Surveyor Plus™

Getting Started with Xcalibur 2.0

60053-97103 Revision D January 2007

Analyze • Detect • Measure • Control™

Thermo ELECTRON CORPORATION

Software Revision: Xcalibur 2.0 and higher

Thermo
ELECTRON CORPORATION

Regulatory Compliance

Thermo Electron San Jose performs complete testing and evaluation of its products to ensure full compliance with applicable domestic and international regulations.

Changes that you make to your instrument 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 Electron. To ensure continued compliance with EMC and safety standards, replacement parts and additional components, options, and peripherals must be ordered from Thermo Electron 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

The following statements apply to all the devices of the Surveyor Plus family of LC instruments.

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 Electron San Jose Instruments

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

Notice on the Proper Use of Thermo Electron San Jose Instruments

In compliance with international regulations: If this instrument is used in a manner not specified by Thermo Electron San Jose, the protection provided by the instrument could be impaired.

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.

Thermo
ELECTRON CORPORATION

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 Electron has contracted with one or more recycling/disposal companies in each EU Member State, and this product should be disposed of or recycled through them. Further information on Thermo Electron's compliance with these Directives, the recyclers in your country, and information on Thermo Electron products which may assist the detection of substances subject to the RoHS Directive are available at www.thermo.com/WEEERoHS.

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 Electron 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 Electron, 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.

Thermo
ELECTRON CORPORATION

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 Electron 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 Electron à ces directives, les recycleurs dans votre pays et les informations sur les produits Thermo Electron qui peuvent aider la détection des substances sujettes à la directive RoHS sont disponibles sur www.thermo.com/WEEERoHS.

Contents

Preface

About This Guide

Welcome to the Surveyor Plus Integrated LC/MS system and the Xcalibur™ data system. The Surveyor Plus™ is part of the Thermo Electron family of LC instruments.

This *Surveyor Plus Getting Started with Xcalibur* manual contains an introduction to the devices of the Surveyor Plus LC System and guides you through the process of preparing your LC devices for a run, creating an instrument method, making your first injection, and reviewing the results in the Qual Browser window of the Xcalibur data system.

Related Documentation

In addition to this guide, Thermo Electron provides the following documents for the Surveyor Plus system:

- *Surveyor Plus Preinstallation Requirements Guide*
- *Surveyor Plus Getting Connected*
- *Surveyor Autosampler Plus Hardware Manual*
- *Surveyor LC Pump Plus Hardware Manual*
- *Surveyor MS Pump Plus Hardware Manual*
- *Surveyor UV/Vis Plus Detector Hardware Manual*
- *Surveyor PDA Plus Detector Hardware Manual*
- *Surveyor UV/Vis Plus Detector Hardware Manual*

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 avoid damage to software, loss of data, invalid test results, or information critical for optimal performance of the system.

Note Highlights information of general interest.

Tip Helpful information that can make a task easier.

Contacting Us

There are several ways to contact Thermo Electron Corporation.

Assistance

For new product updates, technical support, and ordering information, contact us in one of the following ways:

Visit Us on the Web

www.thermo.com/finnigan

Contact Technical Support

Phone: 1-800-685-9535
Fax: 1-561-688-8736
E-mail: techsupport.finnigan@thermo.com

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

Contact Customer Service

In the US and Canada for ordering information:
Phone: 1-800-532-4752
Fax: 1-561-688-8731
Web site: www.thermo.com/finnigan

Changes to the Manual and Online Help

To suggest changes to this guide or to the online Help, use either of the following methods:

- Fill out a reader survey online at www.thermo.com/lcms-techpubs
- Send an e-mail message to the Technical Publications Editor at techpubs.finnigan-lcms@thermo.com

Chapter 1 Introduction to the Surveyor Plus Integrated LC/MS System

The Surveyor Plus LC system, which integrates with the Thermo Electron family of mass spectrometers, consists of an analytical pump, an autosampler, an optional photodiode array detector or dual wavelength UV/Vis detector, and a solvent platform. See Figure 1.

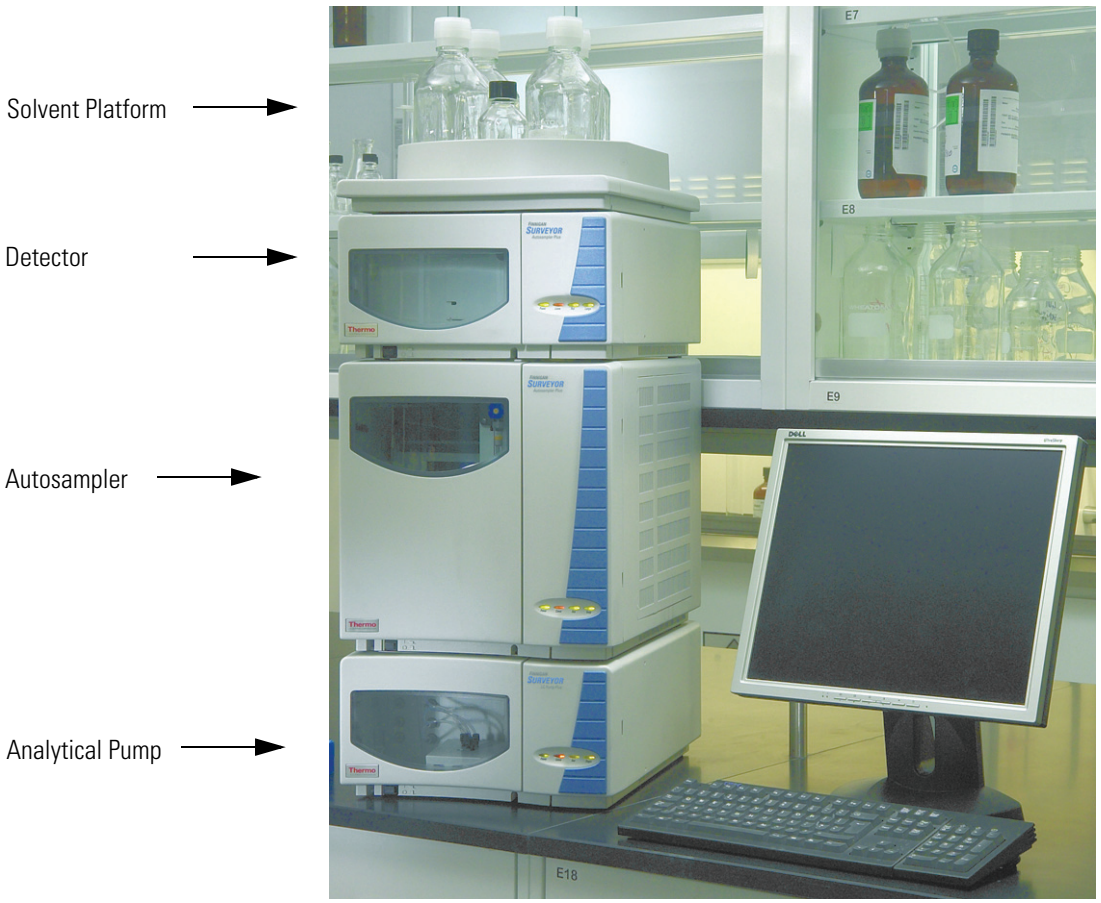


Figure 1. Surveyor Plus LC instrument

Two analytical pumps are available: the Surveyor LC Pump Plus and the Surveyor MS Pump Plus. Each pump is a dual-piston, quaternary, low-pressure mixing solvent delivery system with a built-in vacuum degasser and pulse dampener. The LC pump operates in the flow rate range from 1 to 9999 µL/min. It provides precise gradients over a flow rate range of 200 to 2000 µL/min. The MS pump operates in the flow rate range from 1 to 2000 µL/min. It provides precise gradients in the flow rate range from 25 to 800 µL/min.

Two autosamplers are available: the Surveyor Autosampler Plus and the Surveyor Autosampler Plus Lite. Both autosamplers automate sample injections and sample preparation. Their tray compartments accept various vials, 96-well plates, and 384-well plates. In addition to these features, the full-featured Surveyor Autosampler Plus includes a built-in column oven (5 to 95 °C) and sample compartment temperature control (0 to 60 °C).

Two detectors are available: the Surveyor PDA Plus Detector and the Surveyor UV/Vis Plus Detector. The Surveyor PDA Plus Detector, in combination with the 5 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 dual-lamp optical bench that covers the UV-visible spectrum from 190 to 800 nm.

The solvent platform, which is located on the top of the Surveyor Plus LC stack, holds four 1-L solvent reservoir bottles and one 1-L 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 2-position syringe valve of the autosampler.

This chapter provides a brief description of the Surveyor Plus LC system and contains the following sections:

Analytical Pump

Thermo Electron offers two analytical pumps in the Surveyor Plus series: 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 that is required to purge the lines when you replace an eluent.

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

- 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 0.1 to 2000 µL/min, the range needed to perform LC/MS applications. You can run precise gradients from 25 to 800 µL/min.

The built-in pulse dampening assembly consists of a low volume T-connector through which the mobile phase passes. Attached to the side leg of the T-connector is a permeable isolation membrane made of sintered Teflon followed by a 2 mL coil of stainless steel tubing. The coil is terminated with a priming valve. When the priming valve is open, the dampening coil can be flushed or filled with an appropriate solvent such as methanol or isopropanol. When the priming valve is closed, the diffusion barrier cartridge is shut off from the flow path and the dampening coil absorbs pump pulsations. Even at elevated pressures, the pump will show only minimal pulsation when the pulse dampener is used. Because the 2 mL coil is shut off from the flow path, the low volume T-connector of the pulse dampener adds only 3 µL of delay volume to your system.

The Surveyor MS Pump Plus is remotely controlled by way of an RS232 serial communication link from a PC using Xcalibur software (version 1.3 or higher). The only manual control is the power switch located on the front of the pump in the lower left corner below the door. See Figure 2.

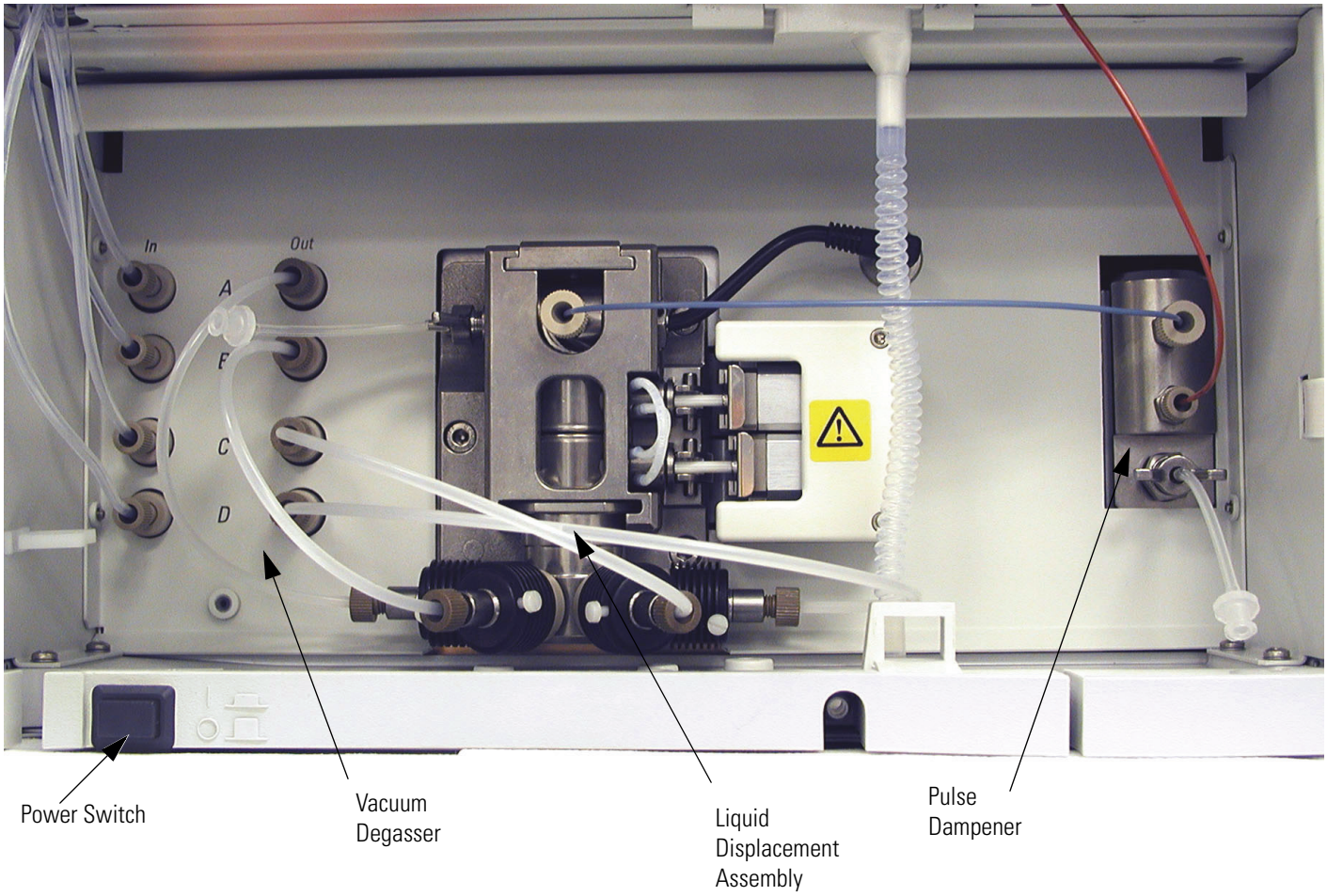


Figure 2. Front view of Surveyor MS Pump Plus

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, the range that is 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). It is remotely controlled by way of an Ethernet communication link from a PC using Xcalibur (version 2.0 or higher). The only manual control is the power switch located on the front of the pump in the lower left corner below the door. Direct control of the pump is achieved through the Xcalibur data system.

The Surveyor LC Pump Plus, shown in Figure 3, 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, which adds 400 µL of gradient delay volume to the system. In addition, the LC pump has status LEDs on the front door, and a low voltage power supply.

Note Do not overtighten the fingertight fitting that connects the LC pump to the autosampler. See Figure 3. Overtightening this fitting will cause leaking.

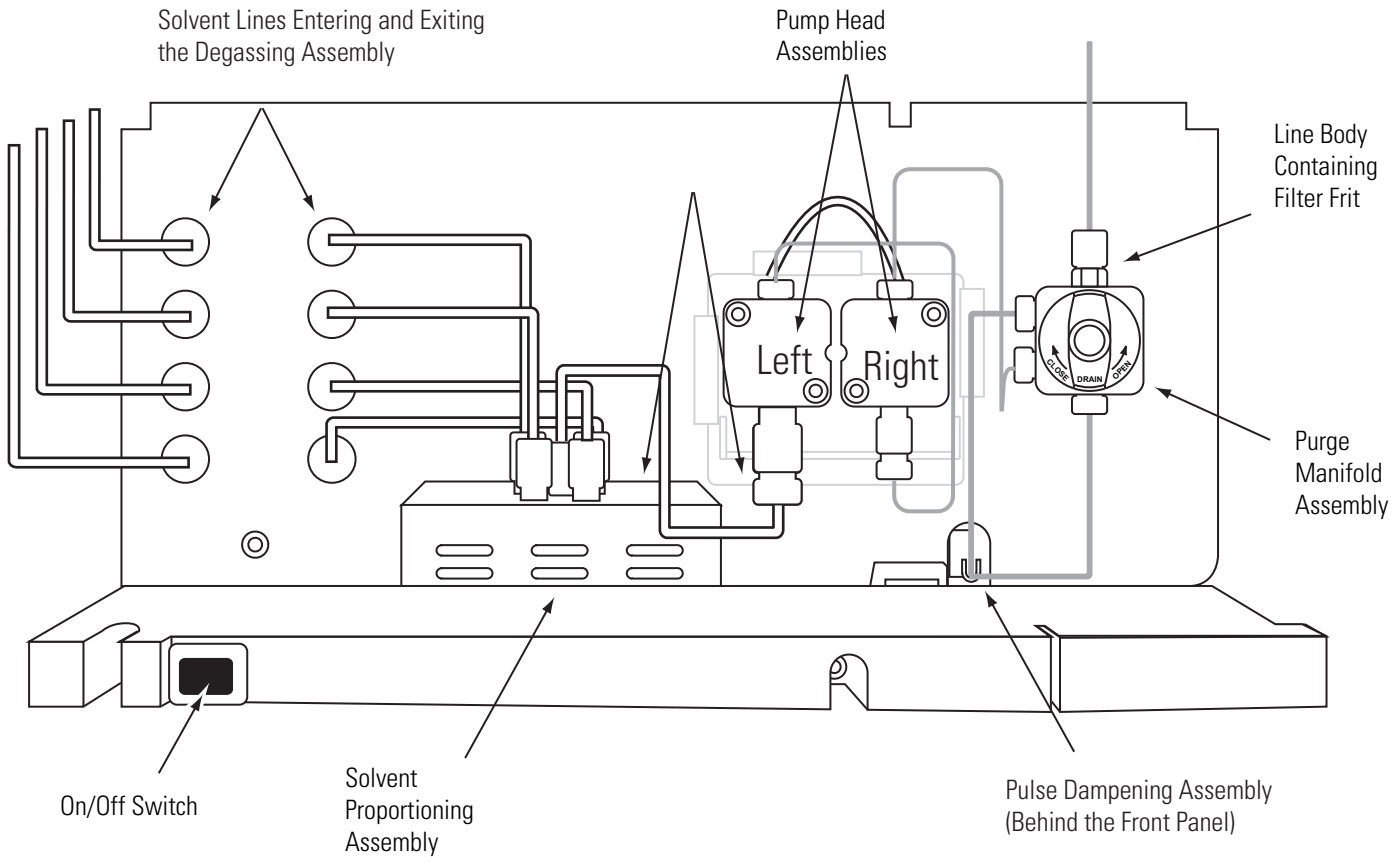


Figure 3. Front view of Surveyor LC Pump Plus

You can purge the LC pump from the Xcalibur data system after you open the purge valve. You open the purge valve by turning the knob on the front of the purge manifold assembly counter-clockwise. See Figure 4.

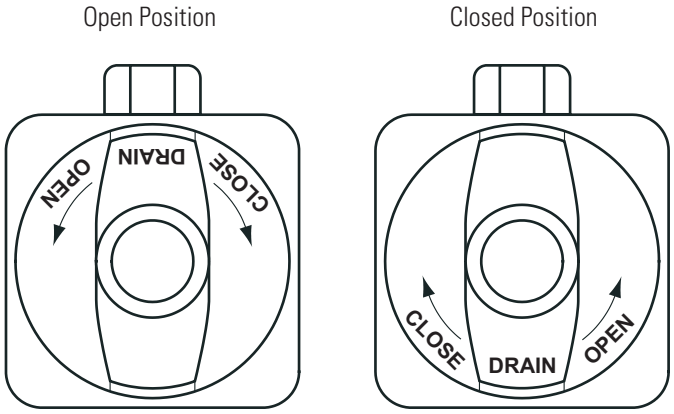


Figure 4. Drain valve positions

Figure 5 shows the line body fitting, located at the top of the purge manifold assembly. The line body fitting contains an in-line 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 frit becomes clogged, your system backpressure rises. Replace this frit when your system backpressure rises above its typical operating limits.

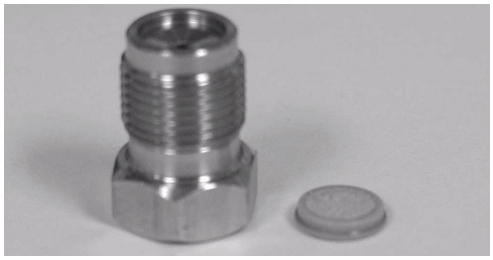


Figure 5. Line body and filter frit

Surveyor Autosampler Plus

The Surveyor Autosampler Plus allows you to inject samples and perform sample dilutions automatically. Thermo Electron offers two Surveyor Plus autosampler models: 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 tray/sample temperature control (0 to 60 °C). The autosampler tray compartment, the injection system, the injection modes, and the temperature control features are described in the following topics:

- Tray Compartment
- Injection System
- Injection Modes
- Temperature Control

Tray Compartment

The tray compartment can hold up to five conventional sample trays or one microwell carrier. The tray compartment also holds up to four 12 mL capacity reservoir vials that can be used to hold solvent, reagent, or diluent. The reservoir vials are located behind the wash station and are designated RV1, RV2, RV3, and RV4. See Figure 6.

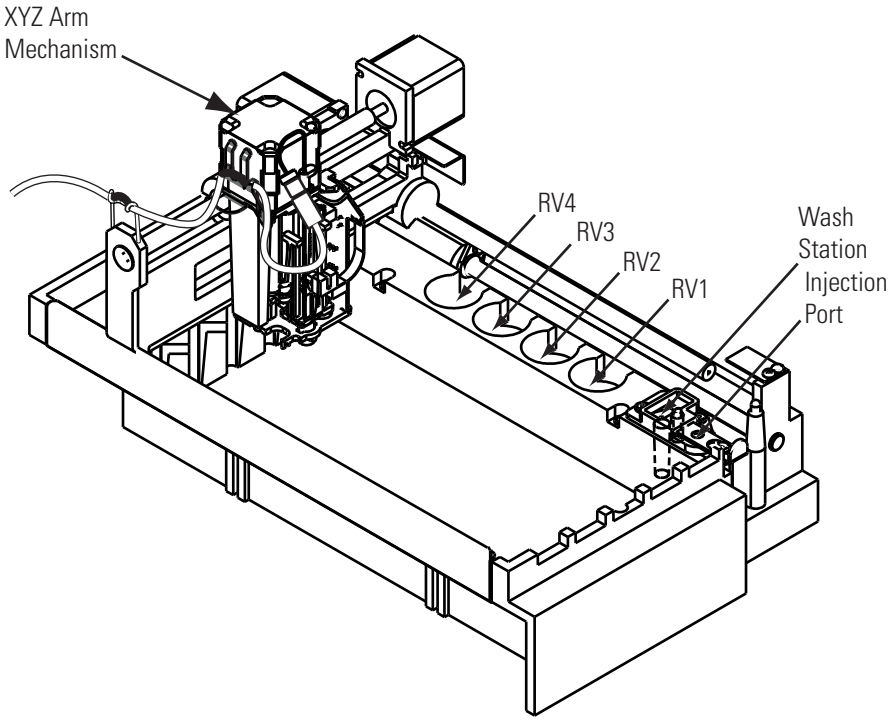


Figure 6. Tray compartment, showing brackets for needle tubing assembly

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.

If you select the Verify Door Is Closed check box when you configure the autosampler, the XYZ arm will automatically move to the back of the tray compartment when you open the door so that you can remove trays or replace vials. Opening the 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 injection is complete, whereupon the sequence is halted and the tray can be removed. After you close the autosampler door, the sequence resumes.

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 consists of a stepper motor drive mechanism, a syringe valve, and fittings that hold the interchangeable syringe. The syringe drive assembly is mounted to the front of the autosampler with three mounting pins. On the back of the assembly, holes with grommets mate with these pins. These rubber grommets minimize the effects of vibration and cause the connection between the syringe drive assembly and the body of the autosampler to feel slack. See Figure 7.

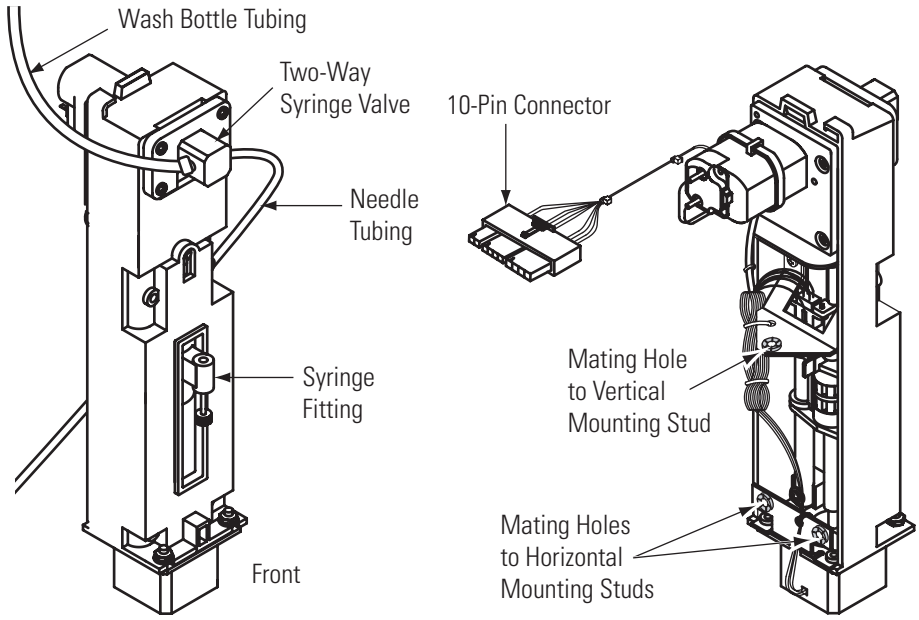


Figure 7. Syringe drive assembly, showing front and back of assembly

The syringe valve is a 2-position rotary valve. In the wash bottle position, solvent is drawn into the syringe barrel as the syringe plunger descends. In the needle position, sample is drawn into the needle tubing as the syringe plunger descends and is pushed out of the needle tubing assembly as the syringe plunger ascends. Sample is never drawn into the barrel of the syringe.

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, ensure that you have also installed 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 Surveyor Plus tack. 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 will fill with air. If the syringe runs dry, the autosampler cannot draw sample into the needle tubing.

Interchangeable Syringe

The standard configuration for the Surveyor Autosampler Plus consists of a 250 µL concentric syringe. The concentric syringe consists of a small, inner plunger, and a larger, outer plunger. See Figure 8.

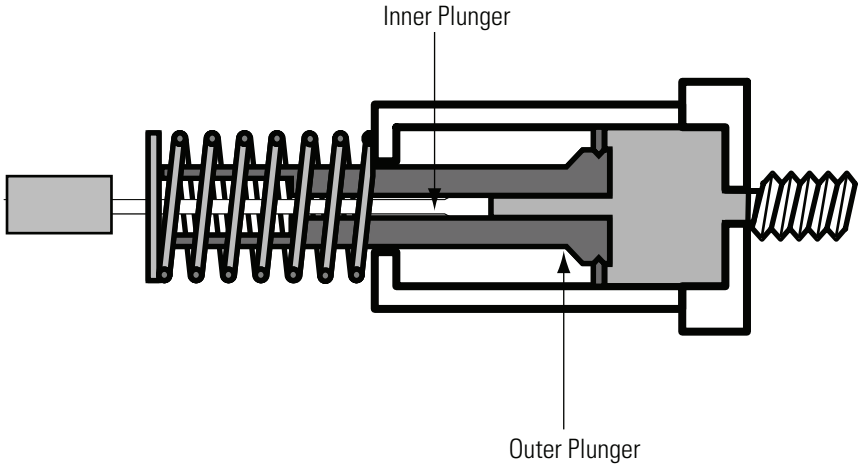


Figure 8. Concentric Syringe

The inner plunger is used to draw and deliver sample amounts equal to or less than the maximum capacity of the syringe, which is 265 µL for the 250 µL concentric syringe. The outer plunger is used to draw and expel large volumes of solvent.

Concentric syringes are available in 100, 250, and 500 µL sizes. In addition, a 2500 µL standard syringe is available. The volume of the outer plunger region, which is 565 µL, is the same for all three concentric syringes. The size of the inner plunger and the injection mode determine the available injection volume range.

Because injections are always performed by using the inner plunger of the syringe, the size of the syringe does not affect the precision of an injection. However, for sample preparation routines, the outer plunger of the syringe is used for solvent transfers larger than the nominal size of the syringe. Solvent transfers performed with the outer plunger of the syringe are less precise than those performed with the inner plunger. Therefore, you might want to order the 500 µL concentric syringe or the 2500 µL standard syringe if you plan to use the autosampler to routinely perform sample dilutions.

XYZ Arm Mechanism

The XYZ arm mechanism moves the needle along the x-y plane to the requested vial or well location. After the needle is positioned above the vial or well, the XYZ arm mechanism lowers the needle along the z-axis to the

requested needle height. After the needle withdraws sample, the XYZ arm mechanism moves along the x-y plane, back to the home position, which is above the injection port of autosampler. The needle is then lowered into the injection port where it expels the sample.

IMPORTANT Because the XYZ arm moves to the sample position to withdraw sample, it is important that you do not place objects taller than 1.8 inches into the tray compartment. Tall objects will stall the XYZ arm.

If you select the Verify Door Is Closed check box when you configure the autosampler, the XYZ arm will automatically move to the back of the tray compartment when you open the door so that you can remove trays or replace vials.

When you submit a Wash Needle direct command from the data system, the XYZ arm mechanism moves the needle to the wash station position. When you submit a Remove Needle direct command from the data system, the XYZ arm mechanism moves to the middle front of the tray compartment to allow easy access to the needle tubing assembly.

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. See Figure 9. The needle is inserted into the needle mount on the XYZ arm.

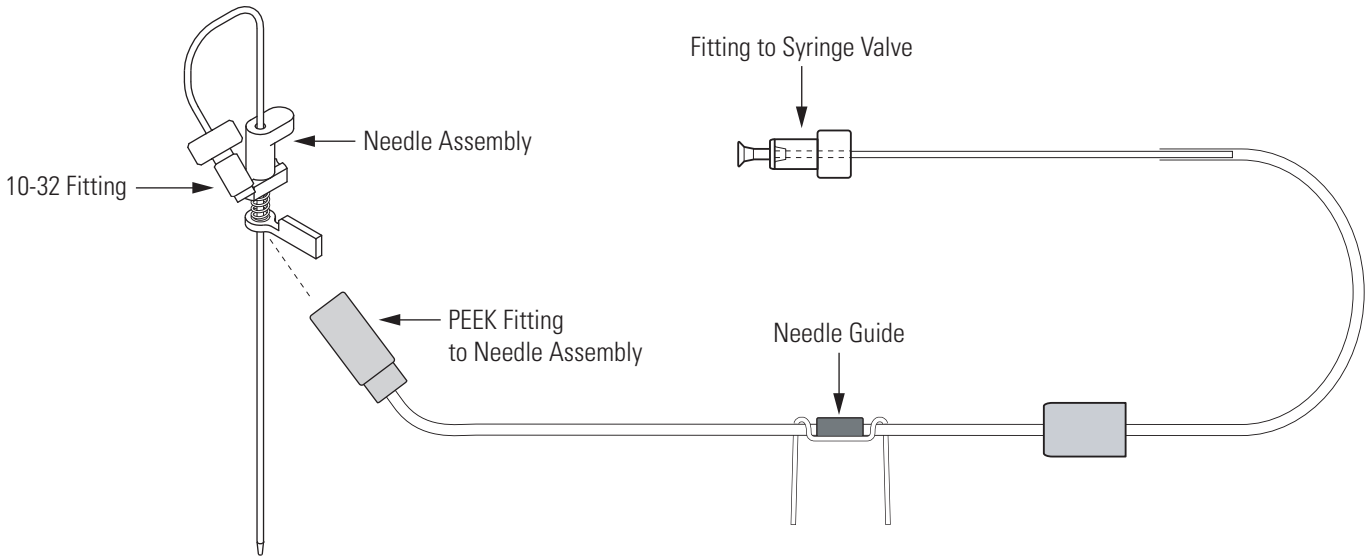


Figure 9. Needle tubing assembly and needle assembly

The needle tube assembly consists of a piece of low-pressure tubing, a PEEK™ fitting that connects to the needle assembly, a fitting and flangeless ferrule that connects to the right side of the syringe valve, and a needle tube guide that is inserted into the X-axis positioning frame. To prevent the needle tubing from interfering with the movement of the XYZ arm, the needle tubing is secured with a clip on the Z-axis of the XYZ arm and a clip on the X-axis positioning frame. In addition to routing the needle tubing through these clips, make sure that you route the needle tubing behind the syringe drive assembly as you mount it.

Injection Port and Transfer Tubing Assembly

The injection port of the autosampler is located behind the syringe drive assembly. A 0.012-in. ID transfer tube connects the injection valve of the autosampler to port 2 of the Valco injection valve. See Figure 10.

Note The label attached to the transfer tube assembly specifies its internal volume. You must enter this value in the Surveyor Autosampler Plus Configuration dialog box. See "Configuring the Surveyor Autosampler Plus" on page 28.

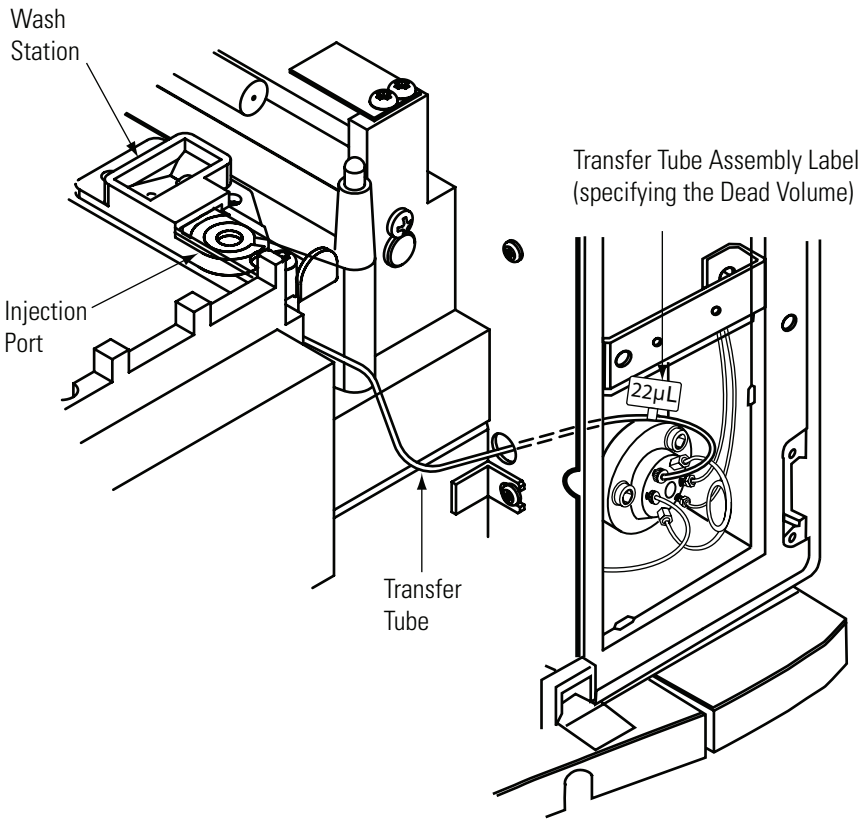


Figure 10. Injection port and transfer tube

Injection Valve and Sample Loop

The injection valve is a six-port Valco 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 the Valco valve at ports 1 and 4. The Surveyor Autosampler Plus ships with a 25 µL sample loop. The available sizes are listed in Table 1.

Table 1. Available sample loop sizes

Sample Loop Size (µL)	Part Number
5	00109-99-00007
10	00109-99-00008
20	00109-99-00009
25	00109-99-000010
50	00109-99-000011
100	00109-99-000012
500	00109-99-000013
1000	00109-99-000014

There are two positions for the six-port injection valve:

- Fill Position
- Inject Position

Fill Position

In the fill position, the sample loop is isolated from the mobile phase stream. As the mobile phase bypasses the sample loop, sample is pushed into the front of the loop that is connected to port 1 of the Valco injection valve. After the loop is filled, excess solution is pushed out the back of the loop to waste. See Figure 11.

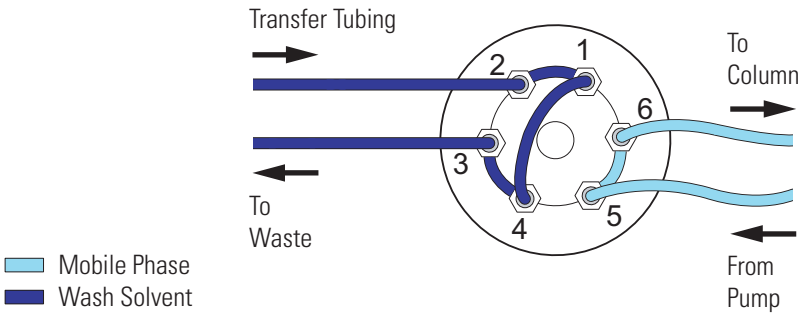


Figure 11. Injection valve in the fill position

Inject Position

In the inject position, mobile phase enters the sample loop from the back, backflushing the contents of the 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. See Figure 12.

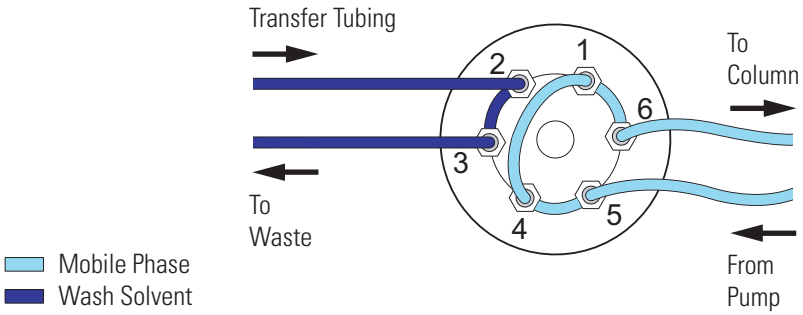


Figure 12. Injection valve in the inject position

Injection Modes

The Surveyor Autosampler Plus 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. The minimum allowable injection volume is 0.1 µL.

Note To perform accurate no waste injections, use a sample loop that is at least 5 µL larger than the injection volume.

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.

Note To make precise partial loop injections, use a sample loop that is at least twice the size of the injection volume.

Full Loop Injection Mode

The full loop injection mode is a technique that withdraws a sample volume from the vial that is sufficient to overfill the sample loop by a minimum factor of two. Because the actual injection volume is determined by the physical size of the sample loop, 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. If you want to change the injection volume, you must change the sample loop size. For the available loop sizes, see Table 1 on page 13.

Note In Xcalibur, 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 maximum capacity of the syringe is reached, 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 approximately 120 cm of 0.020-in. ID stainless steel high-pressure tubing that is located behind the column oven. As it passes through this tubing, the mobile phase equilibrates to the temperature of the column oven before it reaches the Valco injection valve.

Although it is useful for temperature sensitive applications, this additional tubing also adds 250 µL of gradient delay volume to the Surveyor Plus LC system. If you are performing low flow gradient applications, you might want to bypass this tubing by connecting the outlet of the pump directly to port 5 of the Valco injection valve.

Surveyor PDA Plus Detector

The Surveyor PDA Plus Detector is a full-featured, time-programmable, photodiode array detector. Detection can be carried out in the full ultraviolet-visible range from 190 to 800 nm. Data can be taken in this range at a rate of up to 20 Hz with 20-bit digital conversion.

The optimal location for the PDA detector is above the autosampler and below the solvent platform. The PDA detector is controlled remotely over an Ethernet communication link from a PC using the Xcalibur software. 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 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). There is some overlap between the two lamps in the 300 to 500 nm range. Attenuators are used to adjust 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. 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. Position 1 (Open) is used for normal operation. Position 2 contains a sealed, quartz cuvette filled with a holmium oxide / perchloric acid solution (NIST Traceable) and is used for wavelength accuracy verification and calibration. See Figure 13.

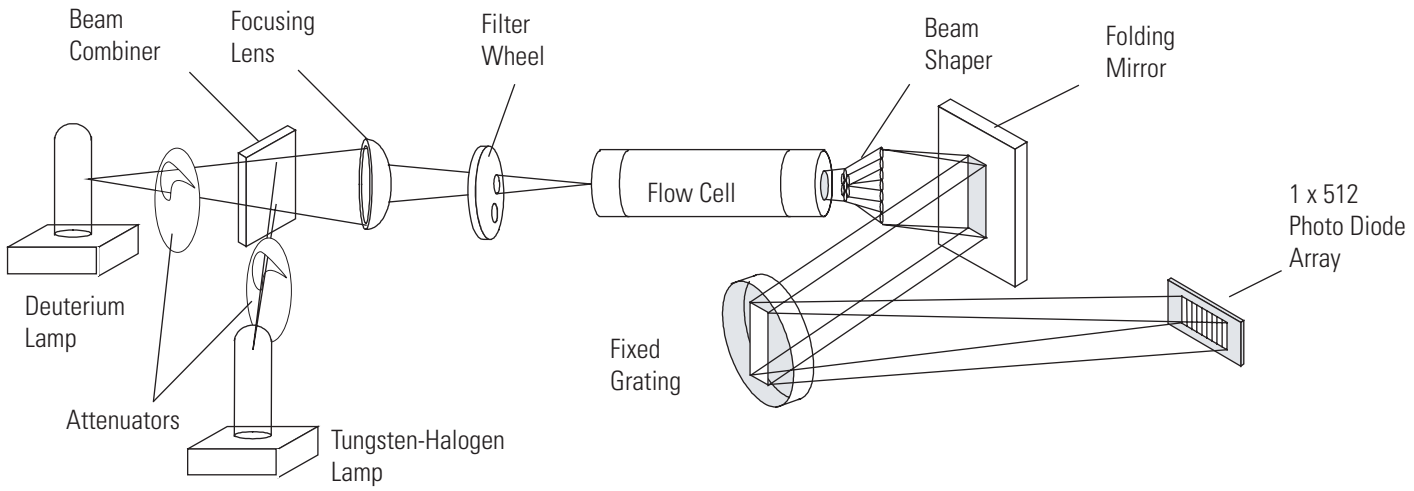


Figure 13. Optical Bench of the Surveyor PDA Plus detector

Surveyor UV/Vis Plus Detector

The Surveyor UV/Vis Plus Detector is a full-featured, time-programmable, variable-wavelength UV/Vis (ultraviolet / visible) absorbance detector. It operates in either the single wavelength mode or the dual wavelength 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 nm to 450 nm. In the dual wavelength Visible mode, the range is 366 nm 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 (190-365 nm) and a tungsten lamp for the visible range (366-800 nm). The lamps are protected by a cover with a special safety interlock to reduce the possibility of human exposure to harmful UV light.

Communication with Xcalibur

Communication with the Xcalibur data system is established with an Ethernet connection for the Surveyor LC Pump Plus, the Surveyor Autosampler Plus, the Surveyor PDA Plus Detector, and the Surveyor UV/Vis Plus Detector. To establish communications with each of these devices, you must connect one end of a Ethernet cable (shielded, category 5, RJ-45, 7-ft. length cable with ferrite) to the Ethernet port on the back panel of the device and the other end of the cable to an Ethernet switch. The Ethernet switch is connected by way of a second Ethernet cable to the PC on which the Xcalibur data system resides.

The Xcalibur data system is connected to the Surveyor MS Pump Plus with a serial communication link. The MS pump accessory kit contains a serial cable with nine pin adaptors on each end.

In addition to connecting the communication cables, you must also enter the appropriate stack addresses when you configure the devices of your instrument. The stack address that you enter when you add a device 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 LC devices, see Chapter 2, “Configuring Your Surveyor Plus LC Instrument.”

Synchronizing the Surveyor LC Devices

A system interconnect cable is used to coordinate the run control signals between the Surveyor Plus devices.

Thermo Electron provides two versions of this cable: the older version has 5-combicon connectors and the newer version has 7-combicon connectors. See the *Surveyor Plus Getting Connecting* manual for details on connecting one of these cables to the devices in your Surveyor Plus stack.

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

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

   The autosampler goes into the Ready state when all of the configuration and Instrument Method conditions are met. These conditions can include the following: the tray door is closed, and the sample tray and column oven temperature zone readings are within tolerance of their setpoints.
3. When the pump pressure stabilizes, the pump issues the Pump Ready signal to the autosampler.

   Note The Surveyor LC Pump Plus issues a Pump Ready signal. The Surveyor LC Pump does not issue this signal.

   The autosampler switches the injection valve to the load position, and then loads 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.

   Note The Surveyor LC Pump Plus issues an Inject Hold Release signal. The Surveyor LC Pump does not issue this signal.
6. The autosampler injects the sample and issues a momentary Inject Out signal to the detector.

   The injection valve of the autosampler switches to the inject position, allowing the mobile phase to backflush the contents of the sample loop onto the column. The detector starts to collect data.

Status LEDs

Each of the Surveyor Plus LC devices has a panel of four status LEDs (light-emitting diodes) located on its front-right door. All of the devices have these three LEDs: Power, Comm, and Run. The fourth LED on the detector is the Lamps LED. The fourth LED on the autosampler in the Temp LED. The fourth LED on the analytical pump is the Degas LED. The states of these status LEDs and their meanings are described in Table 2.

Table 2. States of the status LEDs on the front panels of the Surveyor devices

Location	LED	State	Meaning
All Devices	Power	Steady Green	The Power switch is switched on and the detector is receiving power.
		Amber	The detector is not receiving power.
	Comm	Steady Green	Communication to the data system has been established.
		Amber	There is no communication with the data system.
		Flashing Amber	Firmware is being downloaded to the CPU of the detector. The rotary switches on the back panel of the detector are set to 00.
Detectors	Run	Steady Green	The power is on and no run is in progress.
		Flashing Green	A run is in progress
		Flashing Amber	An error condition has occurred.
	Lamps	Steady Green	One or both lamps are On.
		Amber	The lamp specified in the method is not On.
Autosampler	Run	Steady Green	The autosampler is in the steady state.
		Flashing Green	An injection or a timed event is in progress.
		Flashing Amber	An error condition has occurred.
	Temp	Steady Green	The column oven and tray temperature zones are in equilibrium at the set temperature. You must select the Wait for temperature ready check box when you configure the autosampler for this feature to be active. If the Wait for temperature ready feature is not enabled, the Temp LED remains green.
		Steady Amber	A temperature change is in progress. The column oven, or the tray temperature zones, or both are reaching the set temperature.
Analytical Pump	Run	Steady Green	The pump is On and no run is in progress.
		Flashing Green	An run is in progress.
		Flashing Amber	An error condition has occurred.
		Steady Amber*	There is no communication with the data system PC, the motor is stopped, the pistons are homing, the system pressure is stabilizing, or the pump is being purged.
	Degas	Steady Green	The Degas LED changes to green when sufficient vacuum has developed for chromatography to be performed.
		Steady Amber	The degas unit is building vacuum.
		Flashing Amber	The degasser has lost vacuum.

*This LED state pertains to the Surveyor LC Pump Plus only.

Chapter 2 Configuring Your Surveyor Plus LC Instrument

After you set up and connect your Surveyor Plus LC instrument, you are ready to configure the devices of your instrument. This chapter contains the information for configuring the Surveyor Plus LC devices of your integrated Surveyor Plus LC/MS instrument. For information on configuring your MS detector, see its online Help.

Before you configure your instrument, check the unit ID setting on the rear panel of each device. The unit ID consists of two rotary switches, which are set to 01 at the factory. See Figure 14.

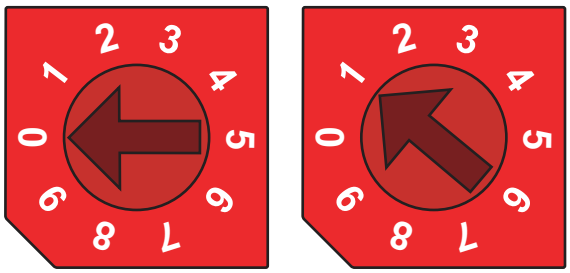


Figure 14. Unit ID with a stack address of 1

This chapter contains the following sections:

- Checking the Communication Hardware
- Configuring the Surveyor Plus LC Devices

Checking the Communication Hardware

The Surveyor LC Pump Plus, Surveyor Autosampler Plus, and Surveyor PDA Plus Detector communicate with the Xcalibur data system by way of an Ethernet connection. Each device has an Ethernet port on its back panel. A shielded, CAT 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 LC pump, autosampler, and detector must match the Stack Number in the configuration.

Unlike the other Surveyor Plus devices, the Surveyor MS Pump Plus communicates with the Xcalibur data system through an RS232 serial connection.

Before you configure your instrument from the data system, do the following:

1. Check the setting of the rotary switches on the back panels of your devices.

2. If you are using the MS pump, verify that the serial communication cable connecting the MS pump to your PC is attached to COM port 1.

Configuring the Surveyor Plus LC Devices

To configure your Surveyor Plus LC Instrument, perform the following procedures in this section as listed:

1. Opening the Instrument Configuration Dialog Box
2. Adding Devices to the Instrument Configuration
3. Configuring the Devices
4. Exiting the Instrument Configuration Dialog Box

Opening the Instrument Configuration Dialog Box

To open the Instrument Configuration dialog box

- From the Windows taskbar, choose **Start > All Programs > Xcalibur > Instrument Configuration**.
- Alternatively, from the Windows desktop, double-click the Instrument Configuration icon.

The Instrument Configuration dialog box is displayed. The Instrument Configuration dialog box shown in Figure 15 contains a list of all the available devices that were installed with the Xcalibur data system when it was loaded onto your computer.

Go to the next topic: Adding Devices to the Instrument Configuration.

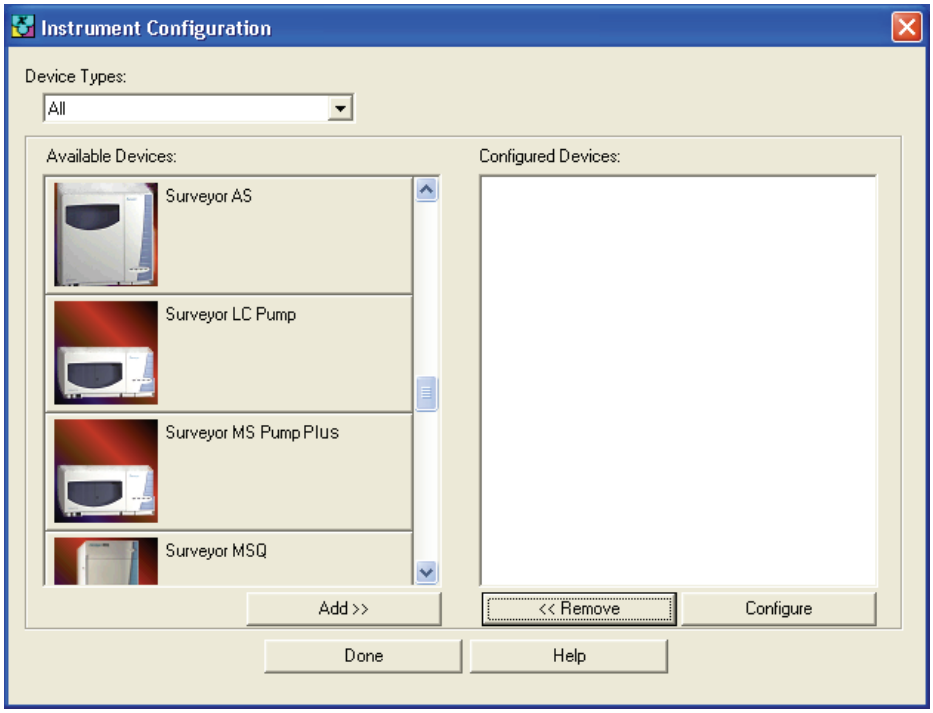


Figure 15. Instrument Configuration dialog box

Adding Devices to the Instrument Configuration

To add the devices of your Surveyor Plus instrument to its configuration, double-click each of your Surveyor Plus devices in the Available Devices list box.

As you double-click a device in the Available Devices list, it is added to the list of configured devices. See Figure 16.

After you add the devices that make up your Surveyor Plus LC system to the Configured Devices list, go to the next topic: Configuring the Devices.

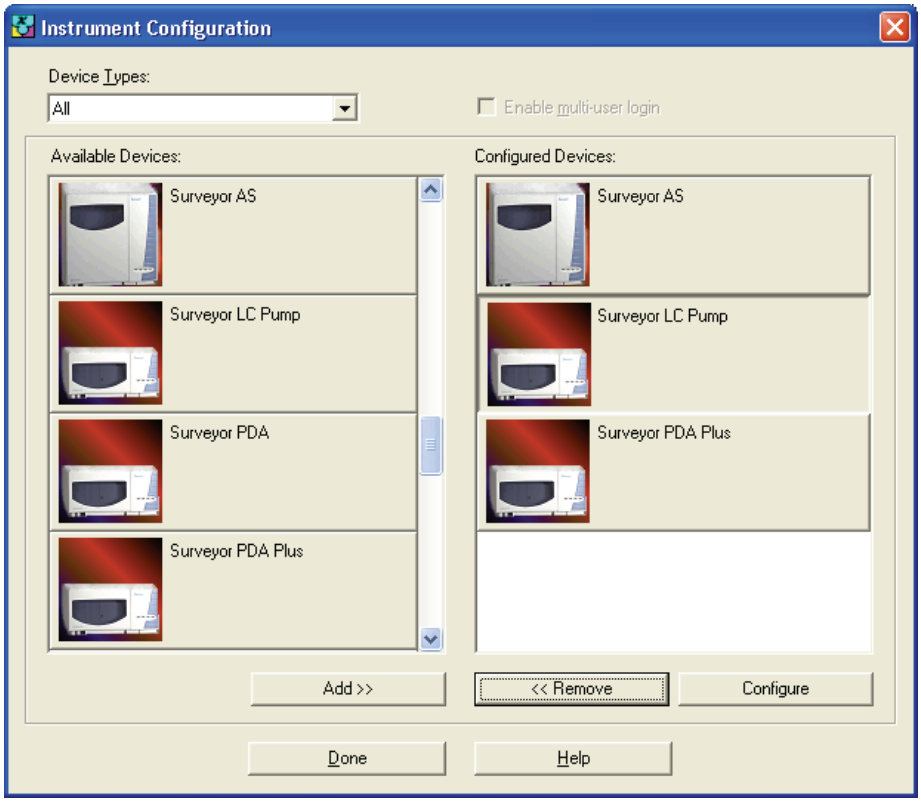


Figure 16. Instrument Configuration dialog box, showing added devices

Configuring the Devices

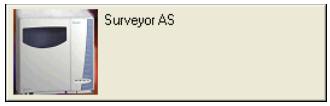
Now that you have added your LC devices to the Configured Devices list, you are ready to specify configuration options for each device.

Perform the procedures provided in this topic that apply to the devices of your instrument:

- Configuring the Surveyor Autosampler Plus
- Configuring the Surveyor MS Pump Plus
- Configuring the Surveyor LC Pump Plus
- Configuring the Surveyor PDA Plus Detector
- Configuring the Surveyor UV/Vis Plus Detector

You can configure the specific devices in any order. After you finish configuring the LC devices, go to "Exiting the Instrument Configuration Dialog Box" on page 38.

Configuring the Surveyor Autosampler Plus



To configure the Surveyor Autosampler Plus

1. Double-click the **Surveyor AS** button in the Configured Devices list box.

   Xcalibur opens the Surveyor Autosampler Configuration dialog box with the Tray page displayed. See Figure 17.

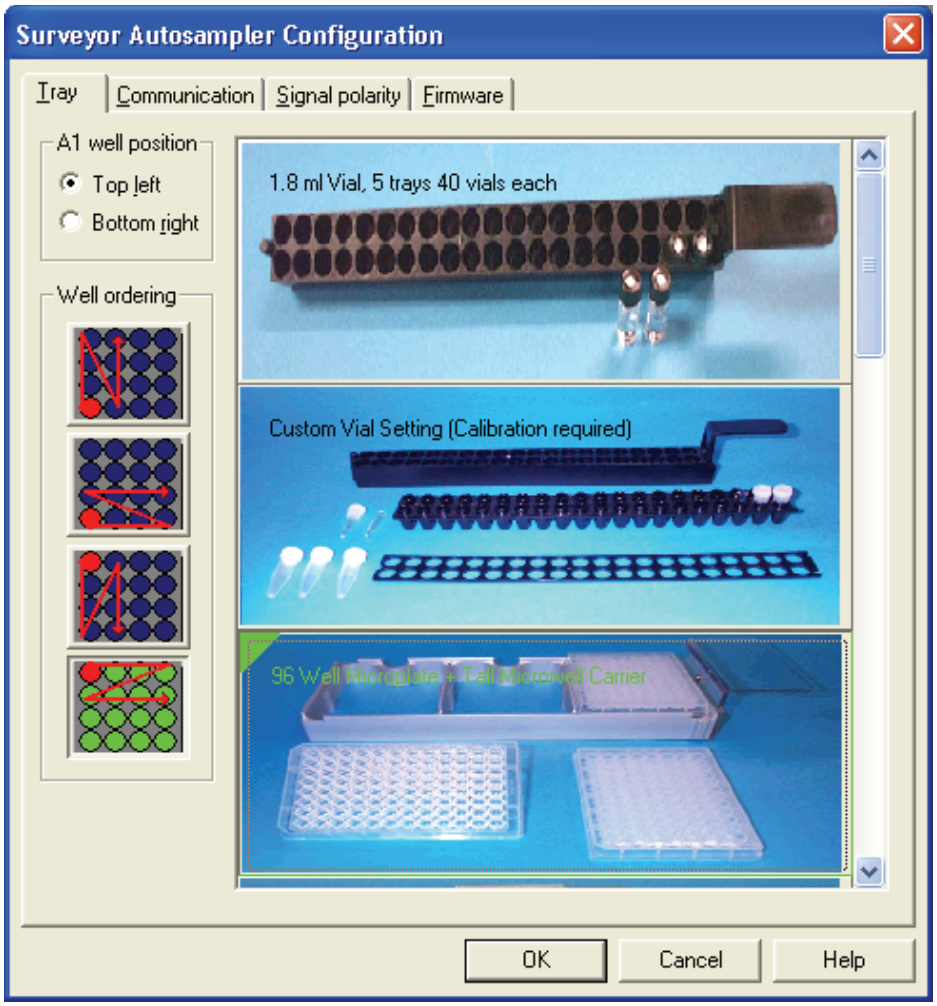


Figure 17. Surveyor Autosampler Configuration dialog box - Tray page

2. Select the tray options:

   a. From the Tray Type list, select the tray type.

   Table 3 lists the 11 tray type selections.

Table 3. Tray type selections

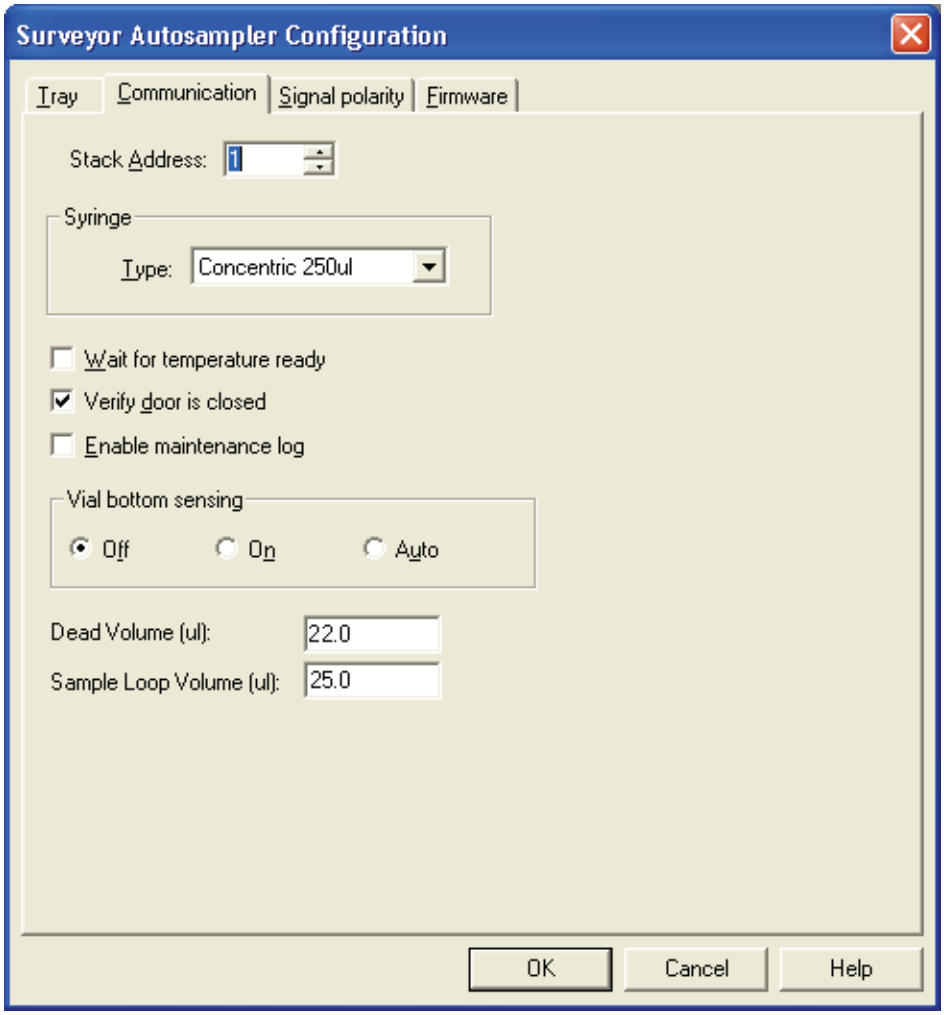
Tray Type	Requires Well Bottom Distance Calibration
1.8 mL Vial, 5 trays, 40 vials each	No
Custom Vial Setting	Yes
96 Well Microplate + Tall Microwell Carrier	No
96 Well Microplate + Short Microwell Carrier +Riser Plate	No
1 mL or 2 mL Deep Well Plate + Short Microwell Carrier	No
96 Well Microplate + Short Microwell Carrie	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

b. If you chose the 1.8 mL Vial option or the Custom Vial Setting, proceed to step 3. Otherwise continue at step 2c.

c. In the **A1 Well Position** area, select either the Top Left or the Bottom Right option button.

d. In the **Well Ordering** area, click the button that represents the order in which you want the sequence injections to occur.

3. Click the **Communication** tab to display the Communication page shown in Figure 18.



Enter the value specified on the label attached to the transfer tube assembly.

Figure 18. Surveyor Autosampler Configuration dialog box – Communication page

4. In the **Communication** page, make the following entries and selections:

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

      The stack address must match the Unit ID setting located on the back panel of the Surveyor Autosampler Plus. The Surveyor Autosampler Plus is shipped with the Unit ID set to 01. The value of 00 is reserved for service functions.

   b. From the **Syringe Type** list, select the size of the syringe that is attached to the autosampler.

c. (optional) To activate the optional Wait For Temperature Ready control, select the **Wait For Temperature Ready** check box.

Activating the Wait For Temperature Ready control prevents the Surveyor Autosampler Plus from triggering a run until the column oven temperature and/or the sample tray temperature have reached their setpoint values.

d. (optional) To activate the Verify Door Is Closed control, select the **Verify Door Is Closed** check box.

Activating the Verify Door Is Closed control prevents the Surveyor Autosampler Plus from starting a run until the tray door is closed. If the tray door is opened during a run, then the XYZ arm moves to its home position.

e. (optional) To activate the maintenance log, select the **Enable Maintenance Log** check box.

Activating the maintenance log allows the Surveyor Autosampler Plus to keep an internal count of the total injections, total valve cycles, total needle usage, and total syringe cycles. If any of the counters exceed the user set Scheduled Maintenance Time (SMT), the autosampler will not start a run until you perform the scheduled maintenance or you clear the check box.

f. In the **Vial Bottom Sensing** area, select the type of vial bottom sensing that is appropriate for your application:

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

Each tray type has a stored value for the distance that the needle must travel to reach the bottom of the vial or well. If 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. Deactivate vial bottom sensing if you do not want the needle to touch the bottom of a vial or well.

g. In the **Dead Volume** box, enter the value specified on the label attached to the transfer tube assembly. See Figure 10 on page 12.

h. In the **Sample Loop Volume** box, type the nominal size of the sample loop attached to the Valco injection valve.

The Surveyor Autosampler Plus ships with a 25 µL sample loop.

5. Click the **Signal Polarity** tab to display the Signal Polarity page.

6. Use the following criteria to select the appropriate check boxes for the Input signal polarities:

   - For the Surveyor LC Pump Plus with firmware versions 2.0 and higher or the Surveyor MS Pump Plus, make the selections shown in Figure 19.

   - For the Surveyor LC pump with firmware versions below 2.0, make the selections shown in Figure 20.

   Note If you select the Input check boxes in the Signal Polarity page and your system has a Surveyor LC Pump with a firmware version below 2.0, the Waiting for Contact Closure status message will be displayed indefinitely for the Surveyor LC Pump after you make an injection. See "Checking the Firmware Version of the Surveyor LC Pump" on page 58.

7. In the **Output** area, leave the check boxes deselected if your instrument consists entirely of Surveyor or Surveyor Plus devices.

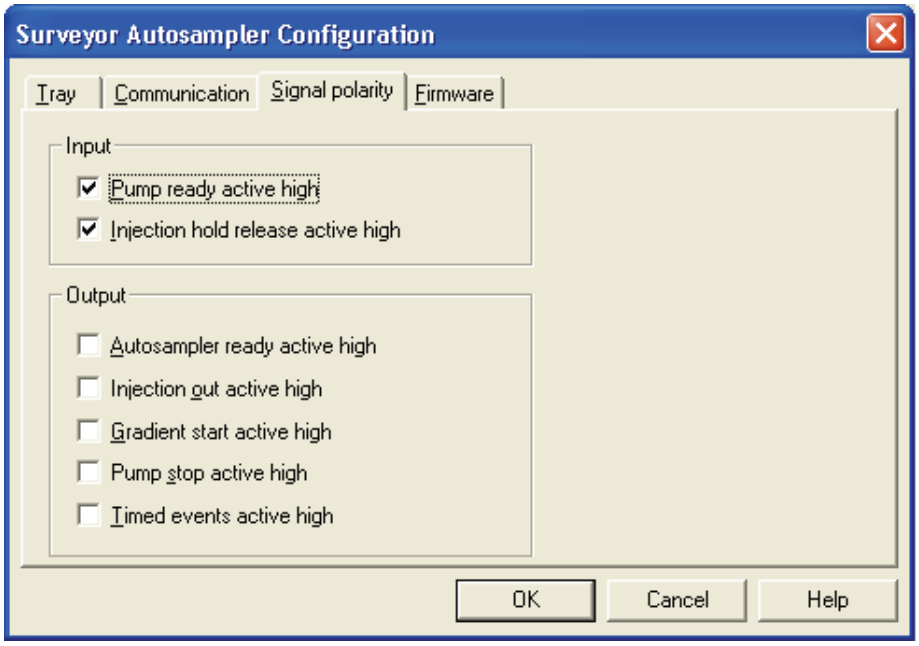


Figure 19. Surveyor Autosampler Configuration dialog box - Signal Polarity page, showing selections for the Surveyor LC Pump Plus or the Surveyor MS Pump Plus

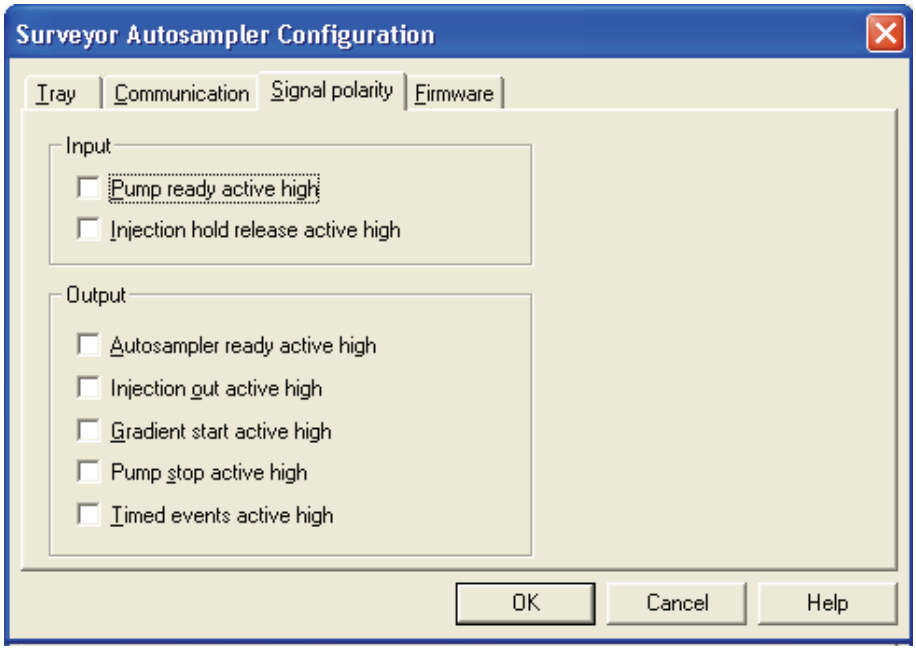


Figure 20. Surveyor Autosampler Configuration dialog box - Signal Polarity page, showing selections for the Surveyor LC Pump

8. Click the **Firmware** tab to display the Firmware page. See Figure 21.

9. If you have upgraded your Surveyor Autosampler Plus firmware, upload the firmware version:

   a. Click **Upload Firmware Version**.

      After you click the Upload Firmware Version button, the firmware version of your Surveyor Autosampler Plus appears next to Current Version. If you have a full-featured Surveyor Autosampler Plus, the temperature controlled features will be listed as installed.

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

If you have finished configuring all of your LC devices, go to "Exiting the Instrument Configuration Dialog Box" on page 38.

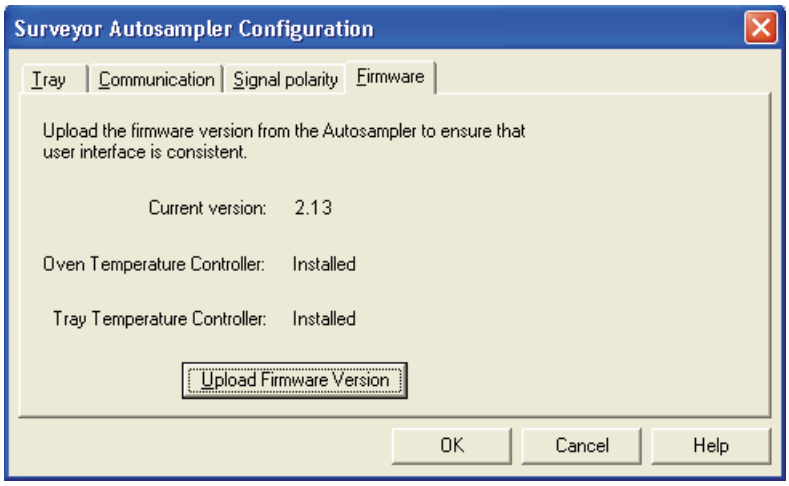
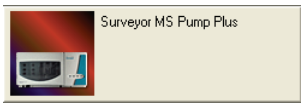


Figure 21. Surveyor Autosampler Configuration dialog box - Firmware page

Configuring the Surveyor MS Pump Plus

The Surveyor MS Pump Plus communicates with the Xcalibur data system by way of an RS232 serial connection. Determine which COM port the serial communication cable for the MS pump is attached to.

To configure the Surveyor MS Pump Plus



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

   The Surveyor MS Pump Plus dialog box appears. See Figure 22.

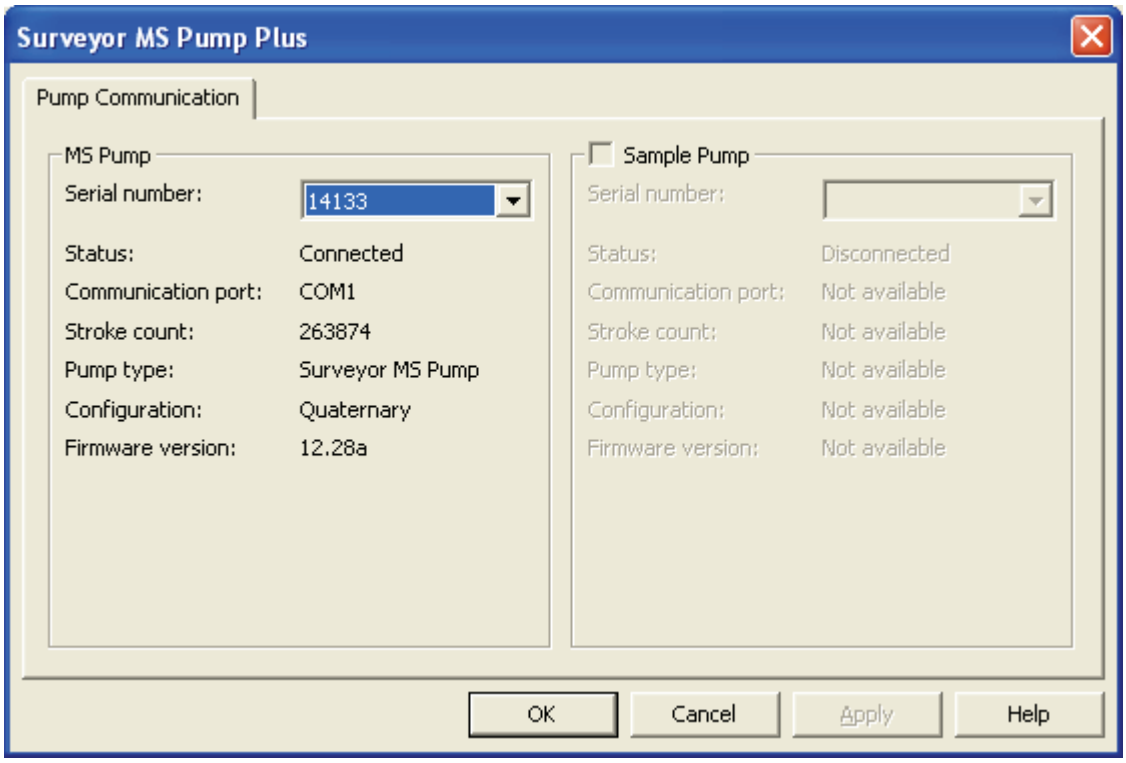


Figure 22. Surveyor MS Pump Plus dialog box

The value in the Serial number box is read from the firmware for the pump. It is not a user-editable parameter.

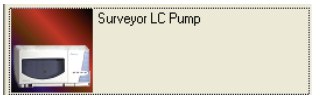
2. Click **OK** to exit the Surveyor MS Pump Configuration dialog box.

If you have finished configuring all of your LC devices, go to "Exiting the Instrument Configuration Dialog Box" on page 38.

Configuring the Surveyor LC Pump Plus

The Surveyor LC Pump communicates with the Xcalibur data system by way of an Ethernet connection. To make this connection, the Ethernet cable on the back of the detector must be connected to the Ethernet switch and the Unit ID setting on the back panel of the pump must match the Stack address in the configuration.

To configure the Surveyor LC Pump Plus



1. Double-click the **Surveyor LC Pump** button to open the Surveyor LC Pump dialog box. See Figure 23.

2. Make the following entries and selections:

   - From the **Pressure Units** list, select the pressure units that you want 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 your Sequences to pause following a degasser error, check the **Pause Sequence Following Degasser Error** check box.

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

Note If you are adding a Surveyor LC Pump (prior to the Plus version) with a firmware version below 2.0 to your instrument, confirm that the Input signal polarities for the autosampler are deselected as shown in Figure 20 on page 33.

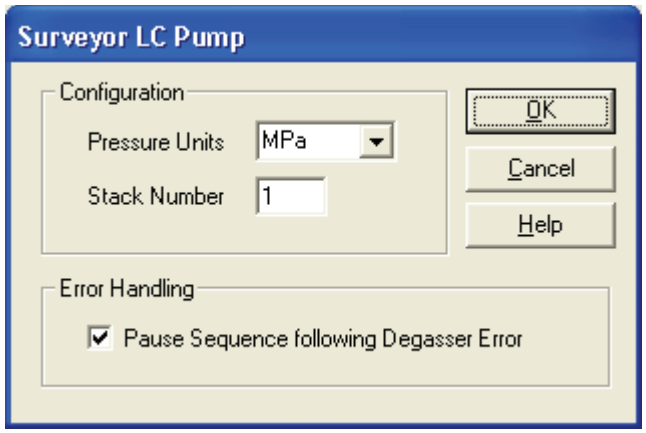
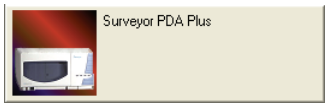


Figure 23. Surveyor LC Pump dialog box

Configuring the Surveyor PDA Plus Detector

The Surveyor PDA Plus Detector communicates with the Xcalibur data system through an Ethernet connection. To make this connection, the Ethernet cable on the back of the detector must be connected to the Ethernet switch and the unit ID setting on the back panel of the detector must match the stack number in the configuration.

To configure the Surveyor PDA Plus Detector



1. Double-click the **Surveyor PDA Plus** button.

   The Surveyor PDA Plus Configuration dialog box shown in Figure 24 appears.

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

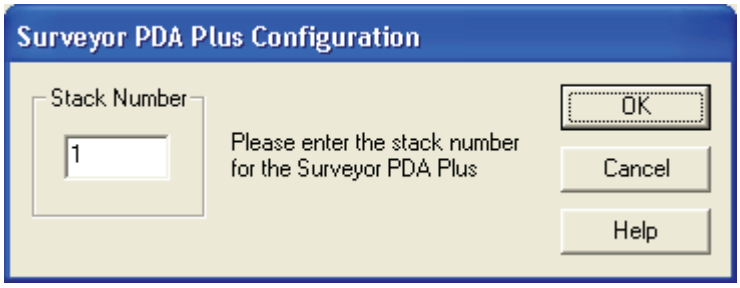


Figure 24. Surveyor PDA Plus Configuration dialog box

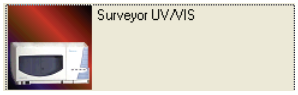
3. Click **OK** to close the Surveyor PDA Plus Configuration dialog box.

   When you have finished configuring all of your LC devices, go to "Exiting the Instrument Configuration Dialog Box" on page 38.

Configuring the Surveyor UV/Vis Plus Detector

LC Devices 2.0.2 supports the Surveyor UV/Vis Plus Detector.

To configure the Surveyor UV/Vis Plus Detector



1. Double-click the **Surveyor UV/Vis** button.

   The Surveyor UV/Vis Configuration dialog box shown in Figure 25 appears.

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

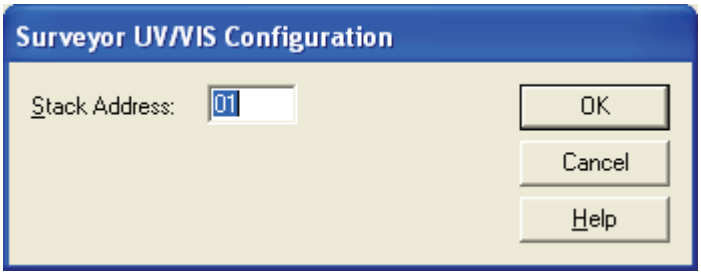


Figure 25. Surveyor UV/Vis Configuration dialog box (for the Surveyor UV/Vis Plus Detector)

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: Exiting the Instrument Configuration Dialog Box.

Exiting the Instrument Configuration Dialog Box

Before you can open the Xcalibur application program, you must close the Instrument Configuration dialog box.

To save your instrument configuration and exit the Instrument Configuration dialog box, click **Done** at the bottom of the Instrument Configuration dialog box.

You return to the Windows desktop.

Note You must exit the Instrument Configuration dialog box before you launch Xcalibur. The two applications cannot be open simultaneously.

Chapter 3 Preparing for Operation

Now that you have installed and configured your Surveyor Plus LC system, you are ready to prepare the system for operation. Not all of the procedures contained in this chapter will apply to your system. For example, if your system contains a Surveyor LC Pump Plus rather than a Surveyor MS Pump Plus, you will not need to prime the pulse dampener. And if your system does not contain an optional Surveyor PDA Plus Detector, you do not need to calibrate it.

Perform the procedures that are provided in this chapter and that apply to your system in the order listed:

1. Turning On the Power to Each LC Device
2. Launching Xcalibur
3. Checking the Status of the LC Devices
4. Turning On the LC Devices from Info View
5. Opening the Instrument Setup Window
6. Removing Air from the Solvent Lines
7. Checking the Firmware Version of the Surveyor LC Pump
8. Priming the Pulse Dampener of the MS Pump
9. Calibrating the PDA Detector

Turning On the Power to Each LC Device

Turn on the power to your LC devices, and then observe their status LEDs. The ON/OFF power switch for each device is located below the left door of the module. Figure 26 shows the location of the status LEDs and the power switches.

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.

If any of the Power LEDs remain amber, make sure that the power line to the affected device(s) is connected. If the Degas LED on the pump is flashing amber, the degas unit has failed to produce a vacuum, and you need to call your local Thermo Electron representative for repairs. If the Lamp LED remains amber, both of the lamps are turned off.

For information on turning on the lamps, see "Warming Up the Deuterium Lamp" on page 121.

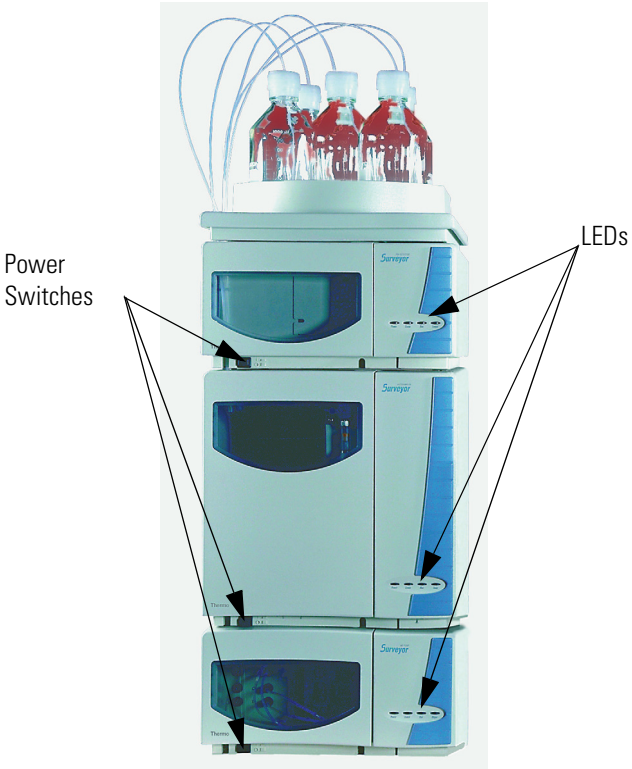


Figure 26. Surveyor Plus LC Stack

Launching Xcalibur

You control the Surveyor devices from the Xcalibur data system. The only manual control for each Surveyor LC device is its ON/OFF power switch.

To launch Xcalibur

1. Double-click the Xcalibur icon on your desktop to display the Home Page shown in Figure 27.

   Shortly after you open Xcalibur, the Comm LEDs on the front panels of your LC devices turn green.

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

   - Check that the communication cable to each module is connected to the data system computer.
   - Check that the stack addresses in the configuration for the autosampler, LC pump, and detector match the setting of the unit IDs on their back panels.
   - Check that the serial communication cable for the MS pump is attached to the Com port selected in the configuration of the pump. For information on configuring your instrument devices, see Chapter 2, "Configuring Your Surveyor Plus LC Instrument."

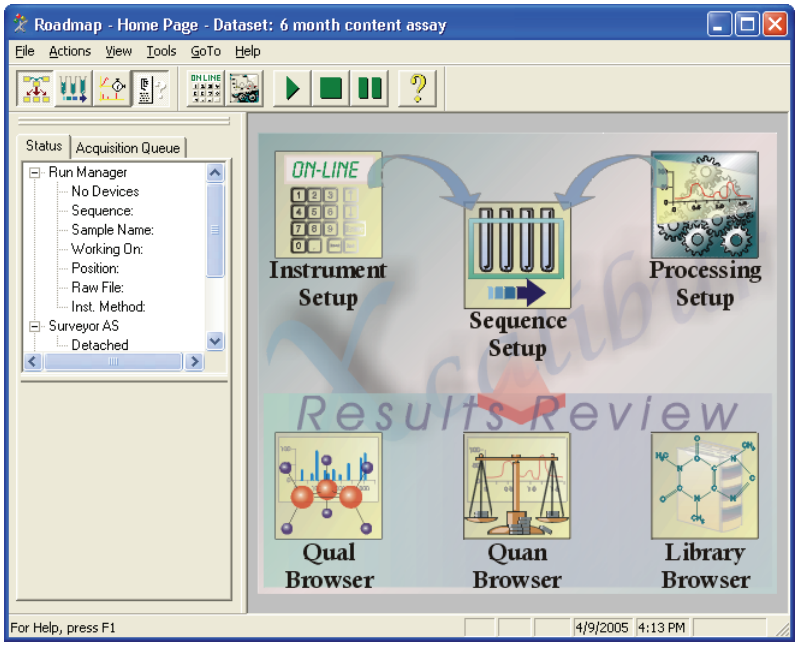


Figure 27. Xcalibur Home Page, showing the Roadmap view and the Info view

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, perform the procedures that are provided in this section in the order listed:

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

Displaying the Information View - Status Page

The status of the Surveyor devices can be monitored 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 turned off. From the Home Page window, choose **View > Info View** to toggle the Information view On and Off.

To display the Information View - Status page

1. In the Home Page window, ensure that the Information View is displayed by choosing **View > Info View**.
2. Click the Status tab to display the Information View – Status page. See Figure 28.

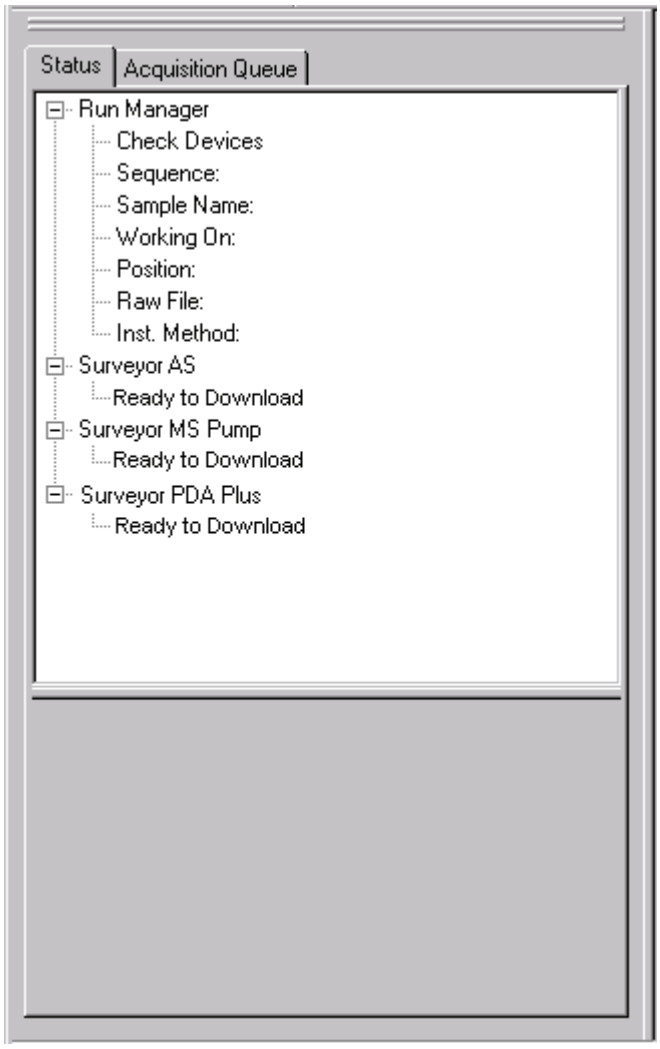


Figure 28. Information View - Status Page

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

- *Initializing* appears while Xcalibur is attempting to connect to an instrument module.
- *Lamp Warm-up* appears for the PDA detector while the deuterium lamp is igniting.
- *Ready to download* appears after communication has been established with an instrument module and after each run has ended.

Viewing the Status of Each Device

The Surveyor Plus Integrated LC system contains an autosampler and an analytical pump. In addition, your system might contain an optional PDA or UV/Vis 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.

The Status view for the full-featured Surveyor Autosampler Plus contains four pages: AS Status, Oven, Tray, and Error Log. See Figure 29. The Status view for the Surveyor Autosampler Plus Lite contains two pages.

The status page for the Surveyor Autosampler Plus displays one of the following eight states:

1. **Initializing** appears while Xcalibur is attempting to connect to the autosampler.

2. **Ready to download** appears after communication has been established with the autosampler and after each run has ended.

3. **Ready to run** appears for a very brief period after a method is downloaded to the autosampler and before the injection process has begun.

4. **Running** appears during the injection process. If the method contains a time function for the autosampler, the running status message is displayed until the time function has expired.

5. **Direct control** appears when the direct control or calibration windows are open.

6. **Busy** appears while the autosampler is performing a direct control operation.

7. **Error** appears when an error condition other than the loss of communication occurs.

8. **Off** appears after Xcalibur has attempted to connect to the autosampler and failed five times. The most common reasons for Xcalibur failing to connect with the Surveyor Autosampler Plus are that the 10-pin connector of the syringe drive assembly is unplugged from the autosampler or that the XYZ arm is stalled.

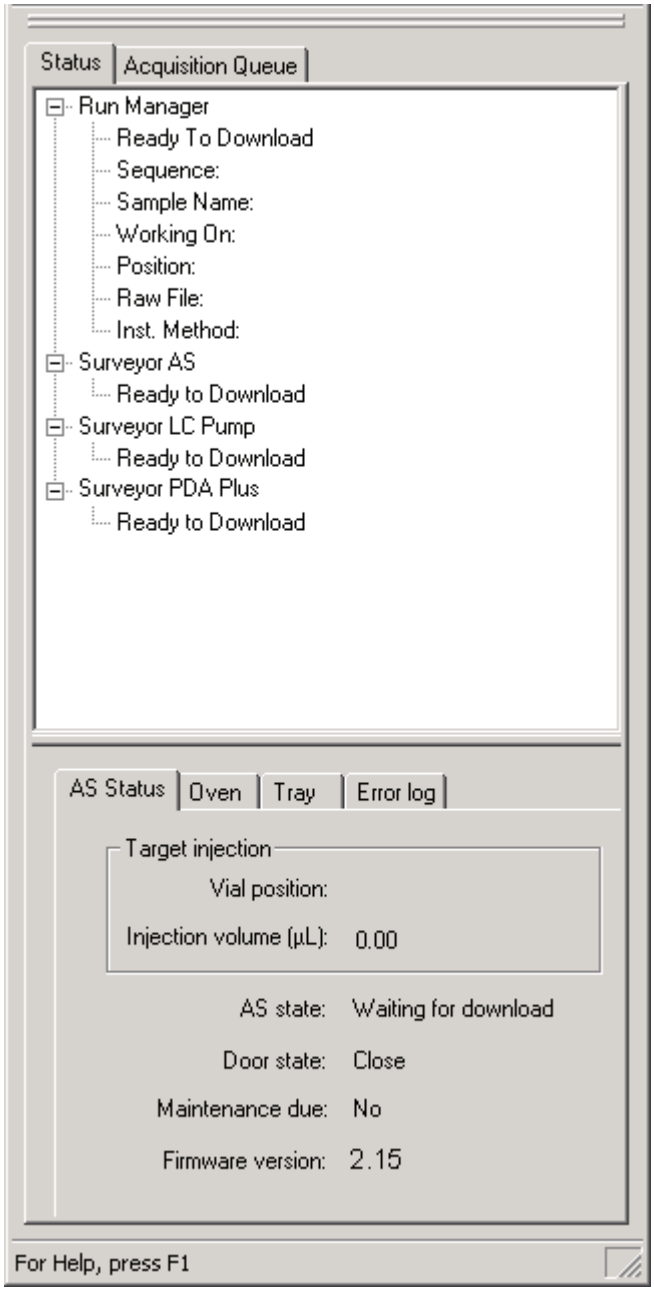


Figure 29. Status view - AS Status page for the Surveyor Autosampler Plus, showing the autosampler in the Ready to Download state

See Figure 30, Figure 31, Figure 32 and Figure 33, which respectively show the Status views for the Surveyor MS Pump Plus, the Surveyor LC Pump Plus, the Surveyor PDA Plus Detector, and the Surveyor UV/Vis Detector.

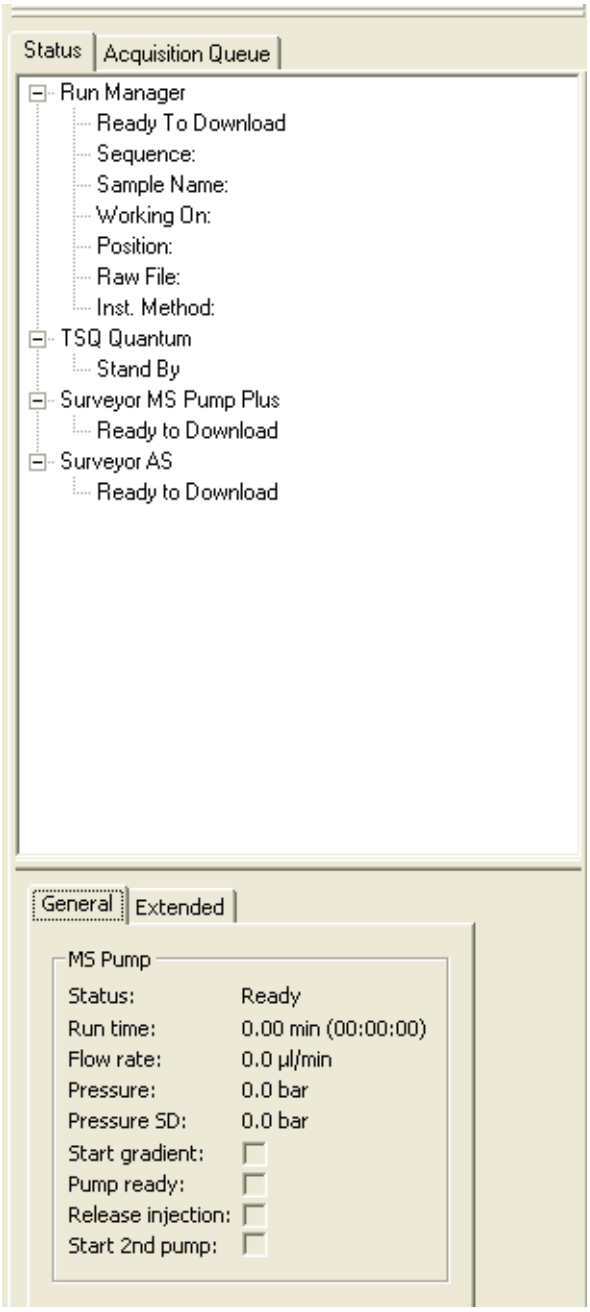


Figure 30. Status view for Surveyor MS Pump Plus

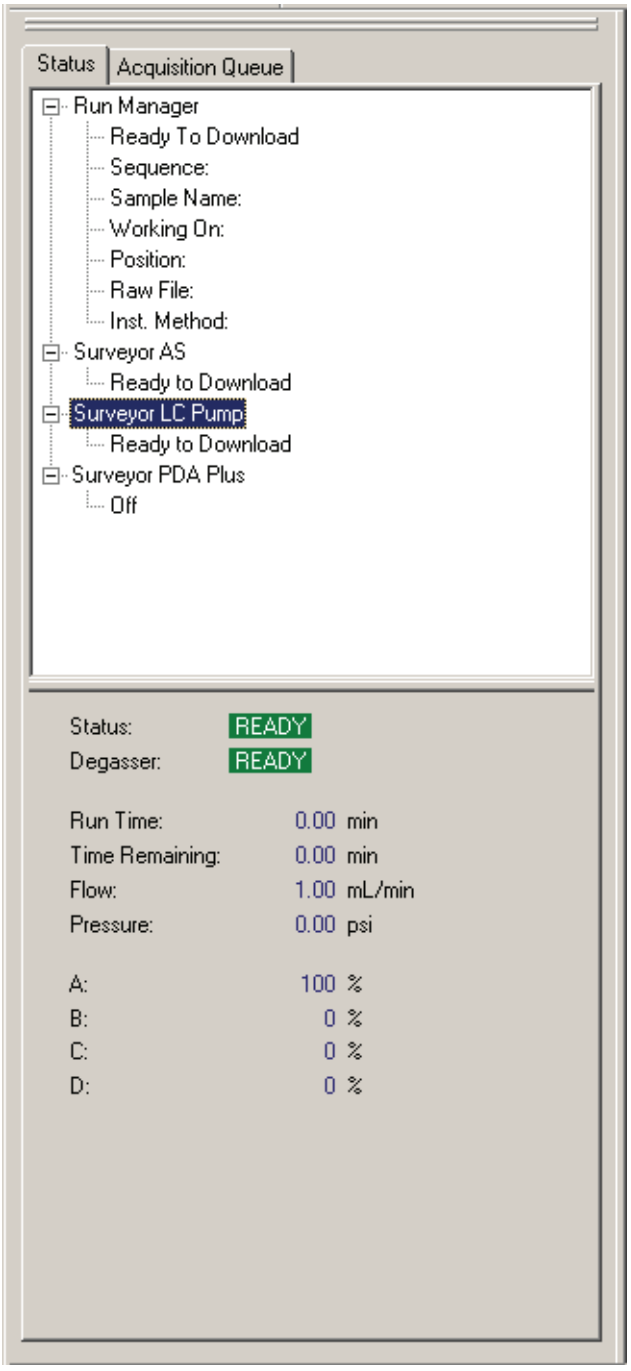


Figure 31. Status view for the Surveyor LC Pump Plus

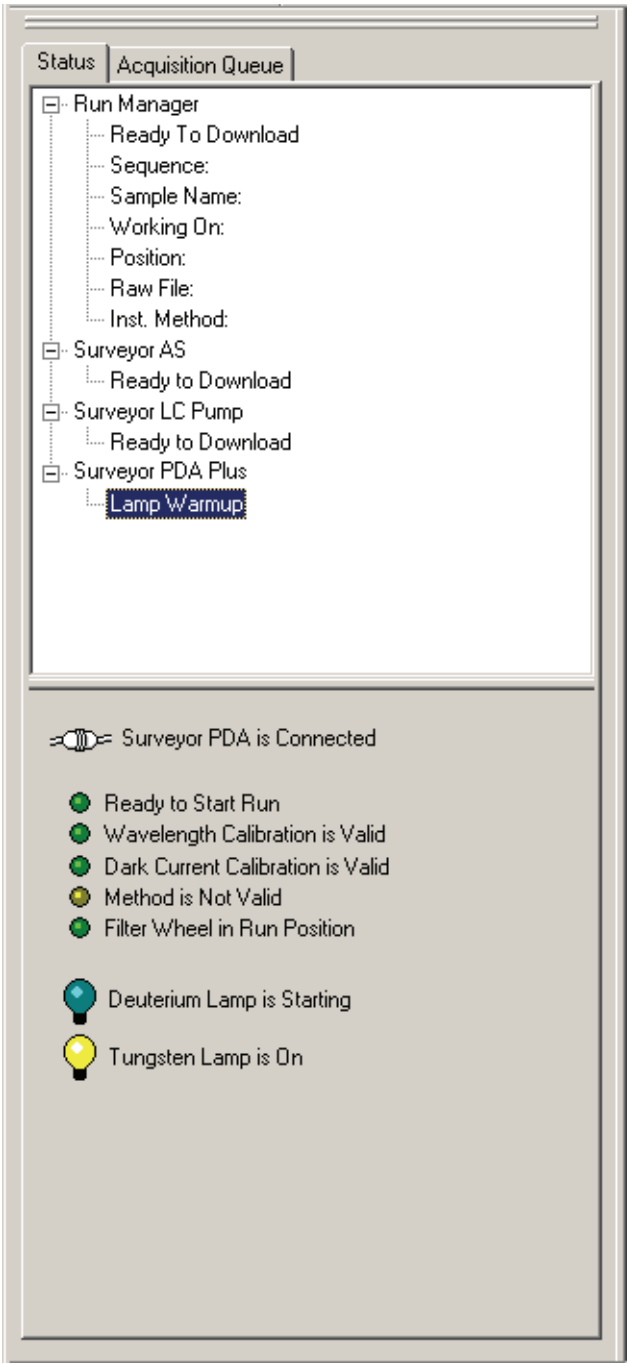


Figure 32. Status view for the Surveyor PDA Plus Detector

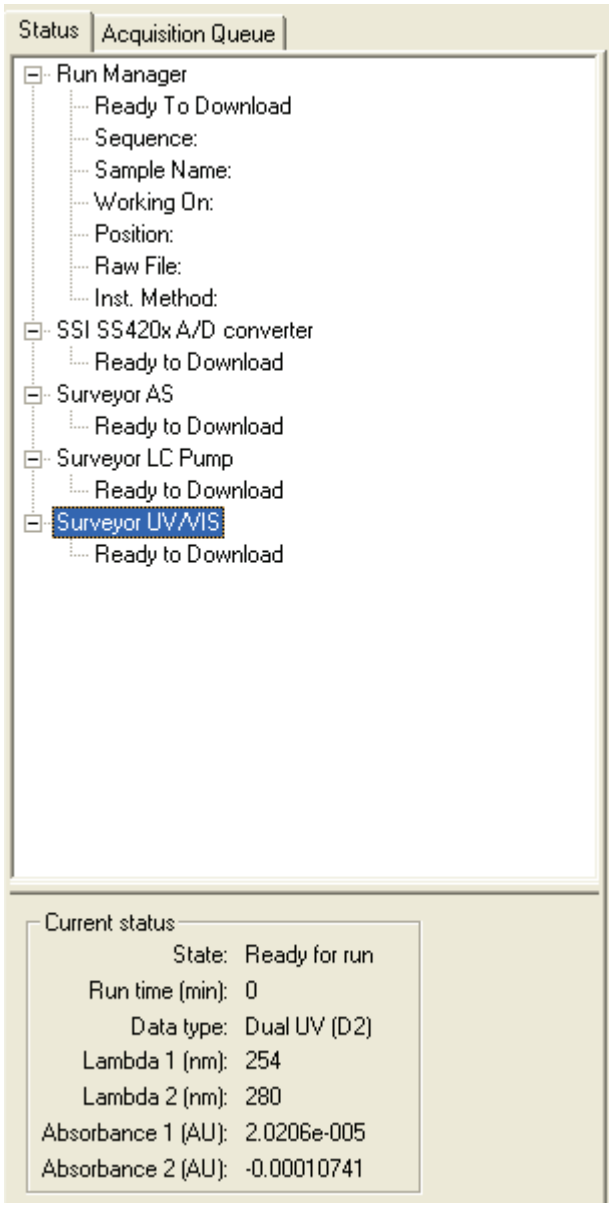


Figure 33. Status view for the Surveyor UV/Vis Detector

Turning On the LC Devices from Info View

You can turn your LC devices On or Off from their respective device listings in Info View.

To turn on a device, right-click its device listing in the Status window. Then, choose **Turn Device On**.

The analytical pump begins pumping solvents from the last set of downloaded parameters. The detector turns on the lamps. The autosampler adjusts its controlled temperature zones to the last set of downloaded parameters. See Figure 34.

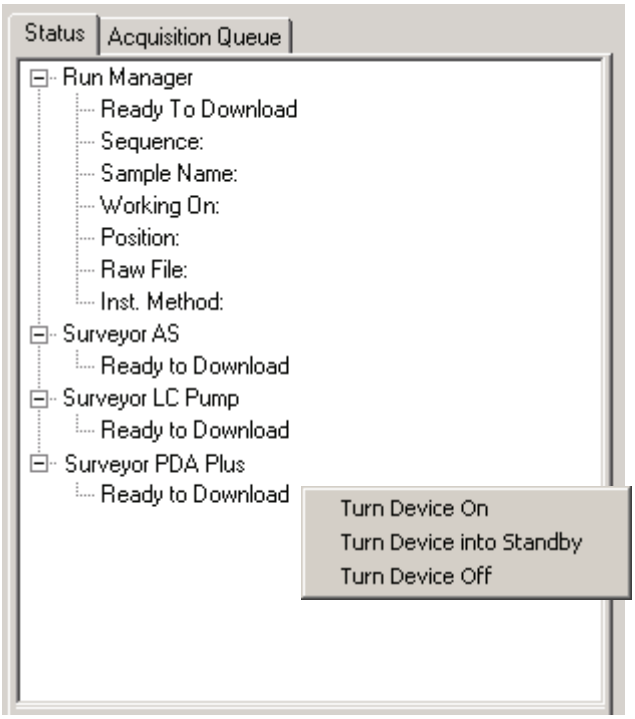


Figure 34. Info view – Status page, showing shortcut menu

Opening the Instrument Setup Window

The Direct Control dialog boxes are accessed through the Instrument Setup window.

To open the Instrument Setup window

1. If it is not already open, open the Roadmap view by clicking the **Roadmap View** 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 that is displayed in the View Bar. The View bar is a vertical bar on the left of the Instrument Setup window. It contains buttons for each of your configured devices. See Figure 35.

Tip To return to Home Page, click the Home Page button in the toolbar.

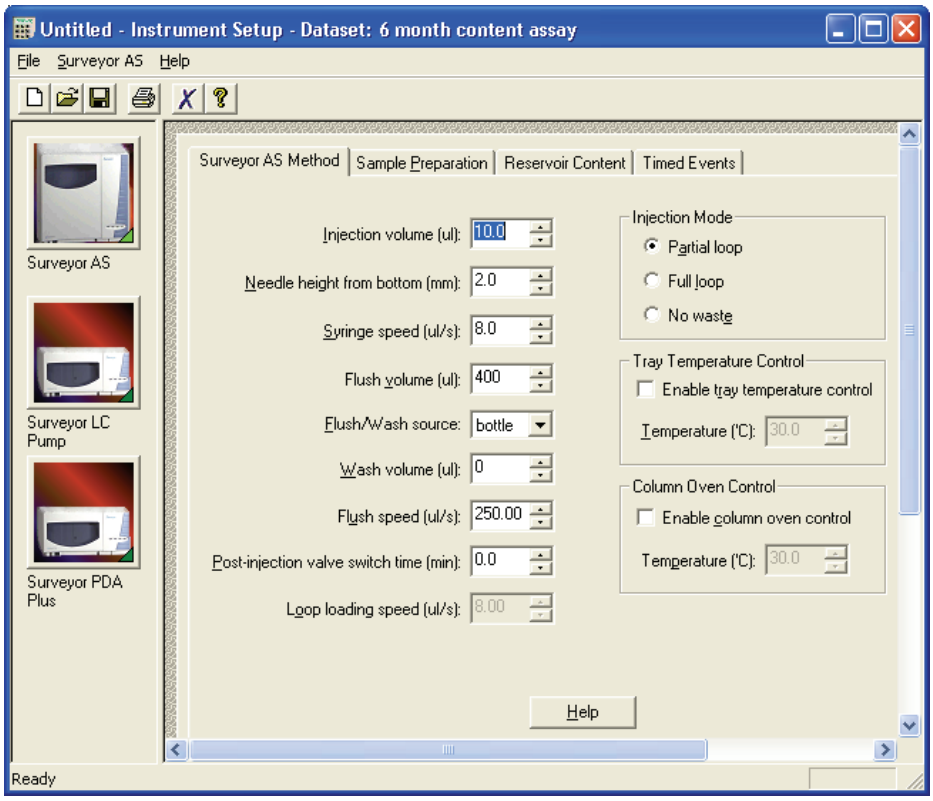


Figure 35. Instrument Setup window – Surveyor AS (full-featured) view

Removing Air from the Solvent Lines

If you have just connected the solvent lines to the built-in degasser of the analytical pump and attached the wash bottle tubing to the left-side of the autosampler syringe valve, you will notice that you have air in these lines. To remove air from these low-pressure solvent lines, perform the procedures provided in this section that apply to your system.

- Purging the LC Pump
- Purging the MS Pump
- Flushing the Autosampler Syringe

Purging the LC Pump

To purge air from the solvent lines or the pump head assemblies

Tip Cyclical baseline noise can be an indication of air in the pump heads.

Note 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.

1. Open the drain valve by turning it counter-clockwise 180° to the purge position. The word DRAIN on the knob will appear upside down as shown in Figure 36.

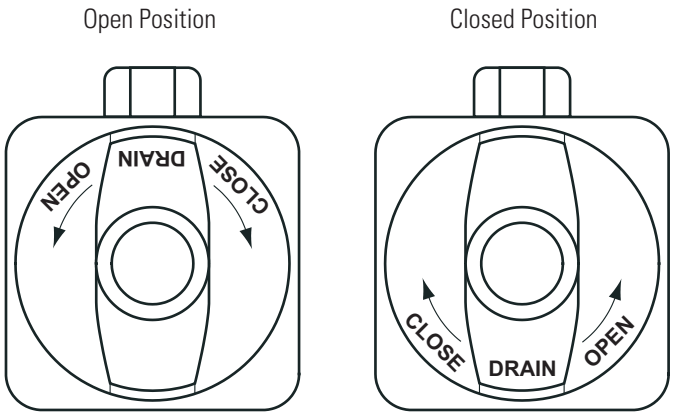


Figure 36. Drain valve knob in purge position

2. In the **Instrument Setup** viewbar, click the **Surveyor LC Pump** button to open its Instrument Setup view.

3. From the menu bar, choose **Surveyor LC Pump > Direct Control > Operation** to open the Operation dialog box for the LC pump. See Figure 37.

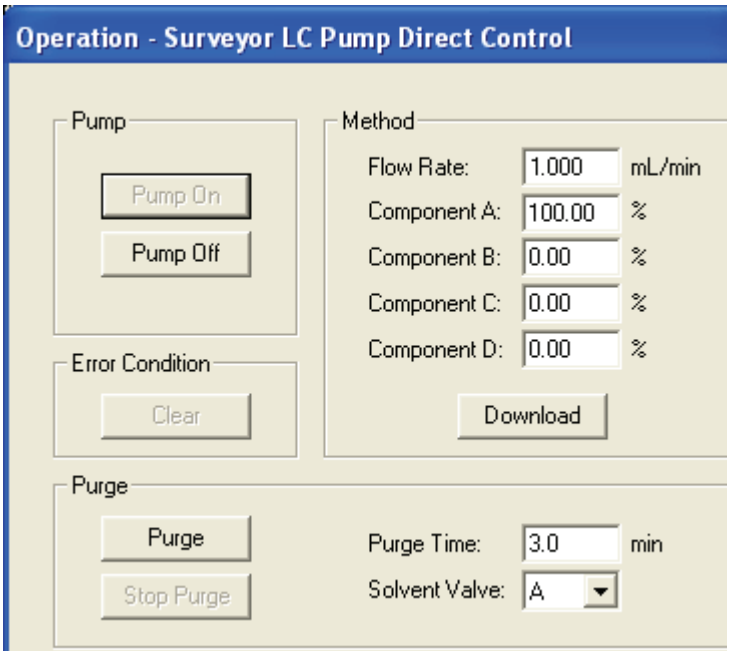


Figure 37. Operation – Surveyor LC Pump Direct Control page

4. In the **Purge** area, do the following:

   a. In the **Purge Time** box, type an appropriate purge time.

      The allowable range is 0.1 to 9.9 min. Each 1/16-in. ID tube connecting a solvent reservoir bottle to the inlet port of the degasser holds approximately 3 mL of solvent.

   b. From the **Solvent Valve** list, select the solvent valve for the solvent line that you want to purge.

      Note Clicking the Pump On button while the drain valve is in the purge position can generate a pump error. You must clear this error before you can run the pump. To clear a pump error, remedy the source of the error, and then click **Clear** in the Error Condition area.

   c. Click **Purge**. The pistons of the pump begin to move rapidly, increasing the flow rate to 9.99 mL/min.

5. After you finish purging the solvent lines, close the drain valve.

Purging the MS Pump

After you have filled the solvent bottles, connected the solvent lines to the degasser, and configured the MS pump to communicate with the Xcalibur data system, prepare the MS pump for operation by removing the air from the solvent lines.

To draw solvent through the solvent lines quickly

1. Insert the tip of the 10 mL syringe into the tubing that is connected to the wingnut located on the left-side of the LDA. See Figure 38.
2. Open the wingnut by turning it counter-clockwise.
3. 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.

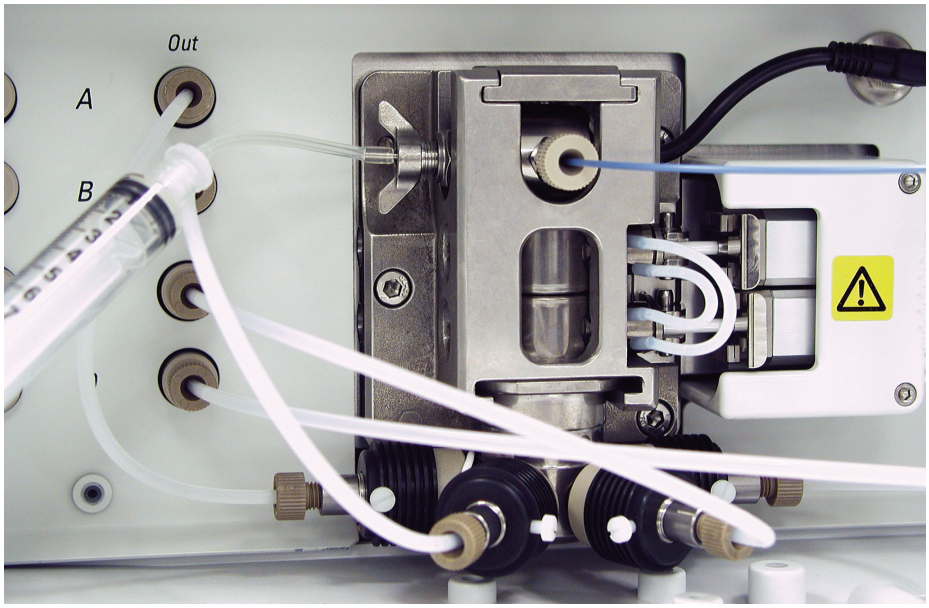


Figure 38. Liquid Displacement Assembly (LDA) of MS pump

4. From the **Instrument Setup View**, open the Direct Control dialog box for the MS pump:
   a. Click the **Surveyor MS Pump Plus** button in the viewbar.

b. Choose **Surveyor MS Pump > Direct Control** to open the Direct Control dialog box for the MS pump. See Figure 39.

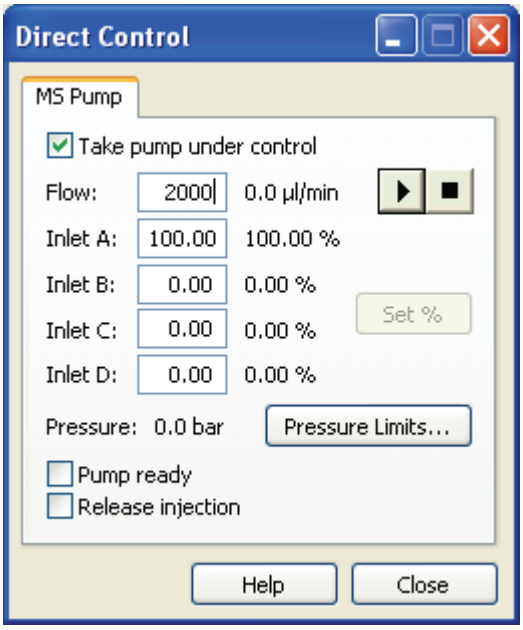


Figure 39. Direct Control dialog box for MS pump

5. In the Direct Control dialog box, do the following:

   a. Select the **Take pump under control** check box.

   b. Type percentages in the Inlet boxes for the solvent lines through which you want to draw solvent.

   c. In the Flow box, type **2000**.

   d. Click the **Start Run** button ▶ to start the pump flow.

      Because each 1.5 m (5-ft.) section of tubing holds approximately 3 mL of solvent, you need to stop the pump flow and empty the syringe periodically.

   e. After you finish purging the solvent lines, click the **Stop** button ■ to stop the pump flow.

6. Verify that the solvent lines are free of air.

7. Turn the wingnut clockwise to close the vent valve.

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.

To flush air out of the wash bottle tubing and the autosampler syringe

1. From the **Instrument Setup** window, click the **Surveyor AS** button to display the Instrument Setup view for the Surveyor Autosampler.

2. Choose **Surveyor AS > Direct Control** to display the Direct Control dialog box for the Surveyor Autosampler. See Figure 40.

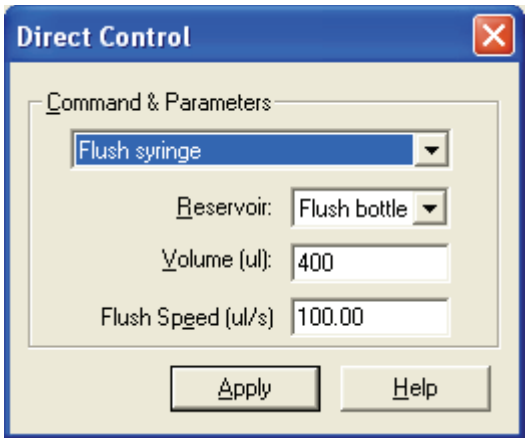


Figure 40. Flush Syringe direct control command

3. Initiate the Flush Syringe direct control command:

   a. Click the down-arrow to display the list of commands.

   b. Select **Flush Syringe** from the list of commands. The parameters for the command appear below the list.

   c. Select **Flush Bottle** from the list of **Reservoir** choices.

   d. Type an appropriate flush volume in the **Volume** box. The maximum allowable flush volume is 6000 µL.

   e. Click **Apply** to download the command to the autosampler.

4. Verify that the wash bottle tubing and syringe are free of air. See Figure 41.

5. Close the Direct Control dialog box.

Note To flush the sample loop as well as the syringe, select the **Flush With Syringe In Fill Position** command from the Command list.

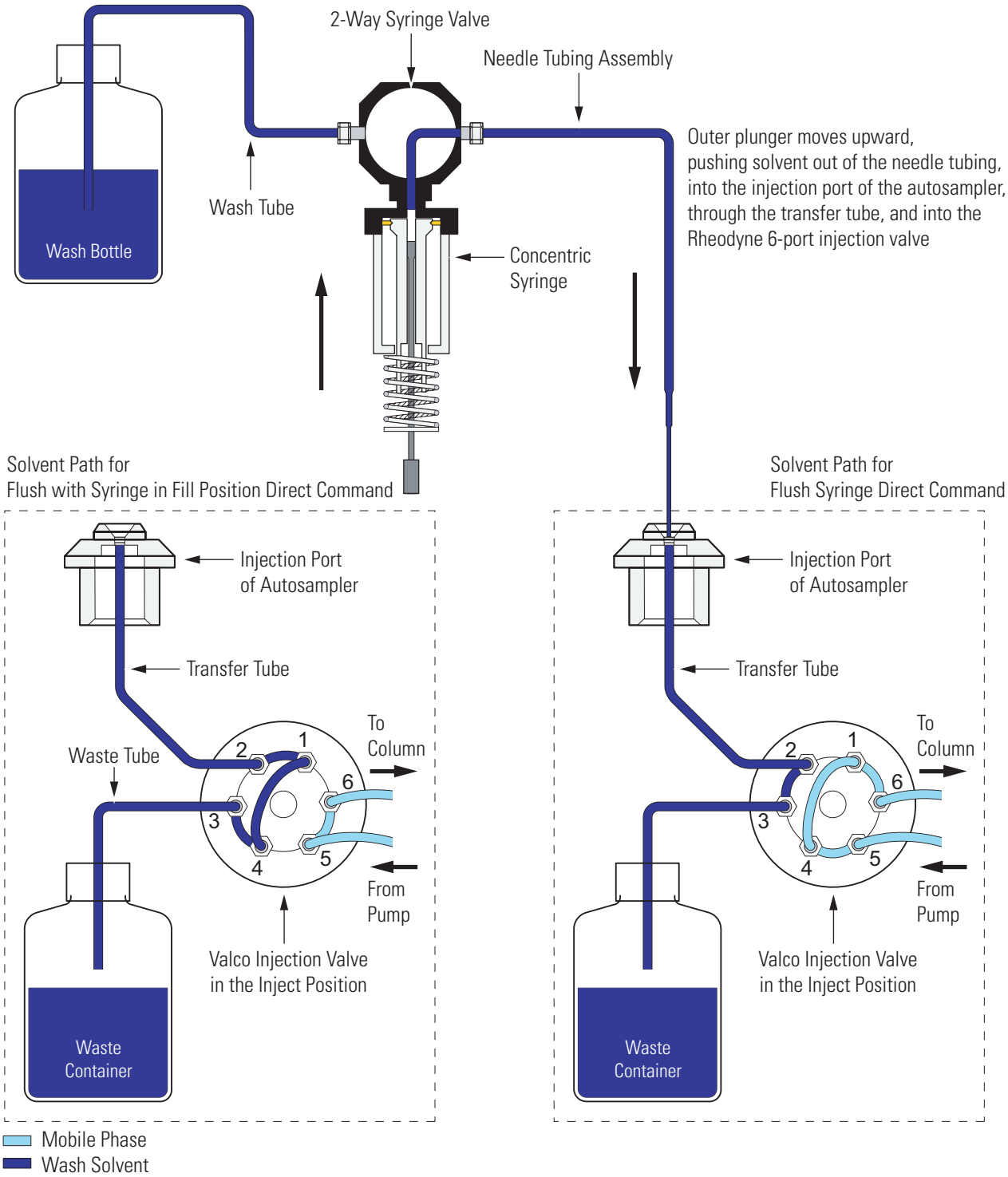


Figure 41. Schematic of solvent path for flushing the syringe

Checking the Firmware Version of the Surveyor LC Pump

If your Surveyor LC Pump has a firmware version below 2.0, it does not send a Pump Ready signal to the Surveyor Autosampler. If you selected either of the Input check boxes in the Signal Polarity page when you configured the Surveyor Autosampler, the status of the Surveyor LC Pump will indefinitely remain at Waiting for Contact Closure after you make an injection. To prevent this mismatch, verify the firmware version of your Surveyor LC Pump. If necessary, modify the configuration for the autosampler signal polarities accordingly.

To check the version of your Surveyor LC Pump Plus

1. Open the Instrument Setup window as described on page 51.

2. In the **Instrument Setup** viewbar, click the **Surveyor LC Pump** button to open its Instrument Setup view.

3. From the menu bar, choose **Surveyor LC Pump > Direct Control > Configuration** to open the Configuration – Surveyor LC Pump Direct Control dialog box. See Figure 42.

4. Note the firmware version of the Surveyor LC Pump. If the version is lower than 2.0, check the configuration of the Surveyor Autosampler. See "Configuring the Surveyor Autosampler Plus" on page 28.

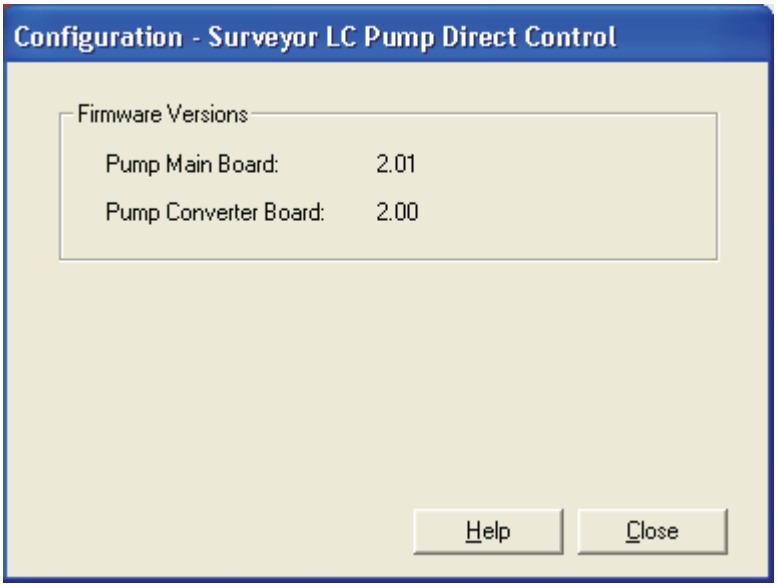


Figure 42. Configuration – Surveyor LC Pump Direct Control dialog box

Priming the Pulse Dampener of the MS Pump

Before you can operate your MS pump, you must prime its built-in pulse dampener. Priming the pulse dampener involves filling the dampening coil of the pulse dampener with a solvent such as methanol or isopropanol. After the coil is filled with solvent, it can effectively dampen pressure pulsations from the rest of your system.

Closing the manual bleeder valve after you fill the coil shuts the dampening coil off from the mobile phase stream. See Figure 43. However, only a permeable membrane separates the mobile phase stream from the dampening coil. Therefore, if you use buffered mobile phases, there will be a gradual buildup of electrolytes in the dampening coil. Due to the pressure gradient, the reverse does not occur. Therefore, you do not need to match the priming solvent to the mobile phase because the solvent in the coil cannot diffuse into the mobile phase stream.

If you are not pumping buffered mobile phases, you need to prime the pulse dampener only before you initially operate the MS pump. In contrast, you will occasionally need to reprime the pulse dampener if you are pumping buffered mobile phases. Flushing the pulse dampener following the use of buffered eluents helps to prevent salt build-up and its associated corrosion.

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.

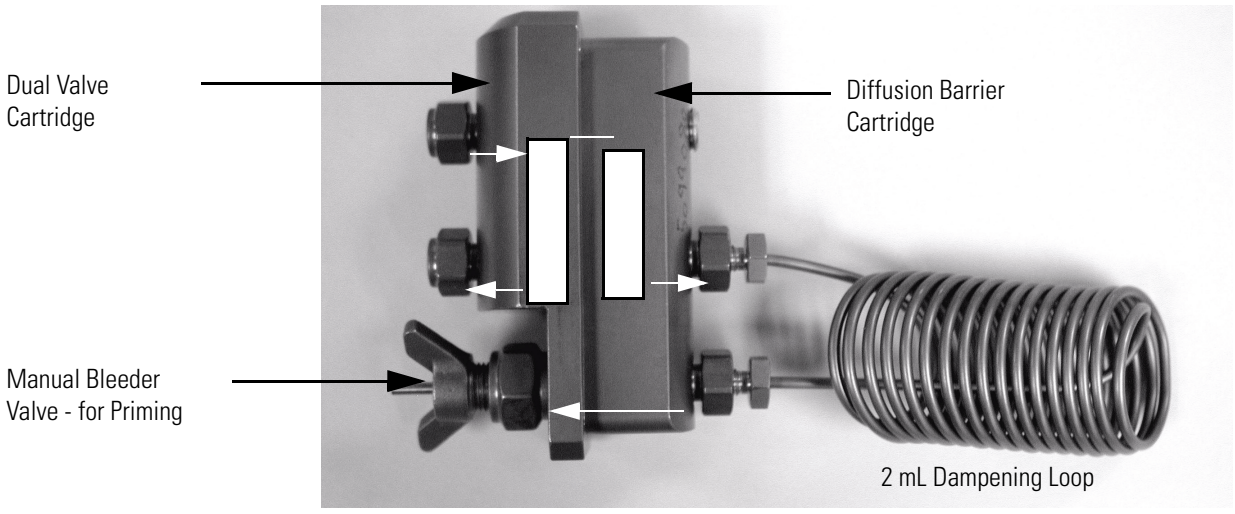


Figure 43. Pulse dampening assembly for MS pump

To prime the pulse dampener

1. Fill a solvent reservoir with particulate-free, HPLC grade methanol.
2. Connect an HPLC column or a flow restrictor to the pulse dampener outlet.
3. Insert the tip of the 10 mL syringe into the tubing that is connected to the manual bleeder valve of the pulse dampener. Then, open the valve by turning it counter-clockwise.
4. Set the flow rate to 1 mL/min (1000 µL/min).
5. Fill the loop completely, to expel any air that might be trapped in the dampener loop.
6. Set the flow rate to one that is appropriate for your system.
7. Close the manual bleeder valve of the pulse dampener (see Figure 44) by turning the valve clockwise.

Unless you need to refill the dampener loop of the pulse dampener with fresh solvent, keep the manual bleeder valve closed.

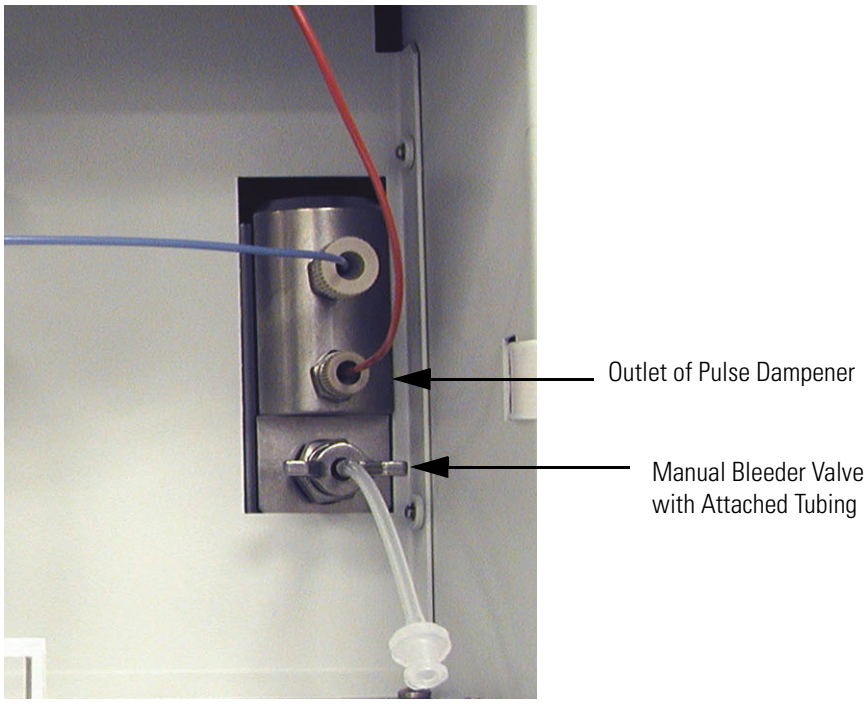


Figure 44. Manual bleeder valve and attached tubing

Calibrating the PDA Detector

There are two types of calibration for the PDA detector:

- Dark Current Calibration
- Wavelength Calibration

You must perform both calibrations after the initial installation of your detector. Thereafter, Thermo Electron recommends that you perform a dark current calibration monthly and a wavelength calibration whenever you move your detector or it is physically jolted. The instructions for performing these calibrations are included in "Verifying the Performance of the PDA Detector" on page 174.

Dark Current Calibration

The function of the dark current calibration is to measure and correct for the background signal that is produced by the photodiode array. Even when both lamps are turned Off, the array still produces a small amount of background signal. Typical dark current values range from 1500 to 3000 counts.

Before intensity values can be converted to absorbance units, the dark current must be subtracted from the intensity measurements. Following dark current calibration, light intensity values are automatically corrected for dark current. Remember that because the dark current signal is a function of temperature both lamps must be On during calibration. You typically warm-up the lamps for one hour before making injections. Therefore, it is important to warm-up the lamps for one hour before performing a dark current calibration to ensure that the temperature within the detector during calibration matches the temperature within the detector during normal operation.

Perform a dark current calibration monthly.

Wavelength Calibration

The alignment of the spectrum on the diode array depends upon the physical alignment of various components of the optical bench. The alignment can become offset if the detector is sharply jolted, for example, in shipping. Such bumps and jars can slightly change the wavelength of light reaching the photodiode array.

The automated wavelength calibration allows you to determine wavelength accuracy. It also allows the PDA detector to mathematically compensate for minor changes in the alignment of the components of the optical bench. Perform a wavelength calibration if you move the detector or if it receives a physical jolt.

Chapter 4 Creating Instrument Methods

To automate the control of your LC devices, you must create an Instrument Method from the Instrument Setup window of the Xcalibur data system.

This chapter describes the instrument control parameters for your Surveyor Plus LC devices. It does not describe the instrument control parameters for your MS detector. For information on the instrument control parameters for your MS detector, see its Getting Started manual.

To create an instrument method containing the appropriate chromatographic conditions, perform the following procedures that are provided in this chapter and that pertain to your LC devices in the order listed:

1. Opening the Instrument Setup Window
2. Entering the Method Parameters for the MS Pump
3. Entering the Method Parameters for the LC Pump
4. Entering the Method Parameters for the Autosampler
5. Entering the Method Parameters for the PDA Detector
6. Entering the Method Parameters for the UV/Vis Detector
7. (Optional) Adding a Sample Preparation Routine to the Method
8. Saving the Method

Opening the Instrument Setup Window

To open the Instrument Setup window

1. Double click the Xcalibur icon on your desktop to display the Xcalibur Home Page.

2. On the Xcalibur Roadmap - Home Page, click the Instrument Setup button in the Road Map or choose **GoTo > Instrument Setup** to open the Instrument Setup window. See Figure 45.

   The Viewbar on the left-side of the window contains an icon for each configured device of your instrument. Clicking an icon for a device opens the Instrument Setup view for that device.

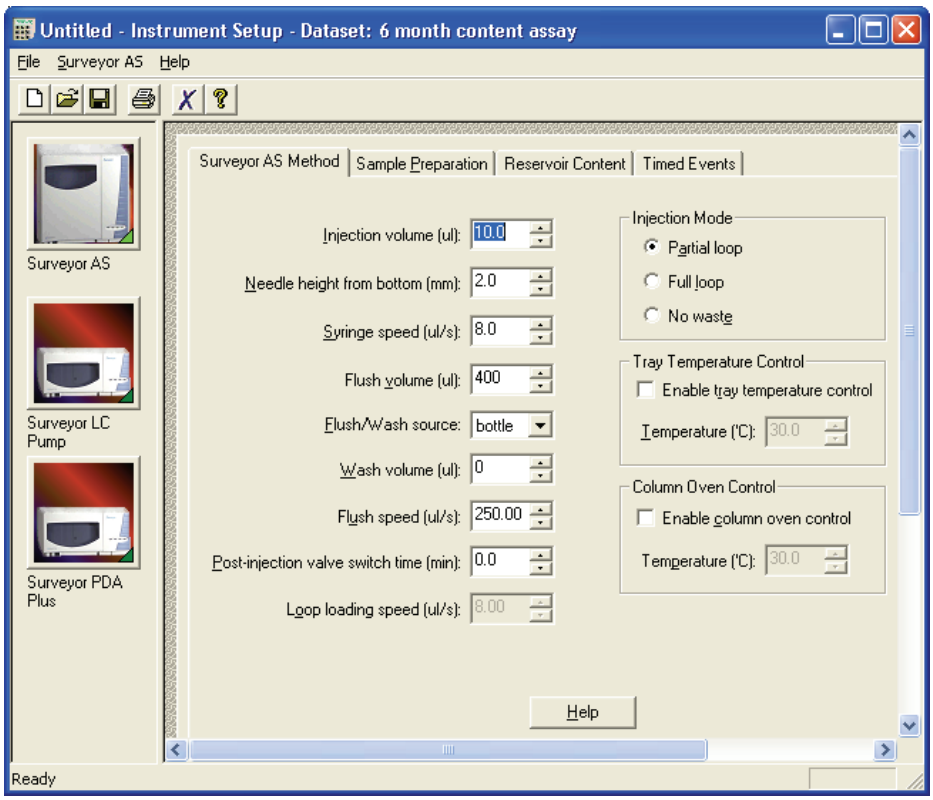


Figure 45. Instrument Setup window

Entering the Method Parameters for the MS Pump

The instrument control parameters for the Surveyor MS Pump Plus that control the composition and flow rate of the mobile phase are entered in the Surveyor MS Pump Instrument Setup view.

To open the Surveyor MS Pump view, click the Surveyor MS Pump icon in the Viewbar of the Instrument Setup window.

If you are unfamiliar with the Surveyor MS Pump Plus, review the description of its instrument control parameters before you create the pump program.

This section contains the following topics:

Parameters for the Surveyor MS Pump Plus

The following topics describe the instrument control parameters for the Surveyor MS Pump Plus:

Surveyor MS Pump View - General Page

The Surveyor MS Pump View – General page has the following areas:

Parameters for the Pump

The General page contains the parameters listed below. The values that you enter in these boxes will be reported in the Instrument Method report attached to the raw file. This report can be accessed in the Qual Browser view by choosing **View > Reports > Instrument Method**.

Name	Use this box to select the pump if you are running a dual pump system.
Comments	Use this box to enter more information about the method, for example, the type of LC column being used.
Solvent	Select the check box to the left of these boxes to make them available, and then type a description of the solvent.
Operating mode	Use this list to select the operating mode: 0 to 7000 PSI (standard) or 0 to 16000 PSI (ultra-high performance).

Start settings	Use this list to select how runs are triggered. The available selections are Autosampler logic and manual. Select **Surveyor AS injection logic** if you want to use the autosampler as the start instrument that triggers the run.
Method finalizing	Use this list to select the mobile phase composition at the end of the run. the available selections are First line conditions, Last line conditions, and Stop after the end. Select **Stop after the end** if you want to use the method to turn off the pump flow.
Min pressure	Use this box to enter a minimum pressure below which the pump will stop operating. Entering an appropriate minimum pressure 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 will quickly ruin the piston seals. The range is 0 to 400 bar (5800 psi) with a default value of 0 bar. Type a value that is well below the typical operating pressure for your application in this box. In the event that the pressure falls below this limit, the pump automatically stops and sends an error message to the computer.
Max pressure	Use this box to enter a maximum operating pressure for the pump. Entering an appropriate maximum pressure 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. The range is 0 to 400 bar (5800 psi) with a default value of 0 bar. In this box, type a value that is well above the typical operating pressure for your application, but also below the pressure that can damage your system. In the event that the pressure rises above this limit, the pump automatically stops and sends an error message to the computer.
Pressure stability	Use this box to adjust 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.
Home before run	This check box is available when you select **Manual** from the Start settings list. Selecting this check box increases the reproducibility of chromatographic runs when you are performing manual injections.
Pressure units	Use this list to select the units for the backpressure readout. The available selections are bar, PSI, and MPa.

Surveyor MS Pump View - Gradient Page

The Surveyor MS Pump View – Gradient page has the following areas:

- Gradient Table Area
- Gradient Profile Area

Gradient Table Area

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 Gradient Table can contain up to 80 time lines.

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.

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. The default composition setting is 100% A.

The flow rate parameter (µL/min) box specifies the flow rate of the mobile phase at the time specified in the associated Min box. The default setting flow rate setting is 1000 µL/min.

Gradient Profile Area

The Gradient Profile area graphically displays the values entered in the Gradient Table. The Y-axis represents percent composition and the X-axis represents time in minutes. Each solvent is color-coded for better visualization of the programmed gradient.

Programming the Surveyor MS Pump Plus

The Surveyor MS Pump Plus 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 MS pump in either the isocratic mode or the gradient mode. In the isocratic mode, the same proportions of solvent are maintained throughout the run.

To program the pump

1. In the General page (see Figure 46), type or select the following:

   a. Type the names of the solvents that make up the mobile phase

   b. Select the operating mode: 0-7000 PSI.

   c. From the Start settings list, select how the system is triggered to start a run.

The available selections are Surveyor AS injection logic and Manual. To trigger a run automatically from the autosampler, select **Surveyor AS injection logic**.

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

   The available selections are First line conditions, Last line conditions, and Stop after the end. If you are creating a shutdown method that turns off the pump flow, select **Stop after the end**.

e. Type or select the minimum allowable operating pressure.

f. Type or select the maximum allowable operating pressure.

g. Type or select an appropriate backpressure stability value.

   The pump will not return a Ready signal until the system pressure reaches this limit.

h. Select the pressure units for the backpressure display

   Note 1 bar = 14.5 psi

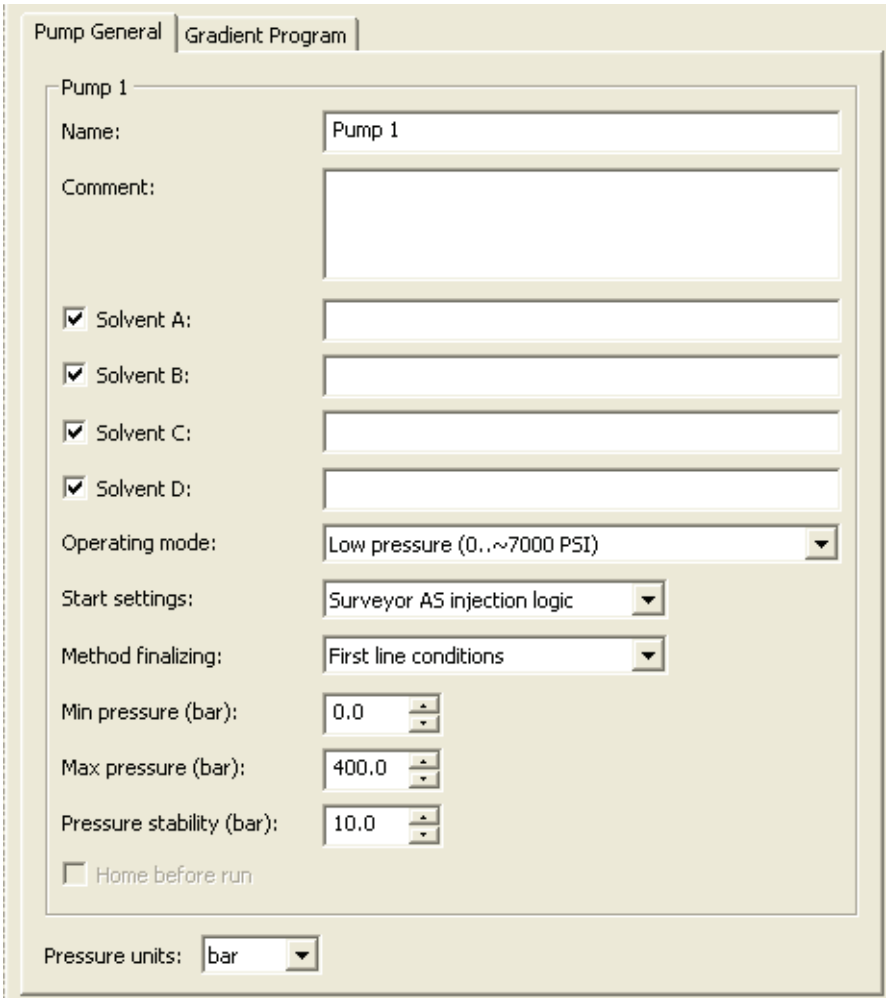


Figure 46. General page for the Surveyor MS Pump Plus

2. Click the **Gradient Program** tab.

3. Type the flow rate and solvent composition in the first time line of the Time Program table, and then press ENTER.

   The allowable flow rate range for the Surveyor MS Pump Plus is 0 to 2000 µL/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 47 will produce a proportioned mobile phase consisting of 80% solvent A and 20% solvent B (v/v). The proportions of the solvents will remain constant throughout the run. The flow rate of the mobile phase will remain at 1 mL/min throughout the run.

4. If you are creating an isocratic pump program, skip the next step, which describes how to create a gradient program, and go to "Entering the Method Parameters for the Autosampler" on page 76.

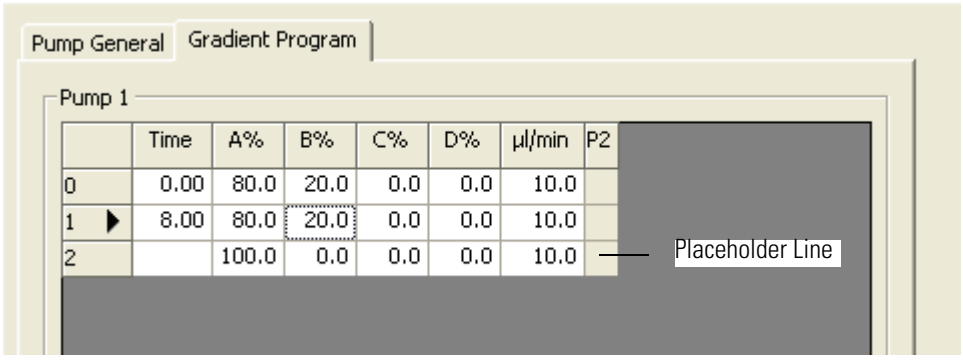


Figure 47. An example of an isocratic pump program for the MS pump

5. To create a gradient pump program, enter at least two steps in the Gradient Table. For each line in the program, type the solvent composition and the flow rate. Then, press ENTER.

   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 last time line in the program is a placeholder line and has no effect on the pumping conditions. The allowable flow rates are 0 to 2000 µL/min.

   The gradient table and gradient profile shown in Figure 48 produce the gradient program described in Table 4.

Table 4. Gradient program

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

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 is a placeholder and has no affect on the pump conditions.

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

After you enter the pump program, go to "Entering the Method Parameters for the Autosampler" on page 76.

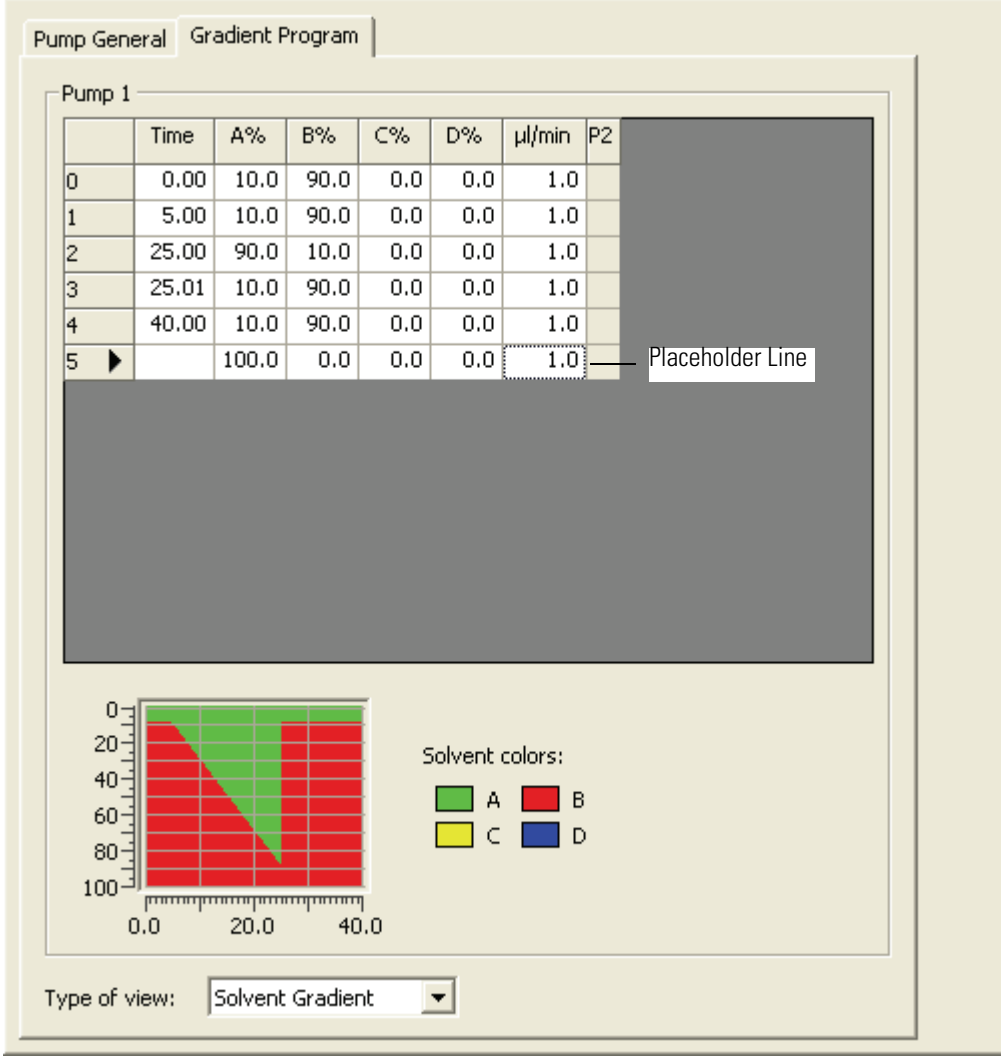


Figure 48. An example of a gradient program for the MS pump

Entering the Method Parameters for the LC Pump

The instrument control parameters for the Surveyor LC Pump Plus that control the composition and flow rate of the mobile phase are entered in the Surveyor LC Pump Instrument Setup view. To open the Surveyor LC Pump Plus view, click the Surveyor LC Pump icon in the Viewbar of the Instrument Setup window.

To create a pump program for the Surveyor LC Pump Plus

1. In the General page (see Figure 49), enter the following:

   - The names of the solvents that make up the mobile phase
   - A description of the LC column
   - The minimum allowable 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(s).
     - An excessive amount of air has entered the pump head assemblies.

   - The maximum allowable 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.

   - The pressure units for the backpressure display

     Note 1 bar = 14.5 psi

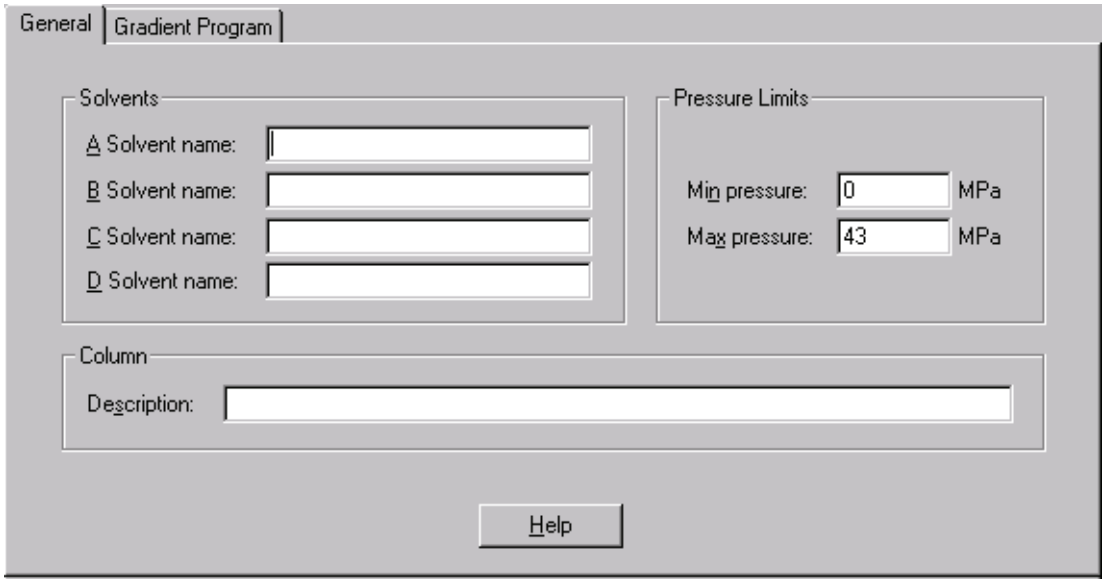


Figure 49. General page for the Surveyor LC Pump Plus

2. Click the Gradient Program tab to open the Gradient Program page.

3. 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.

   To create an isocratic pump program, you need to enter only one time line. If you are not creating a gradient program, skip the next step and go to "Entering the Method Parameters for the Autosampler" on page 76.

   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 affect on the pumping conditions.

For example, the pump program shown in Figure 50 will produce a proportioned mobile phase consisting of 80% methanol and 20% water (v/v). The proportions of the solvents will remain constant throughout the run. The flow rate of the mobile phase will remain at 1 mL/min throughout the run.

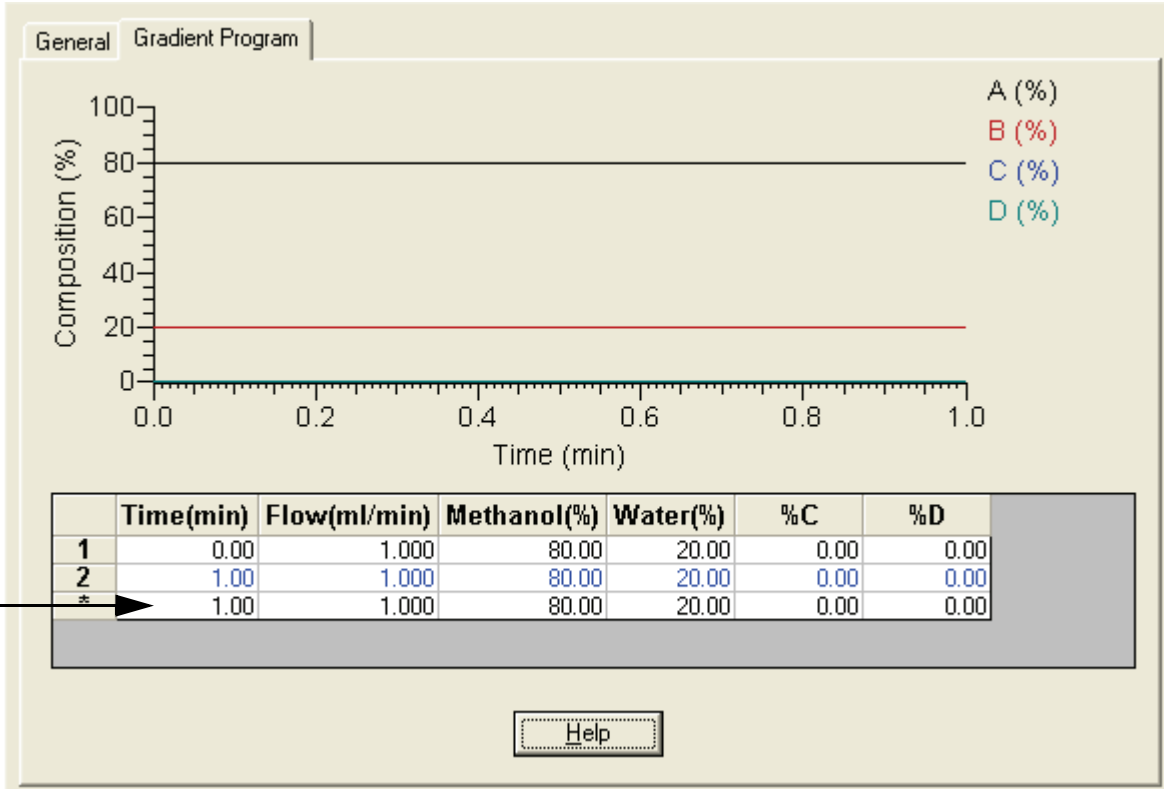


Figure 50. An example of an isocratic pump program for the Surveyor LC Pump Plus

4. To create a gradient pump method, you must 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.

   The Gradient Program table shown in Figure 51 creates the gradient profile shown in Table 5.

Table 5. Gradient program

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

5. After you enter your pump program, go to the next section: "Entering the Method Parameters for the Autosampler" on page 76.

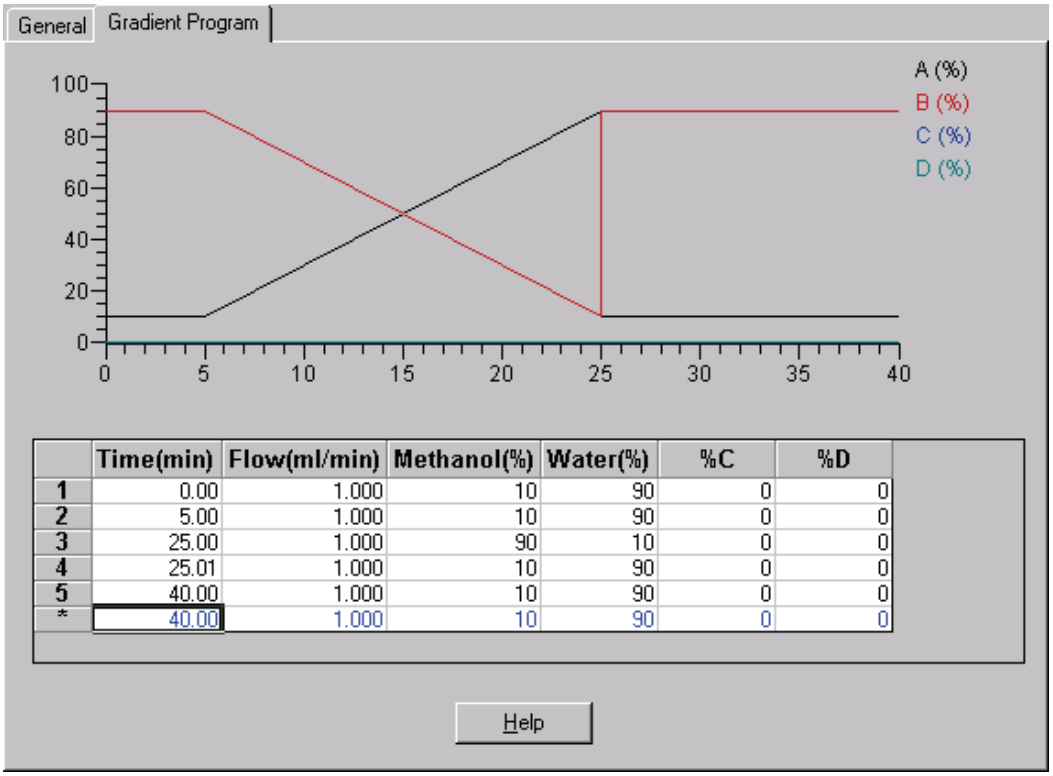


Figure 51. An example of a gradient program for the LC pump

Entering the Method Parameters for the Autosampler

The parameters for the autosampler are entered in the Surveyor Autosampler Instrument Setup view. To open this view, click the Surveyor Autosampler icon in the Viewbar of the Instrument Setup window. If you are unfamiliar the Surveyor Autosampler Plus, review the description of the instrument control parameters for the Surveyor Autosampler before you enter your injection parameters.

This section contains the following topics:

- Parameters for the Surveyor Autosampler Plus
- Programming the Surveyor Autosampler Plus

Parameters for the Surveyor Autosampler Plus

The Surveyor AS Instrument Setup view contains four pages. The following three pages are described in this section. The Sample Preparation page is described in the next section "Adding a Sample Preparation Routine to the Method" on page 98.

- Surveyor AS Method Page
- Reservoir Content Page
- Timed Events Page

Surveyor AS Method Page

You set the injection and the temperature control parameters for the autosampler in the Surveyor AS Method page. See Figure 52.

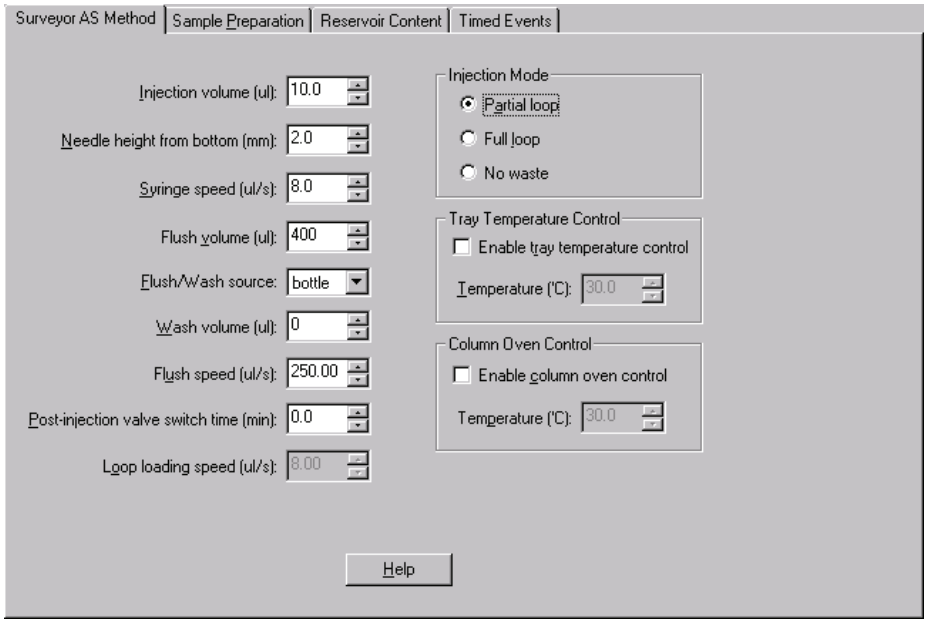


Figure 52. Surveyor AS Instrument Setup view – Surveyor AS Method page

The available parameters are:

- Injection Volume
- Needle Height From Bottom
- Syringe Sample Speed
- Flush Volume
- Flush/Wash Source
- Wash Volume
- Flush Speed
- Post Injection Valve Switch Time
- Loop Loading Speed
- Injection Mode
- Tray Temperature Control
- Column Oven Control

Injection Volume

The Injection Volume box displays the injection volume in microliters of sample that will be placed in the sample loop prior to opening the loop up to the mobile phase stream. The available injection volumes are determined by the size of the syringe. For the No Waste and the Partial Loop injection modes, the sample loop size limits the practical injection volume range.

For optimal performance, refer to the following suggested injection volumes:

- For no waste injections, limit the injection volume range to a minimum of 0.5 µL and a maximum equal to 5 µL less than the nominal sample loop size.
- For partial loop injections, limit the injection volume range to a minimum of 0.1 µL and a maximum equal to one-half the nominal sample loop size.
- For full loop injections, the Injection Volume box is grayed out, limiting the injection volume to the configured sample loop size. The Surveyor Autosampler is shipped with a 25 µL sample loop.

Needle Height From Bottom

The Needle Height From Bottom box displays the height in millimeters from the bottom of the vial or well at which the needle withdraws liquid. The range of values is 0.1 to 18.0 mm in increments of 0.1 mm. The default value is 2.0 mm. Figure 53 shows the needle descending to a depth of 2 mm from the bottom of a standard 1.8 mL vial.

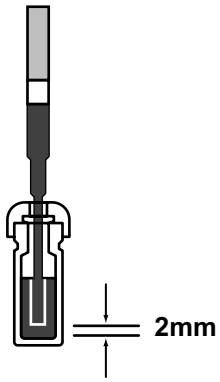


Figure 53. View of needle descending to 2 mm from the bottom of a vial

Syringe Sample Speed

The Syringe Sample Speed box displays the rate in microliters per second at which the syringe withdraws liquid from a tray vial or a plate well. The syringe speed range is determined by the size of the syringe. You select the size of the syringe in the Syringe Type list in the Communication page of the Surveyor Autosampler Configuration dialog box. 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.

Flush Volume

The Flush Volume box displays the volume in microliters of liquid that is used to flush the syringe. During an internal flush, the syringe needle is moved to the injection port to dispense a specified volume of solvent from the bottle or reservoir vial. The injector is switched to the Inject position to prevent solvent from entering the loop. The internal flush is used to remove residual sample. The range of values is 0.0 to 6000.0 µL. The default volume is 400.0 µL.

Note A flush is accomplished by pushing the amount of solvent that you specify into the injection port of the autosampler, through the transfer tubing to the Valco injection valve, and out port 3 of the injection valve to waste. If the specified flush volume exceeds the syringe volume, then the autosampler takes multiple draws from the reservoir or bottle to equal the specified flush volume.

Flush/Wash Source

The Flush/Wash Source list displays the flush/wash source. The flush/wash source can be either the wash bottle or a reservoir vial (RV1 to RV4). To change the flush/wash source, click the arrow to display the list of source options.

Wash Volume

The Wash Volume box displays the volume in microliters of liquid that is used to wash the needle. During an external wash, the syringe needle is moved to the wash station to dispense a specified volume of solvent from the bottle or reservoir vial. The range of volumes is 0.0 to 6000.0 µL. The default value is 0.0 µL.

Note A wash is accomplished by washing the interior and exterior of the syringe needle with solvent. If the specified wash volume exceeds the syringe volume, then the autosampler takes multiple draws from the reservoir or bottle to equal the specified wash volume.

Flush Speed

The Flush Speed box displays the rate in microliters at which the syringe draws flush solvent into its chamber and expels flush solvent through the needle during a flush cycle. The range of values for the concentric syringes is 0.83 to 661.38 µL/s. The default value is 100.0 µL/s. The range of values for the 2500 standard syringe is 8.27 to 330.85 µL/s. The default value is 82.71 µL/s.

Note The default flush speed of 100 µL/s for the 250 µL concentric syringe is the maximum flush speed for a solvent of high viscosity such as water. At high flush speeds, viscous solvents cause the autosampler to make a grinding sound.

Post Injection Valve Switch Time

The default time for the Post Injection Valve Switch Time box is 0. If you leave this value at 0, the valve remains in the inject position throughout the entire run, which allows ample rinsing of the sample loop with mobile phase.

Any nonzero value equal to or greater than 0.1 min is the time at which the injection valve will switch from the inject position to the load position following an injection. 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 entering a nonzero value in this box. In the load position, flow from the pump bypasses the sample loop and goes directly to the column.

Loop Loading Speed

The Loop Loading Speed box displays the speed at which the sample is loaded onto a guard column or a trap. This parameter is only available in the no waste injection mode. The syringe pushes the sample bolus to the injection port at syringe speed. The injection valve then switches to the load position and the syringe meters the sample bolus onto the guard column or trap at loop loading speed.

Injection Mode

The option buttons in the Injection Mode area allow you to select the injection mode. The injection modes are No Waste, Partial Loop, and Full Loop and are described in detail in Chapter 1, "Introduction to the Surveyor Plus Integrated LC/MS System." See "Injection Volume" on page 78 for information on the appropriate injection volumes for the three injection modes.

Tray Temperature Control

You to set the temperature for the sample tray in the Tray Temperature Control area. The temperature control feature performs heating or cooling of the sample tray to establish an isothermal condition.

You select the Enable Tray Temperature Control check box to activate the Temperature box. The Temperature box displays the temperature of the sample tray in degrees Celsius. The range of values is 0 to 60 °C. The default value is 30 °C. The precision is 1 °C. As a safety feature, a thermostat disables power to the sample tray if the temperature reaches 65 °C.

Column Oven Control

You to set the temperature for the column oven in the Column Oven Control area. The temperature control feature performs isothermal heating or cooling of the column oven to establish an isothermal condition.

You select the Enable Column Oven Control check box to activate the Temperature box.

The Temperature box displays the temperature in degrees Celsius of the column oven. The range of values is 5 to 95 °C. The default value is 30 °C. The precision is 1 °C. As a safety feature, a thermostat disables power to the column oven if the temperature reaches 110 °C.

Reservoir Content Page

You enter descriptions for the contents of the four reservoir vials and the wash solvent in the Reservoir Content Page shown in Figure 54. You can type up to 80 characters in each box.

To open the Reservoir Content page, click the Reservoir Content tab in the Surveyor AS Instrument Setup View.

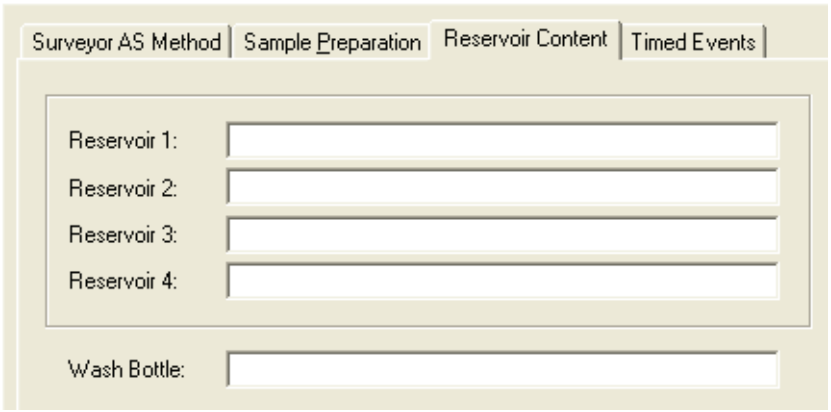


Figure 54. Surveyor AS Instrument Setup view – Reservoir Content page

Timed Events Page

You set timed events for the time function terminals (TF1 to TF4) located on the rear panel of the Surveyor AS (See Figure 56) in the Timed Events page shown in Figure 55. You can use the TF terminals to control peripheral devices that are not controlled by Xcalibur.

Timed Event output signals are issued after the Inject Out signal in the signal sequence. See "Synchronizing the Surveyor LC Devices" on page 20. The TF terminal output signal is LO (Closed) by default. You can change the polarity of the TF output signal to HI (Open) by selecting the Timed Events Active High check box in the Signal Polarity page of the Surveyor Autosampler Configuration dialog box.

To display the Timed Events page, click the Timed Events tab in the Surveyor AS Instrument Setup view. You program timed events by adding entries to the Timed Events table.

The Timed Events table contains Time boxes and event (TF1, TF2, TF3, and TF4) lists. In the Time box, you specify the time in minutes when the Surveyor Autosampler TF terminal (TF1 to TF4) signals an event. Time 0.0 is defined as the time when the Surveyor Autosampler issues an Inject Out signal. The range of values is 0.0 to 9999.9 min.

In the event (TF1, TF2, TF3, and TF4) lists, you select whether the TF1, TF2, TF3, and TF4 output terminal is On or Off at the time specified in the Time box.

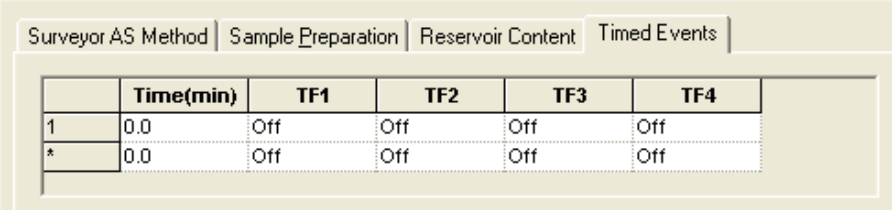


Figure 55. Surveyor AS Instrument Setup view – Timed Events page

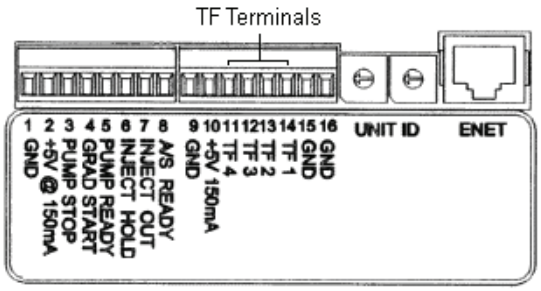


Figure 56. Time function event terminals on the back panel of the Surveyor Autosampler Plus

Programming the Surveyor Autosampler Plus

To program the injection parameters for your Instrument Method

1. Click the Surveyor Autosampler icon in the Viewbar of the Instrument Setup window to open the Surveyor Autosampler view.

2. In the Surveyor AS Method page (see Figure 52 on page 77) make the following entries and selections:

   a. Select an injection mode: Partial Loop, Full Loop, or No Waste. Then, do one of the following:

   - If you selected the Full Loop injection mode, continue at step 2d. The injection volume is automatically set to the configured value for the sample loop size.
   - If you selected the No Waste injection mode, go to step 2b.
   - If you selected the Partial Loop injection mode, go to step 2c.

   b. For the No Waste injection mode, select a loop loading speed from the Loop Loading Speed box. For most applications, leave the loop loading speed at its default value.

c. For the Partial Loop or No Waste variable volume injection modes, type an injection volume in the Injection Volume box. See "Injection Volume" on page 78 for information on the appropriate injection volumes for these injection modes.

d. From the Needle Height From Bottom box, select the depth to which the needle of the autosampler will descend before it withdraws sample from the vial / well location. The allowable values are 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 Surveyor Autosampler have a depth of approximately 20 mm. See Figure 53 on page 78.

e. For most applications, leave the syringe speed at its default value (8 µL/s for the 250 µL concentric syringe). If you notice a break up of the sample in the needle tubing as the autosampler withdraws sample from a vial / well location, adjust the syringe speed.

f. Type a volume from 0 to 6000 µL in the Flush volume box or leave the value at its default of 400 µL.

g. Select a flush / wash solution from the Flush / Wash list. For most applications, select the Wash bottle. If you select one of the reservoir vial locations as your flush / wash source, remember to load the autosampler with a reservoir vial. See "Tray Compartment" on page 7.

h. For most applications, leave the wash volume at its default value of 0 µL. If you want to wash the exterior of the needle after each injection, type a value between 0 to 6000 µL in the Wash Volume box.

i. Leave the flush speed at its default (250 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.

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

j. For most applications, leave the post injection valve switch time at its default of 0 min. This leaves the sample loop in the inject position during the entire run, which generally allows ample rinsing of the sample loop between injections.

k. For the full-featured Surveyor Autosampler Plus, do the following:

- If you want 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.

- If you want 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.

3. In the Reservoir Content page (see Figure 54 on page 82), identify the solvents contained in the 15 mL solvent reservoir vials and the 1 L wash bottle by typing their names in their respective boxes.

4. If you want to use the Surveyor Autosampler Plus to control external devices make the appropriate entries in the Timed Events page (see Figure 55 on page 83).

5. Complete the instrument method:

- If your LC system contains a Surveyor PDA Plus Detector, go to the next section: Entering the Method Parameters for the PDA Detector.

- If your LC system contains a Surveyor UV/Vis Plus Detector, go to

- If your system does not contain a detector, but you want to add sample preparation steps to your method, go to "Adding a Sample Preparation Routine to the Method" on page 98.

- If your system does not contain a detector and you do not want to add sample preparation steps to your method, go to "Saving the Method" on page 109.

Entering the Method Parameters for the PDA Detector

The instrument control parameters for the Surveyor PDA Plus Detector are entered in the Surveyor PDA Instrument Setup view.

To open the Surveyor PDA Plus view

Click the Surveyor PDA Plus icon in the Viewbar of the Instrument Setup window.

This section contains the topics listed below. If you are unfamiliar with the Surveyor PDA Plus Detector, review the description of its instrument control parameters before you program it.

- Parameters for the Surveyor PDA Plus Detector
- Programming the Surveyor PDA Plus Detector

Parameters for the Surveyor PDA Plus Detector

The Surveyor PDA Method page contains parameters that define the data that is collected during an analysis. See Figure 57. The Surveyor PDA Method page contains the following areas:

- Run Area
- Units Area
- Spectra Area
- Channels Area
- Timed Events Area

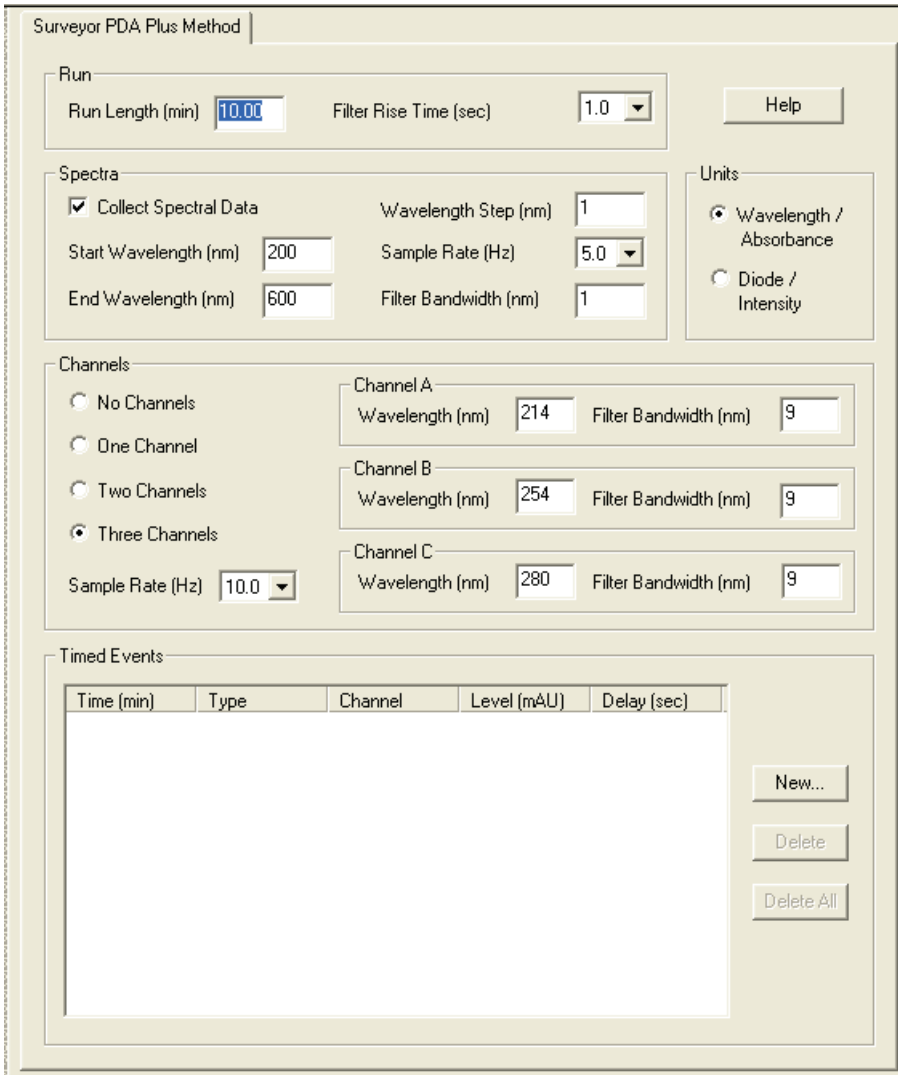


Figure 57. Surveyor PDA Plus Instrument Setup View – Surveyor PDA Plus Method page

Run Area

The run area box contains the following boxes:

- Run Length Box
- Filter Rise Time List

Run Length Box

The Run Length box specifies the time during which the detector will collect spectral and discrete wavelength data. The maximum run length is 600 minutes.

Filter Rise Time List

In the Rise Time list, you select the response time of the detector, in seconds, to the signal. Rise time is inversely proportional to the amount of baseline noise: the longer the rise time, the less noise detected. Setting the rise time to a value greater than one-tenth the width of the chromatographic peak at half-height results in peak broadening as shown in Figure 58. The one-second default value is appropriate for most LC applications.

Note The baseline noise inevitably increases as the deuterium lamp reaches the limit of its expected lifetime. Track the expected signal to noise levels for your chromatographic method to determine an appropriate replacement schedule for the deuterium lamp.

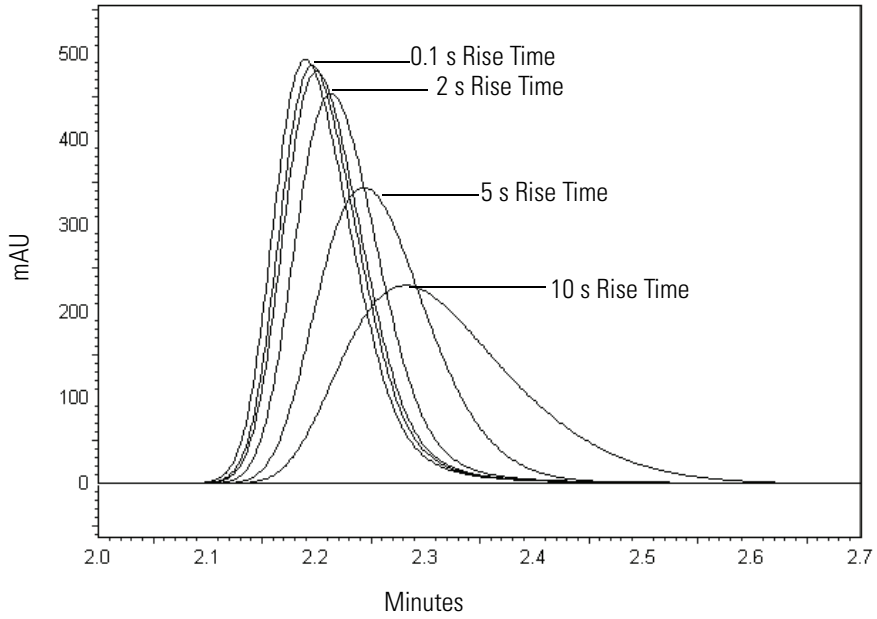


Figure 58. Effect of rise time on band broadening

Units Area

In the Units area, you select the units that are displayed in the spectrum plots. You can select to display either absorbance (default) or intensity. Absorbance displays the absorbance signal from the detector. In this case, the X-axis corresponds to wavelength in nanometers. Intensity displays the light intensity detected by the photo diodes of the diode array. The X-axis, therefore in this case, corresponds to photo diode number.

The controls in this area are the Wavelength/Absorbance option button and the Diode/Intensity option button.

There are 512 diodes in the Surveyor PDA 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 610 /510 diodes = 1.2 nm/diode. The data is interpolated to give integer values.

Spectra Area

To collect spectral scans, select the Collect Spectral Data check box, and then fill in the following scan parameters:

- Start Wavelength
- End Wavelength
- Wavelength Step
- Sample Rate
- Filter Bandwidth

Start Wavelength

In the Start Wavelength box, you enter a starting wavelength for the scan. The range of acceptable values is from 190 to 800 nm. The default starting wavelength is 200 nm.

End Wavelength

In the End Wavelength box, you enter an ending wavelength for the scan data. The range of acceptable values is from 191 to 800 nm. The default ending wavelength is 600 nm. The end wavelength must be greater than the start wavelength and the difference between the end wavelength and the start wavelength must be an integral multiple of the wavelength step.

Wavelength Step

In the Wavelength Step box, you enter an appropriate scan data increment. The default is 1 nm. (At a value of 1 nm, the detector will collect data at every nanometer within the scan range.). Depending on the wavelength range, the allowable values are 1 to 610 nm.

Note If you plan to create Spectrum files from the scan data acquired with this method, use a 1 nm wavelength step.

Sample Rate

In the Sample Rate list, you select an appropriate sample rate for your application. The acceptable values are 0.5, 1, 2, 4, 5, 10, and 20 Hz with 1 Hz being the default. This sampling rate is the rate at which data points are collected for each wavelength in the scan range. The size of your data files increases as the sampling rate and number of wavelengths scanned increases.

Filter Bandwidth

In the Filter Bandwidth box, you enter an appropriate bandwidth for the Savitsky-Golay filter. Increasing the value of the bandwidth filter can improve the signal-to-noise ratio for your chromatograms, but compromises spectral resolution. The default setting is 1.00 nm for the highest spectral resolution. See Figure 59, which shows the loss of spectral resolution for a spectrum of toluene as the bandwidth is raised from 1 nm to 11 nm.

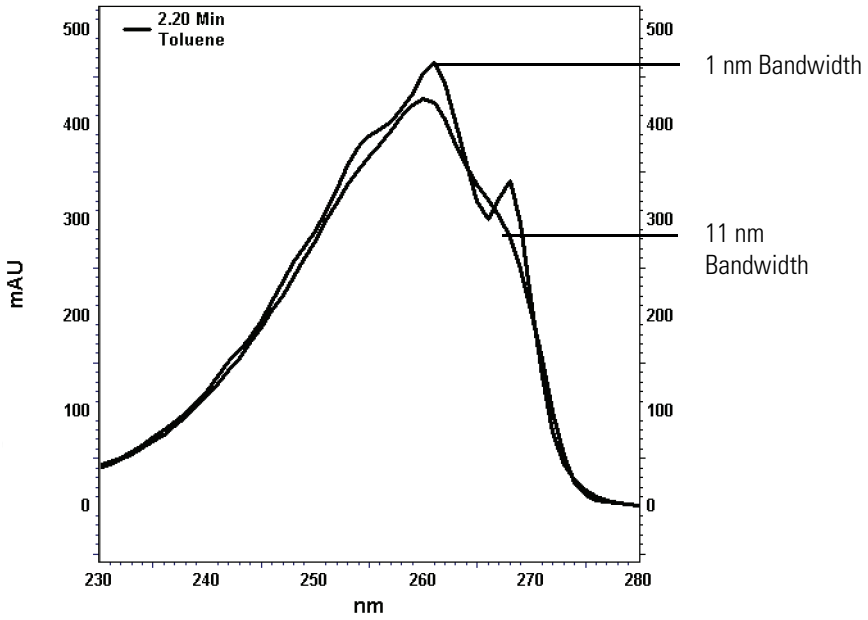


Figure 59. Spectra of toluene, effect of bandwidth on spectral resolution

Bandwidth values are limited to the following subset of odd integers: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 43, 45, 47, and 49. In addition, the allowable scan range for a particular bandwidth is limited as follows:

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

According to these equations, 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 filter bandwidth should not exceed 10% of the bandwidth at half-height of the narrowest spectral feature of interest.

Channels Area

The Channels area contains option buttons to select None, One, Two or Three single-wavelength channels. There is also a sample rate box, which is applied to all chromatogram-channels (A, B, and, C).

Sample Rate

In the Sample Rate list, you select a data-sampling rate for the discrete channels. The available values are: 0.5, 1, 2, 4, 5, 10, and 20 Hz (1 Hz = 1 point per second), with the default of 10 Hz. A sampling rate that is too low can affect the integration of narrow peaks.

None/A/B/C Select

You must select the appropriate channel option button to collect data for that channel. You can select any combination of channels or none. Default is Three Discrete channels.

Wavelength

In the Wavelength box, you enter an appropriate wavelength for your application. The acceptable range of values is from 190 nm to 800 nm, in increments of 1 nm. The default wavelengths are 214, 254, and 280 nm.

Note The combination of the bandwidth and wavelength settings cannot extend beyond the range of the detector. For example, a bandwidth of 49 nm is not allowed for a wavelength of 200 nm because it would require an analysis range from 176 nm to 224 nm. The maximum allowable bandwidth for 200 nm is 21 nm, which requires an analysis range of 190 nm to 210 nm.

Filter Bandwidth

In the Filter Bandwidth box, you enter an appropriate bandwidth for the Savitsky-Golay filtering. The acceptable range is from 1 to 49 nm in odd-number increments, with 1 nm meaning no filtering. The default value is 9 nm.

Note Enter a wider bandwidth to improve the signal-to-noise ratio.

Timed Events Area

In the Timed Events area, you program actuation of a rear panel contact closure as a function of either time or the absorbance level from one of the discrete wavelength channels. This feature can be used to trigger an external device, such as a fraction collector. See your Surveyor *PDA Plus Detector Hardware* manual for information on connecting an external device to the PDA detector.

The Time Events table lists all of the external events in chronological order. The type, time, channel, level, and delay for each event is included for each external event. You can add, modify, and remove events to the external events table by double-clicking the event setting you want to modify, or by using New, Delete, or Delete All buttons.

In the Timed Event dialog box, which is accessed by clicking the New button or an event in the Time Events table, you select the following parameters:

- Type
- Time
- Channel
- Level
- Delay

The Type list describes how the event is actuated. The selections are *Event Off, Event On, Zero Data,* and *Level Trigger*. The *Event Off* option turns the external Event contact Off. The *Event On* option turns the Event On. The *Level Trigger* option turns the Event On when a specified absorbance level is reached. The *Zero Data* option performs a zeroing of the detector signal. The *Event Off* option is the default selection.

The Time box specifies at what point in the analysis the external Event is triggered. The range is 0.00 to 600 min. The maximum allowable time is the run time. The default value is 1.00 min.

The Channel list designates which discrete wavelength channel is monitored for the absorbance level. Either *A, B,* or *C* can be selected. The *A* wavelength channel is the default selection.

The Level box specifies the absorbance level at which the external Event is triggered if the Type is set to Level. The range is from -2000 to 4000.00 mAU. The default value is 0.00 mAU.

The Delay box is used in conjunction with the Level box to compensate for the volume between the detector flow cell and the end of the outlet line going to a fraction collector or other instrument. The range is 0.00 to 65535.00 s. The default value is 0 s.

Programming the Surveyor PDA Plus Detector

To program the Surveyor PDA Plus Detector

1. Type a run time from 0 to 600 min in the Run Time box.
2. Select a rise time from 0 to 10.0 s in the Filter Rise Time list.
3. To collect spectral data, select the Spectra check box. Then, make the following entries and selections:
   a. Type a starting wavelength from 190 to 799 nm in the Start Wavelength box.
   b. Type an ending wavelength from 191 to 800 nm in the End Wavelength box. The ending wavelength must be greater than the starting wavelength.
   c. Type a value for the wavelength interval in the Wavelength Step box. Depending on the wavelength range defined by the starting and ending wavelengths, the allowable values are 1 to 610 nm. The wavelength range must be an integral number of wavelength steps. If you are collecting spectral data for a spectral library, use a wavelength step of 1 nm.
   d. Select a sample rate from the Sample Rate box. As you increase the sampling rate you also increase the data file size.
   e. Type a bandwidth in the Filter Bandwidth box. Depending on the wavelength range defined by the starting and ending wavelengths, the allowable values are the subset of odd integers from 1 to 49 nm. See "Filter Bandwidth" on page 90. Increasing the bandwidth decreases the spectral resolution.
   f. For normal data collection, select the Wavelength / Absorbance option in the Units area.

4. To collect discrete channel data, select one of the option buttons in the Channels area. Then, make the following entries:

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

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

      Depending on the discrete wavelength, the acceptable bandwidth range is from 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 allowable bandwidth is 21 nm. At a bandwidth setting of 21 nm, the reported absorbance value for 200 nm will be a weighted average from 190 nm to 210 nm. A value greater than 21 nm would be outside the lower range limit of the detector, which is 190 nm.

5. If you want to add a timed event to your Instrument Method, such as the triggering of a fraction collector as a peak begins to elute, do the following:

   a. In the Timed Events area (see Figure 57 on page 87), click **New**.

      Xcalibur displays the Timed Event dialog box shown in Figure 60.

   b. Select the appropriate parameters from the Timed Event dialog box. See "Timed Events Area" on page 92.

   c. Click **OK** to exit the Timed Event dialog box.

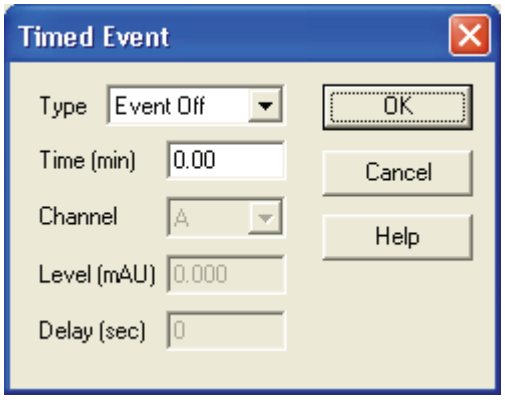


Figure 60. Timed Event dialog box

6. Complete the Instrument Method:

   - If you want to add sample preparation steps to your method, go to "Adding a Sample Preparation Routine to the Method" on page 98.

   - If you do not want to add sample preparation steps to your method, go to "Saving the Method" on page 109.

Entering the Method Parameters for the UV/Vis Detector

The instrument control parameters for the Surveyor UV/Vis Plus Detector are entered in the Surveyor UV/Vis Instrument Setup view shown in Figure 61.

To open the Surveyor UV/Vis Plus view

Click the Surveyor UV/Vis icon in the Viewbar of the Instrument Setup window.

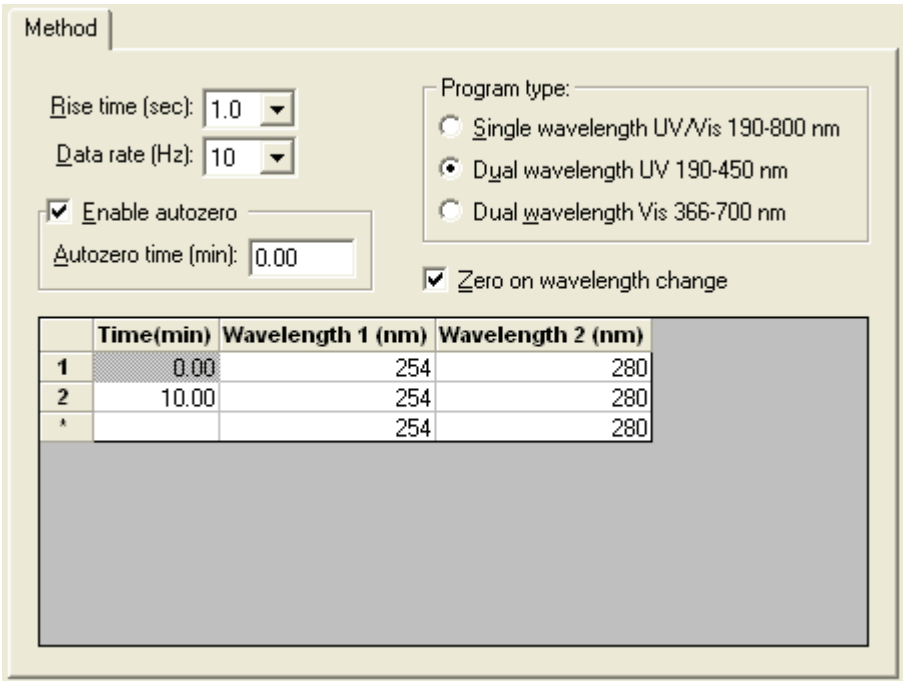


Figure 61. Surveyor UV/Vis Instrument Setup view

To program the Surveyor UV/Vis Plus Detector

1. In the **Rise Time** 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 as shown in Figure 58 on page 88. The one-second default value is appropriate for most LC applications

2. In the **Data Rate** list, select a data rate from 4 to 20 points per second.

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:

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

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

   - Select the Dual wavelength Vis 366 to 700 nm option to collect two chromatograms in the visible range during a run. 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.

Adding a Sample Preparation Routine to the Method

In Xcalibur, sample preparation routines are part of the Instrument Method. In the Sample Preparation page, you create a multi-task routine, which can contain 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 concentric syringe that ships with the Surveyor Autosampler, you must follow an additional set of rules that allow for the proper positioning of the inner and outer plungers of the syringe.

This section contains the following topics:

Opening the Sample Preparation Page

To open the Sample Preparation page

1. Double-click the Xcalibur icon on your desktop to display the Xcalibur Home Page.
2. On the Xcalibur Home Page, click the Instrument Setup button in the Road Map or choose **Goto > Instrument Setup**.
3. Click the Surveyor AS button to display the Instrument Setup view for the Surveyor Autosampler.
4. Click the Sample Preparation tab to display the Sample Preparation page. See Figure 62.

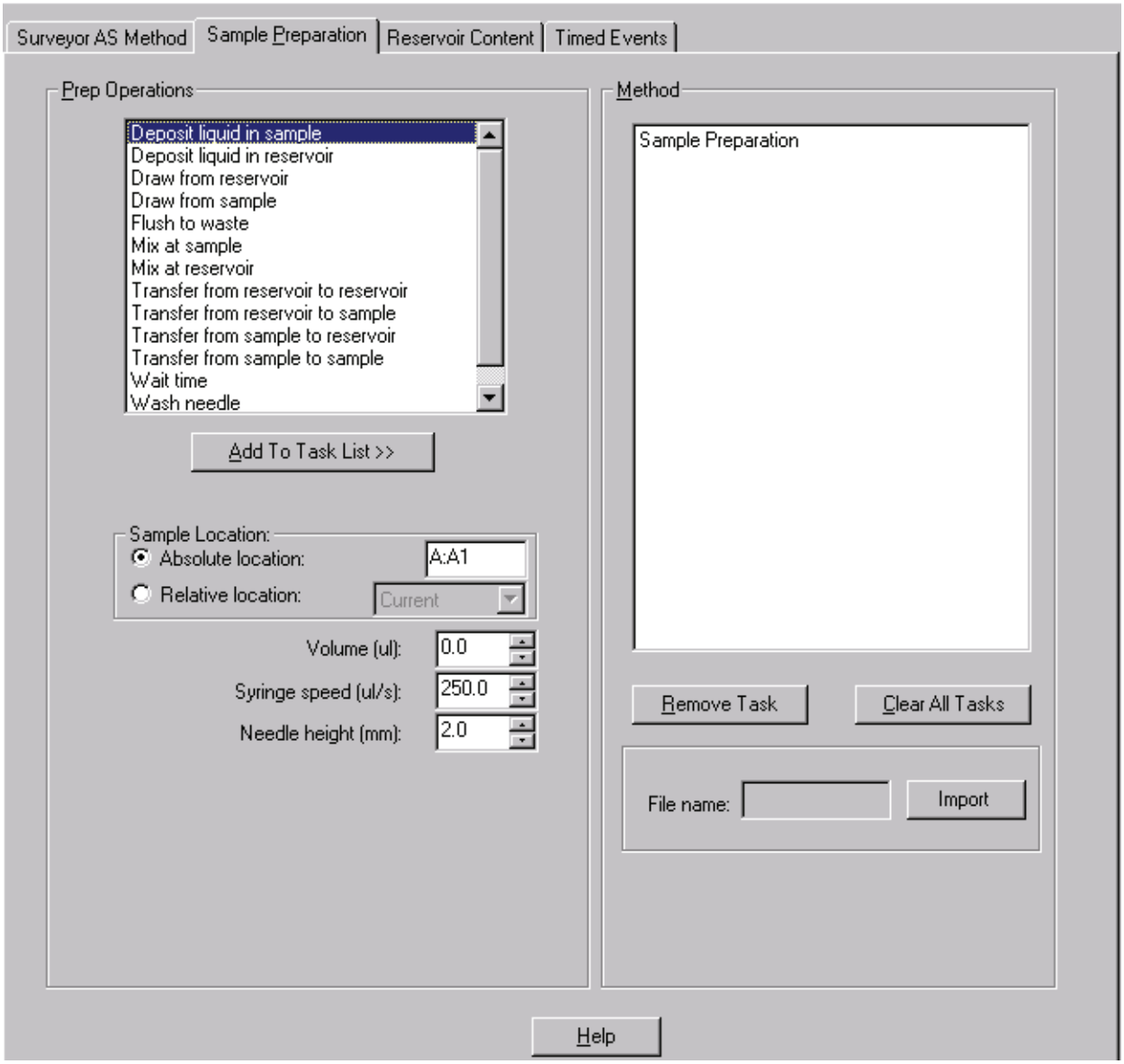


Figure 62. Surveyor AS Instrument Setup view – Sample Preparation page

Building the Sample Preparation List

To build a sample preparation routine

1. If you have a stored instrument method that contains all or part of the sample preparation routine that you want to use, go to step 2. Otherwise, go to step 3.

2. Import the sample preparation routine from a stored instrument method as follows:

   a. Click **Import** to display the Open files dialog box.

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 will be imported into the current Instrument Method.

3. For each task that you want to add to the sample preparation routine, do the following:

   a. Click the individual task in the Prep Operations list.

      Table 6 lists the thirteen tasks that you can use to create a sample preparation routine. The parameters available for the task are displayed below the Prep Operations list.

   b. Enter the appropriate values for each 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 will be limited to the nominal syringe size or less. See "Sample Preparation Rules" on page 101 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**.

Table 6. Pretreatment Tasks

Task	Description
Deposit Liquid in Sample	Deposits liquid into a designated sample vial or well.
Deposit Liquid in Reservoir	Deposits liquid held in the needle tubing into a reservoir vial.
Draw from Sample	Withdraws liquid from a sample vial or a well.
Draw from Reservoir	Withdraws liquid from a reservoir vial.
Flush to Waste	Moves the needle to the injection port and dispense a specified volume of solvent drawn from the wash bottle or a reservoir vial.
Mix at Sample	Mixes the contents at a sample location by aspirating the sample into the needle tubing and expunging it back into the sample location.
Mix at Reservoir	Mixes the contents of a reservoir vial. Mixing is accomplished by aspirating and expunging the contents of the reservoir vial.
Transfer from Sample to Reservoir	Moves liquid from a sample vial or well to a reservoir vial.
Transfer from Sample to Sample	Moves liquid from one sample vial or well to a second sample vial or well.
Transfer from Reservoir to Reservoir	Moves liquid from one reservoir vial to another reservoir vial.
Transfer from Reservoir to Sample	Moves liquid from a reservoir vial to a sample vial or well.
Wait Time	Pauses the sample preparation activity for a specified period of time in min
Wash Needle	Moves the needle to the waste station and dispense a specified volume of solvent drawn from the wash bottle or a reservoir vial.

Sample Preparation Rules

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 the 250 µL concentric syringe, the manner in which the various sample preparation tasks can be linked together is affected by whether the task takes place in only the large bore of the syringe, in only the small bore of the syringe, or in either bore of the syringe.

The sample preparation tasks can be divided into three groups depending their bore usage as shown in Table 7.

Table 7. Sample preparation tasks arranged in groups according to bore usage

Small Bore Only	Large Bore Only	Small or Large 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:

Note To save a method, it must conform to these rules.

- For tasks performed with the small bore of the concentric syringe, the maximum volume (sample + air bubble) that you can draw, deposit, or transfer is limited to the nominal size of the syringe, 250 µL.

  If a task can be performed from either the small or the large bore, the concentric syringe uses the small bore if 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.

- For tasks performed with the large bore of the 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.

  If a task can be performed from either the small or the large bore, the syringe uses the large bore if the requested volume (liquid + air bubble) is greater than the nominal syringe size.

- Crossover between bores is not allowed. Therefore, you cannot add a task that uses the large syringe bore immediately following a task that uses the small syringe bore. To switch from the small bore to the large 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.

An Example of a Sample Preparation Routine

This topic contains an example that describes how to create a sample preparation routine to dilute samples ten-fold.

To add a sample preparation routine that dilutes samples ten-fold

1. From the Instrument Setup window - Surveyor AS view, click the Sample Preparation tab to display the Sample Preparation page.
2. Open the [.meth] file that you want to modify.
3. Create the sample preparation routine:
   a. Add a Transfer From Sample To Sample prep operation to the routine:
      i. In the Prep Operations area, select the *Transfer From Sample To Sample* preparation operation from the list.
      ii. The parameters for the Transfer From Sample To Sample preparation operation are listed below the Add To Task List button.
      iii. Keep all the parameters for the Transfer From Sample To Sample preparation operation set to the default settings except those that are shown below and are reflected in Figure 63.

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

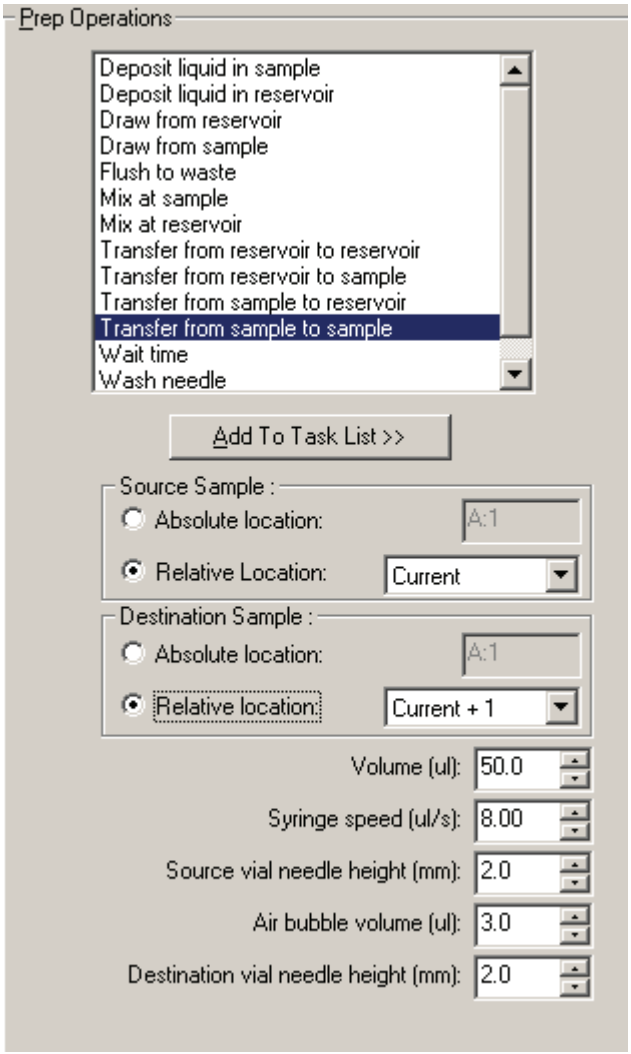


Figure 63. Parameters for Transfer From Sample To Sample preparation operation

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

When this task is performed, 50 µL of sample is withdrawn from the current vial location in the sequence table and deposited in the current + 1 vial location.

b. Add two Transfer from Reservoir To Sample prep operation tasks:

i. In the Prep Operations area, select the *Transfer From Reservoir To Sample* preparation operation from the list.

The parameters for the Transfer From Reservoir To Sample preparation operation are listed below the Add To Task List button.

ii. Keep all the parameters for the Transfer From Reservoir To Sample preparation operation set to the default settings except those that are shown below and are reflected in Figure 64.

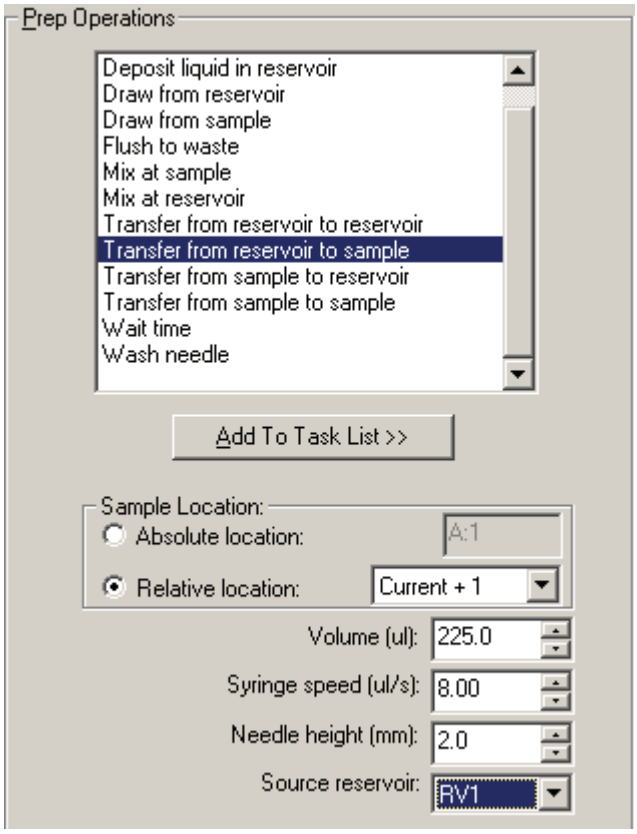


Figure 64. Parameters for the Transfer From Reservoir To Sample preparation operation

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

When this task is performed, 225 µL of diluent is withdrawn from the reservoir vial 1 and deposited 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.

c. Add a Wash Needle prep operation task:

i. In the Prep Operations area, select the *Wash Needle* preparation operation from the list.

The parameters for the Wash Needle preparation operation are listed below the Add To Task List button.

ii. Keep all the parameters for the Wash Needle prep operation set to the default settings as reflected in Figure 65.

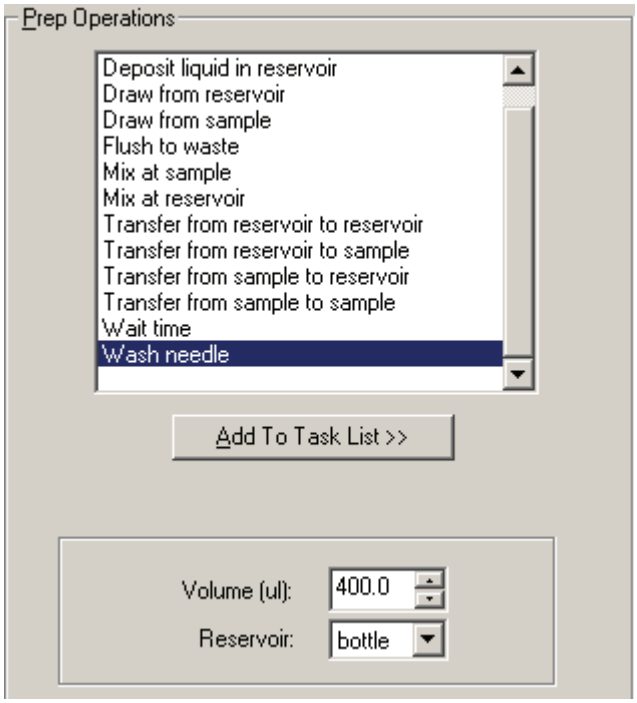


Figure 65. Parameters for the Wash Needle preparation operation

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

The Wash Needle operation is performed at this point in the routine to home the concentric syringe.

d. Add a Mix At Sample prep operation task:

i. In the Prep Operations area, select the *Mix At Sample* prep operation from the list.

The parameters for the Mix At Sample preparation operation are listed below the Add To Task List button.

ii. Keep all the parameters for the Mix At Sample prep operation set to the default settings except those that are shown on page 107 and are reflected in Figure 66.

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

When this operation is performed, the solution in the current + 1 vial location will be aspirated and expunged 10 times.

Parameter	Setting	Result
Sample Location		
Location	◉ Relative Location	Specifies that sample will 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 will be aspirated and expunged
Cycles	10	Specifies that the sample will be aspirated and expunged 10 times

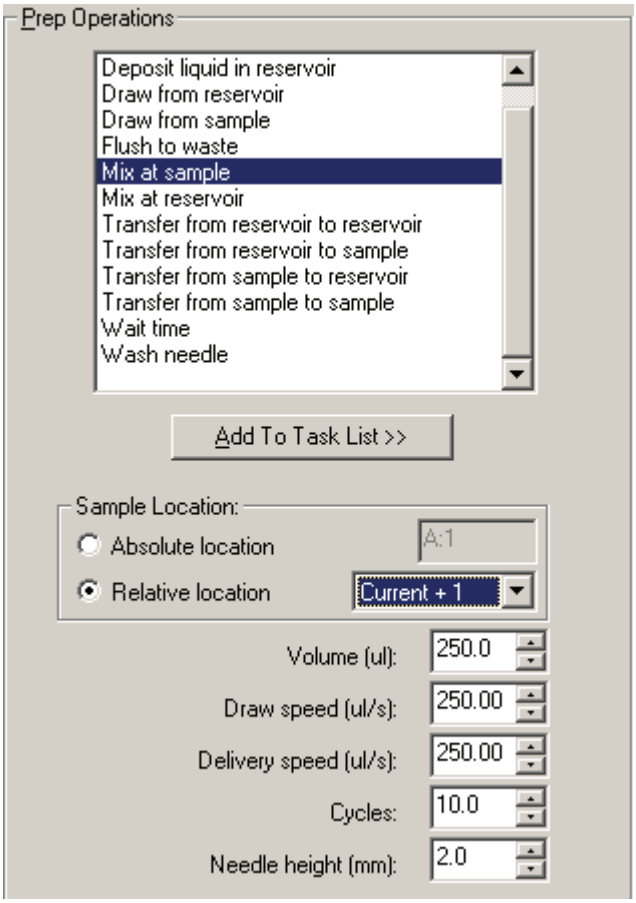


Figure 66. Parameters for the Mix At Sample preparation operation

4. Review the task list by expanding the tasks in the Method list. See Figure 67.

5. Save your method by choosing **File > Save**.

To perform this sample routine, you need to 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 will be injected.

If you want to inject the diluted samples as well, 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.

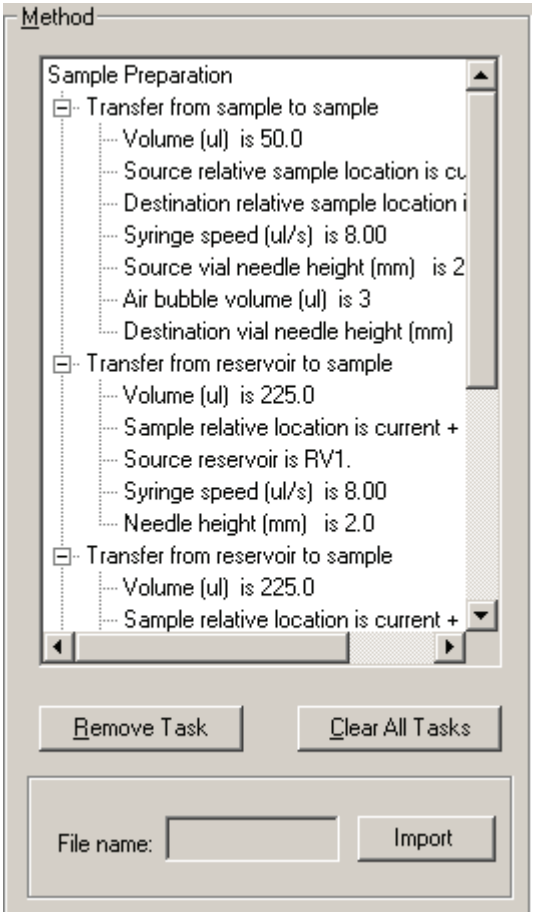


Figure 67. Method area, showing expanded task list

Saving the Method

To save the instrument method as a file of type [.meth]

1. Choose **File > Save As** to open the Save As dialog box. See Figure 68.

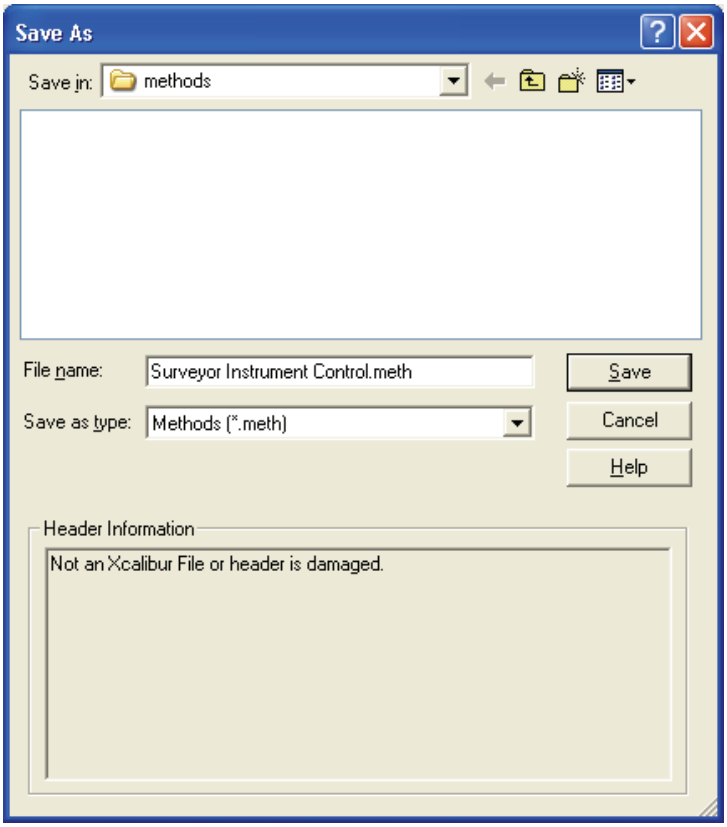


Figure 68. Save As dialog box

2. Browse through the directory tree to find the Drive:\Xcalibur\methods directory.

3. Type a file name in the File Name box.

4. Click **Save** to open the File Summary Information dialog box. See Figure 69.

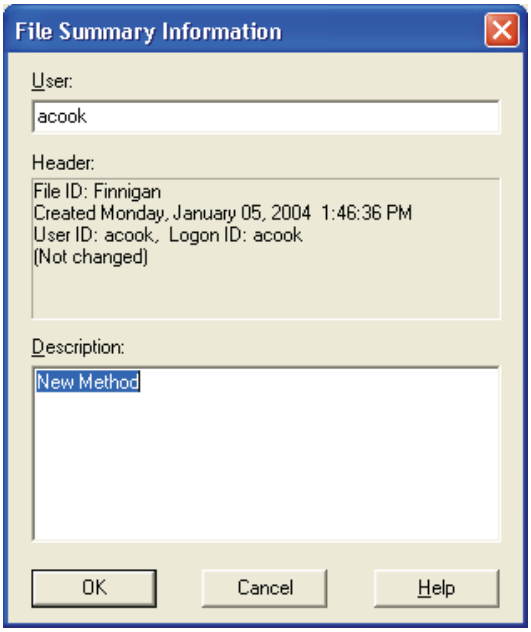


Figure 69. File Summary Information dialog box

5. Type a description of the Method file in the Description box.
6. Click **OK**.

   If the Authorization Manager - Comment check box is enabled, the File Save - Audit Trail dialog box appears. See Figure 70.

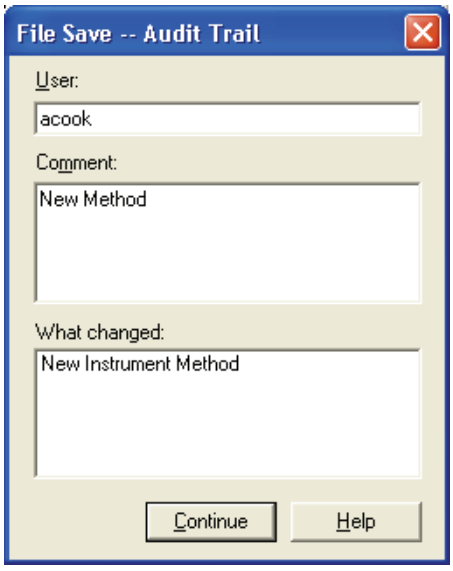


Figure 70. File Save – Audit Trail dialog box

7. Type a comment concerning the changes you made to the instrument method in the Comment box.

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

Chapter 5 Creating and Running Sequences

This chapter describes how to acquire and view chromatographic and PDA spectral data for a single sample and contains the following sections:

Creating a Single Sample Sequence

To set up a Sequence to inject a single sample, perform the following procedures contained in this section in the order listed:

1. Opening the Sequence Setup Window
2. Creating the Sequence
3. Saving the Sequence

Opening the Sequence Setup Window

To open the New Sequence Template Dialog box

1. Click the **Sequence Setup** button on the Home Page to open the Sequence Setup view.

   A view similar to that in Figure 71 is displayed.

2. From the **Sequence Setup** window, choose **File > New**.

   Xcalibur displays the New Sequence Template dialog box. See Figure 72.

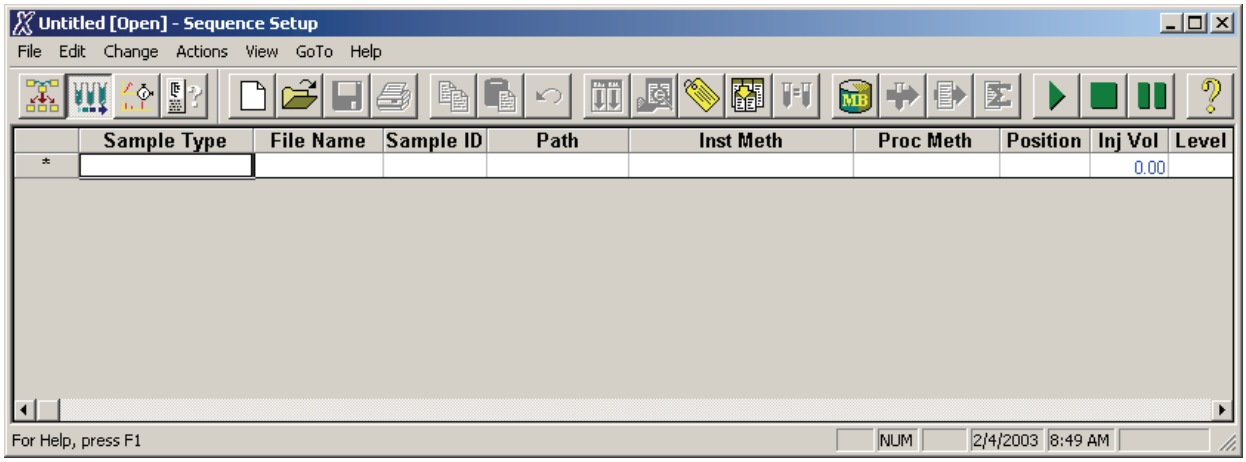


Figure 71. Sequence Setup window

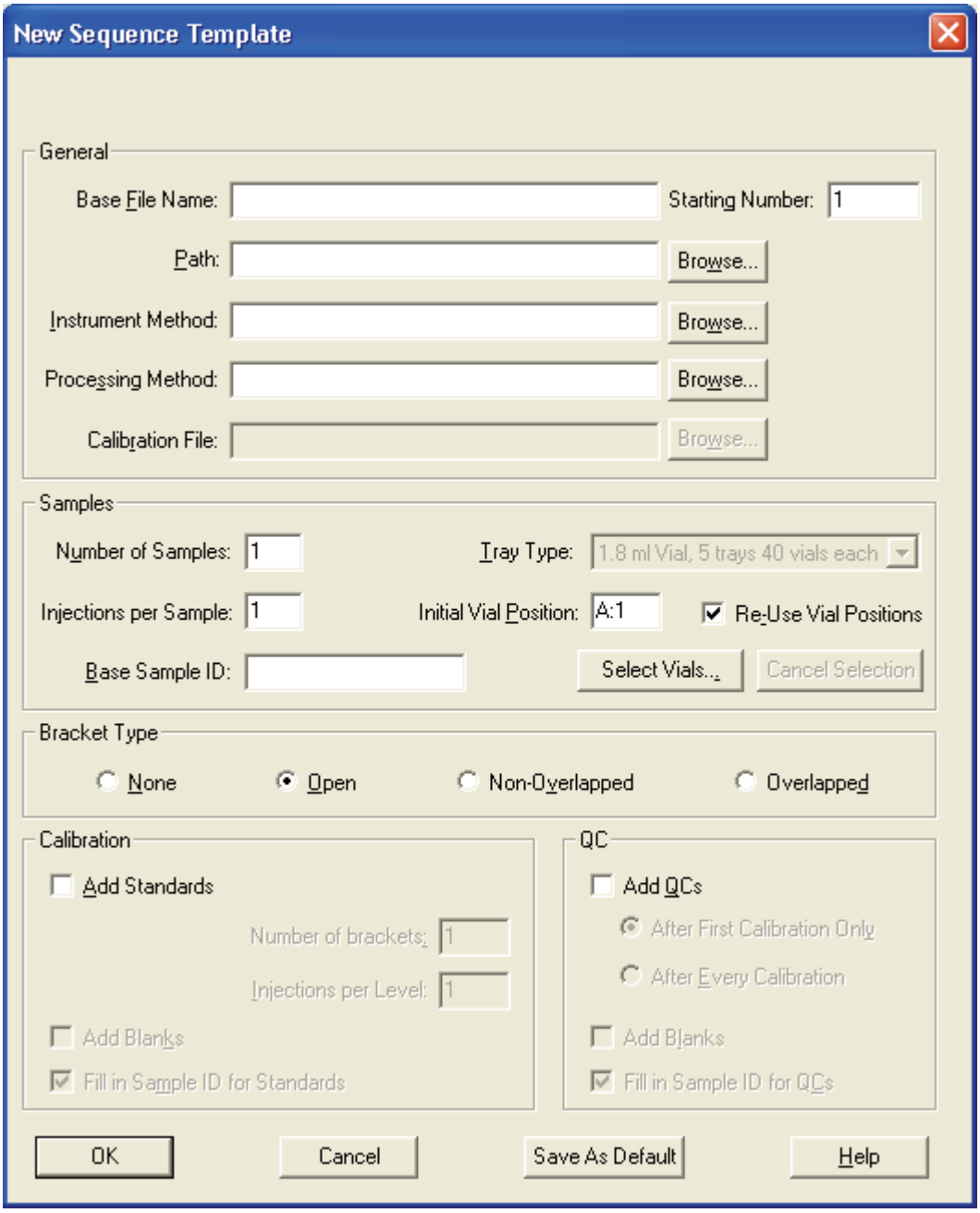


Figure 72. New Sequence Template dialog box

Creating the Sequence

To create a new Sequence

1. In the **General** area, make the following entries and selections:

   a. Type a name for the raw data file in the **Base File Name** box.

   b. Browse to the data file directory where you want to store your raw data files.

      Xcalibur 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 Setup methods have a [.meth] file extension. The Instrument Setup view for the Surveyor devices is described in Chapter 4, "Creating Instrument Methods." .

   d. If you have not yet created a processing method that contains the information needed to quantitate your unknowns, leave the **Processing Method** box blank.

      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, see the *Xcalibur Getting Productive: Quantitative Analysis* manual.

2. In the **Samples** area, type the following:

   a. Type **1** in the **Number Of Samples** box.

   b. Type **1** in the **Number Of Injections** box.

   c. Type the vial position in the **Initial Vial Location** box.

      The vial positions for the conventional trays are shown in Figure 73.

   d. Type an identifying name for the sample in the **Sample ID** box.

      This box entry is optional. If you do not enter a Sample ID, Xcalibur automatically uses the vial position as the Sample ID. If you enter a Sample ID, Xcalibur automatically appends the vial position to your entry.

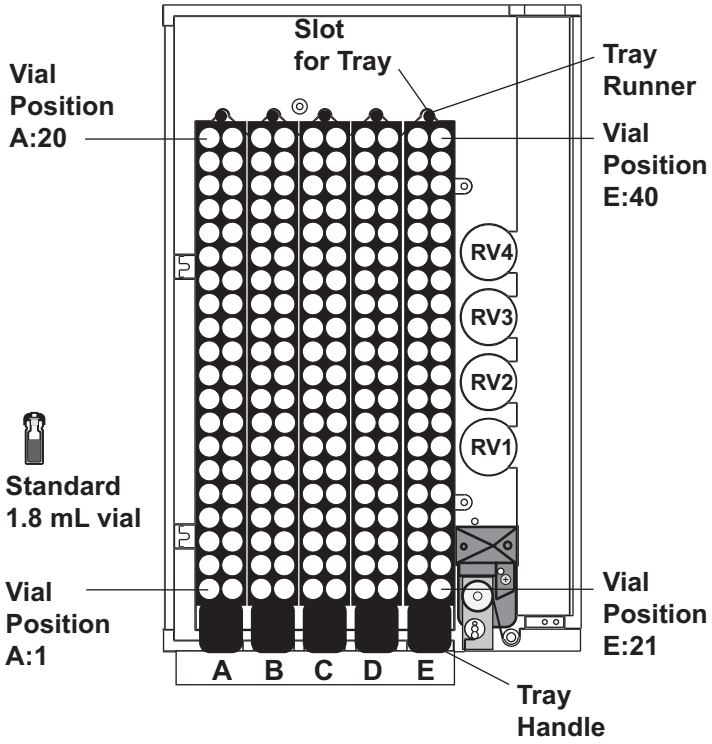


Figure 73. Vial positions for conventional sample trays

3. Click **OK** to display your sequence spreadsheet. See Figure 74.

   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.

4. If you want 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, see the Online Help or your *Xcalibur Getting Productive: Qualitative Analysis* or *Xcalibur Getting Productive: Quantitative Analysis* manuals.

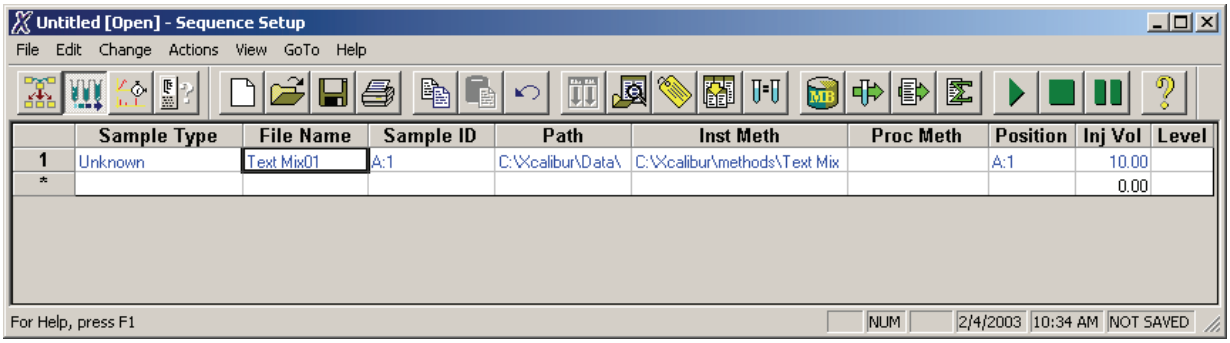


Figure 74. Sequence Setup view, showing newly created one line sequence

5. To alter the current column arrangement, click the **Column Arrangement** toolbar button to display the Column Arrangement dialog box. See Figure 75. Then 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.

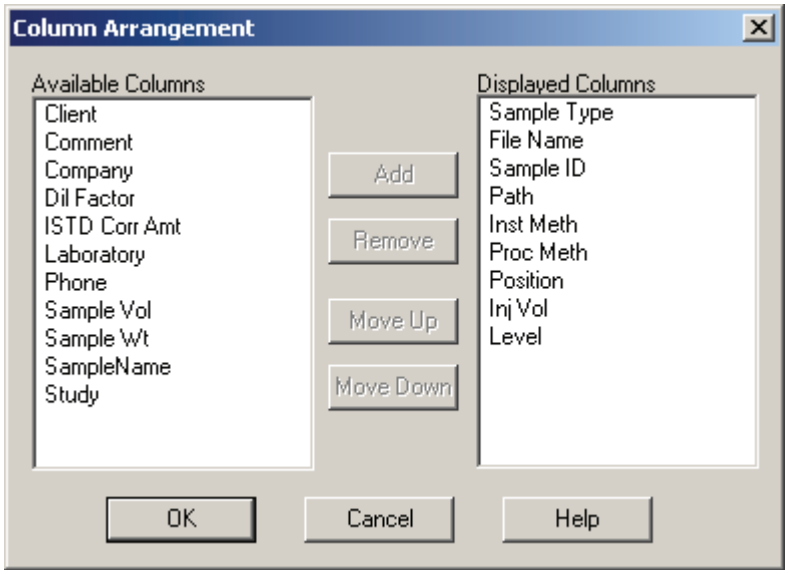


Figure 75. Column Arrangement dialog box

Saving the Sequence

To save the sequence

1. Choose **File > Save As**.

   Xcalibur displays the File Summary Information dialog box. See Figure 76.

2. Type an appropriate description in the **Description** box. Then click **OK**.

   Xcalibur displays the Save As dialog box. See Figure 77.

3. Browse to the appropriate folder in which you want to save the sequence.

4. Type a file name in the **File Name** box.

5. Click **Save**.

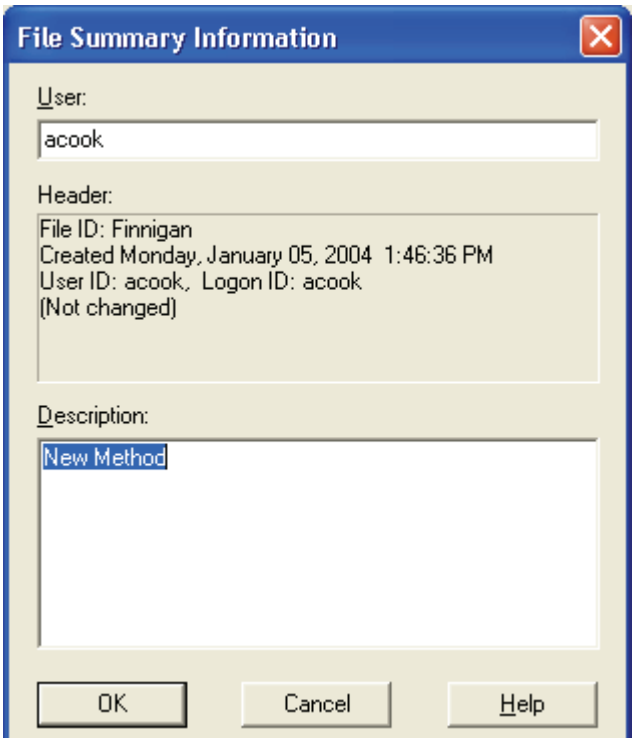


Figure 76. File Summary Information dialog box

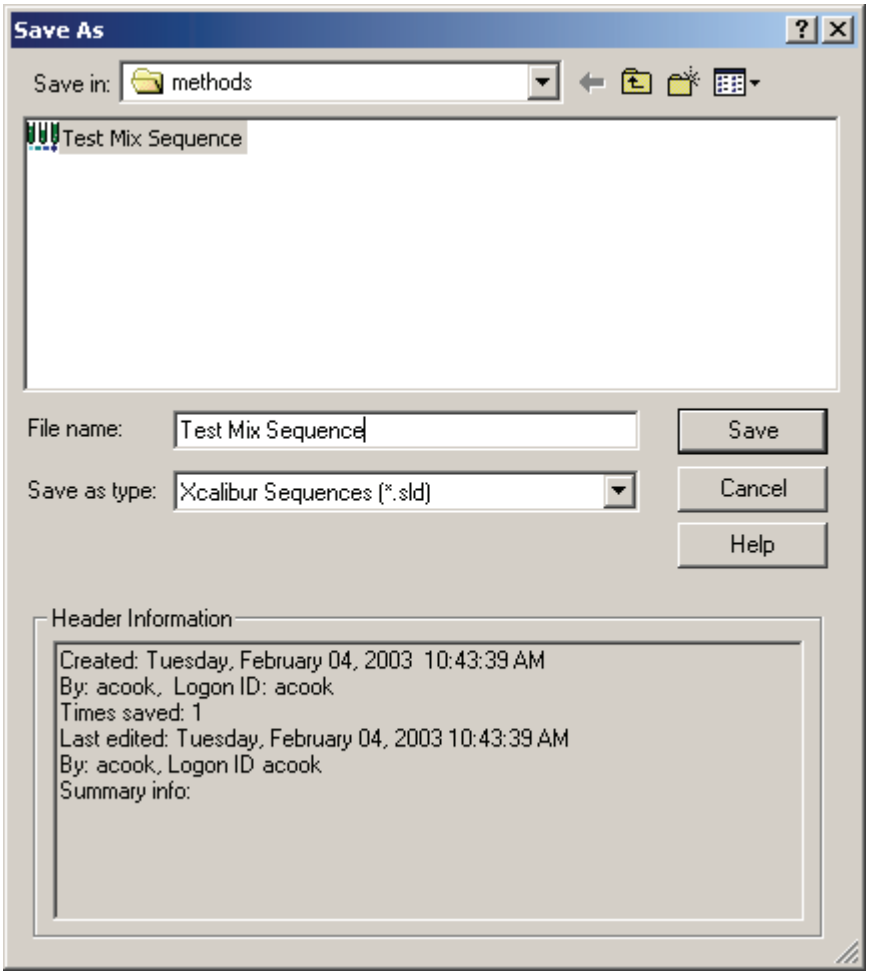


Figure 77. Save As dialog box

Equilibrating Your Column and Warming Up the D2 Lamp

Before you inject your sample, equilibrate your chromatographic column and warm up the deuterium lamp, if necessary. For the best results, warm up the deuterium lamp for a minimum of 1 hour and equilibrate your column for a minimum period of time equivalent to 15 column volumes.

Note Warming up the deuterium lamp and equilibrating your LC column helps to reduce baseline drift.

This section contains the following procedures:

- Warming Up the Deuterium Lamp
- Equilibrating the Chromatographic Column

Warming Up the Deuterium Lamp

To warm up the deuterium lamp of the PDA detector

1. Open the Surveyor PDA Plus Instrument Setup view.
2. Choose **Surveyor PDA Plus > Direct Control**.
3. Click the **Configuration** tab.

   The Configuration page appears as shown in Figure 125 on page 175.
4. In the **Deuterium Lamp** area, click **Turn On**.

To warm up the deuterium lamp of the UV/Vis detector

1. Open the Surveyor UV/Vis Instrument Setup view.
2. Choose **Surveyor UV/Vis > Direct Control**.

   The Direct Control dialog box for the Surveyor UV/Vis Detector appears as shown in Figure 78.
3. In the **Deuterium Lamp** area, click **Lamp On**.

Figure 78. Direct Control dialog box for the Surveyor UV/Vis Detector

Equilibrating the Chromatographic Column

To equilibrate your LC column

1. 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 the LC pump, see Starting the LC Pump Solvent Flow.
   - For the MS pump, see Starting the MS Pump Solvent Flow.

Starting the LC Pump Solvent Flow

To start the solvent flow from the LC pump

1. Click the Surveyor LC Pump button in the viewbar to display the Surveyor LC Pump view.
2. Choose **Surveyor LC Pump > Direct Control > Operation** to open the Operation dialog box. See Figure 79.

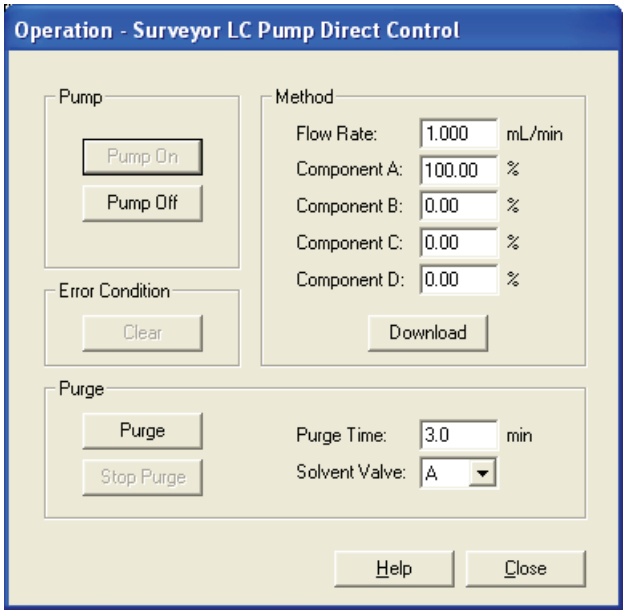


Figure 79. Operation - Surveyor LC Pump Direct Control dialog box

3. In the Method area, enter the appropriate values for the flow rate and the solvent percentages.
4. Click **Download**.
5. Click **Pump On** to start the pump motor.
6. Click **Close** to exit the dialog box.

Starting the MS Pump Solvent Flow

To start the solvent flow from the MS pump

1. Click the **Surveyor MS Pump Plus** button in the viewbar to display the Surveyor MS Pump view.
2. Choose **Surveyor MS Pump Plus > Direct Control** to open the Direct Control dialog box. See Figure 80.

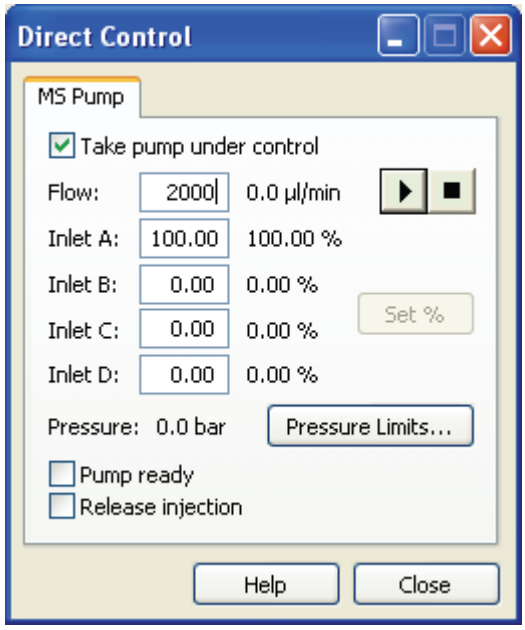


Figure 80. Direct Control dialog box for the MS pump

3. In the Direct Control dialog box, do the following:

   a. Select the **Take pump under control** check box.

   b. Type percentages in the Inlet boxes to create the same mobile phase as the one in the instrument method you plan to use.

   c. In the Flow box, type an appropriate flow rate.

   d. Click the **Start Run** button ▶ to start the pump flow.

   e. After you finish equilibrating the LC column, click the **Stop** button ■ to stop the pump flow.

4. Close the Direct Control dialog box.

Loading the Surveyor 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 injector or the 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-in. If you place vials that fall below this minimum height in the tray, the vial sensor will not detect them, your sequence will be halted, and the message *Vial Not Found* will appear.

To load a conventional tray into the Surveyor Autosampler Plus

1. Open the left door of the autosampler.

   If you selected the Verify Door Is Closed configuration option for the autosampler, the XYZ arm moves to the back of the tray compartment.

2. If the XYZ arm does not move to the back of the tray compartment, use the Position Arm to Access Tray command to move it as follows:

   a. From the Instrument Setup window, click the Surveyor Autosampler button in the Viewbar to display the Surveyor Autosampler view.

   b. Choose **Surveyor AS > Direct Control** to display the Direct Control dialog box.

   c. Click the down-arrow to display the list of commands. Then select **Position Arm To Access Tray** from the list.

   d. Click **Apply** to download and execute the command.

   e. Close the Direct Control dialog box.

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 Home Page – Sequence Setup view, choose **File > Open** to display the Open dialog box. See Figure 81.

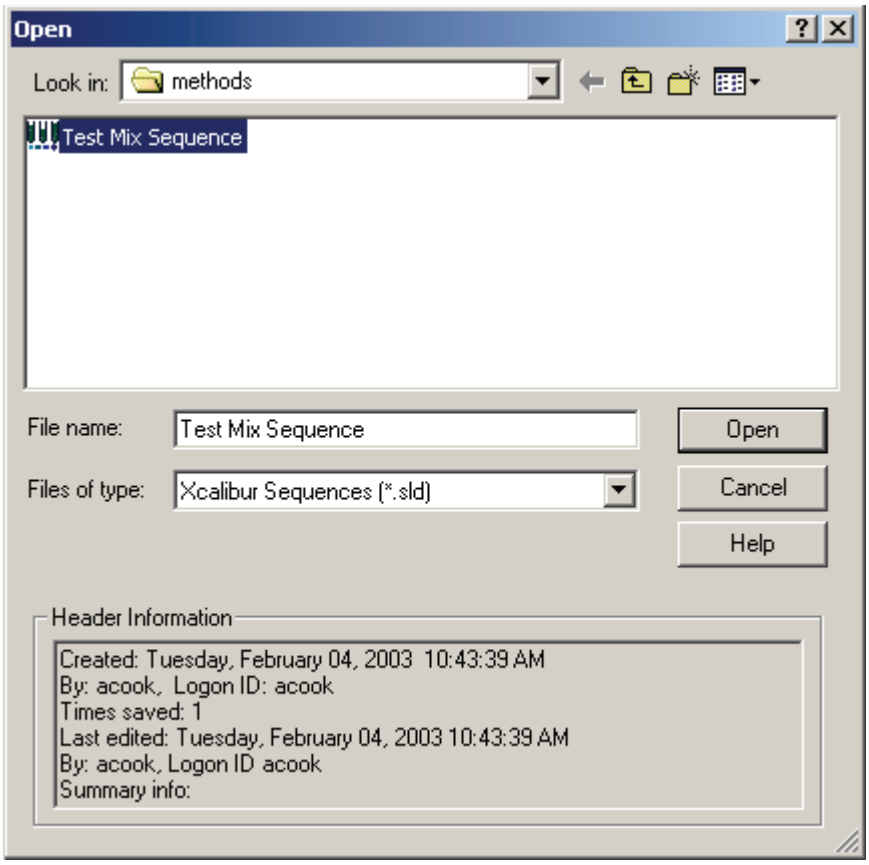


Figure 81. Open dialog box, showing the selection of a Sequence file

   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**.

      Xcalibur opens the selected sequence.

2. Highlight the sequence row that you want to run. Do this even if the sequence contains just one row. See Figure 82.

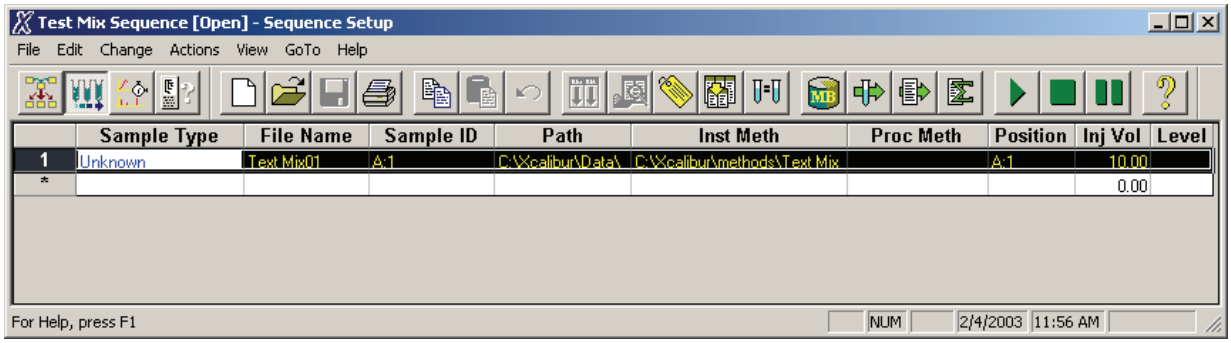


Figure 82. Sequence Setup view, showing sequence with first row selected

3. Confirm that you have a vial in the position specified in the sequence row.

4. From the toolbar, click the **Run Sample** button to open the Run Sequence dialog box. See Figure 83.

   In the Run Sequence dialog box, notice that the User box contains your login name and that the Run Rows box contains the number of the row that you selected in the sequence spreadsheet.

5. In the **Acquisition Options** area, confirm the following:

   a. Confirm that the MS detector and LC components are configured for operation as Xcalibur devices in the Instrument list.

   b. Confirm that the autosampler is configured as the start instrument.

      After the injection valve of the autosampler switches to the inject position, the autosampler sends a signal to the detector to begin data collection.

Note If you want to change the components of your LC system, click Change Instruments to open the Change Instruments In Use dialog box. A list of components that have been configured for operation as Xcalibur devices is displayed. Select the appropriate LC component(s) you want to add to your LC system and deselect the component(s) that you want to remove from your LC system. For more information about the Change Instruments in Use dialog box, see the online Help.

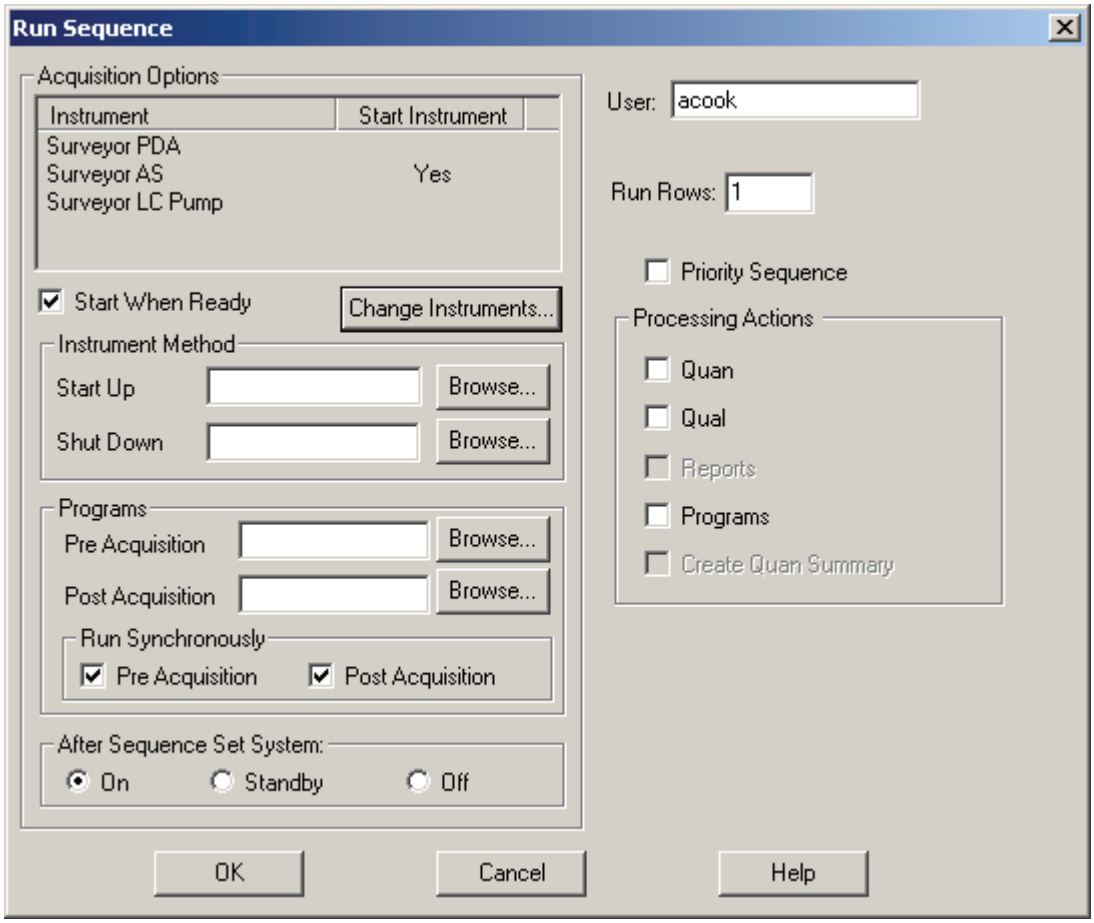


Figure 83. Run Sequence dialog box

c. To start the run automatically, select the **Start When Ready** check box.

   Xcalibur will start the run after you click **OK** in the Run Sequence dialog box. The run begins after the pump sends a pump ready signal to the autosampler. The MS 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**.

d. In the **After Sequence Set System** area, select one of the three options:

- Select the **On** option if you want Xcalibur to leave the devices in the On state.
- Select the **Off** option if you want Xcalibur to turn off the pump flow and the lamps of the PDA detector after the data file is acquired.
- Select the **Standby** option if you want to turn off the pump flow but leave the lamps on after the data file is acquired.

6. Leave the parameters in the other areas at their defaults.

   The Instrument Method and Programs areas in the Run Sequence dialog box are used to specify particular acquisition or processing requirements. For full details of these facilities, see the Xcalibur online Help.

7. Click **OK** to start the run.

Viewing the Data As It Is Acquired

To view the data as it is acquired

1. Choose **View > Real Time Plot View** or click the Real Time Plot View toolbar button on the Home Page.

2. If the display is not already locked, click the Lock Display button to lock the display.

Locked

Unlocked

Locking the display allows you to 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 84 is displayed. The view contains three cells: a Chromatogram cell, a Spectrum cell, and a Total Scan cell.

The sample shown in Figure 84 contains a mixture of uracil, benzene, toluene, anthracene, and pyrene. The peak for pyrene is eluting at 7.5 min. The PDA detector parameters for the data file being acquired are as follows:

- Run Time: 10.0 min
- Rise Time: 1.0 s
- Scan Range: 220 nm - 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

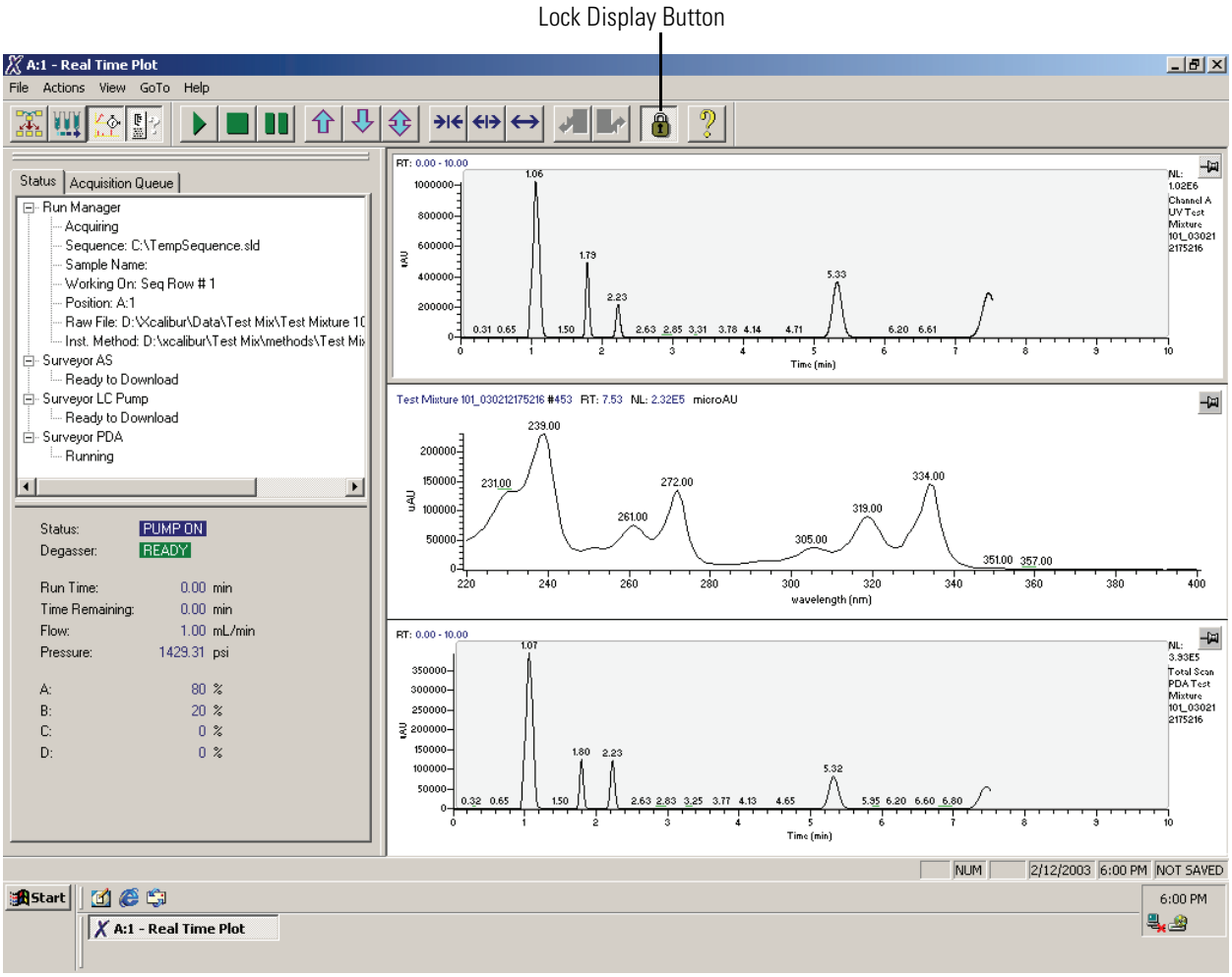


Figure 84. Real Time Plot view, showing the acquisition of PDA scan data and one discrete channel

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. Unlock the display by clicking 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 will now affect the view displayed in the Spectrum cell.

3. Click the peak of interest in the **Chromatogram** cell.

   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 will now affect the view displayed in the Chromatogram cell.

6. Click the wavelength of interest in the **Spectrum** cell.

   In the Chromatogram cell that contained the Total scan chromatogram, a scan chromatogram appears for the specific wavelength that you clicked on.

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 display 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** to open the Chromatogram Ranges dialog box. See Figure 85.

3. For each cell that you want to add, do the following:

   a. Select a **Type** check box.

   b. Select a detector from the **Detector** list.

   c. Select a Plot Type from the **Plot Type** list.

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

5. Choose **View > Lock Display** to resume monitoring real-time data acquisition.

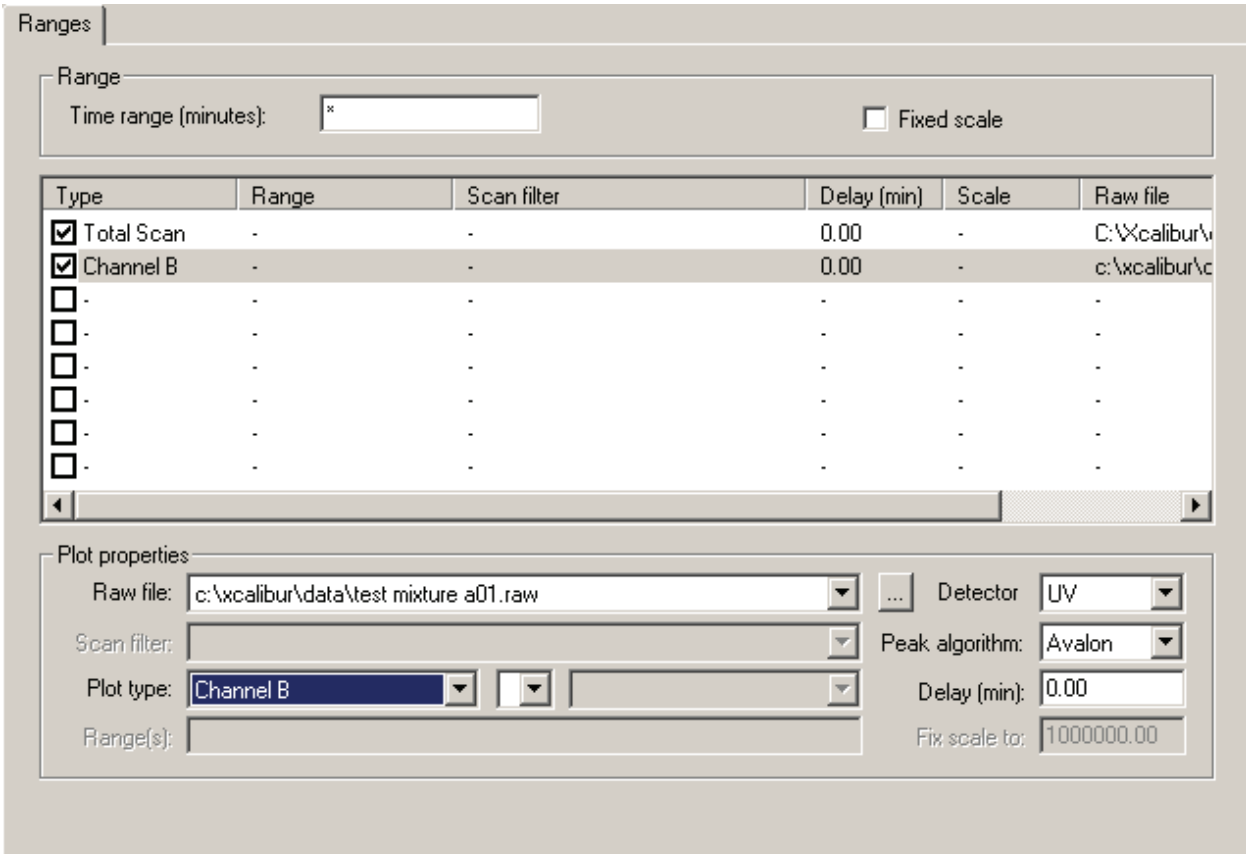


Figure 85. Chromatogram Ranges dialog box

Chapter 6 Using 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.

This chapter contains the following sections:

Opening a Raw Data File in Qual Browser

The 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 on the Home page - Roadmap view or choose **GoTo > Qual Browser** to display the empty Qual Browser window. See Figure 86.

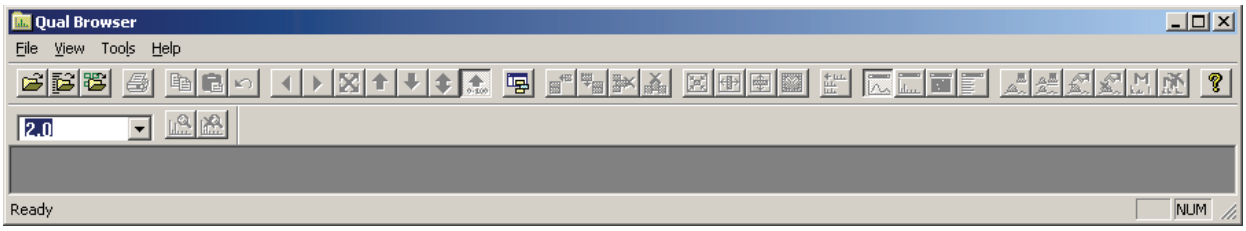


Figure 86. The empty Qual Browser window

2. Choose **File > Open** to display the Open Raw Data File dialog box. See Figure 87.

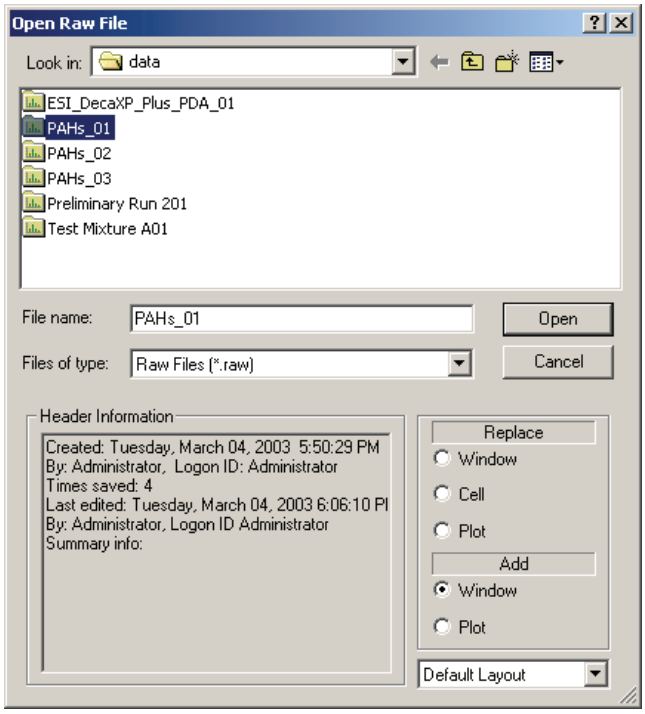


Figure 87. Open Raw File dialog box

3. Select the .raw file that you want to review.

4. Select the window layout from the list at the bottom of the dialog box:

   - Select **Current Layout** if you the current layout for the Qual Browser window is different from the default layout and you want to apply it to your data file.

   - Otherwise, leave the window layout selection at its default of Default Layout.

5. Click **Open** to open the raw file in the Qual Browser window.

   If the default layout has not been modified and your raw file contains MS data in addition to PDA data or UV data, the data file will open 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 140 for an explanation of cells.The Y-axis for these cells is set to Relative Absorbance. See Figure 88.

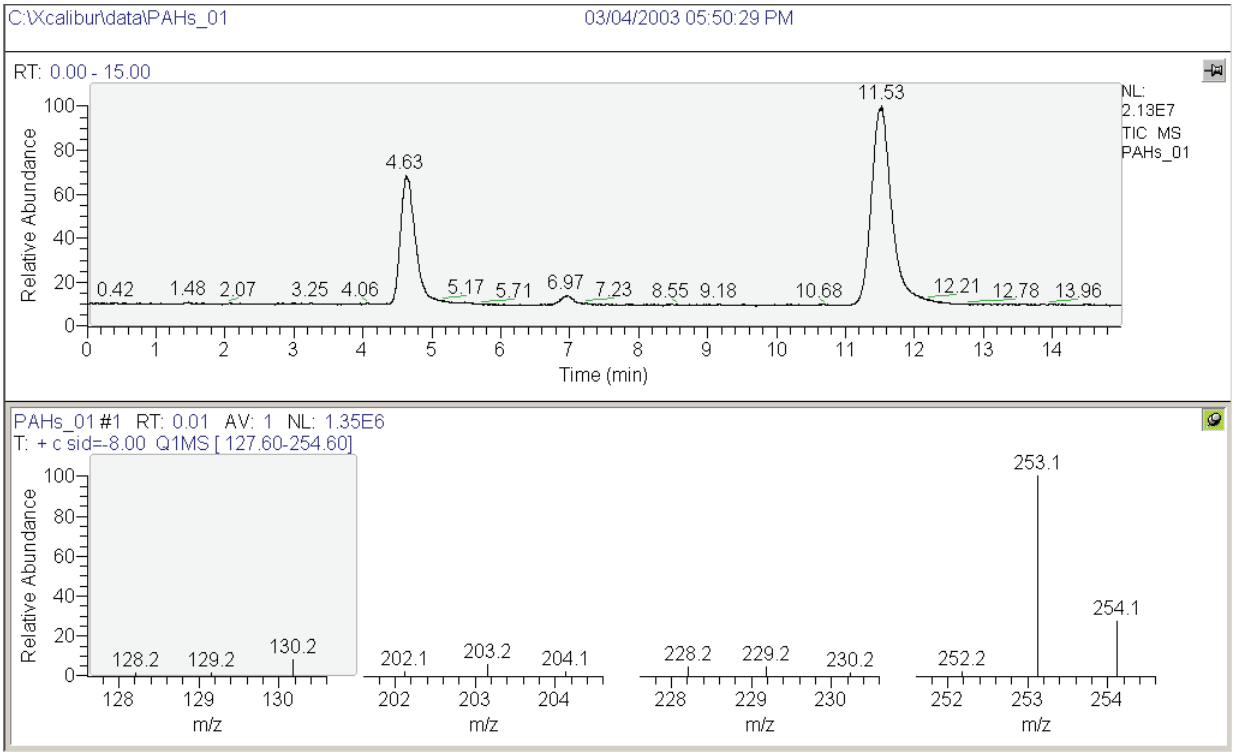


Figure 88. Qual Browser view with a chromatogram cell displaying MS TIC data and a spectrum cell displaying mass range data

6. To apply a custom window layout to the Qual Browser view:

   a. Choose **File > Layout > Apply** to open the Open Layout File dialog box shown in Figure 89.

   b. Select a layout file from the list.

   c. Click **Open** to apply the layout to the [.raw] data file.

      See Figure 90, which 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.

      See "Creating a Layout for PDA Data" on page 149 for instructions on how to create a Layout file for your PDA data.

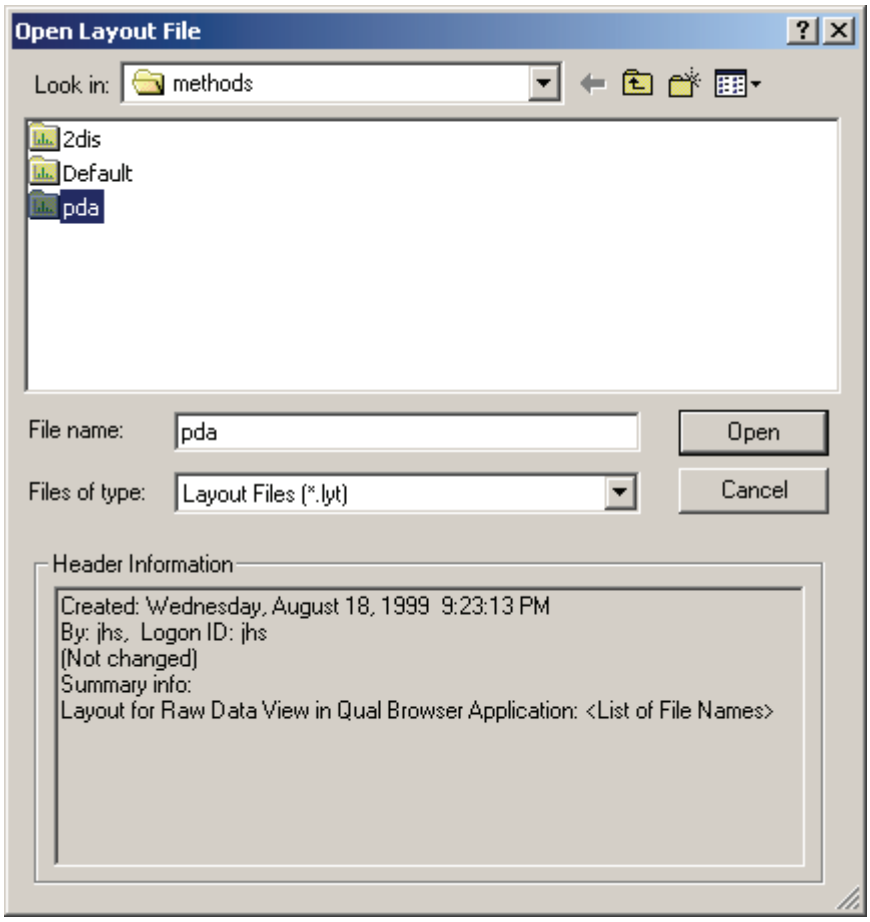


Figure 89. Open Layout File dialog box

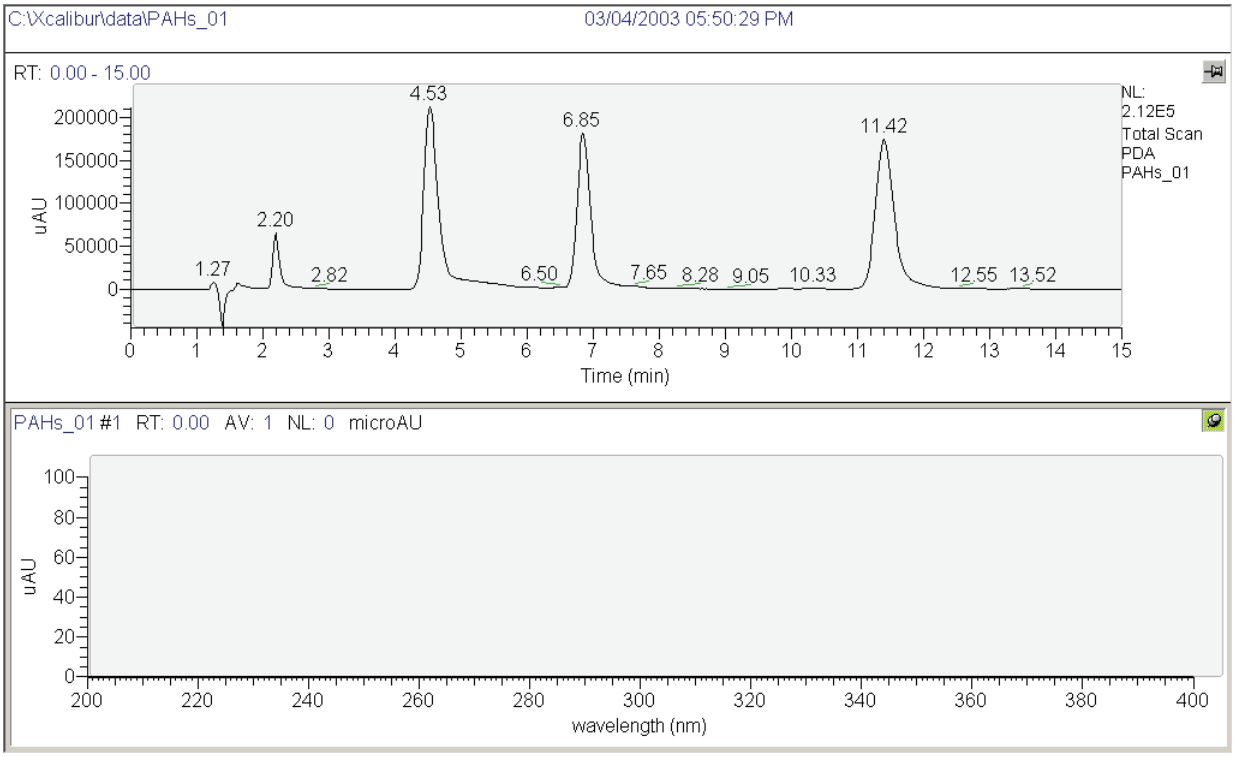


Figure 90. Qual Browser view, showing a chromatogram cell displaying a Total Scan from the PDA detector and a spectrum cell displaying the spectral data from the PDA detector for time 0

Working with the Cell Grid

To use the Qual Browser facility, you need to 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, which are described in this section, 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 91 is inactive as indicated by the absence of a gray border. Menu commands, toolbar buttons, and cursor actions do not affect inactive cells. If you want to zoom in on the contents of a cell or access its shortcut menu, you must make it an active cell.

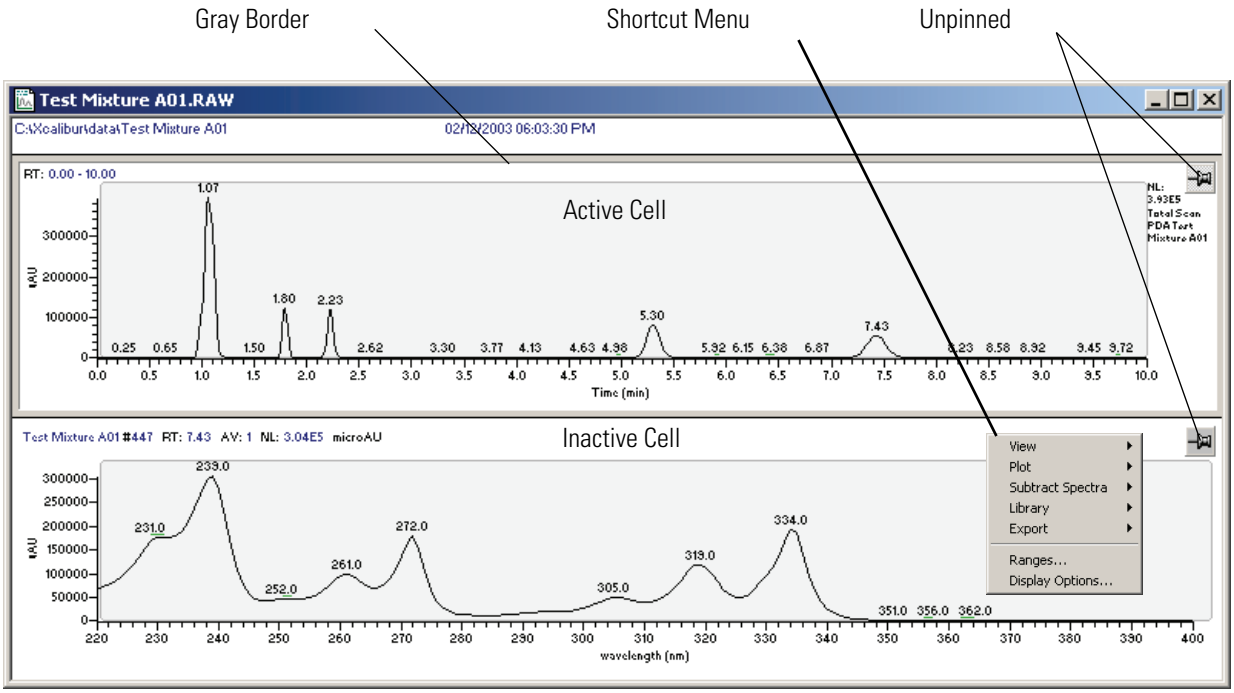


Figure 91. Qual Browser window, displaying an active chromatogram cell and an inactive PDA spectrum cell

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 91 is active but unpinned. Menu commands, toolbar buttons, and cursor actions affect the active cell. Clicking an inactive cell in the grid will make 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 142. The lower cell in Figure 92 is a pinned cell.

If you want to automatically change the range of a cell by clicking in the grid, you must pin the cell. For example, if you want to display the spectrum for the 1 min time point without opening the Spectrum Ranges dialog box, pin the Spectrum cell. Then, click the 1 min time point in the inactive chromatogram cell.

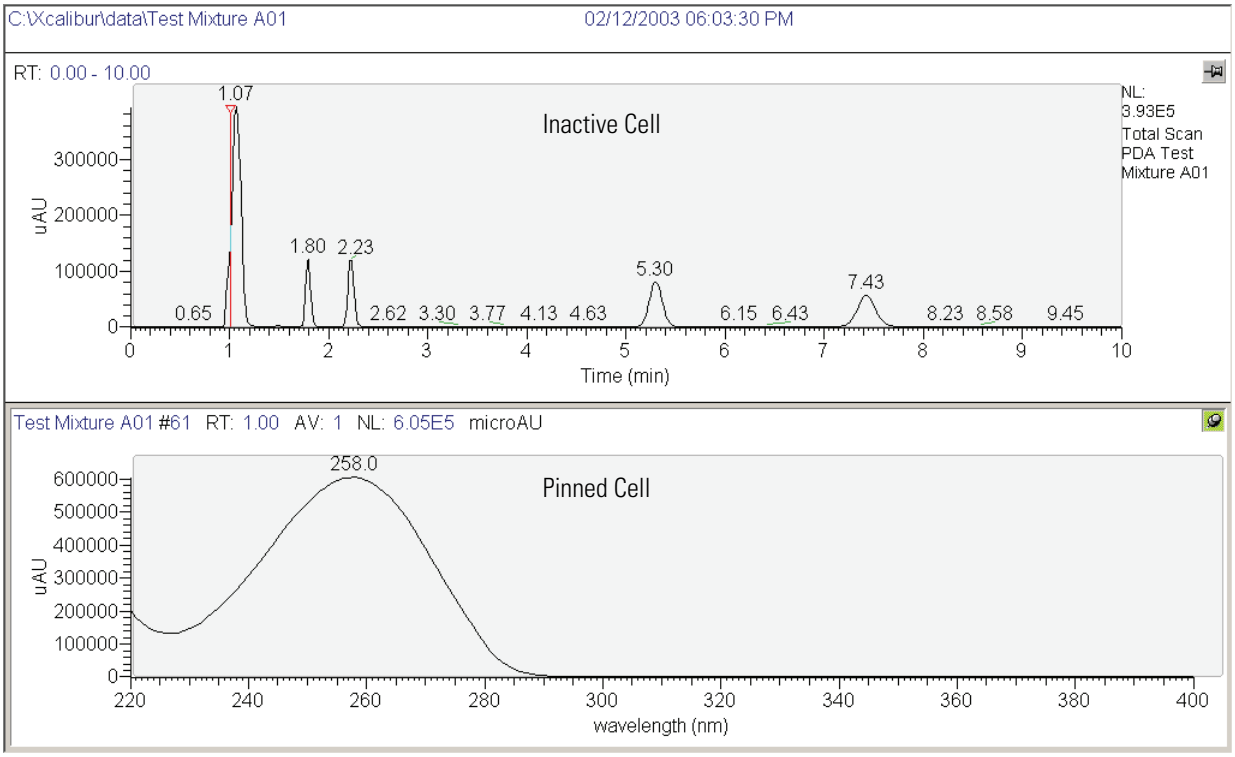


Figure 92. Qual Browser window, displaying an inactive cell and a pinned cell

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 8.

Table 8. Effect of cursor action in an active cell

Cursor Action	Effect
Drag parallel to X-axis	Rescale graph showing selected X range only, same Y range
Drag parallel to Y-axis	Rescale graph showing selected Y range only, same X range
Dragged area	Rescale graph showing both the selected X and Y ranges

If one of the cells is pinned, the cursor action in any of the inactive cells is applied to the pinned cell. See Table 9.

Table 9. 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 93, 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 minute, to be displayed in the pinned Spectrum cell.

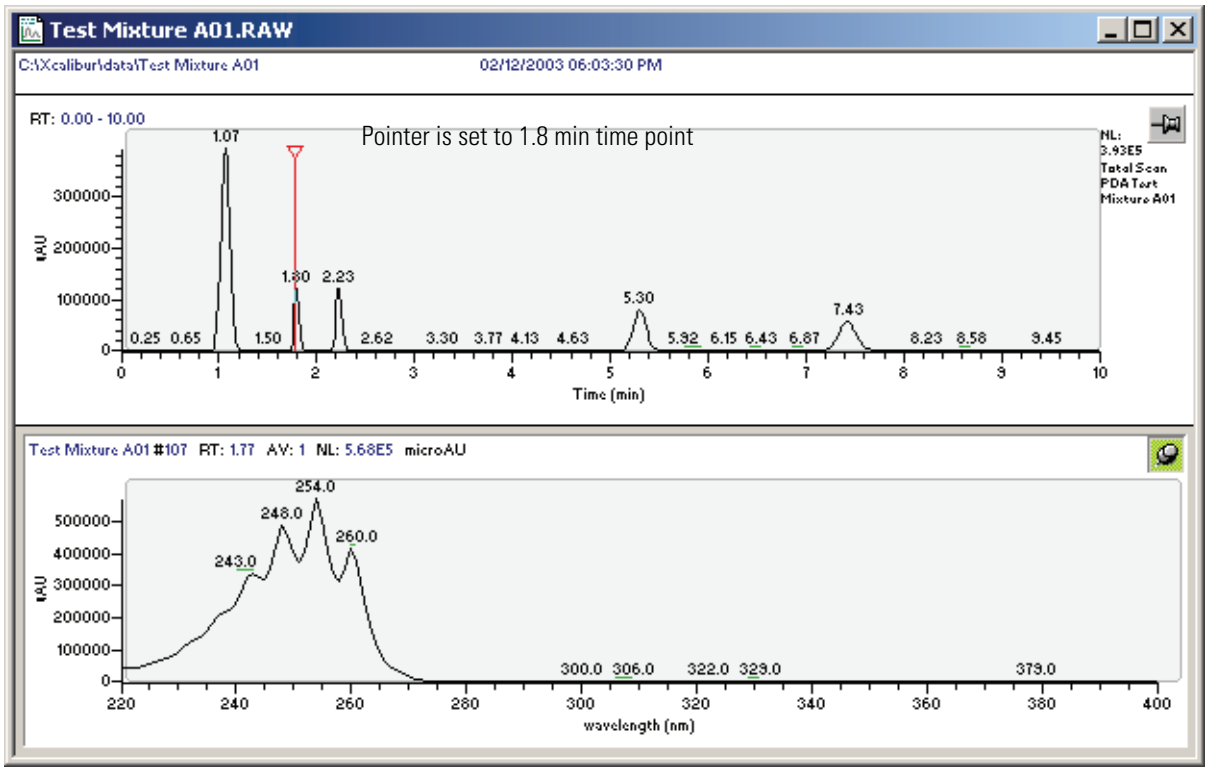


Figure 93. Qual Browser window, showing a pinned Spectrum cell and an unpinned Chromatogram cell, with the cursor pointing to the 1.8 min time point

In Figure 94, the Chromatogram cell on the top of the view is pinned. Clicking the wavelength in the unpinned Spectrum cell displays the chromatogram of the scan wavelength in the pinned Chromatogram cell.

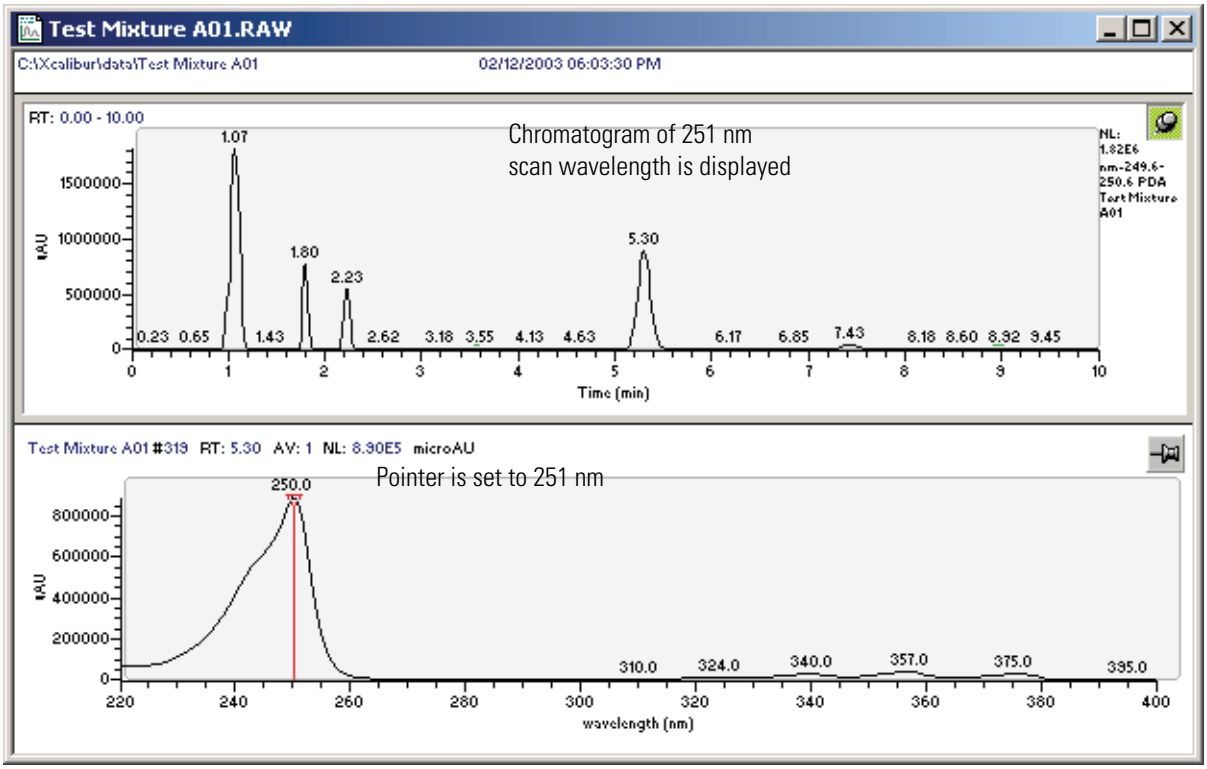


Figure 94. Qual Browser window, showing a pinned Chromatogram cell and an unpinned Spectrum cell, with the cursor pointing to the 251 nm scan wavelength

Clicking the Full Size button in the toolbar sizes the active or pinned cell to the full width and height of the window as shown in Figure 95.

Dragging across a region in the active or pinned cell zooms in on that region as shown in Figure 96.

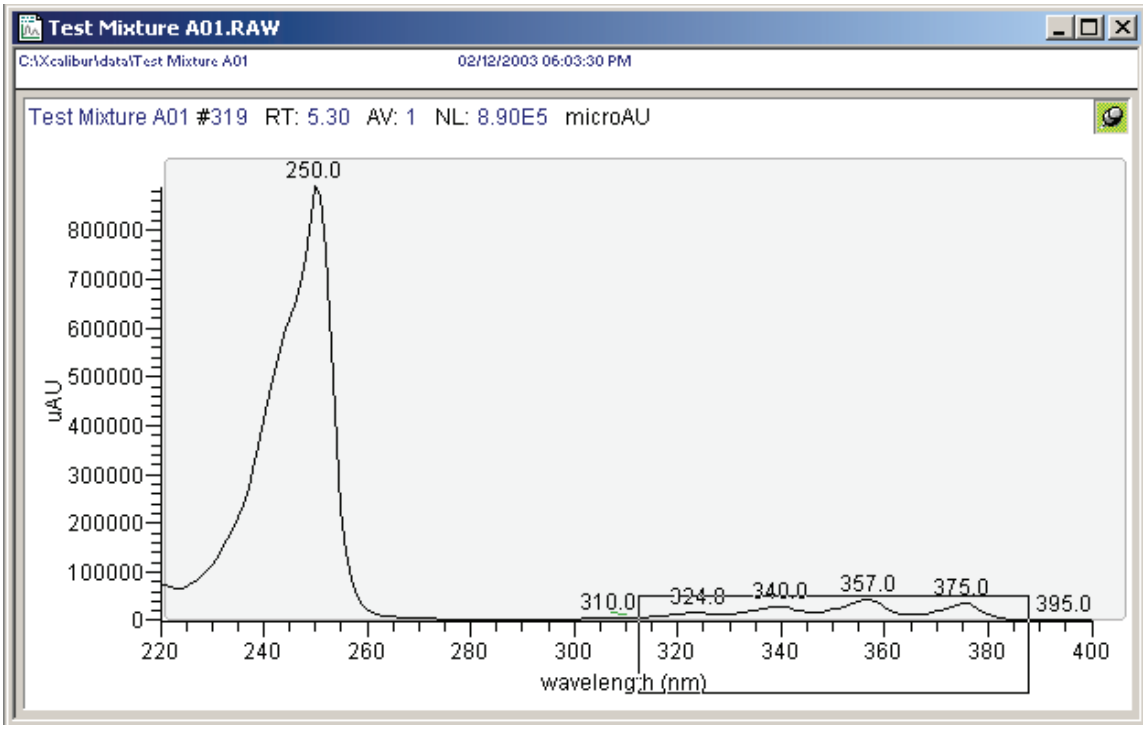


Figure 95. Spectrum sized to full size of window

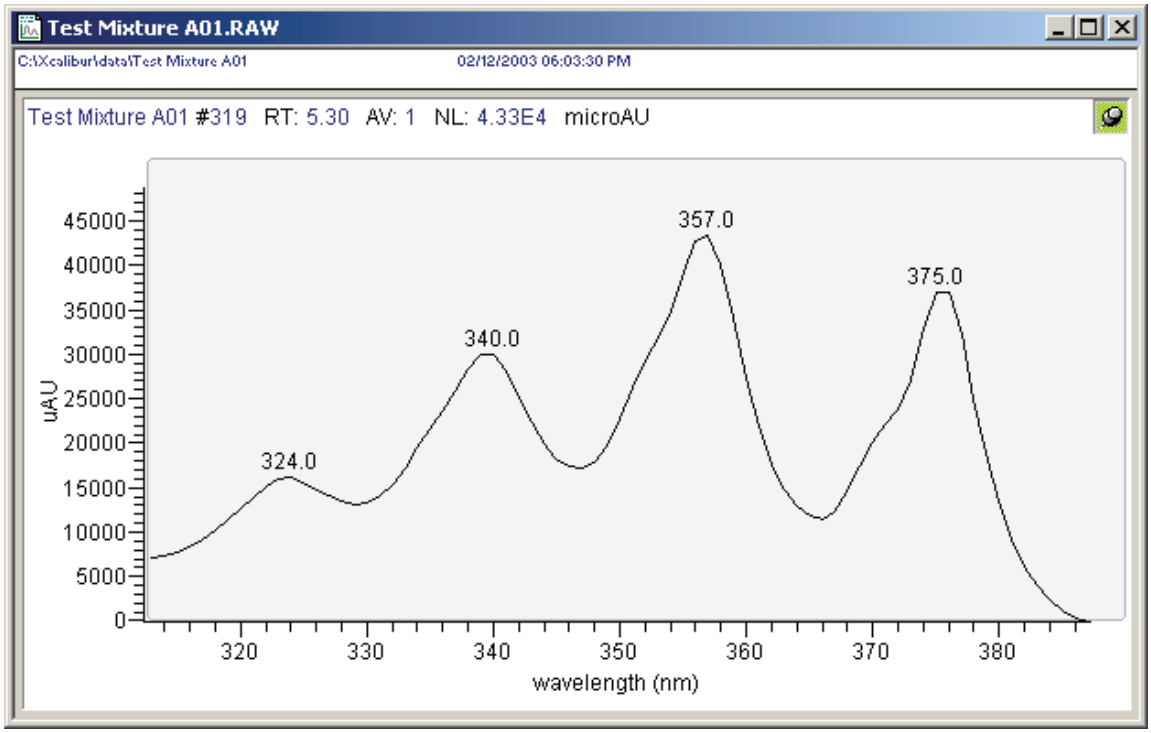


Figure 96. Full size view of Spectrum cell, zoomed in on the 320 nm to 380 nm region

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 Roadmap – Homepage, choose **Tools > Configuration** to open the Xcalibur Configuration dialog box.

2. Click the Fonts tab to display the Fonts page. See Figure 97.

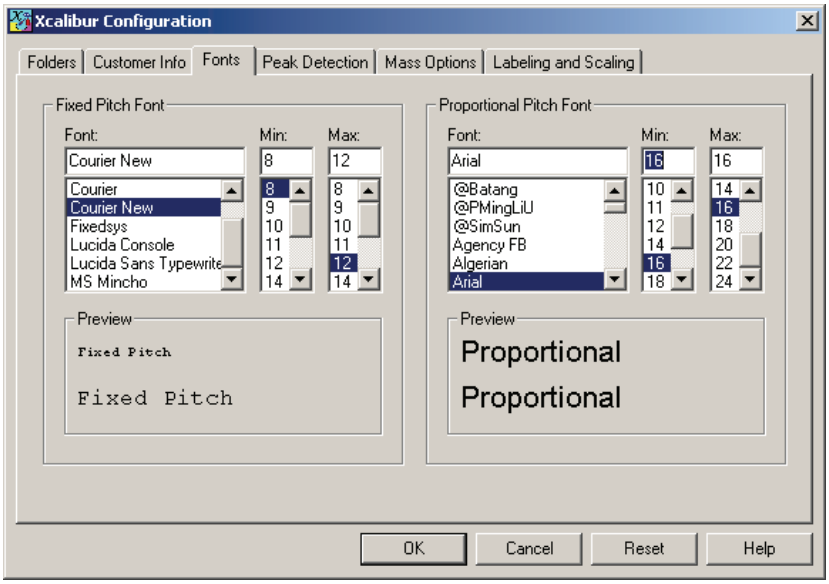


Figure 97. Xcalibur Configuration dialog box – Fonts page

3. To increase the font size of the chromatogram, spectrum, and map axis labels:

   a. In the **Proportional Pitch Font** area, select a larger font size from the **Max** list.

   b. 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, do the following:

   a. In the **Fixed Pitch Font** area, select a larger font size from the **Max** list.

   b. Select a larger font size from the **Min** list.

See Figure 98, which shows the chromatogram axes labeled with a proportional pitch font size of 8 and Figure 99, which shows the chromatogram axes labeled with a proportional pitch font size of 16.

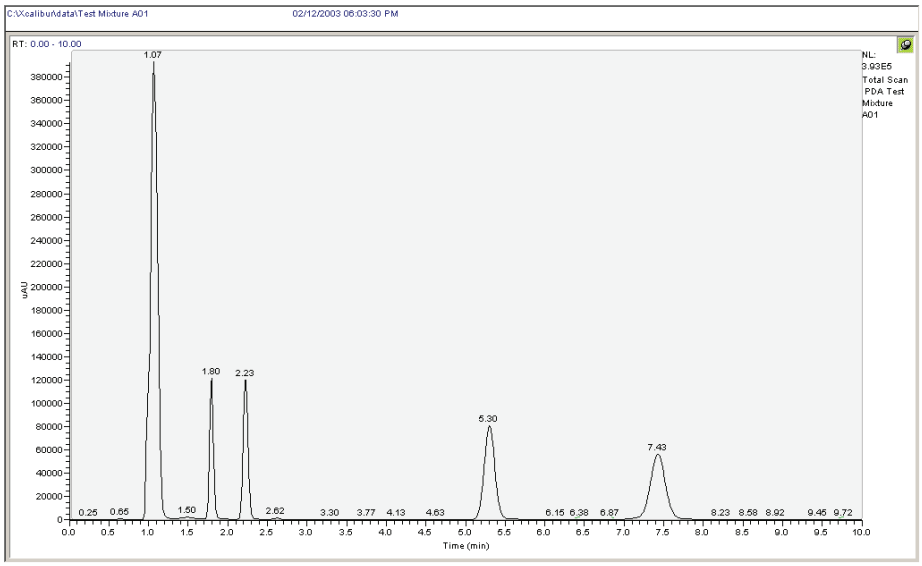


Figure 98. Proportional pitch font size = 8

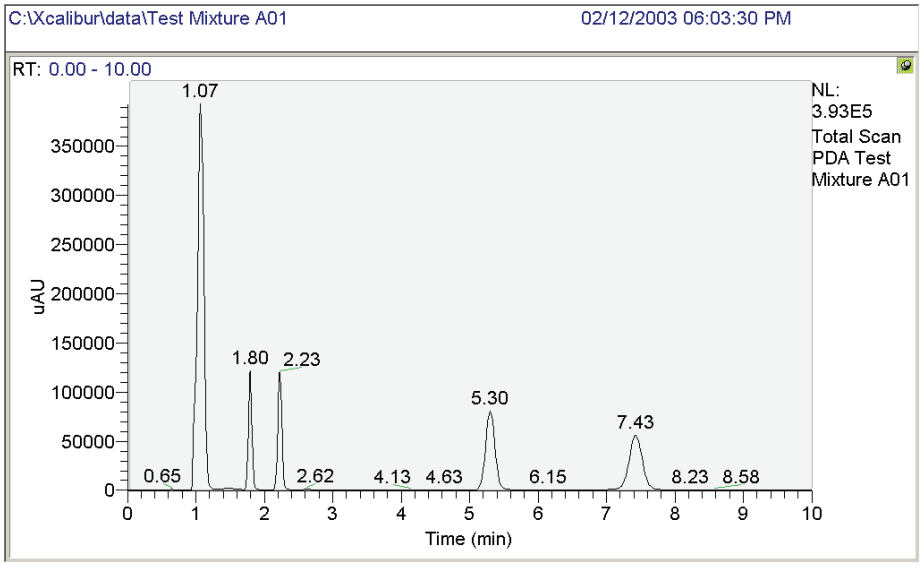


Figure 99. Proportional pitch font size = 16

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 100.

2. Click the **Show Previous** and **Show Next** buttons to move through the pages of your Instrument Method.

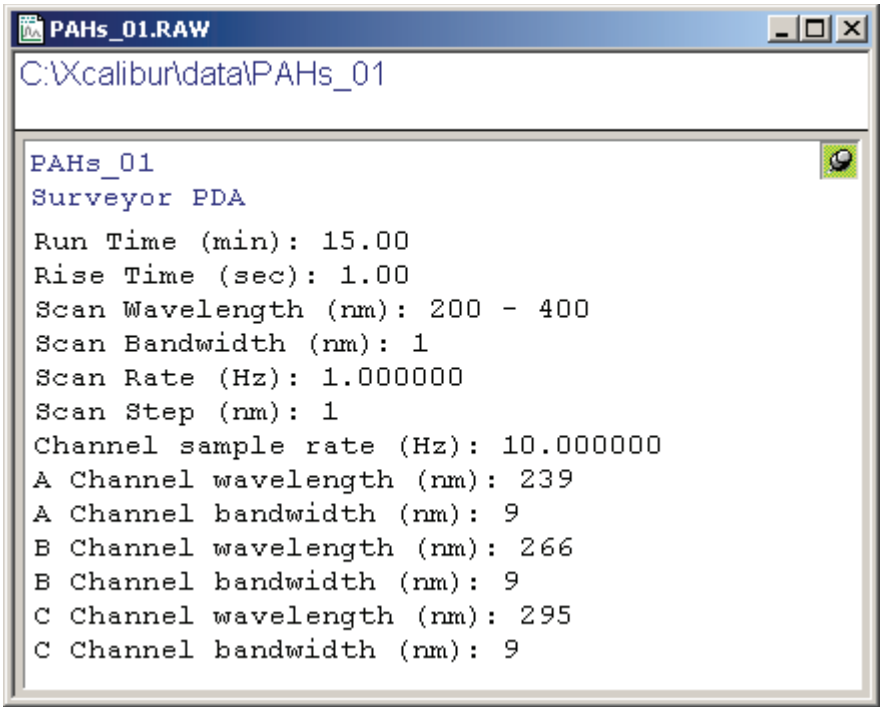


Figure 100. Qual Browser View, displaying the Surveyor PDA page of the Instrument Method Report

Creating a Layout for PDA Data

This topic describes how to view chromatograms and spectral information acquired by the Surveyor PDA detector. In addition, it describes how to save your range and display settings for the PDA data in a Layout file.

Perform the following procedures provided in this section in the order listed:

1. Specifying the Chromatogram Range
2. Setting the Display Options for the Chromatogram Cell
3. Specifying the Spectrum Range
4. Setting the Display Options for the Spectrum View
5. Inserting Cells
6. Saving a New Layout

Specifying the Chromatogram Range

There are two types of chromatograms for the Surveyor PDA: chromatograms acquired from any of the three discrete channels and chromatograms interpolated from the scan data. This topic contains the following procedures that describe how to display the chromatograms acquired by the PDA detector in the Qual Browser window:

- Displaying Scan Chromatograms
- Displaying Discrete Chromatograms

Displaying Scan Chromatograms

To display a scan chromatogram for the PDA detector data

1. Pin the Chromatogram cell.
2. Right-click the **Chromatogram** cell to open a shortcut menu.
3. From the shortcut menu, choose **Ranges** to open the Chromatogram Ranges dialog box. See Figure 101.

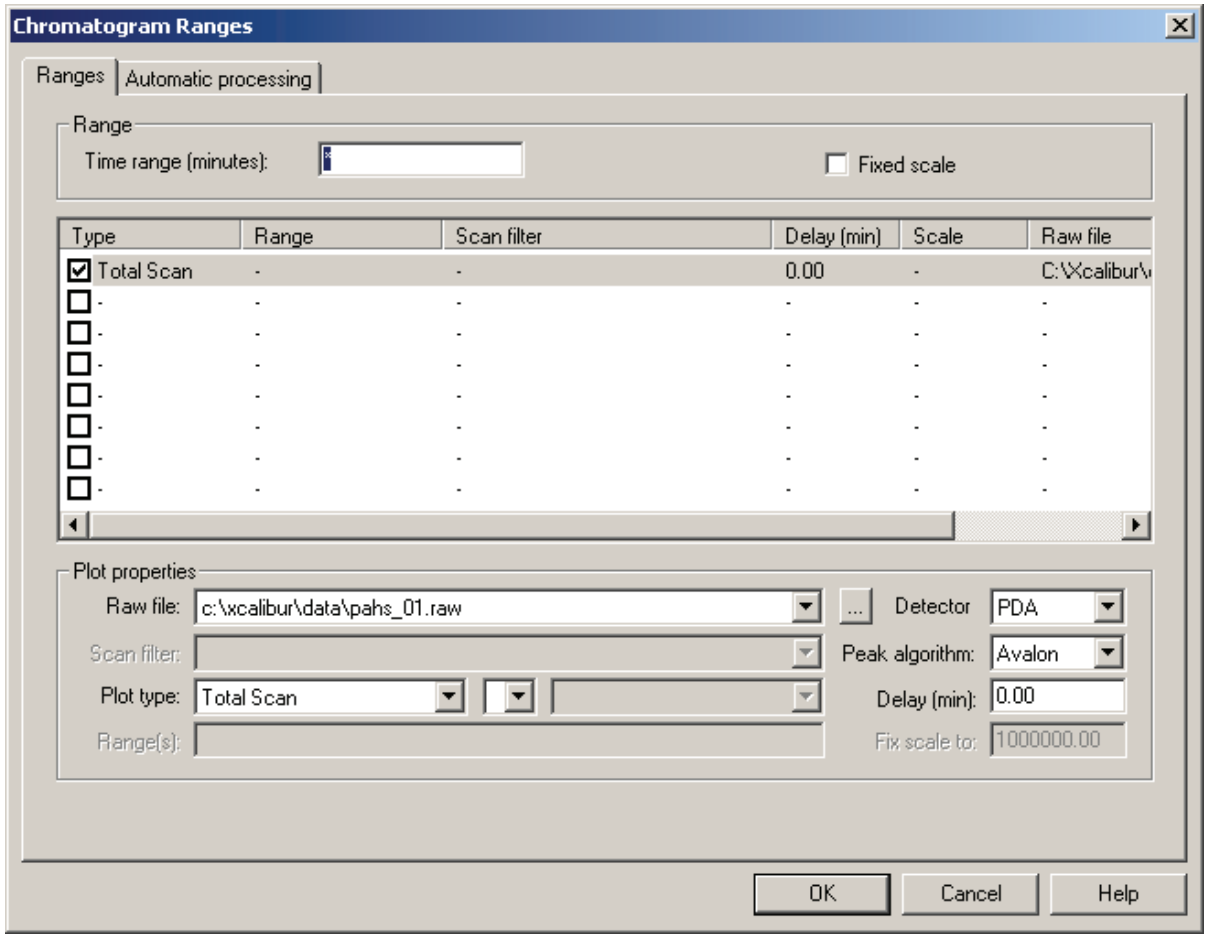


Figure 101. Chromatogram Ranges dialog box, showing the selection of the Total Scan plot type

4. 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.

5. In the **Plot Properties** area, select the following:

   a. Select **PDA** from the **Detector** list.

   b. Select **Avalon** from the **Peak Algorithm** list.

c. Select a Plot Type:

- Select **Wavelength** from the **Plot Type** list 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** from the **Plot Type** list to display the average absorbance for each time point of all the wavelengths in your scan range.

- Select **Spectrum Maximum** from the **Plot Type** list 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** 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** 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.

6. Click **OK** to exit the dialog box and view your scan chromatogram. See Figure 102.

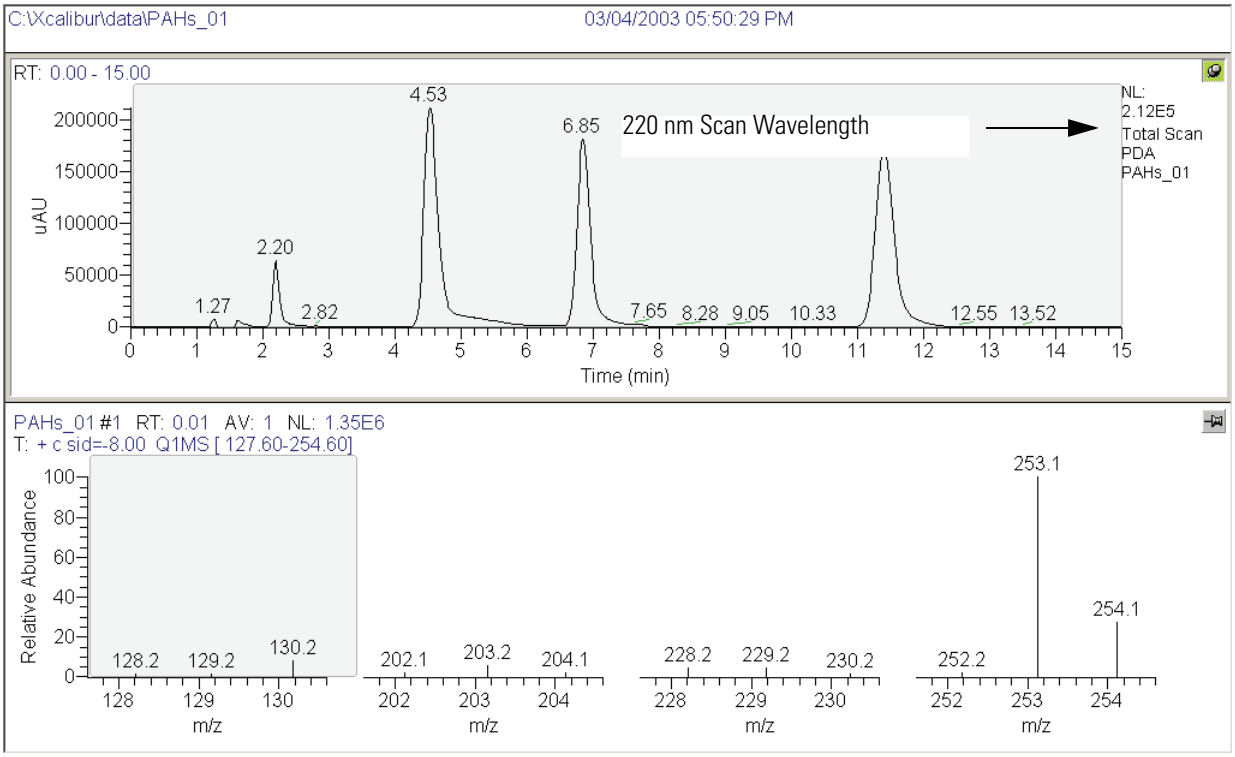


Figure 102. Chromatogram view, showing a chromatogram for a PDA scan wavelength

Displaying Discrete Chromatograms

To display a discrete chromatogram for the Surveyor PDA Plus data

1. Pin the Chromatogram cell.
2. Right-click the Chromatogram cell to open a shortcut menu.
3. From the shortcut menu, choose **Ranges** to open the Chromatogram Ranges dialog box. See Figure 103.

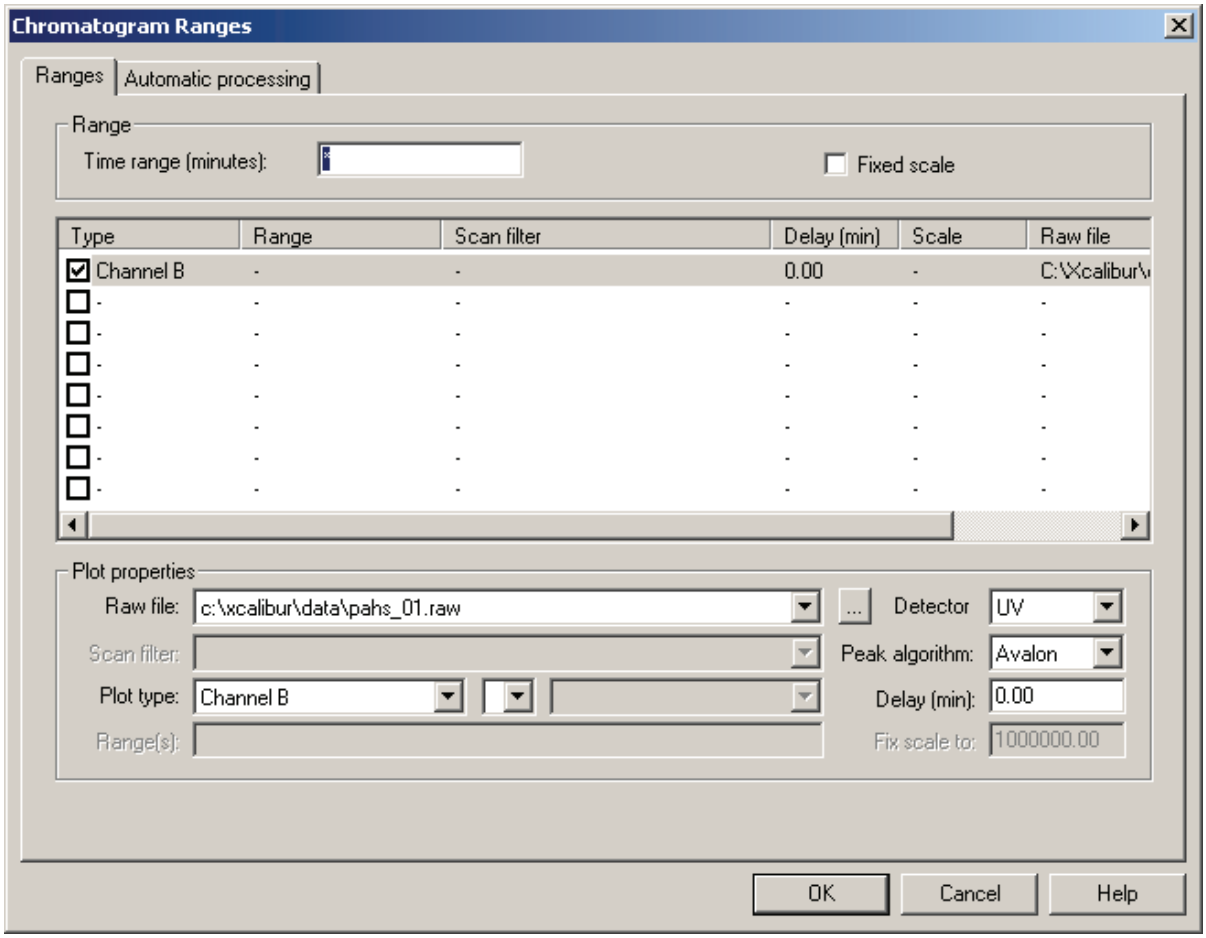


Figure 103. Chromatogram Ranges dialog box, showing the selection of a discrete channel wavelength

4. 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.

5. In the Plot Properties area, select the following:

   a. Select *UV* from the Detector list.

   b. Select *Avalon* from the Peak Algorithm list.

c. Select *Channel A*, *Channel B*, or *Channel C* from the Plot Type list.

See Figure 103, which shows the selection of the discrete channel B wavelength.

6. Click **OK** to exit the dialog box and view your discrete chromatogram. See Figure 104, which shows the display of a discrete channel chromatogram.

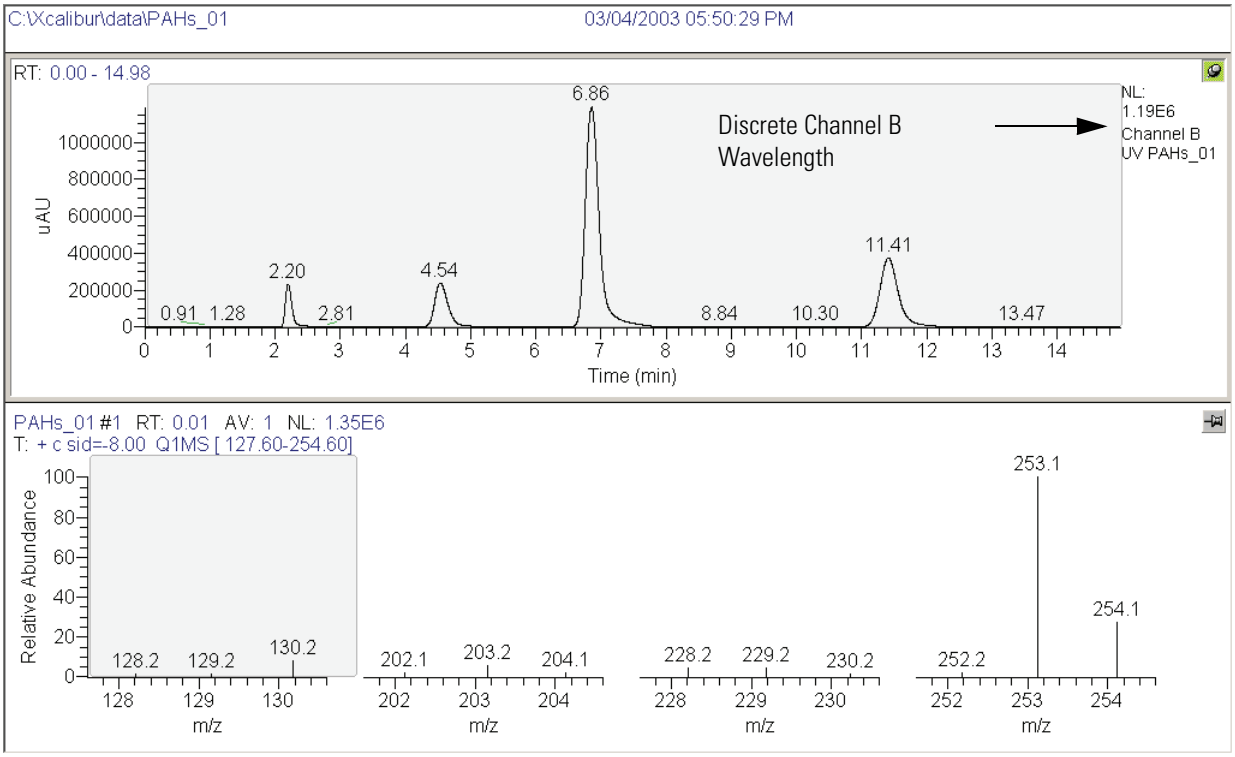


Figure 104. Chromatogram view, showing a chromatogram for a PDA discrete channel

Setting the Display Options for the Chromatogram Cell

To set the display options for the Chromatogram cell

1. Right-click the pinned Chromatogram cell to open a shortcut menu.
2. From the shortcut menu, choose **Display Options** to open the Display Options dialog box.
3. Specify the Normalization parameters:

   a. Click the Normalization tab to open the Normalization page. See Figure 105.

b. In the Normalize Method area, select the Auto Range option button.

This 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 button.

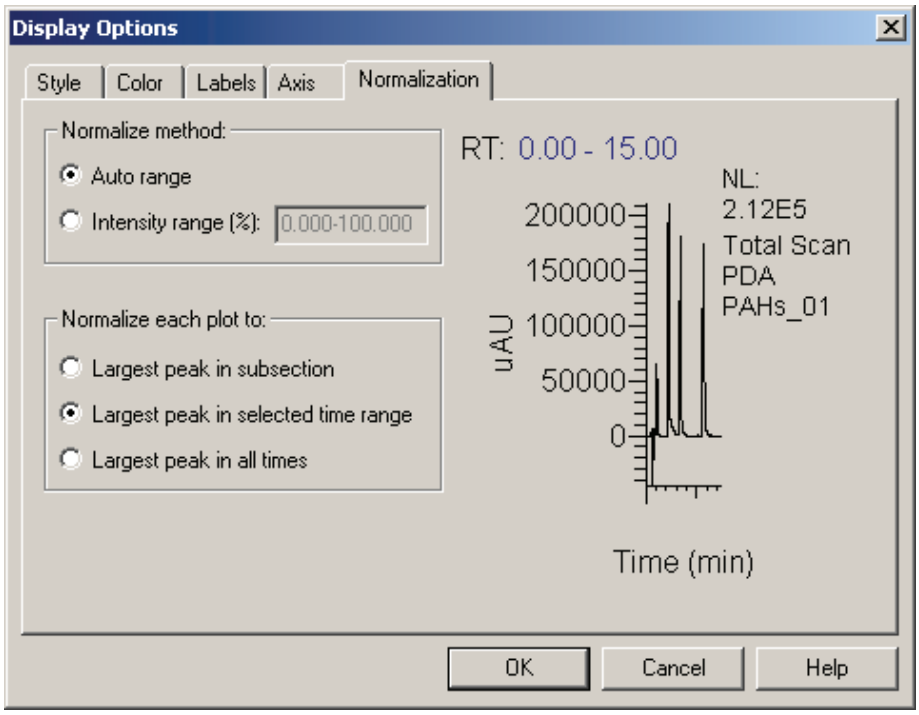


Figure 105. Display Options dialog box – Normalization page

4. Specify the Axis parameters:

a. Click the Axis tab to open the Axis page. See Figure 106.

b. In the Units area, select the Absolute option button.

This sets the Y-axis to the absolute units of µAU.

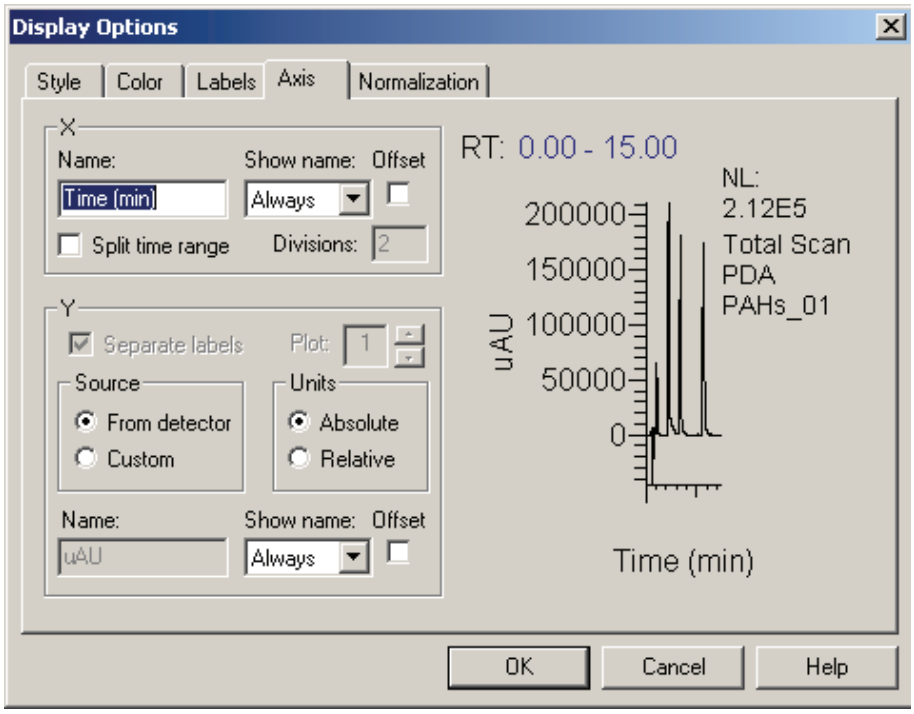


Figure 106. Display Options dialog box – Axis page

5. Specify the labels for the peaks in the chromatogram:

   a. Click the Labels tab.

   b. Select the check boxes associated with the labels that you want to display, such as retention time, area, height, and name.

6. Click **OK** to exit the Display Options dialog box.

Specifying the Spectrum Range

To display a spectrum for the Surveyor PDA Plus data

1. Pin the Spectrum cell.

2. Right-click the Spectrum cell to open a shortcut menu.

3. From the shortcut menu, choose **Ranges** to open the Spectrum Ranges dialog box. See Figure 107.

4. In the Plot Properties area, select *PDA* from the Detector list.

5. For a generic layout file for PDA data, leave the other settings in the Spectrum Ranges dialog box at their defaults as shown in Figure 107.

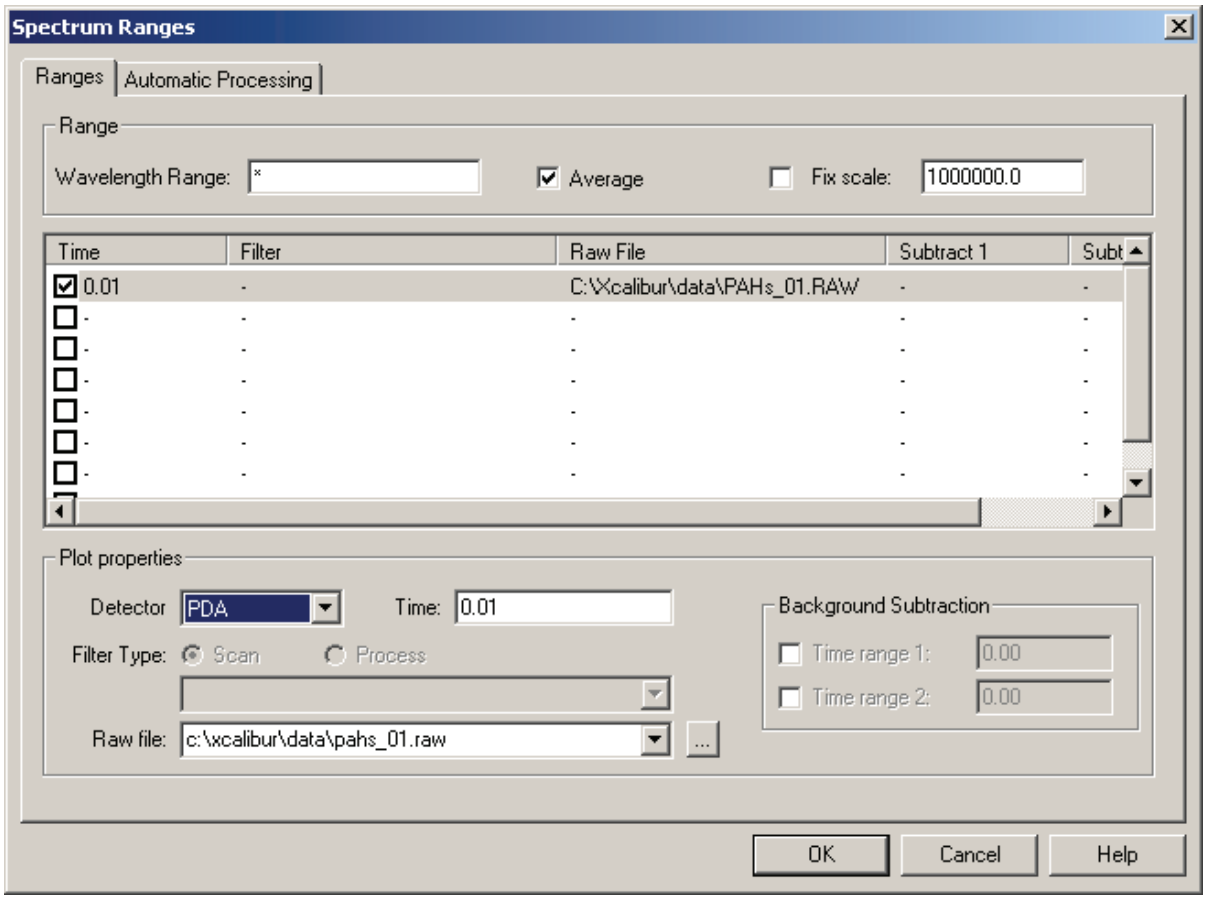


Figure 107. Spectrum Ranges dialog box, showing the selection of the PDA detector

Setting the Display Options for the Spectrum View

To set the display options for the Spectrum cell

1. Right-click the pinned Spectrum cell to open a shortcut menu.
2. From the shortcut menu, choose **Display Options** to open the Display Options dialog box.
3. Specify the Normalization parameters:
   a. Click the Normalization tab to open the Normalization page. See Figure 108.

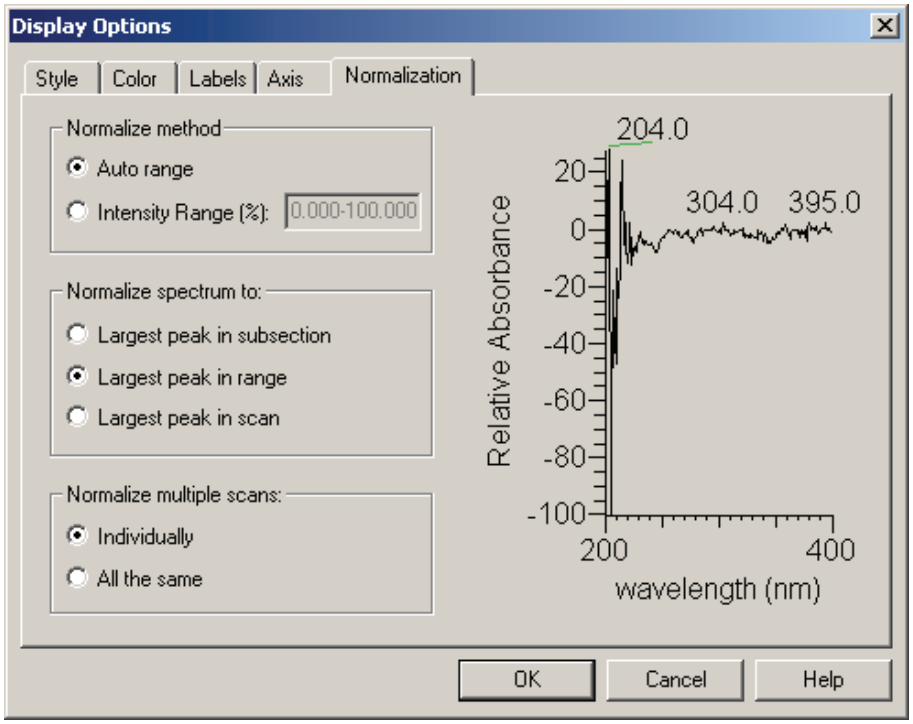


Figure 108. Display Options dialog box - Normalization page

b. In the Normalize Method area, select the Auto Range option button.

   This 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 Each Plot To area, select the Largest Peak In Range option button.

4. Specify the Axis parameters:

   a. Click the Axis tab to open the Axis page. See Figure 109.

   b. In the Units area, select the Absolute option button.

      This sets the Y-axis to the absolute units of µAU.

5. Click **OK** to exit the Display Options dialog box.

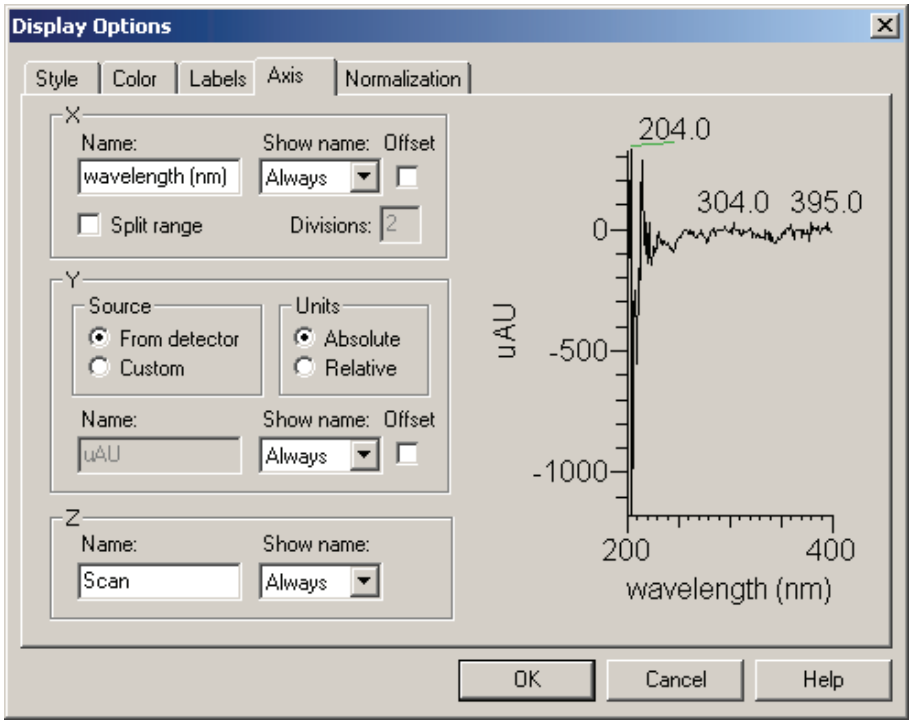


Figure 109. Display Options dialog box – Axis page

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**. Then, make a selection from the following choices: Left, Right, Above, or Below.

   The location of the new cell is relative to the active cell. Initially, the new cell will contain the same information as the existing cell. See Figure 110.

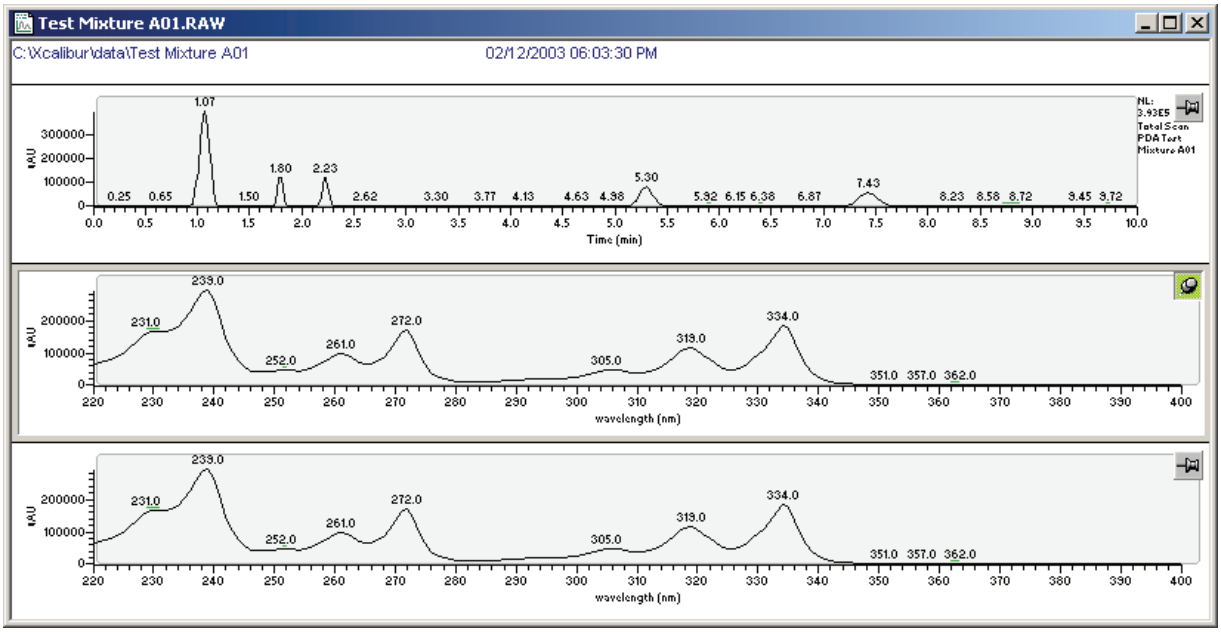


Figure 110. Qual Browser window, showing three cells

3. Change the lower cell so that it displays the Map view (see Figure 111):

   a. Pin the cell by clicking its pin button.

   b. Click the View Map button in the toolbar.

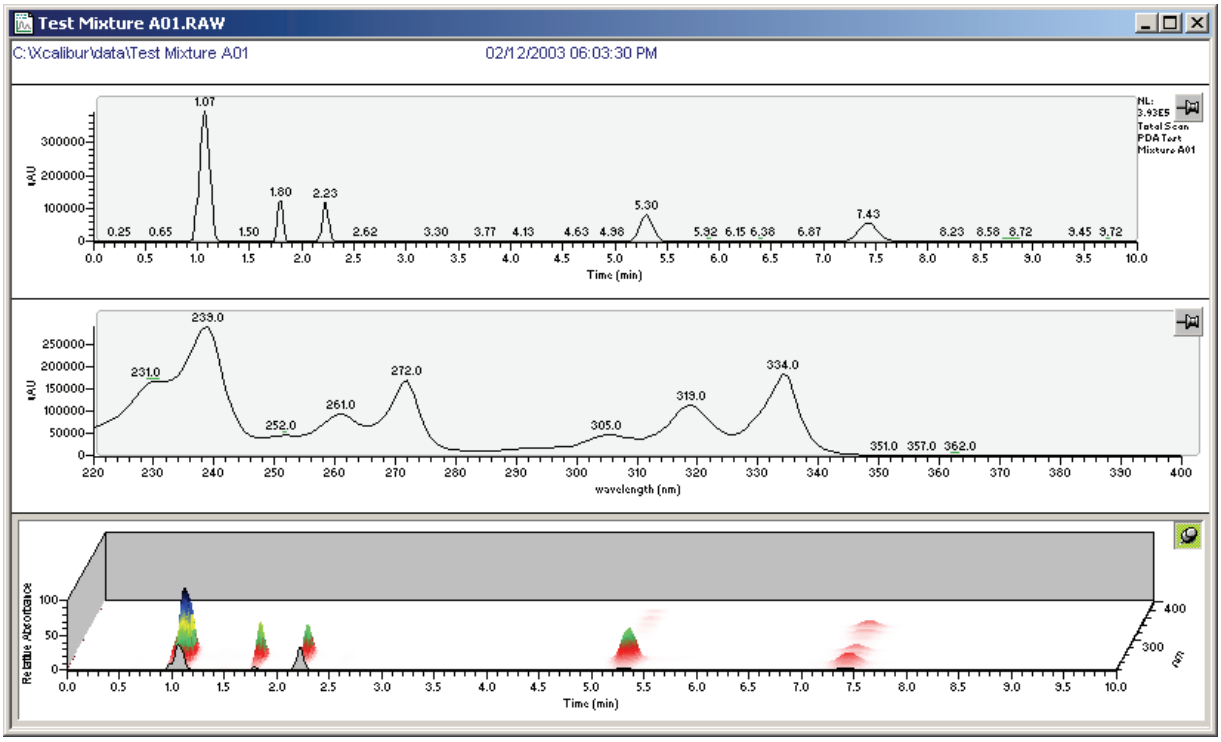


Figure 111. Qual Browser window, showing a Chromatogram view, a Spectrum view, and a Map view

4. Select the appropriate Range options for the Map view:

   a. Right-click the Map cell to open its shortcut menu.

   b. Choose **Ranges** to open the Map Ranges dialog box. See Figure 112.

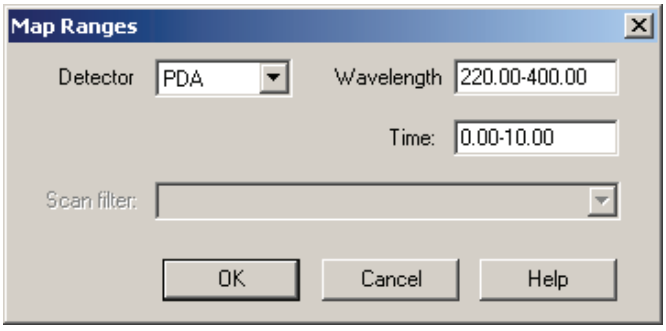


Figure 112. Map Ranges dialog box

   c. Enter the wavelength range that you want to display in the Wavelength box.

d. Enter the time range that you want to display in the Time box.

5. Select the appropriate Display options for the Map view:

   a. Right-click the Map cell to open its shortcut menu.

   b. Choose **Display Options** to open the Display Options dialog box. See Figure 113.

   c. Click the Axis tab.

   d. In the Units area, select the Absolute option button.

   e. Click the Normalization tab.

   f. In the Normalize Method area, select the Auto Range option button.

   g. Click the Style tab.

   h. Select the appropriate style.

   i. Click **OK** to exit the Display Options dialog box.

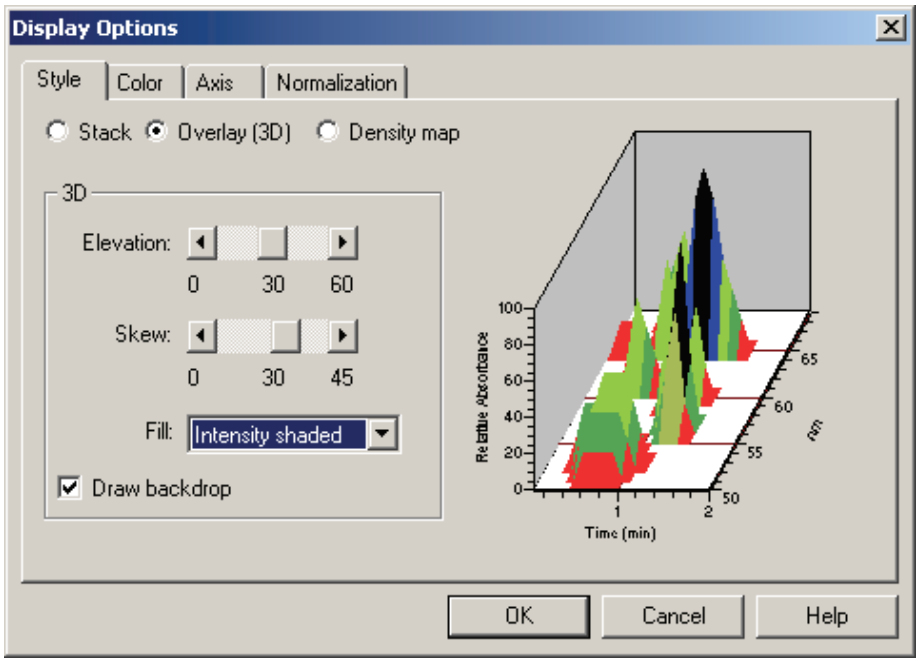


Figure 113. Display Options dialog box for the Map view

Saving a 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. Choose **File > Layout > Save As** to open the Save Layout File dialog box. See Figure 114.
2. Type a file name in the File Name box.
3. Click **Save**.

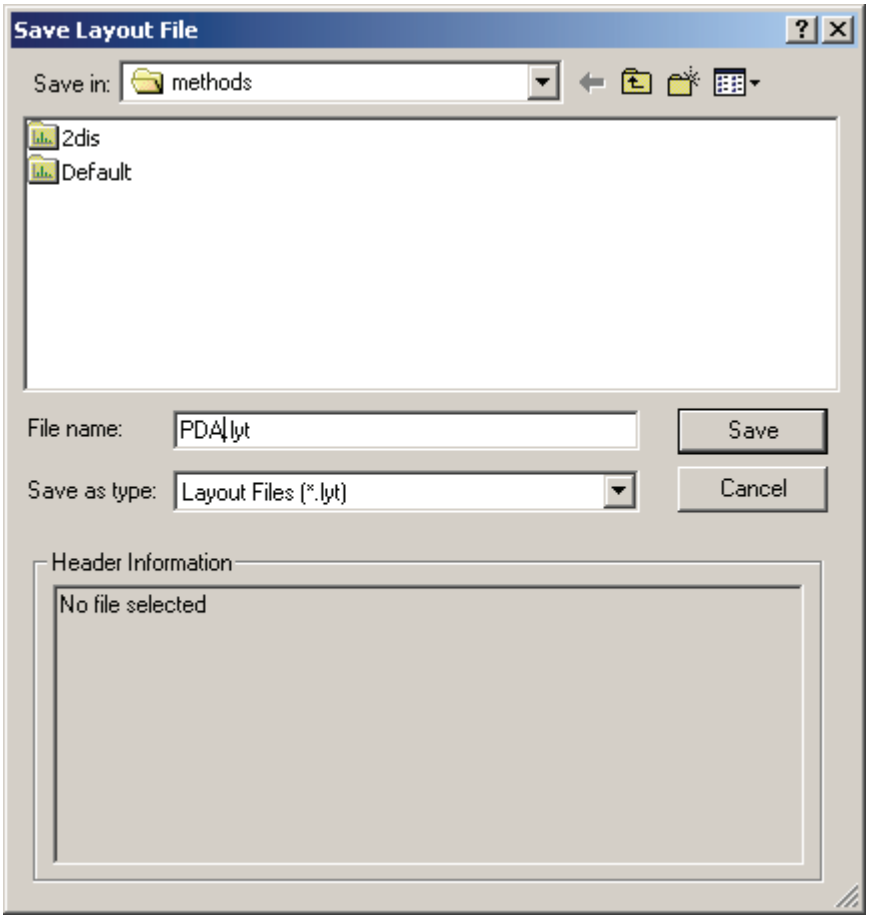


Figure 114. Save Layout File dialog box

Viewing the Spectrum for a Specific Time Point

To view a spectrum for a specific time point

1. Pin the Spectrum cell.
2. Click a time point in the Chromatogram cell.

   The spectrum for the selected time point appears in the Spectrum cell as shown in Figure 115.

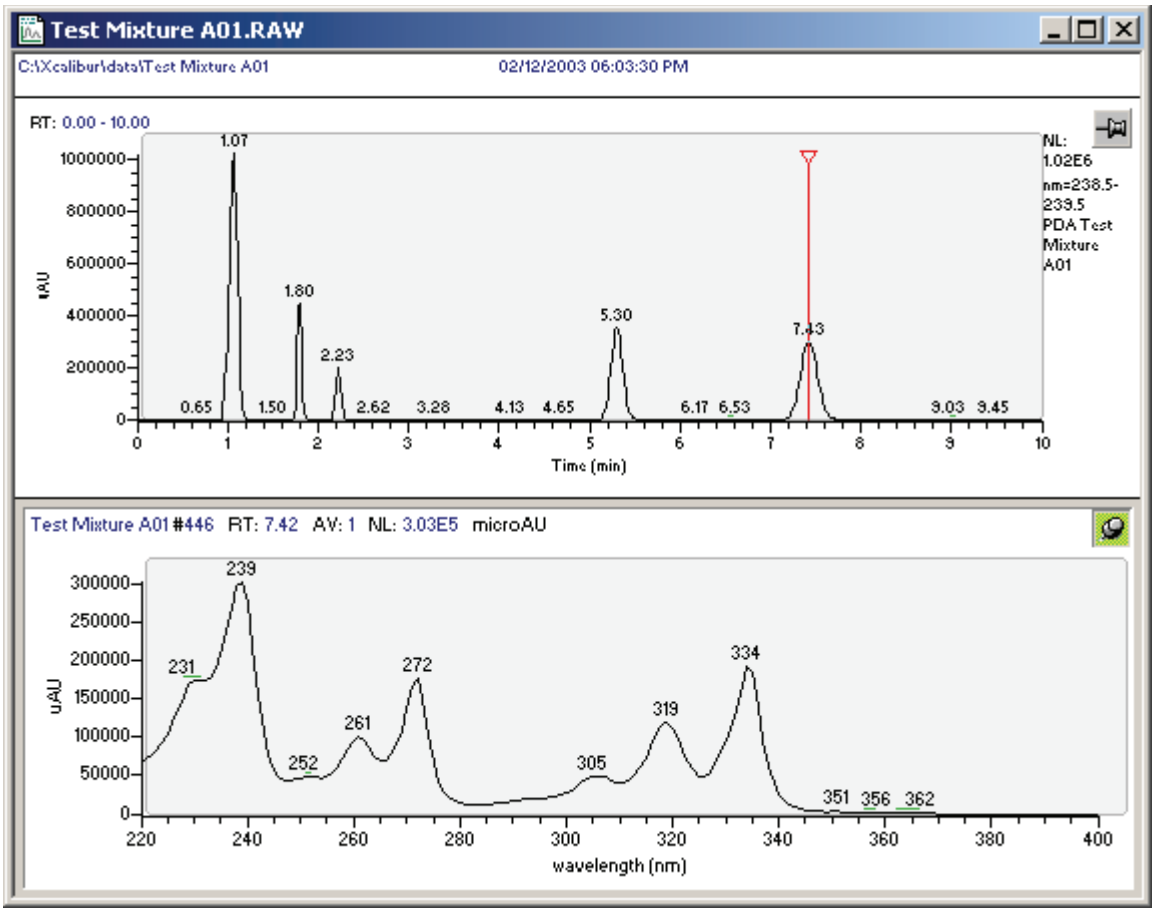


Figure 115. Qual Browser window, displaying a spectrum for the 7.43 min time point

Viewing the Chromatogram for a Specific Wavelength

To view the scan chromatogram for a specific wavelength

1. Pin the Chromatogram cell.
2. Click a wavelength in the Spectrum cell.

   The scan chromatogram for the selected wavelength appears in the Chromatogram cell as shown in Figure 116.

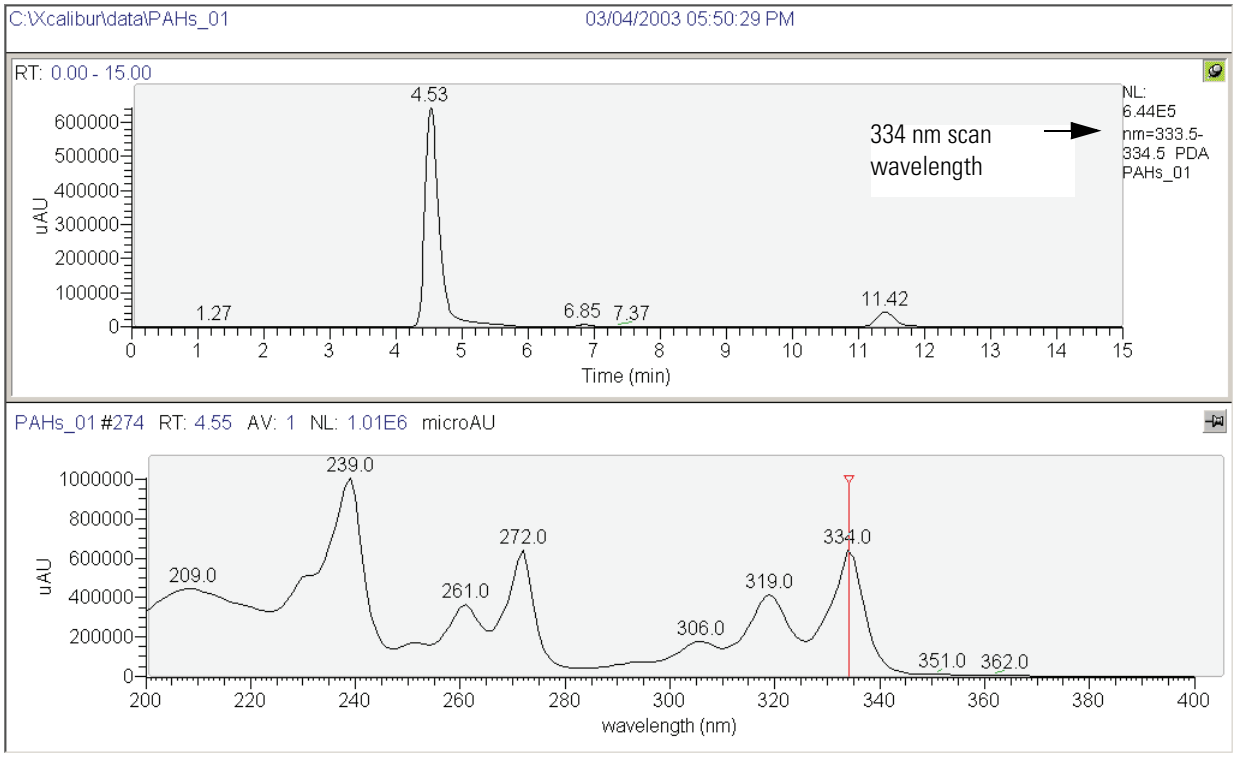


Figure 116. Qual Browser window, displaying a chromatogram cell and a spectrum cell

Determining Peak Areas

In a chromatogram, the area of an isolated peak is directly proportional to the concentration of the analyte. Determining the area of a peak is therefore an important feature of quantitation.

To make a peak area determination using Qual Browser

1. Access Qual Browser and open the [.raw] file that you are interested in. See "Opening a Raw Data File in Qual Browser" on page 136.

2. Ensure that the Chromatogram view is the active cell. An active cell has a gray border.

3. Select the chromatogram that you want to integrate:

   a. Right-click the chromatogram and choose **Ranges** to open the Chromatogram ranges dialog box.

   b. Select the Detector type from the Detector list:

      - 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 text box.
      - To display the chromatogram for a discrete wavelength channel, select *Channel A*, *B*, or *C* from the Plot Type list.

4. Turn on peak detection by right-clicking the Chromatogram view and choosing **Peak Detection > Toggle Detection in This Plot**.

5. To display numerical values for areas of the chromatographic peaks:

   a. Right-click the chromatogram and choose **Display Options** to open the Display Options dialog box. See Figure 117.

   b. Click the Labels tab to display the Labels page.

   c. Select the Area check box.

   d. Click **OK** to return to the Chromatogram view.

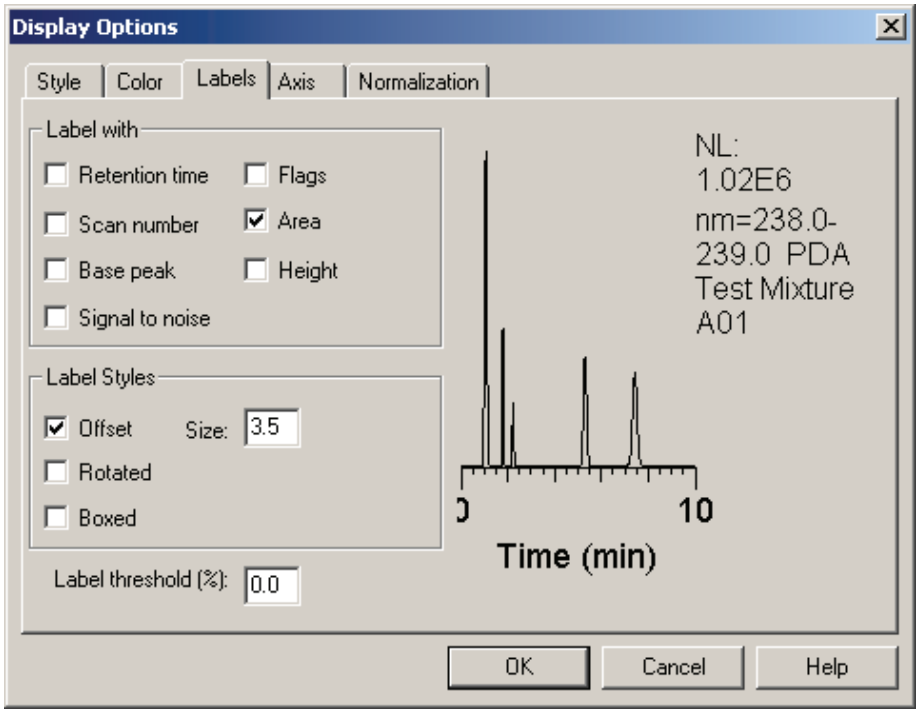


Figure 117. Display Options dialog box – Labels page

6. To set the integration parameters:

   a. Right-click the chromatogram and choose **Peak Detection > Settings** to open the 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.

      See Figure 118, which 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.

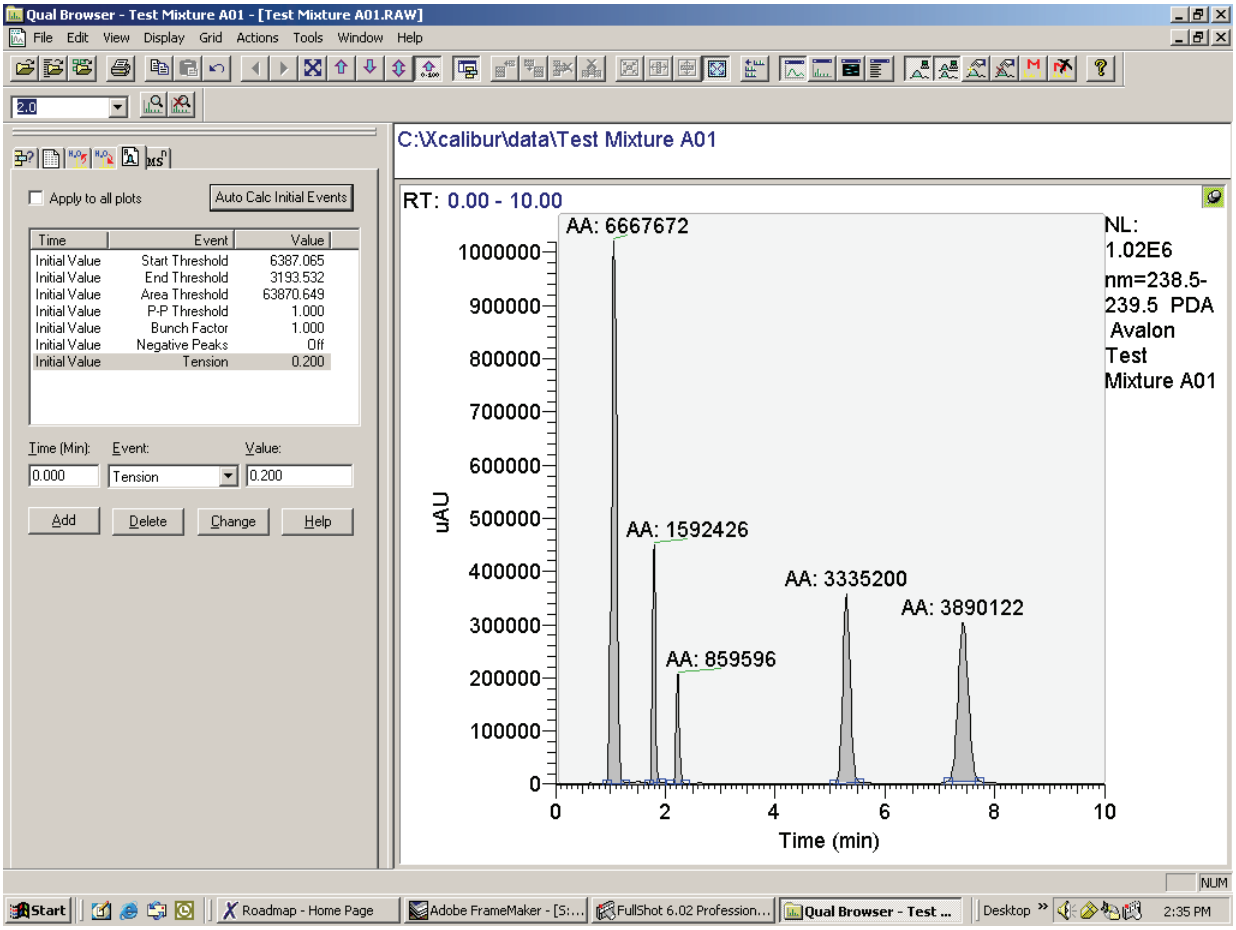


Figure 118. Avalon Peak Detection Settings page and Chromatogram view

Calculating the Purity of the Chromatographic Peaks

Xcalibur 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 as described in "Displaying Scan Chromatograms" on page 149.

2. Set the integration parameters for the chromatogram as described in "Determining Peak Areas" on page 166.

3. Right-click the chromatogram cell and choose **Peak Purity** to open the Peak Purity Settings dialog box. See Figure 119.

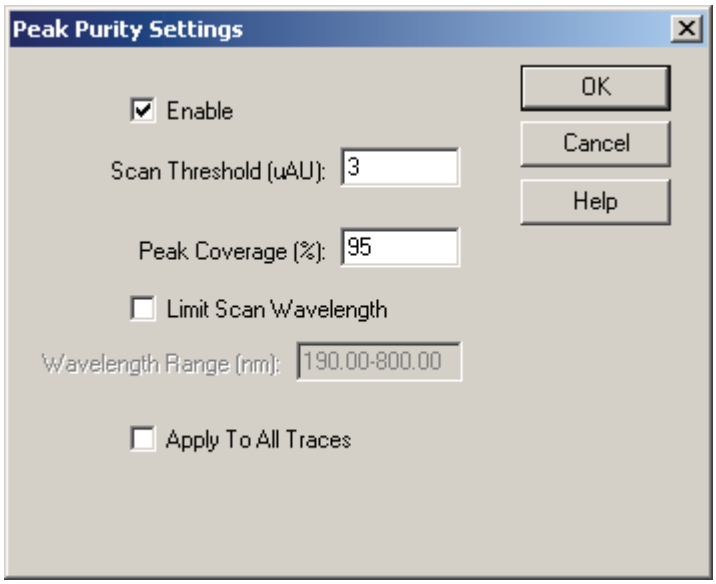


Figure 119. Peak Purity Settings dialog box

4. In the Peak Purity Settings dialog box, select the Enable check box.

5. In the Scan Threshold 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 µAU 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%, Xcalibur will compare all the spectral slices that fall within the beginning and ending tick marks of the integrated peak. If you want to limit the peak purity calculation to a specific range of wavelengths in your scan, select the Limit Scan Wavelength check box, and then enter a wavelength range in the Wavelength Range box.

7. Click **OK** to exit the Peak Purity Settings dialog box. Then, view the effect of your peak purity settings.

   - Figure 120 shows the effect of the scan wavelength on peak purity.
   - You can see the effect of integration on peak purity by comparing Figure 121 and Figure 122.
   - Figure 123 shows the effect of the scan threshold setting on peak purity.
   - Figure 124 shows the effect of the peak coverage setting on peak purity.

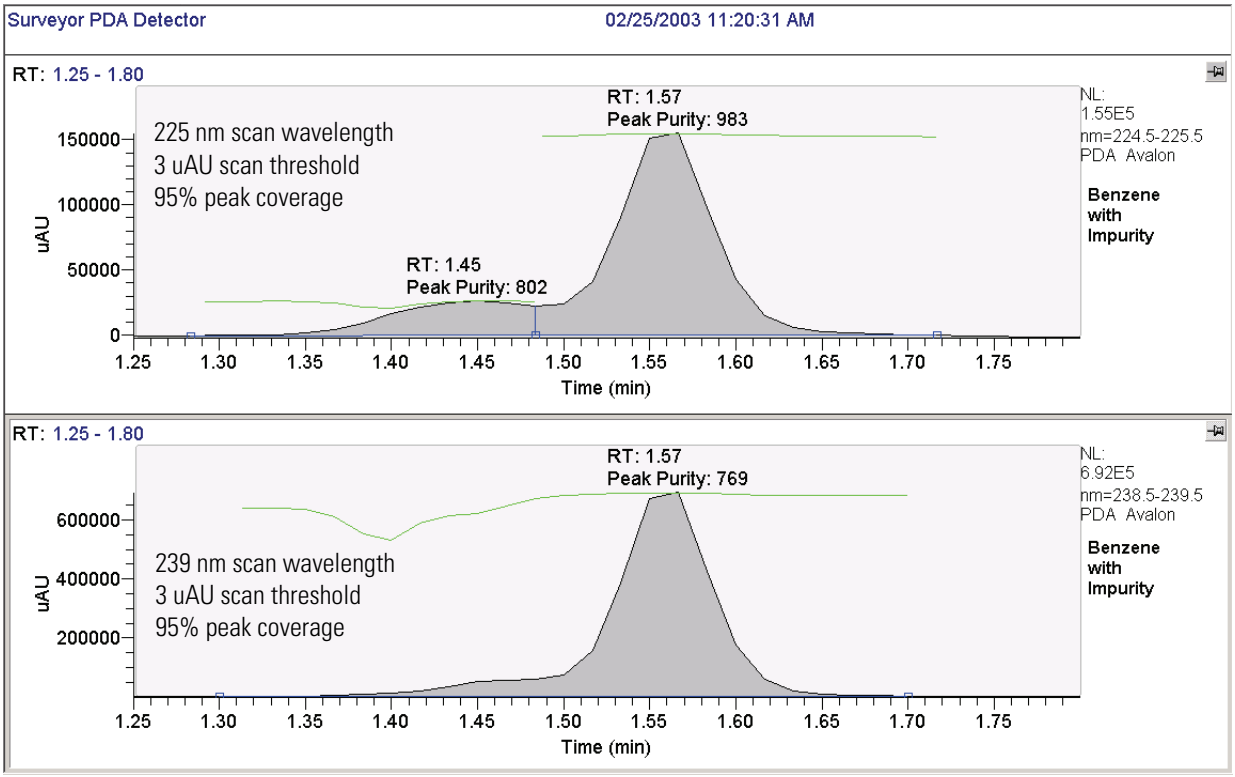


Figure 120. Comparison of peak purity results for two different scan wavelengths

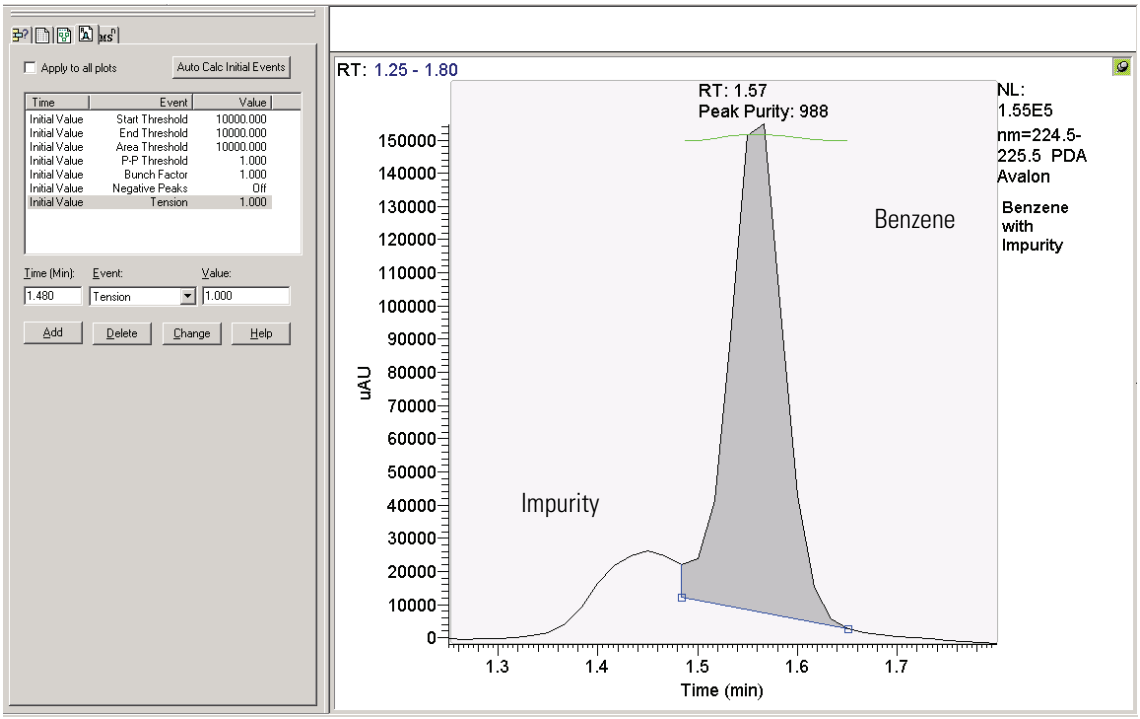


Figure 121. Chromatogram for the 225 nm scan wavelength with default integration parameters

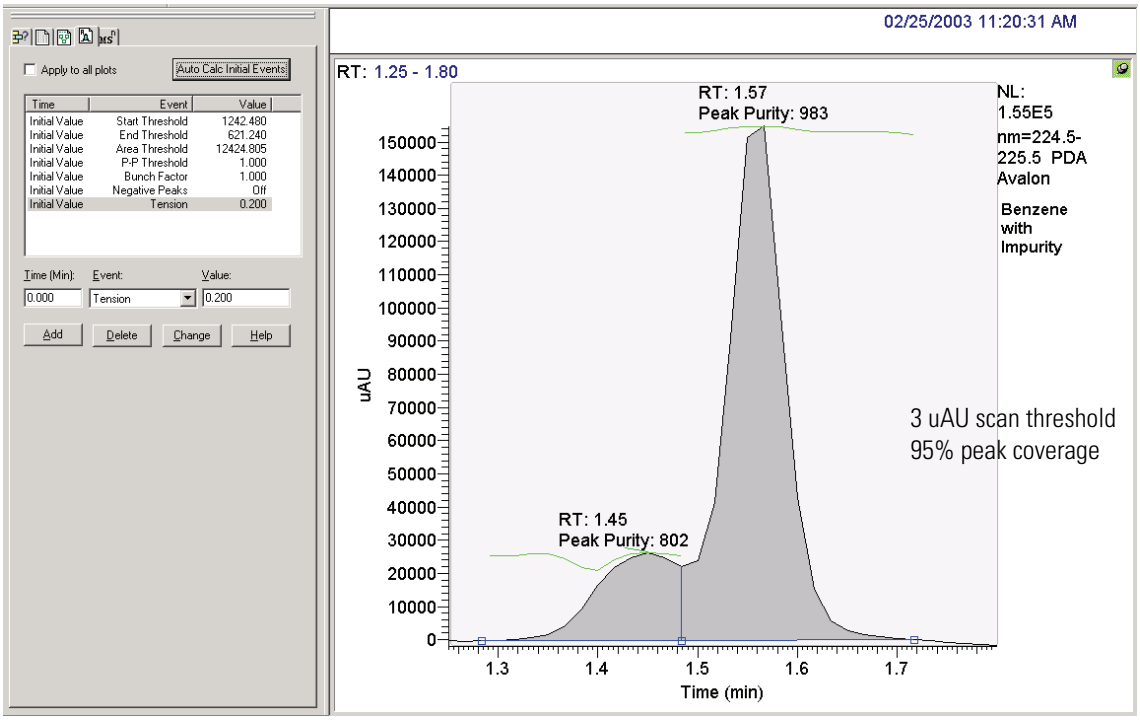


Figure 122. Chromatogram for the 225 nm scan wavelength with Auto calc integration parameters

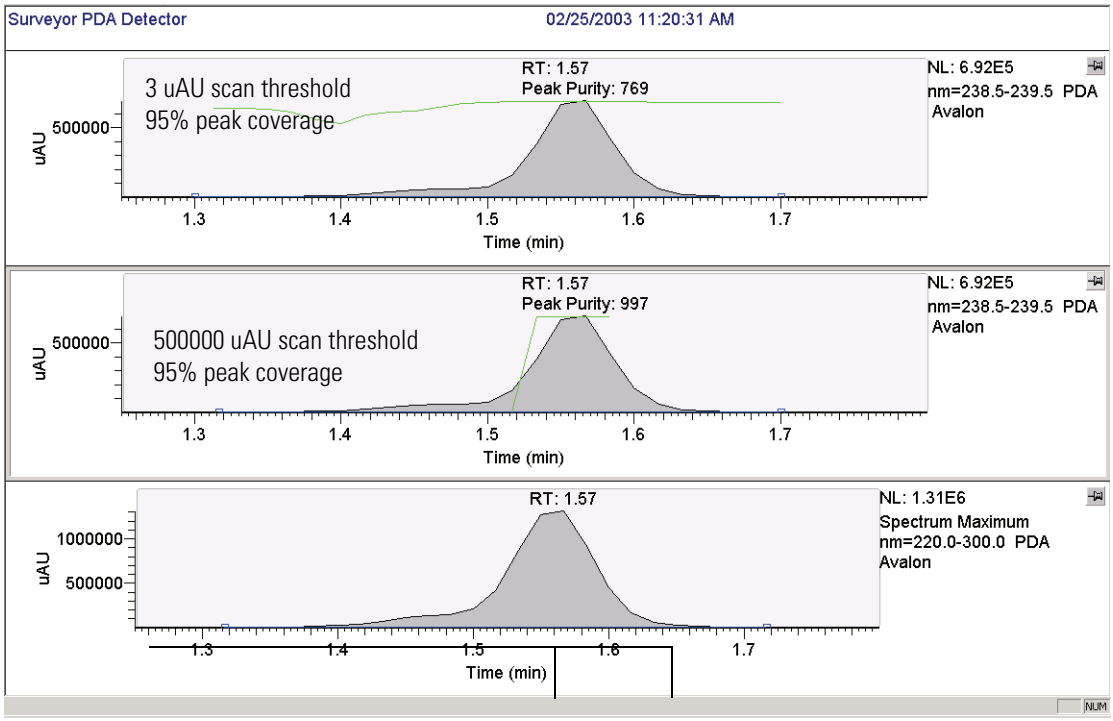


Figure 123. Effect of scan threshold on peak purity calculation

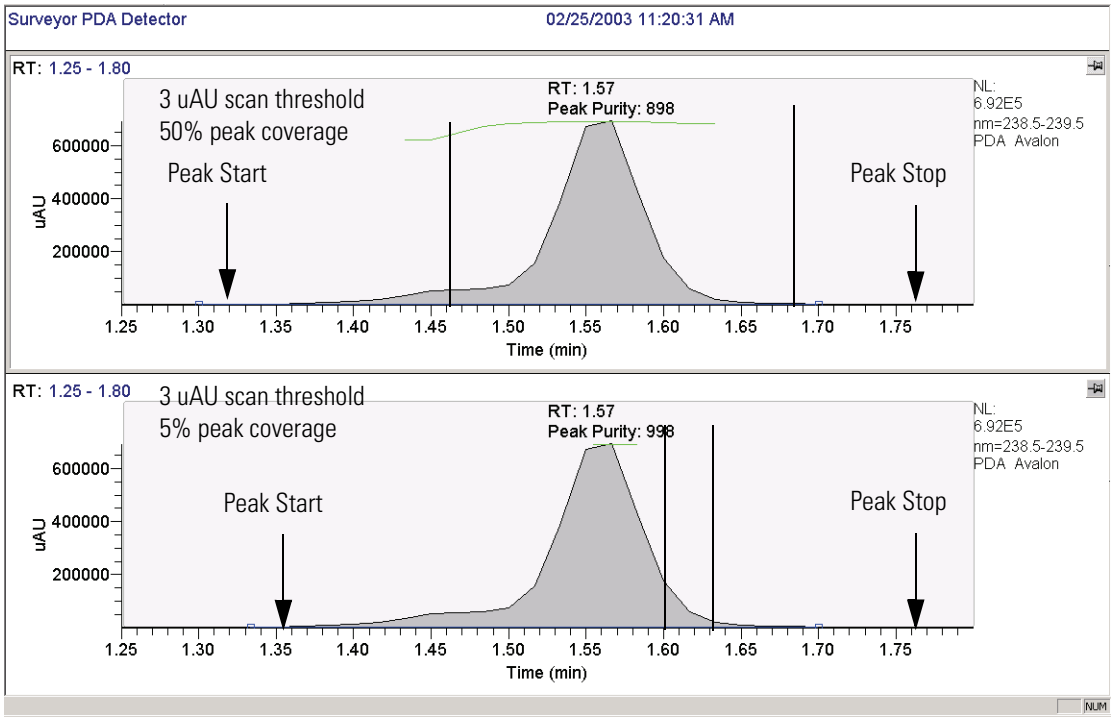


Figure 124. Effect of peak coverage on peak purity calculations

Appendix A Calibration Procedures

This appendix contains the following sections:

The modules of your Surveyor Plus instrument are factory calibrated. The PDA detector is tested for linearity, noise, and drift. The LC pump is 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 column oven and the tray temperature zones of the autosampler are calibrated at 30 °C. The positioning of its XYZ arm mechanism is calibrated for the carrier trays supplied by Thermo Electron.

Because of the sensitivity of its optical bench, it is best to recalibrate the Surveyor PDA Plus Detector after you install it, each time you move it, change its flow cell, or replace either of the lamps. In addition, you might need to increase or decrease the amount of incident light reaching the diode array by adjusting the attenuators.

The Surveyor Autosampler Plus does not require calibration upon arrival at its shipping destination. However, if you use custom vials or custom microplates, you need to perform a Well Bottom Distance calibration, which determines the actual depth of a vial or microplate well. See "Well Bottom Distance Calibration" on page 206. If problems occur with the column oven control, the tray temperature control, or the arm positioning, contact a Thermo Electron service representative. Calibration procedures for these items are included in this appendix for users who prefer to maintain their own instruments.

Like the autosampler, 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, thereby requiring 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.

Verifying the Performance of the PDA Detector

This section contains the following procedures:

Turning On the Lamps

Before you perform either calibration procedure, turn on both lamps and allow the temperature of the detector to stabilize for approx. one hour.

To turn on the lamps

1. From the Windows desktop, select **Start > All Programs > Xcalibur > Xcalibur** to open Xcalibur.
2. In the Xcalibur Roadmap - Home Page view, click the Instrument Setup icon to display the Instrument Setup window.
3. In the Viewbar of the Instrument Setup view, click the Surveyor PDA Plus Detector icon to display the Surveyor PDA Plus Detector Instrument Setup View.
4. From the menu bar of the Surveyor PDA Plus Instrument Setup window, choose **Surveyor PDA Plus > Direct Control**.
5. Click the Configuration tab to display the Configuration Page. See Figure 125.
6. Note the status and usage of each lamp.
7. If they are not already On, turn on both the deuterium (D2) and tungsten (W) lamps.
8. Allow the lamps to equilibrate for one hour before you use the detector.

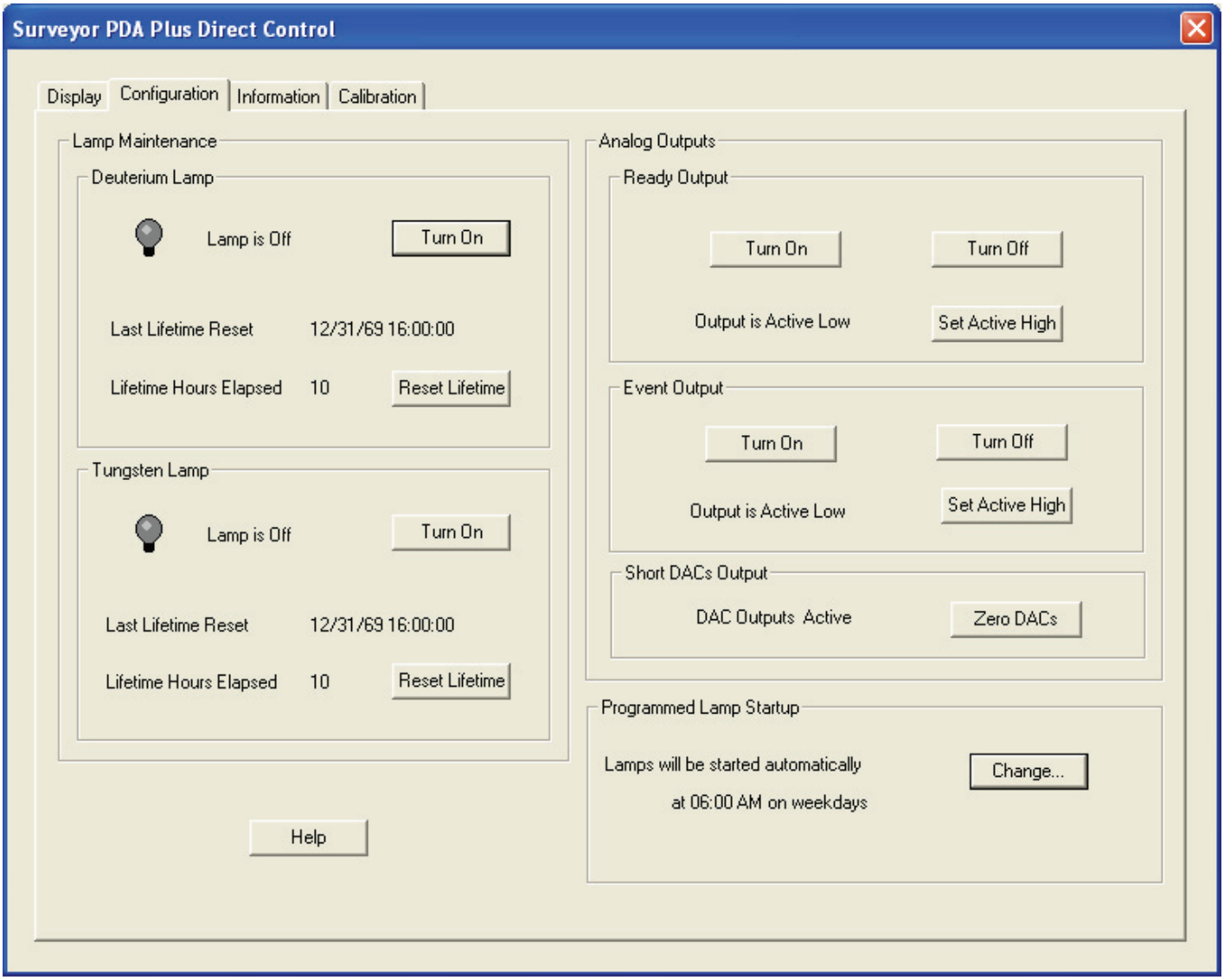


Figure 125. Surveyor PDA Plus Direct Control – Configuration page

Adjusting the Light Output

The Surveyor PDA Plus Detector has two attenuators that control the light output from the lamps. During the lifetime of the Surveyor PDA Plus Detector it might be necessary to adjust the attenuators to increase or decrease the amount of light falling onto the array.

Decreasing light output to the array increases baseline noise. Increasing light output to the array can cause saturation of the diode array. If the array is saturated the response from the Surveyor PDA Plus Detector will be a flat baseline.

The attenuators require adjustment when either lamp is replaced or when the flowcell is replaced. The first time that you adjust the attenuators, you need to create an [.spda] method. After you create the method for adjusting the attenuators, save it with a name that you will associate with adjusting the attenuators and store it for future use. Saving the method will 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, perform the following procedures in the order listed:

1. Preparing the LC for an Adjustment of the Attenuators
2. Accessing the Attenuators
3. Adjusting the Attenuators

Preparing the LC for an Adjustment of the Attenuators

To prepare your LC system for an adjustment of the attenuators

1. Ensure that the lamps are On:
   a. From the Windows taskbar, choose **Start > All Programs > Xcalibur > Xcalibur** to open Xcalibur.
   b. In the Xcalibur Home Page, click the Instrument Setup icon to display the Instrument Setup window.
   c. In the Viewbar, click the Surveyor PDA Plus button to open the Surveyor PDA Plus Detector view.
   d. Choose **Surveyor PDA Plus > Direct Control**.

      The Surveyor PDA Plus Direct Control dialog box is displayed.

e. Click the Configuration tab to display the Configuration page.

f. Verify that both lamps are On. If they are not On, click the **Turn On** buttons for both lamps.

2. Replace the LC column with a flow restrictor.

3. Set your chromatographic pump to deliver HPLC-grade water at a flow rate of 1 mL/min:

   a. In the Viewbar of the Instrument Setup view, click the Surveyor MS Pump or the Surveyor LC Pump icon to display the instrument setup view for your pump.

   b. Choose **Surveyor MS pump > Direct Control** or **Surveyor LC Pump > Direct Control**.

      The Direct Control dialog box for your pump is displayed.

   c. Select a solvent bottle that contains HPLC-grade water and set the flow rate to 1 mL/min.

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 126.

   The left tab is the deuterium lamp attenuator and the right tab is the tungsten lamp attenuator. Pushing the attenuator tab up increases light output and pulling the tab down decreases light output.

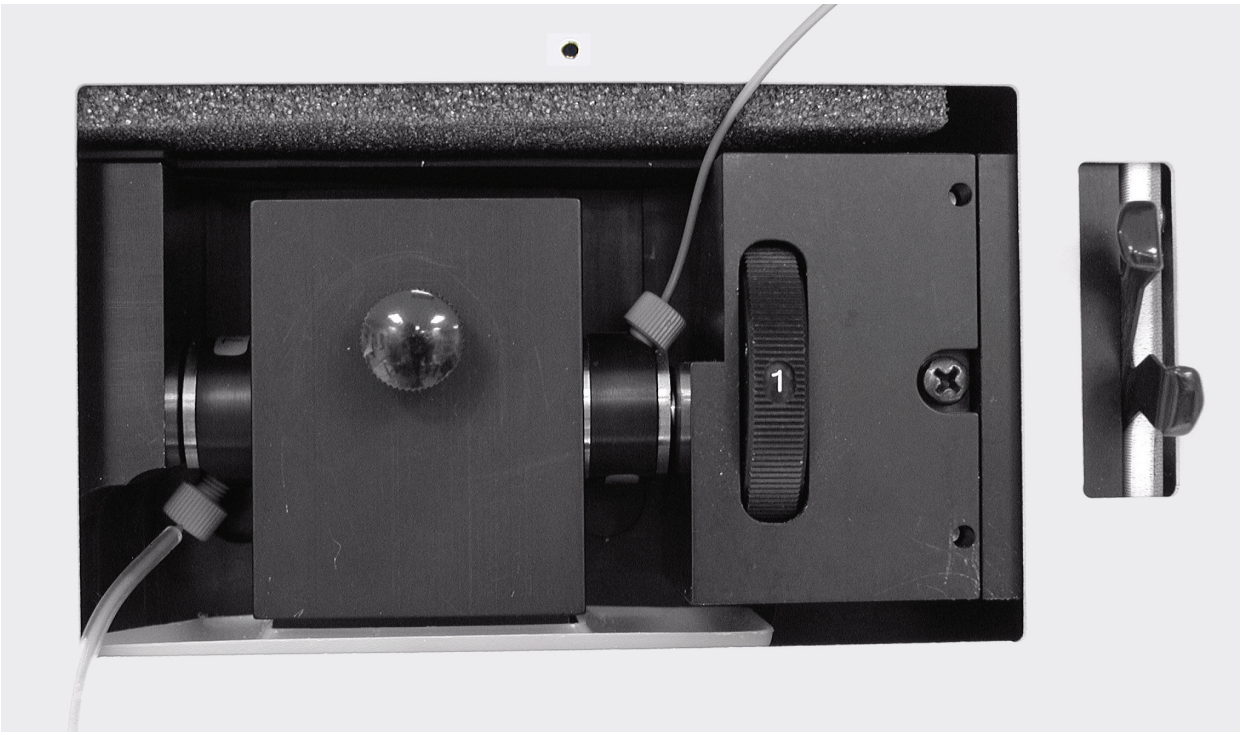


Figure 126. View of attenuators with flow cell access cover removed

Adjusting the Attenuators

To adjust the light output by adjusting the attenuators

1. Create an [.spda] method for the Surveyor PDA Plus Detector:

   a. Click the Surveyor PDA Plus button in the View bar to open the Instrument Setup view for the Surveyor PDA Plus Detector.

   b. Select the Diode/Intensity option button.

   c. Verify that the following parameters are specified in the Surveyor PDA Plus Method Page - Spectra area:

   - Start Diode= 2
   - End Diode= 511
   - Diode Step = 1
   - Sample rate = 1

   d. Choose **File > Save** to save the method. Save the method with a filename, such as attenuator.spda.

Note The .spda file extension is a special file extension used for all method files based on Diode/Intensity units. These methods cannot be used in Sequence Setup. Files with the .spda extension can only be loaded and used in the Direct Control dialog box.

2. Load the [.spda] method to the detector:

   a. In the Surveyor PDA Plus Direct Control dialog box, click the Display tab to open the Display page.

   b. In the Control area, click **Load Method**. Then, choose your attenuator method file from the list.

   c. Click **OK**.

3. Start the data stream and adjust the attenuators:

   a. In the Control area, click **Start**.

      The spectrum of light intensities is displayed in the top window. See Figure 127. For the UV region, the diode of maximum intensity is between diode 30 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.

   b. Adjust the attenuator with the left tab on the PDA (UV attenuation) to achieve a maximum value of between 750000 and 775000 intensity counts in the region between diode number 30 and diode number 50.

   c. Adjust the attenuator with the right tab (Visible attenuation) to achieve a maximum value of between 750000 and 775000 intensity counts in the region between diode number 400 and diode number 500.

4. 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.

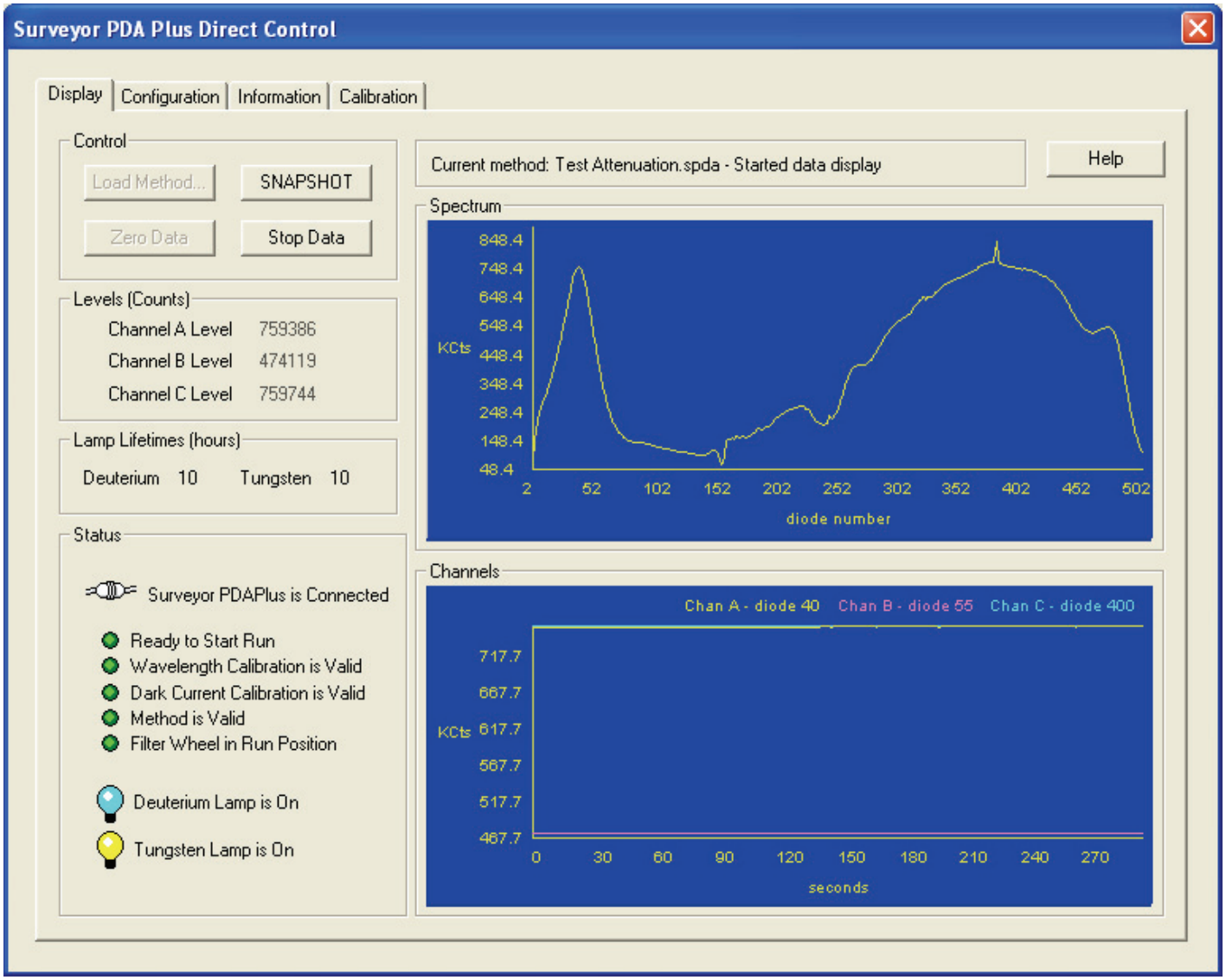


Figure 127. Display page, showing an intensity spectrum

Performing a Wavelength Calibration

The alignment of the spectrum on the diode array is dependent upon the physical alignment of various components of the optical bench. The alignment can become offset if the detector is sharply jolted, for example, in shipping. Such bumps and jars can slightly change the wavelength of light reaching the photodiode array. You can use the automated wavelength calibration to determine the wavelength accuracy of the detector and to correct for any misalignment of the array.

To perform a wavelength calibration

1. Pump HPLC-grade methanol at 1 mL/min through the flow cell.
2. Turn On both lamps and wait 1 hour for the lamps to equilibrate:
   a. From the Instrument Setup window, click the Surveyor PDA Plus button in the View Bar to open the Surveyor PDA Plus Instrument Setup window.
   b. From the menu bar of the Surveyor PDA Plus Instrument Setup window, choose **Surveyor PDA Plus > Direct Control** to open the Surveyor PDA Plus Direct Control dialog box.
   c. Click the Configuration tab to open the Configuration page.
   d. Turn on the deuterium lamp by clicking **Lamp On** in the Deuterium Lamp area.
   e. Turn on the tungsten lamp by clicking **Lamp On** in the Tungsten Lamp area.
3. After the detector has reached a stable temperature (approx. 1 hour after you turn on the lamps), start the Wavelength Calibration Wizard and follow the instructions on each page:
   a. Click the Calibration tab to display the Calibration page. See Figure 128.

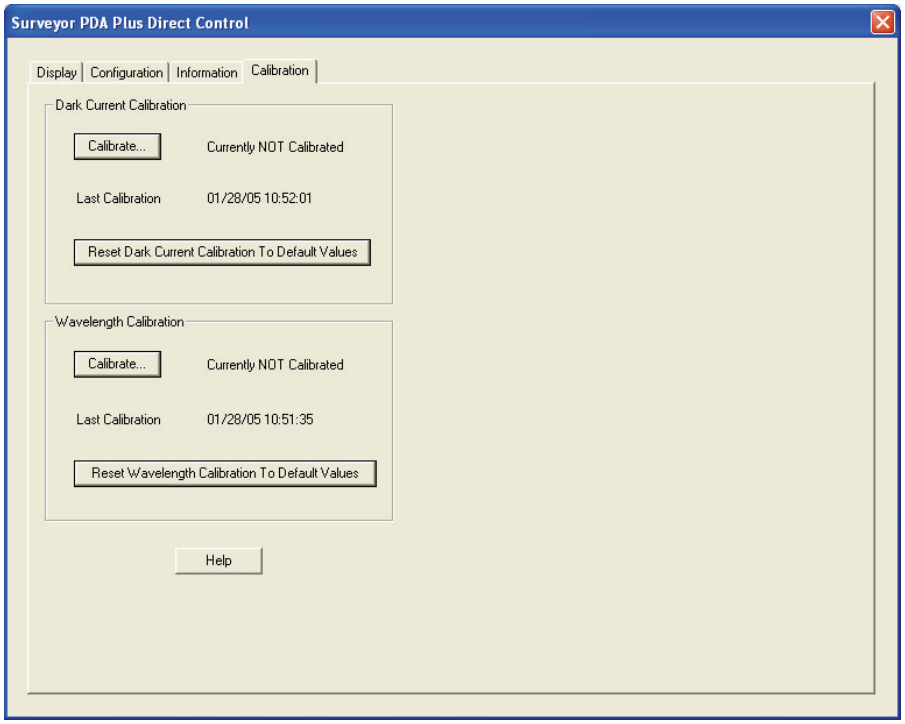


Figure 128. Surveyor PDA Plus Direct Control – Calibration page

b. In the Wavelength Calibration area, click **Calibrate** to start the Wavelength Calibration wizard. The Wavelength Calibration wizard contains nine pages.

   The first page of the Wavelength Calibration wizard, the preconditions page appears. See Figure 129.

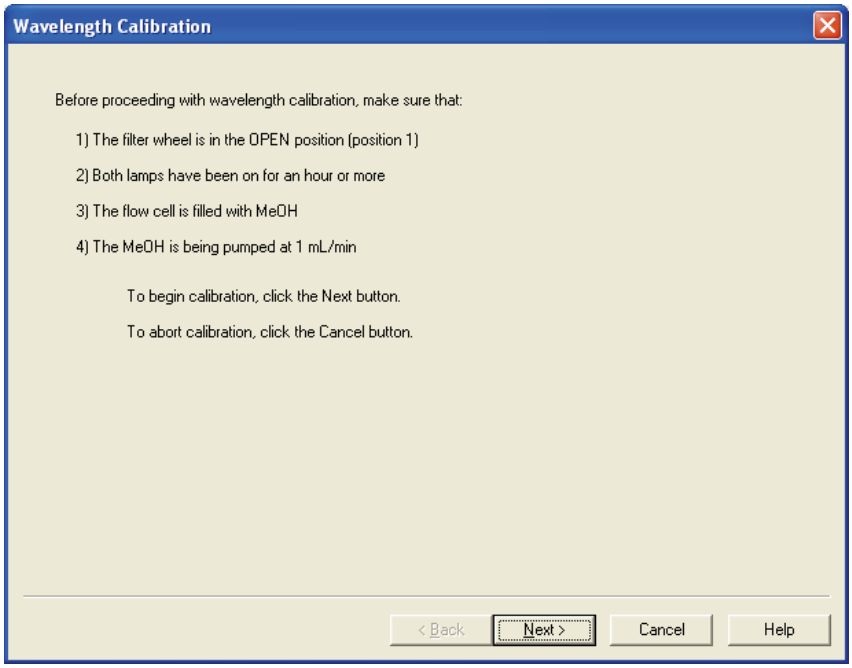


Figure 129. Wavelength Calibration wizard – page 1, listing required preconditions

c. Read the preconditions:

- If all of the preconditions have been met, click **Next** to proceed with the calibration and move on to the second page of the wizard where you will be prompted to select a wavelength file. See Figure 130.

- If the preconditions have not been met, click **Cancel** to exit the wizard and prepare the Surveyor PDA Plus detector for calibration.

Note The Cancel buttons on the pages of the Wavelength Calibration Wizard can be pressed at any time a calibration is in process to abort it.

4. In the second page of the Wavelength Calibration wizard (see Figure 130), select a peak set from the list. The peak set should span the wavelengths you use under normal operating conditions. Then, click **Next** to display the Calibration Status page.

   In Figure 130, you can see that the Holmium Oxide 5 peak set has been chosen. This wavelength list instructs the program to calibrate the detector at each of the five wavelengths shown.

Note Xcalibur has four calibration files to select from. For example, the HolmiumOxideUV 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)."

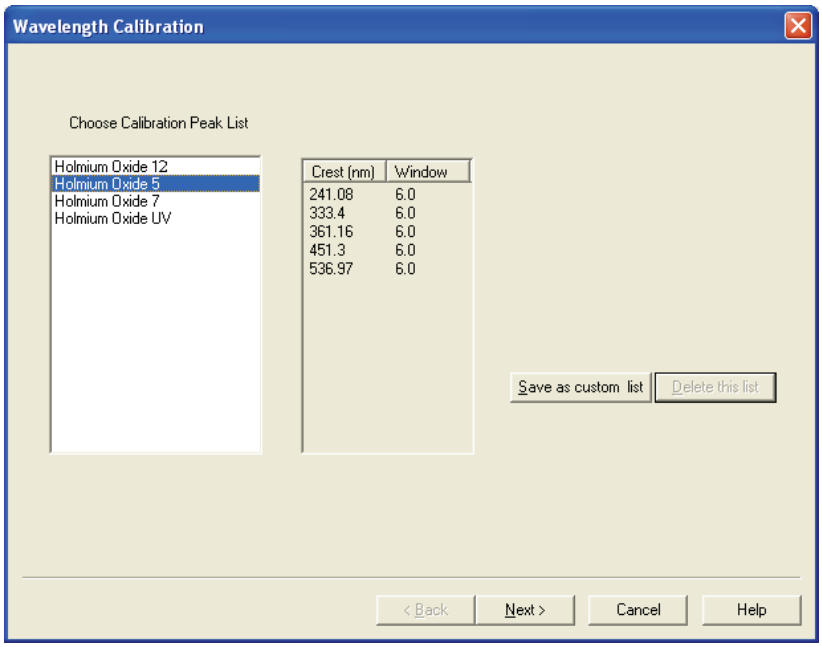


Figure 130. Wavelength Calibration wizard – page 2, showing a list of calibration files

5. In the third page of the Wavelength Calibration wizard, do the following:

   a. Observe the status screen that tells you the wavelength file is being downloaded. See Figure 131.

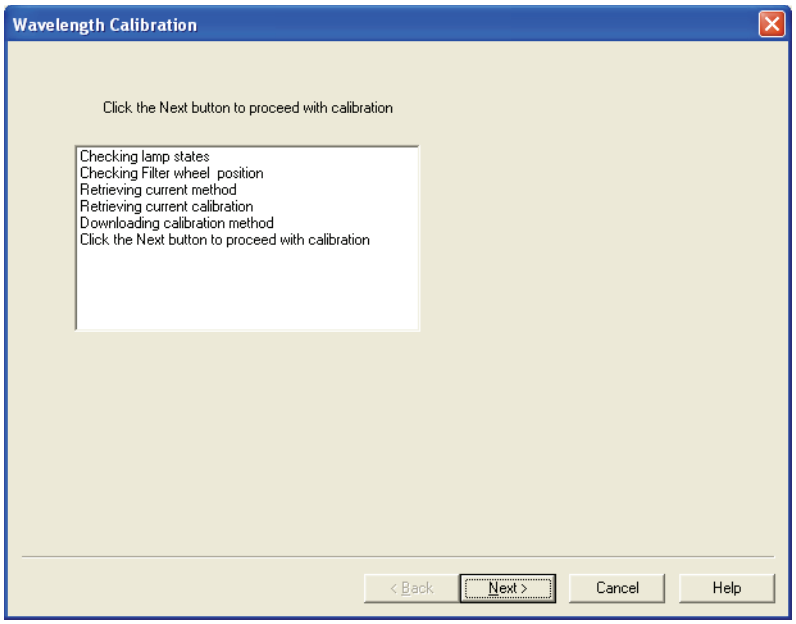


Figure 131. Wavelength Calibration wizard – page 3

b. After the "Click the Next button to proceed with calibration" message appears, click **Next** to display the fourth page of the Wavelength Calibration wizard shown in Figure 132.

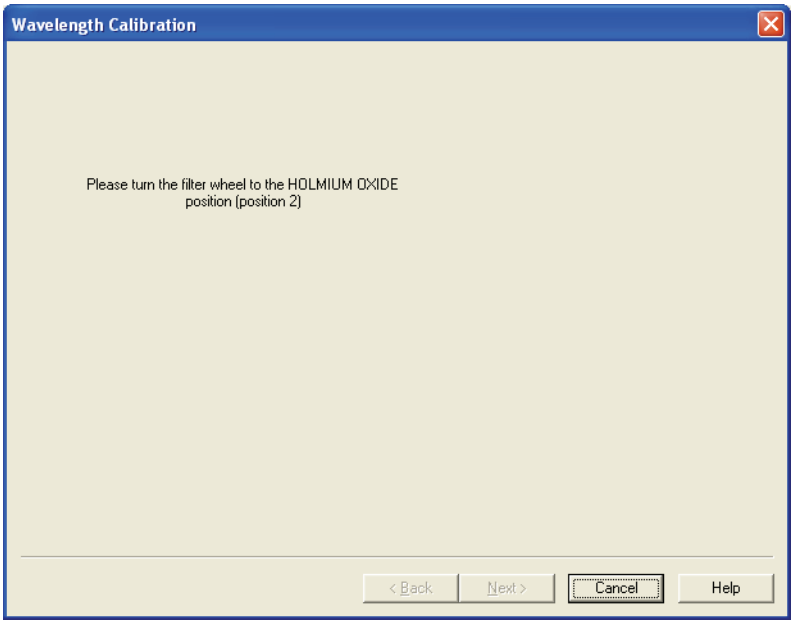


Figure 132. Wavelength Calibration wizard – page 4, directing you to turn the filter wheel

6. Rotate the Holmium Oxide filter wheel to position 2 as directed in the fourth page of the Wavelength Calibration wizard. After you turn the wheel, the Next button becomes active. Click **Next** to proceed.

   Note The Next button is unavailable until the filter wheel is in position 2.

7. In the fifth page of the Wavelength Calibration wizard, observe the status screen that tells you the calibration is being performed. See Figure 133. After the "Click the Next button to proceed with calibration" message appears, click **Next** to proceed.

   The diagnostics program waits for a few seconds for the rise time filter to equilibrate and takes a holmium oxide scan.

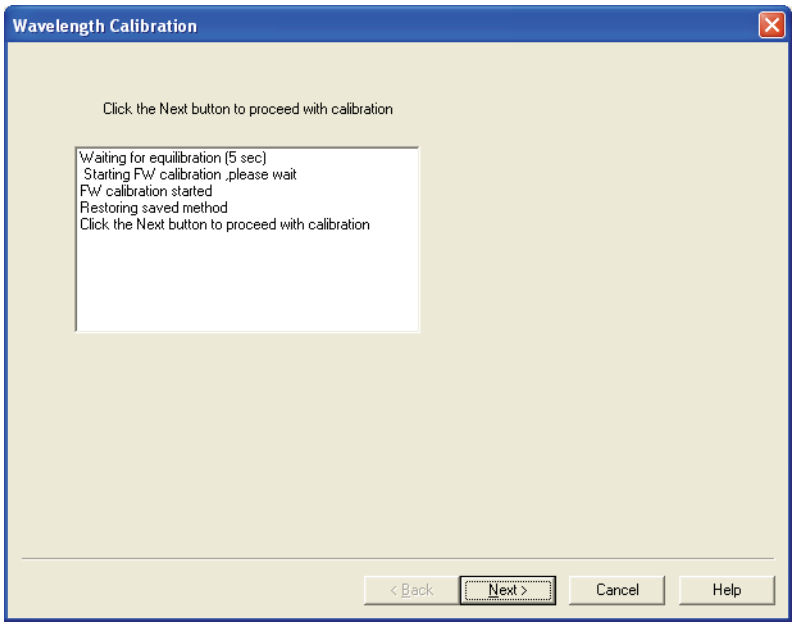


Figure 133. Wavelength Calibration wizard – page 5, showing the status of the rise time filter calibration

8. Rotate the wheel back to position 1 (Open) as instructed on the sixth page of the Wavelength Calibration wizard. See Figure 134. After you rotate the wheel, the next button becomes active as shown in Figure 135. Click **Next** to proceed.

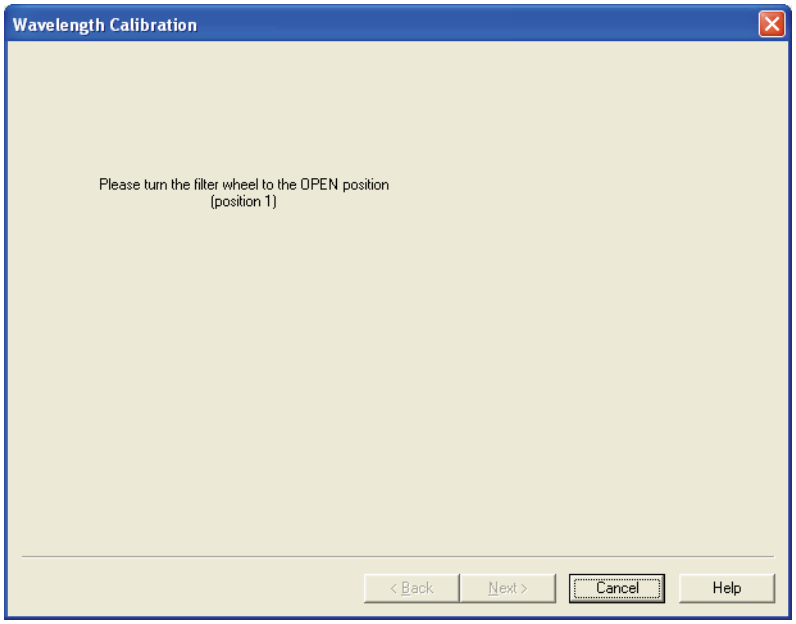


Figure 134. Wavelength Calibration wizard - page 6, directing you to turn the Holmium Oxide filter wheel back to position 1

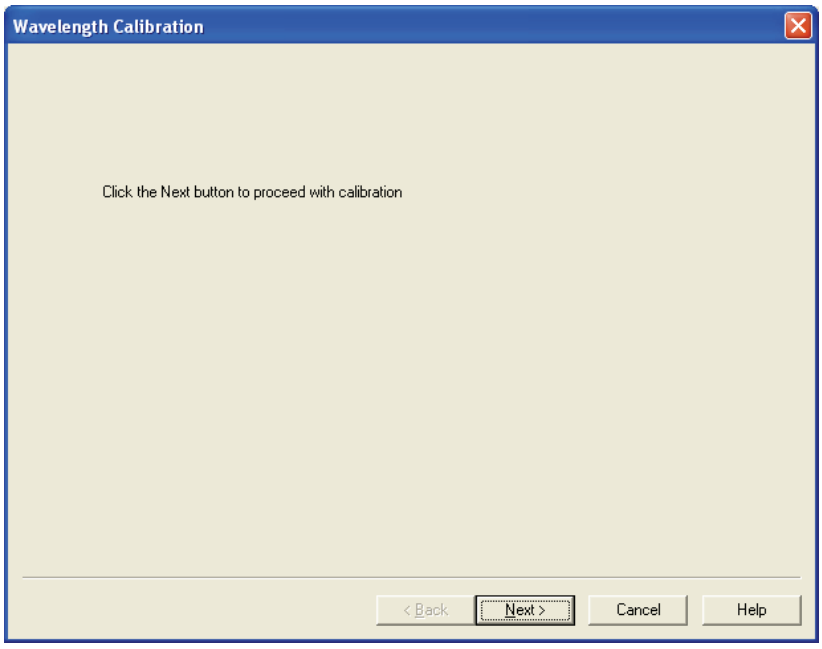


Figure 135. Wavelength Calibration wizard – page 6 with active next button

9. In the seventh page of the Wavelength Calibration wizard, shown in Figure 136, verify that the delta values are within ±1 nm:

   - If the Delta values are acceptable, proceed to step 10.
   - 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 Electron service representative for assistance.

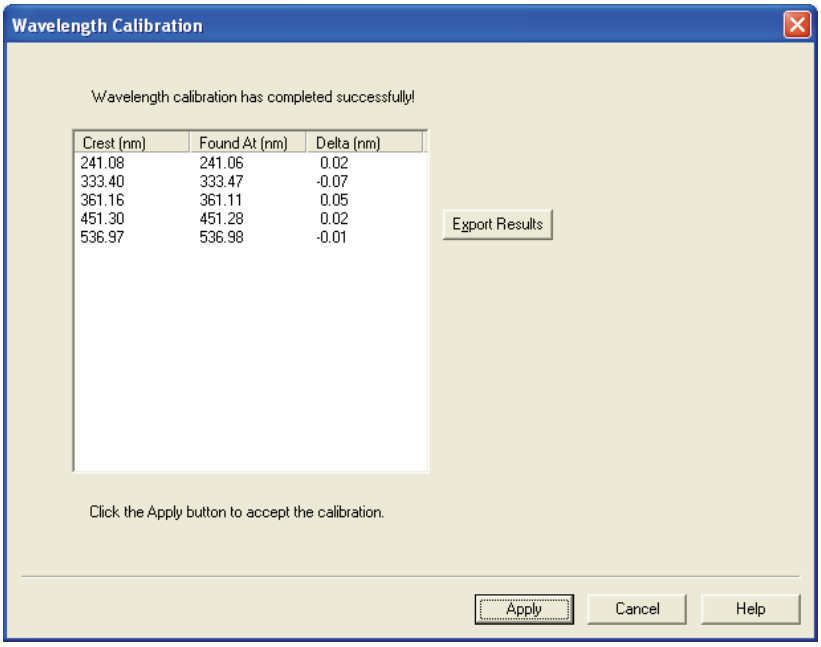


Figure 136. Wavelength Calibration wizard – page 7, showing the Delta values

10. (Optional) Print a report of the calibration results:

    a. Click **Export Results** to print the results to a file.

    Xcalibur opens the Save As dialog box shown in Figure 137.

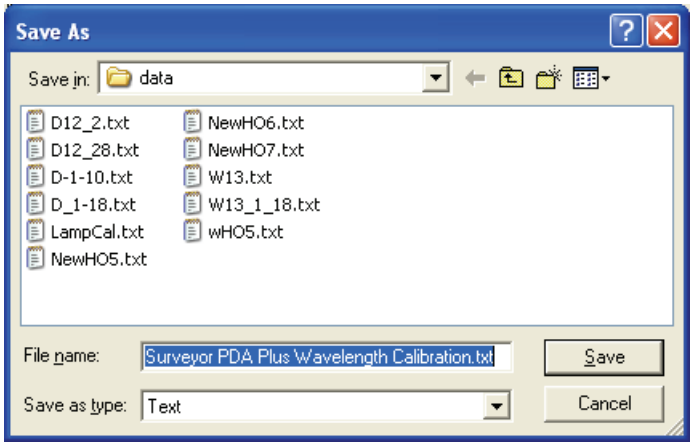


Figure 137. Save As dialog box

b. Type a name in the Save As dialog box. 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 138

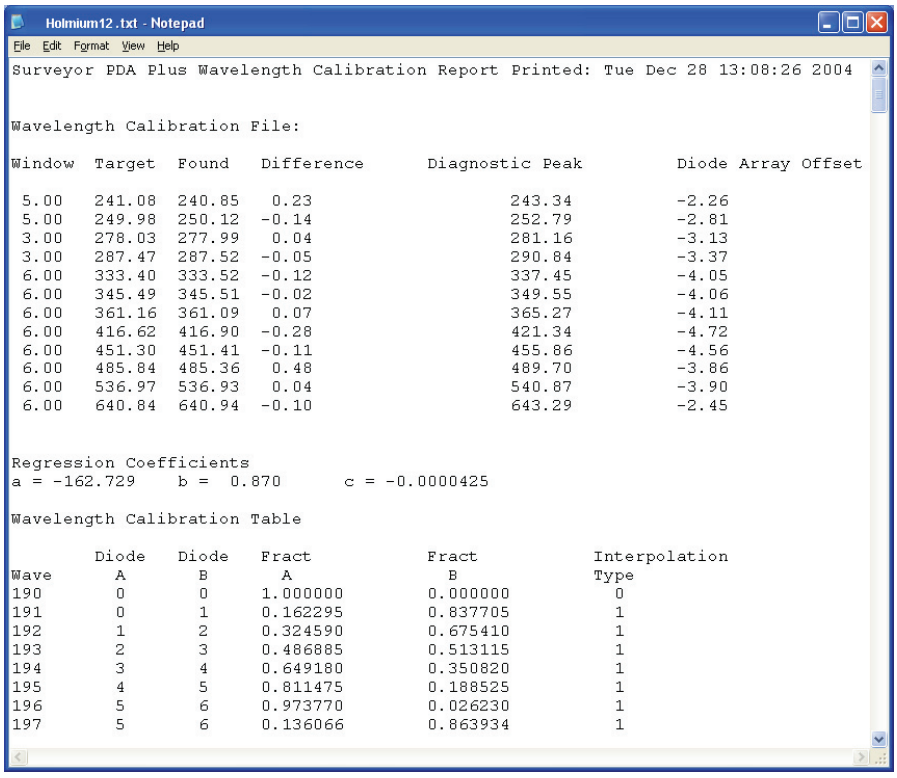


Figure 138. Wavelength Calibration file, viewed in Notepad

11. Click **Apply** in the Delta Values page (see Figure 136) to apply the calibration results to the detector.

    The final page of the Wavelength Calibration wizard is displayed. See Figure 139.

12. In the eighth and final page of the Wavelength Calibration wizard, click **Finish** to complete the 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 128 on page 182.

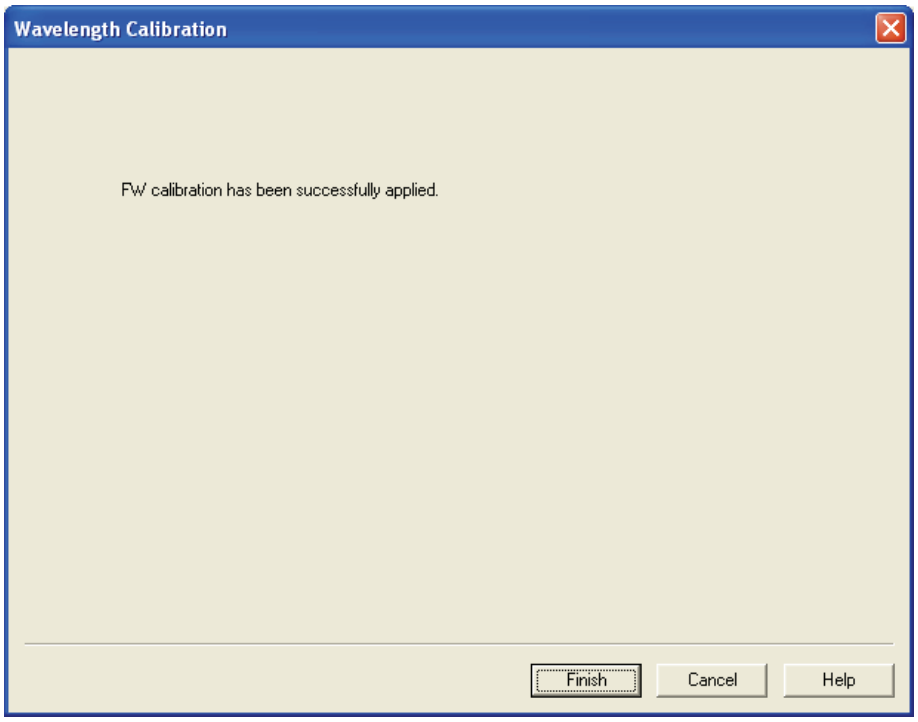


Figure 139. Wavelength Calibration wizard – page 8

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. Typical dark current values range from 1500 to 3000 counts.

The environmental conditions of your laboratory can cause the dark current of the diode array to increase over time. Therefore, we recommend that you perform an array calibration (dark current) after any of the following events occurs:

- After 100 hours of use or monthly, whichever comes first
- Whenever a significant temperature change 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, it is important to warm up the lamps for 1 hour before you perform a dark current calibration. Warming up the lamps for 1 hour equilibrates the detector to its normal operating temperature.

Xcalibur briefly turns the lamps off as it performs the dark current calibration routine. After it completes the dark current calibration routine, Xcalibur turns the lamps 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 1 hour for the lamps to equilibrate.
3. From the menu bar of the Surveyor PDA Plus Instrument Setup window, choose **Surveyor PDA Plus > Direct Control**. Then, click the Calibration tab to display the Calibration page. See Figure 140.

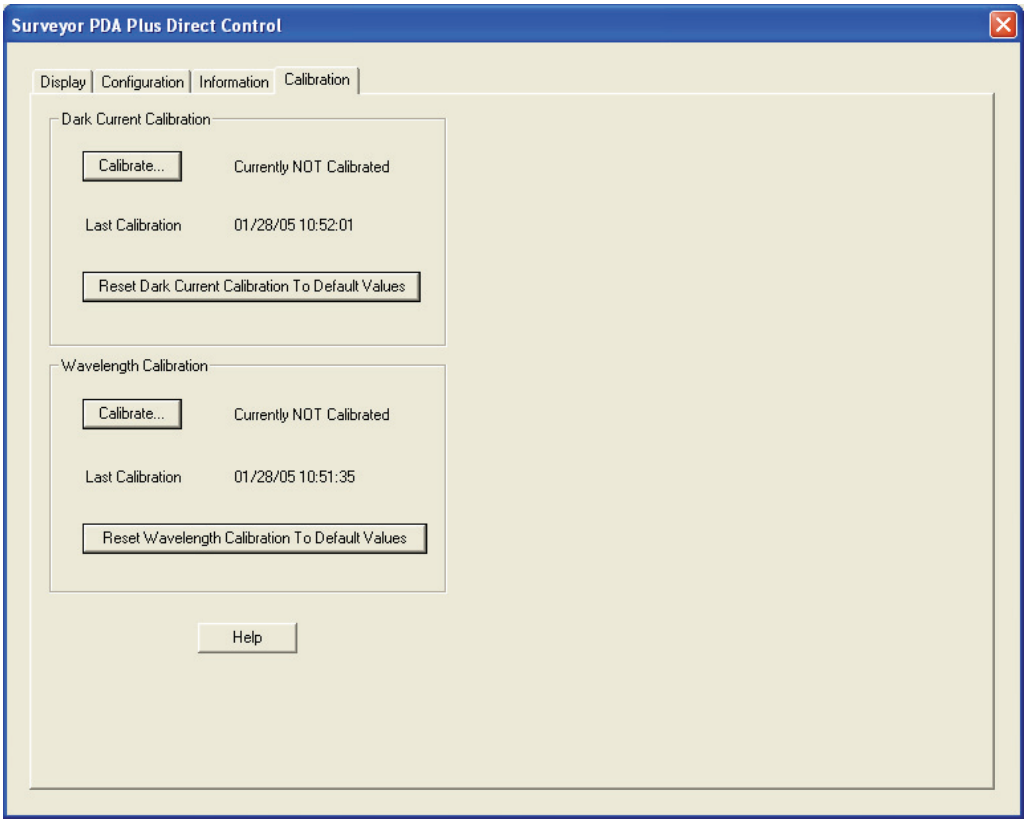


Figure 140. Calibration page, showing that the detector is currently Not calibrated

4. To start the Dark Current Calibration wizard, click **Calibrate** in the Dark Current Calibration area.

   The Dark Current Calibration wizard contains four pages. Xcalibur displays the first page of the Dark Current Calibration wizard, the Preconditions page shown in Figure 141.

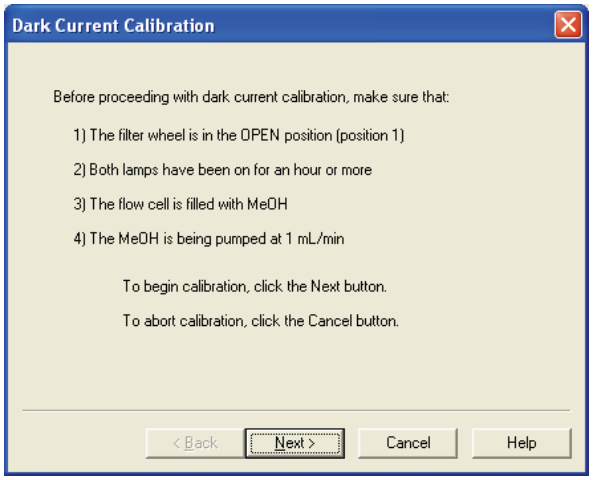


Figure 141. Dark Current Calibration wizard – page 1

5. Read the Preconditions page:

   - If all of the preconditions have been met, click **Next** to proceed with the calibration.
   - If the preconditions have not been met, click **Cancel** to exit the wizard. Then prepare the Surveyor PDA Plus Detector for calibration and begin this procedure again.

6. From page 2 of the Dark Current Calibration wizard, observe the status readback as the calibration proceeds. See Figure 142. After the calibration is complete, click **Next**.

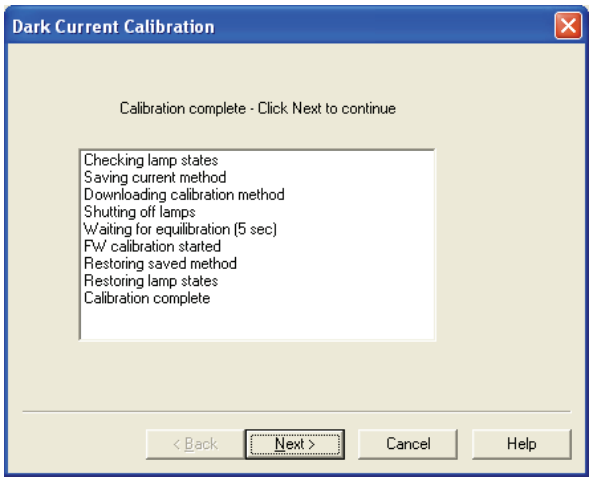


Figure 142. Dark Current Calibration wizard – page 2

Xcalibur displays the third page of the Dark Current Calibration wizard shown in Figure 143. You can export the results of the calibration from this page.

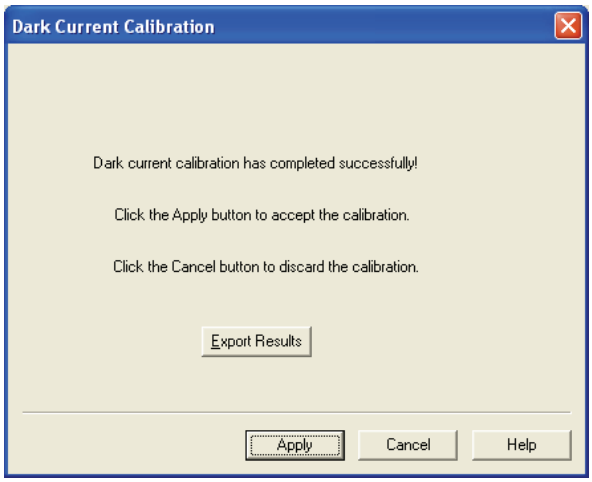


Figure 143. Dark Current Calibration wizard – page 3

7. To print a record of the Dark Current calibration:

   a. Click **Export Results**.

      Xcalibur opens the Save As dialog box shown in Figure 144.

   b. Type a name in the Save As dialog box. 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 145.

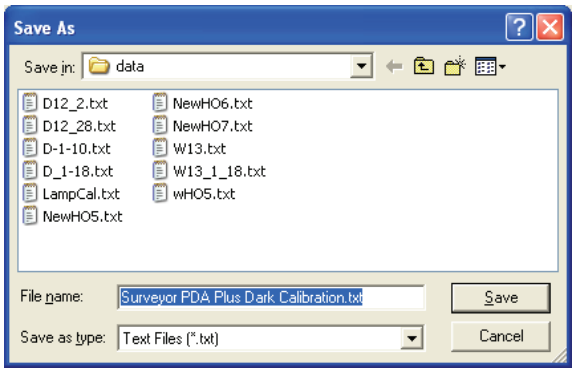


Figure 144. Save As dialog box

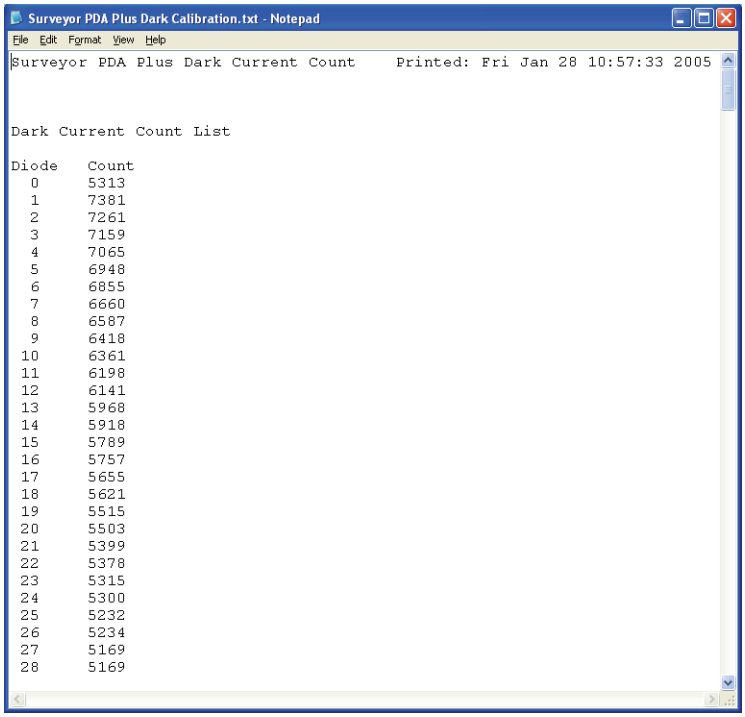


Figure 145. Surveyor PDA Plus Dark Calibration text file

8. In the third page of the Dark Current Calibration wizard, click **Apply** to apply the calibration results to the detector.

9. The Dark Current Calibration wizard proceeds to its fourth and final page. See Figure 146.

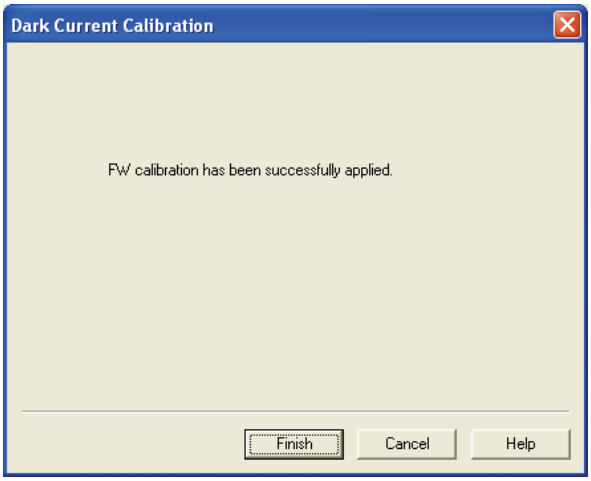


Figure 146. Dark Current Calibration wizard – final page

10. In the final page of the Dark Current Calibration wizard shown in Figure 146, click **Finish** to complete the calibration.

    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 147.

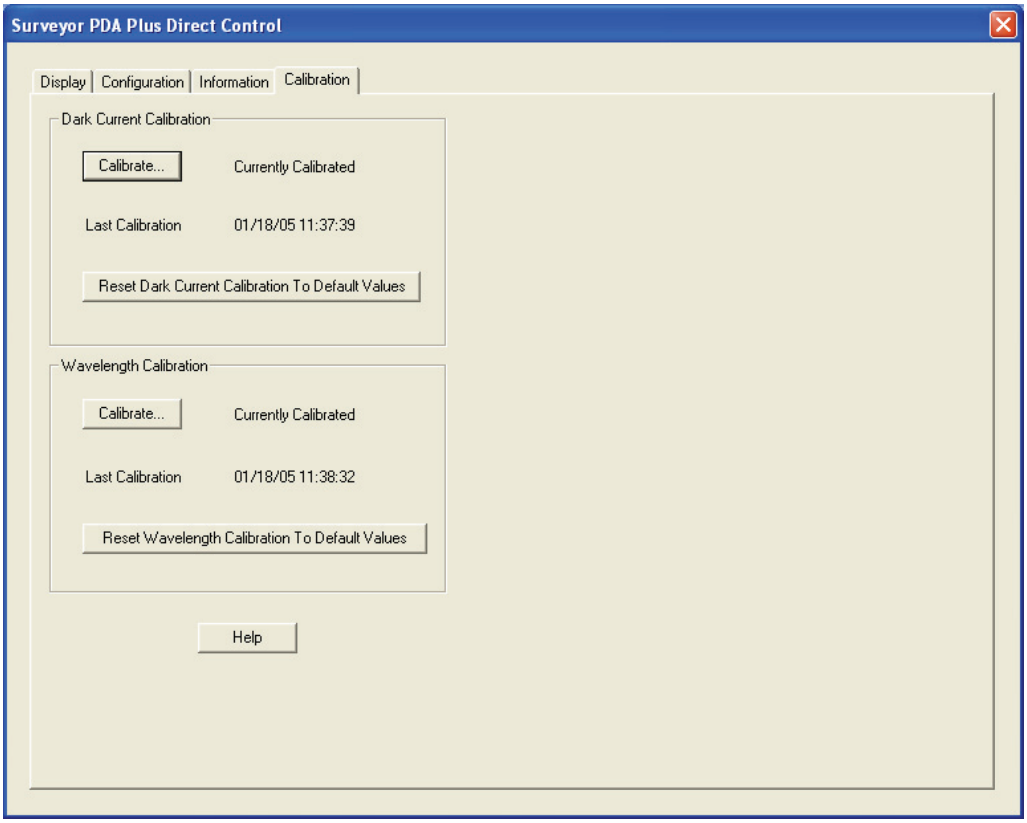


Figure 147. Surveyor PDA Plus Direct Control – Calibration page, showing the status as "Currently Calibrated"

Calibrating the Autosampler

There are four calibration options for the full-featured Surveyor Autosampler Plus and two calibration options available for the Surveyor Autosampler Plus Lite. This section contains the following topics that describe three of these calibration options:

- Column Oven Calibration
- Vial Tray Metal Sensor Calibration
- Well Bottom Distance Calibration

The column oven and oven compartment temperature calibrations are typically performed by a Thermo Electron service representative. To perform these calibrations yourself, you need to order the Field Service Calibration Kit (P/N 60053-62001).

The arm calibration option, which is not listed above, is performed at the factory.

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.

Column Oven Calibration

To perform a column oven air sensor calibration

1. Install the Oven Sensor Test Fixture (869C thermometer):

   a. Open the column oven door, and then loosen the top thumbscrew that holds the column clamp.

   b. With the sensor facing down, slide the metal cable protector under the right side of the clamp.

   c. Verify that the sensor is between the upper and lower column clamps and that it is not touching any metal.

   d. 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.

2. Set the column oven temperature of your Surveyor Autosampler Plus to 30.0° C:

   a. Double-click the Xcalibur icon on your desktop to open the Roadmap – Homepage.

b. If the Information View is closed, click the Information View button to open it. Click the Surveyor AS directory to display the status pages for the Surveyor Autosampler. Verify that the Surveyor AS status reads Ready to Download.

c. Click the Instrument Setup icon to display the Instrument Setup window.

d. Click the Surveyor AS icon in the viewbar.

e. From the Surveyor AS menu, select **Direct Control** to display the Direct Control dialog box shown in Figure 148.

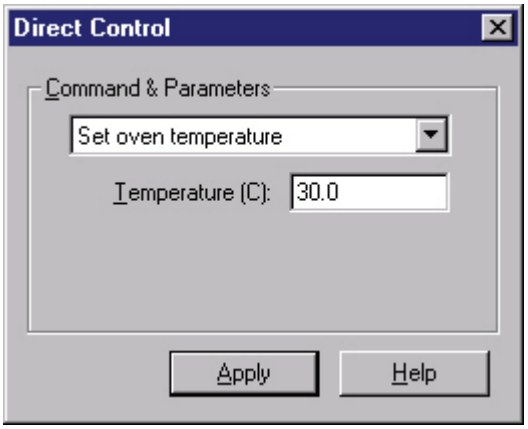


Figure 148. Direct Control dialog box

f. Select **Set Oven Temperature** from the Command and Parameters list.

g. Type **30.0** in the Temperature box, and then click **Apply**.

h. Close the Direct Control dialog box.

3. Open the Column Oven Air Sensor Wizard:

a. From the Surveyor AS menu, choose **Calibration** to display the Diagnostic dialog box shown in Figure 149.

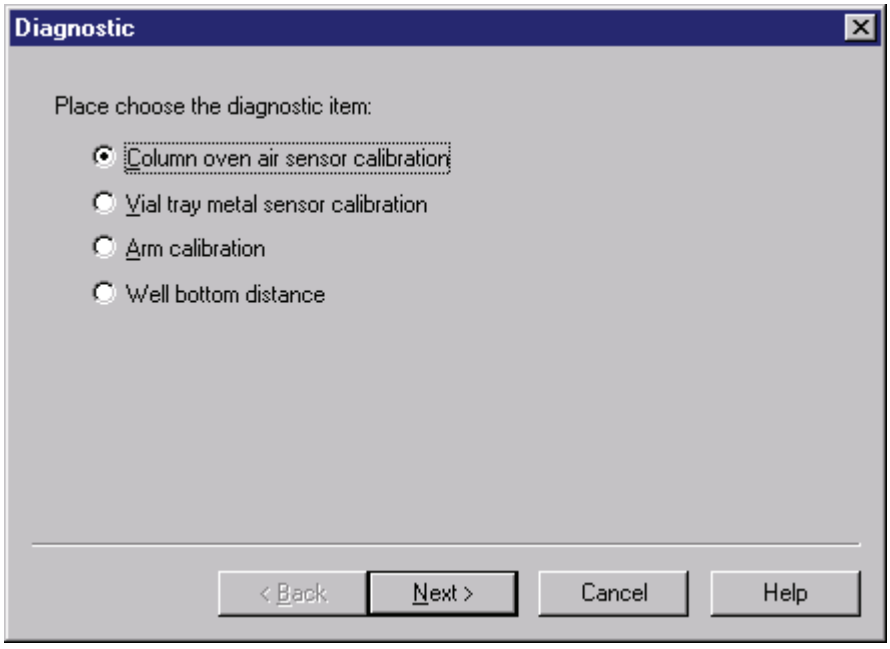


Figure 149. Surveyor Autosampler calibration options

b. Select the Column Oven Air Sensor Calibration option button. Then, click **Next** to display the Diagnostic dialog box - Internal Standard page. See Figure 150.

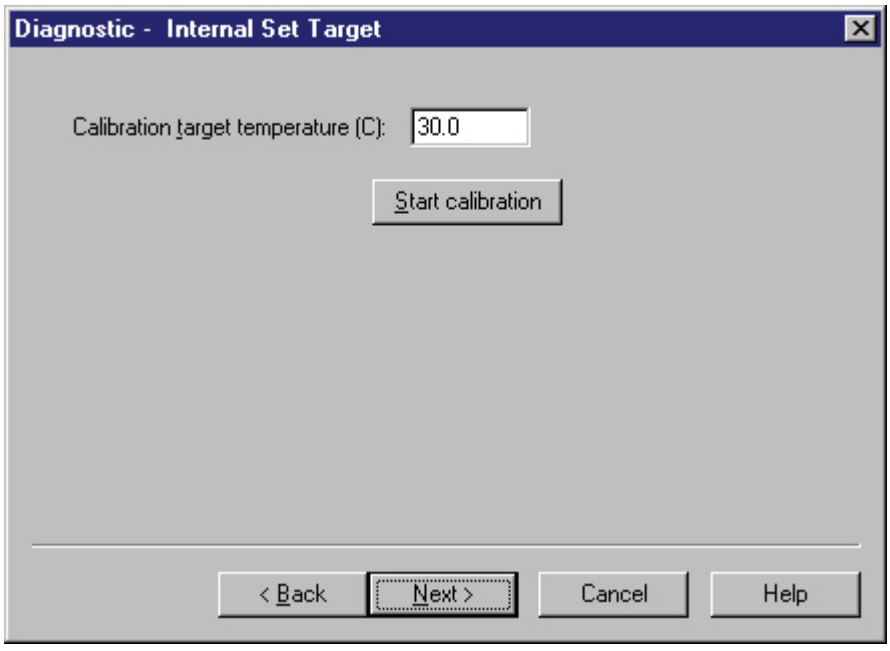


Figure 150. Diagnostic dialog box – Internal Set Target page

4. Type **30.0** in the Calibration Target Temperature box (see Figure 150). Then, click **Start Calibration** to display the Diagnostic dialog box - External Standard page shown in Figure 151.

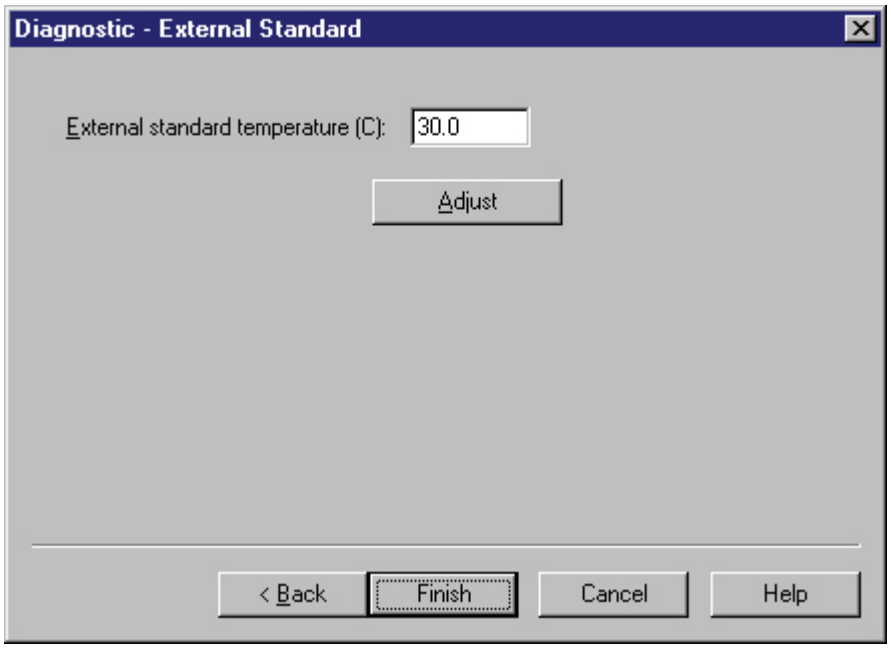


Figure 151. Diagnostic dialog box – External Standard page

5. Verify that the current oven temperature is moving towards the set point of 30.0° C:

   a. Return to the Xcalibur Roadmap - Home Page.

   b. Click the Surveyor AS directory in the Status window. Then, select the Oven tab. See Figure 152.

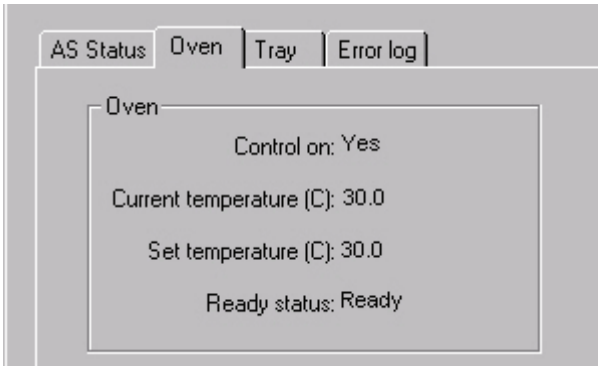


Figure 152. Surveyor Autosampler Oven status

6. When the current temperature readout in the Oven area reaches exactly 30.0° C (see Figure 152):

   a. Type the reading from the 869C thermometer in the External Standard Temperature box (see Figure 151).

   b. Verify that the current temperature in the Oven area is still displaying 30.0° C. Then, click **Adjust** in the Diagnostic - External Standard page (see Figure 151).

   c. Repeat steps 5a and 5b until the readings from the 869C thermometer and the current temperature readout agree within ±0.2° C.

7. After 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 External Standard page (see Figure 151).

8. Verify that the current temperature readout is stable at 30.0° C with a maximum temperature drift of ±0.2° C (see Figure 152).

9. After you verify the stability of the column oven temperature, remove the temperature probe and close Xcalibur.

Vial Tray Metal Sensor Calibration

The vial tray metal temperature sensor calibration wizard is used to calibrate the vial tray temperature control by using an external temperature sensor.

Note The vial tray metal sensor calibration is performed by a Thermo Electron service representative. To perform this calibration yourself, you need to order the Field Service Calibration Kit (P/N 60053-62001).

To perform the vial tray metal sensor calibration procedure, you need the following items that are included in the Field Service Calibration kit:

- Calibrated Omega 869C RTD thermometer
- Surveyor A/S Vial Tray Sensor

To perform a vial tray metal sensor calibration

1. To install the tray temperature sensor:

   a. Open the door to the tray compartment.

b. Install the Tray Temperature sensor (The tray temperature sensor is a standard tray with a temperature sensor potted in a middle vial location of the tray.) into location E of the tray compartment.

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. Set the tray temperature of your Surveyor Autosampler to 30.0° C:

a. If the Information View is closed, click the Information View button to open it. Click the Surveyor AS directory to display the status pages for the Surveyor Autosampler. Verify that the Surveyor AS status reads Ready to Download.

b. Click the Instrument Setup icon to display the Instrument Setup window.

c. Click the Surveyor AS icon in the viewbar.

d. Select **Direct Control** from the Surveyor AS menu to display the Direct Control dialog box. See Figure 153.

e. In the Command and Parameters area, select Set Tray Temperature from the list.

f. Set the temperature to 30.0° C in the temperature box. Then, click **Apply**.

g. Close the Direct Control dialog box.

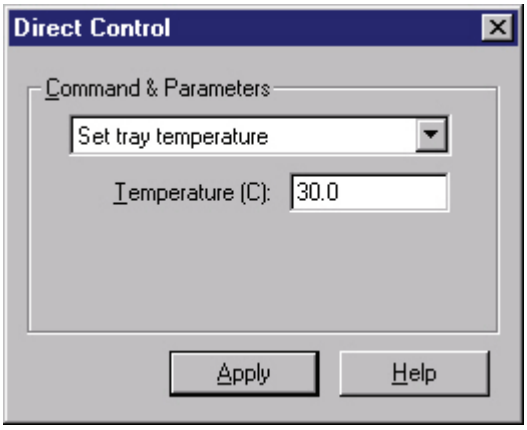


Figure 153. Direct Control – Command and Parameters area

3. Start the Vial Tray Metal Sensor Calibration wizard:

a. From the Surveyor AS menu, choose **Calibration**.

b. Select the Vial Tray Metal Sensor Calibration option button shown in Figure 154, and then click **Next** to display the Diagnostic dialog box – Internal Set Target page shown in Figure 155.

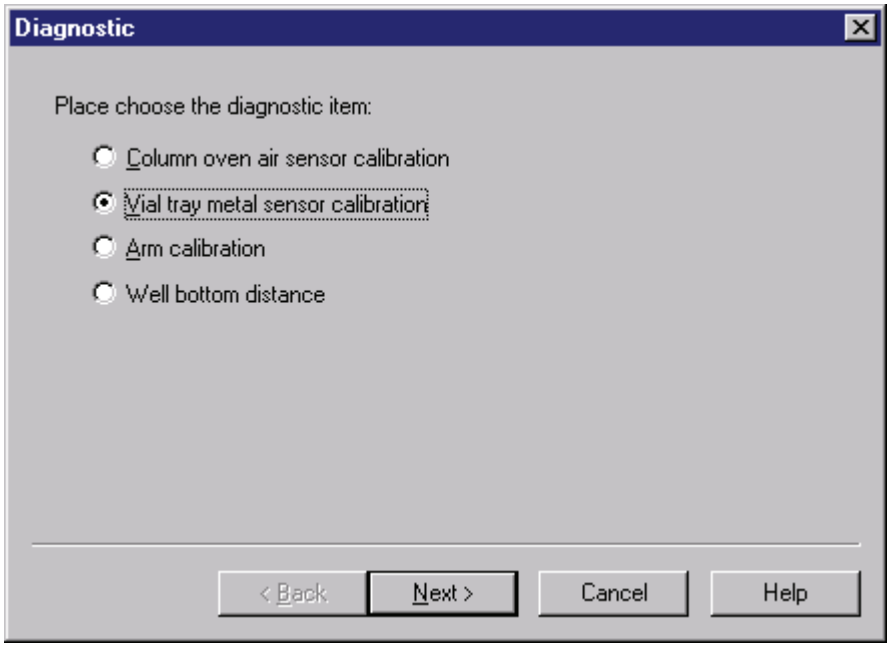


Figure 154. Diagnostic dialog box – Calibration options

4. Type **30.0** in the Calibration Target Temperature box. Then click **Start Calibration** to display the Diagnostic dialog box – External Target page shown in Figure 156.

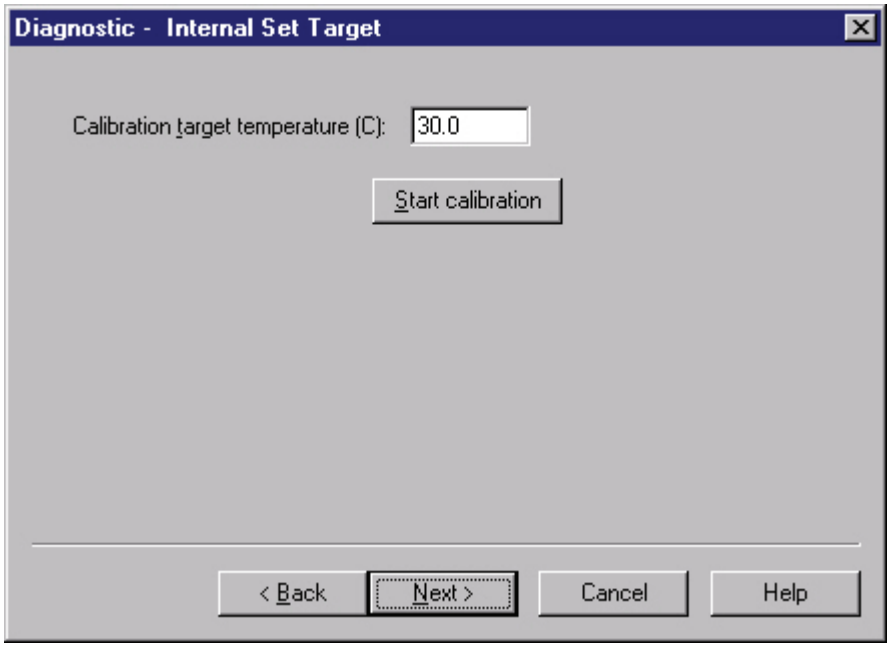


Figure 155. Diagnostic dialog box – Internal Set Target page

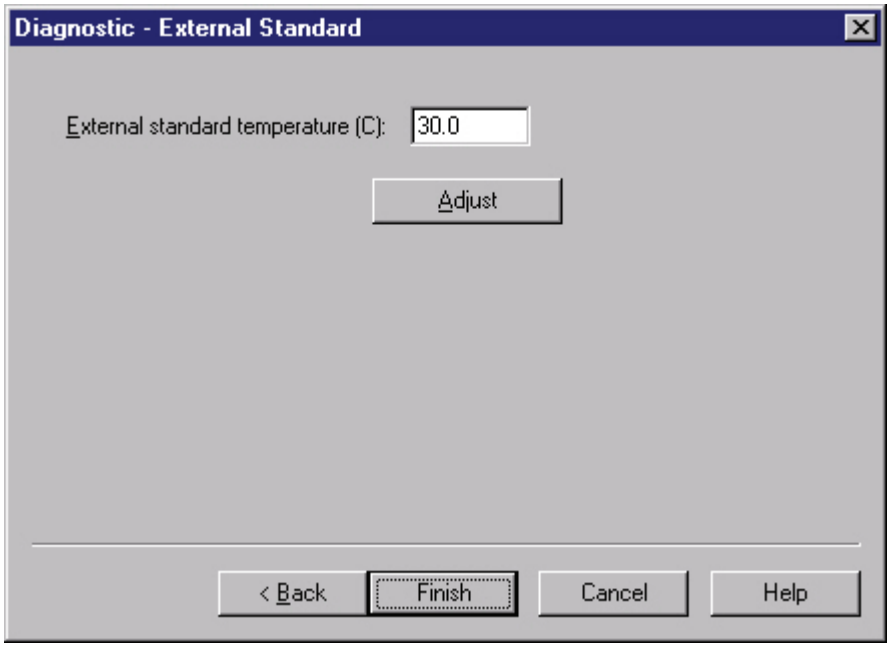


Figure 156. Diagnostic dialog box – External Standard page

5. Verify that the current tray temperature is moving towards the set point of 30.0° C:

   a. Return to the Xcalibur Roadmap – Home Page.

   b. Click the Surveyor AS directory in the status window, and then select the Tray tab to display the Tray page shown in Figure 157.

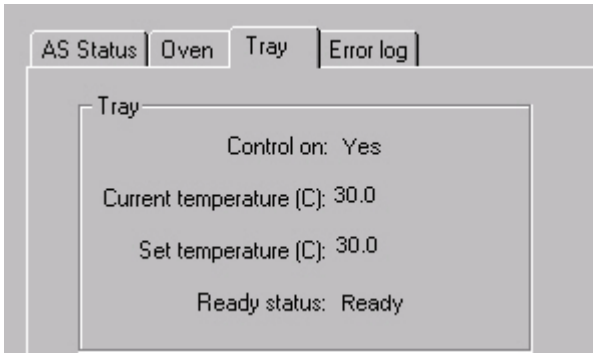


Figure 157. Surveyor AS Status – Tray page

6. When the current temperature readout in the Tray area reaches exactly 30.0° C (see Figure 157):

   a. Type the reading from the 869C thermometer in the External Standard Temperature box (see Figure 156).

   b. Verify that the current temperature in the Tray area is still reading 30.0° C (see step 4). Then, click **Adjust** in the External Standard page (see Figure 156).

   c. Repeat steps a and b until the temperatures on the thermometer and the current temperature readout (see Figure 157) agree within ± 0.2° C

7. After the temperatures on the 869C thermometer and the current temperature readout in the Oven area are within ± 0.2° C of each other, click **Finish** at the bottom of the External Standard page.

8. Verify that the current temperature readout is stable at 30.0° C with a maximum temperature drift of ± 0.2° C.

9. After you have verified the stability of the oven tray temperature, remove the temperature probe and close Xcalibur.

Well Bottom Distance Calibration

The Well Bottom Distance wizard is used to calibrate 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. The well bottom distance wizard consists of two dialog boxes: The Select Calibration Method dialog box and the Well Bottom Distance dialog box.

Note 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 microtitre plate.

To perform a well bottom distance calibration

1. Open the Well Bottom Distance Calibration Wizard:

   a. If the Information View is closed, click the Information View button to open it. Click the Surveyor AS directory to display the status pages for the Surveyor Autosampler. Verify that the Surveyor AS status reads Ready to Download.

   b. Click the Instrument Setup icon to display the Instrument Setup window.

   c. Click the Surveyor AS icon in the viewbar.

   d. From the Surveyor AS menu, choose **Calibration** to display the Diagnostic dialog box shown in Figure 158.

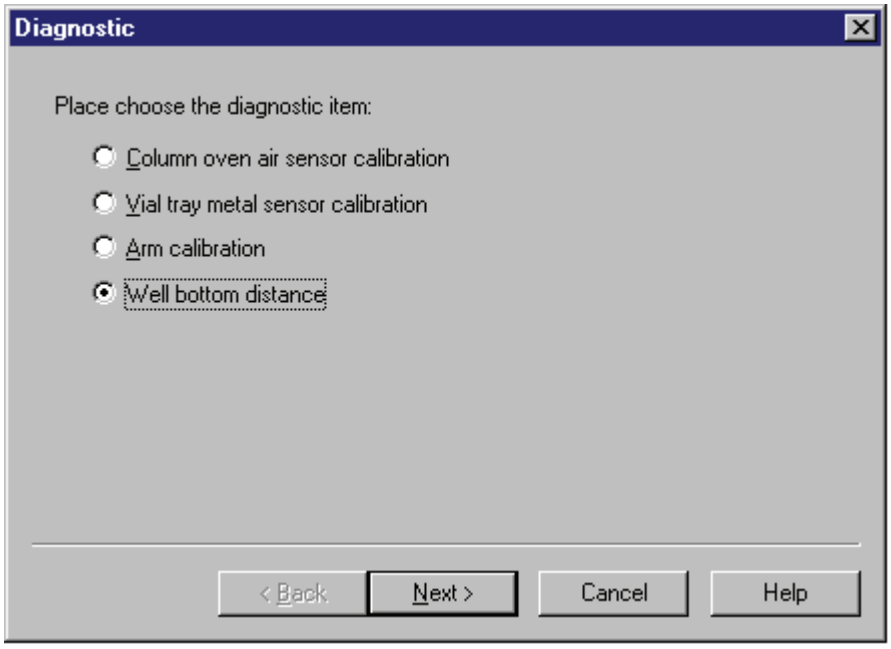


Figure 158. Diagnostic dialog box, showing calibration options

e. Select the Well Bottom Distance option button (see Figure 7-34), and then click **Next** to display the Select Calibration Method page of the Well Bottom Distance wizard. See Figure 159.

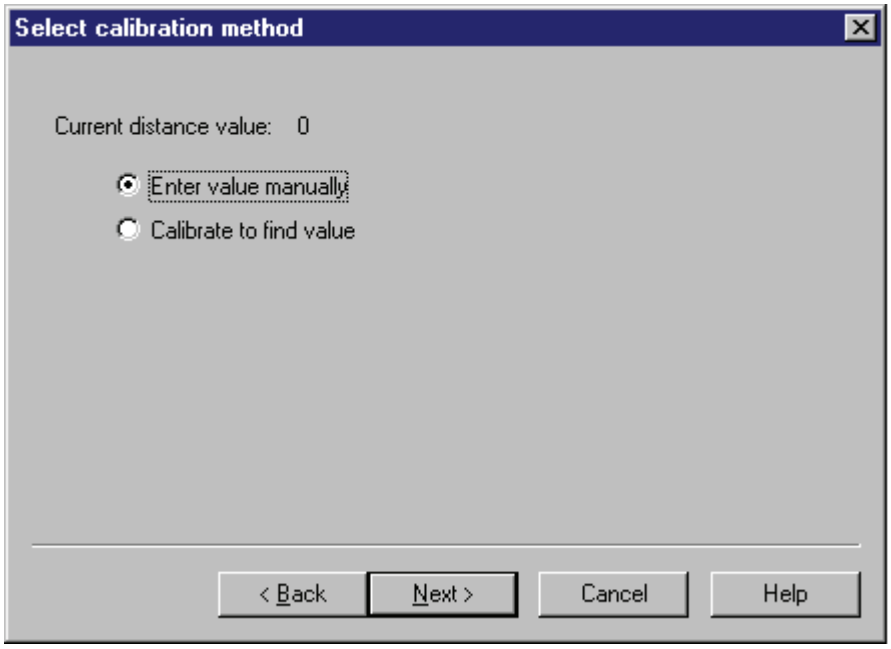


Figure 159. Well bottom Distance Calibration wizard - page 1

2. Select one of the calibration options:

   - Select the Enter The Value Manually option button to enter previously determined value for the well bottom distance. Click **Next**. Then go to step 3.

   - Select the Calibrate To Find Value option button to perform an active calibration. Click **Next**. Then go to step 4.

   Note The Surveyor 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.

3. If you selected the Enter The Value Manually option on the Select Calibration Method page of the wizard, you can enter a new value for the well bottom distance in the Well Bottom Distance dialog box (see Figure 160):

   a. Enter the new value in microns.

      The allowable range is 15000 to 46990 microns (15 mm to 46.9 mm)

   b. Click **Set Calibration**.

      The new value appears at the top of the dialog box.

   c. Click **Finish** to exit.

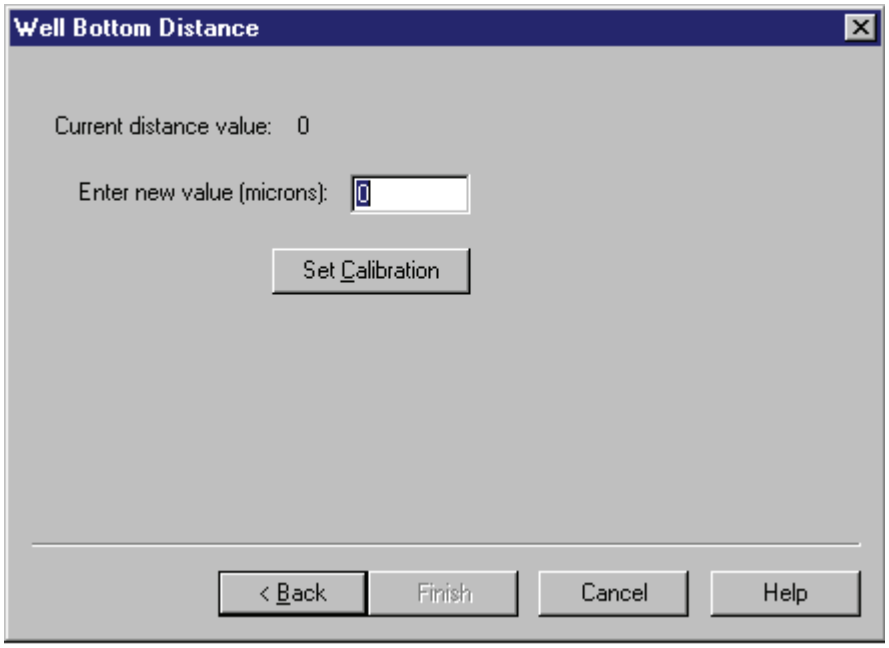


Figure 160. Well Bottom Distance wizard – Manual Calibration

IMPORTANT Remove vial caps or microtitre plate lids before performing an active well bottom distance calibration. As the needle pierces a vial cap or a microtitre 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 tray compartment of the autosampler, check the height limitations for microplates and high-density micro plates listed in the hardware manual for your autosampler. Tall objects will stall the autosampler arm.

4. If you selected the Calibrate To Find Value option button on the Select Calibration Method page of the wizard, you can perform an active calibration using the Well Bottom Distance dialog box shown in Figure 161:

   a. Remove the cap or lid from your vial or microtitre plate.

   b. Place the vial into a tray or the microtitre plate into a carrier, and then place the tray or carrier into the tray compartment of the autosampler.

   c. Type a vial or well location in the Calibrate At Well Position text box.

Note If you are calibrating the bottom distance of a vial, verify that you have placed a vial in the selected location.

d. Click **Set Calibration** to activate the autosampler.

After the autosampler 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 autosampler arm moves to the home position, and the software program displays the new bottom distance calibration value at the top of the dialog box.

5. Click **Finish** to accept the calibration.

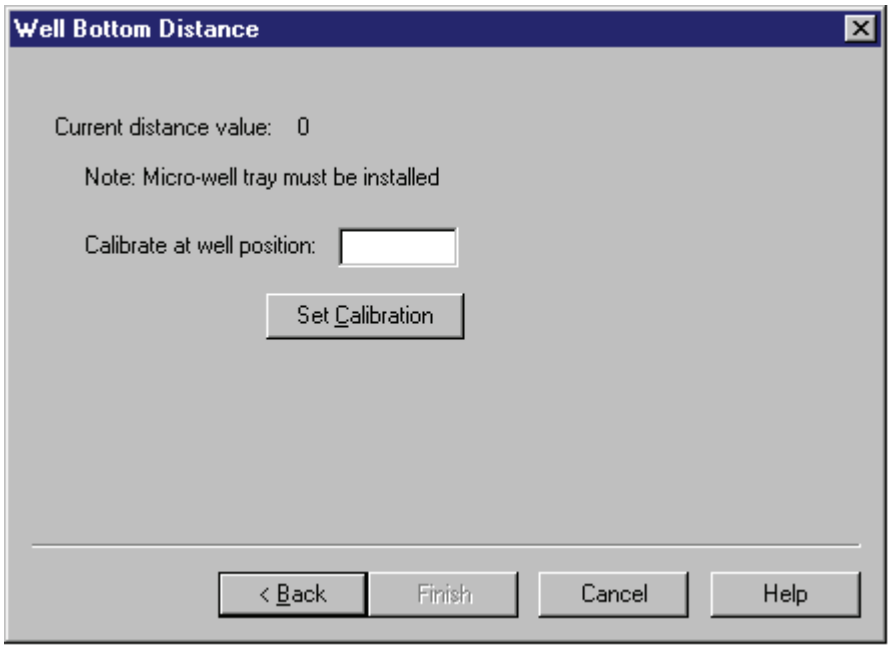


Figure 161. Well Bottom Distance Calibration wizard – Active Calibration

Calibrating the LC Pump

There are two calibration options for the Surveyor LC Pump that affect its performance. These are the alpha setting, which affects the flow rate, and the compressibility setting, which affects the pressure pulsation of the system. The compressibility setting is set to that for water, and then the alpha setting is factory calibrated to produce an accurate flow rate 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. Changing the compressibility setting affects the accuracy of the flow rate. Therefore, after you optimize the compressibility setting to minimize the pressure pulsation of your system, you will want to check the accuracy of the flow rate. If adjusting the compressibility setting has affected the accuracy of your flow rate, you will want to adjust the alpha setting of the LC pump.

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 will need to update the pressure sensor adjustment setting. Even if you never need to replace the pressure sensor, its readout tends to drift under normal usage. Therefore, you need to occasionally re-zero its output.

This section contains the following topics:

Accessing the Calibration Options for the LC Pump

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 Instrument Setup – Surveyor LC Pump view:
   a. Double-click the Xcalibur desktop icon or choose **Start > All Programs > Xcalibur > Xcalibur**.
   b. Click the Instrument Setup icon on the Home Page Roadmap View to display the Instrument Setup window.

c. Click the Surveyor LC Pump icon in the View bar to display the Surveyor LC Pump view.

3. From the Surveyor LC Pump view, choose **Surveyor LC Pump > Direct Control > Calibration** to open the Calibration – Surveyor LC Pump Direct Control dialog box shown in Figure 162.

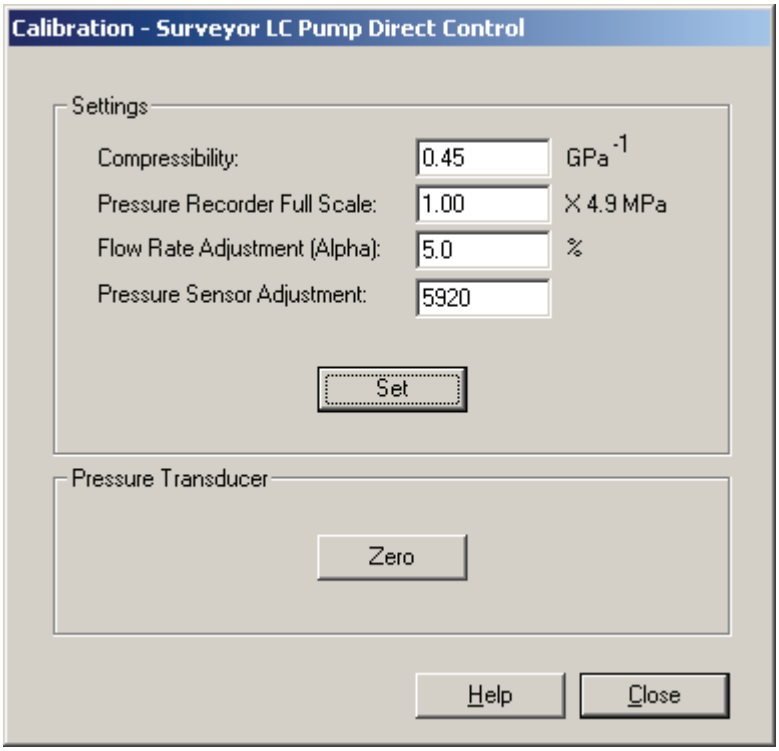


Figure 162. Calibration page for the Surveyor LC Pump

Calibration Options

The Xcalibur data system provides the following calibration options (see Figure 162) for the Surveyor LC Pump Plus. These options are described in this topic.

- Compressibility
- Pressure Recorder Full Scale
- Flow Rate Adjustment (Alpha)
- Pressure Sensor Adjustment
- Pressure Transducer Zero

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.

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 163 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.

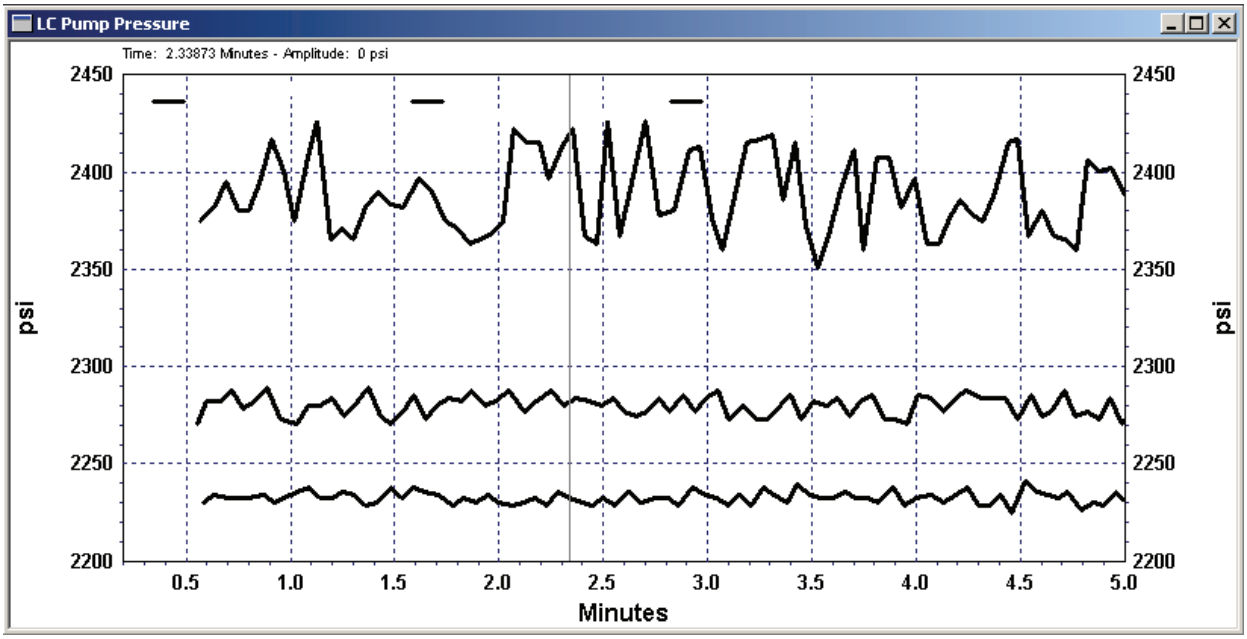


Figure 163. Overlaid LC pump pressure traces, showing the effect of the compressibility setting

Table 10 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 10. Measured flow rate and pressure pulsation vs. 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

Most mobile phases are made up of a mixture of solvents. Therefore, you need to determine the correct compressibility setting for a given mobile phase empirically. Table 11 lists the compressibility ratios for a few common solvents.

Table 11. Compressibility ratios for common solvents

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. See Figure 164. 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.

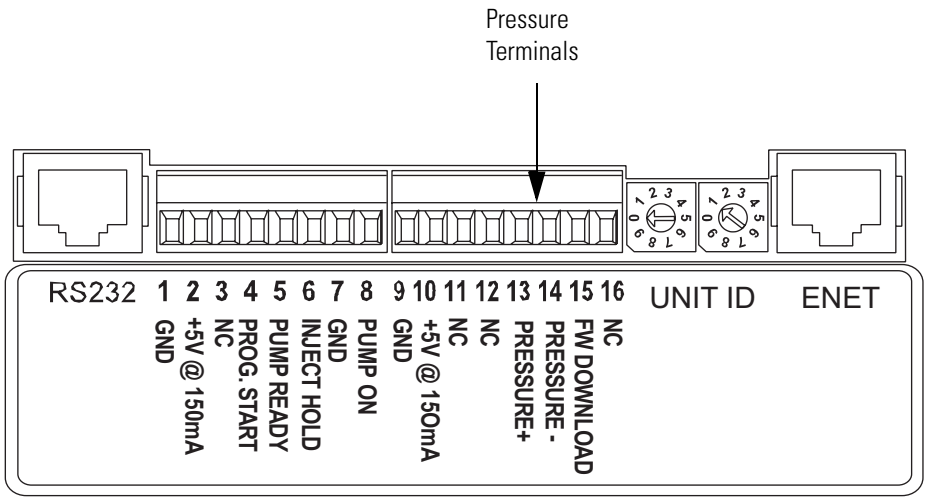


Figure 164. Terminals located on the back of the Surveyor LC Pump Plus

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 allowable 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%. Whereas, increasing the alpha value by 1, increases the flow rate by 1%.

Pressure Sensor Adjustment

Reset the Pressure Sensor Adjustment parameter when the pressure sensor is replaced. 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 10000 and then update the pressure sensor adjustment setting for your Surveyor LC Pump Plus.

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. Therefore, you will occasionally need to re-zero the readout. To update the pressure transducer zero value, turn off the pump flow and open the drain valve knob before clicking the pressure transducer zero button in the software program that operates your pump.

Calibration Procedures

You fine-tune the performance of the Surveyor LC Pump Plus from the Calibration - Surveyor LC Pump Direct Control dialog box (Figure 162 on page 212). For instructions on accessing this dialog box, see "Accessing the Calibration Options for the LC Pump" on page 211.

This topic contains the following calibration procedures for fine-tuning the performance of the Surveyor LC Pump Plus:

- Updating the Compressibility Setting
- Optimizing the Compressibility Setting
- Scaling the Output of the Pressure Recorder Terminals
- Updating the Flow Rate Adjustment Setting
- Updating the Pressure Sensor Adjustment Setting
- Re-zeroing the Pressure Transducer

Updating 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.

To update the compressibility setting

1. Open the Calibration page for the Surveyor LC Pump as described in "Accessing the Calibration Options for the LC Pump" on page 211.

2. Type a new value into the Compressibility setting box. Then click **Set**.

Optimizing the Compressibility Setting

To optimize the compressibility setting for you 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 215.

   b. Scale the output of the pressure terminals as described in "Scaling the Output of the Pressure Recorder Terminals" on page 219.

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. Open the Instrument Setup - Surveyor LC Pump view as described in step 2 on page 211.

   c. From the Surveyor LC Pump view, choose **Surveyor LC Pump > Direct Control > Operation** to open the Operation - Surveyor LC Pump Direct Control dialog box shown in Figure 165.

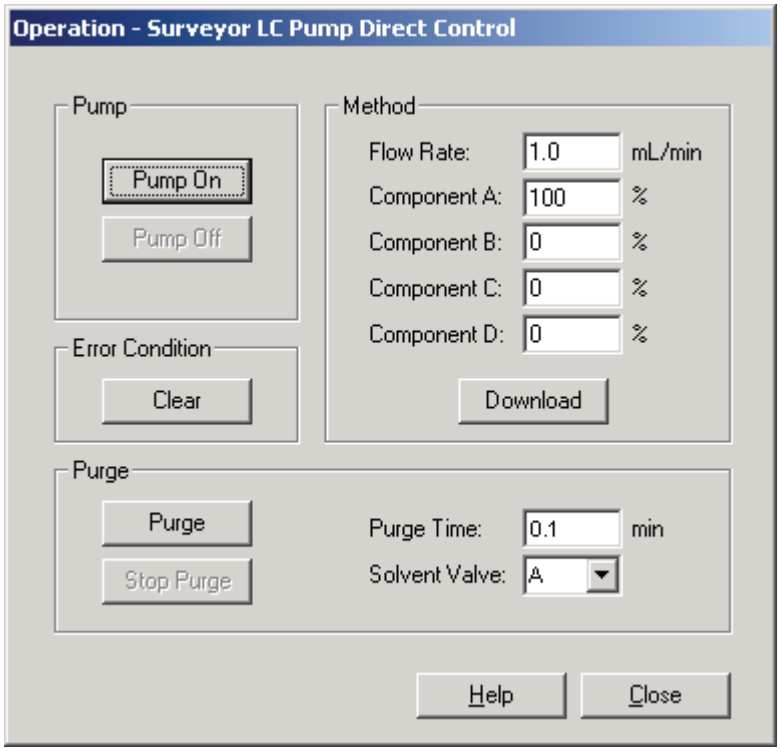


Figure 165. Surveyor LC Pump – Direct Control – Operation page

d. 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

e. Click **Download** to send these parameters to the on-board CPU of the Surveyor LC Pump.

f. 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 will affect 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 219.

Scaling the Output of the Pressure Recorder Terminals

To set the full-scale output of the pressure recorder terminals

1. Open the Calibration page for the Surveyor LC Pump Plus as described in "Accessing the Calibration Options for the LC Pump" on page 211.

2. Enter a value from 1 to 10 in the Pressure Recorder Full-Scale box (Figure 162 on page 212). Then, click **Set**. For information on scaling the output of the pressure recorder terminals, see "Pressure Recorder Full Scale" on page 215.

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 as described in "Accessing the Calibration Options for the LC Pump" on page 211.

2. Enter a new value in the Flow Rate Adjustment (alpha) box (see Figure 162 on page 212):

   - If the actual flow rate is below the set value, increase the alpha value. If you are pumping water, increasing the alpha value by 1, increases 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 the pressure sensor is replaced. 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 as described in "Accessing the Calibration Options for the LC Pump" on page 211.

2. Enter the value on the data sticker "0.XXXX" multiplied by 10000 in the Pressure Sensor Adjustment setting box (see Figure 162 on page 212).

3. Click **Set**.

Re-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 166.

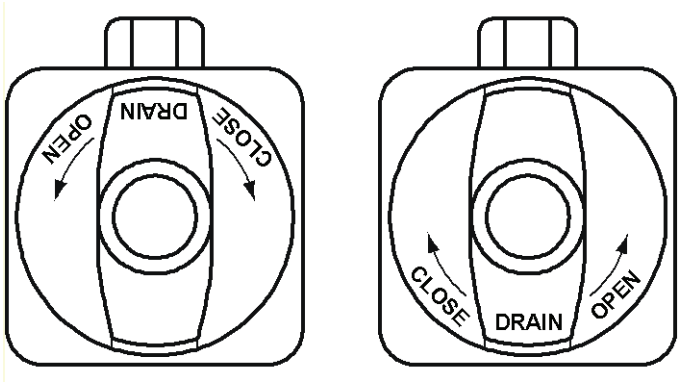


Figure 166. Drain valve in open and closed positions

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 162 on page 212).

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.

Index

A

B

C

D

E

F

G

H

I

L

M

N

O

P

Q

R

S

T

U

V

W

X

Z