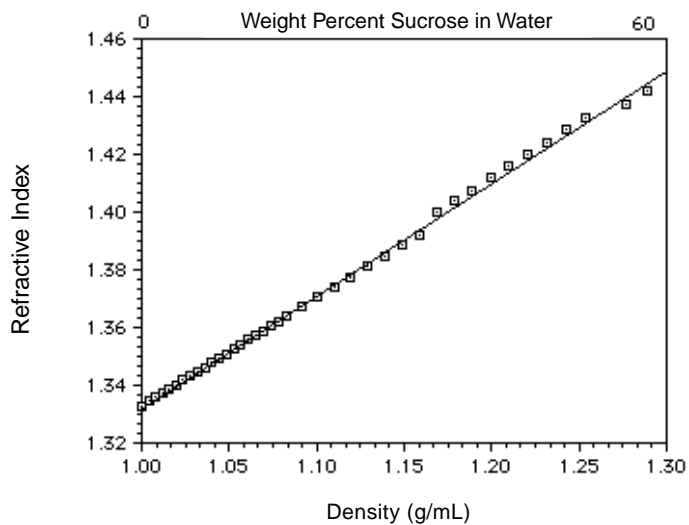


Figure 1-2 Effect of Density on RI

Measuring Refraction

The extent to which a beam of light is refracted when it enters a medium depends on two factors:

- The angle at which the light enters the new medium (the *angle of incidence*)
- The refractive indices of the new media

The angle of a refracted 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.

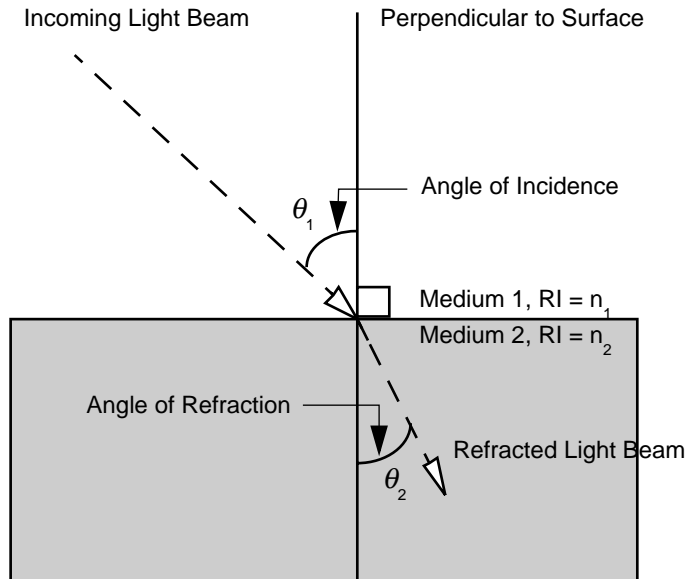


Figure 1-3 Refraction of Light

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

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

where:

- 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 sample solution from the angle of incidence, the RI of the solvent, and the angle of refraction.

Using Changes in RI for Sample Detection

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

- The composition of the sample solution in the flow cell changes.
- The RI of the solution changes.
- The light beam passing through the solution is refracted.

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

Figure 1-4 shows how refraction by the sample in the flow cell changes the proportion of light on each element of the photodiode.

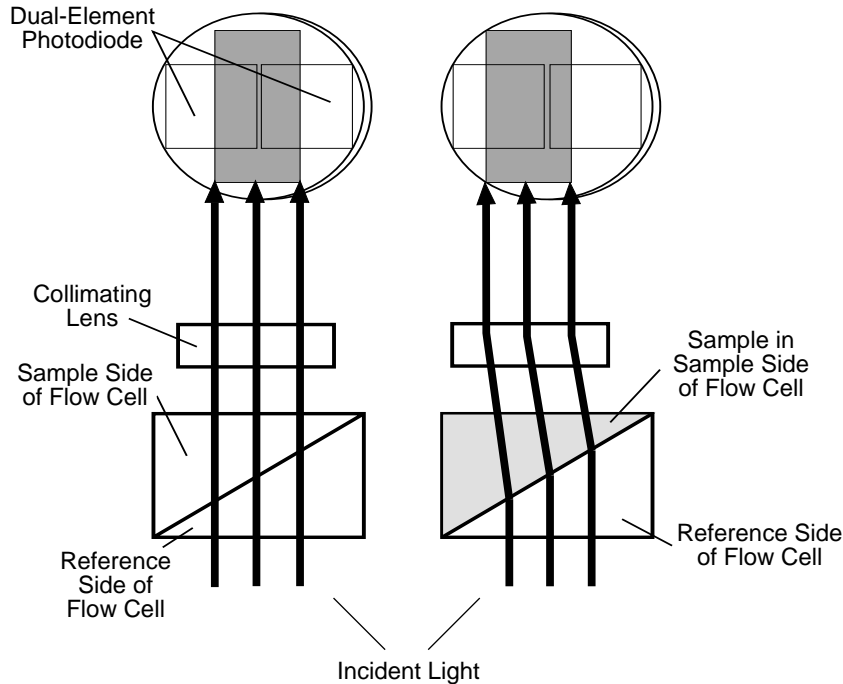


Figure 1-4 Presence of Sample Changes the Photodiode Signal

By keeping wavelength, temperature, and pressure constant, the changes in RI measured by the refractometer are due only to changing sample concentration. A solution with a high concentration of a solute refracts a beam of light more than a dilute solution. Therefore, high concentrations of sample yield large peaks.

1.2.2 Differential Refractometry

The 2410 differential refractometer can measure extremely small changes in refractive index to detect the presence of sample. The small difference in RI between a reference solution and a sample solution is referred to as Δn . Δn is expressed in refractive index units (RIU).

The 2410 differential refractometer measures Δn values as small as 5×10^{-8} RIU by detecting the difference in the amount of light falling upon each of the elements of the dual-element photodiode (see Figure 1-4).

External Angle of Deflection

The amount of light falling upon the elements of the photodiode is determined by the *external angle of deflection* (ϕ), as shown in Figure 1-5. The ϕ determines the magnitude of the shift (Δx) of the image cast on the photodiode by the light beam.

Figure 1-5 illustrates the external angle of deflection (ϕ) and its dependence on the difference in RIs between the reference and sample sides of the flow cell.

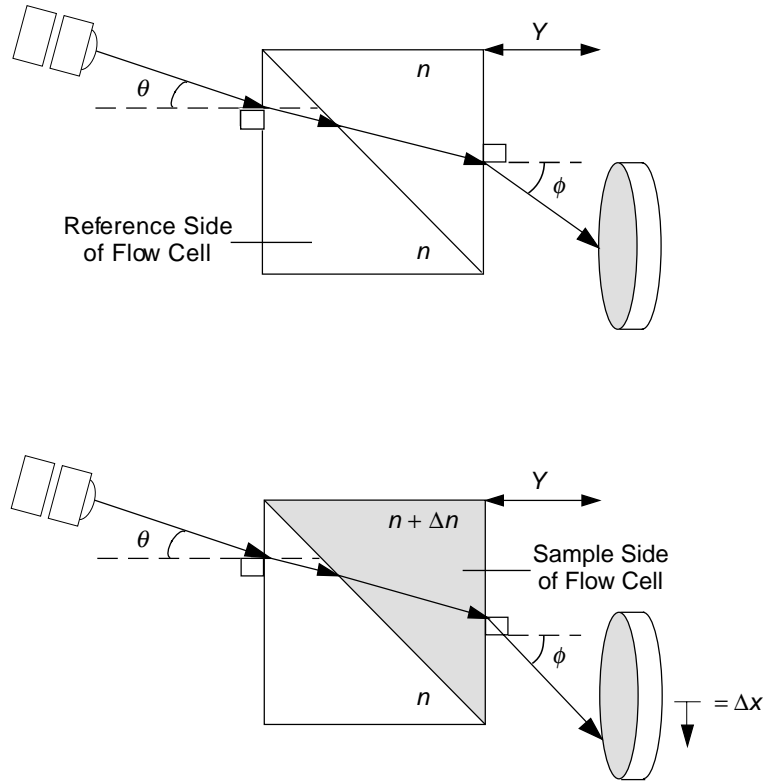


Figure 1-5 How Refraction Changes ϕ

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 optics bench assembly, the fused quartz walls of the flow cell, the solvent in the reference side of the flow cell, and the solution in the sample side of the flow cell.

Of these refractors, only the solution in the sample side of the flow cell changes over the course of a run. As a result, the reference external angle of deflection (ϕ) does not change until a change in the RI of the sample causes the light beam to be refracted from its zero position.

The relationship between the external angle of deflection (ϕ) and the RI of the sample solution is expressed as:

$$\Delta n \cong \phi / \tan \theta$$

where: Δn = Difference in RI between the solvent and the solvent-sample solution

ϕ = External angle of deflection (in radians)

θ = Angle of incidence (in radians)

Effect of Refraction on the Photodiode Signal

The change in ϕ determines the shift (Δx) of the light beam on the photodiode. Because the 2410 differential refractometer uses a dual-pass optics bench assembly, the light beam passes through the flow cell twice before reaching the photodiode, doubling the image shift.

The relationship between the image shift (Δx) at the 2410 detector photodiode and the change in RI of the solution is expressed as:

$$\Delta x = 2Y(\tan \theta) \Delta n$$

where: Δx = Distance of the image shift at the photodiode

Y = Distance from the flow cell to the photodiode

θ = Angle of incidence

Δn = Difference in RI between solvent and sample solution

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

$$\Delta x = C \Delta n$$

Where: C = A constant representing the fixed values

By detecting how far the image shifts (Δx), the refractometer measures the difference in RI (Δn) between the solvent-sample solution and the solvent alone.

The shift in the amount of the light beam striking each element of the dual-element photodiode results in a change in the output voltage from the 2410 detector. The integrator or chart recorder registers the changes in output voltage as peaks in your chromatogram.

1.2.3 Common RI Detection Problems

Changes in solution density caused by factors other than sample concentration are the most common source of problems in RI detection. Changes in solution density can be due to:

- Environmental factors such as changes in temperature or pressure
- Inhomogeneities in the solution

Environmental Factors

Even small changes in ambient temperature can cause baseline drift. Backpressure pulses from a dripping waste tube can cause short-term baseline 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 within a chromatographic band. 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, Preparing Solvents, for more information.

1.3 Principles of Operation

This section describes the design of the 2410 refractometer and its principles of operation, including:

- Fluidics
- Optics
- Electronics

1.3.1 Fluidics

The fluidic path of the 2410 refractometer includes the following components, some of which are shown in Figure 1-6:

- Countercurrent heat exchanger
- Flow cell, with sample and reference sides
- Solenoid valve
- Pressure relief valve
- Inlet and outlet tubing

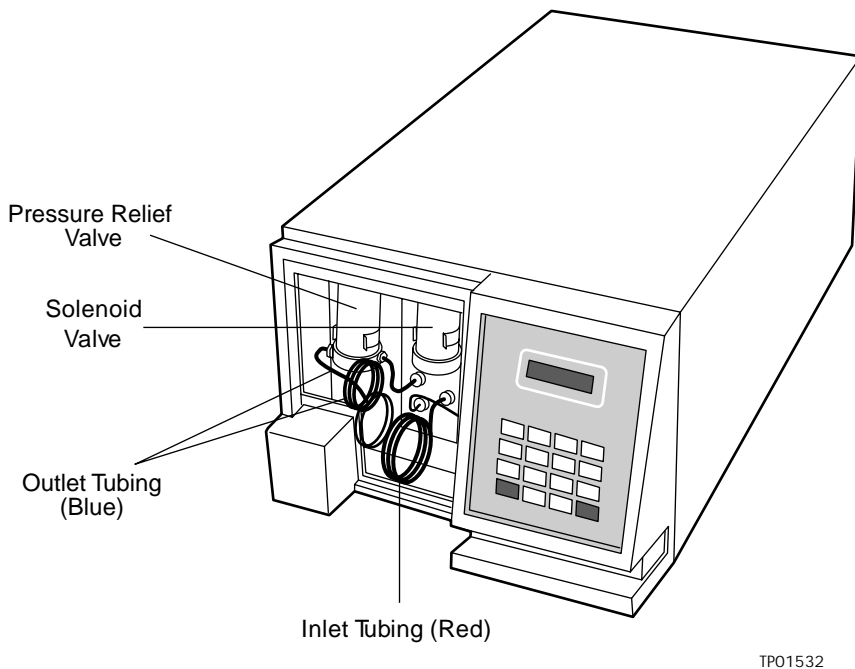


Figure 1-6 Waters 2410 Refractometer Fluidics

Countercurrent Heat Exchanger

The 2410 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 eluent passes during analysis.

The other prism is the reference side of the flow cell. It is filled with clean solvent when you purge the 2410 refractometer during equilibration. When you switch from purge to normal operation, the solenoid valve opens and the pressure relief valve shuts, stopping the flow of solvent through the reference prism but leaving the cell filled with solvent.

Solenoid Valve

During normal operation, the solenoid valve remains open. Fluid that passes through the sample side of the flow cell flows through the solenoid valve and out through the outlet tubing (blue) to the waste reservoir.

When you purge the 2410 refractometer, the solenoid valve closes, causing fluid passing through the sample side of the flow cell to flow out through the reference side of the flow cell, through the purge outlet tubing (blue).

Pressure Relief Valve

During normal operation, the pressure relief valve is closed, opening only if the pressure gets too high. This protects the flow cell, which has a maximum pressure rating of 100 psi.

During purging, fluid moving through the sample and reference sides of the flow cell goes out through the pressure relief valve to the waste reservoir. Figure 1-7 indicates the paths of solvent and sample in the 2410 refractometer during normal operation and during a purge. Table 1-1 provides the inner diameters of the sample and reference fluidic lines.

Table 1-1 Fluidic Line Inner Diameters

| Fluidic Line | Inner Diameter (inches) |
|---------------|-------------------------|
| Sample In | 0.009 |
| Sample Out | 0.040 |
| Reference In | 0.020 |
| Reference Out | 0.040 |

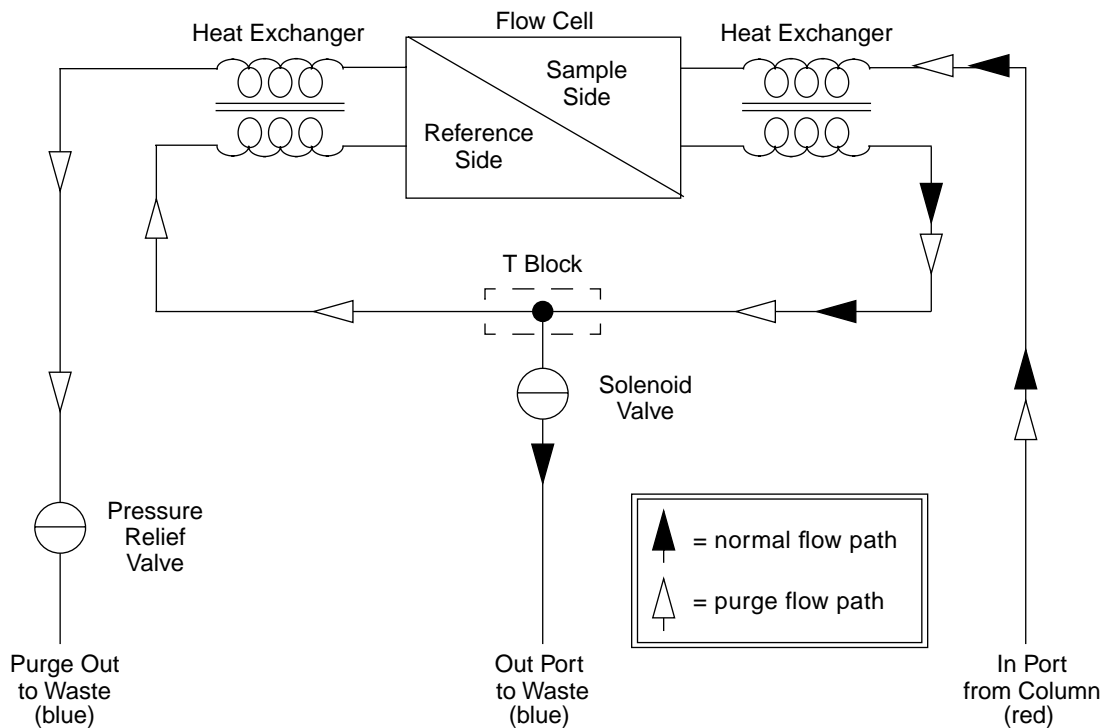


Figure 1-7 Waters 2410 Refractometer Fluidic Paths

Fluidic Path During Analysis

During analysis, the solvent-sample:

1. Flows in through the inlet tubing port.
2. Passes through the Sample In tube of the countercurrent heat exchanger.
3. Flows through the sample side of the flow cell.
4. Flows out through the Sample Out tube of the countercurrent heat exchanger.
5. Passes through the solenoid valve to the outlet tubing port.

Fluidic Path During Purge

When you purge the 2410 refractometer fluidic path, solvent:

1. Flows in through inlet tubing port.
2. Passes through the Sample In tube of the countercurrent heat exchanger.

3. Flows through the sample side of the flow cell.
4. Flows out through the Sample Out tube of the countercurrent heat exchanger to the closed solenoid valve.
5. Passes through the Reference In tube of the countercurrent heat exchanger.
6. Flows through the reference side of the flow cell.
7. Flows out through the Reference Out tube of the countercurrent heat exchanger.
8. Flows out through the pressure relief valve to the purge outlet tubing port.

1.3.2 Optics

The 2410 refractometer optics bench assembly (Figure 1-8) consists of the following components:

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

Figure 1-8 shows the path of the light beam as it passes through the components in the optics bench assembly.

As shown in Figure 1-8:

1. 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 is reflected back through both sides of the flow cell and the collimating lens to the dual-element photodiode.

The difference in the amount of light striking the elements of the photodiode (because of sample refraction) results in a deflection from the baseline on the chromatogram.

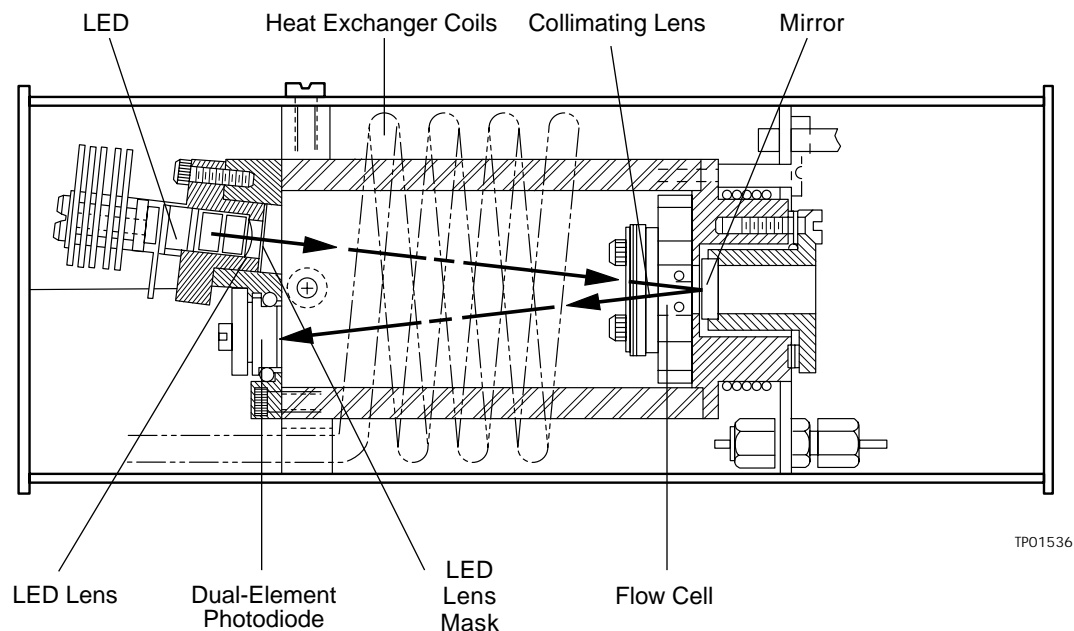


Figure 1-8 Waters 2410 Differential Refractometer Optics Bench Assembly Light Path

1.3.3 Electronics

The 2410 refractometer has both analog and digital components, and includes hardware such as the front panel keyboard and printed circuit (PC) boards and their interconnections. The following PC boards are included in the 2410 refractometer electronics.

- **CPU Board** – Provides the interface between the analog input signals from the optics and the microprocessor, for further signal conditioning. Generates analog output signals, drives the LED, Auto Zero, and signal compensation electronics, and stores and executes input from the front panel keypad and the rear panel contact closures. Provides communication between the 2410 refractometer and external devices through the IEEE-488 interface and terminal strip input/output connections.
- **Front Panel Board** – Controls the keypad, indicators, and display.
- **Power Distribution Board** – Distributes DC voltages to the CPU board, fan, and heaters. Provides the electronic switching for control of the oven compartment.

Chapter 2

Installing the 2410 Refractometer

| | | |
|-----|--|-----|
| 2.1 | Introduction | 2-1 |
| 2.2 | Site Selection and Power Requirements..... | 2-2 |
| 2.3 | Unpacking and Inspection | 2-4 |
| 2.4 | Making Electrical Power Connections | 2-5 |
| 2.5 | Making Fluidic Connections | 2-5 |

2

Installing the 2410 Refractometer

This chapter describes the procedures for selecting the site for installing the Waters 2410 Differential Refractometer, unpacking and inspecting the instrument, installing fuses, and making fluidic connections. For information on connecting the 2410 refractometer to other devices, see Chapter 3.

2.1 Introduction

Figure 2-1 shows the major steps in installing the Waters 2410 Differential Refractometer.

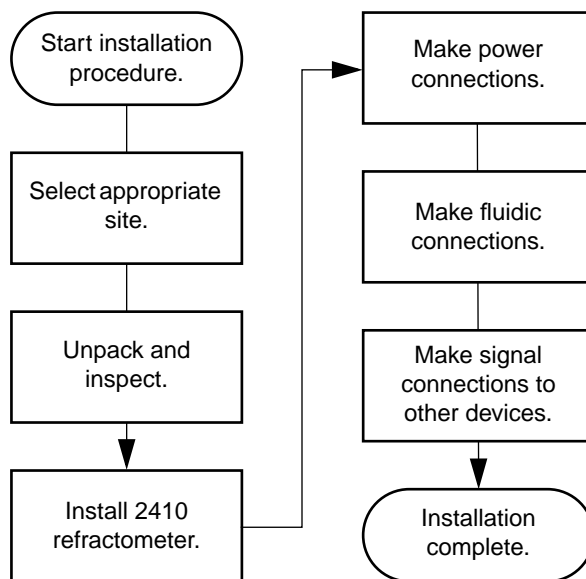
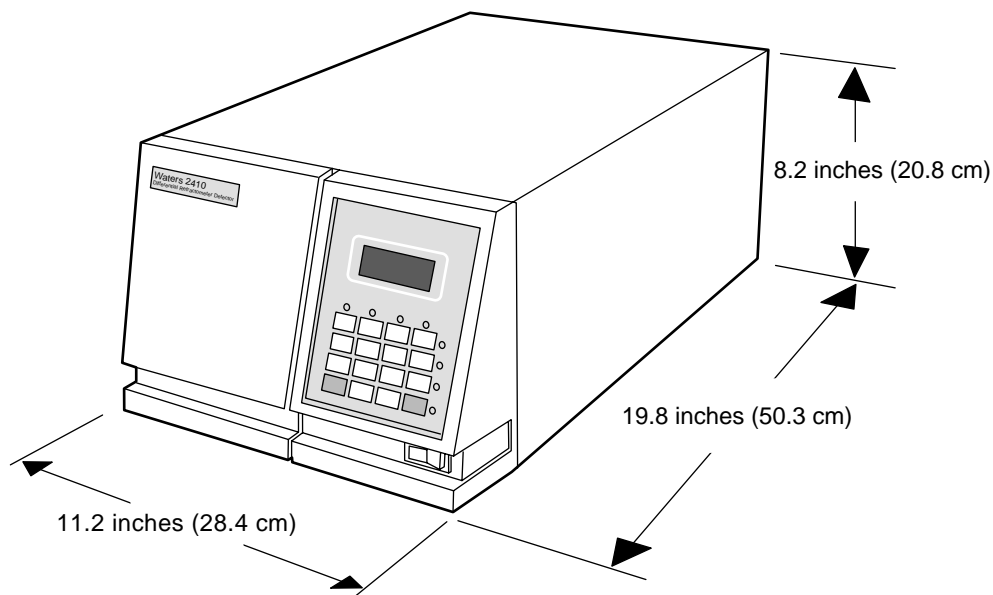


Figure 2-1 Major Steps in Installing the 2410 Differential Refractometer

Figure 2-2 shows the dimensions of the 2410 refractometer.



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Figure 2-2 Dimensions of the 2410 Refractometer



Attention: Access to the instrument inside the top cover is not required. All required access is through the left front panel where the fluidic connections are located (see Section 2.5, Making Fluidic Connections).

2.2 Site Selection and Power Requirements

Reliable operation of your 2410 refractometer depends on a proper installation site and a suitable power supply.

Site Selection Requirements

Install the Waters 2410 Differential Refractometer in an area that meets the requirements listed in Table 2-1.

Table 2-1 Installation Site Requirements

| Parameter | Requirement |
|-----------------------------|--|
| Operating temperature range | +15 °C to +32.2 °C (59 °F to 90 °F); avoid direct exposure to sunlight and heating/cooling vents. |
| Storage temperature range | -40 °C to 70 °C (-104 °F to 158 °F) |
| Relative humidity | 20% to 80%, noncondensing |
| Storage humidity range | 0% to 90%, noncondensing |
| Bench space | At least 11.2 in. (28.4 cm) wide × 24.8 in. (63 cm) deep × 8.2 in. (20.8 cm) high (includes 5 in. [12.7 cm] clearance at rear) |
| Static electricity | < 8 kV contact |
| Power | Grounded ac, 100/240 Vac, 50/60 Hz |
| Surface orientation | Level (ensures proper drip tray function) |

Power Requirements

The 2410 refractometer, which operates over the range 100 Vac to 240 Vac, is shipped from the factory with two 2.0 A fuses.

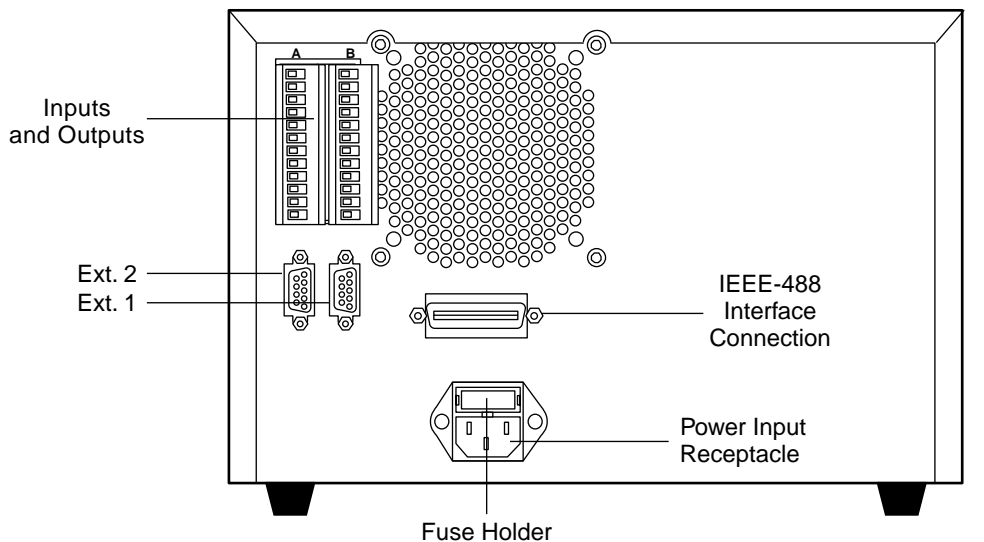


Caution: To avoid electrical shock, power off the 2410 refractometer and unplug the power cord from the rear panel receptacle before you replace a fuse.



Caution: To reduce the risk of fire hazard, always replace the fuse with the same type and rating.

The two fuses are located above the power input receptacle within the power input module on the rear panel (Figure 2-3).



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Figure 2-3 Waters 2410 Refractometer Rear Panel

To replace a fuse in the 2410 refractometer, see Section 6.2, Replacing Fuses.

2.3 Unpacking and Inspection

The Waters 2410 refractometer shipping carton contains:

- Certificate of Structural Validation
- Waters 2410 Differential Refractometer
- Startup Kit
- *Waters 2410 Differential Refractometer Operator's Guide*
- Release Notes

To unpack the 2410 refractometer:

1. Check the contents of the shipping carton against the packing list to ensure you have received all items.
2. Save the shipping carton for future transport or shipment.

If you see any damage or discrepancy when you inspect the contents of the carton, immediately contact the shipping agent. *U.S. and Canadian customers only*, also contact Waters Technical Service at (800) 252-4752. Other customers, call your local Waters subsidiary or your local Waters Technical Service Representative, or call Waters corporate headquarters for assistance at (508) 478-2000 (U.S.).

Note: *Make sure the instrument serial number on the rear panel nameplate or inside the left front panel corresponds to the number on the instrument validation certificate.*

For more information about shipments, damages, and claims, see *Appendix C, Warranty Information*.

2.4 Making Electrical Power Connections

To connect the 2410 refractometer to the ac power supply:

1. Plug the receptacle end of the power cord into the ac power input receptacle on the rear panel of the detector (Figure 2-3).
2. Plug the other end of the power cord into a properly grounded ac power source.

For information about the remaining rear panel electrical connections, see *Chapter 3, Making Signal Connections*.

2.5 Making Fluidic Connections

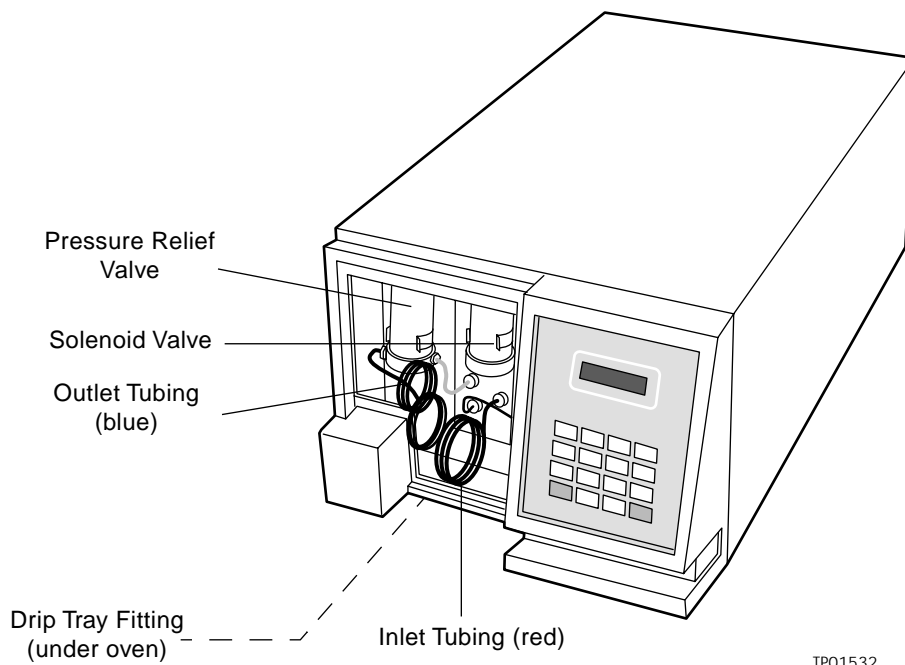


Caution: *To avoid chemical hazards, always observe good laboratory practices when handling solvents. Refer to the Material Safety Data Sheets for solvents in use.*

This section describes the procedures for connecting the 2410 refractometer to:

- A column or another detector
- A waste container
- The drip tray

The fluidic connections for the 2410 refractometer are located to the left of the keypad on the front panel (Figure 2-4).



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Figure 2-4 Fluidic Connections

2.5.1 Connecting a Column or Second Detector

Note: If you are using more than one detector in your system, the Waters 2410 Differential Refractometer must be connected as the last detector in line.

Required Materials

- 1/16-inch stainless steel tubing, 0.009-inch ID (from Startup kit)
- Waters 1/16-inch stainless steel tubing cutter or file
- Pliers, cloth-covered
- Two compression fittings and ferrules (from Startup kit)
- 5/16-inch open-end wrench

To connect a column or other detector to the 2410 refractometer:

1. Measure the minimum length of tubing needed to connect the column or other detector outlet to the inlet tubing port.

2. Cut the tubing to the required length.
 - a. Use the stainless steel tubing cutter or a file with a cutting edge to scribe the circumference of the tubing at the desired end point.
 - b. 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.
 - c. File the ends smooth and straight for maximum column efficiency, and remove all burrs.
3. Slide a compression screw and ferrule over one end of the tubing, as shown in Figure 2-5.

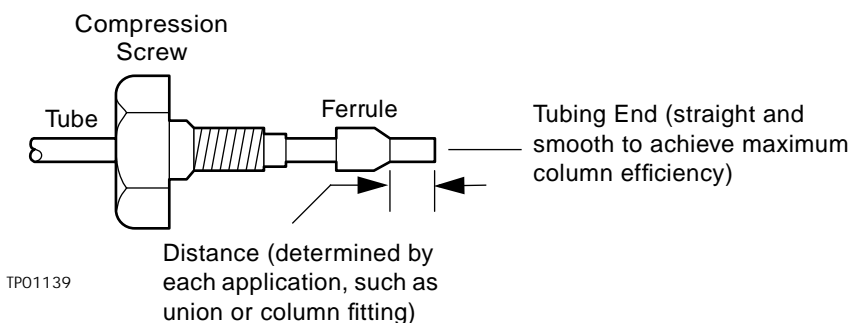


Figure 2-5 Ferrule and Compression Screw Assembly

4. Bottom the tubing in the inlet tubing port fitting of the refractometer, then seat the ferrule by tightening the compression screw 3/4-turn past finger-tight with the 5/16-inch open-end wrench.
5. Repeat steps 3 and 4 to connect the tubing to the outlet fitting of the column or another detector.

2.5.2 Connecting to Waste

Because the 2410 refractometer flow cell is very sensitive to backpressure, be sure to use waste tubing that is 0.040-inch ID and that is no more than 18 to 24 inches (45 to 60 cm) long.

Required Materials

- 1/16-inch stainless steel tubing, 0.040-inch ID (from Startup kit)
- Waters 1/16-inch stainless steel tubing cutter or file
- One compression fitting and ferrule (from Startup kit)

- 5/16-inch open-end wrench
- Waste container

To connect the 2410 refractometer to waste:

1. Cut the minimum length of tubing needed, as described in Section 2.5.1, Connecting a Column or Second Detector.
2. Slide the compression fitting and ferrule over one end of the 0.040-inch tubing, as shown in Figure 2-5.
3. Bottom the tubing in the outlet tubing port fitting of the refractometer, then seat the ferrule by tightening the compression screw 3/4-turn past finger-tight with the 5/16-inch open-end wrench.
4. Place the waste container lower than, or at the same level as, the 2410 refractometer.
5. Place the free end of the tubing in the waste container.



Attention: *The maximum pressure for the 2410 refractometer flow cell is 100 psi. The flow cell could be damaged if this pressure is exceeded.*

2.5.3 Connecting to a Drip Tray

The 2410 refractometer contains a drip tray underneath the flow cell behind the front panel to direct solvent leaks to the front of the unit.

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

Required Materials

- PTFE tubing, 0.063-inch ID (from the Startup kit)
- Razor blade

To connect the drip tray:

1. Cut a length of PTFE tubing sufficient to reach between the drip tray and the waste container.
2. Connect the tubing to the white plastic fitting located under the oven of the 2410 refractometer (see Figure 2-4).
3. Insert the other end of the tubing into the waste container.

Chapter 3

Making Signal Connections

- 3.1 Component Connection Overview 3-1
- 3.2 Making IEEE-488 Signal Connections 3-3
- 3.3 Making Non-IEEE-488 Signal Connections 3-8
- 3.4 Connecting the External Column Heaters 3-19

3

Making Signal Connections

This chapter describes procedures for making signal connections between the Waters 2410 Differential Refractometer and other HPLC system components.

3.1 Component Connection Overview

Table 3-1 summarizes the signal connections needed to connect the 2410 refractometer to other HPLC system components.

Table 3-1 Component Connection Summary

| Connector Type | Component |
|---------------------------------|--|
| IEEE-488 Connections | |
| IEEE-488 Connector | <ul style="list-style-type: none"> • Millennium Chromatography Manager through the busLAC/E card • Waters 845/860 Data System through the LAC/E or busSAT/IN Module • Waters PowerLine™ System Controller • Waters 2690 Separations Module |
| Non-IEEE-488 Connections | |
| Analog outputs | <ul style="list-style-type: none"> • 745/745B/746 Data Module (integrator or data system using the A/D interface) • Chart recorder • Compressed data output |
| Event inputs | <ul style="list-style-type: none"> • System controller (used with the Waters 2690 Separations Module and the 600 Series solvent delivery system) • Waters 700 series or a non-Waters autosampler • Waters or non-Waters manual injector |
| 9-Pin DIN | <ul style="list-style-type: none"> • Waters or non-Waters manual injector • Two optional external column heaters |



Figure 3-1 shows the rear panel locations of the connectors used to operate the 2410 refractometer with external devices.

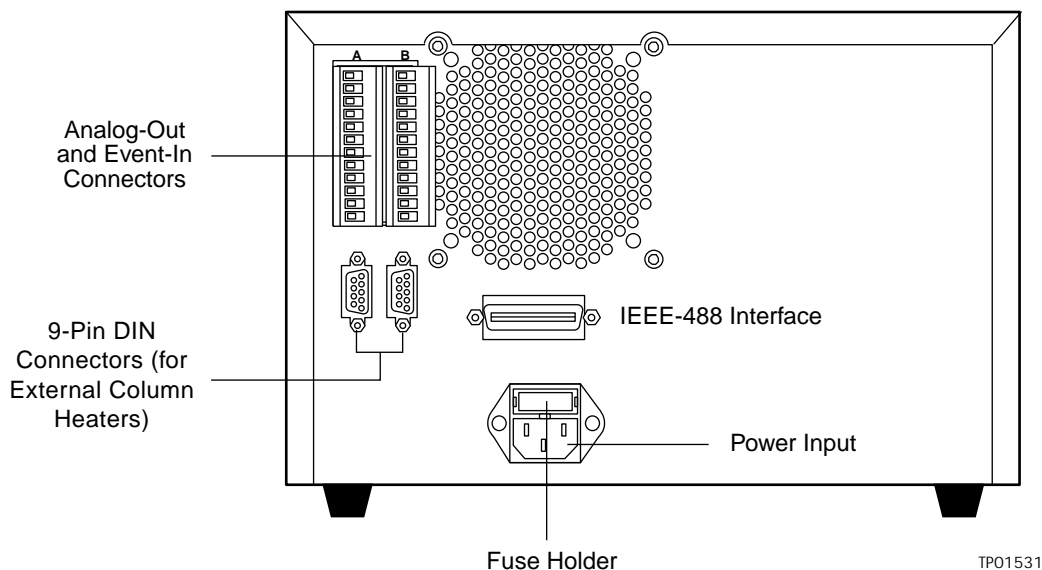


Figure 3-1 Waters 2410 Differential Refractometer Rear Panel

The signal connections you need to make to your 2410 refractometer depend on the signal connections available on the other instruments in your HPLC system.

Figure 3-2 provides an overview of the steps to follow to connect the 2410 refractometer to other instruments in your HPLC system.

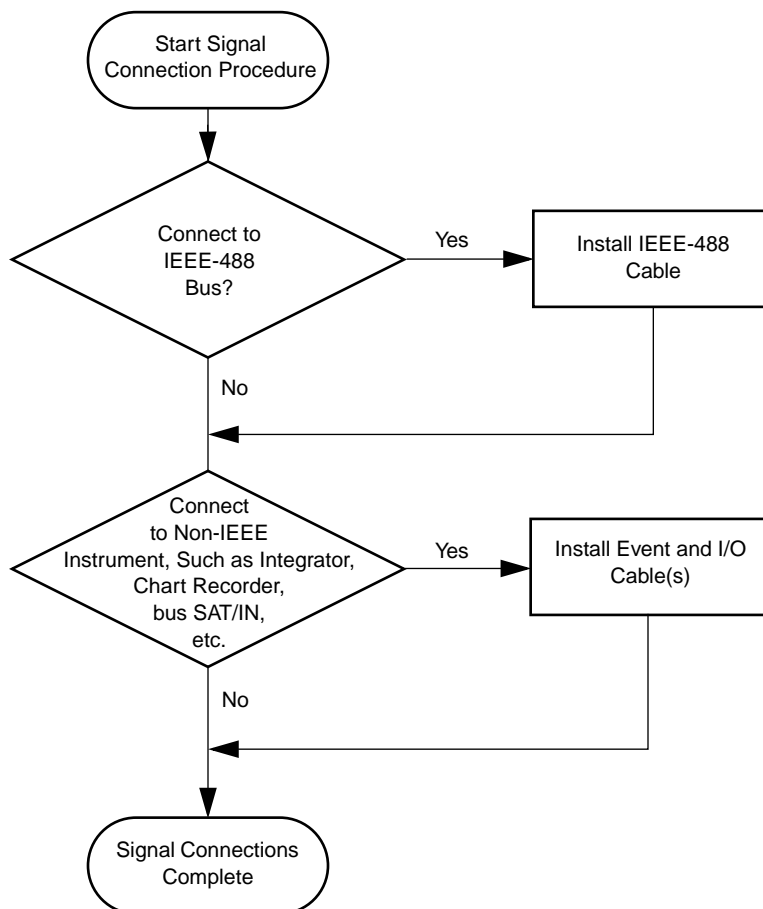


Figure 3-2 Overview of Connecting Components to the 2410 Differential Refractometer

3.2 Making IEEE-488 Signal Connections

You can use the IEEE-488 bus to connect the 2410 refractometer to Waters or third-party data systems.

3.2.1 Connecting to a Waters Data System Using the IEEE-488 Bus

You can use the IEEE-488 bus to connect the 2410 refractometer to a Waters data system in any one of the following configurations (see Figure 3-3, Figure 3-4, and Figure 3-5):

- Millennium Chromatography Manager through the busLAC/E™ card installed on the computer (Figure 3-3)

- Waters 845 or 860 system through a LAC/E module (Figure 3-4)
- Waters 2690 Separations Module as part of an Alliance system (Figure 3-5).

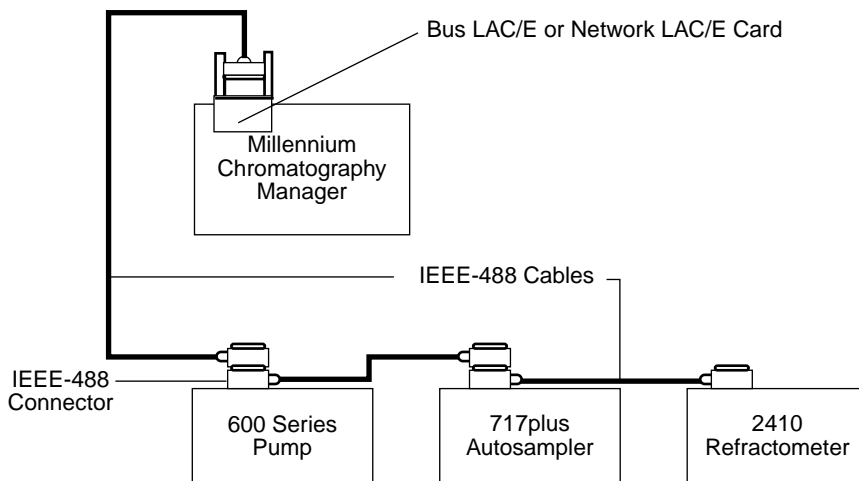


Figure 3-3 Waters Millennium System IEEE-488 Connections

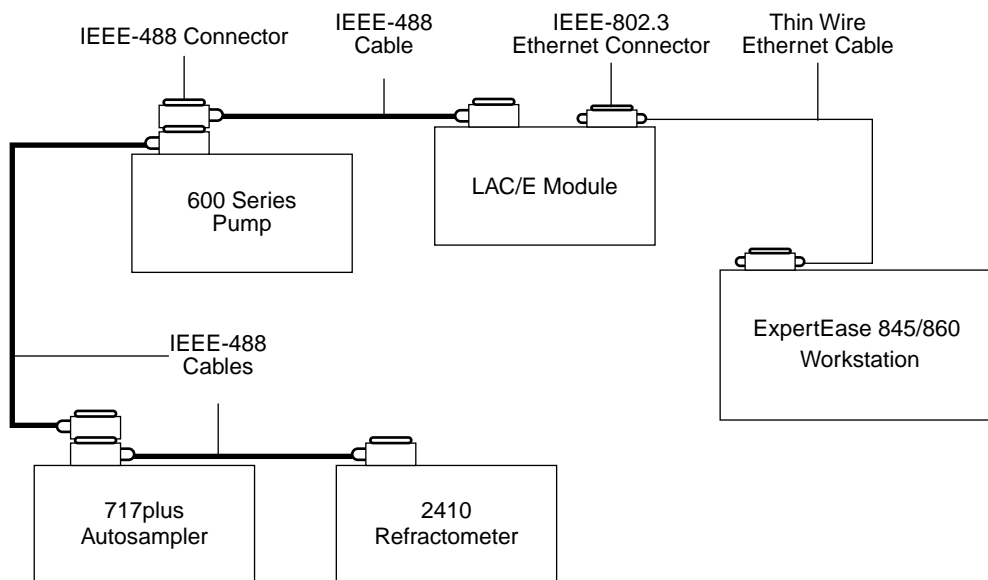


Figure 3-4 Waters 845/860 System IEEE-488 Connections

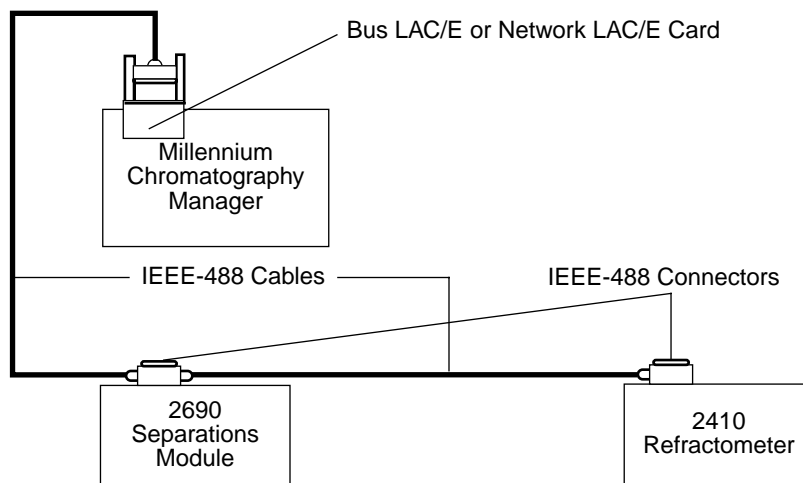


Figure 3-5 Waters Alliance System IEEE-488 Connections

Setting the IEEE-488 Address

Like all other IEEE-488 devices, the 2410 refractometer requires a unique IEEE-488 address to be recognized by an IEEE-488 controller, such as a Millennium Chromatography Manager, busLAC/E module, or an Alliance™ or PowerLine™ System Controller.

The factory-set default IEEE-488 address for the 2410 differential refractometer is 10. To change the IEEE-488 address:

1. Press **2nd Func**, **Clear**, **Clear**, then press **Enter**. The value **diag** is displayed.
2. Press **2nd Func**, **6**, **Enter**.
3. Enter the number corresponding to the desired IEEE-488 address, then press **Enter**.

Note: IEEE-488 addresses must be unique for each instrument in an HPLC system and must be between 2 and 29. Your HPLC system may require that the IEEE-488 address for the 2410 refractometer be greater than that for other devices in the system. Consult your data system or controller operator's manual for more information on IEEE-488 communications.

4. To exit the diagnostic functions, press **2nd Func**, **Clear**, then press **Enter**.

Making Inject Start Signal Connections

When you are using an IEEE-488 data system with the 2410 differential refractometer, the data system or controller must receive an inject start signal from the autosampler or manual injector to initiate the data collection and time-based programs.

Note: Depending on your system configuration, the inject start signal can be transmitted through the IEEE-488 interface or the analog-out/event-in connectors on the 2410 refractometer rear panel. For information on non-IEEE-488 connections, see Section 3.3, Making Non-IEEE-488 Signal Connections.

Table 3-2 summarizes the inject start connections for different system configurations.

Note: If multiple devices in your system require an inject start signal, connect trigger wires from the same (inject out) terminal on the injector to each device.

Table 3-2 Waters 2410 Refractometer Inject Start Connections

| Inject Start Output Source | Inject Start Input Connection (on the 2410 Refractometer) |
|--|--|
| Waters 715, 717, and 717plus, and 2690 Separations Module, on the IEEE-488 bus | IEEE-488 interface (see Section 3.2.1, Connecting to a Waters Data System Using the IEEE-488 Bus) Note: If you are using the Waters 845 or 860 Data System, you must program the multi-method to Start By LAC/E (refer to the ExpertEase Reference Guide for details). |
| Waters 715, 717, and 717plus <i>not</i> on the IEEE-488 bus | Chart Mark and Ground |
| Waters 2690 Separations Module <i>not</i> on the IEEE-488 bus | Chart Mark and Ground or Auto Zero and Ground |
| Waters 712 Autosampler | Chart Mark and Ground |
| Waters manual injector, or third-party manual injector or autosampler | Chart Mark and Ground |

3.2.2 Connecting to a Waters PowerLine System Controller

To connect the 2410 refractometer to a Waters PowerLine system controller, use the IEEE-488 interface cables as shown in Figure 3-6.

Each fluid-handling unit is configured with either of the following:

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

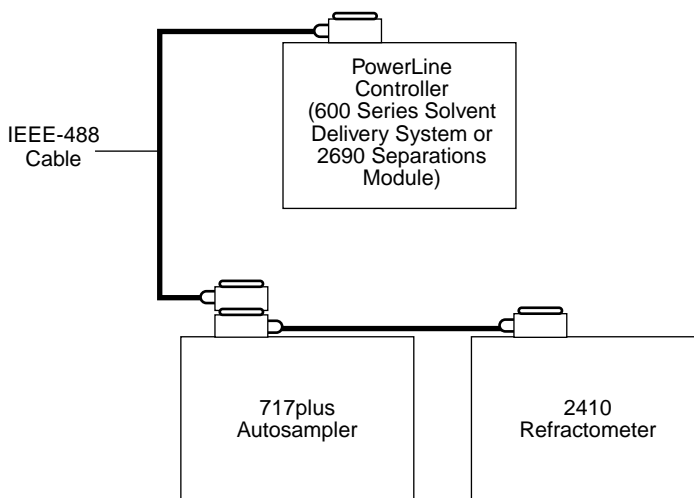


Figure 3-6 Waters PowerLine System Controller IEEE-488 Connections

3.2.3 Connecting to a Manual Injector

If you are using a manual injector with your IEEE-488 system, connect the signal cables from the rear panel connector on the 2410 refractometer to the injector as indicated in Table 3-3.

Table 3-3 Waters 2410 Connections to a Manual Injector

| 2410 Refractometer (Connector B) | Manual Injector |
|-------------------------------------|--|
| Chart Mark + (red) | One set of spade lug Chart Mark terminals (the Waters injector includes two pairs of cables that are functionally identical) |
| Chart Mark - (black) | |

For information on injection trigger signals from a manual injector, see Section 3.3.5, Connecting Injection Trigger Signals.

3.3 Making Non-IEEE-488 Signal Connections

To connect the 2410 refractometer to instruments that lack an IEEE-488 bus, you use the analog-out/event-in (I/O) connectors on the rear panel (Figure 3-7). Figure 3-7 shows the two I/O connectors (and their corresponding pin-outs) on the 2410 refractometer rear panel. Table 3-4 describes the functions of each connector.

This section describes signal connections between the 2410 refractometer rear panel analog-out/event-in connectors and the following:

- Waters 2690 Separations Module (used independently of the IEEE-488 interface)
- Waters 745/745B/746 Integrator
- Chart recorder
- Waters SAT/IN module
- Waters or other manual injector
- Other manufacturer's integrator or A/D interface device



Caution: To avoid electrical shock, power off the 2410 refractometer before making any electrical connections.



Attention: To meet the regulatory requirements of immunity from external electrical disturbances that may affect the performance of this instrument, do not use cables longer than 9.8 feet (3 meters) when you make connections to the analog-out/event-in connectors. In addition, ensure you always connect the shield of the cable to ground at one instrument only.

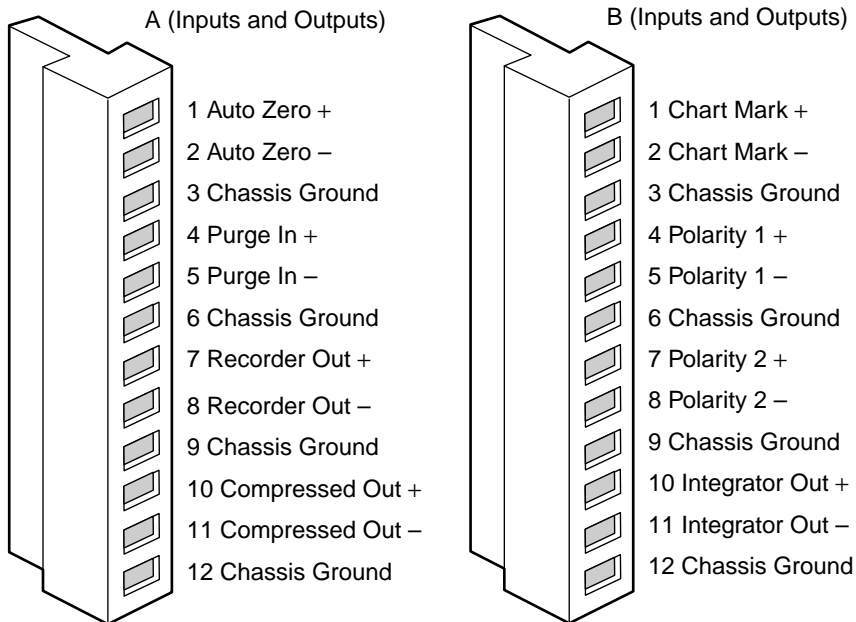


Figure 3-7 Waters 2410 Rear Panel Analog-Out/Event-In Connectors

Table 3-4 describes the functions of the 2410 refractometer analog-out/event-in connectors.

Table 3-4 Waters 2410 Analog-Out/Event-In Connections

| Signal Connections | Description |
|--|--|
| Chart Mark Polarity 1 and 2 Auto Zero Purge | Accept TTL-level (0 to +5 V) or contact closure signals from an external instrument |
| Recorder Out | Sends a ± 2 V (full scale) signal to a chart recorder |
| Integrator Out | Sends a ± 2 V (full scale) signal to an integrator or computer |
| Compressed Out | Sends a compressed (logarithmic) 0 to +10 mV maximum output signal to a chart recorder or integrator |

3.3.1 Connecting to a Stand-Alone 2690 Separations Module

Note: When you use the 2690 Separations Module as the system controller on the IEEE-488 bus, follow the instructions for connecting to a Waters PowerLine system (see Section 3.2.2, Connecting to a Waters PowerLine System Controller).

When you use the 2690 Separations Module as a stand-alone controller (not on the IEEE-488 bus or under Millennium software control), you can make the following signal connections using the 2410 refractometer analog-out/event-in connectors:

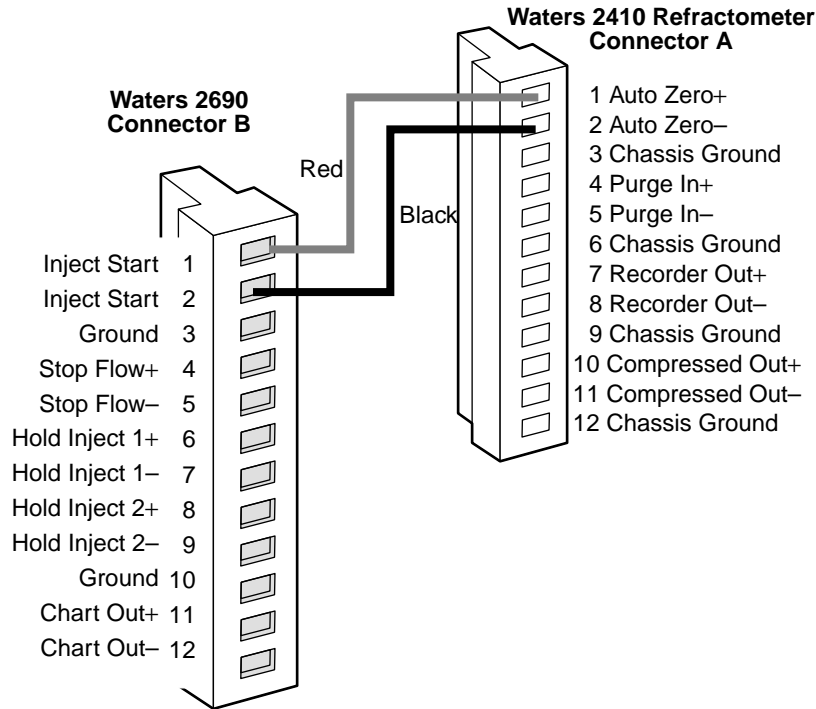
- Auto zero on inject
- Chart mark on inject
- Both chart mark and auto zero on inject

Generating Auto Zero on Inject

To generate the auto zero function on the 2410 refractometer at the start of an injection from the 2690 Separations Module, make the connections shown in Table 3-5 and Figure 3-8.

Table 3-5 Auto Zero Connections Between the 2690 Separations Module and the 2410 Refractometer

| 2690 Separations Module (Connector B) | 2410 Refractometer (Connector A) |
|--|-------------------------------------|
| Pin 1 Inject Start | Pin 1 Auto Zero + |
| Pin 2 Inject Start | Pin 2 Auto Zero – |



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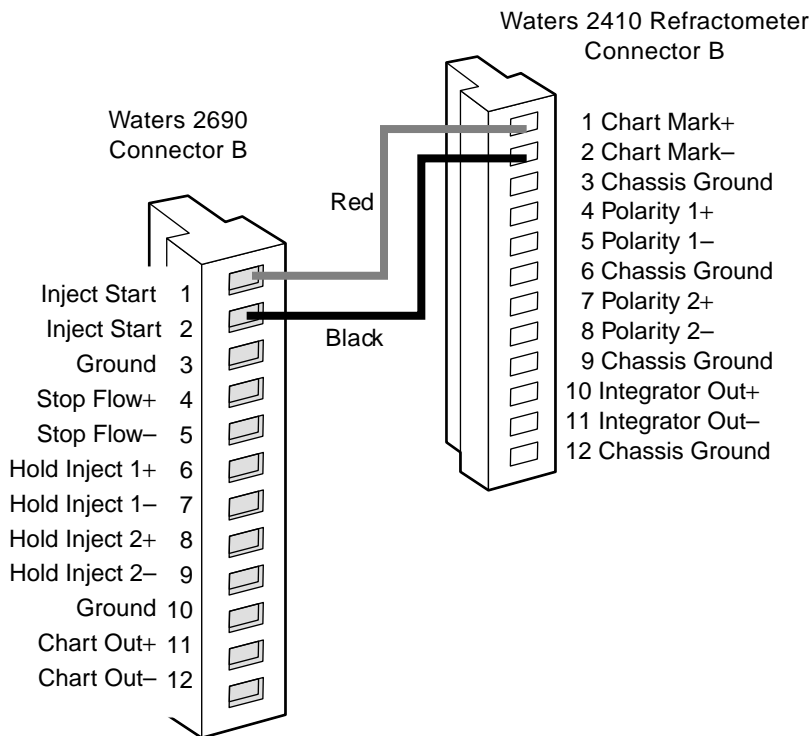
Figure 3-8 Auto Zero Connections Between the 2690 Separations Module and the 2410 Refractometer

Generating Chart Mark on Inject

To generate the chart mark function on the 2410 refractometer at the start of an injection from the 2690 Separations Module, make the connections shown in Table 3-6 and Figure 3-9.

Table 3-6 Chart Mark Connections Between the 2690 Separations Module and the 2410 Refractometer

| 2690 Separations Module (Connector B) | 2410 Refractometer (Connector B) |
|---------------------------------------|----------------------------------|
| Pin 1 Inject Start | Pin 1 Chart Mark + |
| Pin 2 Inject Start | Pin 2 Chart Mark - |



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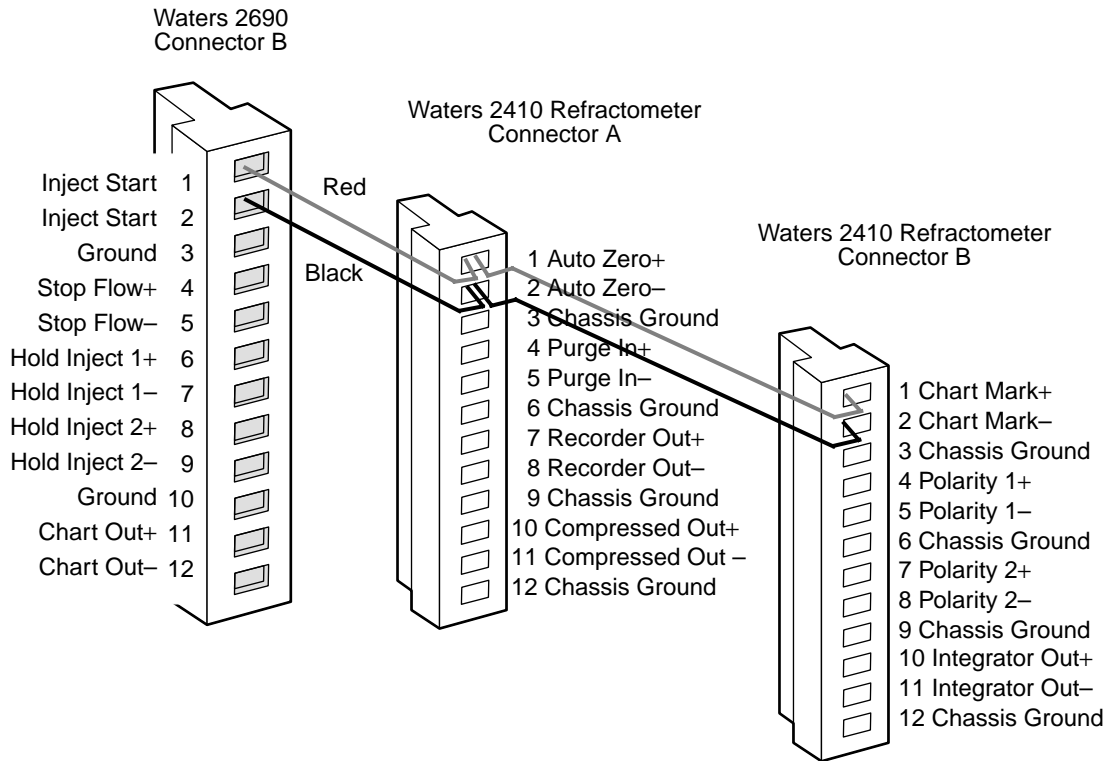
Figure 3-9 Chart Mark Connections Between the 2690 Separations Module and the 2410 Refractometer

Generating Chart Mark and Auto Zero

To generate both a chart mark and an auto zero signal from the 2690 Separations Module to the 2410 refractometer, make the connections shown in Table 3-7 and Figure 3-10.

Table 3-7 Chart Mark and Auto Zero Connections Between the 2690 Separations Module and the 2410 Refractometer

| 2690 Separations Module (Connector B) | 2410 Refractometer (Connector A) | 2410 Refractometer (Connector B) |
|---------------------------------------|----------------------------------|----------------------------------|
| Pin 1 Inject Start | Pin 1 Auto Zero + | Pin 1 Chart Mark + |
| Pin 2 Inject Start | Pin 2 Auto Zero - | Pin 2 Chart Mark - |



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Figure 3-10 Chart Mark and Auto Zero Connections Between the 2690 Separations Module and the 2410 Refractometer

3.3.2 Connecting to the Waters 745/745B/746 Data Module

To send an integrator analog output signal ($-2V$ to $+2V$) from the 2410 refractometer to the Waters 745/745B/746 Data Module, make the connections shown in Table 3-8 and Figure 3-11.

Table 3-8 Analog Output Connections to a 745/745B/746 Data Module

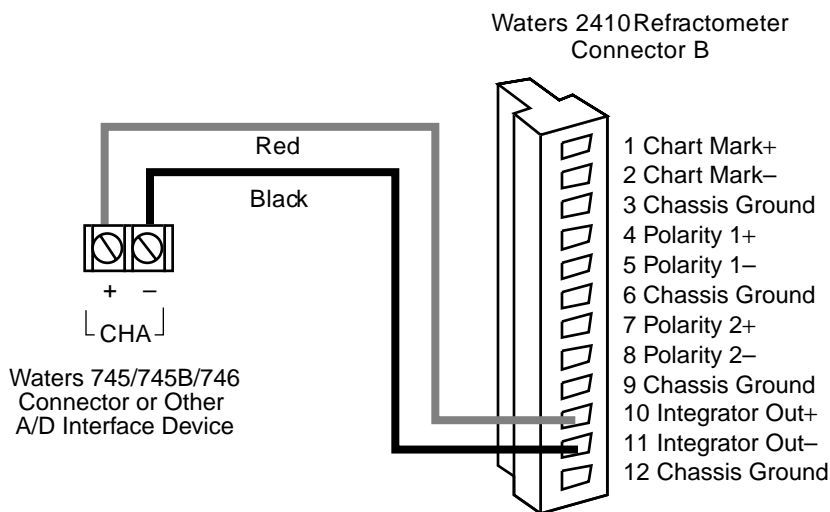
| 745/745B/746 Rear Panel Connectors | 2410 Refractometer (Connector B) |
|------------------------------------|----------------------------------|
| CHA (+) | Pin 10 Integrator Out+ (red) |

Table 3-8 Analog Output Connections to a 745/745B/746 Data Module (Continued)

| 745/745B/746 Rear Panel Connectors | 2410 Refractometer (Connector B) |
|---|----------------------------------|
| CHA (-) | Pin 11 Integrator Out- (black) |
| Shield not used; tape back to prevent shorting. | |

Note: If you use the Waters 745/745B/746 with a chart recorder, use separate channels for plotting and integration. Otherwise, changes in chart recorder attenuation may affect the integration of the peaks.

Note: If you use another manufacturer's integrator or A/D device, you may need to connect the Chassis Ground (pin 12) to the 2410 detector's Integrator Out- (black lead) or an equivalent connection.



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Figure 3-11 Connections to a Waters 745/745B/746 Data Module

3.3.3 Connecting to a Chart Recorder

To send an analog output signal from the 2410 refractometer to a chart recorder, make the connections shown in Table 3-9 and Figure 3-12.

Table 3-9 Analog Output Connections to a Chart Recorder

| Chart Recorder Connectors | 2410 Refractometer (Connector A) |
|---|----------------------------------|
| Pen 1 (+) | Pin 7 Recorder Out + (red) |
| Pen 1 (-) | Pin 8 Recorder Out - (black) |
| Shield not used; tape back to prevent shorting. | |

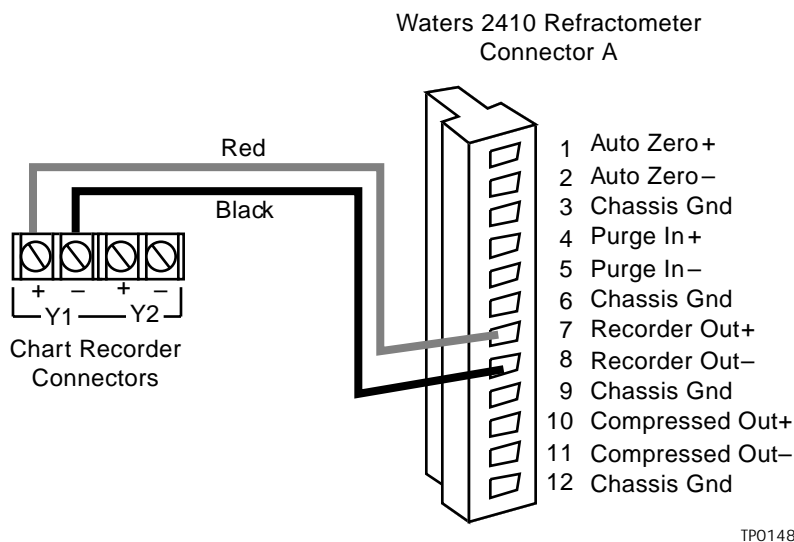


Figure 3-12 Analog Output Connections to a Chart Recorder

Performing Chart Mark with the Chart Recorder

If you are controlling the 2410 refractometer from the 745/745B/746 data module and you want to send a chart mark pulse to the chart recorder at the start of each run, connect the external device (system controller, autosampler, or manual injector) to the 2410 refractometer Chart Mark screw terminals, as described in Section 3.3.2, Connecting to the Waters 745/745B/746 Data Module.

3.3.4 Connecting to the Waters 845/860 ExpertEase System

To send an integrator analog output signal (–2V to +2V) from the 2410 refractometer to an 845/860 ExpertEase System (through a two-channel SAT/IN module), make the connections shown in Table 3-10 and Figure 3-13.

Table 3-10 Analog Output Connections to the Bus SAT/IN Module

| SAT/IN Module Connector | 2410 Refractometer (Connector B) |
|-------------------------|----------------------------------|
| CHANNEL 1 or CHANNEL 2 | Pin 10 Integrator Out + (white) |
| | Pin 11 Integrator Out – (black) |

See Section 3.2.1, Connecting to a Waters Data System Using the IEEE-488 Bus, Figure 3-4, for information on connecting the remaining components of the 845/860 Data System.

3

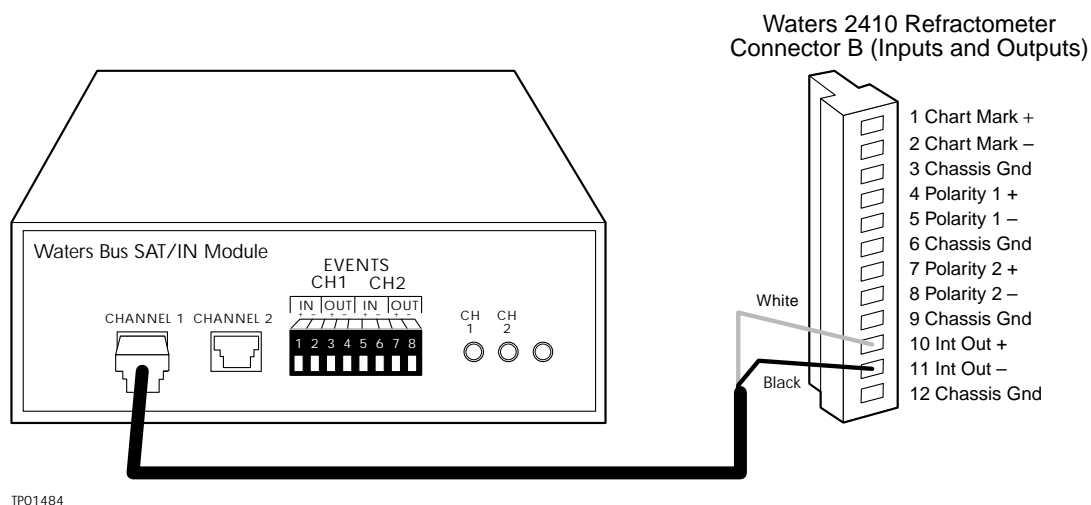


Figure 3-13 Analog Output Connections to the Bus SAT/IN Module

3.3.5 Connecting Injection Trigger Signals

The 2410 refractometer accepts the following injection trigger signals from a manual injector:

- Auto zero signal to automatically adjust the zero offset of the 2410 refractometer each time the injector makes an injection
- Chart mark (inject start) signal from a contact closure signal with each injection

Each time the 2410 refractometer receives a signal from a manual injector, it performs the corresponding auto zero or chart mark function.

To send an auto zero or chart mark signal from a manual injector to the 2410 refractometer, make the connections shown in Table 3-11 and Figure 3-14 and Table 3-12 and Figure 3-15.

Table 3-11 Auto Zero Connections to a Manual Injector

| 2410 Refractometer (Connector A) | Manual Injector Connector |
|-------------------------------------|--|
| Pin 1, Auto Zero + (red) | Two spade lug terminal connectors (both cables may be functionally identical) or similar connectors. |
| Pin 2, Auto Zero – (black) | |

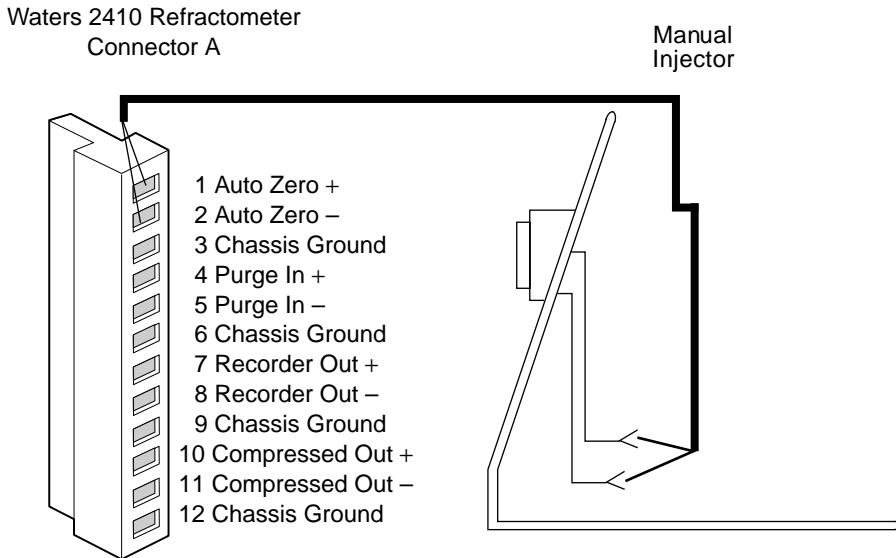


Figure 3-14 Auto Zero Connection to a Manual Injector

Table 3-12 Chart Mark Connections to a Manual Injector

| 2410 Refractometer (Connector B) | Manual Injector Connector |
|-------------------------------------|--|
| Pin 1, Chart Mark + (red) | Two spade lug terminal connectors (both cables may be functionally identical) or similar connectors. |
| Pin 2, Chart Mark – (black) | |

Waters 2410 Refractometer
Connector B

Manual
Injector

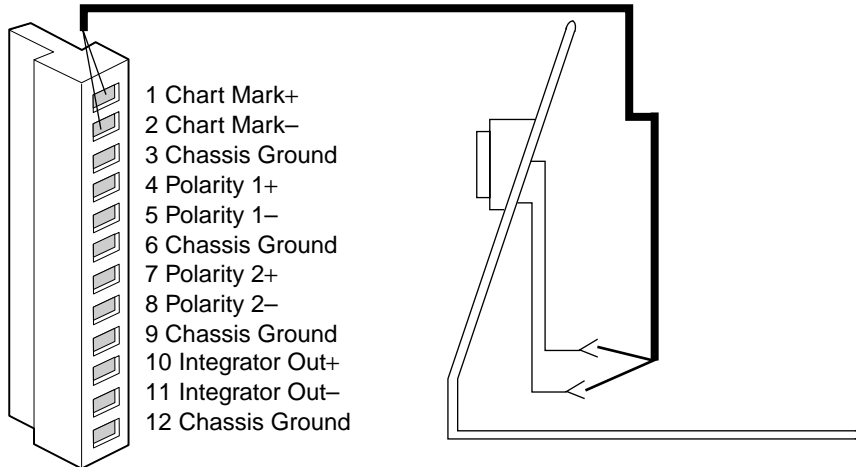


Figure 3-15 Chart Mark Connections to a Manual Injector

3.3.6 Polarity Connections

The Polarity 1 and 2 contact closures on the rear panel of the 2410 refractometer determine the peak polarity of the output signal according to the following conditions (negative polarity results in negative, or inverted, peaks):

- Polarity 1 serves as a positive/negative input
- Polarity 2 serves as an external input (Polarity 1) enable
- When Polarity 2 is open (not connected), the +/- key on the 2410 front panel or an IEEE-488 connected data system (such as the Millennium Chromatography Manager or PowerLine) determines the polarity (see Section 5.2.5, Polarity Guidelines).

- When Polarity 2 is closed (connected to an instrument), Polarity 1 determines peak polarity. Polarity 1 open (disconnected) generates negative polarity. Polarity 1 closed (connected) generates positive polarity.

Table 3-13 summarizes the polarity options.

Table 3-13 Polarity Options

| Polarity 2 | Polarity 1 | Recorder Polarity |
|-------------------|-------------------|--------------------------|
| Open | Open | No Effect |
| Open | Closed | No Effect |
| Closed | Open | Negative (Inverted) |
| Closed | Closed | Unchanged |

3.4 Connecting the External Column Heaters

The Waters 2410 Differential Refractometer can control up to two optional external column heaters through the EXT 1 and EXT 2 ports on the rear panel of the detector (Figure 3-16). The ports are standard 9-pin DIN connectors.

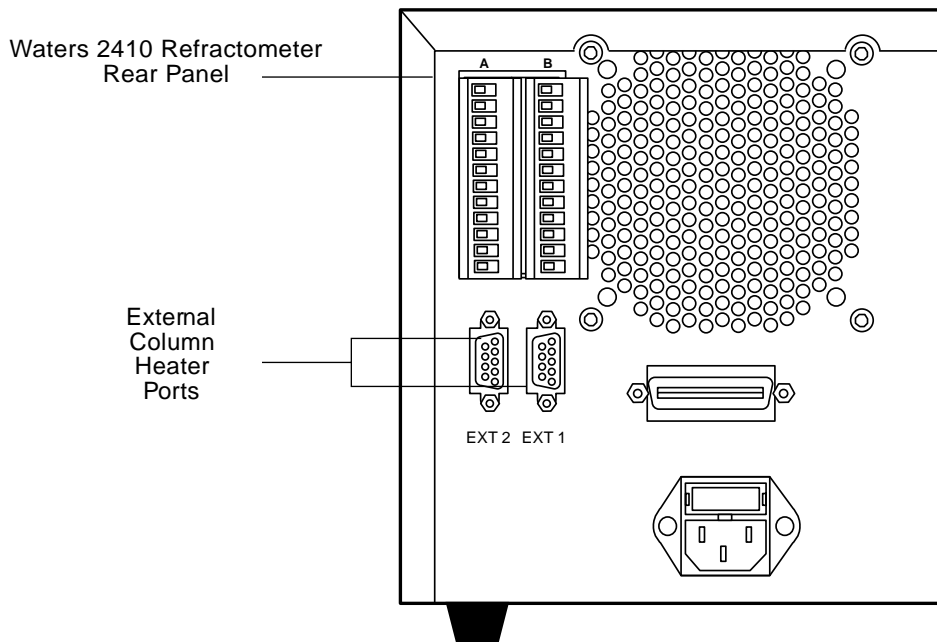


Figure 3-16 2410 Refractometer External Column Heater Ports

3

Chapter 4

Preparing Solvents

| | | |
|-----|-------------------------------|-----|
| 4.1 | Common Solvent Problems | 4-1 |
| 4.2 | Selecting a Solvent | 4-2 |
| 4.3 | Solvent Degassing | 4-4 |

4

Preparing Solvents

Proper solvent selection and preparation are critical in differential refractometry to prevent baseline changes such as drift, noise, or an erratic baseline. This chapter presents information on:

- Common solvent problems
- Selecting a solvent
- Solvent degassing



Caution: *To avoid chemical hazards, always 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 2410 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). However, factors other than the presence of dissolved sample molecules can affect a 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

An ideal solvent for your analysis:

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

Solvent Quality

Use spectral-grade or HPLC-grade solvents to ensure:

- 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 fluidic path

Preparation Checklist

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

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

Water

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

Buffers

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

Tetrahydrofuran (THF)

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



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

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 an RI significantly different from 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 | <i>n</i> -Decane | 1.412 |
| Acetonitrile | 1.344 | Amyl chloride | 1.413 |
| Ethyl ether | 1.353 | Dioxane | 1.422 |
| <i>n</i> -Pentane | 1.358 | Ethyl bromide | 1.424 |
| Acetone | 1.359 | Methylene chloride | 1.424 |
| Ethanol | 1.361 | Cyclohexane | 1.427 |
| Methyl acetate | 1.362 | Ethylene glycol | 1.427 |
| Isopropyl ether | 1.368 | 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 |

Table 4-1 Refractive Indices of Common Solvents (*Continued*)

| Solvent | RI | Solvent | RI |
|---------------------------|-------|---------------------------|-------|
| Acetic acid | 1.372 | Chloroform | 1.443 |
| Isopropyl chloride | 1.378 | Ethylene dichloride | 1.445 |
| Isopropanol | 1.38 | Carbon tetrachloride | 1.466 |
| <i>n</i> -Propanol | 1.38 | Dimethyl sulfoxide (DMSO) | 1.477 |
| Methylethylketone | 1.381 | Toluene | 1.496 |
| Diethyl amine | 1.387 | Xylene | ~1.50 |
| <i>n</i> -Propyl chloride | 1.389 | Benzene | 1.501 |
| Methylisobutylketone | 1.394 | Pyridine | 1.510 |
| Nitromethane | 1.394 | Chlorobenzene | 1.525 |
| 1-Nitropropane | 1.400 | <i>o</i> -Chlorophenol | 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 or solvent delivery system operation

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

4.3.1 Gas Solubility

The amount of gas that can dissolve in a given volume of liquid depends on:

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

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

Effects of Intermolecular Forces

Nonpolar gases (N₂, O₂, CO₂, 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 Temperature

Temperature affects the solubility of gases. If the dissolution is exothermic, the solubility of the gas decreases when you heat the solvent. If the dissolution is endothermic, the solubility increases when you heat the solvent. For example, the solubility of He in H₂O 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

Solvent degassing helps you attain a stable baseline and also improves reproducibility and pump performance.

There are three common methods used to degas solvents:

- Sparging with helium
- Reducing pressure by vacuum
- Sonication

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

Sparging

Sparging removes gases from solution by displacing dissolved gases in 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.

Note: *Sparging may change the composition of mixed solvents.*

Vacuum Degassing

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

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

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 efficient degassing operation for your application. To remove dissolved gas quickly, consider the following:

Sparging

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

Vacuum Degassing

The longer a solvent is exposed to the vacuum, the more dissolved gases are removed. Two factors affect the amount of time the solvent is exposed to the vacuum:

- **Flow rate** – At low flow rates, most of the dissolved gas is removed as the solvent passes through the vacuum chamber. At higher flow rates, lesser amounts of gas per unit volume of solvent are removed.
- **Surface area of the degassing membrane** – The length of the degassing membrane is fixed in each vacuum chamber. To increase the length of membrane, you can connect two or more vacuum chambers in series.

The in-line degasser is available as an option or factory-installed in the Waters® 2690 Separations Module, XE model.

When you are using the 2690 Separations Module with the 2410 refractometer, set the in-line degasser to “continuous” degas mode.

Select the most efficient degassing operation for your application. To remove dissolved gas quickly, consider the following degassing considerations.

Sonication Plus Vacuum

Sonication combined with vacuum degasses solvent very quickly. This technique is less likely to change the composition of mixed solvents because the mixed solvents are held under vacuum for only a short time (less than a minute is usually sufficient).



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

Chapter 5

Using the 2410 Refractometer

| | | |
|-----|--|------|
| 5.1 | Using the Front Panel | 5-1 |
| 5.2 | Selecting Parameter Values..... | 5-9 |
| 5.3 | Starting Up the 2410 Refractometer..... | 5-13 |
| 5.4 | Shutting Down the 2410 Refractometer | 5-15 |

5

Using the 2410 Refractometer

This chapter covers:

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

Stand-Alone Mode

You can use the Waters 2410 Differential Refractometer as a stand-alone module in conjunction with a pump, injector, column, and a recorder or integrator. In this configuration, you control the 2410 refractometer from its front panel. To use the 2410 refractometer in this way, follow the instructions provided in this chapter.

Remote Control Mode

You can use the 2410 refractometer as part of a system configured and controlled by a Waters data system, such as the Millennium Chromatography Manager, or a Waters PowerLine system controller (including the 2690 Separations Module). If you set up the 2410 refractometer in this way, follow the instructions in the appropriate data system or controller operator's guide to set parameters and to control the 2410 refractometer. When the 2410 refractometer is operating in remote control mode, you can continue to run diagnostics from the front panel (see Section 7.3, Diagnostics).

Note: Read Chapter 4, *Preparing Solvents*, before using the 2410 refractometer.

5.1 Using the Front Panel

The 2410 refractometer front panel consists of a four-character LED display, eight LED parameter indicators, and a keypad (Figure 5-1).

Four-Character LED Display

The four-character LED display shows parameter and input values. To display the value of a parameter, press the appropriate parameter key (Figure 5-1 and Table 5-1). The parameter is displayed (in the four-character LED), and its corresponding indicator remains illuminated until you select another parameter.

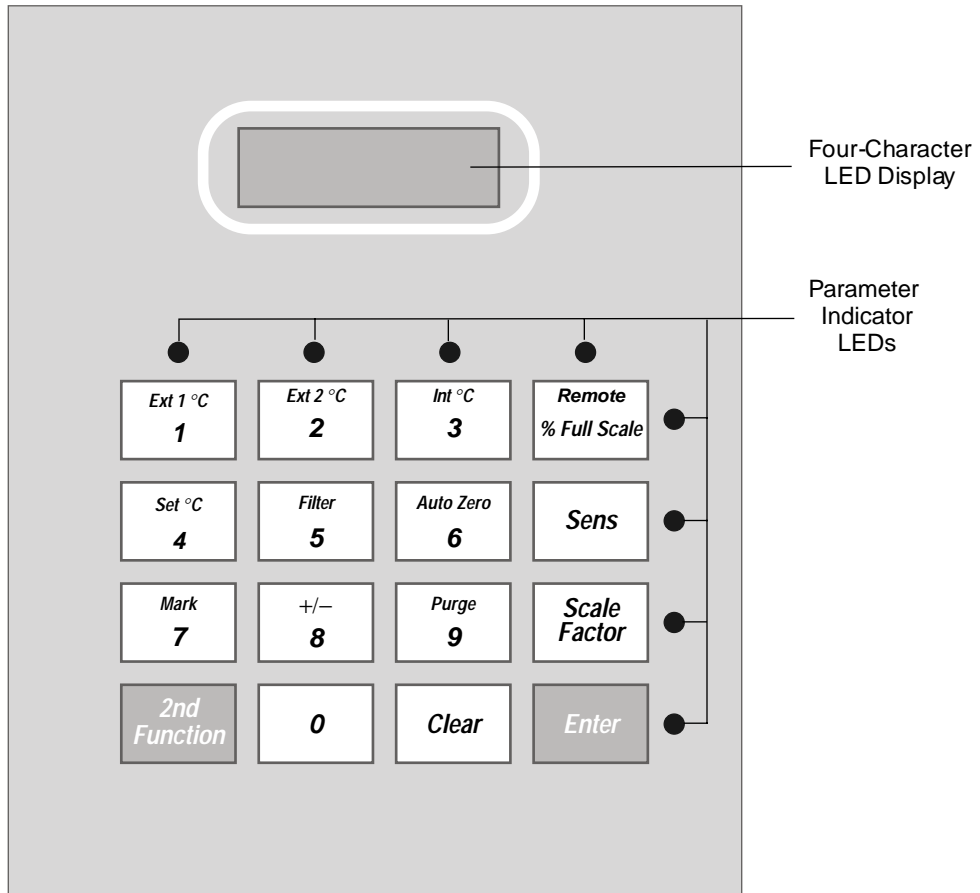


Figure 5-1 Display, LED Indicators, and Keypad

LED Parameter Indicators

Eight parameter indicator LEDs are located above and to the right of the numeric keypad (Figure 5-1). When you select a parameter from those described in Table 5-1, the corresponding LED illuminates.

Table 5-1 Indicator LED Functions

| Parameter Indicator | Description |
|------------------------------------|--|
| Ext 1 °C Ext 2 °C | Illuminates when the current settings for the external column heaters are displayed in the four-character LED; also illuminates when you are changing the settings for the external column heaters. |
| Int °C | Illuminates when the temperature of the internal oven is displayed in the four-character LED; also illuminates when you are changing the temperature. |
| Remote | Illuminates when the 2410 refractometer is under the control of a remote controller. |
| % Full Scale | Illuminates when the chart recorder output of the 2410 differential refractometer (as a percent referenced to 10 mV) is displayed in the four-character LED. |
| Sens | Illuminates when the current sensitivity setting is displayed in the four-character LED; also illuminates when you are changing the sensitivity. |
| Scale Factor | Illuminates when the current scale factor setting is displayed in the four-character LED; also illuminates when you are changing the scale factor. |
| 2nd Func | Illuminates when the 2nd Func key is activated (after pressing the 2nd Func key); stays illuminated for five seconds, waiting for you to press the key whose secondary function you want to access. |

5.1.1 Keypad Functions

You use the keypad (see Figure 5-1) to:

- View the current settings or values of parameters
- Select or enter new parameter settings
- Activate specific operational functions
- Perform diagnostic tests

Some keys scroll through a series of available values. To scroll through the values, you press the key repeatedly until the desired value appears, then release the key and press **Enter**.

Primary and Secondary Functions

Each key is labeled with a *primary function*. When you press a key, the function named on the key is performed. For example, press the **Sens** key and you are prompted to enter a sensitivity value.

Most keys also have a *secondary function*, shown in smaller type (on the key) above the primary function or number. To use a secondary function, press the **2nd Func** key, then the key labeled with the secondary function. For example, press **2nd Func**, then **Purge** to set the 2410 refractometer to purge mode.

Table 5-2 describes how to use the primary and secondary functions.

Table 5-2 Keypad Functions

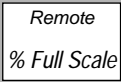
| Key | Description |
|---|--|
|  | Primary |
| | % Full Scale – Displays the chart recorder (REC) output (in millivolts) of the 2410 refractometer as a percent referenced to 10 mV. When the display reads 0001, the output is 1 percent of 10 mV, or 0.1 mV. A value of 0100 means that the output is 100 percent, or 10.0 mV. |
| | Secondary |
| | Remote – When the 2410 refractometer is under active control by a data system or system controller through the IEEE-488 interface, the Remote indicator is illuminated. |

Table 5-2 Keypad Functions (*Continued*)

| Key | Description |
|--------------|---|
| Sens | <p>Sens – Displays the current or selects a new sensitivity value. Repeated pressing of the key scrolls through the allowable values between 1 (least sensitive) and 1024 (most sensitive); or, you can enter a numeric value (only powers of 2 are allowed, such as 2, 4, 8, 16, 32, and so on). The default value is 4. For more information, see Section 5.2.1, Sensitivity Guidelines.</p> |
| Scale Factor | <p>Scale Factor – Selects a scale factor, with allowable values between 1 and 100. The default value is 20.</p> <p>Scale factor affects the magnitude of the peaks on the chart recorder output only. Scale factor does not affect integrator or IEEE-488 data output; it functions as an attenuator for the chart recorder output.</p> <p>See Section 5.2.2, Scale Factor Guidelines, for more information.</p> |
| Enter | <p>Enter – Saves parameter settings in the memory of the 2410 refractometer.</p> |
| Clear | <p>Clear – Erases unsaved parameter entries.</p> |
| 2nd Func | <p>2nd Func – Accesses secondary functions. Pressing the 2nd Func key activates secondary functions. Stays active for five seconds during which the indicator LED located to the right of the Enter key is illuminated.</p> |
| 0-9 | <p>Primary</p> |
| | <p>0-9 – Used to enter values for parameters. After entering a numeric value, press Enter.</p> |

Table 5-2 Keypad Functions (Continued)

| Key | Description |
|--|--|
| <div style="border: 1px solid black; padding: 5px; margin-bottom: 10px; text-align: center;">Ext 1 °C 1</div> <div style="border: 1px solid black; padding: 5px; text-align: center;">Ext 2 °C 2</div> | <p>Secondary</p> <p>Ext1 °C and Ext2 °C – Display the temperature setting of a selected external column heater in degrees Celsius.</p> <p>To change the temperature of a column heater:</p> <ol style="list-style-type: none"> 1. Press 2nd Func followed by Ext1 °C or Ext2 °C. The current temperature setting of the column heater appears in the display, and the corresponding indicator lights up. 2. Press 2nd Func, Set °C, enter the new temperature (ambient to 150 °C), then press Enter. 3. Press Clear to disable. <p>The value of 245.7 appears when no column heater is connected.</p> |
| <div style="border: 1px solid black; padding: 5px; text-align: center;">Int °C 3</div> | <p>Int °C –Displays the current temperature of the internal oven. This is the value that flashes on startup. Press Clear to stop it from flashing.</p> <p>To change the temperature:</p> <ol style="list-style-type: none"> 1. Press 2nd Func followed by Int °C. The temperature of the internal oven appears in the display, and the corresponding indicator lights up. 2. Press 2nd Func, Set °C, enter the new temperature (30 °C to 50 °C), then press Enter. <p>Note: <i>It takes several hours for the optics bench assembly to stabilize at the new temperature. Do not make a run until the temperature has stabilized; the changing temperature causes baseline drift.</i></p> |
| <div style="border: 1px solid black; padding: 5px; text-align: center;">Set °C 4</div> | <p>Set °C – Sets the temperature of a column heater or the internal oven. The range of allowable values (“set points”) for the internal oven is 30 to 50 °C; for the column heaters, it is 0 to 150 °C.</p> <p>Note: <i>The minimum stable set point is 5 °C above the ambient temperature.</i></p> <p>To power off the column heater or internal oven:</p> <ol style="list-style-type: none"> 1. Press 2nd Func followed by Ext1 °C, Ext2 °C, or Int °C (for either column heater or for the internal oven). The temperature of the column heater or oven appears in the display, and the corresponding indicator lights up. 2. Press 2nd Func, Set °C, Clear, then press Enter. |

Table 5-2 Keypad Functions (*Continued*)

| Key | Description |
|---|---|
| <div style="border: 1px solid black; padding: 5px; text-align: center;"> <i>Filter</i> 5 </div> | <p>Filter – Adjusts the time constant of the noise filter to achieve the optimum signal-to-noise ratio. Repeated pressing of the Filter key scrolls through the values 0.2, 1, 3, and 10. Press Enter when you reach the value you want. The default value is 1. For more information, see Section 5.2.3, Time Constant Guidelines.</p> |
| <div style="border: 1px solid black; padding: 5px; text-align: center;"> <i>Auto Zero</i> 6 </div> | <p>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.</p> |
| <div style="border: 1px solid black; padding: 5px; text-align: center;"> <i>Mark</i> 7 </div> | <p>Mark – Sends a chart mark signal to the recorder or data module. The chart mark is always a 10 percent (of full scale) deflection in the positive direction, regardless of chart polarity.</p> |
| <div style="border: 1px solid black; padding: 5px; text-align: center;"> +/- 8 </div> | <p>+/- : Changes the chart polarity. Pressing the +/- key once shows the current setting in the four-character LED. Keeping the +/- key pressed alternates through + (POS) and – (NEG). When the display shows the polarity you want, press Enter.</p> |
| <div style="border: 1px solid black; padding: 5px; text-align: center;"> <i>Purge</i> 9 </div> | <p>Purge – Purges the reference and sample sides of the fluidic path with fresh solvent. Purging requires pressing Purge twice, once to start and then once to finish the purge. During the purge, the display shows the letters PgE. Purge the fluidic path whenever you change solvents or experience an unexpected loss in sensitivity due to excess noise or drift.</p> |

Viewing Parameter Values

To view the current value for a primary function parameter, press the key for the parameter whose value you want to see. To view the current value for a secondary function parameter, press **2nd Func**, then press the key for the secondary function value you want to view.

Changing the Sensitivity or Scale Factor

To change a value for the Sens (sensitivity) or Scale Factor:

1. Press the key for the parameter whose value you want to change.
2. Select a new value by scrolling (Sens only) or by entering the value using the numeric keys.

3. Press **Enter** to save the new value. If you enter an unacceptable value, the 2410 refractometer beeps and returns to the previous value.

Changing the Filter Value

To change the value for the filter:

1. Press the **2nd Func** key.
2. Press the **Filter** key to view the current value.
3. Press the **Filter** key repeatedly to scroll to a new value.
4. Press **Enter** to save the new value. If you do not press **Enter** within 5 seconds or if you enter an unacceptable value, the 2410 refractometer beeps and returns to the previous value.

Changing the Oven or Column Heater Temperature

To change the temperature settings for the oven or the external column heaters:

1. Press the **2nd Func** key, then press the key for the unit whose temperature you want to change (**Ext 1 °C**, **Ext 2 °C**, or **Int °C**).
2. Press the **2nd Func** key, then press the **Set °C** key.
3. Enter a new temperature from the numeric keys (pressing **Clear** powers off the internal oven or column heater).
4. Press **Enter** to save the new temperature. If you enter an invalid temperature, the 2410 refractometer beeps and returns to the previous value.

Changing Polarity

To change output polarity:

1. Press the **2nd Func** key, then press the +/- key.
2. Press the +/- key again to reverse the polarity.
3. Press **Enter** to save the new value.

Using Auto Zero, Mark, and Purge

To use Auto Zero, Mark, or Purge:

1. Press the **2nd Func** key, then press the key for the function you want to access (**Auto Zero**, **Mark**, or **Purge**).

2. Press **Enter**.

When you perform the Auto Zero command, the letters **AX** appear on the display.

When you perform the Mark command, the letters **CH** appear on the display.

When you perform the Purge command, the letters **PgE** appear on the display.

To stop purging, press **2nd Func, Purge**, then press **Enter**. The display returns to the function it displayed before the purge began.

5.2 Selecting Parameter Values

You can adjust the noise level, peak height, peak direction, and the temperatures of the internal oven and column heaters to optimize the performance of the 2410 refractometer. This section provides guidelines and considerations for selecting parameter values that are best suited to your application. The parameters are:

- Sensitivity (Sens key)
- Scale factor
- Time constant (Filter key)
- Temperature (Ext1 °C, Ext2 °C, Int °C, Set °C keys)
- Polarity (+/- key)

5.2.1 Sensitivity Guidelines

Sensitivity affects the magnitude of the output signal to an integrator or a recorder. Increasing the sensitivity (Sens) setting increases the resulting peak areas, but it also increases baseline noise and the response to environmental fluctuations. In addition, an increase in sensitivity reduces the dynamic range over which the refractometer output is useful. Refer to Figure 5-2 for examples of the effects of varying the Sens setting on a chromatogram.

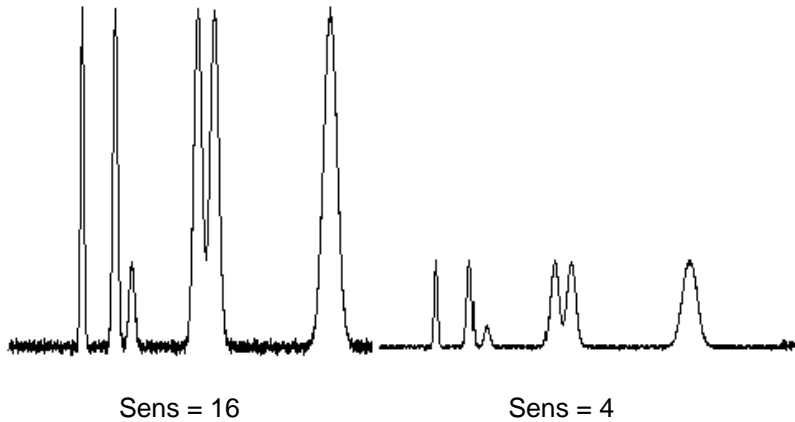


Figure 5-2 Effects of Sensitivity Settings

5.2.2 Scale Factor Guidelines

At high sensitivities, the height of some peaks may be too great to fit on a chart recorder. You can use the scale factor to reduce the plot proportionally. The scale factor affects the refractometer output only to the recorder.

A high scale factor setting results in a large plot, which may cause some peaks to go offscale. A small scale factor setting reduces the height and width of the plot, so small peaks may not be well defined.

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.

To calculate an appropriate scale factor setting, use the equation:

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

where: $\%FS$ = the % Full Scale value displayed in the four-character LED display when the 2410 refractometer detects the largest peak (the % Full Scale display reads 100 for a 10 mV signal at the chart recorder output).

Integrator Output Considerations

The relationship between the sensitivity (S) setting (S), change in refractive index (Δn), and integrator output voltage (V) is expressed by the equation:

$$\text{Integrator Out (V)} = 200 \times S \times \Delta n$$

The maximum change in refractive index (Δn) that the 2410 refractometer can optically measure is 5×10^{-3} RIU. The integrator output range is limited to ± 2 V full scale.

Chart Recorder Output Considerations

Use of the 2410 refractometer with both an integrator and a chart recorder is possible because you can program the 2410 refractometer with an offset from 0 to 50 mV. The maximum voltage on the recorder output is always 2 V regardless of the sensitivity or scale factor setting; a display of 100% Full Scale on the 2410 front panel is equal to 10 mV.

When the detector output is through the recorder output terminals, you can adjust your plot with the 2410 refractometer scale factor function.

The relationship between the chart recorder output (in millivolts) to the difference in refractive index (Δn), the sensitivity setting (S), and the scale factor (SF) is expressed by the equation:

$$\text{Recorder Output (mV)} = 2000 \times SF \times S \times \Delta n$$

Chart recorder output is limited to ± 2 V full scale.

5.2.3 Time Constant Guidelines

The Filter parameter specifies the filter time constant, which adjusts the response time of the noise filter. Adjusting the noise filter (time) allows you to achieve an optimum signal-to-noise ratio by reducing short-time noise.

Low filter time constant settings:

- Produce narrower peaks with minimum peak distortion and time delay
- Increase baseline noise

High filter time constant settings:

- Shorten and broaden peaks
- Decrease baseline noise

The default filter time constant setting of 1.0 second is appropriate for most applications.

You can calculate an appropriate filter time constant using the equation:

$$TC = 0.2 \times PW$$

where: TC = Time constant setting

PW = Peak width in seconds at half height of the narrowest peak

Figure 5-3 illustrates the effects of the time constant (Filter) settings on the signal.

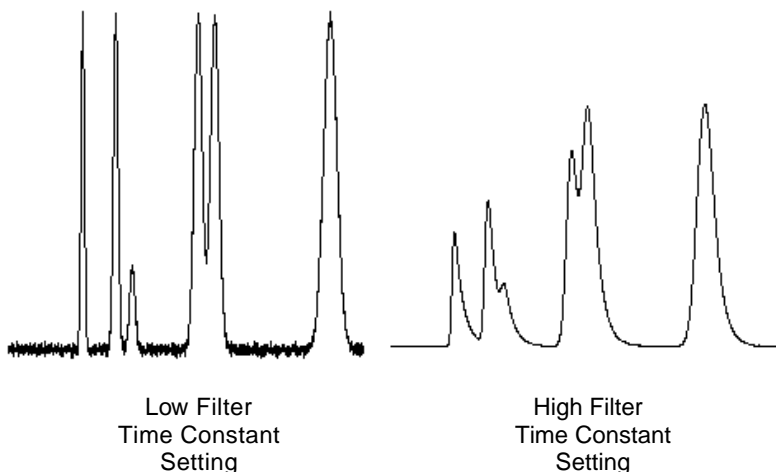


Figure 5-3 Effects of Filter Time Constant Settings

5.2.4 Temperature Guidelines (Ext1 °C, Ext2 °C, Int °C)

The 2410 refractometer permits temperature ranges of 30 to 50 °C for the internal oven (Int °C key), and from 0 to 150 °C for the two external column heaters (Ext1 °C, Ext2 °C). The general operating temperature for the internal oven should be set about 5 °C above the ambient temperature for room temperature applications. This guards against drift caused by variations in the ambient temperature.

Be aware that higher temperature settings generally:

- 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
- Cause dissolved gases to come out of poorly degassed solvents, resulting in bubbles

Internal oven temperatures of 30 to 35 °C are satisfactory for most room-temperature applications. For best performance, the external column heater and the 2410 refractometer oven should be set to the same temperature.

5.2.5 Polarity Guidelines

The polarity key (+/-) inverts the direction of peaks. Samples detected with the 2410 refractometer can yield positive or negative peaks, depending on whether their RIs are greater than or less than the RI of the mobile phase. Polarity affects data sent over the analog output channels and the IEEE-488 interface. Polarity does not affect the % Full Scale display.

The default polarity setting is positive, that is, the polarity is unchanged.

See also Section 3.3.6, Polarity Connections.

5.3 Starting Up the 2410 Refractometer

To start up the 2410 refractometer:

1. Press the **On/Off** switch located on the lower front right corner of the unit. While the 2410 refractometer performs internal tests, the four-character LED display flashes all 8's, goes blank for eight seconds, then displays a flashing “-” in the rightmost slot of the four-character display.
2. If the startup diagnostics fail, one of the following is displayed in the four-character LED display:
 - All blank, indicating that the 2410 refractometer has halted operation during diagnostic testing. Restart the instrument. If the instrument continues to fail, contact Waters Technical Service.
 - An “E” in the leftmost slot, indicating an error condition. Contact Waters Technical Service
3. If the startup diagnostics are successful, the 2410 refractometer checks the integrity of the parameter values stored in battery-backed RAM. If the values are valid, the 2410 refractometer flashes the internal oven setpoint stored in the RAM in the four-character LED display. The 2410 refractometer startup code calibrates its internal sensors while illuminating each LED indicator once in succession. When this calibration is finished, the 2410 refractometer begins to regulate the internal oven and external column heater temperatures to their setpoints, and flashes the current internal oven temperature in the four-character LED display.

4. At this point, the startup sequence has run successfully, and the 2410 refractometer is ready for operation.

Note: Pressing **Clear** does not stop the four-character LED display from flashing while the calibration sequence is in progress.

5. If the battery-backed RAM fails the integrity check (because of a low battery), the software resets the stored parameters to their default values. While the 2410 refractometer calibrates its internal sensors, the “-” continues to flash in the four-character LED display and each LED indicator is illuminated once in succession. When the calibration completes, the % Full Scale parameter is activated and displayed, non-flashing, on the four-character LED display, and the 2410 refractometer is ready for operation.
6. Once the 2410 refractometer startup diagnostics and tests are complete, power on any peripheral equipment.
7. Allow the 2410 refractometer to warm up for 24 hours before operating it.

Remote Mode

The 2410 refractometer operates in remote mode when it is under active control by a system controller through the IEEE-488 interface. You can configure remote control of the 2410 refractometer with Waters systems such as the:

- Millennium Chromatography Manager (see Section 3.2.1, Connecting to a Waters Data System Using the IEEE-488 Bus)
- 600E Multisolvant Delivery System (see Section 3.2.2, Connecting to a Waters PowerLine System Controller)
- 745/745B/746 Data Module (see Section 3.3.2, Connecting to the Waters 745/745B/746 Data Module)
- 845/860 Data Control System (see Section 3.3.4, Connecting to the Waters 845/860 ExpertEase System)
- 2690 Separations Module (see Section 3.3.1, Connecting to a Stand-Alone 2690 Separations Module).

Changing Solvents



Caution: To avoid chemical hazards, always observe safe laboratory practices when you are operating your system. Refer to the Material Safety Data Sheets shipped with solvents for handling information.

When you change solvents, be aware that:

- Changes involving two miscible solvents may be made directly. Changes involving two solvents that are not totally miscible (for example, from chloroform to water), require an intermediate solvent (such as isopropanol).
- Temperature affects solvent miscibility. If you are running a high-temperature application, consider the effect of the higher temperature on solvent solubility.
- Buffers dissolved in water may precipitate when mixed with organic solvents.

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

To change solvents:

1. Make sure the 2410 refractometer Purge Out line goes to waste.
2. To prevent backpressure in the column, replace the column with a union.
3. Set the pump or solvent delivery system flow rate to **5 mL/min**.
4. Press **2nd Func**, then **Purge**.
5. Let the 2410 refractometer purge for a minimum of 5 minutes.
6. Press **2nd Func**, then **Purge** to stop purging.
7. Follow steps 1 through 5 to purge the 2410 refractometer with 10 percent methanol-water before storing it.

5.4 Shutting Down the 2410 Refractometer

Note: Do not power off the 2410 refractometer unless you are storing it.

If you are not storing the 2410 refractometer, set the flow rate to **0.1 mL/min** and keep the pump or solvent delivery system operating. This minimizes the amount of time the 2410 refractometer needs for reequilibration when you use it again.

Do not leave buffers in the system after use. Flush the lines first with HPLC-grade water and then with a suitable solvent (Waters recommends HPLC-grade methanol).



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

Power off peripheral devices before shutting down the 2410 refractometer. To power off the 2410 refractometer, press the **ON/OFF** switch located at the lower right front corner of the unit.

Chapter 6

Maintenance Procedures

| | | |
|-----|---------------------------------|-----|
| 6.1 | Cleaning the Fluidic Path | 6-1 |
| 6.2 | Replacing Fuses..... | 6-3 |

6

Maintenance Procedures

The maintenance of the 2410 refractometer described in this chapter involves cleaning the fluidic path and replacing fuses. When you perform maintenance procedures on your 2410 differential refractometer, keep the following safety considerations in mind:



Caution: To prevent the possibility of electrical shock, never disconnect an electrical assembly while power is applied to the Waters 2410 Differential Refractometer.



Caution: To avoid the possibility of electrical shock, do not open the 2410 differential refractometer cover. The 2410 differential refractometer does not contain user-serviceable components.



Caution: When you handle solvents, change tubing, or operate the 2410 differential refractometer in general, always observe good laboratory practices. Know the physical and chemical properties of the solvents. Refer to the Material Safety Data Sheets for the solvents in use.



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

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

Contacting Waters Technical Service

If you encounter a problem with the 2410 differential refractometer that you cannot troubleshoot, contact Waters Technical Service at 1-800-252-4752, *U.S. and Canadian customers only*. Other customers, call your local Waters subsidiary or your local Waters Technical Service Representative, or call Waters corporate headquarters for assistance at 1-508-478-2000 (U.S.).

6.1 Cleaning the Fluidic Path

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

Before You Begin

If you suspect that the tubing has been contaminated, follow this procedure. Read through it carefully first, and pay strict attention to the warning.

Required Materials

- A wrench suitable for removing and replacing the column
- A solvent miscible in both the mobile phase and water (methanol is commonly used)
- 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 of measuring the pH of acid effluent, if you use an acid as your cleaning solvent



Caution: *Strictly adhere to this procedure. Prepare a separate waste container for the cleaning solution. Be careful to keep organic waste from mixing with acid waste.*

Procedure

To clean the fluidic path:

1. Stop the pump or solvent delivery system 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. Set the 2410 refractometer to Purge mode (see *Chapter 5, Using the 2410 Refractometer*).
4. Restart the pump or solvent delivery system. Set the flow rate to **5 mL/min** to flush the mobile phase from the 2410 differential refractometer. Purge for at least 5 minutes.
5. Switch the pump or solvent delivery system to HPLC-grade water. Flush the 2410 differential refractometer with water for 6 to 10 minutes to remove contaminants from the flow path.
6. Switch the pump or solvent delivery system to the cleaning solvent. Flush for 6 to 10 minutes. Use a clean waste container when pumping cleaning solvent. Do not mix acidic and organic waste.
7. Switch the pump or solvent delivery system back to HPLC-grade water. Flush until the pH of the waste effluent is neutral (a pH value of 6.0 to 7.0).

Note: *If you use 6 N nitric acid, do so with care. If you operate the 2410 refractometer at high sensitivities, you may need to flush the system extensively with water to remove all traces of the nitric acid.*

8. Switch the pump or solvent delivery system back to the water-miscible intermediate solvent. Flush for 10 minutes.
9. Switch the pump or solvent delivery system back to the mobile phase. Flush for 5 minutes.
10. Take the 2410 differential refractometer out of Purge mode and stop the pump or solvent delivery system.
11. Reattach the column and reequilibrate the 2410 refractometer.

6.2 Replacing Fuses

This section describes replacing fuses in the 2410 differential refractometer.

Required Materials

Flat-blade screwdriver

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 ohmmeter.

Suspect a faulty fuse if:

- The 2410 differential refractometer fails to power on.
- The display is blank.
- The fan does not turn on.

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



Caution: To avoid electrical shock, power off and unplug the 2410 refractometer before checking the fuse. For continued protection against fire hazard, replace the fuse only with another fuse of the same type and rating.

Procedure

To replace a fuse:

1. Power off the 2410 refractometer and disconnect the power cable from the rear panel.
2. Insert your fingers into the fuse holder slots on the rear panel of the detector, then pull with minimum force to remove the holder from the rear panel (see Figure 6-1).
3. Remove and discard the old fuse.

4. Make sure that the new fuse is properly rated for your requirements (see Table 6-1).
5. Insert the new fuse into the fuse holder.
6. Insert the fuse holder into the rear panel receptacle and gently push until it locks into position.

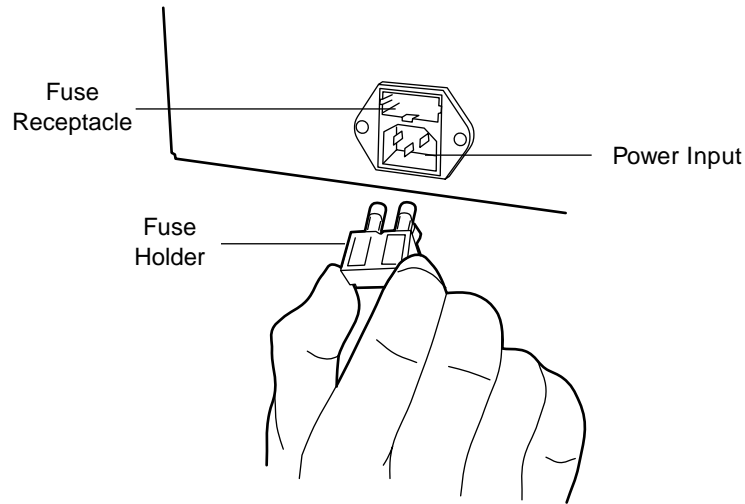


Figure 6-1 Removing and Replacing Fuses

Table 6-1 identifies the 2410 refractometer fuse requirements.

Table 6-1 Voltage and Fuse Requirements

| Nominal Voltage | Frequency | Required Fuse |
|--------------------|-----------|---------------|
| 100 Vac to 240 Vac | 50/60 Hz | F 2.0 A |

Chapter 7

Troubleshooting

| | | |
|-----|--------------------------------------|------|
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| 7.2 | Chromatography Troubleshooting | 7-2 |
| 7.3 | Diagnostics | 7-13 |
| 7.4 | Hardware Troubleshooting | 7-16 |

7

Troubleshooting

Use this section to help you troubleshoot problems with your Waters 2410 Differential Refractometer. Keep in mind that the source of an apparent detector problem may be the chromatography itself or the other instruments in your system.

If you determine that a problem is a general chromatography problem, refer to Section 7.2, Chromatography Troubleshooting.

If you determine that a problem is with the 2410 refractometer, refer to Section 7.3, Diagnostics.

7.1 Troubleshooting Overview

When you troubleshoot your 2410 differential refractometer, keep in mind the following safety considerations:



Caution: To prevent the possibility of electrical shock, never disconnect an electrical assembly while power is applied to the instrument.



Caution: When you handle solvents, change tubing, or operate the 2410 differential refractometer in general, always observe good laboratory practices. Know the physical and chemical properties of the solvents. Refer to the Material Safety Data Sheets for the solvents in use.



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

Most detector problems are relatively easy to correct. If you are unable to correct a problem or a failed condition, contact Waters Technical Service at 1-800-252-4752, U.S. and Canadian customers only. Other customers, call your local Waters subsidiary or your local Waters Technical Service Representative, or call Waters corporate headquarters for assistance at 1-508-478-2000 (U.S.).

When You Contact Waters

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

- 2410 differential refractometer serial number
- Problem symptom(s)
- Operating wavelength(s)
- RIUFS or measurement range
- Flow rate
- Filter setting
- Type of column
- Operating pressure
- Solvent(s)
- System configuration (other components)
- Type and model number(s) of other Waters system components (such as 2690 Separations Module, 600 Multi-Solvent Delivery System, 2700 Autosampler)
- Type and revision number of the data system (for example, Millennium Chromatography Manager, 845/860 Workstation, 746 Data Module)

Diagnostics

Refer to Section 7.3.2, Operating the User-Initiated Diagnostics, for information on using the 2410 refractometer diagnostics to troubleshoot. Refer to Section 5.3, Starting Up the 2410 Refractometer, for information on start-up diagnostics.

Power Surges

Power surges, line spikes, and transient energy sources can adversely affect operation. Be sure that the electrical supply used for the 2410 differential refractometer is properly grounded and free from any of these conditions.

7.2 Chromatography Troubleshooting

This section contains chromatography troubleshooting tables that describe symptoms, possible causes, and suggested corrective actions. These tables can help you isolate the possible causes of problems related to:

- Abnormal baseline (drift, noise, or cycling) – See Table 7-1.

- Erratic or incorrect retention times – See Table 7-2.
- Poor peak resolution – See Table 7-3.
- Incorrect qualitative/quantitative results – See Table 7-4.



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

If your system is exhibiting symptoms *not* addressed in one of the following tables, refer to Table 7-6 in Section 7.4, Hardware Troubleshooting. If you need further help, contact Waters Technical Service.

7.2.1 Abnormal Baseline

Drift, noise, and cycling are common symptoms of an abnormal baseline.

Drift

The most common difficulty with the 2410 differential 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 solvent delivery system or 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.

To troubleshoot problems with your baseline, refer to Table 7-1.

Table 7-1 Abnormal Baseline Troubleshooting

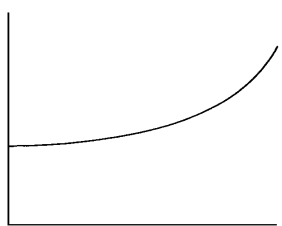
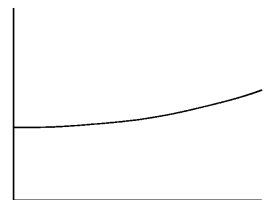
| Symptom | Possible Cause | Corrective Action |
|---|---|--|
| <p>Baseline drift, rapid</p>  | Column not equilibrated | Equilibrate column. |
| | Detector not allowed to warm up | Allow detector to warm up until baseline is stable. Warm-up time varies based on sensitivity. |
| | Solvent contaminated or not HPLC grade | Use fresh solvent. |
| | Solvent not properly degassed (rapid or slow drift) | Degas the solvent. Cap the solvent reservoir. |
| | Tubing contaminated | Clean the tubing using the procedure in Section 6.1, Cleaning the Fluidic Path. |
| | Flow fluctuations (rapid or slow drift) | Fix pump problems, replace pump seals, check valves. |
| <p>Baseline drift, slow</p>  | 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 Fluidic Path). |
| Baseline drift, descending | Leaky flow cell | Call Waters Technical Service. |

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

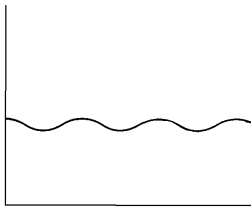
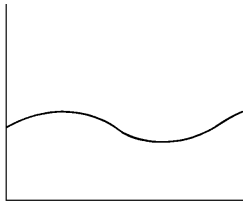
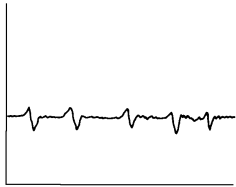
| Symptom | Possible Cause | Corrective Action |
|---|---|--|
| <p>Short-term noise cycling (30 sec to 60 sec)</p>  | Pump pulsing | Add pulse dampener. |
| | Inadequate solvent blending in pump | Connect high-flow pulse dampener. Use gradient mixer. |
| | Flow fluctuating | Stabilize flow (see pump operator's manual). |
| | Solvent not mixed (short- or long-term cycling) | Stir the solvent. |
| | Faulty check valve | Clean/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. |
| <p>Long-term noise cycling (approximately 1 hour)</p>  | 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. |

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

| 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). |
| | 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 fluidic path (see Section 6.1, Cleaning the Fluidic Path). |
| | Analog output cable not properly connected between 2410 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. | |

7.2.2 Erratic or Incorrect Retention Times

When you troubleshoot retention time problems, check if retention times:

- Change from run to run or are constant from run to run, but are outside the allowable range for the assay
- Are due to pressure fluctuations that are short-term (with each pump cycle) or long-term (over the course of several minutes)
- Are associated with an absolute pressure change, that is, if the pressure is constant but higher or lower than the normal operating pressure
- Change suddenly at the end of a series of runs, which may indicate that air is dissolving in the mobile phase, that the mobile phase is degrading, or that the column is contaminated.
- Change early in a series of runs and tend to become constant or within range after 3 to 4 minutes, which may indicate that the column was not equilibrated, or that the solvent is not properly degassed and sparged.

To troubleshoot problems with retention times, refer to Table 7-2.

Table 7-2 Retention Time Troubleshooting

| Symptom | Possible Cause | Corrective Action |
|-------------------------|----------------------------------|--|
| Erratic retention times | Air bubble in pump head | Degas all solvents, prime pump (see Section 4.3.2, Solvent Degassing Methods). |
| | Malfunctioning pump check valves | Clean/replace/rebuild pump check valves. |
| | Leaking pump seals | Replace pump seals. |
| | Separation chemistry | Check mobile phase and column. |
| | Clogged solvent filters | Replace filters. |

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

| Symptom | Possible Cause | Corrective Action |
|---------------------------|---------------------------------------|--|
| Increased retention times | Incorrect flow rate | Verify flow rate. |
| | Incorrect solvent composition | Change solvent composition. |
| | Column heater module not on | Power on column heater module. |
| | 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 (see Section 4.3.2, Solvent Degassing Methods). |
| | Incorrect chemistry/integration | Check chemistry/integration. |
| | Column not equilibrated | Equilibrate column |
| | Injector problem | Troubleshoot injector. |

7.2.3 Poor Peak Resolution

Before you address 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 times are correct, determine if poor resolution occurs:

- Throughout the chromatogram
- At a single peak pair

If efficiency of early peaks is poor, extra-column band broadening, such as autoinjector or guard column failure, may be at fault. If peak efficiency is poor throughout the chromatogram, post-column band-broadening or loss of column efficiency may be the cause.

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

Use Table 7-3 to troubleshoot peak resolution problems that may be affecting your results.



Table 7-3 Resolution Troubleshooting


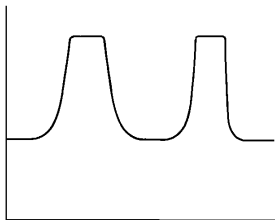
| Symptom | Possible Cause | Corrective Action |
|--|--|--|
| <p>Straight baseline, no peaks</p>  | No pump flow | Set pump flow rate. |
| | LED not on | Use diagnostics to check LED current. Zero current indicates LED not on. |
| | | Call Waters Technical Service. |
| | Detector not zeroed | Auto zero detector baseline. |
| | Improper connection between 2410 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. |
| | Bad flow cell | Use the 2410 detector diagnostics to check the B signal and the A + B signal. A low value may indicate a blocked flow cell. Clean/replace column. Clean/replace flow cell. |

Table 7-3 Resolution Troubleshooting (*Continued*)

| Symptom | Possible Cause | Corrective Action |
|--|---|---|
| Flat-topped peaks  | Detector not zeroed | Auto 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. |

7.2.4 Incorrect Qualitative/Quantitative Results

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

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

To troubleshoot problems with qualification and/or quantitation, refer to Table 7-4.

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 flow cell | Clean the fluidic path (see Section 6.1, Cleaning the Fluidic Path). |
| Increased noise | Electronic noise | Use the shortest lengths of cabling possible. Make sure cables are shielded. |
| | Dirty flow cell | Clean the fluid path (see 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 Diagnostics

This section describes the startup and user-initiated diagnostics for troubleshooting the 2410 refractometer. Use the diagnostics to determine, set, or test:

- Software version
- LED current strength
- Energy transmitted from the photodiode
- Voltage transmitted from the sample side of the photodiode
- IEEE address
- Auto zero offset
- Factory defaults
- Noise and drift
- Keypad and LED display and indicators

This information may be helpful if you need to call Waters Technical Service for assistance.

7.3.1 Operating the Startup Diagnostics

For a complete explanation of the 2410 refractometer startup diagnostic routines, see Section 5.3, Starting Up the 2410 Refractometer.

7.3.2 Operating the User-Initiated Diagnostics

The 2410 differential refractometer provides several user-initiated diagnostics to:

- test the operation of the instrument
- aid in setting operating parameters

Table 7-5 provides a list of diagnostics, the key sequence the user enters to run the diagnostic, and a description of the operation, range of values, and defaults for each diagnostic.

To run the diagnostic functions,

1. Press **2nd Func, Clear, Clear**, then press **Enter**. The value **diag** is displayed. Then enter the appropriate key sequence indicated in Table 7-5.

Table 7-5 User Diagnostics

| Diagnostic Number | Diagnostic Name | Key Sequence | Description |
|-------------------|--------------------------------|---------------------------|---|
| 0 | Software version | 2nd Func, 0, Enter | Displays the version of software running on your 2410 differential refractometer |
| 1 | LED display and indicator test | 2nd Func, 1, Enter | Tests the operation of the 2410 differential refractometer LED display and LED indicators |
| 2 | Keypad test | 2nd Func, 2, Enter | Tests the operation of the 2410 differential refractometer keypad |
| 3 | LED current strength | 2nd Func, 3, Enter | Displays a value between 25 and 150 that indicates the operational current of the LED (see <i>Note</i> below table). |
| 4 | A + B signal | 2nd Func, 4, Enter | Displays a value between 2.4 and 2.6 V. |
| 5 | B signal | 2nd Func, 5, Enter | Displays a value between 1.2 and 1.3 V |
| 6 | Set IEEE address | 2nd Func, 6, Enter | Displays current IEEE-488 address. Range: 2 to 29. Default: 10. |
| 7 | Integrator offset voltage | 2nd Func, 7, Enter | Displays the voltage offset. Range: 0 to 50 mV. Default: 20 mV. |
| 8 | Auto zero delay | 2nd Func, 8, Enter | Sets delayed response for auto zero after an injection (see <i>Note</i> below). Range: 0 to 30 sec. Default: 20 sec |
| 9 | Noise test | 2nd Func, 9, Enter | Displays prior Noise test values. Press Clear, Enter to initiate a new noise test. Displays time in seconds until test is complete, then displays results (RIU) in scientific notation. The Noise test takes 15 min. |

Table 7-5 User Diagnostics (Continued)

| Diagnostic Number | Diagnostic Name | Key Sequence | Description |
|-------------------|-----------------|------------------------------|--|
| 10 | Drift test | 2nd Func, 1, 0, Enter | Displays prior Drift test values. Press Clear, Enter to initiate a new drift test. Displays time in seconds until test is complete, then displays results (RIU/hr) in scientific notation. The Drift test takes 60 min. |
| 11 | Reset defaults | 2nd Func, 1, 1, Enter | Resets system stored parameters as the default parameters |

2. To exit from the diagnostic mode, press **2nd Func, Clear**, then **Enter**. Each parameter indicator lights up for an instant, starting next to the Enter button and ending above the 1 key.
3. After you exit from the diagnostic mode, the operating parameters in effect when you entered the diagnostic mode are retained, except if you reset them using Diagnostic 11.



Attention: *Diagnostics 3, 4, and 5 do not maintain the temperature of the internal oven. Oven temperature falls while these diagnostics are running. When these diagnostics terminate, the oven temperature set point in effect before the diagnostic was run resumes. We strongly recommend that you do not run Diagnostics 9 (Noise test) or 10 (Drift test) immediately after running Diagnostics 3, 4, and 5 because both the Noise and Drift tests require stable oven temperatures to operate correctly.*

Note: *The auto zero delay feature prevents an auto zero signal initiated from either the IEEE-488 connection or from the 2410 refractometer analog-in connectors from activating the auto zero function during the initial injection baseline stabilization period. An auto zero initiated from the front panel of the 2410 detector is effective immediately.*

7.4 Hardware Troubleshooting

This section describes symptoms, causes, and corrective actions related to the 2410 refractometer hardware. Use Table 7-6 when you know the problem you have encountered lies within the instrument.

Table 7-6 Waters 2410 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). |
| Four-character display LEDs fail to illuminate | Weak electrical connections | Check connections. |
| | Fuse blown | Check/replace faulty fuse. |
| | Faulty CPU board | Call Waters Technical Service. |
| Parameter LED indicators do not light | Fuse blown | Check and replace faulty fuse. |
| | Faulty Display board | Call Waters Technical Service. |
| Four-character display shows odd characters or FAIL | Faulty CPU board | Call Waters Technical Service. |
| | Faulty Power Supply | Call Waters Technical Service. |

Table 7-6 Waters 2410 Hardware Troubleshooting (*Continued*)

| Symptom | Possible Cause | Corrective Action |
|--|--|---|
| IEEE-488 communications problems | Incorrect IEEE-488 address | Set correct address (refer to Section 3.2, Making IEEE-488 Signal Connections, the “Setting the IEEE-488 Address” discussion). |
| | IEEE-488 cable not connected | Connect IEEE-488 cable (refer to Section 3.2, Making IEEE-488 Signal Connections, the “Setting the IEEE-488 Address” discussion). |
| | Defective IEEE-488 cable (external) | Replace IEEE-488 cable. |
| Keypad not functioning | Keypad defective | Call Waters Technical Service. |
| LED does not light | Faulty fuse | Call Waters Technical Service. |
| | LED burned out | |
| Unit overheating (2410 beeping) | Faulty fan | Call Waters Technical Service. |
| | Ambient temperature is 5 °C above set oven temperature | Remove external heat sources in the vicinity of the 2410 refractometer. Check column heater settings. |
| Internal oven overheating | Relay stuck | Call Waters Technical Service. |
| | Faulty analog board | |
| No B or A + B signal in diagnostics 4 and 5 | Air bubble in flow cell | Purge the 2410 refractometer. |
| | LED burned out | Call Waters Technical Service. |
| | Bad cable connection | |
| Four-character LED display is greater than 150 for diagnostic 3. | Air bubble in flow cell | Purge the 2410 refractometer. |

Appendix A

Specifications

Table A-1 Operational Specifications

| Conditions | Specifications |
|-------------------------------|---|
| RI Range | 1.00 to 1.75 RIU |
| Measurement Range | 5×10^{-3} RIU maximum FS (SENS = 1, SF = 1) 5×10^{-8} RIU minimum FS (SENS = 1024, SF = 100) |
| Flow Rate | 0.03 to 10 mL/min |
| Noise* | $\leq 2.0 \times 10^{-8}$ RIU, (TC = 1, SF = 80, SENS = 64, THF at 24 °C with restrictor coil) |
| Drift* | 2.5×10^{-7} RIU/hr (static with THF) TC = 1, SF = 20, SENS = 32 |
| Sensitivity Settings | 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024 |
| Time Constant Filter Settings | 0.2, 1, 3, 10 seconds |
| Analog Outputs | -2.0 V to +2.0 V |
| Temperature Control | Internal oven: 30 to 50 °C (86 to 122 °F) External column heaters: <ul style="list-style-type: none"> • Ambient to 150 °C (302 °F), steel • Ambient to 80 °C (176 °F), plastic |

*After a minimum one hour warmup time

Table A-2 Integrator Output

| Sensitivity Setting | Output (V) |
|---------------------|------------|
| 1 | 1 |
| 2 | 2 |
| 4 | 2 |
| 8 | 2 |
| 16 to 1024 | 2 |

Table A-3 Optical Component Specifications

| Conditions | Specifications |
|------------------------|---|
| Light Source | Light emitting diode (LED) |
| Flow Cell | Fused quartz |
| Cell Volume | 10 μ L |
| Pressure Limit | 100 psi |
| Fluidic Path Materials | 316 stainless steel, Dynasil [®] , Suprasil II [®] , PTFE, Kalrez [®] , quartz |

Table A-4 Environmental Specifications

| Conditions | Specifications |
|----------------------------------|-------------------------------------|
| Operating Temperature | 15 °C to 32.2 °C (59 °F to 90 °F) |
| Operating Humidity | 20 to 80%, noncondensing |
| Shipping and Storage Temperature | -40 °C to 70 °C (-104 °F to 158 °F) |
| Shipping and Storage Humidity | 0% to 90%, noncondensing |

Table A-5 Dimensions

| Conditions | Specifications |
|-------------------|-----------------------|
| Height | 8.2 in. (20.8 cm) |
| Length | 19.8 in. (50.3 cm) |
| Width | 11.2 in. (28.4 cm) |
| Weight | 21.7 lbs. (9.7 kg) |

Table A-6 Electrical Specifications

| Conditions | Specifications |
|-------------------|--|
| Line Frequency | 50 Hz, 47 to 53 Hz 60 Hz, 57 to 63 Hz |
| Fuse Rating | F 2 A, 250 Vac |
| Power Consumption | 140 VA (Nominal) |

Table A-7 Power Source Specification

| Nominal Voltage |
|------------------------|
| 100 to 240 V, 50/60 Hz |

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Appendix B

Spare Parts/Accessories

Refer to Table B-1 for spare parts information.

Table B-1 Recommended Spare Parts

| Description | Part Number |
|--|-------------|
| General Parts | |
| Pressure relief valve | WAT070377 |
| Solenoid valve | WAT070376 |
| Ferrule, 1/16-inch, PTFE | WAT070215 |
| Nuts and ferrules (kit of 5 each) | WAT025604 |
| Stainless steel tubing, 0.009-inch ID × 10 feet | WAT026973 |
| Stainless steel tubing, 0.040-inch ID × 10 feet | WAT026805 |
| Recorder cable, 4 ft (1) | WAT048918 |
| Waters Startup Tool Kit | WAT096146 |
| Tubing cutter for 1/16 inch stainless steel tubing | WAT022384 |
| Tubing cutter, spare blades, 3/pkg | WAT022385 |
| Startup kit, 2410 | 200000104 |
| Fuse | |
| Fuse, FAST, 2 Amp, 250V | WAT163-14 |

Table B-1 Recommended Spare Parts (*Continued*)

| Description | Part Number |
|---|------------------------|
| IEEE-488 Cables | |
| 3.3 feet (1 m) | WAT087198 |
| 6 feet (2 m) | WAT087141 |
| 13 feet (3 m) | WAT087191 |
| 26 feet (6 m) | WAT087192 |
| Solvent Preparation Accessories | |
| Solvent Clarification Kit 1 pump, 110 V, 60 Hz 1 L flask 300 mL funnel Clamp Tubulated base 100 Durapore™ filters | WAT085113 |
| 0.45-µm membrane filters Filter, PTFE 47 mm 0.45 (100/pkg) Triton™ free aqueous filters | WAT200534 WAT085147 |

B

Appendix C

Warranty Information

This appendix includes information on:

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

C.1 Limited Express Warranty

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

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

Limited Warranty

Waters Corporation warrants that the Waters 2410 Differential Refractometer is for general purpose use and is not for use in clinical diagnostic procedures, and that during the Warranty period, the performance of all components of the Waters 2410 Differential Refractometer [other than Third-Party Components (non-Waters named)], will not deviate materially from the Specifications for such detectors. Warranties, if any, that may be applicable to Third-Party Components shall be provided by the respective manufacturers or suppliers of such Third-Party Components, and Waters Corporation shall use reasonable efforts to assist Customer in securing the benefits of any such warranties.

Exclusions

The foregoing warranty does not apply to any material deviation from the Specifications by any component of the Waters 2410 Differential Refractometer that results from (a) use of the Waters 2410 Differential Refractometer for any purpose other than general purpose use and specifically excluding use of the Waters 2410 Differential Refractometer in clinical diagnostic procedures, or use of the Waters 2410 Differential Refractometer for investigational use with or without confirmation of diagnosis by another, medically established diagnostic product or procedure, (b) errors or defects in any Third-Party Component, (c) modification of the Waters 2410 Differential



Refractometer by anyone other than Waters Corporation, (d) failure by Customer to install any Standard Enhancement in accordance with an update procedure, release of firmware or any operating system release, (e) any willful or negligent action or omission of Customer, (f) any misuse or incorrect use of the Waters 2410 Differential Refractometer, (g) any malfunction of any information system or instrument with which the Waters 2410 Differential Refractometer may be connected, or (h) failure to establish or maintain the operating environment for the Waters 2410 Differential Refractometer in accordance with the operator's manual.

Exclusive Remedy

In the event of any failure of the Waters 2410 Differential Refractometer to perform, in any material respect, in accordance with the warranty set forth herein, the only liability of Waters Corporation to Customer, and Customer's sole and exclusive remedy, shall be the use, by Waters Corporation, of commercially reasonable efforts to correct for such deviations, in Waters Corporation's sole discretion, replacement of the purchased Waters 2410 Differential Refractometer, or refund of all amounts theretofore paid by Customer to Waters Corporation for the Waters 2410 Differential Refractometer.

Disclaimers

THE LIMITED WARRANTY SET FORTH HEREIN IS EXCLUSIVE AND IN LIEU OF, AND CUSTOMER HEREBY WAIVES, ALL OTHER REPRESENTATIONS, WARRANTIES AND GUARANTEES, EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION ANY IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS OF THE WATERS 2410 DIFFERENTIAL REFRACTOMETER FOR A PARTICULAR PURPOSE, INCLUDING FITNESS FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES OR FOR INVESTIGATIONAL USE WITH OR WITHOUT CONFIRMATION OF DIAGNOSIS BY ANOTHER MEDICALLY ESTABLISHED DIAGNOSTIC PRODUCT OR PROCEDURE, OR NONINFRINGEMENT, AND ANY WARRANTIES ARISING OUT OF COURSE OF DEALING OR COURSE OF PERFORMANCE. CUSTOMER EXPRESSLY ACKNOWLEDGES THAT BECAUSE OF THE COMPLEX NATURE OF THE WATERS 2410 DIFFERENTIAL REFRACTOMETER AND ITS MANUFACTURE, WATERS CORPORATION CANNOT AND DOES NOT WARRANT THAT THE OPERATION OF THE WATERS 2410 DIFFERENTIAL REFRACTOMETER WILL BE WITHOUT DEFECT. CUSTOMER EXPRESSLY ACKNOWLEDGES THAT CUSTOMER IS SOLELY RESPONSIBLE FOR USE OF THE WATERS 2410 DIFFERENTIAL REFRACTOMETER IN CLINICAL DIAGNOSTIC PROCEDURES OR FOR INVESTIGATIONAL USE WITH OR WITHOUT CONFIRMATION OF DIAGNOSIS BY ANOTHER MEDICALLY ESTABLISHED DIAGNOSTIC PRODUCT OR PROCEDURE.

C

Warranty Service

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

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

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

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

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

Warranty Service Exceptions

Warranty service is not performed on:

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

Repair or replacement is not made:

- For expendable items such as gaskets, windows, lenses, and fuses, if such items were operable at the time of initial use.
- Because of decomposition due to chemical action.
- For used equipment.
- Because of poor facilities, operating conditions, or utilities.

Warranty Period

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

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

Table C-1 summarizes the warranty periods for the Waters 2410 and its components.

Table C-1 Waters 2410 Warranty Periods

| Component | Warranty Period |
|--|---|
| Waters 2410 Differential Refractometer, excluding the components listed below | 1 year |
| <ul style="list-style-type: none">• Solenoid valve• Pressure relief valve | 90 days |
| Expendables, Consumables, and Operating Supplies | Warranted to function properly when delivered |

C.2 Shipments, Damages, Claims, and Returns

Shipments

As all shipments are made Free On Board (FOB) shipping point, we suggest insurance be authorized on all shipments. Instruments and major components are packed and shipped via surface, unless otherwise required. Supplies and/or replacement parts are packed and shipped via United Parcel Service (UPS), UPS Blue, air parcel post, or parcel post unless otherwise requested.

Damages

The Interstate Commerce Commission has held that carriers are as responsible for concealed damage as for visible damage in transit. Unpack shipment promptly after receipt as there may be concealed damage even though no evidence of it is apparent. When concealed damage is discovered, cease further unpacking of the unit involved and request immediate inspection by local agent or carrier and secure written report of his findings to support claim. This request must be made within 15 days of receipt. Otherwise, the claim will not be honored by the carrier. Do not return damaged goods to the factory without first securing an inspection report and contacting Waters for a return merchandise authorization number (RMA).

Claims

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

Returns

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

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C

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