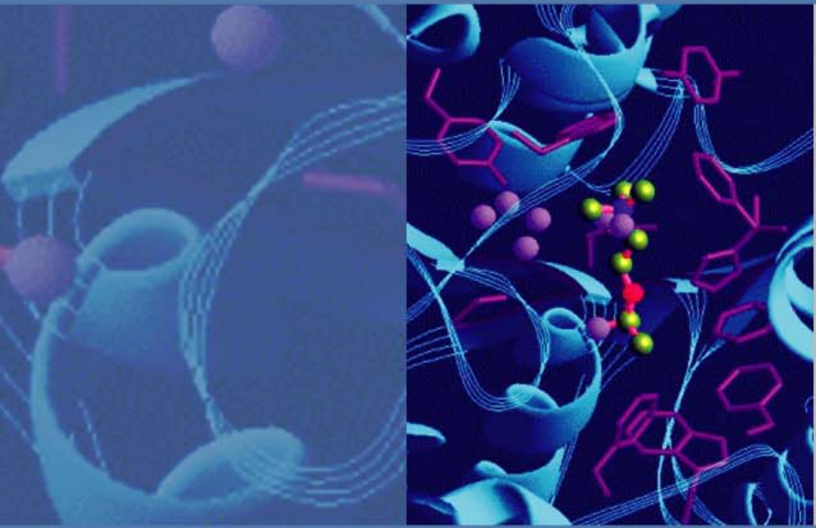

Surveyor Plus

User Guide for the Xcalibur 2.1.x Data System

60053-97221 Revision B April 2010

Thermo SCIENTIFIC

Part of Thermo Fisher Scientific

Software versions: Xcalibur 2.1 or higher and LC Devices 2.2.0 or higher
Firmware versions: Surveyor Autosampler Plus 2.15, Surveyor LC Pump Plus 3.02, Surveyor MS Pump Plus 12.47a, Surveyor PDA Plus Detector 2.02, Surveyor UV/Vis Plus Detector N/A
Revision history: Revision A, January 2009; Revision B, April 2010

For Research Use Only. Not for use in diagnostic procedures.

Regulatory Compliance

Thermo Fisher Scientific performs complete testing and evaluation of its products to ensure full compliance with applicable domestic and international regulations. When the system is delivered to you, it meets all pertinent electromagnetic compatibility (EMC) and safety standards as described in the next section or sections by product name.

Changes that you make to your system may void compliance with one or more of these EMC and safety standards. Changes to your system include replacing a part or adding components, options, or peripherals not specifically authorized and qualified by Thermo Fisher Scientific. To ensure continued compliance with EMC and safety standards, replacement parts and additional components, options, and peripherals must be ordered from Thermo Fisher Scientific or one of its authorized representatives.

This section contains regulatory compliance information for the following devices of the Surveyor Plus family of LC instruments:

Surveyor LC Pump Plus
Surveyor MS Pump Plus
Surveyor Autosampler Plus
Surveyor PDA Plus Detector
Surveyor UV/Vis Plus Detector

Surveyor LC Pump Plus

When the Surveyor LC Pump Plus is delivered to you, it meets all pertinent electromagnetic compatibility (EMC) and safety standards as described below.

EMC Directive 89/336/EEC amended by 92/31/EEC and 93/68/EEC

EMC compliance has been evaluated by Underwriters Laboratories Inc.

EN 55011	1998	EN 61000-4-3	2002
EN 61000-3-2	1995, A1; 1998, A2; 1998, A14; 2000	EN 61000-4-4	1995, A1; 2001, A2; 2001
IEC 61000-3-2	2000	EN 61000-4-5	1995, A1; 2001
EN 61000-3-3	1995	EN 61000-4-6	1996, A1; 2001
IEC 61000-3-3	1994	EN 61000-4-11	1994, A1; 2001
EN 61326-1	1997		
EN 61000-4-2	1995 A1; 1998 A2; 2001	CISPR 11	1999, A1; 1999, A2; 2002

FCC Class A, CFR 47 Part 15 Subpart B: 2004

Low Voltage Safety Compliance

Low voltage safety compliance has been evaluated by TUV Rheinland of North America, Inc. This device complies with Low Voltage Directive 73/23/EEC and harmonized standard EN 61010-1:2001.

Surveyor MS Pump Plus

When the Surveyor MS Pump Plus is delivered to you, it meets all pertinent electromagnetic compatibility (EMC) and safety standards as described below.

EMC Directive 89/336/EEC amended by 92/31/EEC and 93/68/EEC

EMC compliance has been evaluated by Underwriters Laboratories Inc.

EN 55011	1998	EN 61000-4-3	2002
EN 61000-3-2	1995, A1; 1998, A2; 1998, A14; 2000	EN 61000-4-4	1995, A1; 2001, A2; 2001
IEC 61000-3-2	2000	EN 61000-4-5	1995, A1; 2001
EN 61000-3-3	1995	EN 61000-4-6	1996, A1; 2001
IEC 61000-3-3	1994	EN 61000-4-11	1994, A1; 2001
EN 61326-1	1997		
EN 61000-4-2	1995 A1; 1998 A2; 2001	CISPR 11	1999, A1; 1999, A2; 2002

FCC Class A, CFR 47 Part 15 Subpart B: 2004

Low Voltage Safety Compliance

Low voltage safety compliance has been evaluated by TUV Rheinland of North America, Inc.This device complies with Low Voltage Directive 73/23/EEC and harmonized standard EN 61010-1:2001.

Surveyor Autosampler Plus

When the Surveyor Autosampler Plus is delivered to you, it meets all pertinent electromagnetic compatibility (EMC) and safety standards as described below.

EMC Directive 89/336/EEC amended by 92/31/EEC and 93/68/EEC

EMC compliance has been evaluated by Underwriters Laboratories Inc.

EN 55011	1998	EN 61000-4-3	2002
EN 61000-3-2	1995, A1; 1998, A2; 1998, A14; 2000	EN 61000-4-4	1995, A1; 2001, A2; 2001
IEC 61000-3-2	2000	EN 61000-4-5	1995, A1; 2001
EN 61000-3-3	1995	EN 61000-4-6	1996, A1; 2001
IEC 61000-3-3	1994	EN 61000-4-11	1994, A1; 2001
EN 61326-1	1997		
EN 61000-4-2	1995 A1; 1998 A2; 2001	CISPR 11	1999, A1; 1999, A2; 2002
FCC Class A, CFR 47 Part 15 Subpart B: 2004			

Low Voltage Safety Compliance

Low voltage safety compliance has been evaluated by TUV Rheinland of North America, Inc. This device complies with Low Voltage Directive 73/23/EEC and harmonized standard EN 61010-1:2001.

Surveyor PDA Plus Detector

When the Surveyor PDA Plus Detector is delivered to you, it meets all pertinent electromagnetic compatibility (EMC) and safety standards as described below.

EMC Directive 89/336/EEC amended by 92/31/EEC and 93/68/EEC

EMC compliance has been evaluated by Underwriters Laboratories Inc.

EN 55011	1998	EN 61000-4-3	2002
EN 61000-3-2	1995, A1; 1998, A2; 1998, A14; 2000	EN 61000-4-4	1995, A1; 2001, A2; 2001
IEC 61000-3-2	2000	EN 61000-4-5	1995, A1; 2001
EN 61000-3-3	1995	EN 61000-4-6	1996, A1; 2001
IEC 61000-3-3	1994	EN 61000-4-11	1994, A1; 2001
EN 61326-1	1997		
EN 61000-4-2	1995 A1; 1998 A2; 2001	CISPR 11	1999, A1; 1999, A2; 2002
FCC Class A, CFR 47 Part 15 Subpart B: 2003			

Low Voltage Safety Compliance

Low voltage safety compliance has been evaluated by TUV Rheinland of North America, Inc.This device complies with Low Voltage Directive 73/23/EEC and harmonized standard EN 61010-1:2001.

Surveyor UV/Vis Plus Detector

When the Surveyor UV/Vis Detector is delivered to you, it meets all pertinent electromagnetic compatibility (EMC) and safety standards as described below.

EMC Directive 89/336/EEC amended by 92/31/EEC and 93/68/EEC

EMC compliance has been evaluated by Underwriters Laboratories Inc.

EN 55011	1998	EN 61000-4-3	2002
EN 61000-3-2	1995, A1; 1998, A2; 1998, A14; 2000	EN 61000-4-4	1995, A1; 2001, A2; 2001
IEC 61000-3-2	2000	EN 61000-4-5	1995, A1; 2001
EN 61000-3-3	1995	EN 61000-4-6	1996, A1; 2001
IEC 61000-3-3	1994	EN 61000-4-11	1994, A1; 2001
EN 61326-1	1997		
EN 61000-4-2	1995 A1; 1998 A2; 2001	CISPR 11	1999, A1; 1999, A2; 2002
FCC Class A, CFR 47 Part 15 Subpart B: 2004			

Low Voltage Safety Compliance

Low voltage safety compliance has been evaluated by TUV Rheinland of North America, Inc.This device complies with Low Voltage Directive 73/23/EEC and harmonized standard EN 61010-1:2001.

FCC Compliance Statement

THIS DEVICE COMPLIES WITH PART 15 OF THE FCC RULES. OPERATION IS SUBJECT TO THE FOLLOWING TWO CONDITIONS: (1) THIS DEVICE MAY NOT CAUSE HARMFUL INTERFERENCE, AND (2) THIS DEVICE MUST ACCEPT ANY INTERFERENCE RECEIVED, INCLUDING INTERFERENCE THAT MAY CAUSE UNDESIRED OPERATION.

CAUTION Read and understand the various precautionary notes, signs, and symbols contained inside this manual pertaining to the safe use and operation of this product before using the device.

Notice on Lifting and Handling of Thermo Scientific Instruments

For your safety, and in compliance with international regulations, the physical handling of this Thermo Fisher Scientific instrument ***requires a team effort*** to lift and/or move the instrument. This instrument is too heavy and/or bulky for one person alone to handle safely.

Notice on the Proper Use of Thermo Scientific Instruments

In compliance with international regulations: Use of this instrument in a manner not specified by Thermo Fisher Scientific could impair any protection provided by the instrument.

Notice on the Susceptibility to Electromagnetic Transmissions

Your instrument is designed to work in a controlled electromagnetic environment. Do not use radio frequency transmitters, such as mobile phones, in close proximity to the instrument.

For manufacturing location, see the label on the instrument.

WEEE Compliance

This product is required to comply with the European Union's Waste Electrical & Electronic Equipment (WEEE) Directive 2002/96/EC. It is marked with the following symbol:

Thermo Fisher Scientific has contracted with one or more recycling or disposal companies in each European Union (EU) Member State, and these companies should dispose of or recycle this product. See www.thermo.com/WEEERoHS for further information on Thermo Fisher Scientific's compliance with these Directives and the recyclers in your country.

WEEE Konformität

Dieses Produkt muss die EU Waste Electrical & Electronic Equipment (WEEE) Richtlinie 2002/96/EC erfüllen. Das Produkt ist durch folgendes Symbol gekennzeichnet:

Thermo Fisher Scientific hat Vereinbarungen mit Verwertungs-/Entsorgungsfirmen in allen EU-Mitgliedsstaaten getroffen, damit dieses Produkt durch diese Firmen wiederverwertet oder entsorgt werden kann. Mehr Information über die Einhaltung dieser Anweisungen durch Thermo Fisher Scientific, über die Verwerter, und weitere Hinweise, die nützlich sind, um die Produkte zu identifizieren, die unter diese RoHS Anweisung fallen, finden sie unter www.thermo.com/WEEERoHS.

Conformité DEEE

Ce produit doit être conforme à la directive européenne (2002/96/EC) des Déchets d'Equipements Electriques et Electroniques (DEEE). Il est marqué par le symbole suivant:

Thermo Fisher Scientific s'est associé avec une ou plusieurs compagnies de recyclage dans chaque état membre de l'union européenne et ce produit devrait être collecté ou recyclé par celles-ci. Davantage d'informations sur la conformité de Thermo Fisher Scientific à ces directives, les recycleurs dans votre pays et les informations sur les produits Thermo Fisher Scientific qui peuvent aider la détection des substances sujettes à la directive RoHS sont disponibles sur www.thermo.com/WEEERoHS.

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Preface

This guide describes how to use the Xcalibur 2.1.x data system to control the Surveyor Plus high performance liquid chromatography system.

Contents

Tip The default lamp startup time for the PDA detector is 6:55 PM. For information on changing the lamp startup time, see “Setting the Lamp Startup Time” on page 160.

Tip The default setting for the autosampler maintenance counters is 0. If you turn on the autosampler maintenance log (see “Communication Page” on page 45), check the counter settings on the Maintenance Information page, see “Autosampler Maintenance Information” on page 310.

Related Documentation

In addition to Help that you can access from the data system, these manuals are provided with LC Devices as PDF files for the Surveyor family of high performance liquid chromatography instruments:

- *Surveyor Plus User Guide for the Xcalibur Data System*
- *Surveyor Plus Preinstallation Requirements Guide*
- *Surveyor Plus Getting Connected Guide*
- *Surveyor Autosampler Plus Hardware Manual*
- *Surveyor LC Pump Plus Hardware Manual*
- *Surveyor MS Pump Plus Hardware Manual*
- *Surveyor PDA Detector Plus Hardware Manual*
- *Surveyor UV/Vis Detector Plus Hardware Manual*

❖ **To view manuals for your Surveyor Plus LC devices**

Go to **Start** > **Programs** > **Thermo Instruments** > **Manuals** >**LC Devices** > **Surveyor** > ***product name***.

❖ **To open Help**

- From the Instrument Setup window, choose **Help** >***product name*** **Help**.
- If available for a specific window or dialog box, click **Help** or press **F1** for information about setting parameters.

For more information, visit www.thermo.com. You can find application notes at www.thermo.com\appnotes.

Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

Safety and special notices include the following:

CAUTION Highlights hazards to humans, property, or the environment. Each CAUTION notice is accompanied by an appropriate CAUTION symbol.

IMPORTANT Highlights information necessary to prevent damage to software, loss of data, or invalid test results; or might contain information that is critical for optimal performance of the system.

Note Highlights information of general interest.

Tip Highlights helpful information that can make a task easier.

Contacting Us

There are several ways to contact Thermo Fisher Scientific for the information you need.

❖ To contact Technical Support

Phone	800-532-4752
Fax	561-688-8736
E-mail	us.techsupport.analyze@thermofisher.com
Knowledge base	www.thermokb.com

Find software updates and utilities to download at mssupport.thermo.com.

❖ To contact Customer Service for ordering information

Phone	800-532-4752
Fax	561-688-8731
E-mail	us.customer-support.analyze@thermofisher.com
Web site	www.thermo.com/ms

❖ To copy manuals from the Internet

Go to mssupport.thermo.com and click **Customer Manuals** in the left margin of the window.

❖ To suggest changes to documentation or to Help

Do the following:

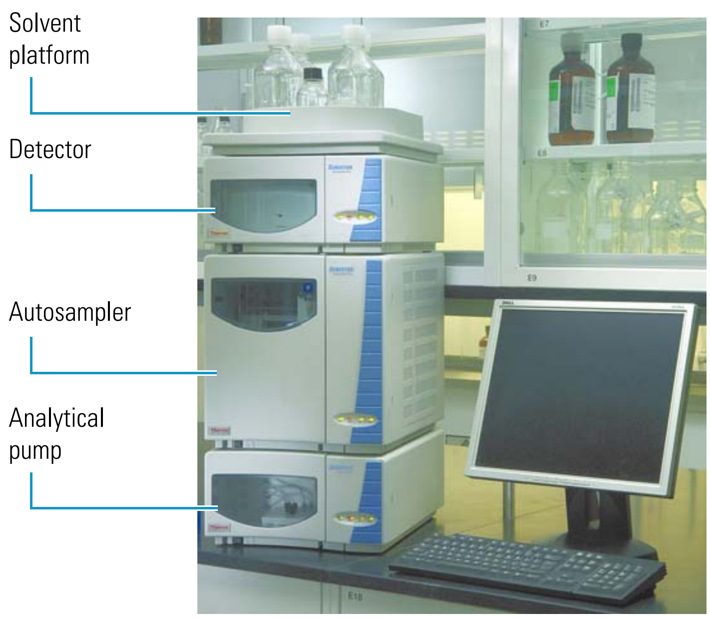
- Fill out a reader survey online at http://www.surveymonkey.com/s.aspx?sm=R7gKOvhLXn3NTkpK2BefHQ_3d_3d.
- Send an e-mail message to the Technical Publications Editor at techpubs-lcms@thermofisher.com.

1

Introduction to the Surveyor Plus LC System

The Surveyor Plus LC system (see Figure 1) which integrates with the Thermo Scientific family of mass spectrometers, consists of an analytical pump, an autosampler, an optional detector, and a solvent platform.

Figure 1. Surveyor Plus LC instrument



Contents

Analytical Pump

Thermo Scientific, part of Thermo Fisher Scientific Inc., offers two analytical pumps in the Surveyor Plus product line: the Surveyor MS Pump Plus, which provides optimum performance in the lower flow rate ranges needed for mass spectrometry; and the Surveyor LC Pump Plus, which provides optimum performance in the flow rate ranges needed for chromatography.

Both analytical pumps contain a built-in solvent degassing system that consists of four independent chambers maintained at a constant vacuum of approximately 27 in. Hg. Each chamber contains an 18 in. length of 0.045 in. ID Teflon™ AF tubing. This translates to a volume of less than 500 µL per channel, adding very little to the quantity of solvent required to purge the lines when you replace an eluent.

The distinguishing features of these analytical pumps are described in the following topics:

- Surveyor MS Pump Plus
- Surveyor LC Pump Plus

Surveyor MS Pump Plus

The Surveyor MS Pump Plus is a dual-piston, quaternary, low-pressure mixing pump with a built-in vacuum degasser and pulse dampener. The pumping system provides flow rates from 1 to 2000 µL/min, which is the range needed to perform LC and LC/MS applications. You can run precise gradients from 200 to 2000 µL/min, while the extremely low gradient delay volume of 65 µL ensures minimum system cycle times.

The pulse dampening assembly consists of a low volume T-connector that the mobile phase passes through. Attached to the side leg of the T-connector is a 2 mL loop of stainless steel tubing. The loop terminates with a priming valve (pulse dampener flush valve). When the priming valve is open, you can flush or fill the dampening loop with an appropriate solvent such as methanol or isopropanol. When the priming valve is closed, the loop is shut off from the flow path and absorbs pump pulsations. Because the 2 mL loop is shut off from the flow path, it adds no gradient delay volume to the LC system.

The priming solvent in the loop does not interfere with the purity of the mobile phase, and its composition does not need to match the mobile phase.

The data system remotely controls the pump with an RS-232 communication link from the data system computer. The only manual control is the power switch located on the front of the pump in the lower-left corner below the door.

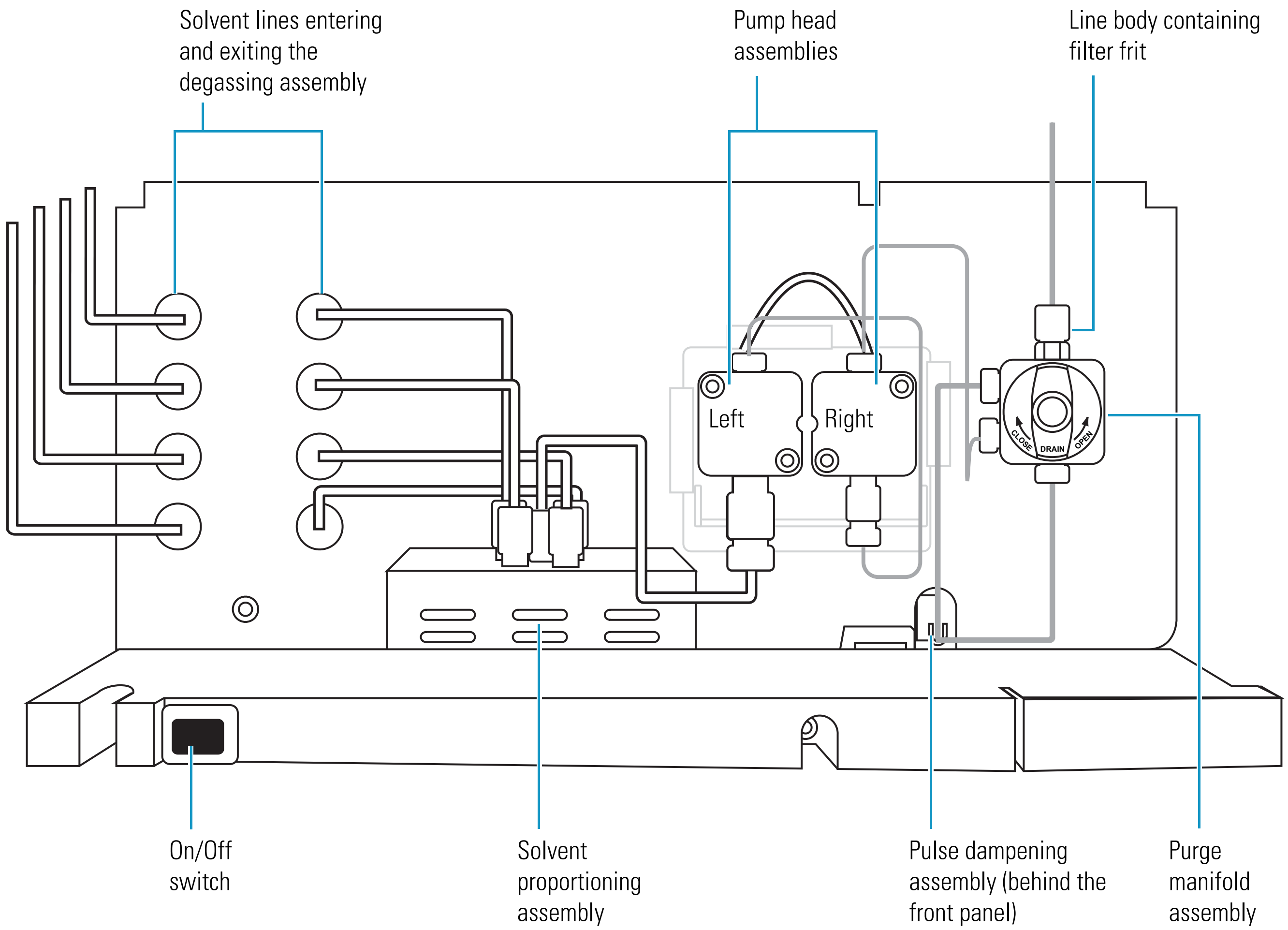
Surveyor LC Pump Plus

The Surveyor LC Pump Plus is a dual-piston, quaternary, low-pressure mixing pump with a built-in vacuum degasser and pulse dampener. The pumping system provides flow rates from 0.001 to 9.999 mL/min, which is the range needed to perform high performance liquid chromatography. You can run precise gradients from 0.200 to 2.000 mL/min.

The Surveyor LC Pump Plus is a bench-top unit for inclusion in the Surveyor Plus high performance liquid chromatograph (HPLC). The Xcalibur data system controls the pump through an Ethernet communication link with the data system computer. The only manual controls are the power switch located on the front of the pump in the lower-left corner below the door and the drain valve.

The Surveyor LC Pump Plus has five major components: the vacuum degassing assembly, the solvent proportioning assembly, the pump head assemblies, the purge manifold assembly, and the pulse dampening assembly (see Figure 2). The pulse dampening assembly adds 400 µL of gradient delay volume to the system. The Surveyor LC Pump Plus has status LEDs on the front door and a low-voltage power supply.

Figure 2. Front view of the Surveyor LC Pump Plus



You can use the Purge command from the data system to draw solvent at a rate of 10 mL/min through the solvent lines connected to the pump after you open the purge valve. To open the purge valve, turn the knob on the front of the purge manifold assembly counterclockwise (see Figure 3). When the valve is in the open position, look for the word DRAIN to appear upside down on the knob.

Figure 3. Drain valve positions

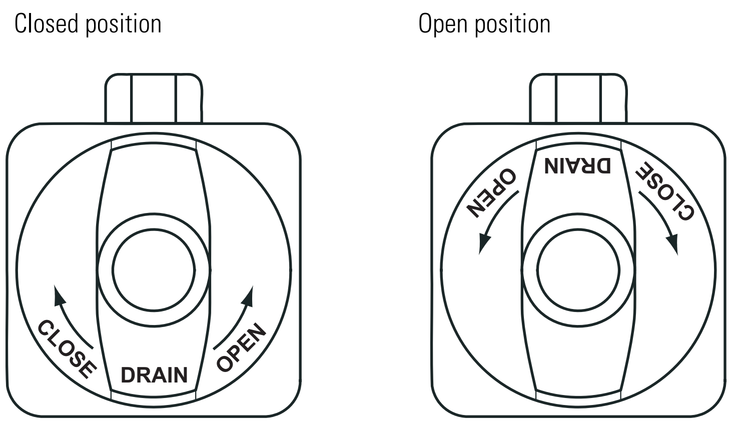
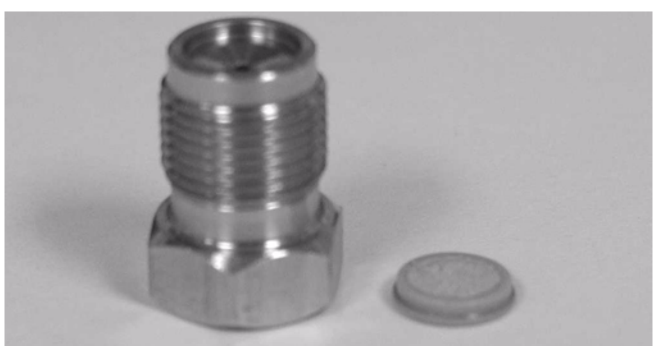


Figure 4 shows the line body fitting removed from the top of the purge manifold assembly. The line body fitting contains an inline filter frit that captures particulate matter shed by the pump seals. This prevents particulate matter from contaminating the tubing that connects the pump to the autosampler. As this filter frit becomes clogged, the system backpressure rises. Replace this filter frit when the system backpressure rises above its typical operating limits.

Figure 4. Line body and filter frit



Autosampler

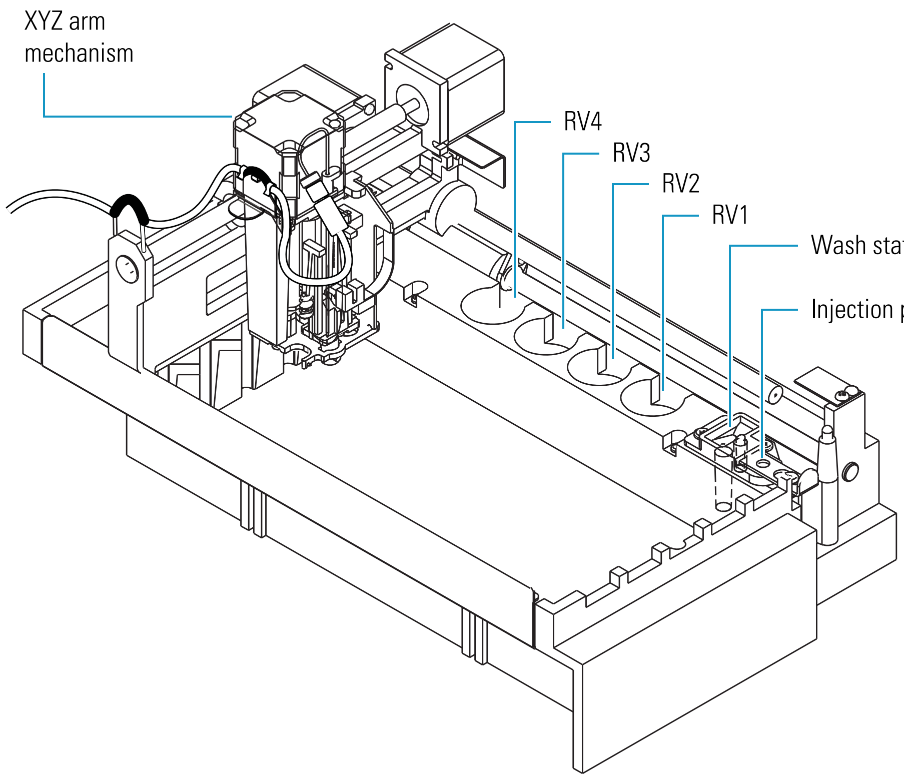
Thermo Fisher Scientific offers two autosampler models in the Surveyor Plus product line: the full-featured Surveyor Autosampler Plus and the Surveyor Autosampler Plus Lite. The full-featured autosampler includes a built-in column oven (5 to 95 °C) and a temperature controlled tray/sample compartment (0 to 60 °C). Both models let you inject samples and perform sample preparation tasks automatically.

- Tray Compartment
- Vial and Well Notation
- Injection System
- Injection Modes
- Temperature Control

Tray Compartment

The tray compartment (see Figure 5) can hold up to five conventional sample trays or one carrier that holds three microwell plates. The tray compartment also holds up to four 16 mL capacity reservoir vials that you can use to hold solvent, reagent, or diluent. The reservoir vials are located behind the wash station and are designated RV1, RV2, RV3, and RV4.

Figure 5. Tray compartment



The five conventional sample trays, from the left side to the right side of the tray compartment, are designated A, B, C, D, and E. Each sample tray holds up to 40 standard 1.8 mL vials, for a total capacity of 200 samples. Overlays allow the sample trays to accommodate different vial sizes. The microwell carrier can hold up to three low-density 96-well microplates or up to three high-density 384-well microplates. The microplates are designated A, B, and C.

The tray compartment door contains a magnetic switch. The magnet is located in the door and the switch is attached to the chassis. When you open the door, the switch signals the autosampler that the door is open.

When you configure the autosampler (from the data system) to verify whether the tray compartment door is open or closed, the XYZ arm automatically moves to the back of the tray compartment when you open the door, allowing you to remove trays or replace vials. Opening the tray compartment door while the autosampler is making an injection does not interrupt the current run. The XYZ arm moves to the back of the compartment after the current injection is complete. The programmed sequence of injections then halts. When you close the tray compartment door, the sequence resumes.

Vial and Well Notation

You specify the vial or microplate well location where you want the autosampler to withdraw sample in the sequence table or the Inject sample direct command. You also specify the vial or microplate well locations for sample preparation tasks.

These topics describe the notation for specifying vial and well locations:

- Vial Notation
- 96-Well Microplate Notation
- 384-Well Microplate Notation

Vial Notation

The notation for the location of vials is as follows:

Tray location in tray compartment:*Vial location in tray*

Where:

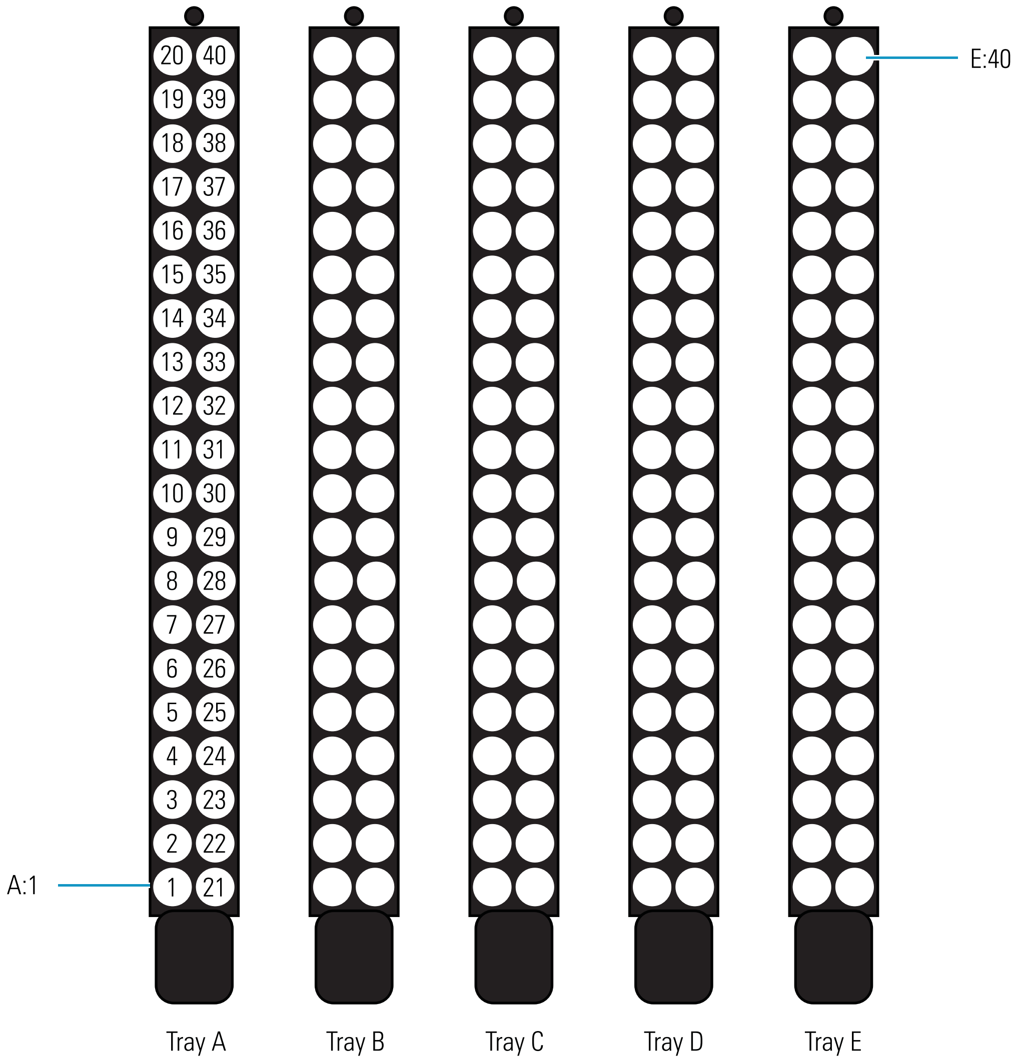
Tray location in tray compartment = A, B, C, D, or E

Vial location in tray = 1 through 40

A colon separates the tray location in the tray compartment from the vial location in the tray.

Figure 6 shows the tray locations.

Figure 6. Standard trays



96-Well Microplate Notation

The notation for the location of 96-well microplates is as follows:

Plate:*RowColumn*

Where:

Plate = A, B, or C

Row = A through H

Column = 1 through 12

Figure 7 shows the notation for 96-well microplates when you select the top left orientation.

Figure 7. Notation for 96-well plates—Top left orientation

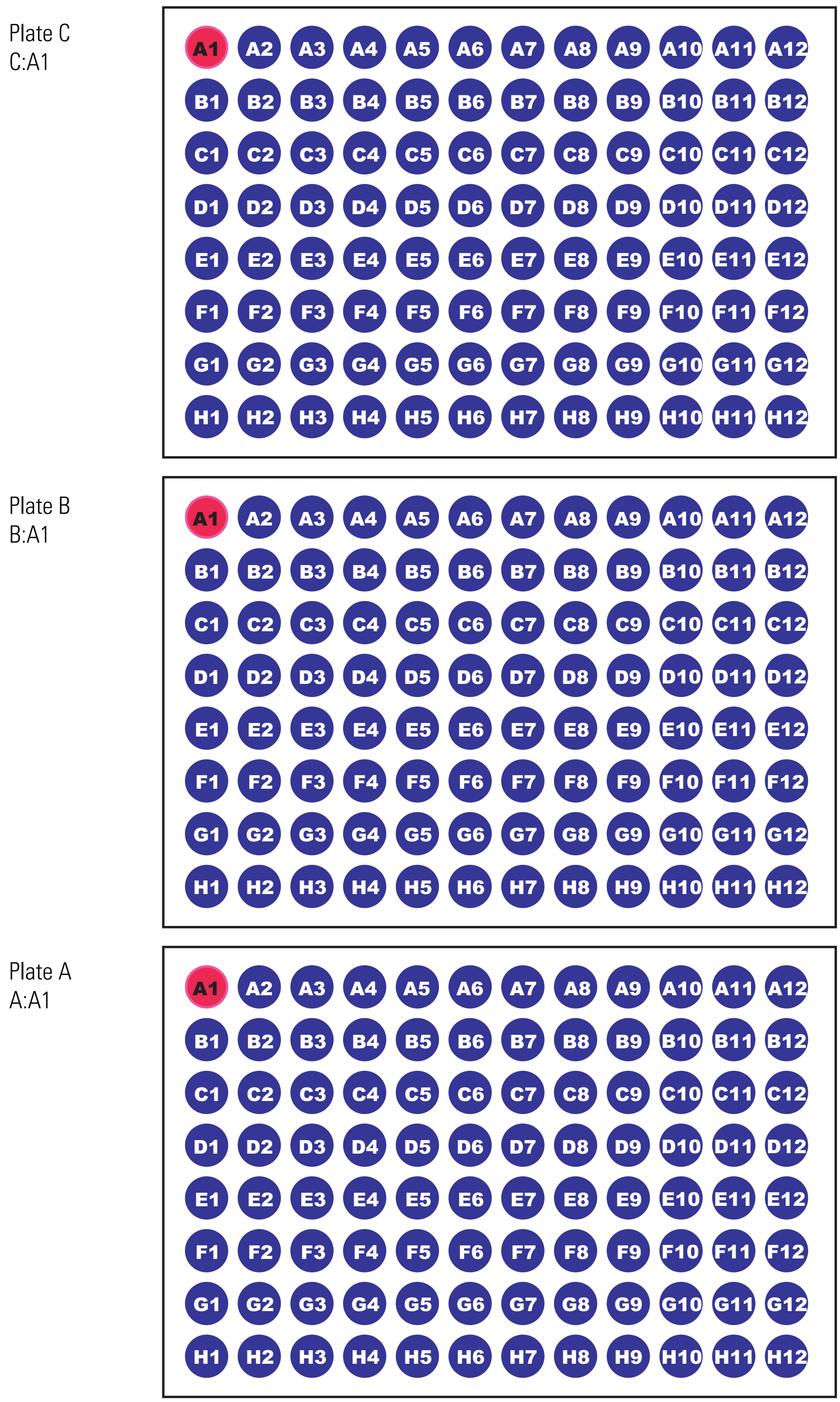


Figure 8 shows the notation for 96-well microplates when you select the top left orientation.

Figure 8. Notation for 96-well plates—Bottom right orientation

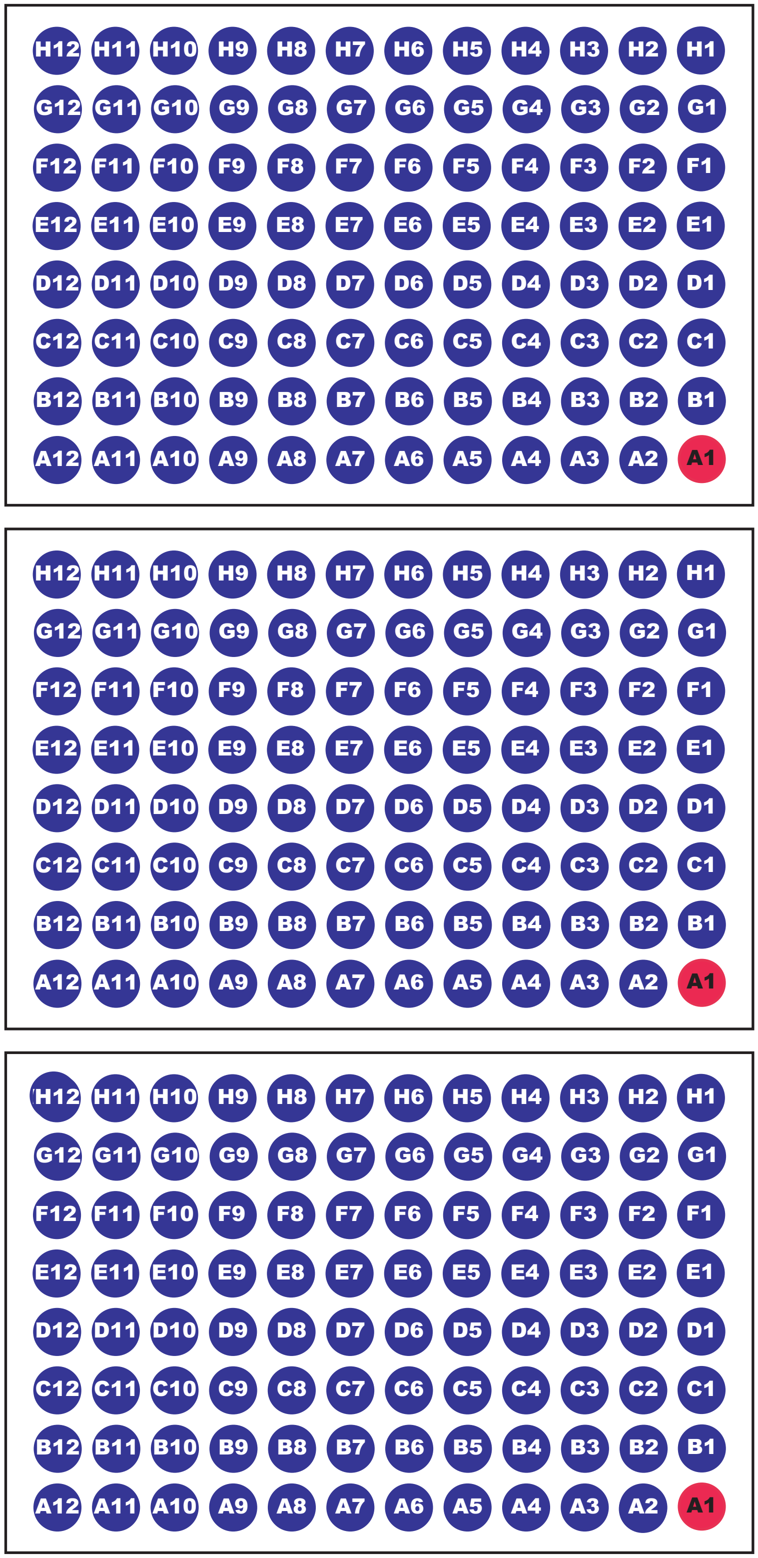


Plate C
C:A1

Plate B
B:A1

Plate A
A:A1

384-Well Microplate Notation

The notation for the location of 384-well microplates is as follows:

Plate:*RowColumn*

Where:

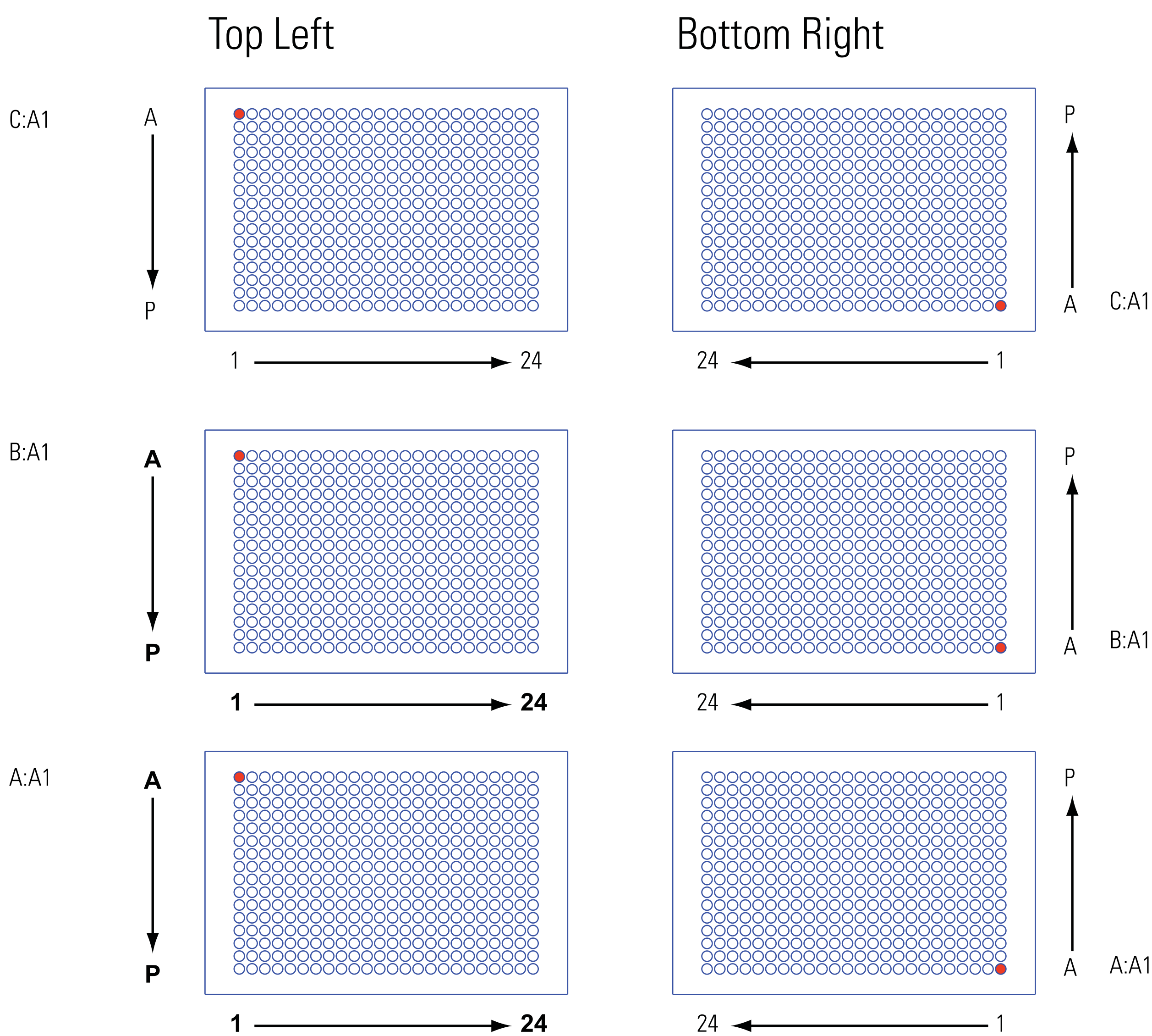
Plate = A, B, or C

Row = A through P

Column = 1 through 24

Figure 9 shows the notation for 384-well microplates.

Figure 9. Notation for 384-well microplates



Injection System

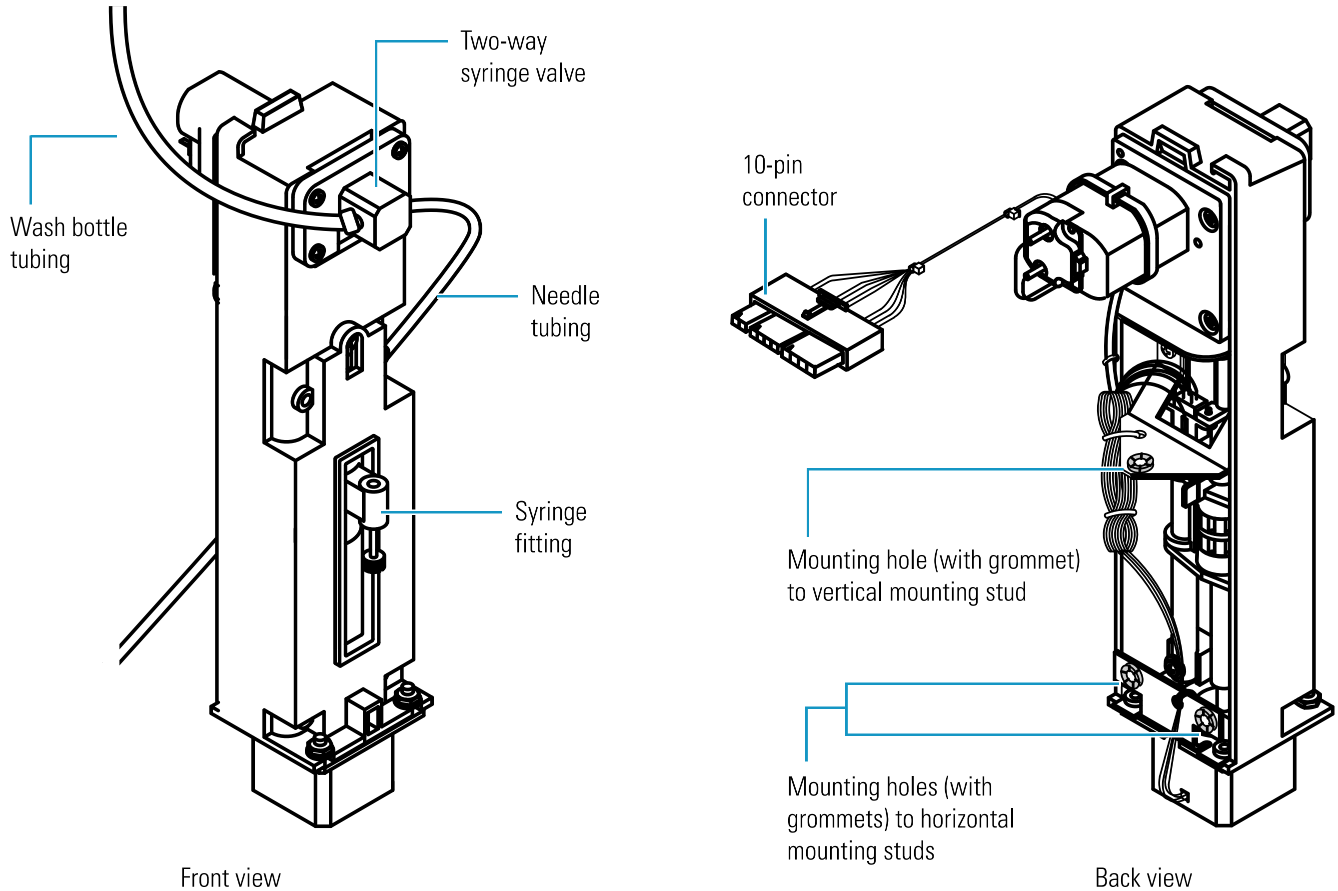
The main components of the injection system include the following:

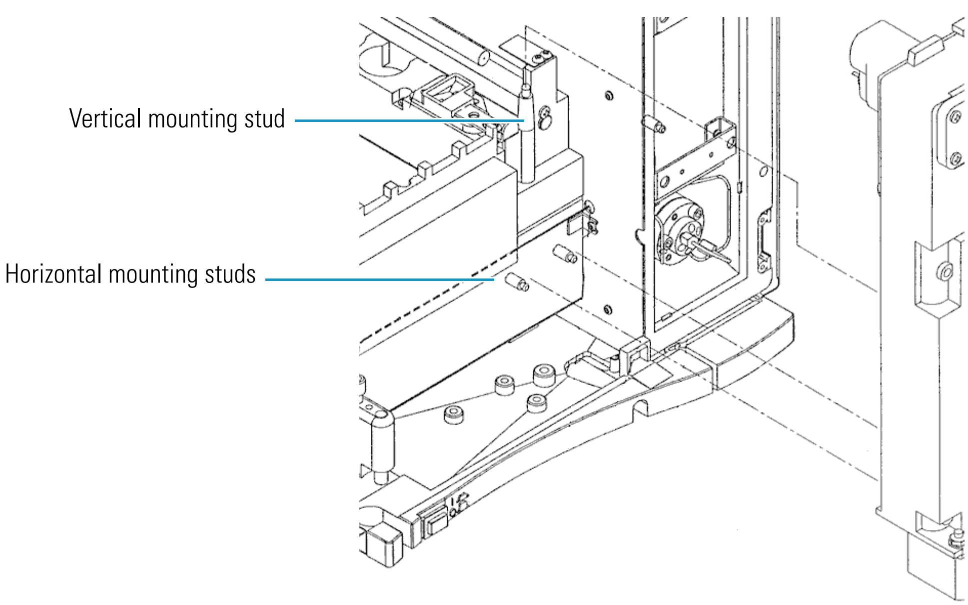
- Syringe Drive Assembly and Syringe Valve
- Wash Bottle Reservoir and Tubing
- Interchangeable Syringe
- XYZ Arm Mechanism
- Needle Assembly and Needle Tubing Assembly
- Injection Port and Transfer Tubing Assembly
- Injection Valve and Sample Loop

Syringe Drive Assembly and Syringe Valve

The syringe drive assembly holds the autosampler syringe. There are three holes with grommets on the back of the syringe drive assembly. You mount the syringe drive assembly to the front of the autosampler by matching these holes to one vertical mounting pin and two horizontal mounting pins located in front of the autosampler injection port. The rubber grommets minimize the effects of vibration, but also cause the connection between the syringe drive assembly and the body of the autosampler to feel slack (see Figure 10).

Figure 10. Syringe drive assembly for the Surveyor Autosampler Plus, showing the front and the back of assembly





The syringe valve is a two-position rotary valve. In the wash bottle position, the syringe draws wash solvent into its syringe barrel as its plunger descends. In the needle position, the syringe draws sample solution into the needle tubing as its plunger descends and pushes sample solution out of the needle tubing as its plunger ascends. The syringe never draws sample solution into its barrel.

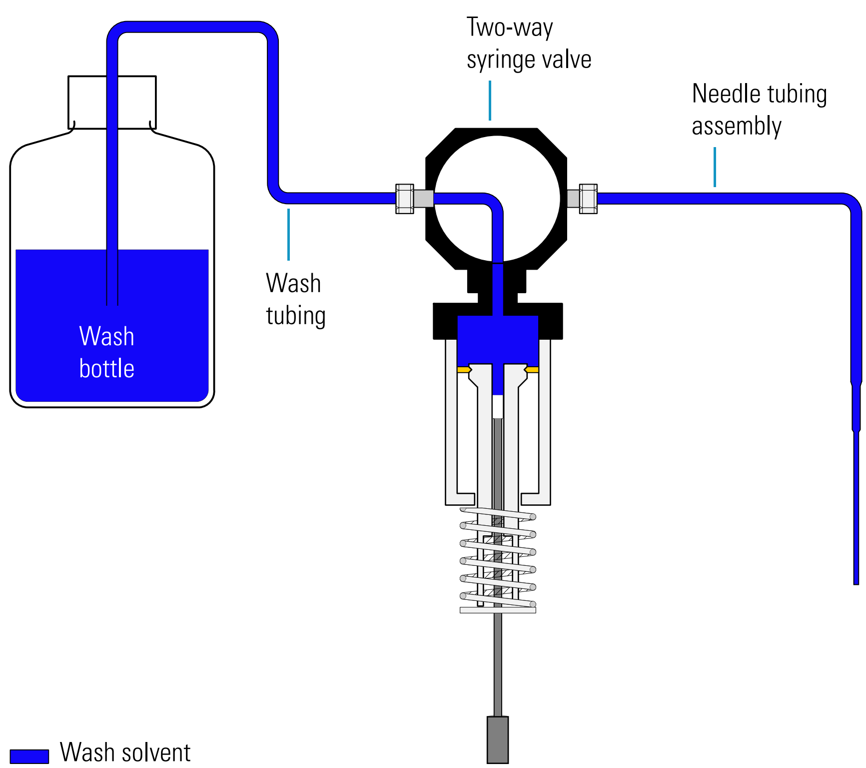
Note The needle tubing holds approximately 560 µL of solvent. If you have installed the 2500 µL standard syringe (which must be special ordered) and you plan to make large volume injections, install the 1-mL needle tubing extension that is shipped with this syringe.

Wash Bottle Reservoir and Tubing

The wash bottle rests in the solvent platform on the top of the LC stack. It is connected to the syringe valve by way of the wash bottle tube. Both of the direct commands, Flush (from bottle) and Wash Needle (from bottle), draw solvent from the wash bottle. In addition, both the partial loop injection and the no waste injection modes draw transfer solution from the wash bottle. If the wash bottle runs dry, the wash bottle tubing and the syringe barrel fill with air. If the syringe runs dry, the autosampler cannot draw sample into the needle tubing.

Figure 11 shows the syringe valve in the wash bottle position.

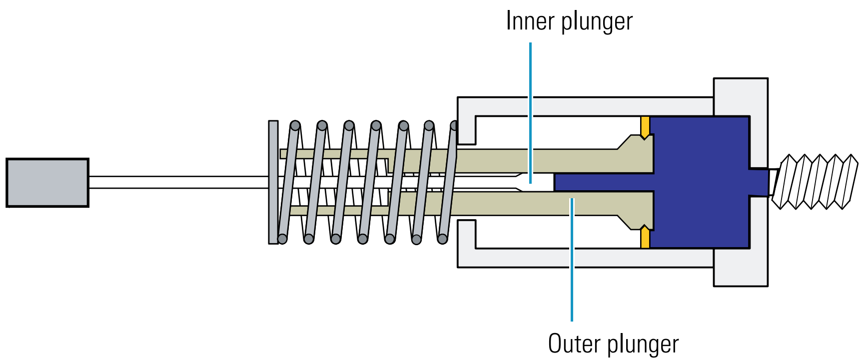
Figure 11. Drawing wash solvent from the wash bottle



Interchangeable Syringe

The standard configuration for the autosampler consists of a 250 µL dual-concentric syringe. The dual-concentric syringe consists of a small, inner plunger, and a larger, outer plunger (see Figure 12).

Figure 12. Dual-concentric syringe



The syringe uses its inner plunger to draw and deliver sample amounts equal to or less than its maximum capacity, which is 265 µL for the 250 µL concentric syringe. The syringe uses its outer plunger to draw and expel large volumes of solvent, for example, during a flush or wash cycle.

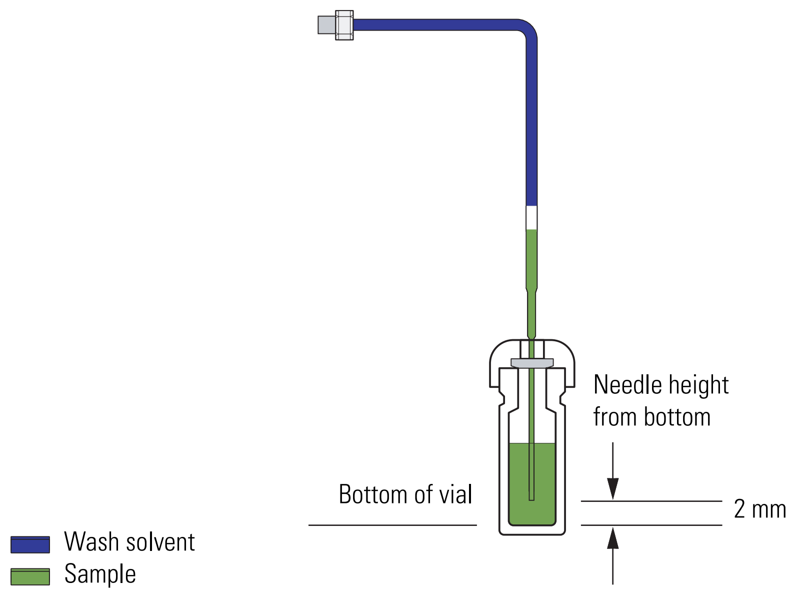
Dual-concentric syringes are available in 100, 250, and 500 µL sizes. The volume of the outer plunger region, 565 µL, is the same for all three dual-concentric syringes. The size of the inner plunger and the injection mode determine the available injection volume range. In addition to offering three sizes of concentric syringes, Thermo Scientific also offers a 2500 µL standard (single plunger) syringe.

XYZ Arm Mechanism

The XYZ arm mechanism moves the needle along the X-Y plane to the requested vial or well location. After it positions the needle above the vial or well, the XYZ arm mechanism lowers the needle along the Z-axis to the requested needle height.

Figure 13 shows the needle descending to a depth of 2 mm from the bottom of a standard 1.8 mL vial.

Figure 13. View of needle descending to 2 mm from the bottom of a vial



After the autosampler withdraws the sample from the sample vial or well into the needle tubing, the XYZ arm mechanism moves along the x-y plane, back to the home position, which is above the injection port of autosampler, and then lowers the needle into the injection port where it expels the sample. The sample travels through transfer tubing and into the sample loop of the injection valve.

> **IMPORTANT** Because the XYZ arm moves to the sample position to withdraw sample, do not place objects taller than 1.8 inches into the tray compartment, as they will stall the XYZ arm.

Do not move the XYZ arm manually. Instead, use the following commands and options provided by the data system to control the position of the XYZ arm:

- To make the XYZ arm automatically move to the back of the tray compartment when you open the tray door, select the **Verify Door is Closed** option, which is available when you configure the autosampler device driver (see “Communication Page” on page 45).
- To move the XYZ arm to the back of the tray compartment, use the **Position Arm to Access Tray** direct command (see “Removing and Installing Sample Trays” on page 193).
- To move the XYZ arm mechanism to its home position above the injection port, use the **Set Arm to Home Position** direct command.
- To move the XYZ arm mechanism to the middle front of the tray compartment, allowing easy access to the needle, use the **Remove Needle** direct command.

Needle Assembly and Needle Tubing Assembly

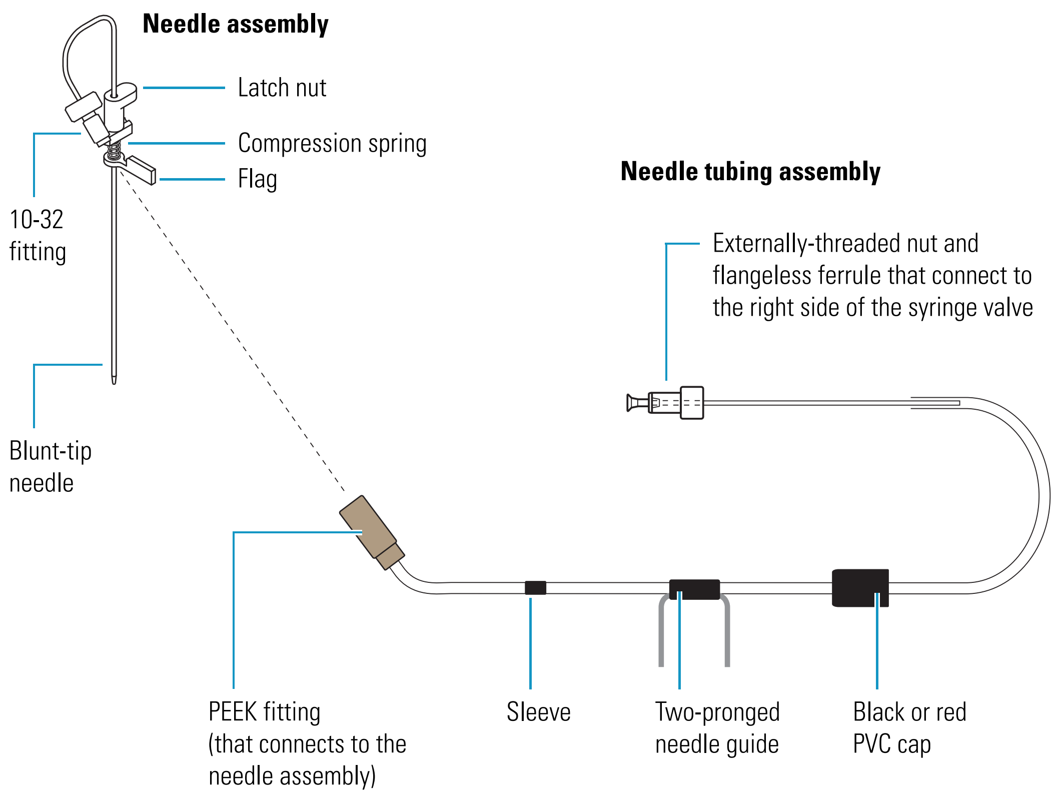
The needle assembly consists of a blunt-tip needle, a latch nut, a flag, a compression spring, and a 10-32 fitting that connects to the needle tube assembly. The needle fits into the needle mount on the XYZ arm.

The needle tube assembly connects the solvent path between the needle and the syringe valve and consists of low-pressure tubing, an internally-threaded fitting that connects to the needle assembly fitting, a sleeve, a black or red PVC cap, a needle tube guide that attaches to the *x*-axis positioning frame, and an externally-threaded fitting with a flangeless ferrule that connects to the right side of the syringe valve.

IMPORTANT To prevent damage to the needle tubing, take care when you connect the needle tubing guide to the back of the syringe drive assembly. Pinched tubing causes performance problems.

Figure 14 shows the needle assembly and the needle tubing assembly.

Figure 14. Needle assembly and needle tubing assembly



Injection Port and Transfer Tubing Assembly

Figure 15 shows the autosampler injection port.

Figure 15. Autosampler injection port

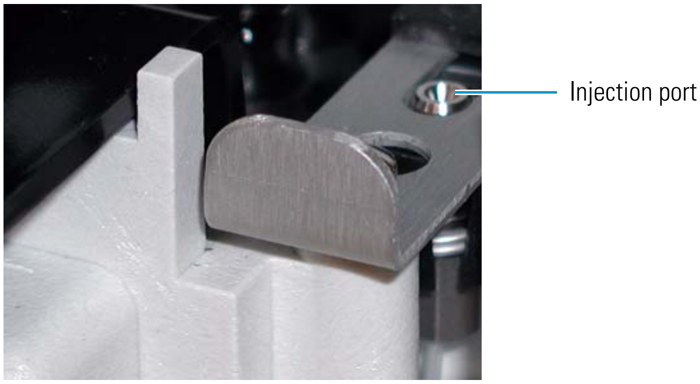
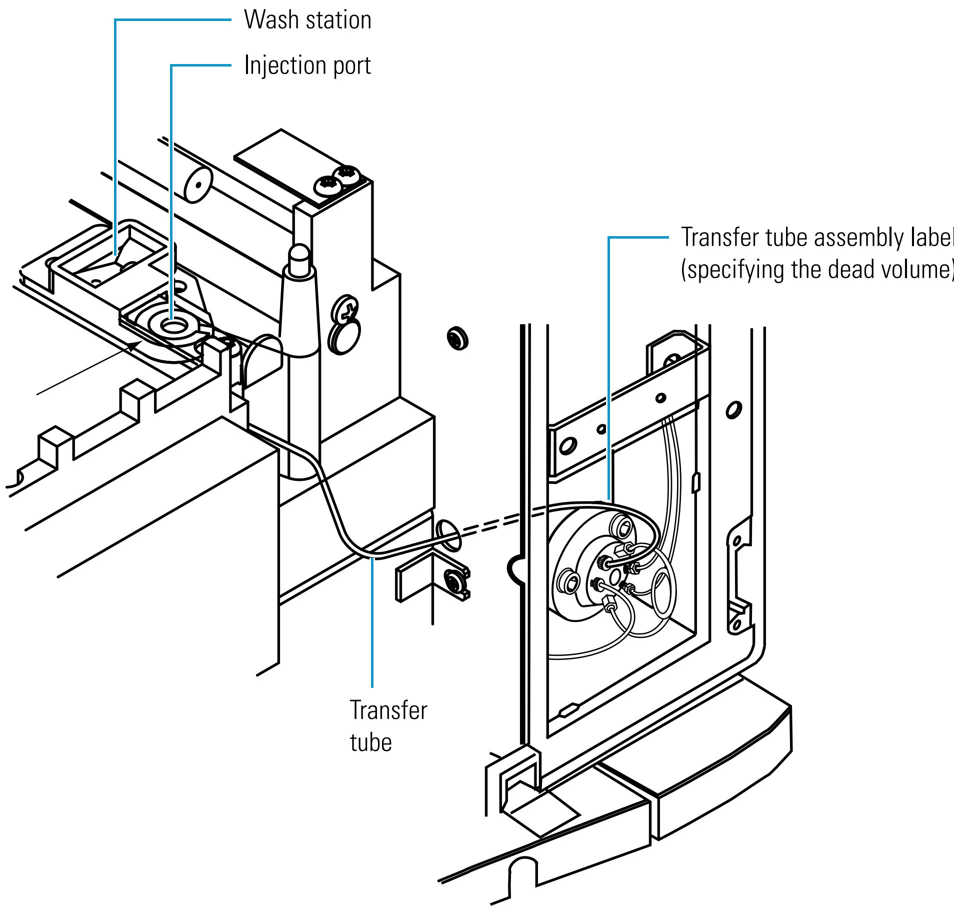


Figure 16 shows the 0.012 in. ID transfer tube that connects the autosampler injection port to port 2 of the injection valve.

> **IMPORTANT** The label attached to the transfer tube assembly specifies its internal volume. You must enter this value when you specify the configuration settings for the autosampler.

Figure 16. Injection port and transfer tube



Injection Valve and Sample Loop

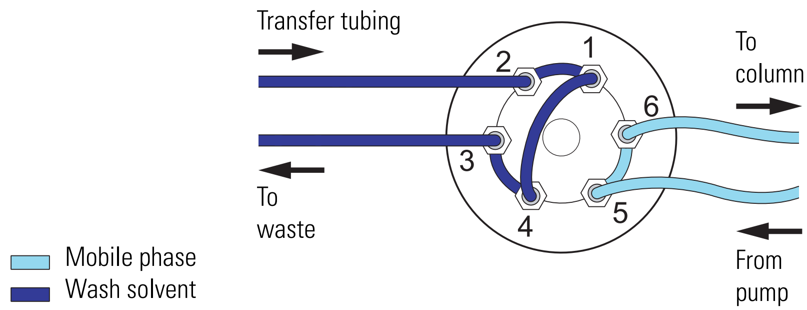
The injection valve is a two-position, six-port valve that introduces sample onto the column by way of the sample loop.

The sample loop is a section of stainless steel tubing with end fittings. It is an interchangeable part that is attached to ports 1 and 4 of the injection valve. The autosampler comes with a 25 µL sample loop.

There are two positions for the six-port injection valve: fill and inject.

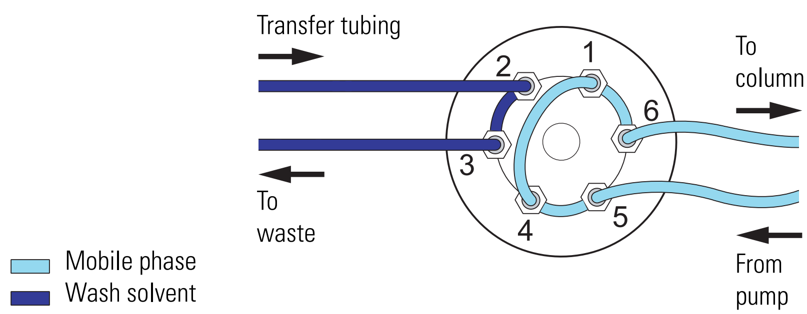
In the fill position (see Figure 17), the sample loop is isolated from the mobile phase stream. As the mobile phase bypasses the sample loop, the upward movement of the autosampler syringe plunger pushes sample into the front of the sample loop, connected to port 1 of the injection valve. Once the sample loop is filled, excess solution exits the injection valve through port 3 to waste.

Figure 17. Injection valve in the fill position



In the inject position (see Figure 18), mobile phase enters the sample loop from the back, backflushing the contents of the sample loop onto the column. Excess sample left in the transfer tube is expelled directly to waste. To allow ample rinsing of the sample loop with mobile phase, the injection valve remains in the inject position during the entire run.

Figure 18. Injection valve in the inject position



Injection Modes

The autosampler can operate in any of the following three modes:

- No Waste Injection Mode
- Partial Loop Injection Mode
- Full Loop Injection Mode

The optimum injection mode depends upon the amount of sample that you have and the degree of precision that your application requires.

No Waste Injection Mode

The no waste injection mode is a technique that withdraws only the exact amount of sample requested from the sample vial. Of the three injection modes, the no waste injection mode uses the least amount of sample, but it is also the least precise. Use this injection mode to conserve sample.

Approximately 0.25 µL of the sample is lost as it travels from the injection port, through the transfer tubing, and into the injection valve. Because of this loss, the minimum recommended injection volume is 1.0 µL.

The quantity of lost sample depends on the syringe rate. Decreasing the syringe rate decreases the sample loss. For best results, use a syringe rate no greater than 4 µL/s for the no waste injection mode.

IMPORTANT For no waste injections, do the following:

- Use a sample loop that is at least 5 µL larger than the injection volume. Because the accuracy of the nominal size is ±20%, use an estimate of 80% for the actual size. For example, use 20 µL as an estimate for the actual volume of a 25 µL loop, and inject no more than 15 µL with this loop size.
- Consider matching the chemistry of the sample matrix, the flush solution, and the mobile phase. For no waste injections, the autosampler loads approximately 2 µL of flush solvent and 3 µL of air into the sample loop, regardless of the requested injection volume.
- Inject at least 1.0 µL of sample.

Partial Loop Injection Mode

The partial loop injection mode is a technique that withdraws 22 µL of excess sample from the vial in addition to the requested injection volume. Approximately one-half of the excess volume is expelled to waste before the center of the sample bolus is metered into the front of the sample loop. The second portion of excess sample is expelled to waste after the sample bolus is backflushed onto the column.

Partial loop injections are useful when you have a limited volume of sample. Using the partial loop injection mode, you can inject variable amounts of sample, ranging from a minimum of 0.1 µL to a working maximum of one-half the volume of your sample loop. This maximum volume limitation is caused by the laminar flow of fluid within the stainless steel sample loop.

IMPORTANT To make precise partial loop injections, use a sample loop that is at least twice the size of the injection volume. The accuracy of the nominal sample loop volume is ±20%. Because the actual volume of the 25 µL sample loop (provided with the autosampler) is anywhere from 20 to 30 µL, limit the maximum injection volume with this loop to 10 µL. To inject more sample, use a larger sample loop.

Full Loop Injection Mode

The full loop injection mode is a technique that withdraws a sample volume from the vial sufficient to overfill the sample loop by a minimum factor of two. Because the physical size of the sample loop determines the actual injection volume, not the metering action of the stepper motor, a full loop injection is very reproducible. However, because the intent of the full loop injection mode is to completely fill the sample loop, you cannot inject variable amounts of sample.

Full loop injection is useful when you want maximum precision and have unlimited sample. To change the injection volume, you must change the sample loop size.

Note Full loop injections are limited to the size of the configured sample loop.

In the full loop injection mode, the autosampler withdraws a large excess of solution from the sample vial according to the following equation:

Amount Withdrawn = 3 × Injection Volume + Dead Volume + 7.5 µL

Where:

Dead Volume = Volume of Transfer Tube + Volume of Injection Port and Rotor Slot

This equation is valid until the syringe reaches its maximum capacity, at which point only the maximum capacity of the syringe is withdrawn. The maximum capacity of the 250 µL concentric syringe is 265 µL.

Temperature Control

The full-featured Surveyor Autosampler Plus has two built-in temperature control features:

- Tray Temperature Control
- Column Oven Control

Tray Temperature Control

The tray temperature control feature provides temperature control of the samples in the range from 0 to 60 °C. A Peltier device maintains the tray temperature.

Column Oven Control

The built-in column oven controls the temperature of the air surrounding the chromatographic column. Isothermal temperature control is achieved using a Peltier device. The Peltier device is a solid-state, heat-transferring assembly used to heat or cool the column oven. The range of temperature control is 5 to 95 °C.

Between the analytical pump and the autosampler injection valve, the mobile phase is diverted through a heat exchanger located behind the column oven. As it passes through the heat exchanger, the mobile phase equilibrates to the temperature of the column oven before it reaches the injection valve. The heat exchanger adds only 3 µL of gradient delay volume to the LC system.

Detector

The Surveyor Plus product line includes two detectors that you can control from the Xcalibur data system: the Surveyor PDA Plus Detector and the Surveyor UV/Vis Plus Detector. The Surveyor PDA Plus Detector, in combination with the 5 cm or 1 cm LightPipe flowcell, provides the highest level of sensitivity available in photodiode array detection for HPLC. The Surveyor UV/Vis Plus Detector is a dual-wavelength UV/Vis detector. Both detectors have a dual-lamp optical bench that covers the UV-visible spectrum from 190 to 800 nm.

This section contains the following topics:

- Surveyor PDA Plus Detector
- Surveyor UV/Vis Plus Detector

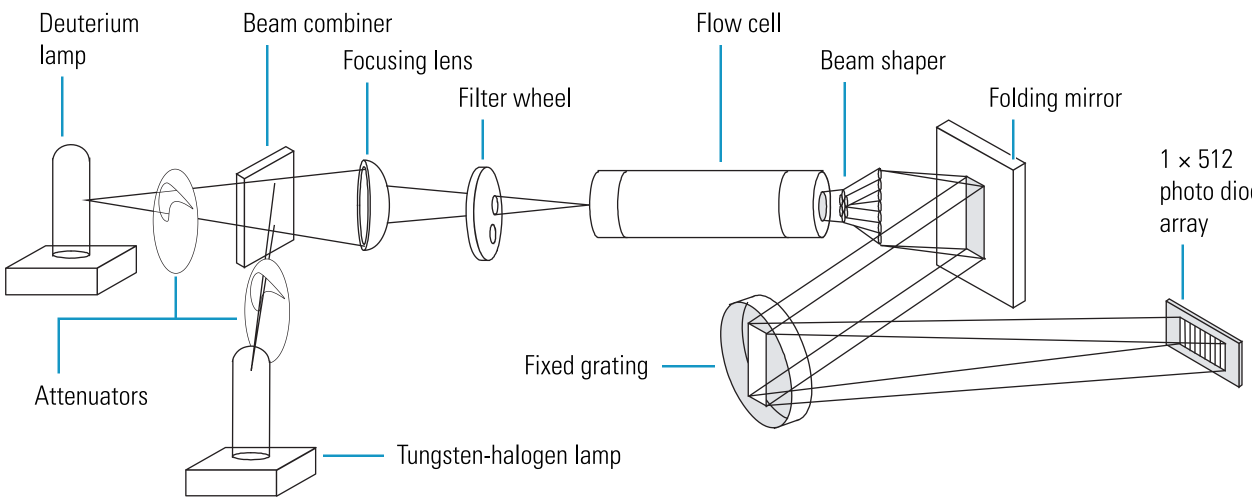
Surveyor PDA Plus Detector

The Surveyor PDA Plus Detector is a full-featured, time-programmable, photodiode array detector that can scan the full ultraviolet-visible range from 190 to 800 nm. The optimal location for the PDA detector is above the autosampler and below the solvent platform. The data system computer controls the PDA detector through an Ethernet communication link. The PDA detector consists of a dual-light source, an optical bench, a photodiode array, a low voltage power supply, several printed circuit boards (PCBs), and four status light-emitting diodes (LEDs).

The dual-light source includes a deuterium lamp for detection in the ultraviolet wavelength range (190 to 360 nm) and a tungsten-halogen lamp for detection in the visible wavelength range (360 to 800 nm). The light emitted by the two lamps overlaps in the 300 to 500 nm range. A pair of attenuators that you can manually adjust controls the intensity of light reaching the photodiode array.

The optical bench contains a beam combiner, focusing lens, filter wheel, flow cell, beam shaper, folding mirror, and grating (see Figure 19). The beam combiner reflects the light coming from the tungsten-halogen lamp so that it is parallel to and coincident with the light from the deuterium lamp. The combined beam is then focused on the inlet window of the flow cell through the filter wheel. The standard filter wheel has two positions. Use position 1(Open), which places a clear, quartz window in the optical path, for normal operation. Use position 2 (Holmium Oxide), which places a sealed, quartz cuvette filled with a holmium oxide/perchloric acid solution (NIST™ traceable) in the optical path, for wavelength accuracy verification and calibration.

Figure 19. Optical bench of the PDA detector



The PDA detector does not have independent controls, such as a keypad, to create data acquisition methods. Instead, you create instrument methods for data acquisition with the data system. The only manual controls for the PDA detector are the On/Off switch that controls line power, the attenuators that control the light throughput to the diode array, and the holmium oxide wheel that controls the position of the wavelength calibration solution.

Surveyor UV/Vis Plus Detector

The UV/Vis detector is a full-featured, time-programmable, variable-wavelength UV/Vis (ultraviolet/visible) absorbance detector. It operates in either the single wavelength mode, the dual wavelength UV wavelength mode, or the dual wavelength Visible mode. The wavelength range in the single wavelength mode is 190 nm to 800 nm. In the dual wavelength UV mode, the range is 190 to 450 nm and in the dual wavelength Visible mode, the range is 366 to 700 nm.

The wavelength time table is available in all three modes. The time table can contain up to 10 lines. If the Zero On Wavelength Change feature is enabled, the absorbance of the baseline is re-zeroed between each line in the time table, even if the wavelengths remain the same. The absorbance of the baseline is not zeroed between the last two lines in the table.

To provide a complete spectrum of ultraviolet and visible light, the detector uses a deuterium lamp for the UV range (190 to 365 nm) and a tungsten lamp for the visible range (366 to 800 nm). The lamps are protected by a cover with a special safety interlock to reduce the possibility of human exposure to harmful UV light.

To control the UV/Vis Detector, you must first add the detector to the instrument configuration by using the Thermo Foundation Instrument Configuration application (see "Surveyor UV/Vis Plus Detector Configuration Settings" on page 59).

After you add the UV/Vis detector to the instrument configuration, you can set up the data acquisition parameters for automated instrument control from the UV/Vis detector view of the Thermo Xcalibur Instrument Setup window (see "UV-Vis Detector Instrument Method Parameters" on page 101).

You can control the detector's lamps from the Information view of Thermo Xcalibur (see "Turning Devices On, Off, or into Standby from the Information View" on page 148).

Solvent Platform

The solvent platform, located on the top of the LC stack, holds four 1 liter solvent reservoir bottles and one 1 liter wash bottle. Four 1/8 in. OD × 1/16 in. ID, FEP solvent lines carry solvent from the reservoir bottles down to the vacuum membrane degasser, which is built into the analytical pump. One 1/8 in. OD × 1/16 in. ID, FEP solvent line carries solvent from the wash bottle to the two-position syringe valve of the autosampler.

Communication with the Data System PC

The Surveyor Autosampler Plus, Surveyor LC Pump Plus, Surveyor PDA Plus Detector, and the Surveyor UV/Vis Plus Detector use an Ethernet link to communicate with the data system computer. To establish communication with these devices, you connect one end of an Ethernet cable to the Ethernet port on the back panel of the device and the other end of the cable to an Ethernet switch. You use a second Ethernet cable to connect the Ethernet switch to the PC that has the data system installed. The accessory kits include a shielded, Category 5, RJ-45, 7 ft length Ethernet cable with ferrite.

The Surveyor MS Pump Plus uses an RS-232 serial link to communicate with the data system computer. The accessory kit for the Surveyor MS Pump Plus includes a USB cable.

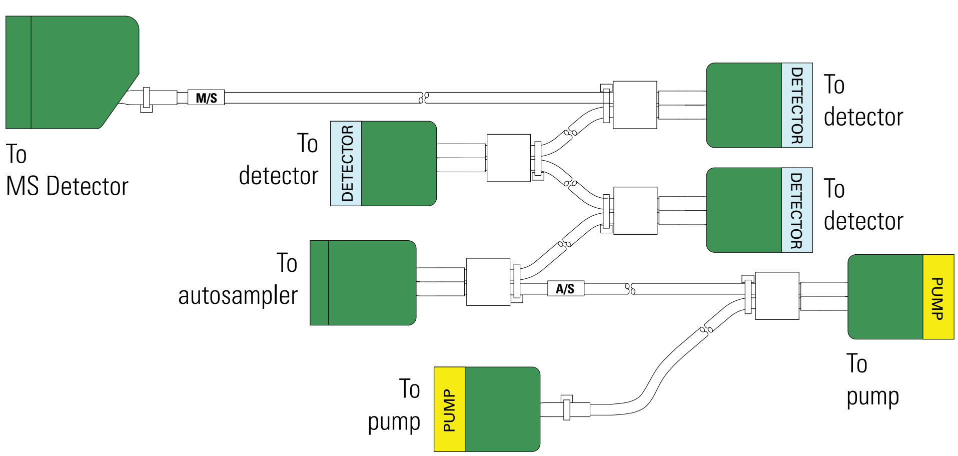
In addition to connecting the communication cables, you must enter the appropriate stack addresses for the Surveyor Autosampler Plus, Surveyor PDA Plus Detector, Surveyor UV/Vis Plus Detector, and Surveyor LC Pump Plus when you configure the device drivers. The stack address that you enter when you add one of these devices to the configuration for your instrument must match the unit ID setting on the back panel of the device. For more information on configuring the Surveyor Plus device drivers, see Chapter 2, "Thermo Foundation Instrument Configuration."

Synchronization of the LC Devices

A system interconnect cable provided in the Surveyor System Kit coordinates the run control signals between the Surveyor Plus devices.

Figure 20 shows the cable assembly with seven green combicon connectors: two are labeled PUMP; three are labeled DETECTOR; and two are unlabeled. You can identify the connector for the autosampler by the small, A/S tag on its adjacent cable. You can identify the M/S connector by its shape and by the small, M/S tag on its adjacent cable.

Figure 20. 7-connector system interconnect cable



To use the 7-connector cable with the Surveyor MS Pump Plus, you need an additional adapter cable. Figure 21 shows the interconnect cable connections for a Surveyor LC system.

Figure 21. Back panels of Surveyor devices, showing the 7-connector system interconnect cable

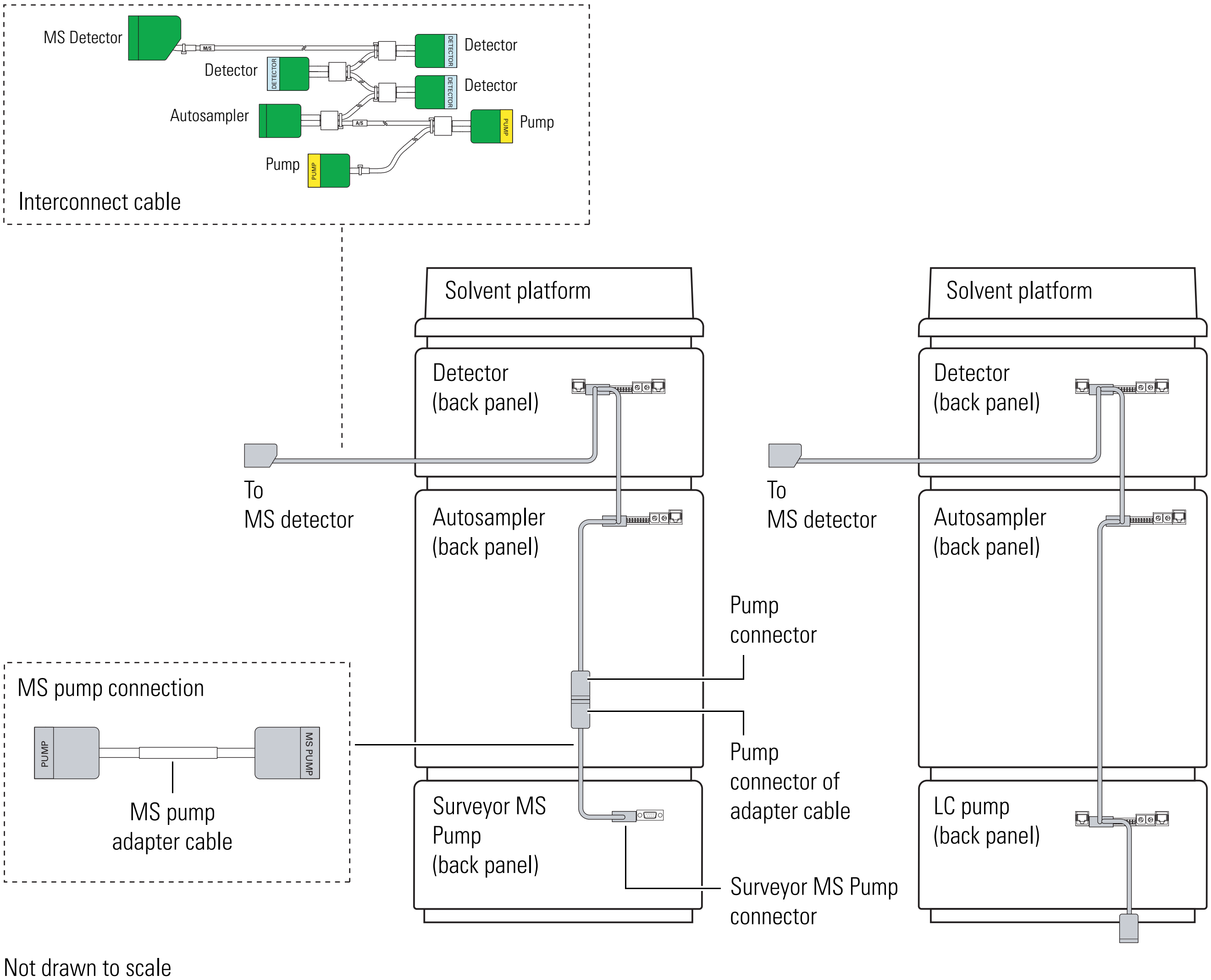


Figure 22 shows the cable assembly with five green combicon connectors. The assembly has two pump connectors: one is labeled MS PUMP and one is labeled LC Pump. You can identify the connectors for the autosampler, detector and MS detector by the small tags on their adjacent cables. To connect the MSQ Plus Mass Detector, you must modify the wiring connections inside the MS detector connector.

Figure 22. 5-connector system interconnect cable (P/N F5049-010)

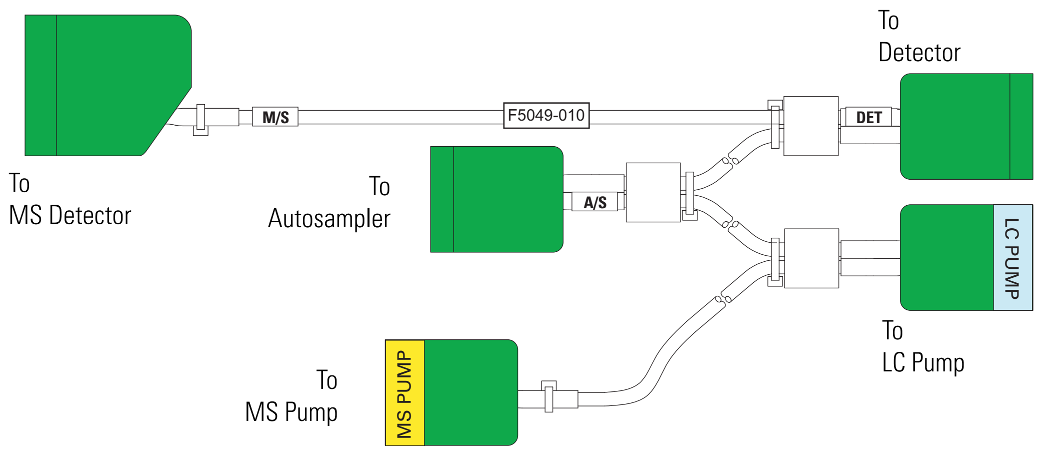
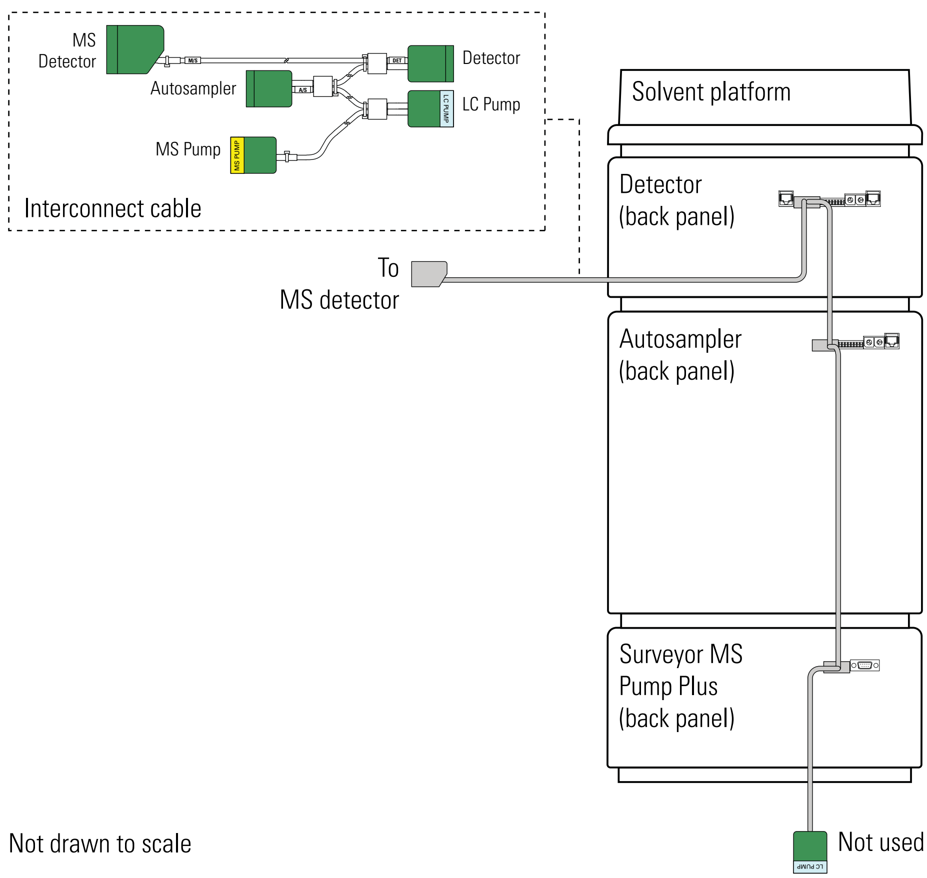


Figure 23 shows the interconnect cable connections for a Surveyor LC system.

Figure 23. Back panels of the Surveyor devices, showing the 5-connector interconnect cable



For more information on connecting the interconnect cable to the devices of your LC system, refer to the *Surveyor Plus LC System Getting Connected Guide*.

During a run, the system issues the following sequence of run control signals:

1. The data system PC issues a request to perform an injection.
2. When the autosampler becomes ready, it issues the A/S Ready signal.

   The autosampler goes into the Ready state when all the configuration and instrument method conditions are met, including the closing of the tray compartment door, and the sample tray and column oven temperature zone readings showing they are within tolerance of their setpoints.
3. When the pump pressure stabilizes, the pump issues the Pump Ready signal to the autosampler.

   The autosampler injection valve switches to the fill position, and then the autosampler pushes sample into the sample loop.
4. The autosampler issues the Gradient Start signal to the pump. This signal commands the pump to start its gradient program.
5. When its piston cam reaches the home position, the pump issues the Inject Hold release signal to the autosampler.
6. The autosampler injection valve switches to the inject position, allowing the mobile phase to backflush the contents of the sample loop onto the column. The autosampler then issues a momentary Inject Out signal to the detector.
7. The detector starts acquiring data.

Status LEDs

Each of the Surveyor Plus LC devices has a panel of four status LEDs located on the front of the left door. All of the devices have these three LEDs: Power, Comm, and Run. In addition, the detector has a Lamps LED; the autosampler has a Temp LED; and the analytical pump has a Degas LED.

These topics describe the meanings of the LED states:

- Surveyor MS Pump Plus LED States
- Surveyor LC Pump Plus LED States
- Autosampler LED States
- UV-Vis Detector LED States
- PDA Detector LED States

Surveyor MS Pump Plus LED States

Table 1 lists the states of the Surveyor MS Pump Plus LEDs.

Table 1. Thermo pump status LEDs and meaning (Sheet 1 of 2)

LED	Status	Meaning
Power	Green	The pump is on and receiving power.
Comm	Amber	The pump is not communicating with the data system.
	Green	Communication to the data system has been established.
	Flashing green	An pump program is downloading from the data system PC.
Run	Amber	The power is switched on, but the p pistons are idle, producing no flow.
	Flashing amber	A firmware download is in progress or an error condition has occurred.
	Green	The pump pistons are moving, but a pump program is not running. This state can occur when the pump is under direct control or a pump program has ended.
	Flashing green	The pump is running a pump program from a downloaded method.

Table 1. Thermo pump status LEDs and meaning (Sheet 2 of 2)

LED	Status	Meaning
Degas	Amber	The degas unit is building vacuum.
	Flashing amber	A failure, such as a loss of vacuum, has occurred.
	Green	Sufficient vacuum has developed to perform chromatography.

Surveyor LC Pump Plus LED States

Table 2 list the LED states for the Surveyor LC Pump Plus.

Table 2. Surveyor LC Pump Plus status LEDs and meaning

LED	Status	Meaning
Power	Green	The pump is on and receiving power.
Comm	Amber	The pump is not communicating with the data system PC.
	Flashing amber	The rotary switches on the back panel of the LC pump are set to 00 for downloading firmware.
	Green	The pump is communicating with the data system PC.
Run	Amber	The pump is not communicating with the data system PC, the pump's motor is stopped, the pistons are homing, or the pump is stabilizing (except during a run).
	Flashing amber	The pump is experiencing an error condition.
	Green	The pump is in the Ready state.
	Flashing green	The pump is performing a run or the pump is stabilizing during a run.
Degas	Amber	The pump is powering-up and the degas unit is building vacuum.
	Flashing amber	The degas unit is experiencing a failure, amber, such as a loss of vacuum.
	Green	The degas unit has developed vacuum sufficient for chromatography to be performed.

Autosampler LED States

Table 3 lists the LED states for the autosampler.

Tip To ensure that the controlled temperature zones are in equilibrium at the set temperature before the autosampler makes an injection, select the **Wait for temperature ready** check box when you add the autosampler to the data system instrument configuration.

When you select this check box, the autosampler waits for the controlled temperature zones are in equilibrium at the set temperature before it makes an injection. While the temperature zones are equilibrating to the set temperature, the Temp LED remains amber.

When do not select this check box, the autosampler does not wait for the temperature zones to equilibrate to the set temperature before making an injection and the Temp LED remains green.

Table 3. Surveyor Autosampler status LEDs and meaning

LED	State	Meaning
Power	Green	The autosampler is on and receiving power.
Comm	Amber	Communication with the data system has not been established.
	Green	Communication with the data system has been established.
Run	Flashing Amber	An error condition, such as an XYZ arm jam or initialization startup error, has occurred.
	Green	The autosampler is in the Ready state.
	Flashing Green	An injection or timed event is in progress.
Temp	Amber	A temperature change within the column oven or tray temperature zones is in progress.
	Green	The column oven and tray temperature zones are in equilibrium at the set temperature. –or– The Wait for temperature ready check box on the Communication page of the Instrument Configuration application is not selected.

UV-Vis Detector LED States

Table 4 lists the LED states for the UV/Vis detector.

Table 4. Surveyor UV/Vis Plus Detector status LEDs and meaning

LED	State	Meaning
Power	Green	The detector is on.
Comm	Green	Communication to the data system PC has been established.
	Amber	There is no communication with the data system.
Run	Green	The detector is ready for a run.
	Flashing green	A run is in progress and the detector is sending data to the data system computer.
	Flashing Amber	An error has occurred during a run.
Lamps	Green	One or both lamps are turned on.
	Amber	The lamps are off.

PDA Detector LED States

Table 5 lists the LED states for the Surveyor PDA Plus Detector.

Table 5. Surveyor PDA Plus Detector status LEDs and their meaning

LED	State	Amber
Power	Green	The detector is turned on and receiving power.
Comm	Amber	There is no communication with the data system.
	Flashing amber	The rotary switches are set to 00 and firmware is being downloaded to the CPU of the detector.
	Green	Communication to the data system PC has been established.
Run	Amber	The PDA detector is not ready to start a run for one of the following reasons: • A valid method has not been downloaded (following power on). • Both lamps are off, or one of the lamps is failing to turn on. • The lamp or wavelength calibration is not valid.
	Flashing amber	The PDA detector is in an error state while in the Run mode.
	Green	The detector is ready for a run.
	Flashing green	A run is in progress and the detector is sending data to the PC.
Lamps	Amber	The lamps are off or the D2 lamp is starting. It takes approximately 10 s for the D2 lamp to turn on.
	Green	One or both lamps are turned on.

2

Thermo Foundation Instrument Configuration

This chapter provides information on configuring the LC device drivers.

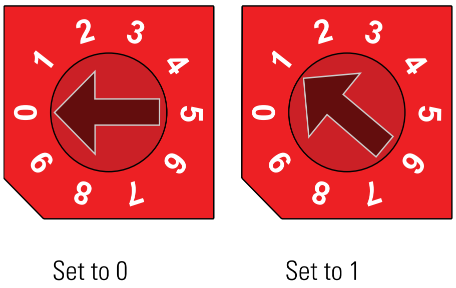
Contents

Checking the Communication Hardware

The autosampler and PDA detector communicate with the Xcalibur data system through an Ethernet connection. Each device has an Ethernet port on its back panel. A shielded, Category 5 Ethernet cable with ferrite is used to connect each device to an Ethernet switch, which in turn is connected by way of an Ethernet cable to the data system computer. The unit ID setting on the back panel of the autosampler and PDA detector must match the stack number in the configuration.

Before you specify the configuration settings, check the unit ID setting on the back panels of the autosampler, Surveyor LC Pump Plus, UV/Vis detector, and PDA detector. The unit ID consists of two rotary switches that are factory-set to 01 (see Figure 24).

Figure 24. Unit ID with a stack address of 01



Unlike the other Surveyor Plus devices, the Surveyor MS Pump Plus communicates with the Xcalibur data system through an RS-232 serial connection.

❖ To check the communication connections

1. Check the settings of the rotary switches on the back panels of the Surveyor Autosampler Plus, the Surveyor UV/Vis Plus Detector or the Surveyor PDA Plus Detector, and the Surveyor LC Pump Plus.

2. For the Surveyor MS Pump Plus, verify that the serial communication cable connecting the MS pump to your PC is attached to COM port 1.

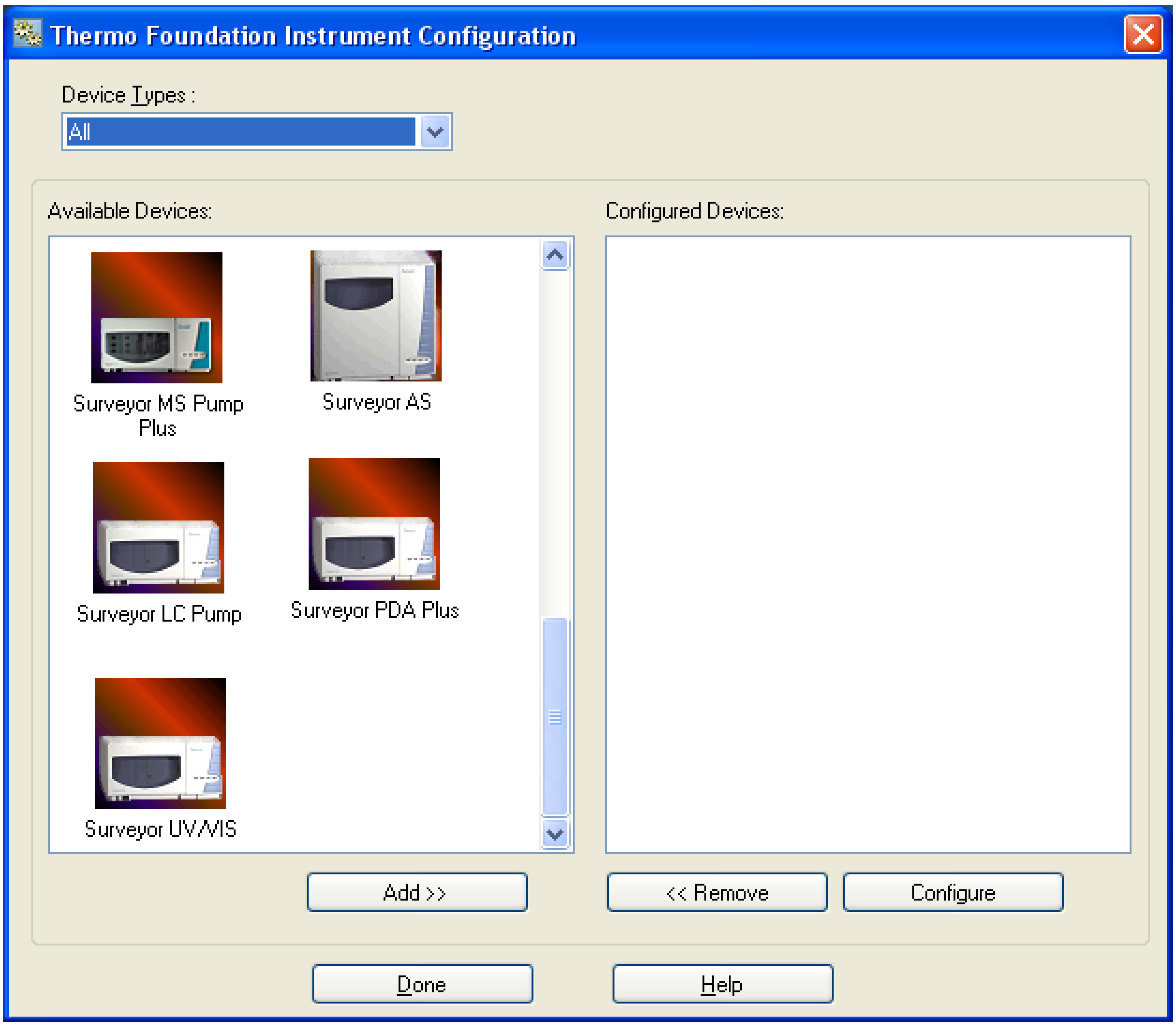
Opening the Thermo Foundation Instrument Configuration Application

❖ To open the Thermo Foundation Instrument Configuration Application

From the Windows taskbar, choose **Start > Programs > Thermo Foundation 1.0 > Instrument Configuration**.

The Thermo Foundation Instrument Configuration application appears (see Figure 25). This window lists the installed device drivers.

Figure 25. Thermo Foundation Instrument Configuration window



Go to the next procedure, "Adding Devices to the Instrument Configuration" on page 38.

Adding Devices to the Instrument Configuration

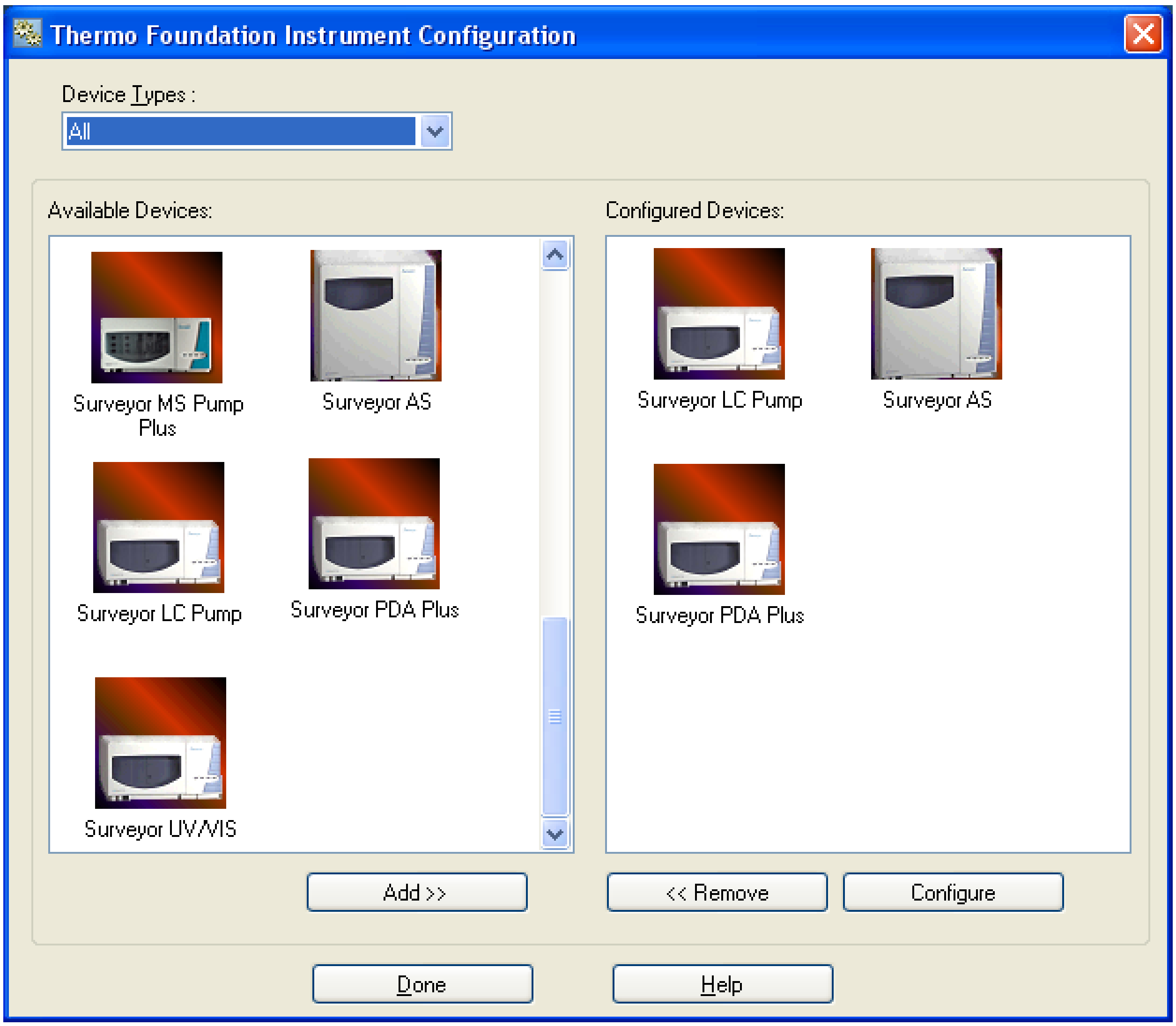
To control a device from the data system, the device must be listed in the Configured Devices pane of the Thermo Foundation Instrument Configuration application.

❖ **To add the LC device drivers to the Thermo Foundation instrument configuration**

In the Available Devices list, double-click the icons for your LC devices.

A copy of the icon appears in the Configured Devices list (see Figure 26).

Figure 26. Thermo Foundation Instrument Configuration window with devices added to the Configured Devices list



Go to the next topic, "Specifying the Configuration Settings" on page 39.

Specifying the Configuration Settings

After you add the devices to the Configured Devices list, specify the configuration settings for each device.

These topics describe the configuration settings:

- "Surveyor Autosampler Plus Configuration Settings," on this page
- "Surveyor MS Pump Plus Configuration Settings" on page 54
- "Surveyor LC Pump Plus Configuration Settings" on page 56
- "Surveyor PDA Plus Detector Configuration Settings" on page 58
- "Surveyor UV/Vis Plus Detector Configuration Settings" on page 59

After specifying the configuration options for the instrument devices, you must close the Instrument Configuration application before you can open the Xcalibur data system.

Surveyor Autosampler Plus Configuration Settings

Use the Surveyor Autosampler Configuration dialog box to specify the configuration settings for the autosampler.

For information on specifying the configuration settings for the autosampler, see these topics:

- "Tray Page" on page 40
- "Communication Page" on page 45
- "Signal Polarity Page" on page 49
- "Firmware Page" on page 53

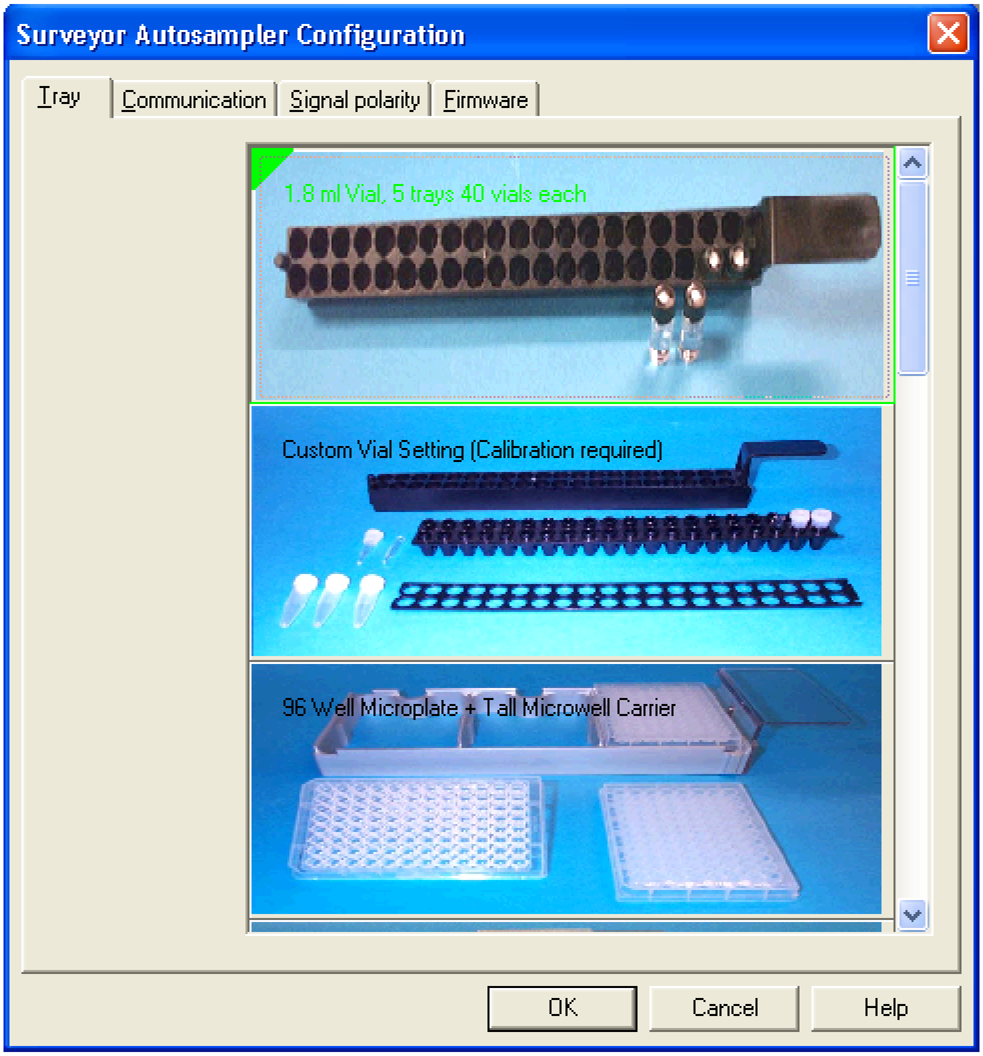
❖ To open the Surveyor Autosampler Configuration dialog box

1. If it is not already open, open the Thermo Foundation Instrument Configuration window (see "Opening the Thermo Foundation Instrument Configuration Application" on page 37).
2. In the Configured Devices list, double-click the **Surveyor AS** button.

Surveyor AS

The Surveyor Autosampler Configuration dialog box appears with the Tray page displayed (see Figure 27).

Figure 27. Tray page of the Surveyor Autosampler Configuration dialog box



Tray Page

The autosampler accessory kit contains several tray types, including trays that hold 1.8 mL vials, trays that hold 96-well microplates, and trays that hold 384-well microplates.

Use the Tray page (see Figure 28) to specify the tray type that you are using.

For information on opening the Tray page, see "Surveyor Autosampler Plus Configuration Settings" on page 39.

❖ To select the tray type and orientation

1. In the list of tray types, select the tray type.

   Table 6 lists the available selections. For the three custom selections, you must calibrate the well bottom distance before you use the trays.

   Note The Autosampler Accessory Kit contains the vial trays and microwell carriers.

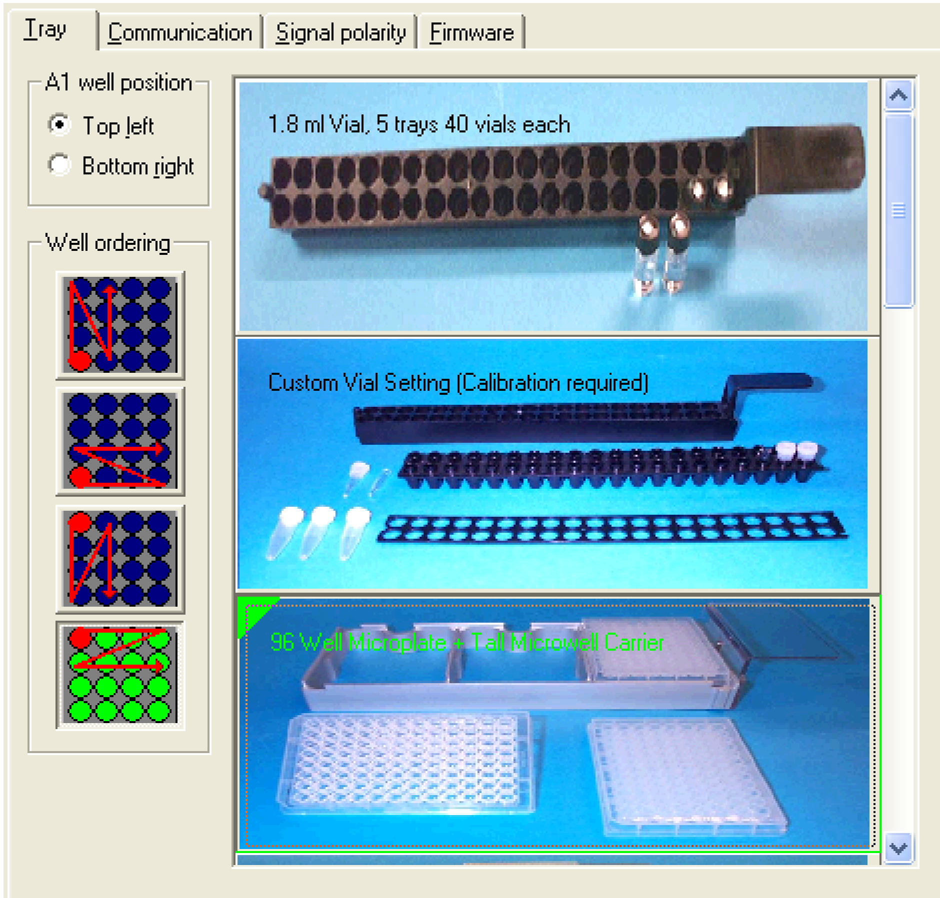
   Table 6. Tray type selections

Tray type	Requires well bottom distance calibration
1.8 mL Vial, 5 trays, 40 vials each	No
Custom Vial Setting	Yes
96 Well Microplate + Tall Microwell Carrier	No
96 Well Microplate + Short Microwell Carrier + Riser Plate	No
1 mL or 2 mL Deep Well Plate + Short Microwell Carrier	No
96 Well Microplate + Short Microwell Carrier	No
96 Well PCR Plate + Cooling Adapter + Short Microwell Carrier	No
Custom 96 Well Setting	Yes
384 Well Microplate + Tall Microwell Carrier	No
384 Well Microplate + Short Microwell Carrier + Riser Plate	No
Custom 384 Well Setting	Yes

2. For the 1.8 mL Vial option or the Custom Vial Setting, go to step 3. Otherwise, go to step 2c.

3. In the A1 well position area, select either the **Top left** or the **Bottom right** option (see Figure 28).

Figure 28. Tray page with a 96-well plate selection



4. In the Well ordering area, click the button that represents your preference for the order of sequence injections.

Autosampler Tray Page Parameter Descriptions

Table 7 describes the configuration parameters on the Tray page.

Table 7. Tray page parameters (Sheet 1 of 3)

<table>
<tr><th>Parameter</th><th colspan="2">Description</th></tr>
<tr><td rowspan="5">Tray Type</td><td colspan="2">The Tray Type list displays pictures of the 11 sample tray configurations. When you choose 96-well plates or 384-well plates, two additional options appear: A1 Well Position and Well Ordering.</td></tr>
<tr><td colspan="2">The conventional autosampler tray holds 40 standard 1.8 mL vials. For other types of vials, select the custom vial setting and the appropriate tray adapter.</td></tr>
<tr><td colspan="2">The autosampler accommodates a variety of microwell plates. You can use the tall microwell carrier, short microwell carrier, a riser plate, and a cooling adapter to accurately position the various microwell plates at an appropriate location in the autosampler tray compartment. The short microwell carrier + riser plate combination is equivalent to the tall microwell carrier.</td></tr>
<tr><td colspan="2">The distance that the needle travels to reach the bottom of the vial is pre-calibrated for the eight standard configurations. You must perform a Well Bottom Distance calibration (see “Well Bottom Distance Calibration” on page 306) to use one of the three custom configurations.</td></tr>
<tr><td colspan="2">Note Do not use the short microwell carrier to hold the 384 well high density microtitre plates. Do not use the tall microwell carrier to hold PCR plates.</td></tr>
<tr><td rowspan="14">Height Limitations</td><td colspan="2">Placing objects taller than 1.87-in. into the autosampler tray compartment will stall the autosampler arm. For custom vials, use the appropriate tray insert to ensure correct positioning. In addition, to trigger the vial sensor, vials must be positioned in the tray such that the top of the vial reaches the minimum height of 1.55 in.</td></tr>
<tr><td colspan="2">Do not use microtitre plates that exceed the following heights:</td></tr>
<tr><td>Tray</td><td>Maximum plate height</td></tr>
<tr><td>Conventional (standard 1.8 mL vials)</td><td>N/A</td></tr>
<tr><td>Custom Vial Setting</td><td>N/A</td></tr>
<tr><td>96 Well Microplate + Tall Microwell Carrier</td><td>0.77 in.</td></tr>
<tr><td>96 Well Microplate + Short Microwell Carrier + Riser Plate</td><td>0.77 in.</td></tr>
<tr><td>1 mL or 2 mL Deep Well Plate + Short Microwell Carrier</td><td>1.80 in.</td></tr>
<tr><td>96 Well Microplate + Short Microwell Carrier</td><td>1.80 in.</td></tr>
<tr><td>96 Well PCR Plate + Short Microwell Carrier</td><td>1.80 in.</td></tr>
<tr><td>Custom 96 Well Setting</td><td>1.80 in.</td></tr>
<tr><td>384 Well Microplate + Tall Microwell Carrier</td><td>0.77 in.</td></tr>
<tr><td>384 Well Microplate + Short Microwell Carrier + Riser Plate</td><td>0.77 in.</td></tr>
<tr><td>Custom 384 Well Setting</td><td>0.77 in.</td></tr>
</table>

Table 7. Tray page parameters (Sheet 2 of 3)

Parameter	Description
A1 Well Position (for the microplate trays)	Use the A1 Well Position box to select the orientation of the microplate when it is mounted in the autosampler plate holder. You can mount the plate in the plate holder so that the A1 well is located at the top left or located at the bottom right of the plate (see the figure below). • Top Left Select this option button to specify that the A1 well is located at the top left of the microplate. • Bottom Right Select this option button to specify that the A1 well is located at the bottom right of the microplate.

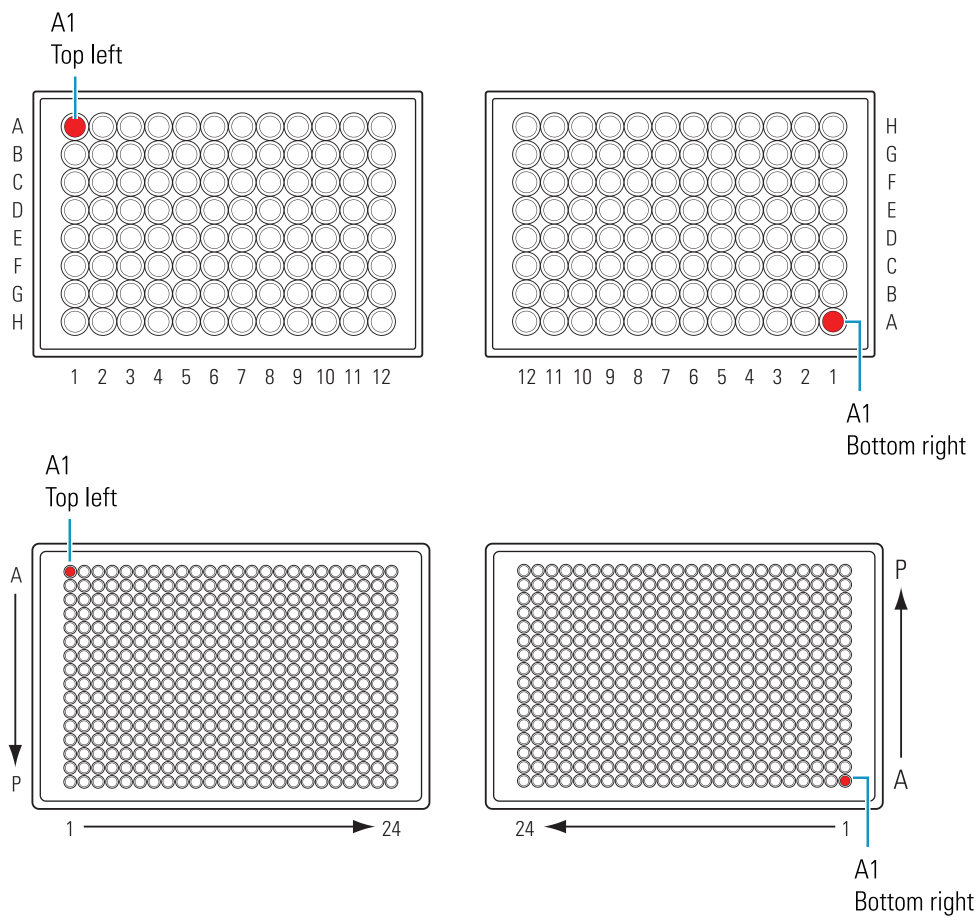
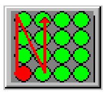
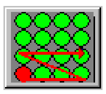
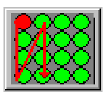
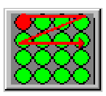


Table 7. Tray page parameters (Sheet 3 of 3)

Parameter	Description
Well Ordering (for the microplate trays)	Use the buttons in the Well ordering box to select the sampling path that the autosampler follows during a sequence run. The sampling path you select is independent of the A1 well position. The path options are shown below:

Graphic	Description
	The autosampler samples wells from bottom to top and then from left to right, as you view the plate from the top.
	The autosampler samples wells from left to right and then from bottom to top, as you view the plate from the top.
	The autosampler samples wells from top to bottom and then from left to right, as you view the plate from the top.
	The autosampler samples wells from left to right and then from top to bottom, as you view the plate from the top.

Communication Page

Use the Communication page to specify these options:

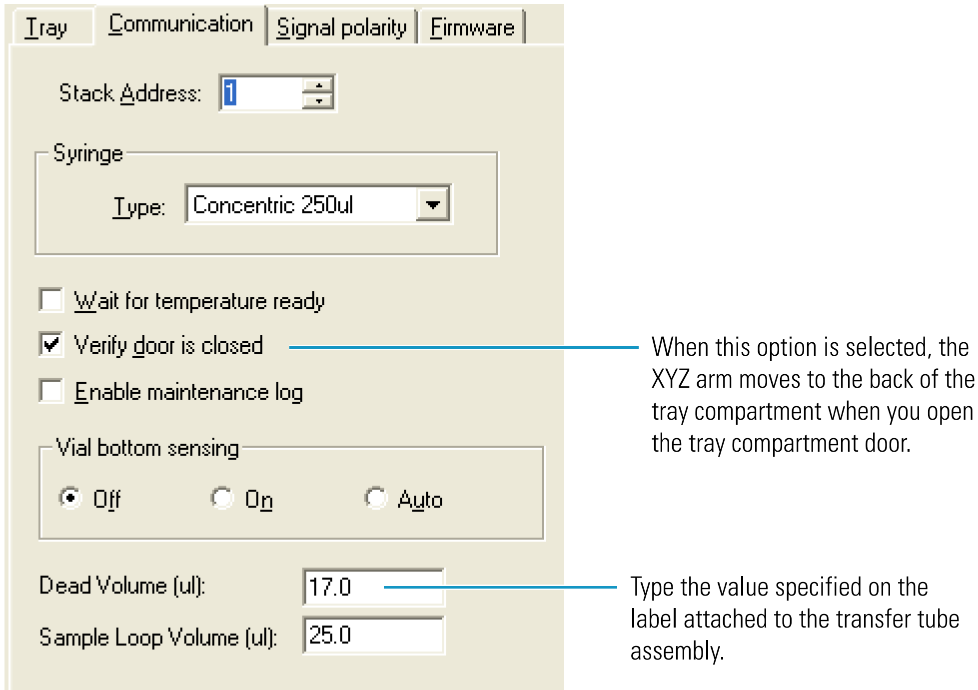
- The autosampler's stack address
- The syringe size and the sample loop size
- The dead volume of the transfer tubing that connects the autosampler injection port to the injection valve
- Whether the controlled temperature zones must be at their set temperature, the tray compartment door must be closed, or both before the autosampler makes an injection
- Whether the autosampler uses the bottom distance for the configured tray type or the stored custom value or determines the bottom distance of sample vials or wells for each injection or at the start of a sequence
- Whether the maintenance log is enabled

❖ To specify the configuration settings on the Communication page

1. Open the Surveyor Autosampler Configuration dialog box (see "Surveyor Autosampler Plus Configuration Settings" on page 39).
2. Click the **Communication** tab.

   The Communication page appears (see Figure 29).

Figure 29. Communication page



3. In the Stack Address box, type the appropriate stack address or use the up and down arrows to select the appropriate stack address.

   The stack address must match the unit ID setting located on the back panel of the autosampler. The rotary switches of the unit ID are factory-set to 01 and the default Stack Address is 1. The value of 00 is reserved for service functions.

4. Under Syringe, in the Type list, select the size of the syringe that is attached to the autosampler.

   The default is Concentric 250 µL, the syringe that ships with the autosampler.

5. (Optional) To prevent the autosampler from making an injection when the temperature zones are not equilibrated at the set temperature, select the **Wait for temperature ready** check box.

   When the Wait for temperature ready check box is selected, the autosampler does not trigger a run until the column oven temperature, the sample tray temperature, or both have reached their setpoint values.

6. (Optional) To prevent the autosampler from making an injection when the tray compartment door is open, select the **Verify door is closed** check box.

   When the Verify door is closed check box is selected, the autosampler cannot trigger a run when the tray compartment door is open. If the tray door is opened during a sequence run, the XYZ arm moves to the back of the tray compartment at the end of the current run, and the Xcalibur sequence halts.

7. (Optional) To activate the maintenance log, select the **Enable maintenance log** check box.

   The maintenance log keeps an internal count of the total injections, total valve cycles, total needle usage, and total syringe cycles. When any of the counters exceed the user set scheduled maintenance time (SMT), the autosampler cannot trigger a run until you perform the scheduled maintenance or you clear the check box.

8. In the Vial bottom sensing area, select the type of vial bottom sensing that is appropriate for your application:

   - To activate vial bottom sensing for every injection in a sequence, select the **On** option.
   - To activate vial bottom sensing for only the first injection in a sequence, select the **Auto** option.
   - To deactivate vial bottom sensing, select the **Off** option.

   Each tray type has a stored value for the distance that the needle must travel to reach the bottom of the vial or well. When you activate vial bottom sensing, the autosampler performs a search routine to determine the actual location of the vial or well bottom. If the search routine determines a new value for the bottom distance, it is stored until the tray type is modified. If you do not want the needle to touch the bottom of a vial or well, deactivate vial bottom sensing.

9. In the Dead Volume (µL) box, type the value specified on the label attached to the transfer tube assembly.

   Tip When you use the No Waste injection mode, for best results, calibrate the transfer tube volume, and then type the empirically determined value in the Dead Volume box. To calibrate the transfer tube volume, refer to the hardware manual for the autosampler.

10. In the Sample Loop Volume (µL) box, type the nominal size of the sample loop attached to the injection valve.

    The autosampler ships with a 25 µL sample loop.

Autosampler Communication Page Parameter Descriptions

Table 8 describes the parameters on the Communication page.

Table 8. Communication page parameters (Sheet 1 of 2)

Parameter	Description
Communication	
Stack Address	Specifies the stack address of the autosampler. The stack address must match the unit ID setting. The unit ID setting is specified by two rotary switches on the back panel of the autosampler. The rotary switches are set to 01 at the factory.
Syringe	Specifies the syringe type. The selections are 100, 250, 500, and 2500 µL.
Wait for temperature ready	Selecting this check box specifies that the autosampler waits for the column oven temperature and the tray compartment temperature to reach their set temperatures before going to the Ready state. Select this check box if you want the heated zones to reach their setpoint before the autosampler makes an injection.
Verify door is closed	Selecting this check box specifies that the autosampler waits for the door to be closed before starting a run and that the XYZ arm moves to the back of the tray compartment when you open the tray compartment door. Select this check box if you want the autosampler to pause a sequence and send the XYZ arm to the back of the tray compartment when you open the tray compartment door.
Enable maintenance log	Selecting this check box activates the maintenance log. The maintenance log keeps an internal count of the total injections, total valve cycles, total needle usage, and total syringe cycles. When any of the counters exceed the user set scheduled maintenance time (SMT), the autosampler cannot trigger a run until you perform the scheduled maintenance or you clear the check box. For information on setting up the scheduled maintenance time for the autosampler's hardware components, see "Autosampler Maintenance Information" on page 317. **Tip** When you select the Enable maintenance log check box, check the maintenance schedule and make sure that the settings are appropriate for your autosampler.
Vial bottom sensing	Specifies whether the vial bottom sensing feature is turned on or off or is set to determine the distance to the bottom of the first vial or well in a sequence of injections. The selections are as follows: • **Off**. Deactivates the vial bottom sensing feature. • **On**. Activates the vial bottom sensing feature for every injection. • **Auto**. Activates the vial bottom sensing feature for the first injection of a sequence.

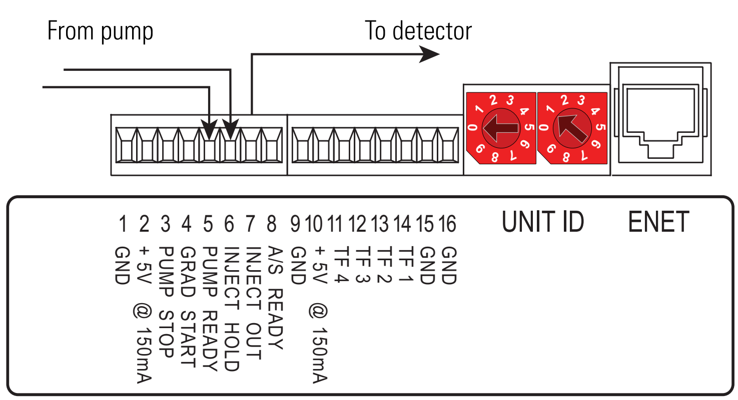
Table 8. Communication page parameters (Sheet 2 of 2)

Parameter	Description
Dead volume (µL)	Specifies the volume of the transfer tubing that connects the autosampler's injection port to the autosampler's injection valve. The transfer tubing has a label that specifies its dead volume.
Sample Loop Volume	Specifies the nominal size of the sample loop that is connected to the injection valve. The autosampler cannot detect the sample loop size; it uses the sample loop size that you type in this box.

Signal Polarity Page

During a run, the autosampler receives a Pump ready signal and an Injection Hold release signal from the pump. When the autosampler switches its injection valve to the inject position, it sends an inject out signal to the detector. Figure 30 shows the signal terminals on the autosampler's back panel.

Figure 30. Signal terminals on the autosampler's back panel



Use the Signal Polarity page of the Surveyor Autosampler Configuration dialog box to specify the signal polarities for the input and output run signals. For the Surveyor LC Pump Plus and the Surveyor MS Pump Plus, ensure that the Pump read active high and the Injection hold release active high check boxes in the Input area are selected.

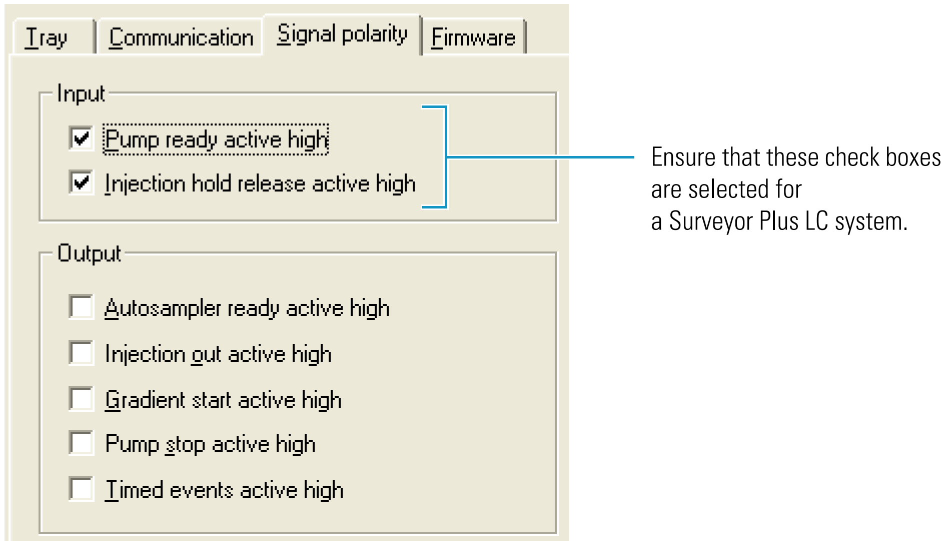
❖ To specify the signal polarities for the autosampler

1. Open the Surveyor Autosampler Configuration dialog box (see "Surveyor Autosampler Plus Configuration Settings" on page 39).

2. Click the **Signal polarity** tab.

   The Signal polarity page appears (see Figure 31).

Figure 31. Signal polarity page parameters



3. In the Input area, ensure that the **Pump ready active high** and **Injection hold release active high** check boxes are selected (see Figure 31). These check boxes are selected in the default instrument method.

4. In the Output area, if your instrument consists entirely of Surveyor Plus devices, do **not** select the check boxes.

Autosampler Signal Polarity Page Parameter Descriptions

Table 9 describes the parameters on the Signal Polarity page.

Table 9. Signal polarity parameters (Sheet 1 of 3)

Parameter	Description
Input	
The Pump ready active high and the Injection hold release check boxes are selected in the default instrument method. Leave these check boxes selected for a Surveyor Plus pump.	
Pump ready active high	Use this check box to specify the polarity of the input signal from the pump: • If the signal from the pump is LO (Closed) and goes HI (Open) when the pump is ready, select the **Pump ready active high** check box. • If the signal from the pump is HI (Open) and goes LO (Closed) when the pump is ready, clear the **Pump ready active high** check box.

Table 9. Signal polarity parameters (Sheet 2 of 3)

Parameter	Description
Injection hold release active high	Use this check box to specify the polarity of the input signal from the pump: • If the signal from the pump is LO (Closed) and goes HI (Open) when the pump is ready, select the **Injection hold release active high** check box. • If the signal from the pump is HI (Open) and goes LO (Closed) when the pump is ready, clear the **Injection hold release active high** check box.
Output	
These check boxes are clear in the default instrument method. For a Thermo Scientific LC/MS system, leave these check boxes clear.	
Autosampler ready active high	Use this check box to set the polarity of the A/S Ready output signal: • When this check box is selected, the signal from the autosampler is LO (Closed) and goes HI (Open) when the autosampler is ready. • When this check box is clear, the signal from the autosampler is HI (Open) and goes LO (Closed) when the autosampler is ready. **Note** The A/S Ready terminal sends a signal that indicates that the autosampler is in the Ready state. The autosampler is ready when your selected ready-conditions are met. The Ready conditions you select include sample tray temperature, column oven temperature, door is closed, maintenance log, and vial bottom sensing (see “Communication Page” on page 45).
Injection out active high	Use this check box to set the polarity of the Inject Out output signal: • When this check box is selected, the signal from the autosampler is LO (Closed) and goes HI (Open) when the autosampler injects a sample. • When this check box is clear, the signal from the autosampler is HI (Open) and goes LO (Closed) when the autosampler injects a sample. **Note** The Injection Out terminal sends a signal to the other LC modules or peripheral devices when the autosampler injects a sample.
Gradient start active high	Use this check box to set the polarity of the Gradient Start output signal: • When the check box is selected, the signal from the autosampler is LO (Closed) and goes HI (Open) to trigger the start of the pump’s gradient program. • When the check box is clear, the signal from the autosampler is HI (Open) and goes LO (Closed) to trigger the start of the pump’s gradient program. **Note** The Grad Start terminal sends a signal to the pump to start the gradient program.

Table 9. Signal polarity parameters (Sheet 3 of 3)

Parameter	Description
Pump stop active high	Use this check box to set the polarity of the Pump Stop output signal: • When the check box is selected, the signal from the autosampler is LO (CLosed) and goes HI (Open) when the pump is to stop. • When the check box is clear, the signal from the autosampler is HI (Open) and goes LO (Closed) when the pump is to stop. **Note** The Pump Stop terminal sends a signal to the pump to stop. The Pump Stop output signal is not active during the injection sequence. The computer must make an explicit request to issue the Pump Stop signal.
Timed events active high	Use this check box to set the polarity of the Timed Events output signals: • When the check box is selected, the signal from the autosampler is LO (Closed) and goes HI (Open) when the timed events program is to start. • When the check box is clear, the signal from the autosampler is HI (Open) and goes LO (Closed) when the timed events program is to start. **Note** The TF terminals send signals for events entered in the timed events table of the instrument method. For information on setting up the timed events table for the autosampler, see "Timed Events Page for the Autosampler" on page 88.

Firmware Page

Use the Firmware page to check the firmware version of the autosampler.

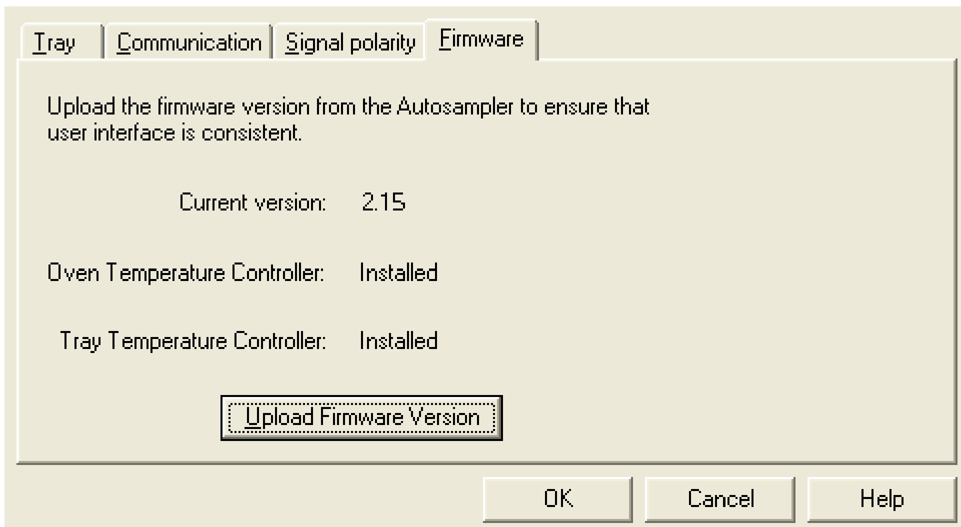
❖ To check the firmware version of the autosampler

1. Open the Surveyor Autosampler Configuration dialog box (see "Surveyor Autosampler Plus Configuration Settings" on page 39).

2. Click the **Firmware** tab.

   The Firmware page appears (see Figure 32).

   Figure 32. Firmware page

3. If you have upgraded your autosampler, upload the firmware version as follows:

   a. Click **Upload Firmware Version**.

      The firmware version of the autosampler appears next to Current Version.

   b. Click **OK** to save the settings and close the dialog box.

If you have finished configuring all of your LC devices, go to "Closing the Foundation Application" on page 59.

Surveyor MS Pump Plus Configuration Settings

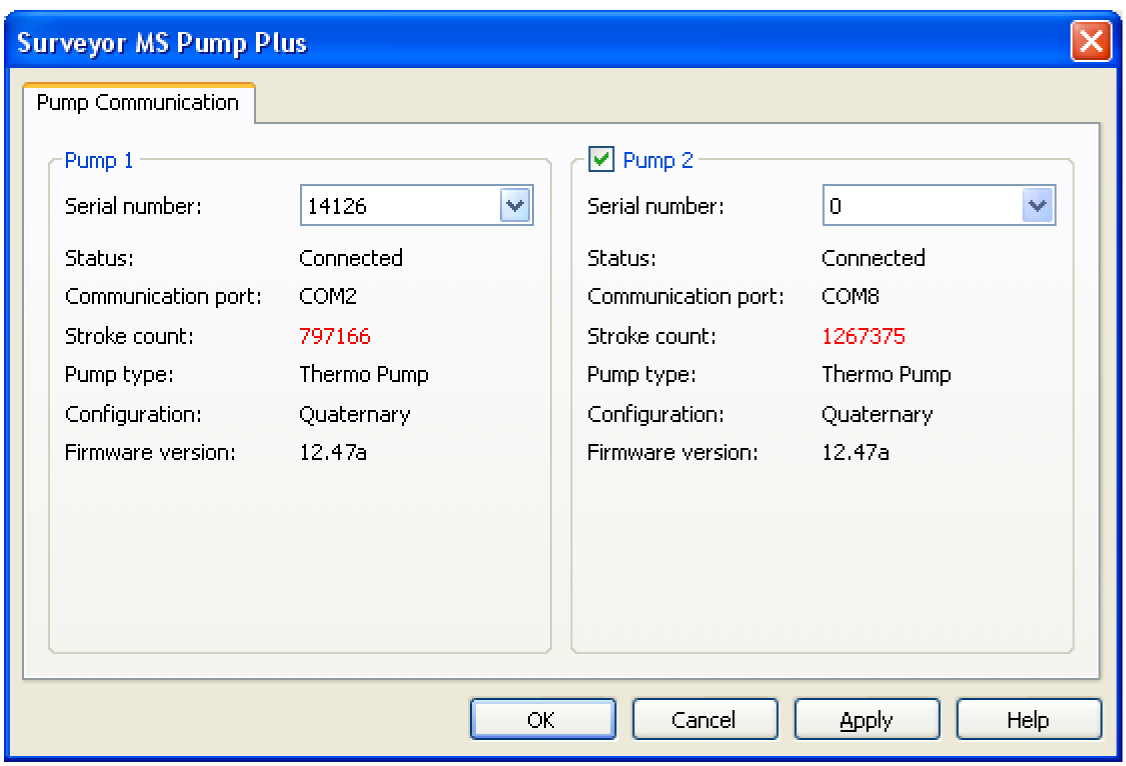
During normal operation, the Surveyor MS Pump Plus communicates with the Xcalibur data system through an RS-232 link. Before you configure the pump device driver, connect the pump to the data system computer. If your LC system contains two pumps, connect both pumps to the data system computer.

❖ To specify the configuration settings for the Surveyor MS Pump Plus

1. Ensure that the pump is connected to the data system computer and powered on.
2. In the Configured Devices list, double-click the **Surveyor MS Pump Plus** button.

The Surveyor MS Pump Plus dialog box appears (see Figure 33).

Figure 33. Surveyor MS Pump Plus dialog box



3. If two pumps are connected to the data system computer, make sure that the appropriate pumps are selected in the Pump 1 and Pump 2 area.

   You can use two pumps of the same pump type to perform two-dimensional chromatography or high-pressure gradient mixing.

4. Click **OK** to close the dialog box.

If you have finished configuring the LC devices, go to "Closing the Foundation Application" on page 59.

Surveyor MS Pump Configuration Parameter Descriptions

Table 10 provides descriptions of the parameters in the Surveyor MS Pump Configuration dialog box.

Table 10. Surveyor MS Pump Plus Configuration dialog box parameters

Parameter	Description
Serial number	Lists the serial number stored by the Surveyor MS Pump Plus.
Status	Displays the pump's status. The states are Not connected or Connected.
Communication port	Displays the type of communication port on the data system computer where the pump is connected.
Stroke count	Displays the total stroke counts for the pump's pistons.
Pump type	Displays the pump type: Thermo Pump.
Configuration	Displays Quaternary.
Firmware version	Displays the firmware version.
Use leak sensor	When this check box is selected, the leak sensor is in use.

Surveyor LC Pump Plus Configuration Settings

The Surveyor LC Pump communicates with the data system by way of an Ethernet connection. To make this connection, connect the Ethernet cable to the Ethernet port on the back of the pump and to the Ethernet switch. The unit ID setting on the back panel of the pump must match the stack address in the configuration.

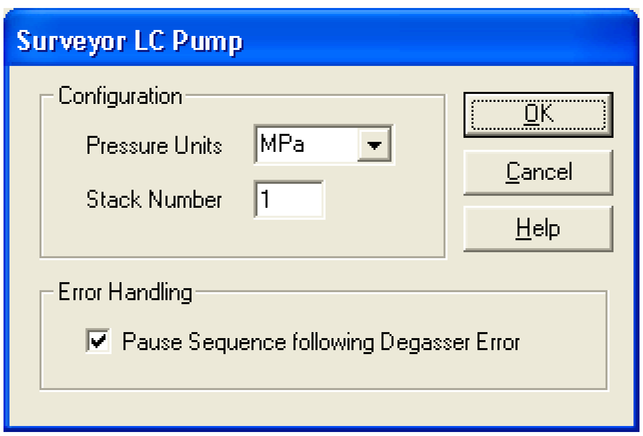
❖ To specify the configuration options for the Surveyor LC Pump Plus

1. In the Configured Devices list, double-click the **Surveyor LC Pump** icon.

Surveyor LC Pump

The Surveyor LC Pump dialog box appears (see Figure 34).

Figure 34. Surveyor LC Pump dialog box



2. Make the following entries and selections:

   - From the Pressure Units list, select the unit of measurement that you want to use to display the backpressure of your system.

     Note 1 MPa = 10 bar = 145 psi

   - In the Stack Number box, type an appropriate value.

     The value must match the Unit ID setting on the back panel of the Surveyor LC Pump.

   - If you want sequence runs to pause following a degasser error, select the **Pause Sequence Following Degasser Error** check box.

3. Click **OK** to exit the Surveyor LC Pump dialog box.

Surveyor LC Pump Plus Configuration Parameter Descriptions

Table 11 describes the configuration parameters for the Surveyor LC Pump Plus.

Table 11. Surveyor LC Pump Plus configuration parameters

Parameter	Description
Configuration	
Pressure Units	Specifies the pressure units for the status display. The selections are MPa, bar and psi. To convert psi to bar, multiply by 0.0689476. To convert bar to psi, multiply by 14.5038.
Stack Number	Specifies the unit ID value for the Surveyor LC Pump Plus. The unit ID on the back panel of the pump consists of two rotary switches. Each switch contains ten positions. The arrow on the left switch points to the first digit of the unit ID. The arrow on the right switch points to the second digit of the unit ID. The rotary switches are set to 01 at the factory.
Error Handling	
Pause Sequence following Degasser Error	Select this check box to pause the injection sequence when the pump detects a degassing error. Clear this check box if you do not want the injection sequence to pause when the pump detects a degassing error.

Surveyor PDA Plus Detector Configuration Settings

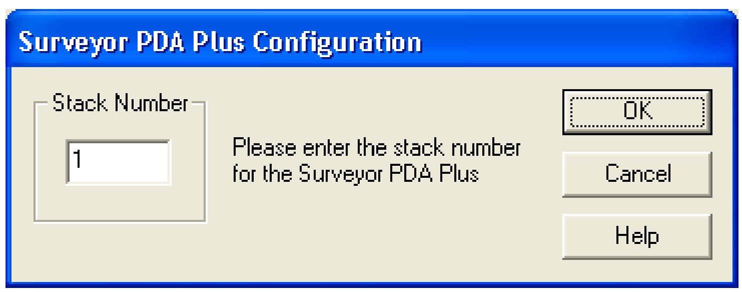
The PDA detector communicates with the Xcalibur data system through an Ethernet connection. To make this connection, connect the Ethernet cable to the Ethernet port on the back of the detector and to the Ethernet switch. The unit ID setting on the back panel of the detector must match the configured stack number.

❖ To specify the configuration options for the PDA detector

1. In the Configured Devices list, double-click the **Surveyor PDA Plus** button.

The Surveyor PDA Plus Configuration dialog box appears (see Figure 35).

Figure 35. Surveyor PDA Plus Configuration dialog box



2. In the Stack Number box, type the appropriate number (unit ID).

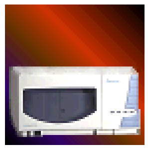
3. Click **OK** to close the Surveyor PDA Plus Configuration dialog box.

When you have finished configuring the instrument devices, go to the next topic: Closing the Foundation Application.

Surveyor UV/Vis Plus Detector Configuration Settings

❖ To specify the configuration settings for the UV/Vis Plus Detector

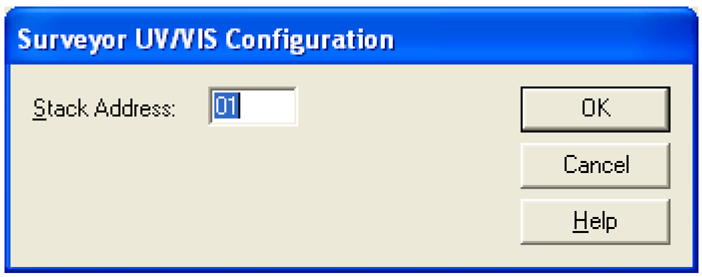
1. In the Configured Devices list, double-click the **Surveyor UV/Vis** button.

   Surveyor UV/VIS

   The Surveyor UV/Vis Configuration dialog box appears (see Figure 36).

   Figure 36. Surveyor UV/Vis Configuration dialog box

2. In the Stack Address box, type the appropriate number (unit ID). The Stack Address setting must match the rotary switches on the back panel of the detector.

3. Click **OK** to close the Surveyor UV/Vis Configuration dialog box.

   When you have finished configuring all of your LC devices, go to the next topic: Closing the Foundation Application.

Closing the Foundation Application

Before you can open the Xcalibur (or equivalent Thermo Scientific) data system, you must close the Foundation application.

Note In addition to the Xcalibur data system, you can control the Surveyor Plus LC devices from other Thermo Scientific data systems such as LCQuan and MetWorks.

❖ To save the instrument configuration and close the Instrument Configuration window

Click **Done** at the bottom of the Thermo Foundation Instrument Configuration window.

The Windows desktop appears.

Tip You must close the Instrument Configuration window before you open the Xcalibur data system. The two applications cannot be open at the same time.

3

Instrument Method Setup

This chapter describes the instrument control parameters for the Surveyor Plus LC devices. It does not describe the instrument control parameters for your Thermo Scientific mass spectrometer. For information on setting up the data acquisition parameters for a Thermo Scientific mass spectrometer, refer to the Getting Started Guide or Help for the mass spectrometer.

To automate control of the liquid chromatography devices, you must create an instrument method, and then specify the instrument method to be used for each run in an acquisition sequence from the Sequence Setup window.

Instrument methods contain the analysis wavelengths, the chromatographic conditions, and the autosampler injection parameters, such as the injection mode, required for data acquisition.

❖ To create an instrument method

1. Open the Instrument Setup window.
2. Specify the instrument method settings for each device.
3. Save the method.

Contents

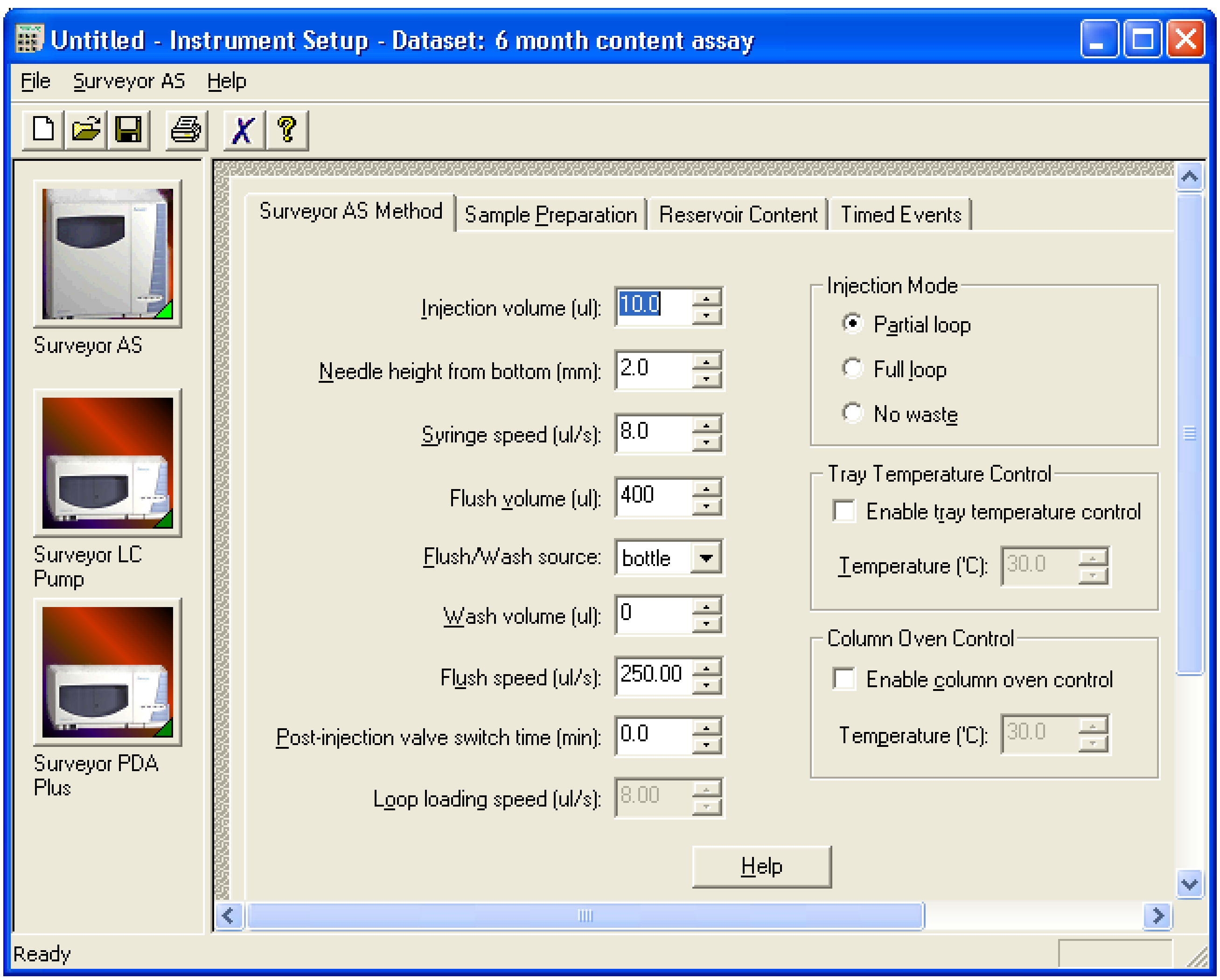
Opening the Instrument Setup Window

❖ To open the Instrument Setup window

1. Start the Xcalibur data system.
2. On the Thermo Xcalibur Roadmap window, click the **Instrument Setup** button in the Road Map or choose **GoTo > Instrument Setup**.

   The Instrument Setup window appears (see Figure 37). The view bar on the left side of the window contains a button for each configured device of your instrument. Clicking the button for a device opens the view where you can specify the instrument method parameters for that device.

Figure 37. Instrument Setup window



Surveyor LC Pump Plus Instrument Method Parameters

Use the Surveyor LC Pump view to set up the instrument method parameters for the pump.

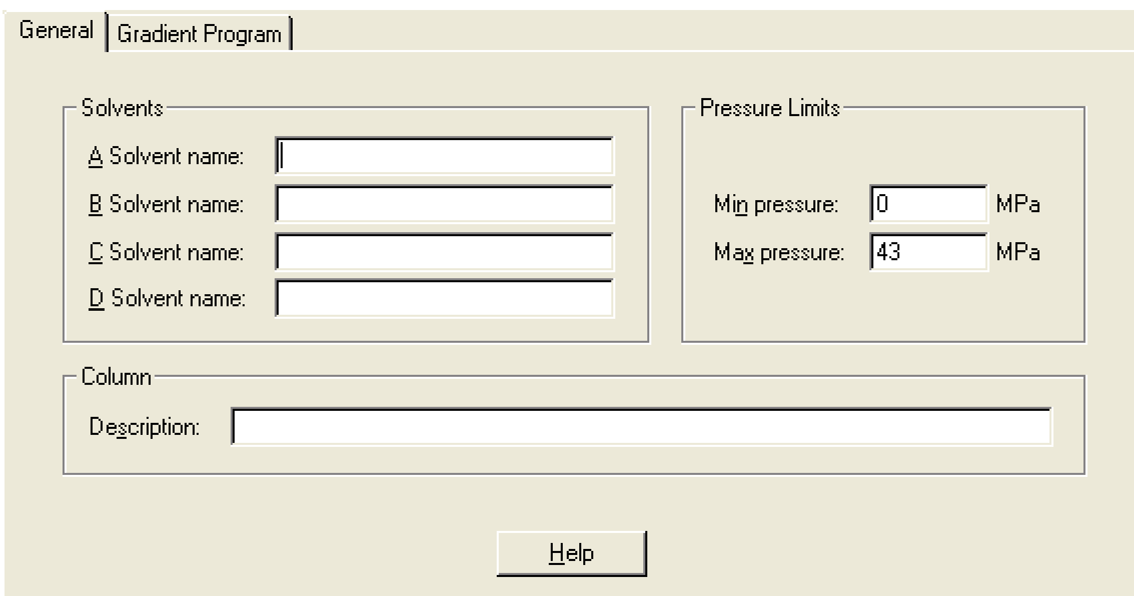
To set up the instrument method parameters for the Surveyor LC Pump Plus, see these topics:

- "Surveyor LC Pump Plus General Page," on this page
- "Surveyor LC Pump Plus Gradient Program Page" on page 65

Surveyor LC Pump Plus General Page

Use the General page for the Surveyor LC Pump Plus to identify the solvents used to create the mobile phase and the LC column used to perform the chromatographic separation. In addition, use the General page to specify the operation pressure limits for the pump (see Figure 38).

Figure 38. General page for the Surveyor LC Pump Plus



❖ **To specify the instrument method settings on the General page**

1. In the solvent name boxes in the Solvents area, type the solvent names.
2. In the Description box in the Column area, type a description of the LC column.

3. In the Pressure limits area, do the following:

   a. In the Min pressure box, type the minimum operating pressure.

   > **Note** Pressure drops can indicate the following problems:
   >
   > - Mobile phase in the solvent reservoir bottles has run out.
   > - A leak has occurred in the flow line or lines.
   > - An excessive amount of air has entered the pump head assemblies.

   b. In the Max pressure box, type the maximum operating pressure.

   > **Note** The maximum pressure setting is 43 MPa, 431 bar, or 6258 psi. Reduce the maximum pressure setting for PEEK fittings to 28 MPa, 276 bar, or 4000 psi.

   > **Note** 1 bar = 14.5 psi = 10 MPa

Surveyor LC Pump Plus General Page Parameter Descriptions

Table 12 describes the parameters on the General page.

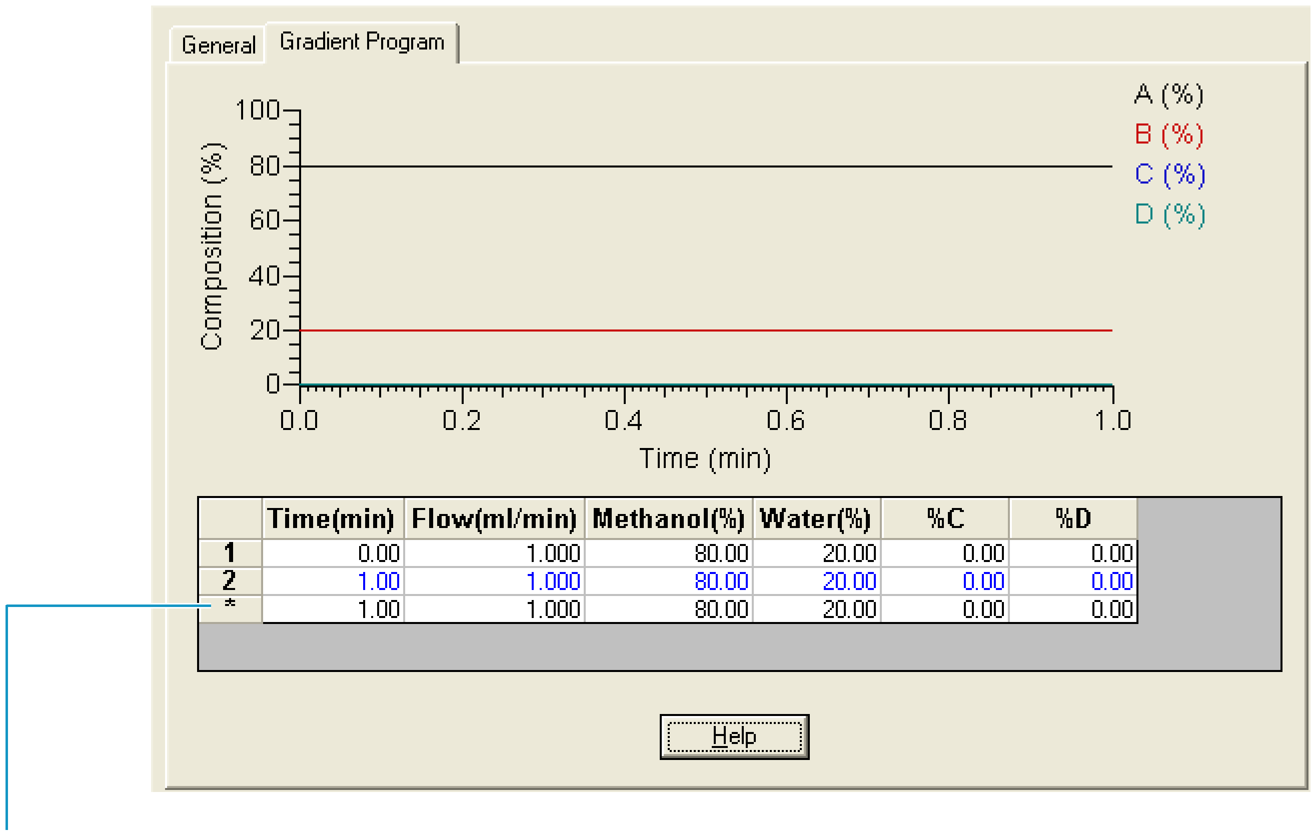
Table 12. General page parameters for the Surveyor LC Pump Plus

Parameter	Description
Solvents	
A Solvent name	Specifies the solvent contained in the solvent A reservoir bottle.
B Solvent name	Specifies the solvent contained in the solvent B reservoir bottle.
C Solvent name	Specifies the solvent contained in the solvent C reservoir bottle.
D Solvent name	Specifies the solvent contained in the solvent D reservoir bottle.
Column	
Description	User-specified description for the LC column.
Pressure Limits	
Min pressure	Specifies the minimum operating pressure for the pump. If the system pressure falls below this limit, the data system turns off the solvent flow from the pump.
Max pressure	Specifies the maximum operating pressure for the pump. If the system pressure rises above this limit, the data system turns off the solvent flow from the pump. The maximum pressure setting must be greater than the minimum pressure setting. Range: 1 to 43 MPa (10 to 431 bar or 145 to 6258 psi).

Surveyor LC Pump Plus Gradient Program Page

Use the Gradient Program page (see Figure 39) to specify the solvent composition and flow rate for your chromatographic method.

Figure 39. An example of an isocratic pump program for the Surveyor LC Pump Plus



❖ To specify the solvent conditions for the Surveyor LC Pump Plus

1. Click the **Gradient Program** tab.

   The Gradient Program page appears (see Figure 39).

2. Type the initial flow rate and the initial solvent composition for your pump program in the first time line of the time program table, and then press ENTER.

   If you are not creating a gradient program, skip the next step.

> **Note** If you set the limit for the maximum pressure to less than 21 MPa, the allowable flow rate range is 0.000 to 9.999 mL/min. If you set the maximum pressure limit to 21 MPa (215 bar, 3129 psi) or higher, the maximum allowable flow rate is 5.000 mL/min.

The first time line in the time program table is set to 0.00 min. This time value of 0.00 min cannot be changed. The second time line in the program is a placeholder line and has no effect on the pumping conditions.

For example, the pump program shown in Figure 39 produces a proportioned mobile phase consisting of 80% methanol and 20% water (v/v). The proportions of the solvents remain constant throughout the run. The mobile phase flow rate remains constant at 1 mL/min throughout the run.

3. To create a gradient pump method, enter at least two steps in the Gradient Program table. For each additional line in the program, type a time value in the Time column, a flow rate in the Flow column, and the solvent percentages for the mobile phase in the solvent columns. Then press ENTER.

   Table 13 lists the gradient conditions used to create the gradient profile shown in Figure 40.

Table 13. Gradient program

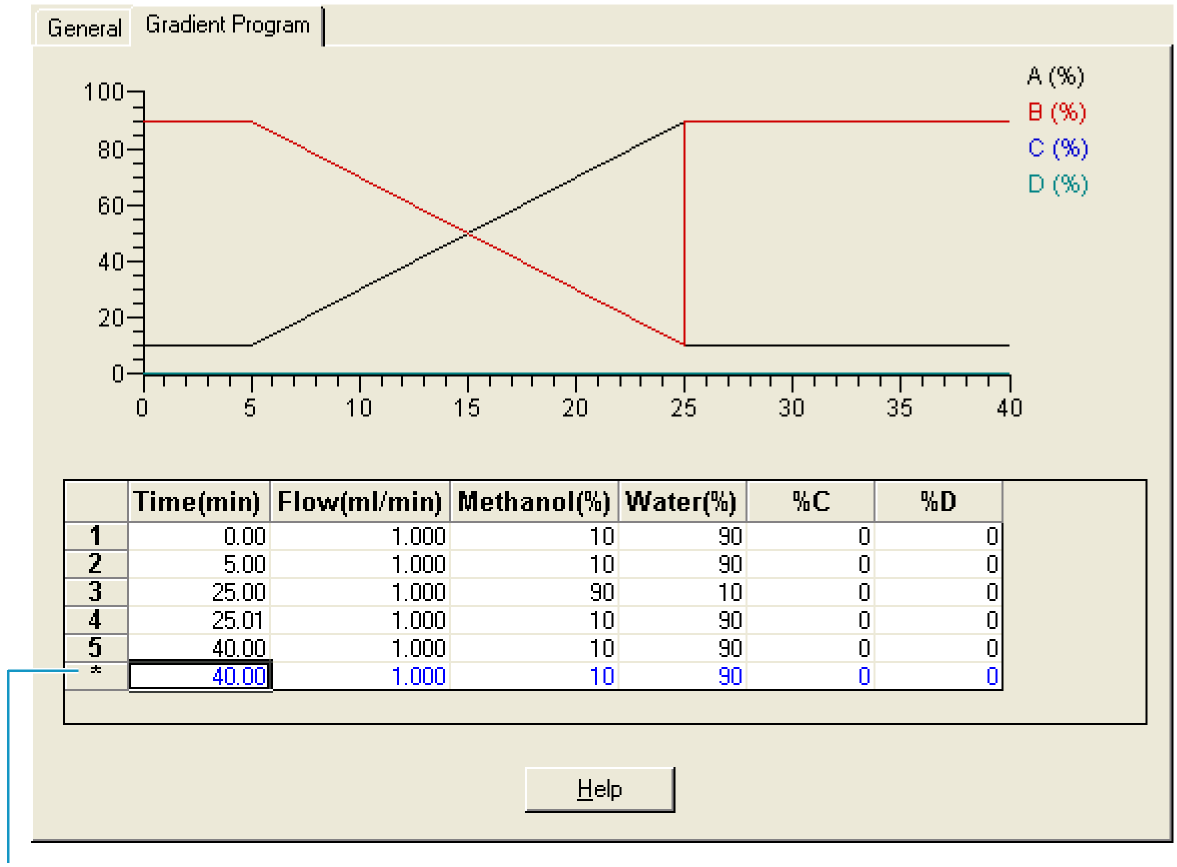
Time (min)	Solvent composition
0.00 to 5.00	Held constant at 90:10 water/methanol
5.00 to 25.00	Linear ramp from 10% to 90% methanol
25.00 to 25.01	Stepped down to initial solvent composition of 90:10 water/methanol
25.01 to 40.00	Column is equilibrated at the initial solvent composition of 90:10 water/methanol

At 25.01 minutes, the program returns to its initial solvent composition. To equilibrate the column prior to the next run, the solvent composition is held at the initial solvent composition for a period of 15 minutes.

The last line in the gradient table, which has an * in place of a line number, is a placeholder and has no effect on the pump conditions.

Note When you are performing a sequence of gradient runs, add an equilibration step at the end of the Gradient Program.

Figure 40. An example of a gradient program for the Surveyor LC Pump Plus



Placeholder line

Surveyor LC Pump Gradient Program Page Parameter Descriptions

Table 14 describes the parameters on the Gradient Program page.

Table 14. Gradient Program page parameters for the Surveyor LC Pump Plus (Sheet 1 of 2)

Parameter	Description
Graph	
Displays the percent composition of solvents A, B, C and D as a function of time.	
Table	
Specifies the gradient steps. The table can contain up to 80 rows. The solvent composition boxes are interactive. After you type a value in column A and press ENTER or click another box, the value in column B changes to maintain a total of 100%, and so on. The solvent composition changes linearly between time points.	
Time (min)	Specifies the time duration for each gradient step. The initial time is always 0 minutes. Range: 0 to 655.35 minutes

Table 14. Gradient Program page parameters for the Surveyor LC Pump Plus (Sheet 2 of 2)

Parameter	Description
Flow (mL/min)	Specifies the flow rate. Range: 0.000 to 9.999 mL/min
%A	Specifies the percent composition of solvent A for each time point.
%B	Specifies the percent composition of solvent B for each time point.
%C	Specifies the percent composition of solvent C for each time point.
%D	Specifies the percent composition of solvent D for each time point.

Surveyor MS Pump Plus Instrument Method Settings

Use the Surveyor MS Pump Plus view in the Instrument Setup window to specify the chromatographic conditions for your instrument method.

To open the Surveyor MS Pump Plus view, click the **Surveyor MS Pump Plus** button in the view bar of the Instrument Setup window.

To set up the chromatographic conditions, see these topics:

- Specifying the Chromatographic Conditions for a Single-Pump System
- Programming the Pumps in a Dual-Pump System

Specifying the Chromatographic Conditions for a Single-Pump System

To specify the chromatographic conditions for a single pump system, follow these procedures in order:

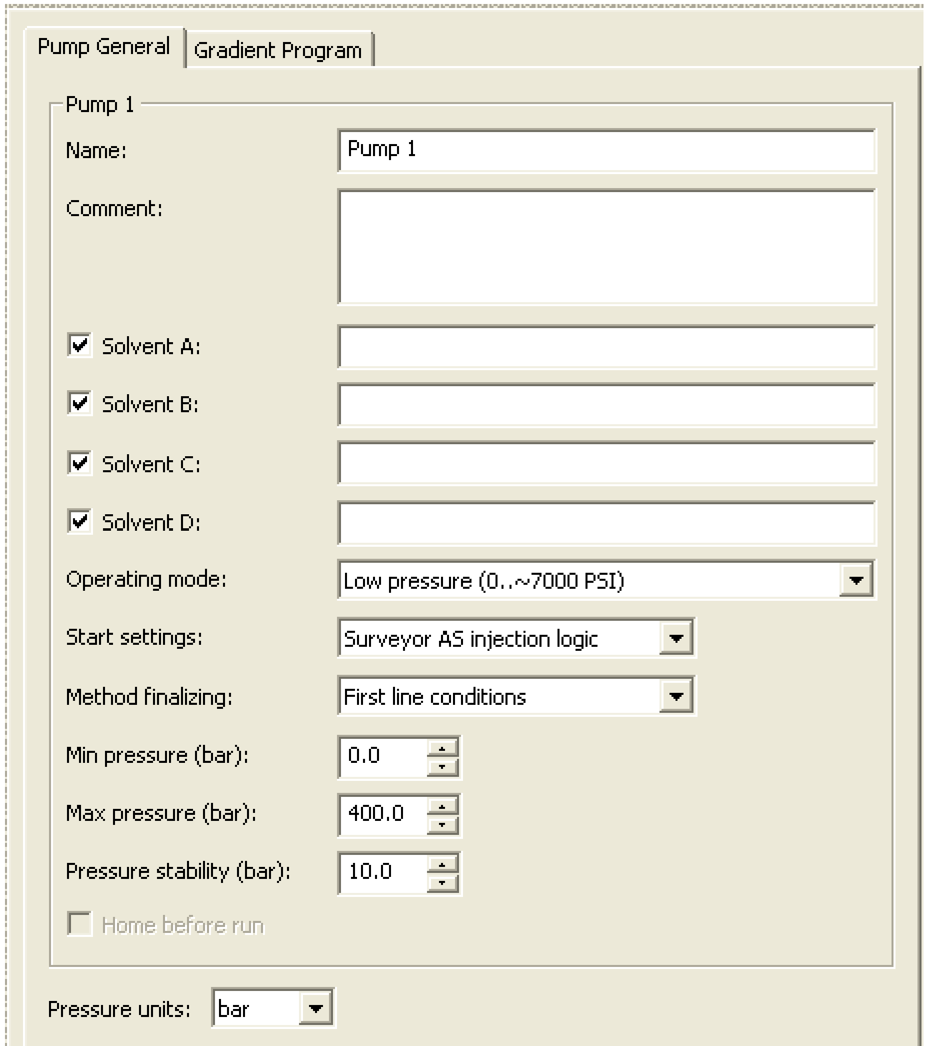
1. "Setting Up the Pump General Parameters," on this page
2. "Setting Up the Gradient Program" on page 74

Note After you specify the solvents that make up the mobile phase, you can switch back and forth between the Pump General and Gradient Program pages.

Setting Up the Pump General Parameters

Use the Pump General page to specify the solvents that the pump uses to create the mobile phase and operating conditions for the pump (see Figure 41).

Figure 41. General page for the Surveyor MS Pump Plus



When you connect two pumps to the data system computer, you must specify the general parameters for both pumps. Pump 1 is triggered by the autosampler or by a manual trigger and triggers the gradient program for Pump 2. You can use a dual-pump system to perform two-dimensional chromatography or to create a high-pressure gradient. For more information on a dual-pump system, see "Programming the Pumps in a Dual-Pump System" on page 78.

The Instrument Method report attached to the raw file contains the values that you select or enter on this page. You can access this report in the Qual Browser view by choosing **View** > **Reports** > **Instrument Method**.

❖ To specify the parameters on the Pump General page

1. Open the view for your pump.

   For information on opening the device views from the Xcalibur 2.1.x data system, see "Opening the Instrument Setup Window" on page 62.

   The pump view includes a Pump 1 area for a single-pump system or a Pump 1 area and a Pump 2 area for a dual-pump system.

2. On the Pump General page, specify the parameters as follows:

   a. In the Name box, use the default label (Pump 1 for a single-pump system) or type a label.

      The label can be up to 16 alphanumeric characters. The data system uses this label for the pump view on the Status page of the Information view.

   b. In the Comment box, type additional information about the chromatographic method (for example, the specifications for the liquid chromatography column).

   c. Identify the solvents that make up the mobile phase.

      All of the solvent check boxes are selected by default. Clearing a solvent check box makes the solvent unavailable on the Gradient Program page.

      In the boxes to the right of the solvent check boxes, type the names of the solvents that make up the mobile phase.

   - In the Operating mode list, the only selection for the Surveyor MS Pump Plus is Low pressure.

   d. In the Start settings list, select how the system is triggered to start a run: Surveyor AS Autosampler injection logic or Manual.

      To trigger a run when the autosampler makes an injection, select **Surveyor AS Autosampler injection logic**.

      IMPORTANT

   e. In the Method finalizing list, select the ending conditions for the run:

      - For a gradient method, do one of the following:
        – Select **First line conditions** to return the mobile phase conditions to the first line of the gradient program following the last time line in the gradient program table.
        – Select **Last line conditions** to let the mobile phase conditions remain at those of the last time line in the gradient program.
      - For a shutdown method that turns off the solvent flow at the end of a run, select **Stop after the end**.

Note The Idle Settings list is available for a dual-pump system on the Pump 2 area of the Pump General page.

f. In the Min pressure (bar) box, type or select the minimum operating pressure for the pump.

   An appropriate minimum pressure setting prevents the pump from operating when the solvent reservoirs have run dry or the system plumbing has developed a leak. Running the pump without solvent quickly ruins the piston seals. Select a value that is well below the typical operating pressure for your application. In the event that the pressure falls below this limit for more than one minute, the pump automatically stops and sends an error message to the computer.

   The range is 0 to 400 bar (5800 psi) with a default value of 0 bar.

g. In the Max pressure (bar) box, type or select the maximum operating pressure for the pump.

   An appropriate maximum pressure setting prevents the pump from operating with a restriction on the outlet side of the pump. Excess pressure can damage the HPLC column and any other component between the restriction and the pump. Select a value that is well above the typical operating pressure for your application, but also below the pressure that can damage your system. If the pressure rises above this limit, the pump automatically stops and sends an error message to the computer.

   The range is 0 to 400 bar (5800 psi) with a default value of 400 bar.

h. In the Pressure stability (bar) box, type or select an appropriate backpressure stability value from 1 to 100.

   The MS pump sends a Ready signal when the system pressure reaches this limit. A lower value requires greater pressure stability before the pump becomes ready. A higher value is more forgiving of pressure pulsations.

i. When you select Manual in the Start settings list, select the Home before run check box if you want the pump to start the gradient program when the cams that control the pump pistons reach the home position. Selecting this check box increases the reproducibility of chromatographic runs when you make manual injections.

j. In the Pressure units list, select the pressure units for the pressure readback.

Note 1 bar = 14.5 psi

Thermo Pump General Page Parameter Descriptions

Table 15 describes the parameters on the General page.

Table 15. Thermo pump method parameters (Sheet 1 of 2)

<table>
<tr><th>Parameter</th><th colspan="2">Description</th></tr>
<tr><td>Name</td><td colspan="2">Identifies the pump on the pump view of the Status page of the Info view.

The text string can consist of up to 16 alphanumeric characters.</td></tr>
<tr><td>Comment</td><td colspan="2">User-text that provides additional information. The text string can consist of up to 255 alphanumeric characters.</td></tr>
<tr><td>Solvent A, B, C, and D check boxes and adjacent boxes</td><td colspan="2">When these check boxes are selected, the associated solvent column on the Gradient Program page is available. Select the check boxes corresponding to the solvent bottles to be used to create the mobile phase. Type a description of the solvent in the adjacent box.

Clearing the check box associated with a solvent (A, B, C, or D) makes the solvent column unavailable on the Gradient Program page.</td></tr>
<tr><td>Operating mode</td><td colspan="2">Specifies the operating mode for the pump. The selection is (0~7000 PSI).</td></tr>
<tr><td rowspan="3">Start settings</td><td colspan="2">Specifies the device that triggers the start of a run and the run synchronization signals between the pump and the autosampler.

The selections are as follows:</td></tr>
<tr><td>Surveyor AS injection logic</td><td>At the start of a run, the Surveyor Autosampler Plus waits for the pump to issue the Pump Ready and the Release Injection signal before making an injection. The pump then waits for the Start gradient signal from the autosampler before starting the pump program.

When you use this injection logic, set up the Surveyor Autosampler Plus as the start instrument that triggers the run.</td></tr>
<tr><td>Manual</td><td>At the start of a run, the pump program starts immediately.

When you use this injection logic, set up the pump as the start instrument.</td></tr>
<tr><td>Min pressure</td><td colspan="2">Specifies the minimum operating pressure of the pump. If the system backpressure falls below this level for more than one minute, the data system triggers turns off the mobile phase flow.

Default: 0 bar</td></tr>
</table>

Table 15. Thermo pump method parameters (Sheet 2 of 2)

Parameter	Description
Max pressure	Specifies the maximum operating pressure of the pump. If the system backpressure rises above this level for more than one minute, the data system triggers the pump to turn off the mobile phase flow. Default: 400 bar
Pressure stability	Specifies the stability condition required for the pump to go into a Ready mode. A lower value requires greater pressure stability before the pump becomes Ready. A higher value is more forgiving of pressure pulsations. Default: 10 The range depends on the pressure units selection. **Units** / **Range** psi: 1 to 1450 MPa: 1 to 10 bar: 1 to 100
Home before run	This check box becomes available when you select Manual in the Start Settings list. Select this check box if you want the pump to wait for the pistons to return to the home position before starting the pump program.
Pressure units	Specifies the units for the pressure readout. The available selections are bar, PSI, and MPa. 1.00 MPa = 10.0 bar = 145 psi
Idle Settings (Pump 2 of a dual-pump system)	Specifies the conditions for Pump 2 while it is waiting to be triggered by Pump 1. Default: Standby The selections are Standby, First line conditions, and Last line conditions. In the Standby mode, the pump remains Idle.

Setting Up the Gradient Program

The pump contains a built-in solvent proportioning assembly that is capable of proportioning up to four solvents to create binary, tertiary, and quaternary mobile phases. This capability reduces the need to make premixed mobile phases. You can run the pump in either the isocratic mode or the gradient mode. In the isocratic mode, you maintain the same proportions of solvent throughout the run.

Use the Gradient Program page to specify the solvent composition and flow rate for your chromatographic method.

The Gradient Program page contains a gradient table with time lines for the solvent composition and flow rate and an interactive view that displays a graphical profile of the solvent gradient or flow rate gradient.

Each row in the gradient table defines the solvent composition and flow rate for a specific time point. The solvent composition columns (%A, %B, %C, and %D) work interactively with each other, maintaining a total solvent composition of 100%.

Between time points, the solvent composition changes linearly, whereas the flow rate changes as a discontinuous step-function.

The gradient table can contain 2 to 398 time lines.

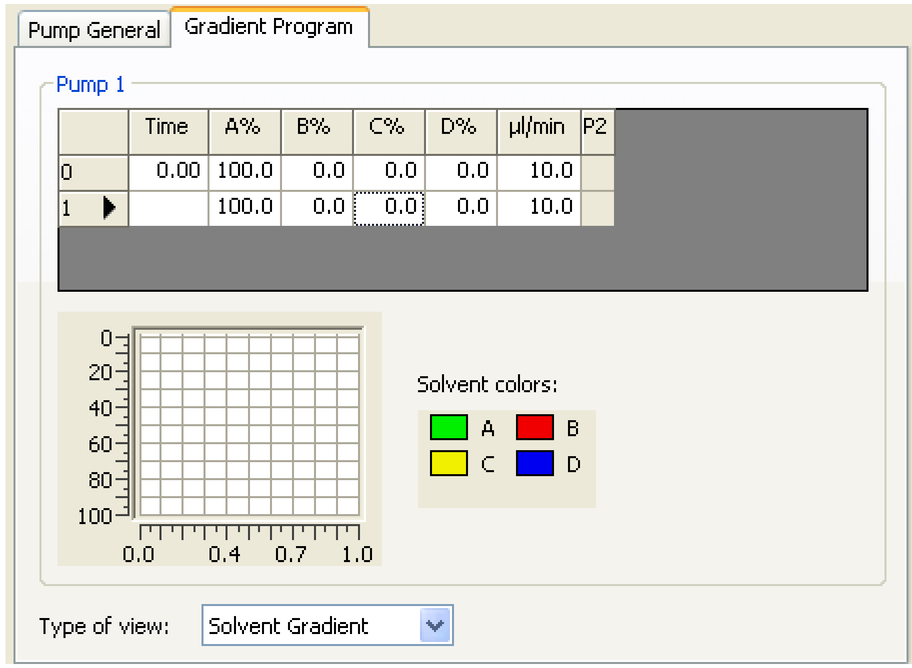
IMPORTANT The gradient table must contain at least two time lines. For an isocratic gradient program, the two time lines specify the same solvent composition and flow rate.

❖ To set up the gradient program for the Surveyor MS Pump Plus

1. Open the view for your pump.
2. Specify the general pump parameters (see “Specifying the Chromatographic Conditions for a Single-Pump System” on page 68).
3. Click the **Gradient Program** tab.

   The Gradient Program page appears (see Figure 42). The columns that represent the solvents selected on the Pump General page are available for editing.

Figure 42. Gradient Program page



4. For time = 0.00, do the following:

   a. Type the initial solvent composition in the A%, B%, C%, and D% columns. For each solvent, after you type the percent composition, press Enter or click another box in the gradient program table.

      The first row in the gradient program table is set to 0.00 min. You cannot change this time value. As you change the percent composition for each solvent, the data system maintains a total solvent composition of 100%.

   b. Type the initial flow rate in the µL/min column, and then press ENTER or click another box in the gradient program table.

      The minimum flow rate setting depends on the start setting for the gradient program as follows:

      - When you select Surveyor AS injection logic or Manual and the Home before run check box, the minimum flow rate is 10.0 µL/min.
      - When you select Manual and leave the Home before run check box cleared, the minimum flow rate is 0.0 µL/min.

      The maximum flow rate setting is 2000 µL/min for the Surveyor MS Pump Plus.

5. For an isocratic separation method, do the following on the Gradient Program page for the Surveyor MS Pump Plus:

   a. Double-click the Time column in the second row of the gradient program table, and then type a time value in this cell.

      The time range is 0.01 to 655.00 minutes.

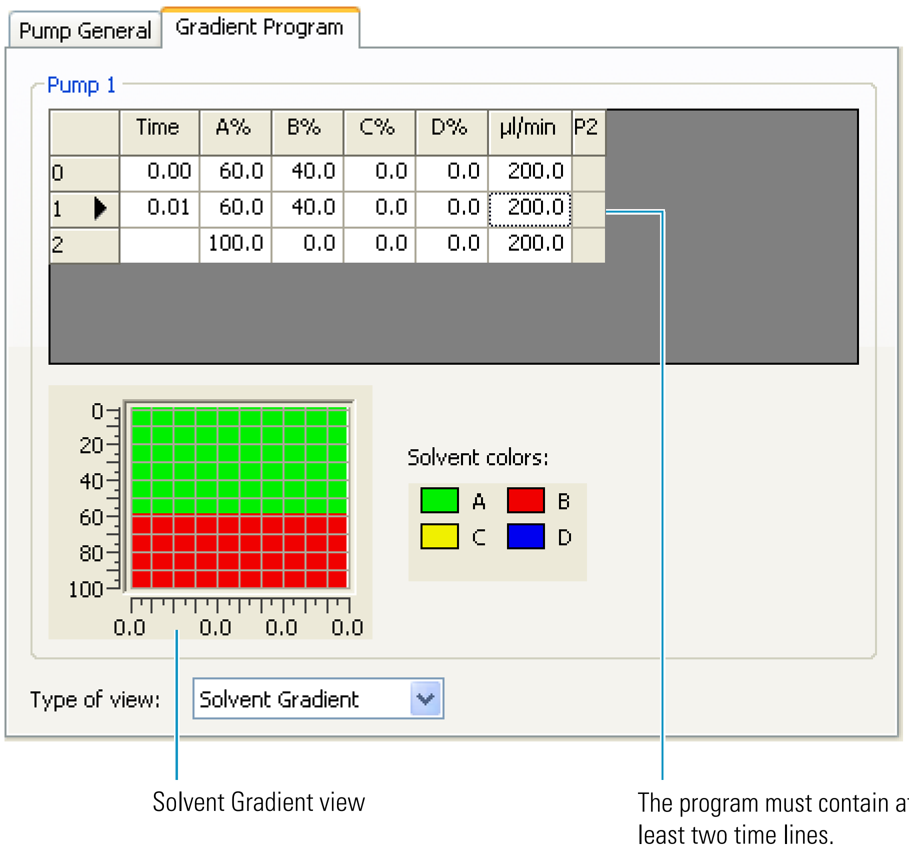
If the time value for the second time point is longer than the data acquisition time for the PDA detector or the MS detector, the data system waits for the gradient program to finish before proceeding to the next row in the acquisition sequence.

b. Type the same values for the solvent composition and the flow rate that you specified for the initial time point. Press ENTER to accept the values.

> **IMPORTANT** You must specify the flow rate and solvent composition for at least two time lines.

c. Verify the gradient program in the Solvent Gradient view (see Figure 43).

Figure 43. Isocratic gradient program



6. For a gradient separation method, do the following:

a. For each row in the program, type the solvent composition and the flow rate, and then press ENTER.

The pump program can contain from 2 to 398 rows (time lines). The last row in the gradient program is a placeholder and has no effect on the pump conditions. The last row does not have a time entry in the Time column.

The flow rate range is the same as for an isocratic run (see step 4b on page 75). To produce a stable gradient, enter a flow rate of at least 25 µL/min.

b. To return the solvent conditions to those specified in the first line of the gradient program, select **First line conditions** in the Method finalizing list. First line conditions is the default selection.

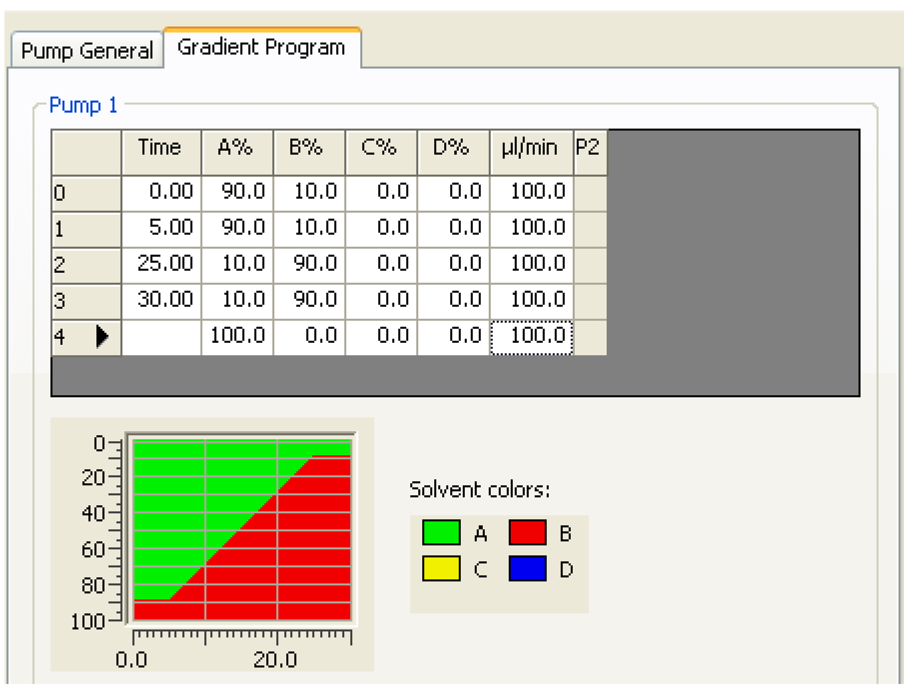
Figure 44 shows the gradient program entries for the gradient program in Table 16. After 30 minutes the gradient program returns to the first line conditions for the remainder of the run. To equilibrate the LC column at the first line conditions for 10 minutes, the run time for the PDA detector or MS detector must be set to 40 minutes.

Tip If you do not want the detector to acquire data during the column equilibration period, you can add an equilibration period to the end of the gradient program and make the gradient program time for the pump longer than the data acquisition time for the detector.

Table 16. Gradient program

Time (min)	Solvent composition
0.00 to 5.00	Held constant at 90% solvent A /10% solvent B
5.00 to 25.00	Linear ramp from 10% solvent B to 90% solvent B
25.00 to 30.00	Held constant at 10% solvent B /90% solvent B
30.00 to 40.00	Column is equilibrated at the initial solvent composition of 90% solvent A /10% solvent B

Figure 44. An example of a gradient program for the pump



Thermo Pump Gradient Program Page Parameter Descriptions

Table 17 describes the parameters on the Gradient Program page.

Table 17. Thermo pump method parameters

Parameter	Description
Gradient table	
Each row in the gradient table defines the solvent composition and flow rate for a specific time point. Between time points, the solvent composition changes linearly, whereas the flow rate changes as a discontinuous step-function. The pump program can contain up to 398 time lines.	
Time	The time parameter (min box) specifies at what point in the run the associated solvent composition and flow rate become effective. The first time line remains set to 0 min.
%A. %B, %C, and %D	The solvent composition parameters (%A, %B, %C, and %D boxes) work interactively with each other and specify the mobile phase composition at the time point specified in the associated Min box. The mobile phase composition changes linearly between two consecutive time points. Default: 100% A
µL/min	The µL/min box specifies the flow rate of the mobile phase at the time specified in the associated Time box. Default: 10 µL/min
P2 (Dual-pump system)	For a dual-pump system, the time line position of the ball in the P2 column specifies the time when Pump 1 triggers Pump 2.
Graph view	
View type	Select Solvent Gradient to view the solvent gradient profile created by the pump program. Select Flow Gradient to view the flow rate changes created by the pump program.
	The Gradient Profile area graphically displays the values entered in the pump program. The *y* axis represents percent composition or flow rate and the *x* axis represents time in minutes. Each solvent is color-coded for better visualization of the programmed gradient.

Programming the Pumps in a Dual-Pump System

❖ **To program the pumps in a dual-pump system**

1. On the Pump General page, do the following:

    a. Follow the instructions in "Setting Up the Pump General Parameters" on page 68.

    b. In the Pump 2 area, select the Idle settings for the pump that you configured as the sample pump (Pump 2) as follows:

    In the Idle Settings list, select the solvent conditions for Pump 2 before it starts its gradient program:

- If you want Pump 2 to remain idle until Pump 1 sends the start signal, select **Standby**.
- If you want Pump 2 to pump solvent at the solvent composition and flow rate specified in the first line of its gradient program until Pump 1 sends the start signal, select **First line**.
- If you want Pump 2 to pump solvent at the solvent composition and flow rate specified in the last line of its gradient program until Pump 1 sends the start signal, select **Last line**.

c. Specify the remaining parameters for Pump 2.

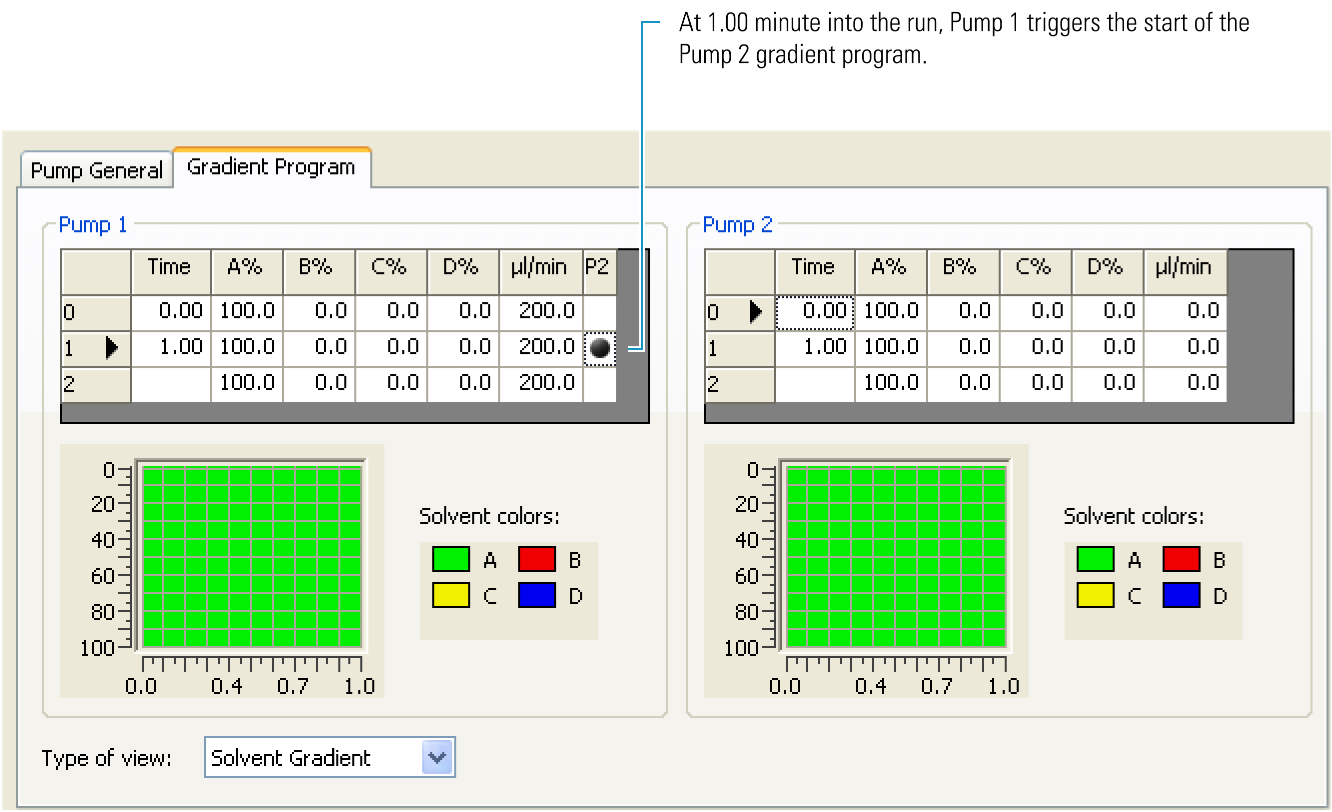
2. On the Gradient Program page (see Figure 45), do the following:

a. To specify the gradient programs for both pumps, follow the instructions in "Setting Up the Gradient Program" on page 74.

b. In the Pump 1 area, in the P2 column, select the time line that corresponds to the time that you want the start pump to trigger the sample pump.

To select the trigger time for Pump 2, click the appropriate row in the P2 column. The icon appears in the P2 column.

Figure 45. Gradient page for a dual-pump system consisting of two pumps



Autosampler Instrument Method Parameters

Use these three pages of the Surveyor AS view to specify the injection settings, the temperatures of the controlled temperature zones, the solvents contained in the wash bottle and the autosampler's 16 mL reservoir bottles, and any external events triggered by the autosampler.

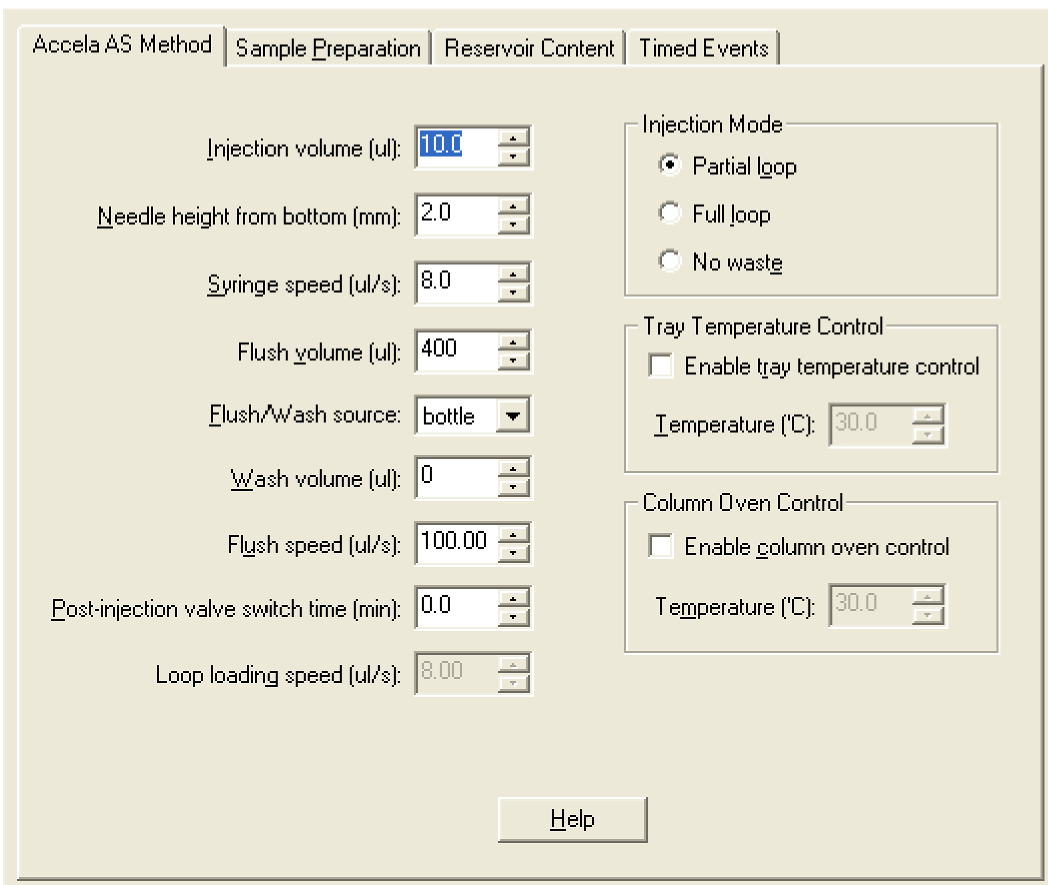
- "Surveyor AS Method Page," on this page
- "Reservoir Content Page" on page 87
- "Timed Events Page for the Autosampler" on page 88

For information on the Sample Preparation page, see Chapter 4, "Sample Preparation Routines."

Surveyor AS Method Page

Use the Surveyor AS Method page to set the injection and the temperature control parameters for the autosampler (see Figure 46).

Figure 46. Surveyor AS Method page



❖ To specify the injection and temperature control settings for your instrument method

1. Select the injection mode:

   - Select the Partial loop option if you want to vary the volume of your sample injections.

   - Select the Full loop option if you want a high degree in injection-to-injection precision. When you select Full loop, the Injection volume (µL) box becomes unavailable.

   - Select the No waste option if you have a limited amount of sample. When you select No Waste, the Loop loading speed (µL/s) box becomes available.

   Note For more information on the injection modes, see "Injection Modes" on page 20.

2. For the No waste injection mode, type or select a loop loading speed in the Loop loading speed box.

   For most applications, keep the loop loading speed at its default value.

3. For the Partial loop and No waste injection modes, type or select an injection volume in the Injection volume box.

   The injection volume range depends on the syringe size see "Injection Volume Range" on page 86.

4. In the Needle height from bottom (mm) box, type or select the height from the bottom of the vial or well that the needle of the autosampler descends to before withdrawing sample from the vial/well location.

   The default value is 2 mm and the range is 0.1 to 18 mm. Entering a lower value causes the needle to descend closer to the bottom of the vial or well. The standard 1.8 mL vials supplied with the autosampler have a depth of approximately 20 mm.

   Figure 13 on page 15 on shows the needle descending to a depth of 2 mm from the bottom of a standard 1.8 mL vial

5. In the Syringe speed (µL/s) box, for most applications, keep the syringe speed at its default value (8 µL/s for the 250 µL concentric syringe).

   If you see the sample in the needle tubing breakup as the autosampler withdraws sample from a vial or well location into the needle tubing, adjust the syringe speed.

6. In the Flush volume (µL) box, type or select a volume from 0 to 6000 µL or keep the value at its default of 400 µL.

7. In the Flush/Wash source list, select a flush/wash solution.

   For most applications, select bottle (wash bottle). If you select one of the reservoir vial locations as your flush/wash source, make sure that you load the autosampler with a reservoir vial.

For information on the location of the 16 mL reservoir vials, see “Tray Compartment” on page 5. For information on specifying the contents of the reservoir vials and the wash bottle, see “Reservoir Content Page” on page 87.

8. In the Wash volume (µL) box, keep the wash volume at its default value of 0 µL.

   To wash the exterior of the needle after each injection, type a value from 1 to 6000 µL in the Wash volume box.

9. In the Flush speed (µL/s) box, keep the flush speed at its default (100 µL/s for the 250 µL concentric syringe) if you are using a flush solvent of low viscosity such as methanol. Reduce the flush speed if you are using water or a methanol/water mixture as the flush solvent.

   > **Tip** If the flush speed is too high, the autosampler makes a grinding noise as it performs a flush.

10. In the Post-injection valve switch time (min) box, keep the time value at its default of 0 minutes for most applications. This leaves the sample loop in the inject position during the entire run, allowing ample rinsing of the sample loop between injections.

11. In the Tray Temperature Control area, to control the temperature of the tray compartment, select the **Enable tray temperature control** check box. Then type an appropriate temperature from 0 to 60 °C in the Temperature box.

12. In the Column Oven Control area, to control the temperature of the LC column, select the **Enable column oven control** check box. Then type an appropriate temperature from 5 to 95 °C in the Temperature box. Controlling the temperature of the LC column increases the reproducibility of the chromatographic retention times.

Autosampler Method Page Parameter Descriptions

Table 18 describes the parameters on the Surveyor AS Method page.

The autosampler ships with a 250 µL concentric plunger syringe, but you can replace this syringe with a 100 µL or 500 µL concentric plunger syringe or a 2500 µL single plunger syringe.

The injection volume range and the syringe speeds depend on the syringe size as described in these topics:

- “Injection Volume Range” on page 86
- “Syringe and Loop Loading Speeds for Injections” on page 86

Table 18. Autosampler Method page parameters (Sheet 1 of 3)

Parameter	Description
Injection volume (µL)	Specifies the sample volume that the autosampler loads into the sample loop. The default value and the range depend on the syringe size (see "Injection Volume Range" on page 86).
Needle height from bottom (mm)	Specifies the height from the bottom of the vial or well where the needle withdraws liquid (see Figure 13 on page 15). Range: 0.1 to 18.0 mm Default: 2 mm
Syringe speed (µL/s)	Specifies the rate at which the syringe withdraws liquid from a tray vial or a plate well. The default value and the range depend on the syringe size (see "Syringe and Loop Loading Speeds for Injections" on page 86).
Flush volume (µL)	Specifies the volume of liquid that the autosampler flushes through the inside of the needle, the injection port, and the transfer tube. During a flush cycle, the injection valve is in the inject position to prevent the flush solvent from entering the sample loop. To reduce carryover, increase the flush volume. When the specified flush volume exceeds the syringe capacity, the autosampler takes multiple draws from the reservoir or bottle. Range: 0.0 to 6000.0 µL Default: 400 µL
Flush/Wash source	Specifies the flush/wash source. Selections: bottle, RV1, RV2, RV3, or RV4 Default: bottle (wash bottle)
Wash volume (µL)	Specifies the volume of liquid used to wash the internal and external surfaces of the needle. During a wash cycle, the autosampler lowers the needle into the wash port. When the specified wash volume exceeds the syringe capacity, the autosampler takes multiple draws from the reservoir vial or wash bottle. Range: 0.0 to 6000.0 µL Default: 0 µL

Table 18. Autosampler Method page parameters (Sheet 2 of 3)

<table>
<tr><th>Parameter</th><th>Description</th></tr>
<tr><td>Flush speed (µL/s)</td><td>Specifies the rate at which the syringe draws flush solvent into its chamber and expels flush solvent through the needle during a flush cycle.
<table>
<tr><th>Syringe type</th><th>Default</th><th>Range</th></tr>
<tr><td>Concentric dual-plunger</td><td>1.65 to 661.38 µL/s</td><td>100.0 µL/s</td></tr>
<tr><td>Standard single-plunger</td><td>8.27 to 330.85 µL/s</td><td>82.71 µL/s</td></tr>
</table>
IMPORTANT A flush speed of 250 µL/s for the 250 µL concentric syringe is the maximum flush speed for a solvent of low viscosity such as 100% methanol. If you are using a solvent of higher viscosity, reduce the flush speed accordingly. For example, because water has approximately twice the viscosity as methanol, if you are using water as your flush solvent, lower the flush speed to 100 µL/s. At high flush speeds, viscous solvents can cause the autosampler to make a grinding sound.</td></tr>
<tr><td>Post-injection valve switch time (min)</td><td>At the default post-injection valve switch time of 0 minutes, the injection valve remains in the inject position during the run.

A time value greater than 0.1 minutes specifies the time when the injection valve switches from the inject position to the load position at the end of an injection cycle.

To reduce the gradient delay volume for low-flow gradient applications, consider switching the injection valve from the inject position to the load position during the run by typing a nonzero value in this box. Allow enough time for the mobile phase to backflush the sample out of the sample loop.

Default: 0 minutes
Range: 0 to 999.0 minutes</td></tr>
<tr><td>Loop loading speed (µL/s)</td><td>Specifies the rate at which the autosampler meters the sample into the sample loop for no waste injections. During a no waste injection, the autosampler meters the sample through the transfer tubing to the injection valve at the specified syringe speed. The injection valve then switches to the load position, and the autosampler meters the sample into the sample loop at the specified loop loading speed.

This parameter is only available in the No waste injection mode.

The default value and range depend on the syringe size (see “Syringe and Loop Loading Speeds for Injections” on page 86).</td></tr>
<tr><td>Injection Mode</td><td></td></tr>
<tr><td>Partial loop</td><td>Select this option if you want to inject variable volumes.

IMPORTANT To make precise partial loop injections, limit the maximum injection volume to less than half the nominal sample loop size. Because the accuracy of the nominal volume of the sample loop is ± 20% (which means that the actual volume of the standard 25 µL sample loop is between 20 µL and 30 µL), for best results, limit the maximum injection volume to 10 µL for the standard 25 µL sample loop.</td></tr>
</table>

Table 18. Autosampler Method page parameters (Sheet 3 of 3)

Parameter	Description
Full loop	Select this option when you need a high degree of accuracy. The Injection Volume box is unavailable with this selection.
No waste	Select this option when you have a limited amount of sample. **IMPORTANT** For no waste injections, do the following: • Use a sample loop that is at least 5 µL larger than the injection volume. Because the accuracy of the nominal size is ± 20%, use an estimate of 80% for the actual size. • Consider matching the chemistry of the sample matrix, the flush solution, and the mobile phase. • Inject at least 1.0 µL of sample.
Tray Temperature Control	
Enable tray temp control	Select this check box to enable temperature control for the tray compartment. In the default instrument method, this check box is clear; temperature control for the tray compartment is not enabled. To maintain a stable temperature, set the temperature to a value that is a least 10 °C above or below the ambient room temperature. As a safety feature, a thermostat turns off power to the sample tray if the temperature reaches 65 °C.
Temperature (° C)	Specifies the tray temperature. Default: 30 °C Range: 0 to 60 °C
Column oven control	
Enable tray temp control	Select this check box to enable temperature control for the column oven. In the default instrument method, this check box is clear; temperature control for the column compartment is not enabled.
Temperature (° C)	Specifies the column oven temperature. To maintain a stable, set the temperature to a value that is a least 10 °C above or below the ambient room temperature. As a safety feature, a thermostat turns off power to the column oven if the temperature reaches 110 °C. Default: 30 °C Range: 5 to 95 °C

Injection Volume Range

The injection volume for full loop injections is automatically set to the value in the Sample Loop Volume box on the Communication page of the Surveyor Autosampler dialog box (see “Communication Page” on page 45).

For partial loop and no waste injections, the minimum injection volume is 0.1 µL. The maximum injection volume depends on the syringe size (see Table 19).

For optimal performance, follow these guidelines:

- For no waste injections, do not inject less than 1.0 µL and limit the maximum injection volume to 80 percent of the nominal loop size minus an additional 5 µL.
- For partial loop injections, limit the maximum injection volume to 40 percent of the nominal loop size. For the standard 25 µL sample loop, limit the maximum injection volume to 10 µL.

Table 19. Maximum injection volume

Syringe size (µL)	Maximum injection volume (µL)
100	20
250	125
500	300
2500	1250

Syringe and Loop Loading Speeds for Injections

During an injection sequence, the inner plunger of the concentric syringe descends, pulling liquid from a sample vial or well into the needle tubing. After the autosampler withdraws sample, the XYZ arm moves the needle to the injection port. The inner plunger of the concentric syringe ascends, pushing liquid through the transfer tubing and into the sample loop. A stepper motor controls the speed at which the plunger moves.

You can specify the rate, in microliters per second, at which the syringe withdraws liquid from a tray vial or plate well, and then expels liquid into the autosampler injection port. The default syringe speed and the syringe speed range depend on the syringe size (see Table 20).

The appropriate syringe speed depends on the sample. For viscous samples, use a syringe speed lower than the default value to avoid stalling the syringe. For samples of low viscosity or surface tension, use a lower syringe speed to prevent the sample bolus from breaking apart during the transport process.

Tip Select the syringe size on the Communication page of the Surveyor Autosampler Configuration dialog box. The autosampler ships with a 250 µL concentric syringe.

Table 20. Syringe speeds for injections

Syringe Size (µL)	Minimum Speed (µL/s)	Maximum Speed (µL/s)	Default Speed (µL/s)
100	0.3	13.2	3.00
250	0.8	33.0	8.0
500	1.6	66.1	8.0
2500	8.2	330.8	25.0

For no waste injections, you can use a higher syringe speed to push the sample from the autosampler injection port to the injection valve and a lower loop loading speed to meter the sample into the sample loop. The range for the loop loading speed depends on the syringe size (see Table 21).

Table 21. Loop loading speeds

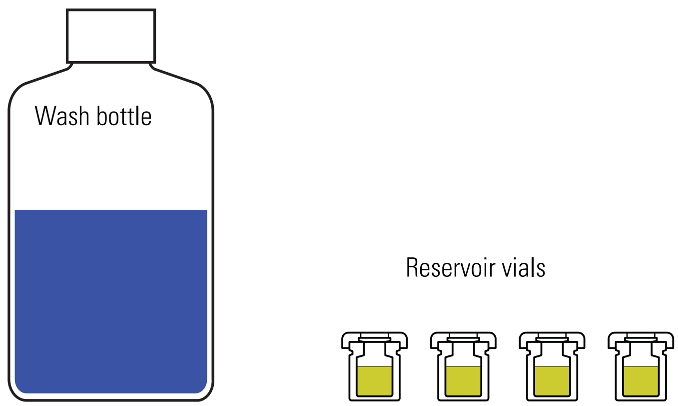
Syringe Size (µL)	Minimum Speed (µL/s)	Maximum Speed (µL/s)	Default Speed (µL/s)
100	0.01	13.20	3.00
250	0.04	33.00	8.00
500	0.08	66.11	8.00
2500	0.41	330.80	25.00

Reservoir Content Page

Use the Reservoir Content page (see Figure 48) to enter descriptions for the contents of the four 16 mL reservoir vials located on right side of the tray compartment and the wash solvent bottle located in the solvent platform at the top of the LC system stack. You can type up to 80 characters in each box.

Figure 47 shows the relative size of the 1 liter wash bottle and the 16 mL reservoir vials.

Figure 47. Reservoir vials and wash solvent bottle

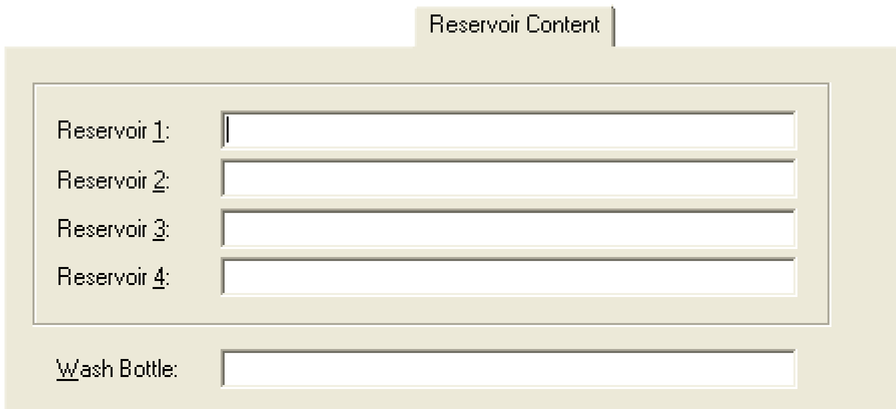


❖ To specify the solvents in the wash bottle and reservoir vials

1. Click the **Reservoir Content** tab.

   The Reservoir Content page appears (see Figure 48).

   Figure 48. Reservoir Content page for the autosampler



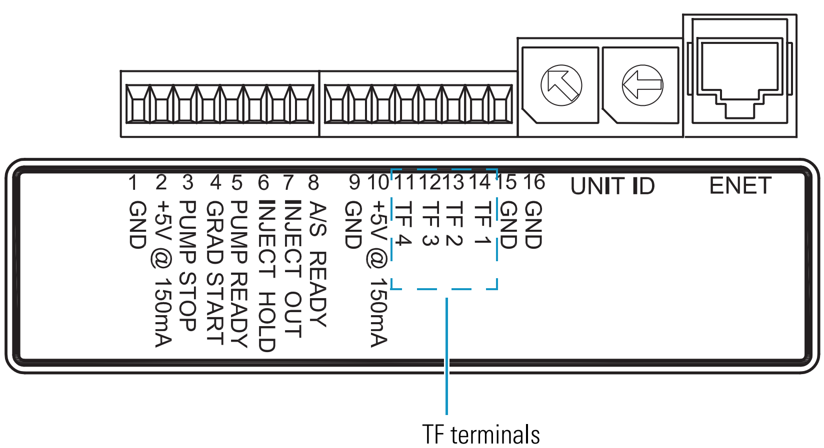
2. Identify the solvents contained in the 16 mL reservoir vials and the 1 L wash bottle by typing their names in their respective boxes.

Timed Events Page for the Autosampler

You can use the TF terminals (see Figure 49) on the back panel of the autosampler to control peripheral devices that the data system does not control.

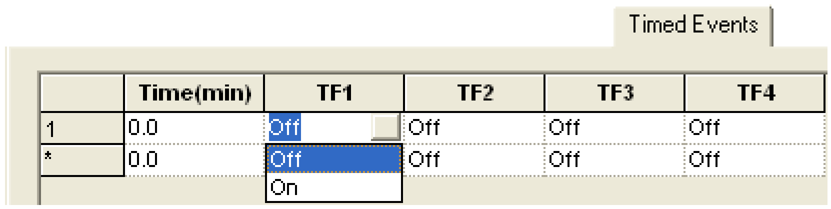
The TF terminal output signal is LO (Closed) by default. To change the polarity of the TF output signal to HI (Open), select the Timed Events Active High check box in the Signal Polarity page of the Surveyor Autosampler Configuration dialog box (see "Signal Polarity Page" on page 49).

Figure 49. Time function event terminals on the back panel of the autosampler



Use the Timed Events page (see Figure 50) to specify the timed events for the time function terminals (TF1 to TF4).

Figure 50. Timed Events page for the autosampler



❖ To control external devices (not controlled by your Thermo Scientific data system)

1. Click the **Timed Events** tab.

   The Timed Events page appears (see Figure 50).

2. Specify the appropriate timed events in the table.

3. Make the appropriate hardwire connections between the TF terminals on the back panel of the autosampler and the external device.

Autosampler Timed Events Page Parameter Descriptions

Table 22 describes the columns in the timed events table for the autosampler.

Table 22. Autosampler timed events table

Parameter	Description
Table of timed events	Use this table to set up timed events.
Time	Specifies the time (in minutes) when the autosampler TF terminal (TF1 to TF4) signals an event. Time 0.00 is defined as the time when the autosampler issues an INJECT OUT signal. The autosampler issues the specified Timed Event signals after issuing the Inject Out signal. Default: 0 Range: 0 to 9999.0 minutes
TF1, TF2, TF3, and TF4	Specifies whether the event is On or Off at the specified time.

PDA Detector Instrument Method Parameters

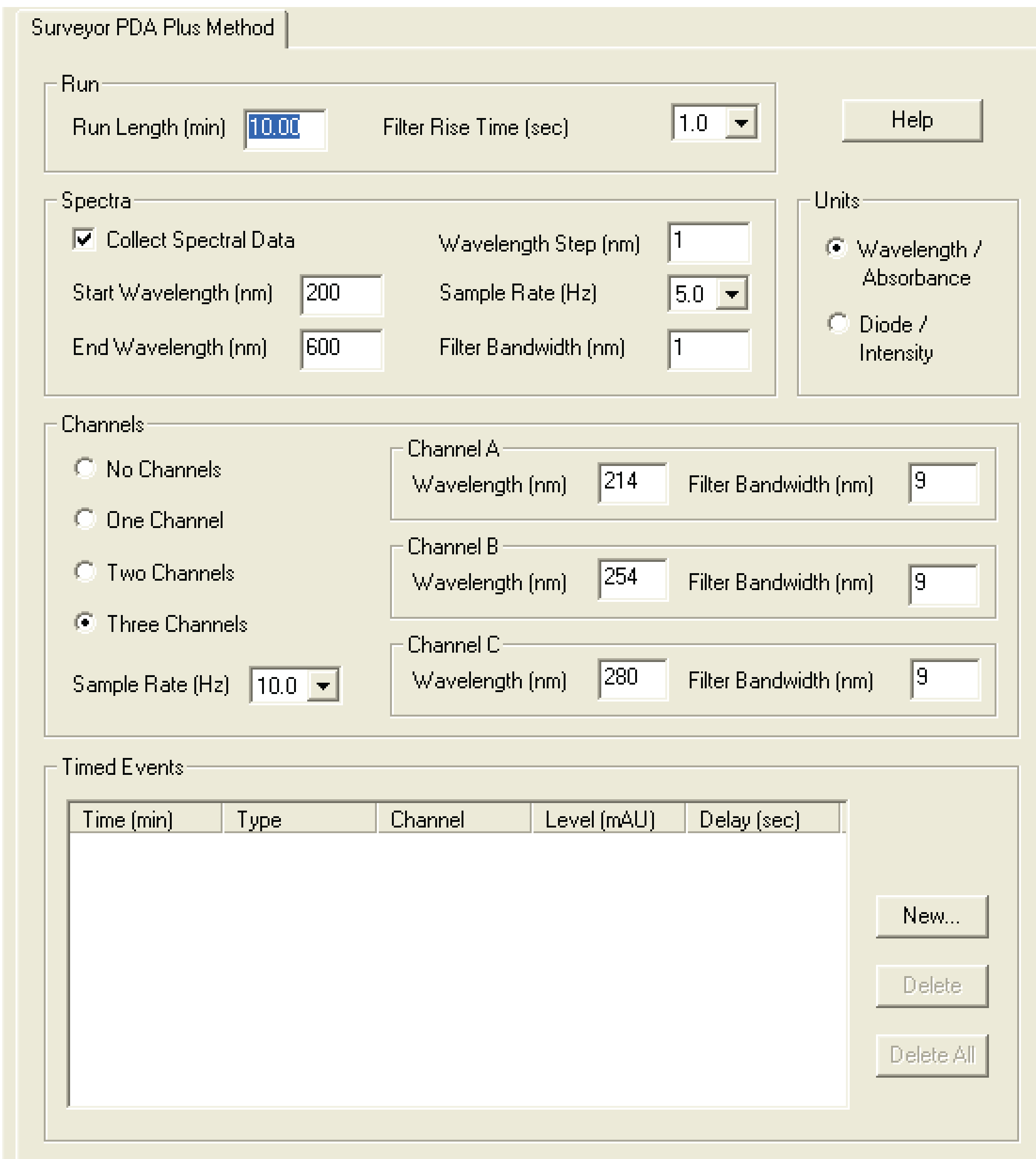
Use the Surveyor PDA Plus Method page to specify the data acquisition settings for the detector and to trigger external devices from the detector.

❖ To program the PDA detector to acquire absorbance data

1. In the view bar, click the **Surveyor PDA Plus** icon.

   The view for the Surveyor PDA Plus Detector appears with the Surveyor PDA Plus Method page displayed (see Figure 51).

Figure 51. Surveyor PDA Plus Method page with the default settings



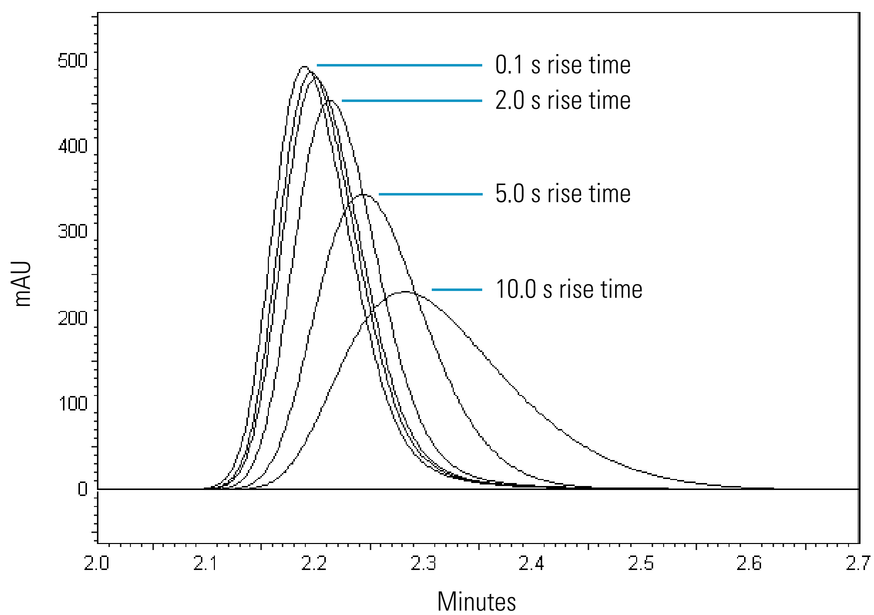
2. In the Run Length (min) box, type a run time from **0** to **600** minutes.

3. In the Filter Rise Time (sec) list, select a rise time from **0** to **10.0** seconds.

   Rise time is inversely proportional to the amount of baseline noise. If your chromatogram contains closely eluting peaks, minimize baseline noise while retaining maximum resolution by selecting a rise time that is approximately one-tenth of the narrowest peak's width at half-height (FWHM). Increasing the rise time above this level increases peak tailing, which can reduce the resolution of closely eluting peaks.

   Figure 52 shows the effect of rise time on peak tailing.

   Figure 52. Effect of rise time on band broadening



4. In the Units area, select the **Wavelength/Absorbance** option.

> **Note** For information on using the Diode/Intensity option to perform diagnostics, see "Adjusting the Attenuators" on page 249. Methods that record light intensities for the PDA detector have the .spda file extension.

5. To collect a wavelength scan, select the **Collect Spectra Data** check box, and then make the following entries and selections:

   a. In the Start Wavelength (nm) box, type a starting wavelength from **190** to **799** nm.

   b. In the End Wavelength (nm) box, type an ending wavelength from **191** to **800** nm. The ending wavelength must be greater than the starting wavelength.

   c. In the Wavelength Step (nm) box, type a value for the wavelength interval.

The default value is 1 nm. The maximum step size depends on the wavelength range defined by the starting and ending wavelengths. The range is 1 to 610 nm for a scan from 190 to 800 nm. If you are collecting spectral data for a spectral library, use a wavelength step of **1** nm.

d. In the Sample Rate (Hz) list, select a sample rate.

For optimal integration of the chromatographic peaks, select a sample rate that acquires a minimum of 20 points across the baseline width of the narrowest peak of interest. For example, if the baseline width of the narrowest peak is 20 seconds, select a sample rate of 1 Hz or higher.

Increasing the sampling rate increases the data file size.

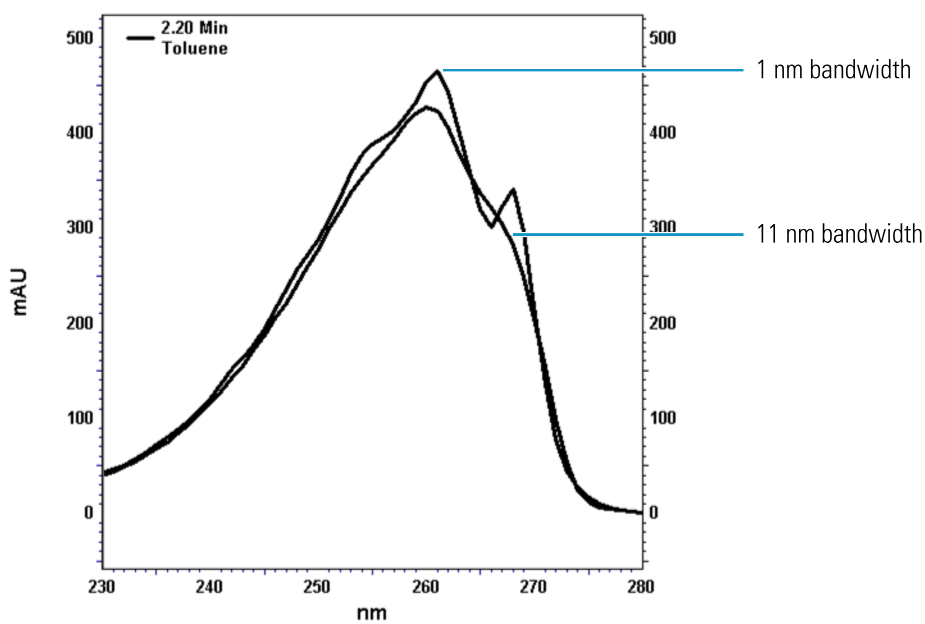
e. In the Filter Bandwidth (nm) box, type a bandwidth.

Depending on the wavelength range defined by the starting and ending wavelengths, the range is the subset of odd integers from 1 to 49 nm. The maximum scan range for a particular bandwidth is limited as follows:

- Start Wavelength (minimum) = 190 nm + (bandwidth - 1)/2
- End Wavelength (maximum) = 800 nm - (bandwidth -1)/2

Increasing the bandwidth decreases the spectral resolution (see Figure 53).

Figure 53. Spectra of toluene, effect of bandwidth on spectral resolution



6. To collect discrete channel data, select one of the options in the Channels area, and then make the following entries:

   a. In the Sample Rate list, select a sampling rate (in data points per second). Higher data rates for discrete channels do not add significantly to the data file size.

b. For each discrete channel (A, B, and C), type a wavelength from 190 to 800 nm in the Wavelength (nm) box and a filter bandwidth in the Filter Bandwidth (nm) box.

Depending on the discrete wavelength, the acceptable bandwidth range is 1 to 49 nm in odd-number increments with 1 nm meaning no filtering. Bandwidth values outside the range of the detector are not allowed. For example, for the discrete wavelength of 200 nm, the maximum bandwidth setting is 21 nm. At a bandwidth setting of 21 nm, the reported absorbance value for 200 nm is a weighted average from 190 to 210 nm. A value greater than 21 nm would be outside the lower range limit of the detector, which is 190 nm.

For information on the Method page parameters, see these topics:

- "PDA Detector Instrument Method Parameters," on this page
- "Display Method Parameter Descriptions" on page 96
- "Timed Events Table for the PDA Detector" on page 97

For information on adding timed events to trigger external devices, see "Triggering an External Device with the PDA Detector" on page 99.

PDA Detector Instrument Method Parameters

Table 23 describes the parameters for an instrument method. These parameters are available when you select the Wavelength/Absorbance option in the Units area.

Table 23. PDA detector Method page parameters for an instrument method (Sheet 1 of 3)

Parameter	Description
Run	
Run Length	Specifies the time in minutes that the PDA detector acquires data. Default: 10.00 minutes Range: 0.00 to 600.00 minutes. At the end of the run time, the PDA detector goes to the Ready for Download state.
Filter Rise Time	Specifies the detector's response time in seconds to the signal. The selections are 0.50, 1.00, 2.00, 4.00, 5.00, and 10.00 seconds.
Units	
Wavelength/ Absorbance	Displays absorbance spectra with the wavelength in nanometers on the *x* axis and the absorbance in milli-absorbance units (mAU) on the Y-axis. The Wavelength/Absorbance option is the default selection. Select the Wavelength/Absorbance option for instrument methods.

Table 23. PDA detector Method page parameters for an instrument method (Sheet 2 of 3)

Parameter	Description
Spectra (with Wavelength/Absorbance Option)	
Collect Spectral Data	To collect scans having a specific wavelength range (nm), bandwidth (nm), scan rate (Hz), and step (nm), select this check box.
Start Wavelength (nm)	Specifies the first wavelength in nanometers of the scan range. Default: 200 nm Range: 190 to 799 nm
End Wavelength (nm)	Specifies the last wavelength in nanometers of the scan range. Default: 600 nm Range: 191 to 800 nm The end wavelength must be greater than the start wavelength.
Wavelength Step	Specifies the wavelength interval between data points across the wavelength range. Default: 1 nm The maximum step size is the full width of the scan. The highest spectral resolution is 1 nm and the lowest resolution is the full width of the scan. When you specify a spectral scan from 200 to 300 nm at a step of 10 nm, the PDA detector sends 11 scan wavelengths to the data system computer.
Sample Rate	Specifies the number of data points per second that the PDA detector sends to the data system for each scan wavelength. For optimal integration of the chromatographic peaks, acquire 20 points across the baseline width of the peak. For the Surveyor PDA Detector Plus, the sample rates are 0.5, 1.0, 2.0, 4.0, 5.0, 10.0, and 20 Hz.
Filter Bandwidth	Specifies the bandwidth for the scan wavelengths. Bandwidth values are limited to the subset of odd integers from 1 to 49. In addition, the maximum scan range for a particular bandwidth is limited as follows: Lower range = 190 nm + (bandwidth - 1)/2 Upper range = 800 nm – (bandwidth - 1)/2 The maximum scan range for a bandwidth of 49 nm is 214 nm to 776 nm. **Note** Wider bandwidths decrease both spectral noise and the resolution of the spectra. In general, the detector bandwidth should not exceed 10 percent of the bandwidth at half-height of the narrowest spectral feature of interest.

Table 23. PDA detector Method page parameters for an instrument method (Sheet 3 of 3)

Parameter	Description
Channels (with Wavelength/ Absorbance Units)	
No Channels	Specifies that no discrete channel measurements are to be acquired. When you select this option, the parameters in the Channels area are unavailable.
One Channel	Specifies the acquisition of one discrete channel. The data for Channel A are displayed in the bottom view of the Display page in black.
Two Channels	Specifies the acquisition of two discrete channels. The data for Channel B are displayed in the bottom view of the Display page in red.
Three Channels	Specifies the acquisition of three discrete channels. The data for Channel C are displayed in the bottom view of the Display page in green.
Sample Rate	Specifies the sample rate in Hz for all of the discrete channels selected (see sample rate in the Spectra area).
[Ch. A, B, C] Wavelength	Specifies the discrete channel wavelength to be acquired. Channel A default: 214 Channel B default: 254 Channel C default: 280 Range: 190 to 800 nm
[Ch. A, B, C] Filter Bandwidth	Specifies the bandwidth for the corresponding discrete channel. Bandwidth values are limited to the subset of odd integers from 1 to 49. Default: 9 nm

Display Method Parameter Descriptions

Table 24 describes the parameters for a display method. These parameters are available when you select the Diode/Intensity option in the Units area.

Table 24. Surveyor PDA Plus Method page parameters for a display method (Sheet 1 of 2)

Parameter	Description
Run	
Run Length	Not applicable
Filter Rise Time	Not applicable
Units	
Units: Diode/Intensity	Specifies the display units for the spectrum plots. When you select the Diode/Intensity option, you can view the light intensity detected by the photo diodes of the diode array. The *x* axis corresponds to the photo diode number (2 to 511). There are 512 diodes in the diode array. Diodes 0 and 1 are not used. Diode 2 corresponds to wavelength 190 nm and diode 511 corresponds to 800 nm. This gives a spacing of 611 nm/510 diodes = 1.2 nm/diode. The PDA detector interpolates the data to give integer values.
Spectra (with Diode/Intensity Option)	
Collect Spectral Data	Select this check box to scan a range of diodes.
Start Diode (diode num)	Specifies the first diode in the scan range. Default: 2 Range: 2 to 510, in increments of 1 Diode 2 corresponds to 190 nm.
End Diode (diode num)	Specifies the last diode in the scan range. Default: 511 Range: 3 to 511, in increments of 1 Diode 511 corresponds to 800 nm.
Diode Step	Specifies the interval between data points across the range of diode numbers. The selections are 1, 2, 4, 5, or 10 diodes.
Sample Rate	Not applicable

Table 24. Surveyor PDA Plus Method page parameters for a display method (Sheet 2 of 2)

Parameter	Description
Channels (with Intensity Units)	
No Channels	Specifies that no discrete channel measurements are to be acquired. When you select this option, the parameters in the Channels area are unavailable.
One Channel	Specifies the acquisition of one discrete channel. The data for Channel A are displayed in the bottom view of the Display page in black.
Two Channels	Specifies the acquisition of two discrete channels. The data for Channel B are displayed in the bottom view of the Display page in red.
Three Channels	Specifies the acquisition of three discrete channels. The data for Channel C are displayed in the bottom view of the Display page in green.
Sample Rate	Not applicable
[Ch. A, B, C] Diode Number	Specifies the diode that you want the detector to monitor. Channel A default: 40 Channel B default: 55 Channel C default: 450 Range: 2 to 511, in increments of 1

Timed Events Table for the PDA Detector

Table 25 describes the timed events area at the bottom of the Surveyor PDA Plus Method page.

Use the Timed Events area to program actuation of a back panel contact closure as a function of either time or the absorbance level from one of the discrete wavelength channels. You can use this feature to trigger an external device, such as a fraction collector, and to zero the absorbance level during an acquisition run.

Refer to the *Surveyor PDA Plus Detector Hardware Manual* for information on connecting an external device to the PDA detector.

Table 25. Timed events table on the Surveyor PDA Plus Method page (Sheet 1 of 2)

Parameter	Description
Timed Events	
Use this area to add, modify, and remove timed events. You can specify the time at which the event occurs, the event type, the channel, the absorbance level (mAU), and the delay settings. Click the New button to open the Timed Events dialog box.	
Timed Events table	This table lists all the events in chronological order. You can add, modify, and remove events by double-clicking the event setting you want to modify, or by using the New button, the Delete button, and the Delete All button.

Table 25. Timed events table on the Surveyor PDA Plus Method page (Sheet 2 of 2)

Parameter	Description
Time	Specifies the time in minutes when the detector triggers the event. The maximum time is the data acquisition time specified in the Run time box. Default: 0.00 minutes
Type	Specifies an event type: Event Off, Event On, Zero Data, or Level Trigger. Use a Level Trigger event to specify an absorbance threshold level at which an event is triggered. When you select a Level type event, you must specify the channel of the event, the absorbance level that is to trigger the event, and the delay time that the event is to occur after the absorbance level trigger event. Use a Zero event to zero the absorbance from the detector.
Channel	**Note** This list is available if Level Trigger is selected in the [Event] Type list. Specifies the discrete wavelength channel (Channel A, Channel B, or Channel C) that the PDA detector monitors to activate the external event.
Level	**Note** This box is available if Level Trigger is selected in the [Event] Type list box. Specifies the absorbance level that triggers the external event. Range: -2000 to 4000 milli-absorbance units (mAU)
Delay	**Note** This box is available if Level Trigger is selected in the [Event] Type list. Specifies the delay time in seconds that is to occur between the activation of the event (by the preset absorbance level being exceeded) and the actual change of the event output state (at the back panel terminals of the PDA detector). This delay compensates for the time interval between, for example, when a compound leaves the detector and when it reaches a fraction collector. Default: 0 second Range: 0 to 65 535, in increments of 1
Buttons	
New	Click this button to open the Timed Event dialog box where you can add external events to the external events table.
Delete	Click this button to remove an external event from the external events table. Select the external event that you want to remove, and then click the Delete button.
Delete All	Click this button to remove all external events from the external events table. To remove all events, click the Delete All button.

Triggering an External Device with the PDA Detector

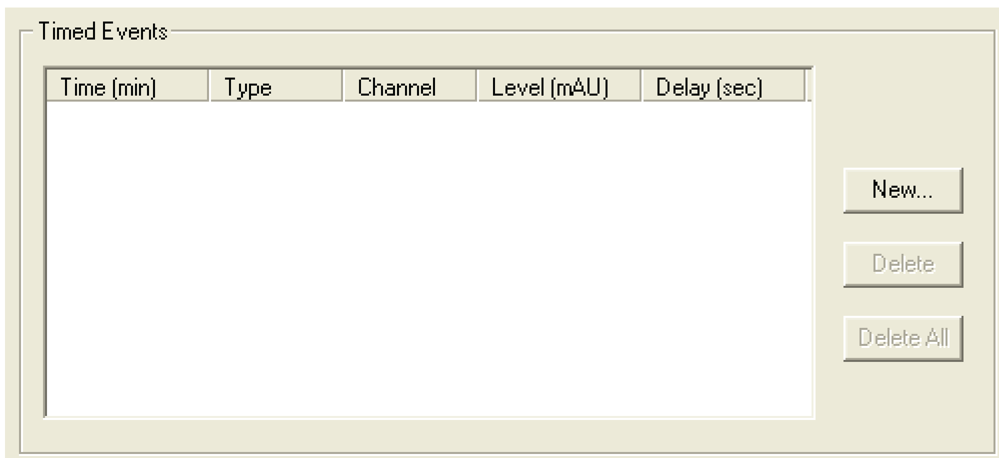
The detector can trigger an external device during a chromatographic run. For example, you can program the PDA detector to trigger a fraction collector as an analyte begins to elute from the LC column.

Note You can also zero the absorbance level for a discrete channel during an acquisition run by adding a zero event to the timed events table.

❖ **To add a timed event to the instrument method**

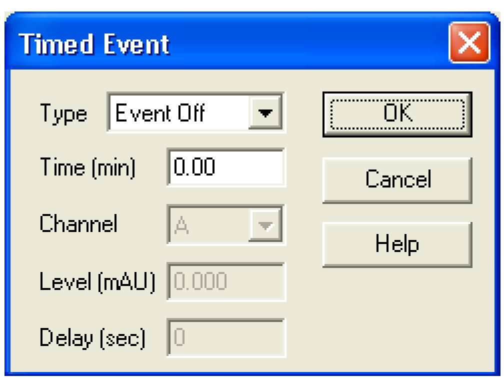
1. In the Timed Events area of the Surveyor PDA Plus Method page (see Figure 54), click **New**.

   Figure 54. Timed Events area of the Method page



   The Timed Event dialog box appears (see Figure 55).

   Figure 55. Timed Event dialog box



2. In the Type list, select an event type.

   The selections are Event Off, Event On, Zero Data, or Level Trigger.

3. In the Time (min) box, type the length of time after the chromatographic run starts that the event is to occur.

   The range is 0.00 to the run length time for the detector. The Event Off, Event On, and Zero Data event types occur at this time. Because the Level Trigger event type occurs after this time is exceeded, do not enter a time value that exceeds the expected time (for example, the expected retention time of the analyte that you want a fraction collector to collect) of the triggering event.

   The Channel list, Level box, and Delay box are available for the Level Trigger event type. For more information on the Level trigger type, see the next procedure.

4. To trigger an external device when the absorbance for a discrete channel reaches a specific level, do the following:

   a. In the type list, select **Level Trigger**.

   b. In the Channel list, select the discrete wavelength channel to be used to trigger the event.

      The available selections are A, B, or C.

   c. In the Level (mAU) box, type a value for the absorbance value that triggers the event.

      The default value is 0.00 mAU and the range is -2000 to 4000.00 mAU.

   d. In the Delay (sec) box, type a time in seconds.

      The delay time compensates for the time interval between, for example, when a compound leaves the detector and when it reaches a fraction collector. The default value is 0 and the range is the set of integers from 0 to 65 535.

5. To zero the absorbance level for all the wavelength channels that the detector is monitoring at a particular time in the run, do the following:

   a. In the type list, select **Zero Data**.

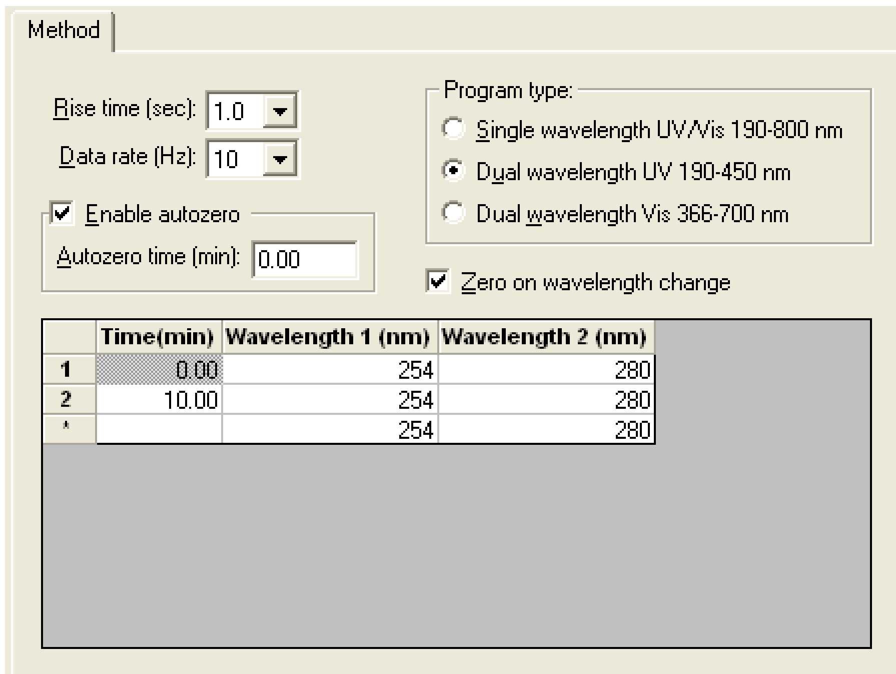
   b. In the Time box, type the time that you want the absorbance level to be zeroed.

6. Click **OK** to accept the new event and close the Timed Event dialog box.

UV-Vis Detector Instrument Method Parameters

In the UV/Vis detector view, use the Method page for the UV/Vis detector to set up the method parameters for the UV/Vis detector (see Figure 56).

Figure 56. Instrument Setup view for the UV/Vis detector



❖ To open the Surveyor UV/Vis Detector view

Click the **Surveyor UV/Vis** icon in the view bar.

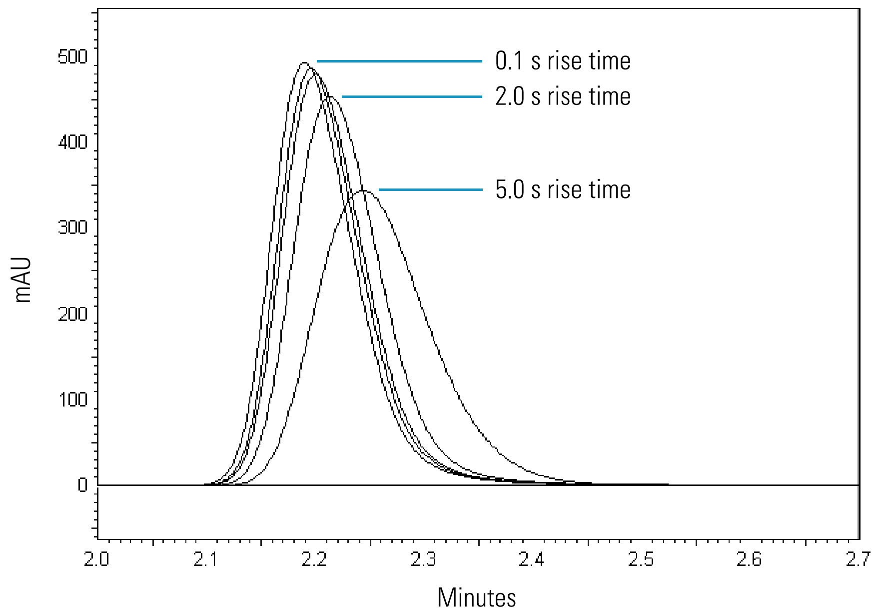
❖ To set up the method parameters for the UV/Vis detector

1. In the Rise time (sec) list, select a rise time from 0 to 10.0 seconds.

   Rise time is the response time of the detector, in seconds, to the signal. Increasing the rise time decreases the baseline noise; however, setting the rise time to a value greater than one-tenth the width of the chromatographic peak at half-height results in peak broadening. The one-second default value is appropriate for most LC applications.

   Figure 57 shows the effect of rise time on peak tailing.

Figure 57. Effect of rise time on band broadening



2. In the Data rate (Hz) list, select a data rate from 4 to 20 points per second.

   The optimal data rate depends on the expected baseline width of your chromatographic peaks. For optimal integration, acquire a minimum of 20 data points across the baseline width of the narrowest peak. For example, if the narrowest chromatographic peak has a baseline width of 10 seconds, select a data rate of 2 Hz or higher.

3. To zero the baseline at a specific time point, select the **Enable autozero** check box and type a time in the Autozero time (min) box.

   The detector resets its output voltage to zero at this time point.

4. In the Program type area, select one of the following options:

   - To collect one chromatogram during a run, select the **Single wavelength 190 to 800 nm** option. You can program time wavelength changes in the program table.

   - To collect two chromatograms in the UV range during a run, select the **Dual wavelength UV 190 to 450 nm** option. You can program time wavelength changes in the program table.

   - To collect two chromatograms in the visible range during a run, select the **Dual wavelength Vis 366 to 700 nm** option. You can program time wavelength changes in the program table.

5. To re-zero the baseline when a programmed wavelength change occurs, select the **Zero on wavelength change** check box.

The detector does not zero the baseline on the first or last rows of a wavelength program. If the wavelength program contains three or more rows, the detector zeroes the baseline on the second row and all successive rows until it reaches the last row. The detector zeroes its output signal even if the same wavelength is listed in the second through second to last rows of the table.

UV-Vis Detector Instrument Method Parameter Descriptions

Table 26 describes the parameters on the Method page for the UV/Vis detector.

Table 26. UV/Vis detector method parameters (Sheet 1 of 2)

Parameter	Description
Rise time	Specifies the filtering in the time domain. Default: 2.0 seconds The selections are 0.0, 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 seconds.
Data rate	Specifies the number of points per second acquired per wavelength. Default: 10 Hz The selections are 20, 10, 6.67, 5, and 4 Hz.
Enable Autozero	
Enable Autozero	When this check box is selected, the detector zeros the absorbance data at the specified time.
Time	Specifies the time when the detector zeros the absorbance data. Default: 0 minutes Range: 0 minutes to the run length
Program type	
Single wavelength UV/Vis 190-800 nm	Specifies that the detector is to acquire data on one wavelength channel. The acquisition wavelength is time programmable.
Dual wavelength UV 190-365 nm	Specifies that the detector is to acquire data on two wavelength channels in the UV range. The acquisition wavelengths are time programmable.
Dual wavelength Vis 366-700 nm	Specifies that the detector is to acquire data on two wavelength channels in the visible range. The acquisition wavelengths are time programmable.
Other options	
Zero on wavelength change	When this check box is selected, the detector zeros the absorbance data at the times specified in the Time column for rows 2 through the last time line.
Programmable wavelength table	
Time	Specifies the time when the detector is to start monitoring the wavelength or wavelengths listed in the Wavelength 1 and Wavelength 2 columns.

Table 26. UV/Vis detector method parameters (Sheet 2 of 2)

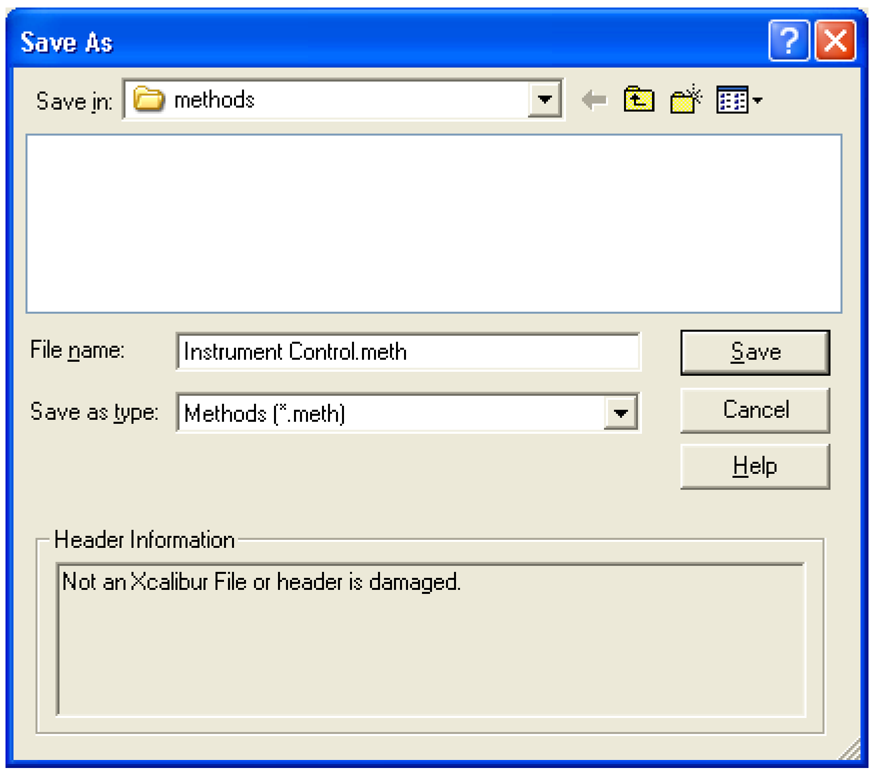
Parameter	Description
Wavelength 1	Specifies the first wavelength that the detector is to start monitoring at the time specified in the Time column.
Wavelength 2	Specifies the second wavelength that the detector is to start monitoring at the time specified in the Time column.

Saving the Instrument Method

❖ **To save the instrument method**

1. Choose **File > Save As**. The Save As dialog box appears (see Figure 58).

Figure 58. Save As dialog box

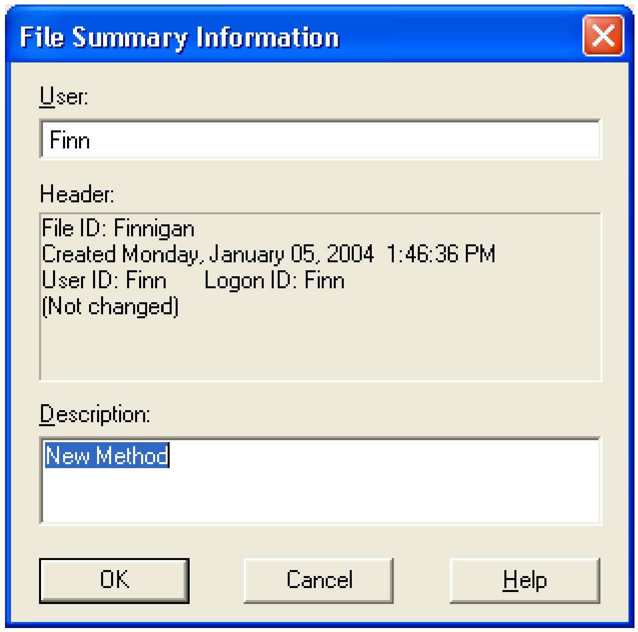


2. Browse to the following folder:

   C:\Xcalibur\methods

3. In the File name box, type a file name.
4. Click **Save**. The File Summary Information dialog box appears (see Figure 59).

Figure 59. File Summary Information dialog box

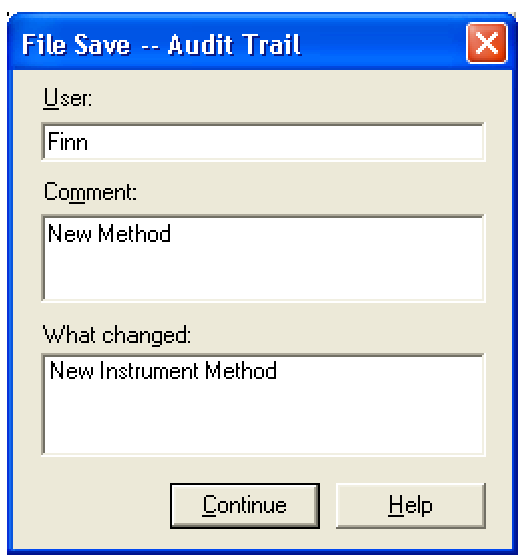


5. In the Description box, type a description of the method file.

6. Click **OK**.

   If the Authorization Manager - Comment check box is selected, the File Save - Audit Trail dialog box appears (see Figure 60).

Figure 60. File Save – Audit Trail dialog box



7. In the Comment box, type a comment concerning the changes you made to the instrument method.

8. Click **Continue** to close the File Save – Audit Trail dialog box and save the instrument method.

Sample Preparation Routines

Sample preparation routines are part of the instrument method. Use the Sample Preparation page to create a multi-task routine, containing up to 512 tasks. A task consists of a sample preparation operation and its associated parameters.

The ability to add 512 tasks to a sample preparation routine gives you considerable flexibility; however, the arrangement of the tasks in the task list must follow a logical order. For example, you cannot add a task that deposits liquid before you add a task that draws liquid. In addition, if you are using the 250 µL dual-concentric syringe that ships with the autosampler, you must follow an additional set of rules that maintain the proper positioning of the inner and outer plungers of the syringe.

Contents

Opening the Sample Preparation Page

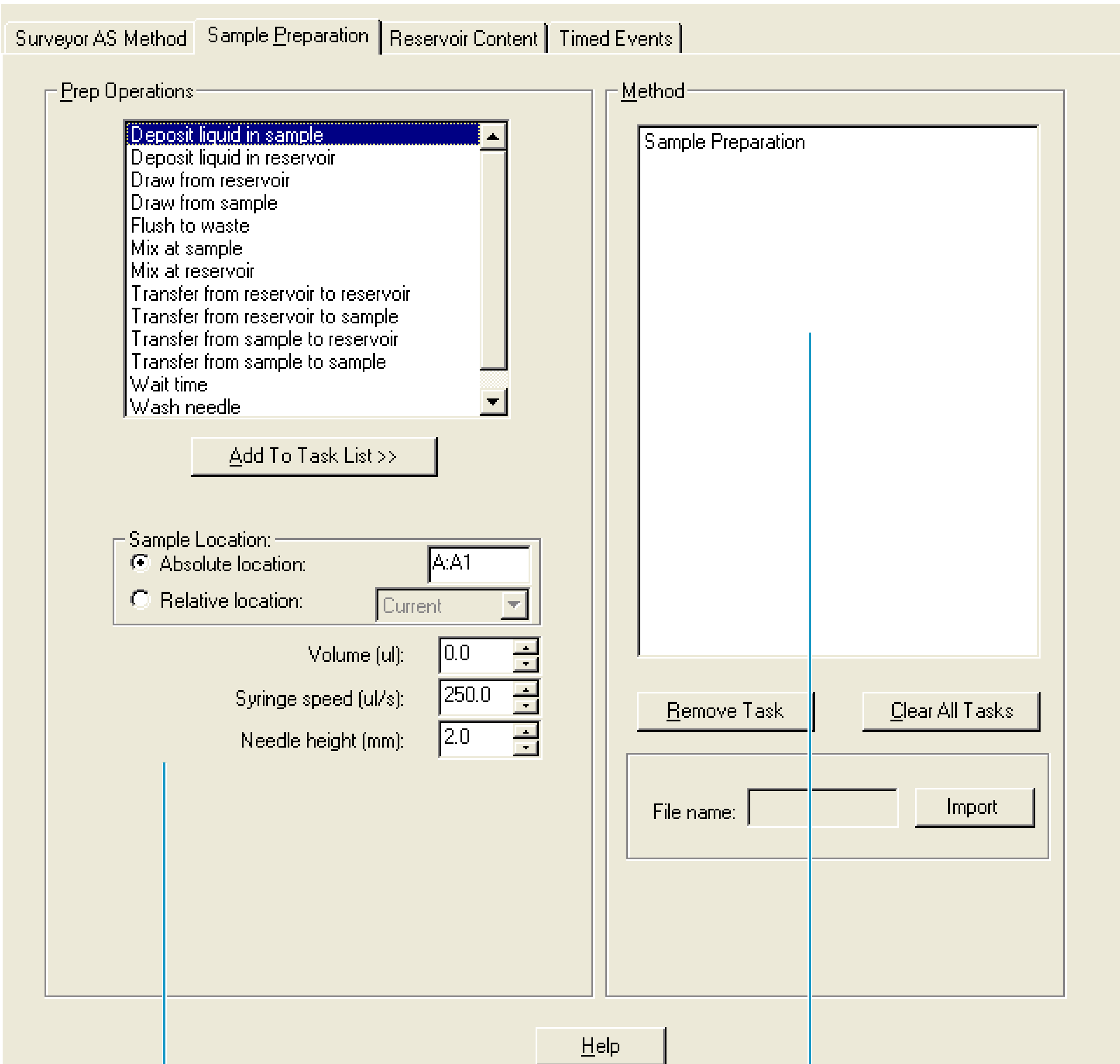
The Sample Preparation page is part of the instrument method.

❖ **To open the Sample Preparation page**

1. Open the autosampler view from your data system.
2. Click the **Sample Preparation** tab.

   The Sample Preparation page appears (see Figure 61). For a new method, in the Prep Operations list, the Deposit liquid in sample task is highlighted, and its parameters are listed below the Add To Task List button.

Figure 61. Sample Preparation page of the Surveyor Autosampler view



Sample Preparation Rules

The maximum volume that the autosampler can deposit, draw, or transfer during a sample preparation task depends on the syringe type, the task requested, and the previous step in the sample preparation method.

Table 27 categorizes the sample preparation tasks into three groups based on whether the task uses only the inner bore, only the outer bore, or either bore of the dual-plunger concentric syringe. For information on the dual-plunger concentric syringe, see "Interchangeable Syringe" on page 14.

Table 27. Sample preparation tasks arranged in groups according to bore usage

Inner bore only	Outer bore only	Inner or outer bore
Draw from sample	Mix at sample	Draw from reservoir
Transfer from sample to sample	Mix at reservoir	Deposit liquid in sample
Transfer from sample to reservoir	Wash needle	Deposit sample in reservoir
	Flush to waste	Transfer from reservoir to sample
		Transfer from reservoir to reservoir

For the 250 µL concentric syringe, the arrangement of the tasks in the sample preparation list is restricted by the following rules:

- For tasks performed with the inner bore of the dual-concentric syringe, the maximum volume (sample + air bubble) that the autosampler can draw, deposit, or transfer is limited to the nominal size of the syringe, 250 µL.
- For tasks performed with either bore, if the sum of the requested volume (liquid + air bubble) plus any volume left in the needle tubing from a previous step is less than the nominal syringe size, the dual-plunger concentric syringe uses the small bore. If this volume is greater than the nominal syringe size, the syringe uses the outer bore.
- For tasks performed with the outer bore of the dual-plunger concentric syringe, the maximum volume (sample + air bubble) that you can draw, deposit, or transfer is limited to 500 µL, except for the Wash Needle and Flush to Waste tasks.
- Crossover between bores is not allowed. This means that you cannot add a task that uses the outer syringe bore immediately following a task that uses the inner syringe bore. To switch from the inner bore to the outer bore of the syringe, you must insert a Flush to Waste step or a Wash Needle step. These tasks home the position of the syringe plungers.

Building the Sample Preparation Routine

Sample preparation routines are part of the instrument method (see "Autosampler Instrument Method Parameters" on page 80). A sample preparation routine consist of a list of preparation operations.

❖ To build a sample preparation routine

1. Do one of the following:
   - To modify an existing sample preparation routine, go to step 2.

     –or–
   - To create a new sample preparation routine, go to step 3.
2. Import the sample preparation routine from a stored instrument method as follows:
   a. Click **Import**. The Open files dialog box appears.

   b. Browse through your Methods folder and select the appropriate instrument method. Then click **Open**.

   Only the sample preparation task list contained in the stored instrument method is imported into the current instrument method.
3. For each task that you want to add to the sample preparation routine, do the following:
   a. In the Prep Operations list, select a task.

   Table 28 lists the thirteen tasks that you can use to create a sample preparation routine. When you select a task, the parameters available for the task appear below the Add to Task List button.

   b. Make the appropriate selections and entries for each task parameter.

   c. Click **Add To Task List**.

   The task is copied to the Task List list in the Method area.

   > **Note** If the previous task used the small bore of the syringe, you must add a Flush to Waste or a Wash Needle task before you can add a task that requires the use of the large bore of the syringe. In addition, if the previous task used the small bore of the syringe, the transfer volume of the current task is limited to the nominal syringe size or less. See "Sample Preparation Rules" on page 109 for more information.
4. Edit the task list as needed:
   - To remove the last task in the list, click **Remove Task**.
   - To clear the entire task list, click **Clear All Tasks**.

Tip To save a method, the sample preparation routine must conform to the rules described in "Sample Preparation Rules" on page 109.

Sample Preparation Parameter Descriptions

Table 28. Sample Preparation page (Sheet 1 of 6)

Parameter	Description	
Prep Operations		
To add a task to the Sample Preparation routine, click the task in the Prep Operations list to display the parameters available for the task. Enter the appropriate values for each parameter, and then click the **Add To Task List** button. The task is copied to the Method list. You can add up to 512 tasks to a sample preparation routine; however, the arrangement of the tasks in the task list must follow a logical order. For example, you must add tasks in a logical order or you will not be able to save the method. For example, a draw task must precede a deposit task. In addition, if you are using the 250 µL concentric syringe that comes with the autosampler, you must follow an additional set of rules that allow for the proper positioning of the inner and outer plungers of the syringe. (See "Sample Preparation Rules" on page 109).		
Task List	**Description**	
Deposit Liquid in Sample	Deposits liquid in a sample vial or well.	
	Parameter	**Reference, Range, or Selections**
	Sample Location	"Sample Locations for the Sample Preparation Tasks" on page 116
	Volume	"Draw, Eject, and Transfer Volumes for Sample Preparation Tasks" on page 117
	Syringe Speed	"Syringe Speed for Tasks that Can Use Either Plunger" on page 119
	Needle Height	Default: 2 mm Range: 0 to 18 mm
Deposit Liquid in Reservoir	Deposit liquids in a reservoir vial.	
	Parameter	**Reference, Range, or Selections**
	Volume	"Draw, Eject, and Transfer Volumes for Sample Preparation Tasks" on page 117
	Destination Reservoir	RV1, RV2, RV3, or RV4
	Syringe Speed	"Syringe Speed for Tasks that Can Use Either Plunger" on page 119

Table 28. Sample Preparation page (Sheet 2 of 6)

Parameter	Description	
Draw from Reservoir	Draws liquid from a reservoir vial or the wash bottle.	
	Parameter	**Reference, Range, or Selections**
	Volume	"Draw, Eject, and Transfer Volumes for Sample Preparation Tasks" on page 117
	Source Reservoir	RV1, RV2, RV3, RV4, or Bottle
	Syringe Speed	"Syringe Speed for Tasks that Can Use Either Plunger" on page 119
	Air Bubble	Range: 0 to 10 µL
Draw from Sample	Draws liquid from a vial or well.	
	Parameter	**Reference, Range, or Selections**
	Sample Location	"Sample Locations for the Sample Preparation Tasks" on page 116
	Volume	"Draw, Eject, and Transfer Volumes for Sample Preparation Tasks" on page 117
	Syringe Speed	"Syringe Speed for Tasks that Can Use Either Plunger" on page 119
	Needle Height	Default: 2 mm Range: 0 to 18 mm
	Air Bubble	Range: 0 to 10 µL
Flush to Waste	Flushes the needle tubing and homes the syringe. After the concentric syringe is homed, the autosampler can switch between tasks that use the inner plunger and tasks that use the outer plunger.	
	Parameter	**Reference, Range, or Selections**
	Volume	Range: 100 to 6000 µL
	Reservoir	bottle, RV1, RV2, RV3, or RV4
	Syringe Speed	1.65 to 661.38 µL/sec for the concentric syringes 0.83 to 330.85 µL/sec for the 2500 µL standard syringe

Table 28. Sample Preparation page (Sheet 3 of 6)

<table>
<tr><th>Parameter</th><th>Description</th></tr>
<tr><td>Mix at Sample</td><td>Mixes the contents of a vial or well.

To mix a sample, the autosampler draws a specified volume of the sample into the needle tubing at a specified speed, and then ejects the sample back into the same location at a specified speed.
<table>
<tr><th>Parameter</th><th>Reference, Range, or Selections</th></tr>
<tr><td>Sample Location</td><td>“Sample Locations for the Sample Preparation Tasks” on page 116</td></tr>
<tr><td>Volume</td><td>The maximum volume is 490 µL for the concentric syringes and 1490 µL for the standard 2500 µL syringe.</td></tr>
<tr><td>Draw Speed
Delivery Speed</td><td>The draw or delivery speed range is from 1.65 to 661.38 µL/s for the concentric syringes, and from 0.83 to 330.85 µL/s for the standard 2500 µL syringe.</td></tr>
<tr><td>Cycles</td><td>Range: 1 to 10</td></tr>
<tr><td>Needle Height</td><td>Default: 2 mm
Range: 0 to 18 mm</td></tr>
</table>
Note To transfer volumes larger than 500 µL, add an optional tubing extension assembly to the needle tubing.</td></tr>
<tr><td>Mix at Reservoir</td><td>Mixes the contents of a reservoir vial.
<table>
<tr><th>Parameter</th><th>Reference, Range, or Selections</th></tr>
<tr><td>Volume</td><td>The maximum volume is 490 µL for the concentric syringes and 1490 µL for the standard 2500 µL syringe.</td></tr>
<tr><td>Destination Reservoir</td><td>RV1, RV2, RV3, or RV4</td></tr>
<tr><td>Draw Speed
Delivery Speed</td><td>The draw or delivery speed range is from 1.65 to 661.38 µL/s for the concentric syringes, and from 0.83 to 330.85 µL/s for the standard 2500 µL syringe.</td></tr>
<tr><td>Cycles</td><td>Range: 1 to 10</td></tr>
<tr><td>Needle Height</td><td>Default: 2 mm
Range: 0 to 18 mm</td></tr>
</table>
Note To transfer volumes larger than 500 µL, you must add an optional tubing extension assembly to the needle tubing.</td></tr>
</table>

Table 28. Sample Preparation page (Sheet 4 of 6)

Parameter	Description	
Transfer from Reservoir to Reservoir	Transfers liquid from one reservoir to another.	
	Parameter	**Reference, Range, or Selections**
	Volume	"Draw, Eject, and Transfer Volumes for Sample Preparation Tasks" on page 117
	Source Reservoir	bottle, RV1, RV2, RV3, or RV4
	Destination Reservoir	RV1, RV2, RV3, or RV4
	Syringe Speed	"Syringe Speed for Tasks that Can Use Either Plunger" on page 119
Transfer from Reservoir to Sample	Transfers liquid from a reservoir vial or the wash bottle to a vial or well.	
	Parameter	**Reference, Range, or Selections**
	Sample Location	"Sample Locations for the Sample Preparation Tasks" on page 116
	Volume	"Draw, Eject, and Transfer Volumes for Sample Preparation Tasks" on page 117
	Syringe Speed	"Syringe Speed for Tasks that Can Use Either Plunger" on page 119
	Needle Height	Default: 2 mm Range: 0 to 18 mm
	Source Reservoir	bottle, RV1, RV2, RV3, or RV4
Transfer from Sample to Reservoir	Transfers liquid from a vial or well to a reservoir vial.	
	Parameter	**Reference, Range, or Selections**
	Sample Location	"Sample Locations for the Sample Preparation Tasks" on page 116
	Volume	"Draw, Eject, and Transfer Volumes for Sample Preparation Tasks" on page 117
	Destination Reservoir	RV1, RV2, RV3, or RV4
	Syringe Speed	"Syringe Speed for Tasks that Can Use Either Plunger" on page 119
	Needle Height	Default: 2 mm Range: 0 to 18 mm
	Air Bubble	0 to 10 µL

Table 28. Sample Preparation page (Sheet 5 of 6)

Parameter	Description	
Transfer from Sample to Sample	Transfers liquid from one vial or well to another vial or well.	
	Parameter	**Reference, Range, or Selections**
	Source Sample	“Sample Locations for the Sample Preparation Tasks” on page 116
	Destination Sample	“Sample Locations for the Sample Preparation Tasks” on page 116
	Volume	“Draw, Eject, and Transfer Volumes for Sample Preparation Tasks” on page 117
	Syringe Speed	“Syringe Speed for Tasks that Can Use Either Plunger” on page 119
	Source Vial Needle Height	Default: 2 mm Range: 0 to 18 mm
	Air Bubble	0 to 10 µL
	Destination Vial Needle Height	Default: 2 mm Range: 0 to 18 mm
Wait Time	Pauses the sample preparation routine for a period of time; for example, for a reaction to occur. Range: 0 to 1000 minutes.	
Wash Needle	Washes the exterior of the needle with the solvent in a reservoir vial or the wash bottle.	
	Parameter	**Reference, Range, or Selections**
	Volume	Range: 100 to 6000 µL
	Reservoir	bottle, RV1, RV2, RV3, or RV4
Button		
Add To Task List	Use the Add To Task List button to add a task to the sample preparation routine. To add a task, click the task in the Prep Operations list box to display the parameters available for the task. Enter the appropriate values for each parameter, and then click the **Add To Task List** button. The task is copied to the list box in the Method group box.	
Method		
You use the Method group box to display the parameters of a task, remove a task, clear all tasks in the task list, or import a method. The Method group box consists of the (Sample Preparation) Task List list box, the Remove Task button, and the Clear All Tasks button, and the File name (read only) box and Import (method) button.		
Remove Task	Use this button to remove a task from the Task List list box. To remove a task, select the task, and then click the **Remove Task** button.	

Table 28. Sample Preparation page (Sheet 6 of 6)

Parameter	Description
Clear All Tasks	Use this button to clear all tasks from the Task List list box. To remove all tasks, click the **Clear All Tasks** button.
Import	The File name (read only) box and Import [method] button displays the file name of the method you import. To import method information, click **Import**, and then use the displayed dialog box.

Sample Locations for the Sample Preparation Tasks

For the sample preparation tasks, you can select an Absolute or a Relative Location.

For the Relative Location option, the selections are Current, Current + 1, Current + 2, and Current + 3. Current is the sample location in the current sequence row. Current + 1 is the next adjacent vial or well location in the tray, and so on. For example, if the vial location in the current sequence row is B:1, Current is B:1, then Current + 1 is B:2, and so on.

For the Absolute Location option, you specify an absolute vial or well location. The vial or well locations depend on the tray configuration (see Table 29).

Note Remember to place a vial at the selected location.

Table 29. Vial and well positions

Tray configuration	Vial or well positions
Standard 1.8 mL vials	A:1 to E:20
96 Well Plate	A:A1 to C:H12
384 Well Plate	A:A1 to C:P24

Draw, Eject, and Transfer Volumes for Sample Preparation Tasks

The maximum volume that can be deposited, drawn, or transferred during a sample preparation task depends on the syringe type, the task requested, and the previous step in the sample preparation method:

- For tasks performed with the inner plunger of the concentric syringe, the maximum volume (liquid + air bubble) that you can draw, deposit, or transfer is limited to the nominal size of the syringe.
- For tasks performed with the outer plunger of the concentric syringe, the maximum volume (liquid + air bubble) that you can draw, deposit, or transfer is limited to 500 µL, except for the Wash Needle and Flush to Waste tasks, which are limited to a range of 100 µL to 6000 µL, and the Mixing tasks, which are limited to a maximum of 490 µL.

Table 30 lists the maximum volumes by task.

Table 30. Draw, eject, and transfer volumes for sample preparation tasks (Sheet 1 of 2)

Task	Maximum Volume (µL) with the Inner Plunger	Maximum Volume (µL) with the Outer Plunger
Draw from Sample	Nominal size of the concentric syringe minus the current contents (liquid + air bubble) of the needle tubing	N/A
Transfer from Sample to Sample	Nominal size of the concentric syringe minus the current contents (liquid + air bubble) of the needle tubing	N/A
Transfer from Sample to Reservoir	Nominal size of the concentric syringe minus the current contents (liquid + air bubble) of the needle tubing	N/A
Mix at Sample	N/A	490
Mix at Reservoir	N/A	490
	Note If the tooltip specifies a range of 0.0 to 0.0, the contents of the needle tubing already equals 500 µL or the previous task used the inner plunger of the syringe. If the previous task used the inner plunger, insert a Flush to Waste or a Needle Wash task to home the syringe.	

Table 30. Draw, eject, and transfer volumes for sample preparation tasks (Sheet 2 of 2)

Task	Maximum Volume (µL) with the Inner Plunger	Maximum Volume (µL) with the Outer Plunger
Wash Needle	N/A	6000
Flush to Waste	N/A	6000
Draw from Reservoir	Nominal size of the concentric syringe minus the current contents (liquid + air bubble) of the needle tubing	500 µL minus the current contents of the needle tubing
Deposit Liquid in Sample	Total contents (liquid + air bubble) of the needle tubing from previous steps	Total contents (liquid + air bubble) of the needle tubing from previous steps
Deposit Sample in Reservoir	Total contents (liquid + air bubble) of the needle tubing from previous steps	Total contents (liquid + air bubble) of the needle tubing from previous steps
Transfer from Reservoir to Sample	Nominal size of the concentric syringe minus the current contents (liquid + air bubble) of the needle tubing	500 µL
Transfer from Reservoir to Reservoir	Nominal size of the concentric syringe minus the current contents (liquid + air bubble) of the needle tubing	500 µL

Syringe Speed Range for Sample Preparation Tasks

The syringe speed range for sample preparation tasks depends on whether the task can use either syringe plunger or only the inner plunger.

If you enter a speed that is invalid for the volume you are transferring, an error message appears.

For information on the syringe speed range for the sample preparation tasks, see these topics:

- "Syringe Speed for Tasks that Can Use Either Plunger" on page 119
- "Syringe Speed for Tasks that Use the Inner Plunger" on page 120

Syringe Speed for Tasks that Can Use Either Plunger

The syringe speed range is determined by the type of syringe and the volume requested. For a concentric syringe, the autosampler can use either the outer or inner plunger to draw liquid into or expel liquid from the needle tubing.

For the tasks listed in Table 31, the autosampler uses the following:

- The outer plunger if the volume of liquid + air being transported is greater than the nominal syringe size
- The inner plunger if the volume of liquid + air being transported is less than or equal to the nominal syringe size

Table 31. Tasks that can use either syringe plunger

Task
Draw from Reservoir
Deposit Liquid in Sample
Deposit Sample in Reservoir
Transfer from Reservoir to Sample
Transfer from Reservoir to Reservoir

Table 32 lists the speed range for the sample preparation tasks that can use either plunger of the concentric syringe.

Note The tooltips for the speed parameters list a range of allowable speeds. The minimum speed listed is valid for only the inner plunger, and the maximum speed listed is valid for only the outer plunger of the configured syringe.

If you enter a volume that triggers the use of the outer plunger and a speed that is valid for only the inner plunger, an error message appears when you attempt to add the task to the sample preparation list.

Table 32. Syringe speed ranges for tasks that can use either syringe plunger

Syringe Size (µL)	Volume (liquid + air)	Minimum Speed (µL/s)	Maximum Speed (µL/s)	Default Speed (µL/s)
100	<=100	0.33	13.25	3.0
100	>100	1.65	661.38	250
250	<=250	0.83	33.09	8.0
250	>250	1.65	661.38	250
500	<=500	1.65	66.10	8.0
500	>500	1.65	661.38	250
2500 (standard)	0 to 1500	0.83	331	25.0

Syringe Speed for Tasks that Use the Inner Plunger

The autosampler uses the inner plunger of the concentric syringe to perform the following tasks:

- Draw From Sample
- Transfer From Sample To Sample
- Transfer From Sample To Reservoir

The default syringe speed and the available syringe speed range is determined by the size of the syringe (see Table 33).

Use a syringe speed lower than the default for viscous samples. Also use a syringe speed lower than the default for samples of very low viscosity or surface tension to prevent the sample bolus from breaking apart during the transport process.

Note You select the size of the syringe by using the Syringe Type list box in the Communication page of the Surveyor AS Configuration dialog box.

Table 33. Syringe speeds for tasks that use the inner plunger of the concentric syringe

Syringe Size (µL)	Minimum Speed (µL/s)	Maximum Speed (µL/s)	Default Speed (µL/s)
100	0.33	13.25	3
250	0.33	33.10	8
500	1.65	66.11	8

Sample Preparation Routine Example

The following procedure describes how to create a sample preparation routine to dilute samples tenfold.

❖ To add a sample preparation routine that dilutes samples tenfold

1. From the Surveyor AS view, click the **Sample Preparation** tab.

   The Sample Preparation page appears.

2. Open the .meth file that you want to modify.

   In step 3 through step 6, you add the sample preparation tasks to the task list.

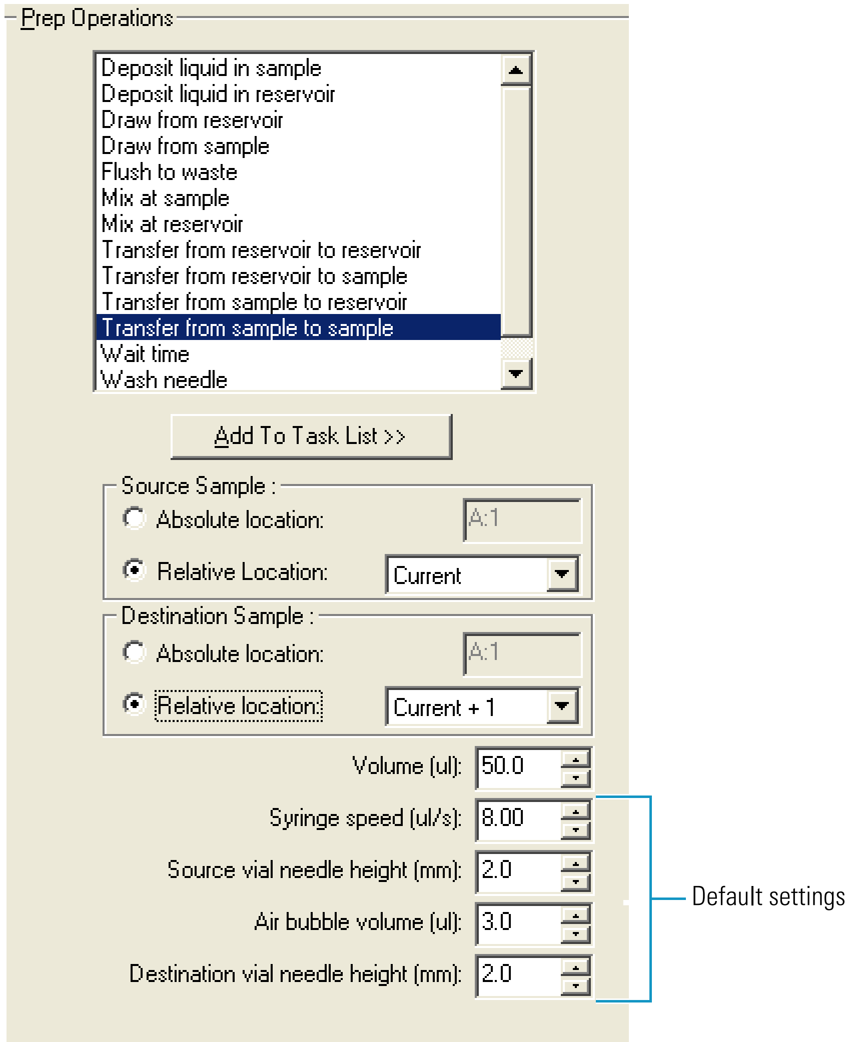
3. Add a task that transfers an aliquot of sample solution from the original sample location to another sample location:

   a. In the Prep Operations list, select **Transfer from sample to sample**. The task parameters appear below the Add To Task List button.

   b. Keep all the default parameter settings for the Transfer from sample to sample task, except those that are shown below (see Figure 62).

Parameter	Setting	Result
Source Sample		
Location	⦿ Relative Location	Specifies that sample is to be withdrawn from the current vial location listed in the sequence table.
Location	Current	
Destination Sample		
Location	⦿ Relative Location	Specifies that sample is to be deposited in the current + 1 vial location listed in the sequence table.
Location	Current + 1	
Volume	50	Specifies that 50 µL of sample is to be transferred.

Figure 62. Settings for the transfer from sample to sample task



c. Click **Add To Task List** to add the task to the sample preparation routine. When the autosampler performs this task, it withdraws 50 µL of sample from the current vial location in the sequence table and deposits it in the current + 1 vial location.

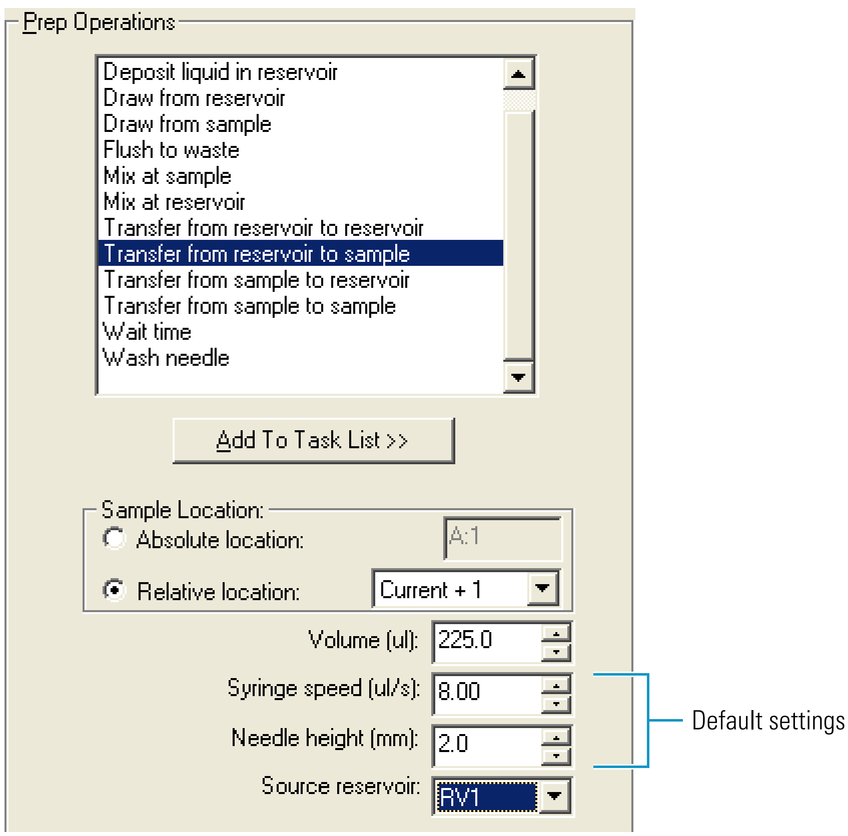
4. Add a task that transfers diluent from a reservoir vial to the sample location:

   a. In the Prep Operations list, select **Transfer from reservoir to sample.** The parameters for the task appear below the Add To Task List button.

   b. Keep all the default parameter settings for the Transfer from reservoir to sample task, except those that are shown below (see Figure 63).

Parameter	Setting	Result
Source reservoir	RV1	Draws solvent from reservoir vial 1
Destination Sample		
Location	◉ Relative Location	Specifies that solvent is to be deposited in the current + 1 vial location listed in the sequence table.
Location	Current + 1	
Volume	225	Specifies that 225 µL of solvent is to be transferred.

Figure 63. Settings for the Transfer from reservoir to sample task



c. Click **Add To Task List** twice to add this task to the sample preparation routine twice.

When the autosampler performs this task, it withdraws 225 µL of diluent from the reservoir vial 1 and deposits the diluent in the current + 1 vial location. This task is performed twice to transfer a total of 450 µL of diluent to the current + 1 vial location.

Two aliquots of 225 µL each are transferred, rather than one aliquot of 450 µL, because fluid transfers performed by the inner plunger of the concentric syringe are more precise than those performed with the outer plunger.

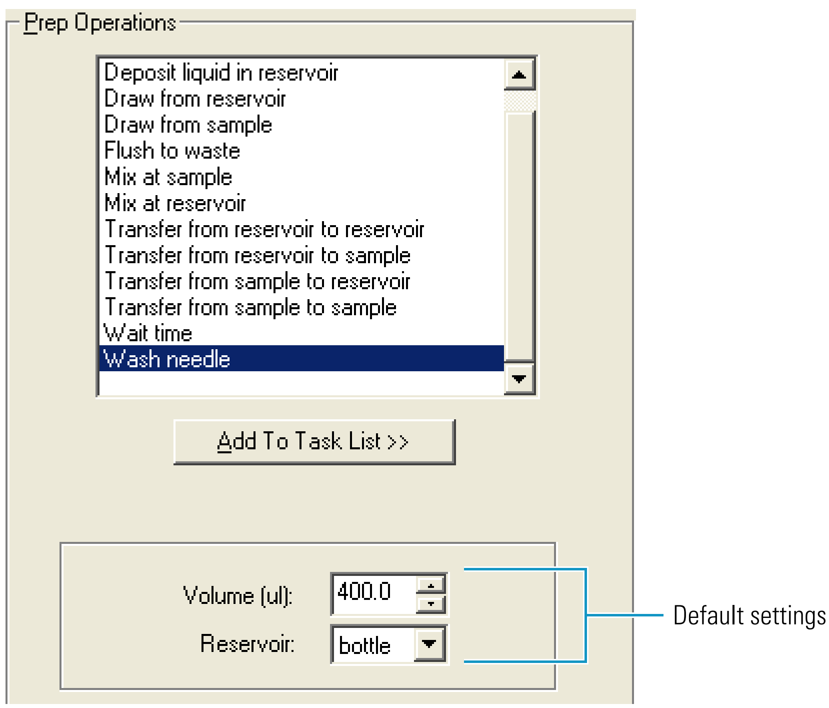
5. Add a task that homes the position of the concentric syringe plungers:

Note The syringe cannot switch between bores without first homing the position of the two syringe plungers. The Transfer from reservoir to sample task that you added in step 4 uses the inner syringe bore. The Mix at sample task that you add in step 6 uses the outer syringe bore. The Wash needle task that you add in this step homes the position of the two syringe plungers.

   a. In the Prep Operations list, select **Wash needle**. The task parameters appear below the Add To Task List button.

   b. Keep all the default parameter settings for the Wash needle task (see Figure 64).

Figure 64. Default parameter settings for the Wash needle task



   c. Click **Add To Task List** to add this task to the sample preparation routine.

6. Add a task that mixes the solution in the new sample location:

   a. In the Prep Operations list, select **Mix at sample**. The task parameters appear below the Add To Task List button.

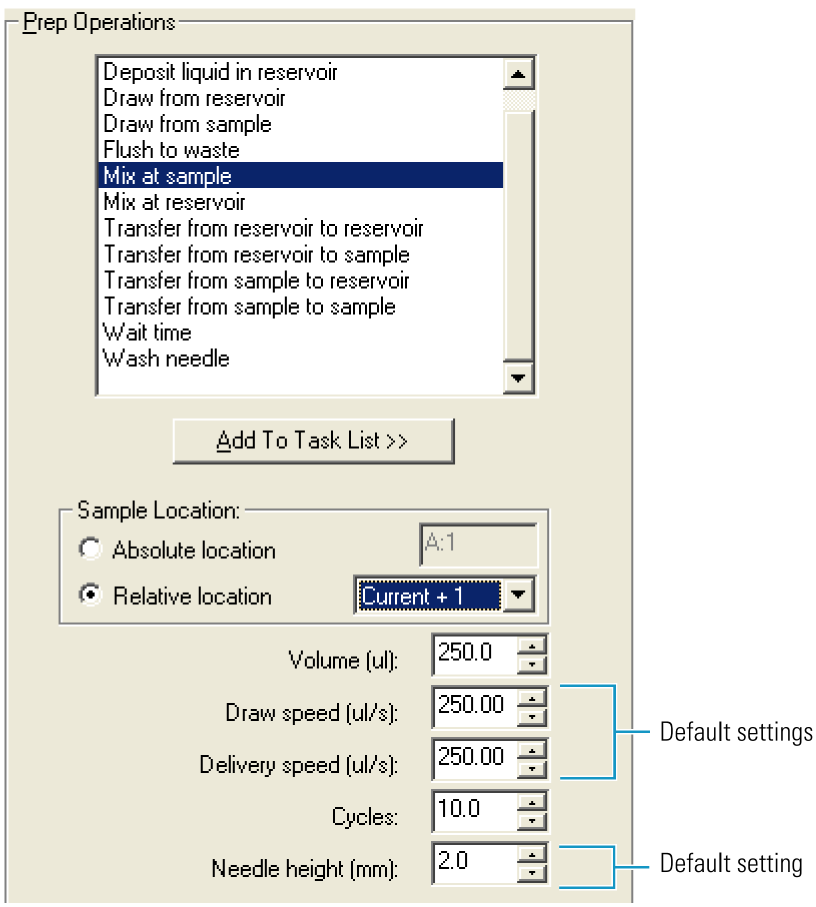
   b. Keep all the default parameter settings for the Mix at sample task, except those listed below (see Figure 65).

Parameter	Setting	Result
Sample Location		
Location	◉ Relative Location	Specifies that sample is to be aspirated and expunged from the current + 1 vial location listed in the sequence table.
Location	Current + 1	
Volume	250	Specifies that 250 µL of sample is to be aspirated and expunged.
Cycles	10	Specifies that the sample is to be aspirated and expunged 10 times.

c. Click **Add To Task List** to add this task to the sample preparation routine.

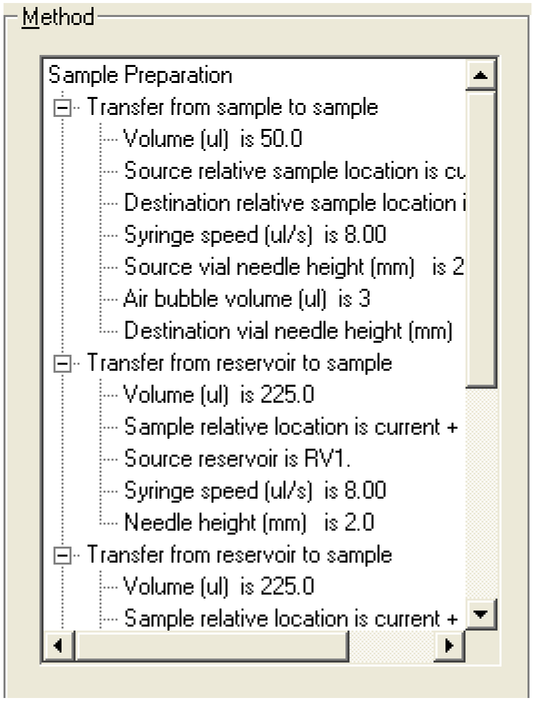
When the autosampler performs this task, it aspirates and expunges 250 µL of the sample solution in the current + 1 vial location 10 times.

Figure 65. Settings for the Mix at sample task



7. Review the task list by expanding the tasks in the Method list (see Figure 66).

Figure 66. Method area with an expanded task list



8. Save the instrument method (see “Saving the Instrument Method” on page 104).

   To perform this sample routine, you must insert empty vials into your sample tray. For example, to dilute five samples, place the samples in vial locations A1, A3, A5, A7, and A9. Place empty vials in vial locations A2, A4, A6, A8, and A10. Fill reservoir vial 1 with an appropriate diluent that matches the sample matrix.

   If you do not want to inject the diluted samples, create a five-line sequence that lists the vial locations of the original samples (A1, A3, A5, A7, and A9). The original samples are not injected.

   If you want to inject the diluted samples, create a ten-line sequence that lists all the vial locations (A1 to A10). Ensure that you use the method that contains the sample preparation routine on only the odd rows.

5

Daily Operation

During the initial installation of the Surveyor Plus LC system, a Thermo Fisher Scientific field service engineer sets up the solvent lines, primes the pulse dampener of the Surveyor MS Pump Plus, and calibrates the PDA detector.

Note The pulse dampener of the Surveyor LC Pump Plus does not require priming.

To prepare the system for daily operation, check the status of the devices, warm up the detector's lamps, and remove air from the solvent lines.

To maintain optimal performance of the system, calibrate the PDA detector on a monthly basis or whenever you move the detector, and check the light throughput to the diode array whenever you move the detector, observe an increase in detector noise, or replace the flowcell or lamps (see "Verifying the Performance of the PDA Detector" on page 244).

Prime the pulse dampener of the Surveyor MS Pump Plus on a monthly basis or when you notice an increase in pump pulsation.

Contents

Turning On the Power to Each LC Device

❖ To turn on the power to the LC devices

1. Depress the power switch on each device.
2. Observe the status LEDs for each device.

   Shortly after you turn on the power, all the LEDs except the Comm LEDs turn green. In addition, the autosampler syringe goes through its initialization process.

3. Verify that the Power LEDs, the Lamp LED, and the Degas LED turn green:

   - If any of the Power LEDs remain amber, make sure that the power line to the affected device or devices is connected.
   - If the Degas LED on the pump is flashing amber, the degas unit has failed to produce a vacuum, and you must call your local Thermo Fisher Scientific representative for repairs.
   - If the Lamp LED remains amber, both of the lamps are turned off. If the lamps are off, turn them on when you check the detector's status from the data system.

Initiating Communication with the Data System

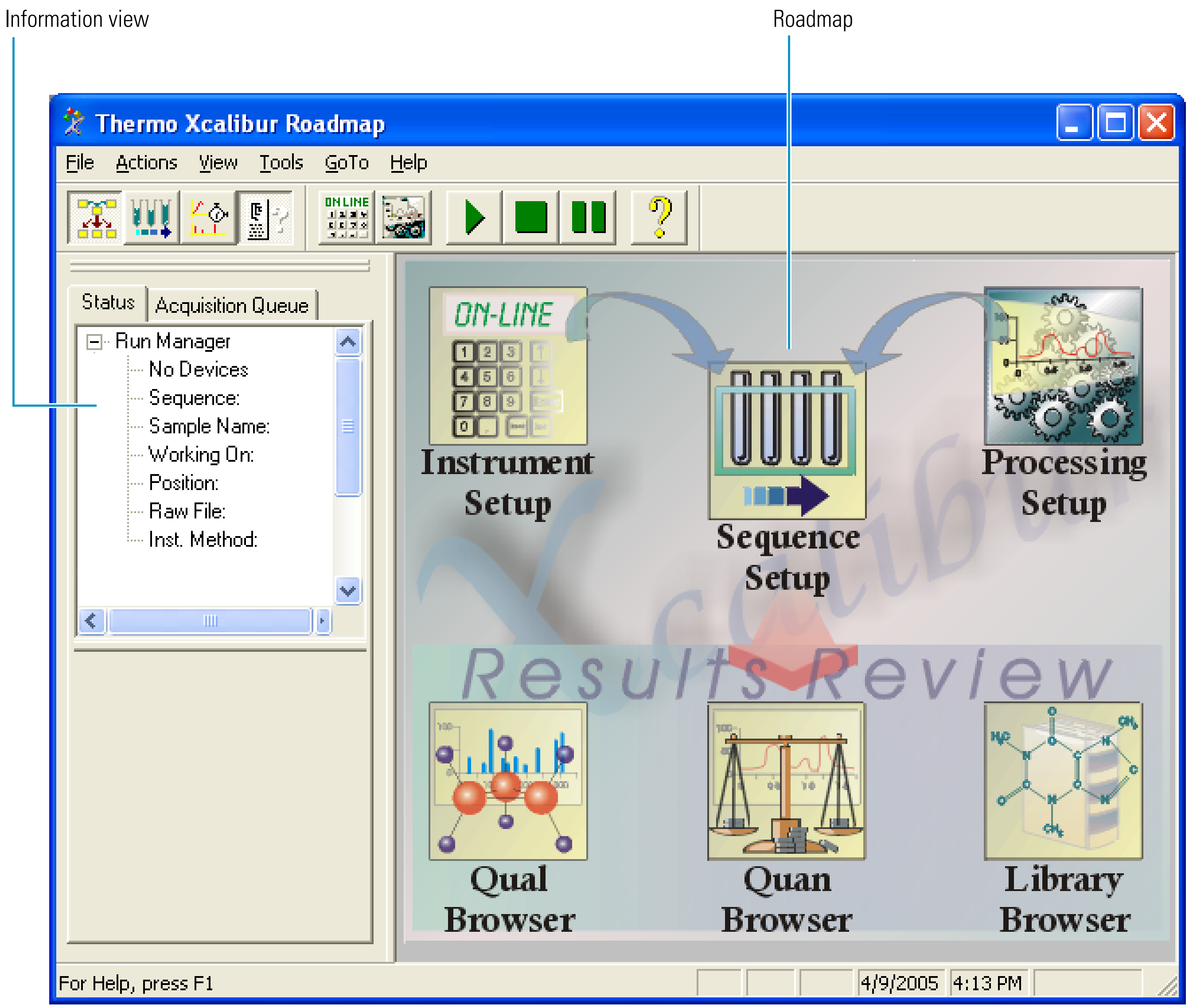
You control the LC devices from the Xcalibur data system. The only manual control for the autosampler and UV/Vis detector are their ON/OFF power switches. The PDA detector has two additional manual controls: the filter wheel and the attenuators. The filter wheel controls the position of the holmium oxide calibration solution. The attenuators control the light throughput to the diode array. The pump has a purge valve.

❖ To open the data system and verify instrument communications

1. From your computer desktop, choose **Start** > **Programs** > **Thermo Xcalibur** > **Xcalibur**.

   The Thermo Xcalibur Roadmap window appears (see Figure 67).

Figure 67. Thermo Xcalibur Homepage window with the Roadmap and Info view displayed



Shortly after you open the data system, the Comm LEDs on the front panels of your LC devices turn green if the instruments are powered on and connected to the data system computer.

2. If the Comm LEDs remain amber, do the following:

   - Verify that the Ethernet communication cable for the Surveyor PDA Plus Detector or Surveyor UV/Vis Plus Detector, Surveyor Autosampler Plus, and Surveyor LC Pump Plus are connected to the Ethernet switch and that the Ethernet switch is connected to the data system computer. Verify that RS-232 cable for the Surveyor MS Pump Plus is connected to the data system computer.

   - Verify that the stack addresses in the configuration for the Surveyor Autosampler Plus, Surveyor PDA Plus Detector or Surveyor UV/Vis Plus Detector, and Surveyor LC Pump Plus

     For information on configuring your instrument devices, see Chapter 2, "Thermo Foundation Instrument Configuration."

Checking the Status of the LC Devices

After you turn on the power to the LC devices and open the Xcalibur data system, check the status of each device.

To check the status of the LC devices, follow these procedures:

1. Opening the Status Page of the Information View
2. Viewing the Status of Each Device

Opening the Status Page of the Information View

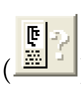
You can monitor the status of the LC devices from the Information View. This view is normally displayed on the left side of the Home Page window. If this view is not displayed, the view has been closed. From the Home Page window, choose **View > Info View** to open the Information view.

❖ To display the Status page of the Information View

1. In the Home Page window, open the Information View by doing one of the following:

   - Choose **View > Info View**.

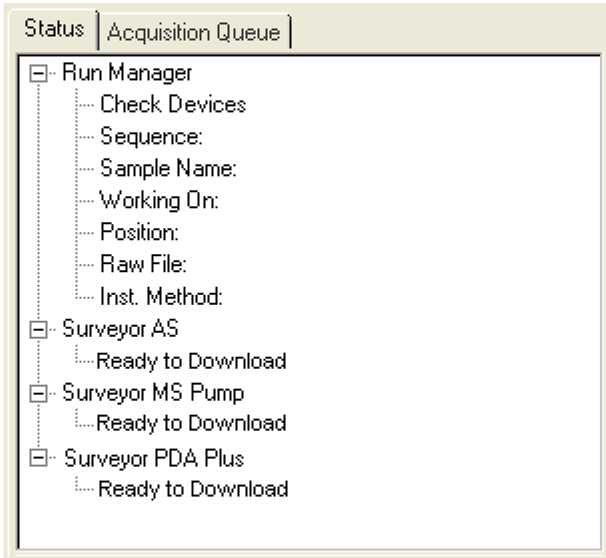
     –or–

   - Click the Information view icon () in the toolbar.

2. Click the **Status** tab.

   The Status page appears (see Figure 68).

Figure 68. Status page of the Information View



If you have just recycled the power and have not yet downloaded a method, you see the following status readouts on the Status page:

- *Initializing* is displayed while the data system attempts to connect to an instrument module.
- *Lamp Warm-up* is displayed for the detector while the deuterium lamp is igniting.
- *Ready to download* is displayed after the data system establishes communication with an instrument device and after each run has ended.

Viewing the Status of Each Device

The Surveyor Plus LC system contains an autosampler and an analytical pump. In addition, your system might contain an optional detector.

Check the status of each device by clicking its name in the device tree list. The status information for a specific device is displayed in the lower portion of the Status page.

These topics describe the status views for the LC devices:

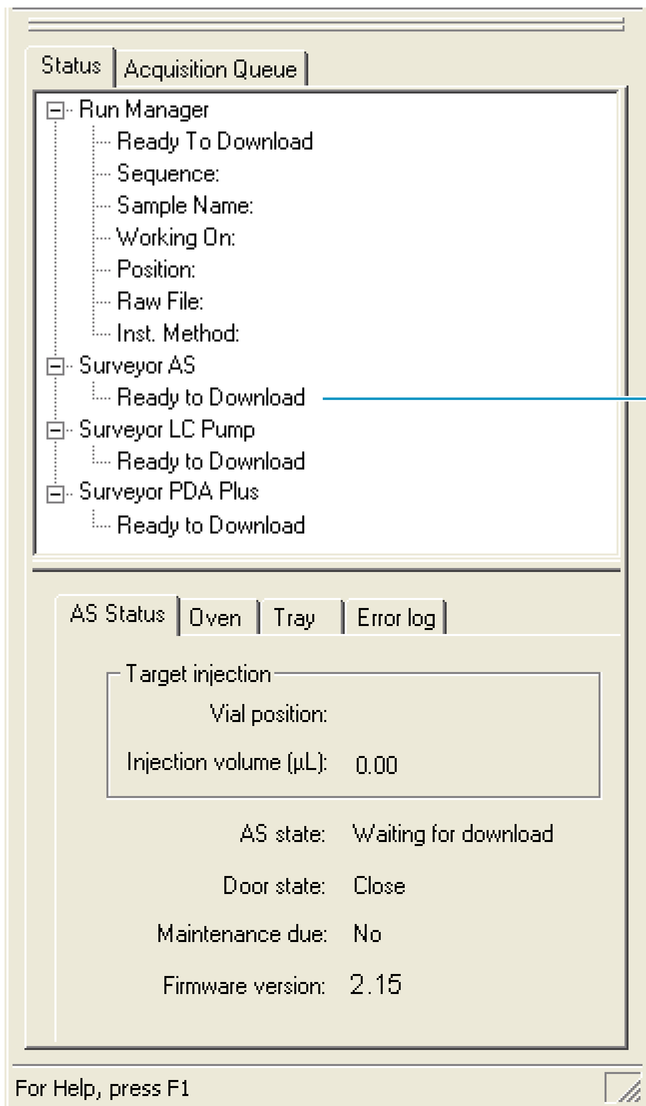
- "Autosampler Status View" on page 132
- "Surveyor MS Pump Plus" on page 138
- "Surveyor LC Pump Plus" on page 143
- "Surveyor PDA Plus Detector Status View" on page 145
- "Surveyor UV/Vis Plus Detector Status View" on page 146

Autosampler Status View

The Status view for the autosampler (see Figure 69) contains four pages:

- "AS Status Page" on page 133
- "Oven Page" on page 135
- "Tray Page" on page 137
- "Error Log (Autosampler) Page" on page 138

Figure 69. Status page showing the AS Status page for the Surveyor Autosampler



See Table 34.

Table 34 describes the states that might appear below the Surveyor AS listing.

Table 34. Autosampler states

State	Meaning
Initializing	The data system is attempting to establish communication with the autosampler.
Ready to download	The autosampler is ready to start a run.
Ready to run	This state appears momentarily when the data system is downloading an instrument method for an LC/MS system.
Running	The autosampler is making an injection. If the instrument method contains a time function for the autosampler, this state is displayed until the time function has expired.
Direct control	The direct control or calibration windows are open.
Busy	The autosampler is performing a direct control operation.
Error	An error condition other than the loss of communication has occurred.
Off	The data system has attempted to connect to the autosampler and failed five times.

AS Status Page

Table 35 describes the readbacks on the AS Status page of the autosampler status view

Table 35. Status readbacks for the autosampler (Sheet 1 of 3)

Readback	Description
Target injection	
Vial position	Displays the current vial or microplate well position. For information on the vial and well notation, see “Vial and Well Notation” on page 6.
Injection volume	Displays the injection volume for the current injection.

Table 35. Status readbacks for the autosampler (Sheet 2 of 3)

Readback	Description	
Other		
AS state	Displays the following run conditions:	
	Waiting for download	Waiting for the data system to download an instrument method.
	Waiting for door close	The Verify door is closed check box is selected (see “Communication Page” on page 45) and the tray compartment door is open.
	Waiting for temperature	The Wait for temperature ready check box is selected (see “Communication Page” on page 45) and the tray compartment temperature, column oven temperature, or both have not yet reached the set temperature. This state remains until the temperature controlled areas are equilibrated to the set temperature.
	Injecting	The autosampler is making an injection.
	Waiting for pump	The autosampler has loaded the sample loop and is waiting for the Inject Hold Release signal from the pump.
	Ready	The autosampler is ready to make an injection.
	Error	An error condition other than the loss of communication has occurred. For example, if you enable the maintenance log and you attempt to start a run when one or more of the maintenance counters has exceeded its limit, an error condition occurs.
Door state	Displays one of the following:	
	Open	The autosampler is configured to verify whether the tray compartment door is open or closed and the tray compartment door is open. For information on selecting the Verify door is closed check box, see “Communication Page” on page 45.
	Close	The tray compartment door is closed, the Verify door is closed check box is not selected, or both.

Table 35. Status readbacks for the autosampler (Sheet 3 of 3)

Readback	Description	
Maintenance due	Displays Yes or No.	
	No	The Enable Maintenance Log check box is clear, none of the maintenance counters has exceeded its limit, or both.
	Yes	The Enable Maintenance Log check box is selected and one or more of the maintenance counters has exceeded its limit. The autosampler cannot start a run until you zero the counter or clear the Enable Maintenance Log check box. For information on setting up the maintenance counters, see "Autosampler Maintenance Information" on page 310. For information on clearing the Enable Maintenance Log check box, see "Communication Page" on page 45.
Firmware version	Displays the firmware version of the autosampler.	

Oven Page

Figure 70 shows the Oven page of the status view for the autosampler.

Figure 70. Oven page

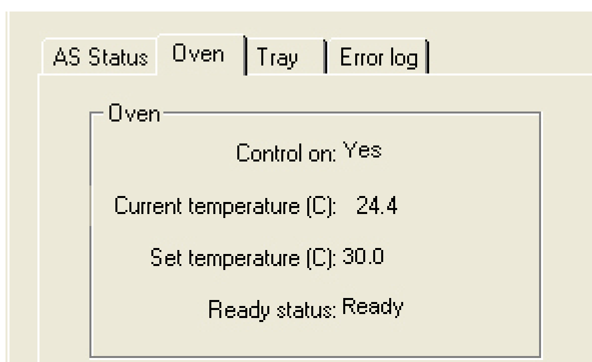


Table 36 describes the readbacks on the Oven page of the autosampler status view

Table 36. Oven page readbacks

<table>
<tr><th>Readback</th><th colspan="2">Description</th></tr>
<tr><td>Control on</td><td colspan="2">Display Yes or No.

You can turn off the oven temperature control by doing one of the following:

• Using the Turn off oven temperature command (see “Controlling the Tray and Oven Compartment Temperatures” on page 193).

• Running an instrument method that has the Enable column oven control check box cleared.</td></tr>
<tr><td>Current temperature (C)</td><td colspan="2">Displays the temperature monitored by the autosampler’s internal temperature sensor.</td></tr>
<tr><td>Set temperature (C)</td><td colspan="2">Displays the user-specified temperature.

You can change the set temperature by doing one of the following:

• Using the Set oven temperature command (see “Controlling the Tray and Oven Compartment Temperatures” on page 193).

• Running an instrument method that has a temperature setting for the column oven.</td></tr>
<tr><td rowspan="3">Ready status</td><td colspan="2">Displays one of the following:</td></tr>
<tr><td>Ready</td><td>The oven compartment temperature has reached the set temperature, you did not select the Wait for temperature ready check box when you specified the configuration settings for the autosampler, or both.

For information on the Wait for temperature ready check box, see “Communication Page” on page 45.</td></tr>
<tr><td>Not Ready</td><td>The oven compartment temperature has not reached the set temperature.</td></tr>
</table>

Tray Page

Figure 71 shows the Tray page of the autosampler status view.

Figure 71. Tray page

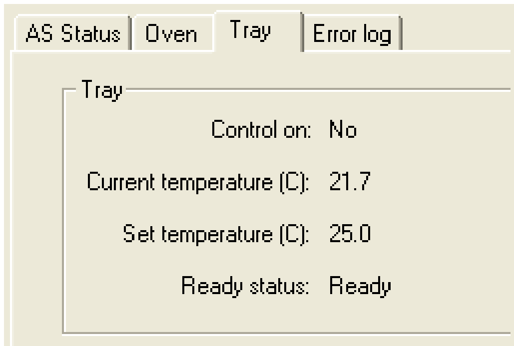


Table 37 describes the readbacks on the Tray page of the autosampler status view

Table 37. Tray page parameters

Readback	Description
Control on	Display Yes or No. You can turn off the tray temperature control by doing one of the following: • Using the Turn off tray temperature command (see "Controlling the Tray and Oven Compartment Temperatures" on page 193). • Running an instrument method that has the Enable column oven control check box cleared.
Current temperature (C)	Displays the temperature monitored by the autosampler's internal temperature sensor.
Set temperature (C)	Displays the user-specified temperature. You can change the set temperature by doing one of the following: • Using the Set tray temperature command (see "Controlling the Tray and Oven Compartment Temperatures" on page 193). • Running an instrument method that has a temperature setting for the column oven.
Ready status	Displays one of the following: **Ready**: The oven compartment temperature has reached the set temperature, you did not select the Wait for temperature ready check box when you specified the configuration settings for the autosampler, or both. For information on the Wait for temperature ready check box, see "Communication Page" on page 45. **Not Ready**: The oven compartment temperature has not reached the set temperature.

Error Log (Autosampler) Page

Figure 72 shows the Error log page for the autosampler.

Figure 72. Error log page

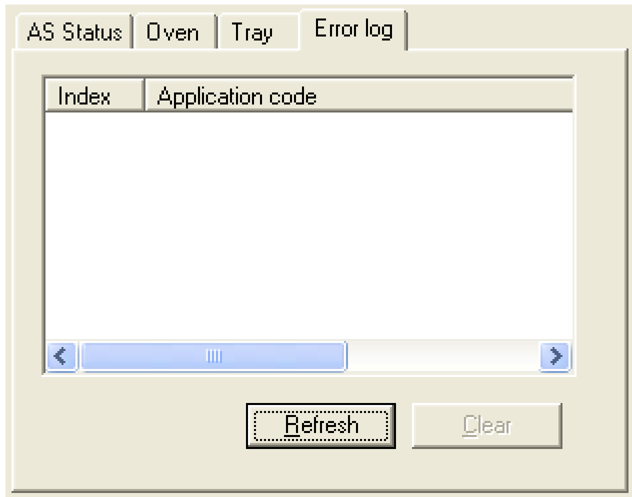


Table 38 describes the parameters on the Error log page.

Table 38. Error log page parameters

Parameter	Description
Index	Lists the order of the error events, in increments of 1. The index number for the first error event that occurs after you clear the log is 1.
Application code	Refer to your autosampler hardware manual for information on the application codes.
Buttons	
Refresh	Loads the error log messages stored by the autosampler.
Clear	Clears the error log.

Surveyor MS Pump Plus

For information on the status of the pump and the degassing unit, see these topics:

- "General Page of the Pump Status View" on page 139
- "Extended Page of the Pump Status View" on page 140
- "Degasser Page of the Pump Status View" on page 141

General Page of the Pump Status View

Figure 73 shows the General page of the pump status view.

Figure 73. General page pump status view

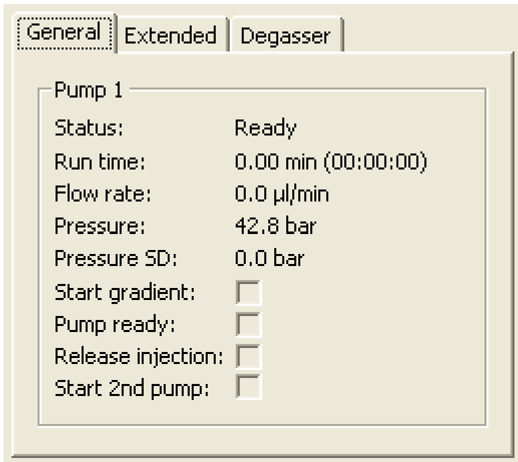


Table 39 describes the status readbacks on the General page of the pump status view.

Table 39. General page of the pump status view (Sheet 1 of 2)

Readback	Description	
Status	Displays the pump status as follows:	
	Ready	The pump is ready for a run.
	Running isocratic	The solvent flow is on, but the pump is not running a gradient program.
	Homing	The pump pistons are moving toward the home position.
	Running gradient	The pump is running the solvent conditions specified in the gradient program of an instrument method. The gradient program can contain isocratic or gradient solvent conditions.
	Low pressure error	The system pressure has fallen below the minimum pressure setting for more than one minute. If you are controlling the pump from the Direct Control dialog box, the status returns to Ready after the pump stops pumping. The error is posted to the pump's event log.
	High pressure error	The system pressure has risen above the maximum pressure setting.

Table 39. General page of the pump status view (Sheet 2 of 2)

Readback	Description
	Tip When a pressure error occurs, the following occur: • If you are controlling the pump from the Direct Control dialog box, the status returns to Ready after the pump stops pumping. The error is posted to the pump's event log. • If you are controlling the pump from the Sequence Setup window, the Acquisition Server dialog box appears.
Run time	Displays the elapsed time since the last instrument method downloaded, you turned the device on from the Status page of the Information view, or you started the pump from the Direct Control dialog box.
Flow rate	Displays the user-specified flow rate. For an instrument method, the flow rate changes linearly between time lines
Pressure	Displays the pressure monitored by the pump.
Pressure SD	Displays the standard deviation of the pressure monitored by the pump.
Start gradient	A check appears in this box when the pump receives the Start gradient signal from the autosampler.
Pump ready	A check appears in this box when the pressure monitored by the pump reaches the stability level specified in the instrument method.
Release injection	A check appears in this box when the pump sends the Release injection signal to the autosampler.
Start 2nd pump	A check appears in this box when Pump 1 triggers Pump 2 to start its pump program.

Extended Page of the Pump Status View

Figure 74 shows the Extended page of the pump status view.

Figure 74. Extended page of the pump status view

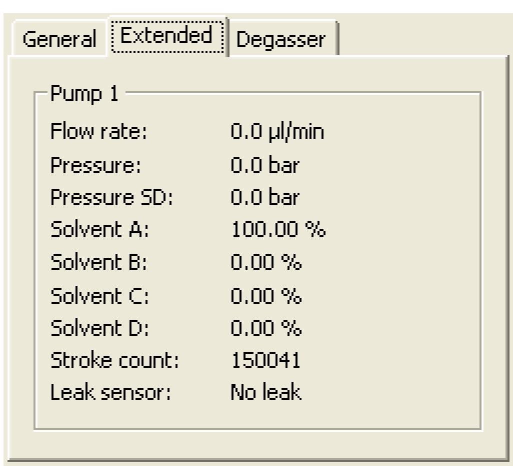


Table 40 describes the status readbacks on the Extended page of the pump status view.

Table 40. General page of the pump status view

Readback	Description
Flow rate	Displays the user-specified flow rate. Flow rate changes are linear between time lines in the gradient program. **Tip** You can change the percent composition of solvents A, B, C, and D by running an instrument method or by downloading new solvent conditions from the (Inlet) Direct Control dialog box.
Pressure	Displays the pressure monitored by the pump.
Pressure SD	Displays the standard deviation of the pressure monitored by the pump.
Solvent A	Displays the user-specified percent composition of solvent A.
Solvent B	Displays the user-specified percent composition of solvent B.
Solvent C	Displays the user-specified percent composition of solvent C.
Solvent D	Displays the user-specified percent composition of solvent D.
Stroke count	Displays the stoke count for the pump pistons. When the stoke count exceeds 1 000 000, the number is displayed in red.

Degasser Page of the Pump Status View

Figure 75 shows the Degasser page of the pump status view.

Figure 75. Degasser page of the pump status view

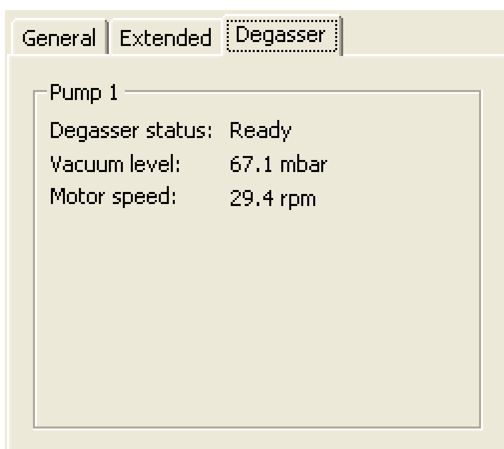


Table 41 describes the status readbacks on the Degasser page of the pump status view.

Table 41. General page of the pump status view

Readback	Description	
Degasser status	The states of the degasser are as follows:	
	Ready	The vacuum level has reached a sufficient level to degas the mobile phase solvents.
	Not connected	The pump is not communicating with the data system computer.
	Error	An error condition has occurred.
Vacuum level	Displays the vacuum level of the solvent degassing chambers.	
Motor speed	Displays the motor speed of the vacuum degasser.	

Surveyor LC Pump Plus

Figure 76 shows the Status view for the Surveyor LC Pump Plus. You can view the system pressure from this view.

Figure 76. Surveyor LC Pump Plus Status view

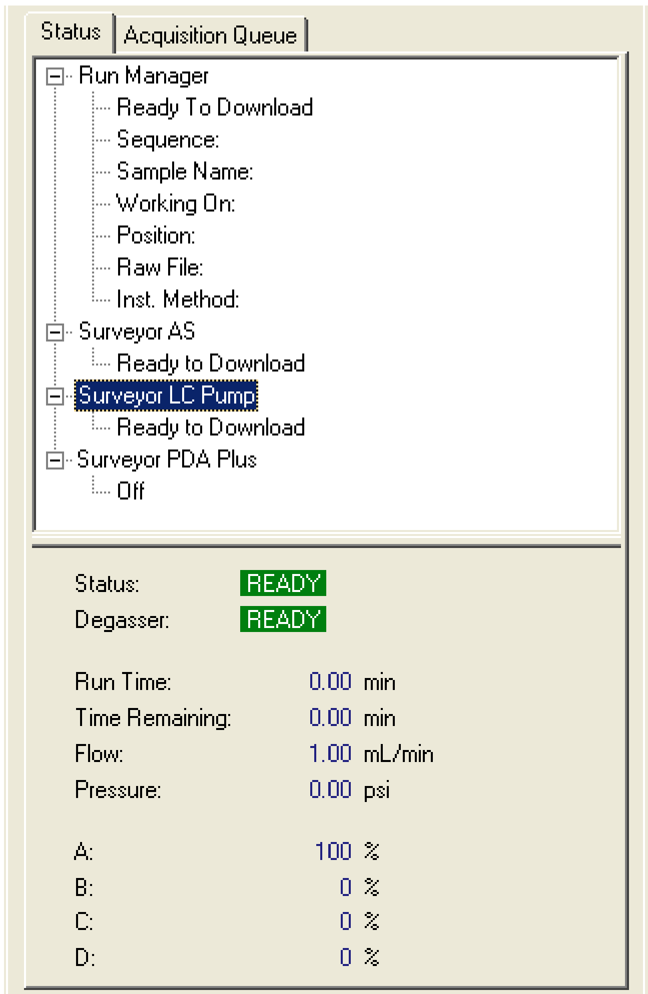


Table 42 describes the readbacks on the pump status view.

Table 42. Surveyor LC Pump Plus status readbacks

Readback	Description	
Status	Displays the following pump states:	
	READY	The pump is ready to take a command.
	Not Connected	The pump is not communicating with the data system computer.
	Off	The pump pistons are stopped.
	Running Isocratic	The pump flow is on.
	Running Gradient	The pump is running a gradient program.
Degasser	Displays the following degasser states: READY or Not Connected.	
Run time	Displays the run time.	
Time remaining	Displays the remaining time for the run.	
Flow	Displays the user-specified flow rate.	
Pressure	Displays the pressure monitored by the pump.	
A	Displays the user-specified percent composition for solvent A.	
B	Displays the user-specified percent composition for solvent B.	
C	Displays the user-specified percent composition for solvent C.	
D	Displays the user-specified percent composition for solvent D.	

Surveyor PDA Plus Detector Status View

Figure 77 shows the Status view for the Surveyor PDA Plus Detector. You can view the status of the lamps and the wavelength and dark current calibrations from this view.

Figure 77. Surveyor PDA Plus Detector Status view

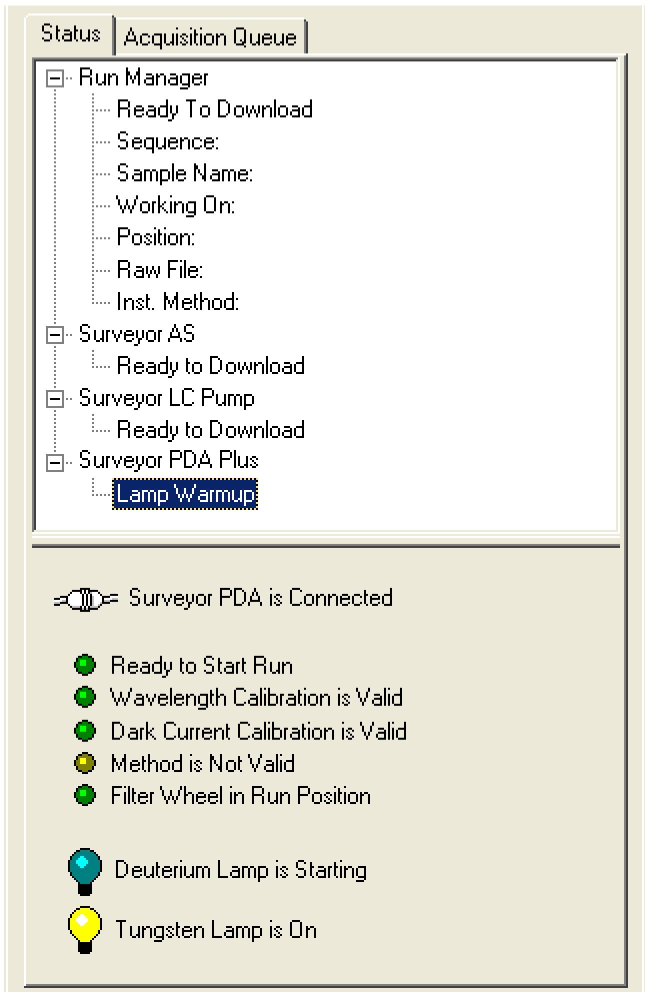


Table 43 describes the readbacks for the PDA detector.

Surveyor UV/Vis Plus Detector Status View

Table 43. PDA detector status readbacks

Readback	Description	
Connection status	Displays whether the PDA detector is communicating with the data system computer. The connection states are Connected or Not connected.	
Ready or Not Ready to Start Run	Ready to Start Run	The detector is ready to start a run or is currently acquiring data.
	Not Ready to Start Run	The deuterium lamp is warming up.
Wavelength Calibration	The wavelength calibration is valid or invalid	
Dark Current Calibration	The dark current calibration is valid or invalid.	
Method is valid	N/A	
Filter wheel position	The filter wheel has two positions:	
	Position 1 (Run)	Light passes through the flowcell onto the diode array.
	Position 2 (Closed)	Light passes through the holmium oxide filter onto the diode array.
Deuterium lamp	The deuterium lamp states are as follows:	
		The deuterium lamp is off.
		The deuterium lamp is starting up, which requires about 1.5 min.
		The deuterium lamp is on.
Tungsten lamp	The tungsten lamp states are as follows:	
		The tungsten lamp is On.
		The tungsten lamp is Off.

Figure 78 shows the Status view for the Surveyor UV/Vis Plus Detector. You can view the absorbance level of the chromatographic baseline from this view.

Figure 78. Surveyor UV/Vis Plus Detector Status view

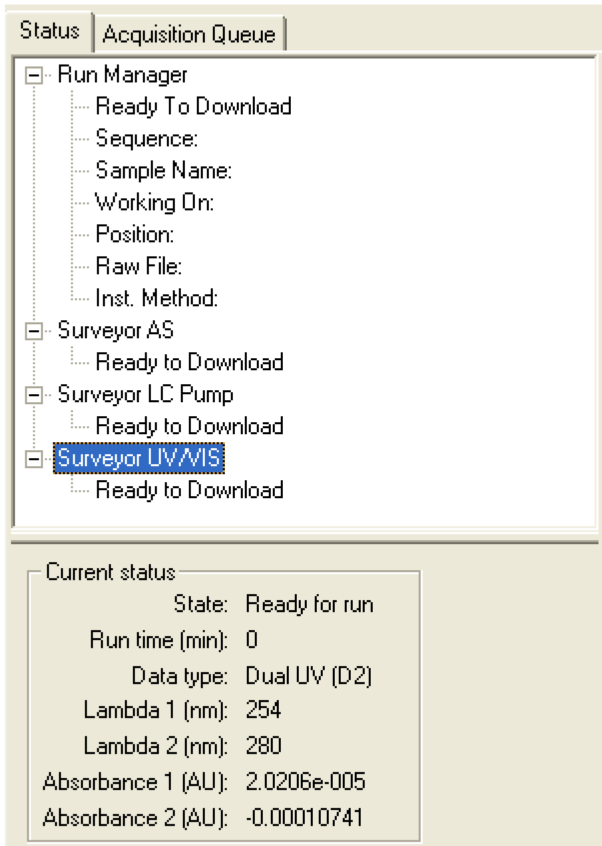


Table 44 describes the status readbacks for the Surveyor UV/Vis Plus Detector.

Table 44. UV/Vis detector status readbacks

Readback	Description
State	Displays the following states: Ready for run and Off.
Run time	Displays the elapsed run time.
Data type	Displays the program type specified in the current instrument method: Dual UV (D2), Dual Visible (W), or Single Wavelength.
Lambda 1 (nm)	Displays the current wavelength setting for the Wavelength 1 channel.
Lambda 2 (nm)	Displays the current wavelength setting for the Wavelength 2 channel.
Absorbance 1 (AU)	Displays the current absorbance level for the Wavelength l channel.
Absorbance 1 (AU)	Displays the current absorbance level for the Wavelength 2 channel.

Turning Devices On, Off, or into Standby from the Information View

You can turn on certain activities for a device from its device listing in the Information view. To use this feature, the device must be powered on and communicating with the data system.

❖ To turn on a device from the Info view

Right-click the device listing on the Status page and choose **Turn Device On**.

Depending on the device, the following actions occur:

- When you turn on the pump, it begins pumping solvents from the last set of downloaded parameters.
- When you turn on the detector, its lamps turn on.
- When you turn on the autosampler, it adjusts its controlled temperature zones to the last set of downloaded parameters.

❖ To place a device in Standby

Right-click the device listing on the Status page and choose **Turn Device into Standby**.

Depending on the device, the following actions occur:

- When you place the pump in Standby, it stops pumping.
- When you place the detector in Standby, nothing happens.
- When you place the autosampler in Standby, nothing happens.

❖ To turn a device off

Right-click the device listing on the Status page and choose **Turn Device Off.**

Depending on the device, the following actions occur:

- When you turn off the pump, it stops pumping.
- When turn off the detector, the lamps turn off.
- When turn off the autosampler, nothing happens.

Filling the Solvent Reservoir Lines with Fresh Solvent

After filling the solvent bottles, connecting the solvent lines to the degasser, and configuring the pump to communicate with the data system, prepare the pump for operation by removing the air from the solvent lines that connect the solvent reservoir bottles to the pump's degassing unit.

For information on using the direct controls to start the solvent flow from the Surveyor MS Pump Plus, see "Pump Direct Controls" on page 179.

❖ To quickly draw fresh solvent into the solvent reservoir lines

1. For any solvent line that you want to pull solvent through, disconnect the tubing that is connected to the corresponding degasser outlet port.
2. Connect a Luer-Lok fitting to the degasser outlet port.
3. Connect a 10 mL syringe to the Luer-Lok fitting, and pull solvent through the line.
4. Remove the Luer-Lok fitting from the outlet port.
5. Reconnect the solvent line to the degasser outlet port.

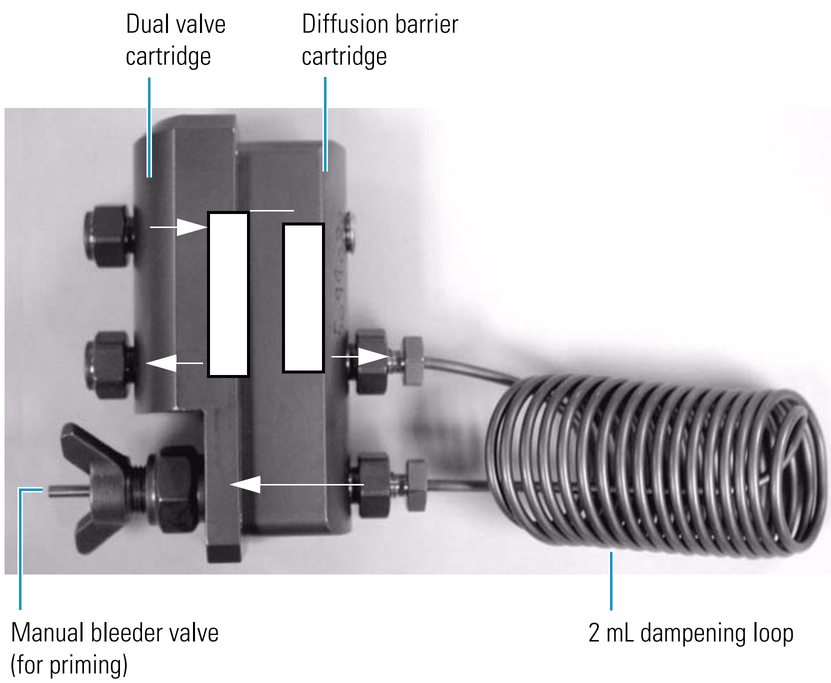
Priming the Pulse Dampener of the Surveyor MS Pump Plus

Before you can operate the Surveyor MS Pump Plus, you must prime its built-in pulse dampener. Priming the pulse dampener involves filling the dampening coil (see Figure 79) with a solvent, such as methanol or isopropanol. After the coil is filled with solvent, it effectively dampens pressure pulsations from the rest of your system.

Closing the pulse dampener flush valve after you fill the coil shuts the dampening coil off from the mobile phase stream. Because a permeable membrane separates the mobile phase stream from the dampening coil, the solvent in the coil slowly diffuses into the mobile phase stream. When the pump is in continuous or frequent use, prime the pulse dampener on a monthly basis.

Note Do not fill the pulse dampener with an aggressive acid or a buffered solution. The recommended filling solvents are methanol, acetonitrile, or isopropyl alcohol. If you accidentally fill the pulse dampener with an acidic or buffered solvent, flush the loop with a miscible solvent, and then refill it with methanol, acetonitrile, or isopropyl alcohol.

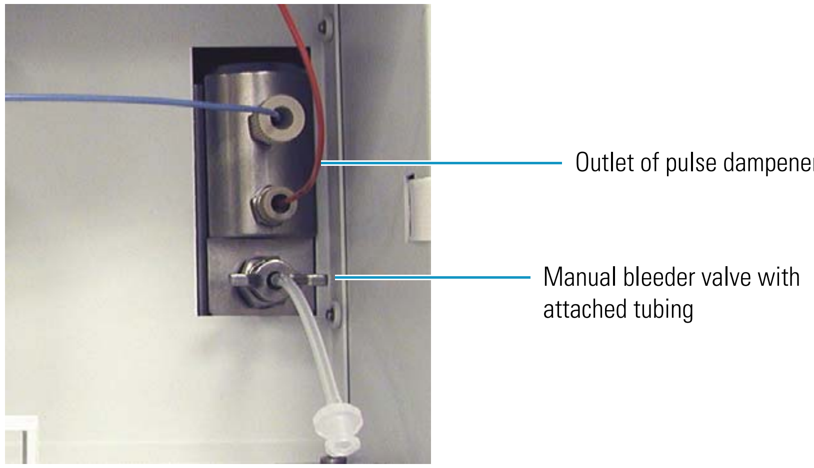
Figure 79. Pulse dampening assembly of the Surveyor MS Pump Plus



❖ To prime the pulse dampener of the Surveyor MS Pump Plus

1. Fill a solvent bottle with particulate-free, HPLC-grade methanol.
2. Connect an HPLC column or a flow restrictor to the LDA outlet.
3. Insert the tip of the 10 mL syringe into the tubing connected to the manual bleeder valve (see Figure 80).

Figure 80. Manual bleeder valve with attached tubing



4. Open the valve by turning it counterclockwise.

5. To open the direct controls for the Surveyor MS Pump Plus, do one of the following:

   - From the Surveyor MS Pump Plus view, choose **Surveyor MS Pump Plus > Direct Control**. The Direct Control dialog box appears.

     Note For information on using the Direct Control dialog box for the Surveyor MS Pump Plus, see "Pump Direct Controls" on page 179.

   –or–

   - From the tune window for your Thermo Scientific mass spectrometer, choose **Setup > Inlet Direct Control**. The Inlet Direct Control dialog box that contains tabbed pages for each configured LC device appears. Click the **Surveyor MS Pump Plus** tab.

     Note For information on using the Inlet Direct Control dialog box, see Chapter 12, "Making a Single Injection from the Tune Window."

6. Start pumping 100% methanol at a flow rate of 1000 µL/min.

7. Fill the loop completely to expel any air that might be trapped in the dampener loop.

8. Set the flow rate to one that is appropriate for your system.

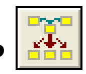
9. Close the manual bleeder valve (see Figure 79 on page 150) by turning the valve clockwise. Always keep the pulse dampener valve closed during normal operation.

Accessing the Direct Controls

You can access the direct controls for each instrument from the Instrument Setup window. You can use the direct controls to start and stop the solvent flow from the LC pump, move the autosampler's XYZ arm, turn the detector's lamps on and off, flush the autosampler's syringe, and so on.

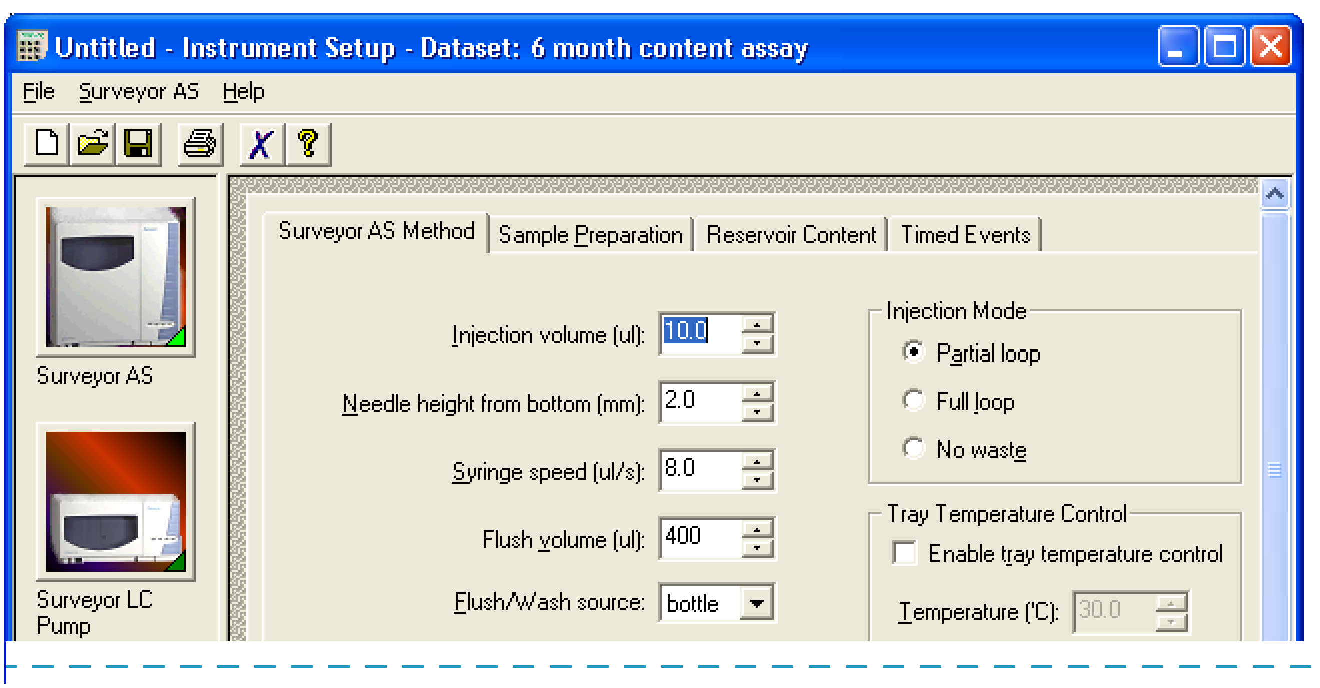
For information on using the direct controls to prepare your LC system for daily operation, see the Chapter 6, "Direct Controls."

❖ To access the direct controls from the Xcalibur data system

1. If it is not already open, open the Roadmap view by clicking the **Roadmap**  button in the toolbar.
2. Click the **Instrument Setup**  button on the toolbar or the larger **Instrument Setup** icon in the Roadmap View.

   The Instrument Setup window opens to the first module displayed in the view bar (see Figure 81). The view bar is a vertical bar on the left side of the Instrument Setup window. It contains a button for each configured device. When you click a device button, a green triangle appears in the lower-right corner of the button, and the device view appears.

Figure 81. Instrument Setup window open to the Surveyor AS Method page of the Surveyor AS view



3. To open the view for the device of interest, click its corresponding button in the view bar.

   The view for the specific device appears.

4. Access the direct controls for each device from the menu bar of the device view as follows:

Device	Menu	Reference
Surveyor LC Pump Plus	Choose > **Surveyor LC Pump** > **Direct Control** > **Operation**.	"Surveyor LC Pump Plus Direct Controls" on page 179
Surveyor MS Pump Plus	Choose **Surveyor MS Pump> Direct Control**.	"Surveyor MS Pump Plus Direct Controls" on page 184
Surveyor PDA Plus Detector	Choose **Surveyor PDA Plus > Direct Control**. Then click the **Configuration** tab.	"PDA Detector Direct Controls" on page 156
Surveyor UV/Vis Plus Detector	Choose **Surveyor UV/Vis > Direct Control**.	"UV/Vis Detector Direct Controls" on page 177
Surveyor Autosampler Plus	Choose **Surveyor AS > Direct Control**.	"Autosampler Direct Controls" on page 186

6

Direct Controls

You can control some device features without downloading an instrument method. For example, you can turn the lamps on or off, set the solvent conditions for the pump, start and stop the solvent flow from the pump, and control the autosampler's temperature zones and its XYZ arm by using the direct controls provided by the data system.

For information on accessing the direct controls for each device, see "Accessing the Direct Controls" on page 152.

Contents

Note For instructions on loading samples into the autosampler, see "Loading the Autosampler" on page 205.

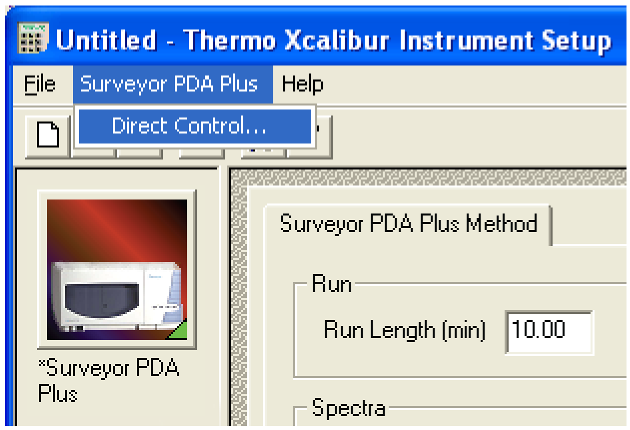
PDA Detector Direct Controls

The Direct Control dialog box for the PDA detector contains the following pages: Display, Configuration, Information, and Calibration.

❖ **To open the Direct Control dialog box for the PDA detector**

1. Open the PDA detector view.
2. From the menu bar, choose **Surveyor PDA Plus > Direct Control** (see Figure 82).

Figure 82. Surveyor PDA Plus menu



The Surveyor PDA Plus Direct Control dialog opens to the Display page (see Figure 83).

Figure 83. Surveyor PDA Plus Control dialog box tabs

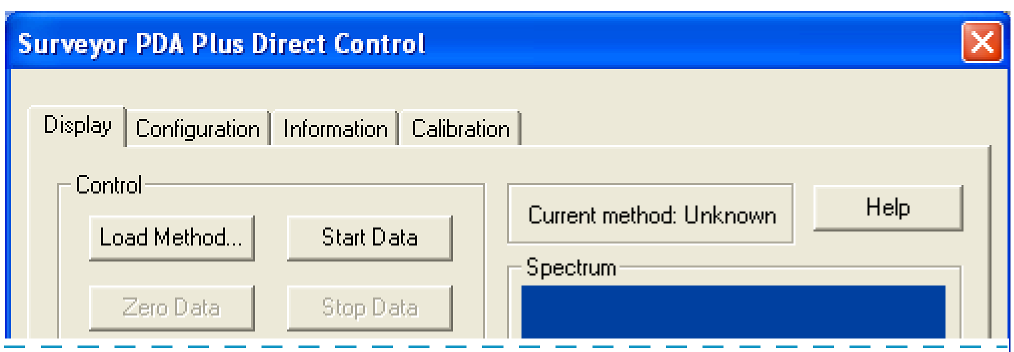


Table 45 lists the direct control procedures by the page where they are performed.

Table 45. Direct control procedures

Page	Procedure
Display Page	"Checking the Chromatographic Baseline" on page 162
	"Verifying the Performance of the PDA Detector" on page 244
Configuration Page	"Turning the Lamps On or Off" on page 157
	"Resetting the Lamp Usage Hours" on page 158
	"Changing the Sense of the Analog Outputs" on page 158
	"Testing the Analog Outputs" on page 159
	"Setting the Lamp Startup Time" on page 160
Information Page	"Viewing, Exporting, and Clearing the Error Log for the PDA Detector" on page 165
	"Checking the Firmware Version of the PDA Detector" on page 165

For information on using the Calibration page to determine the wavelength accuracy of the detector and the dark current produced by the diode array, see "Performing a Wavelength Calibration" on page 261 and "Performing a Dark Current Calibration" on page 256.

Turning the Lamps On or Off

Use the Configuration page of the Surveyor PDA Plus Direct Control dialog box to turn each lamp on or off.

❖ **To turn the lamps on or off**

1. Open the Configuration page of the Surveyor PDA Plus Direct Control dialog box (see "Configuration Page" on page 165).
2. Click **Turn On** for the associated lamp.

   When you turn on the deuterium lamp, its Status readback reads *Starting* during the 30-second ignition period, and then it changes to *On*. If there is a problem with either lamp, its Status readback reads *Failed*.

   Note The intensity of the deuterium lamp falls off very slightly over a period of time after the lamp is turned on. Plan to wait at least one hour for the lamp to stabilize after a cold start before collecting data in the spectral range of the deuterium lamp.

Resetting the Lamp Usage Hours

Use the Configuration page of the Surveyor PDA Direct Control dialog box to reset the lamp usage hours.

❖ To reset the lamp usage hours (lifetime hours elapsed)

1. Open the Configuration page of the Surveyor PDA Direct Control dialog box (see "Configuration Page" on page 165).
2. Click **Reset Lifetime** for the associated lamp.

   The stored total run time for the associated lamp resets to zero, and the Last Lifetime Reset readback is updated to the current date and time.

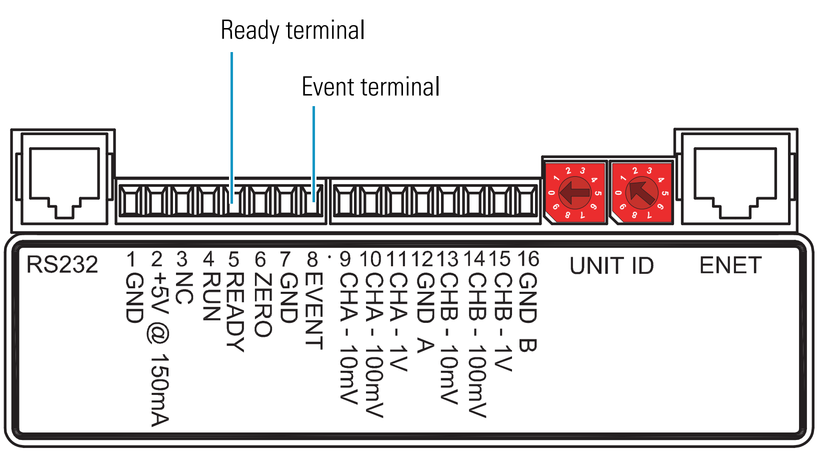
   > **Note** Avoid indiscriminately clicking the Reset buttons. Click Reset only after you replace the associated lamp with a new one.

Changing the Sense of the Analog Outputs

Use the Configuration page of the Surveyor PDA Direct Control dialog box to change the sense of the analog outputs.

There are two output signal terminals on the back panel of the PDA detector: Event and Ready (see Figure 84).

Figure 84. Event and Ready terminals on the back panel of the PDA detector



❖ To change the sense of the analog outputs

1. Open the Configuration page of the Surveyor PDA Direct Control dialog box (see "Configuration Page" on page 165).

   If the output sense is Active Low, the Set Active High button is available. If the output sense is Active High, the Set Active Low button is available.

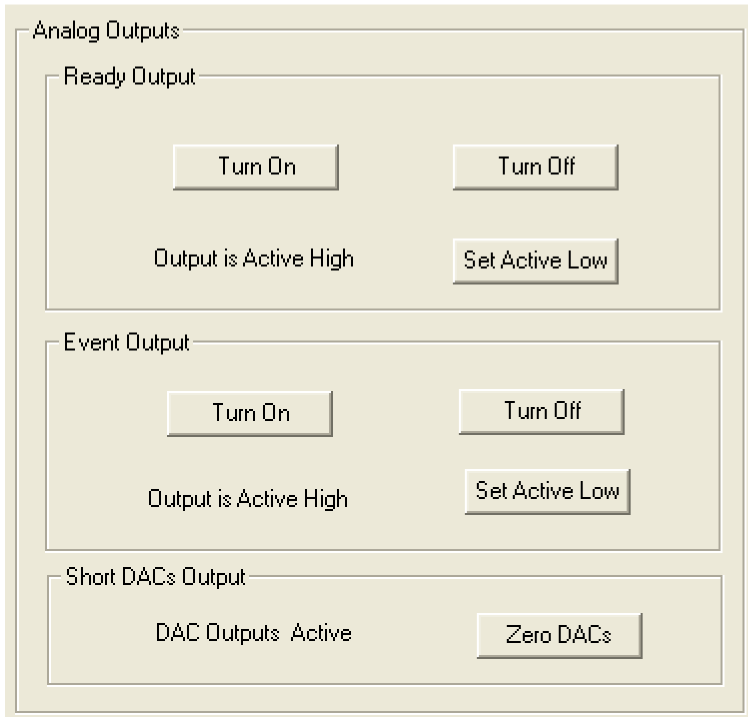
2. Under Analog Outputs (see Figure 85), do one of the following:

   - Click **Set Active High** to change the output sense from Active Low to Active High.

   –or–

   - Click **Set Active Low** to change the output sense from Active High to Active Low.

Figure 85. Analog Outputs area on the Configuration page



Testing the Analog Outputs

Use the Configuration page of the Surveyor PDA Plus Direct Control dialog box to change the sense of the analog outputs.

❖ To test the analog outputs

1. Open the Configuration page of the Surveyor PDA Plus Direct Control dialog box (see "Configuration Page" on page 165).

2. Under Analog Outputs (see Figure 85), do the following:

   - Click **Turn On** or **Turn Off** to trigger the external device.

   - Click **Zero DACs** to calibrate external instruments, such as an SS420X analog to digital converter.

     When you click Zero DACs, the DACs outputs are set to zero for about 20 seconds. You can extend this calibration time by clicking the button again.

Setting the Lamp Startup Time

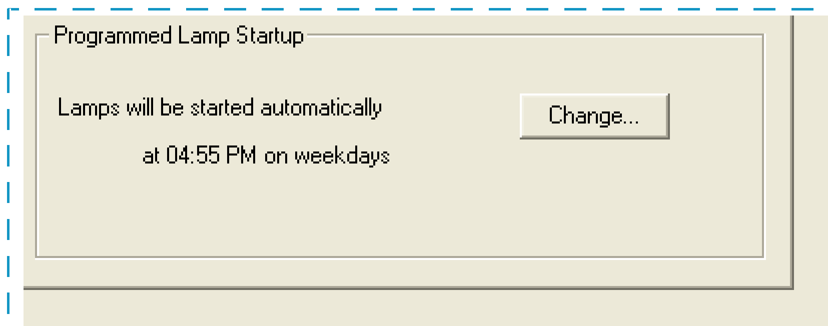
Use the Lamp Startup Time dialog box to set the startup time for the lamps.

If you do not change the lamp startup time, the data system automatically turns the lamps on at 6:55 PM on weekdays. If the data system is closed, it does not turn on the lamps.

❖ To open the Lamp Startup Time dialog box

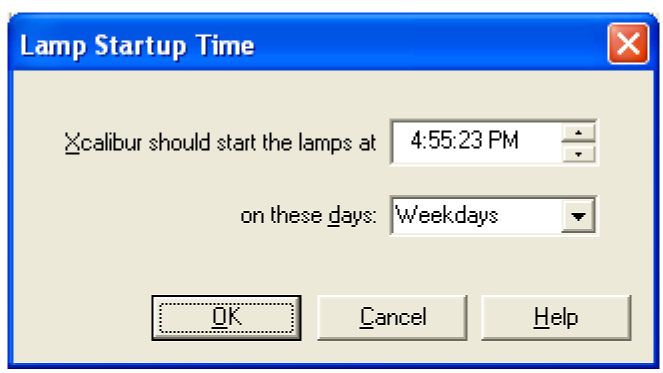
1. Open the Configuration page of the Surveyor PDA Plus Direct Control dialog box (see “Configuration Page” on page 165).
2. In the Programmed Lamp Startup area, click **Change** (see Figure 86).

Figure 86. Programmed Lamp Startup area of the Configuration page



The Lamp Startup Time dialog box appears (see Figure 87).

Figure 87. Lamp Startup Time dialog box



❖ To set the startup time for the lamps

1. Open the Lamp Startup Time dialog box.
2. In the Xcalibur Should Start The Lamps At box, click the hours or minutes text to highlight it.

> **Tip** To change both the hours and the minutes, change one time field, and then click **OK** to accept the setting and close the dialog box. Then open the dialog box a second time and change the other time field.

3. To change the numeric values, do one of the following:

   - Use the up and down arrows to the left of the time entry to scroll to the time value that you want.

     –or–

   - Press the up and down keys on your computer keyboard to scroll to the time value that you want.

     –or–

   - Type the numeric value that you want.

4. To change AM to PM or the reverse, highlight the current setting, and then type AM or PM.

5. To change the day of the week or to turn off the lamp startup feature, in the on these days list, select **Never**, **Weekdays**, or **Everyday**.

6. Click **OK** to apply the new lamp startup time.

Lamp Startup Time Parameter Descriptions

Table 46 describes the parameters in the Lamp Startup Time dialog box.

Table 46. Lamp Startup Time dialog box parameters

Parameter	Description
Xcalibur should start the lamps at	Specifies the time that the Xcalibur data system automatically turns the lamps on. Default: 6:55 PM.
on these days	Specifies the days that the Xcalibur data system automatically turns the lamps on. The selections are Never, Weekdays, and Every Day. Default: Weekdays.
Buttons	
OK	Applies the settings and closes the dialog box.
Cancel	Cancels the applied time value or date.

Checking the Chromatographic Baseline

Use the Display page of the Direct Control dialog box to check the chromatographic baseline (see “Display Page” on page 171).

❖ To check the stability of the chromatographic baseline before starting a sequence run

1. Open the Instrument Setup window of the Xcalibur data system.
2. In the view bar, click the icon for the LC pump, and then open the Direct Control dialog box for the LC pump.
3. Start the solvent flow from the LC pump. Use the chromatographic conditions specified on first line pump program in the instrument method.

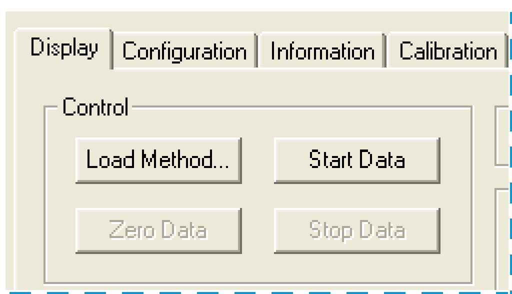
   For information on starting the solvent flow from the LC pump, refer to the Help for the pump.
4. In the view bar of the Instrument Setup window, click the icon for your PDA detector.

   The PDA detector view of the Instrument Setup window appears with the Method page displayed.
5. From the menu bar, choose **Surveyor PDA Plus > Direct Control**.

   The Direct Control dialog box appears.
6. Click the **Display** tab.

   The Display page appears (see Figure 88).

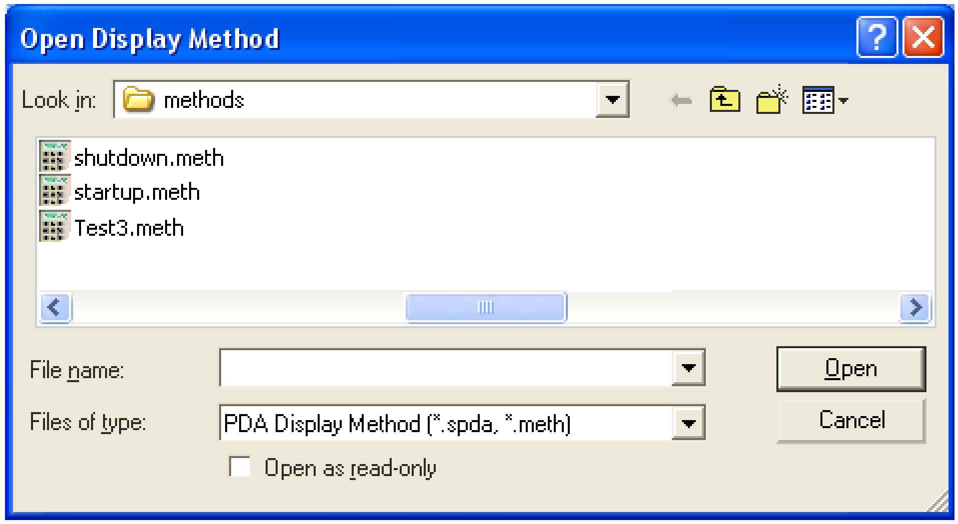
   Figure 88. Control area buttons on the Display page

7. In the Control area, click **Load Method**.

   The Open Display Method dialog box appears (see Figure 89).

Figure 89. Open Display Method dialog box

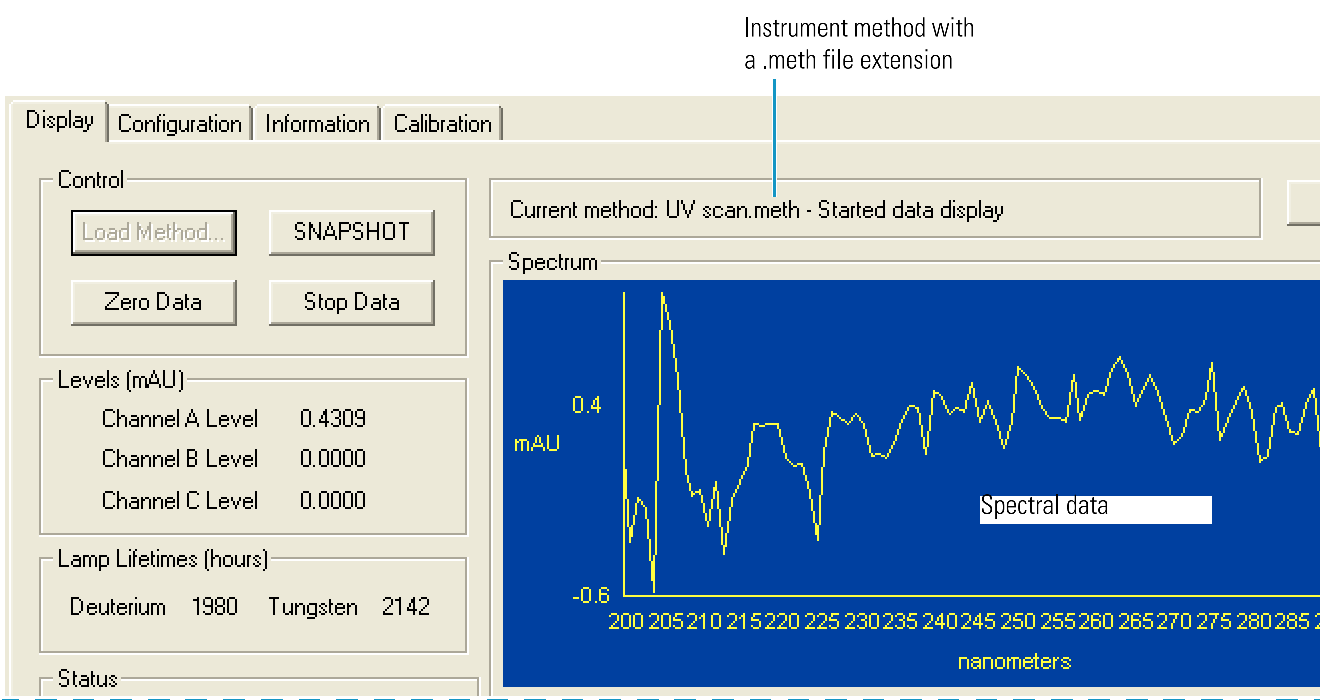


8. Select the instrument method that you plan to use in your acquisition sequence.
9. Click **Open** to download the method to the PDA detector and close the dialog box.
10. In the Control area, click **Start Data**.

    The graphical display begins to update. The spectral scan appears in the top pane and the chromatographic data for the discrete channel wavelengths appear in the bottom pane.

    When you click Start Data, the Start Data button changes to the SNAPSHOT button and the Zero Data button is available (see Figure 90).

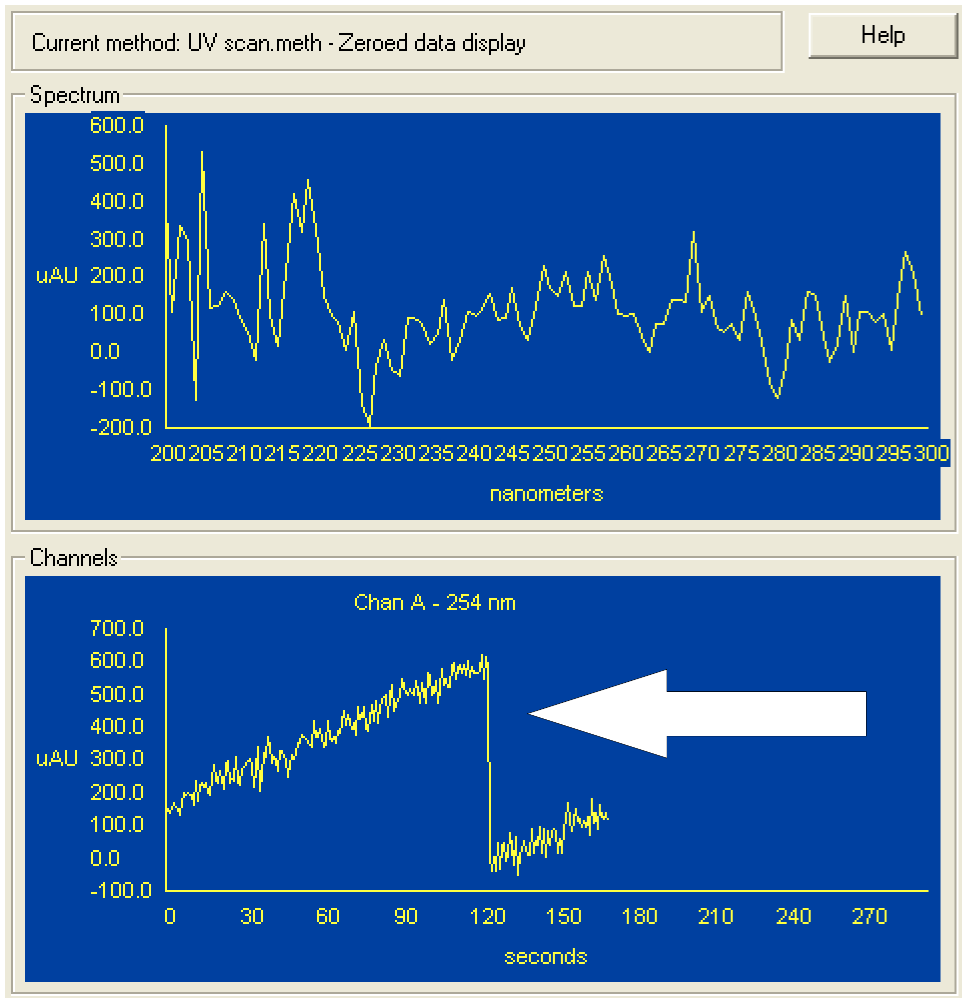
Figure 90. Display page with data displaying for an instrument method



11. To zero the absorbance level, click **Zero Data**.

    The Current method readback displays Zeroed data display (see Figure 91).

Figure 91. Effect of clicking the Zero Data button



12. To stop data acquisition, click **Stop Data**.

    If the noise level is too high, check the light throughput to the diode array.

Viewing, Exporting, and Clearing the Error Log for the PDA Detector

You can access an error log for the PDA detector from the Xcalibur data system.

❖ **To view the error log**

1. Open the Information page of the Surveyor PDA Direct Control dialog box (see "Information Page" on page 175).
2. Click **Request Log**.

❖ **To export the log to a file that you can open in Microsoft Excel**

Click **Export Log**.

❖ **To clear the error log**

Click **Clear Log**.

Checking the Firmware Version of the PDA Detector

❖ **To check the firmware version of the PDA detector**

Open the Information page of the Direct Control dialog box for the PDA detector (see "Information Page" on page 175).

The firmware version of the PDA detector is displayed in the lower-right portion of the Information page.

Configuration Page

Use the Configuration page of the Surveyor PDA Plus Direct Control dialog box to perform these procedures:

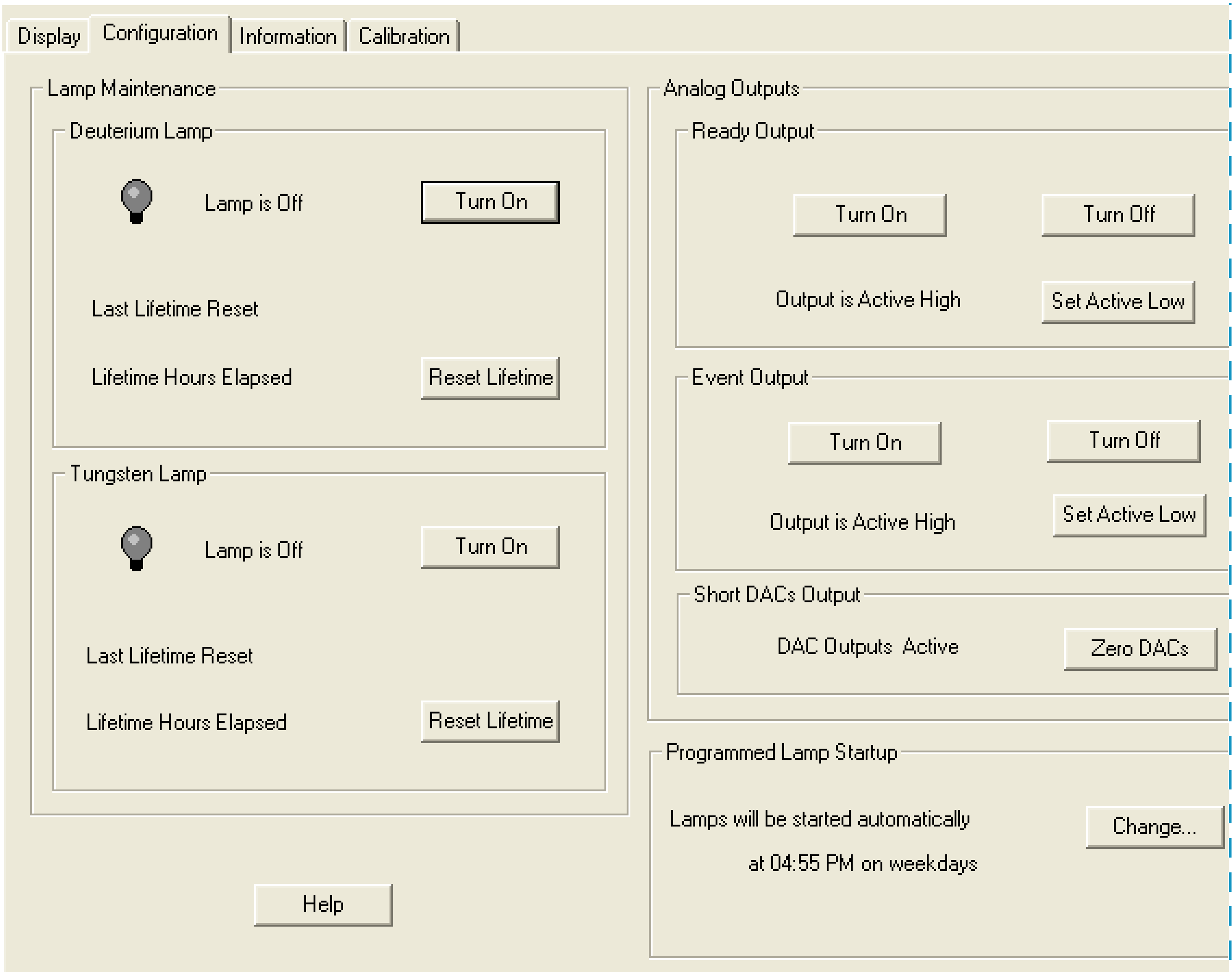
- "Turning the Lamps On or Off" on page 157
- "Resetting the Lamp Usage Hours" on page 158
- "Changing the Sense of the Analog Outputs" on page 158
- "Testing the Analog Outputs" on page 159
- "Setting the Lamp Startup Time" on page 160

❖ **To open the Configuration page of the Direct Control dialog box**

1. Open the Surveyor PDA Direct Control dialog box (see "PDA Detector Direct Controls" on page 156).
2. Click the **Configuration** tab.

   The Configuration page appears (see Figure 92).

Figure 92. Configuration page



Configuration Page Parameter Descriptions

Table 47 describes the parameters on the Configuration page.

Table 47. Configuration page of the Direct Control dialog box for the PDA detector (Sheet 1 of 4)

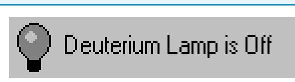
Parameter	Description		
Lamp Maintenance			
Deuterium Lamp			
[Deuterium Lamp] Status	Indicates the status of the deuterium lamp.		
	Graphic	**Text**	**Meaning**
		Off	The deuterium lamp is off.
		Starting	The deuterium lamp is starting up, which requires about 1.5 min.
		On	The deuterium lamp is on. When the status is On, the Turn Off button is available. When the status is Off, the Turn On button is available.
[Deuterium Lamp] Last Lifetime Reset	Displays the date and time that the deuterium lamp was last reset.		
	Note Click the Reset button when you replace the deuterium lamp. This resets the Last Reset readback to the current date and time and restarts the Lifetime readback at zero.		
[Deuterium Lamp] Lifetime Hours Elapsed	Displays the cumulative time in hours that the Deuterium lamp has been in the On state since you clicked the Reset button.		
	Note Click the Reset button whenever you replace the Deuterium lamp. This resets the Last Reset readback to the current date and time and restarts the Lifetime readback at zero.		
[Deuterium Lamp] Turn On/Off button		Turns on the deuterium lamp if its current Status is off.	
		Turns off the deuterium lamp if its current status is on.	
[Deuterium Lamp] Reset Lifetime button	Resets the date and time monitored by the Last Reset readback and the Lifetime readback.		
	Note Click the Reset button whenever you replace the Deuterium lamp. This resets the Last Reset readback to the current date and time and restarts the Lifetime readback at zero.		

Table 47. Configuration page of the Direct Control dialog box for the PDA detector (Sheet 2 of 4)

<table>
<tr><th>Parameter</th><th colspan="3">Description</th></tr>
<tr><td colspan="4">Tungsten Lamp</td></tr>
<tr><td rowspan="4">[Tungsten Lamp] Status</td><td colspan="3">Indicates the status of the tungsten lamp, as follows:</td></tr>
<tr><td>Graphic</td><td>Test</td><td>Meaning</td></tr>
<tr><td>
</td><td>On</td><td>The tungsten lamp is On.</td></tr>
<tr><td>
</td><td>Off</td><td>The tungsten lamp is Off.

When the status is On, the Turn Off button is available. When the status is Off, the Turn On button is available.</td></tr>
<tr><td rowspan="2">[Tungsten Lamp] Last Lifetime Reset</td><td colspan="3">Displays the date and time that the tungsten lamp was last reset.</td></tr>
<tr><td colspan="3">Note Click the Reset button whenever you replace the tungsten lamp. This resets the Last Lifetime Reset readback to the current date and time and restarts the Lifetime readback at zero.</td></tr>
<tr><td rowspan="2">[Tungsten Lamp] Lifetime Hours Elapsed</td><td colspan="3">Displays the cumulative time in hours that the tungsten lamp has been in the On state since it was last reset (replaced).</td></tr>
<tr><td colspan="3">Note Click the Reset button whenever you replace the Tungsten lamp. This resets the Last Lifetime Reset readback to the current date and time and restarts the Lifetime readback at zero.</td></tr>
<tr><td rowspan="2">[Tungsten Lamp] Turn On/Off button</td><td>
</td><td colspan="2">Turns on the tungsten lamp if its current status is Off.</td></tr>
<tr><td>
</td><td colspan="2">Turns off the tungsten lamp if its current status is On.</td></tr>
<tr><td rowspan="2">[Tungsten Lamp] Reset Lifetime button</td><td colspan="3">Resets the date and time monitored by the Last Reset readback and the Lifetime readback.</td></tr>
<tr><td colspan="3">Note Click the Reset button whenever you replace the tungsten lamp. This resets the Last Reset readback to the current date and time and restarts the Lifetime readback at zero.</td></tr>
</table>

Table 47. Configuration page of the Direct Control dialog box for the PDA detector (Sheet 3 of 4)

Parameter	Description		
Analog Outputs			
Analog Outputs	Use the controls in this area to configure and test the Event and Ready outputs. The controls are Event readback and Turn On/Turn Off button, Ready readback and Turn On/Turn Off button, Run readback and Turn On/Turn Off button, Spare readback and Turn On/Turn Off button. The back panel of the PDA detector has the output Event terminal that you can use to trigger external devices, such as a fraction collector. Determine if the external device is triggered by contact closure or a TTL (transistor - transistor - logic) signal. If the external device requires a TTL signal, determine the sense of the signal: active high or active low. **Note** For TTL connections: • Logic level zero (0) is less than +0.8VDC (reading from Ground). • Logic level one (5) is approximately +5VDC (+2.4VDC minimum from Ground). Possible configurations of the Event and Ready outputs include the following:		
	Output State	**Output Sense**	**VDC**
	Off	Active High	+5
Ready Output			
[Ready Output] State/Turn On/ Turn Off	Indicates whether the Ready output state is On or Off. If the Ready output state is On, the Turn Off button is available. If the Ready output state is Off, the Turn On button is available.		
Ready Output [Sense/Set Active High/Set Active Low]	**Note** The output sense used must match the sense (either Active High or Active Low) of the instrument connected to the Event output connection on the back panel of the PDA detector. The signal terminal is the high connection and the GND terminal is the low connection. The autosampler requires active low remote inputs. Indicates whether the Ready output sense is Active High or Active Low. If the Ready output sense is Active Low, the Set Active High button is available. If the Ready output sense is Active High, the Set Active Low button is available.		

Table 47. Configuration page of the Direct Control dialog box for the PDA detector (Sheet 4 of 4)

Parameter	Description
Event Output	
Event Output	Use the controls in this area to specify the parameters for output events. The controls in this area are Event Output State and Turn On/Off buttons, and the Event Output Sense and Set Active High/Low button. If the external device is triggered by a contact closure, connect the PDA EVENT terminal (pin 8) to the positive pin on the external device input, and connect one of the PDA GND terminals (either of pins 1 or 7) to the external device negative pin. If the external device is triggered using a TTL signal, connect the PDA +5V Output (pin2) to the positive Input terminal of the external device, and connect the PDA EVENT Output (pin 8) to the negative Input terminal of the external device.
[Event Output] State/Turn On/Turn Off	Indicates whether the Event output state is On or Off. If the Event output state is On, the Turn Off button is available. If the Event output state is Off, the Turn On button is available.
[Event Output] Sense/Set Active High/ Set Active Low	**Note** The output sense used must match the sense (either Active High or Active Low) of the instrument connected to the Event output connection on the back panel of the PDA detector. The signal terminal is the high connection and the GND Terminal is the low connection. The autosampler requires Active Low remote inputs. Indicates whether the Event output sense is Active High or Active Low. If the Event output sense is Active Low, the Set Active High button is available. If the Event output sense is Active High, the Set Active Low button is available.
Short DACs Output	
Zero DACs button	To calibrate external instruments, such as an SS420X analog to digital converter, use the controls in this area to short the DACs outputs to zero for about 20 seconds.
Programmed Lamp Startup	
Programmed Lamp Startup	Displays the current lamp startup program and provides controls to create a lamp startup program that automatically turns on the lamps for operation at a specified time and day (never, weekdays, every day).
Change	Opens the Lamp Startup Time dialog box where you can specify a lamp startup time (see "Setting the Lamp Startup Time" on page 160).

Display Page

Use the Display page to perform these procedures:

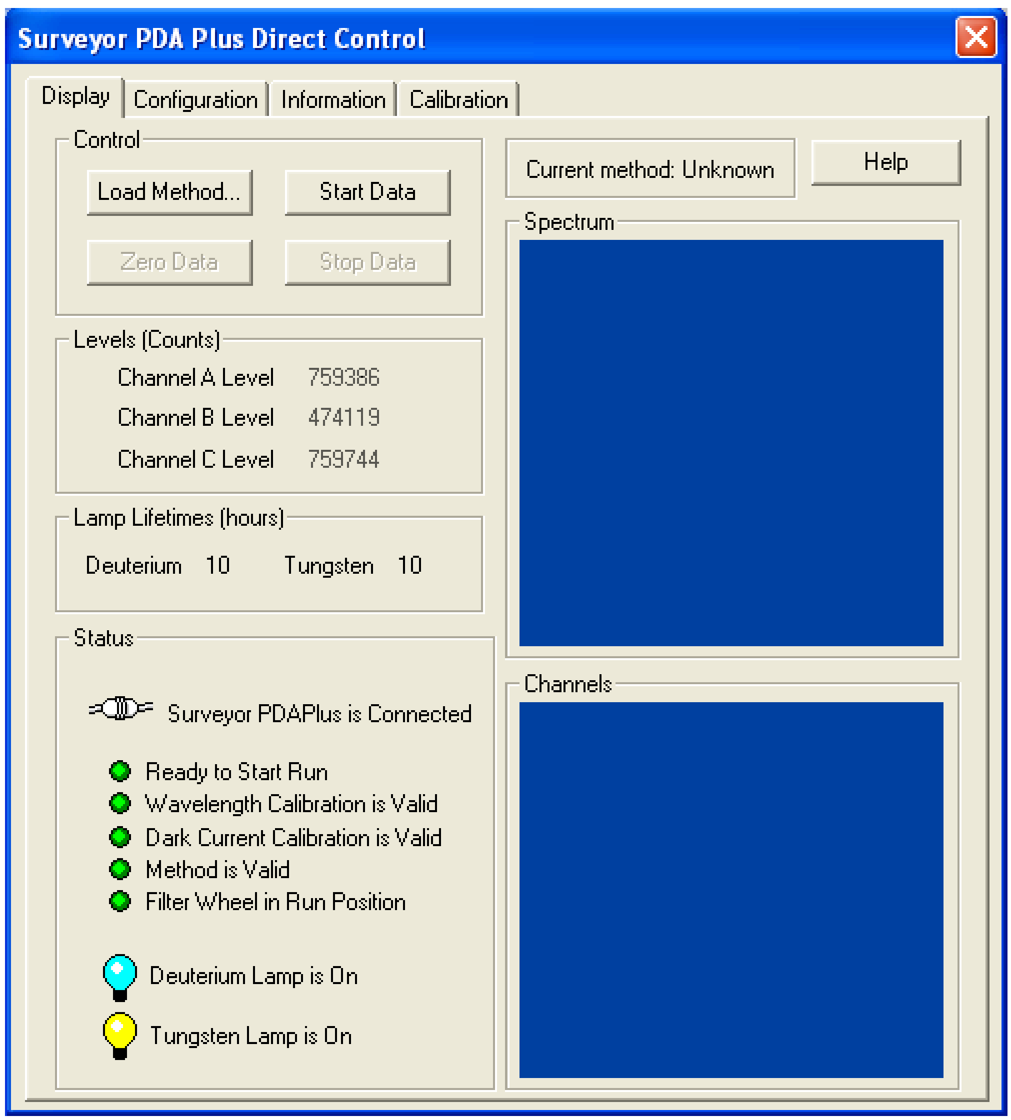
- "Checking the Chromatographic Baseline" on page 162
- "Adjusting the Light Throughput to the Diode Array" on page 255

❖ To open the Display page

1. Open the Surveyor PDA Direct Control dialog box (see "PDA Detector Direct Controls" on page 156).
2. Click the **Display** tab.

   The Display page appears (see Figure 93).

Figure 93. Display page



Display Page Parameter Descriptions

Table 48 describes the parameters on the Display page.

Table 48. Display page parameters (Sheet 1 of 3)

Parameter	Description
Control	
Control	The buttons in this area provide control of the display method (*.spda).
Load Method	Loads the display method or the data acquisition parameters of an instrument method to the detector.
Start Data	Initiates the graphical display of the intensity data or the wavelength data.
Snapshot	Saves a comma-separated values file named **PDASnapshot.csv** to the following folder on the data system computer: C:\Xcalibur\data. A date and time stamp is appended to the file name. The format of the date and time stamp is MMDDYYHHMMSS. MM is the month. DD is the day of the month. YY is the last two digits of the year. HH is the hour in military time. MM is minutes. SS is seconds. You can view this file with Microsoft Excel. For a wavelength/absorbance method, the file contains the absorbance values for the spectral scan and the discrete channel wavelengths at the moment that you clicked Snapshot. For an intensity/diode method, the file contains the intensity values for the scan of the diode array and the intensity values for up to three individual diodes. **Note** The Snapshot button is available only after you click the Start Data button and the graphical display has started updating.
Zero Data	Zeros the absorbance data. After you click Zero Data, the Current Method status box displays the message Zeroed Data Display. **Note** This button is only available in the wavelength/absorbance mode.
Stop Data	Stops the updating of the graphical display. After you click Stop Data, the Current Method status box displays the message Stopped Data Display.
Levels (mAU)	
Channel A/B/C level	This graphical display shows the intensity or absorbance level of each discrete channel A, B or C specified in the method.

Table 48. Display page parameters (Sheet 2 of 3)

Parameter	Description
Lamp Lifetimes (hours)	
Deuterium	Displays the cumulative time in hours that the Deuterium lamp has been in the On state since you clicked the Reset button. **Note** Click the Reset button on the Configuration page whenever you replace the deuterium lamp. This resets the Last Reset readback to the current date and time and sets the Lifetime readback to zero.
Tungsten	Displays the cumulative time in hours that the tungsten lamp has been in the On state since you clicked the Reset button. **Note** Click the Reset button on the Configuration page whenever you replace the tungsten lamp. This resets the Last Lifetime Reset readback to the current date and time and restarts the Lifetime readback to zero.
Status (bottom left)	
Status area	Displays the status of the PDA detector and the lamps.
Connection	Displays the communication status of the PDA detector. Depending on whether the data system can connect to the PDA detector, the status displays Connected or Not Connected. If this readback displays Not Connected, make sure that the PDA detector is powered on. Check the Ethernet connections and the instrument configuration settings for the PDA detector.
Diode array scan rate	Displays the current configuration setting for the diode array scan rate. The possible diode array scan rates are 20, 40, and 80 Hz.
PDA status	Displays the current state of the PDA detector. The possible states are Not Ready for a Run and Ready for a Run. This readback displays Not Ready for a Run if both lamps are off or if the deuterium lamp is warming up.
Wavelength calibration	Displays whether the wavelength calibration has been applied.
Dark current calibration	Displays whether the dark current calibration has been applied.
Method validity	Displays whether a method has been downloaded to the PDA detector.
Filter wheel position	Displays the position of the filter wheel. The filter wheel has two positions: 1 and 2. When the filter wheel is in position 1, the readout displays Run Position. When the filter wheel is in position 2, the readout displays Calibration Position.
Deuterium lamp	Displays whether the deuterium lamp is on or off.
Tungsten lamp	Displays whether the tungsten lamp is on or off.
Current method (top right)	
Current method	Displays the status of the current method.

Table 48. Display page parameters (Sheet 3 of 3)

Parameter	Description
Spectrum	
Spectrum Plot	Depending on the method type (*.meth or *spda), these scan plots are displayed: • For display methods (*.spda), the scan plot shows intensity (counts) normalized to 100% on the Y-axis and diode number (2 to 511) on the X-axis. • For instrument methods (*.meth), the scan plot shows absorbance (mAU) normalized to 100% on the Y-axis and wavelength (nanometers) on the X-axis.
Channels	
Channels Plot	Depending on the method type (*.meth or *spda), these discrete channel plots are displayed: • For display methods (*.spda), the plot shows intensity (counts) normalized to 100% on the Y-axis and time on the X-axis. • For instrument methods (*.meth), the scan plot shows absorbance (mAU) normalized to 100% on the Y-axis and time on the X-axis. Each discrete channel is color coded. Channel A is yellow, Channel B is purple, and Channel C is blue.

Information Page

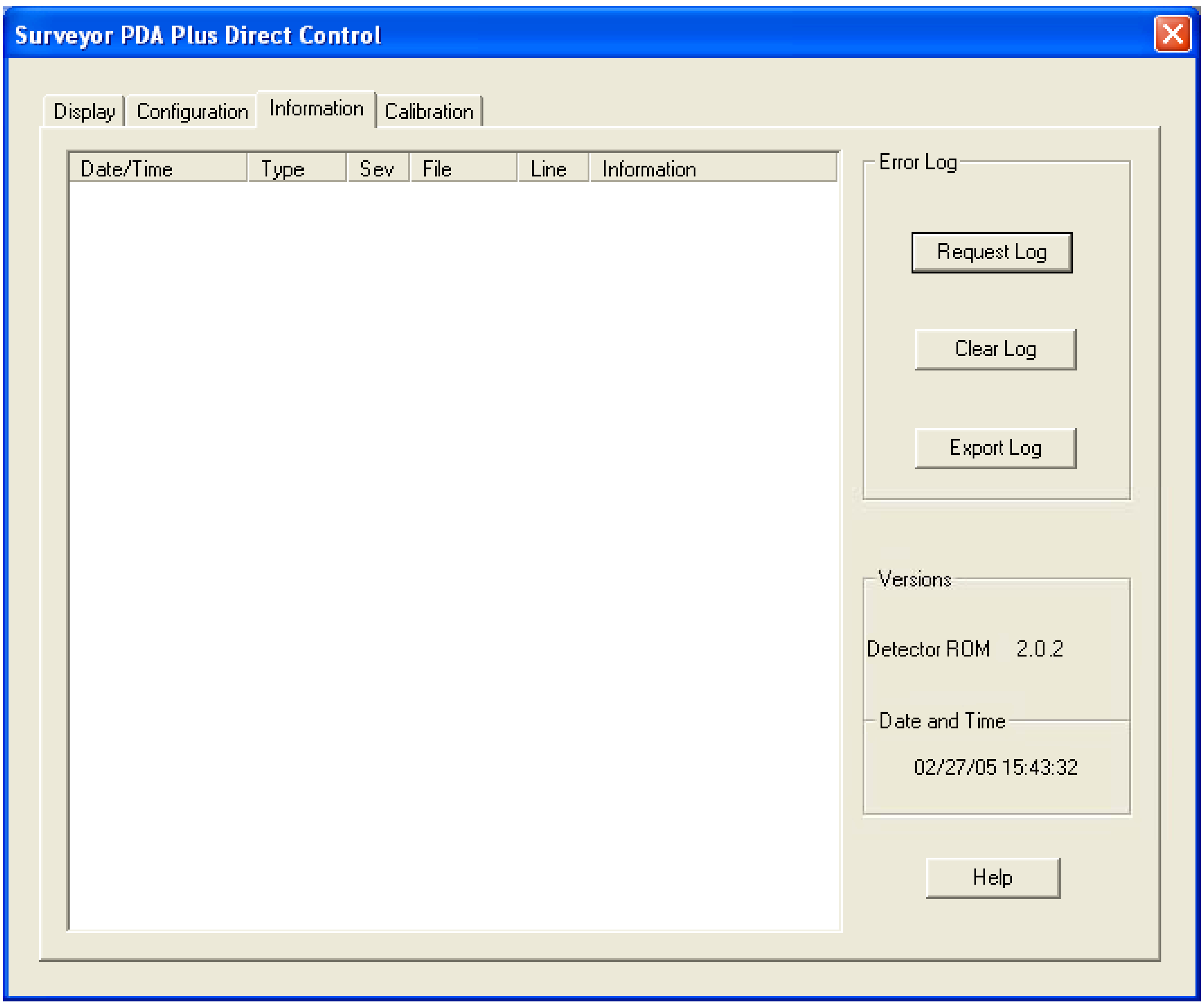
Use the Information page to monitor the chronological run log, instrument version, and system date and time. The error log is an ongoing record of critical and non-critical errors in the PDA Detector. The log is generated and stored in the detector. This data is useful for troubleshooting hardware and software problems.

❖ To open the Information page

1. Open the Direct Control dialog box for the PDA detector (see “PDA Detector Direct Controls” on page 156)
2. Click the **Information** tab.

   The Information page appears (see Figure 94).

Figure 94. Information page



Information Page Parameter Descriptions

Table 49 describes the parameters on the Information page.

Table 49. Information page parameters

Parameter	Description
Error Log	
Error Log	This box displays errors that are generated by the PDA detector. The error log can hold up to 100 errors. When the error log is full, the newest error log entry replaces the oldest error log entry. The error log is protected by a backup battery when the detector is turned off.
	Note For best results, save the error log (print to file) before you clear it. The error log is useful during preventive and problem maintenance.
Buttons	
Request Log	Downloads the error log that is stored in the PDA detector and displays the results in the Error Log box.
Clear Log	Erases the current error log.
	Note For best results, save the error log (print to file) before you clear it. The error log is useful during preventive and problem maintenance.
Export Log	Exports the log to a comma-separated values file that you can open in Microsoft Excel.
Versions	
Versions display box	Displays the version number for the detector firmware. This information is useful to determine if firmware upgrades are necessary. Have this information handy whenever you contact your Thermo Fisher Scientific service representative.
Date and Time	
Date and time	Displays the current system date and time. This is the time that the data system uses for entries in the error log and the time stamp on the snapshot file.

UV/Vis Detector Direct Controls

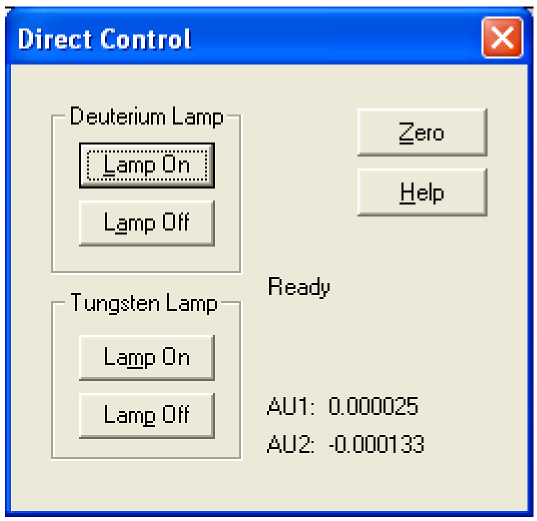
Use the Direct Control dialog box for the UV/Vis detector to turn the lamps on or off and to zero the chromatographic baseline.

❖ To turn the UV/Vis detector's lamps on or off

1. Open the Surveyor UV/Vis Instrument Setup view.
2. From the menu bar, choose **Surveyor UV/Vis > Direct Control**.

   The Direct Control dialog box for the UV/Vis Detector appears (see Figure 95).

   Figure 95. Direct Control dialog box for the UV/Vis Detector

3. In the Deuterium Lamp area, click **Lamp On**.

❖ To zero the absorbance

Click **Zero**.

UV/Vis Direct Control Parameter Descriptions

Table 50 describes the buttons and readbacks in the Direct Control dialog box for the UV/Vis detector.

Table 50. Direct control parameters for the UV/Vis detector (Sheet 1 of 2)

Parameter	Description
Deuterium Lamp	
Lamp On	Turns the deuterium lamp on.
Lamp Off	Turns the deuterium lamp off.

Table 50. Direct control parameters for the UV/Vis detector (Sheet 2 of 2)

Parameter	Description	
Tungsten Lamp		
Lamp On	Turns the tungsten lamp on.	
Lamp Off	Turns the tungsten lamp off.	
Other Options		
Zero	Zeros the absorbance reading from the detector.	
Readbacks		
Status	Displays the state of the UV/Vis detector:	
	Ready	The detector is ready to start data acquisition.
	Not ready	The detector is not ready to start data acquisition.
	Running	The detector is acquiring data.
AU1	Displays the current absorbance value for the first wavelength channel.	
AU2	Displays the current absorbance value for the second wavelength channel.	

Pump Direct Controls

The Surveyor product line includes the Surveyor LC Pump Plus and the Surveyor MS Pump Plus.

These topics describe the direct controls for your Surveyor Plus pump:

- "Surveyor LC Pump Plus Direct Controls," on this page
- "Surveyor MS Pump Plus Direct Controls" on page 184

Surveyor LC Pump Plus Direct Controls

Use the Surveyor LC Pump Direct Control dialog box to quickly draw fresh solvent through the solvent lines, clear an error condition, download new solvent conditions to the pump, and start and stop the solvent flow from the pump.

For information on opening the Direct Control dialog box and using the direct controls for the Surveyor LC Pump Plus, see these topics:

- "Opening the Direct Control Dialog Box for the Surveyor LC Pump Plus" on page 179
- "Operation - Surveyor LC Pump Direct Control Parameter Descriptions" on page 180
- "Removing Air from the Solvent Lines Connected to the Surveyor LC Pump Plus" on page 181
- "Downloading New Solvent Conditions to the Pump" on page 182
- "Starting and Stopping the Solvent Flow" on page 183
- "Clearing an Error Condition for the Pump" on page 183

Opening the Direct Control Dialog Box for the Surveyor LC Pump Plus

In the pump view, the direct controls are on the Operation - Surveyor LC Pump Direct Control dialog box. In the Tune window for your Thermo Scientific mass spectrometer, the direct controls for the pump are on the pump page of the Inlet Direct Control dialog box.

❖ **To open the Operation - Surveyor LC Pump Direct Control dialog box**

1. Open the pump view.
2. From the menu bar, choose **Surveyor LC Pump > Direct Control > Operation**.

   The Operation - Surveyor LC Pump Direct Control dialog box appears.

❖ To access the direct controls for the pump from the Tune window

1. Open the Tune window for your Thermo Scientific mass spectrometer.
2. Choose **Setup > Inlet Direct Control**.

   The Inlet Direct Control dialog box that contains tabbed pages for each configured LC device appears.
3. Click the **Surveyor LC Pump** tab.

Operation - Surveyor LC Pump Direct Control Parameter Descriptions

Table 51 describes the direct control parameters for the Surveyor LC Pump Plus.

Table 51. Operation - Surveyor LC Pump Direct Control parameters (Sheet 1 of 2)

Parameter	Description
Pump	
Pump On	Turns on the solvent flow from the pump.
Pump Off	Turns off the solvent flow from the pump.
Error Condition	
Clear	Clears the error log. You must clear the error log before you can restart the solvent flow from the pump.
Method	
Flow Rate	Specifies the flow rate, in mL/min. Range: 0.000 to 9.999 mL/min.
Component A, B, C, or D	Specifies the percent composition of solvent A, B, C, or D.
Download	Downloads the new mobile phase settings to the on-board CPU of the Surveyor LC Pump Plus.
Purge	
Purge Time	Specifies the length of time that the pump draws solvent through the system at approximately 10 mL/min. Range: 0.1 to 9.9 minutes
Solvent Valve	Specifies the solvent proportioning valve that is activated. The pump draws solvent from the solvent line connected to this proportioning valve. **Tip** The Surveyor LC Pump Plus is a quaternary gradient pump that can be set up to pump four solvents. The solvent lines are labeled A, B, C, and D. You must purge each separately.

Table 51. Operation - Surveyor LC Pump Direct Control parameters (Sheet 2 of 2)

Parameter	Description
Purge	Draws the specified solvent at a speed of approximately 10 mL/min through the pump for the specified purge time. **CAUTION** Before you click the Purge button, open the drain valve by turning the drain valve knob 180° counterclockwise.
Stop Purge	Stops the solvent flow from the pump. **Tip** After you finish purging the pump, close the drain valve by gently turning the drain valve knob clockwise until you feel resistance.

Removing Air from the Solvent Lines Connected to the Surveyor LC Pump Plus

Use the purge feature to remove air bubbles from the solvent lines or the pump heads. If the solvent line connecting the solvent reservoir bottle to the degassing assembly is completely dry, attach a syringe to the drain line and open the drain valve. Make sure that the pump power is on, and then draw the syringe barrel back, pulling solvent into the line.

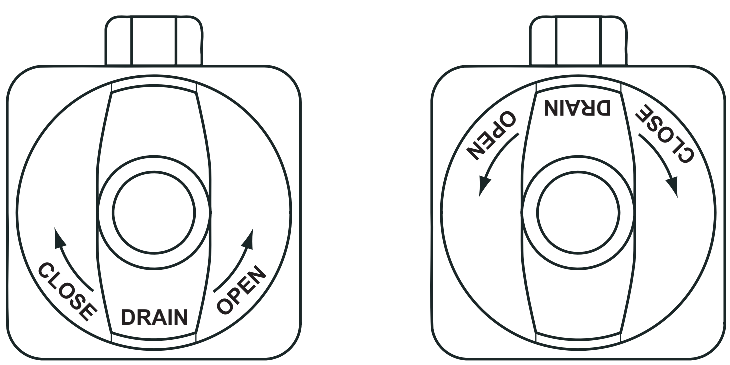
Tip Cyclical baseline noise can be an indication of air in the pump heads.

❖ To remove air from the solvent lines or the pump head assemblies

1. Open the drain valve by turning it counterclockwise 180° to the purge position. Look for the word DRAIN to appear upside down on the knob (see Figure 96).

   Figure 96. Drain valve knob in the closed and open positions

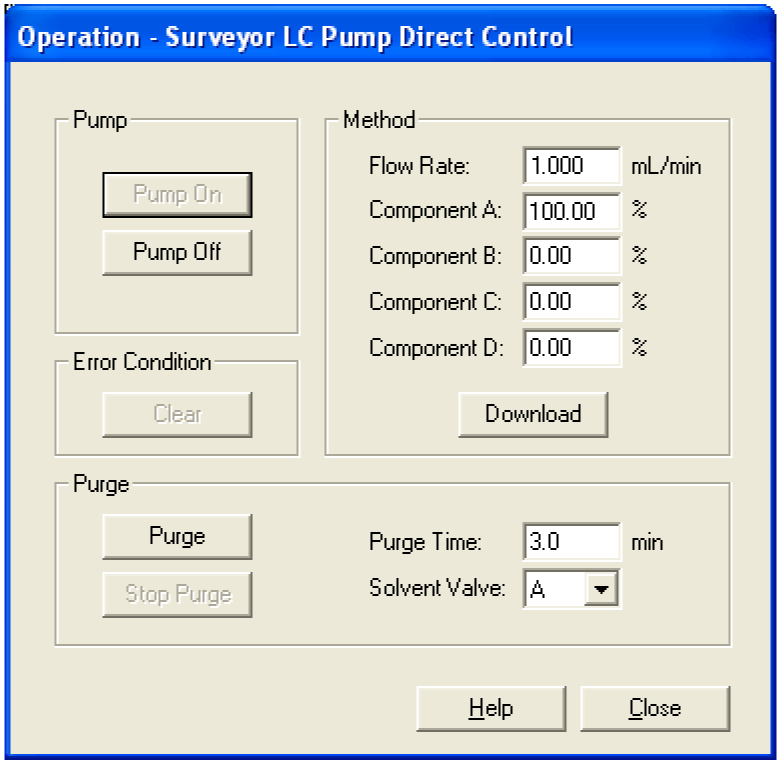
   Closed position Open position



2. Open the view for the Surveyor LC Pump Plus.
3. From the menu bar, choose **Surveyor LC Pump > Direct Control > Operation**.

   The Operation - Surveyor LC Pump Direct Control dialog box appears (see Figure 97).

Figure 97. Operation page of the Surveyor LC Pump Direct Control dialog box



4. In the Purge area, do the following:

   a. In the Purge Time box, type an appropriate purge time.

      The range is 0.1 to 9.9 minutes. Each solvent line holds approximately 3 mL of solvent.

   b. From the Solvent Valve list, select the solvent valve for the solvent line that you want to draw solvent through.

   c. Click **Purge**.

      The pistons of the pump begin to move rapidly, increasing the flow rate to 9.99 mL/min.

5. After you drawing fresh solvent through the solvent lines, close the drain valve.

Downloading New Solvent Conditions to the Pump

❖ To download new solvent conditions to the pump

1. Open the Surveyor LC Pump Plus view.

2. From the menu bar, choose **Surveyor LC Pump > Direct Control > Operation**.

   The Operation - Surveyor LC Pump Direct Control dialog box appears.

3. In the Method area, do the following:

   a. In the Flow rate box, type the flow rate.

      The range is 0.001 to 9.999 mL/min.

   b. In the Component A, B, C, and D boxes, type the solvent composition.

   c. Click **Download**.

Starting and Stopping the Solvent Flow

❖ To start or stop the solvent flow

1. Open the Operation - Surveyor LC Pump Direct Control dialog box (see “Surveyor LC Pump Plus Direct Controls” on page 179).

2. Do one of the following:

   - To start the solvent flow, click **Pump On**.

   - To stop the solvent flow, click **Pump Off**.

Clearing an Error Condition for the Pump

When an error condition occurs, the solvent flow from the pump stops. Before you can restart the solvent flow, you must clear the error condition.

❖ To clear an error condition for the pump

1. Open the Surveyor LC Pump Plus view.

2. From the menu bar, choose **Surveyor LC Pump > Direct Control > Operation**.

   The Operation - Surveyor LC Pump Direct Control dialog box appears.

3. Click **Clear**.

Surveyor MS Pump Plus Direct Controls

Use the Direct Control dialog box to download new solvent conditions to the pump, start and stop the solvent flow from the pump, and change the global pressure limits.

Note To open the direct controls for the pump from the Tune window, choose **Setup > Inlet Direct Control** from the menu bar. Then click the pump tab.

❖ To download new solvent conditions to the pump

1. Turn on the power to the pump.

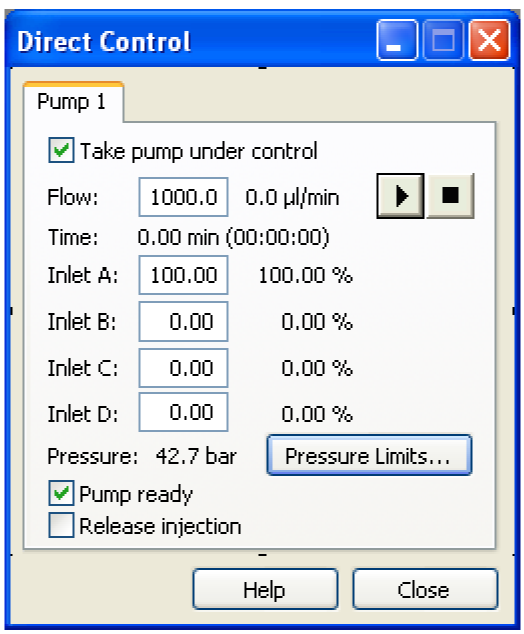
   Tip When the power to the pump is off, the proportioning valves are closed. Before you take the LDA apart to perform maintenance, turn off the power to the pump to prevent solvent from leaking out of the proportioning assembly.

2. To open the Direct Control dialog box from the pump view, do the following:

   a. From the menu bar of the pump view, choose **Surveyor MS Pump Plus > Direct Control**.

   The Direct Control dialog box for the pump appears (see Figure 98).

   Figure 98. Direct Control dialog box for the Surveyor MS Pump Plus



3. In the Direct Control dialog box, do the following:

   a. Select the **Take pump under control** check box.

   b. In the Flow box, type an appropriate flow rate for the pump. If you are drawing fresh solvent through the lines and the liquid displacement assembly, type the maximum flow rate for the pump.

The maximum flow rate for the Surveyor MS Pump Plus is 1000 µL/min.

c. Type percentages for the solvent lines that you want to draw solvent through in the Inlet boxes (A, B, C, and D).

❖ To start the solvent flow

Click  (Start).

❖ To stop the solvent flow

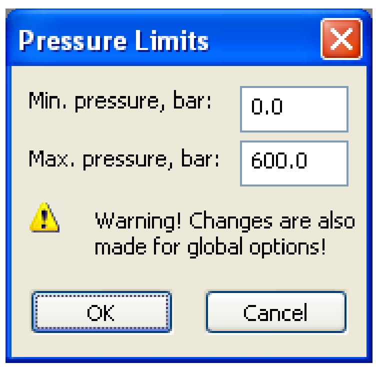
Click  (Stop).

❖ To change the global pressure limits

1. Click **Pressure Limits**.

   The Pressure Limits dialog box appears (see Figure 99).

   Figure 99. Pressure Limits dialog box

2. In the Min. pressure, bar box, type the minimum operating pressure for the pump.

3. In the Max. pressure, bar box, type the maximum operating pressure for the pump.

   The maximum pressure for the Surveyor MS Pump Plus is 1000 bar (100 MPa or 14 504 psi).

4. Click **OK** to accept the settings and close the dialog box.

Autosampler Direct Controls

The On/Off switch is the only manual control provided with the autosampler. To perform tasks such as moving the XYZ arm to the back of the tray compartment, use the direct control commands in the autosampler view or the tune application for your Thermo Scientific mass spectrometer.

These topics describe the direct controls for the autosampler:

- "Applying a Direct Command" on page 188
- "Direct Control Commands for the Autosampler" on page 188
- "Flushing the Autosampler Syringe" on page 190
- "Removing and Installing Sample Trays" on page 193
- "Controlling the Tray and Oven Compartment Temperatures" on page 193

You can access the direct control commands for the autosampler from the autosampler view of your data system or the tune application for your mass spectrometer.

❖ To open the Inlet Direct Control dialog box from the tune application

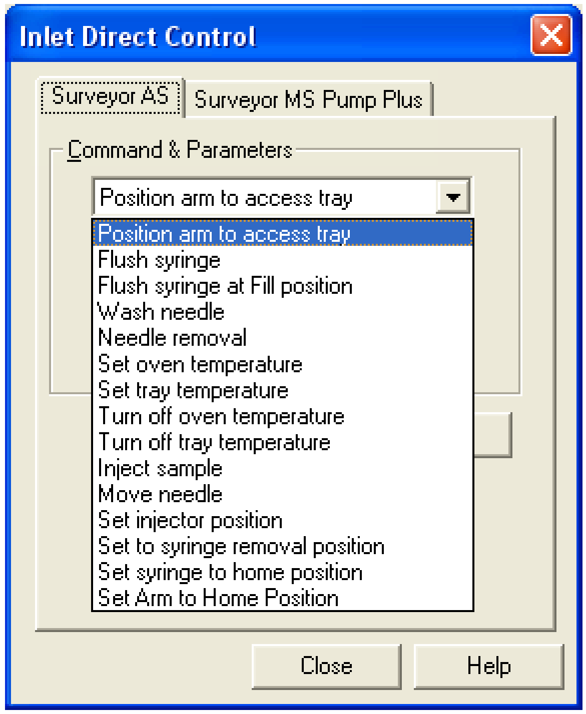
1. In the Tune window, choose **Setup > Inlet Direct Control**.

   The Inlet Direct Control dialog box appears with tabbed pages for each configured LC device.

2. Click the **Surveyor AS** tab.

   The Surveyor AS page appears (see Figure 100).

Figure 100. Inlet Direct Control dialog box for the Surveyor pump and autosampler



❖ To open the Direct Control dialog box

1. Open the autosampler view.
2. From the menu bar, choose **Surveyor AS > Direct Control**.

   The Direct Control dialog box appears (see Figure 101).

Figure 101. Direct Control dialog box (Instrument Setup window)

Applying a Direct Command

❖ **To apply a direct command**

1. Select a command from the list of commands.

   If the command requires additional parameters, these parameters appear below the list. Make the appropriate entries and selections.

2. To execute the command, click **Apply**.

Direct Control Commands for the Autosampler

Table 52 describes the direct control commands for the autosampler.

Table 52. Direct control commands for the autosampler (Sheet 1 of 3)

<table>
<tr><th>Command</th><th colspan="3">Description</th></tr>
<tr><td rowspan="2">Position arm to access tray</td><td colspan="3">Moves the XYZ arm to the back of the tray compartment so that you can remove trays from or place trays into the tray compartment.</td></tr>
<tr><td colspan="3">Note. If the tray compartment door is open and you selected the Verify Door Is Closed check box when you configured the autosampler, the autosampler does not execute this command until you close the tray compartment door.</td></tr>
<tr><td rowspan="7">Flush syringe</td><td colspan="3">Flushes the needle tubing and the interior of the needle with flush solvent.</td></tr>
<tr><td>Parameter</td><td colspan="2">Selections or range</td></tr>
<tr><td>Reservoir</td><td colspan="2">RV1, RV2, RV3, RV4, or Bottle</td></tr>
<tr><td>Volume</td><td colspan="2">0 to 6000 µL</td></tr>
<tr><td rowspan="3">Flush Speed</td><td colspan="2">Depends on the syringe type as follows:</td></tr>
<tr><td>Syringe type</td><td>Range</td></tr>
<tr><td>Concentric syringes</td><td>1.65 to 661.38 µL/s</td></tr>
<tr><td></td><td></td><td>Standard 2500 µL syringe</td><td>0.83 to 330.85 µL/s</td></tr>
<tr><td>Flush syringe at Fill position</td><td colspan="3">Flushes the needle tubing, the interior of the needle, and the sample loop with flush solvent. For the parameter descriptions, see Flush syringe.</td></tr>
<tr><td rowspan="4">Wash needle</td><td colspan="3">Washes the exterior of the needle with solvent.</td></tr>
<tr><td>Parameter</td><td colspan="2">Selections or range</td></tr>
<tr><td>Reservoir</td><td colspan="2">RV1, RV2, RV3, RV4, or Bottle</td></tr>
<tr><td>Volume</td><td colspan="2">0 to 6000 µL</td></tr>
</table>

Table 52. Direct control commands for the autosampler (Sheet 2 of 3)

<table>
<tr><th>Command</th><th colspan="3">Description</th></tr>
<tr><td>Needle removal</td><td colspan="3">Sets the needle to the needle removal position. Apply this command before you remove the needle from the XYZ arm.</td></tr>
<tr><td rowspan="4">Set oven temperature</td><td colspan="3">Sets the temperature of the column oven compartment without downloading an instrument method.</td></tr>
<tr><td>Parameter</td><td colspan="2">Range</td></tr>
<tr><td>Temperature</td><td colspan="2">5 to 95 °C</td></tr>
<tr><td colspan="3">Important. Avoid setting the temperature above the boiling point of the mobile phase.</td></tr>
<tr><td rowspan="3">Set tray temperature</td><td colspan="3">Sets the temperature of the tray compartment without downloading an instrument method. The temperature range is 0 to 60 °C.</td></tr>
<tr><td>Parameter</td><td colspan="2">Range</td></tr>
<tr><td>Temperature</td><td colspan="2">0 to 60 °C</td></tr>
<tr><td>Turn off
oven temperature</td><td colspan="3">Turns off the oven temperature control, allowing the temperature of the column oven compartment to return to ambient.</td></tr>
<tr><td>Turn off
tray temperature</td><td colspan="3">Turns off the tray temperature control, allowing the temperature of the tray compartment to return to ambient.</td></tr>
<tr><td rowspan="14">Inject sample</td><td colspan="3">Injects a sample.</td></tr>
<tr><td colspan="3">Parameter</td></tr>
<tr><td rowspan="5">Vial</td><td colspan="2">The vial or well entries depend on the tray configuration:</td></tr>
<tr><td>Tray type</td><td>Vial locations</td></tr>
<tr><td>Conventional trays</td><td>A:01 to E:40</td></tr>
<tr><td>96-well plates</td><td>A:A1 to C:H12</td></tr>
<tr><td>384-well plates</td><td>A:A1 to C:P24</td></tr>
<tr><td rowspan="6">Volume</td><td colspan="2">For the no waste and partial loop injection modes, the minimum injection volume is 0.1 µL.

The maximum volume depends on the syringe type:</td></tr>
<tr><td>Syringe</td><td>Maximum volume</td></tr>
<tr><td>100 µL concentric syringe</td><td>20 µL</td></tr>
<tr><td>250 µL concentric syringe</td><td>125 µL</td></tr>
<tr><td>500 µL concentric syringe</td><td>300 µL</td></tr>
<tr><td>2500 µL standard syringe</td><td>1250 µL</td></tr>
<tr><td>Injection
Mode</td><td colspan="2">Full Loop, Partial Loop, and No Waste.</td></tr>
<tr><td>Move needle</td><td colspan="3">Moves the XYZ arm to a specific vial or well location.</td></tr>
</table>

Table 52. Direct control commands for the autosampler (Sheet 3 of 3)

Command	Description
Set injector position	Switches the position of the injection valve. The injection valve has two positions: fill and inject.
Set syringe to removal position	Sets the syringe to its removal position.
Set syringe to home position	Sets the syringe to its home position.
Set Arm to Home Position	Moves the XYZ arm to its home position, which is just above the injection port.
Button	
Apply	Executes the command.

Flushing the Autosampler Syringe

To ensure the proper performance of the autosampler, remove air from the wash bottle tubing and the autosampler syringe before you make your first injection. Once you have your system running, periodically check the level of solvent in the wash bottle and remove air from the syringe as needed.

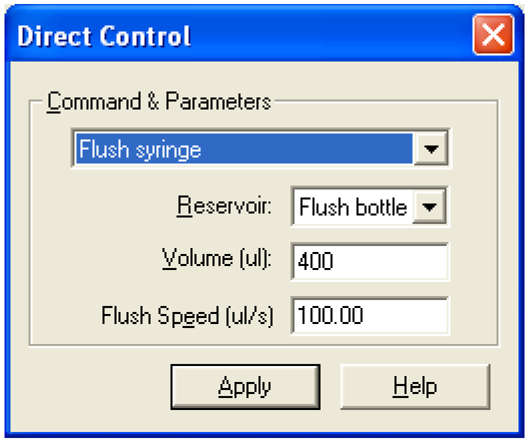
Tip During a flush operation, the autosampler draws wash solvent into the syringe and then pushes wash solvent through the needle tubing and the injection port to waste.
If you want to remove residual sample from the sample loop, use the Flush syringe at Fill position command.

❖ **To flush air out of the wash bottle tubing and the autosampler syringe**

1. Open the view for the autosampler.
2. From the menu bar, choose **Surveyor AS > Direct Control**.

   The Direct Control dialog box for the Surveyor Autosampler Plus appears (see Figure 102).

Figure 102. Flush syringe direct control command

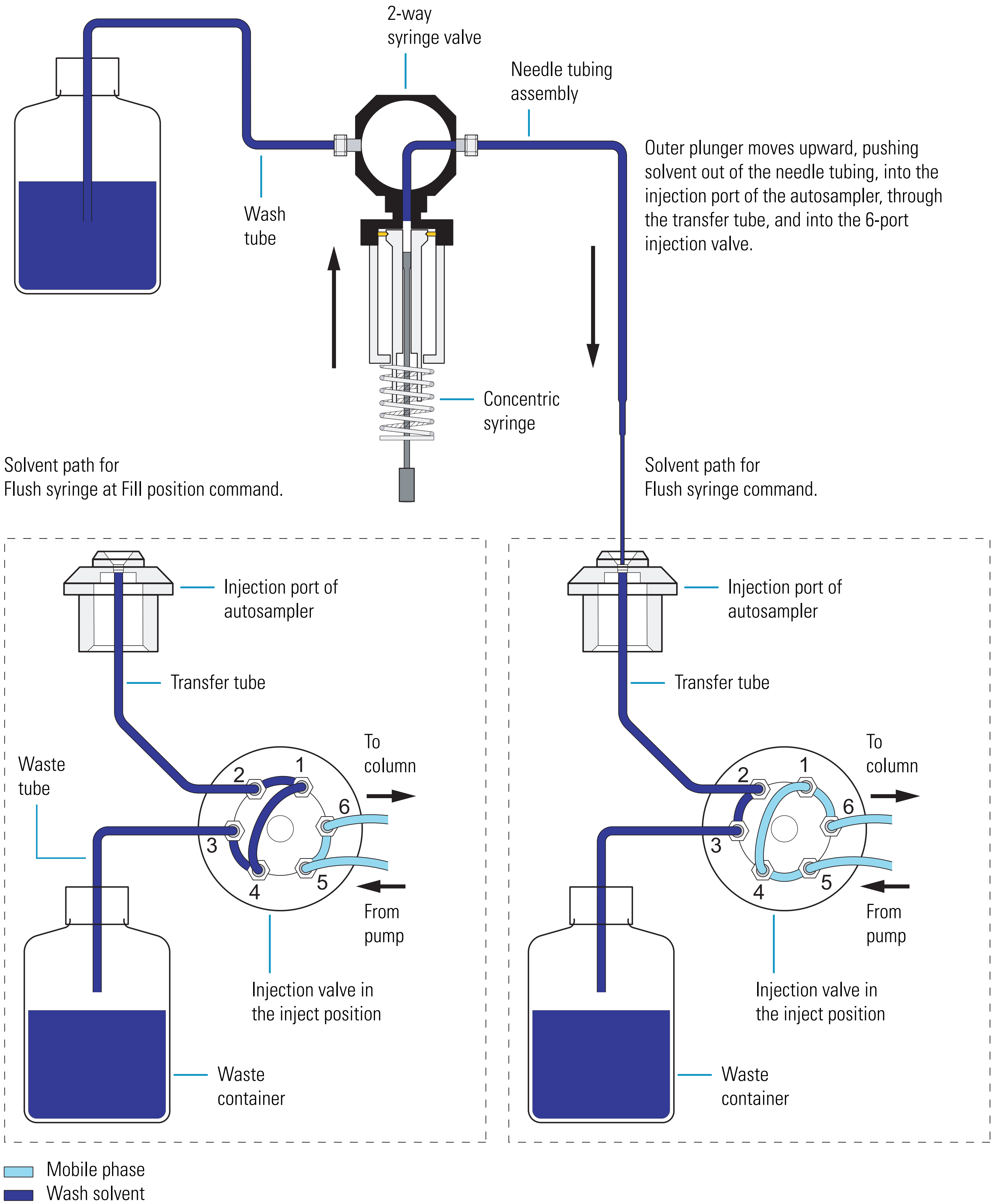


3. Initiate the Flush syringe direct control command:
   a. Select **Flush syringe** from the list of commands.

      The parameters for the command appear below the list.
   b. In the Reservoir list, select **Flush bottle**.
   c. In the Volume box, type an appropriate flush volume.

      The maximum flush volume is 6000 µL.
   d. Click **Apply** to download the command to the autosampler.
4. Verify that the wash bottle tubing and syringe are free of air (see Figure 103).

Figure 103. Close the Direct Control dialog box.Schematic of solvent path for flushing the syringe



Removing and Installing Sample Trays

If you did not select the Verify door is closed check box when you specified the configuration options for the autosampler, the XYZ arm does not move to the back of the tray compartment when you open the tray compartment door.

For information on specifying the configuration settings for the Surveyor Autosampler Plus, see “Surveyor Autosampler Plus Configuration Settings” on page 39.

❖ To remove or install a sample tray

1. Open the Direct Control dialog box for the autosampler.
2. In the Direct Command list, select **Position Arm to Access Tray.**
3. Click **Apply**.

   The XYZ arm moves to the back of the tray compartment.

Controlling the Tray and Oven Compartment Temperatures

The autosampler has two controlled temperature zones: the tray compartment and the column oven compartment. Before you start a sequence run, equilibrate the controlled temperature zones at the temperature specified in the instrument method.

Use the direct commands in the Direct Control dialog box or the Inlet Direct Control dialog box to control the oven and tray compartment temperatures. For information on opening the Direct Control and Inlet Direct Control dialog boxes, see “Autosampler Direct Controls” on page 186.

❖ To download a new column oven temperature

1. Select **Set oven temperature** from the list of commands.
2. In the Temperature box, type an appropriate value for the column oven temperature.

   The range is 5 to 95 °C.

   IMPORTANT Do **not** set the oven temperature above the boiling point for the mobile phase solvent.

3. Click **Apply**.

❖ To turn the column oven off

1. Select Turn off oven temperature from the list of commands.
2. Click **Apply**.

❖ To download a new tray compartment temperature

1. Select **Set tray temperature** from the list of commands.
2. In the Temperature box, type an appropriate value for the tray compartment temperature.

   The range is 0 to 60 °C.
3. Click **Apply**.

❖ To turn the column oven off

1. Select **Turn off tray temperature** from the list of commands.
2. Click **Apply**.

Sequence Setup

This chapter describes how to acquire and view chromatographic and PDA spectral data.

Contents

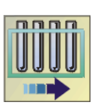
Creating a Single Sample Sequence

To set up a sequence to inject a single sample, follow these procedures in order:

1. "Opening the Sequence Setup Window," on this page
2. "Creating the Sequence" on page 197
3. (Optional) "Selecting the Vial Locations Interactively" on page 200
4. "Saving the Sequence" on page 202

Opening the Sequence Setup Window

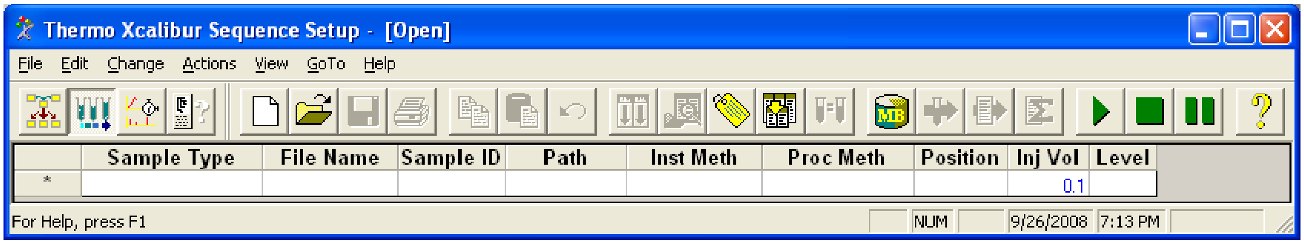
❖ **To open the New Sequence Template dialog box**



1. Click the **Sequence Setup** button on the Home Page.

   The Thermo Xcalibur Sequence Setup window appears (see Figure 104).

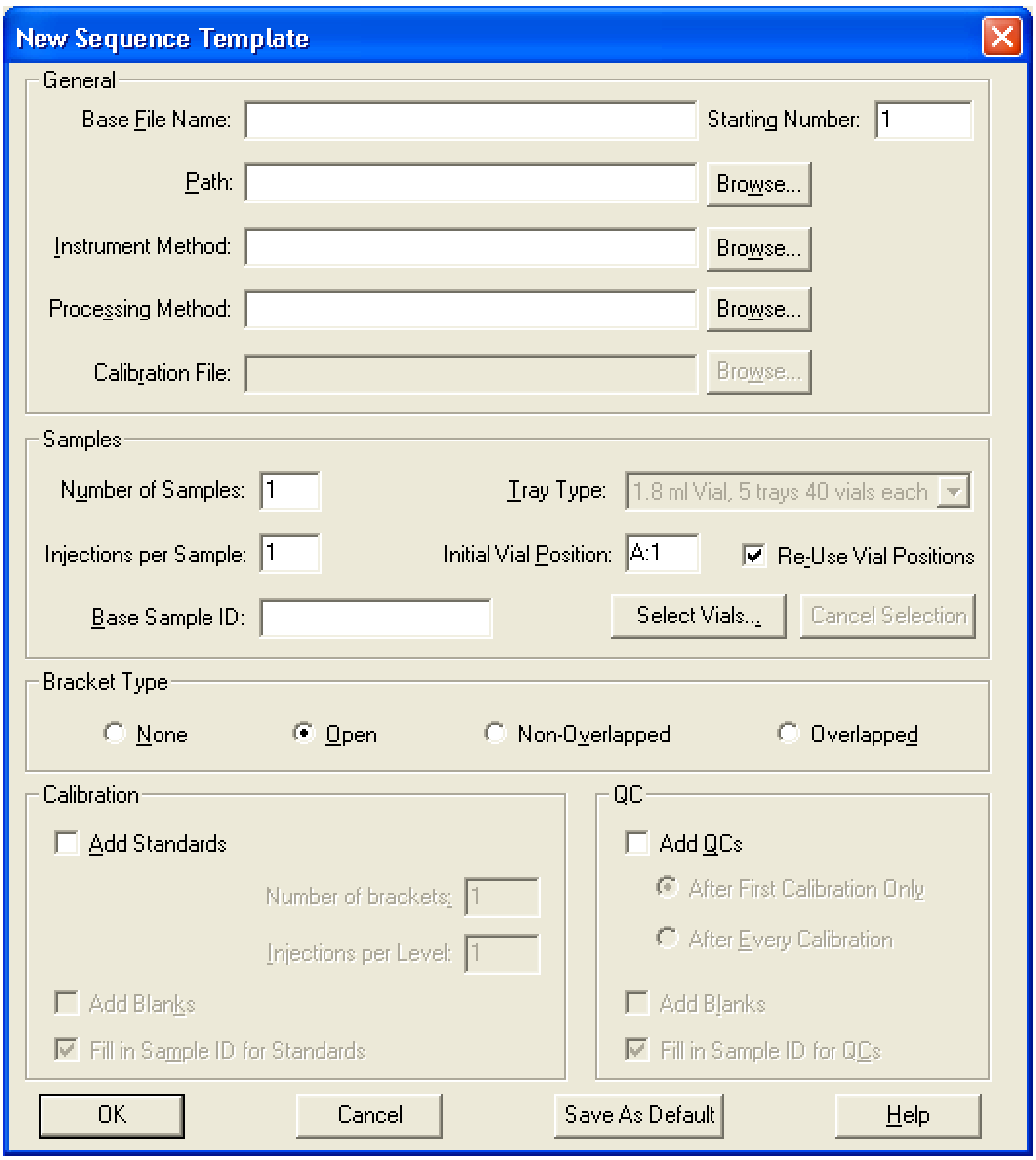
Figure 104. Sequence Setup window



2. From the Sequence Setup window, choose **File > New**.

   The New Sequence Template dialog box appears (see Figure 105). Go to the next topic, "Creating the Sequence" on page 197.

Figure 105. New Sequence Template dialog box



Creating the Sequence

❖ To create a new sequence

1. If it is not already open, open the New Sequence Template dialog box (see "Opening the Sequence Setup Window" on page 195).

2. In the General area, make the following entries and selections:

   a. In the Base File Name box, type a name for the raw data file.

   b. Browse to the data file directory where you want to store your raw data files.

      The data system adds the .raw file extension to the data files that contain the chromatographic and spectral data.

   c. Browse to the instrument method that you want to use to acquire your raw data files.

      Instrument methods have a .meth file extension. The Instrument Setup view for the Surveyor Plus devices is described in Chapter 3, "Instrument Method Setup."

   d. If you have not yet created a processing method that contains the information needed to quantitate your unknowns, do not select a processing method.

      You can create a processing method and reprocess your stored data files at a later date. Processing methods have a .pmd file extension. For information on performing tests to determine the suitability of your chromatographic method and on creating calibration curves to quantitate your unknowns, refer to the *Thermo Xcalibur Quantitative Analysis User Guide*.

3. In the Samples area, specify the vial locations by doing one of the following:

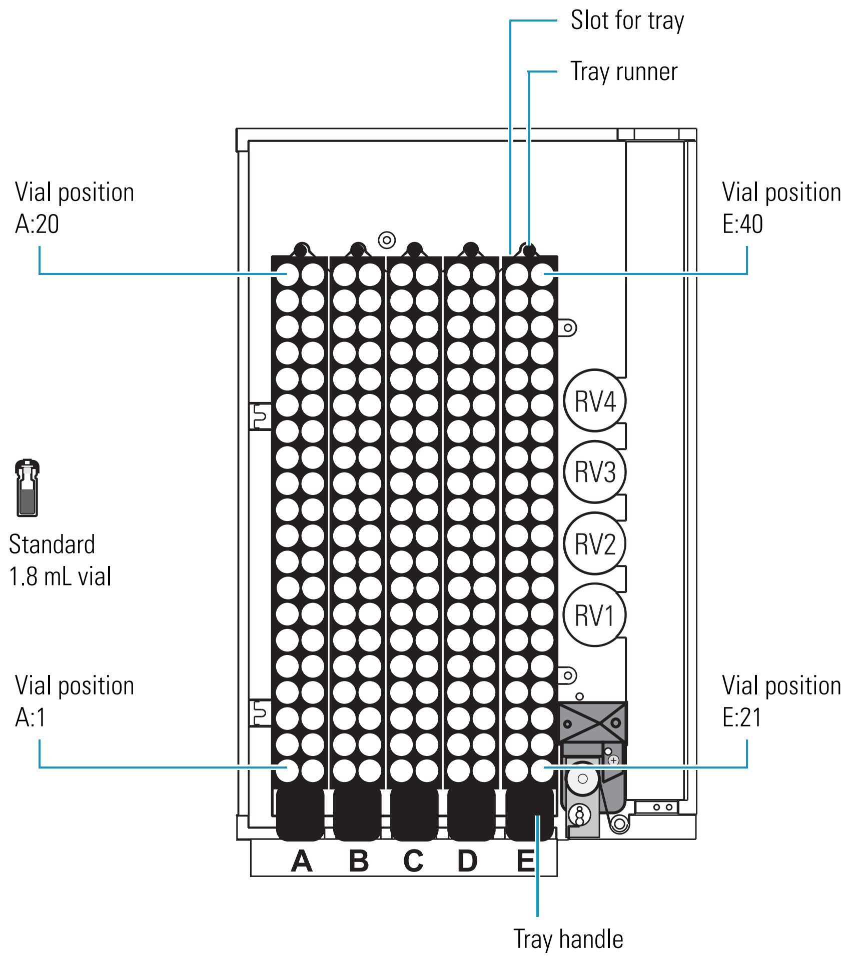
   - To specify a contiguous set of sample vials or well locations:

     – In the Number of Samples box, type the number of samples that you want to analyze.

     – In the Initial Vial Position box, type the vial position.

     The vial positions for the conventional trays are shown in Figure 106.

Figure 106. Vial positions for conventional sample trays



For more information on the vial locations for conventional trays and the well locations for microwell plates, see "Vial and Well Notation" on page 6.

- To select a non-contiguous set of sample vials or well locations, click Select Vials, and then select the vial or well locations by using the Vial Selection dialog box.

  For information on using the Vial Selection dialog box, see "Selecting the Vial Locations Interactively" on page 200.

4. In the Injections per Sample box, type the number of injections per sample that you want the autosampler to make per vial or well location.
5. In the Base Sample ID box, type an identifying name for the sample.

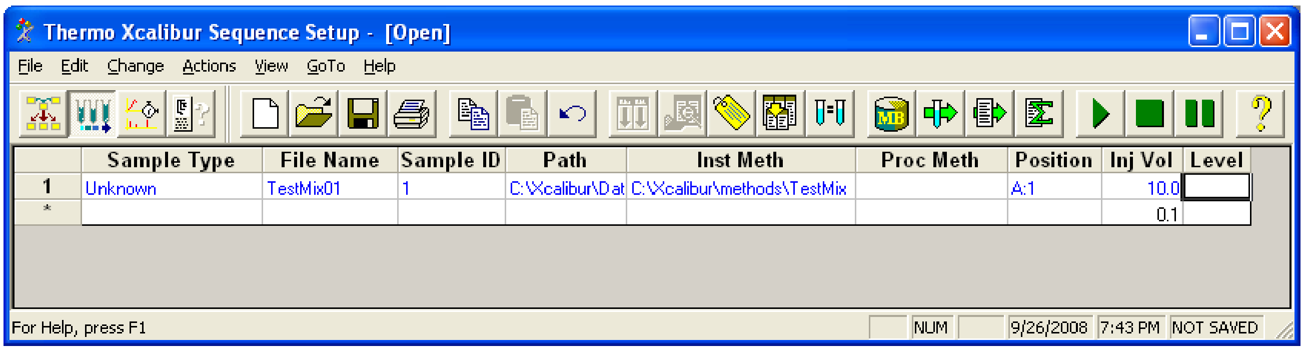
   Base sample IDs are optional. If you do not enter a sample identification, the data system automatically uses the vial position as the sample identification. If you enter a sample identification, the data system automatically appends the vial position to your entry.
6. Click **OK** to display your sequence spreadsheet (see Figure 107).

The injection volume displayed in the Inj Vol column matches the injection volume contained in your instrument setup method. You can override this injection volume value.

7. To change the injection volume, double-click the spreadsheet cell containing the injection volume value that you want to change, highlight the current value, and then type a new value in the cell.

   For full details of all the parameters in the New Sequence Template dialog box, refer to the Help or the Xcalibur manual set.

Figure 107. Sequence Setup view, showing newly created one line sequence

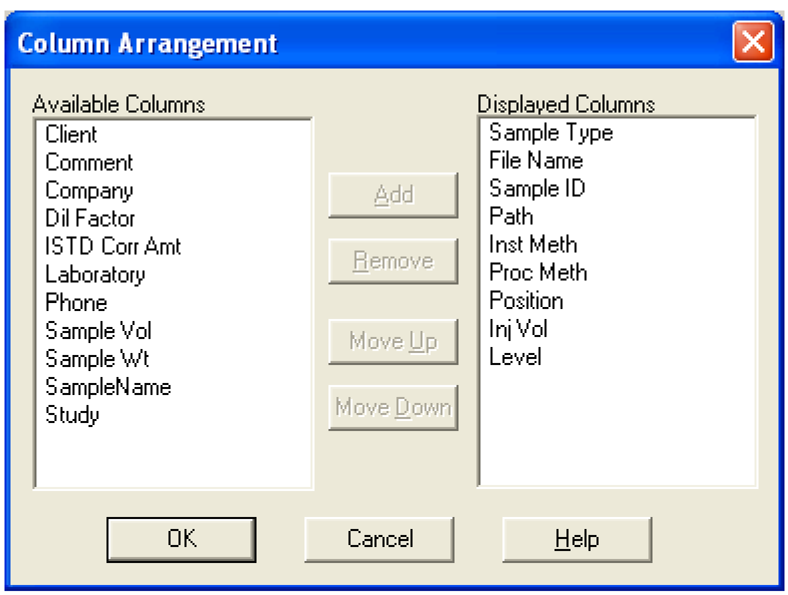


8. To alter the current column arrangement:

   a. Click the **Column Arrangement** toolbar button.

      The Column Arrangement dialog box appears (see Figure 108).

Figure 108. Column Arrangement dialog box



b. Do one of the following:

- To add a column to the sequence, select the column from the Available Columns list, and click **Add**.
- To remove a column from the sequence, select the column from the Displayed Columns list, and click **Remove**.
- To alter the position of the columns in the sequence, select the column from the Displayed Columns list, and click either **Move Up** or **Move Down** as appropriate.

Selecting the Vial Locations Interactively

Use the Vial Selection dialog box to select specific vials for the sequence template.

From the Home Page window, click the Sequence Setup button, and then click File New to open the New Sequence Template. In the Samples group box, click the Select Vials button to open the Vial Selection dialog box.

❖ To interactively select vial or well locations for the sequence list

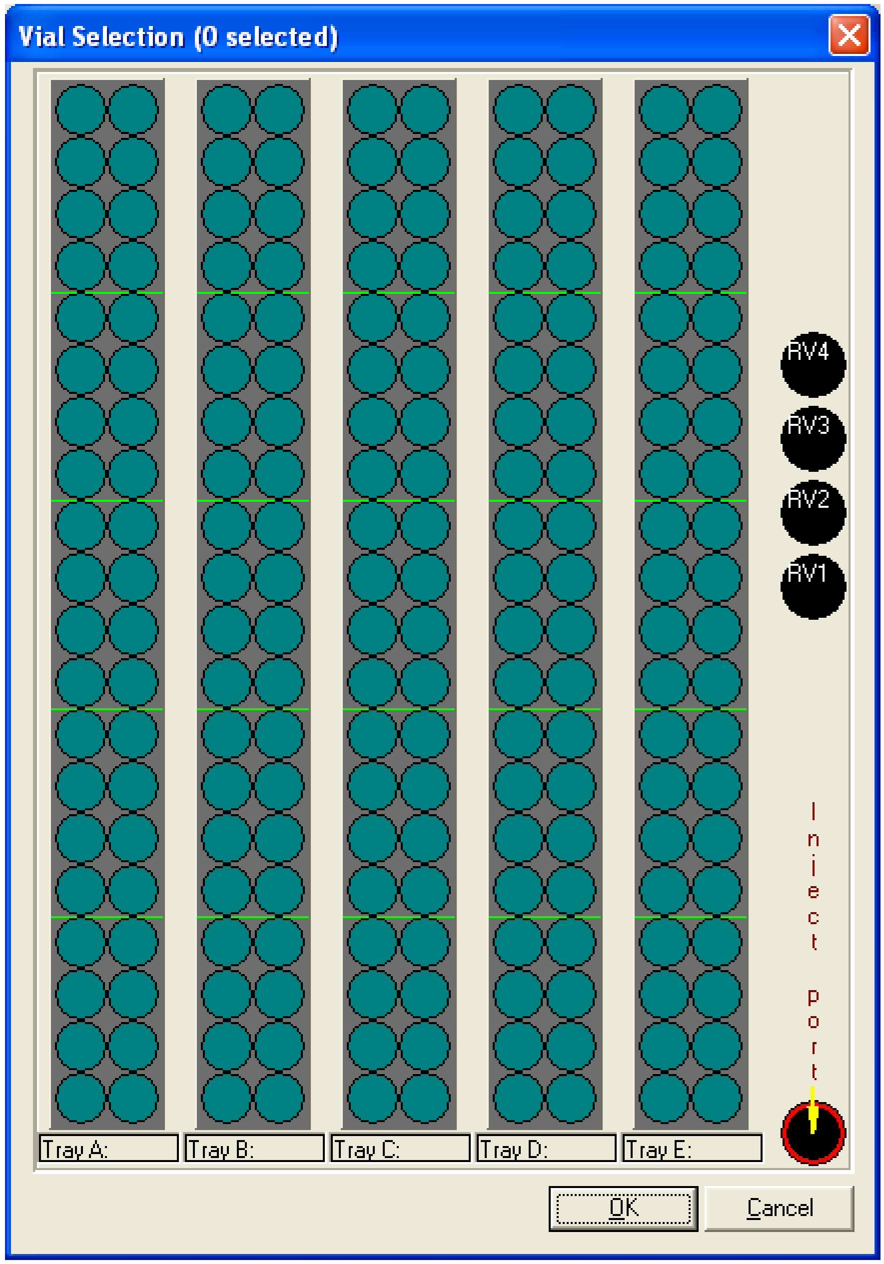
1. In the Sequence Setup window, choose **File > New**.

   The New Sequence Template dialog box appears.

2. In the Samples area, click **Select Vials**.

   The Vial Selection dialog box appears (see Figure 109).

Figure 109. Vial Selection dialog box



3. Click on a vial position to add it to the sequence template.

4. When you have finished making your selection, click **OK**.

   The vial or well locations appear in the position column in the order that you selected them.

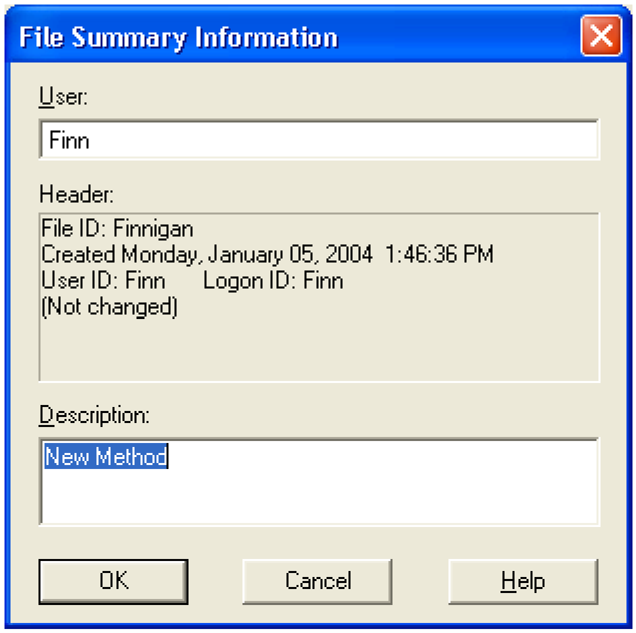
Saving the Sequence

❖ To save the sequence

1. Choose **File > Save As**.

   The File Summary Information dialog box appears (see Figure 110).

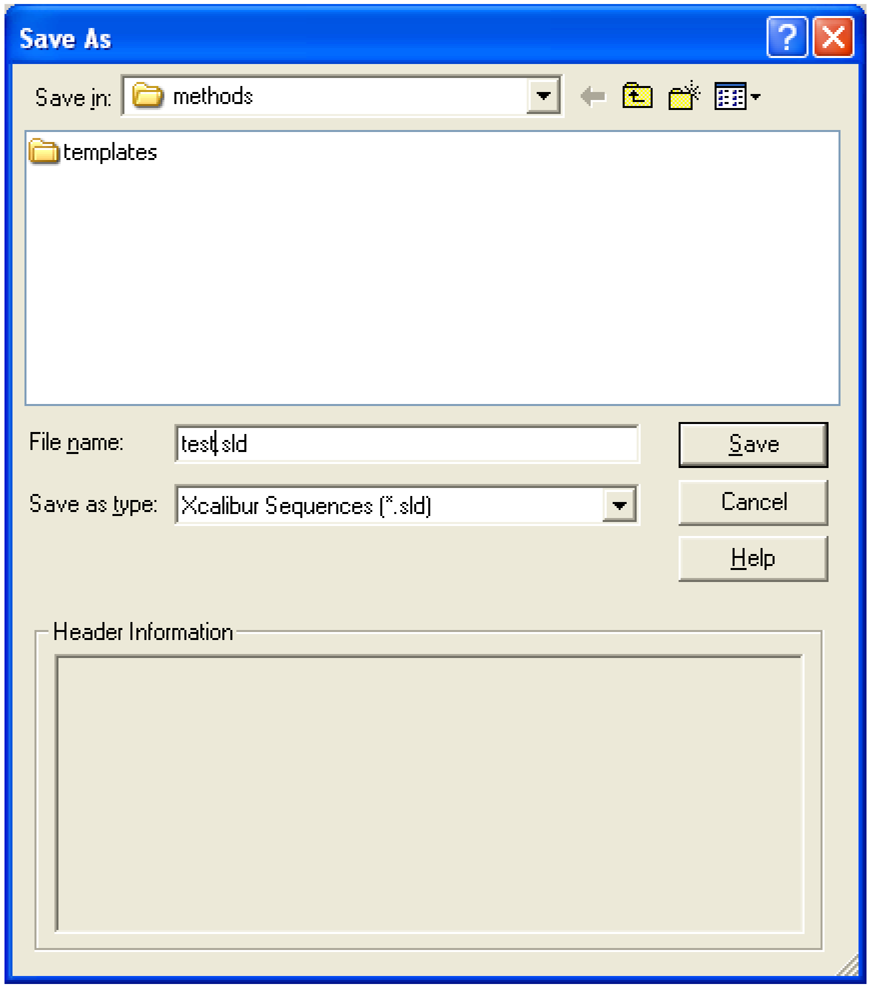
   Figure 110. File Summary Information dialog box



2. In the Description box, type an appropriate description. Then click **OK**.

   The Save As dialog box appears (see Figure 111).

Figure 111. Save As dialog box



3. Browse to the appropriate folder where you want to save the sequence.
4. In the File Name box, type a file name.
5. Click **Save**.

Equilibrating the Chromatographic Column

Warming up the deuterium lamp and equilibrating the LC column reduces baseline drift. For information on warming up the D2 lamp, see “Turning the Lamps On or Off” on page 157.

An LC column requires 10 to 20 column volumes to equilibrate to the initial mobile phase conditions of an instrument method. For example, it takes approximately 17 mL of mobile phase to equilibrate a typical 4.6 mm ID×10 cm length column.

To calculate the volume of an LC column, use the following equation:

$$V_m = \Pi r^2 \times L$$

Where:

V_m = volume in mL

r = column radius in cm (radius = inner diameter/2)

L = column length in cm

❖ To equilibrate your LC column

1. From the Thermo Xcalibur Roadmap menu, choose **GoTo > Instrument Setup** to display the Instrument Setup window.

2. Download the same solvent percentages and flow rate as those contained in your method to the pump:

   - For information on downloading new solvent conditions to the Surveyor LC Pump Plus and starting the solvent flow, see “Surveyor LC Pump Plus Direct Controls” on page 179.

   - For information on downloading new solvent conditions to the Surveyor MS Pump Plus and starting the solvent flow, see “Surveyor MS Pump Plus Direct Controls” on page 184.

3. In the status view for your pump (available on the Status page of Info View), monitor the readings in the Pressure Status area to ensure that the pressure is appropriate for your application. See “Viewing the Status of Each Device” on page 131.

Loading the Autosampler

Before you load your samples into the autosampler, ensure that your samples are completely soluble in the mobile phase and that you have filtered your samples through a 0.5 micron filter (if necessary). These techniques minimize sample precipitation in the lines and remove particulate matter that could obstruct the flow through the autosampler's injection valve or the LC column. In addition, make sure that the vial caps are securely fastened onto the vials.

Before you start a sequence run, ensure that you have samples in the locations specified in your sequence.

Note To trigger the vial sensor, custom vials must be positioned in the tray such that the top of the vial reaches the minimum height of 1.55 inches. If you place vials that fall below this minimum height in the tray, the vial sensor does not detect them. When the sequence reaches a vial that is below the minimum height, the sequence halts, and the Vial Not Found message appears.

❖ To load a conventional tray into the autosampler

1. Open the left door of the autosampler.

   If you configured the Surveyor AS device driver to verify that the tray compartment door is closed, the XYZ arm automatically moves to the back of the tray compartment.

   Figure 29 on page 46 shows the location of the Verify door is closed check box, available in the Instrument Configuration window.

2. If necessary, use the direct controls to move the XYZ arm to the back of the tray compartment (see "Autosampler Direct Controls" on page 186).

3. Hold the tray handle, tilting the back end of the tray down. Insert the tray runner into the slot at the rear of the tray compartment. Lower the front of the tray into place. Then press down firmly to seat the tray.

Starting Data Acquisition

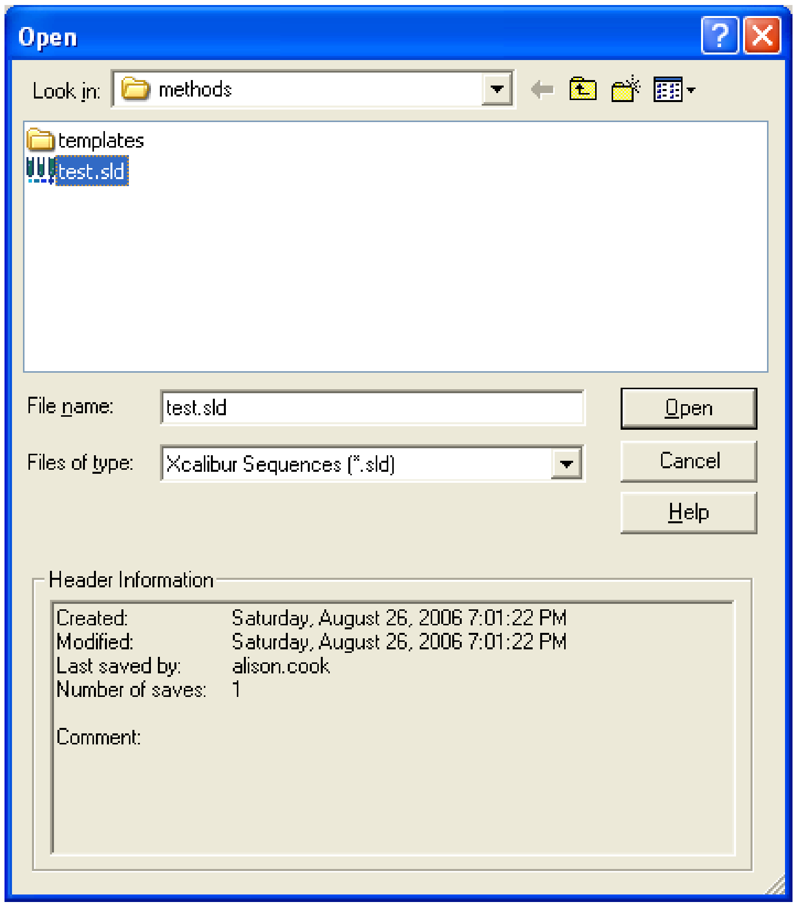
❖ To inject a sample and start data acquisition

1. Open the sequence file containing the information for the sample that you want to inject:

   a. From the Sequence Setup view, choose **File > Open**.

   The Open dialog box appears (see Figure 112).

   Figure 112. Open dialog box, showing the selection of a sequence file

   b. Browse to the appropriate folder.

   c. Select the sequence that contains the sample you want to run.

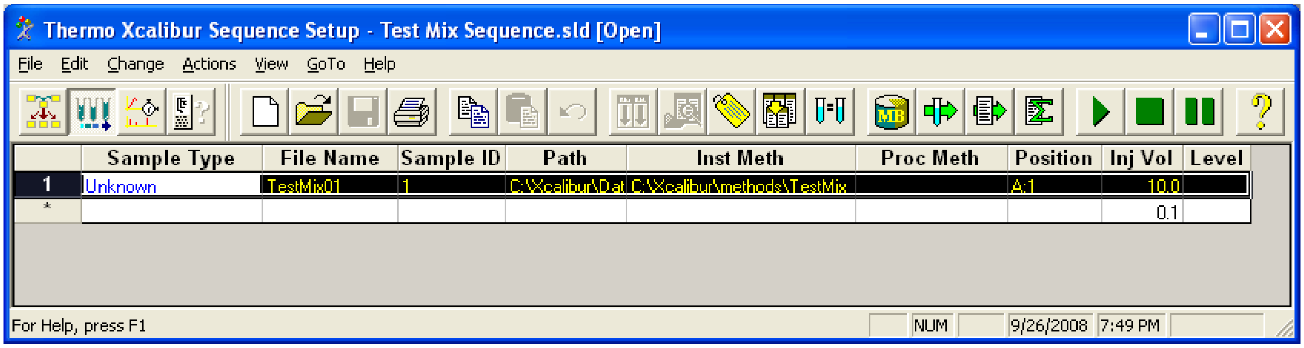
   Sequence files are identified by their .sld file extension.

   d. Click **Open**.

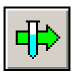
   The selected sequence appears.

2. Highlight the sequence row that you want to run. Do this even if the sequence contains just one row. Figure 113 shows a highlighted sequence row.

Figure 113. Sequence Setup view, showing the first row selected



3. Confirm that you have a vial in the position specified in the sequence row.

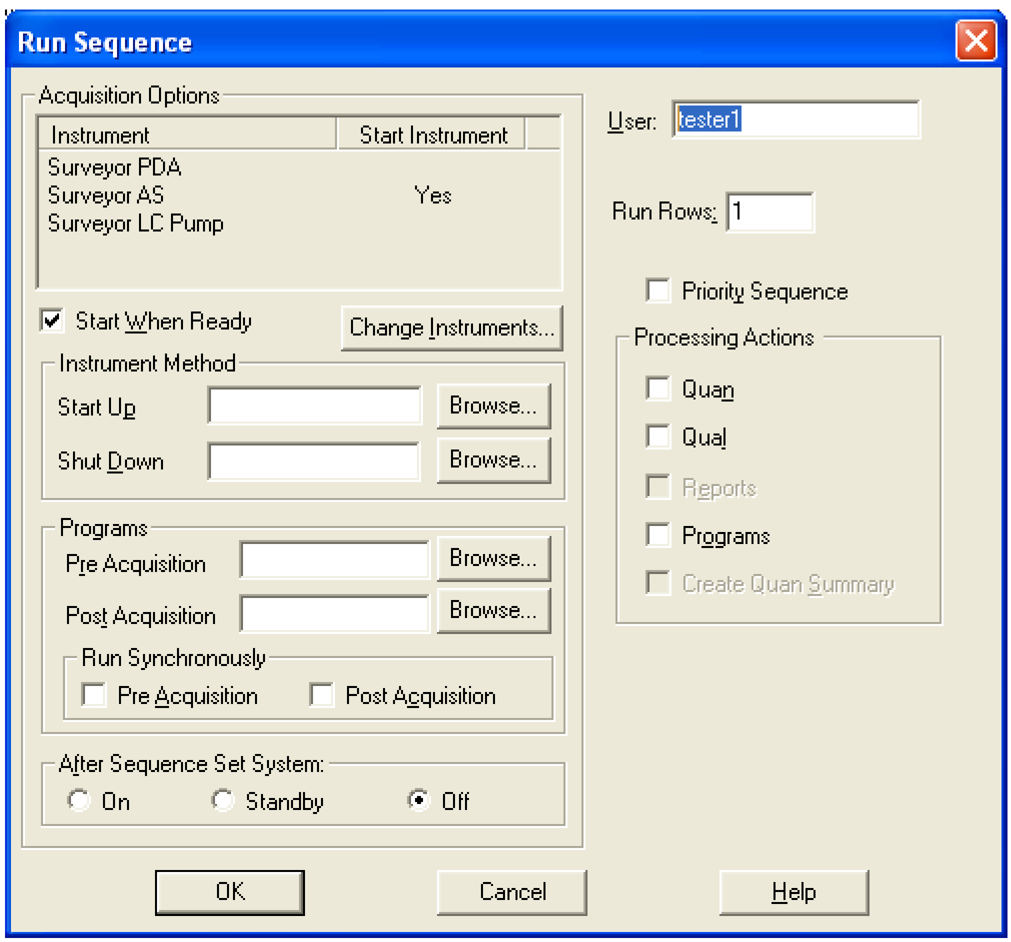


4. From the toolbar, click the **Run Sample** button.

   The Run Sequence dialog box appears (see Figure 114).

   The User box contains your login name, and the Run Rows box contains the row number that you selected in the sequence spreadsheet.

Figure 114. Run Sequence dialog box



5. Confirm the following:

   - The MS detector and LC components are configured for operation as Xcalibur devices in the Instrument list.

   - The autosampler is specified as the start instrument. Yes is displayed in the Start Instrument column next to Surveyor AS (see Figure 115).

   Figure 115. Surveyor AS specified as the start instrument

   After the autosampler injection valve switches to the inject position, the autosampler sends a signal to the detector to begin data acquisition.

6. To start the run automatically, select the **Start When Ready** check box.

   After you complete the entries and selections in the Run Sequence dialog box and click **OK**, the run begins after the pump sends a pump ready signal to the autosampler. The pump does not indicate the Ready state until it monitors a stable backpressure as defined in your instrument method.

   Note To manually start an acquisition, clear the **Start When Ready** check box and choose **Actions > Start Analysis** from the Sequence Setup menu. To subsequently control the acquisition, choose **Actions > Pause Analysis** or **Actions > Stop Analysis**.

7. In the After Sequence Set System area, select one of these three options:

   - To keep the devices in the On state, select the **On** option.

   - To automatically turn off the solvent flow from the pump and the detector's lamps at the end of the sequence run, select the **Off** option.

   - To turn off the solvent flow from the pump but keep the lamps at the end of the sequence run, select the **Standby** option.

8. Keep the parameters in the other areas at their defaults.

   You can use the Instrument Method and Programs areas in the Run Sequence dialog box to specify particular acquisition or processing requirements. For full details of these features, refer to the Xcalibur Help.

9. Click **OK** to start the run.

Working with the Real Time Plot View

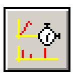
You can view and review data as it is being acquired in the Real Time Plot view.

This section contains these topics:

- Viewing Data Acquisition
- Reviewing Real-Time Data
- Adding Cells to the Display

Viewing Data Acquisition

❖ To view the data as it is acquired



1. Choose **View > Real Time Plot View** or click the **Real Time Plot View** button on the Home Page toolbar.

Locked

Unlocked

2. If the display is not already locked, click the **Lock Display** button to lock the display.

   Locking the display lets you monitor the real-time progress of your run.

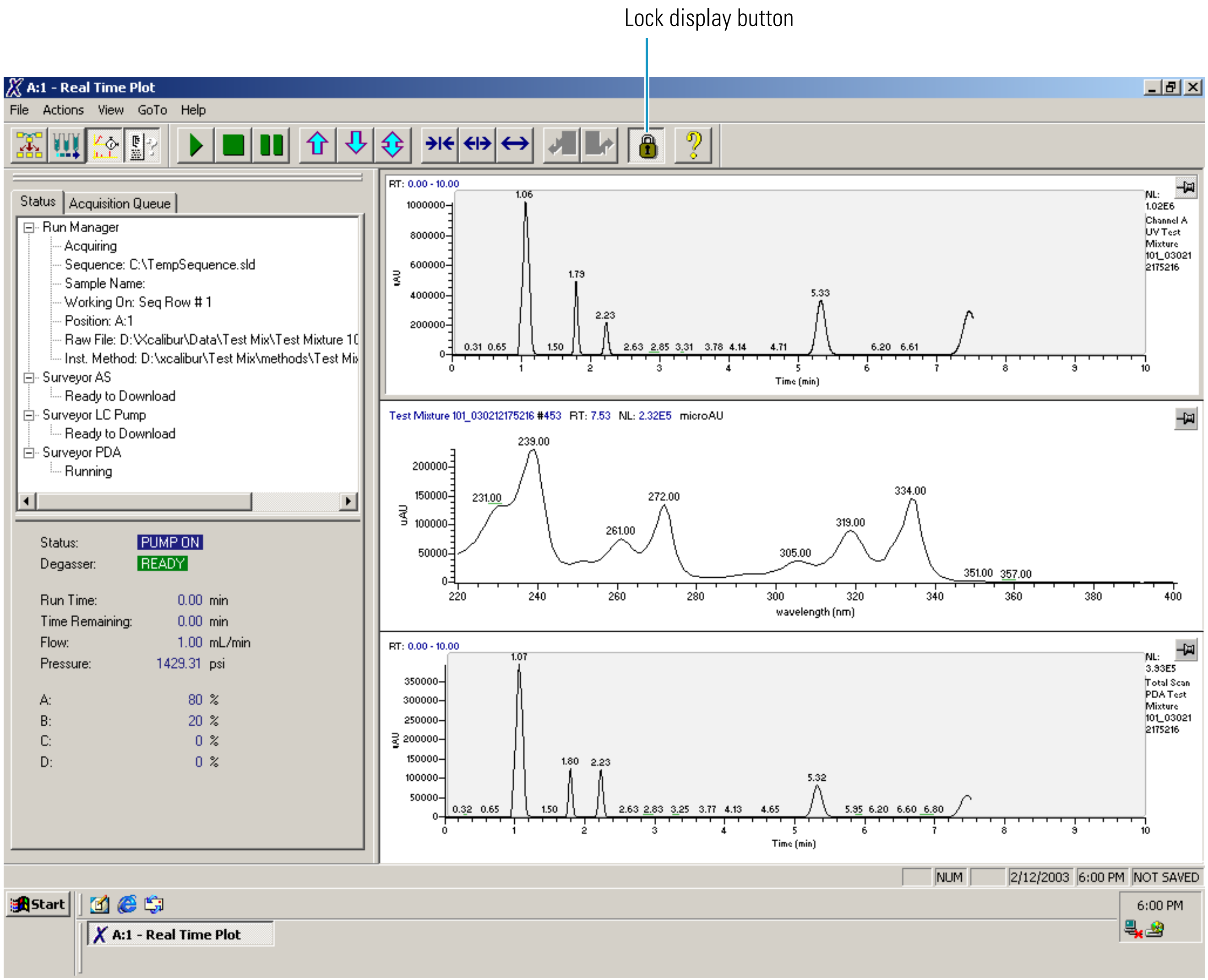
   In the unlocked position, you cannot monitor the real-time progress of your run, but you can review your data. For example, you can display the spectrum for a particular peak that has already eluted. Data collection continues off screen as you review your data.

   If you are collecting PDA scan data, a view similar to that shown in Figure 116 is displayed. The view contains three cells: a chromatogram cell, a spectrum cell, and a Total Scan cell.

The sample shown in Figure 116 contains a mixture of uracil, benzene, toluene, anthracene, and pyrene. The peak for pyrene is eluting at 7.5 minutes. The PDA detector parameters for the data file being acquired are as follows:

- Run Length: 10.0 minutes
- Rise Time: 1.0 seconds
- Scan Range: 220 nm to 400 nm
- Wavelength Step: 1 nm
- Bandwidth: 1 nm
- Three Channels:
  - 239 nm, Bandwidth: 9 nm
  - 254 nm, Bandwidth: 9 nm
  - 357 nm, Bandwidth: 9 nm

Figure 116. Real Time Plot view, showing the acquisition of PDA scan data and one discrete channel



Reviewing Real-Time Data

You can review the data as it is being collected.

❖ To view the spectrum for a particular peak in the chromatogram

1. To unlock the display, click the **Lock Display** button.

   After you unlock the display, data collection continues off screen.

2. Pin the spectrum cell by clicking the pin in the upper-right corner of the cell.

   The pin in the upper-right corner of the spectrum cell turns green. Cursor actions in other cells, such as the chromatogram cell, affect the view displayed in the pinned spectrum cell.

3. In the chromatogram cell, click the peak of interest.

   In the spectrum cell, a spectrum appears for the time point that you clicked.

4. Click the **Lock Display** button to resume monitoring real-time data acquisition.

5. Pin the chromatogram cell by clicking the pin in the upper-right corner of the cell.

   The pin in the upper-right corner of the chromatogram cell turns green. Cursor actions in other cells such as the spectrum cell affect the view displayed in the pinned chromatogram cell.

6. In the spectrum cell, click the wavelength of interest.

   In the chromatogram cell that contained the Total scan chromatogram, a scan chromatogram appears for the specific wavelength that you clicked.

7. Click the **Lock Display** button to resume monitoring real-time data acquisition.

Adding Cells to the Display

You can display multiple cells in the Real Time Plot view.

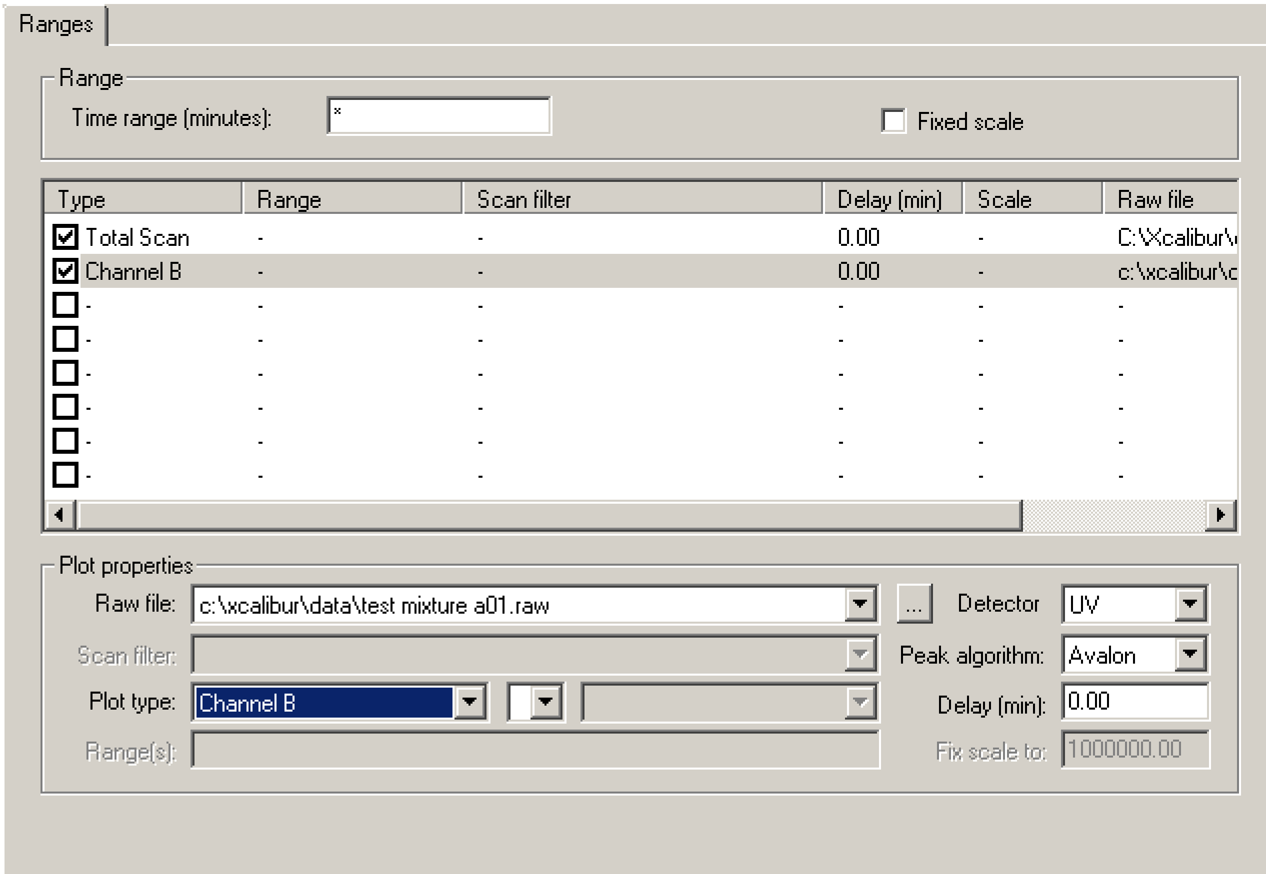
❖ To display multiple chromatogram cells

1. Click the chromatogram cell to make it the active cell with a gray border.

2. Choose **View > Ranges**.

   The Chromatogram Ranges dialog box appears (see Figure 117).

Figure 117. Chromatogram Ranges dialog box



3. For each cell that you want to add, do the following:
   a. In the Type column, select its corresponding check box.
   b. In the Detector list, select a detector.
   c. In the Plot Type list, select a plot type.
4. Click **OK** to close the Chromatogram Ranges dialog box.
5. Choose **View > Lock Display** to resume monitoring real-time data acquisition.

Qual Browser

This chapter provides an introduction to the Xcalibur Qual Browser facilities that you can use to review the PDA data contained in your .raw data files.

Contents

Opening a Raw Data File in Qual Browser

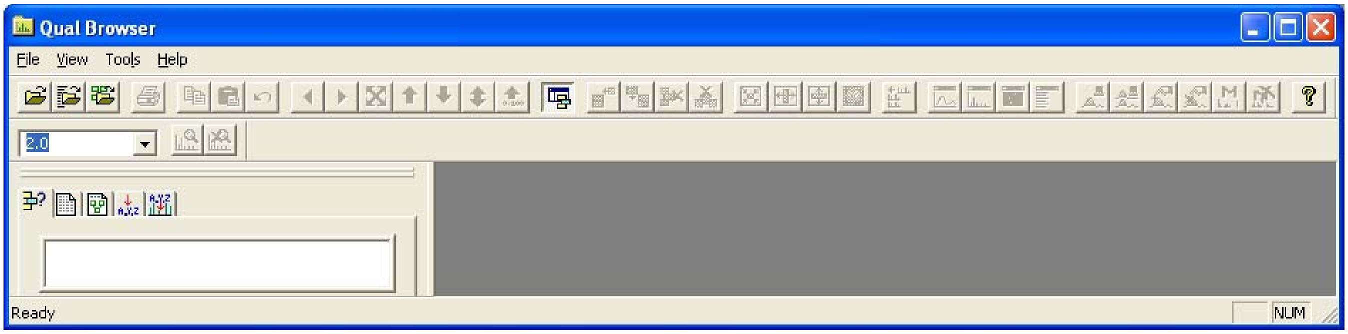
Data files containing the raw chromatographic and spectral data have the .raw file extension.

❖ **To open a .raw file in Qual Browser**

1. Click the Qual Browser icon in the Roadmap view of the Homepage window or choose **GoTo > Qual Browser**.

   The empty Qual Browser window appears (see Figure 118).

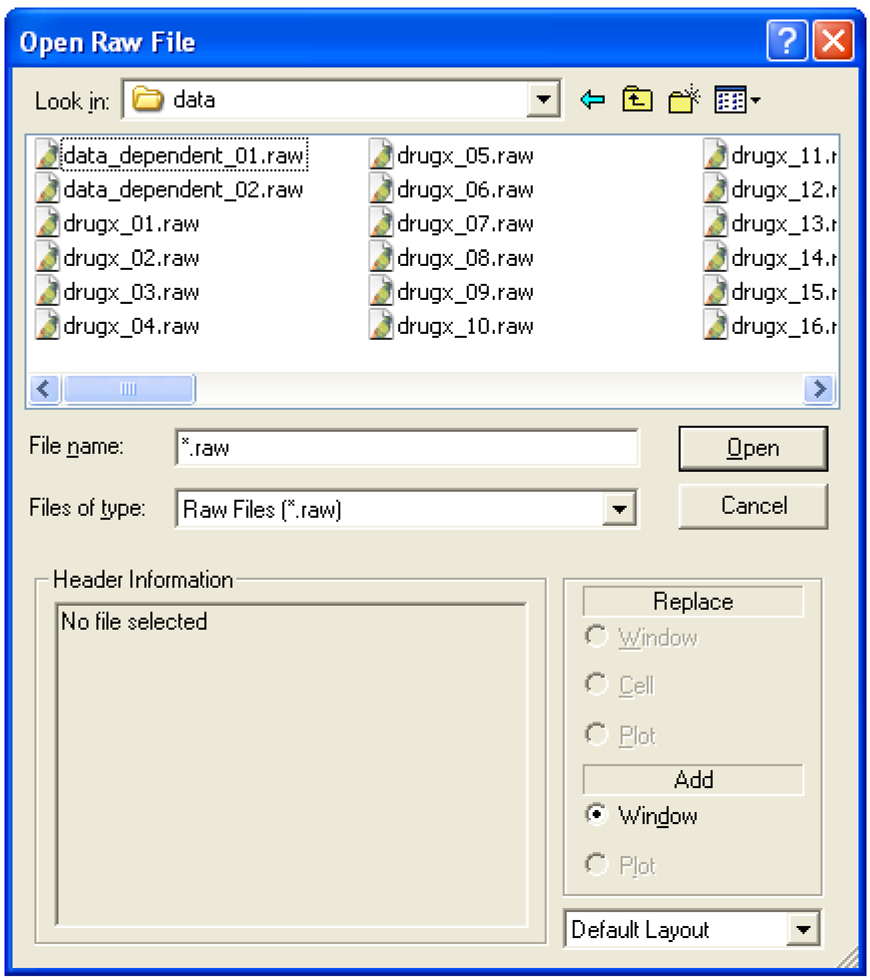
Figure 118. Empty Qual Browser window



2. Choose **File > Open**.

   The Open Raw Data File dialog box appears (see Figure 119).

   Figure 119. Open Raw File dialog box



3. Select the .raw file that you want to review.

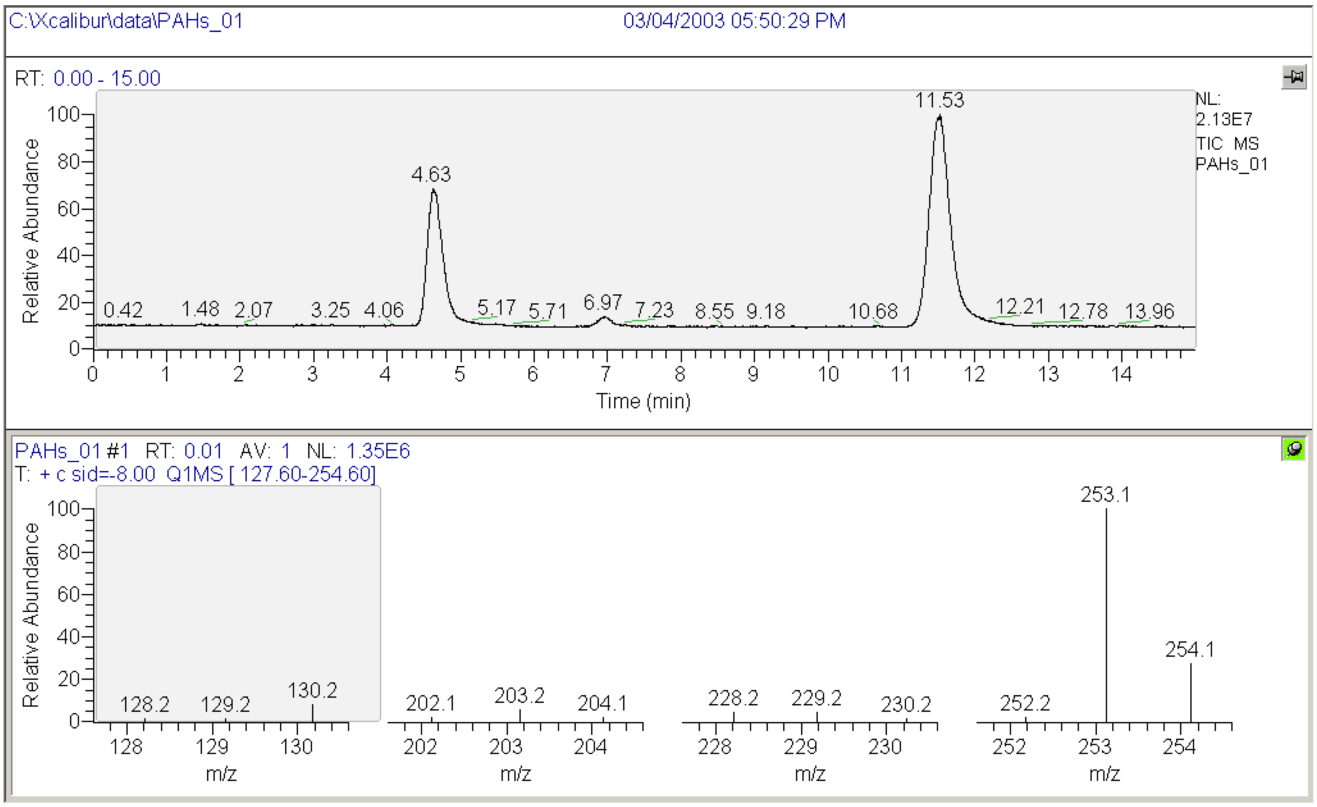
4. Select the layout from the list at the bottom of the dialog box.

   The available selections are Default Layout and Current Layout. Select **Current Layout** if the current layout for the Qual Browser window is different from the default layout and you want to apply it to your data file.

5. Click **Open**.

   If the default layout has not been modified and your raw file contains MS data in addition to PDA or UV data, the data file opens with the MS TIC chromatogram in the upper cell and a mass range spectrum in the lower cell. See "Working with the Cell Grid" on page 217 for an explanation of cells.The Y-axis for these cells is set to relative absorbance (see Figure 120).

Figure 120. Qual Browser view with a chromatogram cell that displays MS TIC data and a spectrum cell that displays mass range data

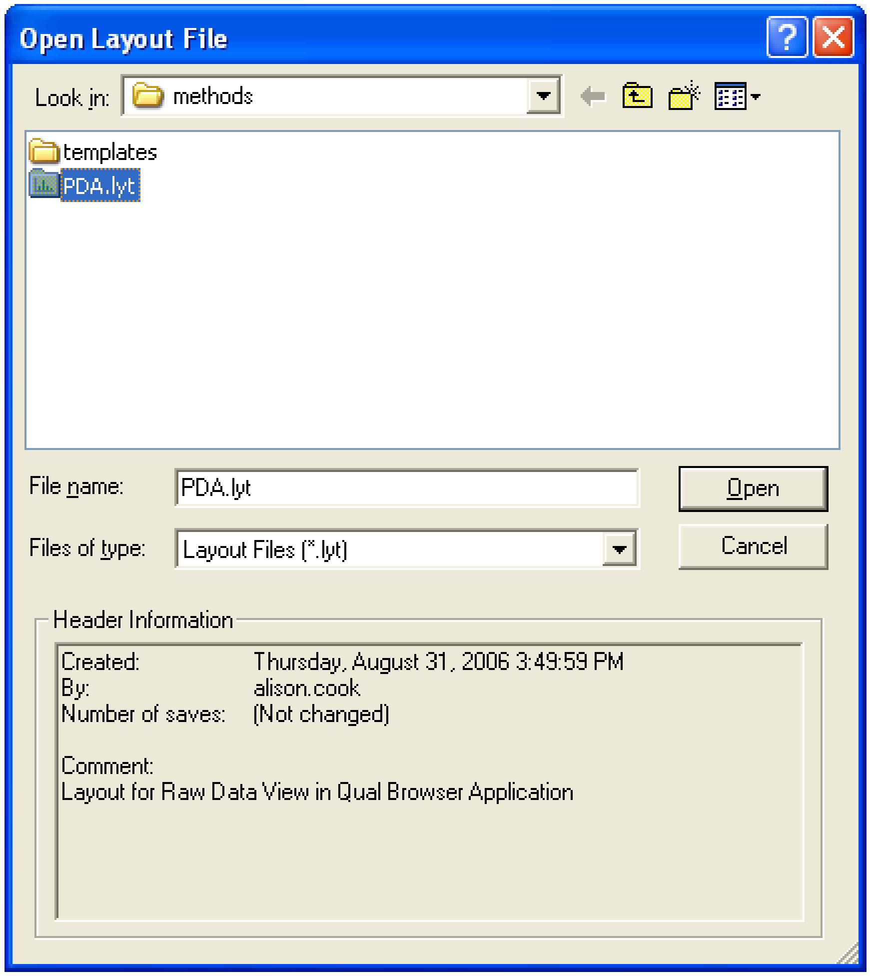


6. To apply a custom window layout to the Qual Browser view, do the following:

   a. Choose **File > Layout > Apply**.

      The Open Layout File dialog box appears (see Figure 121).

Figure 121. Open Layout File dialog box



b. Select a layout file from the list.

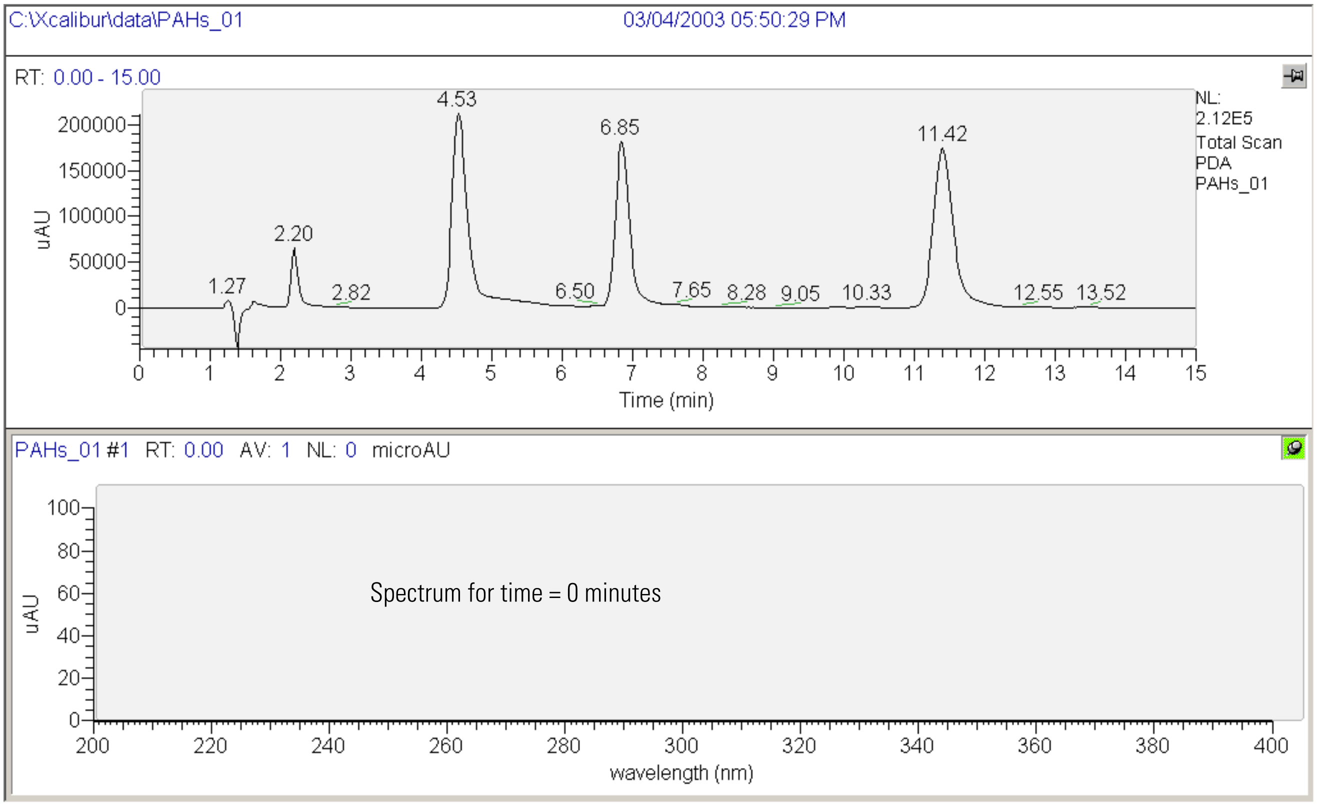
   Layout files have the file extension .lyt.

c. Click **Open** to apply the layout to the raw data file.

   Figure 122 shows a custom layout file applied to a raw data file containing both MS data and PDA data. The custom layout replaces the MS TIC chromatogram with a Total Scan chromatogram for the PDA data. It also replaces the mass range spectrum data from the MS detector with the spectral data from the PDA detector.

   For instructions on how to create a Layout file for your PDA data, see "Creating a Layout for PDA Data" on page 227.

Figure 122. Qual Browser view with a chromatogram cell that displays a Total Scan from the PDA detector and a spectrum cell that displays the spectral data from the PDA detector for time 0



Working with the Cell Grid

To use the Qual Browser facility, you must understand the concept of cell states and the effect of cursor actions in a cell. This section contains the following topics:

- Cell States
- Cursor Actions

Cell States

When you open a raw data file in the Qual Browser window, the information within the data file is displayed as a grid of cells.

There are three hierarchal states for a cell within the grid:

- Inactive Cells
- Active but Unpinned Cells
- Active and Pinned Cells

The grid always contains either one active but unpinned cell or one pinned cell. If the grid contains more than one cell, only one cell can be active and the rest of the cells are inactive.

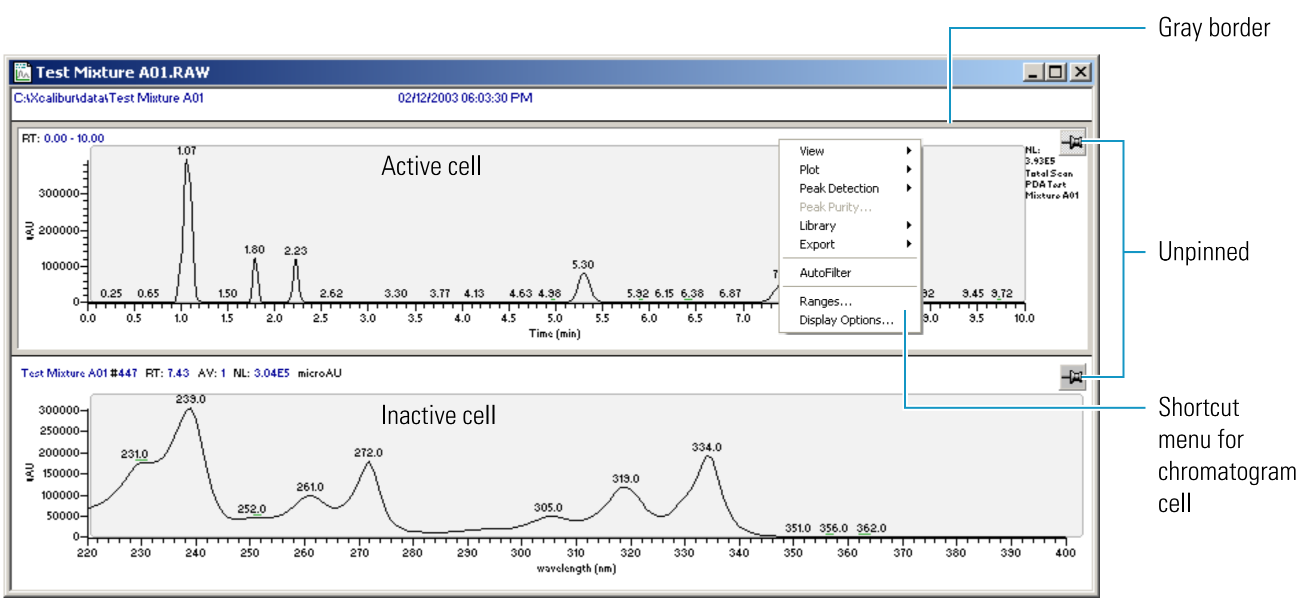
An unpinned cell has a gray pin icon in its upper-right corner.

A pinned cell has a green pin icon in its upper-right corner.

Inactive Cells

Inactive cells are not highlighted with a gray border and the pin icon in their upper-right corners are gray. The cell in the lower portion of Figure 123 is inactive as indicated by the absence of a gray border. Menu commands, toolbar buttons, and cursor actions do not affect inactive cells. To zoom in on the contents of a cell or access its shortcut menu, you must make the cell active.

Figure 123. Qual Browser window, displaying an active chromatogram cell and an inactive PDA spectrum cell



Active but Unpinned Cells

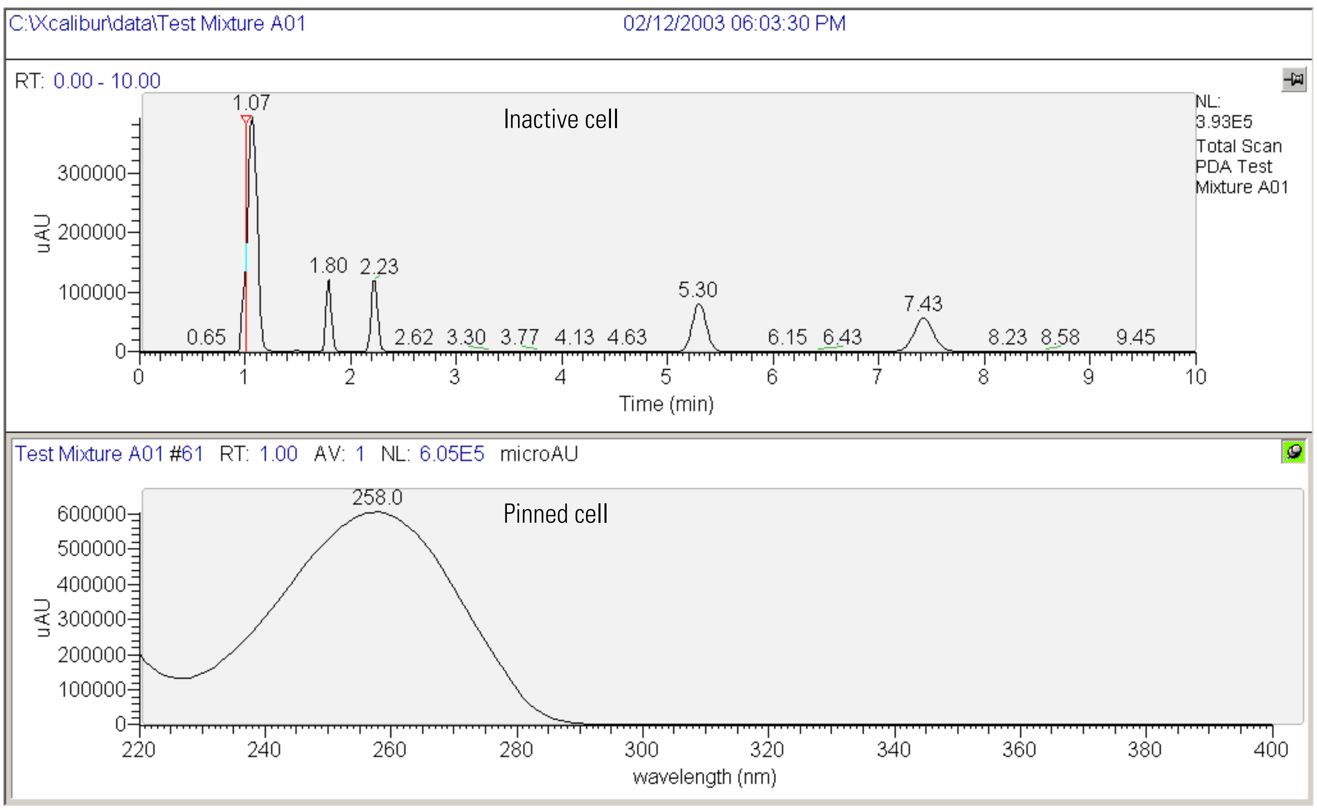
An active but unpinned cell is highlighted with a gray border, and the pin icon in its upper-right corner is gray. The cell in the upper portion of Figure 123 is active but unpinned. Menu commands, toolbar buttons, and cursor actions affect the active cell. If the grid does not contain a pinned cell, clicking an inactive cell in the grid makes it the active cell.

Active and Pinned Cells

Clicking the pin in the upper-right corner of a cell makes it the pinned cell within the grid. A pinned cell is an active cell that cannot be made inactive by clicking in another cell. Instead, actions performed in the inactive cells affect the pinned cell as described in the next topic, "Cursor Actions" on page 220. The lower cell in Figure 124 is a pinned cell.

To automatically change the range of a cell by clicking in the grid, you must pin the cell. For example, to display the spectrum for the 1 minute time point without opening the Spectrum Ranges dialog box, pin the spectrum cell, and then click the 1 minute time point in the inactive chromatogram cell.

Figure 124. Qual Browser window, displaying an inactive cell and a pinned cell



Cursor Actions

Within the cells of the grid, you can use the cursor in three ways:

- A click picks a point on the cell.
- A line dragged parallel to any axis picks a range.
- A line dragged in any diagonal direction selects an area.

The effect of these actions depends on the state of the cell. Within an active cell, cursor actions rescale the plot (see Table 53).

Table 53. Effect of cursor action in an active cell

Cursor action	Effect
Drag parallel to X-axis	Rescales graph showing selected X-axis range only, same Y-axis range.
Drag parallel to Y-axis	Rescales graph showing selected Y-axis range only, same X-axis range.
Dragged area	Rescales graph showing both the selected X-axis and Y-axis ranges.

If one of the cells is pinned, the cursor action in any of the inactive cells affects the pinned cell (see Table 54).

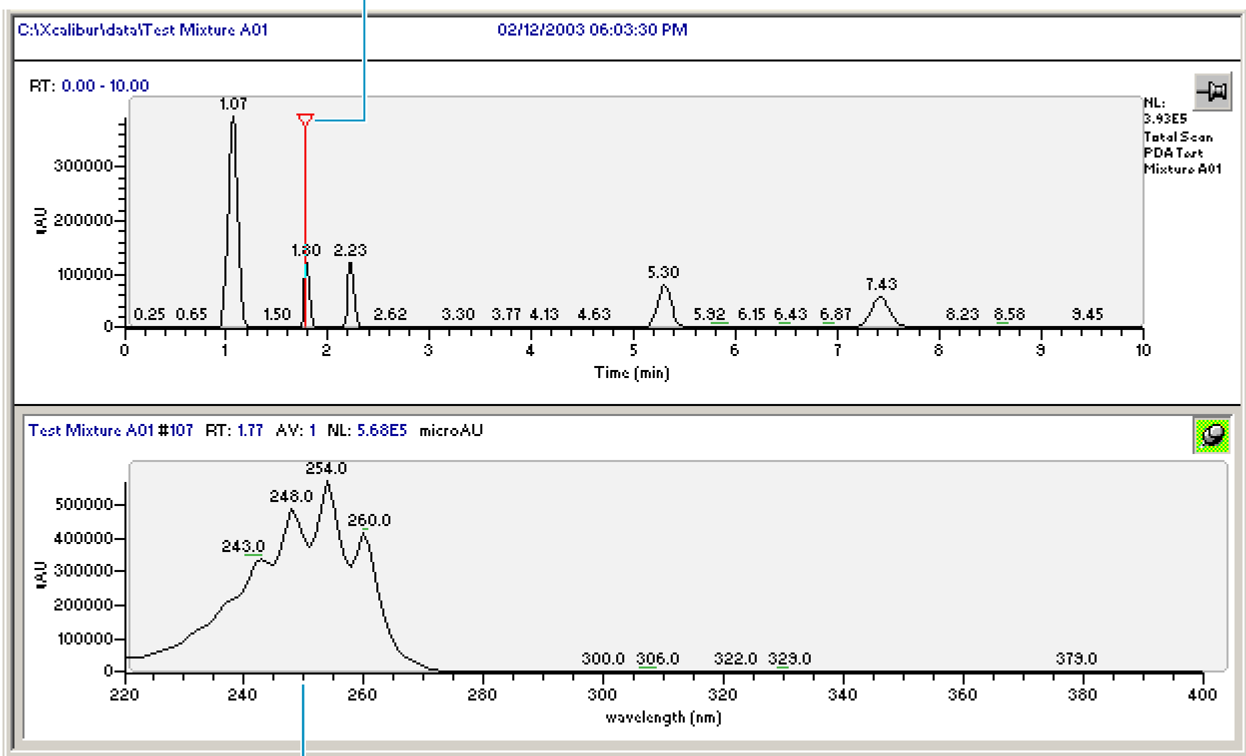
Table 54. Effect of cursor action in an inactive cell on the pinned cell

Pinned cell	Cursor action	Effect
Spectrum	Click in a chromatogram cell	The spectrum cell displays the spectrum at that retention time.
Chromatogram	Click in a spectrum cell	The chromatogram cell displays chromatogram for the wavelength selected in the spectrum cell.

In Figure 125, the spectrum cell on the bottom of the view is pinned. Clicking the 1.80 minute time point in the unpinned chromatogram cell causes the spectrum of benzene, which elutes at 1.8 minutes, to be displayed in the pinned spectrum cell.

Figure 125. Qual Browser window with a pinned spectrum cell and an unpinned chromatogram cell

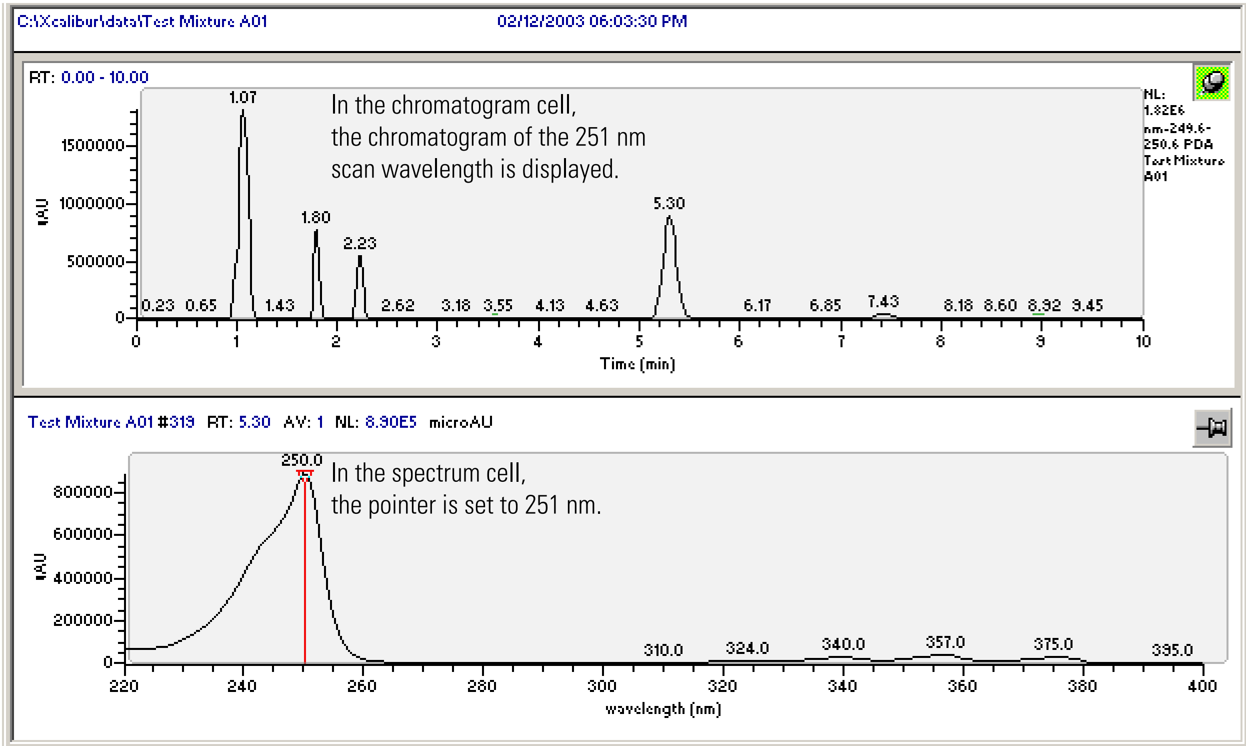
In the chromatogram cell, the pointer is set to 1.8 minutes, the peak apex for benzene.



In the spectrum cell, the spectrum of benzene, which elutes at 1.8 minutes under the chromatographic conditions used, is displayed.

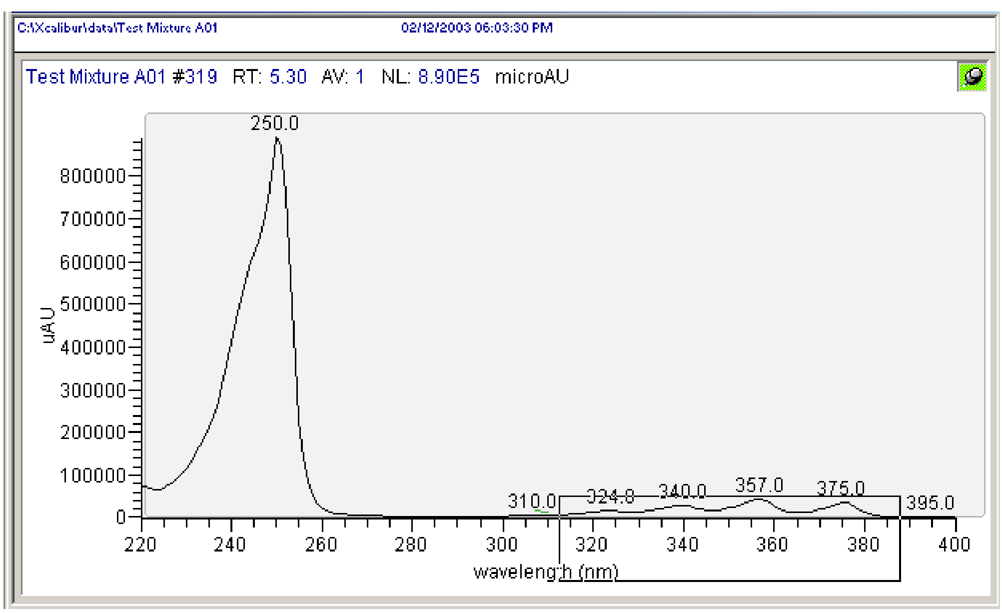
In Figure 126, the chromatogram cell on the top of the view is pinned. Clicking a specific wavelength in the unpinned spectrum cell displays the chromatogram of the scan wavelength in the pinned chromatogram cell.

Figure 126. Qual Browser window with a pinned chromatogram cell and an unpinned spectrum cell



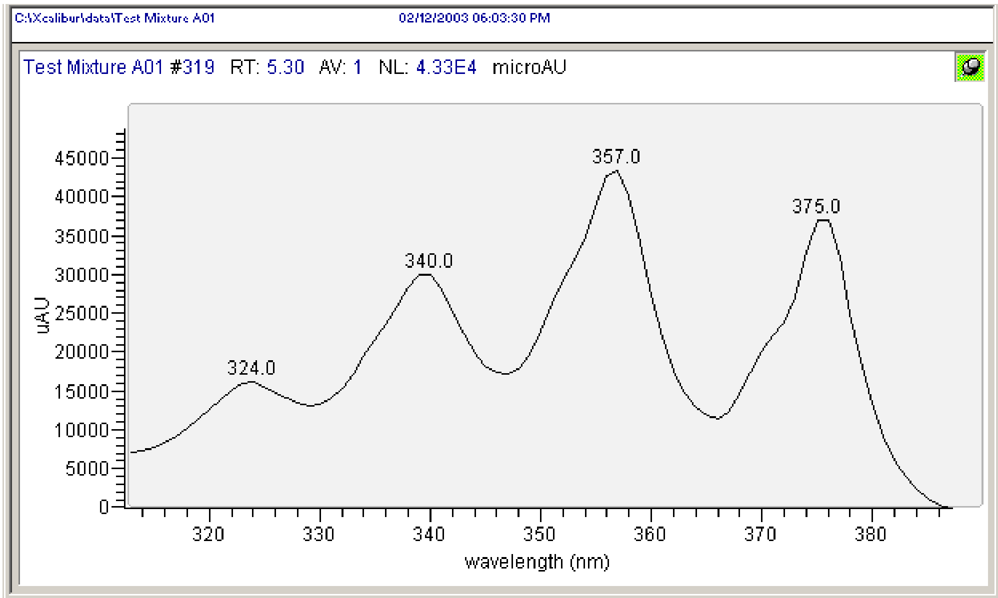
Clicking (Full Size) in the toolbar sizes the active or pinned cell to the full width and height of the window as shown in Figure 127.

Figure 127. Spectrum cell sized to full size of window



Dragging across a region in the active or pinned cell zooms in on that region as shown in Figure 128.

Figure 128. Full size view of the spectrum cell zoomed in on the 320 nm to 380 nm region



Changing the Font Size of the Display

Occasionally, you might want to change the font size of the data displayed in the Xcalibur data system. For example, you might want to increase the font size for screen captures that you plan to use for presentations.

❖ To increase the font size

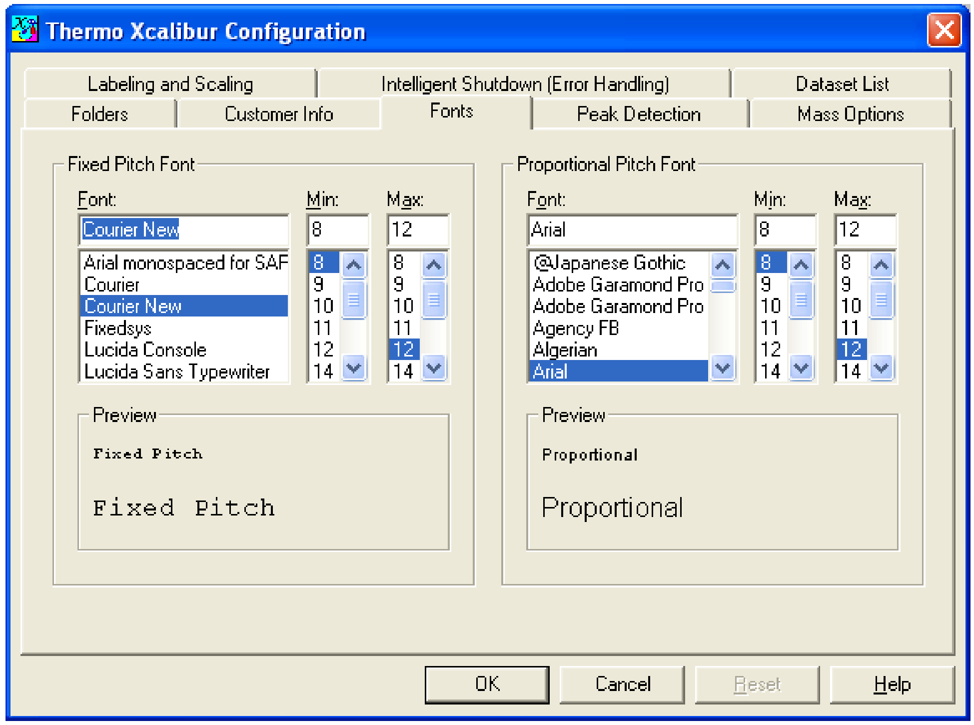
1. From the Thermo Xcalibur Roadmap window, choose **Tools > Configuration**.

   The Thermo Xcalibur Configuration dialog box appears.

2. Click the **Fonts** tab.

   The Fonts page appears (see Figure 129).

Figure 129. Fonts page in the Thermo Xcalibur Configuration dialog box



3. To increase the font size of the chromatogram, spectrum, and map axis labels, in the Proportional Pitch Font area, select a larger font size from the Max list, and select a larger font size from the Min list.

4. To increase the font size for the Spectrum List, Scan Header, Scan Filters, or Report, in the Fixed Pitch Font area, select a larger font size from the Max list, and select a larger font size from the Min list.

Figure 130 shows the chromatogram axes labeled with a font size of 8. Figure 131 shows the chromatogram axes labeled with a font size of 16.

Figure 130. Proportional pitch font size = 8

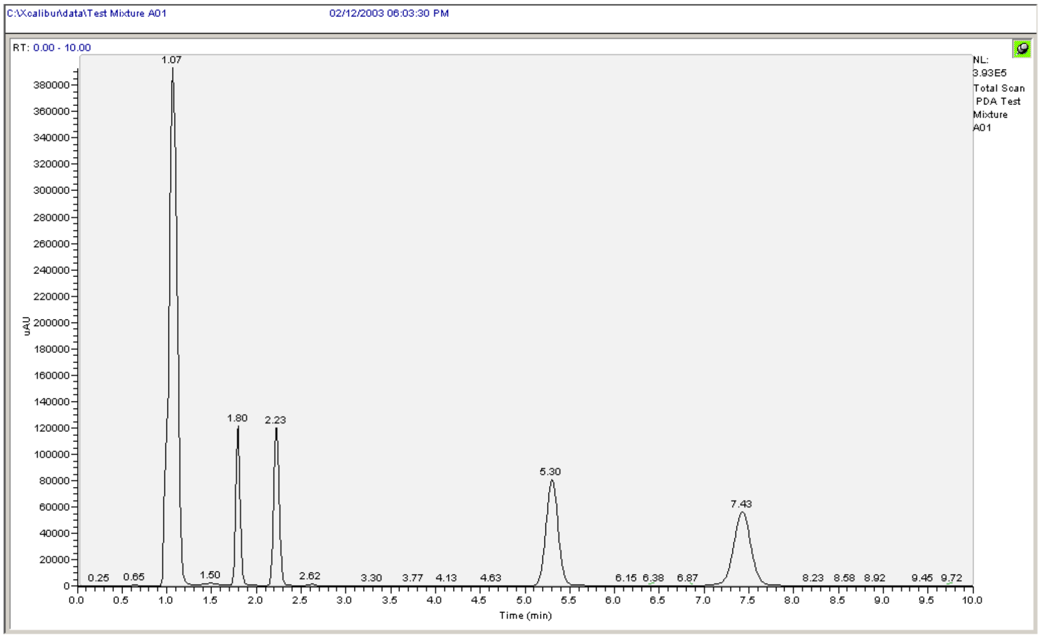
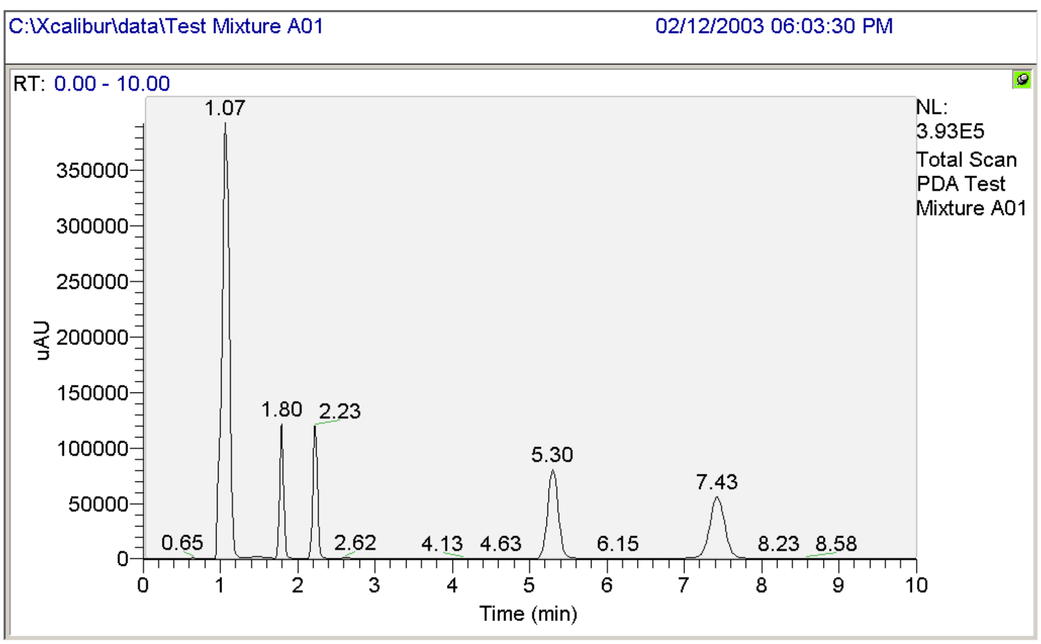


Figure 131. Proportional pitch font size = 16



Viewing a Report of the Instrument Method

After you open a data file, you might want to check the instrument parameters that were used to acquire it.

❖ To view a report that lists the instrument control parameters

1. Choose **View > Report > Instrument Method**.

   The instrument method is displayed in the top cell of the window. The instrument method is divided by device, with the parameters for each device displayed on a separate page (see Figure 132).

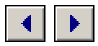
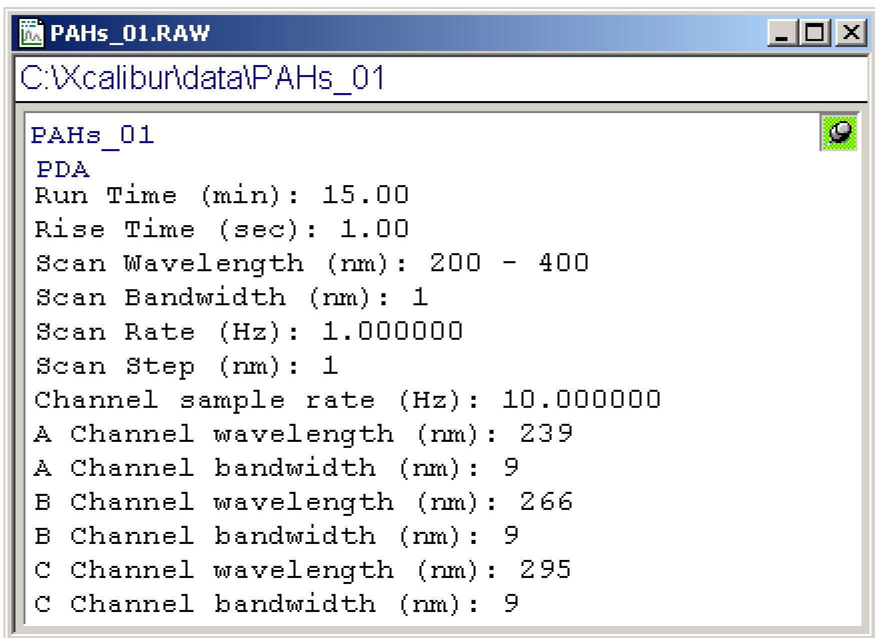
2. Click  (Show Previous and Show Next) to move through the pages of your Instrument Method.

Figure 132. Instrument Method Report (PDA page) in the Qual Browser view



Creating a Layout for PDA Data

This section describes how to view chromatograms and spectral information acquired by the PDA detector. In addition, it describes how to save the range and display settings for the PDA data in a layout file.

To create a layout file for PDA data, follow these procedures in order:

1. Specifying the Chromatogram and Spectrum Ranges
2. Specifying the Display Options for the Chromatogram and Spectrum Cells
3. (Optional) Inserting Cells
4. Saving the New Layout

Specifying the Chromatogram and Spectrum Ranges

When you open a .raw data file in Qual Browser using the default layout, the data file opens with the MS TIC chromatogram in the upper cell and a mass range spectrum in the lower cell.

Follow these topics to change the upper cell to a chromatogram and the lower cell to spectral data acquired by the PDA detector:

- Specifying the Chromatogram Ranges
- Specifying the Spectrum Range

Note You can modify the cells in any order.

Specifying the Chromatogram Ranges

There are two types of chromatograms for the PDA detector: chromatograms acquired from any of the three discrete channels and chromatograms interpolated from the scan data. Follow these procedures to display the two types of chromatograms acquired by the PDA detector in the Qual Browser window:

- Displaying Scan Wavelength Chromatograms
- Displaying Discrete Wavelength Chromatograms

Displaying Scan Wavelength Chromatograms

❖ **To display a scan chromatogram for the PDA detector data**

1. Open a data file (*.raw) that has PDA data and MS data (see “Opening a Raw Data File in Qual Browser” on page 213).

   The data file opens with the MS TIC chromatogram in the upper cell and a mass range spectrum in the lower cell.

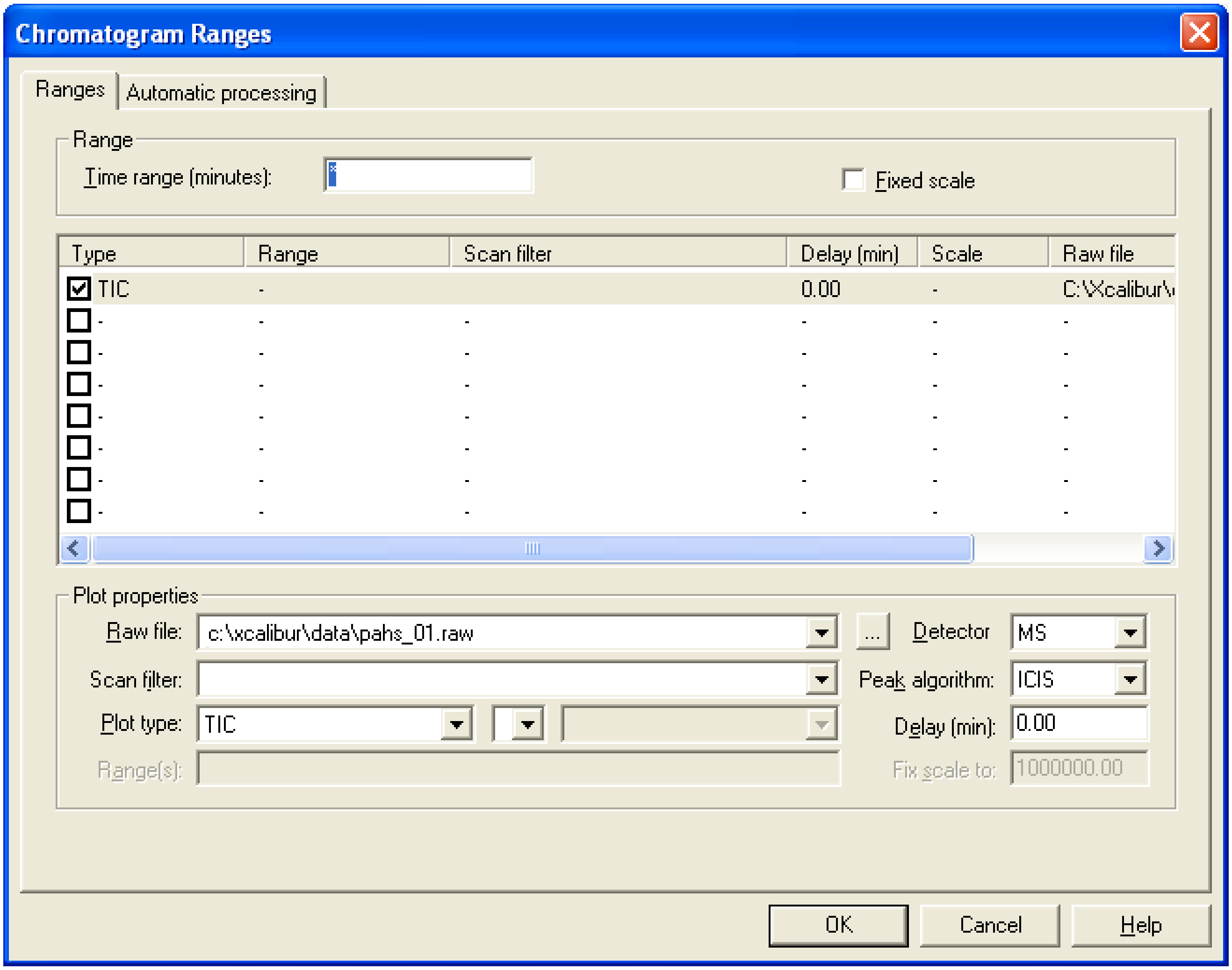
2. Pin the chromatogram cell (see “Active and Pinned Cells” on page 219).

3. Right-click the chromatogram cell.

4. From the shortcut menu, choose **Ranges**.

   The Chromatogram Ranges dialog box appears (see Figure 133).

Figure 133. Chromatogram Ranges dialog box with the default layout settings



5. In the Range area, set the displayed time range of the chromatogram:

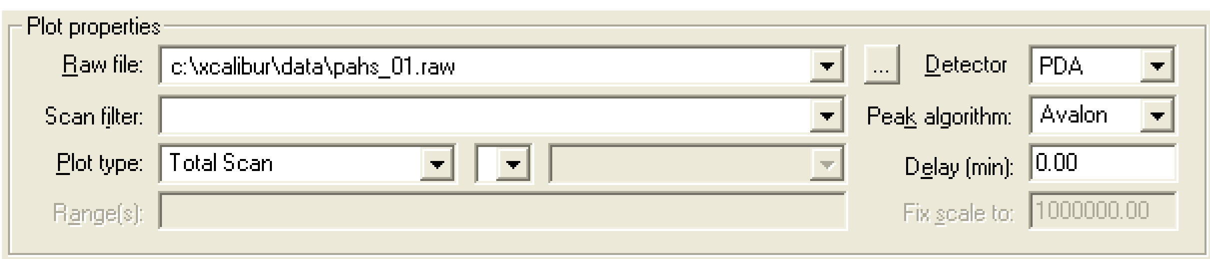
   - For a generic layout file, leave an asterisk in the Time Range box.

     When you open a .raw data file, the X-axis of the chromatogram is scaled to the run time for your detector in the instrument setup method used to acquire the data.

   - To specify a specific time range, type a beginning time point and an ending time point separated by a dash in the Time range (minutes) box.

6. In the Plot properties area (see Figure 134), make the following selections:
   a. In the Detector list, select **PDA**.
   b. In the Peak algorithm list, select **Avalon**.
   c. In the Plot type list, select a plot type:
      - Select **Wavelength** to display the chromatogram for a specific wavelength within your scan range or to display the averaged results from a range of wavelengths in your scan range.
      - Select **Total Scan** to display the average absorbance for each time point of all the wavelengths in your scan range.
      - Select **Spectrum Maximum** to display a plot of the maximum absorbance values in your scan range for each time point.
   d. Select a wavelength range (if you selected the Total Scan plot type, this box is unavailable):
      - To display the chromatogram for a specific scan wavelength, type the wavelength number in the Range(s) box.
      - To display a plot of the average absorbance values for a range of wavelengths, type the beginning wavelength number and the ending wavelength number separated by a dash in the Range(s) box. For example, type 200-300 to display a plot of the average absorbance values for the scanned wavelengths from 200 nm to 300 nm.

Figure 134. Plot properties area of the Chromatogram Ranges dialog box

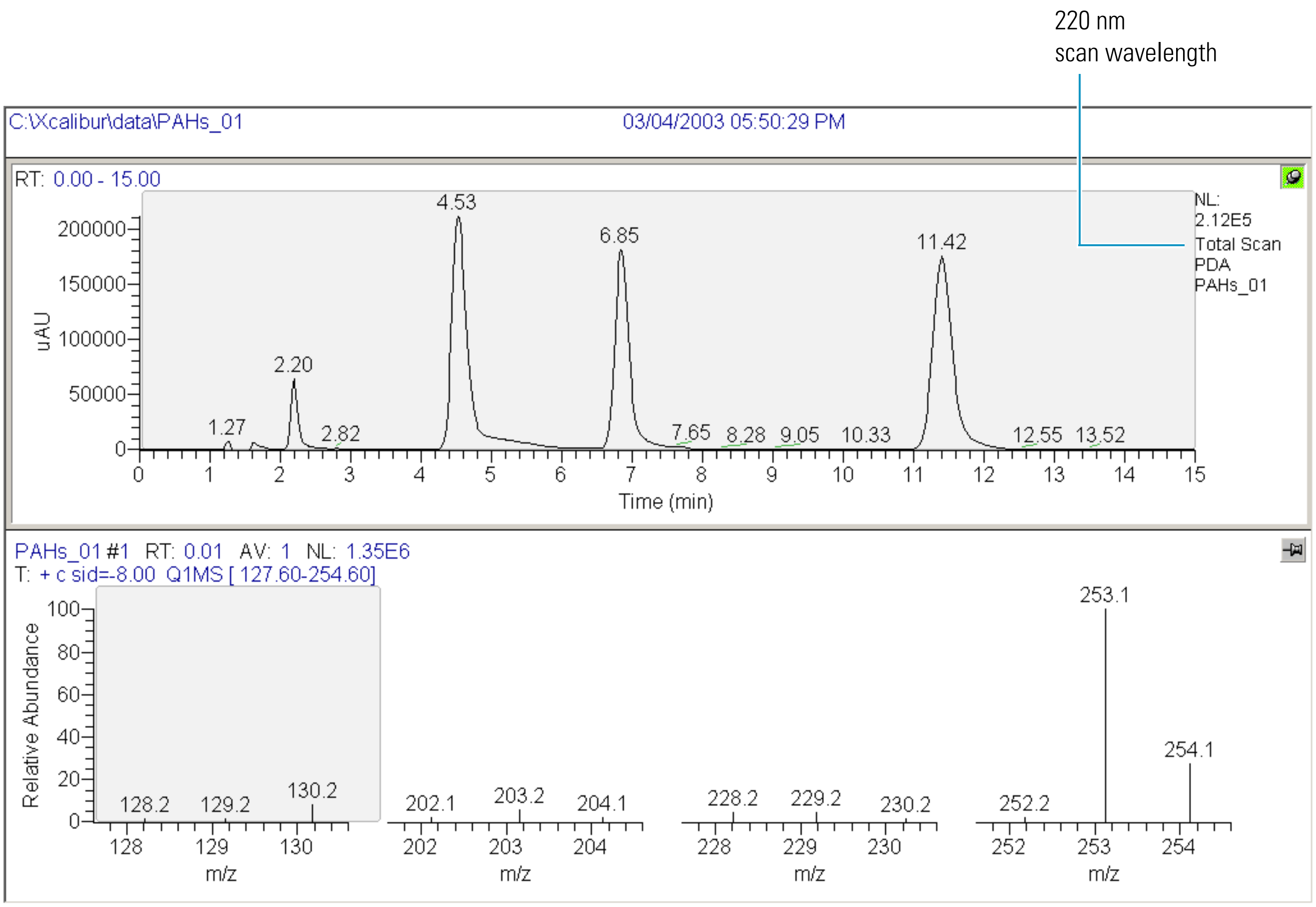


7. Click **OK** to close the dialog box and view your scan chromatogram.

   Figure 135 shows a chromatogram for a wavelength in the PDA scan.

To modify the layout for the spectrum cell, go to "Specifying the Spectrum Range" on page 232.

Figure 135. Chromatogram view with a chromatogram for a PDA scan wavelength



Displaying Discrete Wavelength Chromatograms

❖ To display a discrete chromatogram for the PDA data

1. If you have not already done so, open a data file (*.raw) that has PDA data and MS data (see “Opening a Raw Data File in Qual Browser” on page 213).

   The data file opens with the MS TIC chromatogram in the upper cell and a mass range spectrum in the lower cell.

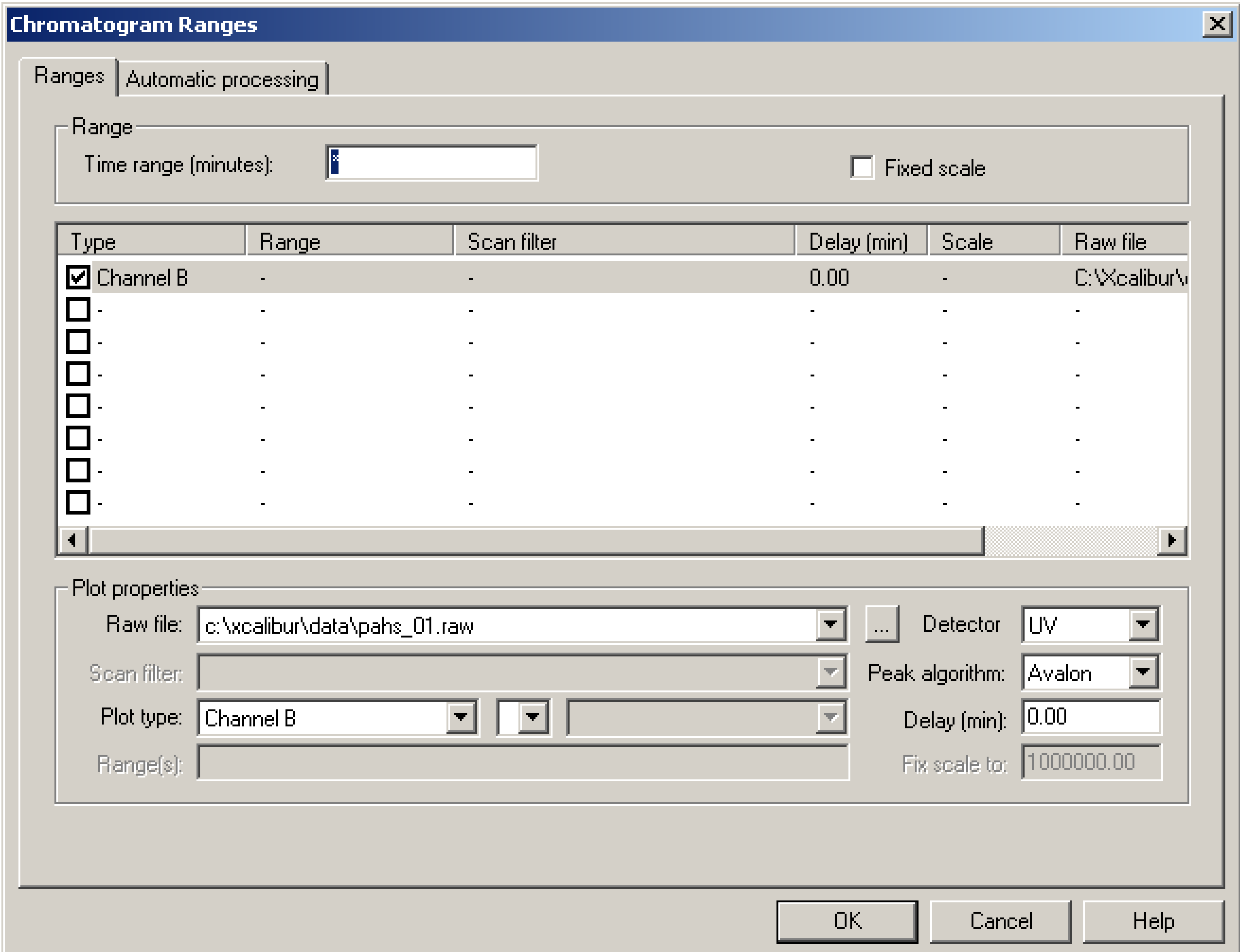
2. Pin the chromatogram cell (see “Active and Pinned Cells” on page 219).

3. Right-click the chromatogram cell.

4. From the shortcut menu, choose **Ranges**.

   The Chromatogram Ranges dialog box appears. Figure 133 on page 228 shows the default settings. Figure 136 shows the settings for a discrete channel wavelength.

Figure 136. Chromatogram Ranges dialog box with the selection of a discrete channel wavelength



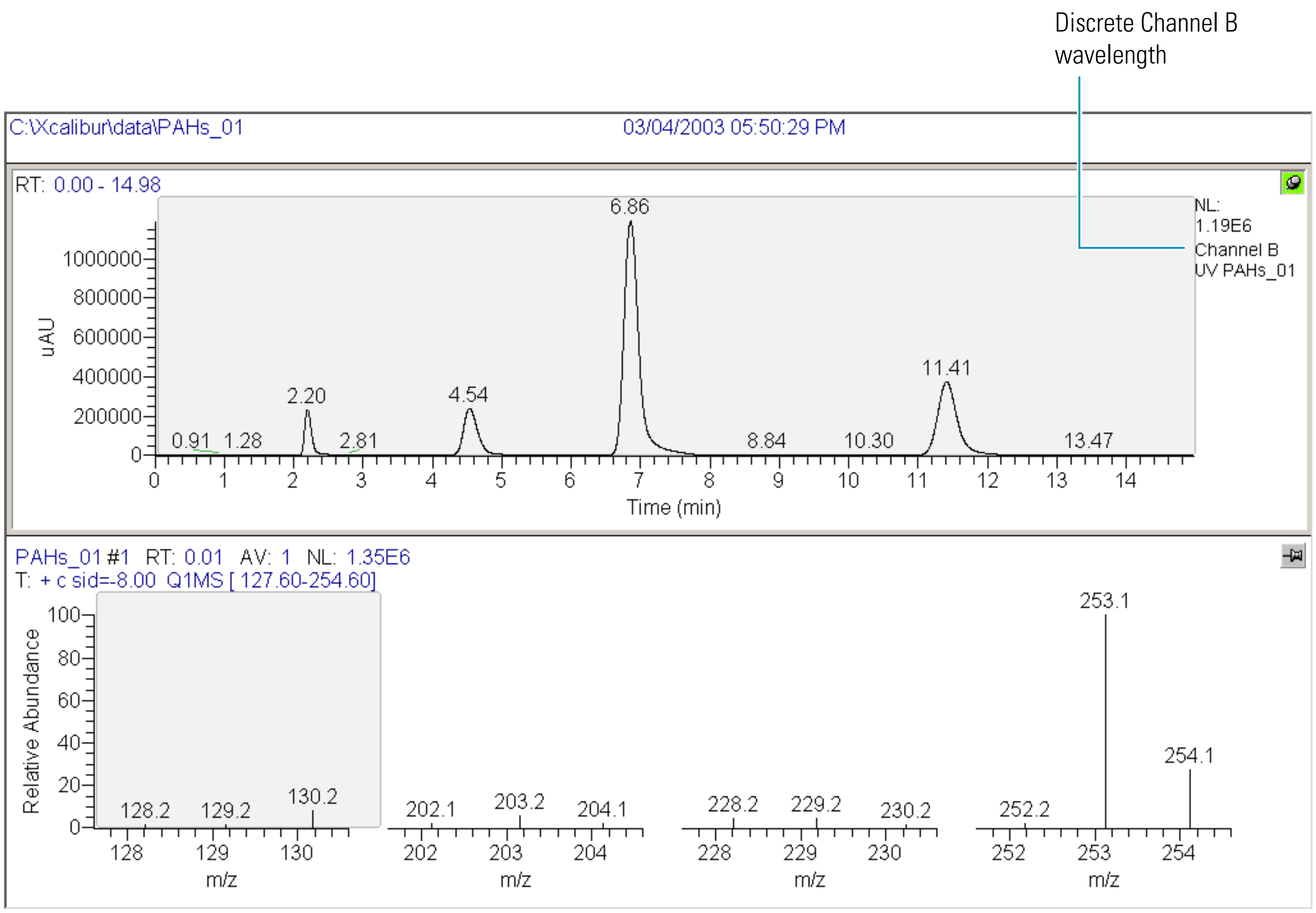
5. In the Range area, set the displayed time range of the chromatogram:
   - For a generic layout file, leave an asterisk in the Time Range box.

     When you open a .raw data file, the X-axis of the chromatogram is scaled to the run time for your detector in the instrument setup method used to acquire the data.
   - To specify a specific time range, type a beginning time point and an ending time point separated by a dash in the Time Range box.
6. In the Plot properties area, select the following:
   a. In the Detector list, select **UV**.
   b. In the Peak Algorithm list, select **Avalon**.
   c. In the Plot type list, select **Channel A**, **Channel B**, or **Channel C**.
7. Click **OK** to exit the dialog box and view your discrete chromatogram.

Figure 137 shows a discrete channel chromatogram.

To modify the layout for the spectrum cell, go to "Specifying the Spectrum Range" on page 232.

Figure 137. Chromatogram view with a chromatogram for a PDA discrete channel



Specifying the Spectrum Range

❖ To display a spectrum for the PDA data

1. If you have not already done so, open a data file (*.raw) that has PDA data and MS data (see "Opening a Raw Data File in Qual Browser" on page 213).

   The data file opens with the MS TIC chromatogram in the upper cell and a mass range spectrum in the lower cell.

2. Pin the spectrum cell.

3. Right-click the spectrum cell.

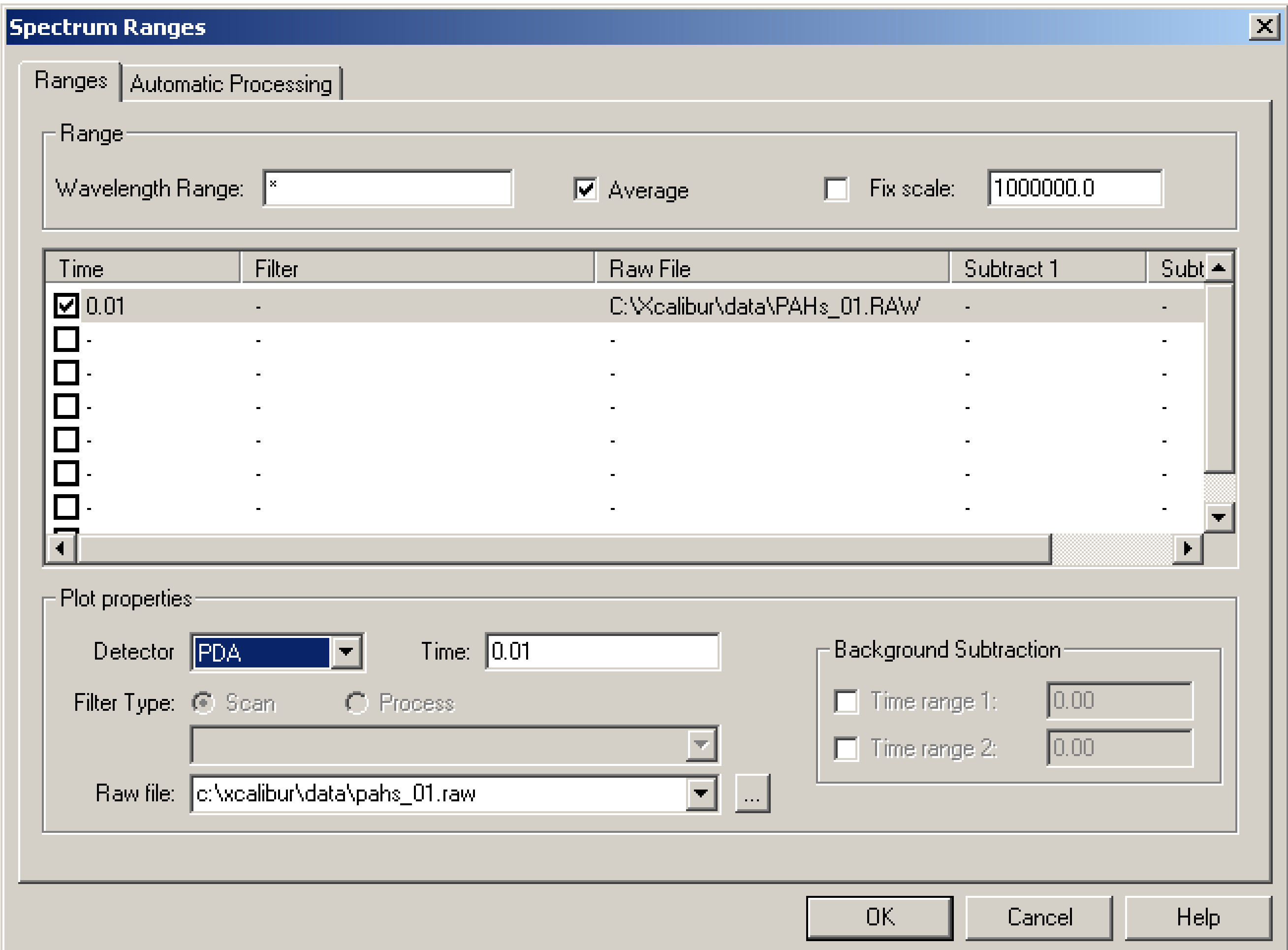
4. From the shortcut menu, choose **Ranges**.

   The Spectrum Ranges dialog box appears (see Figure 138).

5. In the Plot properties area, select **PDA** in the Detector list.

6. For a generic layout file for PDA data, keep the other settings in the Spectrum Ranges dialog box at their defaults as shown in Figure 138.

Figure 138. Spectrum Ranges dialog box with the selection of the PDA detector



If you have not already done so, modify the layout for the chromatogram cell as described in "Specifying the Chromatogram Ranges" on page 227.

If you have specified the ranges for the chromatogram cell and the spectrum cell, go to the next procedure, "Specifying the Display Options for the Chromatogram and Spectrum Cells."

Specifying the Display Options for the Chromatogram and Spectrum Cells

After you specify the chromatogram and spectrum ranges, specify the display options for the chromatogram and spectrum cells as described in these topics:

- Setting the Display Options for the Chromatogram Cell
- Setting the Display Options for the Spectrum Cell

Setting the Display Options for the Chromatogram Cell

❖ **To set the display options for the chromatogram cell**

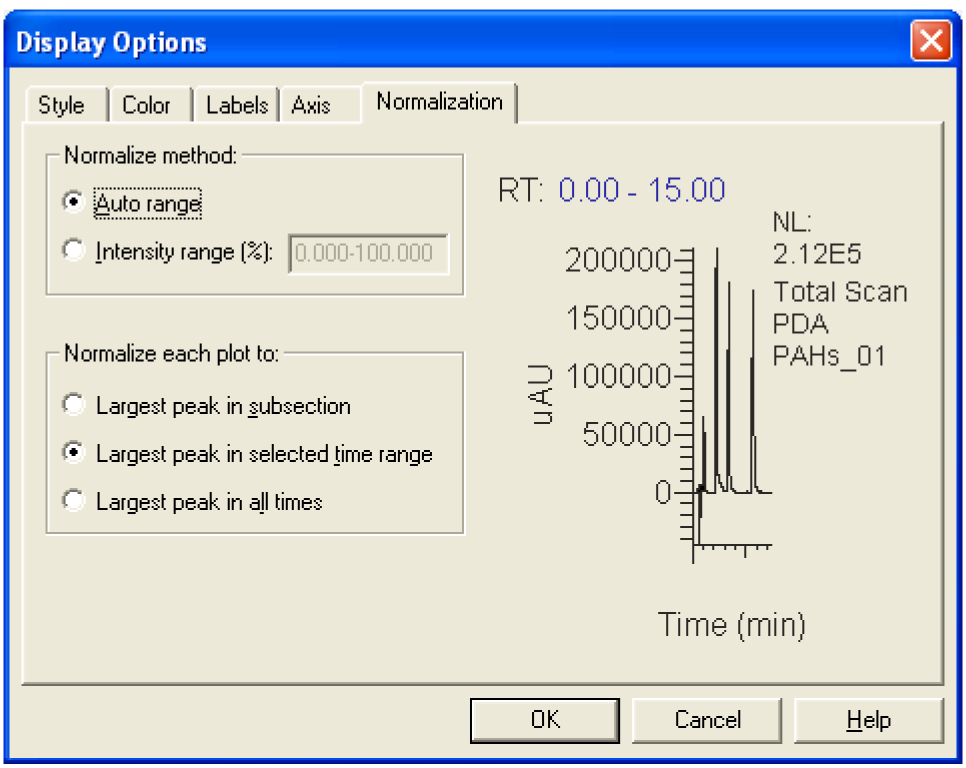
1. Pin the chromatogram cell.
2. Right-click the pinned chromatogram cell.
3. From the shortcut menu, choose **Display Options**.

   The Display Options dialog box appears.
4. Specify the Normalization parameters:

   a. Click the **Normalization** tab.

   The Normalization page appears (see Figure 139).

Figure 139. Normalization page in the Display Options dialog box



b. In the Normalize method area, select the **Auto range** option.

Selecting Auto range ensures that the entire dynamic range of the chromatogram is displayed in the active view, normalized over the full range of the Y-axis.

c. In the Normalize each plot to area, select the **Largest peak in selected time range** option.

5. Specify the Axis parameters as follows:

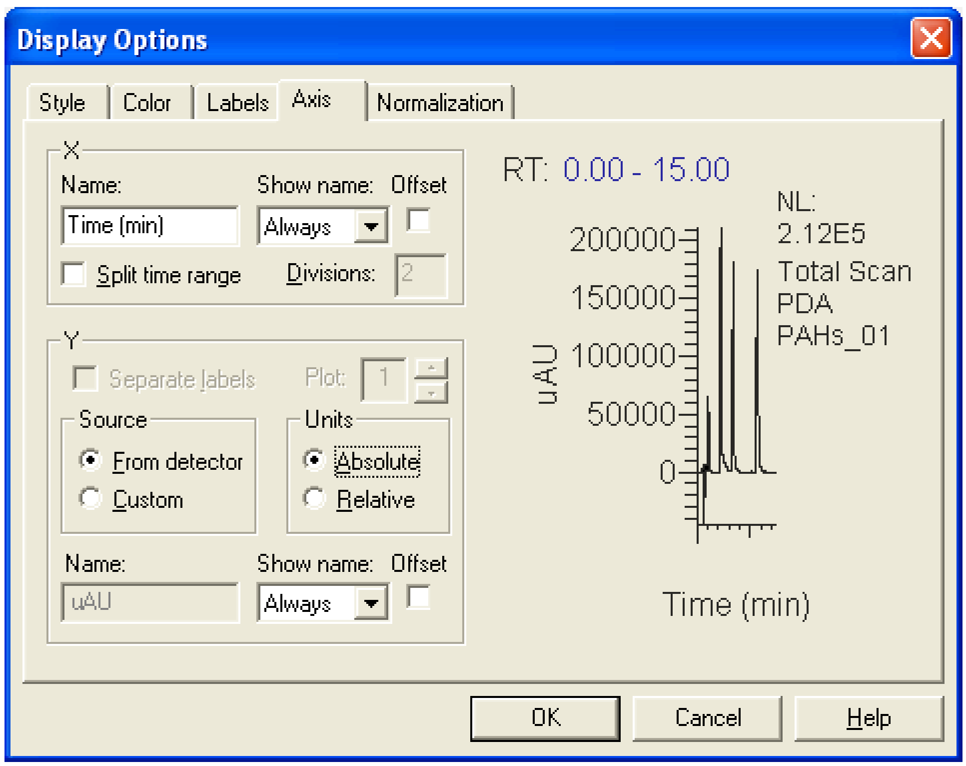
a. Click the **Axis** tab.

The Axis page appears (see Figure 140).

b. In the Units area, select the **Absolute** option.

Selecting Absolute sets the Y-axis to the absolute units of µAU.

Figure 140. Axis page in the Display Options dialog box

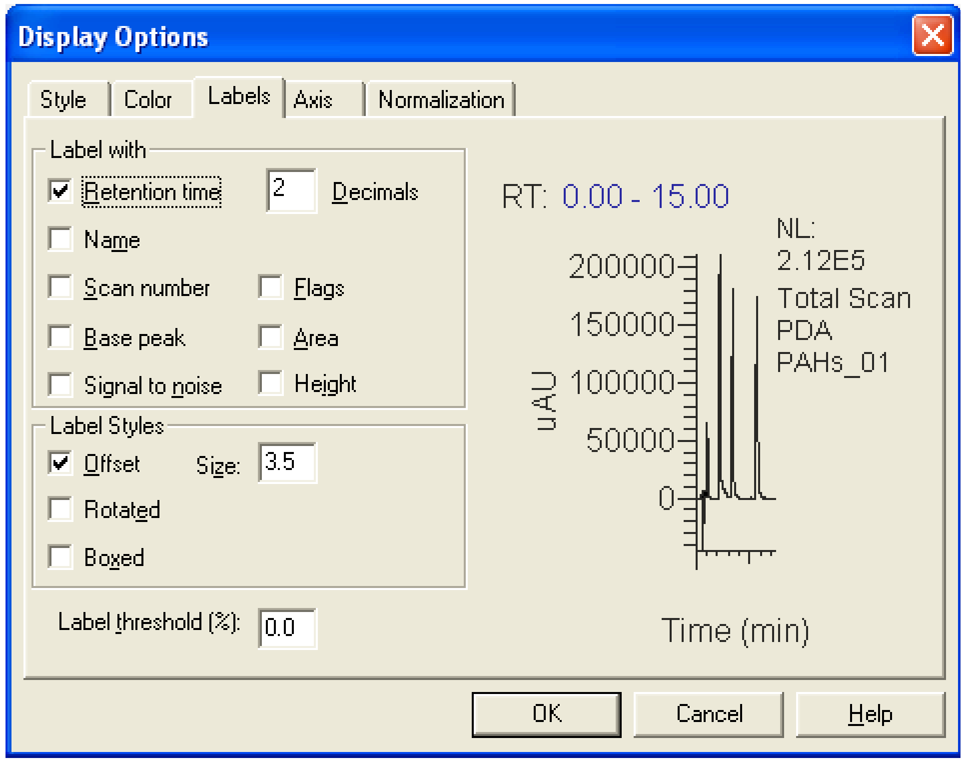


6. Specify the labels for the peaks in the chromatogram as follows:

a. Click the **Labels** tab.

The Labels page appears (see Figure 141).

Figure 141. Labels page



b. Select the check boxes associated with the labels that you want to display, such as retention time, area, height, and name.

7. Click **OK** to accept the settings and close the Display Options dialog box.

Setting the Display Options for the Spectrum Cell

❖ To set the display options for the spectrum cell

1. Pin the spectrum cell.
2. Right-click the pinned spectrum cell.
3. From the shortcut menu, choose **Display Options**.

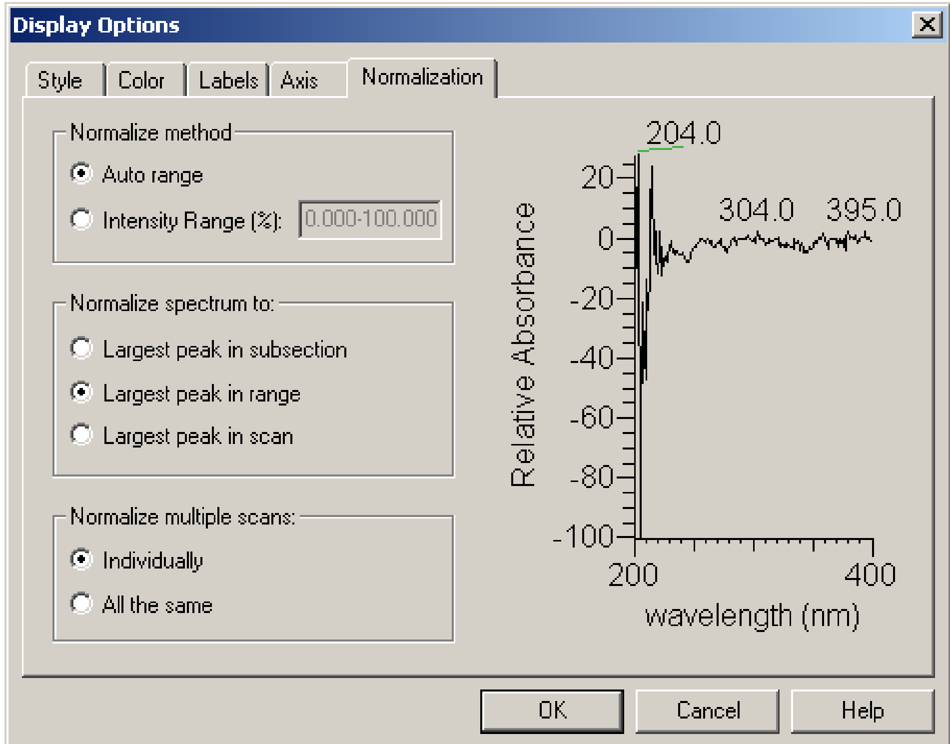
   The Display Options dialog box appears.

4. Specify the Normalization parameters as follows:

   a. Click the **Normalization** tab.

      The Normalization page appears (see Figure 142).

Figure 142. Normalization page in the Display Options dialog box



b. In the Normalize method area, select the **Auto range** option.

Selecting Auto range ensures that the entire dynamic range of the spectrum is displayed in the active view, normalized over the full range of the Y-axis.

c. In the Normalize spectrum to area, select the **Largest peak in range** option.

5. Specify the Axis parameters as follows:

a. Click the **Axis** tab.

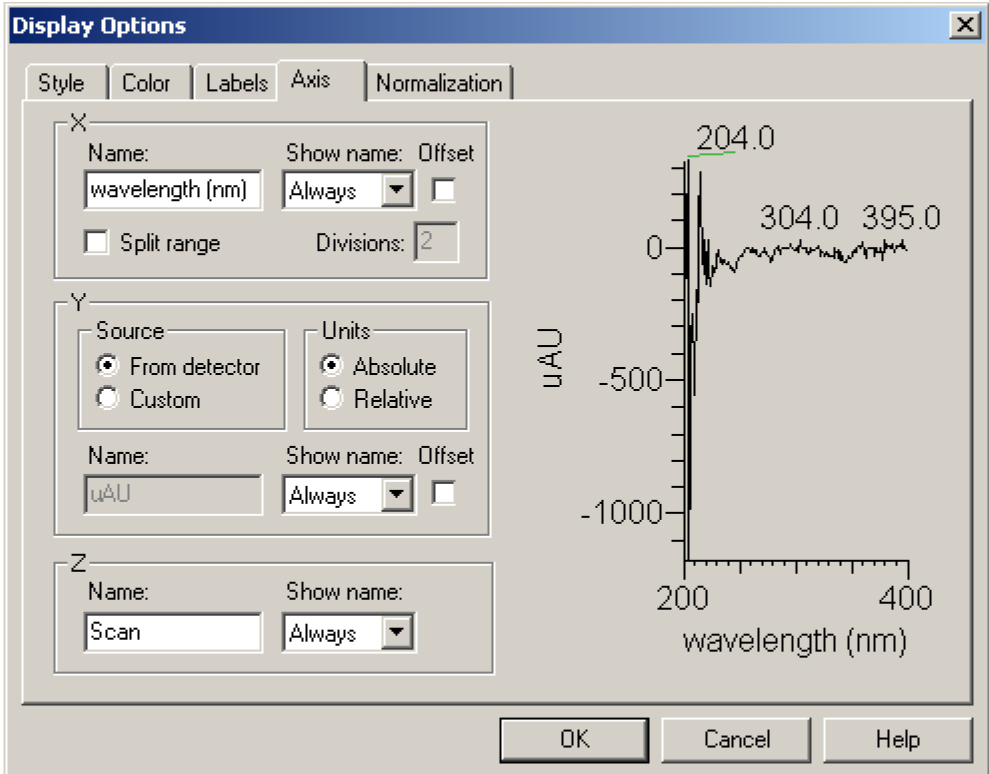
The Axis page appears (see Figure 143).

b. In the Units area, select the **Absolute** option.

Selecting Absolute sets the Y-axis to the absolute units of µAU.

6. Click **OK** to close the Display Options dialog box.

Figure 143. Axis page in the Display Options dialog box



Inserting Cells

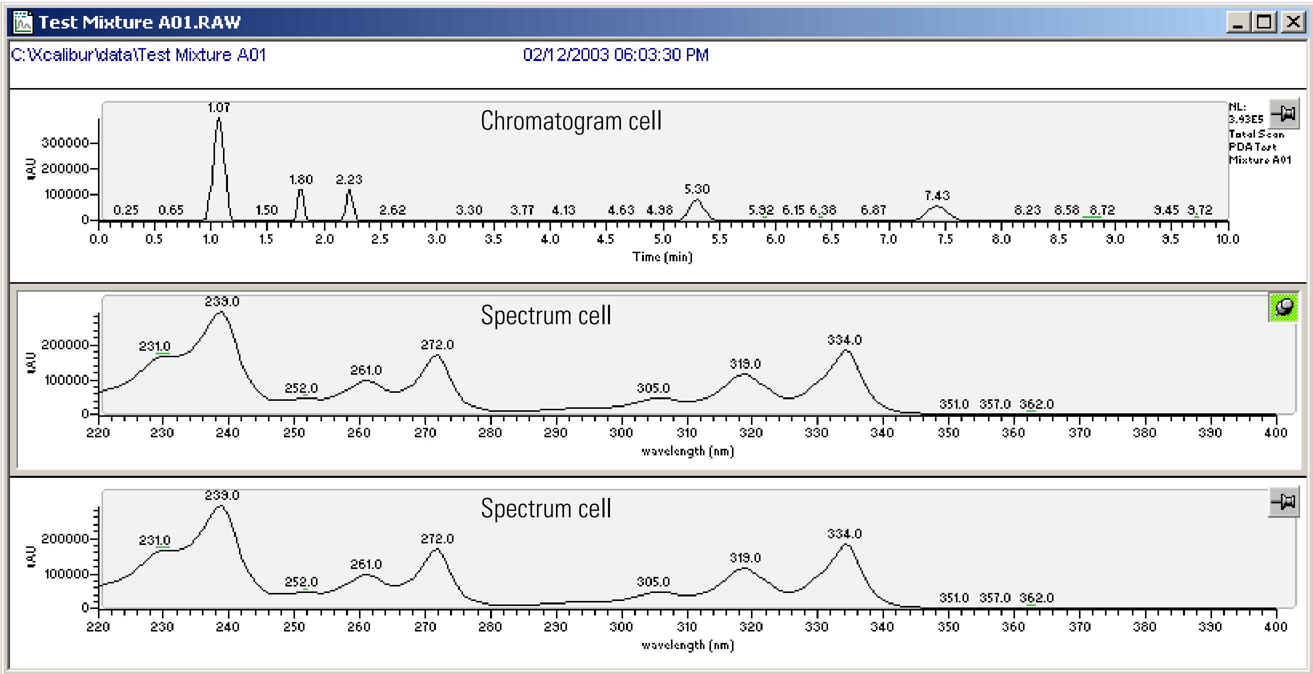
Occasionally, you might want to add more cells to the Qual Browser window. For example, you might want to add a cell containing a Map plot (contour or 3D) to the view screen, or you might want to display several discrete or scan wavelengths in separate cells.

❖ To add a cell containing a Map plot to the window

1. Click a cell to make it the active cell.
2. Choose **Grid > Insert Cells > Left**, **Right**, **Above**, or **Below**.

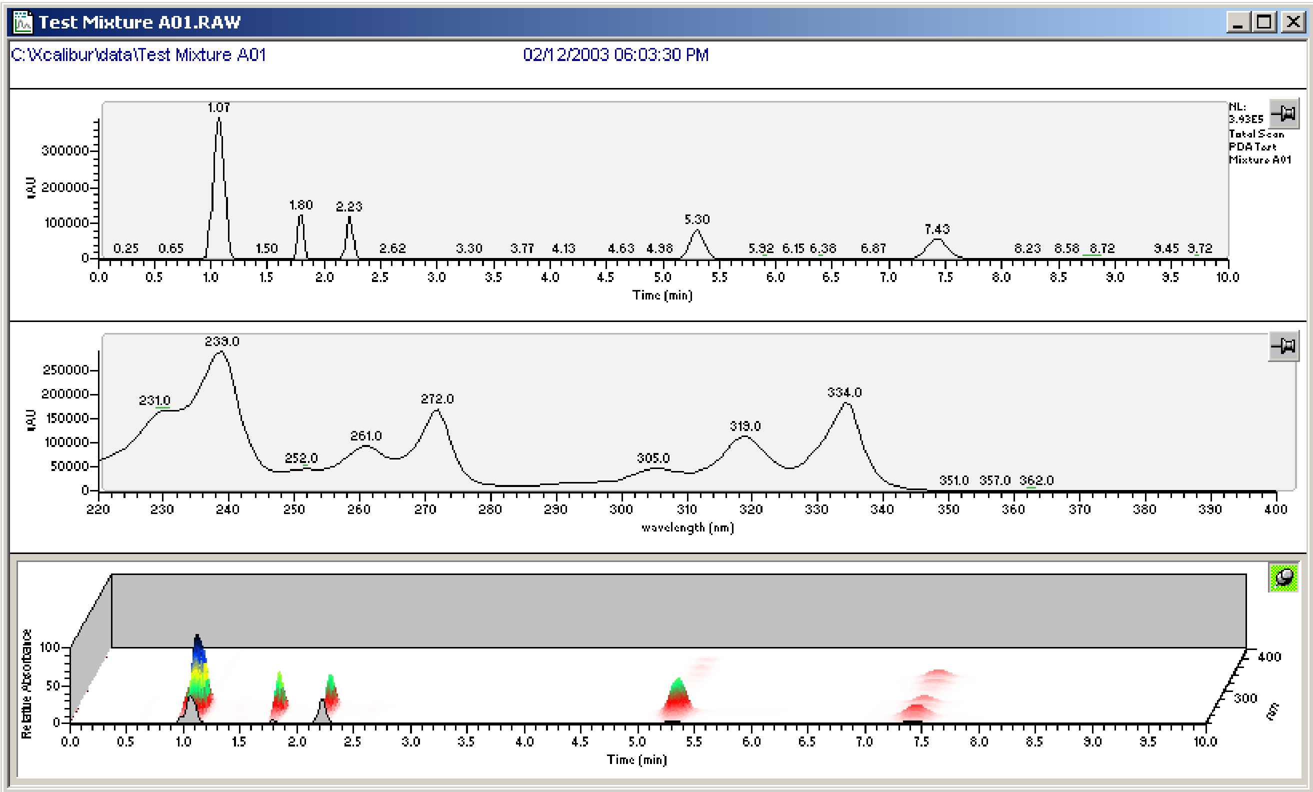
   The location of the new cell is relative to the active cell. Initially, the new cell contains the same information as the existing cell (see Figure 144).

Figure 144. Qual Browser window with three cells



3. Change the lower cell so that it displays the Map view (see Figure 145) as follows:
   a. Pin the cell by clicking its pin button.
   b. Click the **View Map** button in the toolbar.

Figure 145. Qual Browser window with a chromatogram cell, spectrum cell, and map cell



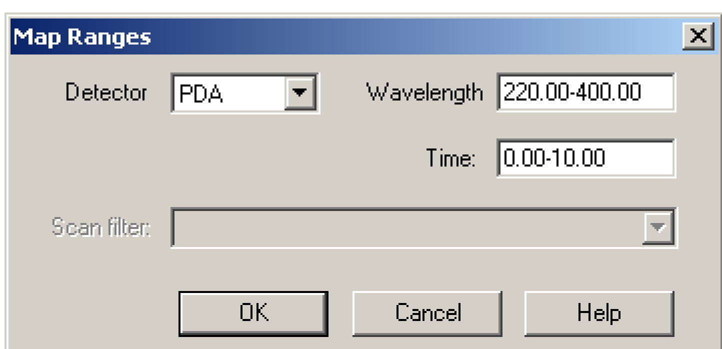
4. Select the appropriate range options for the map cell as follows:

   a. Right-click the map cell.

   b. From the shortcut menu, choose **Ranges**.

   The Map Ranges dialog box appears (see Figure 146).

   Figure 146. Map Ranges dialog box



   c. In the Wavelength box, type the wavelength range that you want to display.

   d. In the Time box, type the time range that you want to display.

5. Select the appropriate display options for the map cell as follows:
   a. Right-click the map cell.
   b. From the shortcut menu, choose **Display Options**.

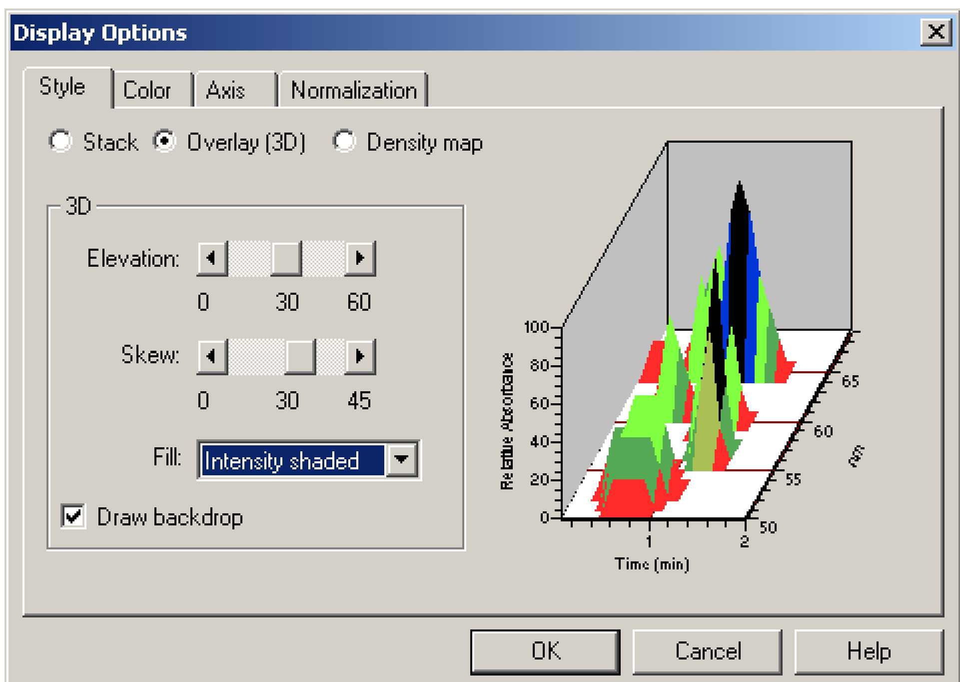
      The Display Options dialog box appears.
   c. Click the **Axis** tab.

      The Axis page appears.
   d. In the Units area, select the **Absolute** option.
   e. Click the **Normalization** tab.

      The Normalization page appears.
   f. In the Normalize method area, select the **Auto range** option.
   g. Click the **Style** tab.

      The Style page appears (see Figure 147).

Figure 147. Style page in the Display Options dialog box for the map cell



   h. Select the appropriate style.
   i. Click **OK** to save your settings and close the Display Options dialog box.

Saving the New Layout

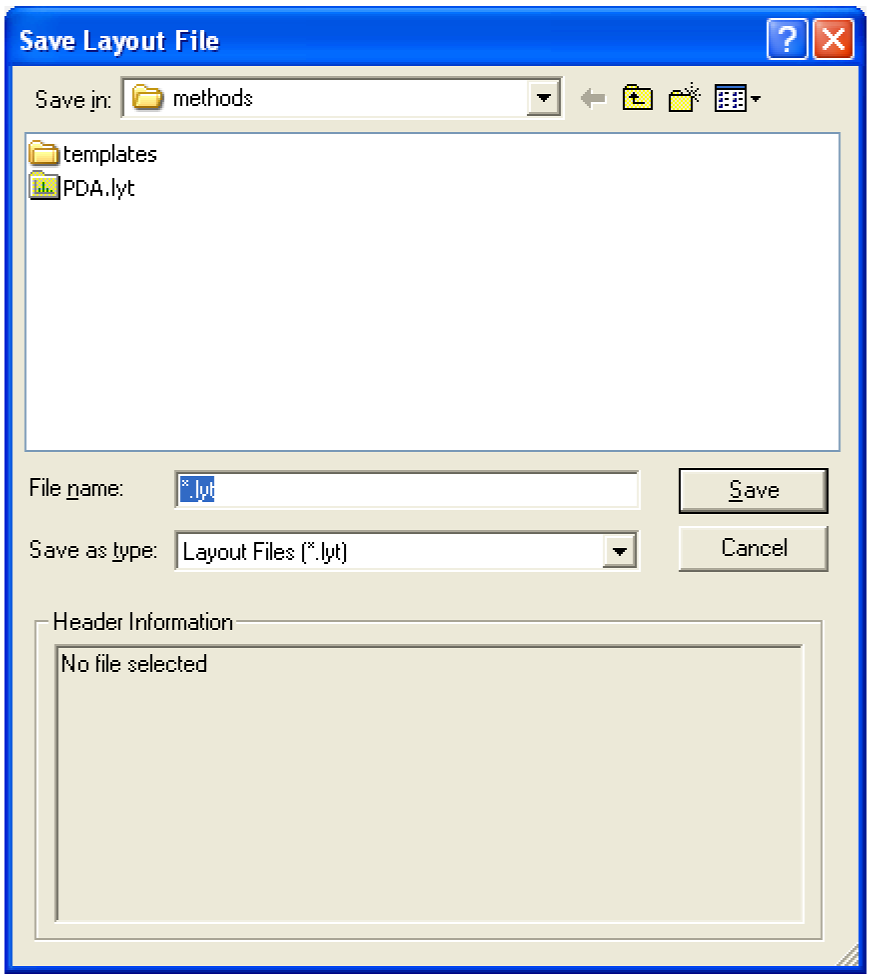
Now that you have created a layout for displaying your PDA data, save the layout so that you can apply it to other data files containing PDA data.

❖ To save the layout

1. From the Qual Browser window, choose **File > Layout > Save As**.

   The Save Layout File dialog box appears (see Figure 148).

   Figure 148. Save Layout File dialog box

2. Type a file name in the File name box.
3. Click **Save**.

Viewing the Spectrum for a Specific Time Point

❖ **To view a spectrum for a specific time point**

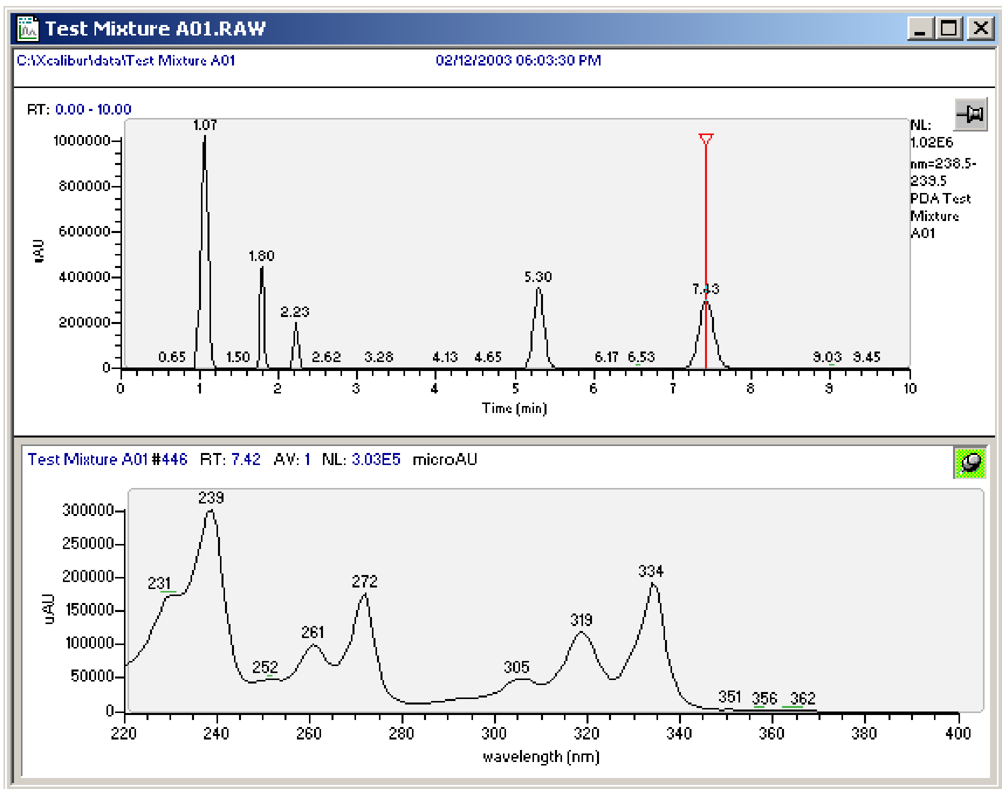
1. Open a raw data file with PDA data and apply an appropriate layout file (see “Opening a Raw Data File in Qual Browser” on page 213).

2. Pin the spectrum cell.

3. Click a time point in the chromatogram cell.

   The spectrum for the selected time point appears in the spectrum cell (see Figure 149).

Figure 149. Qual Browser window with a chromatogram of the PDA test mixture and a spectrum for the 7.42 minute time point



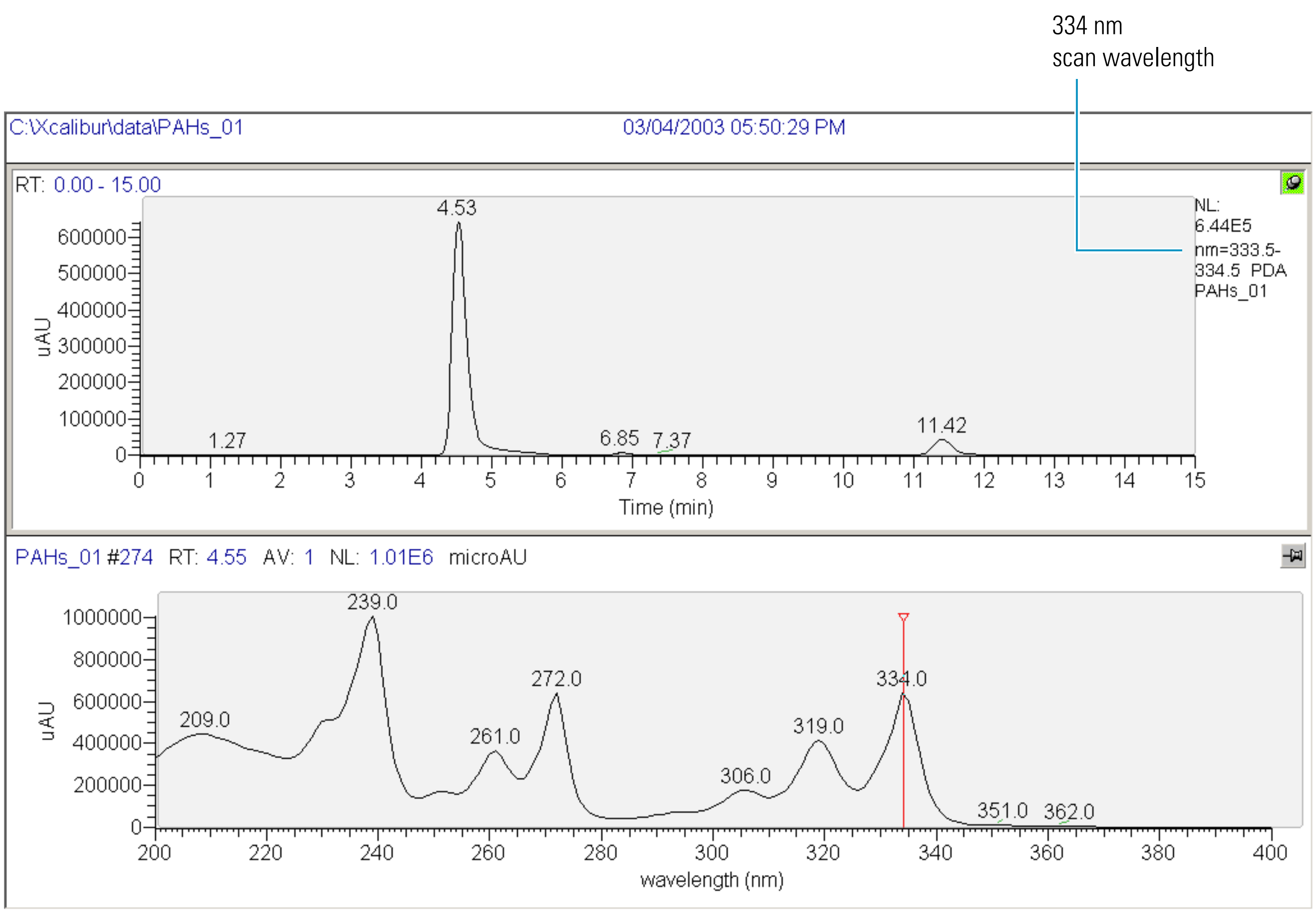
Viewing the Chromatogram for a Specific Wavelength

❖ **To view the scan chromatogram for a specific wavelength**

1. Open a raw data file with PDA data and apply an appropriate layout file (see "Opening a Raw Data File in Qual Browser" on page 213).
2. Pin the chromatogram cell.
3. Click a wavelength in the spectrum cell.

   The scan chromatogram for the selected wavelength appears in the chromatogram cell as shown in Figure 150.

Figure 150. Qual Browser window, displaying a chromatogram cell and a spectrum cell



Determining Peak Areas

In a chromatogram, the area of an isolated peak is directly proportional to the concentration of the analyte.

❖ To make a peak area determination using Qual Browser

1. Open Qual Browser.
2. Open the data file (.raw) of interest (see "Opening a Raw Data File in Qual Browser" on page 213).
3. Ensure that the chromatogram cell is the active cell, as indicated by a gray border.
4. Select the chromatogram that you want to integrate as follows:
   a. Right-click the chromatogram and from the shortcut menu choose **Ranges**.

      The Chromatogram ranges dialog box appears.

   b. In the Detector list, select the detector type:
      - To integrate a scan wavelength, select **PDA**.
      - To integrate a discrete wavelength channel, select **UV**.

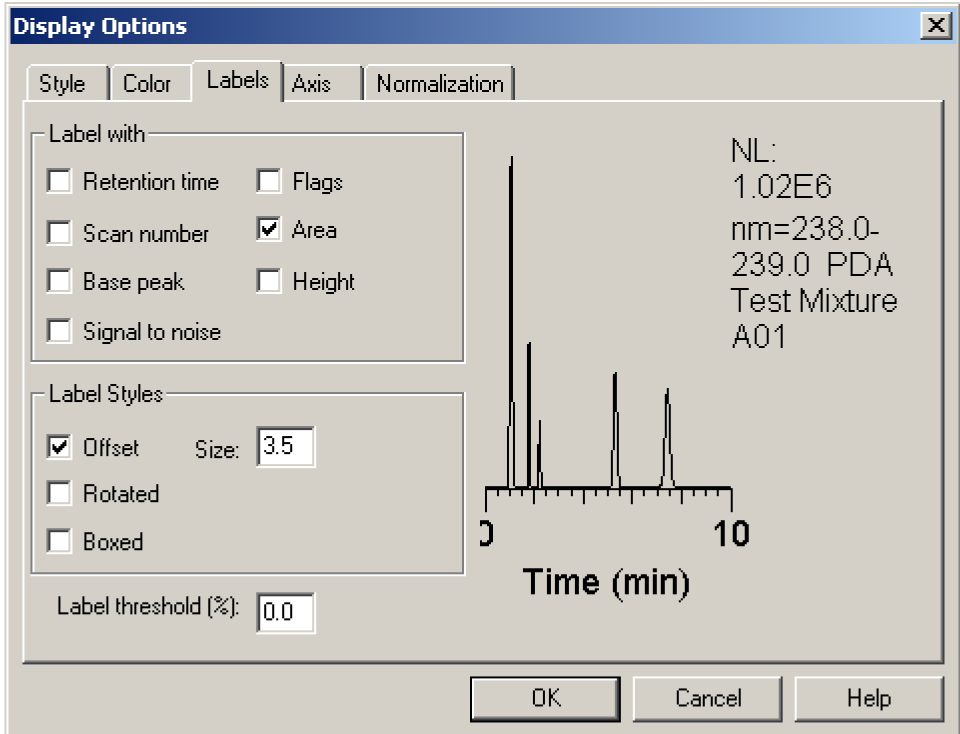
   c. Ensure that **Avalon** is selected as the Peak Algorithm.
   d. Select the appropriate wavelength:
      - To display the chromatogram for a scan wavelength, select **Wavelength Range** from the Plot type list, and then type a value for a wavelength within your scan range in the Range box.
      - To display the chromatogram for a discrete wavelength channel, select **Channel A**, **B**, or **C** from the Plot type list.
5. Turn on peak detection by right-clicking the chromatogram cell and choosing **Peak Detection > Toggle Detection in This Plot f**rom the shortcut menu.
6. To display numerical values for areas of the chromatographic peaks, do the following:
   a. Right-click the chromatogram cell and choose **Display Options**.

      The Display Options dialog box appears.

   b. Click the **Labels** tab.

      The Labels page appears (see Figure 151).

Figure 151. Labels page in the Display Options dialog box



c. Select the **Area** check box.

d. Click **OK** to return to the chromatogram cell.

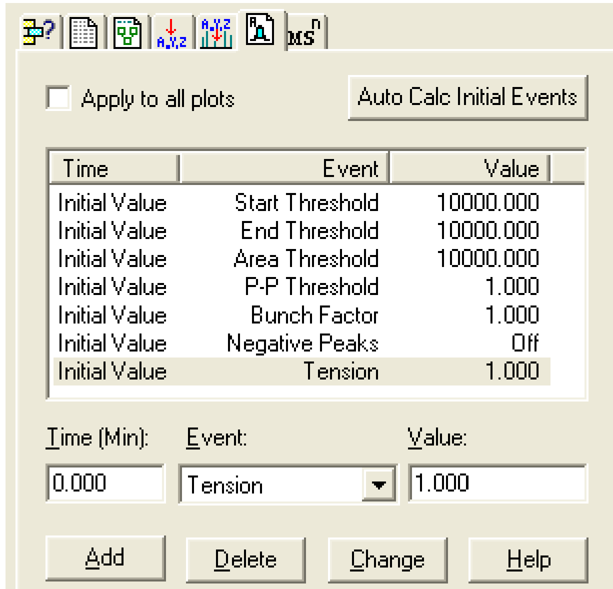
7. To set the integration parameters, do the following:

   a. Right-click the chromatogram and choose **Peak Detection** > **Settings** from the shortcut menu.

      The Avalon Peak Detection Settings page appears on the left side of the window (see Figure 152).

> **Note** The Avalon Peak Detection Settings page appears only when Avalon is selected as the peak algorithm in the Chromatogram Ranges dialog box.

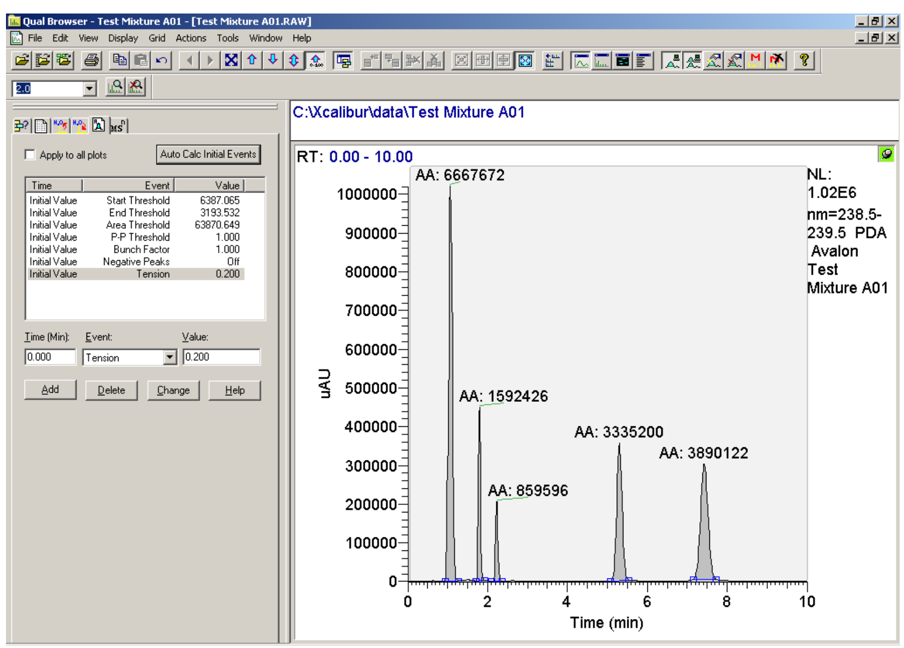
Figure 152. Avalon Peak Detection Settings page



b. Click **Auto Calc Initial Events** to force the Avalon peak integration algorithm to determine the "best" values for the following initial events: Start Threshold, End Threshold, Peak Threshold, P-P Threshold, Bunch Factor, Negative Peaks, and Tension.

Figure 153 shows a chromatogram for the 239 nm scan wavelength that is integrated with the "best" initial integration values as determined by the Avalon peak integration algorithm. The peaks areas are shown above the apexes of the integrated peaks.

Figure 153. Avalon Peak Detection Settings page displaying a chromatogram acquired with the PDA detector



Calculating the Purity of the Chromatographic Peaks

The data system can calculate the spectral purity of your chromatographic peaks by comparing the similarity of the spectra across the peak to a spectrum from the peak apex. The calculation is affected by the integration of the scan chromatogram and by the scan threshold, peak coverage, and scan wavelengths that you set in the Peak Purity Settings dialog box.

❖ To display the purity values for the integrated peaks

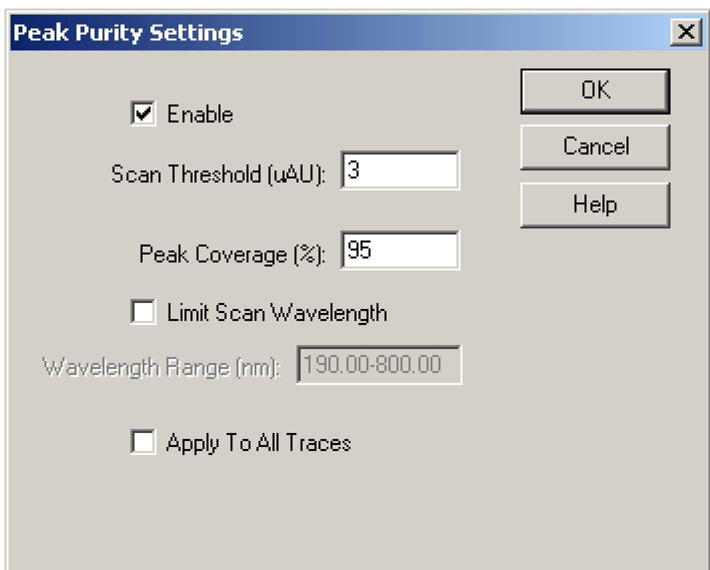
1. Select a chromatogram for a scan wavelength (see “Displaying Scan Wavelength Chromatograms” on page 227).

2. Set the integration parameters for the chromatogram (see “Determining Peak Areas” on page 245).

3. Right-click the chromatogram cell and choose **Peak Purity** from the shortcut menu.

   The Peak Purity Settings dialog box appears (see Figure 154).

   Figure 154. Peak Purity Settings dialog box

4. Select the **Enable** check box.

5. In the Scan Threshold (µAU) box, type an appropriate scan threshold.

   The scan threshold limits the portion of the peak included in the analysis to spectral slices that have a lambda max above the scan threshold. You can set this limit to eliminate noise from the analysis. The limits for this box are 0 to 2000000 µAU.

6. In the Peak Coverage % box, type an appropriate value for your application. The limits for this parameter are 1 to 100% coverage.

At a setting of 100%, the data system compares all the spectral slices that fall within the beginning and ending tick marks of the integrated peak. To limit the peak purity calculation to a specific range of wavelengths in the scan, select the Limit Scan Wavelength check box, and then enter a wavelength range in the Wavelength Range box.

7. Click **OK** to close the Peak Purity Settings dialog box, and then view the effect of your peak purity settings.

   The following figures show the effect of various settings on peak purity:

   - Figure 155 shows the effect of the scan wavelength on peak purity.
   - A comparison of Figure 156 and Figure 157 on page 250 demonstrates the effect of integration on peak purity.
   - Figure 158 on page 251 shows the effect of the scan threshold setting on peak purity.
   - Figure 159 on page 251 shows the effect of the peak coverage setting on peak purity.

Figure 155. Comparison of peak purity results for two different scan wavelengths

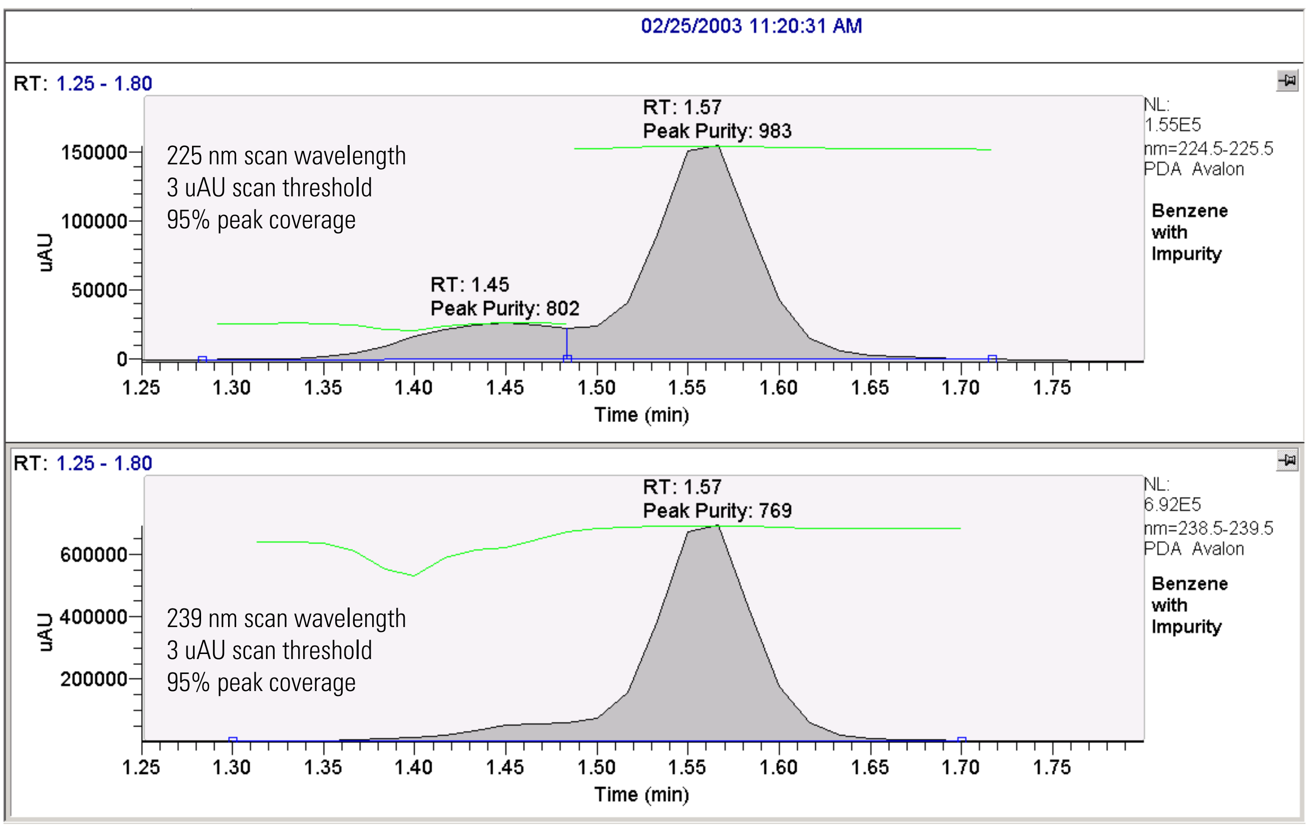


Figure 156. Chromatogram for the 225 nm scan wavelength with default integration parameters

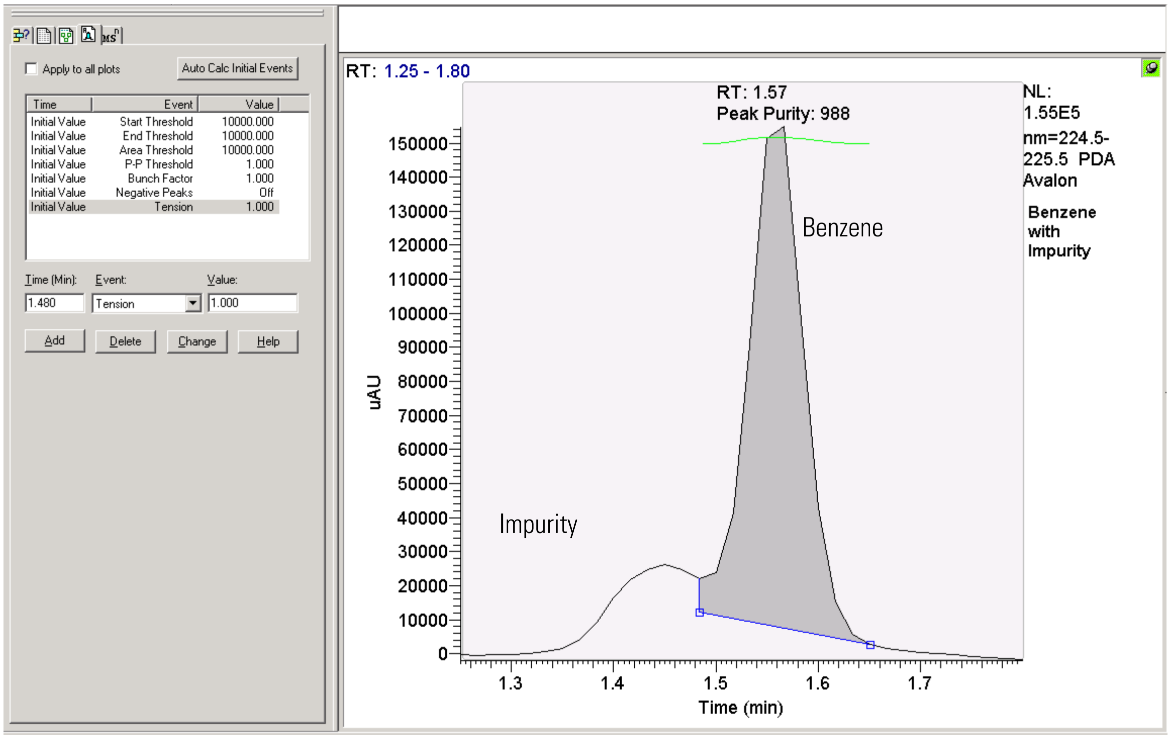


Figure 157. Chromatogram for the 225 nm scan wavelength with Auto calc integration parameters

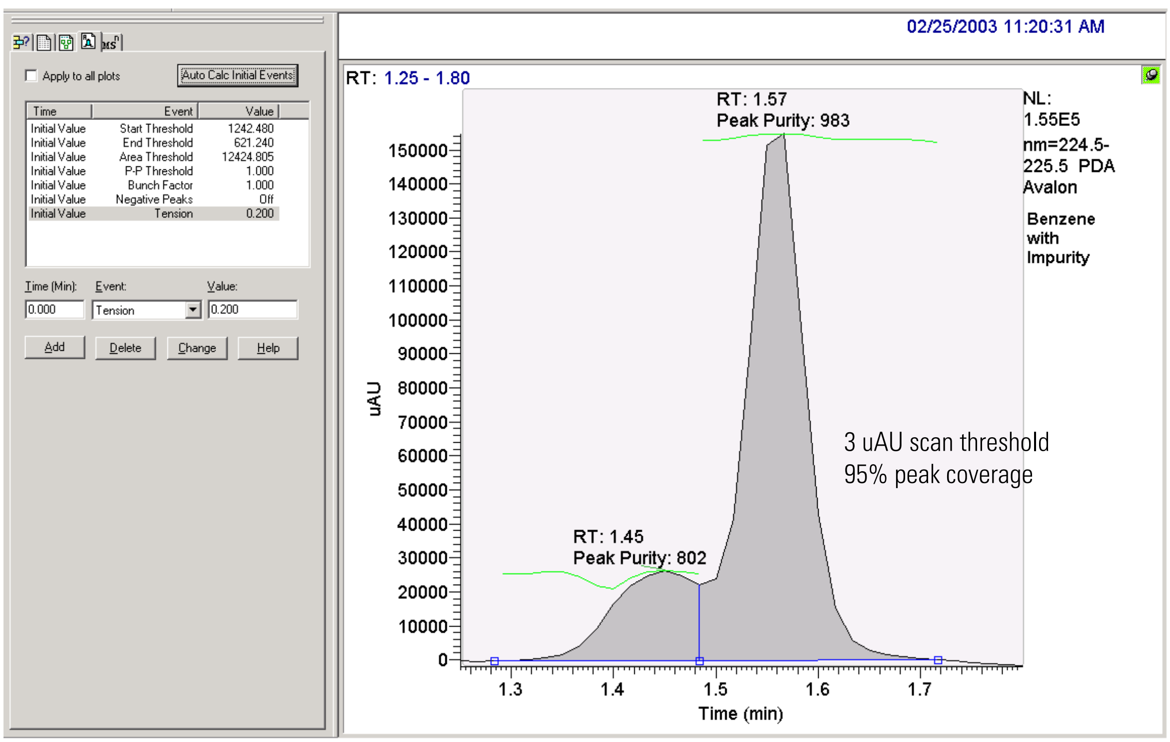


Figure 158. Effect of scan threshold on peak purity calculation

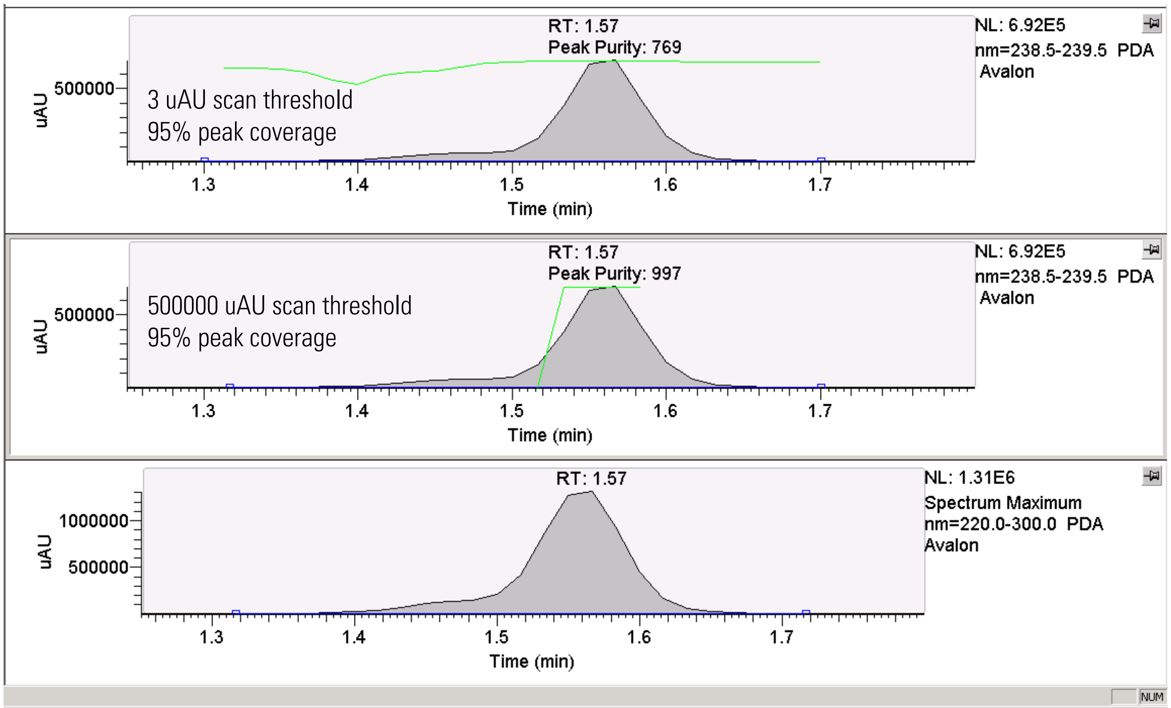
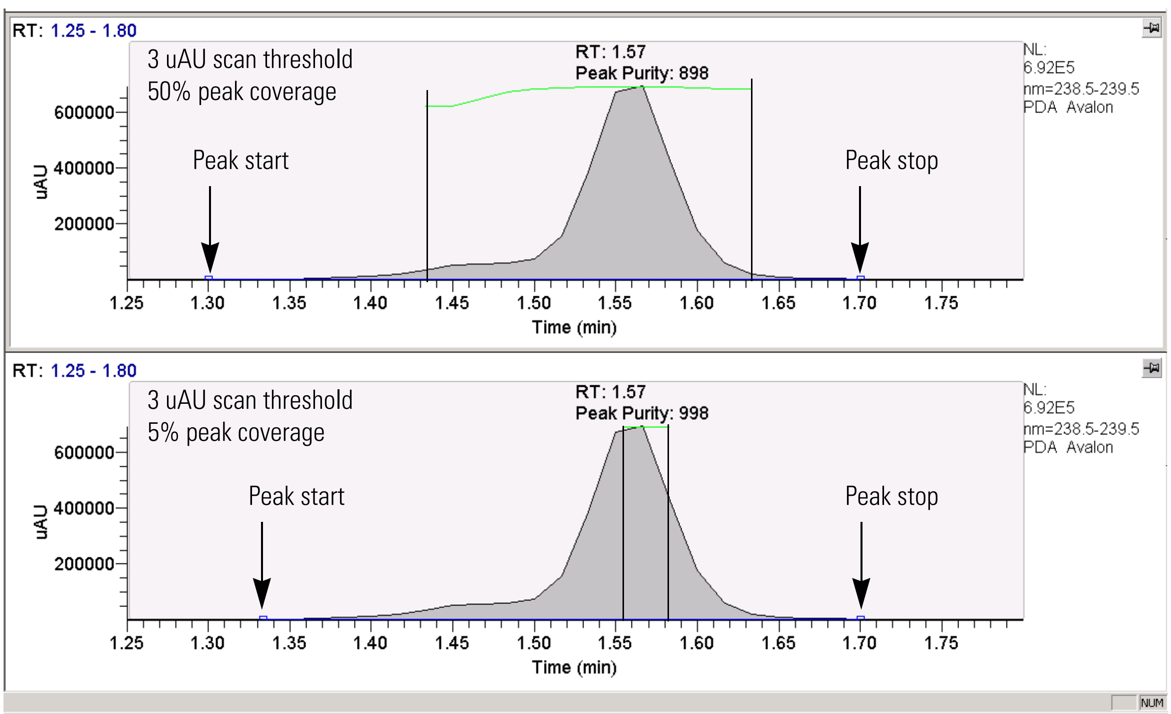


Figure 159. Effect of peak coverage on peak purity calculations



9

PDA Detector Performance Check and Calibration

This chapter describes the performance verification and calibration procedures for the PDA detector.

Contents

The PDA detector is factory tested for linearity, noise, and drift. Because of the sensitivity of its optical bench, adjust the light throughput to the diode array and recalibrate the PDA detector after you install it, each time you move it, change its flow cell, or replace either of the lamps.

Verifying the Performance of the PDA Detector

Before you perform either calibration procedure, turn on both lamps and let the temperature of the detector stabilize for approximately one hour (see "Turning the Lamps On or Off" on page 157).

This section contains the following procedures:

- "Creating a Display Method," on this page
- "Adjusting the Light Throughput to the Diode Array" on page 255

Creating a Display Method

Methods that displays a plot of integrated light intensity versus diode number have a .spda file extension.

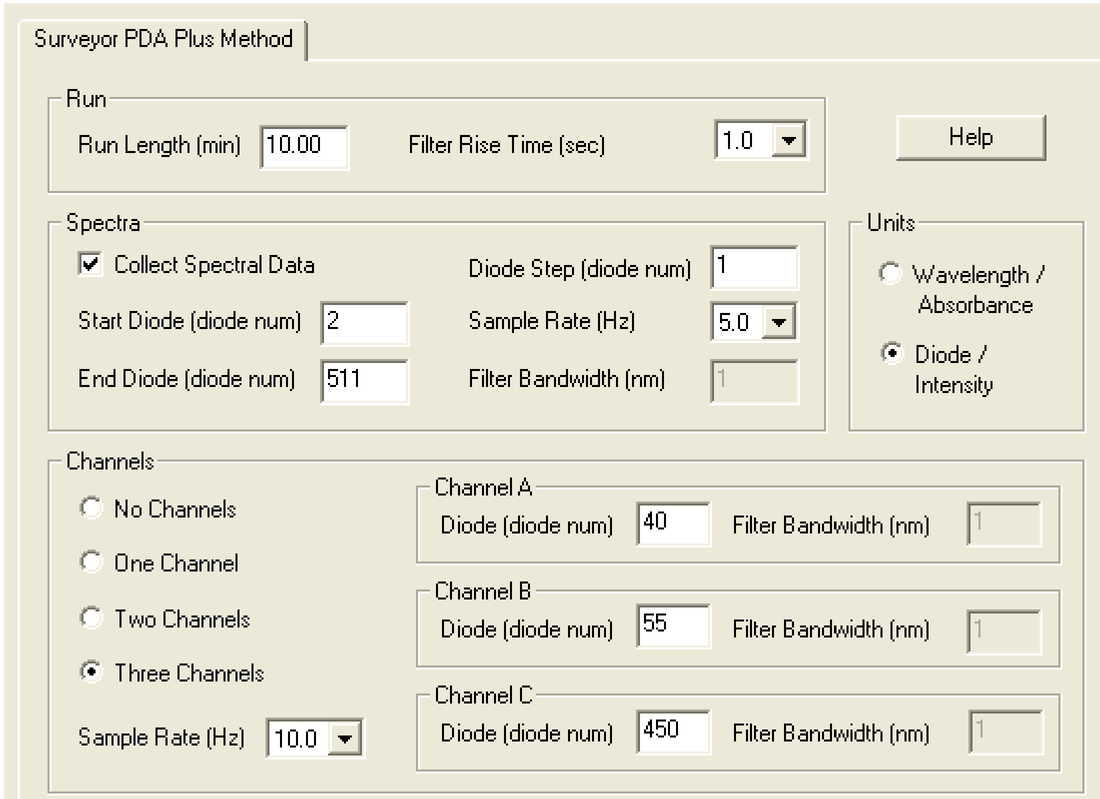
❖ **To create a display method for the PDA detector**

1. In the view bar of the Instrument Setup window, click the **Surveyor PDA Plus** button.

   The view for the PDA detector appears.

2. In the Units area, select the **Diode/Intensity** option (see Figure 160).

Figure 160. Surveyor PDA Plus Method page with the Diode/Intensity option selected



3. Verify that the following parameters are specified on the Surveyor PDA Method page in the Spectra area:

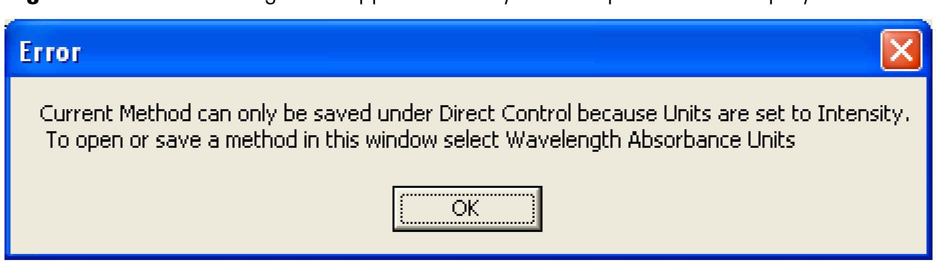
   - Start Diode (diode num) = 2
   - End Diode (diode num) = 511
   - Diode Step (diode num) = 1

Note For a display method, the following parameters are not downloaded to the PDA detector: run length, filter rise time, and sample rate.

4. Choose **File > Save** to save the display method.

   An error message appears (see Figure 161).

Figure 161. Error message that appears when you attempt to save a display method



5. Click **OK**.

6. From the menu bar, choose **Surveyor PDA > Direct Control**.

7. Click **Yes**.

   The Save PDA Display Method dialog box appears.

8. Save the display method.

   The data system saves the method with a .spda file extension.

   Note The .spda file extension is a special file extension used for all method files based on Diode/Intensity units. Files with the .spda extension can only be loaded and used in the Direct Control dialog box.

   After you save the method, the Direct Control dialog box appears with the Display page open.

9. To adjust light throughput to the diode array, go to "Adjusting the Light Throughput to the Diode Array" on page 255.

Adjusting the Light Throughput to the Diode Array

The PDA Detector has two attenuators that control the light throughput from the lamps. Manually adjusting the position of the attenuators increases or decreases the amount of light falling onto the array.

Adjust the light throughput to the diode array whenever you do the following:

- Observe an increase in the detector noise level
- Move the detector
- Replace either lamp or the flowcell

The first time that you adjust the attenuators, you must create a display method that records light intensities. Display methods are identified by their .spda file extension. After you create the method for adjusting the attenuators, save it with a descriptive name, such as diagnostics.spda, and store it for future use to simplify future adjustments of the attenuators.

Note Before you adjust the attenuators, replace the column with a flow restrictor, and set the pump to deliver HPLC-grade water at a flow rate of 1 mL/min through the flowcell.

To adjust the light output from the lamps, follow these procedures in order:

1. "Preparing the LC System for an Attenuator Adjustment" on page 256
2. "Adjusting the Attenuators" on page 257
3. "Adjusting the Attenuators" on page 257

Preparing the LC System for an Attenuator Adjustment

❖ To prepare the LC system for an attenuator adjustment

1. Ensure that the lamps are on.

   You can view the lamp status from the Configuration page of the Direct Control dialog box or from the PDA detector view of the Status page of the Information view. For information on the Configuration page, see “Configuration Page” on page 165.

2. Replace the LC column with a flow restrictor.

3. Start pumping HPLC-grade water at a flow rate of 1 mL/min through the flowcell.

Go to the next procedure, “Accessing the Attenuators.”

Accessing the Attenuators

❖ To access the attenuators

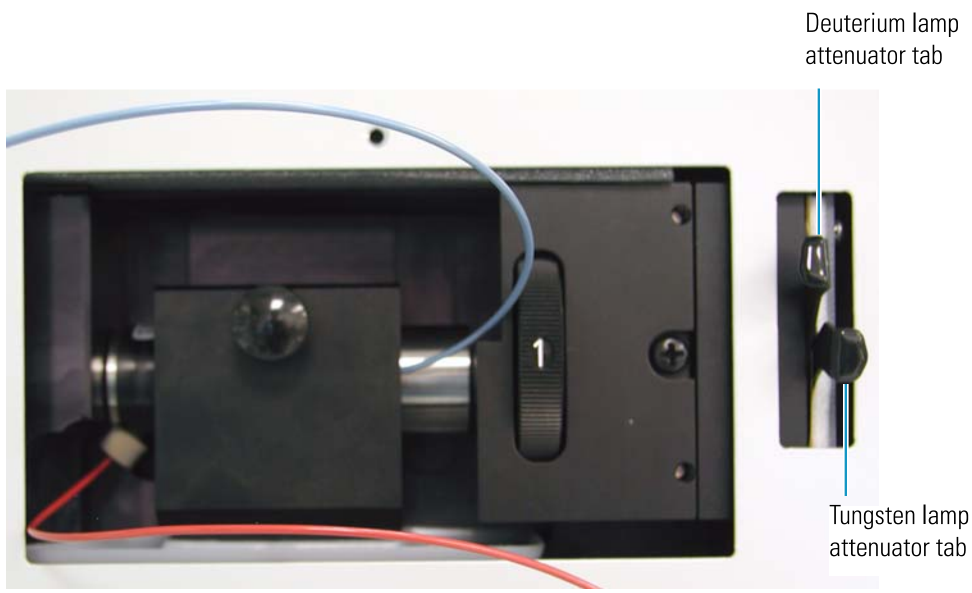
1. Open the front doors of the detector.

2. Unscrew the captive screw, and then remove the flowcell cover.

   The attenuators are located on the right side of the front panel. There are two black tabs attached to the attenuators for manual adjustments (see Figure 162).

   The left tab controls the light throughput from the deuterium lamp (UV region) and the right tab controls the light throughput from the tungsten lamp (Visible region). Moving an attenuator tab up increases the light throughput, and moving the tab down decreases the light throughput to the diode array.

Figure 162. Attenuator tabs for the Surveyor PDA Plus Detector



Go to the next procedure, “Adjusting the Attenuators” on page 257.

Adjusting the Attenuators

❖ To adjust the attenuators

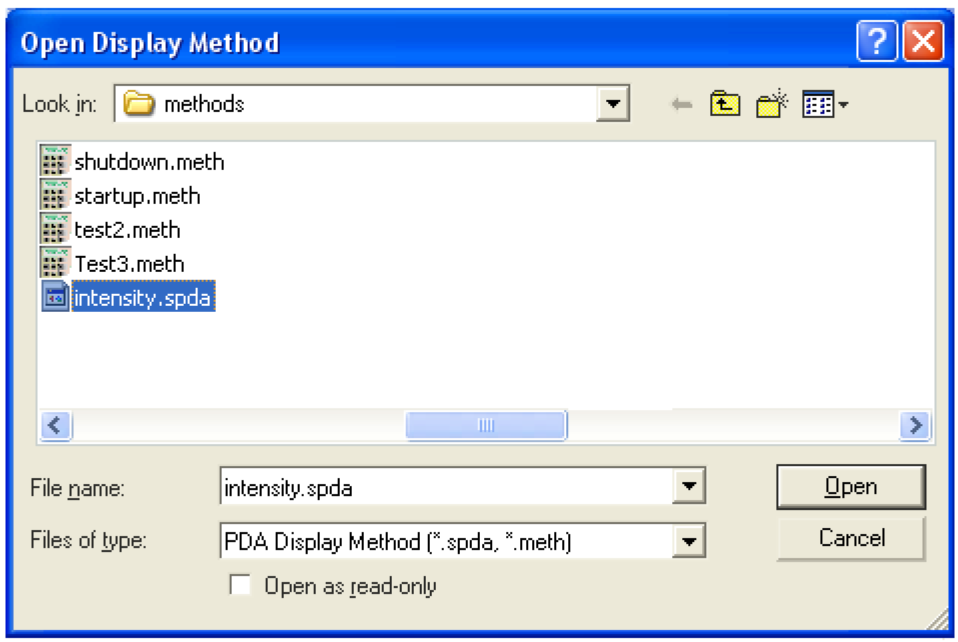
1. If you have not already done so, create a display method to view the light intensity from the lamps (see “Creating a Display Method” on page 253).
2. Load the display method to the detector as follows:
   a. In the Direct Control dialog box for the PDA detector, click the **Display** tab.

      The Display page appears.

   b. In the Control area, click **Load Method**.

      The Open Display Method dialog box appears (see Figure 163).

      Figure 163. Open Display Method dialog box for the PDA detector



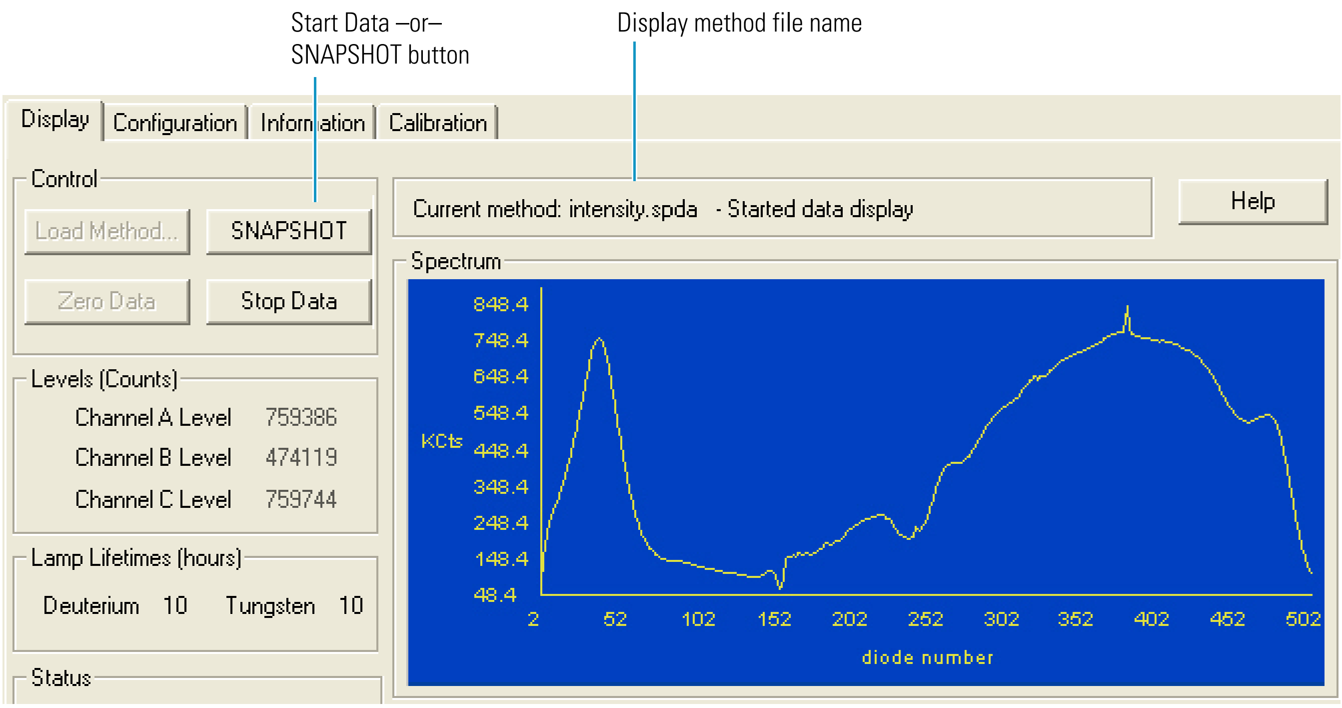
   c. Choose your display method file (*.spda).
   d. Click **Open**.

      The file name of the display method appears in the Current method readback on the Display page.

3. Start the data stream and adjust the attenuators as follows:
   a. In the Control area, click **Start Data**.

      The spectrum of light intensities appears in the top window (see Figure 164). For the UV region, the diode of maximum intensity is between diode 10 and diode 50. For the Visible region, the diode of maximum intensity is between diode 400 and diode 500. Ignore the spike at approximately diode number 380. This spike is due to the deuterium lamp.

Figure 164. Display page for the PDA detector with an intensity spectrum



b. Adjust the attenuators (see Figure 162 on page 256) as follows:

- Adjust the attenuator with the left tab on the PDA (UV range attenuation) to achieve a maximum value from 400000 to 900000 intensity counts in the region between diode number 10 and diode number 40.

- Adjust the attenuator with the right tab (Visible range attenuation) to achieve a maximum value from 400000 to 900000 intensity counts in the region between diode number 400 and diode number 500.

4. To record the light intensities, do the following:

- To save a picture of the scan to the Clipboard, press ALT+PRINT SCREEN. Paste this picture into a text editor such as Microsoft™ Word. Keep this scan for future comparisons to see if there is degradation in light intensity. Date the printout and add it to your maintenance records.

- To save the data to a file, click **SNAPSHOT**.

  The data system saves a comma-separated values file named **PDASnapshot.csv** to the following folder on the data system computer:

  C:\Xcalibur\data

  A date and time stamp is appended to the file name. The format of the date and time stamp is MMDDYYHHMMSS. MM is the month. DD is the day of the month. YY is the last two digits of the year. HH is the hour in military time. MM is minutes. SS is seconds. You can view this file with Microsoft Excel.

For a wavelength/absorbance method, the file contains the absorbance values for the spectral scan and the discrete channel wavelengths at the moment that you clicked Snapshot.

For an intensity/diode method, the file contains the intensity values for the scan of the diode array and the intensity values for up to three individual diodes.

5. After you finish adjusting the attenuators, replace the flowcell access cover, close the front doors of the detector, and replace the flow restrictor with your LC column.

Calibrating the PDA Detector

For information on calibrating the PDA detector, see these topics:

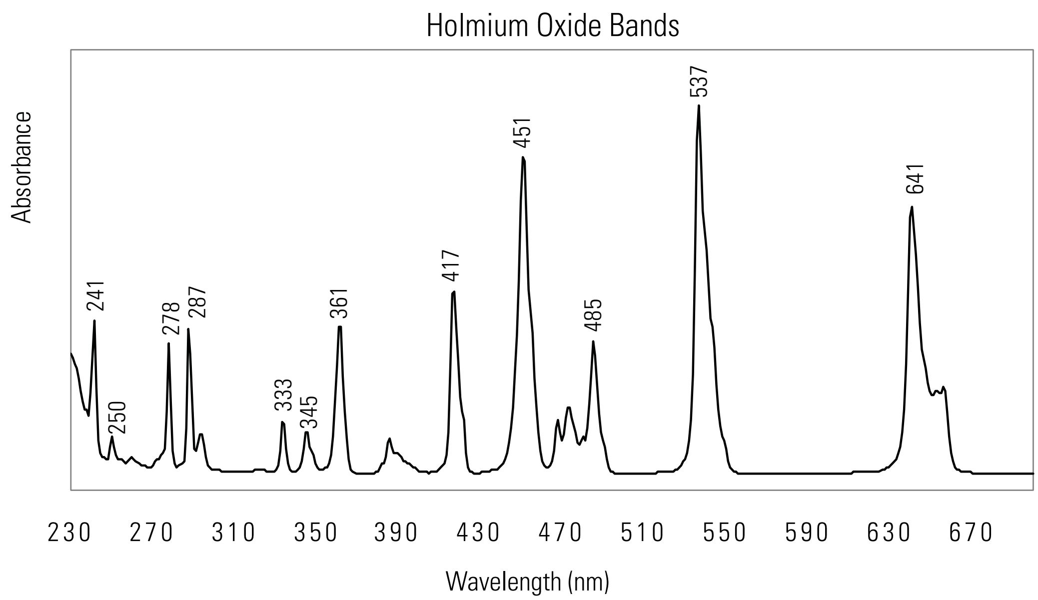
- "Calibration Page for the PDA Detector" on page 260
- "Performing a Dark Current Calibration" on page 263
- "Performing a Wavelength Calibration" on page 268
- "Creating and Editing a Custom Wavelength Calibration List" on page 278

To calibrate the wavelength accuracy of the optical bench, the PDA detector uses a holmium oxide reference solution, one of the four calibration files provided with the Xcalibur data system, or your own custom wavelength calibration file.

IMPORTANT Before you perform a wavelength calibration, verify that the diode array is not saturated (see "Adjusting the Light Throughput to the Diode Array" on page 255).

Figure 165 shows the holmium oxide spectrum.

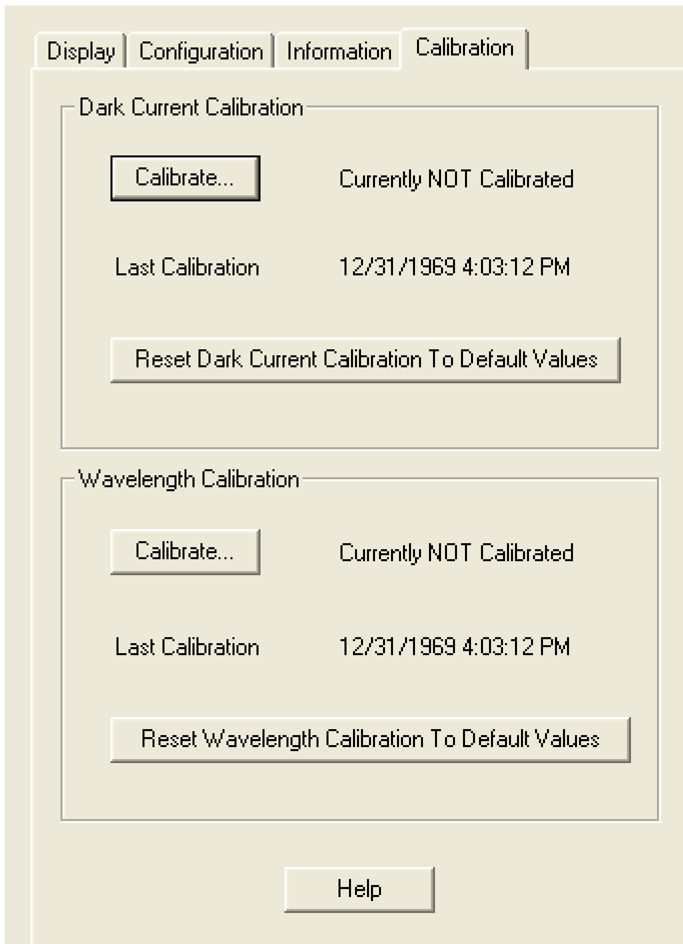
Figure 165. Holmium oxide spectrum



Calibration Page for the PDA Detector

Use the Calibration page (see Figure 166) to perform the dark current and wavelength accuracy calibrations. Perform these calibrations on a regular basis and as part of the operation verification procedure.

Figure 166. Calibration page, showing that the detector is not calibrated



Before you perform a wavelength calibration, verify that the diode array is not saturated (see "Adjusting the Light Throughput to the Diode Array" on page 255).

❖ To open the Calibration page

1. From the PDA detector view, choose **Surveyor PDA Plus > Direct Controls**.

   The Direct Control dialog box appears.

2. Click the **Calibration** tab.

Note The diode array used in the PDA detector has a background count level at each diode even when both lamps are off. This background count level is called the dark current. This background or dark current must be subtracted from the intensity counts before the intensity values are converted to absorbance units (AU). After you perform a dark current calibration, the PDA detector corrects the spectrum intensity values for the dark current.

❖ To start a dark current calibration

1. Turn on both lamps and wait one hour for the lamps to equilibrate (see "Turning the Lamps On or Off" on page 157).
2. In the Dark Current Calibration area, click **Calibrate**.

   The Dark Current Calibration wizard appears.
3. Follow the instructions provided by the wizard. For more information, click **Help** at the bottom of the Dark Current Calibration dialog box.

❖ To start a wavelength calibration

1. Disconnect the LC column from the system, and then pump HPLC-grade methanol through the flowcell to remove any contaminants.
2. Turn on both lamps and wait one hour for the lamps to equilibrate (see "Turning the Lamps On or Off" on page 157).
3. Verify that the array is not saturated ("Adjusting the Light Throughput to the Diode Array" on page 255).
4. In the Wavelength Calibration area, click **Calibrate**.

   The Wavelength Calibration wizard appears.
5. Follow the instructions provided by the wizard. For more information, click **Help** at the bottom of the Wavelength Calibration dialog box.

❖ To reset the wavelength or dark current calibration to the default values

- To reset the dark current calibration values, in the Dark Current Calibration area, click **Reset Dark Current Calibration To Default Values**.
- To reset the wavelength calibration values, in the Wavelength Calibration area, click **Reset Wavelength Calibration To Default Values**.

Calibration Page Parameter Descriptions

Table 55 describes the parameters on the Calibration page for the PDA detector.

Table 55. Calibration page parameters for the PDA detector

Parameter	Description
Dark Current Calibration	
Calibrate	Starts the dark current calibration.
Last Calibration	Indicates whether or not the PDA detector is currently calibrated. If the detector has not been calibrated, the readback is Not Calibrated. If the detector has been calibrated, the date and time of the last calibration is displayed.
Reset Dark Current Calibration To Default Values	Reloads the default instrument dark current calibration values. When you perform a dark current calibration, the Xcalibur data system compares the results to the last saved calibration file. This can cause successive calibrations to deviate from the default. Occasionally you need to reset to the default calibration values before performing a new dark current calibration to get calibration values as close to the default as possible.
Wavelength Calibration	
Calibrate	Starts the wavelength calibration.
Last Calibration [Wavelength]	Indicates whether the PDA detector is calibrated. If the detector has not been calibrated, Not Calibrated is displayed. If the detector has been calibrated, the date and time of the last calibration is displayed.
Reset Wavelength Calibration To Default Values	Reloads the default instrument wavelength calibration values. When you perform a wavelength calibration, the Xcalibur data system compares the results to the last saved calibration file. This can cause successive calibrations to deviate from the default. Occasionally, you need to reset to the default calibration values before performing a new wavelength calibration to get calibration values as close as possible to the default.

Performing a Dark Current Calibration

The function of the array calibration is to measure and correct for the dark current produced by the diodes of the photodiode array. The dark current is the small amount of background signal that is produced by the diodes of the array even when both lamps are turned off.

The environmental conditions of your laboratory can cause the dark current of the diode array to increase over time. For best results, perform an array calibration (dark current) after any of the following events occur:

- After 100 hours of use or monthly, whichever comes first
- Whenever a 5 °C change in the operating environment occurs
- After you move the detector
- After you replace the lamp
- After you download a new firmware file

Because the dark current produced by the diodes rises as the temperature within the detector rises, warm up the lamps for one hour before you perform a dark current calibration. Warming up the lamps for one hour allows the detector to equilibrate to its normal operating temperature.

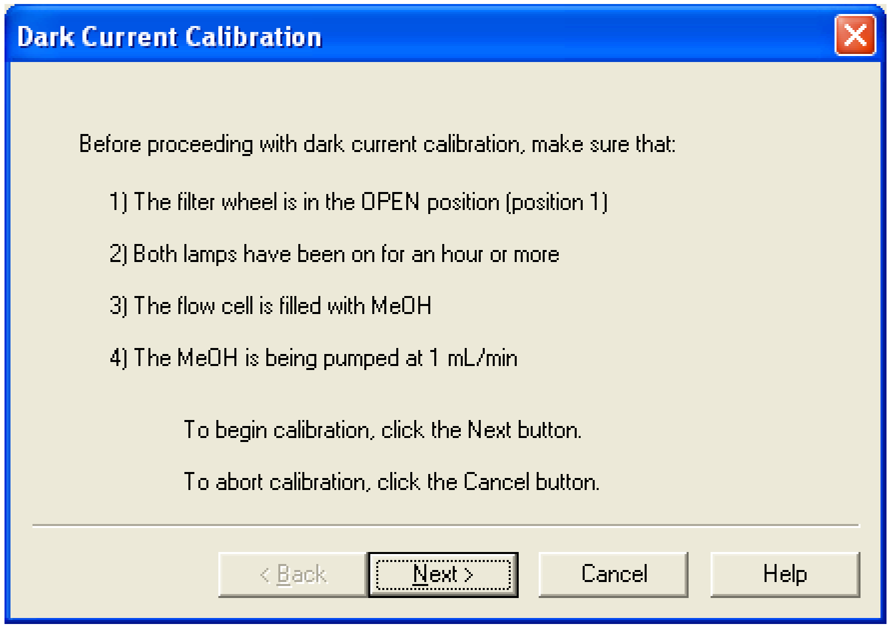
The lamps turn off during the dark current calibration routine. After the dark current calibration routine finishes, the lamps turn back on.

❖ To perform the dark current calibration

1. Pump methanol through the flow cell at 1 mL/min.
2. Turn on both lamps and wait one hour for the lamps to equilibrate.
3. Open the Calibration page for the PDA detector.
4. To start the wizard for the dark current calibration, click **Calibrate** in the Dark Current Calibration area.

   The preconditions page appears (see Figure 167).

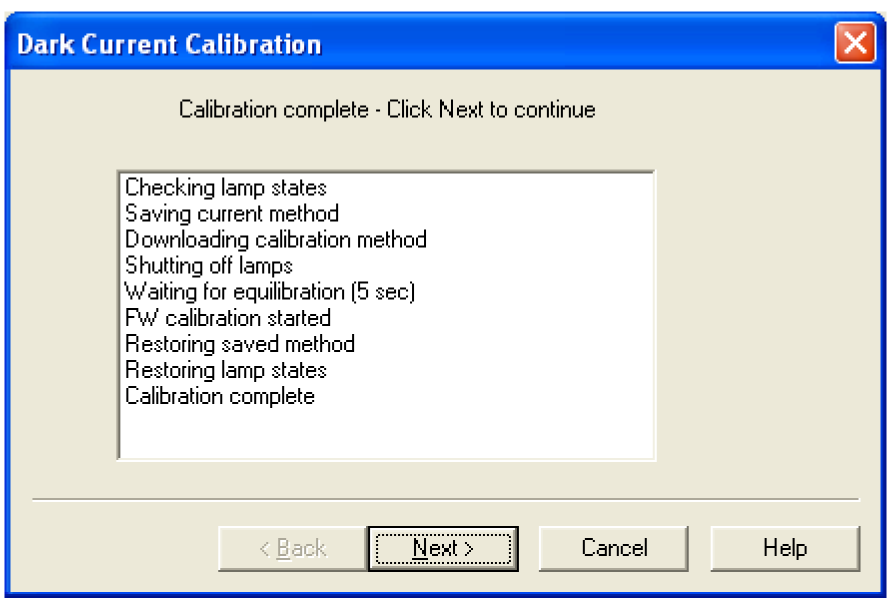
Figure 167. Preconditions page



5. Read the list of preconditions, and determine if the PDA detector is ready:
   - If the detector meets all the preconditions, click **Next** to proceed with the calibration.

     The status page appears.
   - If the detector does not meet all the preconditions, click **Cancel** to exit the wizard. Then prepare the PDA detector for calibration and begin this wizard again.
6. Observe the status pane as the calibration proceeds (see Figure 168).

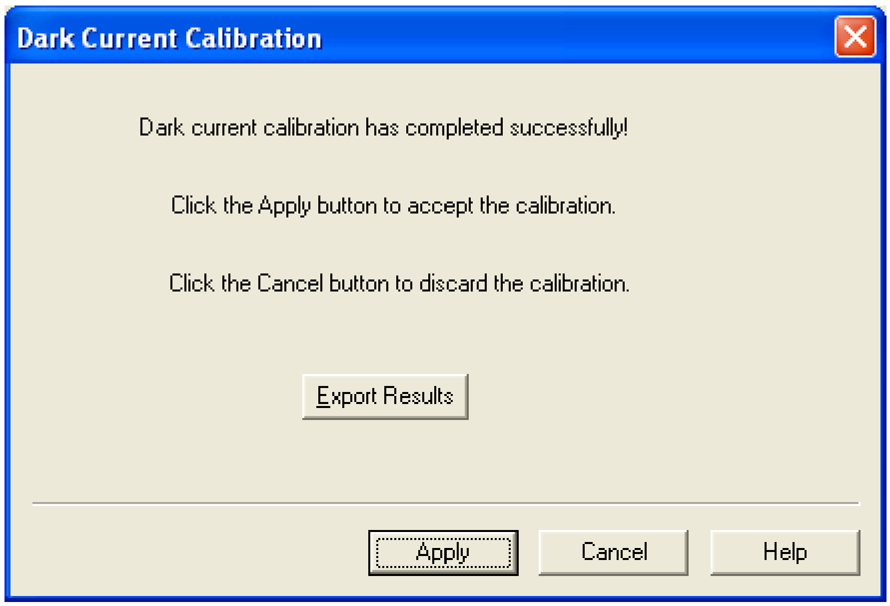
Figure 168. Calibration status page



7. When the calibration is complete, click **Next**.

   The next page of the wizard appears (see Figure 169). You can export the results of the calibration from this page.

   Figure 169. Export results page

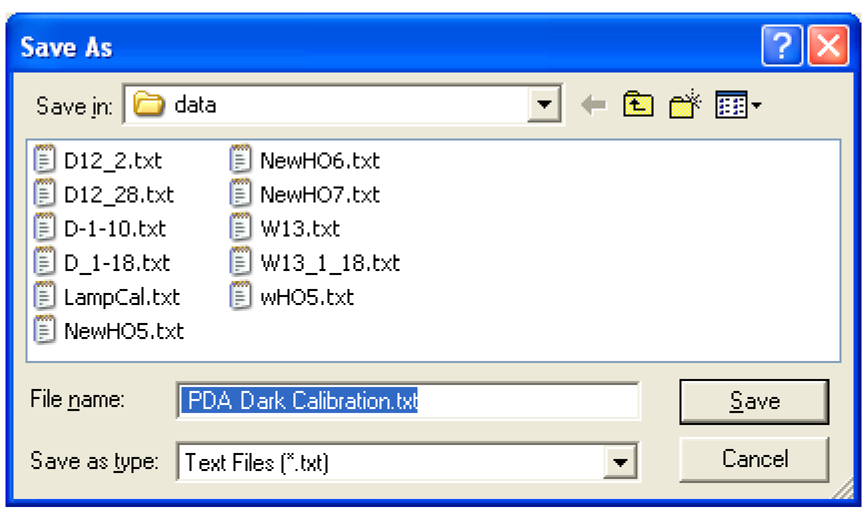
   

8. (Optional) To print a record of the dark current calibration, do the following:

   a. Click **Export Results**.

      The Save As dialog box appears (see Figure 170).

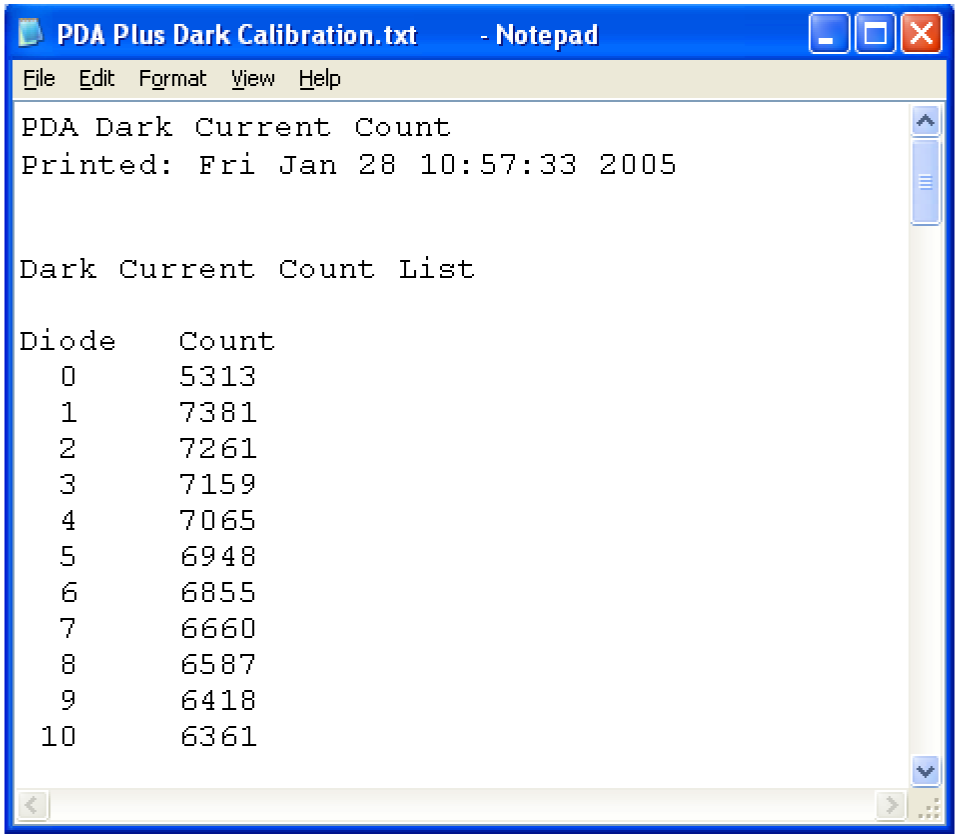
      Figure 170. Save As dialog box

   b. Type a name in the File name box, and then click **Save**.

      Once you have saved the file with a name of your choice, you can view or print the contents of the file using any text editing program (see Figure 171).

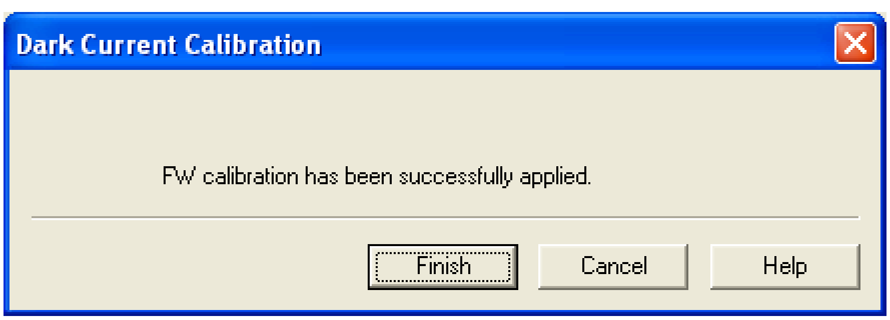
Figure 171. PDA dark current calibration text file



9. Click **Apply** to download the calibration results to the detector.

   The wizard proceeds to the success confirmation page (see Figure 172).

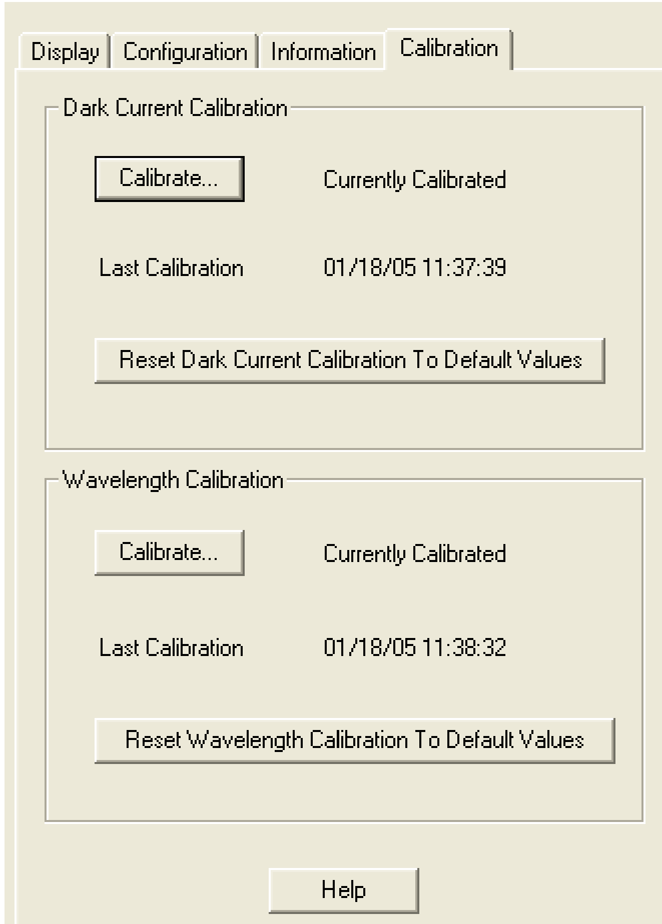
   Figure 172. Success confirmation page



10. Click **Finish** to close the wizard.

    The calibration is saved and the date and time of calibration are displayed in the Dark Current Calibration area of the Calibration page as the Last Calibration (see Figure 173).

Figure 173. Calibration page, showing the Currently Calibrated status



Performing a Wavelength Calibration

The alignment of the spectrum on the diode array depends on the physical alignment of various components of the optical bench. The alignment can become offset if the detector is sharply jolted, in shipping, for example. Such bumps and jars can slightly change the wavelength of light reaching the photodiode array. The automated wavelength calibration procedure determines the detector's wavelength accuracy and adjusts the detector's wavelength algorithm to correct for any misalignment.

❖ To perform a wavelength calibration

1. Pump HPLC-grade methanol at 1 mL/min through the flowcell.
2. Turn on both lamps and wait one hour for the lamps to equilibrate as follows:
   a. From the PDA detector view, choose **Surveyor PDA Plus > Direct Controls**.

      The Direct Control dialog box appears.

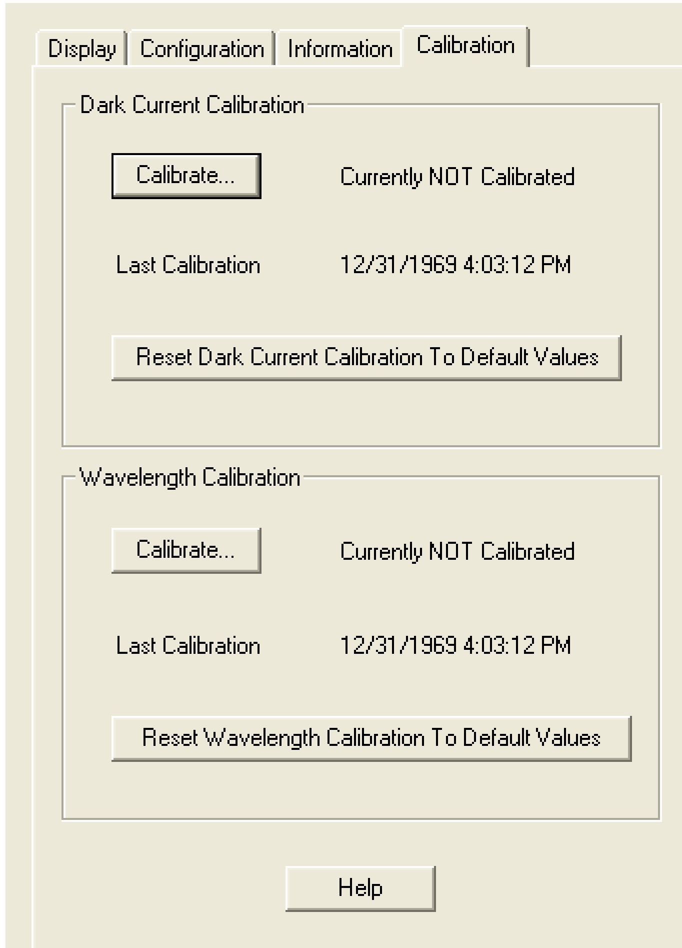
   b. Click the **Configuration** tab.

      The Configuration page appears.

   c. In the Deuterium Lamp area, click **Lamp On** to turn on the deuterium lamp.
   d. In the Tungsten Lamp area, click **Lamp On** to turn on the tungsten lamp.
3. Start the Wavelength Calibration wizard as follows:
   a. Click the **Calibration** tab.

      The Calibration page appears (see Figure 174).

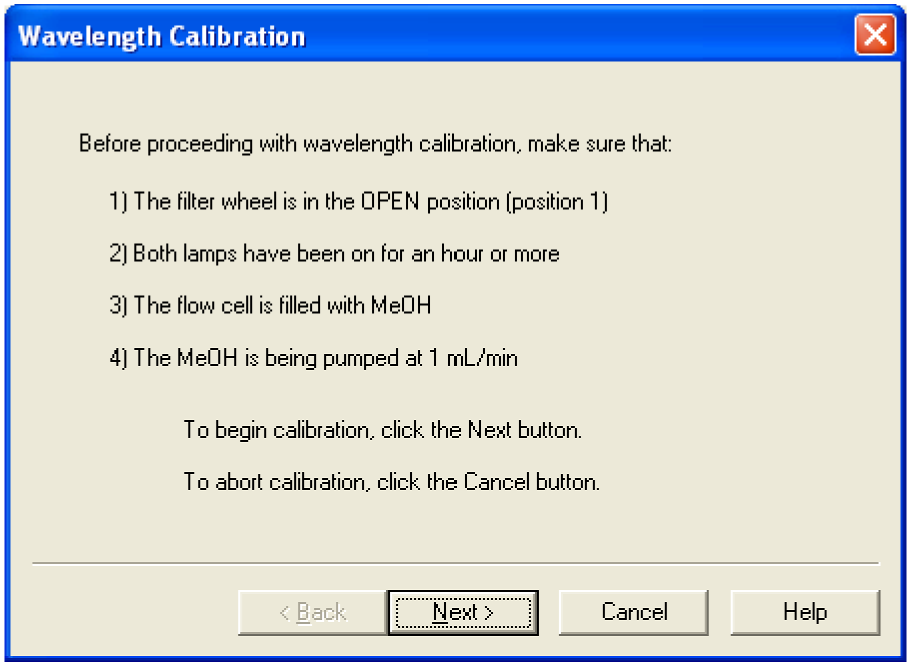
Figure 174. Calibration page for the PDA detector



b. In the Wavelength Calibration area, click **Calibrate**.

Page 1 of the wizard appears. This page lists the preconditions required to perform a wavelength calibration (see Figure 175).

Figure 175. Preconditions page



c. Read the preconditions, and determine if the detector meets these preconditions:

- If the detector meets all of the preconditions, click **Next** to proceed to the next page of the wizard where you are prompted to select a wavelength file.
- If the detector does not meet all the preconditions, click **Cancel** to exit the wizard and prepare the PDA detector for calibration.

Note On any page of the wavelength calibration wizard, you can click Cancel buttons to exit the procedure.

4. Do the following:

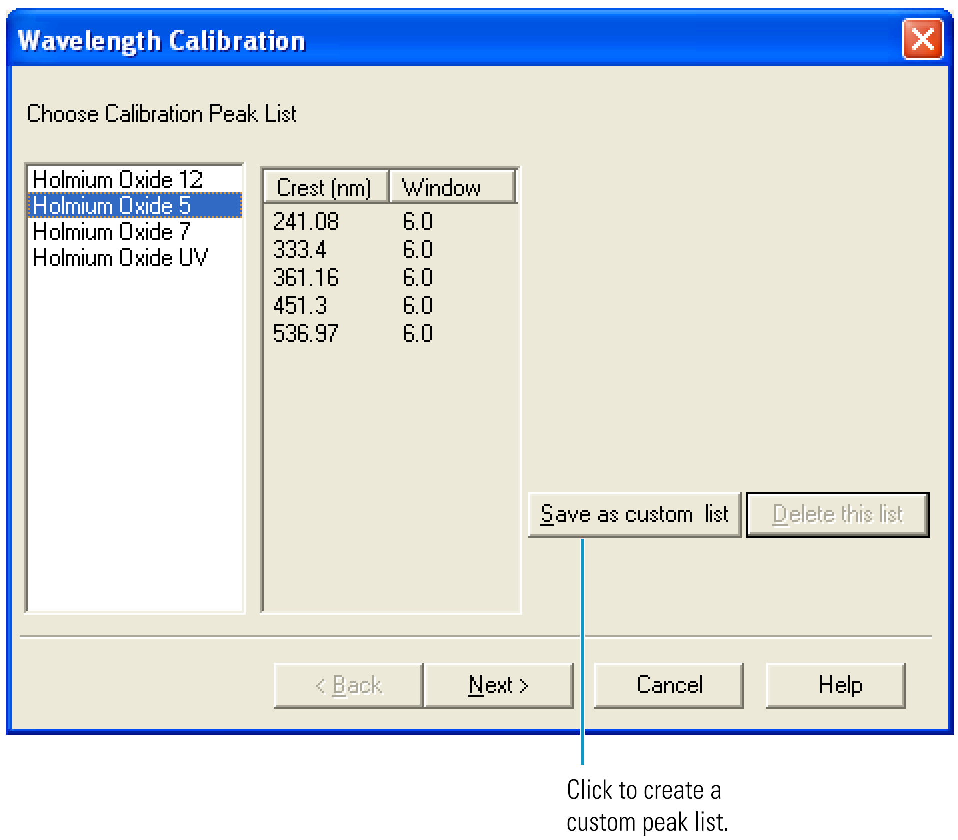
   a. Select a peak set from the list.

      The peak set should span the wavelengths you use under normal operating conditions.

      Figure 176 shows the selection of the Holmium Oxide 5 peak set. This wavelength list instructs the program to calibrate the detector at each of the five wavelengths shown.

      Note The data system has four calibration files to select from. For example, the Holmium Oxide UV file contains five wavelengths in the UV region, while the other files use sets of wavelengths from both the UV and visible wavelength regions. The holmium oxide absorbance maxima are selected from a spectrum published in "Holmium Oxide Solution Wavelength Standard from 240 to 640 nm - SRM 2034 (NIST Special Publication 260-54)."

Figure 176. Choose calibration peak list page



Note For information on creating a custom peak list, see "Creating and Editing a Custom Wavelength Calibration List" on page 278

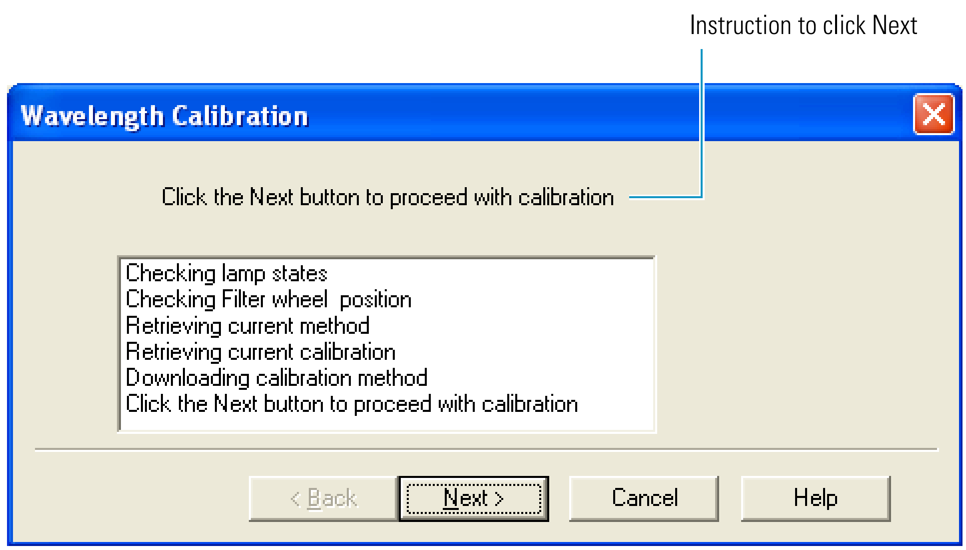
b. Click **Next.**

The next page of the wizard appears (see Figure 177).

5. Do the following:

a. Observe the status pane that tells you the wavelength file is being downloaded (see Figure 177).

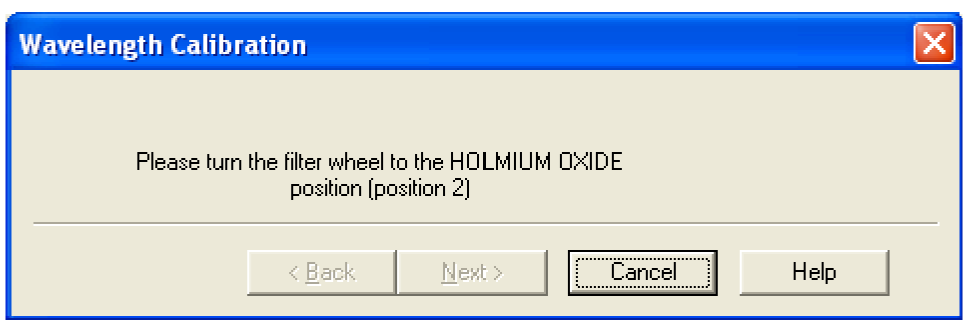
Figure 177. Download status for the selected peak list page



b. After you see the message: Click the Next button to proceed with the wavelength calibration, click **Next**.

The next page of the Wavelength Calibration wizard appears (see Figure 178). This page instructs you to place the filter wheel in position 2.

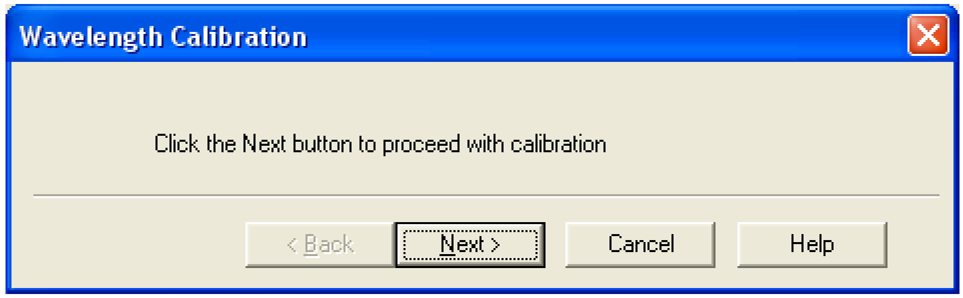
Figure 178. Request to rotate the filter wheel to position 2 page



6. Rotate the Holmium Oxide filter wheel to position 2 as directed.

After you turn the wheel, the Next button becomes available (see Figure 179).

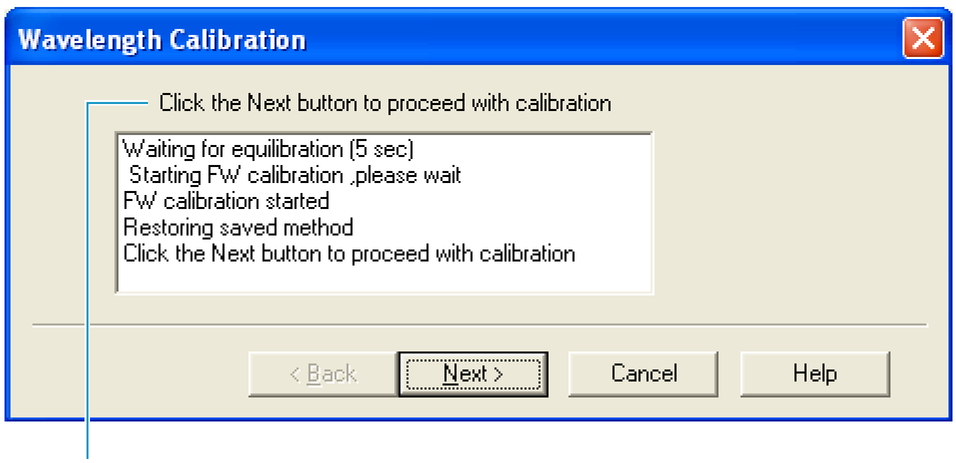
Figure 179. Available Next button



7. Click **Next**.

   The calibration status page of the wizard appears (see Figure 180).

Figure 180. Calibration status page



8. Do the following:

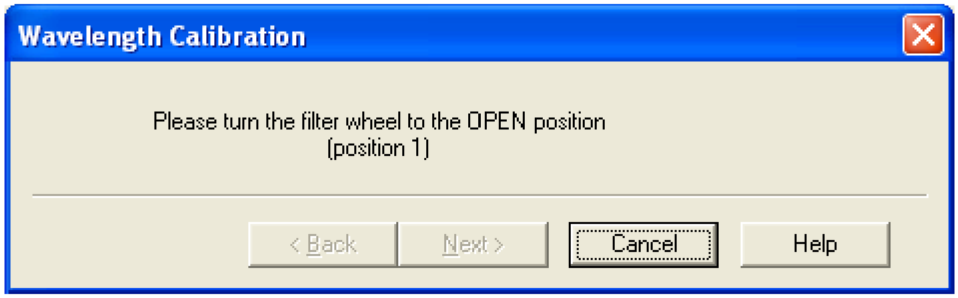
   a. Observe the status pane (see Figure 180).

      The diagnostics program waits for a few seconds for the rise time filter to equilibrate, and then the detector takes a holmium oxide scan.

   b. When you see the message: Click the Next button to proceed with calibration, click **Next**.

      The next page of the wizard appears (see Figure 181).

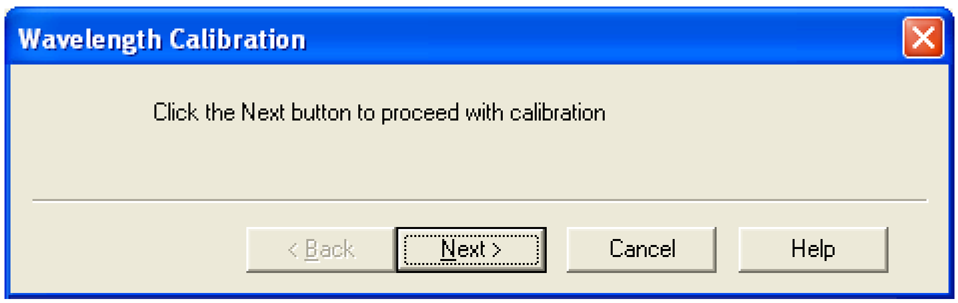
Figure 181. Request to rotate the filter wheel to position 1 page



9. Rotate the wheel back to position 1 (Open) as instructed.

   After you rotate the wheel, the Next button becomes available (see Figure 182).

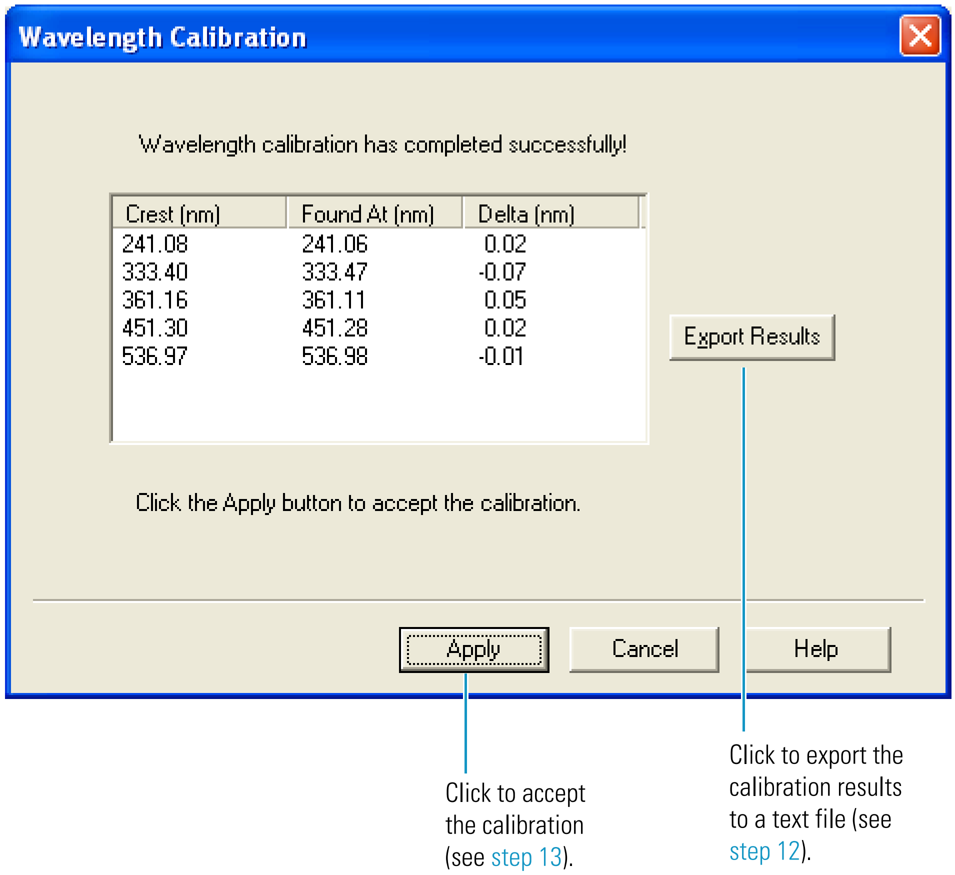
   Figure 182. Available Next button

10. Click **Next** to proceed.

    The results page for the wavelength calibration appears (see Figure 183).

Figure 183. Delta values page



11. Verify that the delta values are within ±1 nm:

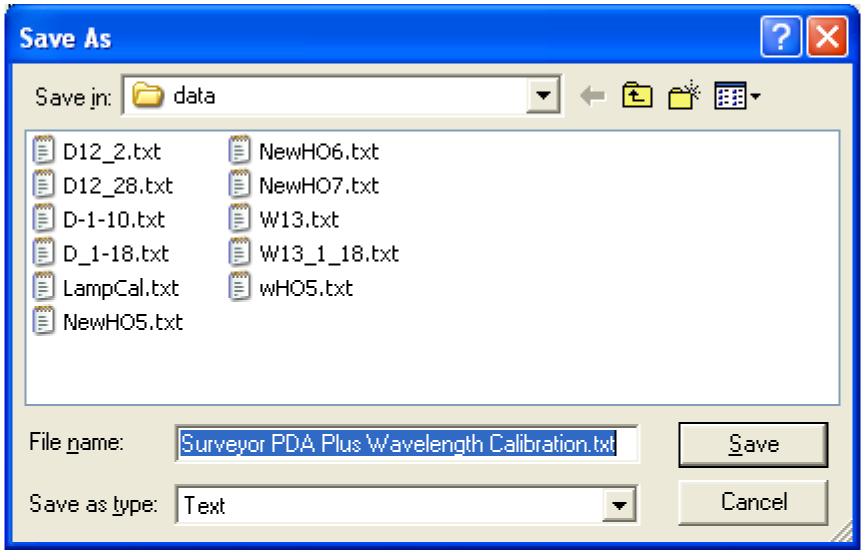
    - If the Delta values are acceptable, go to step 12.
    - If the delta values are not within the range of ±1 nm, do not export the results. Complete the calibration procedure. Then, repeat the wavelength calibration. If, after applying a second calibration, the Delta values are still not within the range of ±1 nm, call your Thermo Fisher Scientific service representative for assistance.

12. (Optional) To print a report of the calibration results, do the following:

    a. Click **Export Results** to print the results to a file.

       The Save As dialog box appears (see Figure 184).

Figure 184. Save As dialog box



b. Type a name in the File name box, and then click **Save**.

After you save the file with a name of your choice, you can view or print the contents of the file using any text editing program (see Figure 185).

Figure 185. Wavelength Calibration file, viewed in Notepad

Holmium12.txt - Notepad

File Edit Format View Help

Wavelength Calibration Report Printed: Tue Dec 28 13:08:26 2004

Wavelength Calibration File:

Window	Target	Found	Difference	Diagnostic Peak	Diode Array Offset
5.00	241.08	240.85	0.23	243.34	-2.26
5.00	249.98	250.12	-0.14	252.79	-2.81
3.00	278.03	277.99	0.04	281.16	-3.13
3.00	287.47	287.52	-0.05	290.84	-3.37
6.00	333.40	333.52	-0.12	337.45	-4.05
6.00	345.49	345.51	-0.02	349.55	-4.06
6.00	361.16	361.09	0.07	365.27	-4.11
6.00	416.62	416.90	-0.28	421.34	-4.72
6.00	451.30	451.41	-0.11	455.86	-4.56
6.00	485.84	485.36	0.48	489.70	-3.86
6.00	536.97	536.93	0.04	540.87	-3.90
6.00	640.84	640.94	-0.10	643.29	-2.45

Regression Coefficients
a = -162.729   b = 0.870   c = -0.0000425

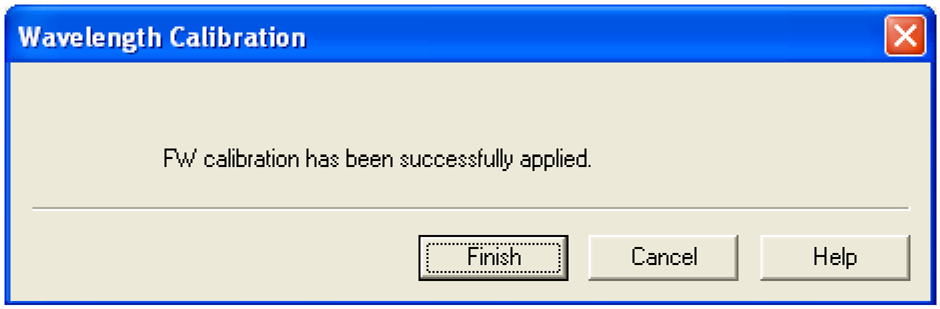
Wavelength Calibration Table

Wave	Diode A	Diode B	Fract A	Fract B	Interpolation Type
190	0	0	1.000000	0.000000	0
191	0	1	0.162295	0.837705	1
192	1	2	0.324590	0.675410	1
193	2	3	0.486885	0.513115	1
194	3	4	0.649180	0.350820	1
195	4	5	0.811475	0.188525	1
196	5	6	0.973770	0.026230	1
197	5	6	0.136066	0.863934	1

13. To apply the calibration results to the detector, click **Apply** on the Delta Values page (see Figure 183 on page 275).

    The success confirmation page appears (see Figure 186).

    Figure 186. Success confirmation page

14. Click **Finish** to complete the wavelength calibration.

    The calibration is saved. The date and time of the calibration are displayed in the Wavelength Calibration area of the Calibration page (see Figure 174 on page 269).

Creating and Editing a Custom Wavelength Calibration List

Use the Custom List Name dialog box to name a custom wavelength calibration list (WCL file).

❖ To open the Custom List Name dialog box and the Edit Peak List area

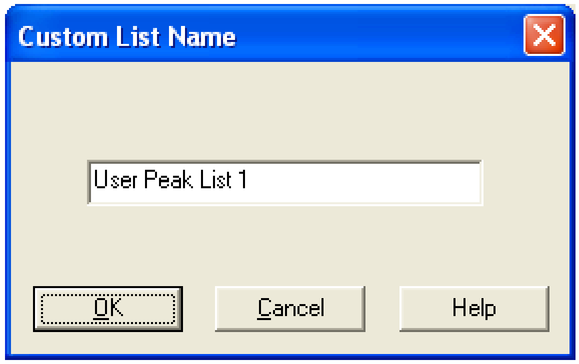
1. Start the Wavelength Calibration wizard (see "Performing a Wavelength Calibration" on page 268).
2. Verify that the PDA detector meets the preconditions for a wavelength calibration and click **Next**.

   The wavelength list page appears (see Figure 176 on page 271).

3. Click **Save As Custom List**.

   The Custom List Name dialog box appears (see Figure 187).

Figure 187. Custom List Name dialog box



4. Type a name for the list in the box, and then click **OK** to add the name to the Choose Calibration Peak List and close the dialog box.

   The name of the custom list appears in the Choose Calibration Peak List box.

5. In the Choose Calibration Peak List box, click the custom list name.

   The wavelength list becomes available.

6. In the wavelength list, click a wavelength that you want to edit.

   The Edit Peak List area appears to the right of the wavelength list.

❖ To create a custom wavelength calibration list

1. Start the Wavelength Calibration wizard (see "Performing a Wavelength Calibration" on page 268).
2. Click **Save As Custom List**.

   The Custom List Name dialog box appears.

3. In the box, type a name.
4. Click **OK**.

   The name of the new wavelength calibration list appears in the Choose Calibration Peak List box.

❖ To edit the wavelength calibration list

1. Click the **Crest/Window** pane.

   The Edit Peak List area appears.

2. To add a wavelength, type a wavelength in the Peak Crest box and a window in the Peak Window box, and then click **Add**.
3. To delete a wavelength, select the wavelength, and then click **Delete**.

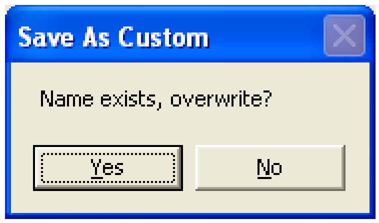
4. Click **Save As Custom List** to save the edited list.

   The Custom List Name dialog box appears.

5. Type the name of the custom list in the box.

   The Save As Custom dialog box appears (see Figure 188).

   Figure 188. Save As Custom dialog box

6. Click **Yes** to save the wavelength calibration list.

Edit Peak List Area

Table 55 describes the parameters in the Edit Peak List area on the Choose calibration peak list page of the Wavelength Calibration wizard.

Table 56. Edit Peak List area of the Wavelength Calibration wizard for the PDA detector

Parameter	Description
Peak Crest	Specifies a spectral band. The holmium oxide wheel contains a solution of holmium oxide in perchloric acid. Range: 190 to 800 nm.
Peak Window	Specifies the absolute wavelength range for the peak crest. Wavelength range = peak crest ± peak window/2 Range: 0.1 to 611.0.
Buttons	
Modify	Modifies the selected spectral band.
Add	Adds a spectral band.
Delete	Deletes a spectral band.

10

Autosampler Calibration and Record Keeping

This chapter describes the calibration and record keeping procedures for the autosampler.

Contents

The autosampler does not require calibration upon arrival at its shipping destination. However, if you use custom vials or custom microplates, you must perform the Well bottom distance calibration, which determines the actual depth of a vial or microplate well (see "Well Bottom Distance Calibration" on page 313). If problems occur with the column oven control, the tray temperature control, or the arm positioning, contact a Thermo Fisher Scientific service representative. This appendix includes calibration procedures for these items; however, to calibrate the autosampler's temperature zones and XYZ arm position, you must have the kit that contains the necessary test fixtures.

Calibrating the Autosampler

There are four calibration options for the autosampler:

- “Column Oven Air Sensor Calibration” on page 283
- “Vial Tray Metal Sensor Calibration” on page 289
- “Arm Calibration” on page 295
- “Well Bottom Distance Calibration” on page 313

The column oven and oven compartment temperature calibrations and the arm calibration are typically performed by a Thermo Fisher Scientific field service representative. To perform these calibrations yourself, you must have the service kit that contains the temperature test fixtures. In addition, to calibrate the temperature sensors for the autosampler’s temperature-controlled zones, your Thermo Scientific application program must be capable of providing feedback for these sensors.

Note Typically, a Thermo Fisher Scientific field service representative calibrates the controlled temperature zones and the XYZ arm position. To perform the temperature calibrations yourself, order the Field Service Calibration Kit (P/N 60053-62001). To calibrate the XYZ arm, order the LED light fixture and the non-reflective target port fixture.

If you choose to use custom vials or custom microwell plates, you must perform the Well Bottom Distance Calibration, which determines the depth of the custom vial or microplate well. This calibration must be performed each time you select a new custom tray configuration and each time you use a new type of custom vial or custom microwell plate.

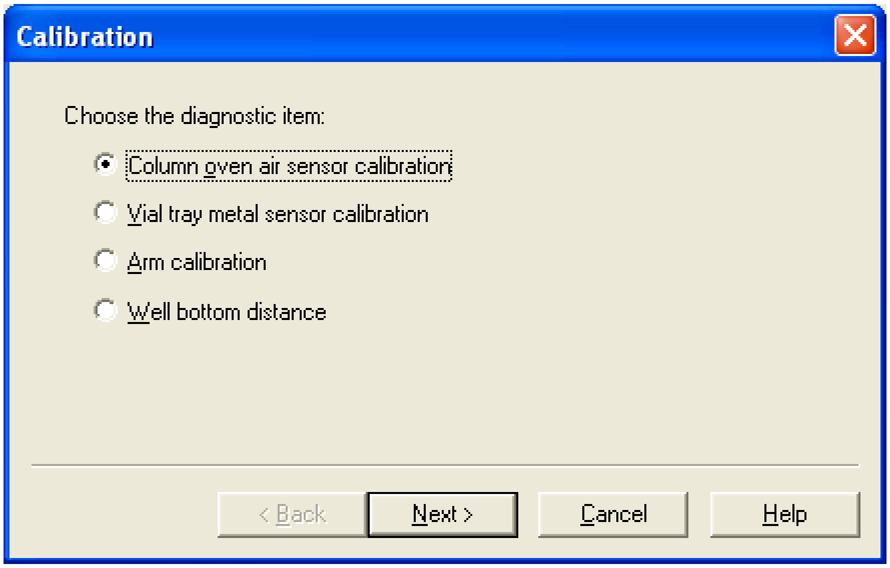
Note If you are controlling the autosampler from a Thermo Scientific data system other than the Xcalibur data system, refer to the Help provided with your data system for information on accessing the Calibration dialog box for the autosampler.

❖ To open the Calibration dialog box for the autosampler

1. Open the view for your autosampler.
2. In the menu bar, choose **Surveyor AS > Calibration**.

   The Calibration dialog box appears (see Figure 189).

Figure 189. Calibration dialog box for the autosampler



Column Oven Air Sensor Calibration

To calibrate the column oven temperature, you must have the oven sensor test fixture provided in the Field Service Calibration Kit (P/N 60053-62001) for the autosampler.

CAUTION Because the column oven compartment can reach temperatures as high 95 °C (203 °F), ensure that the column oven is off and that the column oven compartment is at room temperature before you install the oven sensor test fixture.

To calibrate the column oven temperature, follow these procedures in order:

1. "Preparing to Calibrate the Column Oven Temperature," on this page
2. Specifying the Internal Set Target for the Column Oven Calibration Temperature
3. Adjusting the Temperature Calibration for the Column Oven

Preparing to Calibrate the Column Oven Temperature

❖ **To set up the system for a temperature calibration**

1. Open the status view for the autosampler's oven temperature.

   To open the Oven page from the Xcalibur data system, do the following:

   a. If the Information view is closed, click the **Information View** button () to open the view.

      The Information view opens with the Status page displayed.

b. Verify that the status reads Ready to Download.

   For information on checking the status, see “Checking the Status of the LC Devices” on page 130.

c. Click **Surveyor AS** in the directory tree.

   The status pages for the autosampler appear at the bottom of the Status page.

d. Click the **Oven** tab.

   The Oven status page appears.

2. On the Oven page, check the status of column oven.

3. If the column oven is on and set to a high temperature, turn the column oven off, open the column oven door, and wait for the column oven to cool to room temperature.

   For information on using the direct controls to turn the column oven on or off, see “Controlling the Tray and Oven Compartment Temperatures” on page 193.

4. Install the oven sensor test fixture (869C thermometer or equivalent) as follows:

   a. If it is not already open, open the column oven door.

   b. Loosen the top thumbscrew that holds the column clamp.

   c. With the sensor facing down, slide the metal cable protector under the right side of the clamp.

   d. Verify that the sensor is between the upper and lower column clamps and that it is not touching any metal.

   e. Tighten the thumbscrew on the sensor, route the cable of the sensor so as not to interfere with the door, and then close the column oven door.

5. Open the Calibration dialog box for the autosampler. For information on accessing the Calibration dialog box from the Xcalibur data system, see “Calibrating the Autosampler” on page 282.

Specifying the Internal Set Target for the Column Oven Calibration Temperature

Use the Internal Set Target page of the Column oven air sensor calibration wizard to specify the calibration temperature.

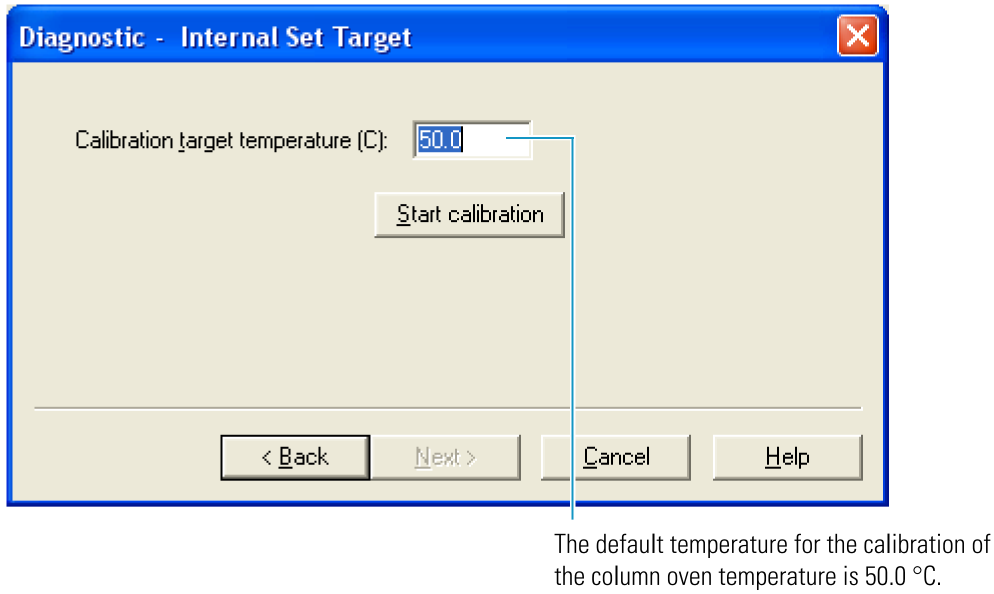
❖ To specify the internal set target for the calibration temperature

1. Set up the system to perform the column oven calibration (see “Preparing to Calibrate the Column Oven Temperature” on page 283).

2. Select the **Column oven air sensor calibration** option, and then click **Next**.

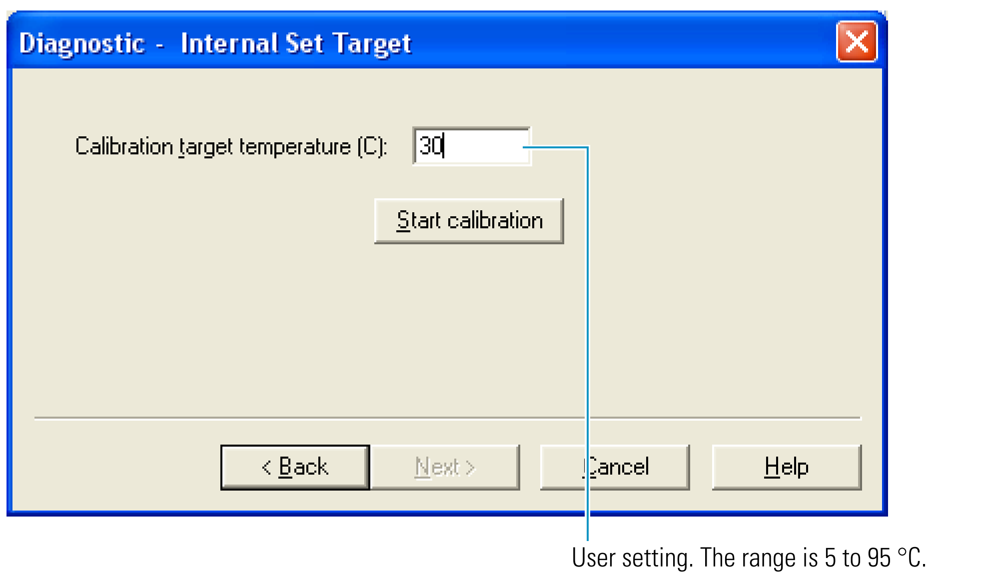
   The Diagnostic - Internal Set Target page of the Column oven air sensor wizard appears (see Figure 190). The default calibration temperature is 50.0 °C.

Figure 190. Diagnostic - Internal Set Target page of the Column oven air sensor calibration wizard with the default temperature setting of 50.0 °C



3. In the Calibration target temperature (C) box, type **30** (see Figure 191).

Figure 191. Diagnostic - Internal Set Target page of the Column oven air sensor calibration wizard with the user setting of 30 °C



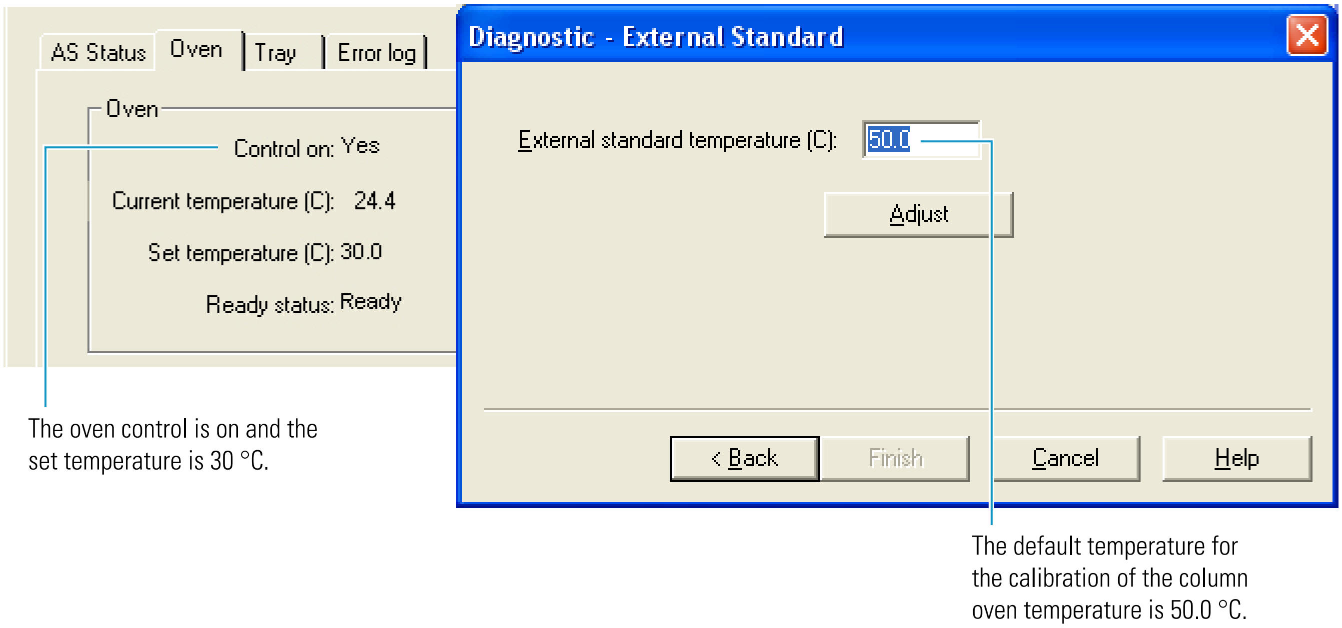
4. Click **Start calibration**.

   The data system turns on the column oven and downloads the target temperature and the Next button becomes available.

5. Click **Next**.

The Diagnostic - External Standard page of the Column oven air sensor wizard appears (see Figure 192). The default calibration temperature for the external standard is 50 °C.

Figure 192. Oven page and the Diagnostic - External Standard page of the Column oven air sensor calibration wizard



Adjusting the Temperature Calibration for the Column Oven

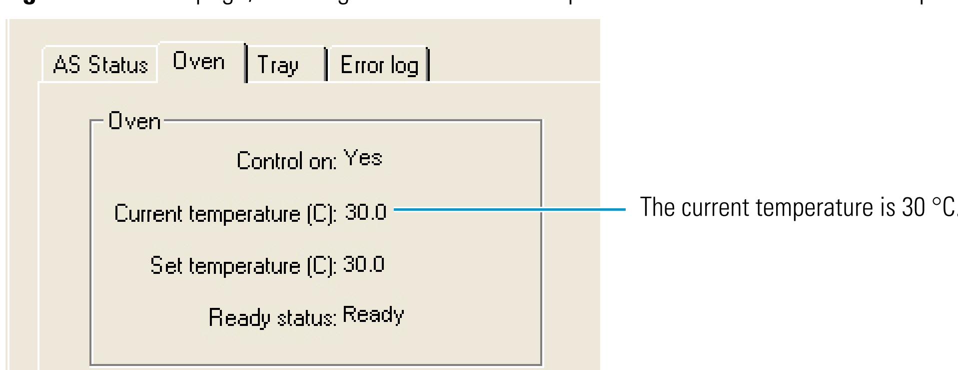
❖ To adjust the temperature calibration for the Column Oven

1. Specify the calibration temperature and start the Column oven air sensor calibration wizard (see "Specifying the Internal Set Target for the Column Oven Calibration Temperature" on page 284).

2. Wait for the current temperature readback on the Oven page to reach 30.0 °C (see Figure 193).

   The Current temperature readback displays the temperature recorded by the autosampler's internal temperature sensor.

Figure 193. Oven page, showing that the current temperature has reached the set temperature



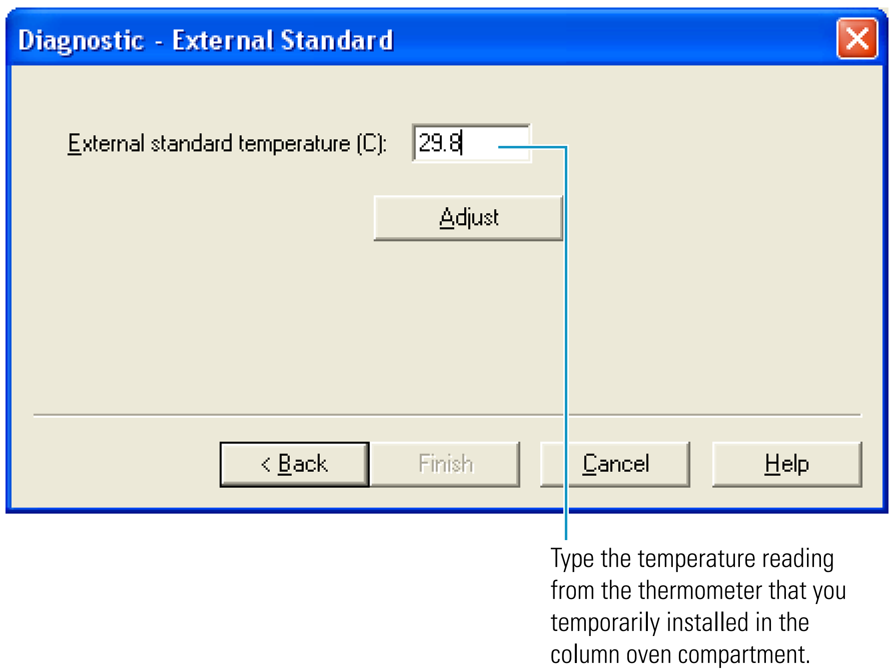
3. When the current temperature readback on the Oven page (autosampler view on the Status page of the Information view) reaches exactly 30.0 °C (see Figure 193), do the following:

   a. Type the reading from the 869C thermometer in the External standard temperature (C) box (see Figure 194).

      For example, if the thermometer reading is 29.8 °C, type 29.8 in the External standard temperature (C) box. The range is 0 to 110.0 °C.

      Note The external standard is the thermometer.

      Figure 194. Diagnostic - External Standard page of the Column oven air sensor calibration wizard

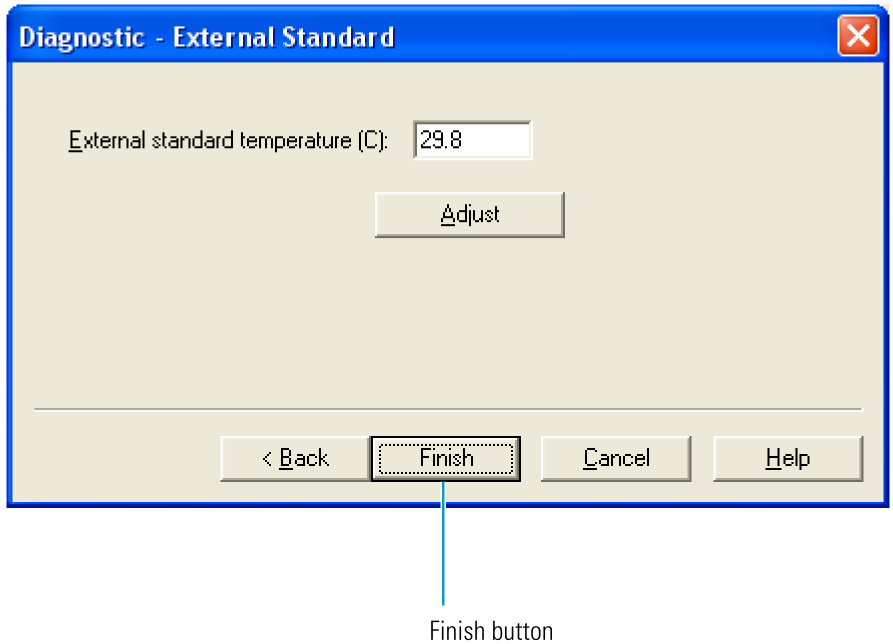


   b. Verify that the current temperature on the Oven page is still displaying 30.0 °C.

   c. On the Diagnostic - External Set Target page (see Figure 194), click **Adjust**.

      The data system downloads the temperature calibration to the autosampler and the Finish button becomes available (see Figure 195).

Figure 195. Diagnostic - External Standard page of the Column oven air sensor wizard



d. Repeat step 3a through step 3c until the readings from the 869C thermometer and the current temperature readout agree within ±0.2 °C.

4. When the temperatures on the 869C thermometer and the Current temperature readback are within ±0.2 °C of each other, click **Finish** (see Figure 195).

5. On the Oven page, verify that the Current temperature readback is stable at 30.0 °C with a maximum temperature drift of ±0.2 °C.

6. After you verify the stability of the column oven temperature, turn off the column oven using the autosampler direct controls and remove the temperature probe.

   For information on the autosampler direct controls, see "Controlling the Tray and Oven Compartment Temperatures" on page 193.

Vial Tray Metal Sensor Calibration

Use the Vial tray metal temperature sensor calibration wizard and an external temperature sensor to calibrate the vial tray temperature control.

To perform the vial tray metal sensor calibration procedure, you must have the following items provided in the Field Service Calibration Kit (P/N 60053-62001):

- Calibrated Omega 869C RTD thermometer or equivalent
- A/S vial tray sensor

To calibrate the column oven temperature, follow these procedures in order:

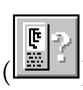
1. "Preparing to Calibrate the Tray Compartment Temperature," on this page
2. "Specifying the Internal Set Target for the Tray Compartment Temperature" on page 291
3. "Adjusting the Tray Temperature Calibration" on page 293

Preparing to Calibrate the Tray Compartment Temperature

❖ **To set up the system to calibrate the temperature of the tray compartment**

1. Install the tray temperature sensor as follows:
   a. Open the door to the tray compartment.
   b. Install the tray temperature sensor into location E of the tray compartment.

      The tray temperature sensor is a standard tray with a temperature sensor potted in a middle vial location of the tray.
   c. Route the cable of the sensor through the notch at the top on the tray compartment so that it does not interfere with the door closure.
   d. Close the door to the tray compartment.
2. Open the status page for the tray temperature.

   To open the Tray page from the Xcalibur data system, do the following:
   a. If the Information view is closed, click the **Information View** button () to open the view.

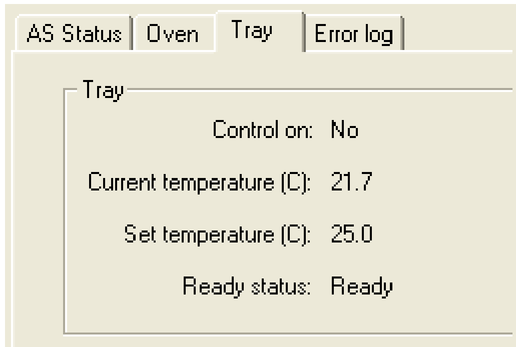
      The Information view opens with the Status page displayed.
   b. Verify that the status reads Ready to Download. For more information, see "Checking the Status of the LC Devices" on page 130.
   c. Click the listing for the autosampler in the directory tree.

      The status pages for the autosampler appear at the bottom of the Status window.

3. Click the **Tray** tab.

   The Tray page appears (see Figure 196).

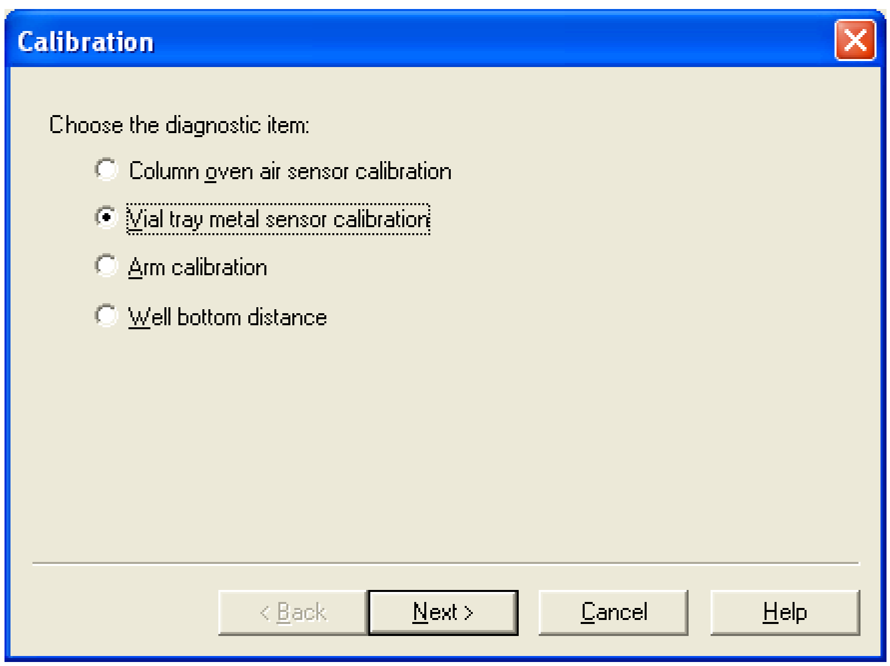
   Figure 196. Tray page



4. Open the Calibration dialog box for the autosampler (see "Calibrating the Autosampler" on page 282).

5. Select the **Vial tray metal sensor calibration** option (see Figure 197).

   Figure 197. Calibration dialog box



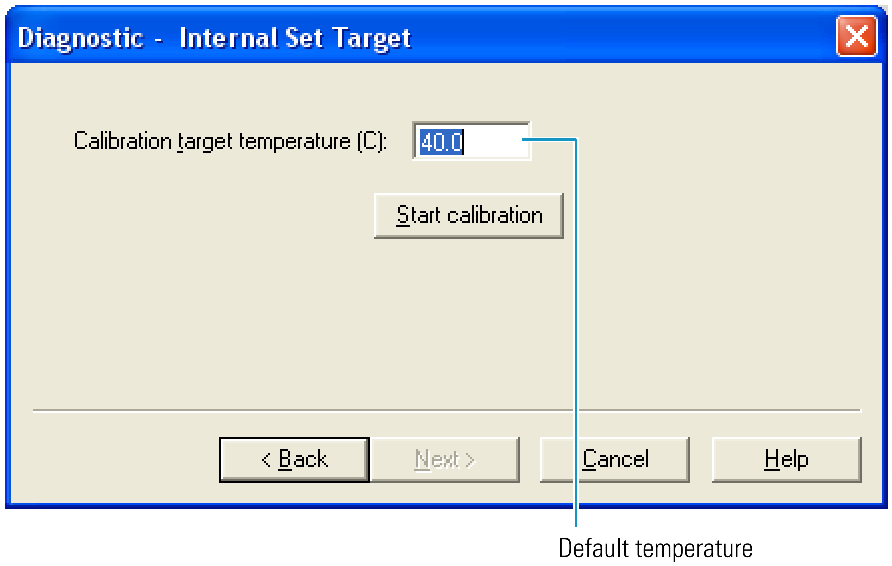
6. Click **Next**.

   The Diagnostic - Internal Set Target page of the Vial tray metal sensor calibration wizard appears (see Figure 198). The default external standard temperature is 40.0 °C.

Specifying the Internal Set Target for the Tray Compartment Temperature

Use the Diagnostic – Internal Set Target page (see Figure 198) of the Vial Tray Metal Sensor Calibration wizard to specify the calibration temperature.

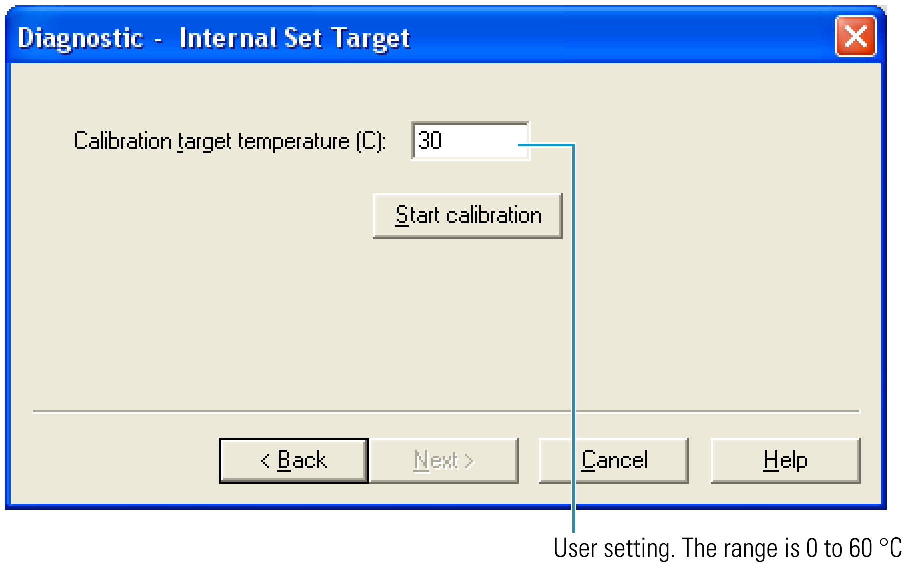
Figure 198. Diagnostic - Internal Set Target page of the Vial tray metal sensor calibration wizard



❖ To specify the target calibration temperature for the tray compartment and start the calibration

1. Prepare the system for the temperature calibration (see "Preparing to Calibrate the Tray Compartment Temperature" on page 289).
2. In the Calibration target temperature box, type **30.0**, and then click **Start calibration** (see Figure 199).

Figure 199. Diagnostic -Internal Set Target page of the Vial tray metal sensor calibration wizard

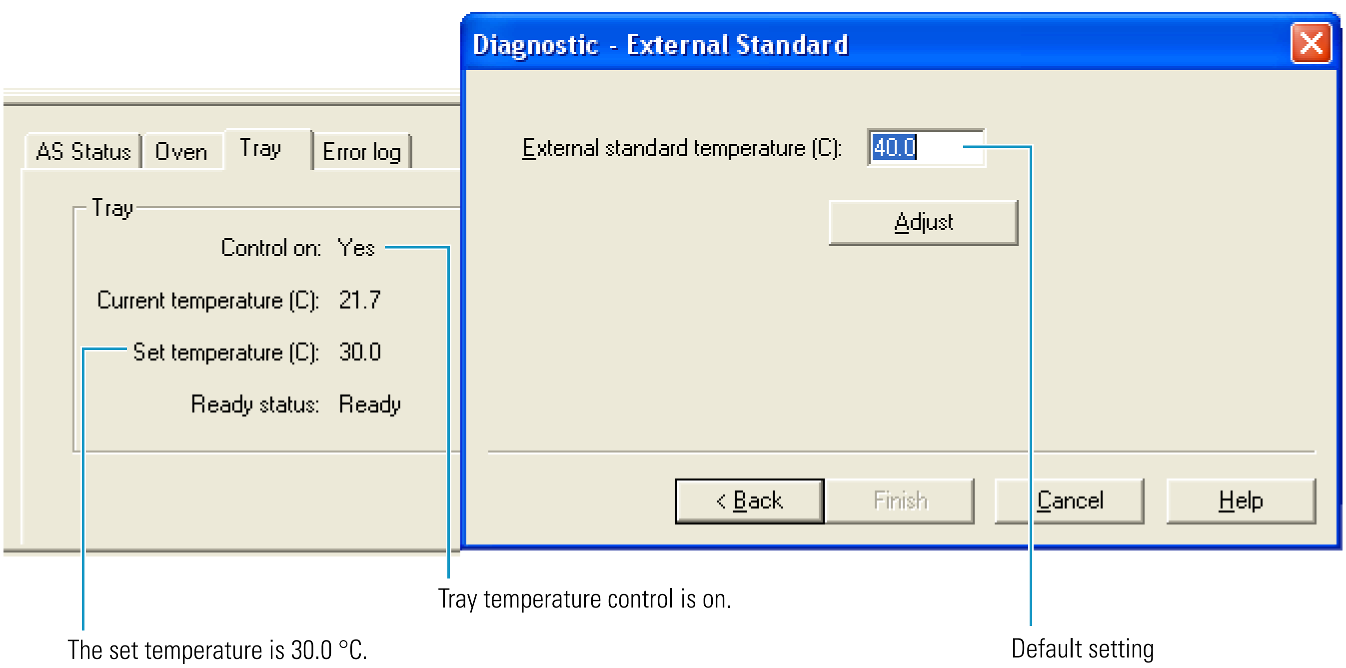


The data system turns on the tray temperature control and downloads the set temperature of 30.0 °C, and the Next bottom becomes available.

3. Click **Next**.

   The Diagnostic - External Standard page appears (see Figure 200). The default external standard temperature is 40.0 °C. The range is 0 to 110.0 °C.

Figure 200. Tray page and the Diagnostic - External Standard page of the Vial tray metal sensor calibration wizard



Adjusting the Tray Temperature Calibration

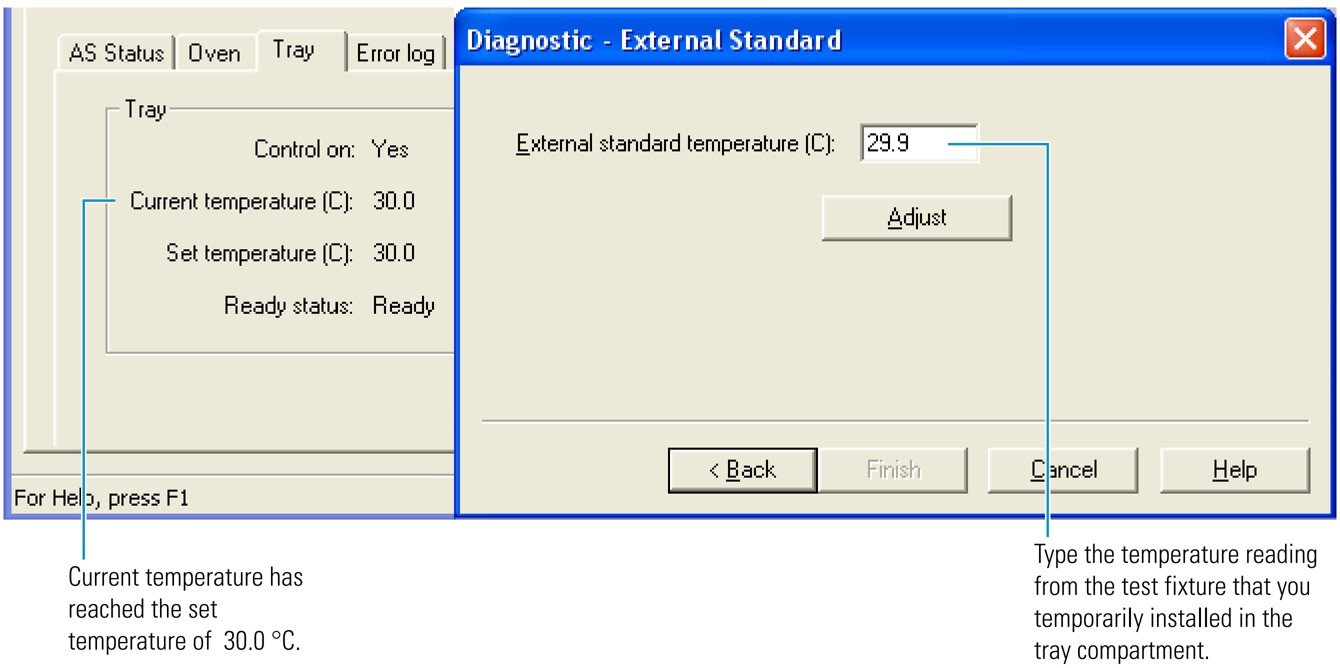
Use the Diagnostic – External Standard page of the Vial tray metal sensor calibration wizard the status readout on the Tray page, and the external temperature sensor to adjust the temperature calibration of the tray compartment.

❖ To adjust the calibration temperature of the tray compartment

1. When the current temperature readback on the Tray page reaches exactly 30.0 °C (see Figure 201), do the following:

   a. In the External standard temperature (C) box (see Figure 201) on the External Set Target page, type the reading from the thermometer.

Figure 201. View of the Tray page and the Diagnostic - External Standard page of the Vial tray metal sensor calibration wizard

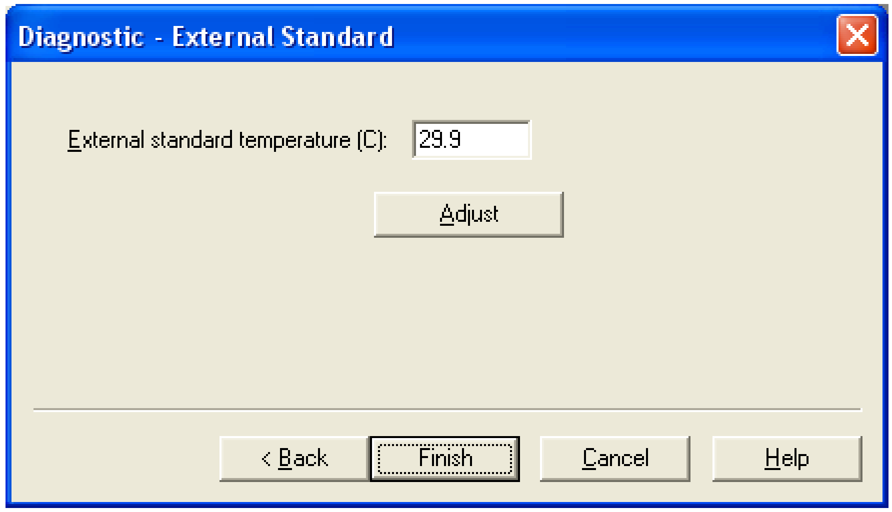


   b. On the Tray page, verify that the Current temperature readback still displays 30.0 °C.

   c. On the Diagnostic - External Standard page (see Figure 201), click **Adjust**.

   The data system downloads the calibration to the autosampler and the Finish button becomes available (see Figure 202).

Figure 202. Diagnostic - External Standard page of the Vial tray metal sensor calibration wizard with an available Finish button



d. Repeat step 1a through step 1c until the temperatures on the thermometer and the current temperature readout (see Figure 201) agree within ± 0.2 °C

2. When the temperatures on the 869C thermometer and the current temperature readout are within ±0.2 °C of each other, click **Finish** at the bottom of the Diagnostic - External Set Target page.

3. On the Tray status page, verify that the current temperature readout is stable at 30.0 °C with a maximum temperature drift of ± 0.2 °C.

4. After you have verified the stability of the oven tray temperature, remove the temperature probe.

Arm Calibration

During an injection, the XYZ arm moves between the specified vial or well location and the injection port. When you move the autosampler, you can jar the XYZ arm, causing it to lose alignment.

Performing the arm calibration procedure requires you to visually align the XYZ arm over the injection port. To make this visual alignment, you must replace the needle with an LED light fixture that shines a small beam of red light onto the port and replace the stainless steel injection port with a non-reflective target fixture. These test fixtures are not provided with the autosampler; you must order them from Thermo Fisher Scientific.

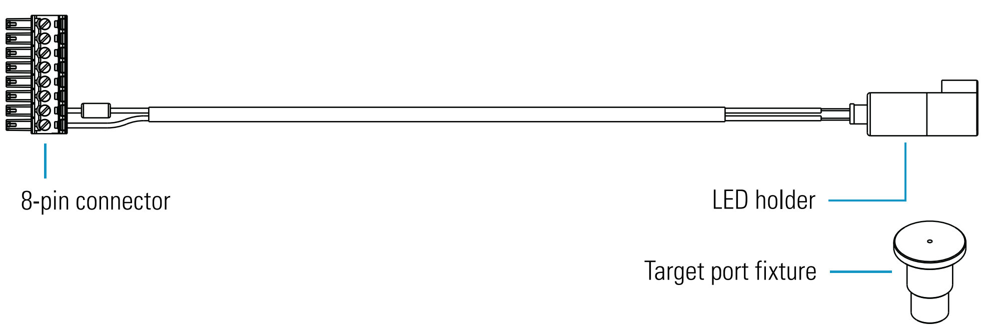
After following the instructions in the Arm Calibration wizard to adjust the XYZ arm alignment, test the alignment by making a set of injections from one or two 384-well microplates. The microplate carrier and the 384-well microwell plates are provided in the Autosampler Accessory Kit.

To calibrate the XY position of the XYZ arm, you must have these items:

- LED light fixture (P/N 60357-60021)
- Target port fixture (P/N 60357-20021)
- Microplate carrier
- One or two 384-well microtitre plates
- Masking tape

Figure 203 shows the LED light and the target port fixtures.

Figure 203. Arm calibration fixtures available by special order



To calibrate the XY position of the XYZ arm and verify the calibration, follow these procedures in order:

1. "Modifying the Autosampler Instrument Configuration," on this page
2. "Starting the Arm Calibration Wizard" on page 298
3. "Moving the Arm to the Needle Removal Position" on page 300
4. "Installing the LED Light Fixture and the Target Port Fixture" on page 300
5. "Moving the XYZ Arm to the Home Position" on page 304
6. "Aligning the Light Beam with the Target" on page 305
7. "Applying the Arm Calibration Setting" on page 307
8. "Checking the Arm Calibration Offset Values" on page 308
9. "Testing the Alignment of the XYZ Arm" on page 310

Modifying the Autosampler Instrument Configuration

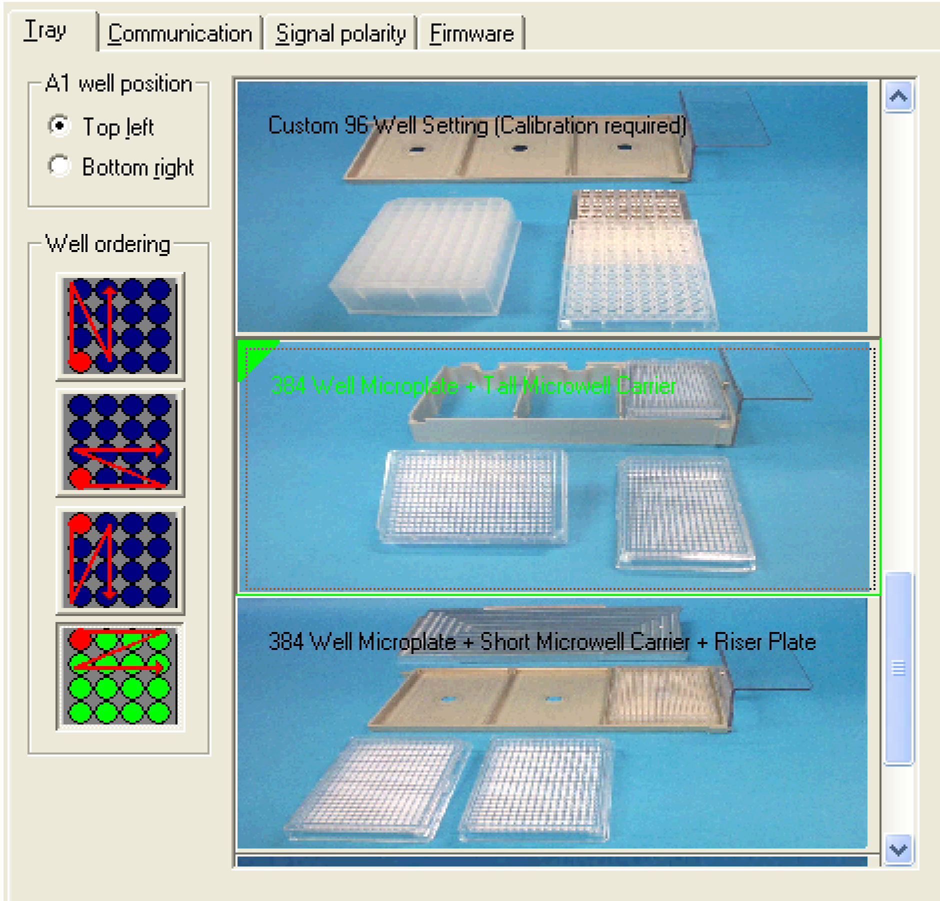
To calibrate the arm position, turn off the feature that prevents control of the autosampler when the tray compartment door is open.

❖ To modify the instrument configuration for the autosampler

1. Open the Surveyor Autosampler Configuration dialog box (see "Surveyor Autosampler Plus Configuration Settings" on page 39).

   The dialog box opens with the Tray page displayed (see Figure 204).

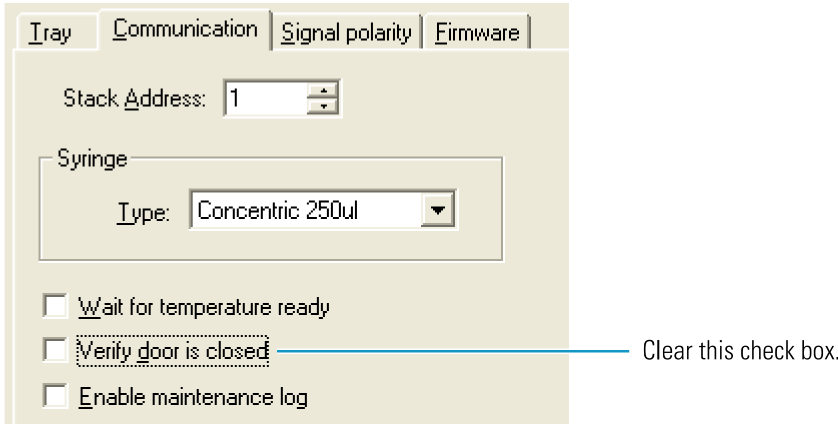
Figure 204. Tray page with the 384-well microplate tray type selected



2. Select the 384-well plate tray type.
3. In the A1 well position area, select the **Top left** option.
4. Click the **Communication** tab.

   The Communication page appears.
5. Clear the **Verify door is closed** check box (see Figure 205).

Figure 205. Communication page with the Verify door is closed check box cleared



When the Verify door is closed check box is selected, the data system prevents you from starting an injection sequence or using some of the direct control commands for the autosampler when the tray compartment door is open.

6. Click **OK** at the bottom of the dialog box to accept the settings and close the dialog box.
7. Click **Done** to close the Thermo Foundation Instrument Configuration window.

Go to the next procedure, "Starting the Arm Calibration Wizard."

Starting the Arm Calibration Wizard

Use the Arm Calibration wizard to adjust the home position of the XYZ arm.

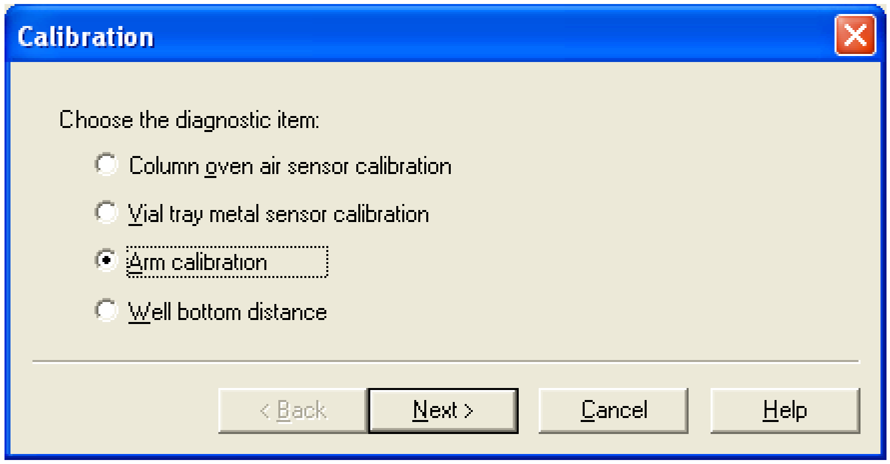
For information on the LED light and non-reflective needle port fixtures that you must have to perform the Arm Calibration wizard, see "Arm Calibration" on page 295.

❖ To start the Arm Calibration wizard

1. If you have not already do so, modify the autosampler instrument configuration as described in "Modifying the Autosampler Instrument Configuration" on page 296.
2. Open the view for the autosampler.
3. From the Surveyor AS menu, choose **Calibration**.

   The Calibration dialog box appears (see Figure 206).
4. Select the **Arm Calibration** option.

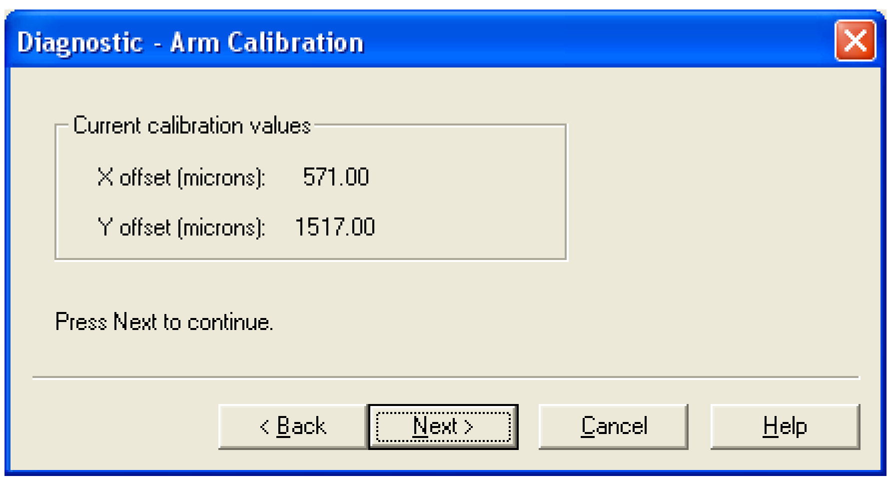
Figure 206. Calibration dialog box with the Arm calibration option selected



The Arm Calibration wizard appears (see Figure 207).

The Current calibration values area displays the current calibration values for the *x* axis and *y* axis position of the XYZ arm over the autosampler injection port.

Figure 207. Current calibration values page



Tip 1000 microns = 0.04 inches

The diameter of the hole in the center of the target port fixture is 0.047 inches.

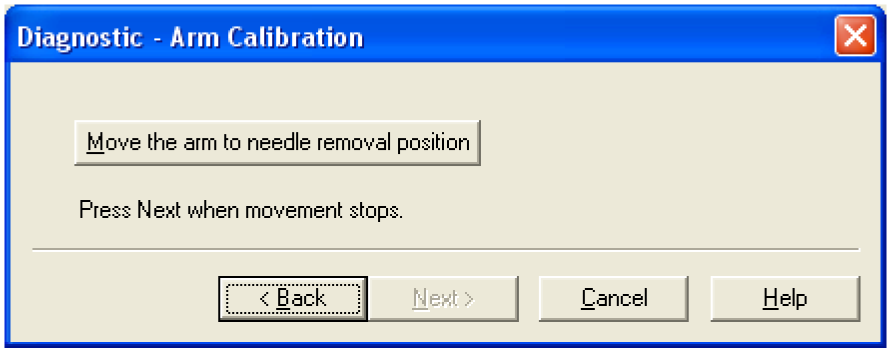
5. Click **Next** to proceed to the next page of the wizard.

Go to the next procedure, "Moving the Arm to the Needle Removal Position."

Moving the Arm to the Needle Removal Position

Use this page of the Arm calibration wizard to move the arm to needle removal position (see Figure 208).

Figure 208. Move the arm to needle removal position button page



❖ **To move the XYZ arm to the needle removal position**

1. Click **Move the arm to needle removal position**.

   The XYZ arm moves to the center front of the tray compartment and the needle holder moves down 1 inch.

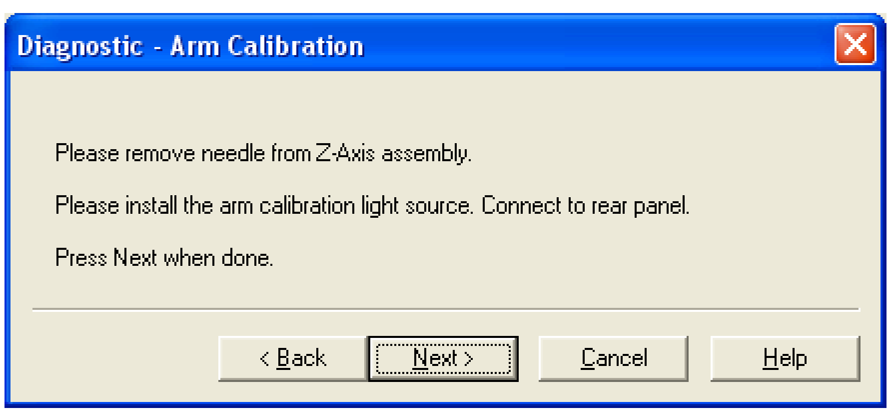
2. Click **Next** to proceed to the next page of the wizard.

Go to the next procedure, "Installing the LED Light Fixture and the Target Port Fixture."

Installing the LED Light Fixture and the Target Port Fixture

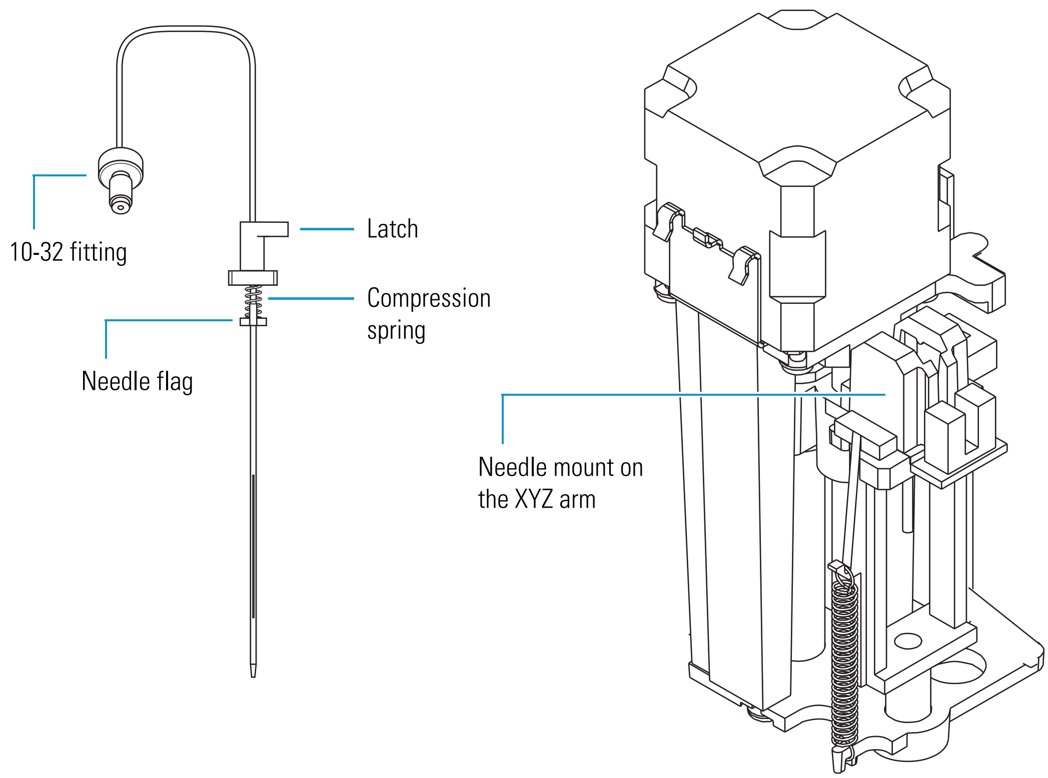
This page of the Arm Calibration wizard instructs you to remove the needle and install the calibration light source (see Figure 209).

Figure 209. Request to install the LED light fixture page



The needle assembly is a welded piece of 0.012 in. ID stainless steel tubing with an externally-threaded fitting, a needle flag, a latch, and a compression spring (see Figure 210). It slides into the needle mount on the XYZ arm and is secured with the latch. The needle tubing assembly has an internally-threaded fitting that connects to the 10-32 externally-threaded fitting of the needle assembly.

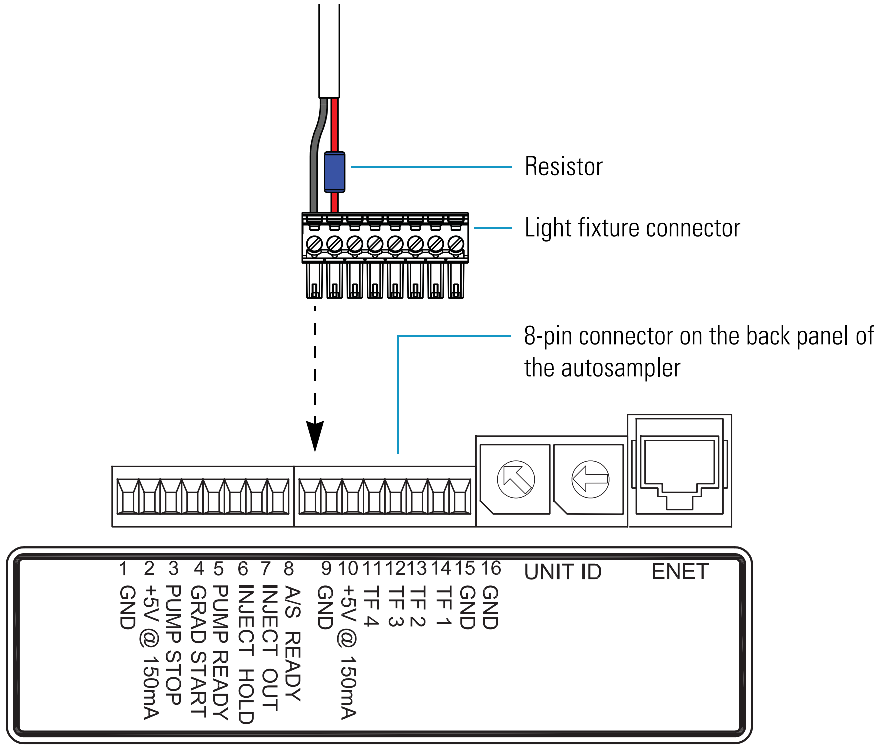
Figure 210. Needle assembly



❖ To install the light fixture and the target port fixture

1. Remove the needle assembly from the needle mount as follows:
   a. Unscrew the needle tubing assembly from the needle assembly.
   b. For the Surveyor Autosampler Plus, remove the syringe drive assembly (refer to the instructions in the Surveyor Autosampler Plus Hardware Manual).
   c. Pull the latch nut of the needle assembly forward.
   d. Pull the needle up from the needle mount on the XYZ arm.
2. Install the LED light fixture as follows:
   a. Connect the 8-pin connector of the light fixture cable to the timed events connector on the back panel of the autosampler (see Figure 211). The red wire with the resistor connects to the +5V autosampler pin and the black wire connects to the autosampler ground pin.

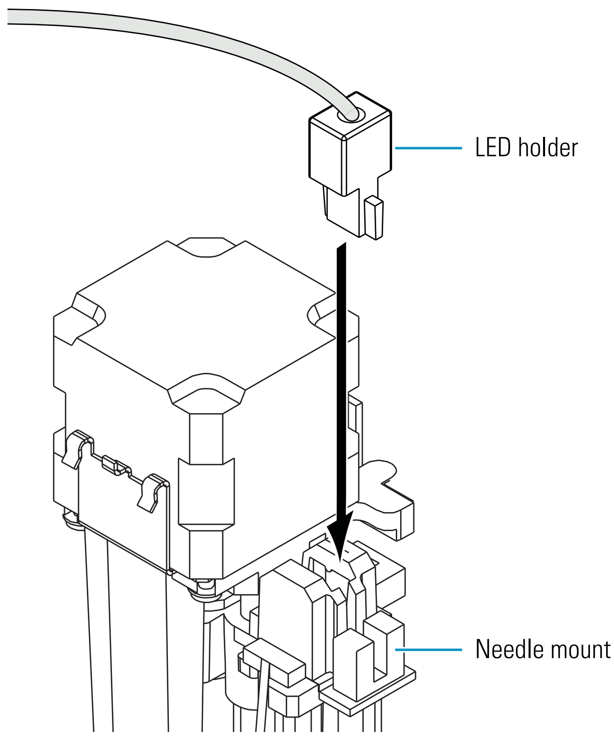
Figure 211. Cable connection for the light fixture



b. Verify that the LED is brightly lit.

c. Insert the LED holder into the needle mount (see Figure 212).

Figure 212. Inserting the LED holder into the needle mount



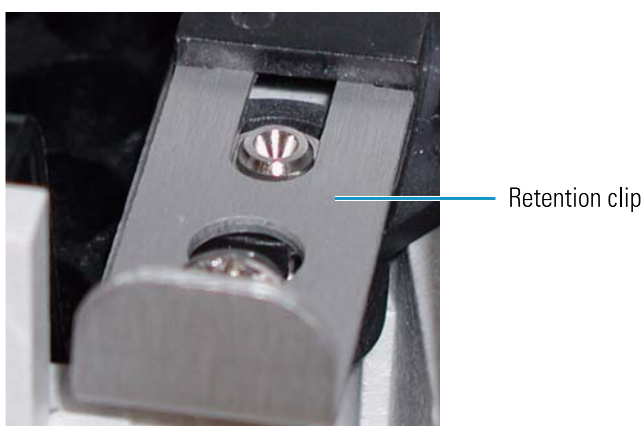
d. Push the LED holder down until it meets resistance from the needle mount.

e. Make sure that the light fixture cable is routed so that it does not obstruct the movement of the XYZ arm.

3. Install the target port fixture as follows:

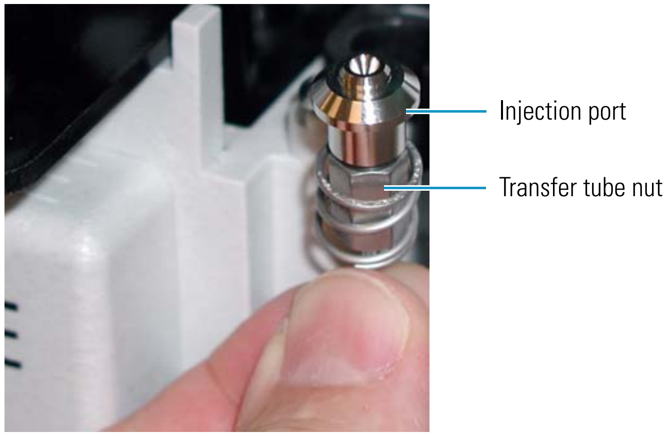
   a. Remove the aluminum retention clip (see Figure 213) from the wash station housing, pull it forward, and then upward.

Figure 213. Removing the retention clip



b. Pull the injection port out from the wash station (see Figure 214).

Figure 214. View of the injection port connected to the transfer tube nut



4. Insert the target port fixture (see Figure 203 on page 295) into the position previously held by the injection port.

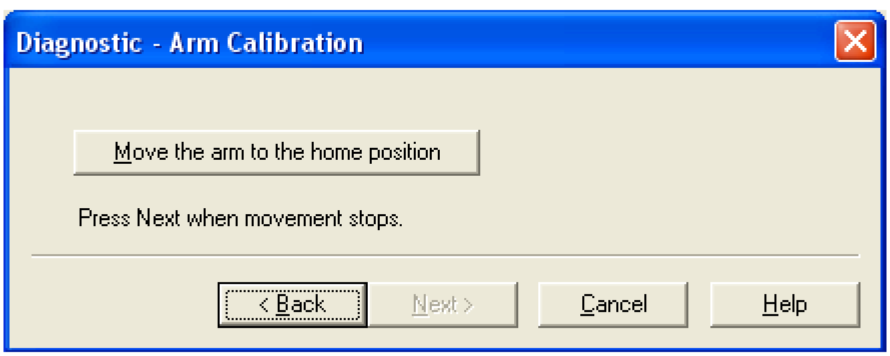
5. Click **Next** to proceed to the next page of the wizard.

Go to the next procedure, "Moving the XYZ Arm to the Home Position."

Moving the XYZ Arm to the Home Position

Use this page of the Arm Calibration wizard (see Figure 215) to move the XYZ arm to the home position. In the home position, the XYZ arm is aligned over the autosampler injection port.

Figure 215. Move the arm to the home position button page



❖ **To move the XYZ arm to the home position**

1. Click **Move the arm to the home position**.

   The XYZ arm moves to the home position, and the Next button becomes available.

2. Click **Next** to proceed to the next page of the Arm Calibration wizard.

Go to the next procedure, "Aligning the Light Beam with the Target."

Aligning the Light Beam with the Target

Use this page of the Arm Calibration wizard (see Figure 216) to adjust the fine position of the XYZ arm.

Two lead screws and two stepper motors control the *x* axis and *y* axis position of the XYZ arm (see Figure 217).

The Left and Right buttons move the XYZ arm along the *x* axis. The Forward and Backward buttons move the XYZ arm along the *y* axis.

Figure 216. Alignment buttons page

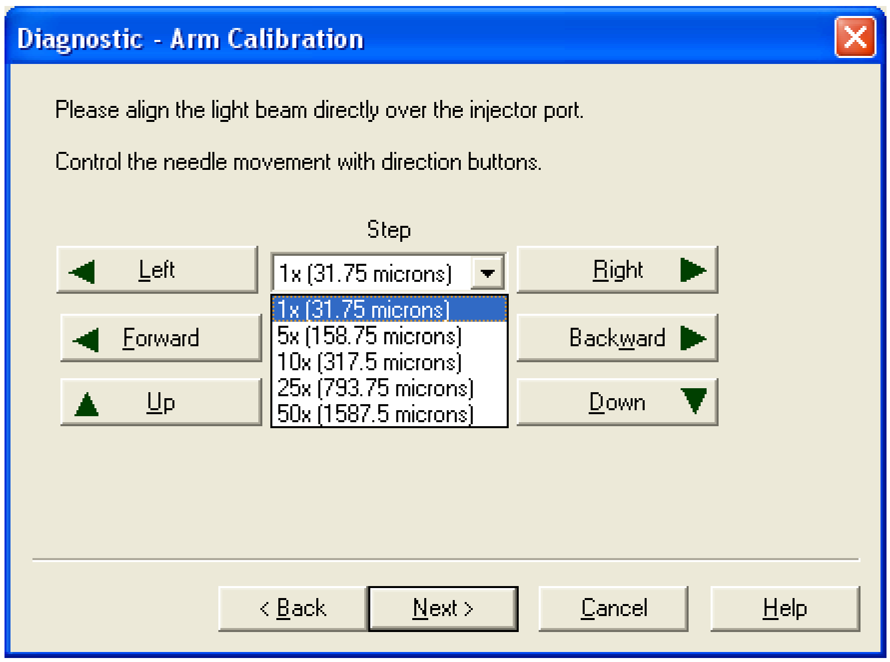
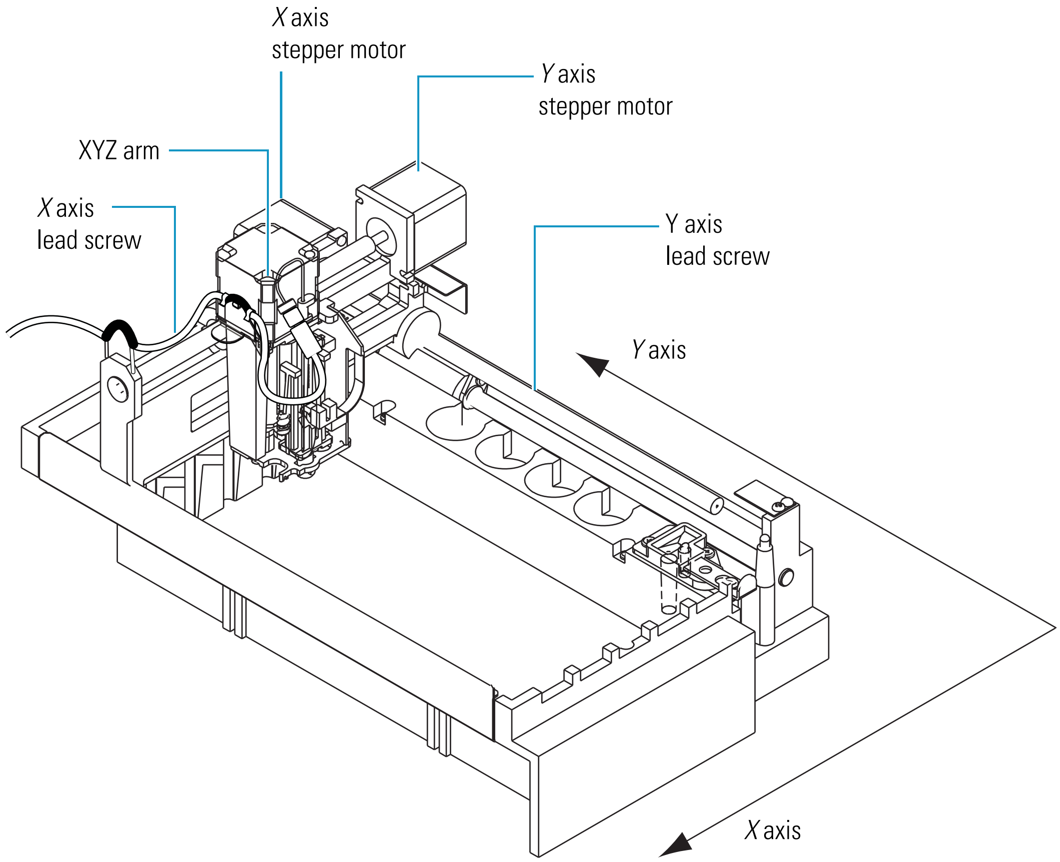


Figure 217. XYZ arm of the Surveyor Autosampler Plus



❖ To align the light beam over the hole in the target port fixture

1. In the Step list of the Arm Calibration wizard, select **25x (793.75 microns)**.

   This selection moves the arm 793.75 microns (0.03125 inches) each time you click Left, Right, Forward, or Backward.

   1 micron = 3.937×10^{-5} inches

2. Click **Left**, **Right**, **Forward**, and **Backward** to align the position of the light beam with the hole in the center of the target port fixture.

3. In the Step list, select a smaller step size, and continue aligning the position of the light beam.

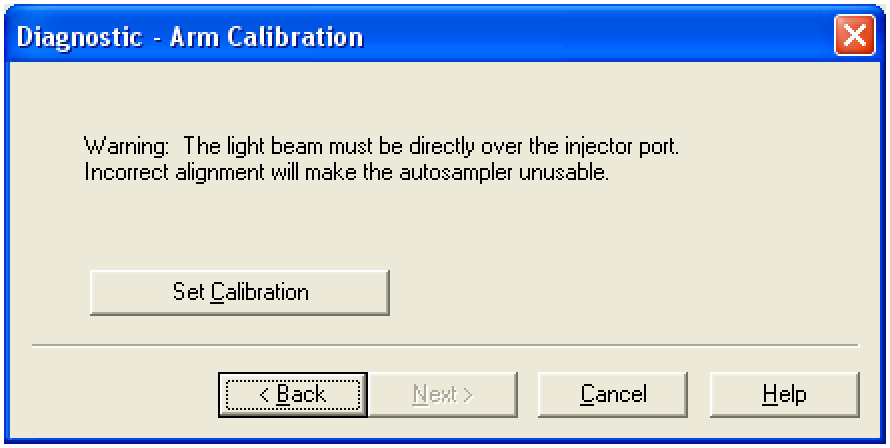
4. After you align the light beam with the hole in the center of the target port fixture, click **Next** to proceed to the next page of the wizard.

Go to the next procedure, "Applying the Arm Calibration Setting."

Applying the Arm Calibration Setting

Use this page of the Arm Calibration wizard (see Figure 218) to download the new arm calibration settings.

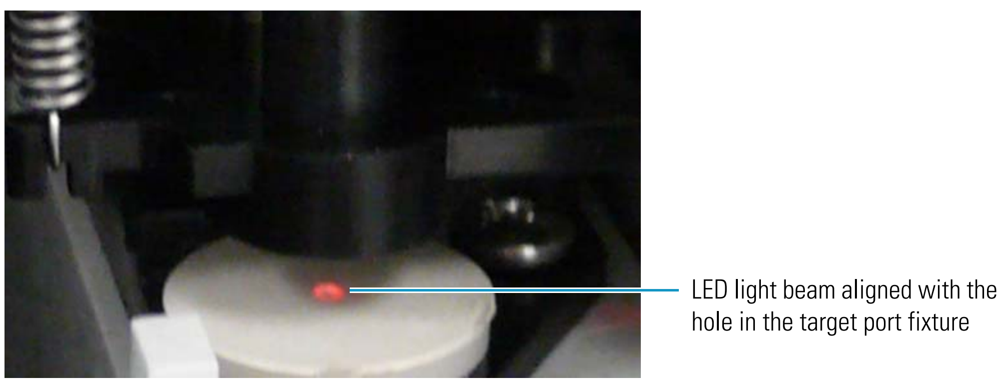
Figure 218. Set Calibration button page



❖ **To apply the current calibration settings for the arm position**

1. Ensure that the light beam is centered over the hole in the target port fixture (see Figure 219).

   Figure 219. View of the LED light beam centered over the hole in the target port fixture

2. Click **Set Calibration**.

   The Next button becomes available.

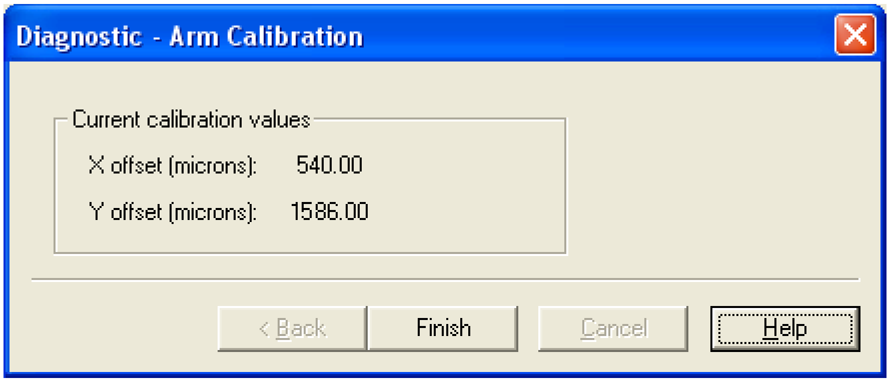
3. Click **Next** to proceed to the final page of the wizard.

Go to the next procedure, "Checking the Arm Calibration Offset Values."

Checking the Arm Calibration Offset Values

Use this page of the Arm Calibration wizard to check the calibration values and close the wizard (see Figure 220).

Figure 220. Final page of the Arm calibration wizard with the Finish button

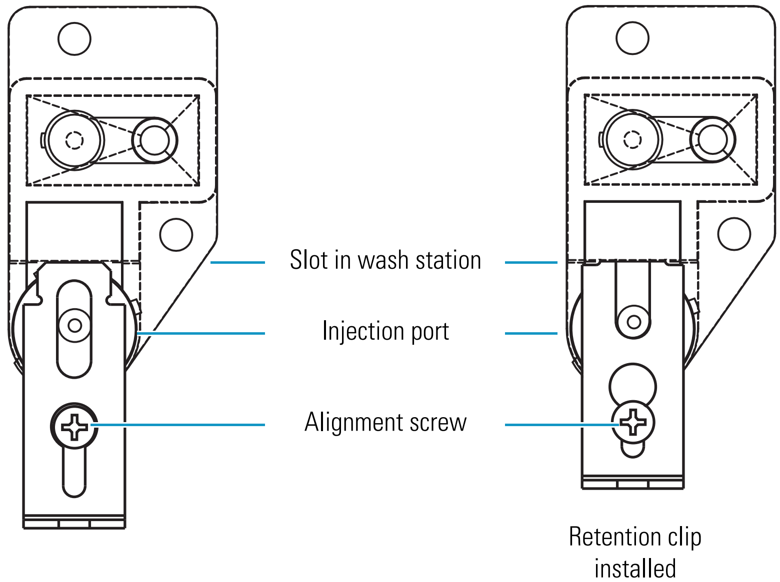


❖ **To verify the calibration setting**

1. In the Current calibration values area, verify the following:
   - The X offset is between +900 and -900 microns
   - The Y offset is between 1500 and 2500 microns
2. Click **Finish** to accept the settings and close the Arm Calibration wizard.
3. Remove the LED and target port calibration fixtures.
4. Reinstall the injection port as follows:
   a. Insert the injection port into the port in front of the wash station.
   b. Align the retention clip with the slot located in front of the wash station.
   c. Using the retention clip to push the injection port down, align the circular cutout in the retention clip over the alignment screw.
   d. Insert the end of the retention clip into the wash station slot as far as it will go.

   Figure 221 shows the installation of the retention clip.

Figure 221. Installing the retention clip



5. Reinstall the needle assembly as follows:
6. Remount the syringe drive assembly.
   a. Slide the needle into the needle mount on the XYZ arm.
   b. Turn the latch to the right.
   c. Reconnect the needle tubing assembly to the needle assembly.

Leave the autosampler view of the Instrument Setup view open and go to the next procedure, "Testing the Alignment of the XYZ Arm."

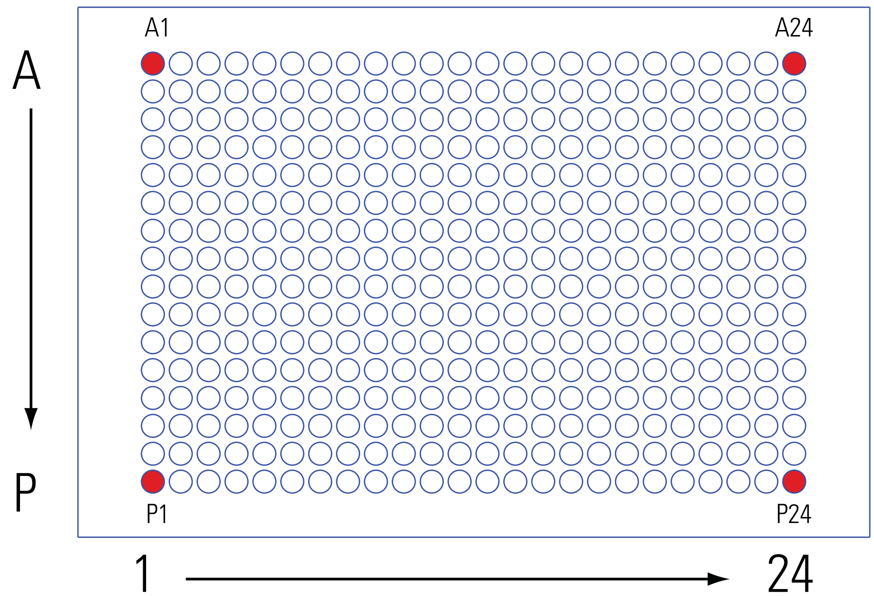
Testing the Alignment of the XYZ Arm

To test the alignment of the XYZ arm, make injections from the four corners of two 384-well microplates.

❖ To test the alignment of the XYZ arm

1. Cover columns 1, 2, 23, and 24 of two 384-well microplates with tape (see Figure 222).

Figure 222. 384-well microplate positions



2. Place the microplates in each end of the microwell carrier, leaving the middle position empty.
3. Load the microwell carrier into the tray compartment of the autosampler as follows:
   a. From the menu bar, choose **Surveyor AS > Direct Control**.

      The Direct Control dialog box appears (see Figure 223).

Figure 223. Direct Control dialog box for the autosampler

b. Select **Position arm to access tray**, and then click **Apply**.

The XYZ arm moves to the back of the tray compartment.

c. Insert the microwell carrier into the tray compartment.

d. Verify that the microwell carrier sits flat and is properly installed.

4. Send the XYZ arm to the home position as follows:

a. In the Direct Control dialog box, select **Go to Home Position**.

b. Click **Apply**.

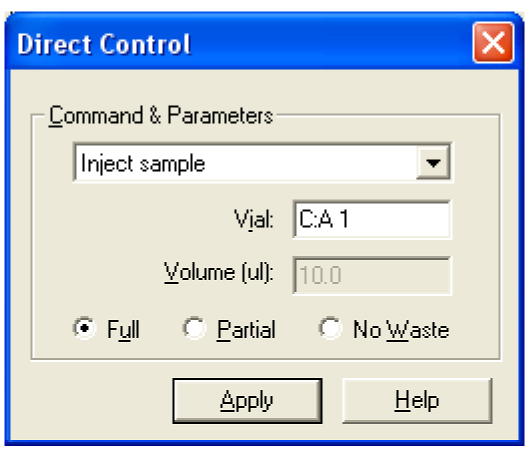
The XYZ arm moves to the home position, which is above the injection port.

5. Make an injection from each corner of the microplate as follows:

a. In the Direct Control dialog box, select **Inject sample**.

The sample injection parameters appear (see Figure 224).

Figure 224. Direct Control dialog box with the Inject sample command selected



b. In the Vial box, type **C:A1**.

c. Click **Apply** and wait for the injection to finish.

   The XYZ arm moves to the location of the A:1 well of the (C) microplate at the back of the tray compartment, and then lowers the needle through the masking tape and into the well.

d. In the Vial box, type **C: A24**.

e. Click **Apply** and wait for the injection to finish.

f. In the Vial box, type **C: P1**.

g. Click **Apply** and wait for the injection to finish.

h. In the Vial box, type **C: P24**.

i. Click **Apply** and wait for the injection to finish.

6. Remove the microplate and check the location of the injections as follows:

   a. In the Direct Control dialog box, select **Position arm to access tray**.

      The XYZ arm moves to the back of the tray compartment.

   b. Remove the tray from the tray compartment, and inspect the holes in the tape. Verify that the holes are at or near the center of the microplate wells. Each hole must be at least 0.02 inches from the edge of the well.

7. If the results are not satisfactory, repeat the arm calibration, and then repeat the alignment test with the second (A) microplate, located at the front of the tray compartment.

Well Bottom Distance Calibration

The autosampler supports custom vials and well plates in addition to the standard 1.8 mL vials, 96-well plates, and 384-well plates. However, to use custom vials or well plates, you must first determine how far the autosampler needle must travel to reach the bottom of the vials or wells.

Use the Well bottom distance wizard to determine the distance that the needle must travel to reach the bottom of a vial or well. The XYZ arm uses this value when you select one of the custom tray configurations.

IMPORTANT Because the autosampler stores only one value for the custom well bottom distance, you must perform a well bottom distance calibration each time you select a new custom tray type configuration and each time you use a different type of custom vial or custom microwell plate.

To perform a well bottom distance calibration, follow these procedures in order:

1. Starting the Well Bottom Distance Wizard
2. Selecting the Calibration Method for the Well Bottom Distance
3. Depending on the selected calibration method, follow one of these procedures:
   - Manually Entering the Well Bottom Distance

     –or–

   - Using the Needle Sensor to Determine the Well Bottom Distance

Starting the Well Bottom Distance Wizard

❖ **To start the well bottom distance wizard**

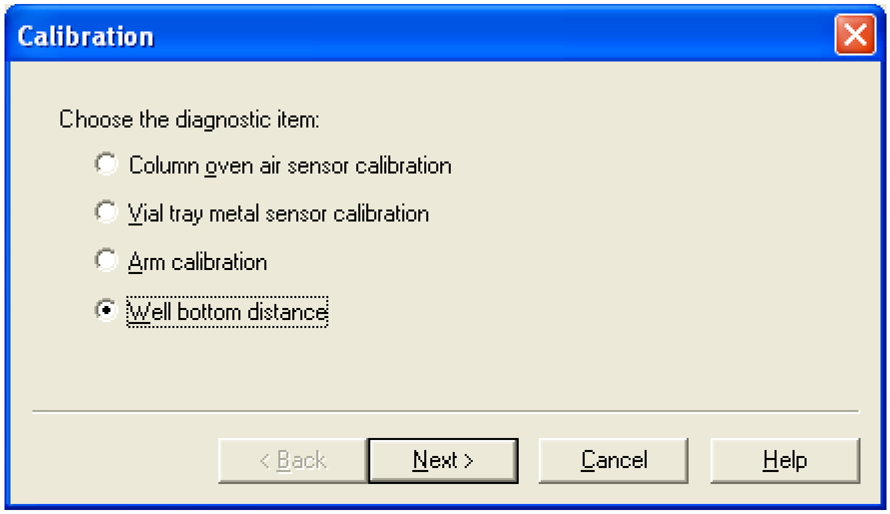
1. Open the status view for the autosampler.

   For information on opening the status view from the Xcalibur data system, see “Checking the Status of the LC Devices” on page 130.

2. Verify that the autosampler status reads Ready to Download.
3. Open the view for the autosampler.
4. From the Surveyor AS menu, choose **Calibration**.

   The Calibration dialog box appears (see Figure 225).

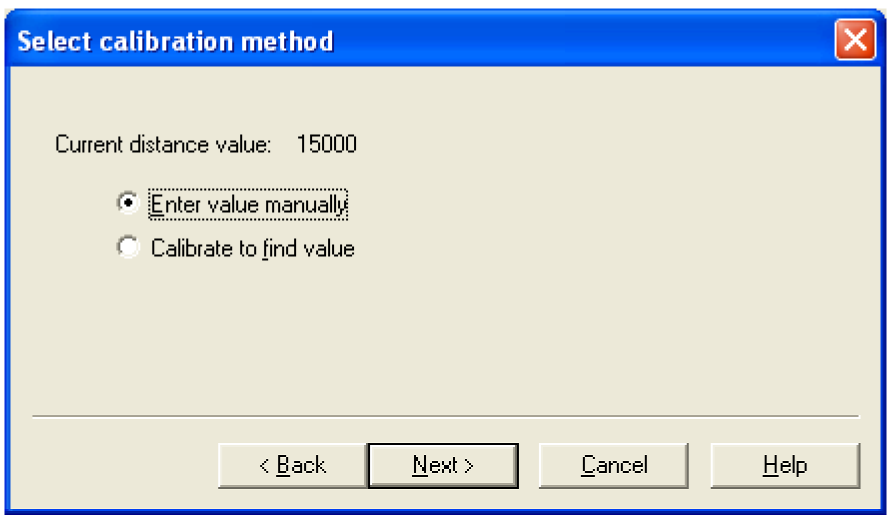
Figure 225. Calibration dialog box with the Well bottom distance option selected



5. Select the **Well bottom distance** option, and then click **Next**.

   The Select calibration method page appears (see Figure 226).

Figure 226. Select calibration method page



Selecting the Calibration Method for the Well Bottom Distance

Use the Select calibration method page of the Well Bottom Distance wizard to select a calibration method. You can manually enter a bottom distance value for your custom vials or microplates or you can use the autosampler's needle sensor feature to determine the correct distance value.

❖ To select the calibration method for the well bottom distance

Do one of the following:

- Select the **Enter value manually** option if you want to enter a previously determined value for the well bottom distance. Click **Next**. Then go to the next procedure, "Manually Entering the Well Bottom Distance."

  –or–

- Select the **Calibrate to find value** option to perform an active calibration. Click **Next**. Then go to "Using the Needle Sensor to Determine the Well Bottom Distance" on page 316.

> **Note** The autosampler stores only one well bottom distance value for custom tray configurations. The number at the top of this dialog box is the current value for the distance.

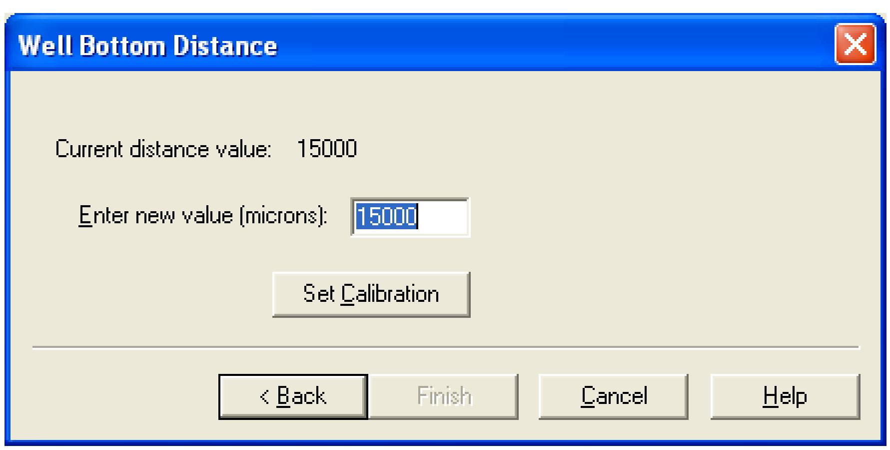
Manually Entering the Well Bottom Distance

❖ To manually enter a new value for the well bottom distance

1. In the Enter new value (microns) box, type the new value in microns (see Figure 227).

   The range is 15 000 to 46 990 microns (15 to 46.9 mm).

   Figure 227. Manual calibration page



2. Click **Set Calibration**.

   The new bottom distance value (in microns) appears at the top of the dialog box and the Finish button becomes available.

3. Click **Finish** to close the Well Bottom Distance dialog box.

Using the Needle Sensor to Determine the Well Bottom Distance

The autosampler has a needle sensor that can determine the bottom distance of custom vials or microplate wells.

❖ To determine the well bottom distance with the autosampler's needle sensor

1. Remove the cap or lid from your vial or well plate.

   IMPORTANT Remove vial caps or well plate lids before performing an active well bottom distance calibration. As the needle pierces a vial cap or a well plate lid, the spring in the needle mechanism is compressed, which can cause premature activation of the needle sensor.

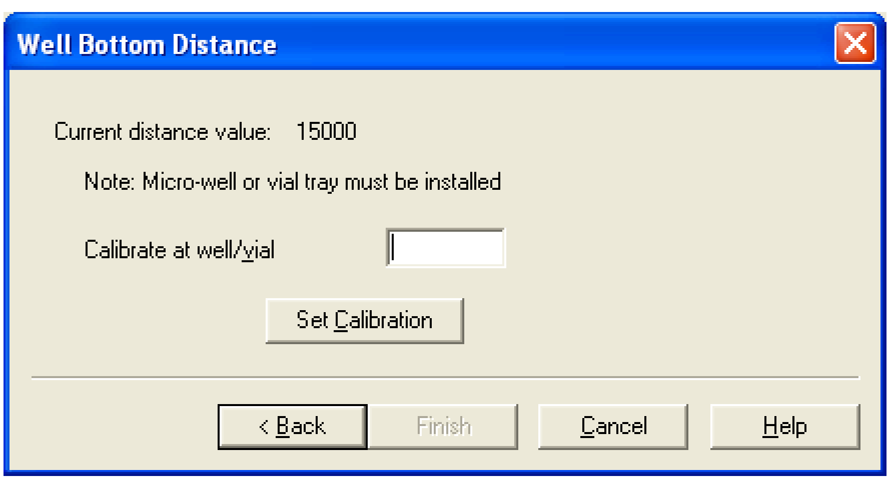
   IMPORTANT Before placing a custom tray into the autosampler tray compartment, check the height limitations for 96-well and 384-well plates listed in the hardware manual for your autosampler. Tall objects will stall the autosampler arm.

2. Place the vial into a tray or the well plate into a carrier, and then place the tray or carrier into the tray compartment of the autosampler.

3. In the Calibrate at well/vial position box (see Figure 228), type a vial or well location.

   Note If you are calibrating the bottom distance of a vial, verify that you have placed a vial in the selected location.

   Figure 228. Calibrate to find page

4. Click **Set Calibration** to activate the autosampler.

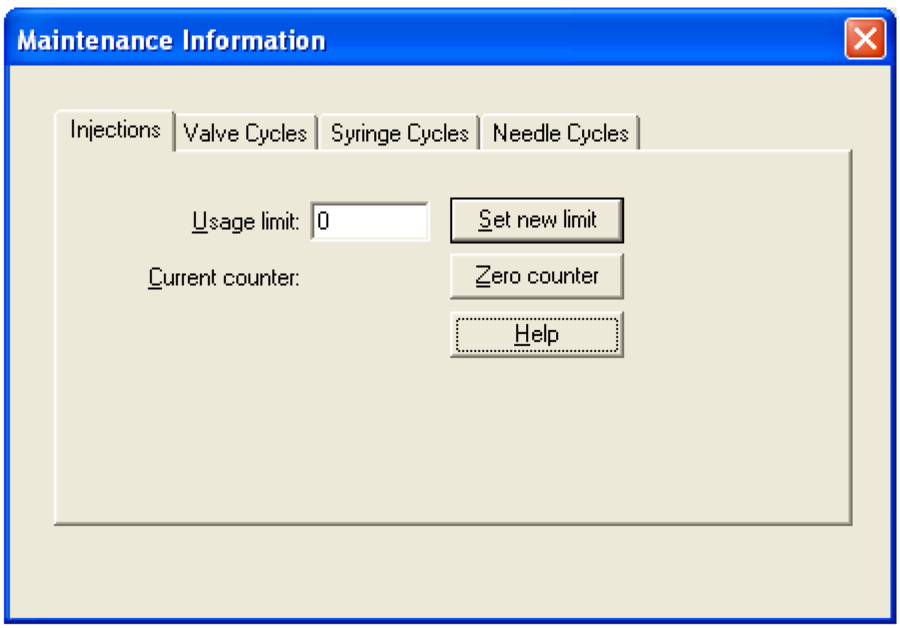
After the XYZ arm moves to the selected location, the needle mechanism descends until it detects the bottom of the vial or well. After it detects the bottom, the XYZ arm moves to the home position. In the Well bottom distance wizard, the new bottom distance calibration value appears at the top of the page.

5. Click **Finish** to accept the calibration.

Autosampler Maintenance Information

Use the Maintenance Information dialog box (see Figure 229) to set the scheduled maintenance time (SMT) for the autosampler maintenance items: Injections, Valve Cycles, Needle Cycles, and Syringe Cycles.

Figure 229. Maintenance Information page



❖ **To open the Maintenance Information dialog box**

1. Open the view for your autosampler.

   The Method page for your autosampler appears.

2. In the menu bar, choose **Surveyor AS > Maintenance**.

❖ **To set a new usage limit**

1. Click the tab for the page that contains the maintenance item that you want to change the usage limit for.

2. In the Usage limit box, type the usage limit.

3. Click **Set new limit**.

❖ To reset the usage counter after performing the scheduled maintenance task

1. Click the tab for the scheduled maintenance task.
2. Click **Zero counter**.

> **Note** If you selected the Enable Maintenance Log check box in the Communication page of the Surveyor AS Autosampler dialog box when you configured the Surveyor Autosampler, you can set the scheduled maintenance times (see "Communication Page" on page 45).

Maintenance Information Parameter Descriptions

Table 57 describes the parameters on the Maintenance Information page.

Table 57. Maintenance Information dialog box pages and parameters

Page or Parameter	Description
Page	
Injections	Use this page to set the scheduled maintenance time for the number of user-defined injections (excluding any aborted injections). When the counter exceeds the usage limit, the Maintenance due readback displays Yes. Before you can start a run, you must perform the scheduled maintenance and zero the counter.
Valve Cycles	Use this page to set the scheduled maintenance time for the number of user-defined valve cycles for the injection valve (from Fill to Inject). If the counter exceeds the usage limit, the autosampler will not start a run until the scheduled maintenance is performed and you have zeroed the counter.
Needle Cycles	Use this page to set the scheduled maintenance time (SMT) for the number of user-defined needle cycles in which the needle is inserted into a septum, injection port, or wash station. If the counter exceeds the usage limit, the autosampler will not start a run until the scheduled maintenance is performed and you have zeroed the counter.
Syringe Cycles	Use this page to set the scheduled maintenance time (SMT) for the number of user-defined syringe cycles in which the syringe is put in the Ready position. If the counter exceeds the usage limit, the autosampler will not start a run until the scheduled maintenance is performed and you have zeroed the counter.
Parameter	
Usage Limit	Use this box to specify the new usage limit for the selected maintenance item. **Note** If the counter exceeds the usage limit, the autosampler will not start a run until either the scheduled maintenance is performed or the Enable maintenance log check box in the Communication page of the Surveyor Autosampler Configuration dialog box is not selected (see "Communication Page" on page 45).
Set New Limit	Downloads the new usage limit to the autosampler.

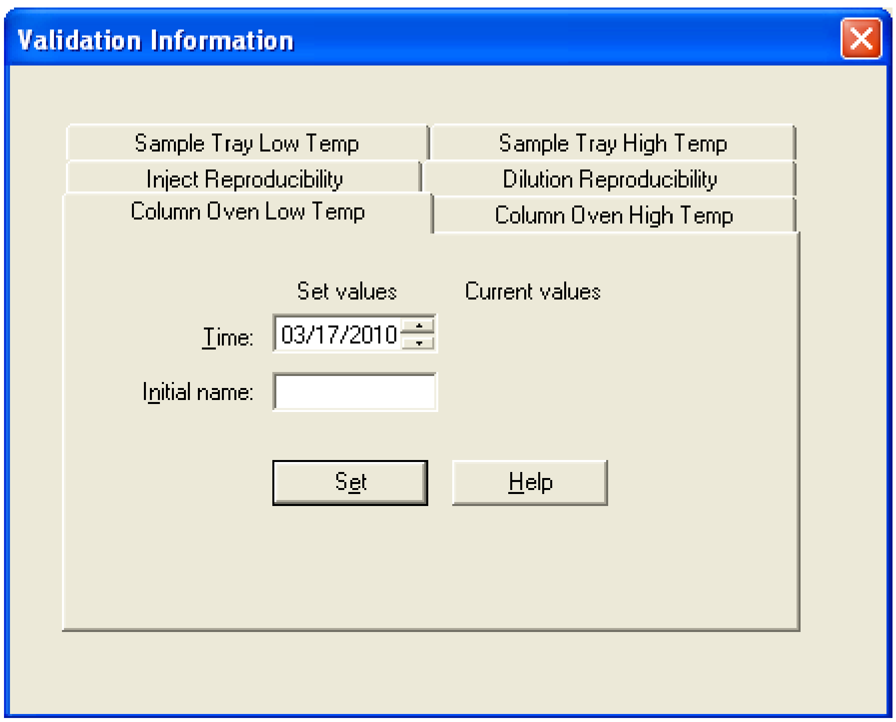
Table 57. Maintenance Information dialog box pages and parameters

Page or Parameter	Description
Current Counter and Zero Counter	Displays the current count. When the counter exceeds the usage limit (scheduled maintenance time), the autosampler can not start a run until you perform the scheduled maintenance or clear the Enable maintenance log check box in the Communication page of the Autosampler Configuration dialog box.
Zero Counter	Zeros the counter for the selected maintenance item.

Autosampler Validation Information

Use the Validation Information dialog box (see Figure 230) to enter the validation date for the indicated parameter and the name of the person who performed the validation.

Figure 230. Validation Information dialog box



❖ To open the Validation Information dialog box

1. Open the view for your autosampler.

   The Method page for your autosampler appears.

2. In the menu bar, choose **Surveyor AS > Validation**.

   The Validation Information dialog box appears.

❖ To specify your user name and the validation date

1. In the Time box, type or select the validation date.
2. In the Initial Name box, type your initials.
3. Click **Set**.

Validation Information Parameter Descriptions

Table 58 describes the parameters on the Validation Information dialog box.

Table 58. Validation Information dialog box parameters

Parameter	Description
Time	To change the date that the validation was performed, click the arrows to increase or decrease the value, and then click the **Set** button.
Initial Name	To change the name of the person who performed the validation, type the new value in the Initial Name box, and then click the **Set** button.
Current Values	This readback displays the current time and initial name.
Button	
Set	Enter the appropriate values in the Time spin box and the Initial Name box, and then click the **Set** button to set the values.

LC Pump Calibration and Record Keeping

This chapter describes the calibration and record keeping procedures for the Surveyor LC Pump Plus.

Contents

The Surveyor LC Pump Plus is factory-calibrated to produce an accurate flow rate while pumping water at 1 mL/min. Its ability to accurately proportion binary mobile phases is also tested at a flow rate of 1 mL/min.

The Surveyor LC Pump Plus does not require calibration upon arrival at its shipping destination. However, over time, the pressure readings from its built-in pressure transducer can drift and require re-zeroing. If you are running the LC pump at high backpressures, you might notice an increase in its pressure pulsation. You can reduce this pressure pulsation by adjusting the compressibility setting of the LC pump.

Calibrating the Surveyor LC Pump Plus

Two calibration options for the Surveyor LC Pump Plus affect its performance: the alpha setting, which affects the flow rate; and the compressibility setting, which affects the pressure pulsation of the system. The alpha setting is factory calibrated to produce an accurate flow rate for water. The compressibility setting is set to 0.45 GPa^{-1}, which is the compressibility value for water.

If you are pumping solvents other than water and your application is sensitive to pressure pulsation, you might want to optimize the compressibility setting. After you optimize the compressibility setting, check the flow rate accuracy. If adjusting the compressibility setting has affected the flow rate accuracy, adjust the alpha setting.

In addition to the alpha setting and the compressibility setting, the pressure transducer that is attached to the back of the purge manifold assembly is also factory calibrated. If you replace the pressure transducer, you must update the pressure sensor adjustment setting. Even if you never need to replace the pressure sensor, its readout tends to drift under normal usage, requiring you to occasionally re-zero its output.

These topics provide information on calibrating the LC pump:

- "Accessing the Calibration Options for the Surveyor LC Pump Plus," on this page
- "Calibration Options for the Surveyor LC Pump Plus" on page 324
- "Calibration Procedures for the Surveyor LC Pump Plus" on page 328

Accessing the Calibration Options for the Surveyor LC Pump Plus

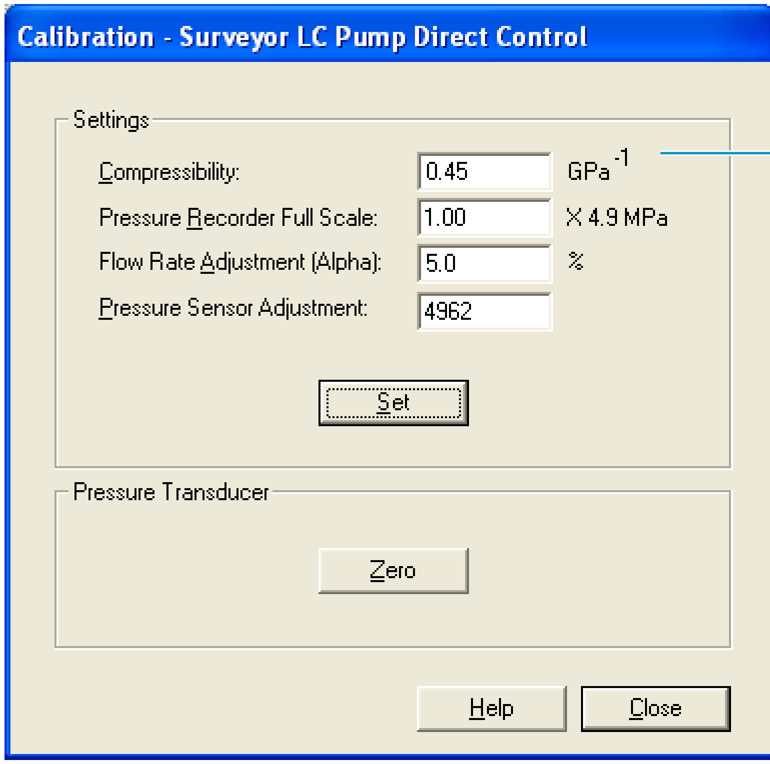
You can fine-tune the performance of the Surveyor LC Pump from the Calibration – Surveyor LC Pump Direct Control dialog box.

❖ To open the Calibration – Surveyor LC Pump Direct Control dialog box

1. Turn on the power to the Surveyor LC Pump.
2. Open the Surveyor LC Pump view in the Instrument Setup window.
3. Choose **Surveyor LC Pump > Direct Control > Calibration**.

   The Calibration – Surveyor LC Pump Direct Control dialog box appears (see Figure 231).

Figure 231. Calibration page for the Surveyor LC Pump



Calibration - Surveyor LC Pump Direct Control Parameter Descriptions

Table 59 describes the calibration parameters for the Surveyor LC Pump Plus.

Table 59. Calibration parameters for the Surveyor LC Pump Plus (Sheet 1 of 2)

Parameter	Description
Settings	
Compressibility	Specifies the compressibility of the mobile phase (see “Compressibility” on page 324). For information on determining the appropriate value for your solvent conditions, see “Updating and Optimizing the Compressibility Setting” on page 328.
Pressure Recorder Full Scale	Specifies the full scale range of the recorder connected to the pressure terminals on the back panel of the pump (see “Pressure Recorder Full Scale” on page 326). Range: 1 to 10, in integer steps. For information on setting the full-scale range for the pressure recorder, see “Scaling the Output of the Pressure Recorder Terminals” on page 330
Flow Rate Adjustment (Alpha)	Makes fine adjustments to the flow rate (see “Flow Rate Adjustment (Alpha)” on page 327). For information on calibrating the alpha value, see “Updating the Flow Rate Adjustment Setting” on page 330.

Table 59. Calibration parameters for the Surveyor LC Pump Plus (Sheet 2 of 2)

Parameter	Description
Pressure Sensor Adjustment	Adjusts the pressure sensor reading (see "Pressure Sensor Adjustment" on page 327) For information on adjusting the pressure sensor reading, see "Updating the Pressure Sensor Adjustment Setting" on page 331.
Set	Downloads the new setting to the pump.
Pressure Transducer	
Zero	Calibrates the current pressure reading as the zero point (see "Pressure Transducer Zero" on page 327). For information on setting the zero point for the pressure transducer, see "Zeroing the Pressure Transducer" on page 331.

Calibration Options for the Surveyor LC Pump Plus

The Xcalibur data system provides the following calibration options (see Figure 231) for the Surveyor LC Pump Plus:

- "Compressibility," on this page
- "Pressure Recorder Full Scale" on page 326
- "Flow Rate Adjustment (Alpha)" on page 327
- "Pressure Sensor Adjustment" on page 327
- "Pressure Transducer Zero" on page 327

Compressibility

The compressibility of a liquid is a measure of its resistance to a decrease in volume caused by an increase in pressure. Compared to gases, most liquids are relatively incompressible. Water, a commonly used mobile phase solvent, is even less compressible than most organic solvents because of its extensive hydrogen bonding and cluster structure.

For information on updating the compressibility setting, see "Updating and Optimizing the Compressibility Setting" on page 328.

Effect of the Compressibility Setting

Even though liquids are relatively incompressible, the compressibility of your mobile phase can affect your chromatography if you are running the LC pump at the upper limit of its pressure range. You can use the compressibility setting for the Surveyor LC Pump to compensate for the compressibility of your mobile phase. However, changing the compressibility setting simultaneously affects both the pressure pulsation of the Surveyor LC Pump and the flow rate of the mobile phase.

The compressibility effect is caused by the portion of the cam cycle in which the pistons are compressing the mobile phase. The compressibility portion of the cam cycle extends from the time at which the primary piston starts discharging until the time at which the secondary piston reaches full intake—approximately 80° of the cam cycle. To minimize the pressure pulsation and maintain a constant flow rate, the on-board CPU compensates for this compression by making fine adjustments to the speed of the stepping motor. If the compressibility setting is incorrect, the LC pump will not be able to effectively minimize the pressure pulsation.

Figure 232 shows the effect of the compressibility setting on the pressure pulsation. The pressure trace was recorded as water was pumped at a flow rate of 3 mL/min. Three runs were recorded and the compressibility setting was changed between the runs. The compressibility settings for the three runs were 0.45 GPa^{-1}, 1.25 GPa^{-1}, and 3.0 GPa^{-1}.

Figure 232. Overlaid LC pump pressure traces, showing the effect of the compressibility setting

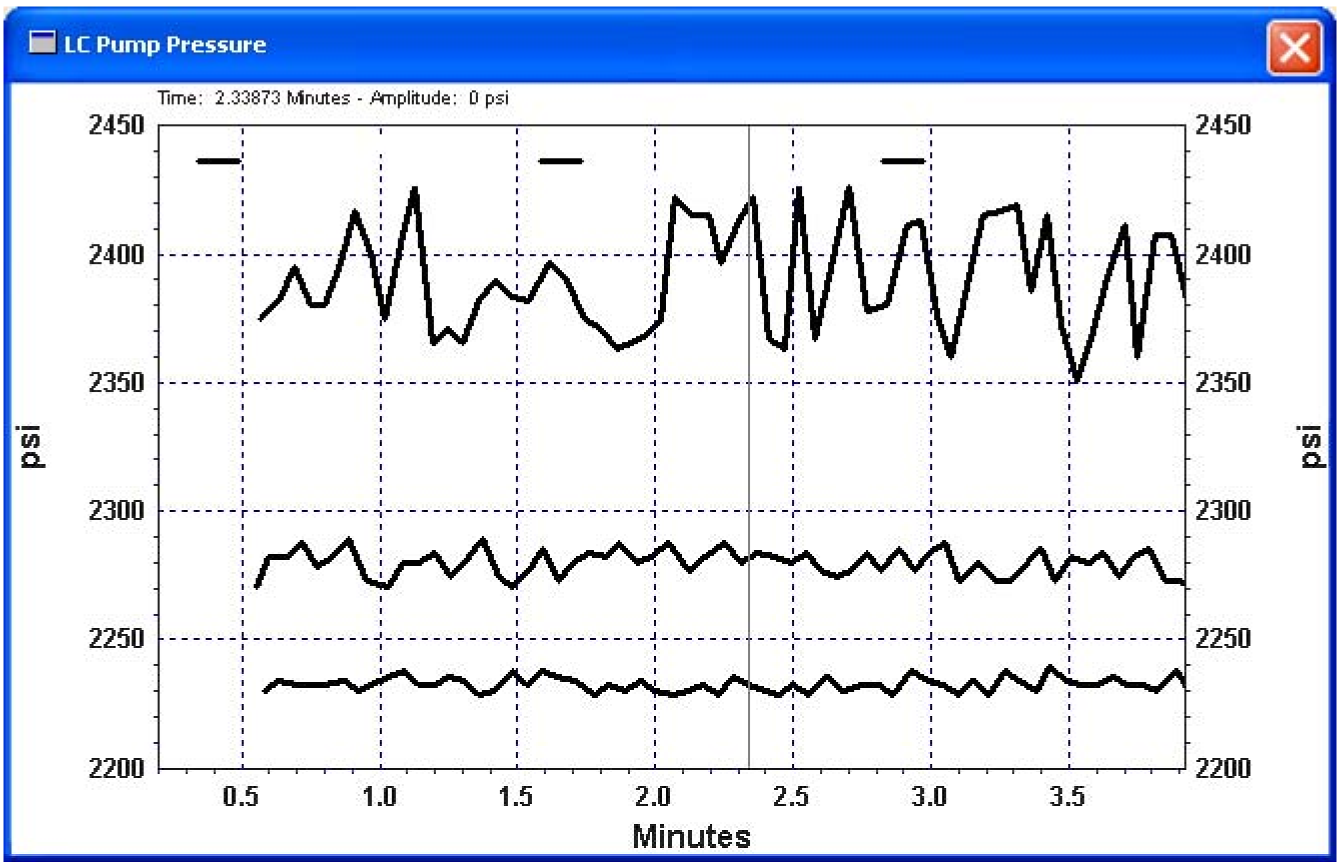


Table 60 shows a comparison of water pumped at three different compressibility settings. The correct compressibility setting for water is 0.45 GPa^{-1}. The pressure pulsation as well as the actual flow rate increased as the compressibility setting increased.

Table 60. Measured flow rate and pressure pulsation versus compressibility setting

Compressibility setting	% Pressure pulsation	Actual flow rate (mL/min)	% Rel diff
0.45 GPa^{-1}	± 0.2%	3.01	+ 0.3%
1.25 GPa^{-1}	± 0.4%	3.05	+ 1.8%
3.0 GPa^{-1}	± 1%	3.18	+ 5.9%

Compressibility Ratios for Common Solvents

Because most mobile phases are made up of a mixture of solvents, you must determine the correct compressibility setting for a given mobile phase empirically. Table 61 lists the compressibility ratios for a few common solvents.

Table 61. Measured flow rate and pressure pulsation versus compressibility setting

Solvent	Compressibility ratio (GPa^{-1})
Water	0.45
Acetonitrile	1.20
Methanol	1.25
Hexane	1.60

Pressure Recorder Full Scale

The pressure recorder full-scale calibration option sets the scaling factor for the pressure trace if you choose to monitor the pressure with a chart recorder. The pressure recorder terminals are located on the back of the Surveyor LC Pump Plus (see Figure 233). Integer values from 1 to 10 are within the allowable range.

Full scale = Set value × 4.9 MPa (50 kgf/cm2) (full-scale voltage = 1 mV)

For example:

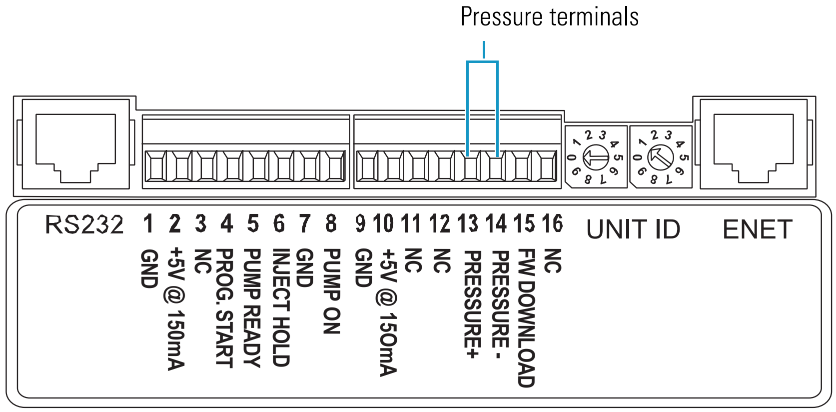
When the set value = 1, the full scale is 4.9 MPa (50 kgf/cm2)

When the set value = 10, the full scale is 49.0 MPa (500 kgf/cm2)

4.9 MPa = 50 kgf/cm2 = 49 bar = 711 psi

Note The value for the scaling factor is stored until you change it or until you turn off the power to the Surveyor LC Pump.

Figure 233. Terminals located on the back of the Surveyor LC Pump Plus



For information on setting the scaling factor for the pressure recorder, see “Scaling the Output of the Pressure Recorder Terminals” on page 330.

Flow Rate Adjustment (Alpha)

The alpha value for your Surveyor LC Pump Plus is factory calibrated based on a flow rate of 1.000 mL/min for distilled water. A sticker containing the factory calibration value is located inside the pump on the motor casing. You can optimize the alpha value for your application by updating the alpha setting. The range for alpha values is 1 to 9.9. If you are pumping water, decreasing the alpha value by 1 decreases the flow rate by 1%. Increasing the alpha value by 1, however, increases the flow rate by 1%.

For information on setting the alpha value, see “Updating the Flow Rate Adjustment Setting” on page 330.

Pressure Sensor Adjustment

Reset the Pressure Sensor Adjustment parameter when you replace the pressure sensor. Each pressure sensor is labeled with a data sticker that contains a “0.XXXX” value for the pressure sensor adjustment setting. Multiply this value by 10 000 and then update the pressure sensor adjustment setting for your Surveyor LC Pump Plus.

For information on updating the pressure sensor adjustment, see “Updating the Pressure Sensor Adjustment Setting” on page 331.

Pressure Transducer Zero

The pressure readout for the Surveyor LC Pump Plus is produced by a cell type potentiometer. This type of device tends to drift by small increments, requiring you to occasionally re-zero the readout. To update the pressure transducer zero value, turn off the pump flow and open the drain valve knob. Then click the pressure transducer zero button in the data system that operates your pump.

For information on zeroing the pressure transducer, see “Zeroing the Pressure Transducer” on page 331.

Calibration Procedures for the Surveyor LC Pump Plus

You fine-tune the performance of the Surveyor LC Pump Plus from the Calibration - Surveyor LC Pump Direct Control page (Figure 231 on page 323).

For instructions on opening the Calibration page, see “Accessing the Calibration Options for the Surveyor LC Pump Plus” on page 322.

To fine-tune the performance of the Surveyor LC Pump Plus, follow these procedures:

- “Updating and Optimizing the Compressibility Setting,” on this page
- “Scaling the Output of the Pressure Recorder Terminals” on page 330
- “Updating the Flow Rate Adjustment Setting” on page 330
- “Updating the Pressure Sensor Adjustment Setting” on page 331
- “Zeroing the Pressure Transducer” on page 331

Updating and Optimizing the Compressibility Setting

If you have already determined the best compressibility setting for your application, update the compressibility setting of the Surveyor LC Pump Plus.

For information on compressibility, see “Compressibility” on page 324.

❖ **To update the compressibility setting**

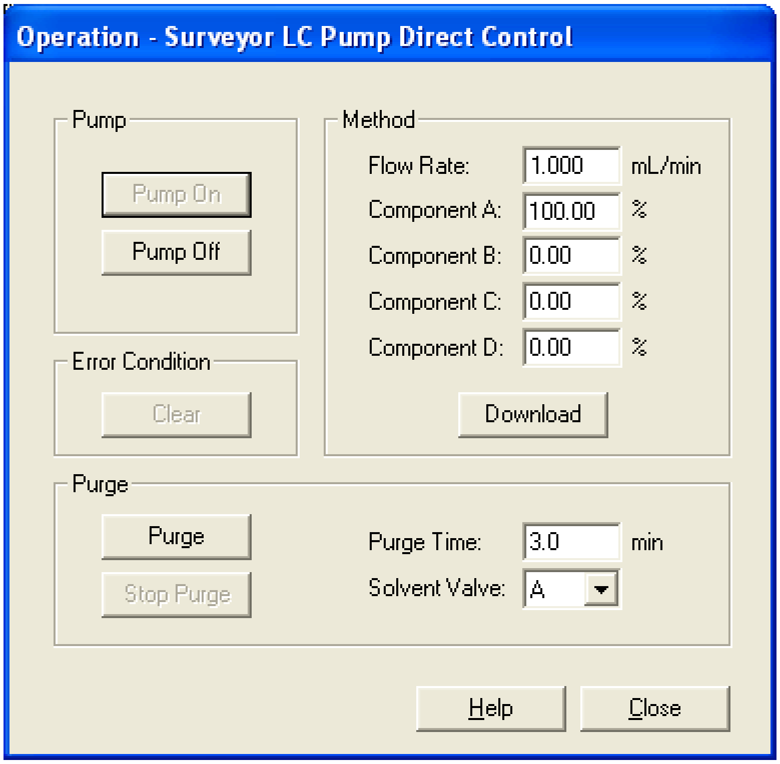
1. Open the Calibration page for the Surveyor LC Pump (see “Accessing the Calibration Options for the Surveyor LC Pump Plus” on page 322).
2. Type a new value into the Compressibility setting box. Then click **Set**.

❖ **To optimize the compressibility setting for your application**

1. Set up your system:
   a. Set up a device to record the pressure output from the back panel pressure terminals. See “Pressure Recorder Full Scale” on page 326.
   b. Scale the output of the pressure terminals as described in “Scaling the Output of the Pressure Recorder Terminals” on page 330.
2. Download the mobile phase parameters specified in your application:
   a. If it is not already on, turn on the power to the Surveyor LC Pump Plus.
   b. From the Surveyor LC Pump view, choose **Surveyor LC Pump > Direct Control > Operation**.

      The Operation - Surveyor LC Pump Direct Control dialog box appears (see Figure 234).

Figure 234. Operation - Surveyor LC Pump Direct Control dialog box



c. In the Method area, enter the specified flow rate in the Flow Rate box and enter the specified mobile phase composition in the Component A through Component D boxes.

d. Click **Download** to send these parameters to the onboard CPU of the Surveyor LC Pump Plus.

e. Click **Pump On** to turn on the pump flow.

3. Record the pressure trace.

4. If the pressure pulsation is too high for your application, change the compressibility setting. Then, repeat steps 2 and 3. Continue to change the compressibility setting until the level of pump pulsation is adequate for your application.

> **Note** Changing the compressibility setting affects the flow rate.

5. After you change the compressibility setting to minimize the pressure pulsation, check the flow rate. If necessary, adjust the flow rate adjustment setting as described in “Updating the Flow Rate Adjustment Setting” on page 330.

Scaling the Output of the Pressure Recorder Terminals

For information on using the pressure terminals on the pump's back panel, see "Pressure Recorder Full Scale" on page 326.

❖ To set the full-scale output of the pressure recorder terminals

1. Open the Calibration page for the Surveyor LC Pump (see "Accessing the Calibration Options for the Surveyor LC Pump Plus" on page 322).
2. Enter a value from 1 to 10 in the Pressure Recorder Full Scale box (see Figure 231 on page 323). Then click **Set**. For information on scaling the output of the pressure recorder terminals, see "Pressure Recorder Full Scale" on page 326.

Updating the Flow Rate Adjustment Setting

If you change the compressibility setting for the Surveyor LC Pump, you might also need to change its flow rate adjustment setting. Changing the flow rate adjustment setting can compensate for minor deviations in the flow rate of approximately ±5% from the expected value.

❖ To update the flow rate adjustment setting

1. Open the Calibration page for the Surveyor LC Pump (see "Accessing the Calibration Options for the Surveyor LC Pump Plus" on page 322).
2. In the Flow Rate Adjustment (alpha) box (see Figure 231 on page 323), type a new value:
   - If the actual flow rate is below the set value, increase the alpha value. If you are pumping water, increasing the alpha value by 1increases the flow rate by 1%.

     For example, if the pump is set to deliver a flow rate of 1 mL/min and the actual flow rate is 0.96 mL/min, which is 4% below the set rate, raise the current setting by 4. If the current setting is 5.0, enter a new setting of 9.0.
   - If the actual flow rate is above the set value, decrease the alpha value. If you are pumping water, decreasing the alpha value by 1 decreases the flow rate by 1%.

     For example, if the pump is set to deliver a flow rate of 1 mL/min and the actual flow rate is 1.04 mL/min, which is 4% above the set rate, lower the current setting by 4. If the current setting is 5.0, enter a new setting of 1.0.
3. Click **Set**.

Updating the Pressure Sensor Adjustment Setting

Reset this parameter when you replace the pressure sensor. Each pressure sensor is labeled with a data sticker that contains a "*0.xxxx*" value for the pressure sensor adjustment setting. Multiply this value by 10 000 and enter the result in the Pressure Sensor Adjustment setting box.

❖ To update the pressure sensor adjustment setting

1. Open the Calibration page for the Surveyor LC Pump (see "Accessing the Calibration Options for the Surveyor LC Pump Plus" on page 322).
2. In the Pressure Sensor Adjustment box (see Figure 231 on page 323), type the value on the data sticker "*0.xxxx*" multiplied by 10 000.
3. Click **Set**.

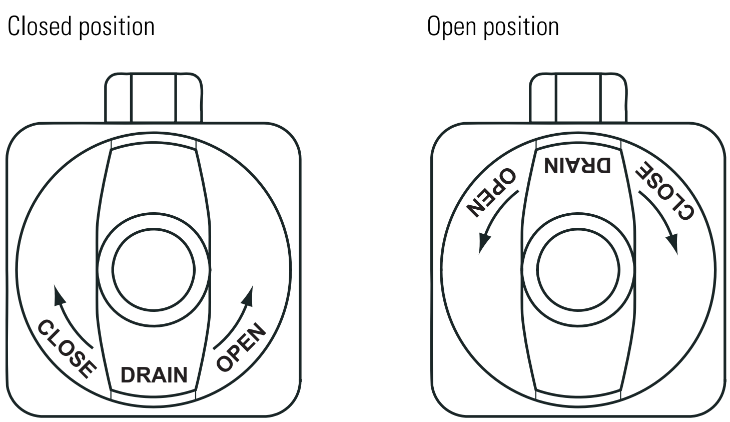
Zeroing the Pressure Transducer

The offset error of the pressure transducer is the value that is displayed when it should be zero, such as when the purge manifold knob is set to the open position. You can correct for this offset error by re-zeroing the pressure readout of the pressure transducer when the purge manifold knob is open.

❖ To re-zero the output of the pressure transducer

1. Stop the flow of solvent from the LC pump:
   a. From the Instrument Setup window, choose **Surveyor LC Pump > Direct Control > Operation**.
   b. Click **Pump Off**.
2. Open the drain valve to ensure that the transducer is actually sensing zero system pressure (see Figure 235). When the drain valve is in the open position, look for the word DRAIN to appear upside down on the knob.

Figure 235. Drain valve in closed and open positions



3. Open the Calibration page of the Direct Control dialog box by choosing **Surveyor LC Pump > Direct Control > Calibration**.

4. Click **Zero** in the Pressure Transducer area (see Figure 231 on page 323).

5. When you are finished zeroing the pressure transducer, close the drain valve by turning the knob clockwise until you feel resistance.

Note Applying excessive force to the drain valve knob reduces the life span of its O-ring.

Recording Maintenance Information for the Surveyor LC Pump Plus

Use the Direct - Control Maintenance dialog box to update the maintenance information for the Surveyor LC Pump Plus and to set the pistons to the maintenance position.

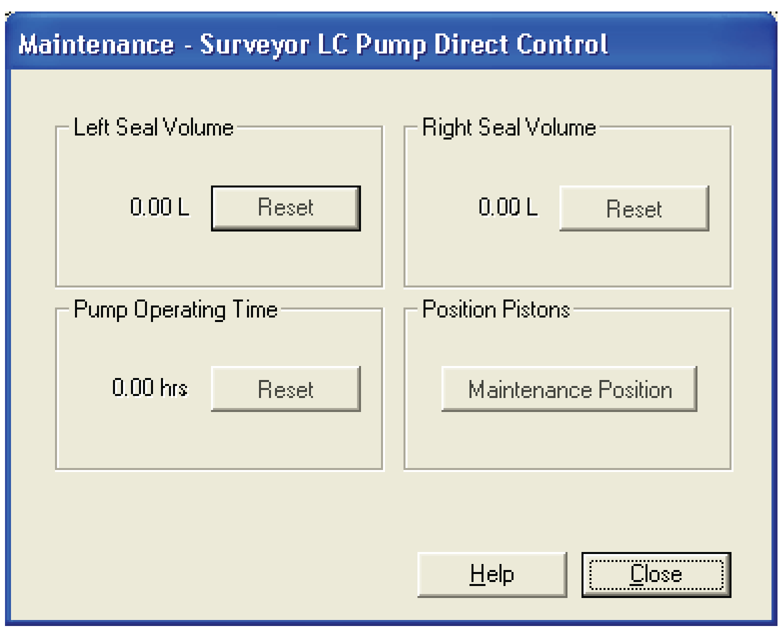
Each pump head assembly contains a piston seal. The two seals can wear at different rates, causing leaks to occur in one or both pump heads. Before dismantling the pump head assemblies, attempt to determine the source of the leakage. To save time and materials, replace only the piston seal in the pump head assembly that is leaking. Or, as a preventive measure, replace the piston seals after pumping 120 L of mobile phase.

❖ To open the Direct Control - Maintenance dialog box

From the menu bar of the Surveyor LC Pump Plus view, choose **Surveyor LC Pump > Direct Control > Maintenance**.

The Maintenance - Surveyor LC Pump Direct Control page appears (see Figure 236).

Figure 236. Direct Control - Maintenance dialog box



❖ To reset the solvent volumes or the pump operating time

Perform the following tasks as specified:

- Every time you install a new piston seal, click the appropriate **Reset** button.

  Clicking Reset sets the readback for the volume of liquid pumped by the respective pump head assembly to zero.

- If you install a new motor, click the Reset button under Pump Operating Time.

  Clicking Reset sets the recorded hours for the pump operating time to zero.

❖ To set the pistons to the maintenance position before removing the pump heads

Click **Maintenance Position**.

Maintenance -Surveyor LC Pump Direct Control Parameter Descriptions

Table 62 describes the parameters in the Maintenance dialog box for the Surveyor LC Pump Plus.

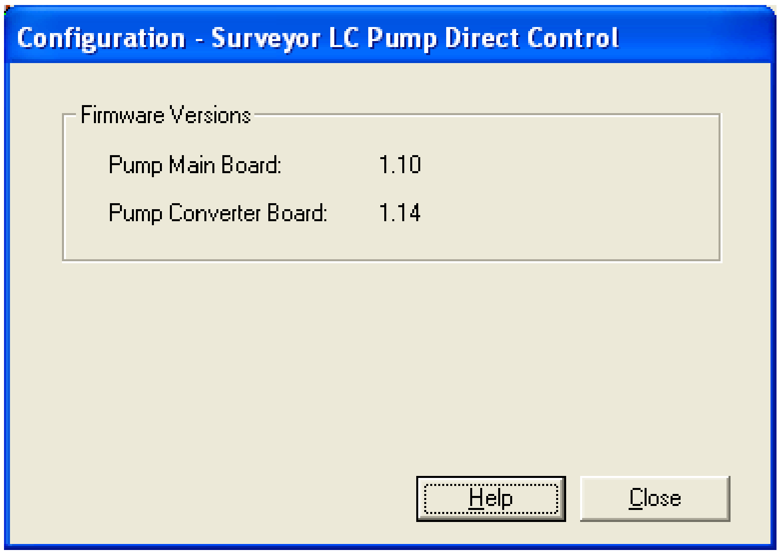
Table 62. Maintenance dialog box for the Surveyor LC Pump Plus

Parameter	Description
Left Seal Volume	
Reset	Zeros the recorded volume of mobile phase pumped by the left pump head. Every time you install a new piston seal in the left pump head, click **Reset** in the Left Seal Volume area to set the recorded volume of liquid pumped by the left pump head assembly back to zero.
Right Seal Volume	
Reset	Zeros the recorded volume of mobile phase pumped by the right pump head. Every time you install a new piston seal in the right pump head, click **Reset** in the Right Seal Volume area to set the recorded volume of liquid pumped by the right pump head assembly back to zero.
Pump Operating Time	
Reset	Resets the recorded operating time for the Surveyor LC Pump Plus to zero. If you install a new motor, click **Reset** in the Pump Operating Time area to set the recorded Pump Operating Time back to zero.
Position Pistons	
Maintenance Position	Sets the pistons to the maintenance position. Set the pump pistons to the maintenance position before removing the pump heads. To set the pistons to the maintenance position, click **Maintenance Position**.

Firmware Version Information for the Surveyor LC Pump Plus

Use the Configuration - Surveyor LC Pump Direct Control dialog box (see Figure 237) to view the firmware versions of the pump's Main board and Pump Converter board.

Figure 237. Configuration - Surveyor LC Pump Direct Control dialog box



❖ **To view the firmware version of your Surveyor LC Pump Plus**

1. Open the pump view.
2. From the menu bar, choose **Surveyor LC Pump > Direct Control > Configuration**.

   The Configuration - Surveyor LC Pump Direct Control page appears.

Configuration Page Parameter Descriptions

Table 63 describes the parameters on the Firmware page.

Table 63. Configuration - Surveyor LC Pump Direct Control page parameters

Parameter	Description
Firmware Versions	
Pump Main Board	Displays the firmware version of the Pump Main printed circuit board.
Pump Converter Board	Displays the firmware version of the Pump Converter printed circuit board.

Making a Single Injection from the Tune Window

If you are using the Surveyor Plus LC system as an inlet to a Thermo Scientific mass spectrometer, you can use the direct control dialog boxes available from the Tune window to make a single injection and acquire data.

For information on setting up the LC/MS system, refer to the Getting Connected Guide for your mass spectrometer. For information on acquiring raw data files from the tune program for your mass spectrometer, refer to the Getting Started Guide for your mass spectrometer.

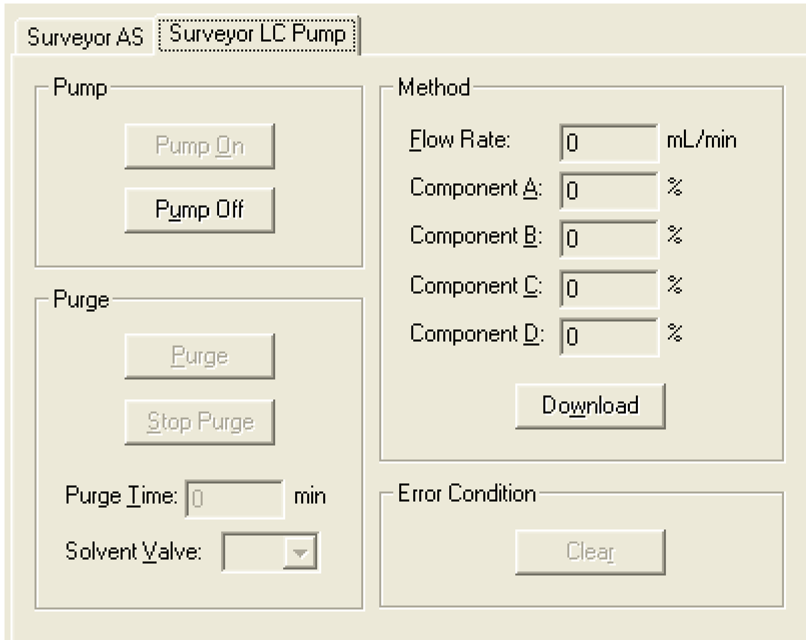
Note You can use the PDA Direct Control dialog box to perform diagnostic tests such as checking the performance of the deuterium lamp. To acquire PDA data for a single injection, you must run a single row sequence from the Sequence window.

❖ **To inject a single sample from the Tune window**

1. Prepare the LC system for operation. See Chapter 5, "Daily Operation."
2. From the Tune window for your Thermo Scientific mass spectrometer, choose **Setup > Inlet Direct Control**.

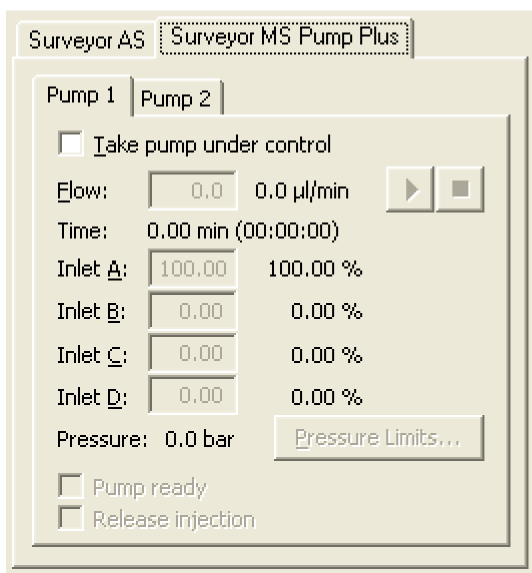
   The Inlet Direct Control dialog box that contains tabbed pages for each configured LC device appears.
3. Start the solvent flow from the pump as follows:
   - For the Surveyor LC Pump Plus, click the **Surveyor LC Pump** tab. In the Method area (see Figure 238), enter the appropriate values for the flow rate and the solvent percentages. Click **Download**. In the Pump area, click **Pump On** to start the solvent flow.

Figure 238. Surveyor LC Pump page of the Inlet Direct Control dialog box



- For the Surveyor MS Pump Plus, click the **Surveyor MS Pump Plus** tab. Select the **Take pump under control** check box (see Figure 239). Type the appropriate flow rate in the Flow box. Type the appropriate solvent proportions in the Inlet boxes. Select the **Pump ready** check box. Click ▶ (Start) to start the solvent flow.

Figure 239. Direct Control dialog box for the pump



4. Start the injection as follows:

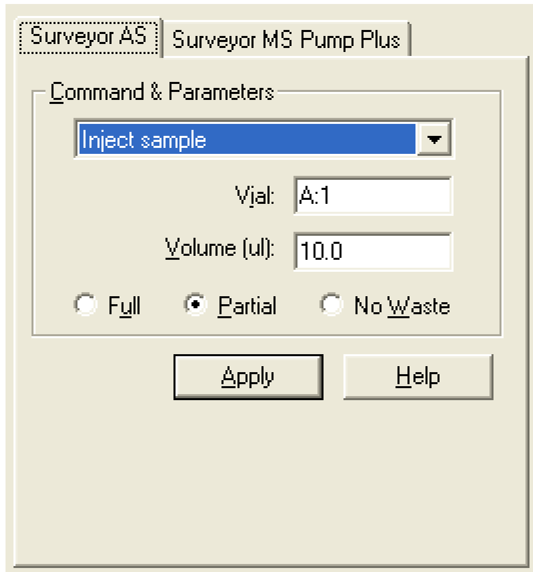
   a. In the Inlet Direct Control dialog box, click the **Surveyor AS** tab.

      The Surveyor AS page appears (see Figure 240).

b. In the list of commands, select **Inject sample**.

The Inject sample parameters appear (see Figure 240).

Figure 240. Surveyor AS page of the Direct Inlet Control dialog box



c. In the Vial box, type the location of the sample. Ensure that you have placed a sample in this location.

For information on the vial and well notation, see "Vial and Well Notation" on page 6.

d. In the Volume (µL) box, type the volume that you want to inject.

e. Select the injection mode: **Full**, **Partial**, or **No Waste**. For more information, see "Injection Modes" on page 20.

f. Click **Apply**.

The autosampler loads the sample into the sample loop connected to the injection valve as follows:

The XYZ arm of the autosampler moves to the sample location, and then lowers the needle into the sample vial. The syringe plunger descends, drawing sample into the needle tubing. The XYZ arm moves back to the injection port, and then lowers the needle into the injection port. The syringe plunger ascends, pushing sample into the injection port, through the transfer tubing, and into the sample loop of the injection valve.

5. On the Surveyor MS Pump Plus page, select the **Release injection** check box.

The injection valve switches to the inject position, allowing mobile phase to backflush the sample out of the sample loop and into the LC column.

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