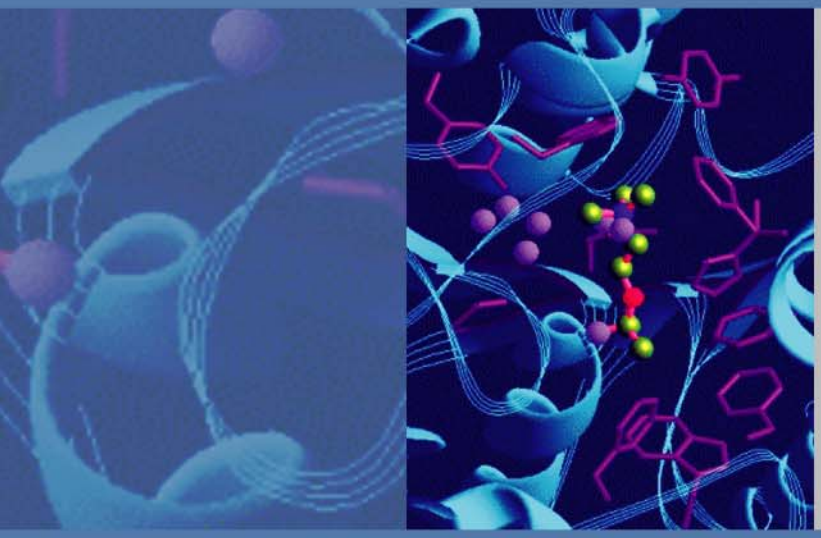


Accela UHPLC System

User Guide for LC Devices

(version 2.5.0 or later)

60057-97050 Revision A March 2011

DOCUMENTATION SURVEY

Part of Thermo Fisher Scientific

Thermo SCIENTIFIC

Software versions: Thermo Foundation 1.0.1 or later, Thermo Xcalibur 2.1.x or later, and LC Devices 2.5.0 or later

Revision history: Revision A, March 2011

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

Regulatory Compliance

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

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

Accela Pump, Accela Autosampler, and Accela PDA Detector (20 Hz)

EMC Directive 89/336/EEC, 92/31/EEC, 93/68/EEC

EMC compliance has been evaluated by TUV Rheinland of North America, Inc.

EN 61326	1997; A1, 1998; A2, 2001; A3, 2003	EN 61000-4-4	1995; A1, 2000; A2, 2001
EN 61000-3-2	2000	EN 61000-4-5	2001
EN 61000-3-3	1995; A1, 2001	EN 61000-4-6	2003
EN 61000-4-2	2001	EN 61000-4-8	2001
EN 61000-4-3	2002	EN 61000-4-11	2001

FCC Class A, CFR 47 Part 15 Subpart B: 2005

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, IEC 61010-1:2002, UL 61010 A-1:2004, CAN/CSA 22.2 61010-1:2004.

Thermo SCIENTIFIC

Accela 600 Pump

EMC Directive 2004/108/EC

EMC compliance has been evaluated by TUV Rheinland of North America Inc.

EN 61326-1: 2006
EN 55011: 2007
EN 61000-3-2: 2006
EN 61000-3-3: 1995, A1: 2001, A2: 2005
EN 61000-4-2: 1995, A!: 1999, A2: 2001
EN 61000-4-3: 2006
EN61000-4-4: 2004
EN61000-4-5: 2005
EN61000-4-6: 2007
EN61000-4-11: 2004

FCC Class A: CFR 42, Part 15: 2007

Low Voltage Safety Compliance

This device complies with Low Voltage Directive 2006/95/EC and the following harmonized standards: EN 61010-1: 2001, IEC 61010-1: 2002, UL 61010A-1: 2004, CAN/CSA 22.2 61010-1: 2004.

Accela 1250 Pump

EMC Directive 2004/108/EC

EMC compliance has been evaluated by TUV Rheinland of North America Inc.

EN 55011: 2007
EN 61000-3-2: 2006
EN 61000-3-3: 1995, A1: 2001, A2: 2005
EN 61000-4-2: 1995, A1: 1999, A2: 2001
EN 61326-1: 2006
FCC Class A: CFR 47, Part 15: 2009
EN 61000-4-3: 2006
EN 61000-4-4: 2004
EN 61000-4-5: 2005
EN 61000-4-6: 2007
EN 61000-4-11: 2004

Low Voltage Safety Compliance

This device complies with Low Voltage Directive 2006/95/EC and the following harmonized standards: EN 61010-1: 2001, IEC 61010-1: 2002, UL 61010A-1: 2004, CAN/CSA 22.2 61010-1: 2004.

Thermo SCIENTIFIC

Accela PDA Detector (80 Hz version)

EMC Directive 2004/108/EC

EMC compliance has been evaluated by TUV Rheinland of North America, Inc.

EN 61326-1	2006	EN 61000-4-3	2006
EN 55011	2007, A2: 2007	EN 61000-4-4	2004
EN 61000-3-2	2006	EN 61000-4-5	2005
EN 61000-3-3	1995, A1: 2001, A2: 2005	EN 61000-4-6	2007
EN 61000-4-2	1995, A1: 1999, A2: 2001	EN 61000-4-11	2001

FCC Class A, CFR 47 Part 15: 2007

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 2006/95/EC and harmonized standard EN 61010-1:2001, IEC 61010-1:2002, UL 61010 A-1:2004, CAN/CSA 22.2 61010-1:2004.

Accela UV/Vis Detector

EMC Directive 2004/108/EC

EMC compliance has been evaluated by TÜV Rheinland of North America, Inc.

EN 55011: 2007	EN 61000-4-3: 2006
EN 61000-3-2: 2006	EN 61000-4-4: 2004
EN 61000-3-3: 1995, A1; 2001, A2; 2005	EN 61000-4-5: 2005
EN 61326-1: 2006	EN 61000-4-6: 2007
EN 61000-4-2: 1995, A1; 1999, A2; 2001	EN 61000-4-11: 2004

FCC Class A, CFR 47 Part 15: 2008

Low Voltage Safety Compliance

Low Voltage Safety Compliance has been evaluated by TÜV Rheinland of North America, Inc.

This device complies with Low Voltage Directive 2006/95/EC and harmonized standard EN 61010-1:2001, IEC 61010-1:2002, UL 61010 A-1:2004, CAN/CSA 22.2 61010-1:2004.

FCC Compliance Statement

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

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

Notice on Lifting and Handling of Thermo Scientific Instruments

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

Notice on the Proper Use of Thermo Scientific Instruments

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

Notice on the Susceptibility to Electromagnetic Transmissions

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

For manufacturing location, see the label on the instrument.

WEEE Compliance

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

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

WEEE Konformität

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

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

Conformité DEEE

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

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

Contents

Preface

This guide describes how to use your Thermo Scientific mass spectrometry application to control the Accela™ ultra-high-performance liquid chromatography (UHPLC) system.

Note For information about controlling the Accela Open Autosampler from your Thermo Scientific data system, refer to the *Accela Open Autosampler User Guide*.

Contents

Tip Use the Lamp Startup Time feature to preserve the useful lifetime of the deuterium lamp. The default lamp startup time for the PDA detector is 4:55 PM. For optimal system performance, reset the lamp startup time to approximately two hours before you plan to start data acquisition. For information about changing the lamp startup time, see "Setting the Lamp Startup Time" on page 170.

Tip The default setting for the autosampler maintenance counters is 0. If you turn on the autosampler maintenance log (see "Communication Page" on page 50) when you specify the configuration settings for the autosampler and leave the counters at the default setting, the autosampler status displays Maintenance Due and the data system prevents you from starting a run. For information about changing the counter settings on the Maintenance Information page, see "Autosampler Maintenance Information" on page 325.

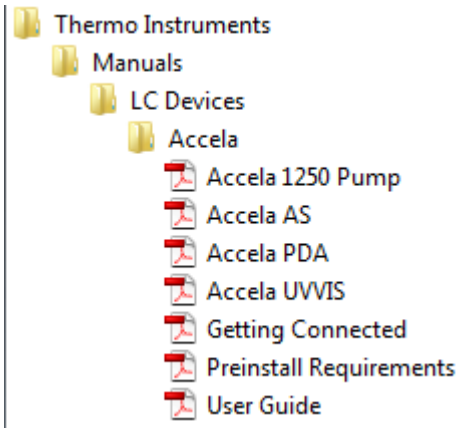
Related Documentation

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

- *Accela User Guide for LC Devices (versions 2.5.0 or later)*
- *Accela LC System Preinstallation Requirements Guide*
- *Accela UHPLC System Getting Connected Guide*
- *Accela Autosampler Hardware Manual*
- *Accela PDA (80 Hz) Detector Hardware Manual*
- *Accela UV/Vis Detector Hardware Manual*
- *Accela Pump Hardware Manual*
- *Accela 600 Pump and Accela 1250 Pump Hardware Manual*
- *Accela Open Autosampler User Guide*
- *Accela Open Autosampler Hardware Manual*

❖ To view manuals for your Accela LC devices

Go to **Start > Programs > Thermo Instruments > Manuals > LC Devices > Accela >**.



❖ To open Help

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

For more information, visit www.thermoscientific.com.

Safety and Special Notices

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

Safety and special notices include the following:

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

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

Note Highlights information of general interest.

Tip Highlights helpful information that can make a task easier.

Contacting Us

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

❖ To contact Technical Support

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

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

❖ To contact Customer Service for ordering information

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

❖ To get local contact information for sales or service

Go to www.thermoscientific.com/wps/portal/ts/contactus.

❖ To copy manuals from the Internet

Go to mssupport.thermo.com, agree to the Terms and Conditions, and then click **Customer Manuals** in the left margin of the window.

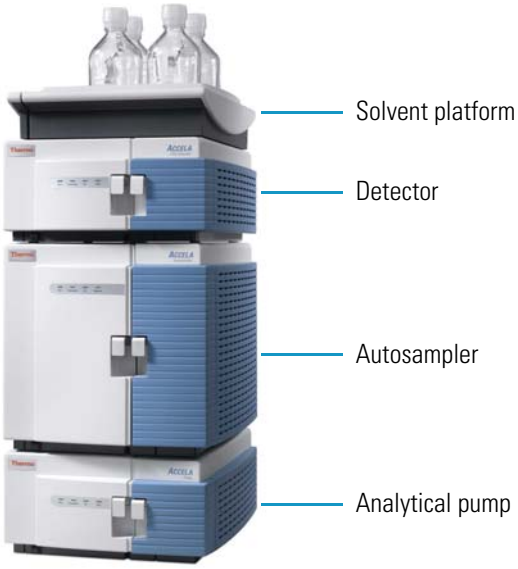
❖ To suggest changes to documentation or to Help

- Fill out a reader survey online at www.surveymonkey.com/s/PQM6P62.
- Send an e-mail message to the Technical Publications Editor at techpubs-lcms@thermofisher.com.

Introduction to the Accela System

The Accela ultra-high-performance liquid chromatography system (see Figure 1), which integrates with the Thermo Scientific family of mass spectrometers, consists of an analytical pump, an autosampler, an optional PDA detector or UV/Vis detector, and a solvent platform.

Figure 1. Accela ultra-high performance liquid chromatography system



Contents

Analytical Pump

Thermo Scientific, part of Thermo Fisher Scientific Inc., offers three analytical pumps in the Accela product line. Each pump has a built-in degassing unit and is remotely controlled with a USB communication link from the data system computer. The only manual control is the power switch located on the front of the pump in the lower-left corner below the door.

These topics describe the pump features:

- Accela Pump
- Accela 600 Pump and Accela 1250 Pump
- Built-in Degassing Unit

Accela Pump

The Accela Pump is a dual-piston, quaternary, low-pressure mixing pump with a built-in vacuum degasser and pulse dampener. The pumping system provides flow rates from 1.0 to 1000 µL/min, which is the range needed to perform LC and LC/MS applications. You can run precise gradients from 50 to 1000 µL/min, while the extremely low gradient delay volume of 65 µL ensures minimum system cycle times.

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

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

Accela 600 Pump and Accela 1250 Pump

The Accela 600 Pump and the Accela 1250 Pump are quaternary, low-pressure mixing pumps with a built-in solvent degassing system and an automatic calibration feature.

Table 1 lists the flow rate ranges where these pumps provide optimal performance as well as their maximum operating pressures. The minimum programmable flow rate range for both pumps is 1.0 µL/min.

Tip For gradient applications, Thermo Fisher Scientific recommends that you use a flow rate equal to or greater than twice the gradient delay volume of the pump's liquid displacement assembly (LDA).

Table 1. Flow rate range and maximum operating pressure

Pump	Flow rate range for optimal performance	Maximum operating pressure
Accela 600 Pump	50 to 5000 µL/min (isocratic) 180[a] to 5000 µL/min (gradient)	600 bar (8702 psi)
Accela 1250 Pump	50 to 2000 µL/min (isocratic) 140[b] to 2000 µL/min (gradient)	1250 bar (18 130 psi)

[a] The gradient delay volume of the LDA is 90 µL.

[b] The gradient delay volume of the LDA is 70 µL.

The pumps use a force sensor feedback controller, which continuously calibrates valve timing and pumping efficiency based on the measured compressibility of the solvent. This patent-pending feature enables the pumps to form accurate gradients virtually pulsation-free; no pulse damping device is required.

Built-in Degassing Unit

The Accela pumps have a built-in solvent degassing system that consists of four independent chambers maintained at a constant vacuum of approximately 50 mm Hg absolute. Each chamber contains an 18 in. length of 0.045 in. ID Teflon™ AF tubing, which translates to a volume of less than 500 µL per channel. This small chamber volume adds very little to the solvent volume required to purge the lines when you replace an eluent.

Autosampler

The Accela Autosampler automates sample injections and sample preparation. The autosampler includes a built-in column oven (5 to 95 °C) and tray/sample temperature control (0 to 60 °C). The following topics describe the autosampler tray compartment, the injection system, the injection modes, and the temperature control features:

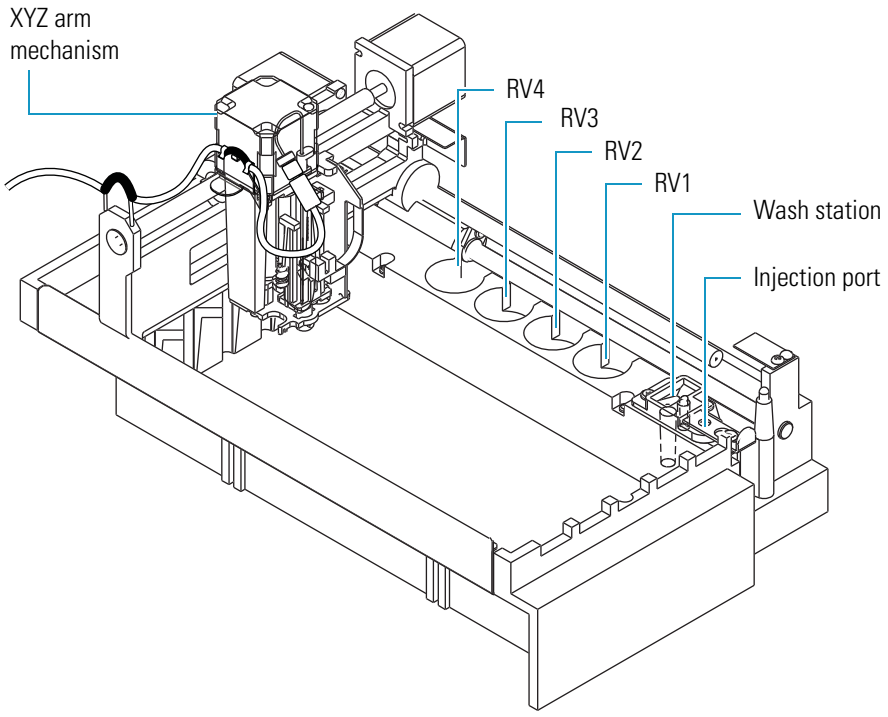
- Tray Compartment
- Vial and Well Notation
- Injection System
- Injection Modes
- Temperature Control

Note For information about the Accela Open Autosampler, refer to the Accela Open Autosampler user guide for your data system.

Tray Compartment

The tray compartment (see Figure 2) can hold up to five conventional sample trays or one carrier that holds three microwell plates. The tray compartment also holds up to four 16 mL capacity reservoir vials that you can use to hold solvent, reagent, or diluent. The reservoir vials are located behind the wash station and are designated RV1, RV2, RV3, and RV4.

Figure 2. Tray compartment



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

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

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

Vial and Well Notation

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

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

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

Vial Notation

The notation for the location of vials is as follows:

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

Where:

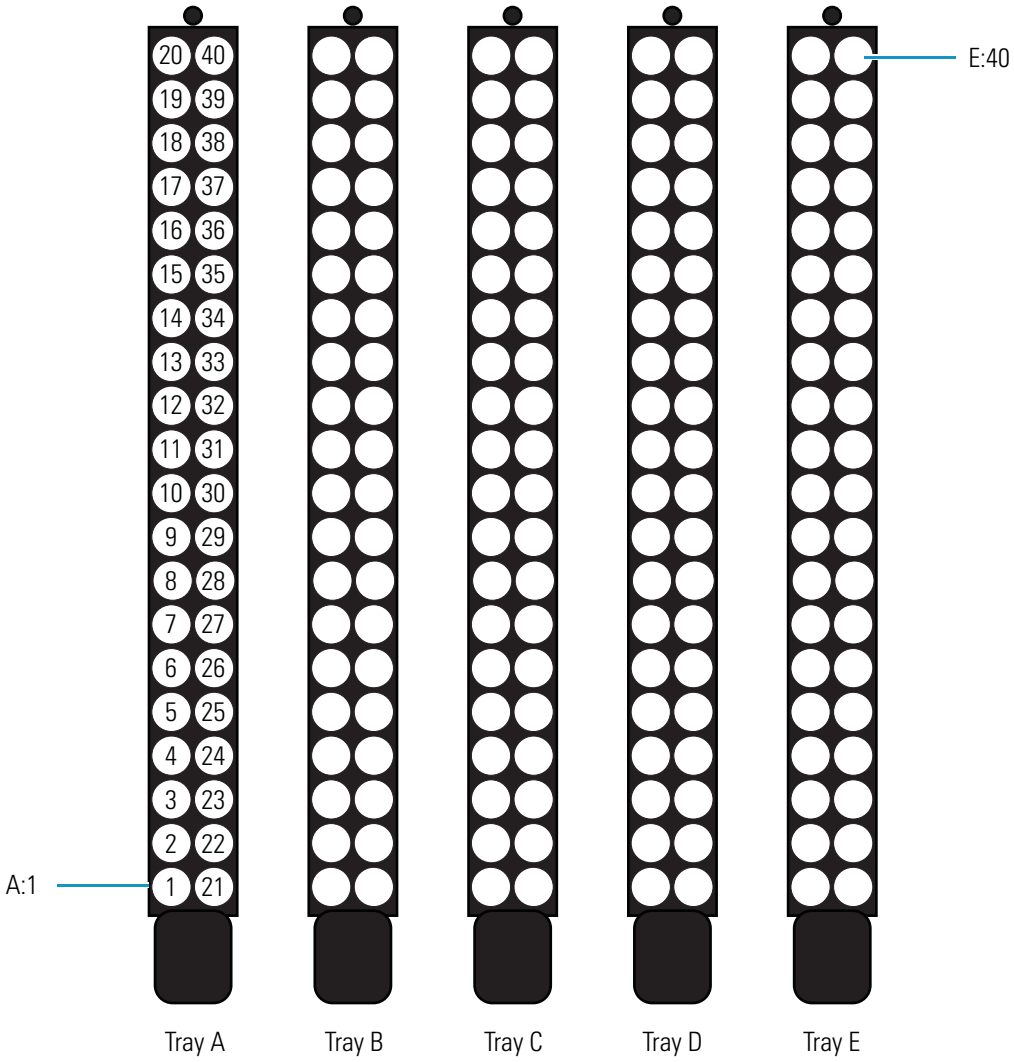
Tray location in tray compartment = A, B, C, D, or E

Vial location in tray = 1 through 40

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

Figure 3 shows the tray locations.

Figure 3. Standard trays



96-Well Microplate Notation

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

Plate:*RowColumn*

Where:

Plate = A, B, or C

Row = A through H

Column = 1 through 12

Figure 4 shows the notation for 96-well microplates when you select the top left orientation.

Figure 4. Notation for 96-well plates—Top left orientation

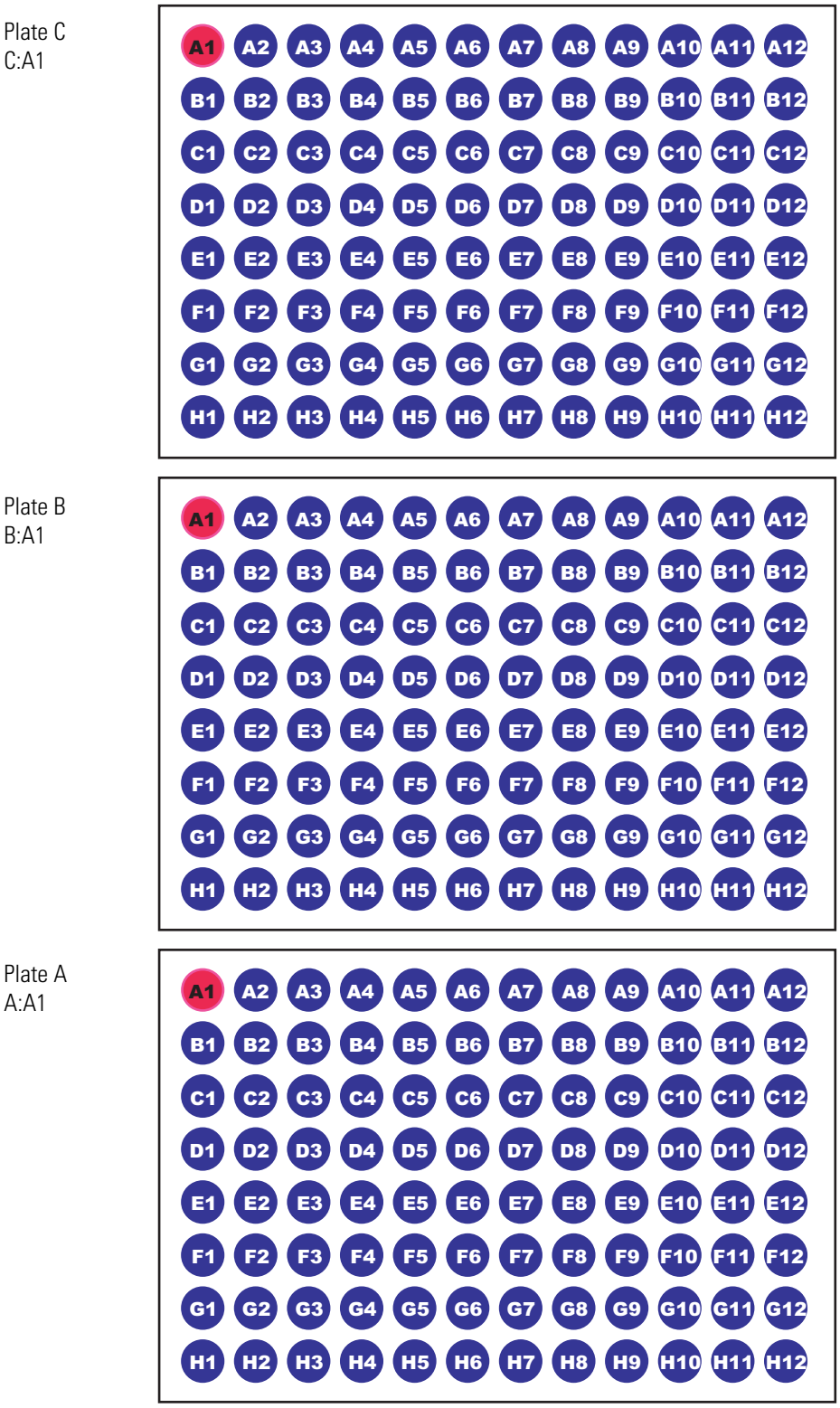
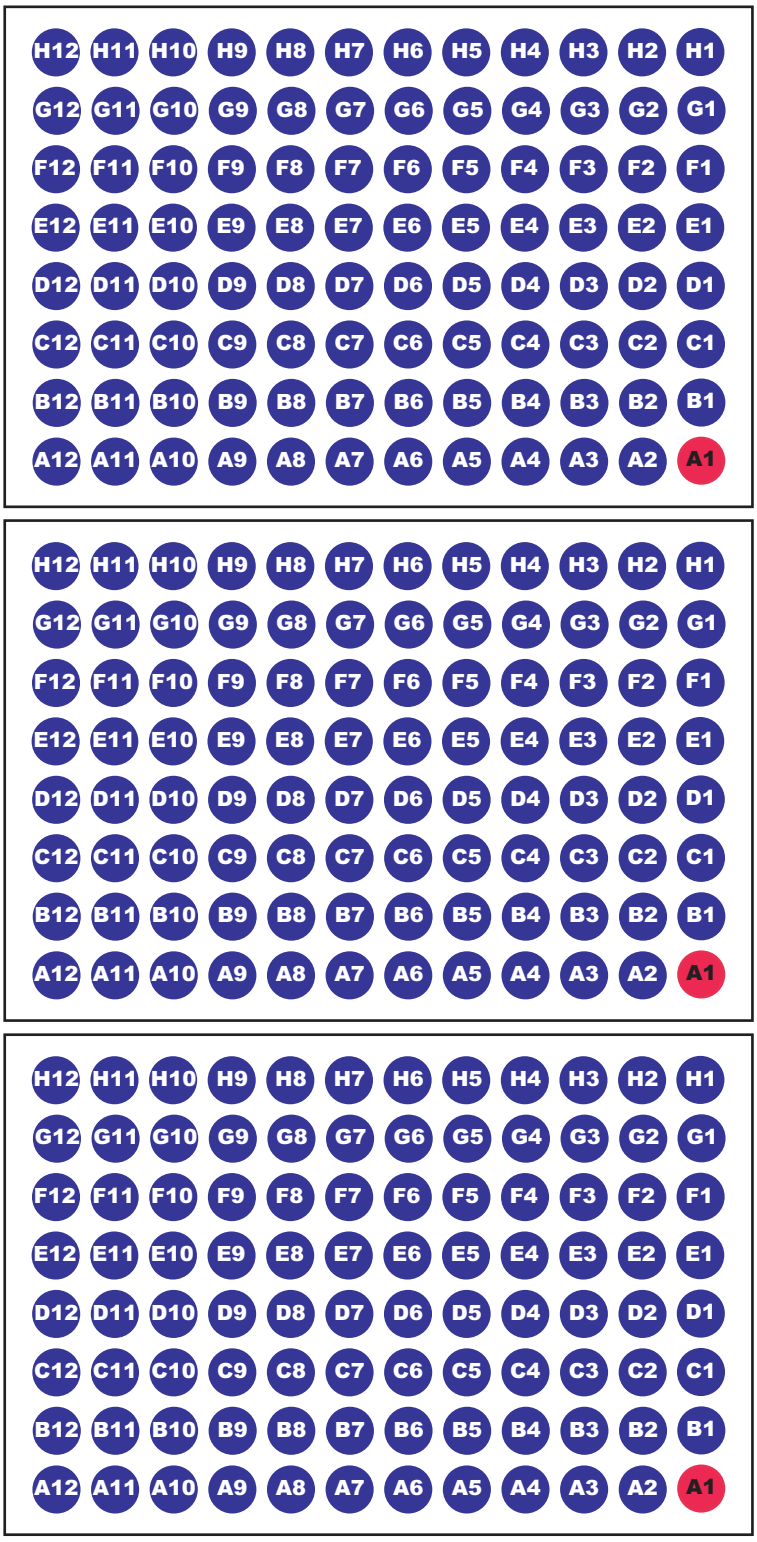


Figure 5 shows the notation for 96-well microplates when you select the bottom right orientation.

Figure 5. Notation for 96-well plates—Bottom right orientation



384-Well Microplate Notation

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

Plate:*RowColumn*

Where:

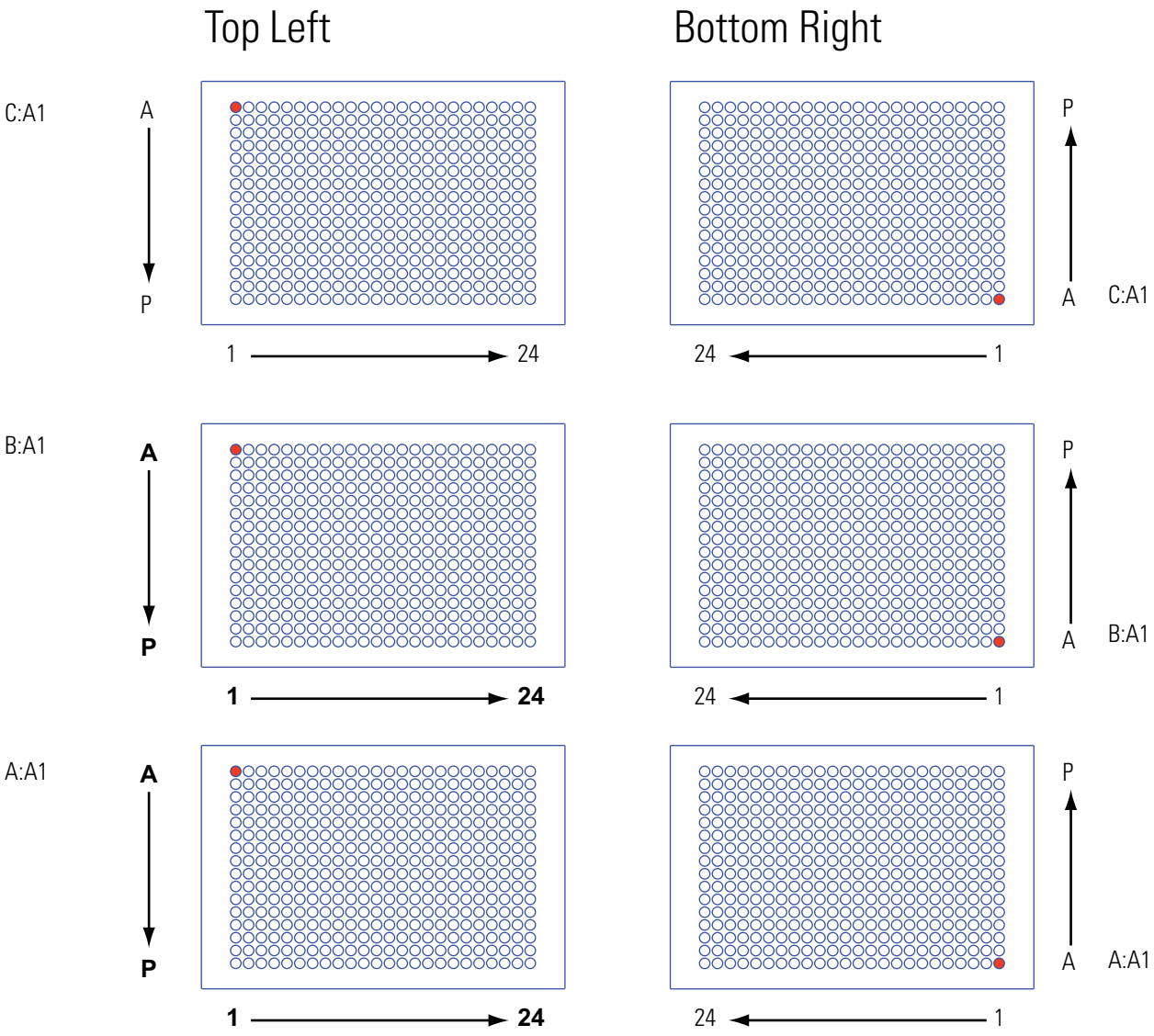
Plate = A, B, or C

Row = A through P

Column = 1 through 24

Figure 6 shows the notation for 384-well microplates.

Figure 6. Notation for 384-well microplates



Injection System

The main components of the injection system include the following:

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

Syringe Drive Assembly and Syringe Valve

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

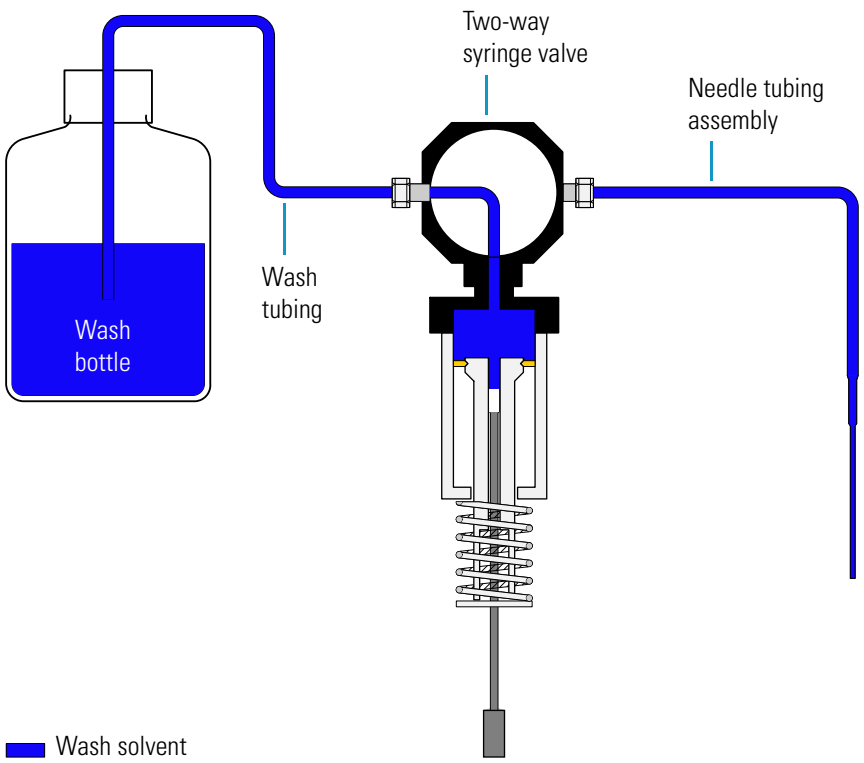
Note The needle tubing holds approximately 560 µL of solvent. If you installed the 2500 µL standard syringe (which must be special ordered) and you plan to make large volume injections, install the 1 mL needle tubing extension that comes with this syringe.

Wash Bottle Reservoir and Tubing

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

Figure 7 shows the syringe valve in the wash bottle position.

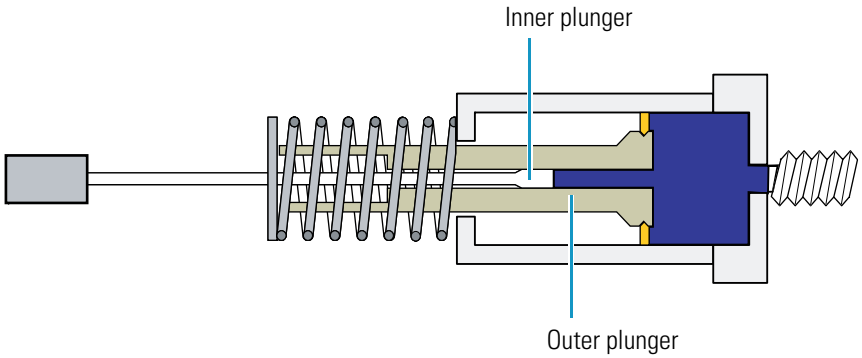
Figure 7. Drawing wash solvent from the wash bottle



Interchangeable Syringe

The standard configuration for the autosampler consists of a 250 µL dual-concentric syringe. The dual-concentric syringe consists of a small, inner plunger, and a larger, outer plunger (see Figure 8).

Figure 8. Dual-concentric syringe



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

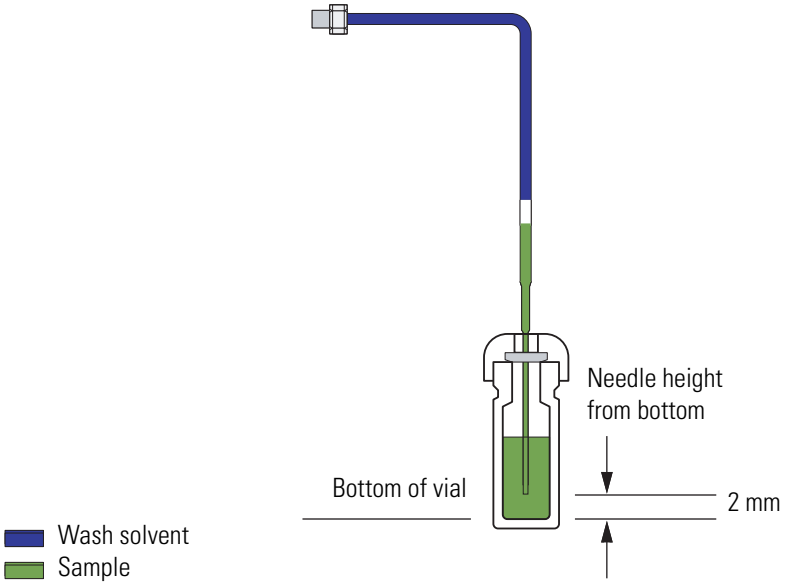
Dual-concentric syringes are available in 100, 250, and 500 µL sizes. The volume of the outer plunger region, 565 µL, is the same for all three dual-concentric syringes. The size of the inner plunger and the injection mode determine the available injection volume range. In addition to offering three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

XYZ Arm Mechanism

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

Figure 9 shows the needle descending to a depth of 2 mm from the bottom of a standard 1.8 mL vial.

Figure 9. Autosampler needle positioned 2 mm from the bottom of a vial



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

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

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

- To make the XYZ arm automatically move to the back of the tray compartment when you open the tray door, select the **Verify Door is Closed** option, which is available when you configure the autosampler device driver (see "Communication Page" on page 50).
- To move the XYZ arm to the back of the tray compartment, use the **Position Arm to Access Tray** direct command (see "Removing and Installing Sample Trays" on page 198).
- To move the XYZ arm mechanism to its home position above the injection port, use the **Set Arm to Home Position** direct command.
- To move the XYZ arm mechanism to the middle front of the tray compartment, allowing easy access to the needle, use the **Remove Needle** direct command.

Needle Assembly and Needle Tubing Assembly

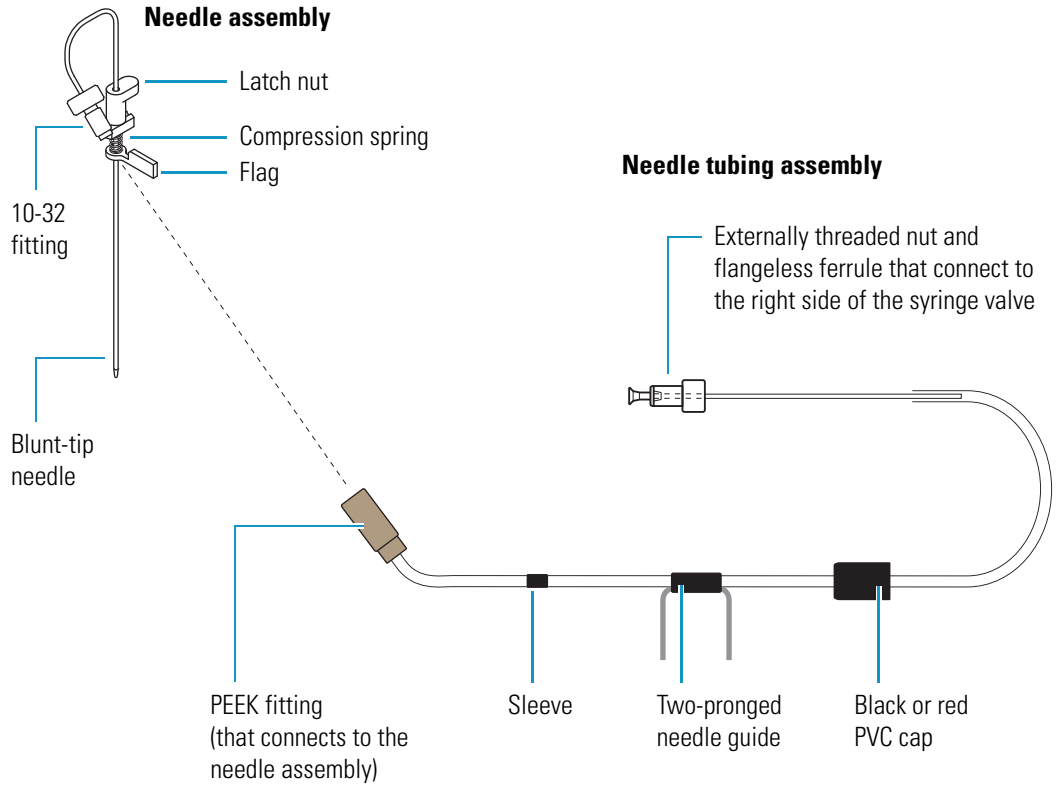
The needle assembly consists of a blunt-tip needle, a latch nut, a flag, a compression spring, and a 10-32 fitting that connects to the needle tube assembly. The needle fits into the needle mount on the XYZ arm.

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

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

Figure 10 shows the needle assembly and the needle tubing assembly.

Figure 10. Needle assembly and needle tubing assembly



Injection Port and Transfer Tubing Assembly

Figure 11 shows the autosampler injection port.

Figure 11. Autosampler injection port

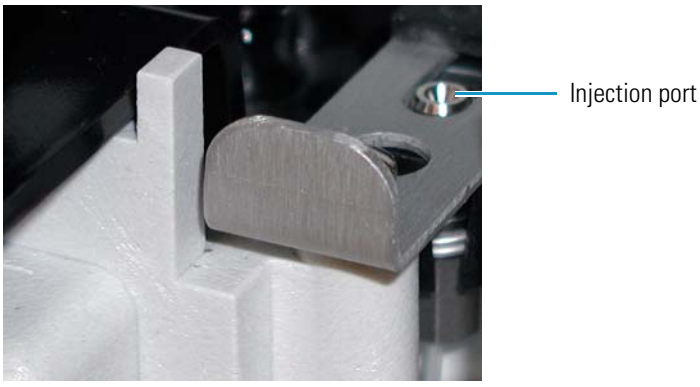
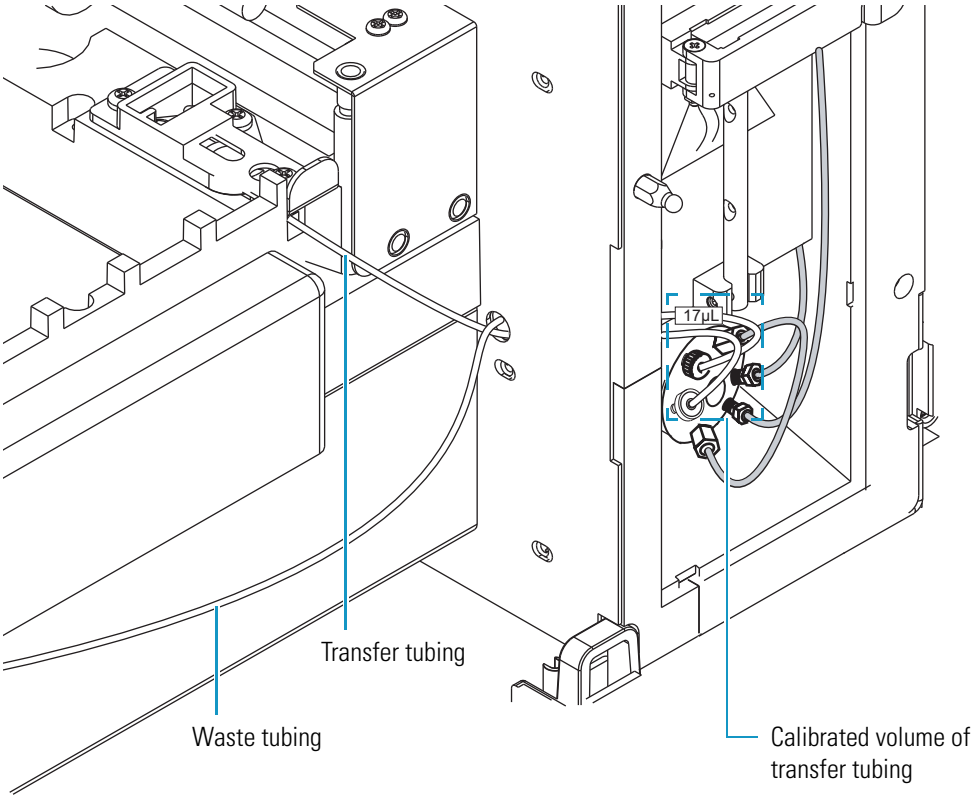


Figure 12 shows the 0.012 in. ID transfer tube that connects the autosampler injection port to port 2 of the injection valve.

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

Figure 12. Transfer tube connections to the autosampler injection port and injection valve



Injection Valve and Sample Loop

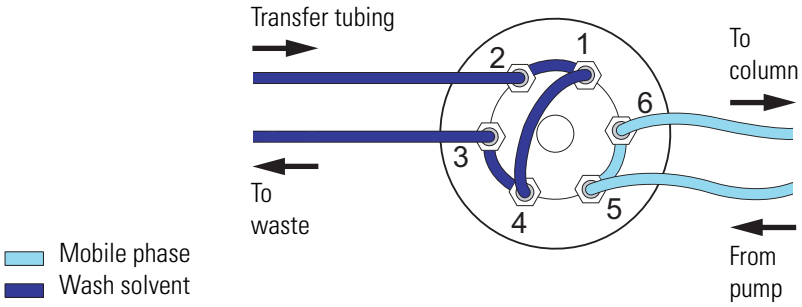
The injection valve is a two-position, six-port valve that introduces sample onto the column by way of the sample loop.

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

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

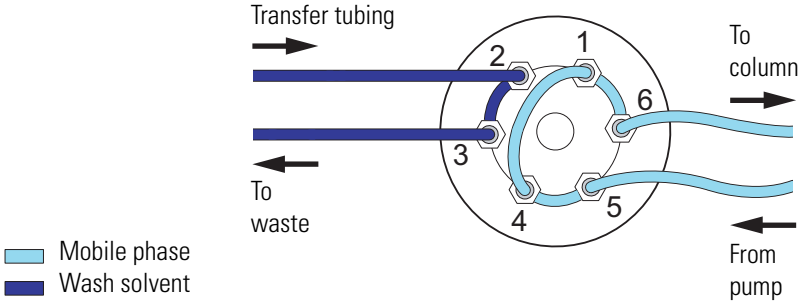
In the fill position (see Figure 13), the sample loop is isolated from the mobile phase stream. As the mobile phase bypasses the sample loop, the upward movement of the autosampler syringe plunger pushes sample into the front of the sample loop, connected to port 1 of the injection valve. Once the sample loop is filled, excess solution exits the injection valve through port 3 to waste.

Figure 13. Injection valve in the fill position



In the inject position (see Figure 14), mobile phase enters the sample loop from the back, backflushing the contents of the sample loop onto the column. Excess sample left in the transfer tube is expelled directly to waste. To allow ample rinsing of the sample loop with mobile phase, the injection valve remains in the inject position during the entire run.

Figure 14. Injection valve in the inject position



Injection Modes

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

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

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

No Waste Injection Mode

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

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

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

IMPORTANT For no waste injections, do the following:

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

Partial Loop Injection Mode

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

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

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

Full Loop Injection Mode

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

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

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

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

$$Amt_w = 3 \times V_{inj} + V_{dead} + 7.5\ \mu L$$

Where:

Amt_w = sample volume withdrawn by the autosampler

V_{inj} = user-specified injection volume

V_{dead} = dead volume held by the transfer tubing, injection port, and rotor slot

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

Temperature Control

The autosampler has two built-in temperature control features:

- Tray Temperature Control
- Column Oven Control

Tray Temperature Control

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

Column Oven Control

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

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

Detector

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

Note The Accela UV/Vis Detector driver is provided with LC Devices 2.5.0 or later.

For more information about the Accela detectors, see these topics:

- PDA Detector
- UV/Vis Detector

PDA Detector

The PDA detector is a full-featured, time-programmable, photodiode array detector that can scan the full ultraviolet-visible range from 190 to 800 nm. The detector can acquire data at a rate of up to 80 Hz with a 20 bit digital conversion.

Note The discontinued version of the PDA detector acquires data at a rate up to 20 Hz.

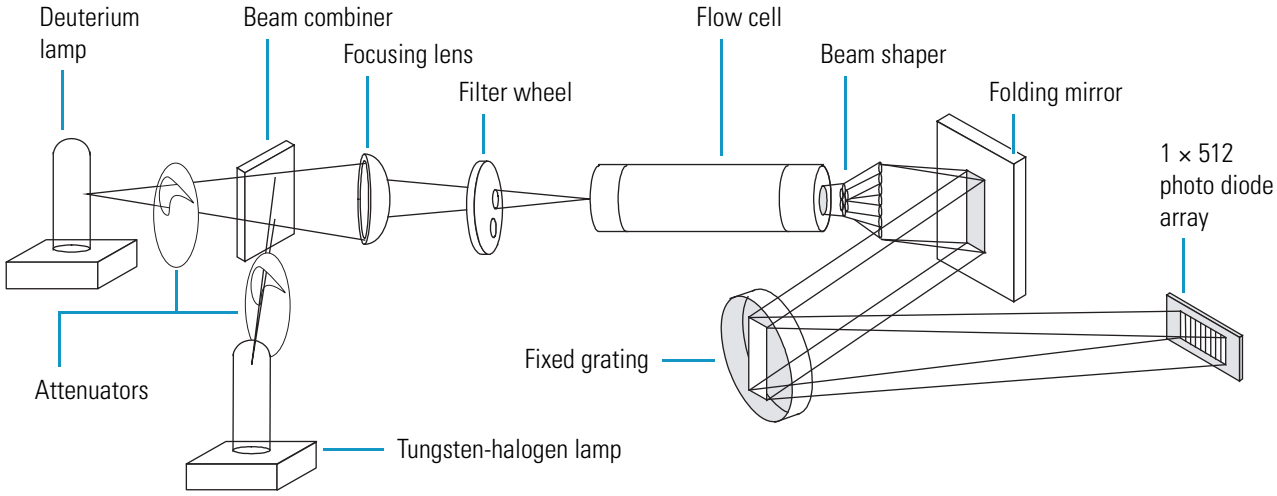
The model type and firmware version of the PDA detector is listed on the back panel of the detector. Figure 39 on page 63 shows the back panel of the Accela PDA (80 Hz) Detector.

The optimal location for the PDA detector is above the autosampler and below the solvent platform. The data system computer controls the PDA detector through an Ethernet communication link. The PDA detector consists of a dual-light source, an optical bench, a photodiode array, a low voltage power supply, several printed circuit boards (PCBs), and four status light-emitting diodes (LEDs).

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

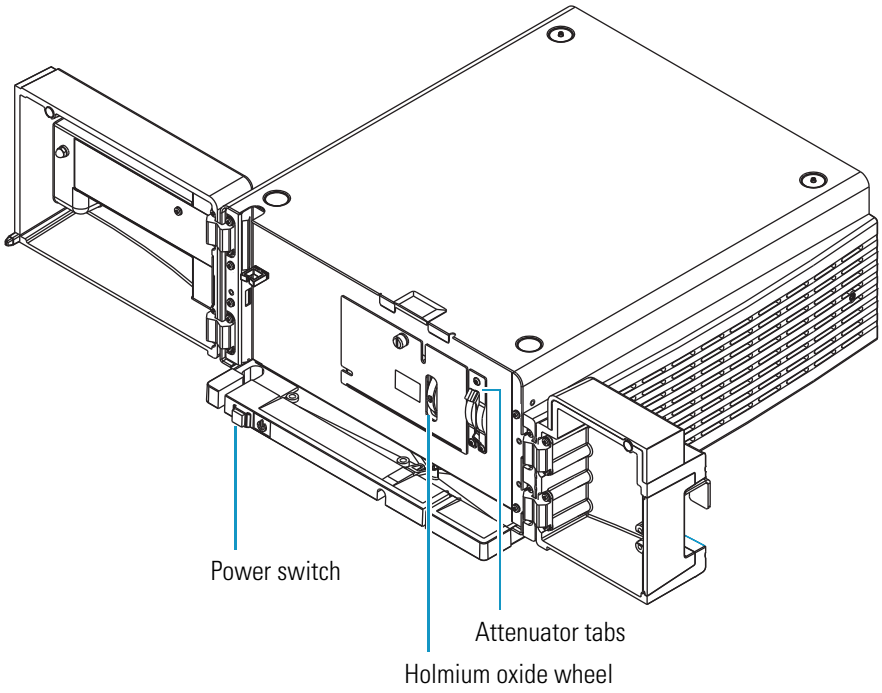
The optical bench contains a beam combiner, focusing lens, filter wheel, flow cell, beam shaper, folding mirror, and grating (see Figure 15). The beam combiner reflects the light coming from the tungsten-halogen lamp so that it is parallel to and coincident with the light from the deuterium lamp. The combined beam is then focused on the inlet window of the flow cell through the filter wheel. The standard filter wheel has two positions. Use position 1 (Open), which places a clear, quartz window in the optical path, for normal operation. Use position 2 (Holmium Oxide), which places a sealed, quartz cuvette filled with a holmium oxide/perchloric acid solution (NIST™ traceable) in the optical path, for wavelength accuracy verification and calibration.

Figure 15. Optical bench of the PDA detector



The PDA detector does not have independent controls, such as a keypad, to create data acquisition methods. Instead, you create instrument methods for data acquisition with the data system. The only manual controls for the PDA detector are the On/Off switch that controls line power, the attenuators that control the light throughput to the diode array, and the holmium oxide wheel that controls the position of the wavelength calibration solution (see Figure 16).

Figure 16. Manual controls for the PDA detector



Note For the discontinued version of the Accela PDA Detector, you must remove the flow cell cover to access the attenuator tabs.

UV/Vis Detector

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

The wavelength time table is available in all three modes. The time table can contain up to 10 lines. If you enable the Zero On Wavelength Change feature, the baseline absorbance returns to zero between each line in the time table, even if the wavelengths remain the same. The baseline absorbance does not return to zero between the last two lines in the table.

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

To control the UV/Vis Detector, you must first add the detector to the software instrument configuration (see "Adding Devices to the Instrument Configuration" on page 41).

After you add the UV/Vis detector to the instrument configuration, you can set up the data acquisition parameters from the UV/Vis detector view of the Thermo Xcalibur Instrument Setup window (see "UV-Vis Detector Instrument Method Parameters" on page 107).

You can turn the detector lamps on or off and zero the detector output from the Direct Control dialog box (see "UV-Vis Detector Direct Controls" on page 187).

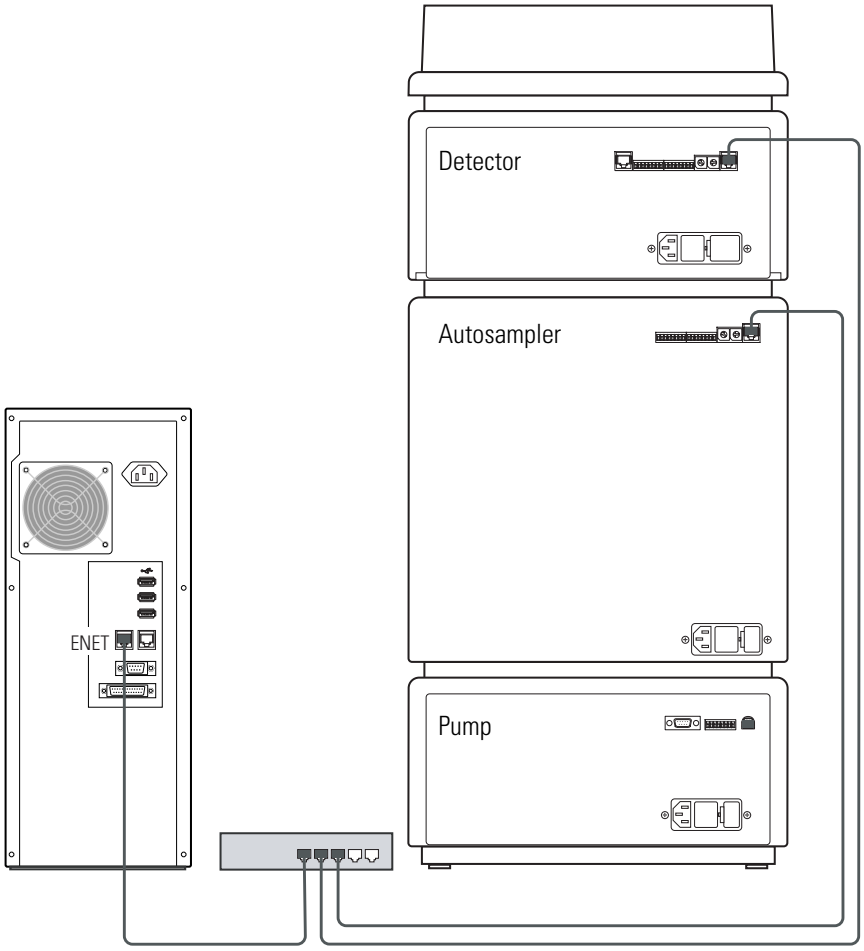
Solvent Platform

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

Communication with the Data System Computer

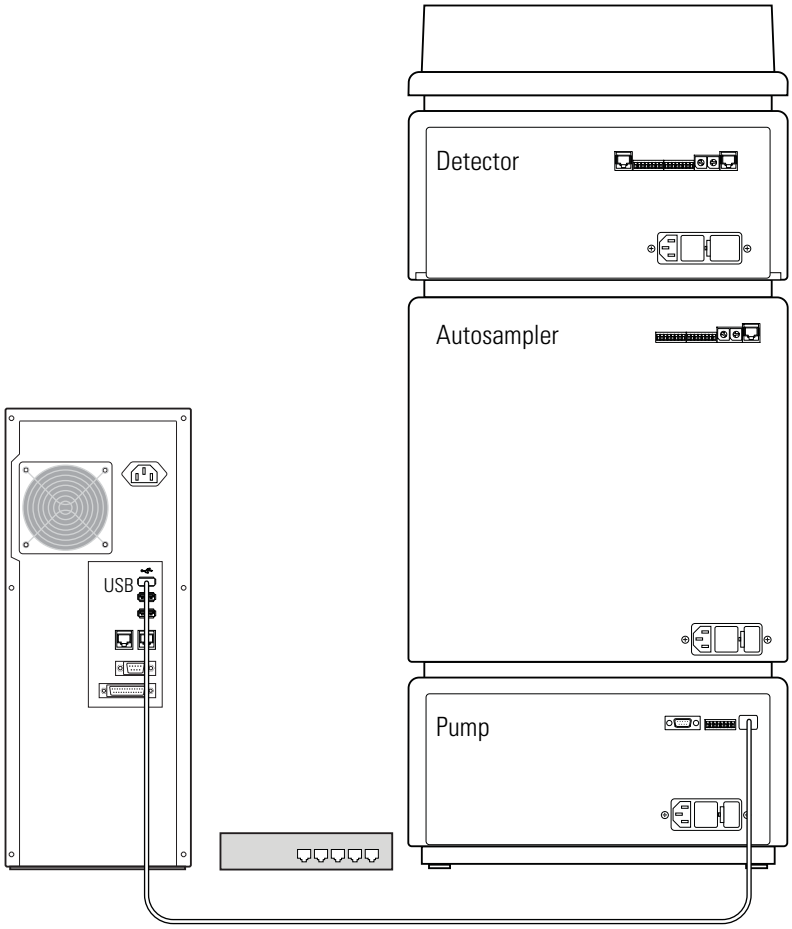
The autosampler and detector use an Ethernet link to communicate with the data system computer (see Figure 17). To establish communication with these devices, you connect one end of an Ethernet cable to the Ethernet port on the back panel of the device and the other end of the cable to an Ethernet switch. You use a second Ethernet cable to connect the Ethernet switch to the computer that has the data system installed. The accessory kits include a shielded, Category 5, RJ-45, 7 ft length Ethernet cable with ferrite.

Figure 17. Ethernet connections for an Accela LC system



The pump uses a USB 1.1 compatible serial link to communicate with the data system computer. To establish communication with the pump, you connect the series B socket of a USB cable to the USB port on the back panel of the pump and the series A socket of the USB cable to one of the USB ports on the data system computer (see Figure 18). The accessory kit for the pump includes an RS-232 cable.

Figure 18. USB connection for the Accela pump



In addition to connecting the communication cables, you must enter the appropriate stack addresses for the autosampler and PDA detector when you configure the device drivers. The stack address or stack number that you enter when you add one of these devices to the configuration for your instrument must match the unit ID setting on the back panel of the device. For more information about configuring the Accela device drivers, see Chapter 2, "Thermo Foundation Instrument Configuration."

Turning Off the Computer's Energy Saving Features

The PDA detector, UV/Vis detector, and autosampler communicate with the data system computer through an Ethernet link. To ensure communication between the data system computer and the LC system, turn off the data system computer's screen saver and energy saving features.

❖ To turn off the screen saver and energy saving features for the Windows XP operating system

1. From the Windows XP desktop, choose **Start > Control Panel**.

   Tip If you selected the Classic Start Menu option for the Start menu properties, choose **Start > Settings > Control Panel**.

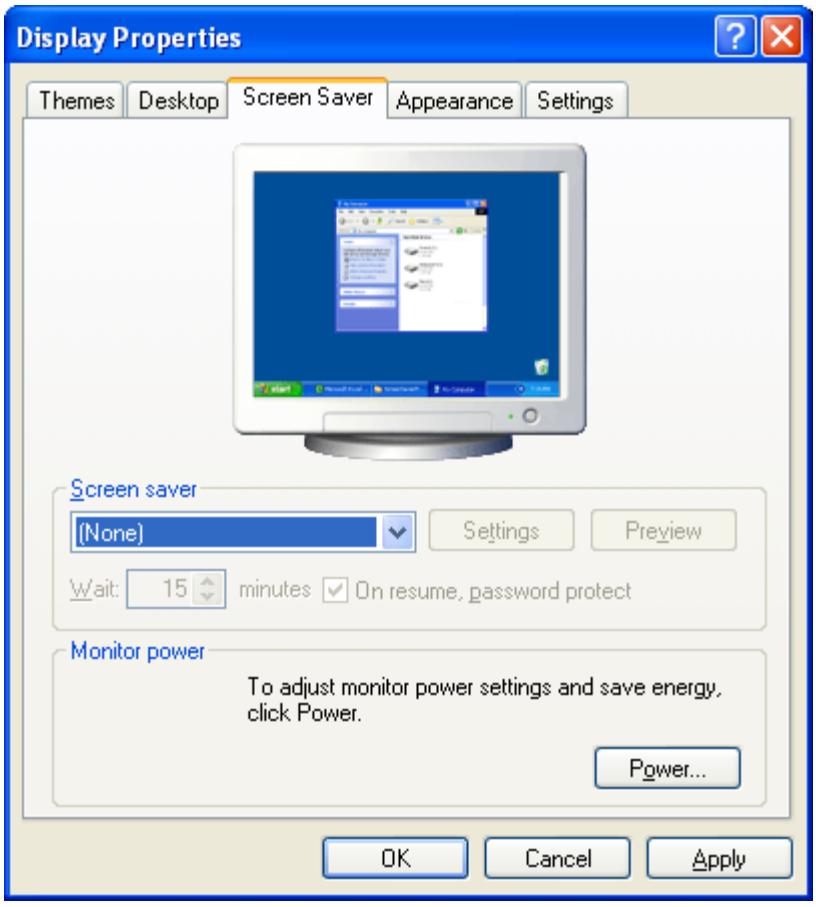
2. Double-click **Display**.

   The Display Properties dialog box appears.

3. Click the **Screen Saver** tab.

   The Screen Saver page appears (see Figure 19).

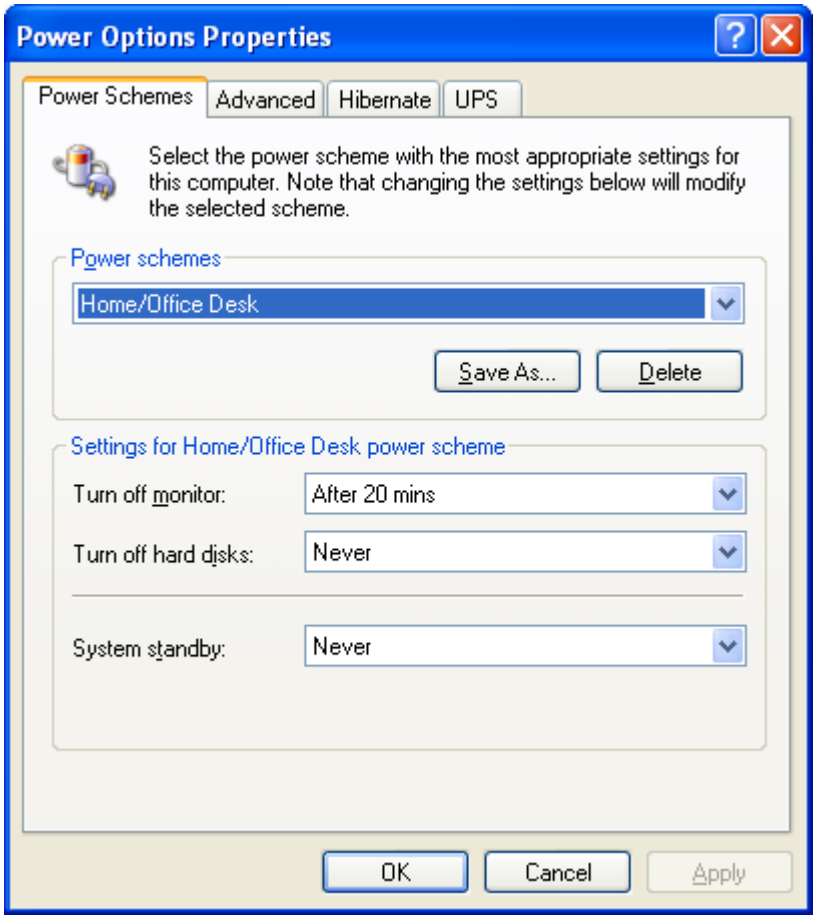
Figure 19. Screen Saver page of the Display Properties dialog box (Windows XP)



4. In the Screen Saver list, select **None**.
5. Click **Apply** to accept this setting.
6. Click **Power**.

   The Power Options Properties dialog box opens to the Power Schemes page (see Figure 20).

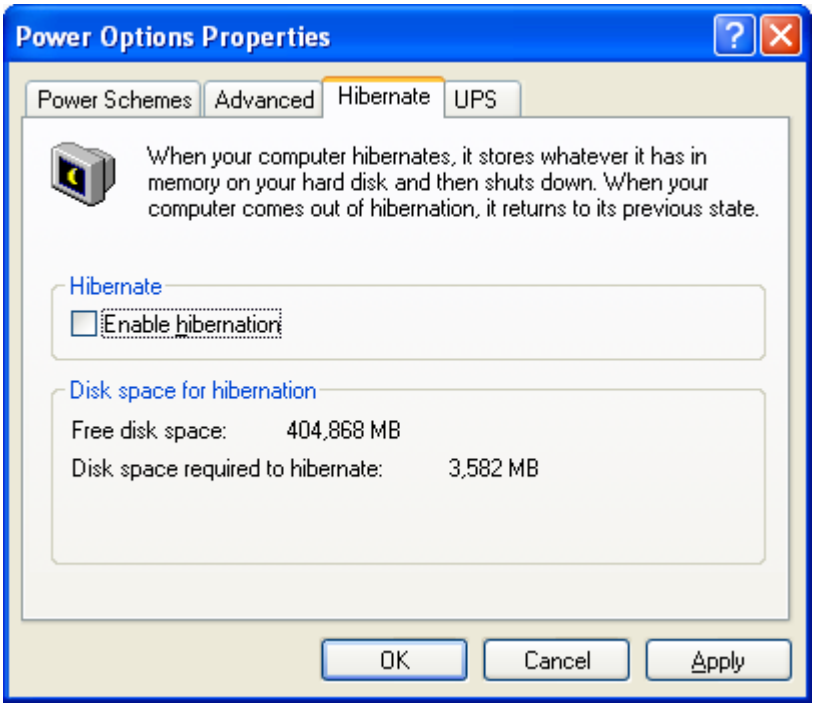
Figure 20. Power Options Properties



7. Modify the power scheme settings as follows:
   - In the Turn Off Monitor list, select **Never**.
   - In the System Standby list, select **Never**.
8. Modify the hibernate setting as follows:
   a. Click the **Hibernate** tab.

      The Hibernate page appears (see Figure 21).

Figure 21. Hibernate page



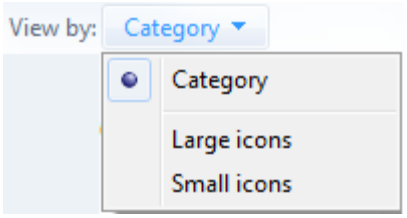
b. If the **Enable Hibernation** check box is selected, clear it.

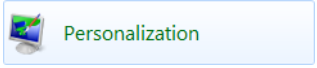
c. Click **Apply** to accept the setting.

9. Click **OK** to close the Power Options Properties dialog box.
10. Click **OK** to close the Display Properties dialog box.
11. Close the Control Panel window.

❖ **To turn off the screen saver an energy saving features for the Windows 7 operating system**

1. From the Windows 7 desktop, choose **Start > Control Panel**.
2. Choose **Large Icons** from the View By menu.

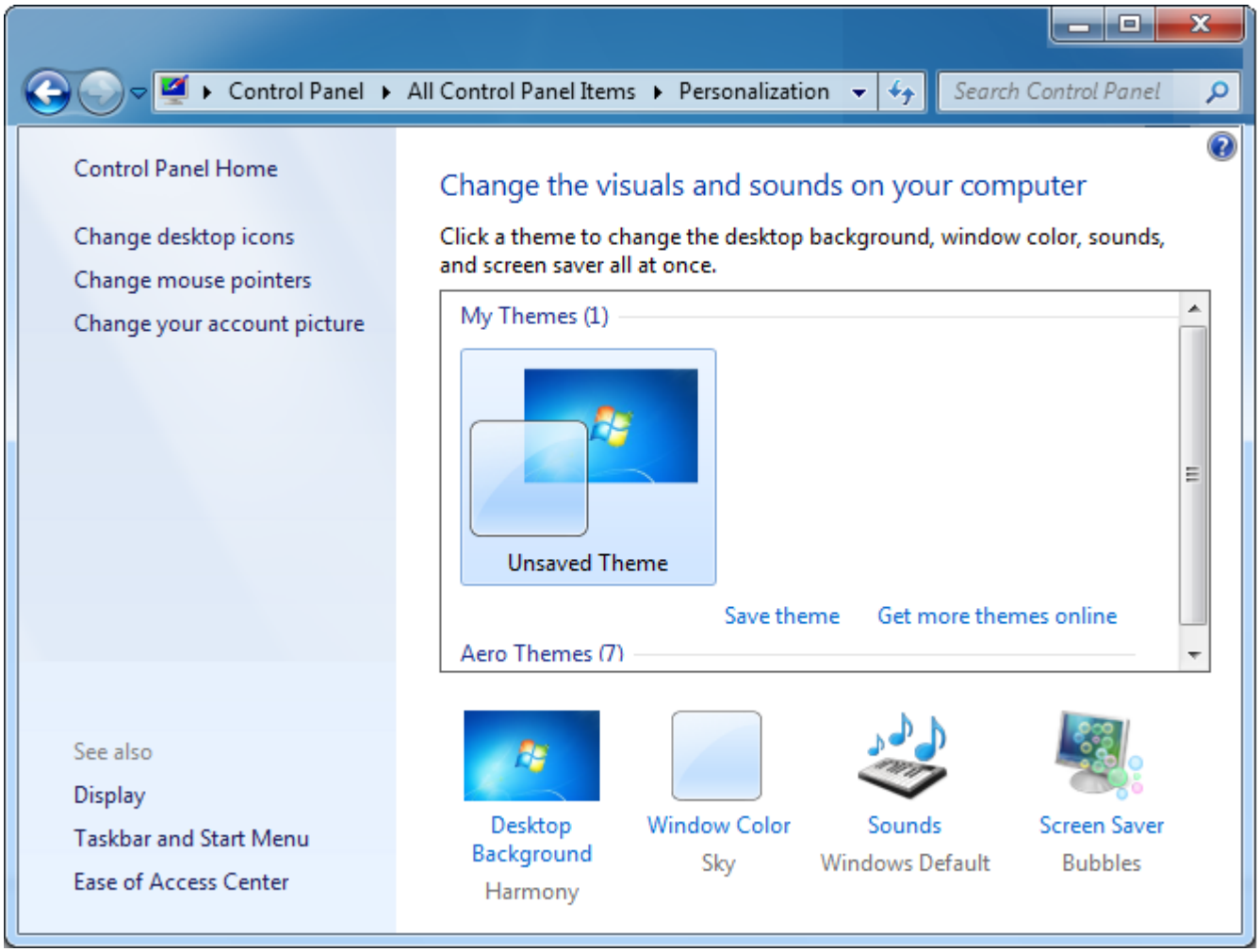


3. Click the **Personalization** icon 

.

The Personalization window appears (see Figure 22).

Figure 22. Personalization window



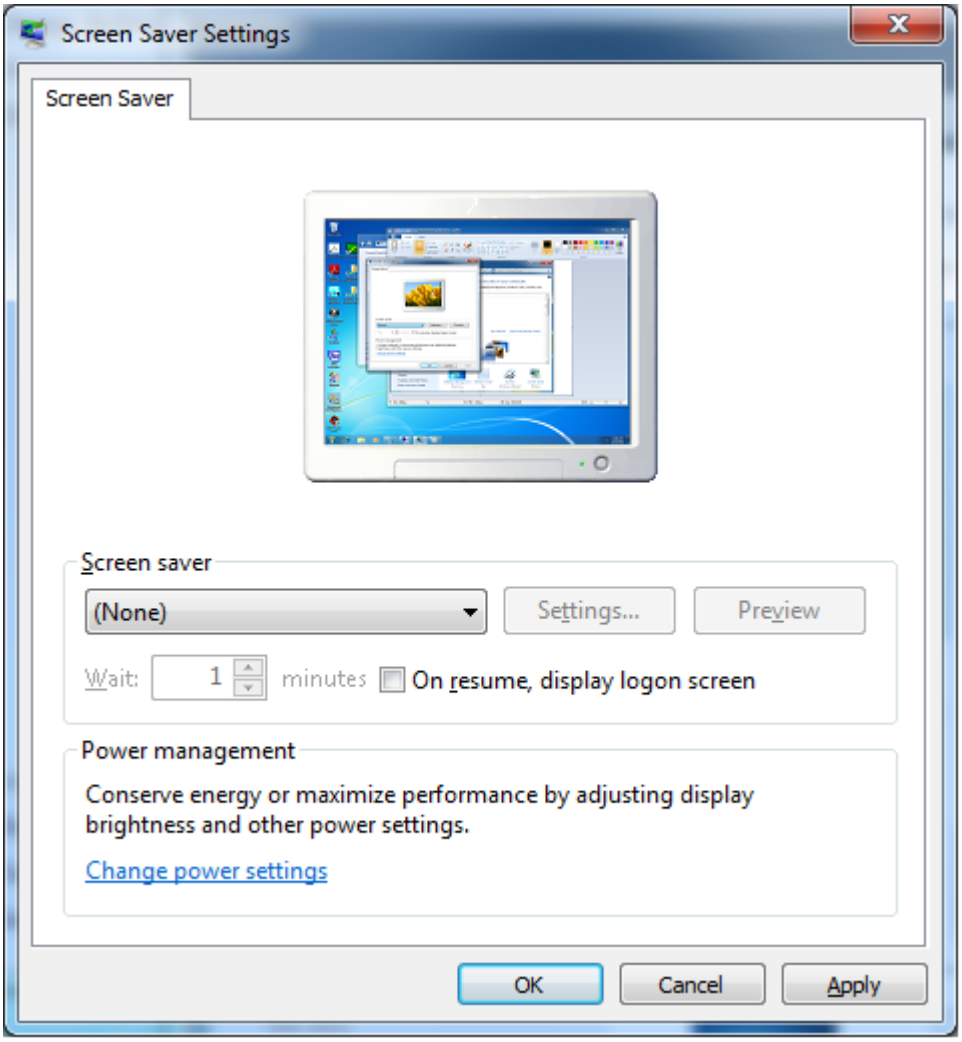
4. Click the **Screen Saver** icon on the bottom-right side of the window.

   The Screen Saver Settings dialog box appears (see Figure 23).

5. Change the screen saver setting as follows:

   a. In the Screen Saver list, select **None**.

   Figure 23. Screen Saver Settings dialog box (Windows 7 operating system)



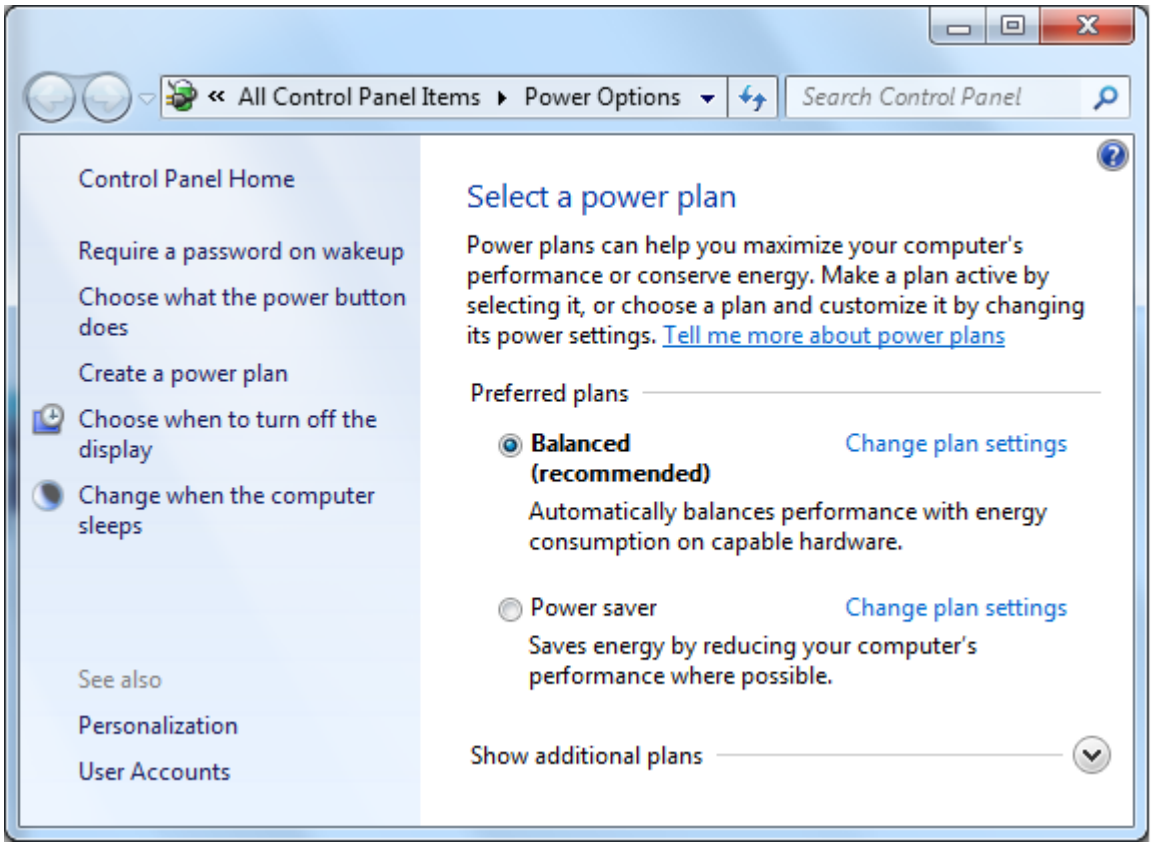
   b. Click **Apply** to accept this setting.

6. Change the power settings as follows:

   a. Click **Change Power Settings**.

   The Select a Power Plan page of the Power Options dialog box appears (see Figure 24).

Figure 24. Select a Power Plan page of the Power Options dialog box

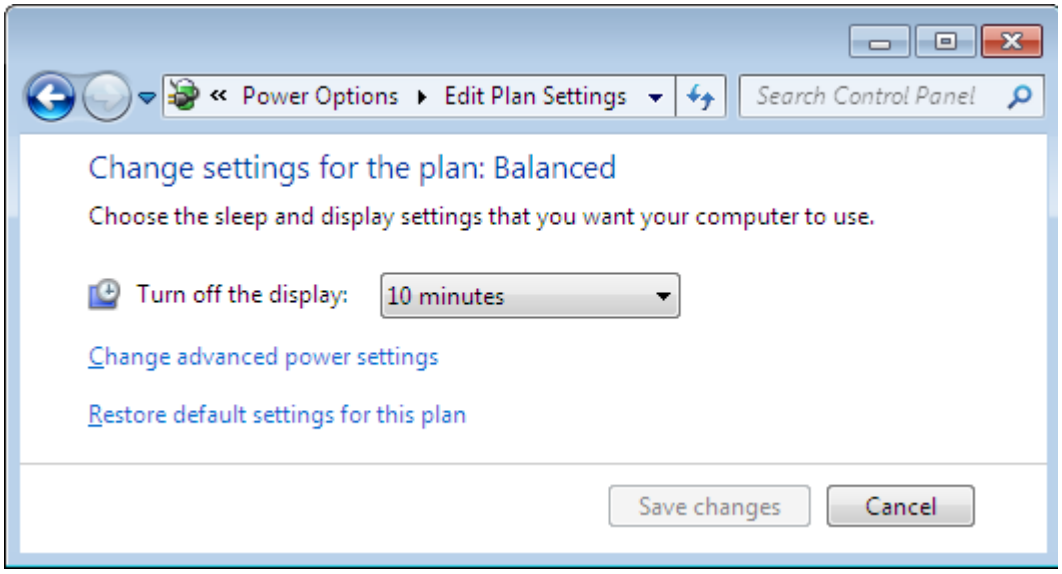


b. Select the **Balanced** option.

c. Click **Change Plan Settings** for the Balanced option.

The Edit Plan Settings dialog box appears (see Figure 25).

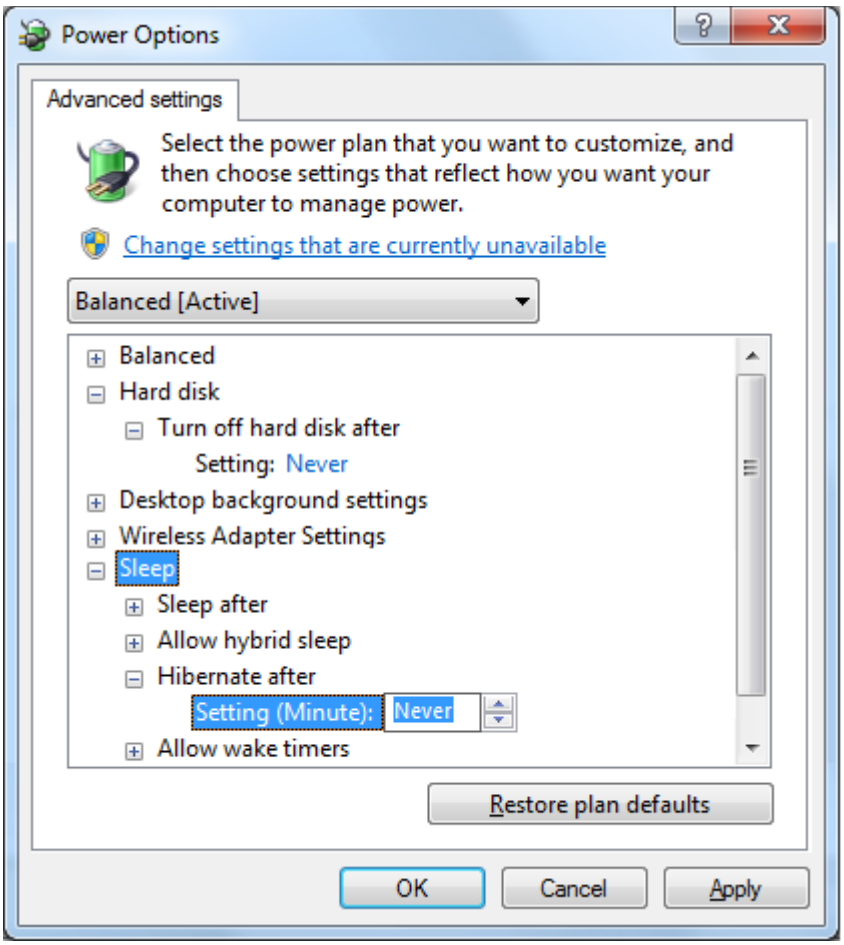
Figure 25. Edit Plan Settings dialog box



d. Click **Change Advanced Power Settings**.

The Advanced Settings page of the Power Options dialog box appears (see Figure 26).

Figure 26. Advanced Settings page of the Power Options dialog box



e. Set the Turn Off Hard Disk After option to Never as follows:

i. Click the ⊞ icon to the left of Hard Disk.

ii. Click the ⊞ icon to the left of Turn Off Hard Disk After.

iii. If the setting is not set to Never, click the setting and select **Never**.

f. Set the Hibernate After option to Never as follows:

i. Click the ⊞ icon to the left of Sleep.

ii. Click the ⊞ icon to the left of Hibernate After.

iii. If the setting is not set to Never, click the setting and select **Never**.

g. Click **Apply** to accept these settings.

h. Click **OK** to close the Advanced Settings page.

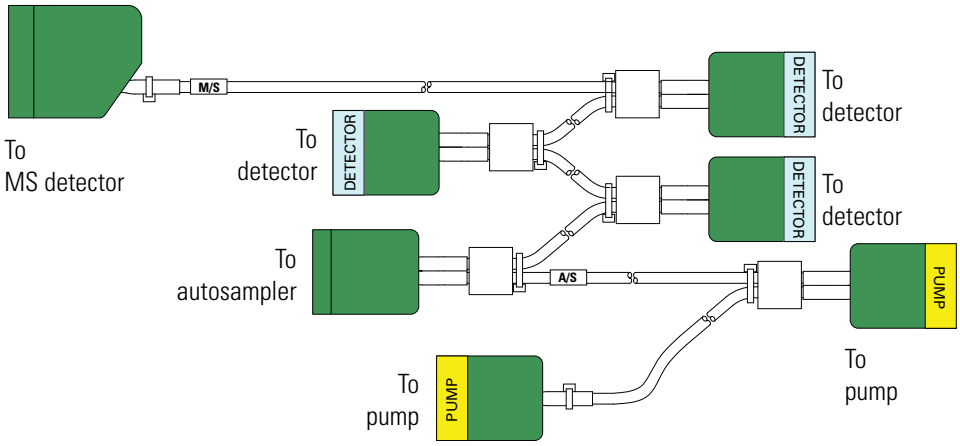
7. Close the Control Panel window.

Synchronization of the LC Devices

A system interconnect cable (contact closure) provided in the Accela System Kit coordinates the run control signals between the Accela devices.

Figure 27 shows the cable assembly with seven green combicon connectors: two are labeled PUMP; three are labeled DETECTOR; and two are unlabeled. You can identify the connector for the autosampler by the small, A/S tag on its adjacent cable. You can identify the M/S connector by its shape and by the small, M/S tag on its adjacent cable.

Figure 27. 7-connector system interconnect cable



You can interconnect one or two Accela Pumps (or Accela 600 Pumps), an Accela PDA Detector, an MSQ Plus Mass Detector, and an Accela Autosampler with this cable. You can also connect other Thermo Scientific MS detectors using additional adapter cables.

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

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

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

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

   The autosampler injection valve switches to the fill position, and then the autosampler pushes sample into the sample loop.

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

Status LEDs

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

These topics describe the meanings of the LED states:

- Pump LED States
- Autosampler LED States
- PDA Detector LED States
- UV-Vis Detector LED States

Pump LED States

Table 2 lists the states of the Accela pump LEDs.

Table 2. Pump status LEDs and their meaning

LED	Status	Meaning
Power	Green	The pump is on and receiving power.
Comm	Amber	The pump is not communicating with the data system.
	Green	Communication to the data system has been established.
	Flashing green	A program is downloading from the data system computer.
Run	Amber	The power is switched on, but the pump pistons are idle, producing no flow.
	Flashing amber	A firmware download is in progress or an error condition has occurred.
	Green	The pump pistons are moving, but a pump program is not running. This state can occur when the pump is under direct control or a pump program has ended.
	Flashing green	The pump is running a pump program from a downloaded method.
Degas	Amber	The degas unit is building vacuum.
	Flashing amber	A failure, such as a loss of vacuum, has occurred.
	Green	Sufficient vacuum has developed to perform chromatography.

Autosampler LED States

Table 3 lists the LED states for the autosampler.

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

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

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

Table 3. Accela Autosampler status LEDs and their meaning

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

PDA Detector LED States

Table 4 lists the LED states for the Accela PDA (80 Hz) Detector.

For information about the status LEDs for the discontinued Accela PDA (20 Hz) Detector, refer to its hardware manual.

Note When the PDA detector fails to establish communication with the data system computer, the Power LED remains amber and the Comm, Lamps, and Run LEDs remain unlit.

Table 4. Accela PDA (80 Hz) Detector status LEDs and their meaning

LED	State	Meaning
Power	Green	The detector is on and has downloaded the operational file.
	Amber	The detector is on but has not yet downloaded the operational file from the data system computer.
Comm	Green	Communication to the data system has been established.
	Amber	There is no communication with the data system.
Run	Green	The detector is ready for a run.
	Flashing green	A run is in progress and the detector is sending data to the data system computer.
	Amber	The PDA detector is not ready to start a run for one of these reasons: • A valid method has not been downloaded (following power-on). • Both lamps are off, or one of the lamps is failing to turn on. • The lamp or wavelength calibration is not valid.
	Flashing amber	The PDA detector is in an error state while in the Run mode.
Lamps	Green	One or both lamps are turned on.
	Amber	The lamps are off or the D2 lamp is starting. The D2 lamp takes approximately 30 seconds to turn on.

UV-Vis Detector LED States

Table 5 lists the LED states for the UV/Vis detector.

Table 5. UV/Vis detector LED states and their meaning

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

Thermo Foundation Instrument Configuration

This chapter provides information about configuring the LC device drivers.

Contents

❖ **To set up the software instrument configuration for your LC system**

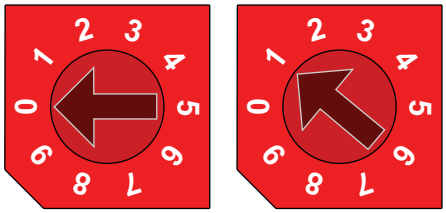
1. Open the Thermo Foundation™ application.
2. Add the devices of your LC system to the instrument configuration.
3. Specify the configuration settings for each device.
4. Close the Foundation application.

Checking the Communication Hardware

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

Before you specify the configuration settings, check the unit ID setting on the back panels of the autosampler UV/Vis detector, and PDA detector. The unit ID consists of two rotary switches that are factory set to 01 (see Figure 28).

Figure 28. Unit ID with a stack address of 01



Set to 0 Set to 1

Unlike the other Accela devices, the pump communicates with the data system through a USB 1.1 compliant USB link.

❖ To check the communication connections

1. Check the setting of the rotary switches on the back panels of the autosampler and the detector.
2. For the Accela pump, verify that the USB cable is connected to the pump and the data system computer.

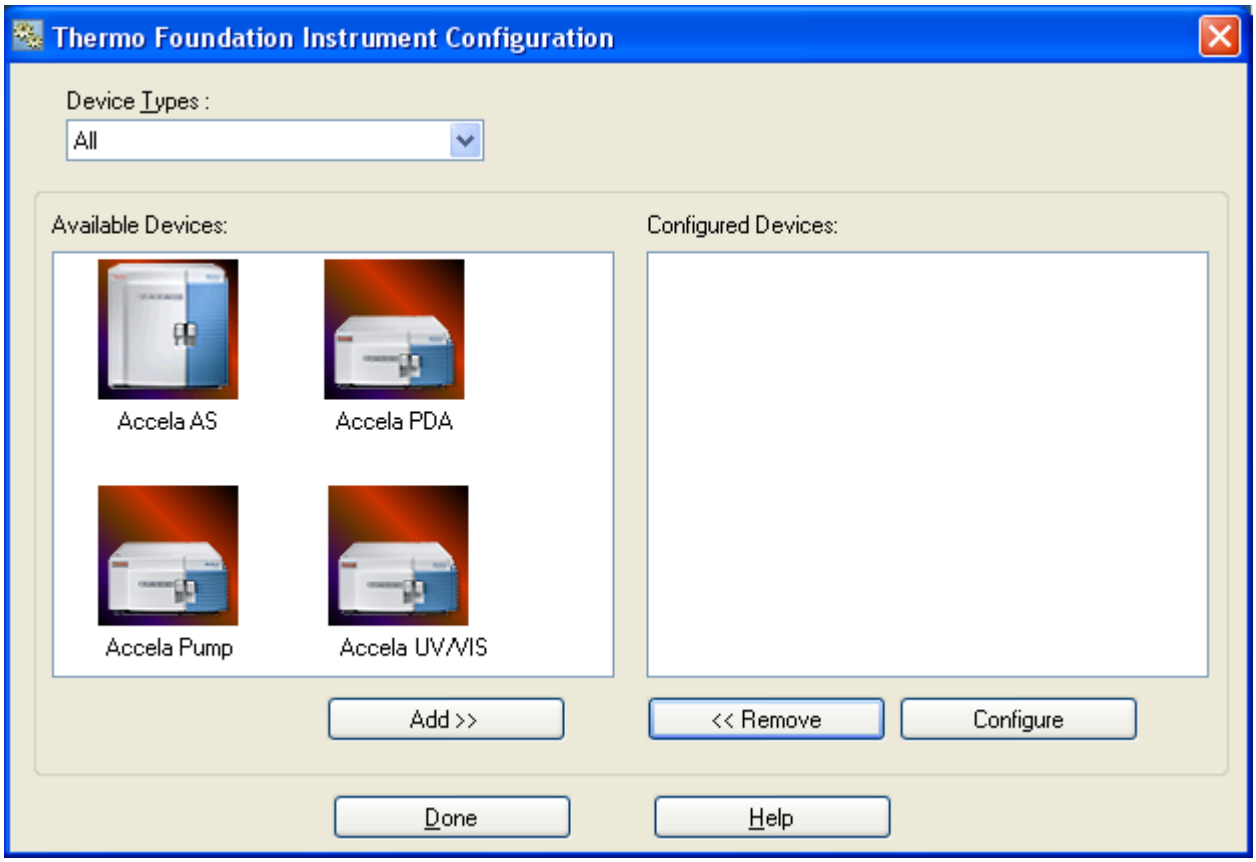
Opening the Thermo Foundation Instrument Configuration Application

❖ **To open the Thermo Foundation Instrument Configuration application**

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

The Thermo Foundation Instrument Configuration window appears (see Figure 29). This window lists the installed device drivers.

Figure 29. Thermo Foundation Instrument Configuration window



Adding Devices to the Instrument Configuration

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

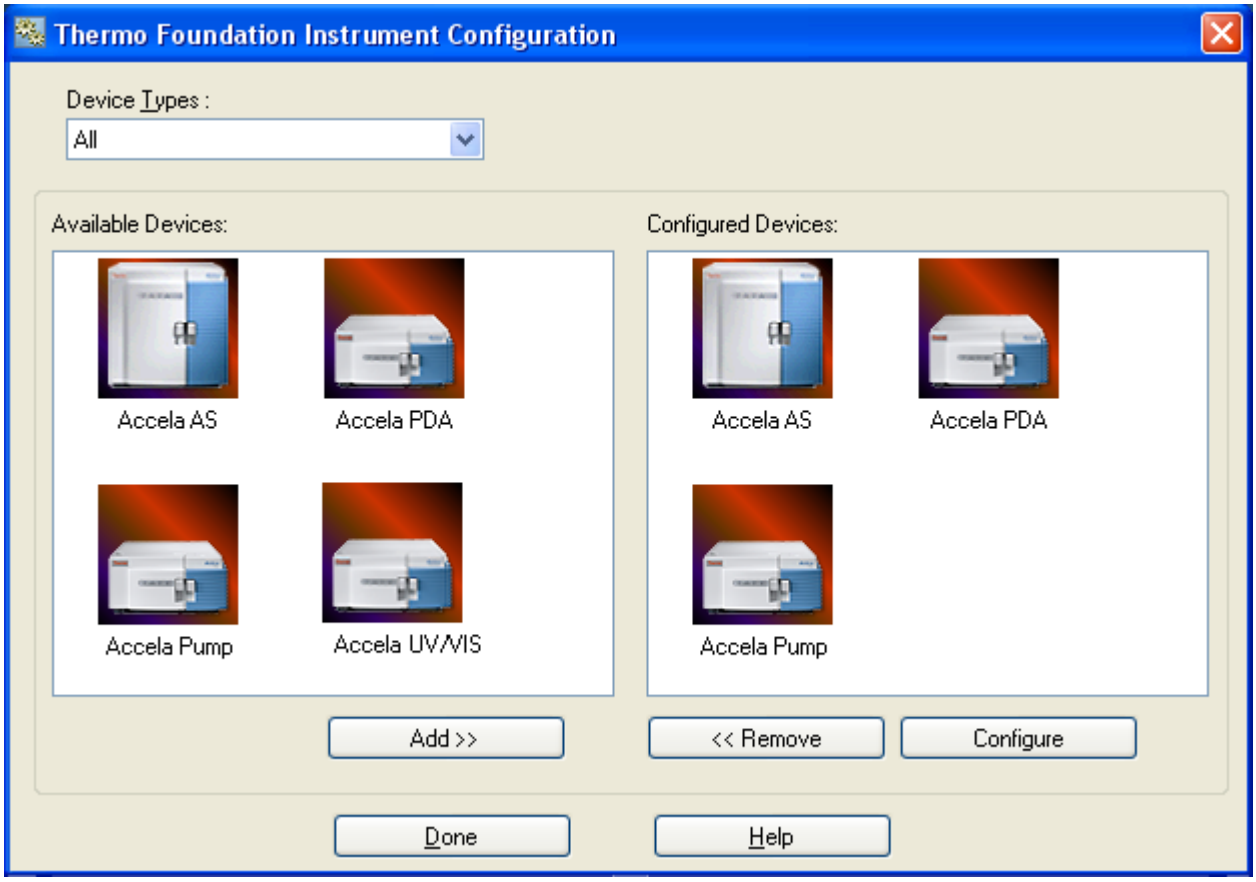
If you are controlling a dual-pump system, follow the procedure in "Pump Configuration Settings" on page 59 to add the pump driver to the Configured Devices pane.

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

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

A copy of the icon appears in the Configured Devices list (see Figure 30).

Figure 30. Thermo Foundation Instrument Configuration window with devices added to the Configured Devices list



Specifying the Configuration Settings

After you add the devices to the Configured Devices list, specify the configuration settings for each device as described in these topics:

- Autosampler Configuration Settings
- Pump Configuration Settings
- PDA Detector Configuration Settings
- UV/Vis Detector Configuration Settings

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

Note The Accela UV/Vis Detector driver is provided with LC Devices 2.5.0 or later.

Autosampler Configuration Settings

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

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

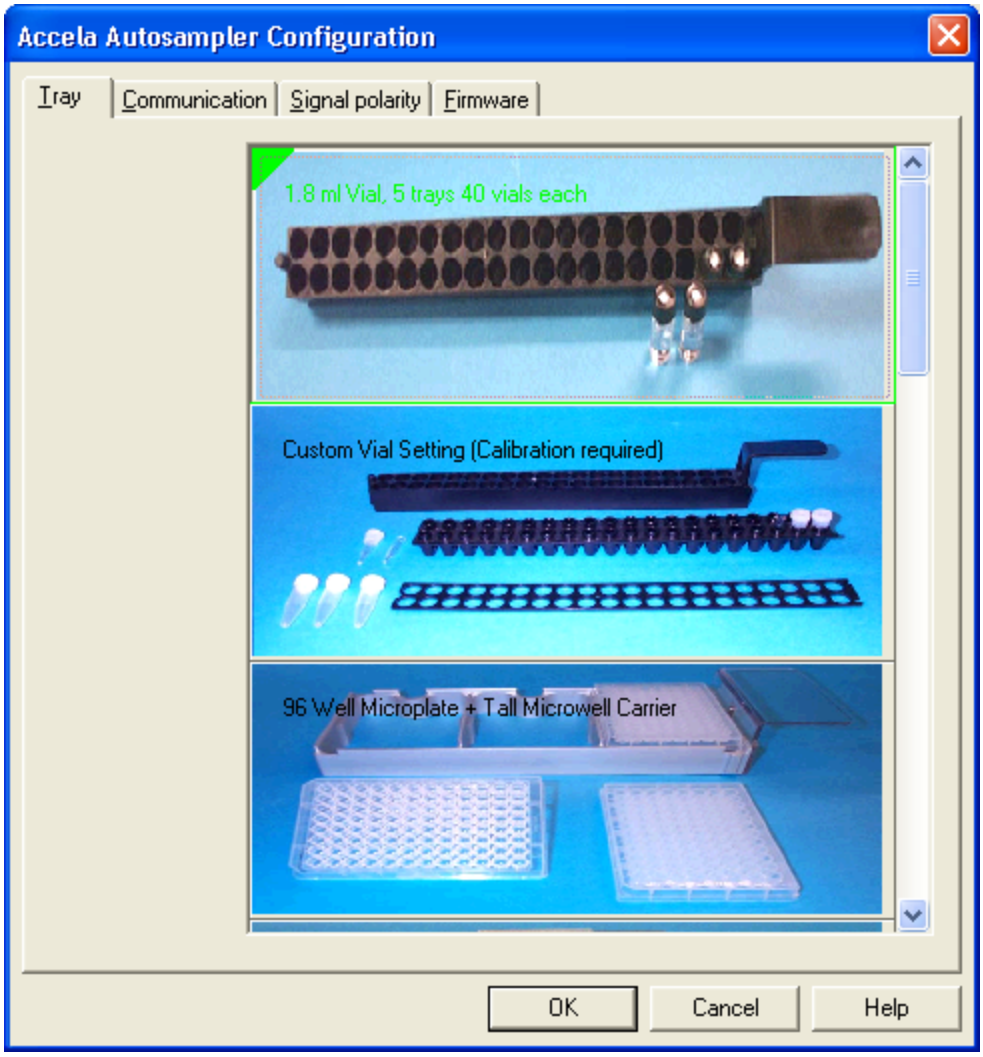
- Tray Page
- Communication Page
- Signal Polarity Page
- Firmware Page

❖ To open the Accela Autosampler Configuration dialog box

1. If it is not already open, open the Thermo Foundation Instrument Configuration window (see "Opening the Thermo Foundation Instrument Configuration Application" on page 41).
2. In the Configured Devices list, double-click the **Accela AS** icon.

The Accela Autosampler Configuration dialog box appears with the Tray page displayed (see Figure 31).

Figure 31. Tray page of the Accela Autosampler Configuration dialog box



Tray Page

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

Use the Tray page (see Figure 32) to specify the tray type that you are using.

❖ To select the tray type and orientation

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

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

> **Note** The Autosampler Accessory Kit contains the vial trays and microplate carriers. The current version of the accessory kit contains a standard tray, a short microwell carrier, and a tall microwell carrier with a solid metal bottom.
>
> Previous versions of the accessory kit contained a riser plate that you could use in combination with the short microwell carrier to load standard depth 96-well or 384-well microplates into the autosampler tray compartment. Thermo Fisher Scientific has replaced the short microwell carrier and riser plate option with a tall microplate carrier that has a solid metal bottom for thermal conduction.

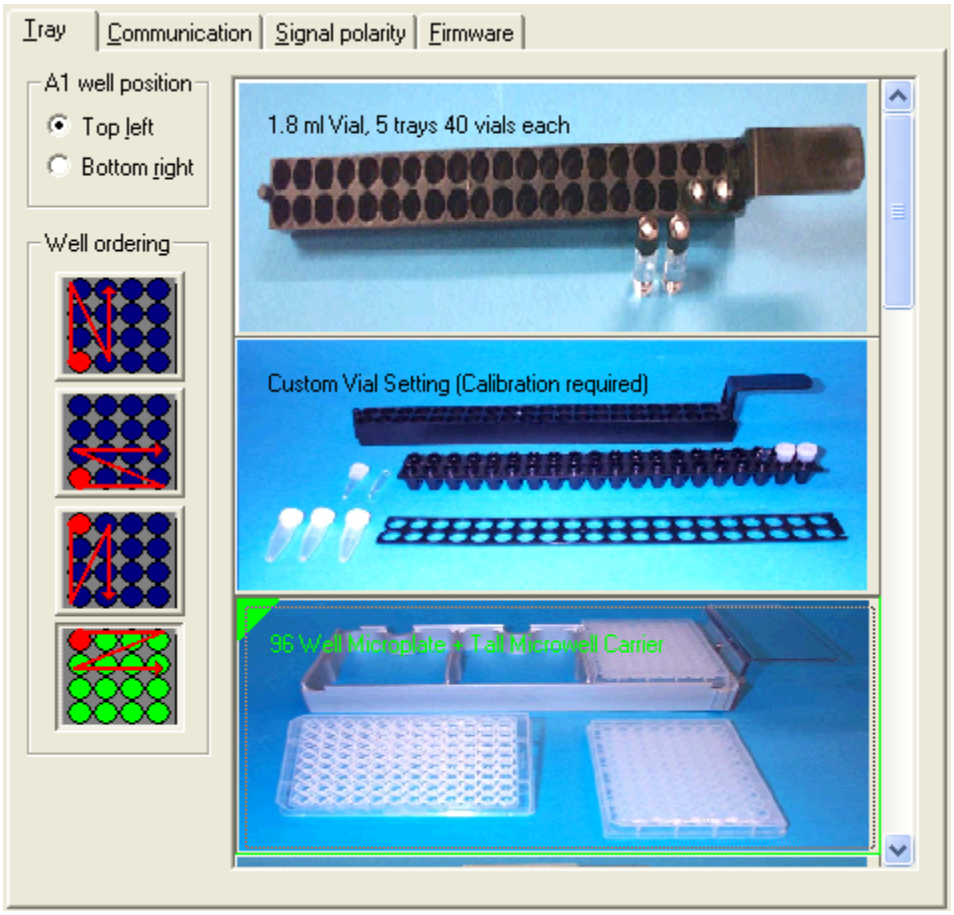
Table 6. Tray type selections

Tray type		Requires well bottom distance calibration
1	1.8 mL Vial, 5 trays, 40 vials each	No
2	Custom Vial Setting	Yes
3	96 Well Microplate + Tall Microwell Carrier	No
4	96 Well Microplate + Tall, Solid Microwell Carrier –or– 96 Well Microplate + Short Microwell Carrier + Riser Plate	No
5	1 mL or 2 mL Deep Well Plate + Short Microwell Carrier	No
6	96 Well Microplate + Short Microwell Carrier	No
7	96 Well PCR Plate + Cooling Adapter + Short Microwell Carrier	No
8	Custom 96 Well Setting	Yes
9	384 Well Microplate + Tall Microwell Carrier	No
10	384 Well Microplate + Tall, Solid Microwell Carrier –or– 384 Well Microplate + Short Microwell Carrier + Riser Plate	No
11	Custom 384 Well Setting	Yes

2. For a microplate tray type, do the following:

   a. In the A1 Well Position area, select either the **Top Left** or the **Bottom Right** option (see Figure 32).

   Figure 32. Tray page with a 96-well plate selection



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

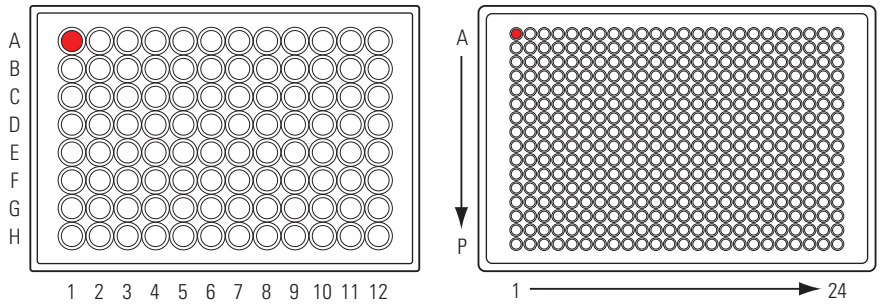
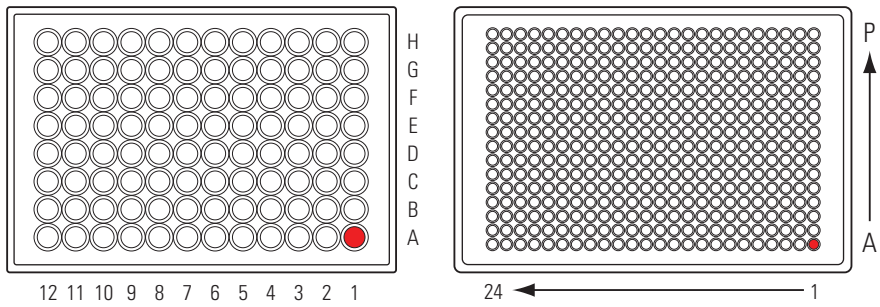
Autosampler Tray Page Parameters

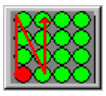
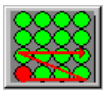
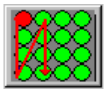
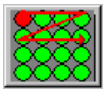
Table 7 describes the configuration parameters on the Tray page.

Table 7. Tray page parameters (Sheet 1 of 2)

Parameter	Description
Tray Type	The Tray Type list displays pictures of the 11 sample tray configurations. When you choose 96-well plates or 384-well plates, two additional options appear: A1 Well Position and Well Ordering. The conventional autosampler tray holds 40 standard 1.8 mL vials. For other types of vials, select the custom vial setting and the appropriate tray adapter. The autosampler accommodates a variety of microwell plates. You can use the tall or short microwell carriers to accurately position the various microwell plates at an appropriate location in the autosampler tray compartment. The tall microwell carrier with a solid metal bottom provides the same height as the short microwell carrier and riser plate combination. The distance that the needle travels to reach the bottom of the vial is pre-calibrated for the eight standard configurations. You must perform a Well Bottom Distance calibration (see "Well Bottom Distance Calibration" on page 321) to use one of the three custom configurations. For information about the height limitations for the various tray selections, see Table 8. **Note** Do not use the short microwell carrier to hold the 384-well, high density microtitre plates. Do not use the tall microwell carrier to hold PCR plates.

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

Parameter	Description
A1 Well Position (for the microplate trays)	Use the options in the A1 Well Position area to select the orientation of the microplate when it is mounted in the autosampler plate holder. You can mount the plate in the plate holder so that the A1 well is located at the top left or located at the bottom right of the plate.
Top Left	Specifies that the A1 well is located at the top left of the microplate. 
Bottom Right	Specifies that the A1 well is located at the bottom right of the microplate. 
Well Ordering (for the microplate trays)	Use the buttons in the Well Ordering area to select the sampling path that the autosampler follows during a sequence run. The sampling path you select is independent of the A1 well position. The path options are shown below:

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

Placing objects taller than 1.87 in. into the autosampler tray compartment will stall the autosampler arm. For custom vials, use the appropriate tray insert to ensure correct positioning. In addition, to trigger the vial sensor, position the vials in the tray so that the top of each vial reaches the minimum height of 1.55 in.

Do not use microtitre plates that exceed the heights listed in Table 8.

Table 8. Autosampler tray compartment height limitations

Tray type	Maximum height
Conventional (standard 1.8 mL vials)	N/A
Custom Vial Setting	N/A
96 Well Microplate + Tall Microwell Carrier	0.77 in.
96 Well Microplate + Tall, Solid Microwell Carrier or 96 Well Microplate + Short Microwell Carrier + Riser Plate	0.77 in.
1 mL or 2 mL Deep Well Plate + Short Microwell Carrier	1.80 in.
96 Well Microplate + Short Microwell Carrier	1.80 in.
96 Well PCR Plate + Short Microwell Carrier	1.80 in.
Custom 96 Well Setting	1.80 in.
384 Well Microplate + Tall Microwell Carrier	0.77 in.
384 Well Microplate + Tall, Solid Microwell Carrier or 384 Well Microplate + Short Microwell Carrier + Riser Plate	0.77 in.
Custom 384 Well Setting	0.77 in.

Communication Page

Use the Communication page to specify these options:

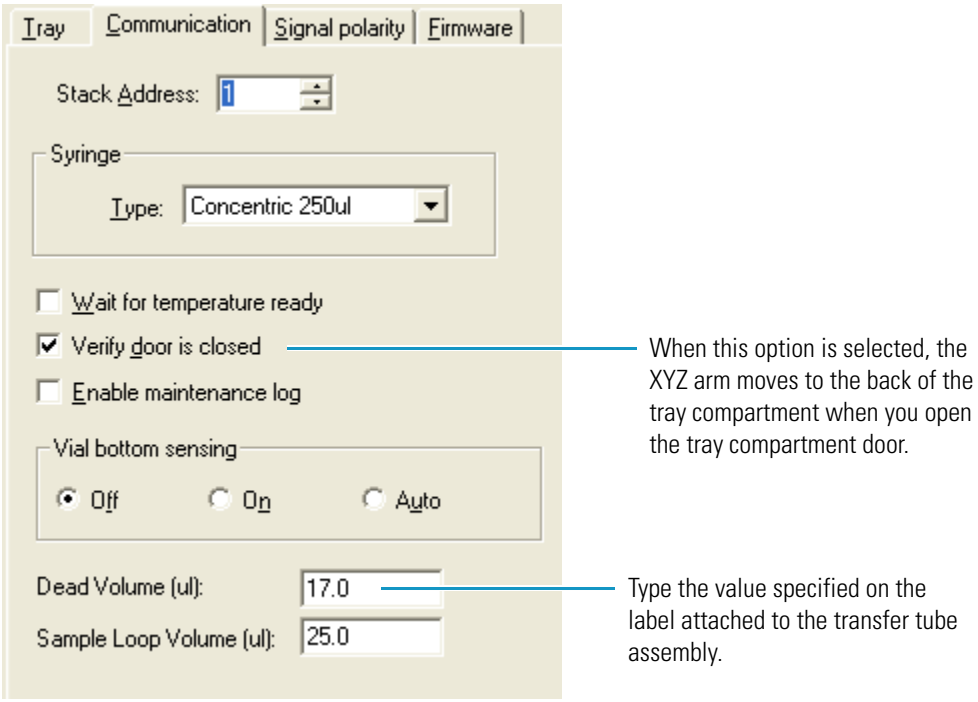
- The autosampler's stack address
- The syringe size and the sample loop size
- The dead volume of the transfer tubing that connects the autosampler injection port to the injection valve
- Whether the controlled temperature zones must be at their set temperature, the tray compartment door must be closed,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

❖ To specify the configuration settings on the Communication page

1. Open the Accela Autosampler Configuration dialog box (see "Autosampler Configuration Settings" on page 43).
2. Click the **Communication** tab.

   The Communication page appears (see Figure 33).

   Figure 33. Communication page



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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

    The autosampler ships with a 25 µL sample loop.

Autosampler Communication Page Parameters

Table 9 describes the parameters on the Communication page.

Table 9. Communication page parameters (Sheet 1 of 2)

Parameter	Description
Communication	
Stack Address	Specifies the stack address of the autosampler. The stack address must match the unit ID setting. The unit ID setting is specified by two rotary switches on the back panel of the autosampler. The rotary switches are set to 01 at the factory.
Syringe	Specifies the syringe type. The selections are 100, 250, 500, and 2500 µL.
Wait for Temperature Ready	Selecting this check box specifies that the autosampler waits for the column oven temperature and the tray compartment temperature to reach their set temperatures before going to the Ready state. Select this check box if you want the heated zones to reach their setpoint before the autosampler makes an injection.
Verify Door Is Closed	Selecting this check box specifies that the autosampler waits for the door to be closed before starting a run and that the XYZ arm moves to the back of the tray compartment when you open the tray compartment door. Select this check box if you want the autosampler to pause a sequence and send the XYZ arm to the back of the tray compartment when you open the tray compartment door.
Enable Maintenance Log	Selecting this check box activates the maintenance log. The maintenance log keeps an internal count of the total injections, total valve cycles, total needle usage, and total syringe cycles. When any of the counters exceed the user-set scheduled maintenance time (SMT), the autosampler cannot trigger a run until you perform the scheduled maintenance or you clear the check box. For information about setting up the scheduled maintenance time for the autosampler's hardware components, see "Autosampler Maintenance Information" on page 325. **Tip** When you select the Enable maintenance log check box, check the maintenance schedule and make sure that the settings are appropriate for your autosampler.
Vial Bottom Sensing	Specifies whether the vial bottom sensing feature is turned on or off or is set to determine the distance to the bottom of the first vial or well in a sequence of injections. The selections are as follows: • **Off**. Deactivates the vial bottom sensing feature. • **On**. Activates the vial bottom sensing feature for every injection. • **Auto**. Activates the vial bottom sensing feature for the first injection of a sequence.

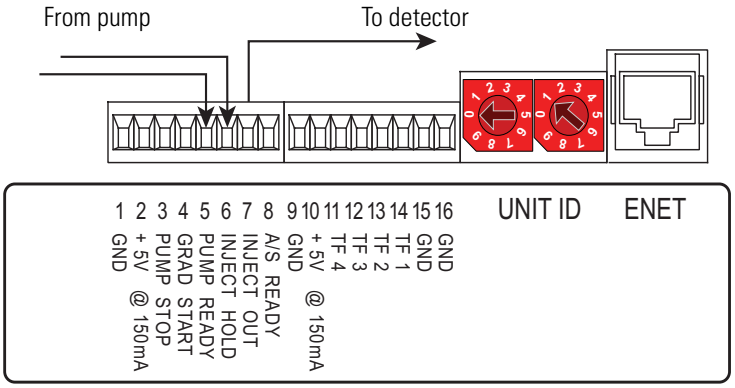
Table 9. Communication page parameters (Sheet 2 of 2)

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

Signal Polarity Page

During a run, the autosampler receives a Pump Ready signal and an Injection Hold release signal from the pump. When the autosampler switches its injection valve to the inject position, it sends an Inject Out signal to the detector. Figure 34 shows the signal terminals on the autosampler's back panel.

Figure 34. Signal terminals on the autosampler's back panel



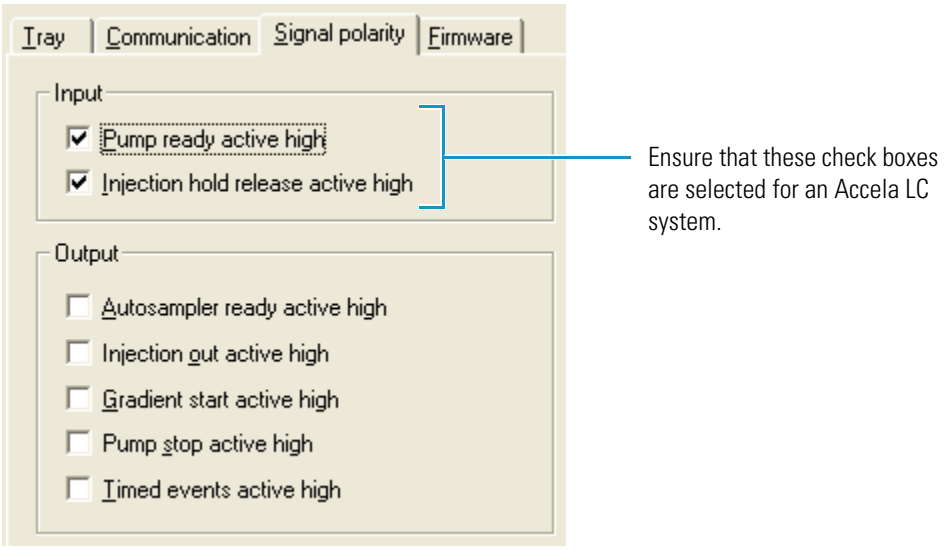
Use the Signal Polarity page of the Accela AS Configuration dialog box to specify the signal polarities for the run signals. For the Accela pumps, select the Pump Read Active High and the Injection Hold Release Active High check boxes in the Input area.

❖ To specify the signal polarities for the autosampler

1. Open the Accela Autosampler Configuration dialog box (see "Autosampler Configuration Settings" on page 43).
2. Click the **Signal Polarity** tab.

   The Signal Polarity page appears (see Figure 35).

Figure 35. Signal Polarity page parameters



3. In the Input area, ensure that the **Pump Ready Active High** and **Injection Hold Release Active High** check boxes are selected (see Figure 35). These check boxes are selected in the default instrument method.
4. In the Output area, if your instrument consists entirely of Accela devices, do **not** select the check boxes.

Autosampler Signal Polarity Page Parameters

Table 10 describes the parameters on the Signal Polarity page.

Table 10. Signal Polarity page parameters (Sheet 1 of 3)

Parameter	Description
Input	
The Pump Ready Active High and the Injection Hold Release check boxes are selected in the default instrument method. Leave these check boxes selected for an Accela pump.	
Pump Ready Active High	Use this check box to specify the polarity of the input signal from the pump: • If the signal from the pump is LO (Closed) and goes HI (Open) when the pump is ready, select the **Pump Ready Active High** check box. • If the signal from the pump is HI (Open) and goes LO (Closed) when the pump is ready, clear the **Pump Ready Active High** check box.

Table 10. Signal Polarity page parameters (Sheet 2 of 3)

Parameter	Description
Injection Hold Release Active High	Use this check box to specify the polarity of the input signal from the pump: • If the signal from the pump is LO (Closed) and goes HI (Open) when the pump is ready, select the **Injection Hold Release Active High** check box. • If the signal from the pump is HI (Open) and goes LO (Closed) when the pump is ready, clear the **Injection Hold Release Active High** check box.
Output These check boxes are clear in the default instrument method. For a Thermo Scientific LC/MS system, leave these check boxes clear.	
Autosampler Ready Active High	Use this check box to set the polarity of the A/S Ready output signal: • When this check box is selected, the signal from the autosampler is LO (Closed) and goes HI (Open) when the autosampler is ready. • When this check box is clear, the signal from the autosampler is HI (Open) and goes LO (Closed) when the autosampler is ready.
	Note The A/S Ready terminal sends a signal that indicates that the autosampler is in the Ready state. The autosampler is ready when it meets the selected ready conditions. The ready conditions you select include sample tray temperature, column oven temperature, door is closed, maintenance log, and vial bottom sensing (see "Communication Page" on page 50).
Injection Out Active High	Use this check box to set the polarity of the Inject Out output signal: • When this check box is selected, the signal from the autosampler is LO (Closed) and goes HI (Open) when the autosampler injects a sample. • When this check box is clear, the signal from the autosampler is HI (Open) and goes LO (Closed) when the autosampler injects a sample.
	Note The Injection Out terminal sends a signal to the other LC modules or peripheral devices when the autosampler injects a sample.
Gradient Start Active High	Use this check box to set the polarity of the Gradient Start output signal: • When the check box is selected, the signal from the autosampler is LO (Closed) and goes HI (Open) to trigger the start of the pump's gradient program. • When the check box is clear, the signal from the autosampler is HI (Open) and goes LO (Closed) to trigger the start of the pump's gradient program.
	Note The Grad Start terminal sends a signal to the pump to start the gradient program.

Table 10. Signal Polarity page parameters (Sheet 3 of 3)

Parameter	Description
Pump Stop Active High	Use this check box to set the polarity of the Pump Stop output signal: • When the check box is selected, the signal from the autosampler is LO (CLosed) and goes HI (Open) when the pump is to stop. • When the check box is clear, the signal from the autosampler is HI (Open) and goes LO (Closed) when the pump is to stop.
	Note The Pump Stop terminal sends a signal to the pump to stop. The Pump Stop output signal is not active during the injection sequence. The computer must make an explicit request to issue the Pump Stop signal.
Timed Events Active High	Use this check box to set the polarity of the Timed Events output signals: • When the check box is selected, the signal from the autosampler is LO (Closed) and goes HI (Open) when the timed events program is to start. • When the check box is clear, the signal from the autosampler is HI (Open) and goes LO (Closed) when the timed events program is to start.
	Note The TF terminals send signals for events entered in the timed events table of the instrument method. For information about setting up the timed events table for the autosampler, see "Timed Events Page for the Autosampler" on page 93.

Firmware Page

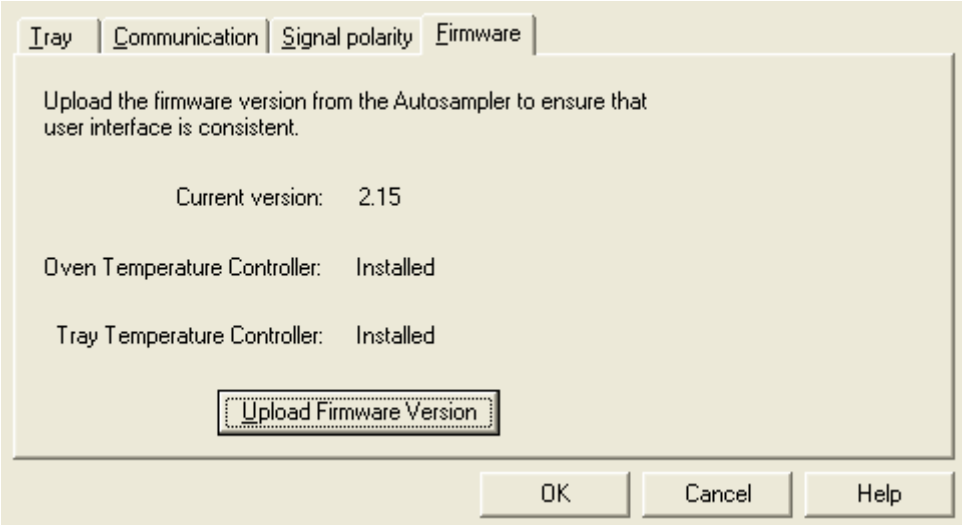
Use the Firmware page to check the firmware version of the autosampler.

❖ To check the firmware version of the autosampler

1. Open the Accela Autosampler Configuration dialog box (see "Autosampler Configuration Settings" on page 43).
2. Click the **Firmware** tab.

   The Firmware page appears (see Figure 36).

   Figure 36. Firmware page



3. If you have upgraded your autosampler, upload the firmware version as follows:
   a. Click **Upload Firmware Version**.

      The firmware version of the autosampler appears next to Current Version.
   b. Click **OK** to save the settings and close the dialog box.

If you have finished configuring all of your LC devices, go to "Closing the Foundation Application" on page 66.

Pump Configuration Settings

The Accela product line includes the following pumps:

Type	Pressure range	Flow rate range
Accela Pump	0 to 1000 bar (14 503 psi)	0.1 to 1000 µL/min
Accela 600 Pump	0 to 600 bar (8702 psi)	1 to 5000 µL/min
Accela 1250 Pump	0 to 1250 bar (18 130 psi)	1 to 2000 µL/min

During normal operation, the Accela pump communicates with the Xcalibur data system through a USB link. Before you configure the pump device driver, connect the pump to the data system computer. If your LC system contains two pumps, connect both pumps to the data system computer. Dual-pump systems can contain two Accela 600 Pumps, two Accela 1250 Pumps, or an Accela 600 Pump and an Accela 1250 Pump.

You can install only one Accela pump driver on your data system computer; however, the Accela 600 Pump and Accela 1250 Pump drivers can recognize and control the Accela 600 Pump and Accela 1250 Pump.

Depending on whether you are adding one or two pumps to your instrument configuration, follow one or both of these procedures:

- Adding Pump 1 to the Instrument Configuration
- Adding Pump 2 to the Instrument Configuration

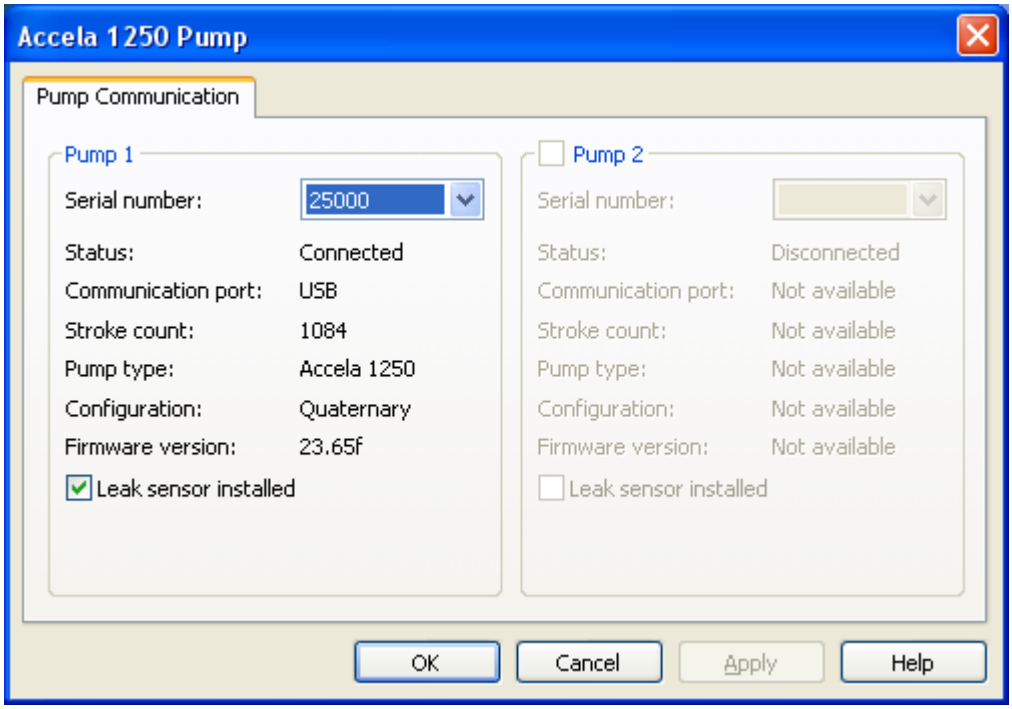
Adding Pump 1 to the Instrument Configuration

❖ **To specify the configuration settings for a single pump or pump 1 in a dual-pump system**

1. Ensure that the pump is connected to the data system computer and powered on.
2. For a dual-pump system, ensure that pump 2 is powered off.
3. In the Configured Devices list, double-click the **Accela Pump, Accela 600 Pump**, or **Accela 1250 Pump** icon.

The Accela *pump type* dialog box appears (see Figure 37).

Figure 37. Accela 1250 Pump dialog box for a single-pump setup



The Thermo Foundation Instrument Configuration application automatically populates the Serial number list with the last five digits of the pump's six-digit serial number.

4. If your pump has a leak sensor, select the **Leak Sensor** check box to activate the leak sensor.
5. Click **OK** to close the dialog box.
6. If you are controlling a dual-pump system, click **Done** to close the Instrument Configuration application, and go to "Adding Pump 2 to the Instrument Configuration."

If you have finished configuring the LC devices, go to "Closing the Foundation Application" on page 66.

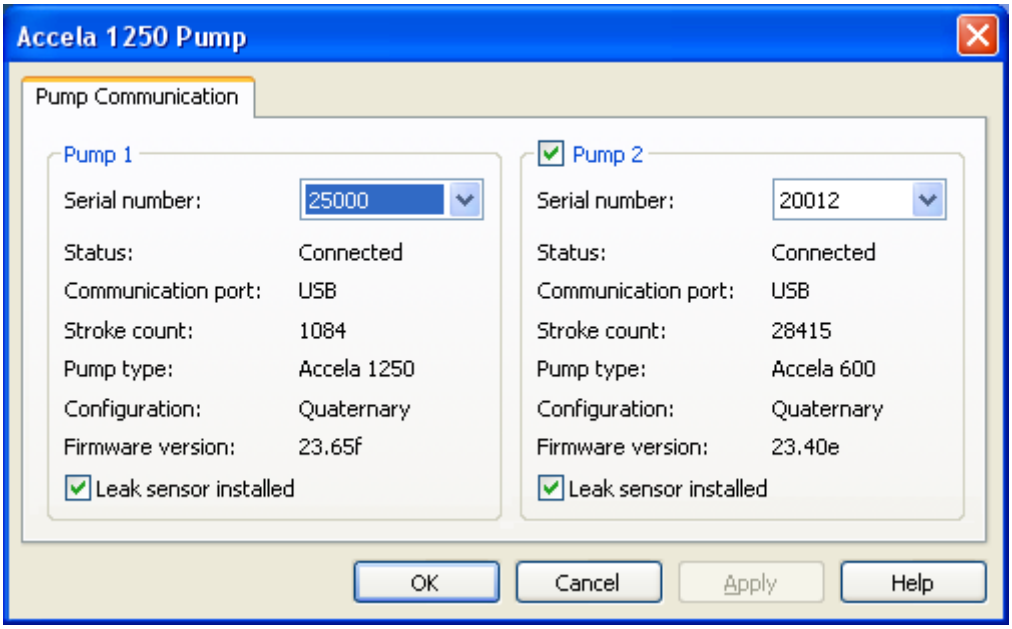
Adding Pump 2 to the Instrument Configuration

❖ To configure pump 2

1. Power on pump 2.
2. Open the Thermo Foundation Instrument Configuration application.
3. In the Configured Devices pane, double-click the icon for the pump.

   The Accela *pump type* dialog box appears.
4. Select the **Pump 2** check box (see Figure 38).

Figure 38. Accela 1250 Pump dialog box with the settings for a dual-pump system



5. Verify the following:
   - The value in the Serial Number list corresponds to the serial number on the pump's back panel.
   - The Status readback displays Connected.
   - The Pump Type readback displays the appropriate pump model.
6. Click **OK** to close the Accela *pump type* dialog box.
7. Click **Done** to accept the configuration and close the Instrument Configuration application.

Accela Pump Configuration Parameters

Table 11 provides descriptions of the parameters in the Accela pump Configuration dialog box.

Table 11. Accela pump Configuration dialog box parameters

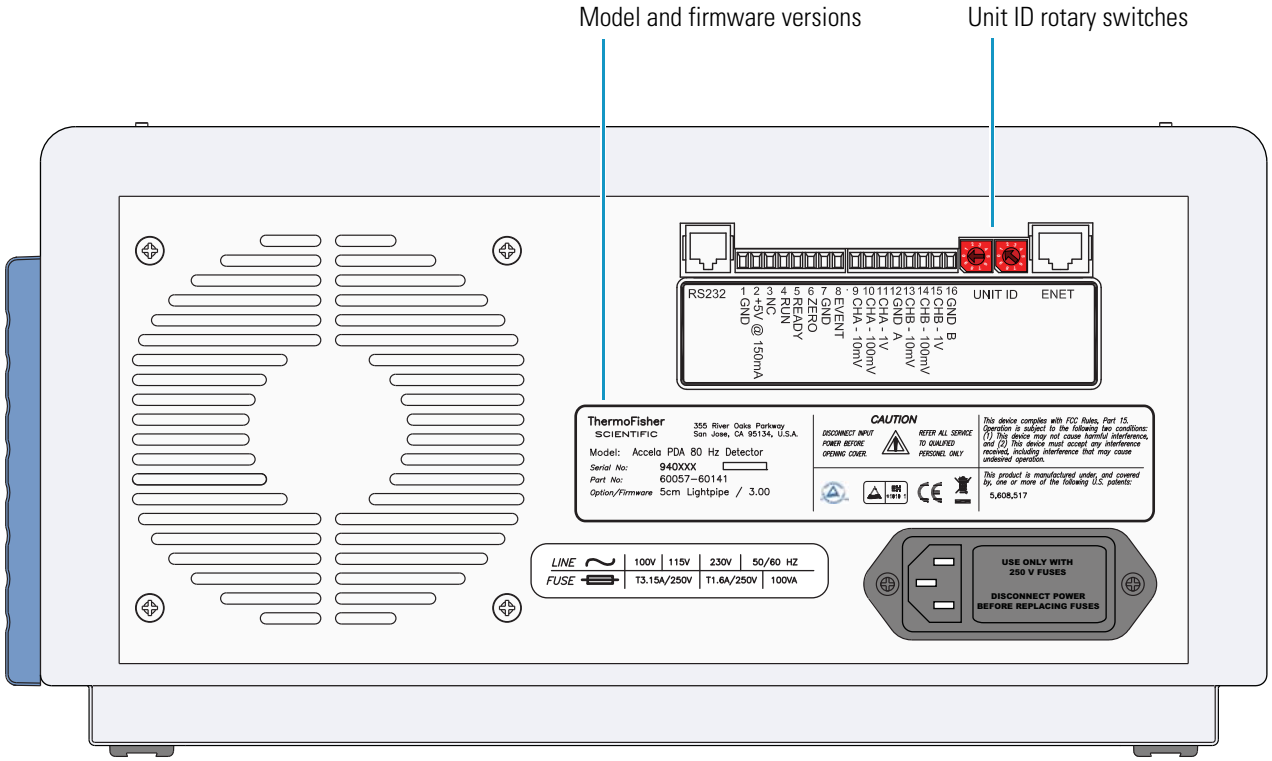
Parameter	Description		
Serial Number	Specifies the last five digits of the serial number stored in the pump's memory. The serial number is also listed on the pump's back panel. The first three digits of the pump's serial number depend on the pump type:		
	Pump type	**Six-digit serial number on the pump's back panel**	**Five-digit number in the Serial Number list**
	Accela 600 Pump	920 *xxx*	20 *xxx*
	Accela 1250 Pump	925 *xxx*	25 *xxx*
Status	Displays the pump's status. The states are Not Connected or Connected.		
Communication Port	Displays the type of communication port on the data system computer where the pump is connected.		
Stroke Count	Displays the total stroke counts for the pump's pistons.		
Pump Type	Displays the pump type: Accela, Accela 600, or Accela 1250.		
Configuration	Displays Quaternary. The Accela Pump, Accela 600 Pump, and Accela 1250 Pump are quaternary solvent pumps.		
Firmware Version	Displays the firmware version.		
Use Leak Sensor	When this check box is selected, the leak sensor is in use.		

PDA Detector Configuration Settings

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

The model version is listed on the back panel of the PDA detector (see Figure 39).

Figure 39. PDA detector (back panel)

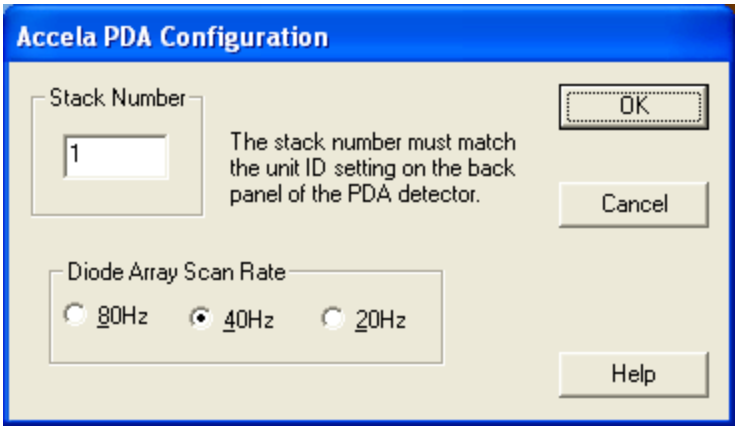


❖ To specify the configuration settings for the PDA detector

1. In the Configured Devices pane, double-click the **Accela PDA** icon.

   The Accela PDA Configuration dialog box appears (see Figure 40).

Figure 40. Accela PDA Configuration dialog box with the default selections



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

   The default value is 1 and the range is 0 through 99. The value of 0 is reserved for service operations.

   The stack number must match the unit ID setting on the back panel of the PDA detector. The unit ID consists of two rotary switches (see Figure 28 on page 40) factory-set to 01.

3. In the Diode Array Scan Rate area, select the appropriate sampling frequency for the diode array.

   The default selection is 40 Hz.

IMPORTANT There are two versions of the PDA detector: the Accela PDA Detector and the Accela PDA (80 Hz) Detector. The Accela PDA (80 Hz) Detector is the current version. For the discontinued PDA detector, you must select the 20 Hz option to establish communication between the data system and the PDA detector.

The model and firmware versions are listed on the back panel of the PDA detector (see Figure 39 on page 63).

IMPORTANT When you change the diode array scan rate, you must adjust the light throughput to the diode array (see "Adjusting the Light Throughput to the Diode Array" on page 262).

IMPORTANT The diode array scan rate affects the detector noise level. When you are developing a validated HPLC method, record the configuration setting for the diode array scan rate.

The appropriate diode array scan rate depends on the detector version and the baseline width (W_b) of your application's chromatographic peaks:

- For the discontinued PDA detector, which is not capable of acquiring data at an 80 Hz rate, you must select 20 Hz. The discontinued PDA detector has a firmware version below 3.00. The firmware version is listed on the back panel of the detector.
- For the Accela PDA (80 Hz) Detector, select the appropriate diode array scan rate based on your chromatographic application.

Baseline peak width (seconds)	Data rate or scan rate (Hz)
$W_b \leq 0.5$	80
$0.5 < W_b < 1$	40
$1 \leq W_b < 2$	20

4. Click **OK** to accept the settings and close the Accela PDA Configuration dialog box.

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

Accela PDA Detector Configuration Parameters

Table 12 describes the instrument configuration parameters for the Accela PDA Detector.

Table 12. Accela PDA Detector configuration parameters

Parameter	Description
Stack Number	The stack address must match the unit ID (rotary switches) setting on the back panel of the PDA detector. The range of values is 1 to 99. If your Accela PDA Detector is connected to a network, use the stack address to select the PDA detector that you want to control.
Diode Array Scan Rate	Frequency that the PDA detector scans the photodiode readouts. The default selection is 40 Hz. The available selections are 20 Hz, 40 Hz, and 80 Hz.
	IMPORTANT For the Accela PDA Detector, select the 20 Hz option. If you do not select the 20 Hz option, the data system cannot establish communication with the PDA detector.
	IMPORTANT When you change the diode array scan rate, you must adjust the light throughput to the diode array (see "Adjusting the Light Throughput to the Diode Array" on page 262).

UV/Vis Detector Configuration Settings

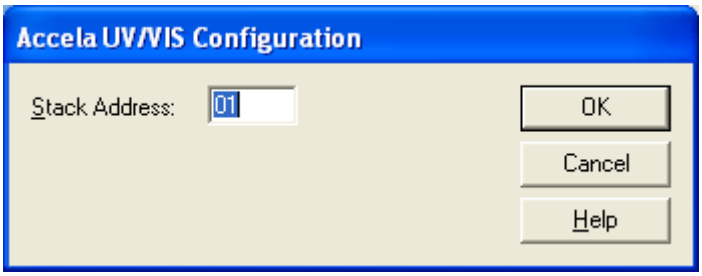
The UV/Vis Detector communicates with the data system computer through an Ethernet connection.

❖ **To specify the configuration settings for the UV/Vis detector**

1. In the Configured Devices list, double-click the **Accela UV/Vis** icon.

The Accela UV/Vis Configuration dialog box appears (see Figure 41).

Figure 41. Accela UV/Vis Configuration dialog box



2. In the Stack Address box, type the appropriate number (unit ID). The Stack Address setting must match the rotary switches on the back panel of the detector.
3. Click **OK** to close the Accela UV/Vis Configuration dialog box.

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

Closing the Foundation Application

Before you can open your Thermo Scientific data system, you must close the Foundation application.

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

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

The Windows desktop appears.

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

Instrument Method Setup

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

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

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

❖ To create an instrument method

1. Open the Instrument Setup window (Xcalibur data system) or equivalent window for your Thermo Scientific mass spectrometry application.
2. Specify the instrument method settings for each device.
3. Save the instrument method.

Contents

Opening the Instrument Setup Window

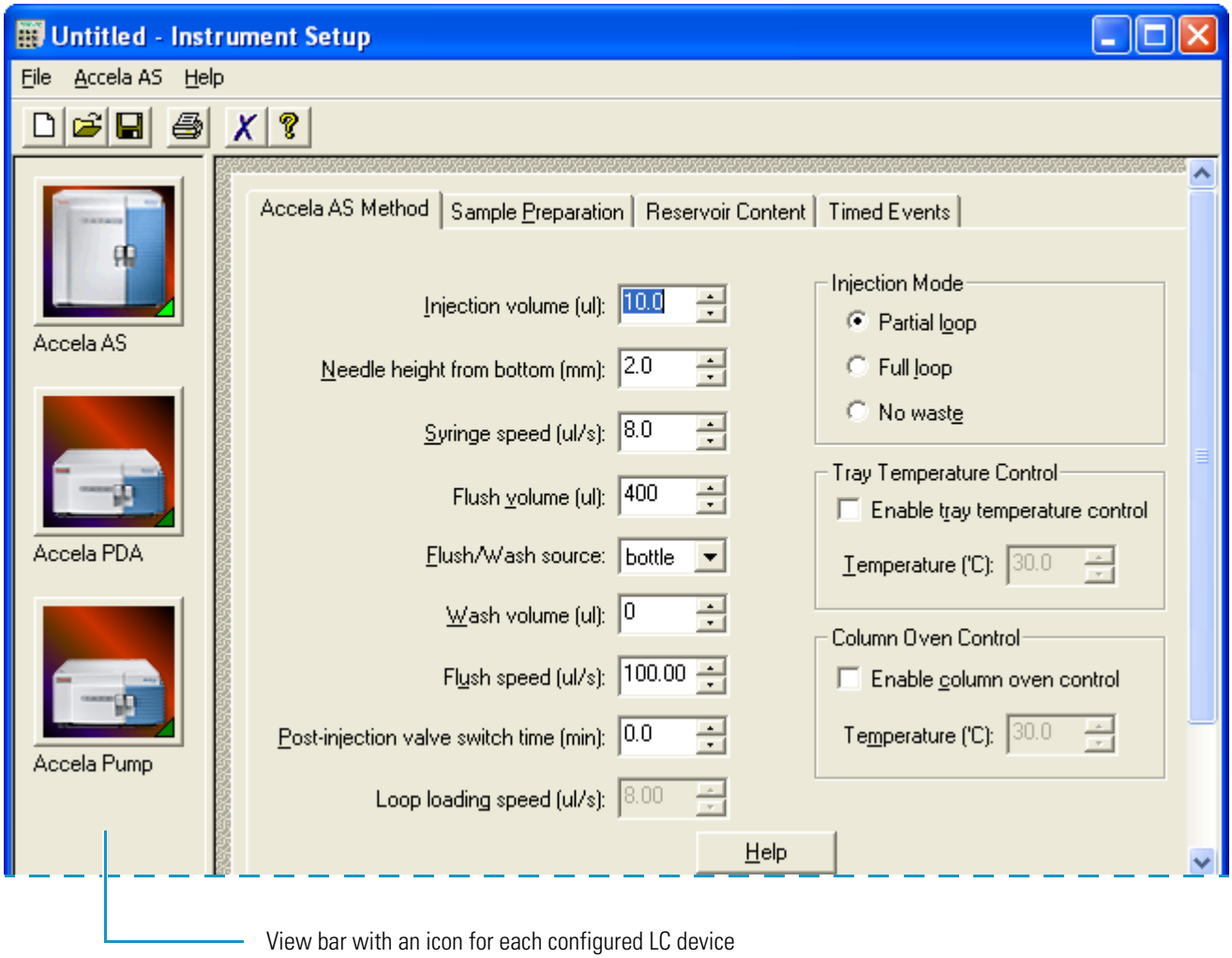
The Instrument Setup window of the Xcalibur data system is where you create instrument methods and access the direct control commands for your instrument devices. If you are controlling your LC/MS instrument from another Thermo Scientific mass spectrometry application, open the equivalent window.

❖ To open the Instrument Setup window

1. Start the Xcalibur data system.
2. In the Thermo Xcalibur Roadmap window, click the **Instrument Setup** icon on the Road Map or choose **GoTo > Instrument Setup**.

   The Instrument Setup window appears (see Figure 42). The view bar on the left side of the window contains an icon for each configured instrument device. Clicking the device icon opens the view for that device.

Figure 42. Instrument Setup window



Pump Instrument Method Settings

Use the Accela Pump, Accela 600 Pump, or Accela 1250 Pump view in the Instrument Setup window to specify the chromatographic conditions for your instrument method.

To open the pump view, click the **Accela Pump**, **Accela 600 Pump**, or **Accela 1250** icon on the view bar of the Instrument Setup window.

To set up the chromatographic conditions, see these topics:

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

Specifying the Chromatographic Conditions for a Single-Pump System

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

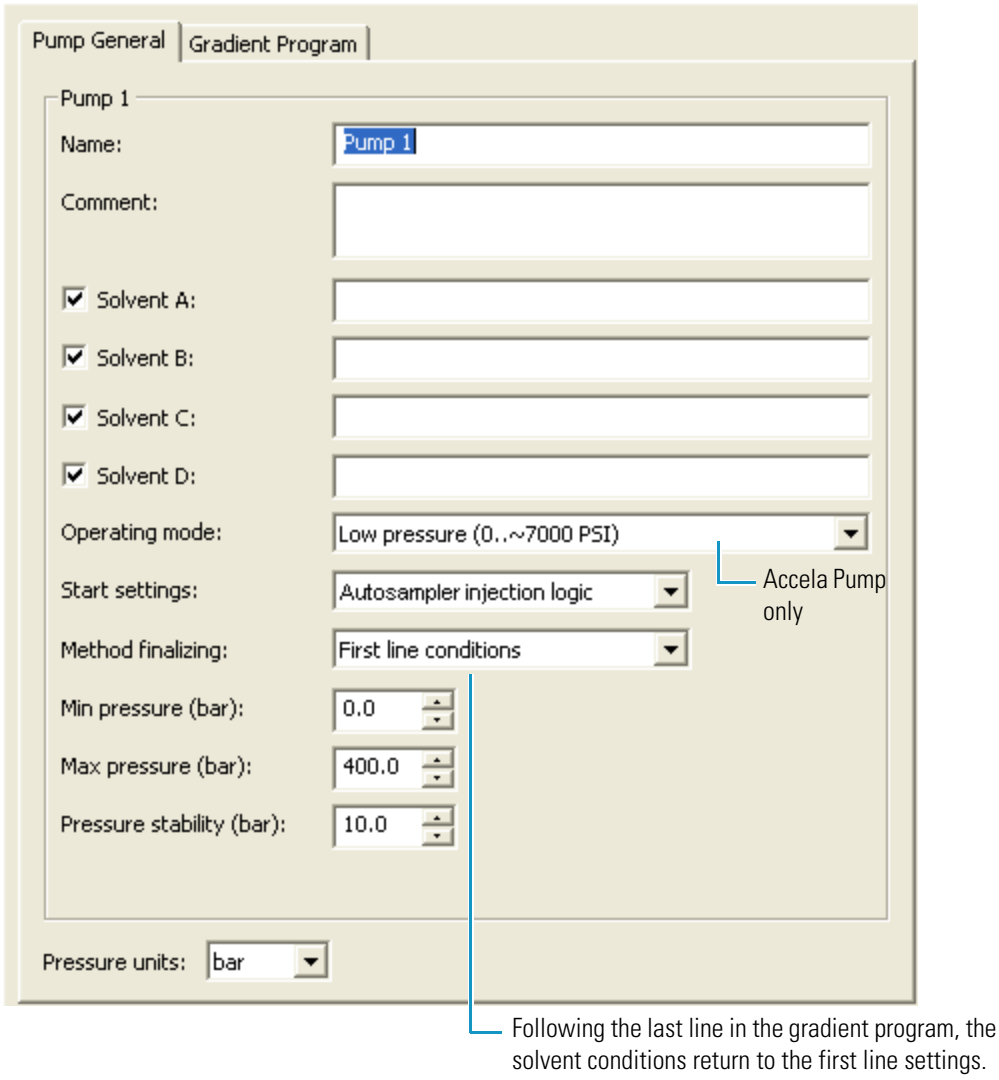
1. Setting Up the Pump General Parameters
2. Setting Up the Gradient Program

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

Setting Up the Pump General Parameters

Use the Pump General page to specify the solvents that the pump uses to create the mobile phase and operating conditions for the pump (see Figure 43).

Figure 43. Pump General page for the Accela Pump with the default settings



When you connect two pumps to the data system computer, you must specify the general parameters for both pumps. The autosampler triggers the gradient program for Pump 1 at the start of a run. Pump 1 triggers the gradient program for Pump 2 at a user-specified time during the run (see "Programming the Pumps in a Dual-Pump System" on page 82).

Note You can use a dual-pump system to perform two-dimensional chromatography or to create a high-pressure gradient.

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

❖ To specify the parameters on the Pump General page

1. Open the view for your pump.

   For information about opening the device views from the Xcalibur 2.1.*x* data system, see "Opening the Instrument Setup Window" on page 68.

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

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

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

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

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

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

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

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

   d. In the Operating Mode list (Accela Pump only), select the operating mode: **Low Pressure (0 ~7000 PSI)** or **High Pressure (~7000 ~15000 PSI)**.

   e. In the Start settings list, select how the system is triggered to start a run: **Autosampler Injection Logic**, **Open Accela AS Injection Logic**, **Accela AS Injection Logic**, or **Manual**.

      When you select Manual, the Home before Run check box becomes available.

      IMPORTANT If your system contains the original Accela Pump and an Accela Open Autosampler, select **Accela AS Injection Logic**.

   f. In the Method Finalizing list, select the ending conditions for the run.

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

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

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

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

Pump type	Range	Default
Accela Pump	0 to 400 bar (5800 psi) 0 to 1000 bar (14 504 psi)	0 bar
Accela 600 Pump	0 to 600 bar (8702 psi)	0 bar
Accela 1250 Pump	0 to 1250 bar (18 130 psi)	0 bar

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

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

Pump type	Range	Default
Accela Pump	0 to 400 bar (5800 psi) 0 to 1000 bar (14 504 psi)	400 bar
Accela 600 Pump	0 to 600 bar (8702 psi)	600 bar
Accela 1250 Pump	0 to 1250 bar (18 130 psi)	1250 bar

i. In the Pressure Stability (bar) box, type or select an appropriate pressure stability value from **1** to **100**.

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

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

Note 1 bar = 14.5 psi

Thermo Pump General Page Parameters

Table 13 describes the parameters on the General page.

Table 13. Thermo pump method parameters (Sheet 1 of 3)

Parameter	Description
Name	Identifies the pump on the pump view of the Status page of the Info view. The text string can consist of up to 16 alphanumeric characters.
Comment	User text that provides additional information. The text string can consist of up to 255 alphanumeric characters.
Solvent A, B, C, and D check boxes and adjacent boxes	When these check boxes are selected, the associated solvent column on the Gradient Program page is available. Select the check boxes corresponding to the solvent bottles to be used to create the mobile phase. Type a description of the solvent in the adjacent box. Clearing the check box associated with a solvent (A, B, C, or D) makes the solvent column unavailable on the Gradient Program page.
Operating Mode (Accela Pump only)	Specifies the operating mode for the pump. The selections are Low Pressure (0~7000 PSI) and High Pressure (~7000 ~15000 PSI).

Table 13. Thermo pump method parameters (Sheet 2 of 3)

Parameter	Description	
Start settings	Specifies the device that triggers the start of a run and the run synchronization signals between the pump and the autosampler.	
	Selection	**Effect**
	Accela AS Injection Logic	At the start of a run, the Accela Autosampler waits for the pump to issue the Pump Ready and the Release Injection signals before making an injection. The pump then waits for the Start gradient signal from the autosampler before starting the gradient program. When you use this injection logic, set up the Accela Autosampler as the start instrument that triggers the run.
	Tip If your LC system contains an Accela Open Autosampler and an Accela Pump, select Accela AS Injection Logic instead of Open Accela AS Logic.	
	Open Accela AS Injection Logic	At the start of a run, the autosampler waits for the pump to issue the Pump Ready signal before making an injection. The pump then waits for the Start gradient signal from the autosampler before starting the pump program. When you use this injection logic, set up the Accela Open Autosampler as the start instrument. If your system contains an Accela Open Autosampler and an Accela 600 Pump or and Accela 1250 Pump, select Open Accela AS Injection Logic.
	Micro AS Injection Logic	The Xcalibur 2.1.x data system does not support the Micro AS autosampler. At the start of a run, the Micro AS autosampler issues the start gradient signal to the pump. When you use this injection logic, do not set up the pump as the start instrument.
	Manual	At the start of a run, the pump program starts immediately. When you use this injection logic, set up the pump as the start instrument.
Method Finalizing	Specifies the mobile phase composition at the end of the run. The 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 mobile phase flow.	
Min Pressure	Specifies the minimum operating pressure of the pump. If the system pressure falls below this level for more than one minute, the data system triggers turns off the mobile phase flow. Default: 0 bar	

Table 13. Thermo pump method parameters (Sheet 3 of 3)

Parameter	Description
Max Pressure	Specifies the maximum operating pressure of the pump. If the system backpressure rises above this level, the data system triggers the pump to turn off the mobile phase flow. The default maximum pressure depends on the pump type.

Pump	Max pressure
Accela Pump	1000 bar (14 503 psi or 100 MPa)
Accela 600 Pump	600 bar
Accela 1250 Pump	1250 bar

Parameter	Description
Pressure Stability	Specifies the stability condition required for the pump to go into a Ready state. A lower value requires greater pressure stability before the pump state goes to Ready. A higher value is more forgiving of pressure pulsations. Default: 10 The range depends on the pressure units selection.

Units	Range
psi	1 to 1450
MPa	1 to 10
bar	1 to 100

Parameter	Description
Pressure Units	Specifies the units for the pressure readout. Selections: PSI and MPa 1.00 MPa = 10.0 bar = 145 psi
Idle Settings (Pump 2 of a dual-pump system)	Specifies the conditions for Pump 2 while it is waiting to be triggered by Pump 1. Default: Standby Selections: Standby, First Line Conditions, and Last Line Conditions In the Standby mode, the pump remains in the Idle state.

Setting Up the Gradient Program

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

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

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

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

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

The gradient table can contain 2 to 398 time lines.

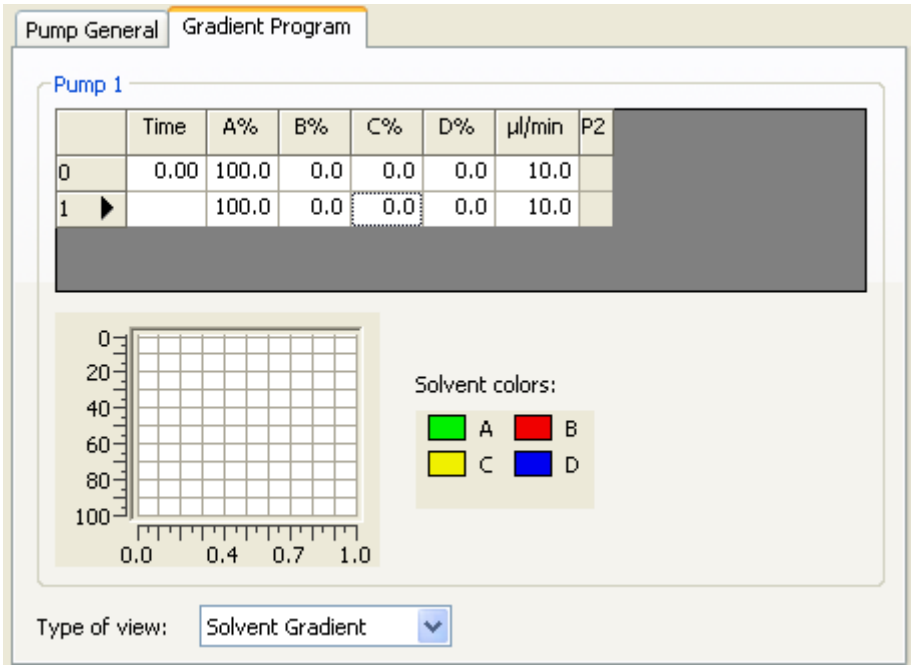
IMPORTANT The gradient table must contain at least two time lines. For an isocratic gradient program, the two time lines specify the same solvent composition and flow rate.

❖ To set up the gradient program for the pump

1. Open the view for your pump.
2. Specify the general pump parameters (see "Specifying the Chromatographic Conditions for a Single-Pump System" on page 69).
3. Click the **Gradient Program** tab.

   The Gradient Program page appears (see Figure 44). The columns that represent the solvents selected on the Pump General page are available for editing.

Figure 44. Gradient Program page



4. For time = 0.00, do the following:

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

      The first row in the gradient program table is set to 0.00 min and is uneditable. As you change the percent composition for each solvent, the data system maintains a total solvent composition of 100%.

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

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

Pump	Minimum flow rate (µL/min)		
	Accela AS injection logic	Manual and Home before run	Manual
Accela Pump	0.1	0.1	0.0
Accela 600 Pump	1.0	1.0	0.0
Accela 1250 Pump	1.0	1.0	0.0

The maximum flow rate for the Accela pump depends on the pump type as follows:

Pump	Maximum flow rate (µL/min)
Accela Pump	1000
Accela 600 Pump	5000
Accela 1250 Pump	2000

5. For an isocratic separation method, do the following on the Gradient Program page for the Accela pump:
   a. Double-click the Time column in the second row of the gradient program table, and then type a time value in this cell.

      The time range is 0.01 to 655.00 minutes.

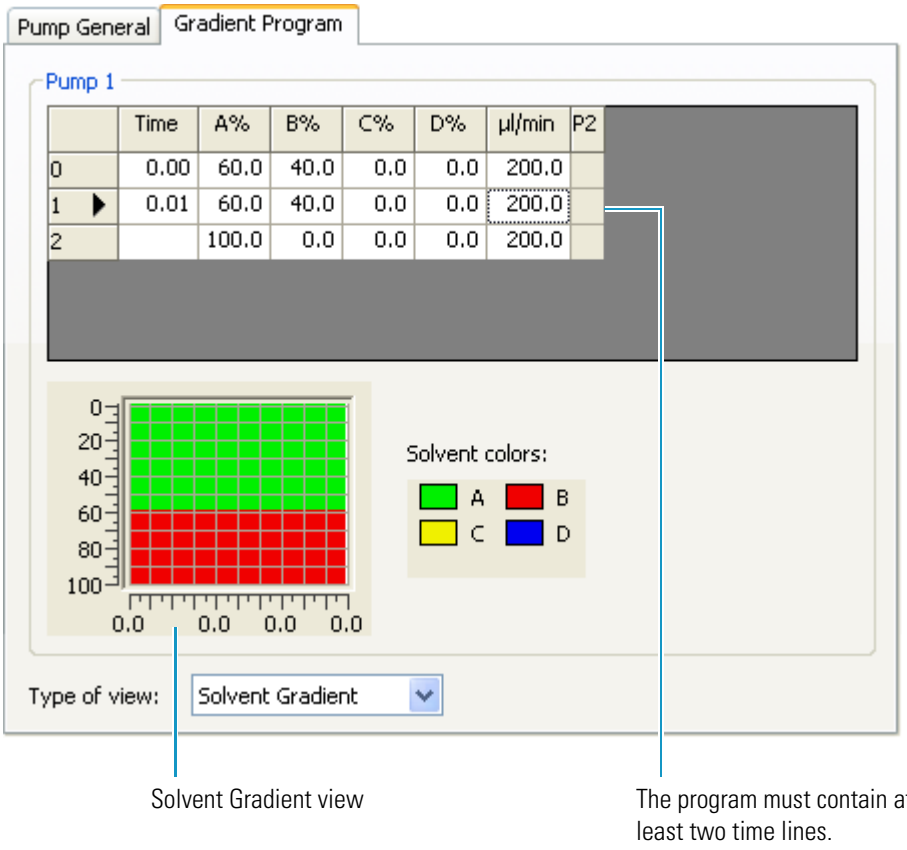
      If the time value for the second time point is longer than the data acquisition time for the PDA detector or the MS detector, the data system waits for the gradient program to finish before proceeding to the next row in the acquisition sequence.

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

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

   c. Verify the gradient program in the Solvent Gradient view (see Figure 45).

Figure 45. Isocratic gradient program



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

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

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

      The flow rate range is the same as for an isocratic run (see step 4b on page 77). To produce a stable gradient, enter a flow rate of at least 25 µL/min.

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

      Figure 46 shows the gradient program entries for the gradient program in Table 14. After 30 minutes the gradient program returns to the first line conditions for the remainder of the run. To equilibrate the LC column at the first line conditions for 10 minutes, you must set the run time for the PDA detector or MS detector to 40 minutes.

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

Figure 46. Example of a pump gradient program

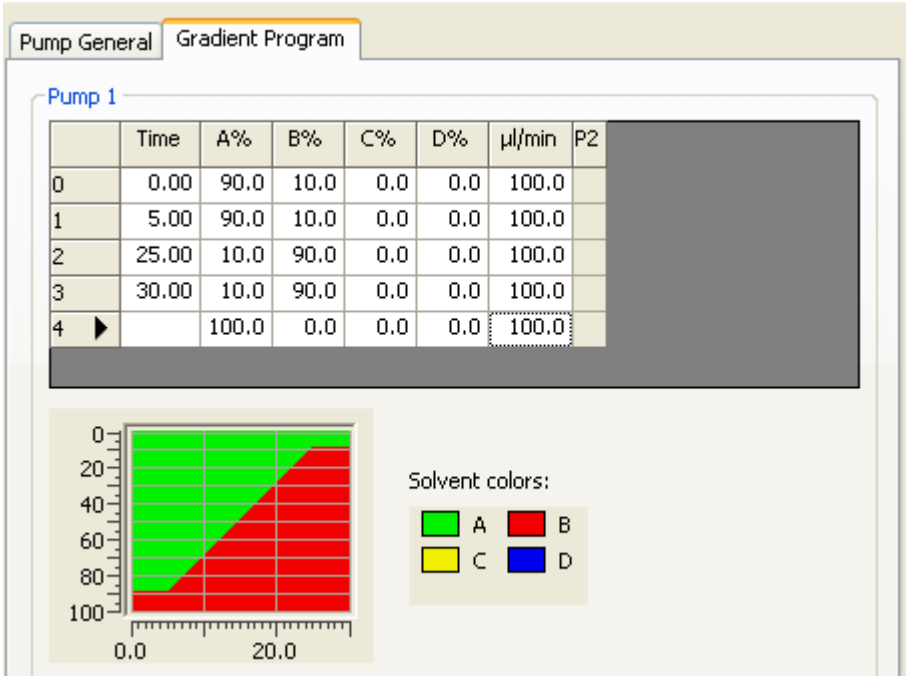


Table 14. Gradient program

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

Thermo Pump Gradient Program Page Parameters

Table 15 describes the parameters on the Gradient Program page.

Table 15. Thermo pump method parameters

Parameter	Description	
Gradient table		
Each row in the gradient table defines the solvent composition and flow rate for a specific time point. Between time points, the solvent composition changes linearly, whereas the flow rate changes as a discontinuous step-function. The pump program can contain up to 398 time lines.		
Time	The time parameter (min box) specifies at what point in the run the associated solvent composition and flow rate become effective. The first time line remains set to 0 min.	
A%, B%, C%, and D%	The solvent composition parameters (A%, B%, C%, and D% columns) work interactively with each other and specify the mobile phase composition at the time point specified in the associated Min box. The mobile phase composition changes linearly between two consecutive time points. Default: 100% A	
µL/min	The µL/min box specifies the flow rate of the mobile phase at the time specified in the associated Time box. The default flow rate setting is 10 µL/min. The range depends on the pump type.	
	Pump	**Range**
	Accela Pump	0.1 to 1000 µL/min
	Accela 600 Pump	1 to 5000 µL/min
	Accela 1250 Pump	1 to 2000 µL/min
P2 (Dual-pump system)	For a dual-pump system, the time line position of the ball in the P2 column specifies the time when Pump 1 triggers Pump 2.	
Graph view		
Type of View	Select Solvent Gradient to view the solvent gradient profile created by the pump program. Select Flow Gradient to view the flow rate changes created by the pump program.	
	The Gradient Profile area graphically displays the values entered in the pump program. The *y* axis represents percent composition or flow rate and the *x* axis represents time in minutes. Each solvent is color-coded for better visualization of the programmed gradient.	

Programming the Pumps in a Dual-Pump System

❖ To program the pumps in a dual-pump system

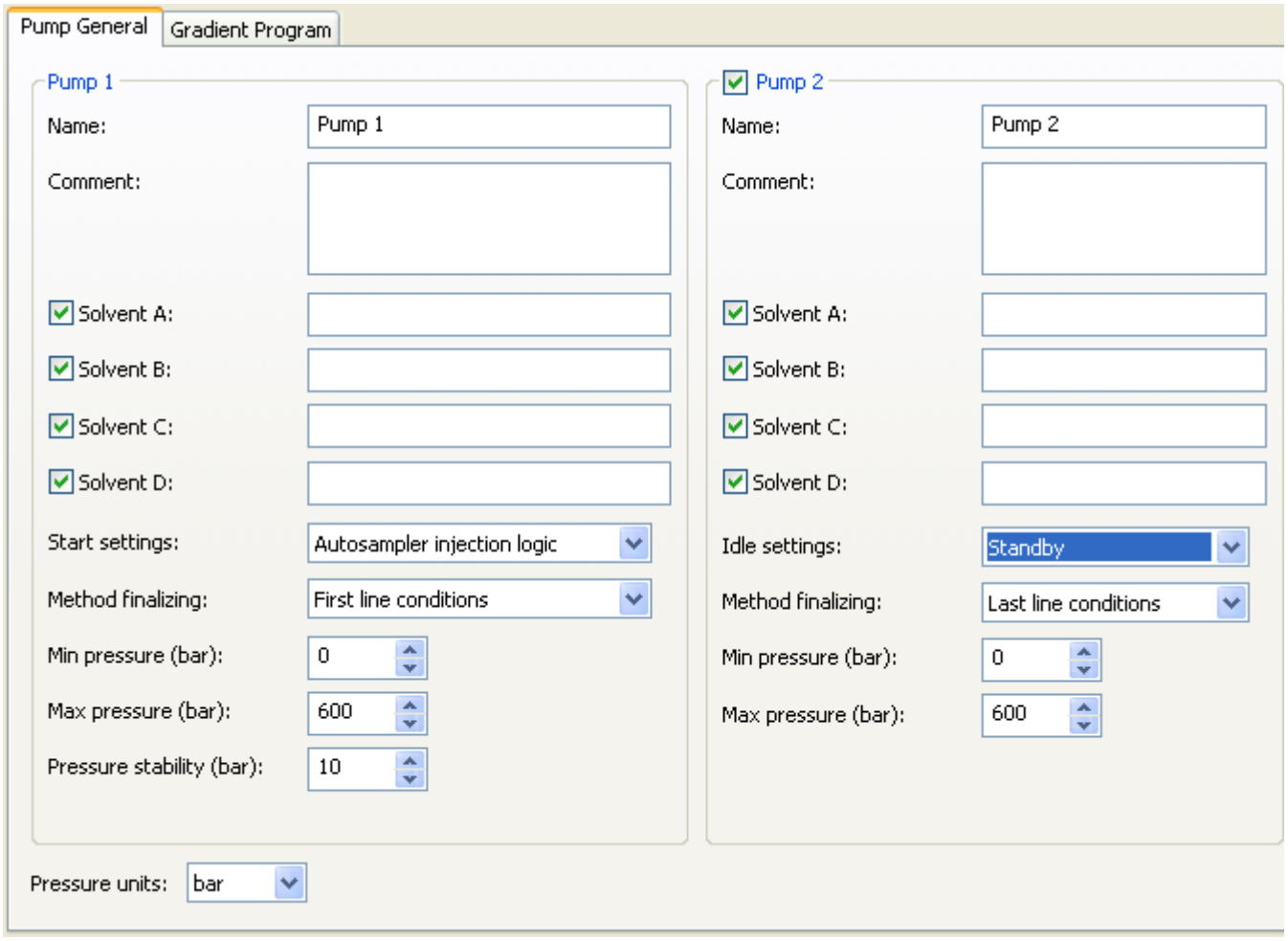
1. On the Pump General page (see Figure 47), do the following:
   a. Follow the instructions in "Setting Up the Pump General Parameters" on page 69.
   b. In the Pump 2 area, select the Idle settings for the pump that you configured as the sample pump (Pump 2) as follows:

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

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

   c. Specify the remaining parameters for Pump 2.

Figure 47. Pump General page for a dual-pump system consisting of two Accela 600 Pumps



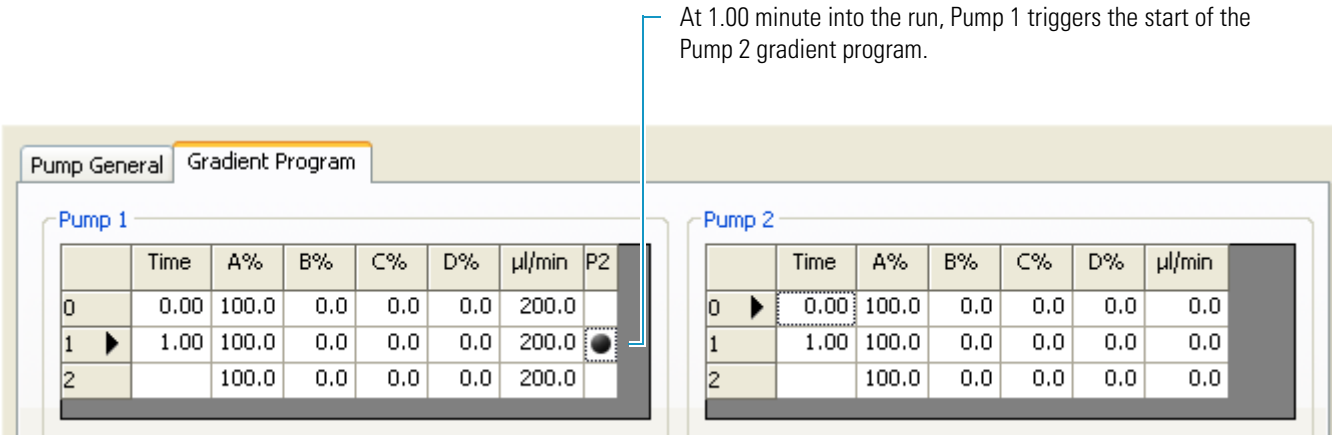
2. On the Gradient Program page (see Figure 48), do the following:

   a. To specify the gradient programs for both pumps, follow the instructions in "Setting Up the Gradient Program" on page 76.

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

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

Figure 48. Gradient page for a dual-pump system



Autosampler Instrument Method Parameters

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

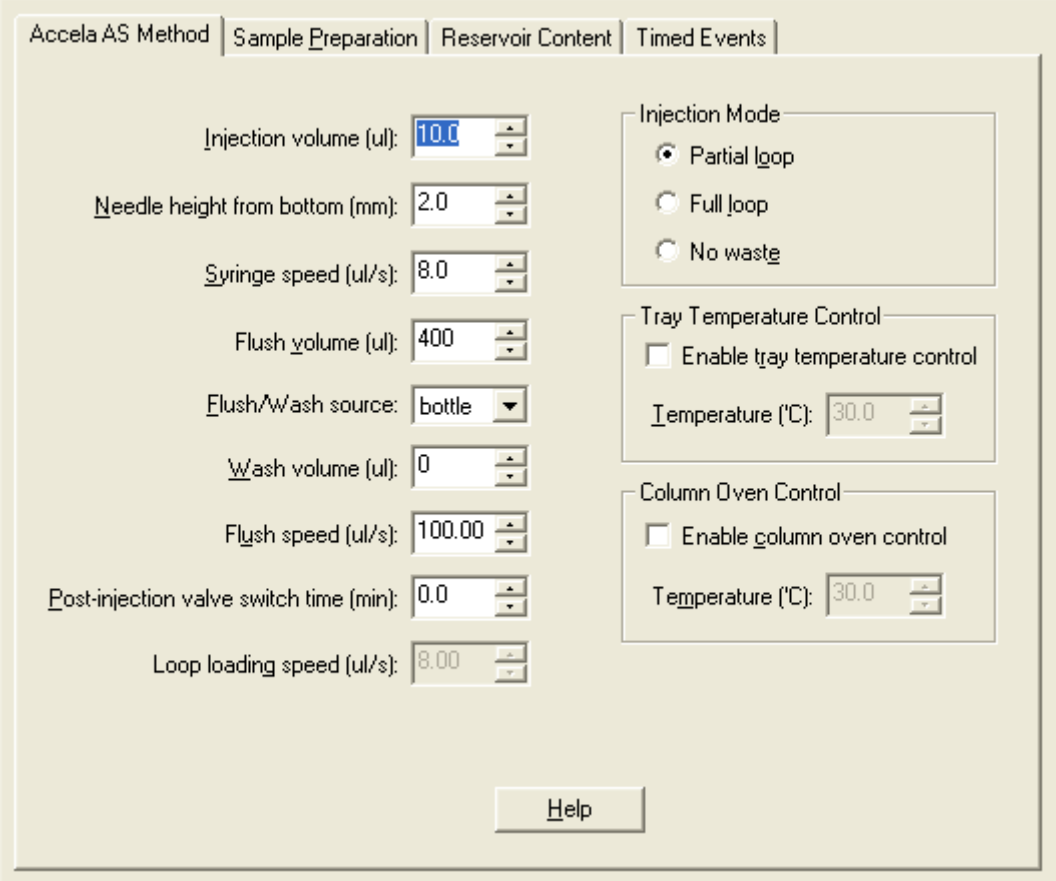
- Accela AS Method Page
- Reservoir Content Page
- Timed Events Page for the Autosampler

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

Accela AS Method Page

Use the Accela AS Method page to set the injection and the temperature control parameters for the autosampler (see Figure 49).

Figure 49. Accela AS Method page



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

1. Select the injection mode:

   - If you want to vary the volume of your sample injections, select the **Partial Loop** option.
   - If you want a high degree in injection-to-injection precision, select the **Full Loop** option. When you select Full loop, the Injection Volume (µL) box becomes unavailable.
   - If you have a limited amount of sample, select the **No Waste** option. When you select No Waste, the Loop Loading Speed (µL/s) box becomes available.

   Note For more information about the injection modes, see "Injection Modes" on page 17.

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

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

3. For the Partial Loop and No Waste injection modes, type or select an injection volume in the Injection Volume box.

   The injection volume range depends on the syringe size (see "Injection Volume Range" on page 91).

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

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

   Figure 9 on page 12 shows the needle descending to a depth of 2 mm from the bottom of a standard 1.8 mL vial.

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

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

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

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

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

For information about the location of the 16 mL reservoir vials, see "Tray Compartment" on page 4. For information about specifying the contents of the reservoir vials and the wash bottle, see "Reservoir Content Page" on page 92.

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

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

9. In the Flush Speed (µL/s) box, set a flush speed appropriate for the flush solvent:

   - If you are using a flush solvent of low viscosity such as methanol, keep the flush speed at its default (**100** µL/s for the 250 µL concentric syringe)
   - If you are using water or a methanol/water mixture as the flush solvent, reduce the flush speed.

   Tip If you hear a grinding noise from the autosampler as it performs a flush, lower the flush speed.

10. In the Post-Injection Valve Switch Time (min) box, keep the time value at its default of **0** minutes for most applications. This leaves the sample loop in the inject position during the entire run, allowing ample rinsing of the sample loop between injections.
11. In the Tray Temperature Control area, to control the temperature of the tray compartment, select the **Enable Tray Temperature Control** check box. Then type an appropriate temperature from 0 to 60 °C in the Temperature box.
12. In the Column Oven Control area, to control the temperature of the LC column, select the **Enable Column Oven Control** check box. Then type an appropriate temperature from 5 to 95 °C in the Temperature box. Controlling the temperature of the LC column increases the reproducibility of the chromatographic retention times.

Autosampler Method Page Parameters

Table 16 describes the parameters on the Accela AS Method page.

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

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

- Injection Volume Range
- Syringe and Loop Loading Speeds for Injections

Table 16. Autosampler Method page parameters (Sheet 1 of 3)

Parameter	Description
Injection Volume (µL)	Specifies the sample volume that the autosampler loads into the sample loop. The default value and the range depend on the syringe size (see "Injection Volume Range" on page 91).
Needle Height from Bottom (mm)	Specifies the height from the bottom of the vial or well where the needle withdraws liquid (see Figure 9 on page 12). Range: 0.1 to 18.0 mm Default: 2 mm
Syringe Speed (µL/s)	Specifies the rate at which the syringe withdraws liquid from a tray vial or a plate well. The default value and the range depend on the syringe size (see "Syringe and Loop Loading Speeds for Injections" on page 91).
Flush Volume (µL)	Specifies the volume of liquid that the autosampler flushes through the inside of the needle, the injection port, and the transfer tube. During a flush cycle, the injection valve is in the inject position to prevent the flush solvent from entering the sample loop. To reduce carryover, increase the flush volume. When the specified flush volume exceeds the syringe capacity, the autosampler takes multiple draws from the reservoir or bottle. Range: 0.0 to 6000.0 µL Default: 400 µL
Flush/Wash source	Specifies the flush/wash source. Selections: bottle, RV1, RV2, RV3, or RV4 Default: bottle (wash bottle)
Wash Volume (µL)	Specifies the volume of liquid used to wash the internal and external surfaces of the needle. During a wash cycle, the autosampler lowers the needle into the wash port. When the specified wash volume exceeds the syringe capacity, the autosampler takes multiple draws from the reservoir vial or wash bottle. Range: 0.0 to 6000.0 µL Default: 0 µL

Table 16. Autosampler Method page parameters (Sheet 2 of 3)

Parameter	Description
Flush Speed (µL/s)	Specifies the rate at which the syringe draws flush solvent into its chamber and expels flush solvent through the needle during a flush cycle.
	Syringe type / **Default** / **Range** Concentric dual-plunger / 1.65 to 661.38 µL/s / 100.0 µL/s Standard single-plunger / 8.27 to 330.85 µL/s / 82.71 µL/s
	IMPORTANT A flush speed of 250 µL/s for the 250 µL concentric syringe is the maximum flush speed for a solvent of low viscosity such as 100% methanol. If you are using a solvent of higher viscosity, reduce the flush speed accordingly. For example, because water has approximately twice the viscosity as methanol, if you are using water as your flush solvent, lower the flush speed to 100 µL/s. At high flush speeds, viscous solvents can cause the autosampler to make a grinding sound.
Post-Injection Valve Switch Time (min)	At the default post-injection valve switch time of 0 minutes, the injection valve remains in the inject position during the run. A time value greater than 0.1 minutes specifies the time when the injection valve switches from the inject position to the load position at the end of an injection cycle. To reduce the gradient delay volume for low-flow gradient applications, consider switching the injection valve from the inject position to the load position during the run by typing a nonzero value in this box. Allow enough time for the mobile phase to backflush the sample out of the sample loop. Default: 0 minutes Range: 0 to 999.0 minutes
Loop Loading Speed (µL/s)	Specifies the rate at which the autosampler meters the sample into the sample loop for no waste injections. During a no waste injection, the autosampler meters the sample through the transfer tubing to the injection valve at the specified syringe speed. The injection valve then switches to the load position, and the autosampler meters the sample into the sample loop at the specified loop loading speed. This parameter is only available in the No Waste injection mode. The default value and range depend on the syringe size (see "Syringe and Loop Loading Speeds for Injections" on page 91).
Injection Mode	
Partial Loop	Select this option if you want to inject variable volumes.
	IMPORTANT To make precise partial loop injections, limit the maximum injection volume to less than half the nominal sample loop size. Because the accuracy of the nominal volume of the sample loop is ± 20% (which means that the actual volume of the standard 25 µL sample loop is between 20 and 30 µL), for best results, limit the maximum injection volume to 10 µL for the standard 25 µL sample loop.

Table 16. Autosampler Method page parameters (Sheet 3 of 3)

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

Injection Volume Range

The injection volume for full loop injections is automatically set to the value in the Sample Loop Volume box on the Communication page of the Accela Autosampler dialog box (see "Communication Page" on page 50).

For partial loop and no waste injections, the minimum injection volume is 0.1 µL. The maximum injection volume depends on the syringe size (see Table 17).

For optimal performance, follow these guidelines:

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

Table 17. Maximum injection volume

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

Syringe and Loop Loading Speeds for Injections

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

You can specify how fast, in microliters per second, the syringe withdraws liquid from a tray vial or plate well, and then expels liquid into the autosampler injection port. The default syringe speed and the syringe speed range depend on the syringe size (see Table 18).

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

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

Table 18. Syringe speeds for injections

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

For no waste injections, you can use a higher syringe speed to push the sample from the autosampler injection port to the injection valve and a lower loop loading speed to meter the sample into the sample loop. The range for the loop loading speed depends on the syringe size (see Table 19).

Table 19. Loop loading speeds

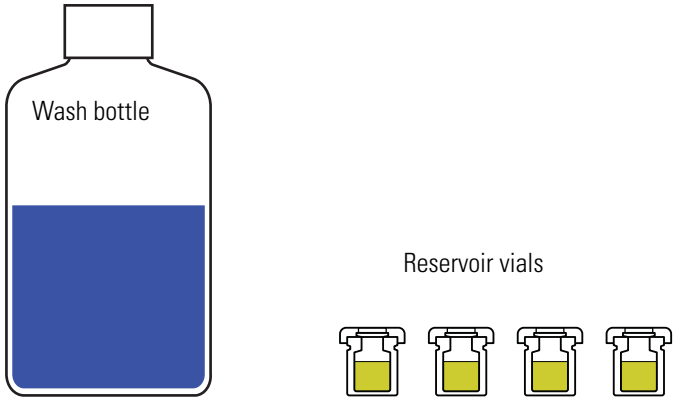
Syringe size (µL)	Minimum speed (µL/s)	Maximum speed (µL/s)	Default speed (µL/s)
100	0.01	13.20	3.00
250	0.04	33.00	8.00
500	0.08	66.11	8.00
2500	0.41	330.80	25.00

Reservoir Content Page

Use the Reservoir Content page (see Figure 51) to enter descriptions for the contents of the four 16 mL reservoir vials located on the right side of the tray compartment and the wash solvent bottle located in the solvent platform at the top of the LC system stack. You can type up to 80 characters in each box.

Figure 50 shows the relative size of the 1 liter wash bottle and the 16 mL reservoir vials.

Figure 50. Reservoir vials and wash solvent bottle

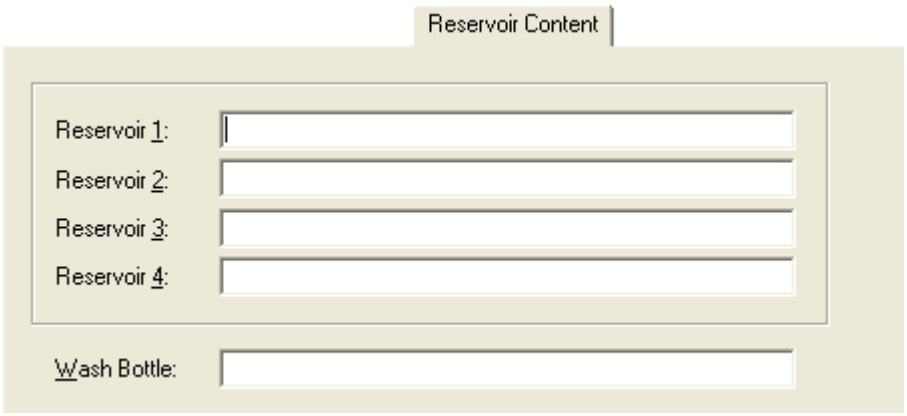


❖ **To specify the solvents in the wash bottle and reservoir vials**

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

   The Reservoir Content page appears (see Figure 51).

   Figure 51. Reservoir Content page for the autosampler



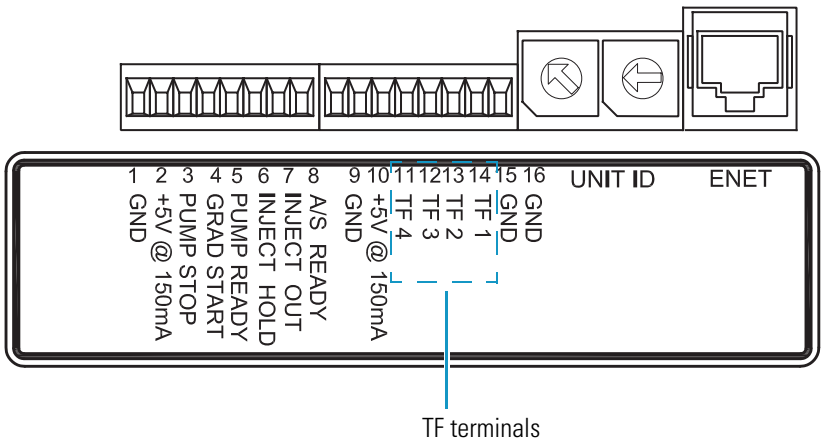
2. Identify the solvents contained in the 16 mL reservoir vials and the 1 L wash bottle by typing their names in their respective boxes.

Timed Events Page for the Autosampler

You can use the TF terminals (see Figure 52) on the back panel of the autosampler to control peripheral devices that the data system does not control.

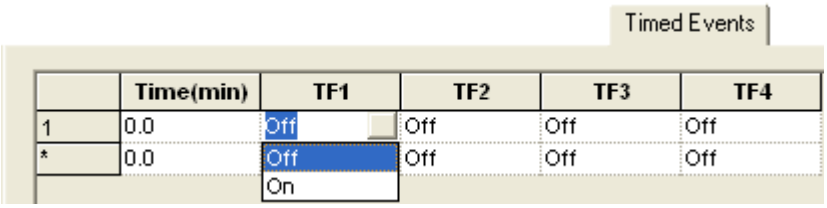
The TF terminal output signal is LO (Closed) by default. To change the polarity of the TF output signal to HI (Open), select the **Timed Events Active High** check box on the Signal Polarity page of the Accela Autosampler Configuration dialog box (see "Signal Polarity Page" on page 54).

Figure 52. Time function event terminals on the back panel of the autosampler



Use the Timed Events page (see Figure 53) to specify the timed events for the time function terminals (TF1 to TF4).

Figure 53. Timed Events page for the autosampler



❖ **To control external devices (not controlled by your Thermo Scientific MS application)**

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

   The Timed Events page appears (see Figure 53).

2. Specify the appropriate timed events in the table.

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

Autosampler Timed Events Page Parameters

Table 20 describes the columns in the timed events table for the autosampler.

Table 20. Autosampler timed events table

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

PDA Detector Instrument Method Parameters

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

When you open the view for the Accela PDA Detector, the following occurs:

- If you have the Accela PDA Detector (80 Hz version), the Accela PDA Setup view appears with the Accela PDA Method page displayed. The diode array scan rate that you specified when you configured the PDA detector appears in the upper-left corner of the Accela PDA Method page.

  Use the Accela PDA Method page to specify the data acquisition parameters for the Accela PDA Detector as part of an instrument method.

- If you have the Accela PDA Detector (20 Hz version) and you accepted the default diode array configuration option of 40 Hz or selected the 80 Hz option when you specified the configuration options for the PDA detector, an error message appears. Close the Xcalibur data system, open the Thermo Foundation Instrument Configuration application, and change the configuration setting for the diode array scan rate to 20 Hz.

Tip The model version is listed on the back panel of the PDA detector.

IMPORTANT In the instrument method, the available sample rates for the spectral scans and the discrete wavelengths depend on the configuration setting for the diode array scan rate.

Methods that specify an 80 Hz sample rate are incompatible with the 20 and 40 Hz diode array scan rate, and methods that specify a 40 Hz sample rate are incompatible with the 20 Hz diode array scan rate.

The acquisition server for the data system validates the instrument method when you start a sequence run. If the method contains a sample rate that exceeds the diode array scan rate, the sequence pauses and the following message appears: Scan rate exceeds current configuration, method invalid.

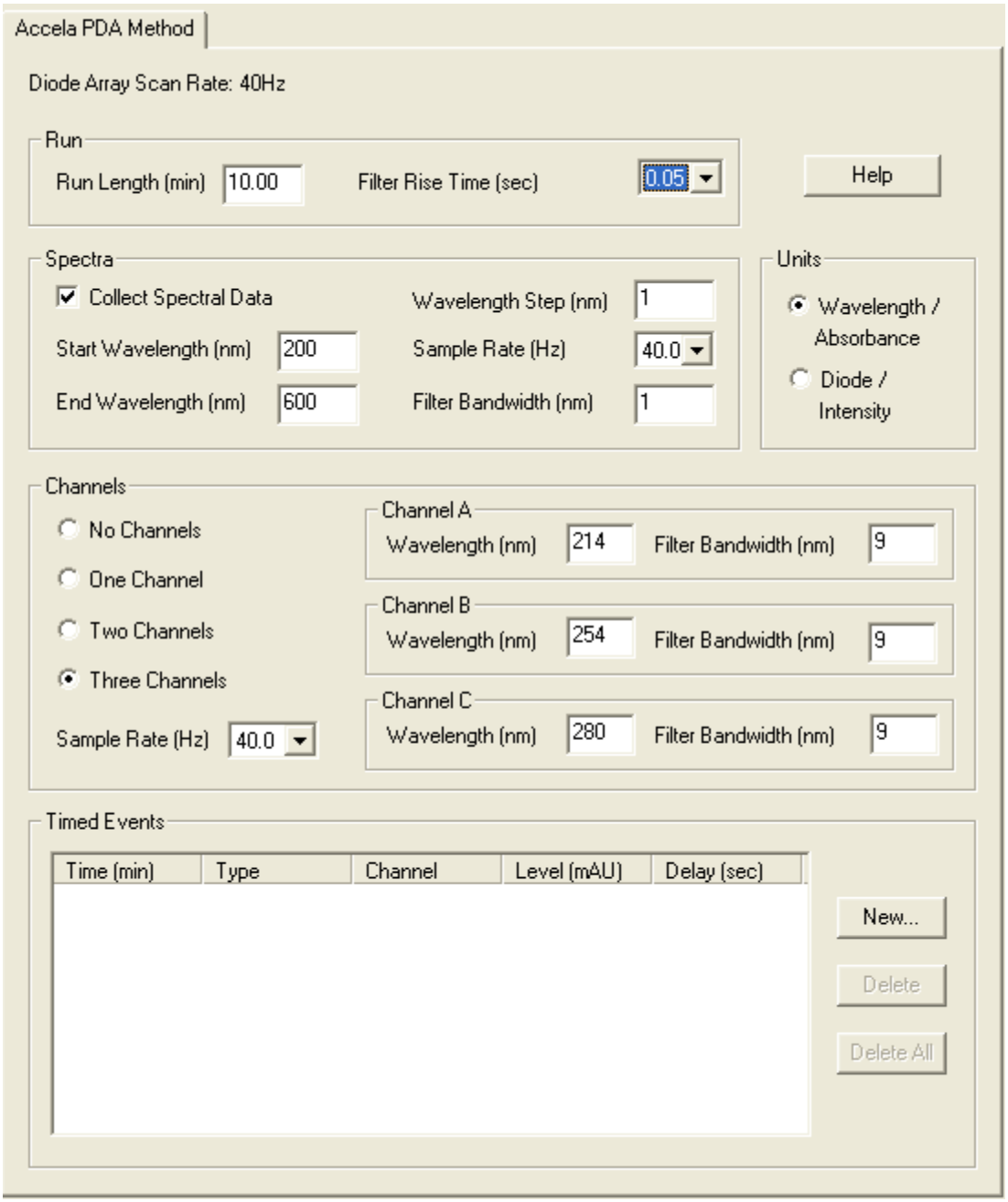
❖ To program the PDA detector to acquire absorbance data

1. In the view bar, click the **Accela PDA** icon.

   The view for the Accela PDA Detector appears with the Accela PDA Method page displayed (see Figure 54).

   The configuration setting for the diode array scan rate is displayed at the top of the page.

Figure 54. Accela PDA Method page with the default settings for Wavelength/Absorbance

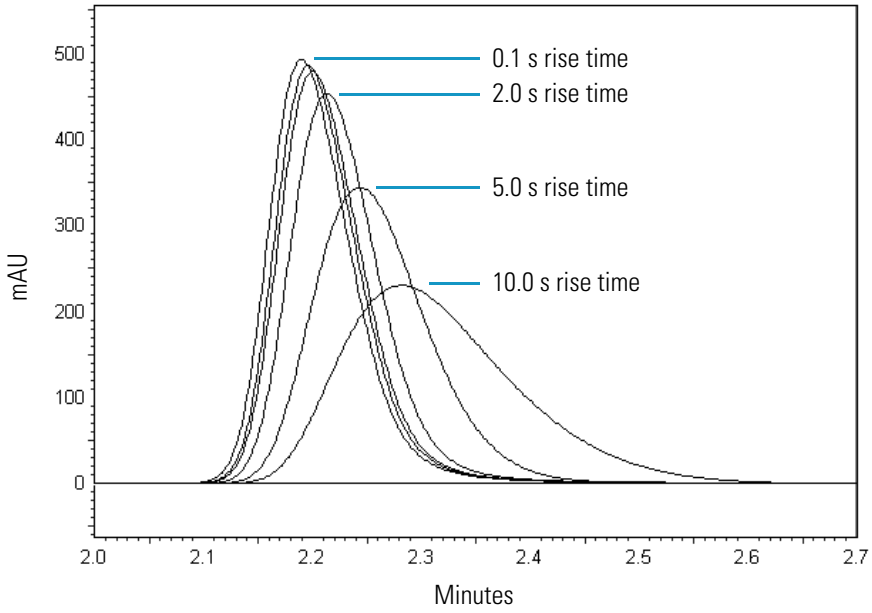


2. In the Run Length (min) box, type a run time from **0** to **600** minutes.
3. In the Filter Rise Time (sec) list, select a rise time from **0** to **10.0** seconds.

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

Figure 55 shows the effect of rise time on peak tailing.

Figure 55. Effect of rise time on peak tailing



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

Note For information about using the Diode/Intensity option to perform diagnostics, see "Creating a Display Method" on page 259. Methods that record light intensities for the PDA detector have the .spda file extension.

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

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

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

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

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

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

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

      Increasing the sampling rate increases the data file size.

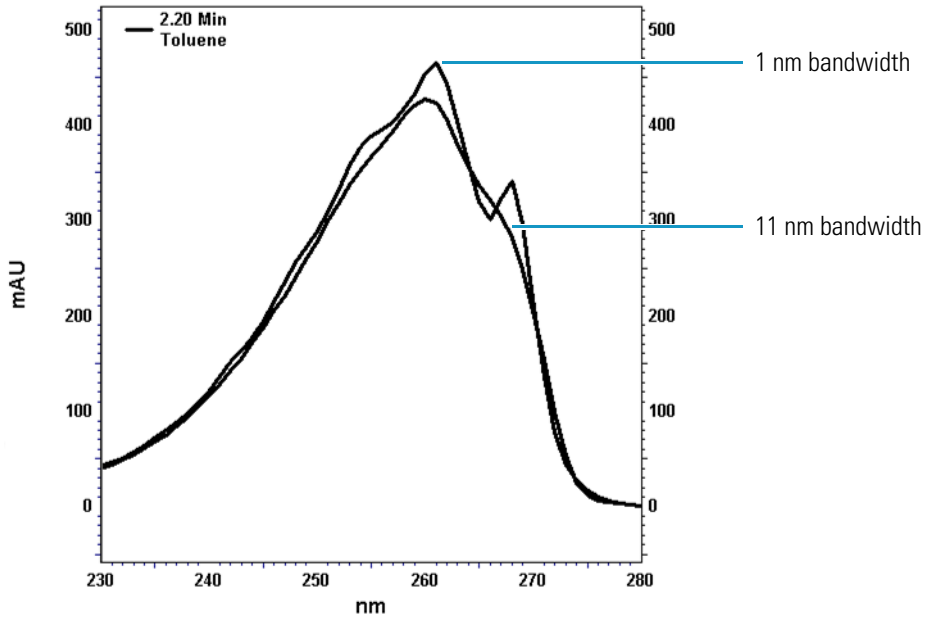
e. In the Filter Bandwidth (nm) box, type a bandwidth.

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

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

Increasing the bandwidth decreases the spectral resolution (see Figure 56).

Figure 56. Spectra of toluene, effect of bandwidth on spectral resolution



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

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

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

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

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

- PDA Detector Instrument Method Parameters
- Display Method Parameters
- Timed Events Table for the PDA Detector

For information about adding timed events to trigger external devices, see "Triggering an External Device with the PDA Detector" on page 105.

PDA Detector Instrument Method Parameters

Table 21 describes the parameters for an instrument method. These parameters are available when you select the Wavelength/Absorbance option in the Units area.

Table 21. PDA detector Method page parameters for an instrument method (Sheet 1 of 4)

Parameter	Description
Run	
Run Length	Specifies the time in minutes that the PDA detector acquires data. Default: 10.00 minutes Range: 0.00 to 600.00 minutes At the end of the run time, the PDA detector goes to the Ready for Download state.
Filter Rise Time	Specifies the detector's response time in seconds to the signal. The selections are 0.02, 0.05, 0.10, 0.20, 0.50, 1.00, 2.00, 5.00, and 10.00 seconds. The default rise time setting depends on the configured Diode Array Scan Rate:

Diode Array Scan Rate	Default Filter Rise Time
80 Hz	0.02 seconds
40 Hz	0.05 seconds
20 Hz	0.10 seconds

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

Table 21. PDA detector Method page parameters for an instrument method (Sheet 2 of 4)

Parameter	Description
Units	
Wavelength/ Absorbance	Displays absorbance spectra with the wavelength in nanometers on the *x* axis and the absorbance in milli-absorbance units (mAU) on the *x* axis. Default: Wavelength/Absorbance. Select the Wavelength/Absorbance option for instrument methods.
Spectra (with Wavelength/Absorbance option)	
Collect Spectral Data	To collect scans having a specific wavelength range (nm), bandwidth (nm), scan rate (Hz), and step (nm), select this check box.
Start Wavelength (nm)	Specifies the first wavelength in nanometers of the scan range. Default: 200 nm Range: 190 to 799 nm
End Wavelength (nm)	Specifies the last wavelength in nanometers of the scan range. Default: 600 nm Range: 191 to 800 nm The end wavelength must be greater than the start wavelength.
Wavelength Step	Specifies the wavelength interval between data points across the wavelength range. Default: 1 nm The maximum step size is the full width of the scan. The highest spectral resolution is 1 nm and the lowest resolution is the full width of the scan. When you specify a spectral scan from 200 to 300 nm at a step of 10 nm, the PDA detector sends 11 scan wavelengths to the data system computer.

Table 21. PDA detector Method page parameters for an instrument method (Sheet 3 of 4)

Parameter	Description
Sample Rate	Specifies the number of data points per second that the PDA detector sends to the data system for each scan wavelength. For optimal integration of the chromatographic peaks, acquire 20 points across the baseline width of the peak.When the sample rate is less than the diode array scan rate, the PDA detector averages consecutive data points. A sample rate equal to the diode array scan rate means that the PDA detector does not filter the data points that it acquires as it scans the diode array. The default sample rate is equal to the configured diode array scan rate. The sample rate selections depend on the diode array scan rate:

Diode array scan rate	Sample rates (Hz)
20 Hz	0.5, 1.0, 2.0, 4.0, 5.0, 10.0, and 20.0 Hz
40 Hz	0.5, 1.0, 2.0, 4.0, 5.0, 10.0, 20.0, and 40.0 Hz
80 Hz	0.5, 1.0, 2.0, 4.0, 5.0, 10.0, 20.0, 40.0, and 80.0 Hz

Filter Bandwidth	Specifies the bandwidth for the scan wavelengths. Bandwidth values are limited to the subset of odd integers from 1 to 49. In addition, the maximum scan range for a particular bandwidth is limited as follows: Lower range = 190 nm + (bandwidth – 1) / 2 Upper range = 800 nm – (bandwidth – 1) / 2 The maximum scan range for a bandwidth of 49 nm is 214 to 776 nm. **Note** Wider bandwidths decrease both spectral noise and the resolution of the spectra. In general, the detector bandwidth should not exceed 10 percent of the bandwidth at half-height of the narrowest spectral feature of interest.
Channels (with Wavelength/ Absorbance Units)	
No Channels	Specifies that no discrete channel measurements are to be acquired. When you select this option, the parameters in the Channels area are unavailable.
One Channel	Specifies the acquisition of one discrete channel. The data for Channel A is displayed in the bottom view of the Display page in black.
Two Channels	Specifies the acquisition of two discrete channels. The data for Channel B is displayed in the bottom view of the Display page in red.
Three Channels	Specifies the acquisition of three discrete channels. The data for Channel C is displayed in the bottom view of the Display page in green.
Sample Rate	Specifies the sample rate in Hz for all of the discrete channels selected (see sample rate in the Spectra area).

Table 21. PDA detector Method page parameters for an instrument method (Sheet 4 of 4)

Parameter	Description
[Ch. A, B, C] Wavelength	Specifies the discrete channel wavelength to be acquired. Channel A default: 214 Channel B default: 254 Channel C default: 280 Range: 190 to 800 nm
[Ch. A, B, C] Filter Bandwidth	Specifies the bandwidth for the corresponding discrete channel. Bandwidth values are limited to the subset of odd integers from 1 to 49. Default: 9 nm

Display Method Parameters

Table 22 describes the parameters for a display method. These parameters are available when you select the Diode/Intensity option in the Units area.

Table 22. PDA Method page parameters for a display method (Sheet 1 of 2)

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

Table 22. PDA Method page parameters for a display method (Sheet 2 of 2)

Parameter	Description
End Diode (diode num)	Specifies the last diode in the scan range. Default: 511 Range: 3 to 511, in increments of 1 Diode 511 corresponds to 800 nm.
Diode Step	Specifies the interval between data points across the range of diode numbers. The selections are 1, 2, 4, 5, or 10 diodes.
Sample Rate	Not applicable
Channels (with Intensity Units)	
No Channels	Specifies that no discrete channel measurements are to be acquired. When you select this option, the parameters in the Channels area are unavailable.
One Channel	Specifies the acquisition of one discrete channel. The data for Channel A is displayed in the bottom view of the Display page in black.
Two Channels	Specifies the acquisition of two discrete channels. The data for Channel B is displayed in the bottom view of the Display page in red.
Three Channels	Specifies the acquisition of three discrete channels. The data for Channel C is displayed in the bottom view of the Display page in green.
Sample Rate	Not applicable
[Ch. A, B, C] Diode Number	Specifies the diode that you want the detector to monitor. Channel A default: 40 Channel B default: 55 Channel C default: 450 Range: 2 to 511, in increments of 1

Timed Events Table for the PDA Detector

Table 23 describes the timed events area at the bottom of the Accela PDA Method page.

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

For information about connecting an external device to the PDA detector, refer to the *Accela PDA Detector Hardware Manual*.

Table 23. Timed events table on the Accela PDA Method page (Sheet 1 of 2)

Parameter	Description
Timed Events	
Use this area to add, modify, and remove timed events. You can specify the time when the event occurs, the event type, the channel, the absorbance level (mAU), and the delay settings. Click New to open the Timed Events dialog box.	
Timed Events table	This table lists all the events in chronological order. You can add, modify, and remove events by double-clicking the event setting you want to modify, or by using the New, Delete, and Delete All buttons.
Time	Specifies the time in minutes when the detector triggers the event. The maximum time is the data acquisition time specified in the Run Length box (see Figure 54 on page 96). Default: 0.00 minutes
Type	Specifies an event type: Event Off, Event On, Zero Data, or Level Trigger. Use a Level Trigger event to specify the absorbance threshold level for triggering an event. When you select a Level type event, you must specify the channel of the event, the absorbance level that is to trigger the event, and the delay time that the event is to occur after the absorbance level trigger event. Use a Zero event to zero the absorbance from the detector.
Channel	**Note** This list is available if you select Level Trigger in the [Event] Type list. Specifies the discrete wavelength channel (Channel A, Channel B, or Channel C) that the PDA detector monitors to activate the external event.
Level	**Note** This box is available if you select Level Trigger in the [Event] Type list. Specifies the absorbance level that triggers the external event. Range: –2000 to 4000 milli-absorbance units (mAU)
Delay	**Note** This box is available if you select Level Trigger in the [Event] Type list. Specifies the delay time in seconds that is to occur between the activation of the event (by the preset absorbance level being exceeded) and the actual change of the event output state (at the back panel terminals of the PDA detector). This delay compensates for the time interval between, for example, when a compound leaves the detector and when it reaches a fraction collector. Default: 0 seconds Range: 0 to 65 535, in increments of 1
Buttons	
New	Click this button to open the Timed Event dialog box where you can add external events to the external events table.

Table 23. Timed events table on the Accela PDA Method page (Sheet 2 of 2)

Parameter	Description
Delete	Click this button to remove an external event from the external events table. Select the external event that you want to remove, and then click Delete.
Delete All	Click this button to remove all external events from the external events table. To remove all events, click Delete All.

Triggering an External Device with the PDA Detector

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

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

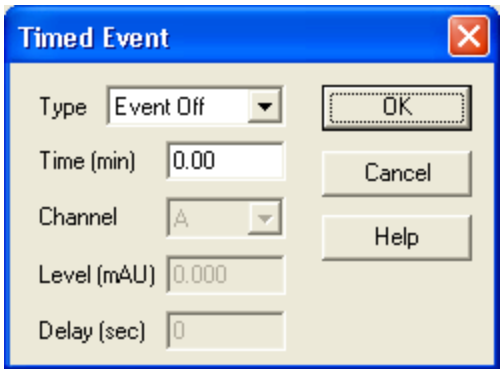
❖ To add a timed event to the instrument method

1. In the Timed Events area of the Accela PDA Method page (see Figure 57), click **New**.

Figure 57. Timed Events area of the Method page

The Timed Event dialog box appears (see Figure 58).

Figure 58. Timed Event dialog box



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

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

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

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

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

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

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

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

      The available selections are A, B, or C.

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

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

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

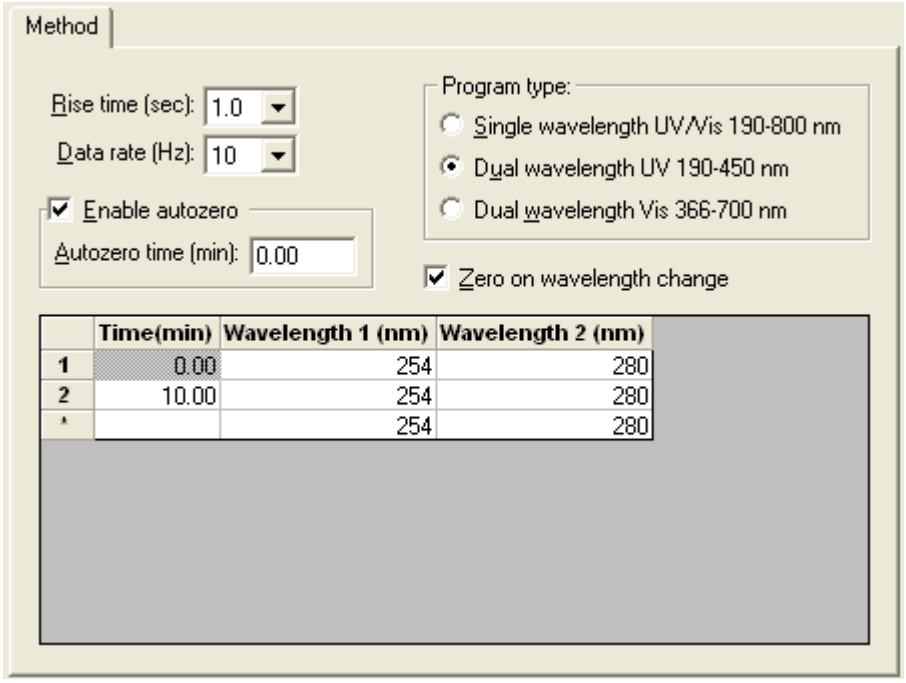
      The delay time compensates for the time interval between, for example, when a compound leaves the detector and when it reaches a fraction collector. The default value is 0 and the range is the set of integers from 0 to 65 535.

5. To zero the absorbance level for all the wavelength channels that the detector is monitoring at a particular time in the run, do the following:
   a. In the Type list, select **Zero Data**.
   b. In the Time box, type the time that you want the absorbance level to be zeroed.
6. Click **OK** to accept the new event and close the Timed Event dialog box.

UV-Vis Detector Instrument Method Parameters

In the UV/Vis detector view, use the Method page to set up the instrument method parameters for the UV/Vis detector.

Figure 59. Instrument Setup view for the UV/Vis detector



❖ To open the UV/Vis Detector view

Click the **Accela UV/Vis** icon on the view bar.

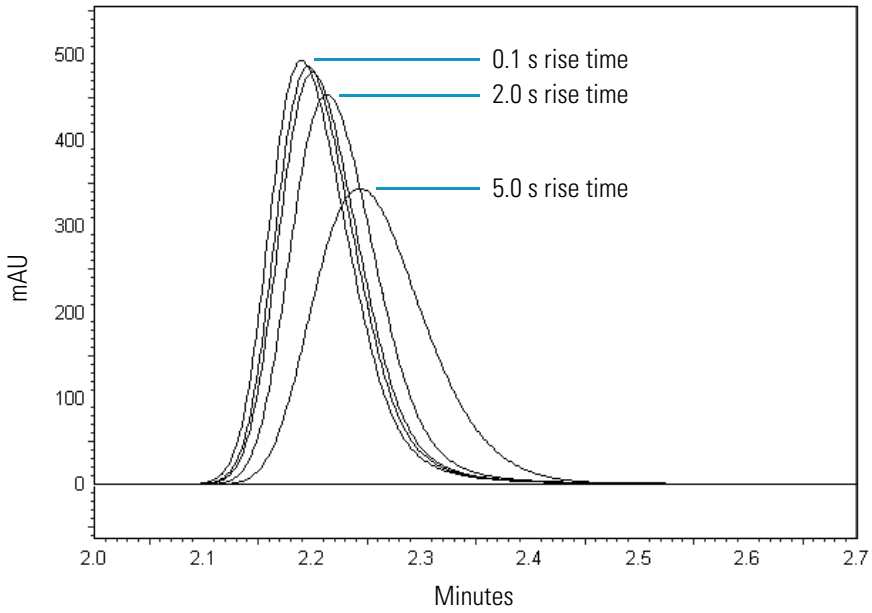
❖ To set up the instrument method parameters for the UV/Vis detector

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

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

   Figure 60 shows the effect of rise time on peak tailing.

Figure 60. Effect of rise time on band broadening



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

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

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

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

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

   - To collect one chromatogram during a run, select the **Single Wavelength 190 to 800 nm** option. You can program time wavelength changes in the program table.
   - To collect two chromatograms in the UV range during a run, select the **Dual Wavelength UV 190 to 450 nm** option. You can program time wavelength changes in the program table.
   - To collect two chromatograms in the visible range during a run, select the **Dual Wavelength Vis 366 to 700 nm** option. You can program time wavelength changes in the program table.

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

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

UV-Vis Detector Instrument Method Parameters

Table 24 describes the parameters on the Method page for the UV/Vis detector.

Table 24. UV/Vis detector method parameters (Sheet 1 of 2)

Parameter	Description
Rise Time	Specifies the filtering in the time domain. Default: 2.0 seconds Selections: 0.0, 0.01, 0.1, 0.2, 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 seconds
Data Rate	Specifies the number of points per second acquired per wavelength. Default: 10 Hz Selections: 20, 10, 6.67, 5, and 4 Hz
Enable Autozero	
Enable Autozero	When this check box is selected, the detector zeros the absorbance data at the specified time.
Autozero Time (min)	Specifies the time when the detector zeros the absorbance data. Default: 0 minutes Range: 0 minutes to the run length
Program Type	
Single Wavelength UV/Vis 190-800 nm	Specifies that the detector is to acquire data on one wavelength channel. The acquisition wavelength is time programmable.
Dual Wavelength UV 190-365 nm	Specifies that the detector is to acquire data on two wavelength channels in the UV range. The acquisition wavelengths are time programmable.
Dual Wavelength Vis 366-700 nm	Specifies that the detector is to acquire data on two wavelength channels in the visible range. The acquisition wavelengths are time programmable.
Other options	
Zero on Wavelength Change	When this check box is selected, the detector zeros the absorbance data at the times specified in the Time column for rows 2 through the last time line.
Programmable wavelength table	
Time	Specifies the time when the detector is to start monitoring the wavelength or wavelengths listed in the Wavelength 1 and Wavelength 2 columns.

Table 24. UV/Vis detector method parameters (Sheet 2 of 2)

Parameter	Description
Wavelength 1	Specifies the wavelength that the detector is to start monitoring at the time specified in the Time column. To view the chromatogram for this wavelength in Qual Browser, select Wavelength 1 in the Range list for the Chromatogram window.
Wavelength 2	Specifies the wavelength that the detector is to start monitoring at the time specified in the Time column. To view the chromatogram for this wavelength in Qual Browser, select Wavelength 2 in the Range list for the Chromatogram window.

Saving the Instrument Method

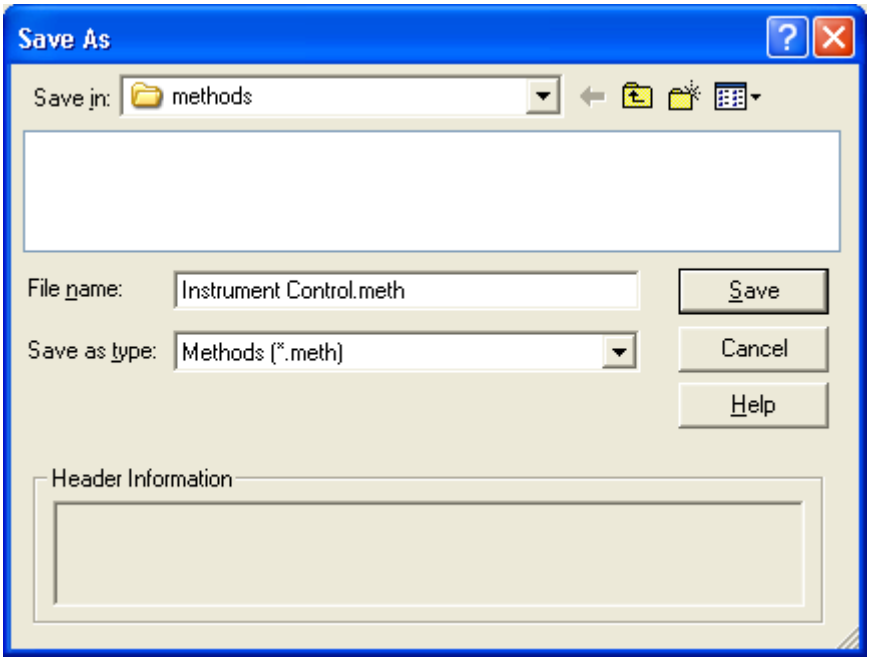
The following procedure applies only to the Xcalibur data system. For other Thermo Scientific mass spectrometry applications, refer to the Help.

❖ To save the instrument method

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

   The Save As dialog box appears (see Figure 61).

   Figure 61. Save As dialog box, showing the file extension for an instrument method



2. Browse to the following folder:

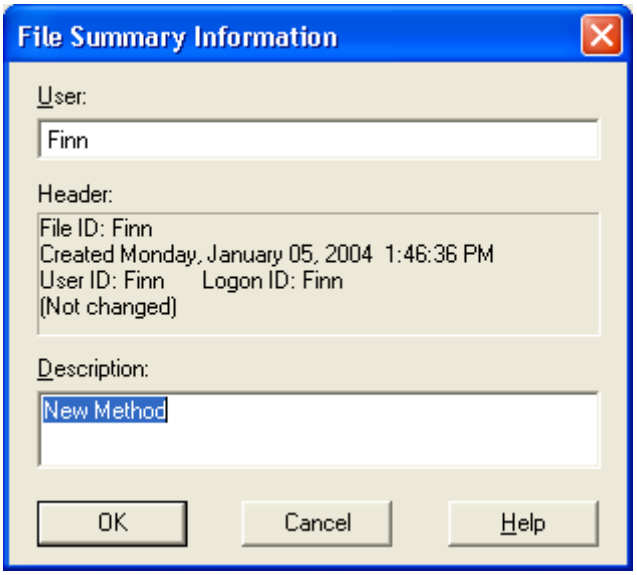
   drive:\Xcalibur\methods

3. In the File Name box, type a file name.

4. Click **Save**.

   The File Summary Information dialog box appears (see Figure 62).

Figure 62. File Summary Information dialog box

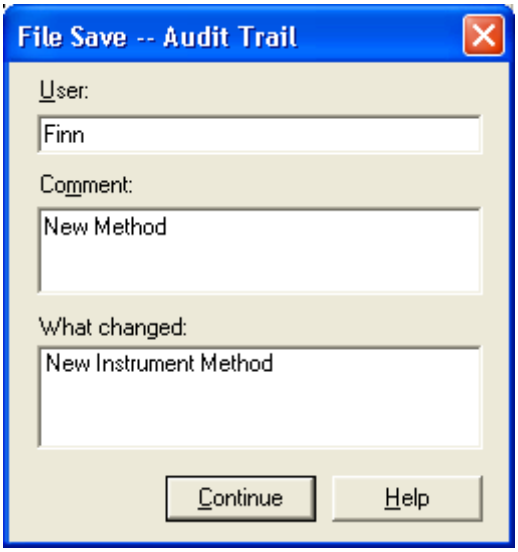


5. In the Description box, type a description of the method file.

6. Click **OK**.

   If the Authorization Manager – Comment check box is selected, the File Save – Audit Trail dialog box appears (see Figure 63).

Figure 63. File Save – Audit Trail dialog box



7. In the Comment box, type a comment concerning the changes you made to the instrument method.
8. Click **Continue** to close the File Save – Audit Trail dialog box and save the instrument method.

Sample Preparation Routines

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

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

Contents

Opening the Sample Preparation Page

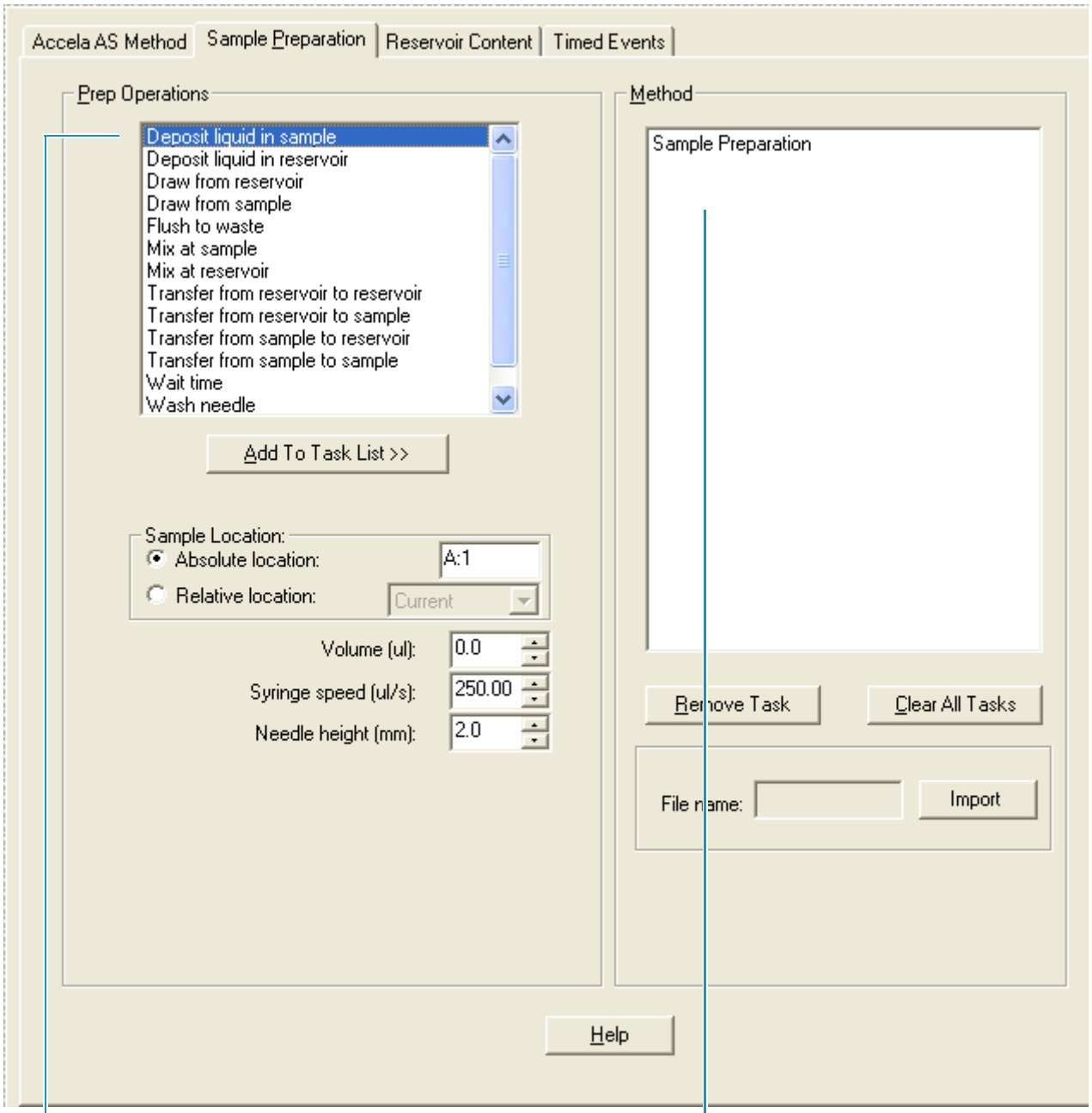
The Sample Preparation page is part of the instrument method.

❖ **To open the Sample Preparation page**

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

   The Sample Preparation page appears (see Figure 64). For a new method, in the Prep Operations list, the Deposit Liquid in Sample task is highlighted, and its parameters are listed below the Add To Task List button.

Figure 64. Sample Preparation page of the Accela Autosampler view



Sample Preparation Rules

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

Table 25 categorizes the sample preparation tasks into three groups based on whether the task uses the inner bore only, the outer bore only, or either bore of the dual-plunger concentric syringe. For information about the dual-plunger concentric syringe, see "Interchangeable Syringe" on page 11.

Table 25. Sample preparation tasks arranged in groups according to bore usage

Inner bore only	Outer bore only	Inner or outer bore
Draw from Sample	Mix at Sample	Draw from Reservoir
Transfer from Sample to Sample	Mix at Reservoir	Deposit Liquid in Sample
Transfer from Sample to Reservoir	Wash Needle	Deposit Sample in Reservoir
	Flush to Waste	Transfer from Reservoir to Sample
		Transfer from Reservoir to Reservoir

For the 250 µL concentric syringe, these rules restrict the arrangement of the tasks in the sample preparation list:

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

Building the Sample Preparation Routine

Sample preparation routines are part of the instrument method (see "Autosampler Instrument Method Parameters" on page 85). A sample preparation routine consists of a list of tasks performed in order by the autosampler before it makes an injection.

❖ To build a sample preparation routine

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

   –or–

   - To create a new sample preparation routine, go to step 3.
2. Import the sample preparation routine from a stored instrument method as follows:
   a. Click **Import**.

      The Open files dialog box appears.

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

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

      Table 26 lists the thirteen tasks that you can use to create a sample preparation routine. When you select a task, the parameters available for the task appear below the Add to Task List button.

      Tip The syringe position and nominal volume limit the task order and transfer volume:

      - 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. These tasks home the concentric syringe plungers.
      - If the previous task used the small bore of the syringe, the transfer volume of the current task is limited to the nominal syringe size or less. See "Sample Preparation Rules" on page 115 for more information.

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

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

      The task appears in the task list in the Method area.

4. Edit the task list as needed:
   - To remove the last task in the list, click **Remove Task**.
   - To remove a specific task, select the task, and then click **Remove Task**.
   - To clear the entire task list, click **Clear All Tasks**.

Tip To save an instrument method, the sample preparation routine must conform to the rules described in "Sample Preparation Rules" on page 115.

Sample Preparation Parameters

Table 26. Sample Preparation page (Sheet 1 of 5)

Parameter	Description
Prep Operations	
Use this area to select and add tasks to the task list in the Method area. You can add up to 512 tasks to a sample preparation routine; however, the arrangement of the tasks in the task list must follow a logical order or you will not be able to save the instrument method. For example, a draw task must precede a deposit task. In addition, if you are using the 250 µL concentric syringe that comes with the autosampler, you must follow an additional set of rules that allow for the proper positioning of the inner and outer plungers of the syringe. (See "Sample Preparation Rules" on page 115). If you are using the 2500 µL, single plunger syringe, to transfer volumes larger than 500 µL, add an optional tubing extension assembly to the needle tubing.	
Task List	**Description**
Deposit Liquid in Sample	Deposits liquid in a sample vial or well.
	Parameter / **Reference, range, or selections**
	Sample Location / "Sample Locations for the Sample Preparation Tasks" on page 122
	Volume / "Draw, Eject, and Transfer Volumes for Sample Preparation Tasks" on page 123
	Syringe Speed / "Syringe Speed for Tasks that Can Use Either Plunger" on page 125
	Needle Height / Default: 2 mm Range: 0 to 18 mm
Deposit Liquid in Reservoir	Deposit liquids in a reservoir vial.
	Parameter / **Reference, range, or selections**
	Volume / "Draw, Eject, and Transfer Volumes for Sample Preparation Tasks" on page 123
	Destination Reservoir / RV1, RV2, RV3, or RV4
	Syringe Speed / "Syringe Speed for Tasks that Can Use Either Plunger" on page 125

Table 26. Sample Preparation page (Sheet 2 of 5)

Parameter	Description	
Draw from Reservoir	Draws liquid from a reservoir vial or the wash bottle.	
	Parameter	**Reference, range, or selections**
	Volume	"Draw, Eject, and Transfer Volumes for Sample Preparation Tasks" on page 123
	Source Reservoir	RV1, RV2, RV3, RV4, or Bottle
	Syringe Speed	"Syringe Speed for Tasks that Can Use Either Plunger" on page 125
	Air Bubble	Range: 0 to 10 µL
Draw from Sample	Draws liquid from a vial or well.	
	Parameter	**Reference, range, or selections**
	Sample Location	"Sample Locations for the Sample Preparation Tasks" on page 122
	Volume	"Draw, Eject, and Transfer Volumes for Sample Preparation Tasks" on page 123
	Syringe Speed	"Syringe Speed for Tasks that Can Use Either Plunger" on page 125
	Needle Height	Default: 2 mm Range: 0 to 18 mm
	Air Bubble	Range: 0 to 10 µL
Flush to Waste	Flushes the needle tubing and homes the syringe. When the concentric syringe plungers are in the home position and the needle tubing is flushed, the autosampler can next perform any of the sample preparation tasks, with the exception of the deposit tasks.	
	Parameter	**Reference, range, or selections**
	Volume	Range: 100 to 6000 µL
	Reservoir	bottle, RV1, RV2, RV3, or RV4
	Syringe Speed	1.65 to 661.38 µL/sec for the concentric syringes 0.83 to 330.85 µL/sec for the 2500 µL standard syringe

Table 26. Sample Preparation page (Sheet 3 of 5)

Parameter	Description	
Mix at Sample	Mixes the contents of a vial or well. To mix a sample, the autosampler draws a specified volume of the sample into the needle tubing at a specified speed, and then ejects the sample back into the same location at a specified speed.	
	Parameter	**Reference, range, or selections**
	Sample Location	"Sample Locations for the Sample Preparation Tasks" on page 122
	Volume	The maximum volume is 490 µL for the concentric syringes and 1490 µL for the standard 2500 µL syringe.
	Draw Speed	The draw or delivery speed range is from 1.65 to 661.38 µL/s for the concentric syringes, and from 0.83 to 330.85 µL/s for the standard 2500 µL syringe.
	Delivery Speed	
	Cycles	Range: 1 to 10
	Needle Height	Default: 2 mm Range: 0 to 18 mm
Mix at Reservoir	Mixes the contents of a reservoir vial.	
	Parameter	**Reference, range, or selections**
	Volume	The maximum volume is 490 µL for the concentric syringes and 1490 µL for the standard 2500 µL syringe.
	Destination Reservoir	RV1, RV2, RV3, or RV4
	Draw Speed	The draw or delivery speed range is from 1.65 to 661.38 µL/s for the concentric syringes, and from 0.83 to 330.85 µL/s for the standard 2500 µL syringe.
	Delivery Speed	
	Cycles	Range: 1 to 10
	Needle Height	Default: 2 mm Range: 0 to 18 mm
Transfer from Reservoir to Reservoir	Transfers liquid from one reservoir to another.	
	Parameter	**Reference, range, or selections**
	Volume	"Draw, Eject, and Transfer Volumes for Sample Preparation Tasks" on page 123
	Source Reservoir	bottle, RV1, RV2, RV3, or RV4
	Destination Reservoir	RV1, RV2, RV3, or RV4
	Syringe Speed	"Syringe Speed for Tasks that Can Use Either Plunger" on page 125

Table 26. Sample Preparation page (Sheet 4 of 5)

Parameter	Description	
Transfer from Reservoir to Sample	Transfers liquid from a reservoir vial or the wash bottle to a vial or well.	
	Parameter	**Reference, range, or selections**
	Sample Location	"Sample Locations for the Sample Preparation Tasks" on page 122
	Volume	"Draw, Eject, and Transfer Volumes for Sample Preparation Tasks" on page 123
	Syringe Speed	"Syringe Speed for Tasks that Can Use Either Plunger" on page 125
	Needle Height	Default: 2 mm Range: 0 to 18 mm
	Source Reservoir	bottle, RV1, RV2, RV3, or RV4
Transfer from Sample to Reservoir	Transfers liquid from a vial or well to a reservoir vial.	
	Parameter	**Reference, range, or selections**
	Sample Location	"Sample Locations for the Sample Preparation Tasks" on page 122
	Volume	"Draw, Eject, and Transfer Volumes for Sample Preparation Tasks" on page 123
	Destination Reservoir	RV1, RV2, RV3, or RV4
	Syringe Speed	"Syringe Speed for Tasks that Can Use Either Plunger" on page 125
	Needle Height	Default: 2 mm Range: 0 to 18 mm
	Air Bubble	0 to 10 µL
Transfer from Sample to Sample	Transfers liquid from one vial or well to another vial or well.	
	Parameter	**Reference, range, or selections**
	Source Sample	"Sample Locations for the Sample Preparation Tasks" on page 122
	Destination Sample	"Sample Locations for the Sample Preparation Tasks" on page 122
	Volume	"Draw, Eject, and Transfer Volumes for Sample Preparation Tasks" on page 123
	Syringe Speed	"Syringe Speed for Tasks that Can Use Either Plunger" on page 125
	Source Vial Needle Height	Default: 2 mm Range: 0 to 18 mm
	Air Bubble	0 to 10 µL
	Destination Vial Needle Height	Default: 2 mm Range: 0 to 18 mm

Table 26. Sample Preparation page (Sheet 5 of 5)

Parameter	Description	
Wait Time	Pauses the sample preparation routine for a period of time, for example, for a reaction to occur. Range: 0 to 1000 minutes	
Wash Needle	Washes the exterior of the needle with the solvent in a reservoir vial or the wash bottle.	
	Parameter	**Reference, range, or selections**
	Volume	Range: 100 to 6000 µL
	Reservoir	bottle, RV1, RV2, RV3, or RV4
Button		
Add To Task List	Use the Add To Task List button to add a task to the sample preparation routine.	
Method		
Use the Method area to display the parameters of a task, remove a task, clear all tasks in the task list, or import the sample preparation routine from a stored instrument method. The Method area consists of the (sample preparation) task list, the Remove Task button, the Clear All Tasks button, and the File Name (read only) box and Import (method) button.		
File Name box	Displays the name of the selected instrument method that contains the sample preparation routine that you want to import into the current instrument method.	
Buttons		
Remove Task	Use this button to remove a task from the task list in the Method area.	
Clear All Tasks	Use this button to clear all tasks from the task list in the Method area.	
Import	Use this button to open the Open dialog box where you can select an instrument method with the sample preparation routine that you want to add to the current instrument method. After you select an instrument method and click Import, the File Name (read only) box displays the file name of the instrument method you want to import.	

Sample Locations for the Sample Preparation Tasks

For the sample preparation tasks, you can select an absolute or a relative location.

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

For the Absolute Location option, you specify an absolute vial or well location. The vial or well locations depend on the tray configuration (see Table 27).

Tip Remember to place a vial at the selected location.

Table 27. Vial and well positions

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

Draw, Eject, and Transfer Volumes for Sample Preparation Tasks

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

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

Table 28 lists the maximum volumes by task.

Table 28. Draw, eject, and transfer volumes for sample preparation tasks (Sheet 1 of 2)

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

Table 28. Draw, eject, and transfer volumes for sample preparation tasks (Sheet 2 of 2)

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

Syringe Speed Range for Sample Preparation Tasks

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

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

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

- Syringe Speed for Tasks that Can Use Either Plunger
- Syringe Speed for Tasks that Use the Inner Plunger

Syringe Speed for Tasks that Can Use Either Plunger

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

For the tasks listed in Table 29, the autosampler uses the following:

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

Table 29. Tasks that can use either syringe plunger

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

Table 30 lists the speed range for the sample preparation tasks that can use either plunger of the concentric syringe.

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

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

Table 30. Syringe speed ranges for tasks that can use either syringe plunger

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

Syringe Speed for Tasks that Use the Inner Plunger

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

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

The syringe size determines the default syringe speed and the syringe speed range (see Table 31).

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

Note You select the size of the syringe by using the Syringe Type list on the Communication page of the Accela Autosampler Configuration dialog box.

Table 31. Syringe speeds for tasks that use the inner plunger of the concentric syringe

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

Sample Preparation Routine Example

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

❖ To add a sample preparation routine that dilutes samples tenfold

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

   The Sample Preparation page appears.

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

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

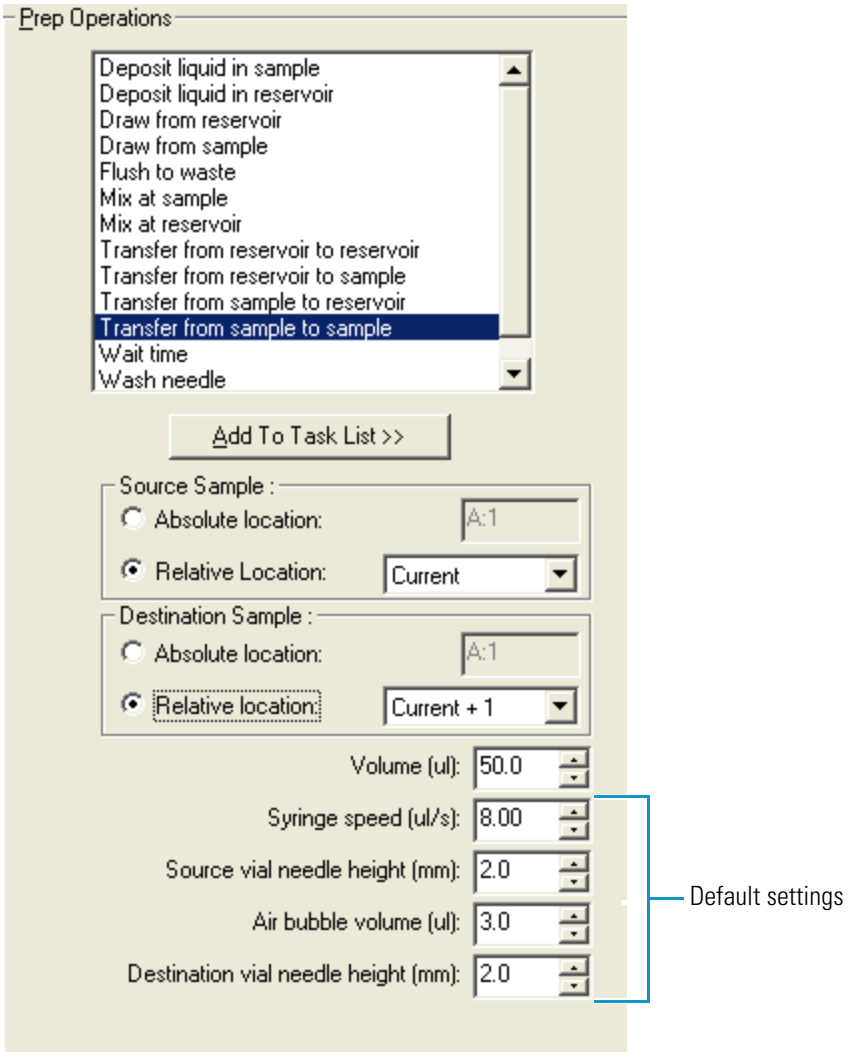
3. Add a task that transfers an aliquot of sample solution from the original sample location to another sample location:

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

   b. Keep all the default parameter settings for the Transfer from Sample to Sample task, except those that are shown below (see Figure 65).

Parameter	Setting	Autosampler action
Source Sample		
Location	◉ Relative Location	Withdraws sample from the current vial location listed in the sequence table.
Location	Current	
Destination Sample		
Location	◉ Relative Location	Deposits sample in the current + 1 vial location listed in the sequence table.
Location	Current + 1	
Volume	50	Transfers 50 µL of sample.

Figure 65. Settings for the Transfer from Sample to Sample task



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

When the autosampler performs this task, it withdraws 50 µL of sample from the current vial location in the sequence table and deposits it in the current + 1 vial location.

4. Add a task that transfers diluent (with the inner syringe plunger) from a reservoir vial to the sample location:

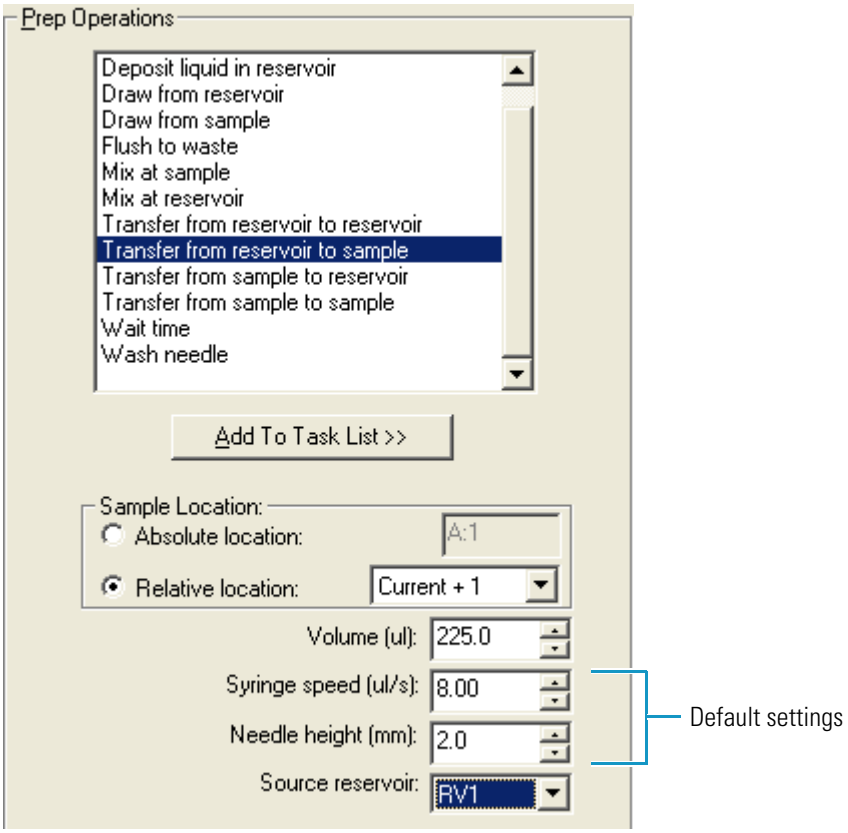
a. In the Prep Operations list, select **Transfer from Reservoir to Sample.** The parameters for the task appear below the Add To Task List button.

b. Keep all the default parameter settings for the Transfer from Reservoir to Sample task, except those that are shown below (see Figure 66).

Tip When accuracy is important, keep the transfer volume below the nominal syringe size, so that the autosampler uses the inner syringe plunger. Solvent transfers made with the inner plunger are more accurate than solvent transfers made with the outer plunger because the stepper motor for the inner plunger takes smaller steps and provides finer control than the stepper motor for the outer plunger.

Parameter	Setting	Autosampler action
Source reservoir	RV1	Draws solvent from reservoir vial 1.
Destination Sample		
Location	Relative Location	Deposits solvent in the current + 1 vial location listed in the sequence table.
Location	Current + 1	
Volume	225	Transfers 225 µL of solvent.

Figure 66. Settings for the Transfer from Reservoir to Sample task



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

When the autosampler performs this task, it uses the inner syringe plunger to withdraw 225 µL of diluent from the reservoir vial 1 and deposit the diluent in the current + 1 vial location. Performing this task twice transfers a total of 450 µL of diluent to the current + 1 vial location.

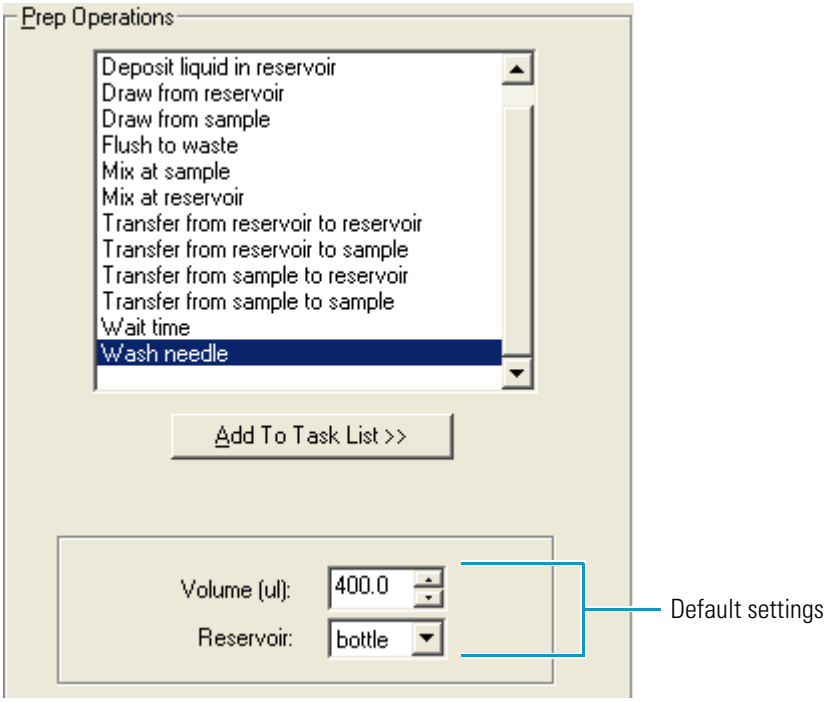
5. Add a task that homes the position of the concentric syringe plungers as follows:

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

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

b. Keep all the default parameter settings for the Wash Needle task (see Figure 67).

Figure 67. Default parameter settings for the Wash Needle task



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

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

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

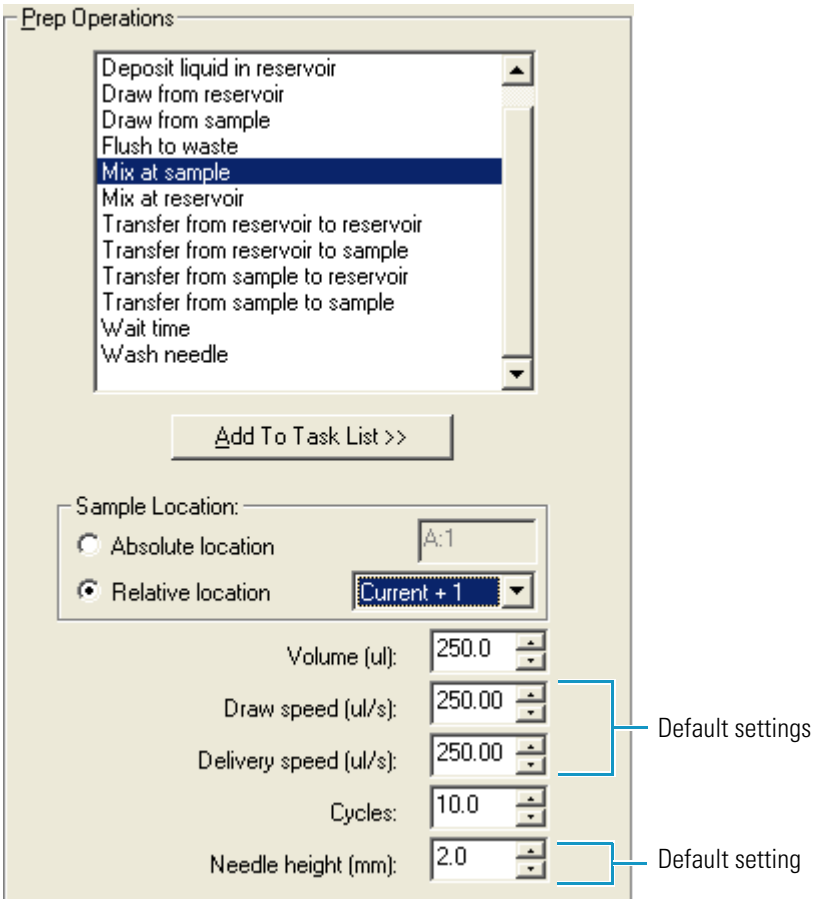
b. Keep all the default parameter settings for the Mix at Sample task, except those listed below (see Figure 68).

Parameter	Setting	Autosampler action
Sample Location		
Location	Relative Location	Aspirates and expunges 250 µL sample from the current + 1 vial location listed in the sequence table 10 times.
Location	Current + 1	
Volume	250	
Cycles	10	

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

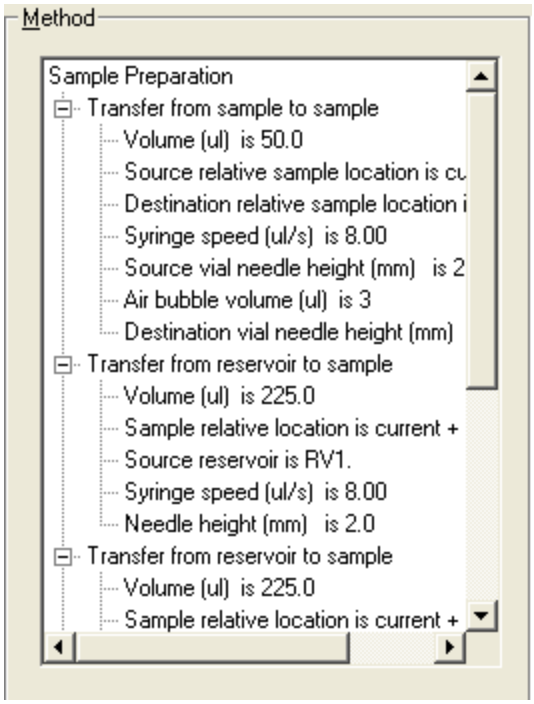
When the autosampler performs this task, it aspirates and expunges 250 µL of the sample solution in the current + 1 vial location 10 times.

Figure 68. Settings for the Mix at Sample task



7. Review the task list by expanding the tasks in the Method list (see Figure 69).

Figure 69. Method area with an expanded task list



8. Save the instrument method (see "Saving the Instrument Method" on page 110).

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

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

   If you want to inject the diluted samples, create a ten-line sequence that lists all the vial locations (A1 to A10). Ensure that you use the method that contains the sample preparation routine on the odd rows only (Rows 1, 3. 5. 7. and 9).

Daily Operation

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

Note The Accela 600 Pump and the Accela 1250 Pump do not have pulse dampeners.

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

To maintain optimal performance of the system, calibrate the PDA detector on a monthly basis or whenever you move the detector, and check the light throughput to the diode array whenever you change the configuration setting for the diode array scan rate (Accela PDA 80 Hz Detector), move the detector, observe an increase in detector noise, or replace the flowcell or lamps (see "Verifying the Performance of the PDA Detector" on page 259).

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

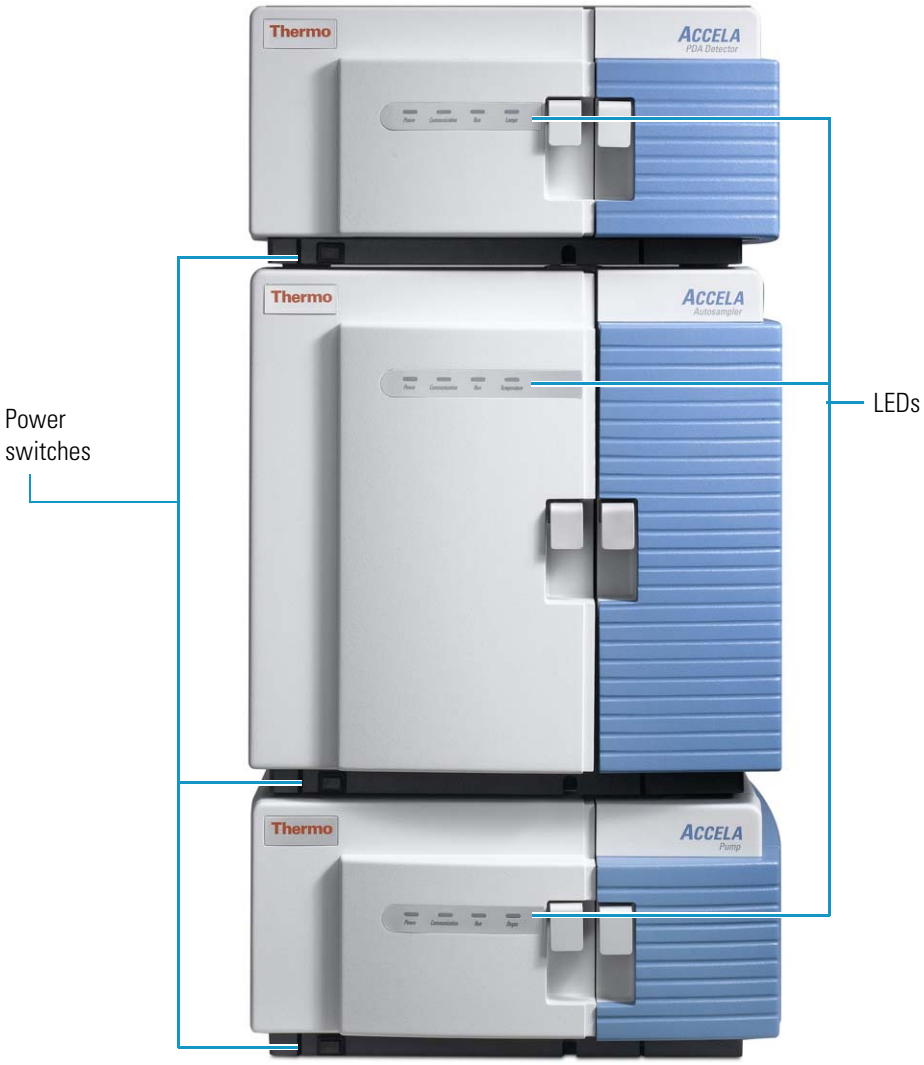
If your Accela 600 Pump or Accela 1250 has an optional seal wash pump, leak sensor, or both, set up the seal wash program and the leak sensor actions.

Contents

Turning On the Power to Each LC Device

The ON/OFF power switch for each device is located below the left door of the module. Figure 70 shows the location of the status LEDs and the power switches.

Figure 70. Accela LC stack



❖ To turn on the power to the LC devices

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

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

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

   - If any of the Power LEDs remain amber, make sure that the power line to the affected device or devices is connected.

     Note For the Accela PDA (80 Hz) Detector, the Power LED remains amber and the Comm, Lamp, and Run LEDs remain unlit until the data system establishes communication with the detector.

   - If the Degas LED on the pump is flashing amber, the degas unit has failed to produce a vacuum. Call your local Thermo Fisher Scientific representative for repairs.

   - If the Lamp LED remains amber, both of the lamps are turned off. If the lamps are off, turn them on when you check the detector's status from the data system.

Initiating Communication with the Data System

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

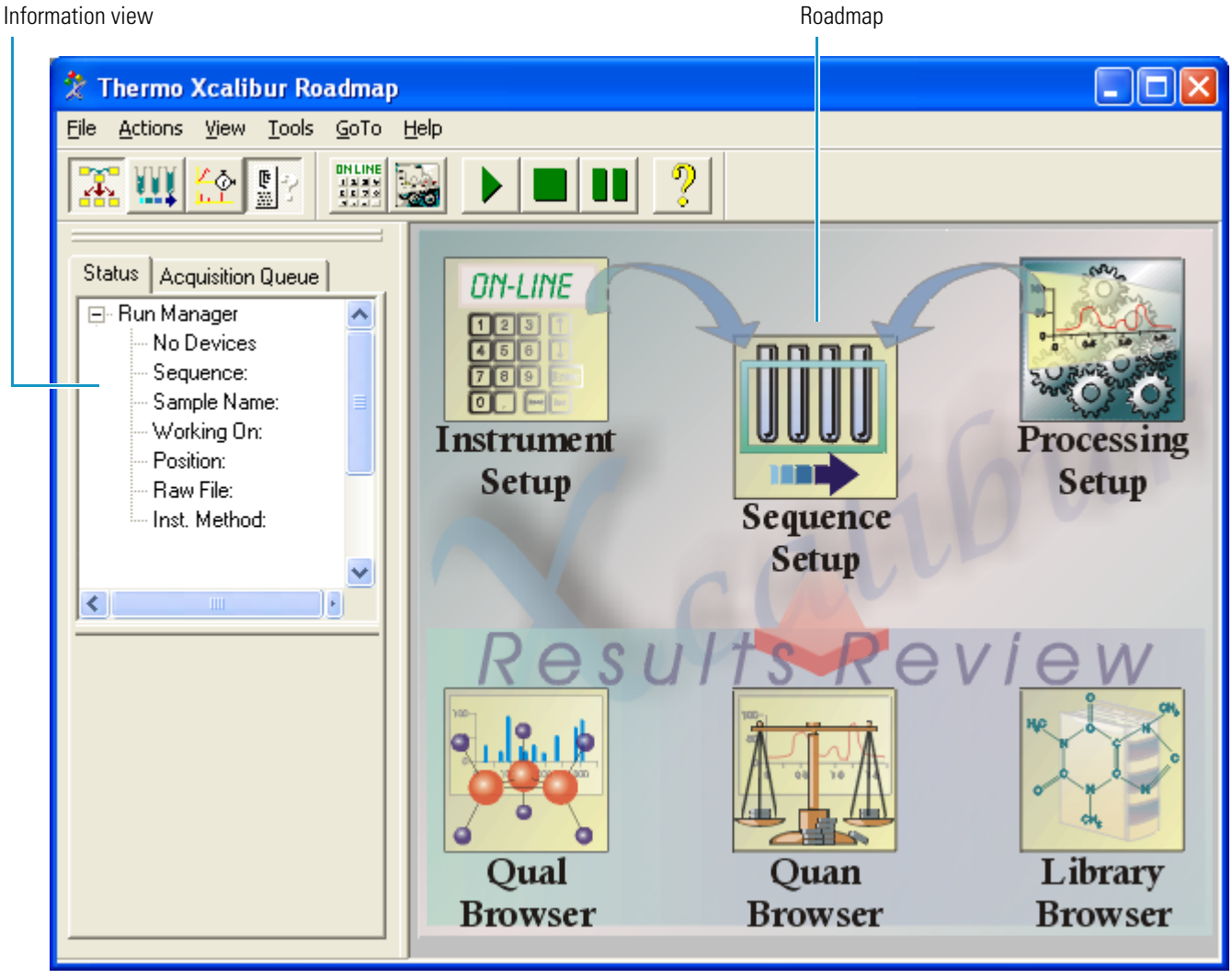
The following procedure describes how to verify instrument communications from the Thermo Xcalibur data system.

❖ To open the data system and verify instrument communications

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

   The Thermo Xcalibur Roadmap window appears (see Figure 71).

   Figure 71. Thermo Xcalibur Homepage window with the Roadmap and Info view displayed



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

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

   - Verify that the Ethernet communication cable for the detector and the autosampler are connected to the Ethernet switch and that the Ethernet switch is connected to the data system computer. Verify that the USB cable for the pump is connected to the data system computer.
   - Verify that the stack addresses in the configuration for the autosampler and the detector match the units IDs (rotary switch settings) on their back panels.

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

Checking the Status of the LC Devices

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

Tip You can also view the system pressure on the pump page of the Inlet Direct Control dialog box in the Tune window for your Thermo Scientific mass spectrometer.

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

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

Opening the Status Page of the Information View

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

❖ To display the Status page of the Information view

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

   - Choose **View > Info View**.

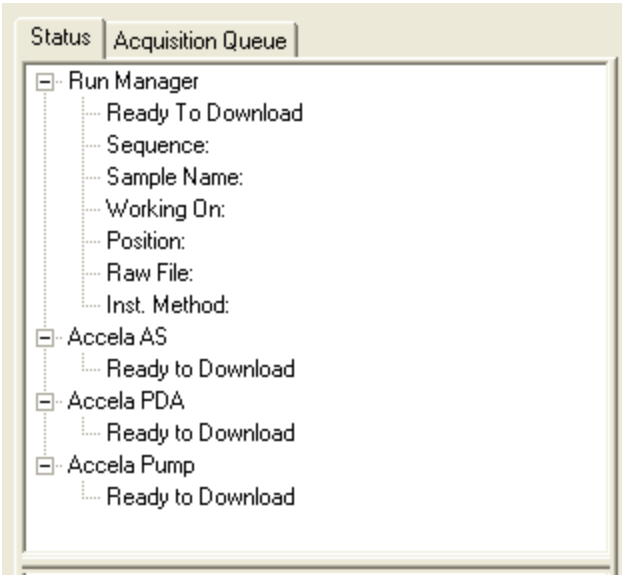
   –or–

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

2. Click the **Status** tab.

The Status page appears (see Figure 72).

Figure 72. Status page of the Information view



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

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

Viewing the Status of Each Device

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

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

These topics describe the status views for the LC devices:

- Autosampler Status View
- Accela Pump, Accela 600 Pump, or Accela 1250 Pump Status View
- Accela PDA Detector Status View
- Accela UV/Vis Detector Status View

Autosampler Status View

The Status view for the autosampler (see Figure 73) contains four pages:

- AS Status Page
- Oven Page
- Tray Page
- Error Log (Autosampler) Page

Figure 73. AS Status page in the Accela AS Status view

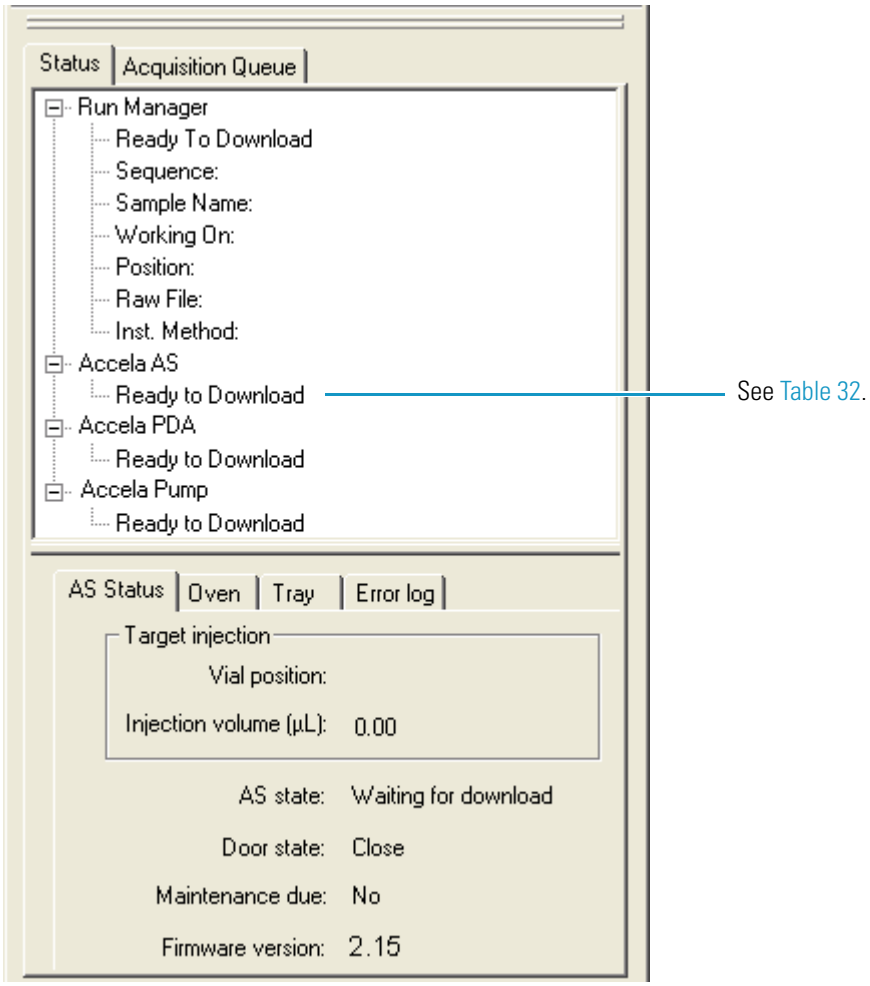


Table 32 describes the states that might appear below the Accela AS listing.

Table 32. Autosampler states (Sheet 1 of 2)

State	Meaning
Initializing	The data system is attempting to establish communication with the autosampler.
Ready to Download	The autosampler is ready to start a run.

Table 32. Autosampler states (Sheet 2 of 2)

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

AS Status Page

Table 33 describes the readbacks on the AS Status page of the autosampler status view.

Table 33. Status readbacks for the autosampler (Sheet 1 of 3)

Readback	Description
Target injection	
Vial Position	Displays the current vial or microplate well position. For information about the vial and well notation, see "Vial and Well Notation" on page 5.
Injection Volume	Displays the injection volume for the current injection.

Table 33. Status readbacks for the autosampler (Sheet 2 of 3)

Readback	Description	
Other		
AS State	Displays the following run conditions:	
	Waiting for Download	The autosampler is waiting for the data system to download an instrument method.
	Waiting for Door Close	The Verify Door Is Closed check box is selected (see "Communication Page" on page 50) and the tray compartment door is open.
	Waiting for Temperature	The Wait for Temperature Ready check box is selected (see "Communication Page" on page 50) and the tray compartment temperature, column oven temperature, or both have not yet reached the set temperature. This state remains until the temperature controlled areas are equilibrated to the set temperature.
	Injecting	The autosampler is making an injection.
	Waiting for Pump	The autosampler has loaded the sample loop and is waiting for the Inject Hold Release signal from the pump.
	Ready	The autosampler is ready to make an injection.
	Error	An error condition other than the loss of communication has occurred. For example, if you enable the maintenance log and you attempt to start a run when one or more of the maintenance counters has exceeded its limit, an error condition occurs.
Door State	Displays one of the following:	
	Open	The Verify Door Is Closed check box on the Communication page of the Accela Autosampler Configuration dialog box in the Instrument Communication window is selected and the tray compartment door is open. For information about selecting the Verify Door Is Closed check box, see "Communication Page" on page 50.
	Closed	The Door State displays Closed for these conditions: • The Verify Door Is Closed Check box is selected and the tray compartment door is closed. • The Verify Door Is Closed check box is clear and the door is open or closed.

Table 33. Status readbacks for the autosampler (Sheet 3 of 3)

Readback	Description	
Maintenance Due	Displays Yes or No.	
	No	The Enable Maintenance Log check box is clear, none of the maintenance counters has exceeded its limit, or both.
	Yes	The Enable Maintenance Log check box is selected and one or more of the maintenance counters has exceeded its limit. The autosampler cannot start a run until you zero the counter or clear the Enable Maintenance Log check box. For information about setting up the maintenance counters, see "Autosampler Maintenance Information" on page 325. For information about clearing the Enable Maintenance Log check box, see "Communication Page" on page 50.
Firmware Version	Displays the firmware version of the autosampler.	

Oven Page

Figure 74 shows the Oven page of the status view for the autosampler.

Figure 74. Oven page

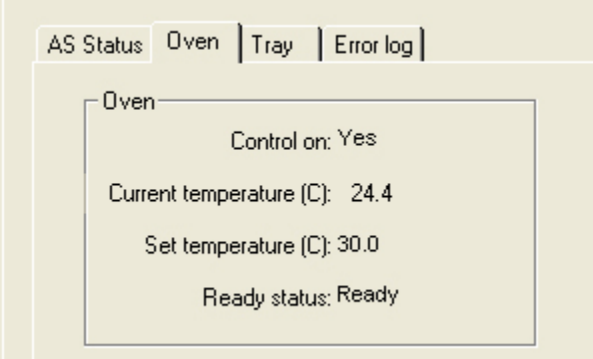


Table 34 describes the readbacks on the Oven page of the autosampler status view.

Table 34. Oven page readbacks

Readback	Description	
Control On	Displays Yes or No. You can turn off the oven temperature control by doing one of the following: • Using the Turn Off Oven Temperature command (see "Controlling the Tray and Oven Compartment Temperatures" on page 198) • Running an instrument method that has the Enable Column Oven Control check box cleared	
Current Temperature (C)	Displays the temperature monitored by the autosampler's internal temperature sensor.	
Set Temperature (C)	Displays the user-specified temperature. You can change the set temperature by doing one of the following: • Using the Set Oven Temperature command (see "Controlling the Tray and Oven Compartment Temperatures" on page 198) • Running an instrument method that has a temperature setting for the column oven	
Ready Status	Displays one of the following:	
	Ready	The oven compartment temperature has reached the set temperature, you did not select the Wait for Temperature Ready check box when you specified the configuration settings for the autosampler, or both. For information about the Wait for Temperature Ready check box, see "Communication Page" on page 50.
	Not Ready	The oven compartment temperature has not reached the set temperature.

Tray Page

Figure 75 shows the Tray page of the autosampler status view.

Figure 75. Tray page

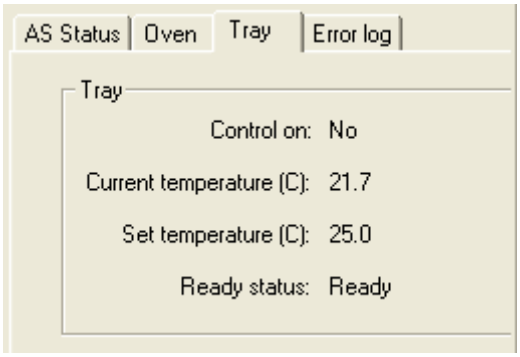


Table 35 describes the readbacks on the Tray page of the autosampler status view.

Table 35. Tray page parameters

Readback	Description	
Control On	Display Yes or No. You can turn off the tray temperature control by doing one of the following: • Using the Turn Off Tray Temperature command (see "Controlling the Tray and Oven Compartment Temperatures" on page 198) • Running an instrument method that has the Enable Column Oven Control check box cleared	
Current Temperature (C)	Displays the temperature monitored by the autosampler's internal temperature sensor.	
Set Temperature (C)	Displays the user-specified temperature. You can change the set temperature by doing one of the following: • Using the Set Tray Temperature command (see "Controlling the Tray and Oven Compartment Temperatures" on page 198) • Running an instrument method that has a temperature setting for the column oven	
Ready Status	Displays one of the following:	
	Ready	The oven compartment temperature has reached the set temperature, you did not select the Wait for Temperature Ready check box when you specified the configuration settings for the autosampler, or both. For information about the Wait for Temperature Ready check box, see "Communication Page" on page 50.
	Not Ready	The oven compartment temperature has not reached the set temperature.

Error Log (Autosampler) Page

Figure 76 shows the Error Log page for the autosampler.

Figure 76. Error Log page

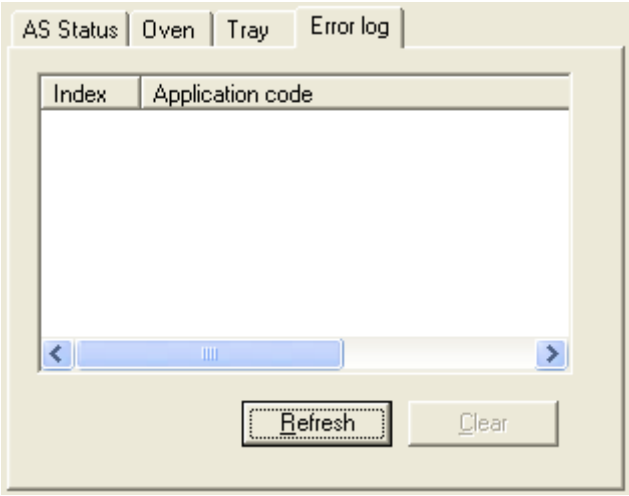


Table 36 describes the parameters on the Error Log page.

Table 36. Error Log page parameters

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

Accela Pump, Accela 600 Pump, or Accela 1250 Pump Status View

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

- General Page of the Pump Status View
- Extended Page of the Pump Status View
- Degasser Page of the Pump Status View

General Page of the Pump Status View

Figure 77 shows the General page of the pump status view.

Figure 77. General page pump status view

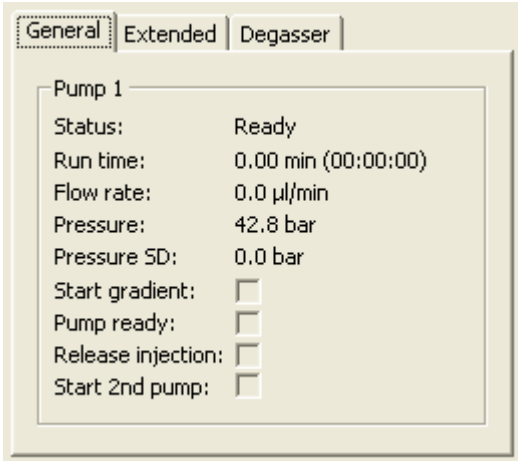


Table 37 describes the status readbacks on the General page of the pump status view.

Table 37. General page of the pump status view (Sheet 1 of 2)

Readback	Description	
Status	Displays the pump status as follows:	
	Ready	The pump is ready for a run.
	Running Isocratic	The solvent flow is on, but the pump is not running a gradient program.
	Homing	The pump pistons are moving toward the home position.
	Running Gradient	The pump is running the solvent conditions specified in the gradient program of an instrument method. The gradient program can contain isocratic or gradient solvent conditions.
	Low Pressure Error	The system pressure has fallen below the minimum pressure setting for more than one minute. If you are controlling the pump from the Direct Control dialog box, the status returns to Ready after the pump stops pumping. The error is posted to the pump's event log.
	High Pressure Error	The system pressure has risen above the maximum pressure setting.
	Note When a pressure error occurs, one of the following occurs: • If you are controlling the pump from the Direct Control dialog box, the status returns to Ready after the pump stops pumping. The error is posted to the pump's event log. • If you are controlling the pump from the Sequence Setup window, the Acquisition Server dialog box appears.	
Run Time	Displays the elapsed time since the last instrument method downloaded, you turned the device on from the Status page of the Information view, or you started the pump from the Direct Control dialog box.	
Flow Rate	Displays the user-specified flow rate. For an instrument method, the flow rate changes linearly between time lines.	
Pressure	Displays the pressure monitored by the pump.	
Pressure SD	Displays the standard deviation of the pressure monitored by the pump.	
Start Gradient	A check appears in this box when the pump receives the Start gradient signal from the autosampler.	
Pump Ready	A check appears in this box when the pressure monitored by the pump reaches the stability level specified in the instrument method.	

Table 37. General page of the pump status view (Sheet 2 of 2)

Readback	Description
Release Injection	A check appears in this box when the pump sends the Release Injection signal to the autosampler.
Start 2nd Pump	A check appears in this box when Pump 1 triggers Pump 2 to start its pump program.

Extended Page of the Pump Status View

Figure 78 shows the Extended page of the pump status view.

Figure 78. Extended page of the pump status view

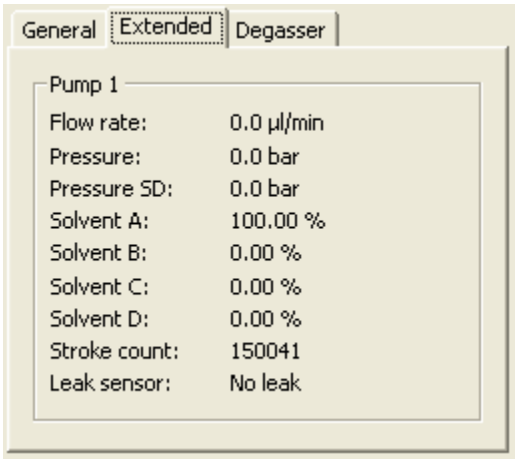


Table 38 describes the status readbacks on the Extended page of the pump status view.

Table 38. General page of the pump status view (Sheet 1 of 2)

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

Table 38. General page of the pump status view (Sheet 2 of 2)

Readback	Description
Leak Sensor	Displays the leak sensor status: No Leak or Leak Detected. The leak sensor is an option for the Accela 600 Pump and the Accela 1250 Pump. This readback appears when you select the Leak Sensor Installed check box in the instrument configuration for the pump (see "Pump Configuration Settings" on page 59). For information about restarting the solvent flow after the leak sensor detects a leak, see "Setting Up the Leak Sensor" on page 159.

Degasser Page of the Pump Status View

Figure 79 shows the Degasser page of the pump status view.

Figure 79. Degasser page of the pump status view

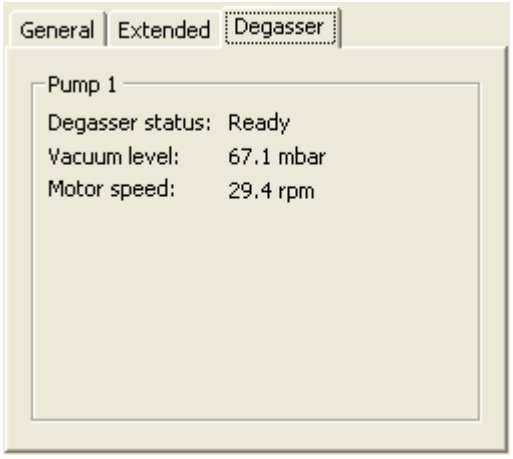


Table 39 describes the status readbacks on the Degasser page of the pump status view.

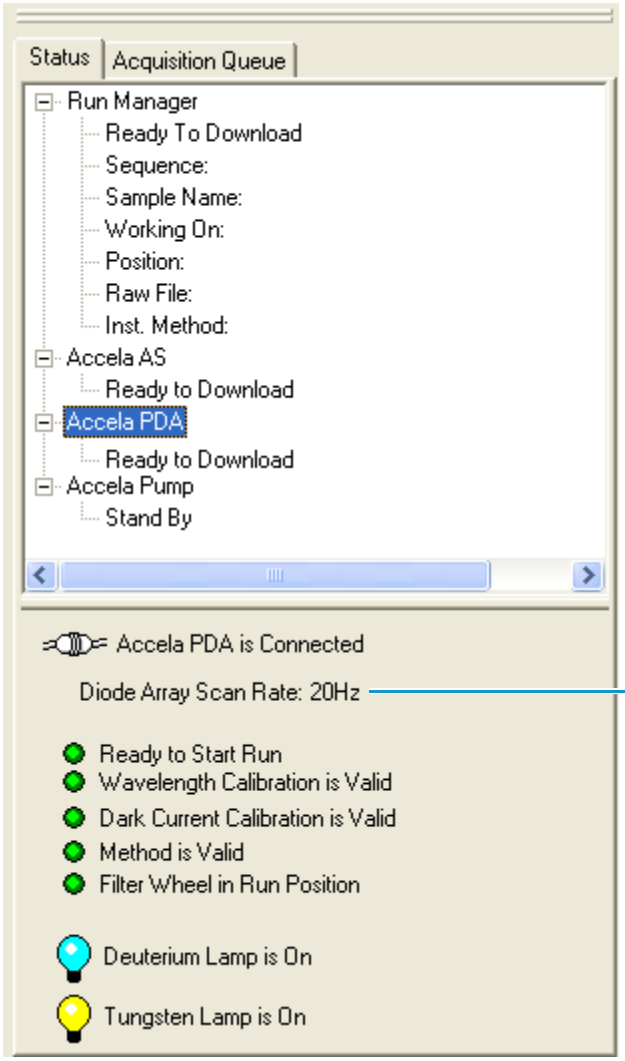
Table 39. General page of the pump status view

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

Accela PDA Detector Status View

Figure 80 shows the Status view for the Accela PDA Detector. You can view the status of the lamps and the configuration setting for the diode array scan rate from this view.

Figure 80. Accela PDA Detector Status view



Configuration setting for the diode array scan rate

Table 40 describes the readbacks for the PDA detector.

Table 40. PDA detector status readbacks

Readback	Description	
Connection status	Displays whether the PDA detector is communicating with the data system computer. The connection states are Connected or Not Connected.	
Diode Array Scan Rate	The Accela PDA (80 Hz) Detector has three diode array scan rate settings: 20 Hz, 40 Hz, and 80 Hz.	
Ready or Not Ready to Start Run	Ready to Start Run	The detector is ready to start a run or is currently acquiring data.
	Not Ready to Start Run	The deuterium lamp is warming up.
Wavelength Calibration	The wavelength calibration is valid or invalid.	
Dark Current Calibration	The dark current calibration is valid or invalid.	
Method is Valid	N/A	
Filter Wheel Position	The filter wheel has two positions:	
	Position 1 (Run)	Light passes through the flowcell onto the diode array.
	Position 2 (Closed)	Light passes through the holmium oxide filter onto the diode array.
Deuterium Lamp	The deuterium lamp states are as follows:	
		The deuterium lamp is off.
		The deuterium lamp is starting up, which requires about 1.5 min.
		The deuterium lamp is on.
Tungsten Lamp	The tungsten lamp states are as follows:	
		The tungsten lamp is on.
		The tungsten lamp is off.

Accela UV/Vis Detector Status View

Figure 81 shows the Status view for the Accela UV/Vis Detector. You can view the absorbance level of the chromatographic baseline from this view.

Figure 81. Accela UV/Vis Detector Status view

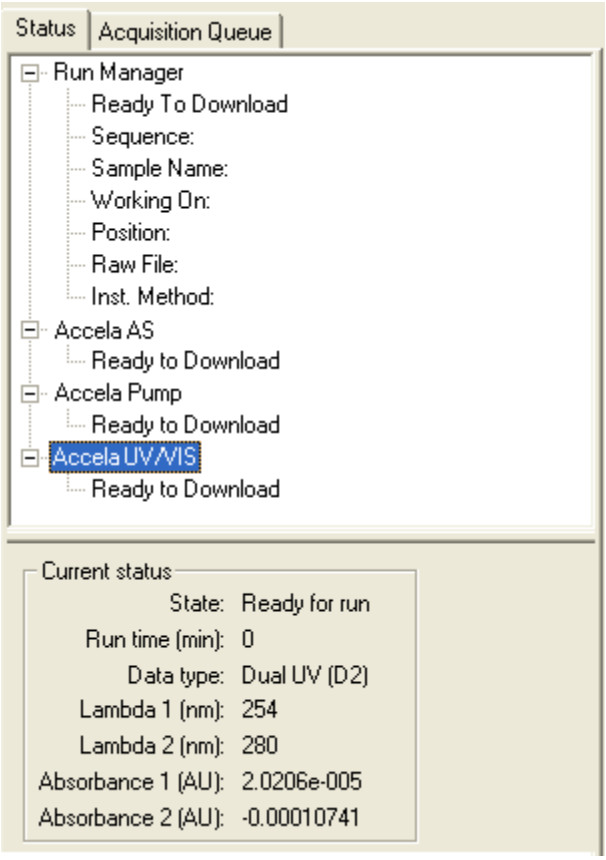


Table 41 describes the status readbacks for the Accela UV/Vis Detector.

Table 41. UV/Vis detector status readbacks

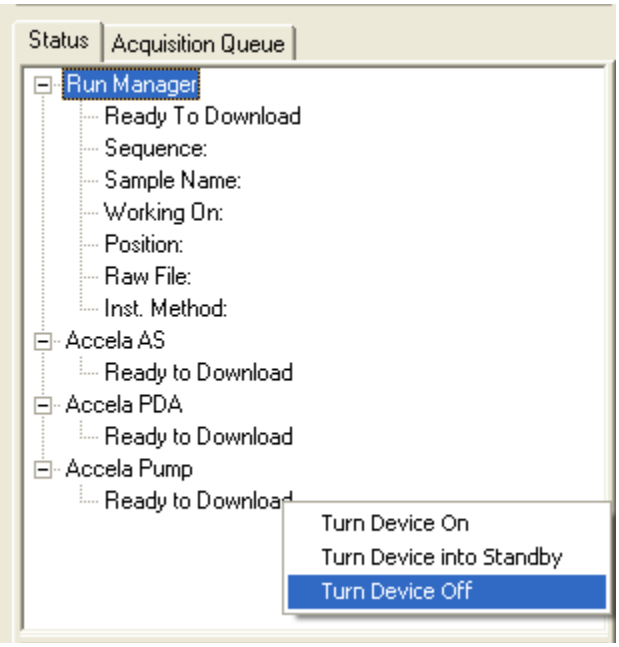
Readback	Description
State	Displays the following states: Ready for Run and Off.
Run Time	Displays the elapsed run time.
Data Type	Displays the program type specified in the current instrument method: Dual UV (D2), Dual Visible (W), or Single Wavelength.
Lambda 1 (nm)	Displays the current wavelength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

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

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

Figure 82 shows the shortcut menu for the Accela Pump.

Figure 82. Status page with a shortcut menu displayed in the Info View



❖ To turn on a device from the Info View

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

Depending on the device, the following actions occur:

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

❖ To place a device in Standby mode

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

Depending on the device, the following actions occur:

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

❖ **To turn a device off**

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

Depending on the device, the following actions occur:

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

Filling the Solvent Reservoir Lines with Fresh Solvent

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

CAUTION Using a syringe or similar suction tool to draw solvent through the LDA can damage the check valves.

To quickly draw solvent into the solvent reservoir lines, follow the procedure below.

For information about using the direct controls to start the solvent flow from the Accela pump, see "Pump Direct Controls" on page 189.

❖ **To quickly draw fresh solvent into the solvent reservoir lines**

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

Priming the Pulse Dampener of the Accela Pump

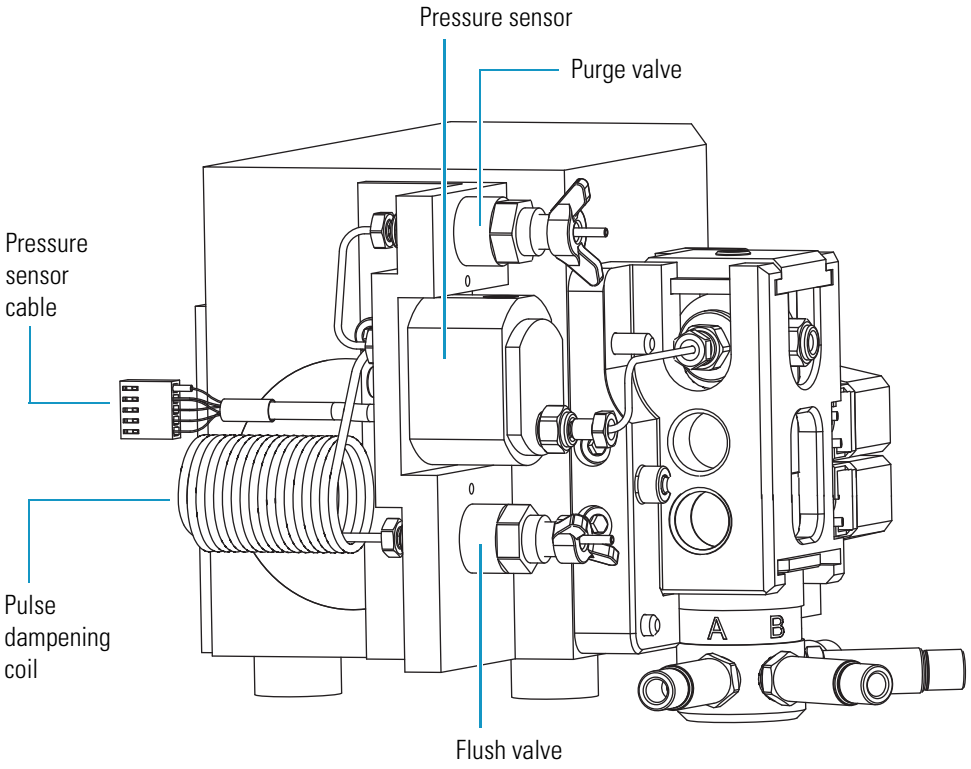
Before you can operate the Accela Pump, you must prime its built-in pulse dampener. Priming the pulse dampener involves filling the dampening coil (see Figure 83) with a solvent, such as methanol or isopropanol. After the coil is filled with solvent, it effectively dampens pressure pulsations from the rest of your system.

Note The Accela 600 Pump and the Accela 1250 Pump do not have a pulse dampener.

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

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

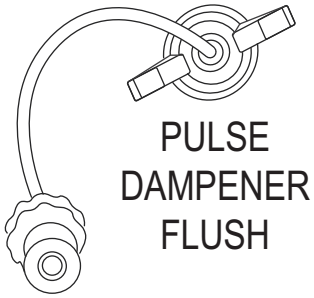
Figure 83. Pulse dampening assembly of the Accela Pump



❖ To prime the pulse dampener of the Accela Pump

1. Fill a solvent bottle with particulate-free, HPLC-grade methanol.
2. Connect an HPLC column or a flow restrictor to the LDA outlet.
3. Insert the tip of the 10 mL syringe into the tubing connected to the pulse dampener flush valve (see Figure 84).

   Figure 84. Pulse dampener flush valve with attached tubing

4. Open the valve by turning it counterclockwise.
5. To open the direct controls for the Accela Pump, do one of the following:

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

     Note For information about using the Direct Control dialog box for the Accela Pump, see "Pump Direct Controls" on page 189.

   –or–

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

     Note For information about using the Inlet Direct Control dialog box, see Chapter 11, "Making a Single Injection from the Tune Window."

6. Start pumping 100% methanol at a flow rate of 1000 µL/min.
7. Fill the loop completely to expel any air that might be trapped in the dampener loop.
8. Set the flow rate to one that is appropriate for your system.
9. Close the flush valve (see Figure 83 on page 155) by turning the valve clockwise. Always keep the pulse dampener valve closed during normal operation.

Setting Up the Seal Wash Pump

The seal wash pump is a hardware option for the Accela 600 Pump and the Accela 1250 Pump.

If your Accela pump has a seal wash pump, set it up for one of these control modes:

- Automatic Control
- Manual Control

Note Thermo Fisher Scientific recommends that you set up the seal wash pump for automated flushing if you use mobile phases with a high concentration of salts.

Automatic Control

❖ **To set up the seal wash pump for automatic control**

1. In the view bar, click the **Accela 600 Pump** or the **Accela 1250 Pump** icon.

   The pump view appears.

2. From the menu bar, choose **Accela 600 Pump** or **Accela 1250 Pump > Options**.

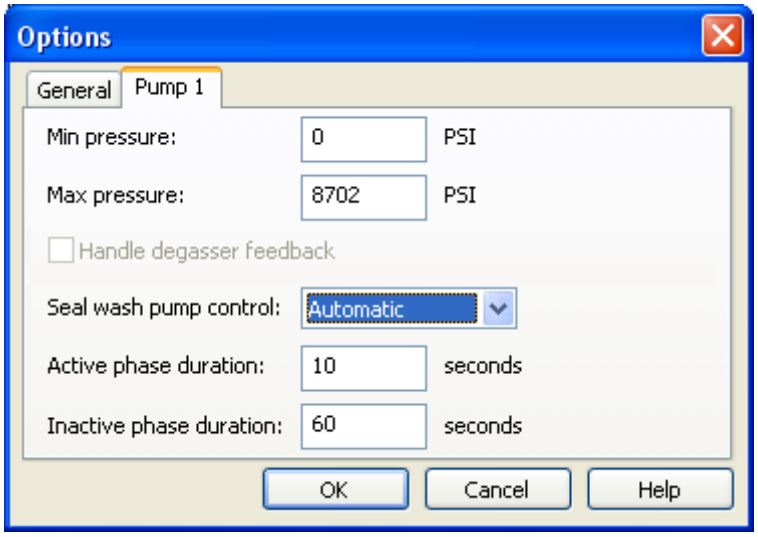
   The Options dialog box appears.

3. Click the **Pump 1** tab.

   The Pump 1 page appears (see Figure 85).

   If you have a dual-pump system and both pumps have the optional seal wash pump installed, the Options dialog box contains a Pump 1 tab and a Pump 2 tab. You can set up different wash settings for the two pumps.

Figure 85. Pump page of the Options dialog box for the pump



4. In the Seal Wash Pump Control list, select **Automatic**.
5. In the Active Phase Duration box, type the length of time in seconds that you want the seal wash pump to flush the pistons.

   The range is 1 to 65 534 seconds (18.2 hours).
6. In the Inactive Phase Duration box, type the length of time in seconds that you want the seal wash pump to remain idle between flush cycles.

   The range is 1 to 65 534 seconds (18.2 hours).
7. Click **OK** to accept the settings and close the dialog box.

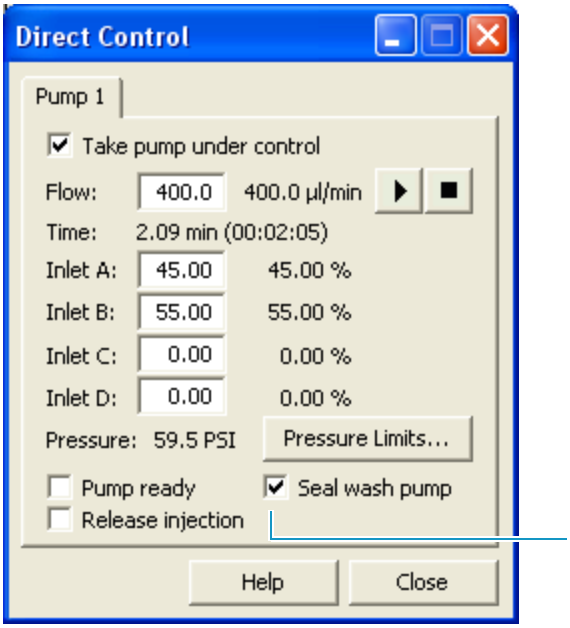
Manual Control

❖ **To set up manual control of the seal wash pump**

1. Open the Pump page of the Options dialog box.
2. In the Seal Wash Pump Control list, select **Manual**.
3. Click **OK** to apply the settings and close the dialog box.
4. To start the seal wash pump, do the following:
   a. From the menu bar, choose **Accela 600 Pump** or **Accela 1250 Pump > Direct Control**.

      The Direct Control dialog box for the pump appears (see Figure 86). If the pump has an optional seal wash pump installed, the Seal Wash Pump check box appears.

Figure 86. Direct Control dialog box for a pump with the optional seal wash pump



b. Select the **Take Pump Under Control** check box.

The direct control parameters for the pump become available.

c. Select the **Seal Wash Pump** check box.

The seal wash pump starts pumping wash solvent through the piston guide bushings.

> **Note** If you select None in the Seal Wash Pump Control list on the Options page, the Seal wash pump check box is unavailable.

5. To stop the seal wash pump, clear the **Seal Wash Pump** check box.

Setting Up the Leak Sensor

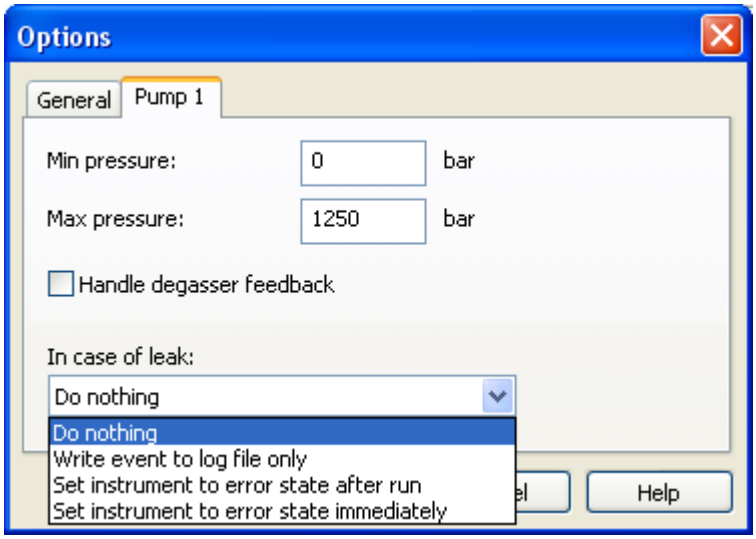
The leak sensor is a hardware option for the Accela 600 Pump or the Accela 1250 Pump that can detect the presence of liquid on the pump drip tray. When liquid comes in contact with the leak sensor, the leak sensor's optical LED turns green and the leak sensor sends a signal to the data system.

❖ To set up the leak sensor actions

1. Check the configuration settings for your Accela pump (see "Pump Configuration Settings" on page 59). Ensure that the **Leak Sensor Installed** check box is selected, and then exit the Thermo Foundation application.
2. From your data system, open the view for your Accela pump.
3. From the menu bar, choose **Accela 600 Pump** or **Accela 1250 Pump > Options**.

The Options dialog box appears (see Figure 87).

Figure 87. Options dialog box



4. Select the action that you want the pump to take when the leak sensor detects a leak.

   The default selection is Do Nothing.

Selection	Meaning
Do Nothing	The pump pistons continue moving.
Write Event to Log File Only	The pump pistons continue moving, but the following text appears in the log file: Leak detected.
Set Instrument to Error State after Run	When the current run ends, the pump pistons stop moving and the sequence pauses.
Set Instrument to Error State Immediately	When the leak sensor detects the presence of liquid on the pump's drip tray, the pump pistons stop moving and the sequence pauses.

Tip To restart the pump after the leak sensor detects a leak, do the following:

1. Stop the sequence and delete it from the run queue.
2. Close the data system.
3. Fix the leak and make sure that the pump's drip tray is dry and that the leak sensor LED is green.
4. Reopen the data system and check the status of the instrument's modules.
5. Resubmit the sequence.

–or–

1. Fix the leak and make sure that the pump's drip tray is dry and that the leak sensor LED is green.
2. Open the Information view in the Xcalibur data system.
3. Right-click the directory listing for the pump and choose **Turn device on** from the shortcut menu.

4. Click **OK** to accept the selection and close the Options dialog box.

Accessing the Direct Controls

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

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

❖ To access the direct controls

1. Depending on the data system, do one of the following:

   - If you are controlling your instrument from the Xcalibur data system, do the following:

     a.  If it is not already open, open the Roadmap view by clicking the **Roadmap** button in the toolbar.

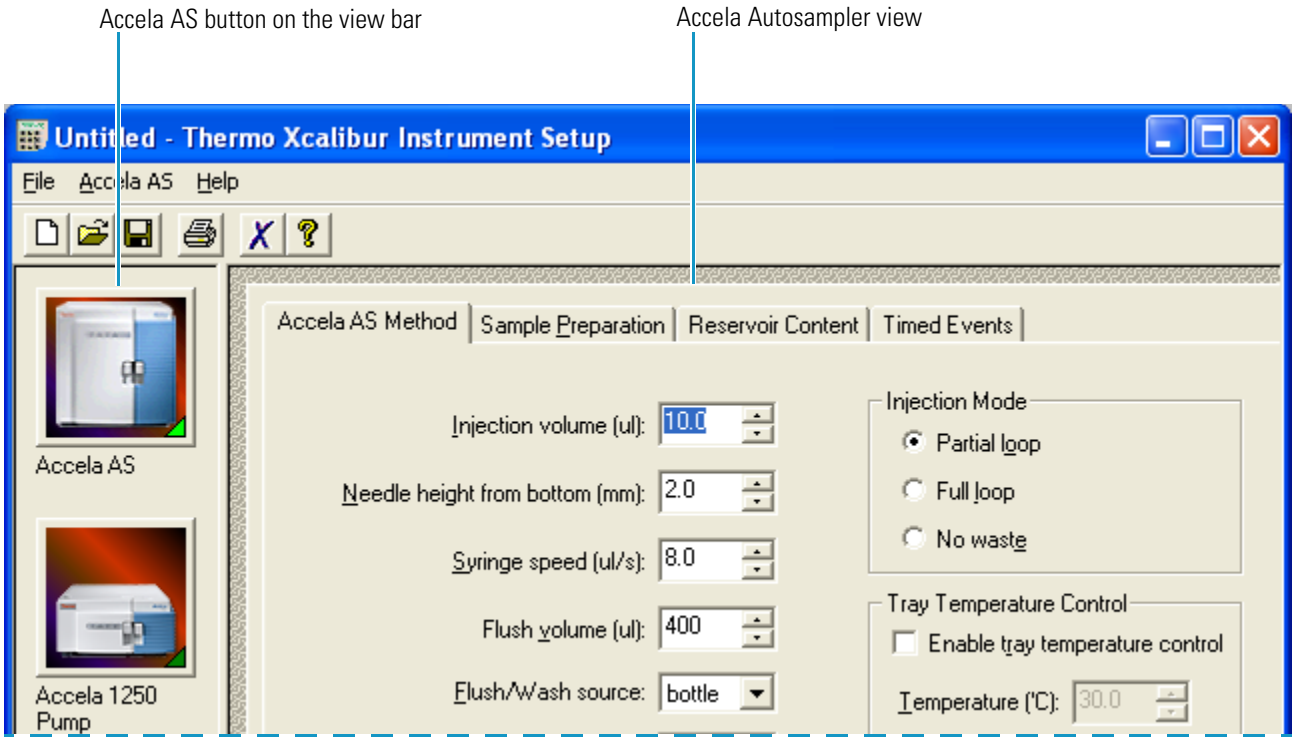
     b.  Click the **Instrument Setup** button on the toolbar or the larger **Instrument Setup** icon in the Roadmap View.

     The Instrument Setup window opens to the first module displayed in the view bar (see Figure 88). The view bar is a vertical bar on the left side of the Instrument Setup window. It contains an icon for each configured device. When you click a device icon, a green triangle appears in the lower-right corner of the icon, and the device view appears.

   - If you are controlling your instrument from a Thermo Scientific data system other than Xcalibur, open the instrument control area.

     The instrument control area opens to the first module displayed in the view bar on the left side of the window.

Figure 88. Instrument Setup window open to the Accela AS Method page of the Accela AS view



2. To open the view for the device of interest, click its corresponding icon in the view bar.

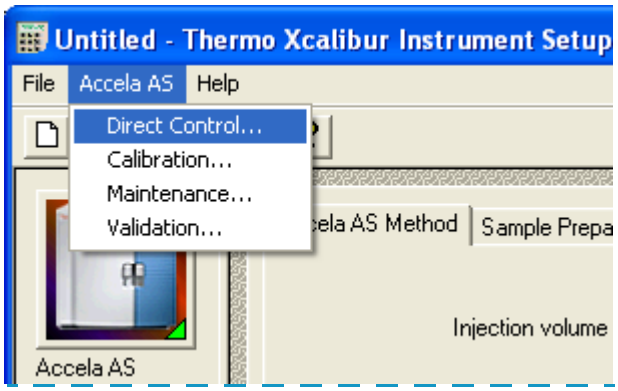
   The view for the specific device appears.

3. Access the direct controls for each device from the menu bar of the device view as follows:

Device	Menu	Reference
Accela Pump	Choose **Accela Pump > Direct Control**.	"Pump Direct Controls" on page 189
Accela 600 Pump	Choose **Accela 600 > Direct Control**.	
Accela 1250 Pump	Choose **Accela 1250 > Direct Control**.	
Accela PDA Detector	Choose **Accela PDA > Direct Control**. Then click the **Configuration** tab.	"PDA Detector Direct Controls" on page 166
Accela UV/Vis Detector	Choose **Accela UV/Vis > Direct Control**.	"UV-Vis Detector Direct Controls" on page 187
Accela Autosampler	Choose **Accela AS > Direct Control**.	"Autosampler Direct Controls" on page 191

Figure 89 shows the Accela AS menu.

Figure 89. Top-left portion of the Accela AS view, showing the Accela AS menu



Direct Controls

You can control some device features without downloading an instrument method. For example, you can turn the lamps on or off, set the solvent conditions for the pump, start and stop the solvent flow from the pump, and control the autosampler's temperature zones and its XYZ arm by using the direct controls provided by the data system.

For information about accessing the direct controls for each device, see "Accessing the Direct Controls" on page 161.

Contents

Note For instructions on loading samples into the autosampler, see "Loading the Autosampler" on page 210.

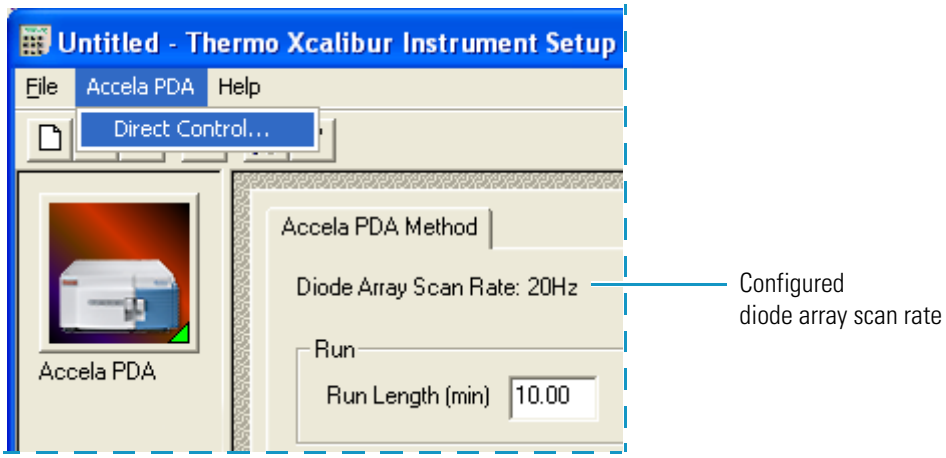
PDA Detector Direct Controls

The Direct Control dialog box for the PDA detector contains the following pages: Display, Configuration, Information, and Calibration.

❖ To open the Direct Control dialog box for the PDA detector

1. Open the PDA detector view.
2. From the menu bar, choose **Accela PDA > Direct Control** (see Figure 90).

Figure 90. Accela PDA menu



The Accela Direct Control dialog opens to the Display page (see Figure 91).

Figure 91. Accela Direct Control dialog box tabs

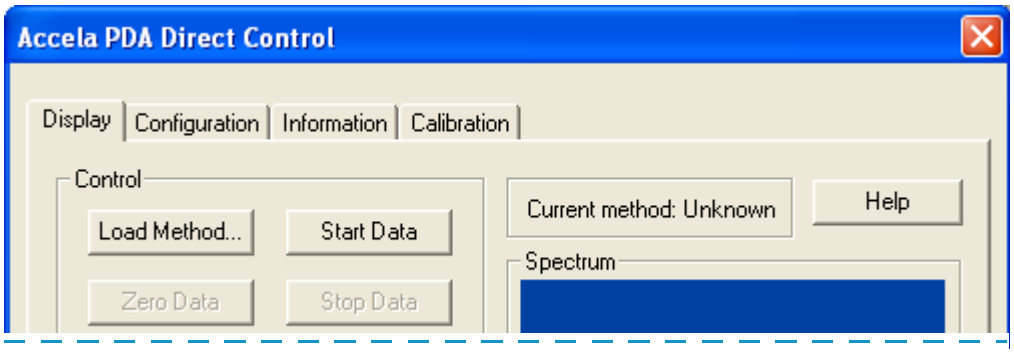


Table 42 lists the direct control procedures by the page where they are performed.

Table 42. Direct control procedures

Page	Procedure
Display Page	"Checking the Chromatographic Baseline" on page 172
	"Verifying the Performance of the PDA Detector" on page 259
Configuration Page	"Turning the Lamps On or Off" on page 167
	"Resetting the Lamp Usage Hours" on page 168
	"Changing the Polarity of the Analog Outputs" on page 168
	"Testing the Analog Outputs" on page 169
	"Setting the Lamp Startup Time" on page 170
Information Page	"Viewing, Exporting, and Clearing the Error Log for the PDA Detector" on page 175
	"Checking the Firmware Version of the PDA Detector" on page 175

For information about using the Calibration page to determine the wavelength accuracy of the detector and the dark current produced by the diode array, see "Calibrating the PDA Detector" on page 267.

Turning the Lamps On or Off

Use the Configuration page of the Accela PDA Direct Control dialog box to turn each lamp on or off.

❖ To turn the lamps on or off

1. Open the Configuration page of the Accela PDA Direct Control dialog box (see "Configuration Page" on page 175).
2. Click **Turn On** for the associated lamp.

   When you turn on the deuterium lamp, its Status readback reads Starting during the 30-second ignition period, and then it changes to On. If there is a problem with either lamp, its Status readback reads Failed.

   Note The intensity of the deuterium lamp falls off very slightly over a period of time after the lamp is turned on. Plan to wait at least one hour for the lamp to stabilize after a cold start before collecting data in the spectral range of the deuterium lamp.

Resetting the Lamp Usage Hours

Use the Configuration page of the Accela PDA Direct Control dialog box to reset the lamp usage hours.

❖ **To reset the lamp usage hours (lifetime hours elapsed)**

1. Open the Configuration page of the Accela PDA Direct Control dialog box (see "Configuration Page" on page 175).
2. Click **Reset Lifetime** for the associated lamp.

   The stored total run time for the associated lamp resets to zero, and the Last Lifetime Reset readback is updated to the current date and time.

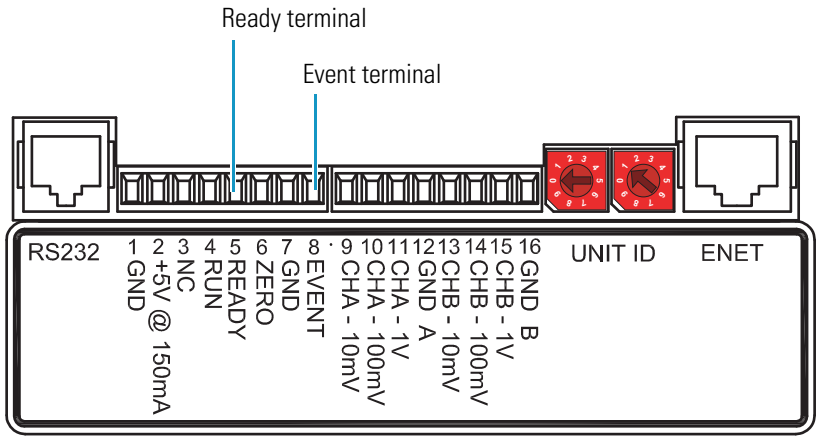
> **Tip** Avoid indiscriminately clicking the Reset buttons. Click Reset only after you replace the associated lamp with a new one.

Changing the Polarity of the Analog Outputs

Use the Configuration page of the Accela PDA Direct Control dialog box to change the polarity of the analog outputs.

There are two output signal terminals on the back panel of the PDA detector: Event and Ready (see Figure 92).

Figure 92. Event and Ready terminals on the back panel of the PDA detector



❖ **To change the polarity of the analog outputs**

1. Open the Configuration page of the Accela PDA Direct Control dialog box (see "Configuration Page" on page 175).

   If the output polarity is Active Low, the Set Active High button is available. If the output polarity is Active High, the Set Active Low button is available.

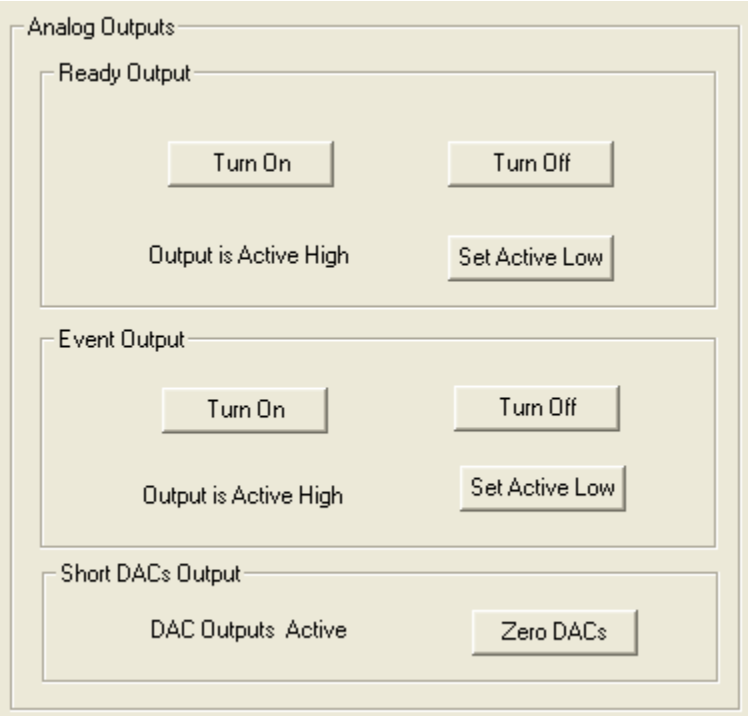
2. Under Analog Outputs (see Figure 93), do one of the following:

   - Click **Set Active High** to change the output polarity from Active Low to Active High.

   –or–

   - Click **Set Active Low** to change the output polarity from Active High to Active Low.

Figure 93. Analog Outputs area on the Configuration page



Testing the Analog Outputs

Use the Configuration page of the Accela PDA Direct Control dialog box to change the polarity of the analog outputs.

❖ To test the analog outputs

1. Open the Configuration page of the Accela PDA Direct Control dialog box (see "Configuration Page" on page 175).
2. Under Analog Outputs (see Figure 93), do the following:

   - Click **Turn On** or **Turn Off** to trigger the external device.
   - Click **Zero DACs** to calibrate external instruments, such as an SS420X analog to digital converter.

     When you click Zero DACs, the DACs outputs are set to zero for about 20 seconds. You can extend this calibration time by clicking the button again.

Setting the Lamp Startup Time

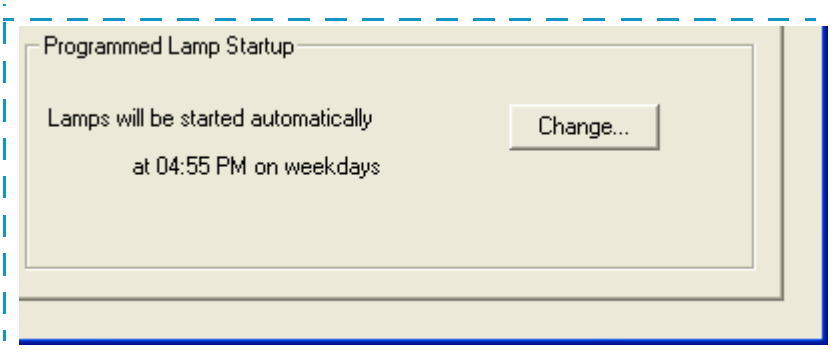
Use the Lamp Startup Time dialog box to set the startup time for the lamps.

If you do not change the lamp startup time, the data system automatically turns the lamps on at 4:55 PM on weekdays.

❖ To open the Lamp Startup Time dialog box

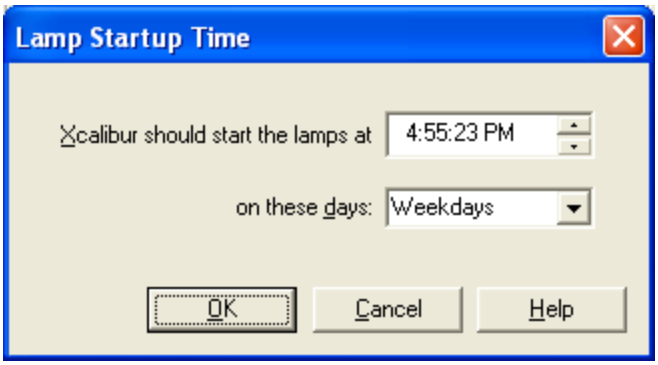
1. Open the Configuration page of the Accela PDA Direct Control dialog box (see "Configuration Page" on page 175).
2. In the Programmed Lamp Startup area, click **Change** (see Figure 94).

Figure 94. Programmed Lamp Startup area of the Configuration page



The Lamp Startup Time dialog box appears (see Figure 95).

Figure 95. Lamp Startup Time dialog box



❖ To set the startup time for the lamps

1. In the Xcalibur Should Start the Lamps At box, click the hours or minutes text to highlight it.

Tip To change both the hours and the minutes, change one time field, and then click **OK** to accept the setting and close the dialog box. Then open the dialog box a second time and change the other time field.

2. To change the numeric values, do one of the following:

   - Use the up or down arrow to the left of the time entry to scroll to the time value that you want.

   –or–

   - Press the UP or DOWN key on your computer keyboard to scroll to the time value that you want.

   –or–

   - Type the numeric value that you want.

3. To change AM to PM or the reverse, highlight the current setting, and then type **AM** or **PM**.
4. To change the day of the week or to turn off the lamp startup feature, in the On These Days list, select **Never**, **Weekdays**, or **Everyday**.
5. Click **OK** to apply the new lamp startup time.

Lamp Startup Time Parameters

Table 43 describes the parameters in the Lamp Startup Time dialog box.

Table 43. Lamp Startup Time dialog box parameters

Parameter	Description
Xcalibur Should Start the Lamps At	Specifies the time that the Xcalibur data system automatically turns the lamps on. Default: 4:55 PM
On These Days	Specifies the days that the Xcalibur data system automatically turns the lamps on. The selections are Never, Weekdays, and Every Day. Default: Weekdays
Buttons	
OK	Applies the settings and closes the dialog box.
Cancel	Cancels the applied time value or date.

Checking the Chromatographic Baseline

Use the Display page of the Direct Control dialog box to check the chromatographic baseline (see "Display Page" on page 180).

❖ **To check the stability of the chromatographic baseline before starting a sequence run**

1. Open the Instrument Setup window of the Xcalibur data system.
2. In the view bar, click the icon for the LC pump, and then open the Direct Control dialog box for the LC pump.
3. Start the solvent flow from the LC pump. Use the chromatographic conditions specified on first line pump program in the instrument method.

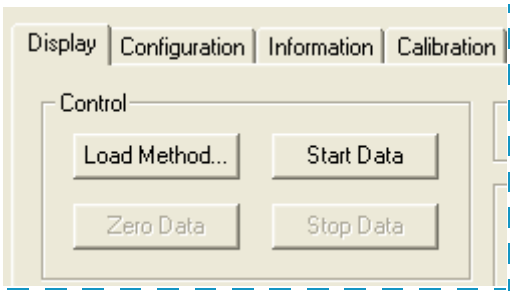
   For information about starting the solvent flow from the LC pump, refer to the Help for the pump.
4. In the view bar of the Instrument Setup window, click the icon for your PDA detector.

   The PDA detector view of the Instrument Setup window appears with the Method page displayed.
5. From the menu bar, choose **Accela PDA > Direct Control**.

   The Direct Control dialog box appears.
6. Click the **Display** tab.

   The Display page appears (see Figure 96).

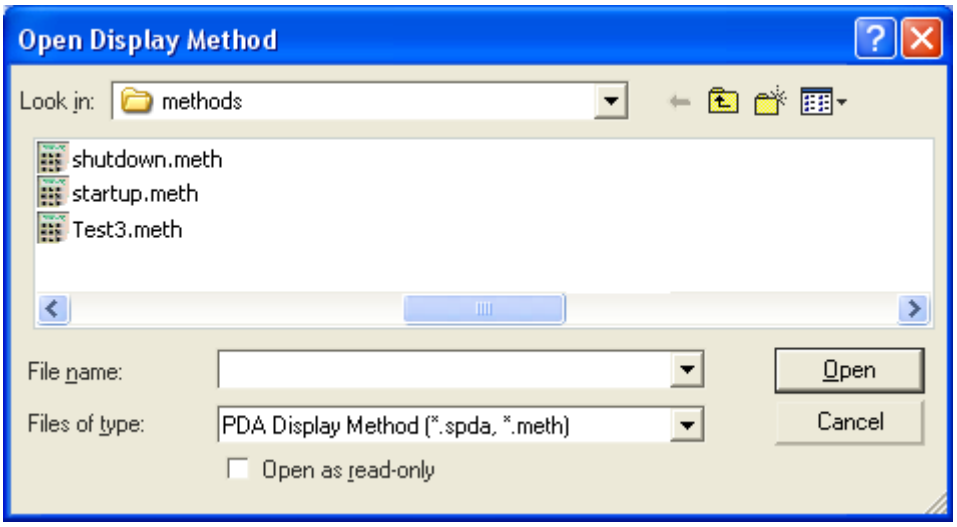
   Figure 96. Control area buttons on the Display page



7. In the Control area, click **Load Method**.

   The Open Display Method dialog box appears (see Figure 97).

Figure 97. Open Display Method dialog box

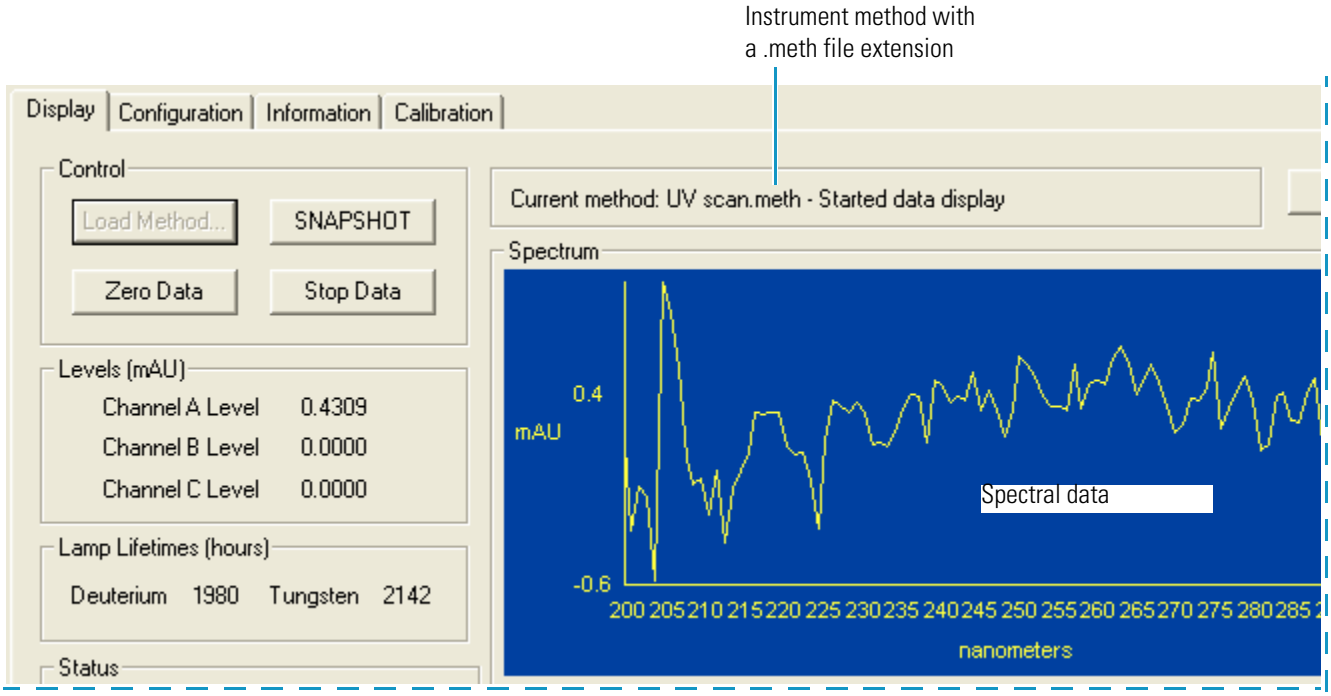


8. Select the instrument method that you plan to use in your acquisition sequence.
9. Click **Open** to download the method to the PDA detector and close the dialog box.
10. In the Control area, click **Start Data**.

    The graphical display begins to update. The spectral scan appears in the top pane and the chromatographic data for the discrete channel wavelengths appears in the bottom pane.

    When you click Start Data, the Start Data button changes to the SNAPSHOT button and the Zero Data button is available (see Figure 98).

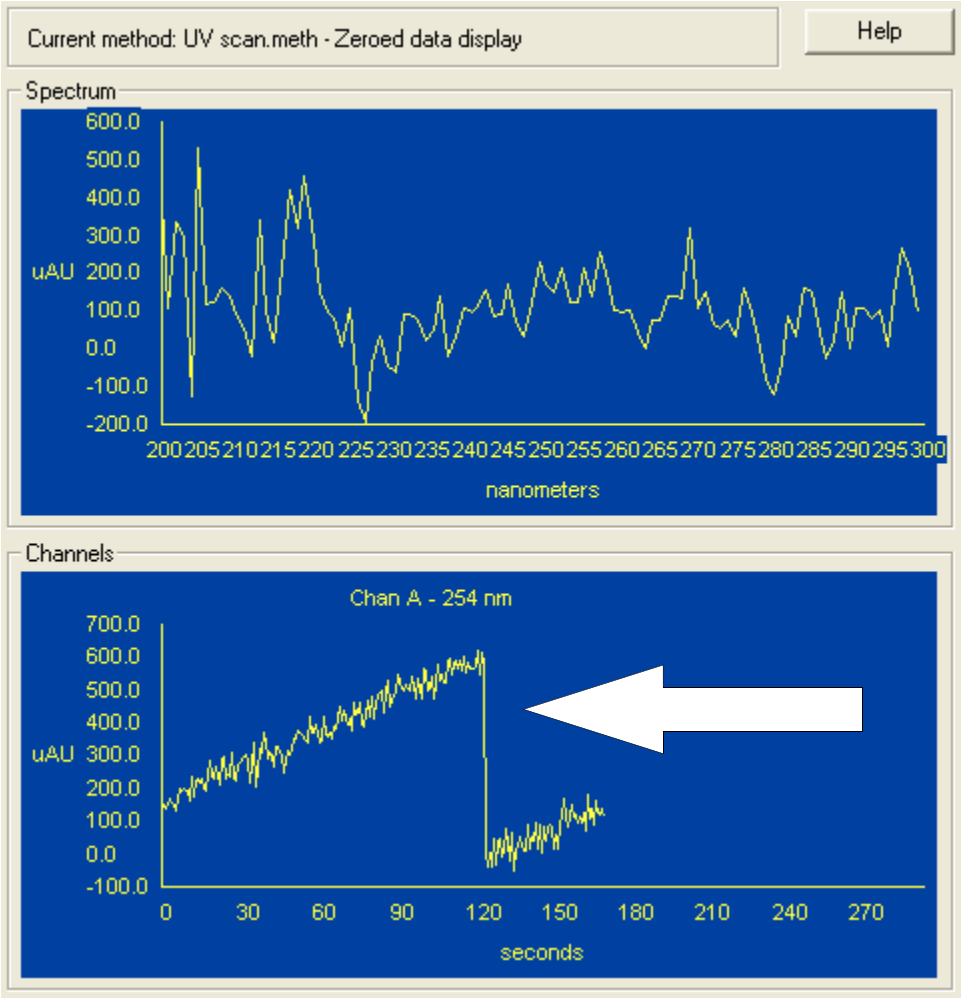
Figure 98. Display page with data displaying for an instrument method



11. To zero the absorbance level, click **Zero Data**.

    The Current method readback displays Zeroed Data Display (see Figure 99).

    Figure 99. Effect of clicking the Zero Data button



12. To stop data acquisition, click **Stop Data**.

    If the noise level is too high, check the light throughput to the diode array.

Viewing, Exporting, and Clearing the Error Log for the PDA Detector

You can access an error log for the PDA detector from the Xcalibur data system.

❖ **To view the error log**

1. Open the Information page of the Accela PDA Direct Control dialog box (see "Information Page" on page 185).
2. Click **Request Log**.

❖ **To export the log to a Microsoft Excel Spreadsheet**

Click **Export Log**.

❖ **To clear the error log**

Click **Clear Log**.

Checking the Firmware Version of the PDA Detector

❖ **To check the firmware version of the PDA detector**

Open the Information page of the Direct Control dialog box for the PDA detector (see "Information Page" on page 185).

The firmware version of the PDA detector is displayed in the lower-right portion of the Information page.

Configuration Page

Use the Configuration page of the Accela PDA Direct Control dialog box to perform these procedures:

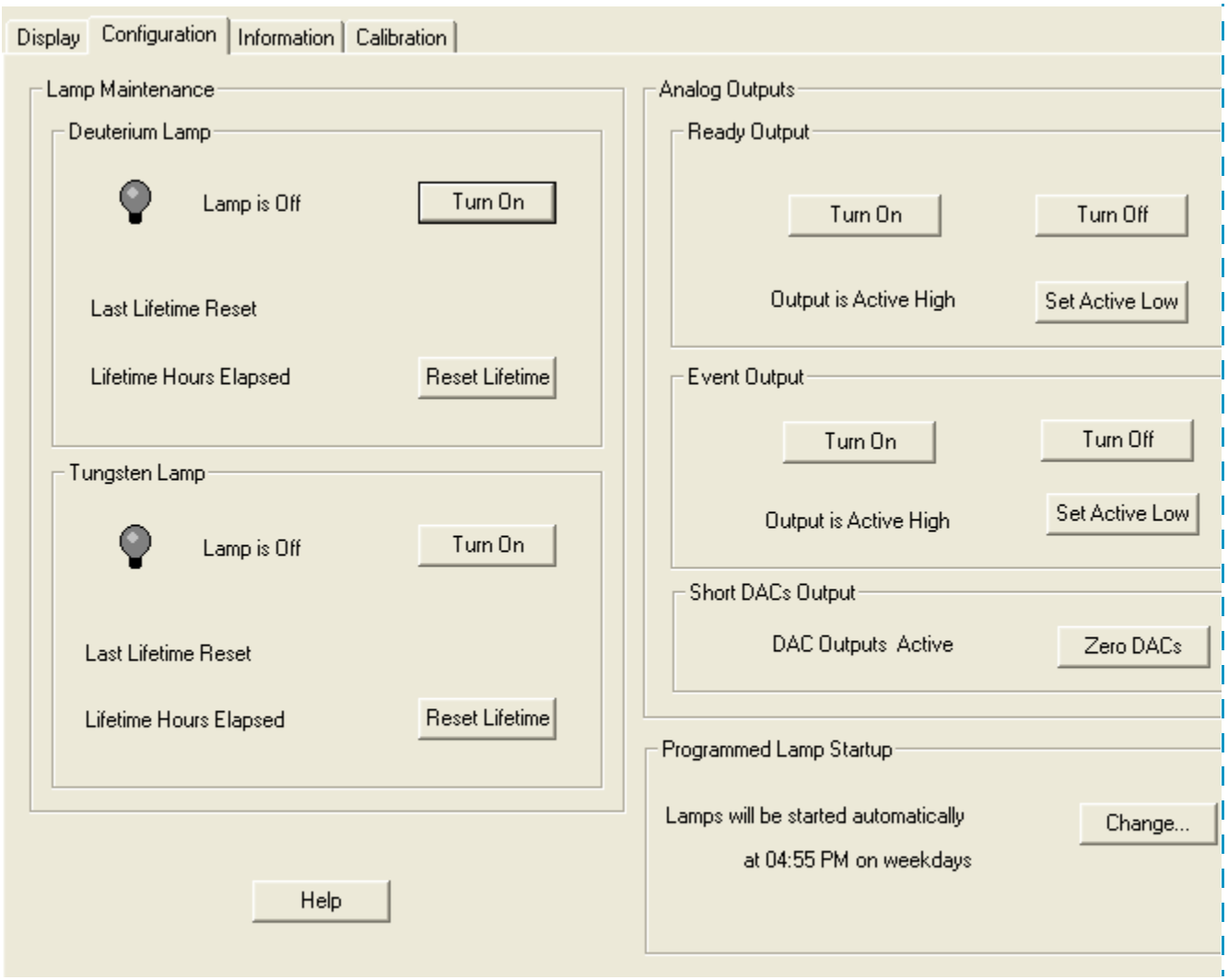
- Turning the Lamps On or Off
- Resetting the Lamp Usage Hours
- Changing the Polarity of the Analog Outputs
- Testing the Analog Outputs
- Setting the Lamp Startup Time

❖ **To open the Configuration page of the Direct Control dialog box**

1. Open the Accela PDA Direct Control dialog box (see "PDA Detector Direct Controls" on page 166).
2. Click the **Configuration** tab.

   The Configuration page appears (see Figure 100).

Figure 100. Configuration page



Configuration Page Parameters

Table 44 describes the parameters on the Configuration page.

Table 44. Configuration page of the Direct Control dialog box for the PDA detector (Sheet 1 of 4)

Parameter	Description		
Lamp Maintenance			
Use the controls in this area to turn the lamps on and off and to reset the lamp lifetime hours after you replace a lamp.			
Deuterium Lamp			
Status readback	Indicates the status of the deuterium lamp.		
	Graphic	**Text**	**Meaning**
		Off	The deuterium lamp is off.
		Starting	The deuterium lamp is starting up, which requires about 1.5 min.
		On	The deuterium lamp is on. When the status is On, the Turn Off button is available. When the status is Off, the Turn On button is available.
Last Lifetime Reset readback	Displays the date and time that the deuterium lamp was last reset.		
Lifetime Hours Elapsed	Displays the cumulative time in hours that the deuterium lamp has been in the On state since you clicked the Reset Lifetime button.		
Turn On/Off button		Turns on the deuterium lamp if its current status is Off.	
		Turns off the deuterium lamp if its current status is On.	
Reset Lifetime button	Resets the date and time monitored by the Last Reset readback and the Lifetime readback.		
	Note Click Reset Lifetime whenever you replace the deuterium lamp. This resets the Last Reset readback to the current date and time and restarts the Lifetime readback at zero.		

Table 44. Configuration page of the Direct Control dialog box for the PDA detector (Sheet 2 of 4)

Parameter	Description		
Tungsten Lamp			
Status readback	Indicates the status of the tungsten lamp, as follows:		
	Graphic	**Test**	**Meaning**
		On	The tungsten lamp is on.
		Off	The tungsten lamp is off. When the status is On, the Turn Off button is available. When the status is Off, the Turn On button is available.
Last Lifetime Reset readback	Displays the date and time that the tungsten lamp was last reset.		
Lifetime Hours Elapsed	Displays the cumulative time in hours that the tungsten lamp has been in the On state since you clicked the Reset Lifetime button.		
Turn On/Off button		Turns on the tungsten lamp if its current status is Off.	
		Turns off the tungsten lamp if its current status is On.	
Reset Lifetime button	Resets the date and time monitored by the Last Reset readback and the Lifetime readback.		
	Note Click Reset Lifetime whenever you replace the tungsten lamp. This resets the Last Reset readback to the current date and time and restarts the Lifetime readback at zero.		
Analog Outputs			
Use the controls in this area to configure and test the Event and Ready outputs. Determine if the external device is triggered by contact closure or a TTL (transistor - transistor - logic) signal. If the external device requires a TTL signal, determine the polarity of the signal: active high or active low.			
Note For TTL connections: • Logic level zero (0) is less than +0.8 Vdc (reading from Ground). • Logic level one (5) is approximately +5 Vdc (+2.4 Vdc minimum from Ground).			
Possible configurations of the Event and Ready outputs include the following:			
Output state	**Output polarity**	**Vdc**	
Off	Active High	+5	

Table 44. Configuration page of the Direct Control dialog box for the PDA detector (Sheet 3 of 4)

Parameter	Description
Ready Output	
Turn On and Turn Off buttons	Indicates whether the Ready output state is On or Off. If the Ready output state is On, the Turn Off button is available. If the Ready output state is Off, the Turn On button is available.
Ready Output readback	Indicates whether the Ready output polarity is Active High or Active Low. If the Ready output polarity is Active Low, the Set Active High button is available. If the Ready output polarity is Active High, the Set Active Low button is available.
Set Active Low or Set Active High buttons	Set the polarity of the Ready Output signal.
Note The output polarity must match the polarity (either Active High or Active Low) of the instrument connected to the Ready output connection on the back panel of the PDA detector. The signal terminal is the high connection and the ground (GND) terminal is the low connection. The autosampler requires active low remote inputs.	
Event Output	
Use the controls in this area to specify the parameters for output events. If the external device is triggered by a contact closure, connect the PDA EVENT terminal (pin 8) to the positive pin on the external device input, and connect one of the PDA GND terminals (either of pins 1 or 7) to the external device negative pin. If the external device is triggered using a TTL signal, connect the PDA +5V output (pin 2) to the positive Input terminal of the external device, and connect the PDA EVENT output (pin 8) to the negative Input terminal of the external device.	
Turn On and Turn Off buttons	Indicates whether the Event output state is On or Off. If the Event output state is On, the Turn Off button is available. If the Event output state is Off, the Turn On button is available.
Event Output readback	Indicates whether the Event output polarity is Active High or Active Low. If the Event output polarity is Active Low, the Set Active High button is available. If the Event output polarity is Active High, the Set Active Low button is available.
Set Active Low or Set Active High buttons	Set the polarity of the Event Output signal.
Note The output polarity must match the polarity (either Active High or Active Low) of the instrument connected to the Event output connection on the back panel of the PDA detector. The signal terminal is the high connection and the GND Terminal is the low connection.	
Short DACs Output	
Zero DACs button	To calibrate external instruments, such as an SS420X analog to digital converter, use the controls in this area to short the DACs outputs to zero for about 20 seconds.

Table 44. Configuration page of the Direct Control dialog box for the PDA detector (Sheet 4 of 4)

Parameter	Description
Programmed Lamp Startup	
Programmed Lamp Startup	Displays the current lamp startup program and provides controls to create a lamp startup program that automatically turns on the lamps for operation at a specified time and day (never, weekdays, every day).
Change	Opens the Lamp Startup Time dialog box where you can specify a lamp startup time (see "Setting the Lamp Startup Time" on page 170).

Display Page

Use the Display page to perform these procedures:

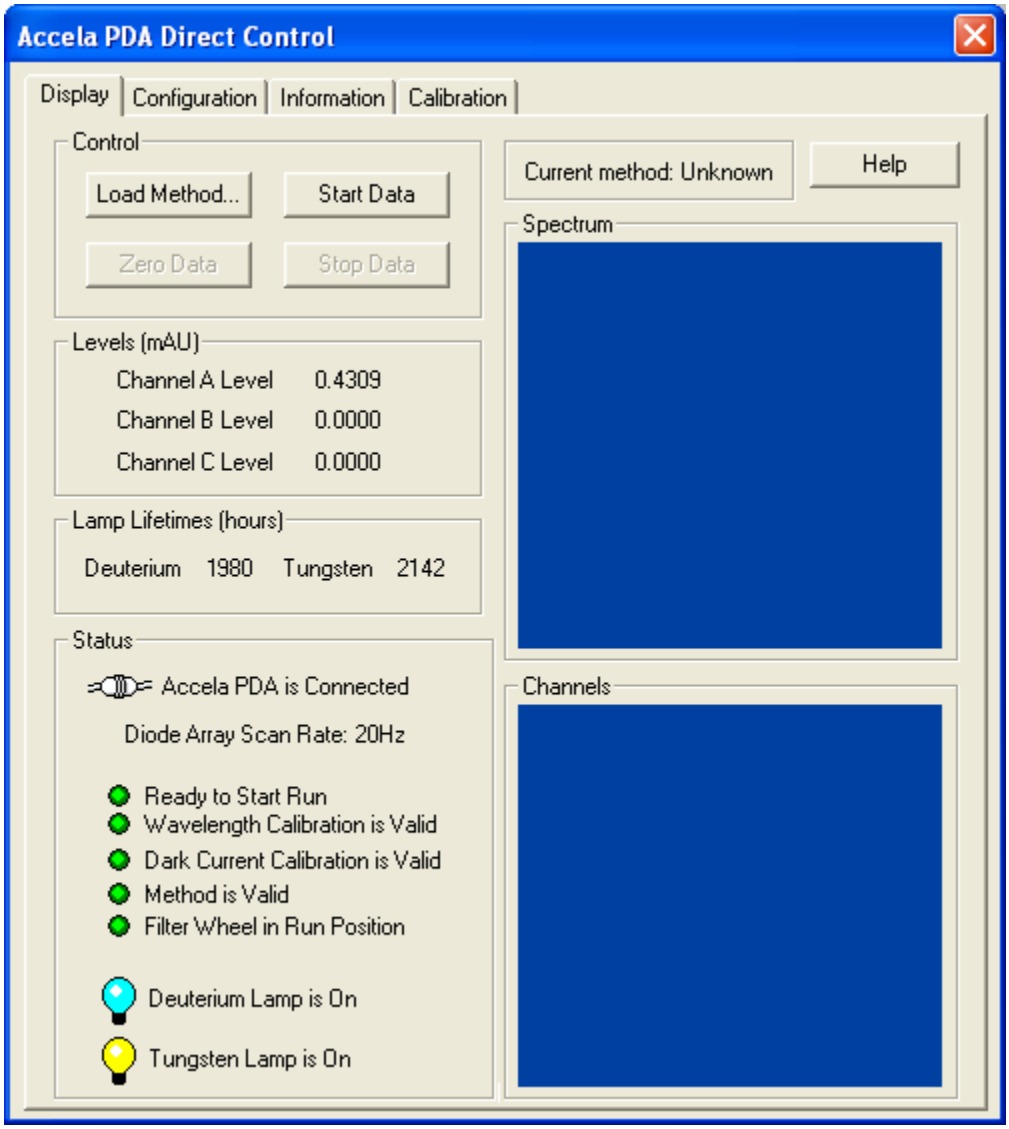
- "Checking the Chromatographic Baseline" on page 172
- "Adjusting the Light Throughput to the Diode Array" on page 262

❖ To open the Display page

1. Open the Accela PDA Direct Control dialog box (see "PDA Detector Direct Controls" on page 166).
2. Click the **Display** tab.

   The Display page appears (see Figure 101).

Figure 101. Display page



Display Page Parameters

Table 45 describes the parameters on the Display page.

Table 45. Display page parameters (Sheet 1 of 4)

Parameter	Description
Control	
Control	The buttons in this area provide control of the display method (.spda).
Load Method	Loads the display method or the data acquisition parameters of an instrument method to the detector.
Start Data	Initiates the graphical display of the intensity data or the wavelength data.

Table 45. Display page parameters (Sheet 2 of 4)

Parameter	Description
Snapshot	Saves a comma-separated values file named **PDASnapshot.csv** to the following folder on the data system computer: C:\Xcalibur\data A date and time stamp are appended to the file name. The format of the date and time stamp is MMDDYYHHMMSS. MM is the month. DD is the day of the month. YY are the last two digits of the year. HH is the hour in military time. MM are minutes. SS are seconds. You can open this file with the Excel™ application. For a wavelength/absorbance method, the file contains the absorbance values for the spectral scan and the discrete channel wavelengths at the moment that you clicked Snapshot. For an intensity/diode method, the file contains the intensity values for the scan of the diode array and the intensity values for up to three individual diodes.
	Note The Snapshot button is available only after you click the Start Data button and the graphical display has started updating.
Zero Data	Zeros the absorbance data. After you click Zero Data, the Current Method status box displays the message Zeroed Data Display.
	Note This button is only available in the wavelength/absorbance mode.
Stop Data	Stops the updating of the graphical display. After you click Stop Data, the Current Method status box displays the message Stopped Data Display.
Levels (mAU)	
Channel A/B/C level	This graphical display shows the intensity or absorbance level of each discrete channel, A, B, or C, specified in the method.
Lamp Lifetimes (hours)	
Deuterium	Displays the cumulative time in hours that the Deuterium lamp has been in the On state since you clicked the Reset button.
	Note Click Reset Lifetime on the Configuration page whenever you replace the deuterium lamp. This resets the Last Reset readback to the current date and time and sets the Lifetime readback to zero.
Tungsten	Displays the cumulative time in hours that the tungsten lamp has been in the On state since you clicked Reset Lifetime.
	Note Click the Reset Lifetime button on the Configuration page whenever you replace the tungsten lamp. This resets the Last Lifetime Reset readback to the current date and time and restarts the Lifetime readback to zero.

Table 45. Display page parameters (Sheet 3 of 4)

Parameter	Description
Status (bottom left)	
Status area	Displays the status of the PDA detector and the lamps.
Connection	Displays the communication status of the PDA detector. Depending on whether the data system can connect to the PDA detector, the status displays Connected or Not Connected. If this readback displays Not Connected, make sure that the PDA detector is powered on. Check the Ethernet connections and the instrument configuration settings for the PDA detector.
Diode array scan rate	Displays the current configuration setting for the diode array scan rate. The possible diode array scan rates are 20, 40, and 80 Hz.
PDA status	Displays the current state of the PDA detector. The possible states are Not Ready for a Run and Ready for a Run. This readback displays Not Ready for a Run if both lamps are off or if the deuterium lamp is warming up.
Wavelength calibration	Displays whether the wavelength calibration has been applied.
Dark current calibration	Displays whether the dark current calibration has been applied.
Method validity	Displays whether a method has been downloaded to the PDA detector.
Filter wheel position	Displays the position of the filter wheel. The filter wheel has two positions: 1 and 2. When the filter wheel is in position 1, the readout displays Run Position. When the filter wheel is in position 2, the readout displays Calibration Position.
Deuterium lamp	Displays whether the deuterium lamp is on or off.
Tungsten lamp	Displays whether the tungsten lamp is on or off.
Current method (top right)	
Current method	Displays the status of the current method.
Spectrum	
Spectrum Plot	Depending on the method type (.meth or .spda), these scan plots are displayed: • For display methods (.spda), the scan plot shows intensity (counts) normalized to 100 percent on the *y* axis and diode number (2 to 511) on the *x* axis. • For instrument methods (.meth), the scan plot shows absorbance (mAU) normalized to 100 percent on the *y* axis and wavelength (nanometers) on the *x* axis.

Table 45. Display page parameters (Sheet 4 of 4)

Parameter	Description
Channels	
Channels Plot	Depending on the method type (.meth or .spda), these discrete channel plots are displayed: • For display methods (.spda), the plot shows intensity (counts) normalized to 100 percent on the *y* axis and time on the x axis. • For instrument methods (.meth), the scan plot shows absorbance (mAU) normalized to 100 percent on the *y* axis and time on the *x* axis. Each discrete channel is color coded. Channel A is yellow, Channel B is purple, and Channel C is blue.

Information Page

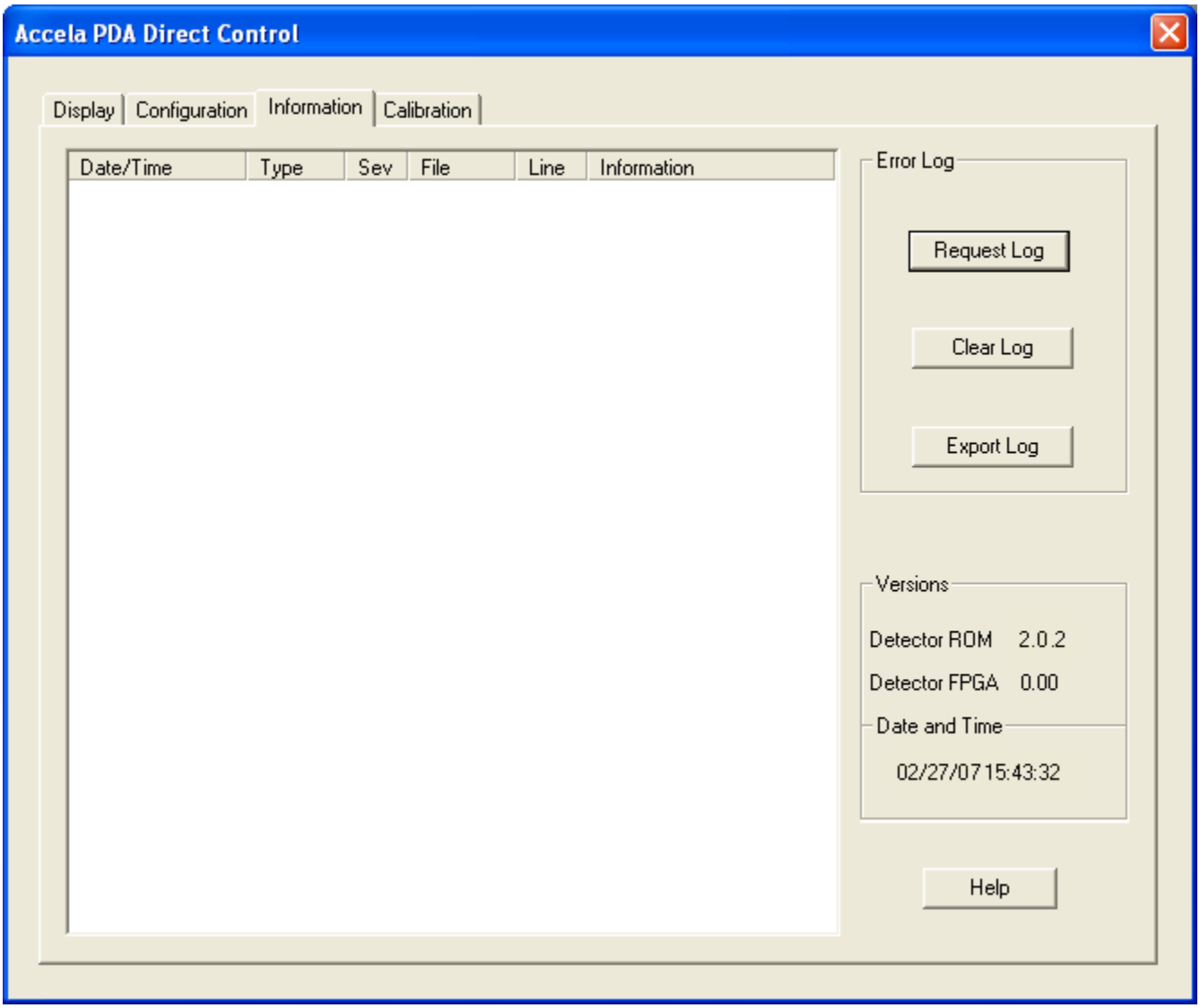
Use the Information page to monitor the chronological run log, instrument version, and system date and time. The error log is an ongoing record of critical and non-critical errors in the PDA detector. The log is generated and stored in the detector. This data is useful for troubleshooting hardware and software problems.

❖ To open the Information page

1. Open the Direct Control dialog box for the PDA detector (see "PDA Detector Direct Controls" on page 166).
2. Click the **Information** tab.

   The Information page appears (see Figure 102).

   Figure 102. Information page



Information Page Parameters

Table 46 describes the parameters on the Information page.

Table 46. Information page parameters

Parameter	Description
Error Log	
Error Log	Displays errors that are generated by the PDA detector. The error log can hold up to 100 errors. When the error log is full, the newest error log entry replaces the oldest error log entry. The error log is protected by a backup battery when the detector is turned off.
	Tip For best results, save the error log (print to file) before you clear it. The error log is useful during preventive and problem maintenance.
Buttons	
Request Log	Downloads the error log that is stored in the PDA detector and displays the results in the Error Log box.
Clear Log	Erases the current error log.
	Tip For best results, save the error log (print to file) before you clear it. The error log is useful during preventive and problem maintenance.
Export Log	Exports the log to a comma-separated values file that you can open with the Excel application.
Versions	
Versions display box	Displays the version number for the detector firmware. This information is useful to determine if firmware upgrades are necessary. Have this information handy whenever you contact your Thermo Fisher Scientific service representative.
Date and Time	
Date and time	Displays the current system date and time. This is the time that the data system uses for entries in the error log and the time stamp on the snapshot file.

UV-Vis Detector Direct Controls

Use the Direct Control dialog box for the UV/Vis detector to turn the lamps on or off and to zero the chromatographic baseline.

❖ **To turn the UV/Vis detector's lamps on or off**

1. Open the Accela UV/Vis Instrument Setup view.
2. From the menu bar, choose **Accela UV/Vis > Direct Control**.

   The Direct Control dialog box for the UV/Vis Detector appears.

   Figure 103. Direct Control dialog box for the UV/Vis Detector

3. In the Deuterium Lamp area, click **Lamp On**.

❖ **To zero the absorbance**

Click **Zero**.

UV/Vis Direct Control Parameters

Table 47 describes the buttons and readbacks in the Direct Control dialog box for the UV/Vis detector.

Table 47. Direct control parameters for the UV/Vis detector (Sheet 1 of 2)

Parameter	Description
Deuterium Lamp	
Lamp On	Turns the deuterium lamp on.
Lamp Off	Turns the deuterium lamp off.

Table 47. Direct control parameters for the UV/Vis detector (Sheet 2 of 2)

Parameter	Description	
Tungsten Lamp		
Lamp On	Turns the tungsten lamp on.	
Lamp Off	Turns the tungsten lamp off.	
Other Options		
Zero	Zeros the absorbance reading from the detector.	
Readbacks		
Status	Displays the state of the UV/Vis detector:	
	Ready	The detector is ready to start data acquisition.
	Off	The detector is not ready to start data acquisition because one or both lamps are off. Depending on the acquisition wavelengths specified in the downloaded instrument method, one or both lamps must be on. • For the UV range, the deuterium lamp must be on. • For the visible range, the tungsten lamp must be on. • When the wavelength table specifies wavelengths in both the UV and visible ranges, both lamps must be on.
	Running	The detector is acquiring data.
AU1	Displays the current absorbance value for the first wavelength channel.	
AU2	Displays the current absorbance value for the second wavelength channel.	

Pump Direct Controls

Use the Direct Control dialog box to download new solvent conditions to the pump, start and stop the solvent flow from the pump, and change the global pressure limits.

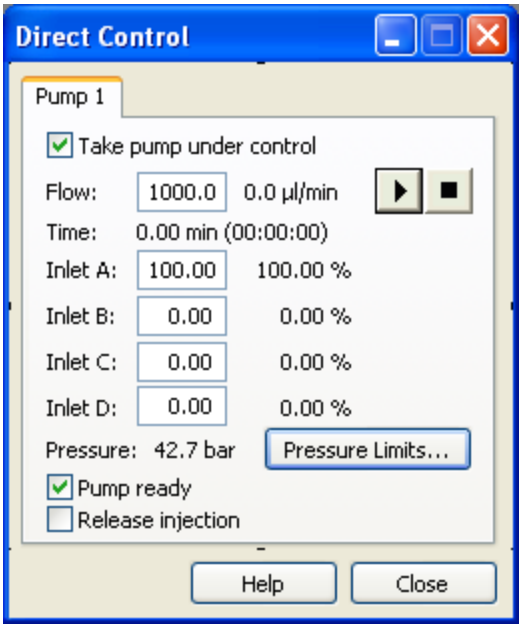
Tip To open the direct controls for the pump from the Tune window, choose **Setup > Inlet Direct Control** from the menu bar. Then click the pump tab.

❖ To download new solvent conditions to the pump

1. Turn on the power to the pump.
2. To open the Direct Control dialog box from the pump view, do the following:
   a. From the menu bar of the pump view, choose **Accela Pump**, **Accela 600 Pump**, or **Accela 1250 Pump > Direct Control**.

      The Direct Control dialog box for the pump appears (see Figure 104).

      Figure 104. Direct Control dialog box for the Accela pump

3. In the Direct Control dialog box, do the following:
   a. Select the **Take Pump Under Control** check box.
   b. In the Flow box, type an appropriate flow rate for the pump. If you are drawing fresh solvent through the lines and the liquid displacement assembly, type the maximum flow rate for the pump.

The flow rate range depends on the pump type:

Pump	Flow rate range
Accela Pump	0.1 to 1000 µL/min
Accela 600 Pump	10 to 5000 µL/min
Accela 1250 Pump	1 to 2000 µL/min

c. Type percentages for the solvent lines that you want to draw solvent through in the Inlet boxes (A, B, C, and D).

❖ **To start the solvent flow**

Click (Start).

❖ **To stop the solvent flow**

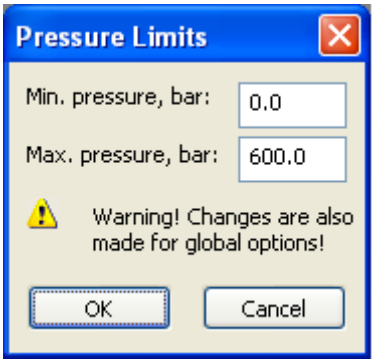
Click (Stop).

❖ **To change the global pressure limits**

1. Click **Pressure Limits**.

   The Pressure Limits dialog box appears (see Figure 105).

   Figure 105. Pressure Limits dialog box



2. In the Min. Pressure box, type the minimum operating pressure for the pump.
3. In the Max. Pressure box, type the maximum operating pressure for the pump.

   The maximum pressure for the Accela pumps depends on the pump type:

Pump	Maximum pressure		
	bar	MPa	psi
Accela Pump	1000	100	14 504
Accela 600 Pump	600	60	8702
Accela 1250 Pump	1250	125	18 130

4. Click **OK** to accept the settings and close the dialog box.

Autosampler Direct Controls

The On/Off switch is the only manual control provided with the autosampler. To perform tasks such as moving the XYZ arm to the back of the tray compartment, use the direct control commands in the autosampler view or the tune application for your Thermo Scientific mass spectrometer.

These topics describe the direct controls for the autosampler:

- Applying a Direct Command
- Direct Control Commands for the Autosampler
- Flushing the Autosampler Syringe
- Removing and Installing Sample Trays
- Controlling the Tray and Oven Compartment Temperatures

You can access the direct control commands for the autosampler from the autosampler view of your data system or the tune application for your mass spectrometer.

❖ To open the Inlet Direct Control dialog box from the tune application

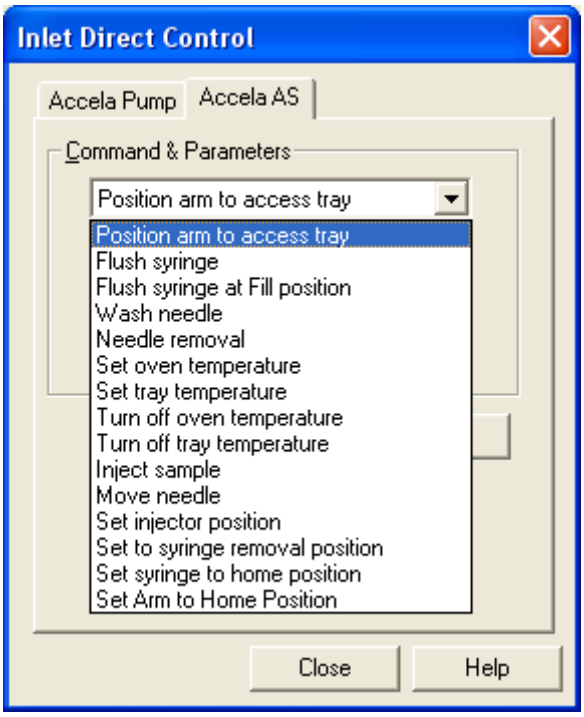
1. In the Tune window, choose **Setup > Inlet Direct Control**.

   The Inlet Direct Control dialog box appears with tabbed pages for each configured LC device.

2. Click the **Accela AS** tab.

   The Accela AS page appears (see Figure 106).

Figure 106. Inlet Direct Control dialog box for the Accela pump and autosampler

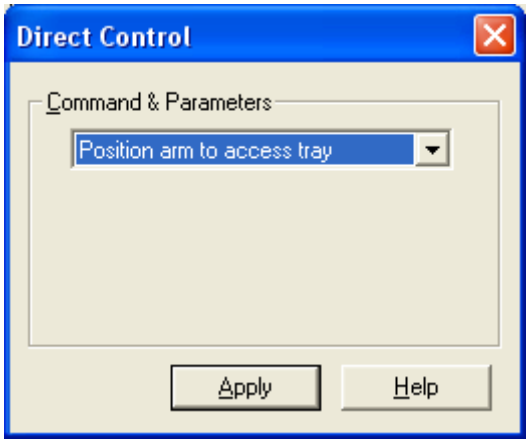


❖ To open the Direct Control dialog box

1. Open the autosampler view.
2. From the menu bar, choose **Accela AS > Direct Control**.

   The Direct Control dialog box appears (see Figure 107).

   Figure 107. Direct Control dialog box (Instrument Setup window)



Applying a Direct Command

❖ **To apply a direct command**

1. Select a command from the list of commands.

   If the command requires additional parameters, these parameters appear below the list. Make the appropriate entries and selections.

2. To execute the command, click **Apply**.

Direct Control Commands for the Autosampler

Table 48 describes the direct control commands for the autosampler.

Table 48. Direct control commands for the autosampler (Sheet 1 of 3)

Command	Description		
Position Arm to Access Tray	Moves the XYZ arm to the back of the tray compartment so that you can remove trays from or place trays into the tray compartment.		
	Note. If the tray compartment door is open and you selected the Verify Door Is Closed check box when you configured the autosampler, the autosampler does not execute this command until you close the tray compartment door.		
Flush Syringe	Flushes the needle tubing and the interior of the needle with flush solvent.		
	Parameter	**Selections or range**	
	Reservoir	RV1, RV2, RV3, RV4, or Bottle	
	Volume	0 to 6000 µL	
	Flush Speed	Depends on the syringe type as follows:	
		Syringe type	**Range**
		Concentric syringes	1.65 to 661.38 µL/s
		Standard 2500 µL syringe	0.83 to 330.85 µL/s
Flush Syringe at Fill Position	Flushes the needle tubing, the interior of the needle, and the sample loop with flush solvent. For the parameter descriptions, see Flush Syringe.		
Wash Needle	Washes the exterior of the needle with solvent.		
	Parameter	**Selections or range**	
	Reservoir	RV1, RV2, RV3, RV4, or Bottle	
	Volume	0 to 6000 µL	

Table 48. Direct control commands for the autosampler (Sheet 2 of 3)

Command	Description		
Needle Removal	Sets the needle to the needle removal position. Apply this command before you remove the needle from the XYZ arm.		
Set Oven Temperature	Sets the temperature of the column oven compartment without downloading an instrument method.		
	Parameter	**Range**	
	Temperature	5 to 95 °C	
	IMPORTANT Avoid setting the temperature above the boiling point of the mobile phase.		
Set Tray Temperature	Sets the temperature of the tray compartment without downloading an instrument method. The temperature range is 0 to 60 °C.		
	Parameter	**Range**	
	Temperature	0 to 60 °C	
Turn Off Oven Temperature	Turns off the oven temperature control, allowing the temperature of the column oven compartment to return to ambient.		
Turn Off Tray Temperature	Turns off the tray temperature control, allowing the temperature of the tray compartment to return to ambient.		
Inject Sample	Injects a sample.		
	Parameter		
	Vial	The vial or well entries depend on the tray configuration:	
		Tray type	**Vial locations**
		Conventional trays	A:01 to E:40
		96-well plates	A:A1 to C:H12
		384-well plates	A:A1 to C:P24
	Volume	For the no waste and partial loop injection modes, the minimum injection volume is 0.1 µL. The maximum volume depends on the syringe type:	
		Syringe	**Maximum volume**
		100 µL concentric syringe	20 µL
		250 µL concentric syringe	125 µL
		500 µL concentric syringe	300 µL
		2500 µL standard syringe	1250 µL
	Injection Mode	Full Loop, Partial Loop, and No Waste.	

Table 48. Direct control commands for the autosampler (Sheet 3 of 3)

Command	Description
Move Needle	Moves the XYZ arm to a specific vial or well location.
Set Injector Position	Switches the position of the injection valve. The injection valve has two positions: fill and inject.
Set Syringe to Removal Position	Sets the syringe to its removal position.
Set Syringe to Home Position	Sets the syringe to its home position.
Set Arm to Home Position	Moves the XYZ arm to its home position, which is just above the injection port.
Button	
Apply	Executes the command.

Flushing the Autosampler Syringe

To ensure the proper performance of the autosampler, remove air from the wash bottle tubing and the autosampler syringe before you make your first injection. Once you have your system running, periodically check the level of solvent in the wash bottle and remove air from the syringe as needed.

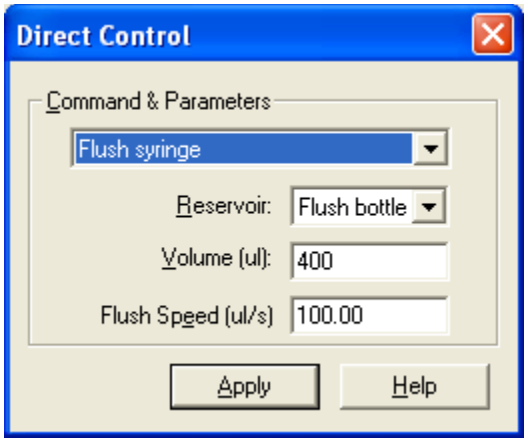
Tip During a flush operation, the autosampler draws wash solvent into the syringe and then pushes wash solvent through the needle tubing and the injection port to waste. If you want to remove residual sample from the sample loop, use the Flush Syringe at Fill Position command.

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

1. Open the view for the autosampler.
2. From the menu bar, choose **Accela AS > Direct Control**.

   The Direct Control dialog box for the Accela Autosampler appears (see Figure 108).

Figure 108. Flush syringe direct control command

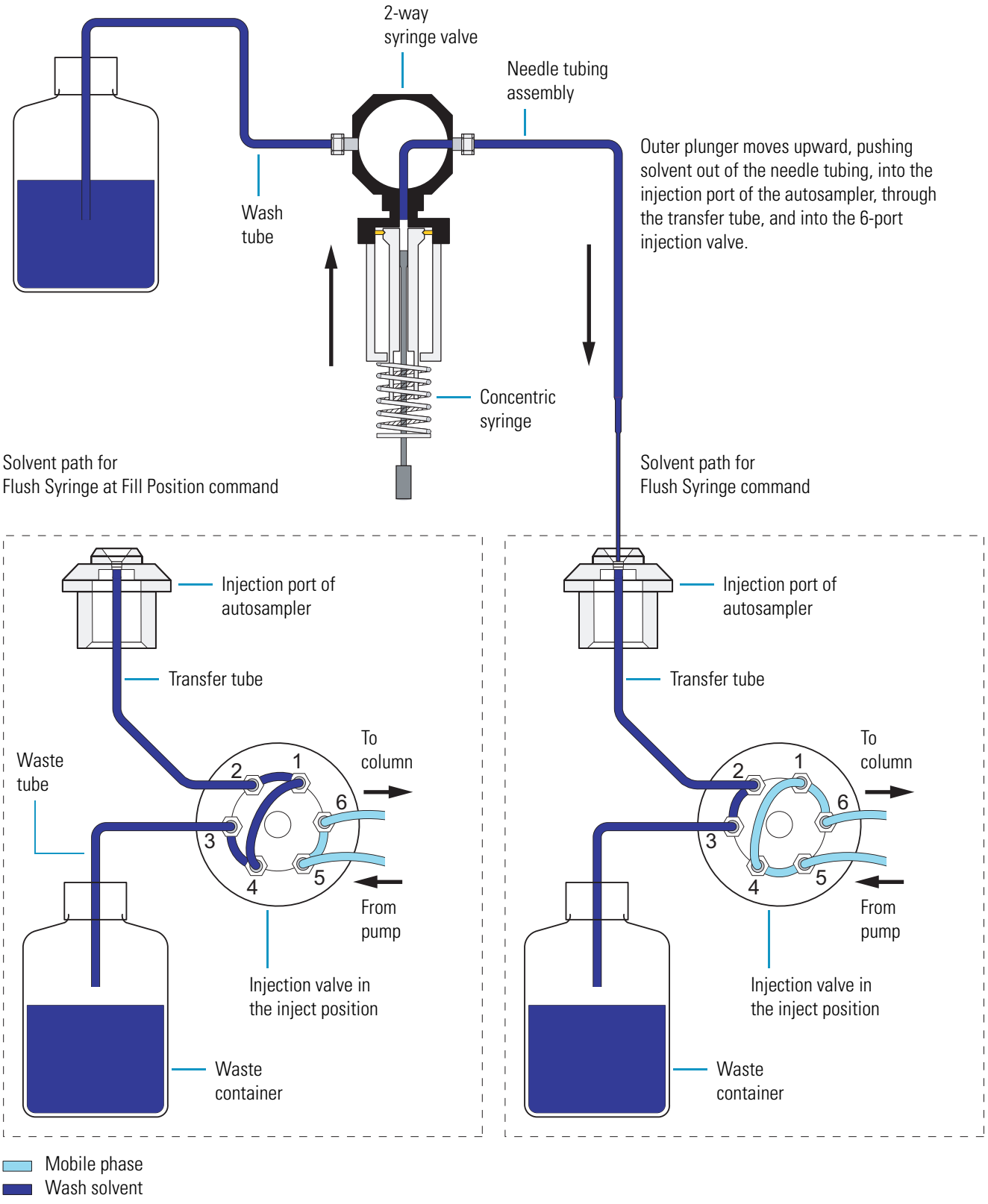


3. Initiate the Flush Syringe command:
   a. Select **Flush Syringe** from the list of commands.

      The parameters for the command appear below the list.
   b. In the Reservoir list, select **Flush Bottle**.
   c. In the Volume box, type an appropriate flush volume.

      The maximum flush volume is 6000 µL.
   d. Click **Apply** to download the command to the autosampler.
4. Verify that the wash bottle tubing and syringe are free of air (see Figure 109).
5. Close the Direct Control dialog box.

Figure 109. Solvent path for flushing the syringe



Removing and Installing Sample Trays

If you did not select the Verify Door Is Closed check box when you specified the configuration options for the autosampler, the XYZ arm does not move to the back of the tray compartment when you open the tray compartment door.

❖ To remove or install a sample tray

1. Open the Direct Control dialog box for the autosampler.
2. In the Direct Command list, select **Position Arm to Access Tray.**
3. Click **Apply**.

   The XYZ arm moves to the back of the tray compartment.

Controlling the Tray and Oven Compartment Temperatures

The autosampler has two controlled temperature zones: the tray compartment and the column oven compartment. Before you start a sequence run, equilibrate the controlled temperature zones at the temperature specified in the instrument method.

Use the direct commands in the Direct Control dialog box or the Inlet Direct Control dialog box to control the oven and tray compartment temperatures. For information about opening the Direct Control and Inlet Direct Control dialog boxes, see "Autosampler Direct Controls" on page 191.

❖ To download a new column oven temperature

1. Select **Set Oven Temperature** from the list of commands.
2. In the Temperature box, type an appropriate value for the column oven temperature.

   The range is 5 to 95 °C.

   IMPORTANT Do **not** set the oven temperature above the boiling point for the mobile phase solvent.
3. Click **Apply**.

❖ To turn the column oven off

1. Select Turn Off Oven Temperature from the list of commands.
2. Click **Apply**.

❖ To download a new tray compartment temperature

1. Select **Set Tray Temperature** from the list of commands.
2. In the Temperature box, type an appropriate value for the tray compartment temperature.

   The range is 0 to 60 °C.
3. Click **Apply**.

❖ To turn the column oven off

1. Select **Turn Off Tray Temperature** from the list of commands.
2. Click **Apply**.

Sequence Setup

This chapter describes how to acquire and view chromatographic and PDA spectral data using the Xcalibur data system.

Contents

Creating a Single Sample Sequence

To set up a sequence to inject a single sample, follow these procedures in order:

1. Opening the Sequence Setup Window
2. Creating the Sequence
3. (Optional) Selecting the Vial Locations Interactively
4. Saving the Sequence

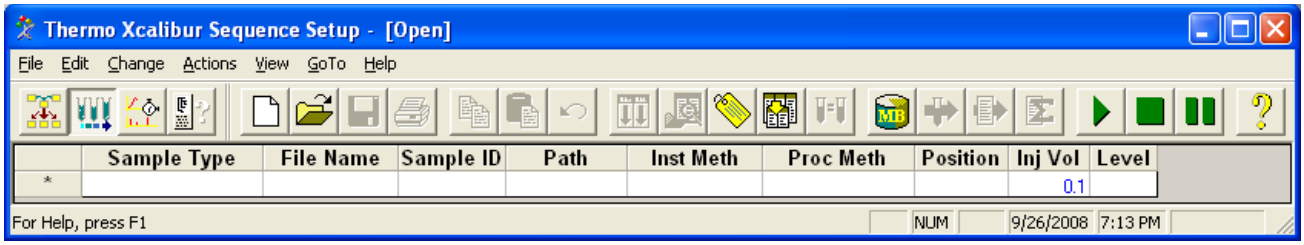
Opening the Sequence Setup Window

❖ **To open the New Sequence Template dialog box**

1. Click the **Sequence Setup** icon on the Home Page.

   The Thermo Xcalibur Sequence Setup window appears (see Figure 110).

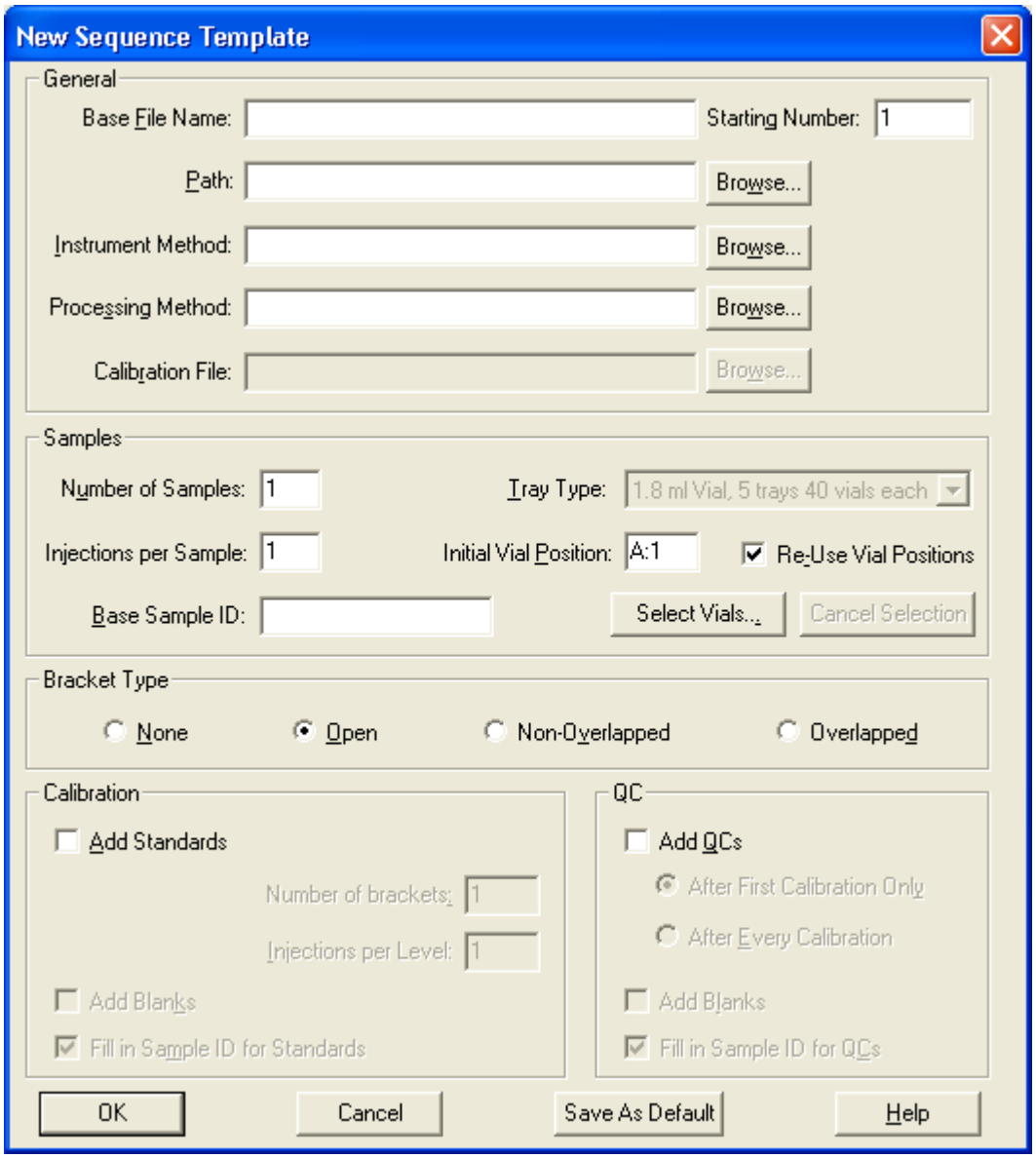
Figure 110. Sequence Setup window



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

   The New Sequence Template dialog box appears (see Figure 111). Go to the next topic, "Creating the Sequence" on page 203.

Figure 111. New Sequence Template dialog box



Creating the Sequence

❖ To create a new sequence

1. If it is not already open, open the New Sequence Template dialog box (see "Opening the Sequence Setup Window" on page 201).
2. In the General area, make the following entries and selections:
   a. In the Base File Name box, type a name for the raw data file.
   b. Browse to the data file directory where you want to store your raw data files.

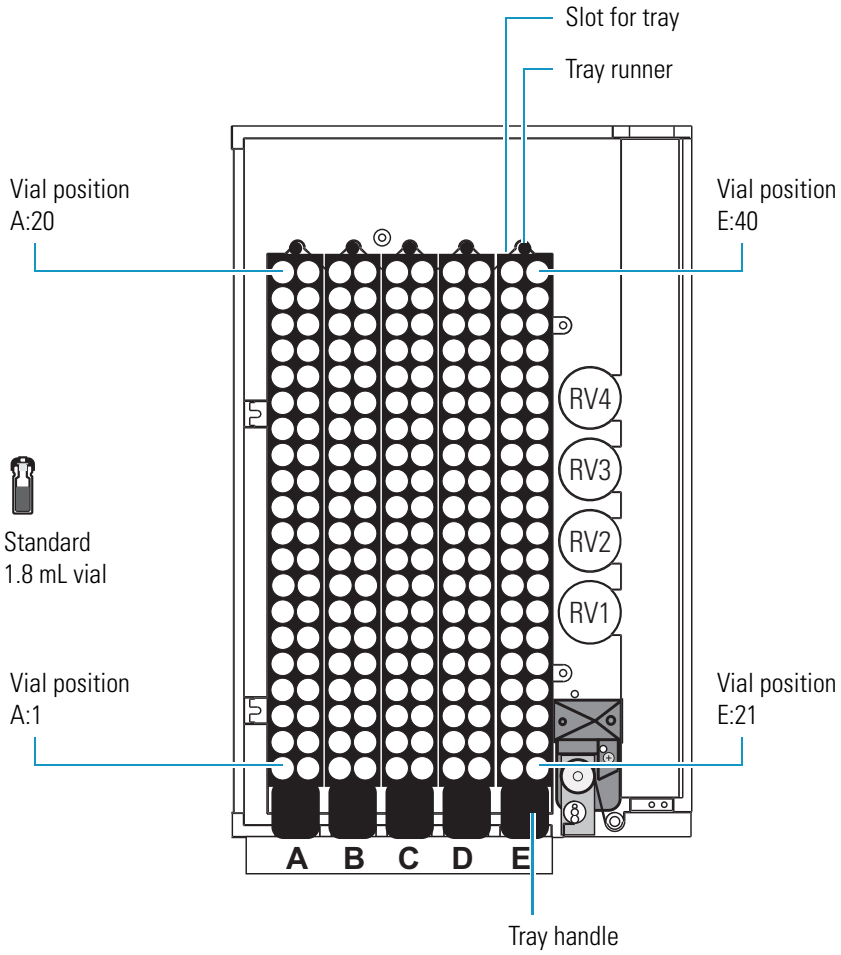
      The data system adds the .raw file extension to the data files that contain the chromatographic and spectral data.
   c. Browse to the instrument method that you want to use to acquire your raw data files.

      Instrument methods have a .meth file extension. The Instrument Setup view for the Accela devices is described in Chapter 3, "Instrument Method Setup."
   d. If you have not yet created a processing method that contains the information needed to quantitate your unknowns, do not select a processing method.

      You can create a processing method and reprocess your stored data files at a later date. Processing methods have a .pmd file extension. For information about performing tests to determine the suitability of your chromatographic method and on creating calibration curves to quantitate your unknowns, refer to the *Thermo Xcalibur Quantitative Analysis User Guide*.
3. In the Samples area, specify the vial locations by doing one of the following:
   - To specify a contiguous set of sample vials or well locations:
     – In the Number of Samples box, type the number of samples that you want to analyze.
     – In the Initial Vial Position box, type the vial position.

     The vial positions for the conventional trays are shown in Figure 112.

Figure 112. Vial positions for conventional sample trays



For more information about the vial locations for conventional trays and the well locations for microwell plates, see "Vial and Well Notation" on page 5.

- To select a non-contiguous set of sample vials or well locations, click **Select Vials**, and then select the vial or well locations by using the Vial Selection dialog box.

  For information about using the Vial Selection dialog box, see "Selecting the Vial Locations Interactively" on page 206.

4. In the Injections per Sample box, type the number of injections per sample that you want the autosampler to make per vial or well location.
5. In the Base Sample ID box, type an identifying name for the sample.

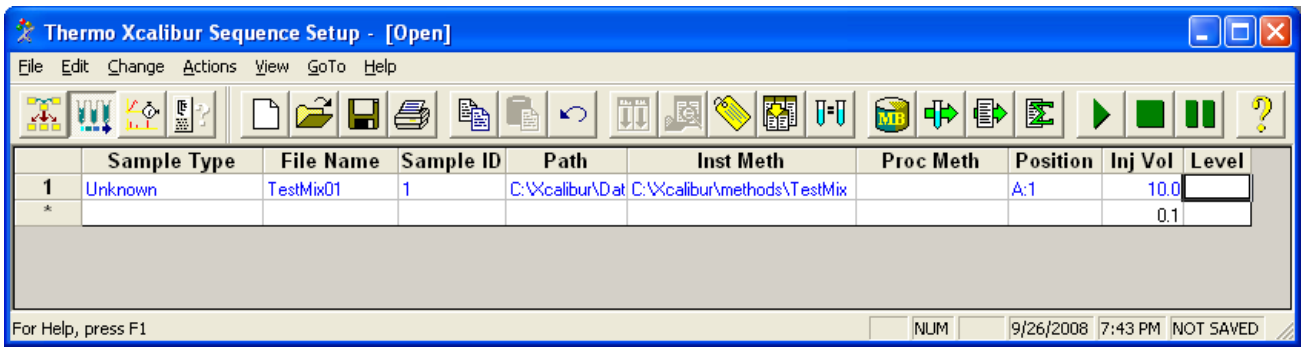
   Base sample IDs are optional. If you do not enter a sample identification, the data system automatically uses the vial position as the sample identification. If you enter a sample identification, the data system automatically appends the vial position to your entry.
6. Click **OK** to display your sequence spreadsheet (see Figure 113).

The injection volume displayed in the Inj Vol column matches the injection volume contained in your instrument setup method. You can override this injection volume value.

7. To change the injection volume, double-click the spreadsheet cell containing the injection volume value that you want to change, highlight the current value, and then type a new value in the cell.

   For full details of all the parameters in the New Sequence Template dialog box, refer to the Help or the Xcalibur manual set.

Figure 113. Sequence Setup view, showing newly created one-line sequence

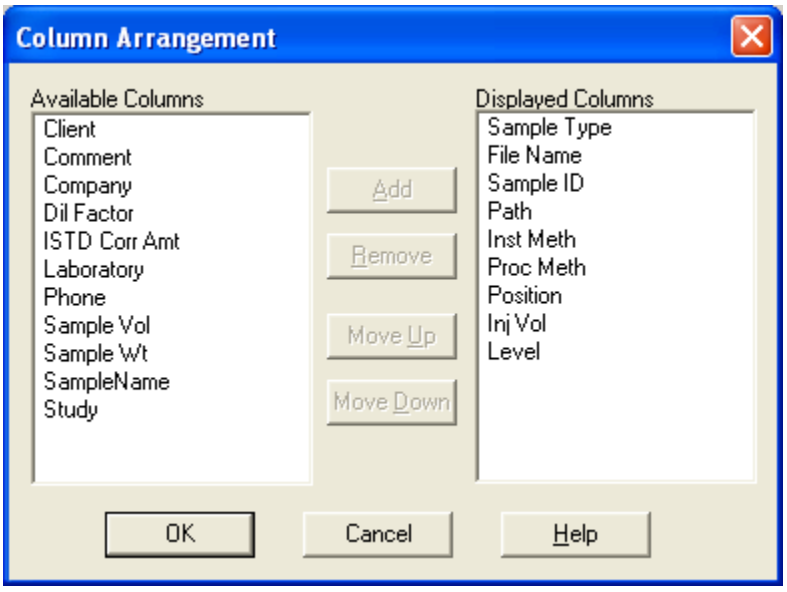


8. To alter the current column arrangement:

   a. Click the **Column Arrangement** toolbar button.

      The Column Arrangement dialog box appears (see Figure 114).

      Figure 114. Column Arrangement dialog box



b. Do one of the following:

- To add a column to the sequence, select the column from the Available Columns list, and click **Add**.
- To remove a column from the sequence, select the column from the Displayed Columns list, and click **Remove**.
- To alter the position of the columns in the sequence, select the column from the Displayed Columns list, and click either **Move Up** or **Move Down** as appropriate.

Selecting the Vial Locations Interactively

Use the Vial Selection dialog box to select specific vials for the sequence template.

❖ To interactively select vial or well locations for the sequence list

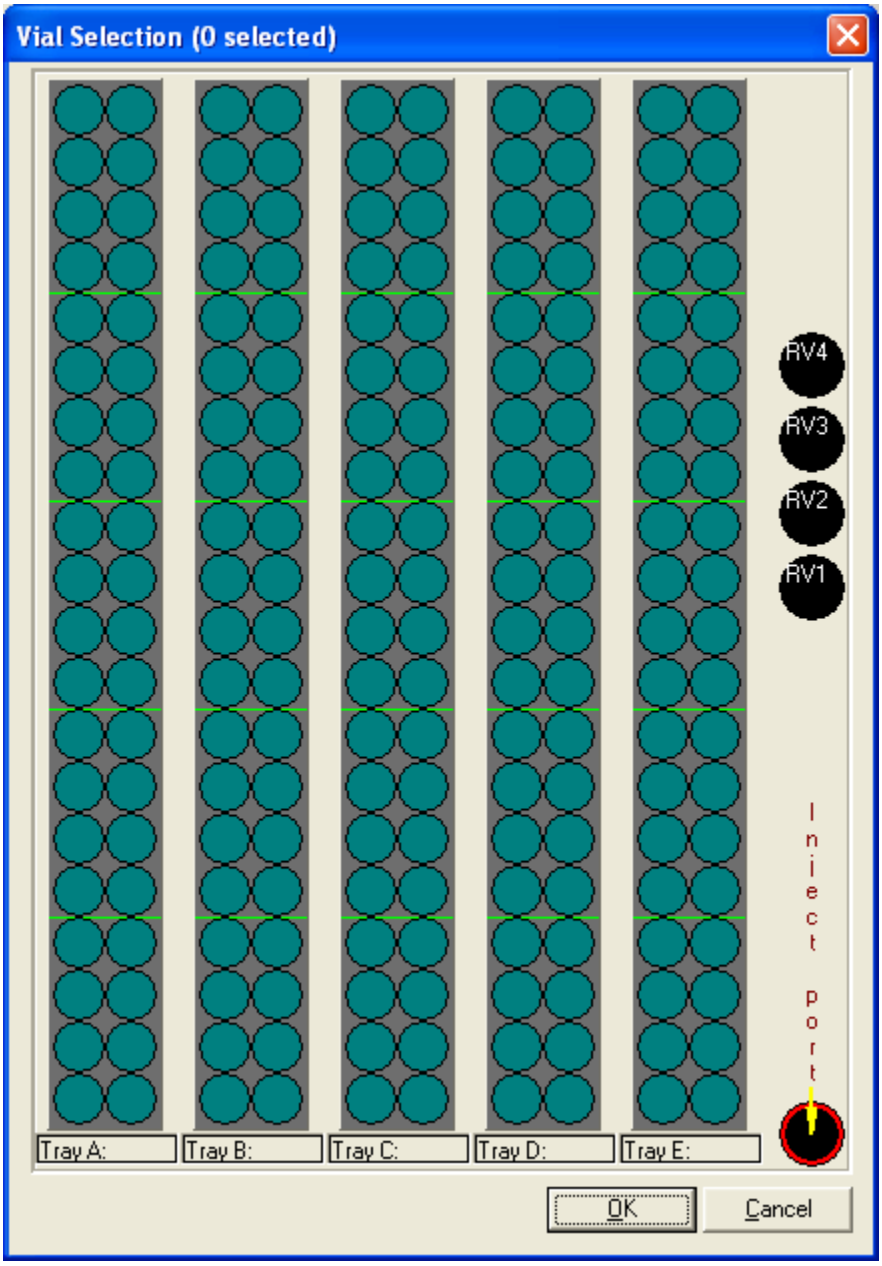
1. In the Sequence Setup window, choose **File > New**.

   The New Sequence Template dialog box appears.

2. In the Samples area, click **Select Vials**.

   The Vial Selection dialog box appears (see Figure 115).

Figure 115. Vial Selection dialog box



3. Click on a vial position to add it to the sequence template.
4. When you have finished making your selection, click **OK**.

   The vial or well locations appear in the position column in the order that you selected them.

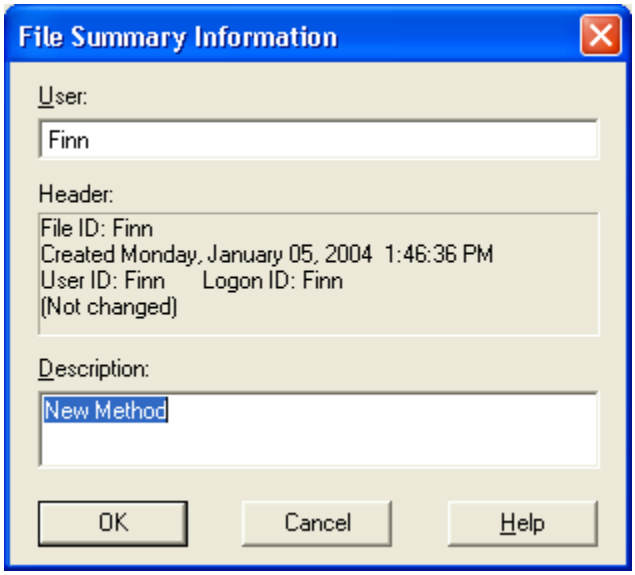
Saving the Sequence

❖ **To save the sequence**

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

   The File Summary Information dialog box appears (see Figure 116).

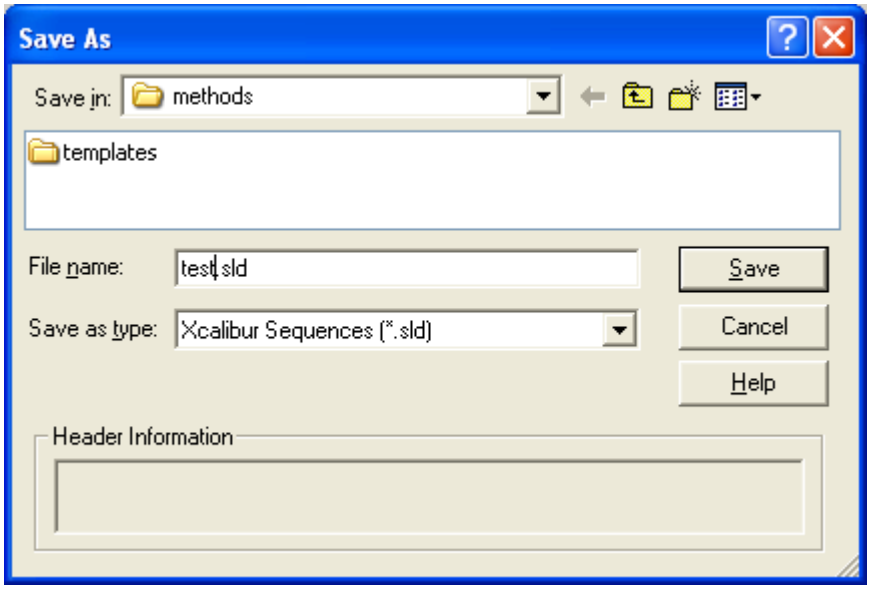
   Figure 116. File Summary Information dialog box



2. In the Description box, type an appropriate description. Then click **OK**.

   The Save As dialog box appears (see Figure 117).

   Figure 117. Save As dialog box, showing the file extension for a sequence file



3. Browse to the appropriate folder where you want to save the sequence.

4. In the File Name box, type a file name.
5. Click **Save**.

Equilibrating the Chromatographic Column

Warming up the deuterium lamp and equilibrating the LC column reduces baseline drift. For information about warming up the D2 lamp, see "Turning the Lamps On or Off" on page 167.

An LC column requires 10 to 20 column volumes to equilibrate to the initial mobile phase conditions of an instrument method. For example, it takes approximately 17 mL of mobile phase to equilibrate a typical 4.6 mm ID×10 cm length column.

To calculate the volume of an LC column, use the following equation:

$$V_m = \pi r^2 \times L$$

Where:

V_m = volume in mL

r = column radius in cm (radius = inner diameter/2)

L = column length in cm

❖ To equilibrate your LC column

1. From the Thermo Xcalibur Roadmap menu, choose **GoTo > Instrument Setup** to display the Instrument Setup window.
2. Download the same solvent percentages and flow rate as those contained in your instrument method to the pump.

   For information about downloading new solvent conditions to the Accela pump and starting the solvent flow, see "Pump Direct Controls" on page 189.
3. In the status view for your pump (available on the Status page of Info View), monitor the readings in the Pressure Status area to ensure that the pressure is appropriate for your application. See "Viewing the Status of Each Device" on page 138.

Loading the Autosampler

Before you load your samples into the autosampler, ensure that your samples are completely soluble in the mobile phase and that you have filtered your samples through a 0.5 µm filter (if necessary). These techniques minimize sample precipitation in the lines and remove particulate matter that could obstruct the flow through the autosampler's injection valve or the LC column. In addition, make sure that the vial caps are securely fastened onto the vials.

Before you start a sequence run, ensure that you have samples in the locations specified in your sequence.

Note To trigger the vial sensor, make sure to position custom vials in the tray so that the top of the vial reaches the minimum height of 1.55 inches. If you place vials that fall below this minimum height in the tray, the vial sensor does not detect them. When the sequence reaches a vial that is below the minimum height, the sequence halts, and the Vial Not Found message appears.

❖ To load a conventional tray into the autosampler

1. Open the left door of the autosampler.

   If you configured the Accela AS device driver to verify that the tray compartment door is closed, the XYZ arm automatically moves to the back of the tray compartment.

   Figure 33 on page 51 shows the location of the Verify Door Is Closed check box, available in the Instrument Configuration window.

2. If necessary, use the direct controls to move the XYZ arm to the back of the tray compartment (see "Autosampler Direct Controls" on page 191).

3. Hold the tray handle, tilting the back end of the tray down. Insert the tray runner into the slot at the rear of the tray compartment. Lower the front of the tray into place. Then press down firmly to seat the tray.

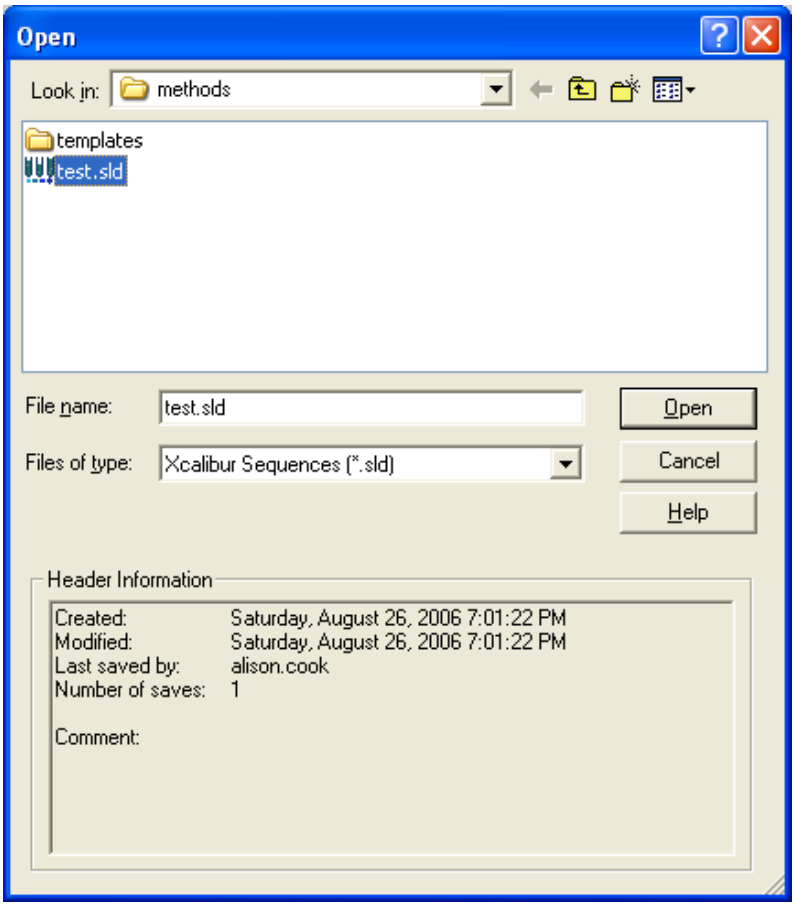
Starting Data Acquisition

❖ **To inject a sample and start data acquisition**

1. Open the sequence file containing the information for the sample that you want to inject:
   a. From the Sequence Setup view, choose **File > Open**.

      The Open dialog box appears (see Figure 118).

      Figure 118. Open dialog box, showing the selection of a sequence file

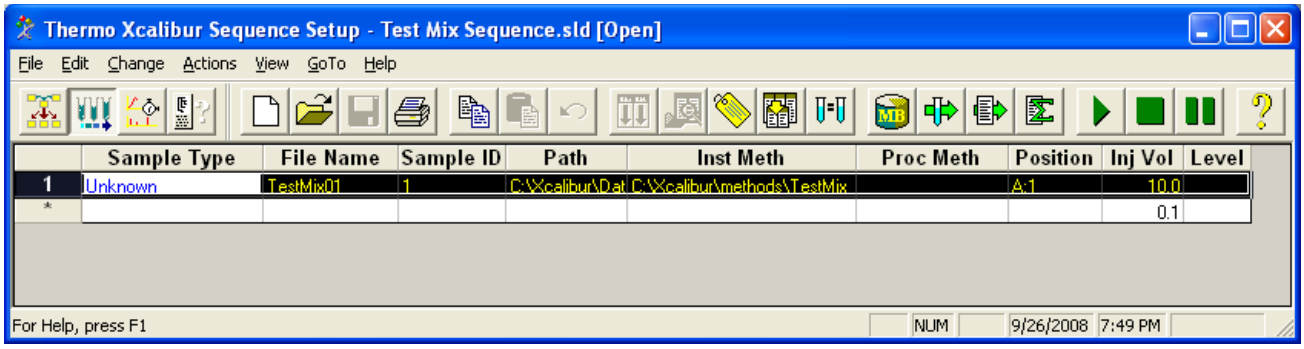


   b. Browse to the appropriate folder.
   c. Select the sequence that contains the sample you want to run.

      You can identify sequence files by their .sld file extension.
   d. Click **Open**.

      The selected sequence appears.
2. Highlight the sequence row that you want to run. Do this even if the sequence contains just one row. Figure 119 shows a highlighted sequence row.

Figure 119. Sequence Setup view, showing the first row selected



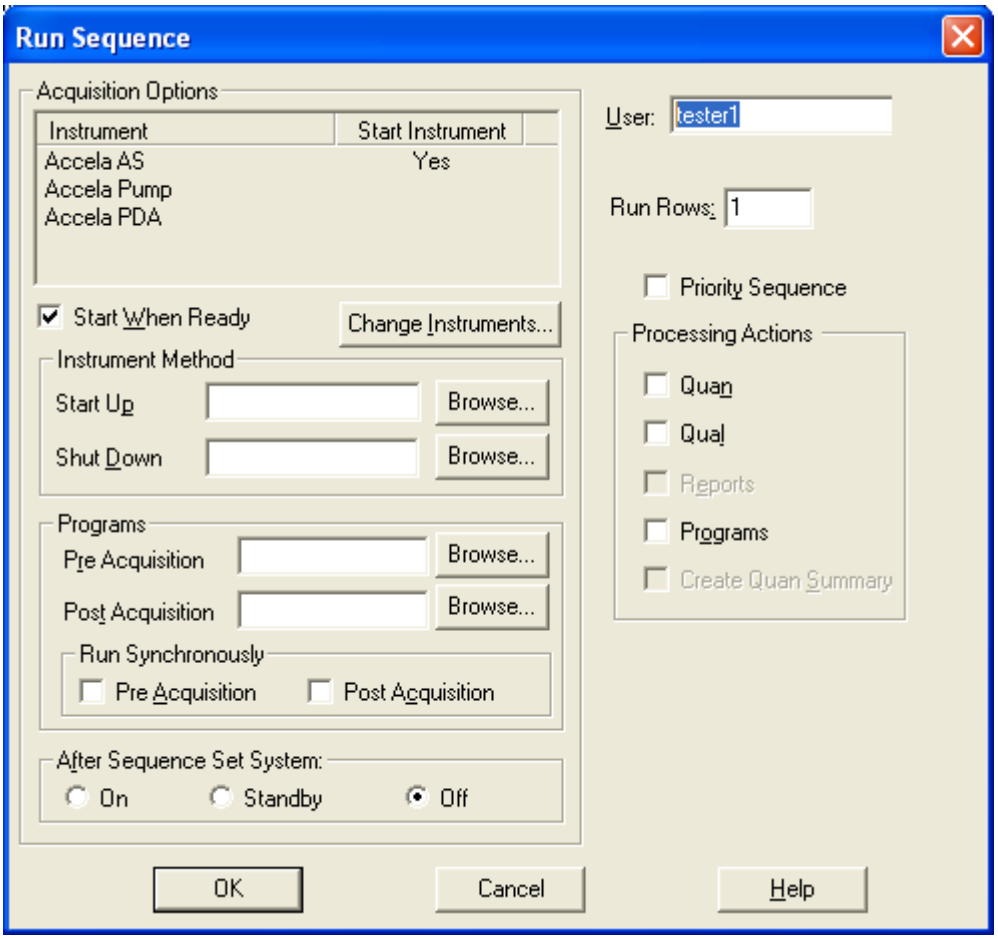
3. Confirm that you have a vial in the position specified in the sequence row.

4. From the toolbar, click the **Run Sample** button.

   The Run Sequence dialog box appears (see Figure 120).

   The User box contains your login name, and the Run Rows box contains the row number that you selected in the sequence spreadsheet.

Figure 120. Run Sequence dialog box



5. Confirm the following:

   - The MS detector and LC components are configured for operation as Xcalibur devices in the Instrument list.
   - The autosampler is specified as the start instrument. Yes is displayed in the Start Instrument column next to Accela AS (see Figure 121).

     Figure 121. Accela AS specified as the start instrument

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

6. Set up how the run is started as follows:

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

     After you complete the entries and selections in the Run Sequence dialog box and click OK, the run begins after the pump sends a pump ready signal to the autosampler. The pump does not indicate the Ready state until it monitors a stable backpressure as defined in your instrument method.

   - To start the run manually, clear the **Start When Ready** check box.

7. In the After Sequence Set System area, select one of these three options:

   - To keep the devices in the On state, select the **On** option.
   - To automatically turn off the solvent flow from the pump and the detector's lamps at the end of the sequence run, select the **Off** option.
   - To turn off the solvent flow from the pump but keep the lamps at the end of the sequence run, select the **Standby** option.

8. Keep the parameters in the other areas at their defaults.

   You can use the Instrument Method and Programs areas in the Run Sequence dialog box to specify particular acquisition or processing requirements. For full details of these features, refer to the Xcalibur Help.

9. Start the run as follows:

   - For automated runs, click **OK** to start the run.
   - For manual runs, click **OK**, and then choose **Actions > Start Analysis** from the Sequence Setup menu. To subsequently control the acquisition, choose **Actions > Pause Analysis** or **Actions > Stop Analysis**.

Working with the Real Time Plot View

To view and review data as it is being acquired in the Real Time Plot view, follow these procedures:

- Viewing Data Acquisition
- Reviewing Real-Time Data
- Adding Cells to the Display

Viewing Data Acquisition

❖ To view the data as it is acquired

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

Locked 

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

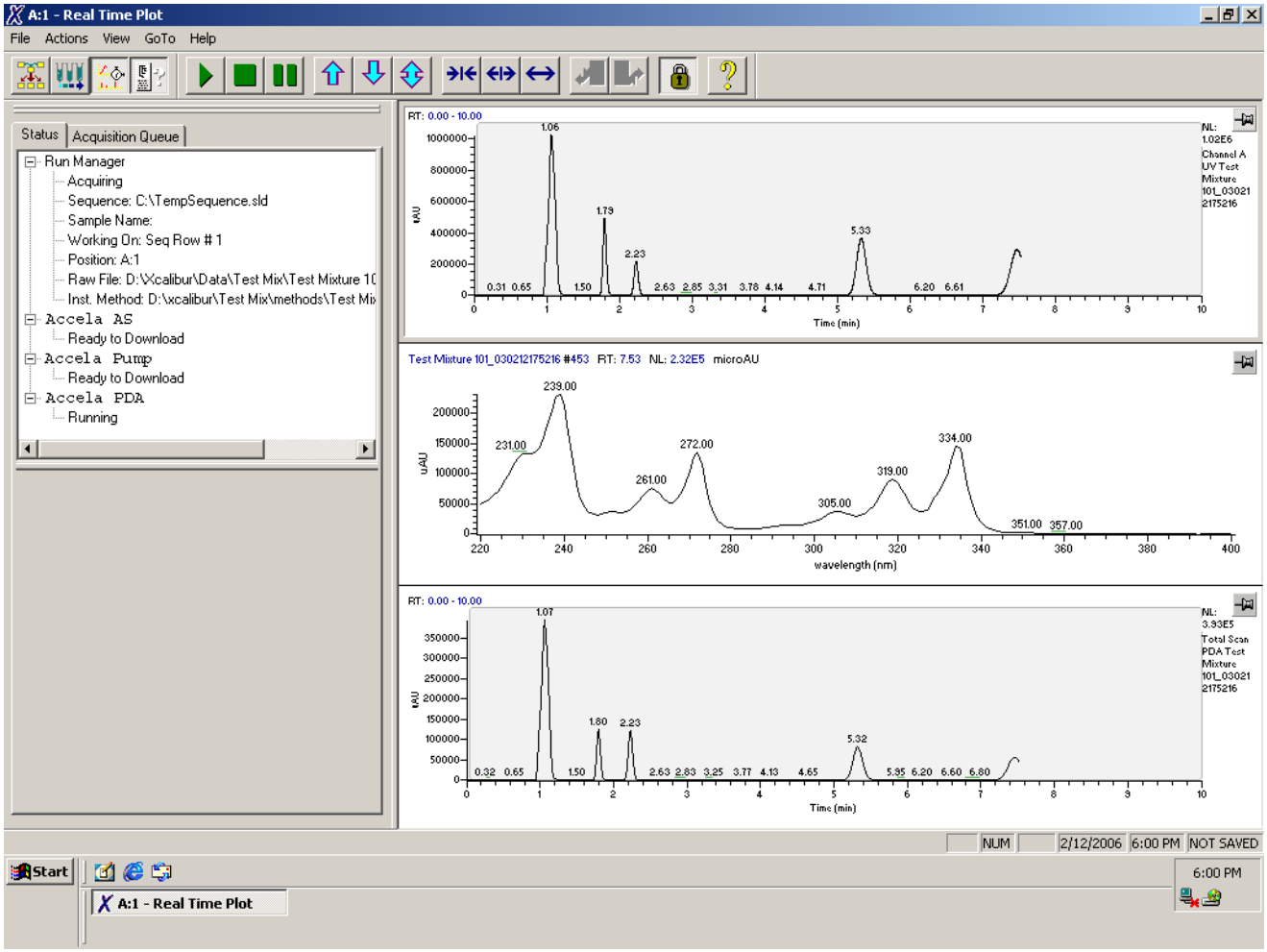
   Locking the display lets you monitor the real-time progress of your run.

Unlocked 

   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 122 is displayed. The view contains three cells: a chromatogram cell, a spectrum cell, and a Total Scan cell.

Figure 122. Real Time Plot view, showing the acquisition of PDA scan data and one discrete channel



Reviewing Real-Time Data

You can review the data as it is being collected.

❖ To view the spectrum for a particular peak in the chromatogram

1. To unlock the display, click the **Lock Display** button.

   After you unlock the display, data collection continues off screen.

2. Pin the spectrum cell by clicking the pin in the upper-right corner of the cell.

   The pin in the upper-right corner of the spectrum cell turns green. Cursor actions in other cells, such as the chromatogram cell, affect the view displayed in the pinned spectrum cell.

3. In the chromatogram cell, click the peak of interest.

   In the spectrum cell, a spectrum appears for the time point that you clicked.

4. Click the **Lock Display** button to resume monitoring real-time data acquisition.
5. Pin the chromatogram cell by clicking the pin in the upper-right corner of the cell.

   The pin in the upper-right corner of the chromatogram cell turns green. Cursor actions in other cells such as the spectrum cell affect the view displayed in the pinned chromatogram cell.
6. In the spectrum cell, click the wavelength of interest.

   In the chromatogram cell that contained the Total Scan chromatogram, a scan chromatogram appears for the specific wavelength that you clicked.
7. Click the **Lock Display** button to resume monitoring real-time data acquisition.

Adding Cells to the Display

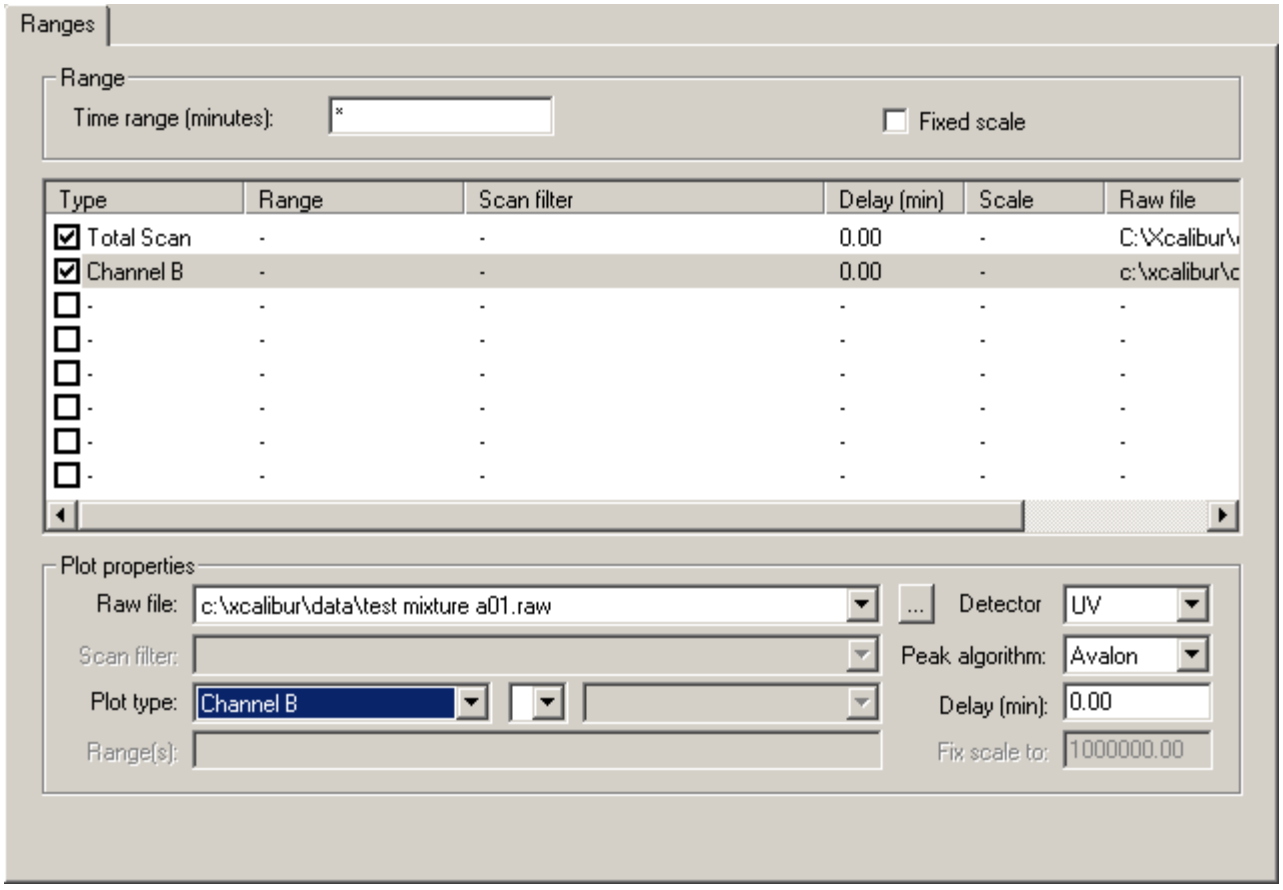
You can display multiple cells in the Real Time Plot view.

❖ To display multiple chromatogram cells

1. Click the chromatogram cell to make it the active cell with a gray border.
2. Choose **View > Ranges**.

   The Chromatogram Ranges dialog box appears (see Figure 123).

Figure 123. Chromatogram Ranges dialog box



3. For each cell that you want to add, do the following:
   a. In the Type column, select its corresponding check box.
   b. In the Detector list, select a detector.
   c. In the Plot Type list, select a plot type.
4. Click **OK** to close the Chromatogram Ranges dialog box.
5. Choose **View > Lock Display** to resume monitoring real-time data acquisition.

Qual Browser

This chapter provides an introduction to the Xcalibur Qual Browser functions that you can use to review the PDA data contained in your .raw data files.

Contents

Opening a Raw Data File in Qual Browser

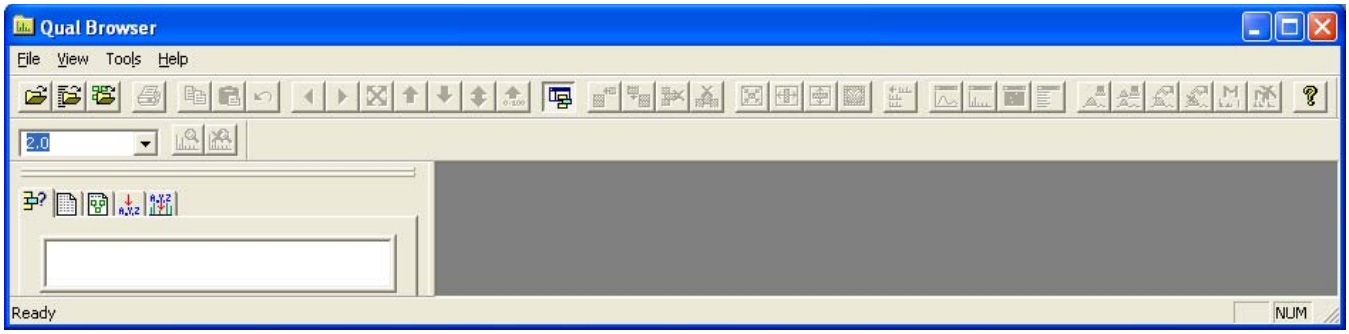
Data files containing the raw chromatographic and spectral data have the .raw file extension.

❖ **To open a .raw file in Qual Browser**

1. Click the **Qual Browser** icon in the Roadmap view of the Homepage window, or choose **GoTo > Qual Browser**.

   The empty Qual Browser window appears (see Figure 124).

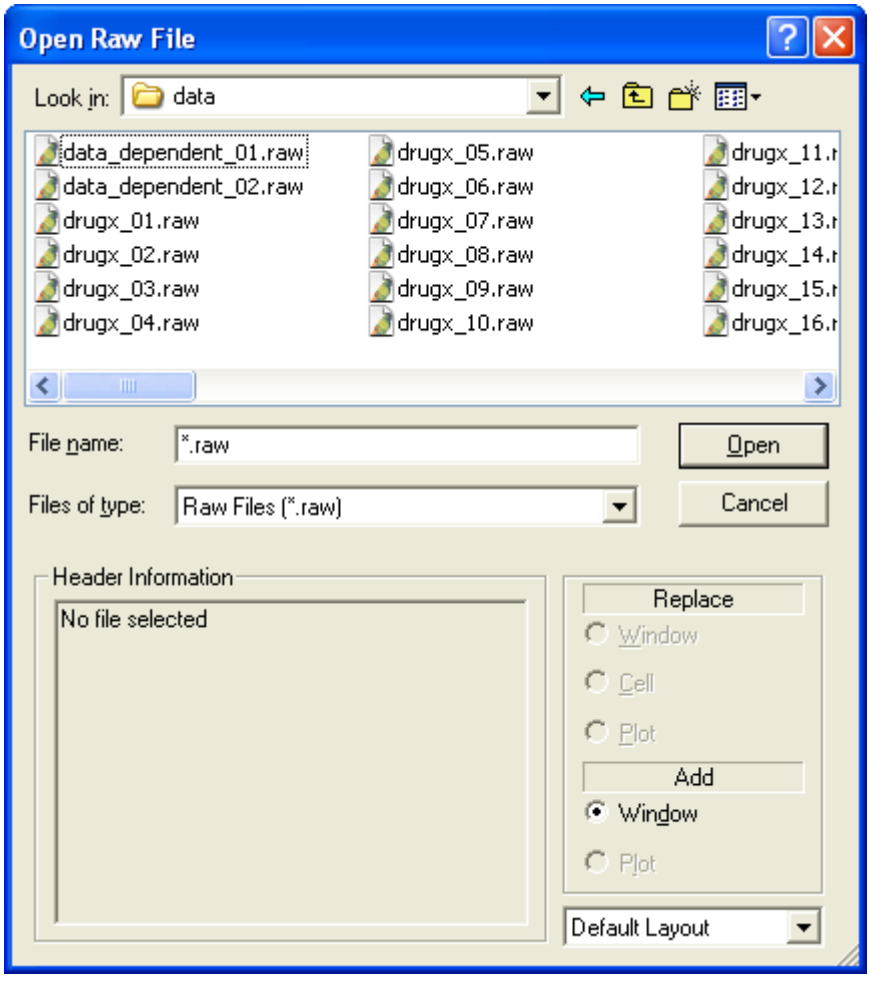
Figure 124. Empty Qual Browser window



2. Choose **File > Open**.

   The Open Raw Data File dialog box appears (see Figure 125).

   Figure 125. Open Raw File dialog box



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

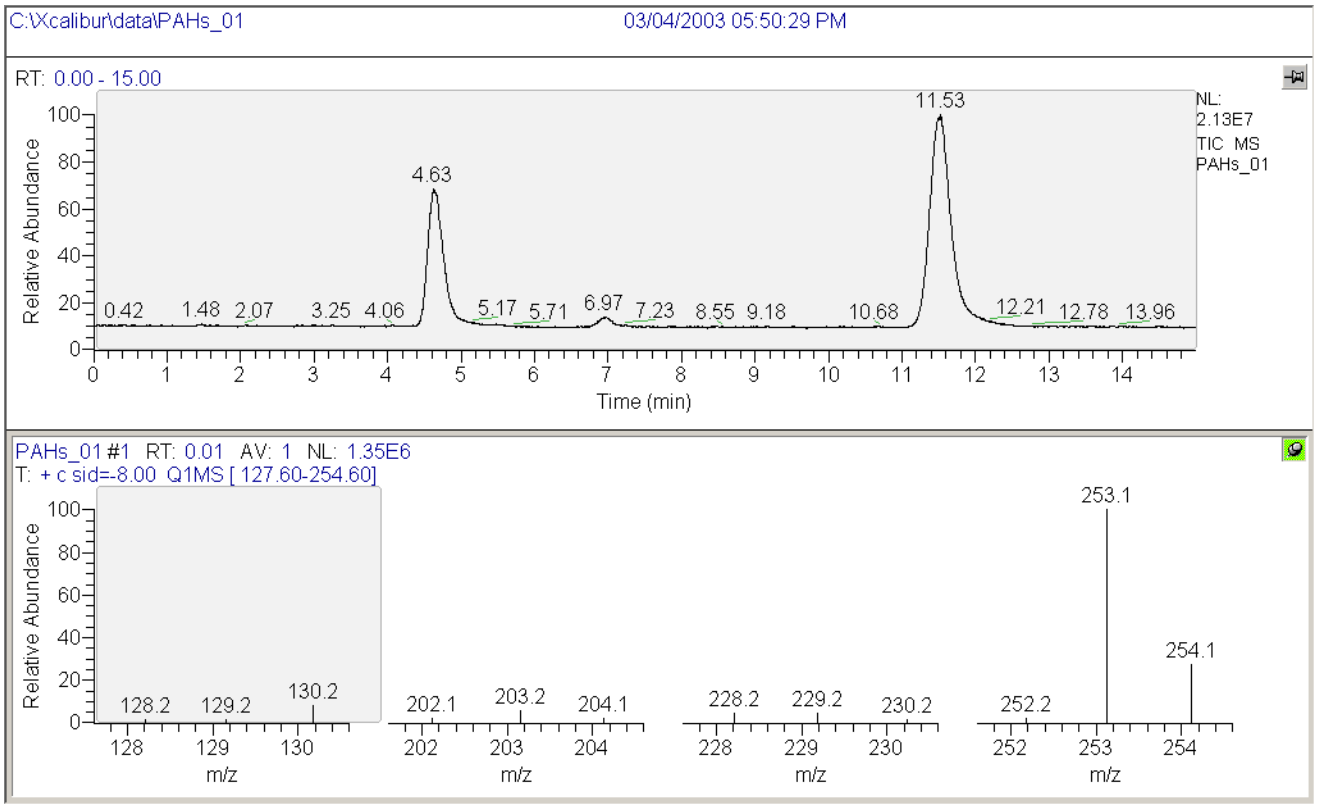
4. Select the layout from the list at the bottom of the dialog box.

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

5. Click **Open**.

   If the default layout has not been modified and your raw file contains MS data in addition to PDA or UV data, the data file opens with the MS TIC chromatogram in the upper cell and a mass range spectrum in the lower cell. For an explanation of cells, see "Working with the Cell Grid" on page 224. The *y* axis for these cells is set to relative absorbance (see Figure 126).

Figure 126. Qual Browser view with a chromatogram cell displaying MS TIC data and a spectrum cell displaying mass range data

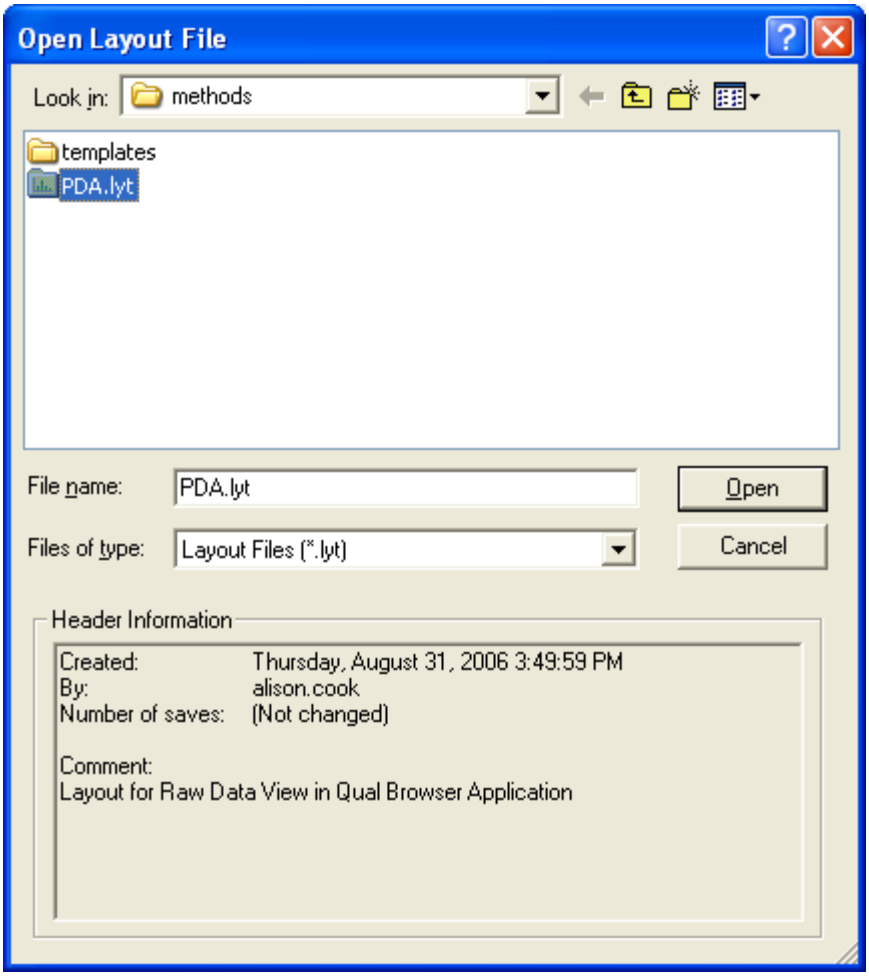


6. To apply a custom window layout to the Qual Browser view, do the following:

   a. Choose **File > Layout > Apply**.

      The Open Layout File dialog box appears (see Figure 127).

Figure 127. Open Layout File dialog box



b. Select a layout file from the list.

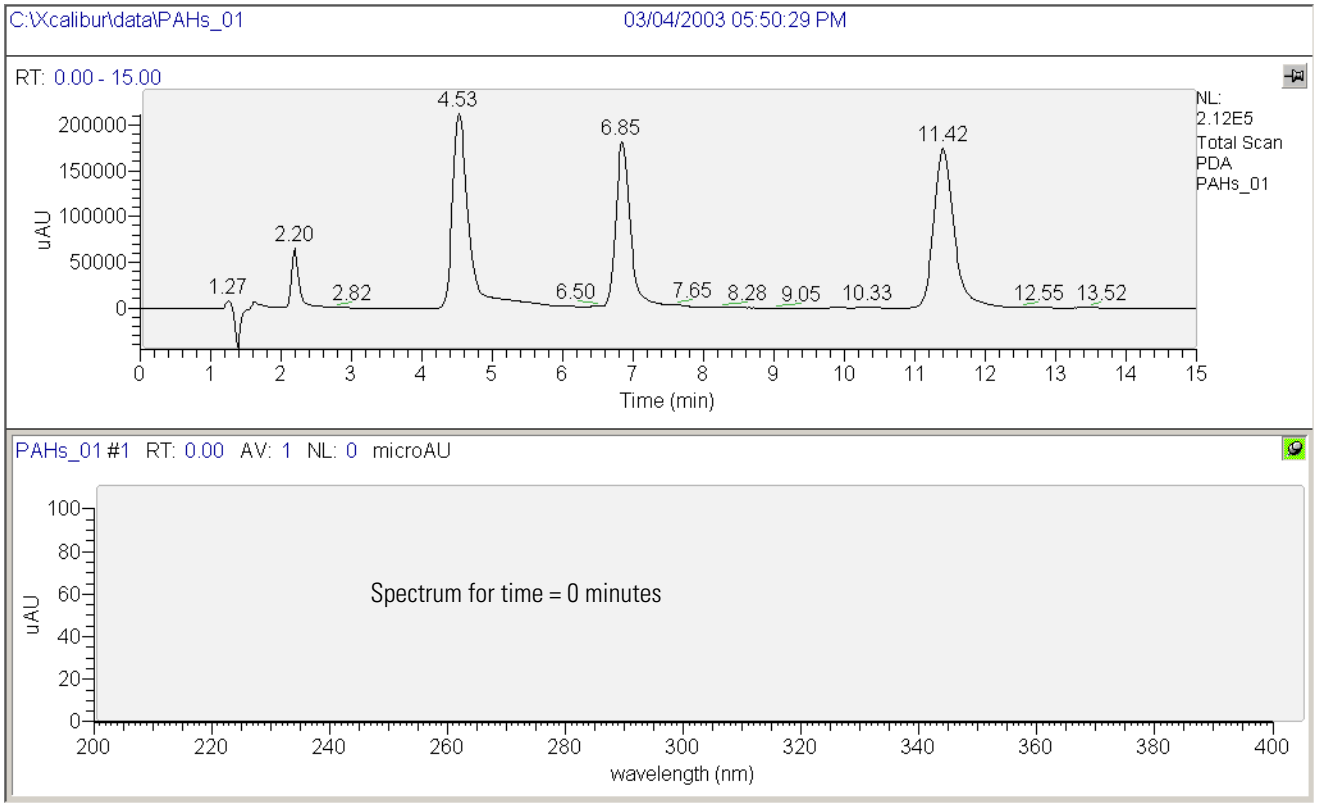
   Layout files have the .lyt file extension.

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

   Figure 128 shows a custom layout file applied to a raw data file containing both MS data and PDA data. The custom layout replaces the MS TIC chromatogram with a Total Scan chromatogram for the PDA data. It also replaces the mass range spectrum data from the MS detector with the spectral data from the PDA detector.

   For instructions on how to create a Layout file for your PDA data, see "Creating a Layout for PDA Data" on page 233.

Figure 128. Qual Browser view with 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 must understand the concept of cell states and the effect of cursor actions in a cell. This section contains the following topics:

- Cell States
- Cursor Actions

Cell States

When you open a raw data file in the Qual Browser window, the information within the data file is displayed as a grid of cells.

There are three hierarchal states for a cell within the grid:

- Inactive Cells
- Active but Unpinned Cells
- Active and Pinned Cells

The grid always contains either one active but unpinned cell or one pinned cell. If the grid contains more than one cell, only one cell can be active and the rest of the cells are inactive.

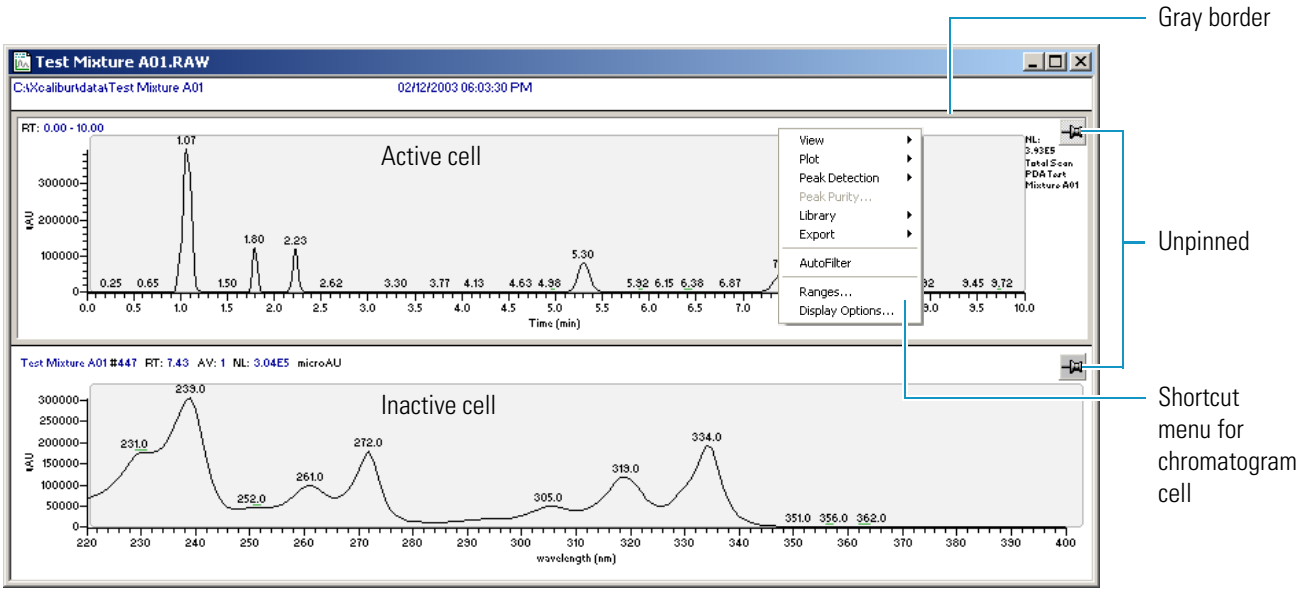
An unpinned cell has a gray pin icon in its upper-right corner.

A pinned cell has a green pin icon in its upper-right corner.

Inactive Cells

Inactive cells are not highlighted with a gray border and the pin icon in their upper-right corners is gray. The cell in the lower portion of Figure 129 is inactive as indicated by the absence of a gray border. Menu commands, toolbar buttons, and cursor actions do not affect inactive cells. To zoom in on the contents of a cell or access its shortcut menu, you must make the cell active.

Figure 129. Qual Browser window, displaying an active chromatogram cell and an inactive PDA spectrum cell



Active but Unpinned Cells

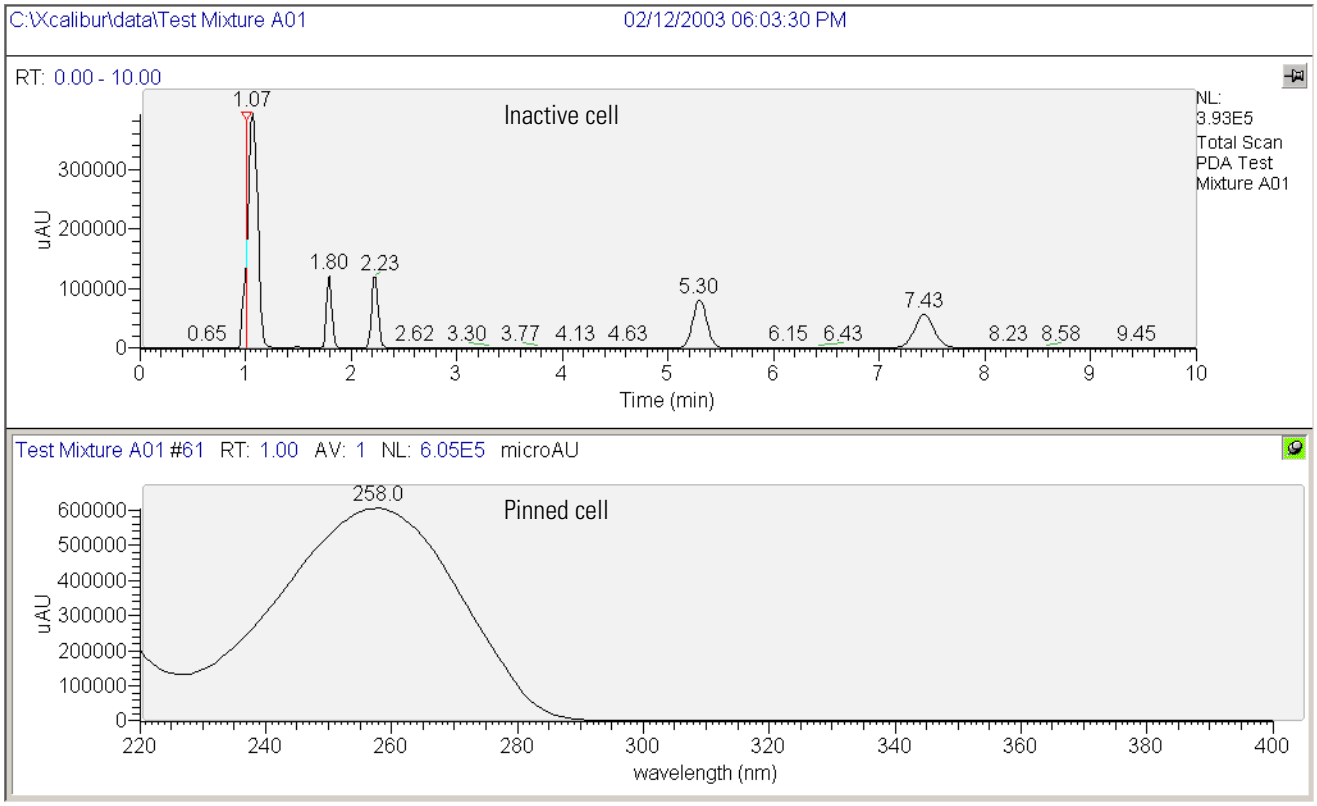
An active but unpinned cell is highlighted with a gray border, and the pin icon in its upper-right corner is gray. The cell in the upper portion of Figure 129 is active but unpinned. Menu commands, toolbar buttons, and cursor actions affect the active cell. If the grid does not contain a pinned cell, clicking an inactive cell in the grid makes it the active cell.

Active and Pinned Cells

Clicking the pin in the upper-right corner of a cell makes it the pinned cell within the grid. A pinned cell is an active cell that cannot be made inactive by clicking in another cell. Instead, actions performed in the inactive cells affect the pinned cell as described in the next topic, "Cursor Actions" on page 226. The lower cell in Figure 130 is a pinned cell.

To automatically change the range of a cell by clicking in the grid, you must pin the cell. For example, to display the spectrum for the 1 minute time point without opening the Spectrum Ranges dialog box, pin the spectrum cell, and then click the 1 minute time point in the inactive chromatogram cell.

Figure 130. 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 selects a point on the cell.
- A line dragged parallel to any axis selects 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 49).

Table 49. Effect of cursor action in an active cell

Cursor action	Effect
Drag parallel to *x* axis	Rescales graph showing selected *x*-axis range only, same *y*-axis range.
Drag parallel to *y* axis	Rescales graph showing selected *y*-axis range only, same *x*-axis range.
Dragged area	Rescales graph showing both the selected *x*-axis and *y*-axis ranges.

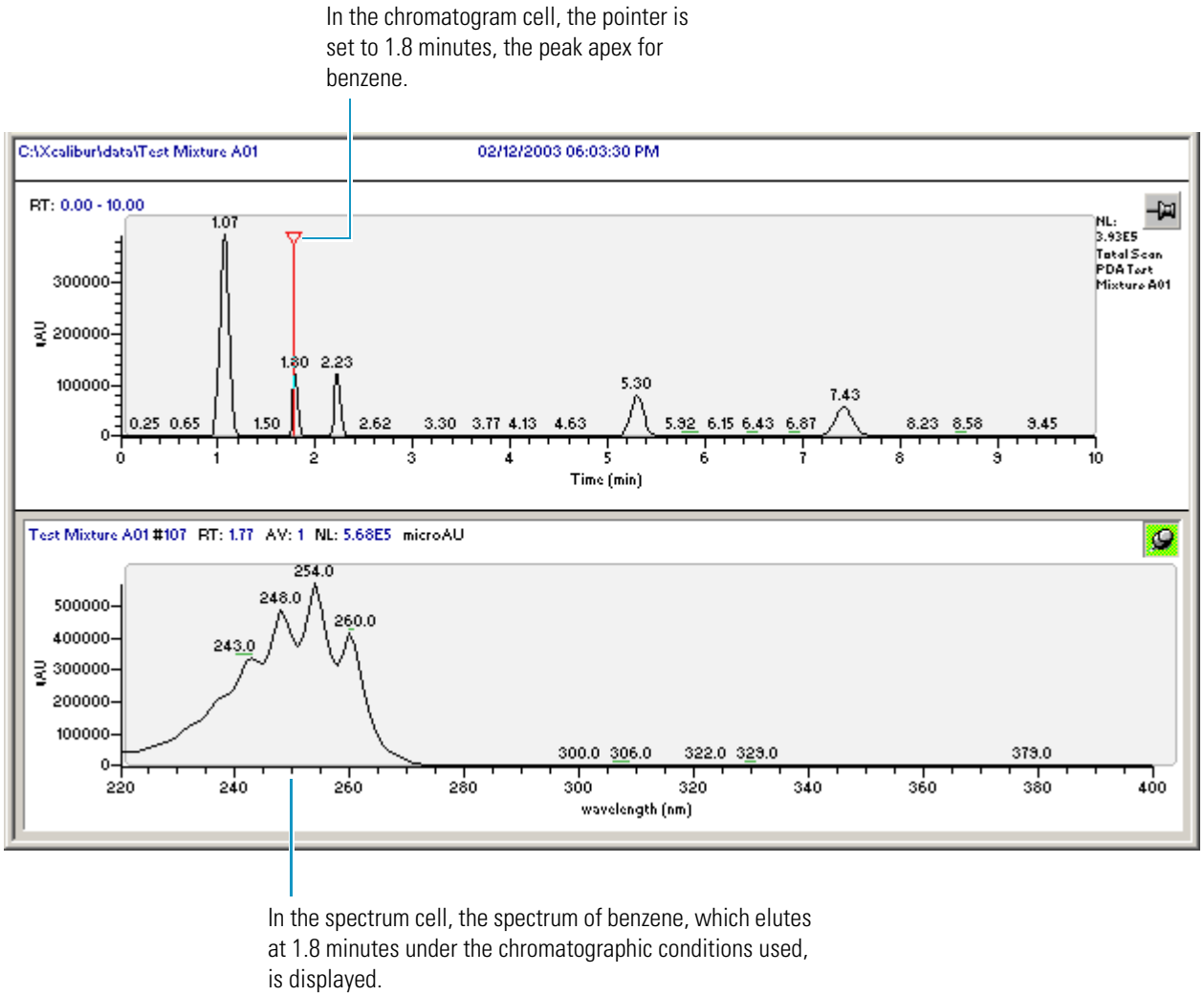
If one of the cells is pinned, the cursor action in any of the inactive cells affects the pinned cell (see Table 50).

Table 50. 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 the chromatogram for the wavelength selected in the spectrum cell.

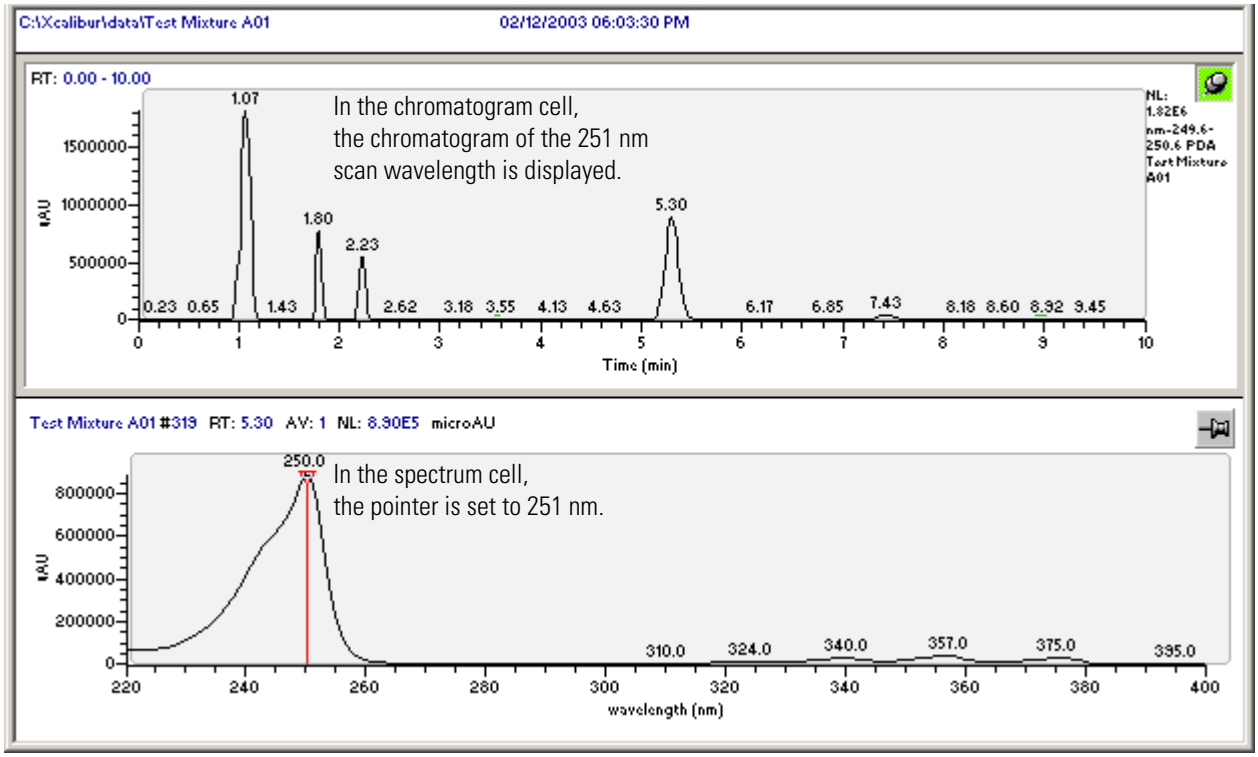
In Figure 131, the spectrum cell on the bottom of the view is pinned. Clicking the 1.80 minute time point in the unpinned chromatogram cell causes the spectrum of benzene, which elutes at 1.8 minutes, to appear in the pinned spectrum cell.

Figure 131. Qual Browser window with a pinned spectrum cell and an unpinned chromatogram cell



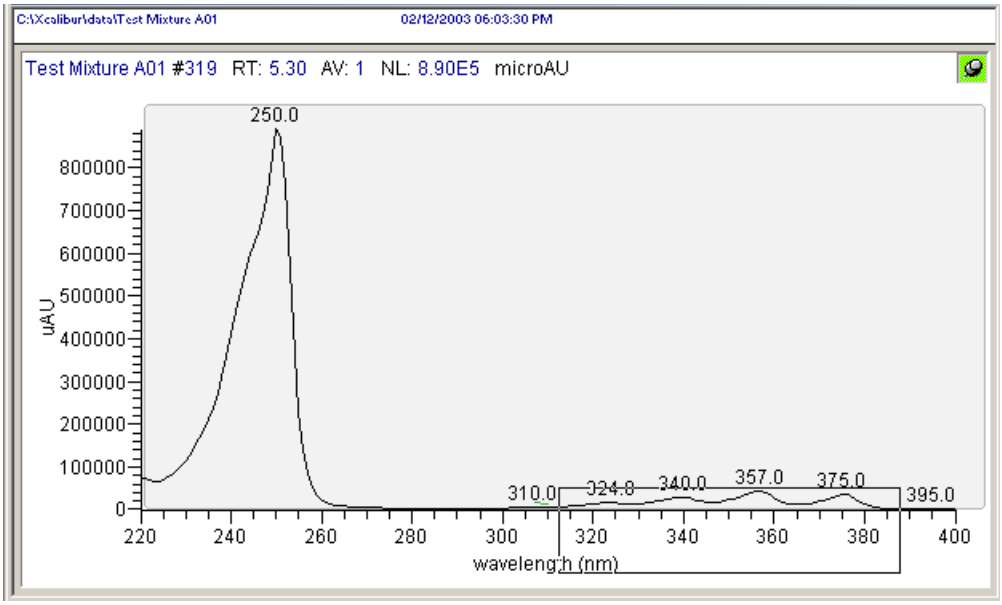
In Figure 132, the chromatogram cell on the top of the view is pinned. Clicking a specific wavelength in the unpinned spectrum cell displays the chromatogram of the scan wavelength in the pinned chromatogram cell.

Figure 132. Qual Browser window with a pinned chromatogram cell and an unpinned spectrum cell



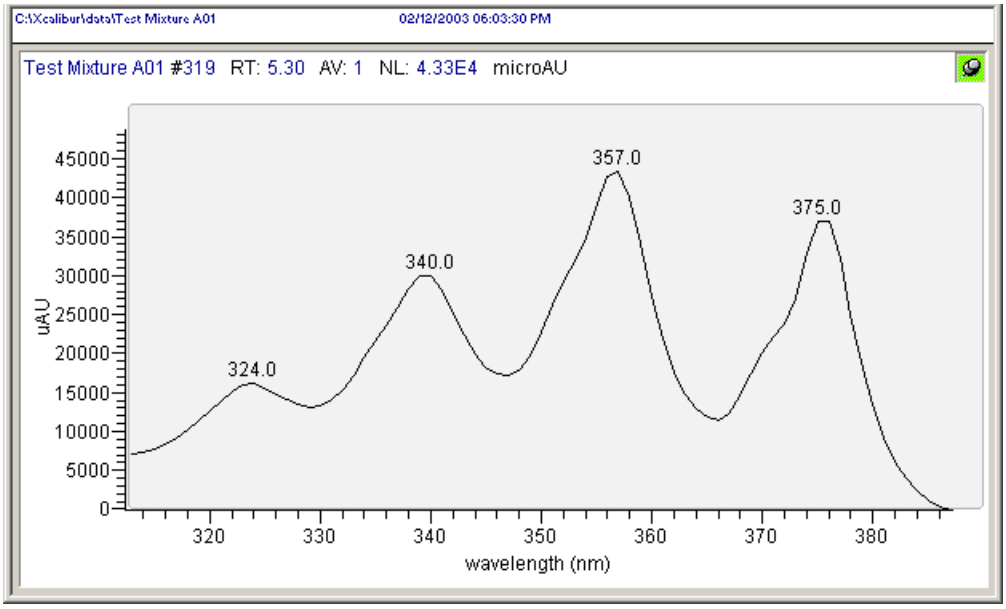
Clicking (Full Size) in the toolbar sizes the active or pinned cell to the full width and height of the window as shown in Figure 133.

Figure 133. Spectrum cell sized to full size of window



Dragging across a region in the active or pinned cell zooms in on that region as shown in Figure 134.

Figure 134. Full size view of the spectrum cell zoomed in on the 320 to 380 nm region



Changing the Font Size of the Display

Occasionally, you might want to change the font size of the data displayed in the Xcalibur data system. For example, you might want to increase the font size for screen captures that you plan to use for presentations.

❖ To increase the font size

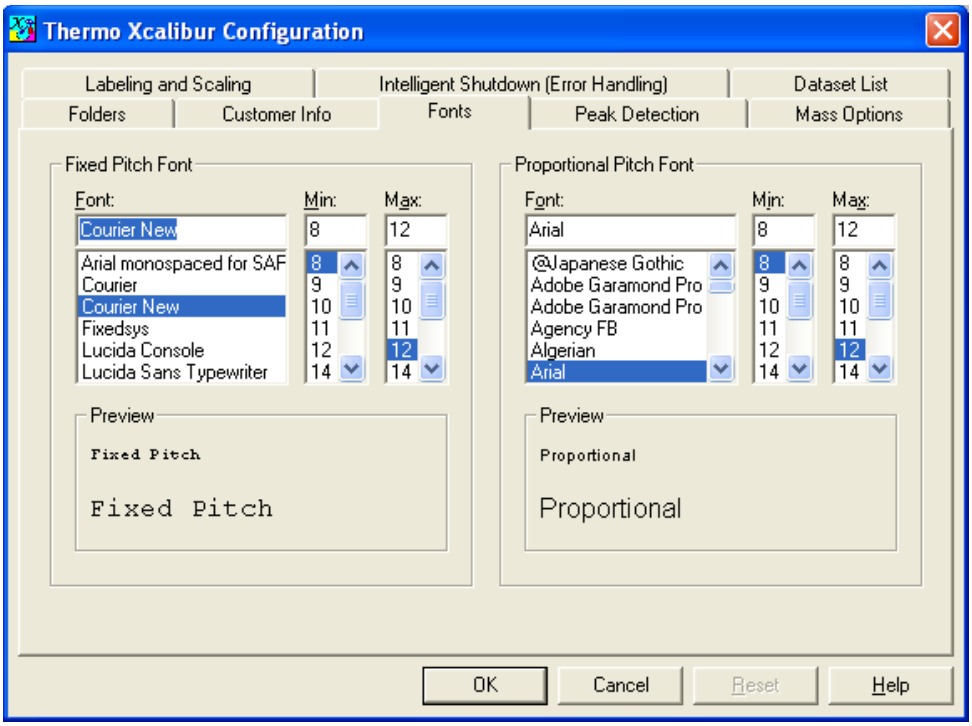
1. From the Thermo Xcalibur Roadmap window, choose **Tools > Configuration**.

   The Thermo Xcalibur Configuration dialog box appears.

2. Click the **Fonts** tab.

   The Fonts page appears (see Figure 135).

   Figure 135. Fonts page in the Thermo Xcalibur Configuration dialog box



3. To increase the font size of the chromatogram, spectrum, and map axis labels, in the Proportional Pitch Font area, select a larger font size from the Max list, and select a larger font size from the Min list.

4. To increase the font size for the Spectrum List, Scan Header, Scan Filters, or Report, in the Fixed Pitch Font area, select a larger font size from the Max list, and select a larger font size from the Min list.

   Figure 136 shows the chromatogram axes labeled with a font size of 8. Figure 137 shows the chromatogram axes labeled with a font size of 16.

Figure 136. Proportional pitch font size = 8

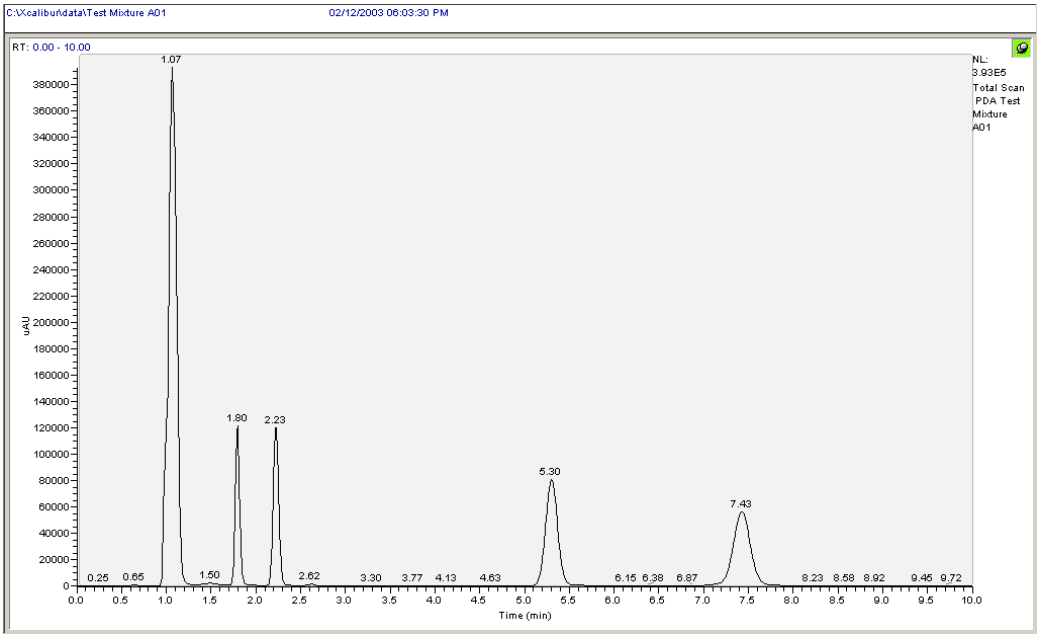
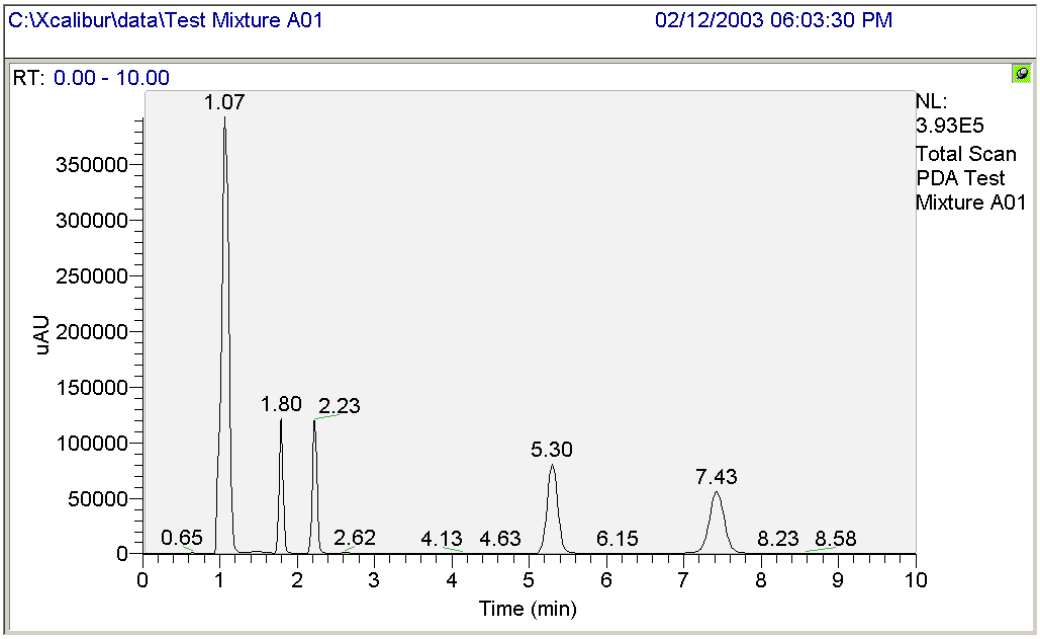


Figure 137. Proportional pitch font size = 16



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.

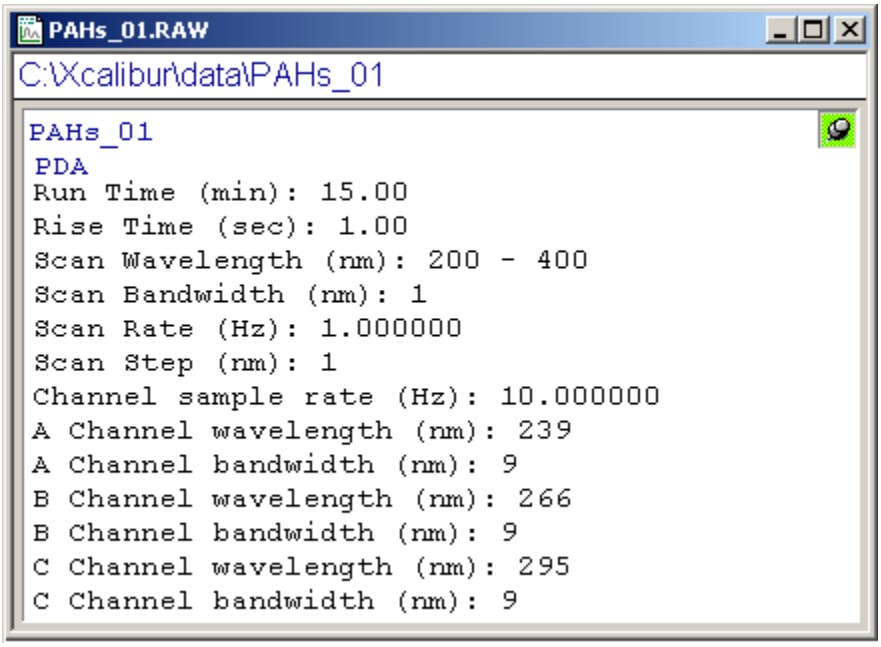
❖ To view a report that lists the instrument control parameters

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 138).

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

Figure 138. Instrument Method Report (PDA page) in the Qual Browser view



Creating a Layout for PDA Data

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

To create a layout file for PDA data, follow these procedures in order:

1. Specifying the Chromatogram and Spectrum Ranges
2. Specifying the Display Options for the Chromatogram and Spectrum Cells
3. (Optional) Inserting Cells
4. Saving the New Layout

Specifying the Chromatogram and Spectrum Ranges

When you open a .raw data file in the Qual Browser application using the default layout, the data file opens with the MS TIC chromatogram in the upper cell and a mass range spectrum in the lower cell.

Follow these topics to change the upper cell to a chromatogram and the lower cell to spectral data acquired by the PDA detector:

- Specifying the Chromatogram Ranges
- Specifying the Spectrum Range

Note You can modify the cells in any order.

Specifying the Chromatogram Ranges

There are two types of chromatograms for the PDA detector: chromatograms acquired from any of the three discrete channels and chromatograms interpolated from the scan data. Follow these procedures to display the two types of chromatograms acquired by the PDA detector in the Qual Browser window:

- Displaying Scan Wavelength Chromatograms
- Displaying Discrete Wavelength Chromatograms

Displaying Scan Wavelength Chromatograms

❖ **To display a scan chromatogram for the PDA detector data**

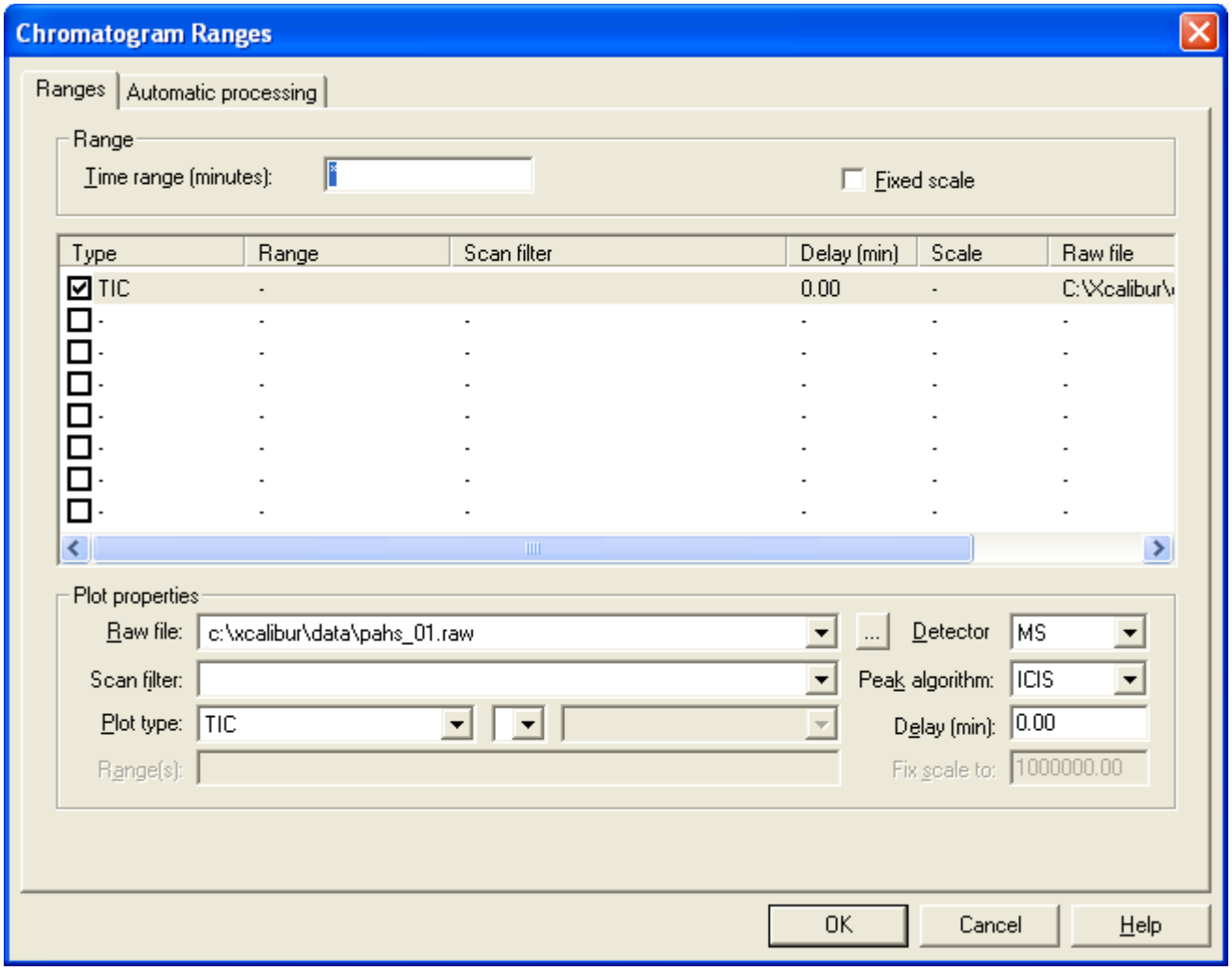
1. Open a data file (*.raw) that has PDA data and MS data (see "Opening a Raw Data File in Qual Browser" on page 219).

   The data file opens with the MS TIC chromatogram in the upper cell and a mass range spectrum in the lower cell.

2. Pin the chromatogram cell (see "Active and Pinned Cells" on page 225).
3. Right-click the chromatogram cell.
4. From the shortcut menu, choose **Ranges**.

   The Chromatogram Ranges dialog box appears (see Figure 139).

Figure 139. Chromatogram Ranges dialog box with the default layout settings

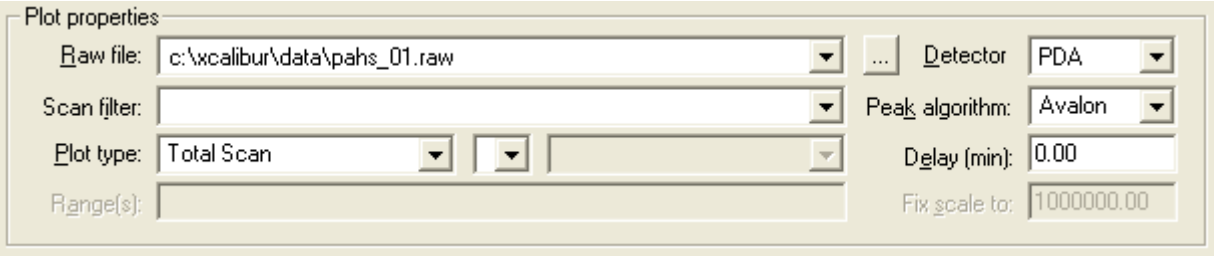


5. In the Range area, set the displayed time range of the chromatogram:
   - For a generic layout file, leave an asterisk in the Time Range box.

     When you open a .raw data file, the *x* axis of the chromatogram is scaled to the run time for your detector in the instrument setup method used to acquire the data.
   - To specify a specific time range, type a beginning time point and an ending time point separated by a dash in the Time Range (minutes) box.

6. In the Plot Properties area (see Figure 140), make the following selections:
   a. In the Detector list, select **PDA**.
   b. In the Peak Algorithm list, select **Avalon**.
   c. In the Plot Type list, select a plot type:
      - Select **Wavelength** to display the chromatogram for a specific wavelength within your scan range or to display the averaged results from a range of wavelengths in your scan range.
      - Select **Total Scan** to display the average absorbance for each time point of all the wavelengths in your scan range.
      - Select **Spectrum Maximum** to display a plot of the maximum absorbance values in your scan range for each time point.
   d. Select a wavelength range (if you selected the Total Scan plot type, this box is unavailable):
      - To display the chromatogram for a specific scan wavelength, type the wavelength number in the Range(s) box.
      - To display a plot of the average absorbance values for a range of wavelengths, type the beginning wavelength number and the ending wavelength number separated by a dash in the Range(s) box. For example, type 200-300 to display a plot of the average absorbance values for the scanned wavelengths from 200 to 300 nm.

Figure 140. Plot Properties area of the Chromatogram Ranges dialog box

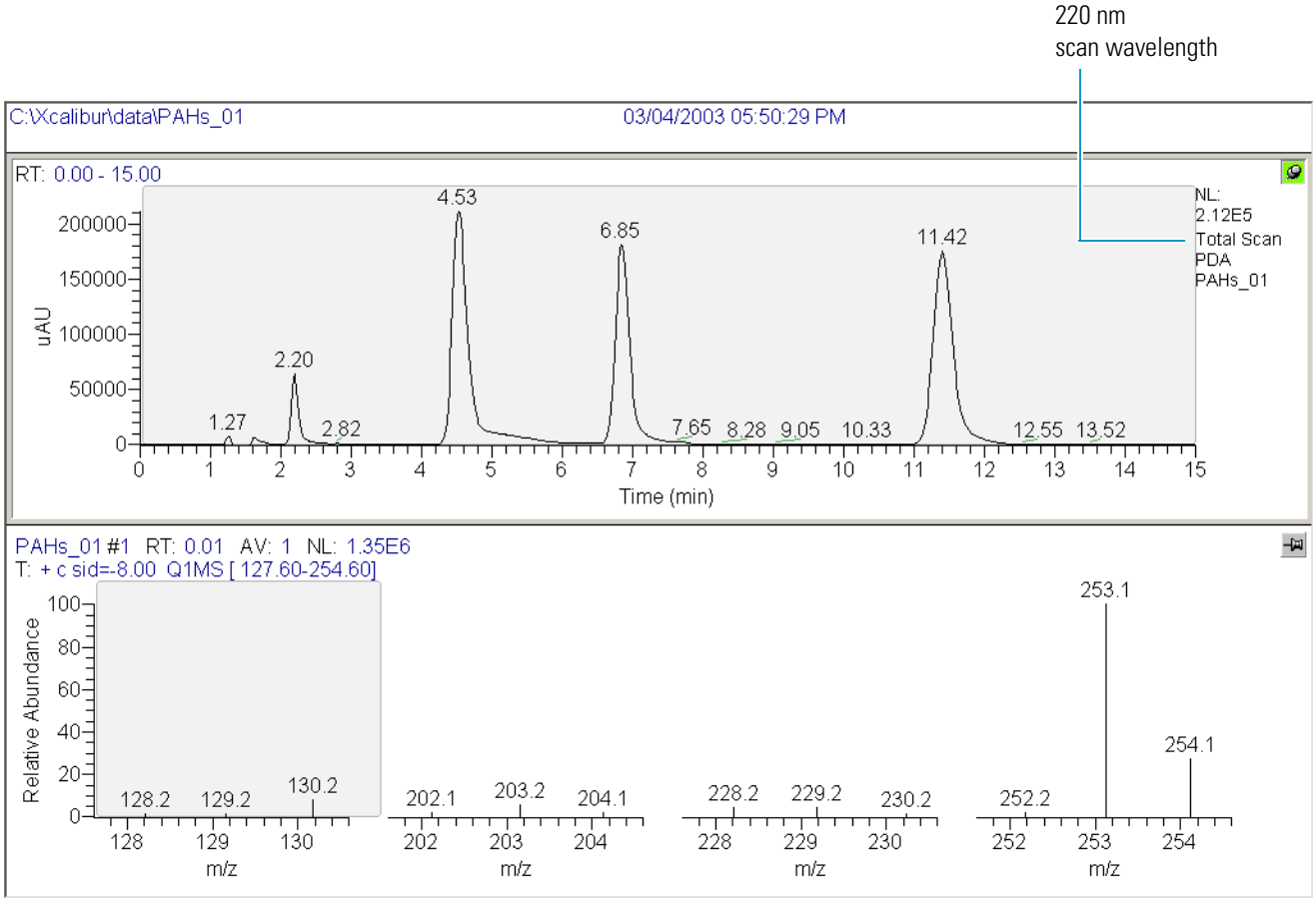


7. Click **OK** to close the dialog box and view your scan chromatogram.

   Figure 141 shows a chromatogram for a wavelength in the PDA scan.

To modify the layout for the spectrum cell, go to "Specifying the Spectrum Range" on page 238.

Figure 141. Chromatogram view with a chromatogram for a PDA scan wavelength



Displaying Discrete Wavelength Chromatograms

❖ To display a discrete chromatogram for the PDA data

1. If you have not already done so, open a data file (.raw) that has PDA data and MS data (see "Opening a Raw Data File in Qual Browser" on page 219).

   The data file opens with the MS TIC chromatogram in the upper cell and a mass range spectrum in the lower cell.

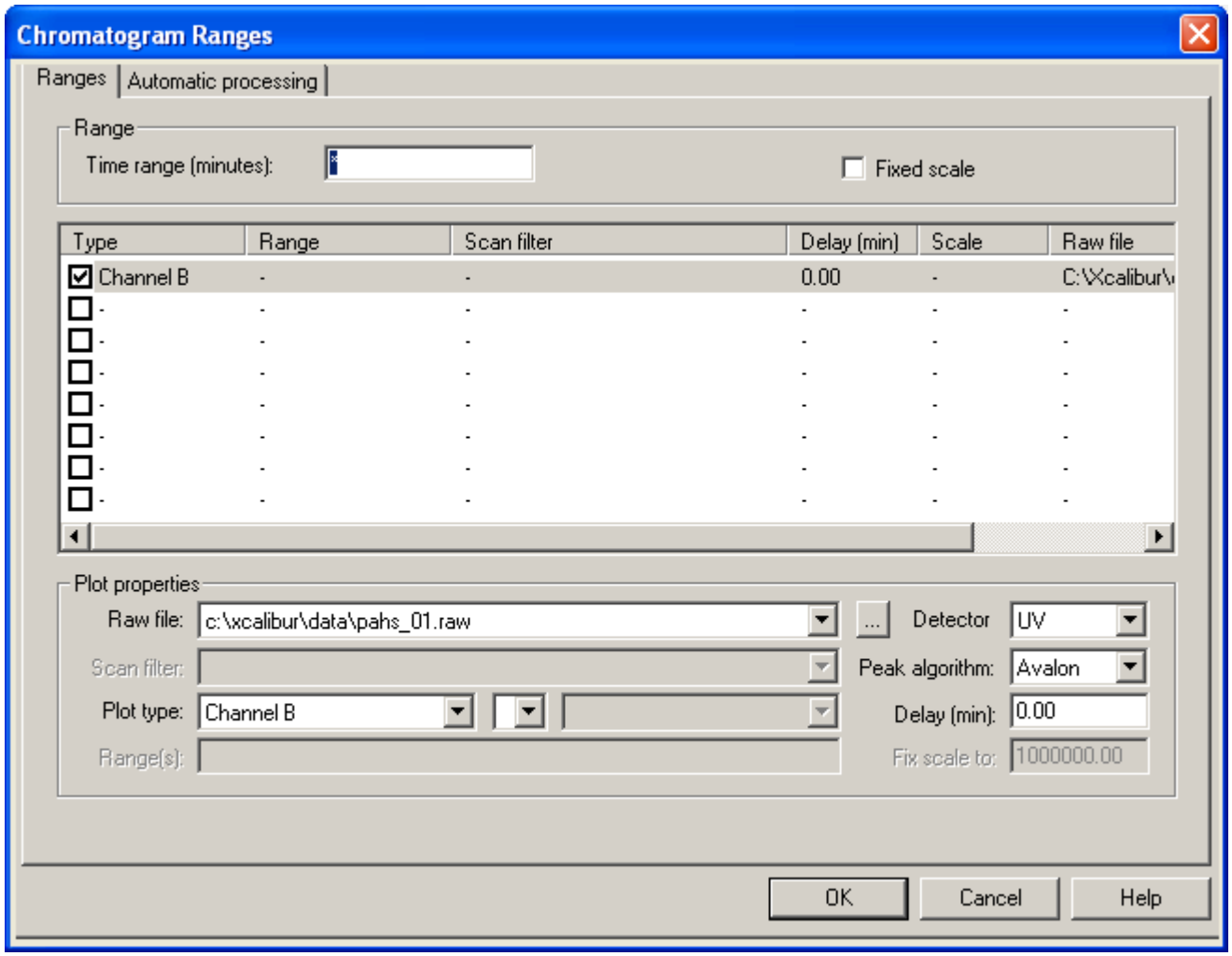
2. Pin the chromatogram cell (see "Active and Pinned Cells" on page 225).

3. Right-click the chromatogram cell.

4. From the shortcut menu, choose **Ranges**.

   The Chromatogram Ranges dialog box appears. Figure 139 on page 234 shows the default settings. Figure 142 shows the settings for a discrete channel wavelength.

Figure 142. Chromatogram Ranges dialog box with the selection of a discrete channel wavelength



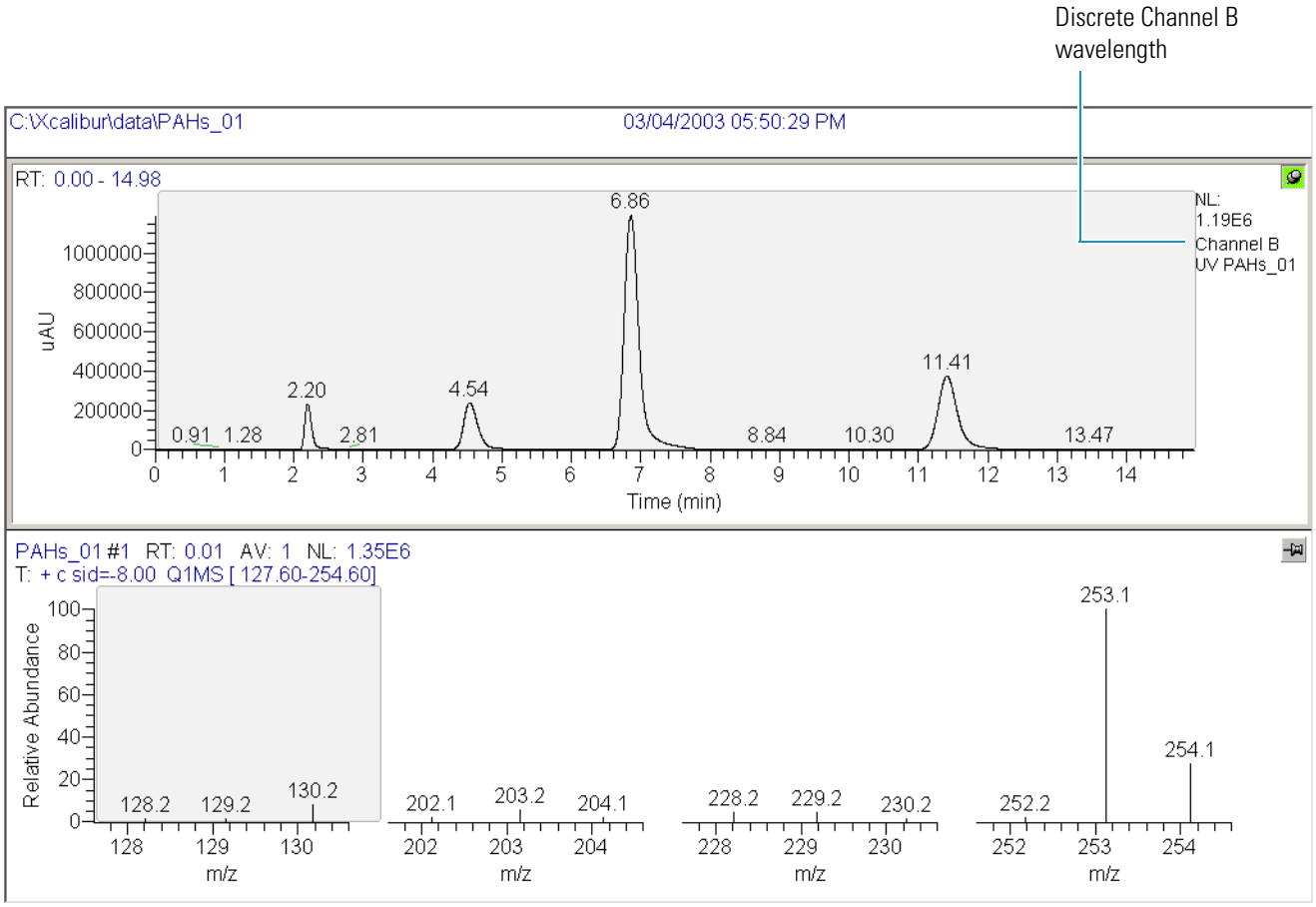
5. In the Range area, set the displayed time range of the chromatogram:
   - For a generic layout file, leave an asterisk in the Time Range box.

     When you open a .raw data file, the *x* axis of the chromatogram is scaled to the run time for your detector in the instrument setup method used to acquire the data.
   - To specify a specific time range, type a beginning time point and an ending time point separated by a dash in the Time Range box.
6. In the Plot Properties area, select the following:
   a. In the Detector list, select **UV**.
   b. In the Peak Algorithm list, select **Avalon**.
   c. In the Plot Type list, select **Channel A**, **Channel B**, or **Channel C**.
7. Click **OK** to exit the dialog box and view your discrete chromatogram.

Figure 143 shows a discrete channel chromatogram.

To modify the layout for the spectrum cell, go to "Specifying the Spectrum Range" on page 238.

Figure 143. Chromatogram view with a chromatogram for a PDA discrete channel



Specifying the Spectrum Range

❖ To display a spectrum for the PDA data

1. If you have not already done so, open a data file (.raw) that has PDA data and MS data (see "Opening a Raw Data File in Qual Browser" on page 219).

   The data file opens with the MS TIC chromatogram in the upper cell and a mass range spectrum in the lower cell.

2. Pin the spectrum cell.

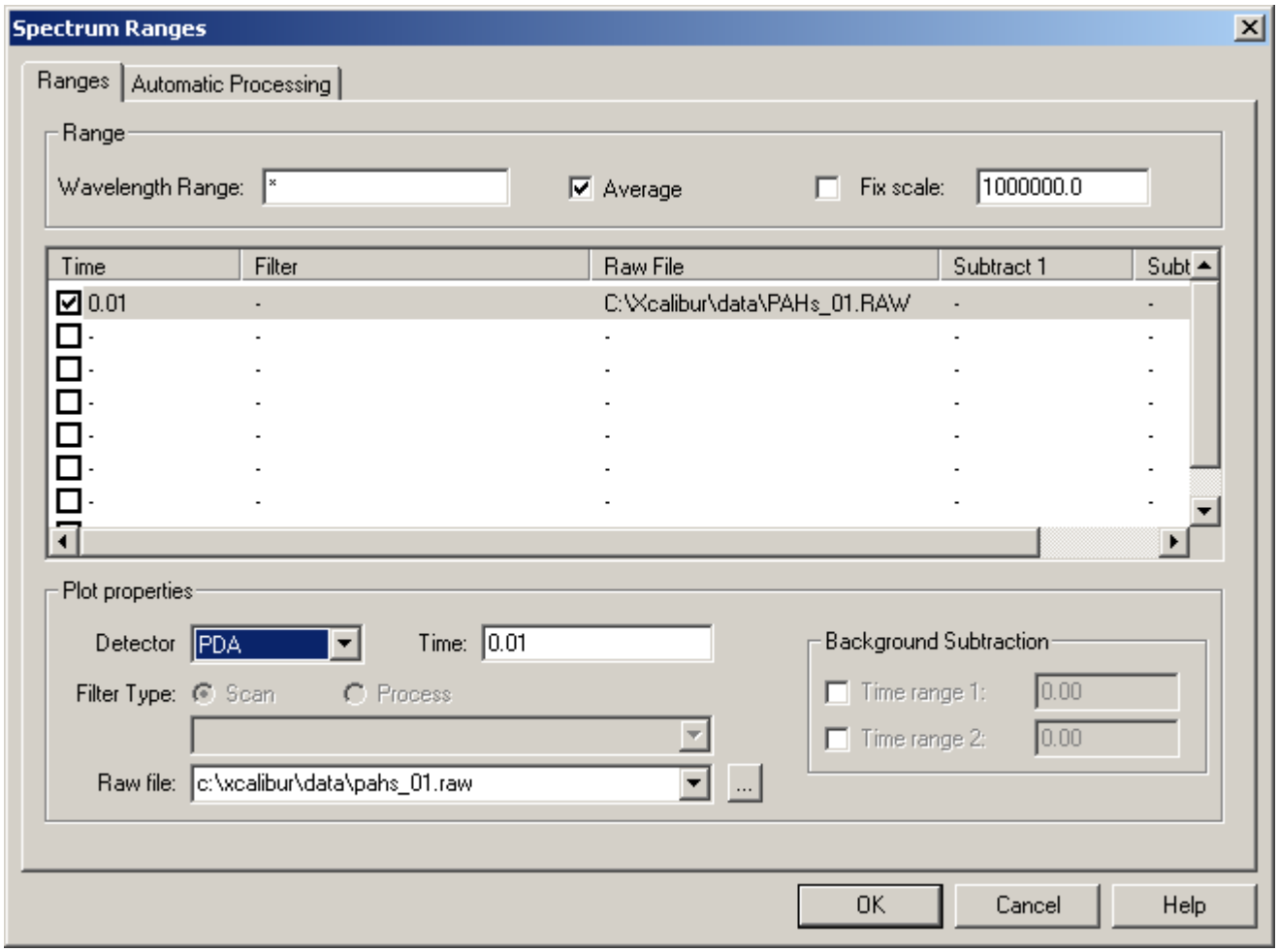
3. Right-click the spectrum cell.

4. From the shortcut menu, choose **Ranges**.

   The Spectrum Ranges dialog box appears (see Figure 144).

5. In the Plot Properties area, select **PDA** in the Detector list.
6. For a generic layout file for PDA data, keep the other settings in the Spectrum Ranges dialog box at their defaults as shown in Figure 144.

Figure 144. Spectrum Ranges dialog box with the PDA detector selected



If you have not already done so, modify the layout for the chromatogram cell as described in "Specifying the Chromatogram Ranges" on page 233.

If you have specified the ranges for the chromatogram cell and the spectrum cell, go to the next procedure, "Specifying the Display Options for the Chromatogram and Spectrum Cells."

Specifying the Display Options for the Chromatogram and Spectrum Cells

After you specify the chromatogram and spectrum ranges, specify the display options for the chromatogram and spectrum cells as described in these topics:

- Setting the Display Options for the Chromatogram Cell
- Setting the Display Options for the Spectrum Cell

Setting the Display Options for the Chromatogram Cell

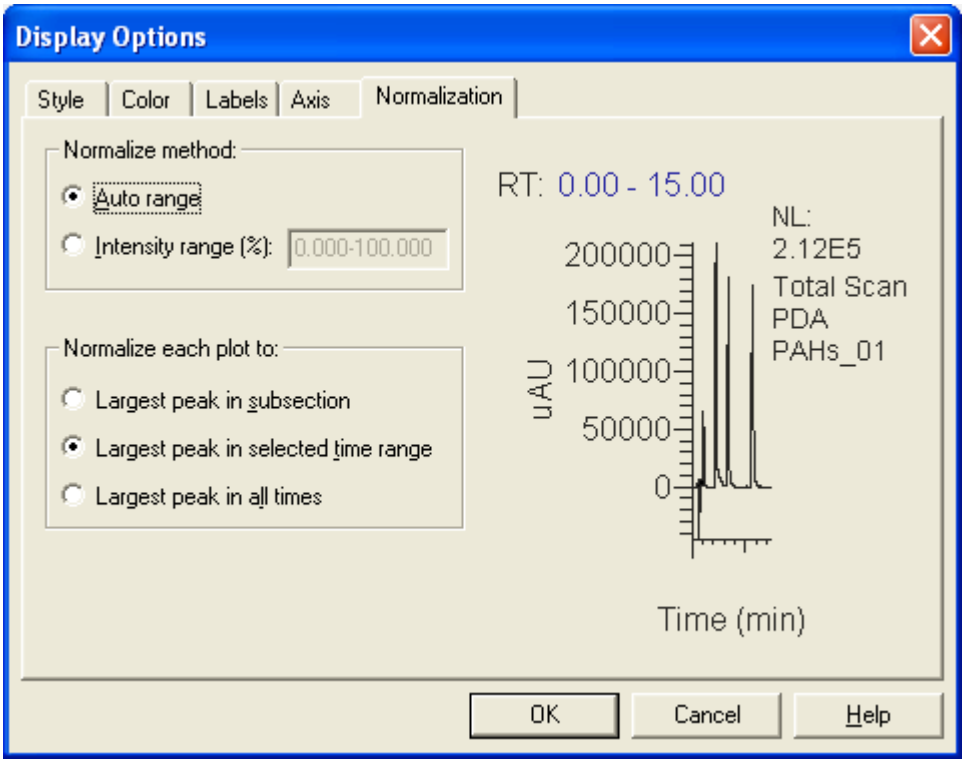
❖ **To set the display options for the chromatogram cell**

1. Pin the chromatogram cell.
2. Right-click the pinned chromatogram cell.
3. From the shortcut menu, choose **Display Options**.

   The Display Options dialog box appears.
4. Specify the Normalization parameters:
   a. Click the **Normalization** tab.

      The Normalization page appears (see Figure 145).

      Figure 145. Normalization page in the Display Options dialog box



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

Selecting Auto Range ensures that the entire dynamic range of the chromatogram is displayed in the active view, normalized over the full range of the *y* axis.

c. In the Normalize Each Plot To area, select the **Largest Peak in Selected Time Range** option.

5. Specify the Axis parameters as follows:

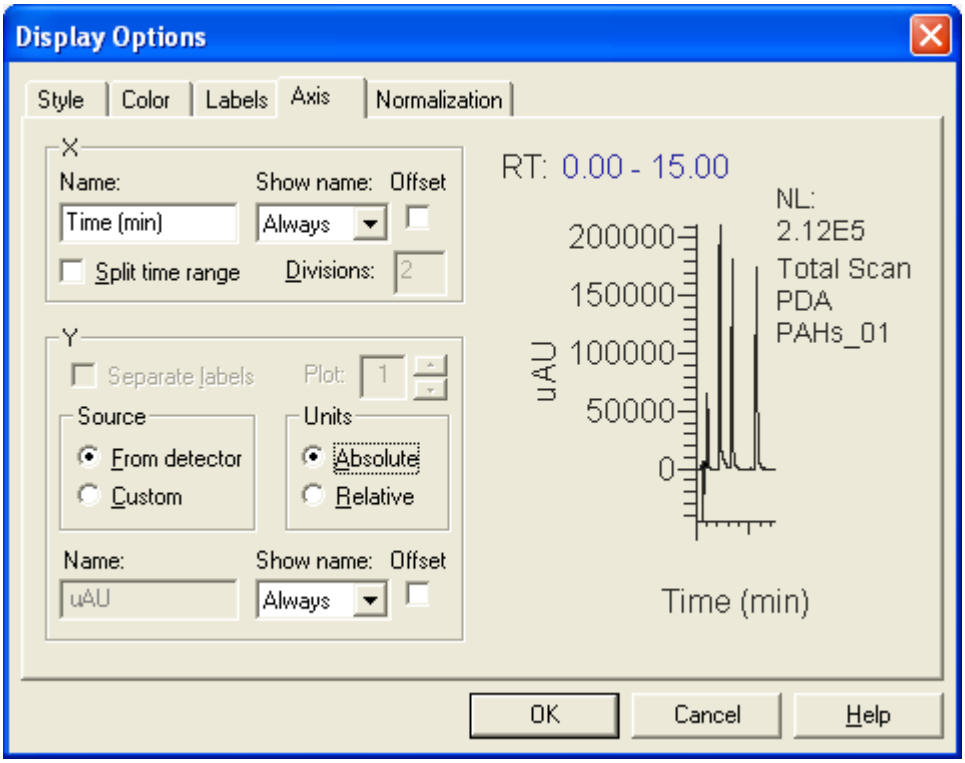
a. Click the **Axis** tab.

The Axis page appears (see Figure 146).

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

Selecting Absolute sets the *y* axis to the absolute units of µAU.

Figure 146. Axis page in the Display Options dialog box

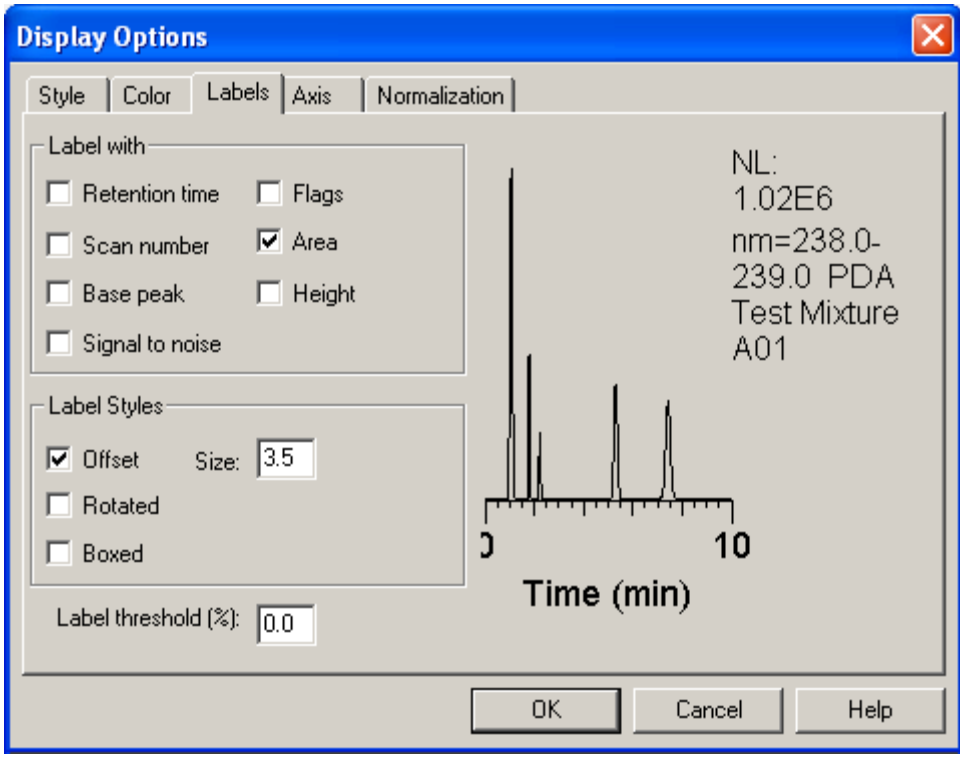


6. Specify the labels for the peaks in the chromatogram as follows:

a. Click the **Labels** tab.

The Labels page appears (see Figure 147).

Figure 147. Labels page in the Display Options dialog box



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

7. Click **OK** to accept the settings and close the Display Options dialog box.

Setting the Display Options for the Spectrum Cell

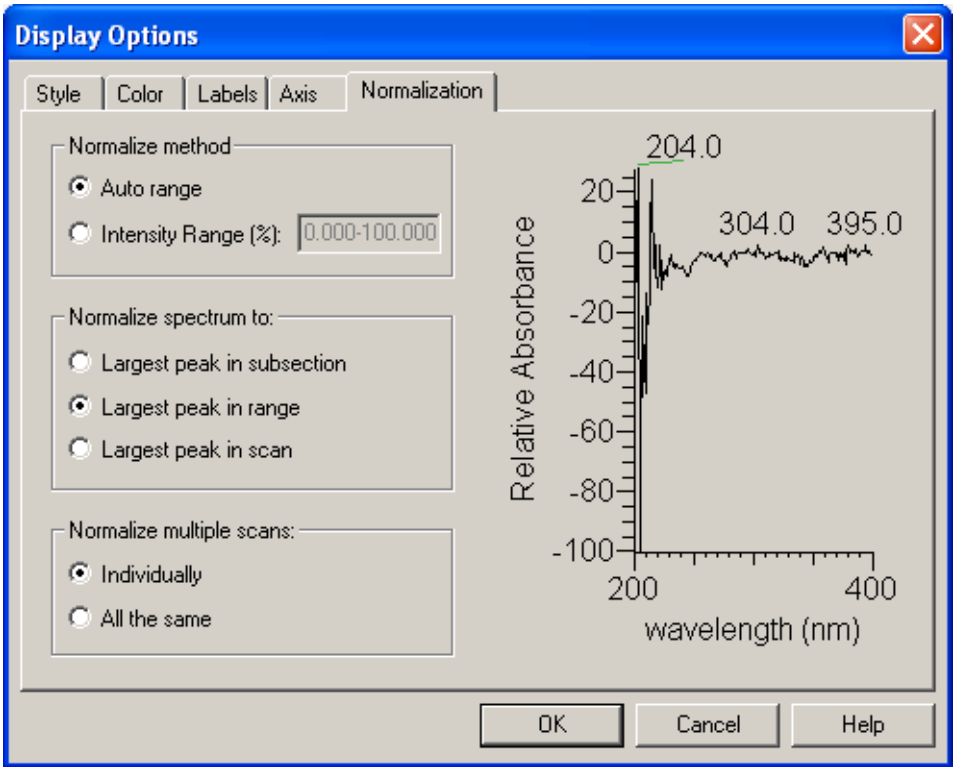
❖ To set the display options for the spectrum cell

1. Pin the spectrum cell.
2. Right-click the pinned spectrum cell.
3. From the shortcut menu, choose **Display Options**.

   The Display Options dialog box appears.
4. Specify the Normalization parameters as follows:
   a. Click the **Normalization** tab.

      The Normalization page appears (see Figure 148).

Figure 148. Normalization page in the Display Options dialog box



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

   Selecting Auto Range ensures that the entire dynamic range of the spectrum is displayed in the active view, normalized over the full range of the *y* axis.

c. In the Normalize Spectrum To area, select the **Largest Peak in Range** option.

5. Specify the Axis parameters as follows:

   a. Click the **Axis** tab.

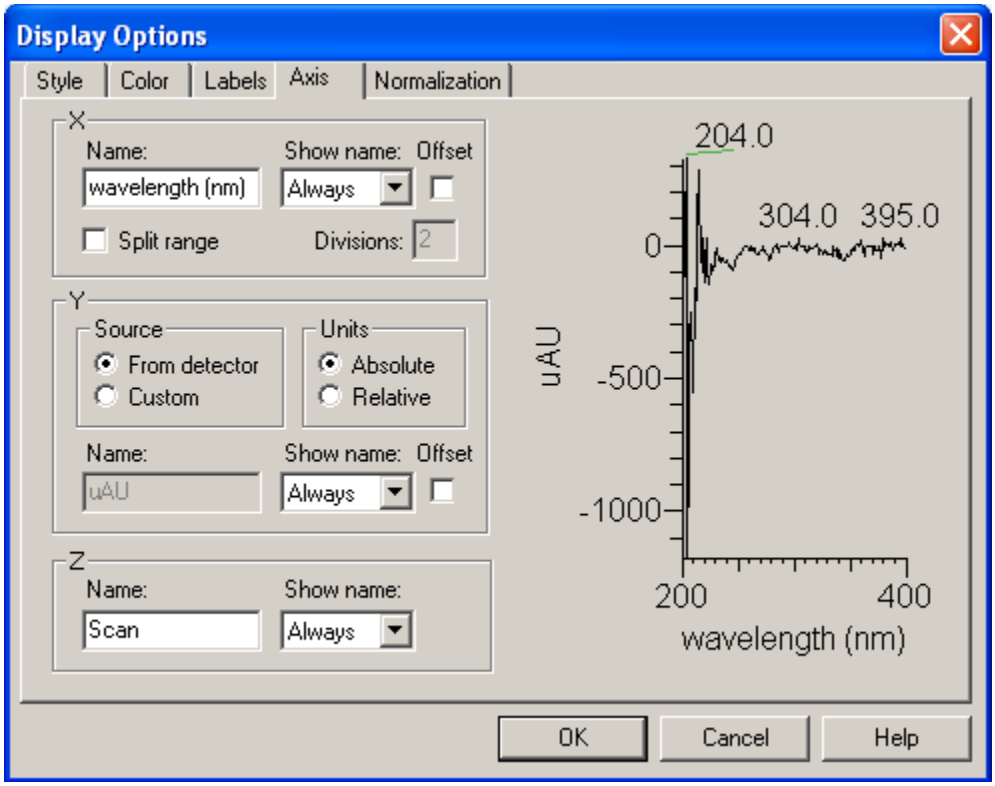
      The Axis page appears (see Figure 149).

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

      Selecting Absolute sets the *y* axis to the absolute units of µAU.

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

Figure 149. Axis page in the Display Options dialog box



Inserting Cells

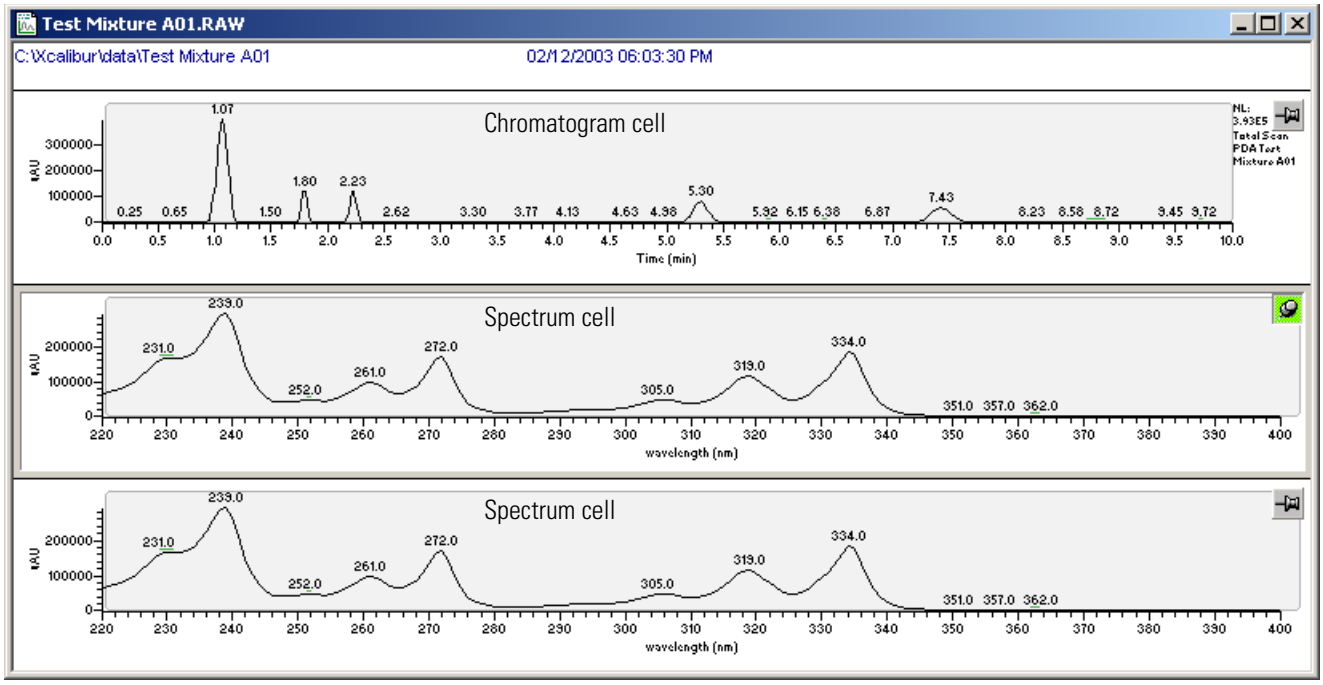
Occasionally, you might want to add more cells to the Qual Browser window. For example, you might want to add a cell containing a map plot (contour or 3D) to the view screen, or you might want to display several discrete or scan wavelengths in separate cells.

❖ To add a cell containing a map plot to the window

1. Click a cell to make it the active cell.
2. Choose **Grid > Insert Cells > Left**, **Right**, **Above**, or **Below**.

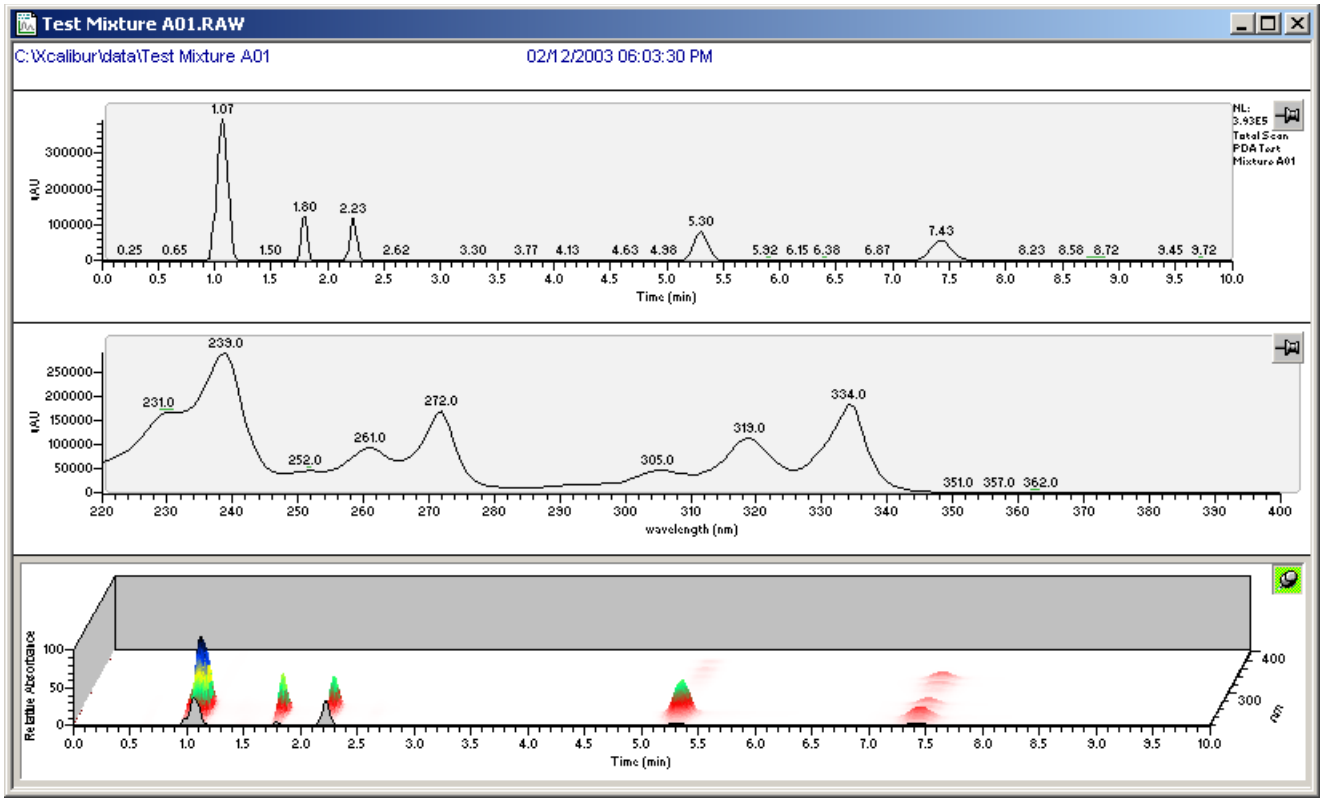
   The location of the new cell is relative to the active cell. Initially, the new cell contains the same information as the existing cell (see Figure 150).

Figure 150. Qual Browser window with three cells



3. Change the lower cell so that it displays the Map view (see Figure 151) as follows:
   a. Pin the cell by clicking its pin button.
   b. Click the **View Map** button in the toolbar.

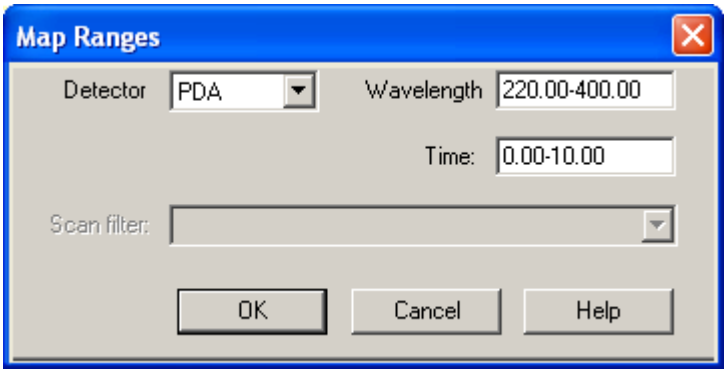
Figure 151. Qual Browser window with a chromatogram cell, spectrum cell, and map cell



4. Select the appropriate range options for the map cell as follows:
   a. Right-click the map cell.
   b. From the shortcut menu, choose **Ranges**.

      The Map Ranges dialog box appears (see Figure 152).

      Figure 152. Map Ranges dialog box



   c. In the Wavelength box, type the wavelength range that you want to display.
   d. In the Time box, type the time range that you want to display.

5. Select the appropriate display options for the map cell as follows:
   a. Right-click the map cell.
   b. From the shortcut menu, choose **Display Options**.

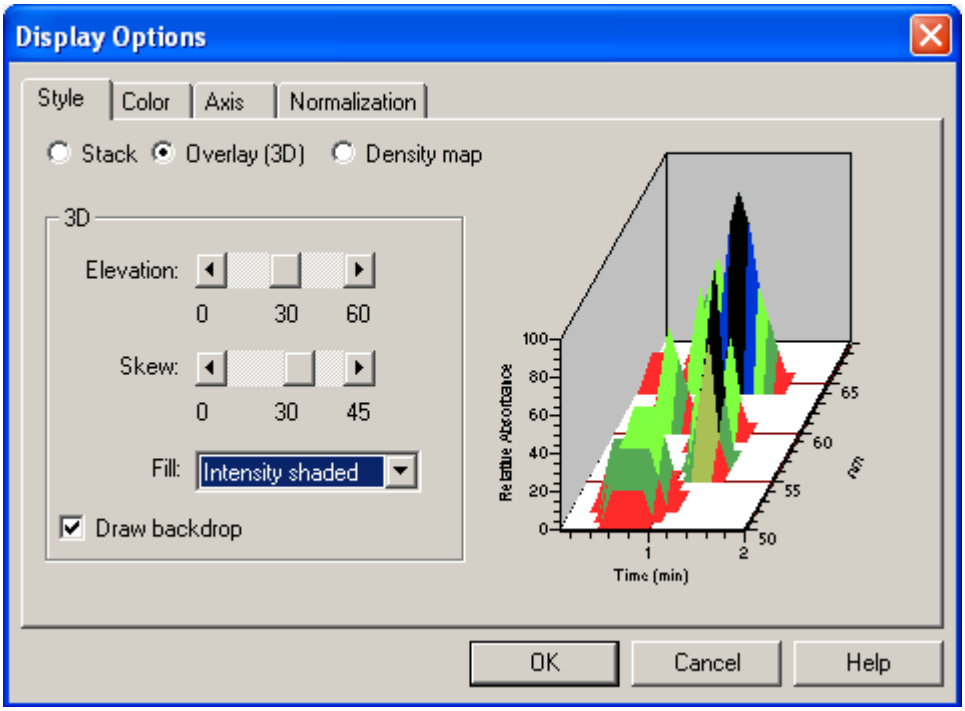
      The Display Options dialog box appears.
   c. Click the **Axis** tab.

      The Axis page appears (see Figure 149 on page 244).
   d. In the Units area, select the **Absolute** option.
   e. Click the **Normalization** tab.

      The Normalization page appears.
   f. In the Normalize Method area, select the **Auto Range** option.
   g. Click the **Style** tab.

      The Style page appears (see Figure 153).

      Figure 153. Style page in the Display Options dialog box for the map cell



   h. Select the appropriate style.
   i. Click **OK** to save your settings and close the Display Options dialog box.

Saving the New Layout

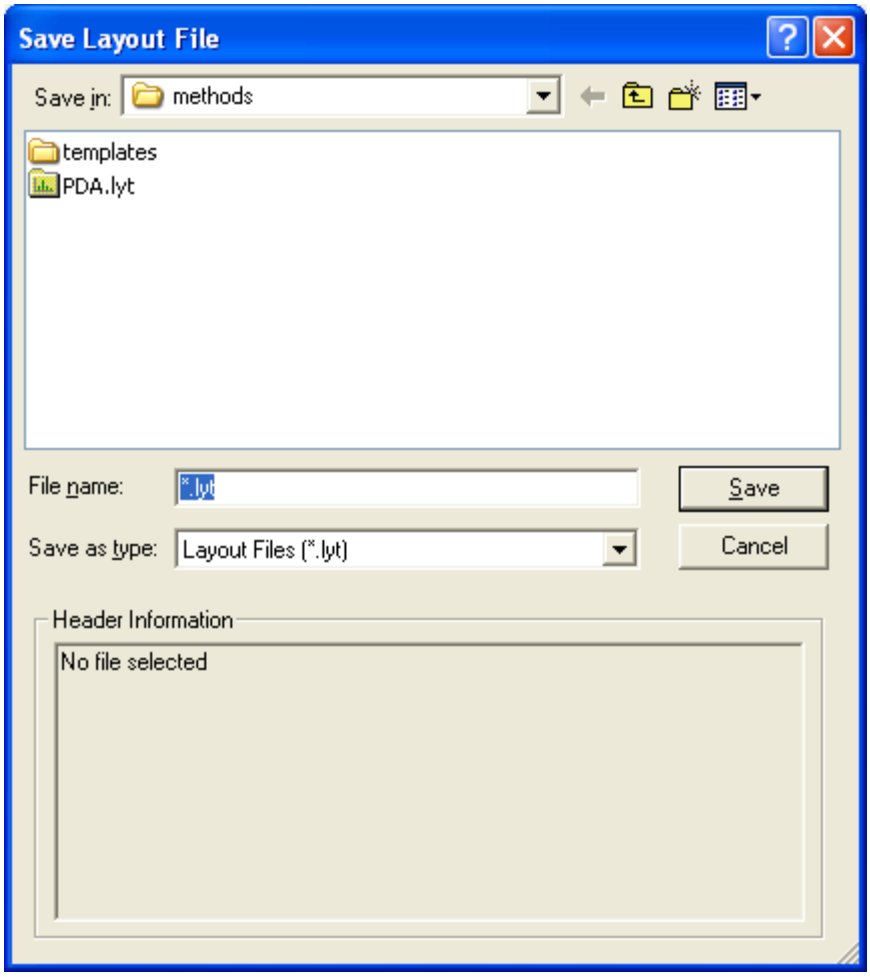
Now that you have created a layout for displaying your PDA data, save the layout so that you can apply it to other data files containing PDA data.

❖ To save the layout

1. From the Qual Browser window, choose **File > Layout > Save As**.

   The Save Layout File dialog box appears (see Figure 154).

   Figure 154. Save Layout File dialog box



2. Type a file name in the File name box.
3. Click **Save**.

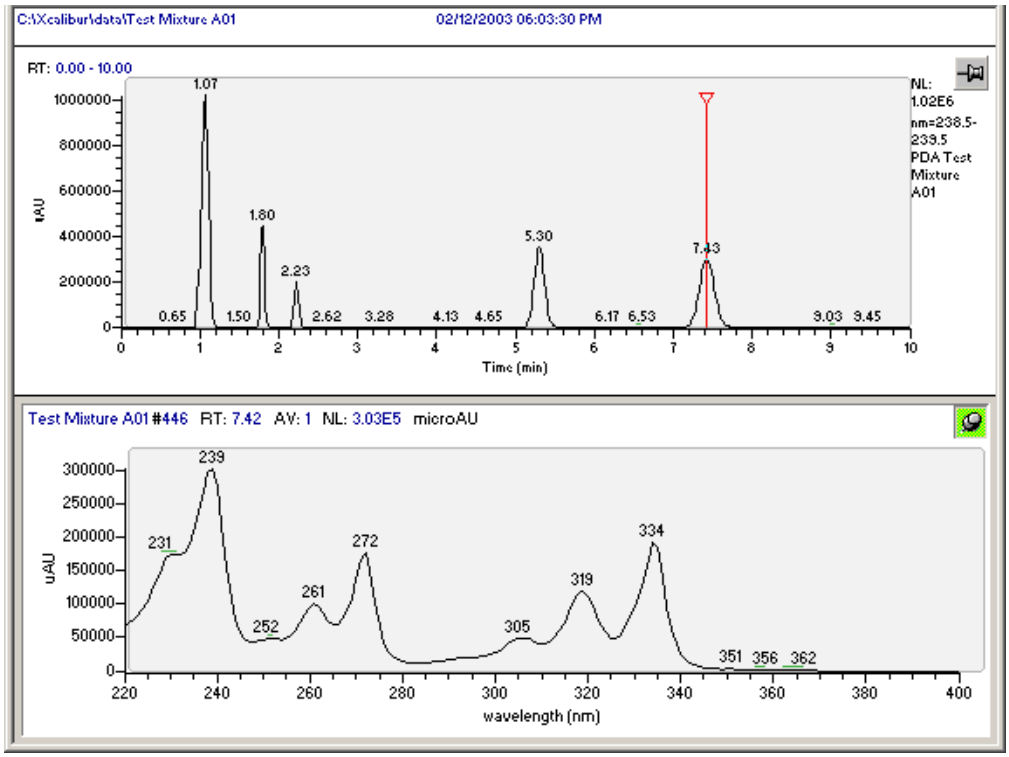
Viewing the Spectrum for a Specific Time Point

❖ To view a spectrum for a specific time point

1. Open a raw data file with PDA data and apply an appropriate layout file (see "Opening a Raw Data File in Qual Browser" on page 219).
2. Pin the spectrum cell.
3. Click a time point in the chromatogram cell.

   The spectrum for the selected time point appears in the spectrum cell (see Figure 155).

   Figure 155. Qual Browser window with a chromatogram of the PDA test mixture and a spectrum for the 7.42 minute time point



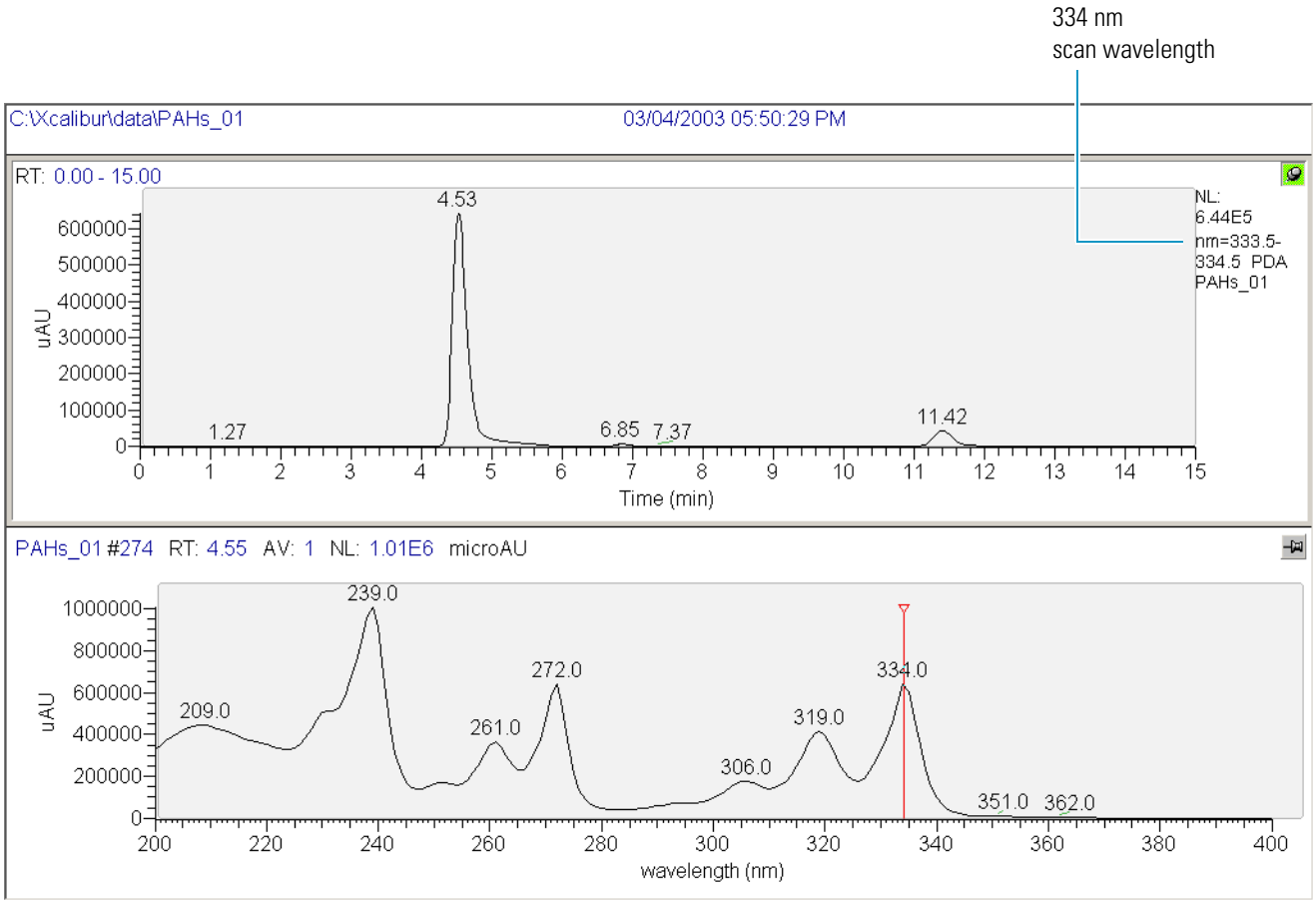
Viewing the Chromatogram for a Specific Wavelength

❖ **To view the scan chromatogram for a specific wavelength**

1. Open a raw data file with PDA data and apply an appropriate layout file (see "Opening a Raw Data File in Qual Browser" on page 219).
2. Pin the chromatogram cell.
3. Click a wavelength in the spectrum cell.

   The scan chromatogram for the selected wavelength appears in the chromatogram cell as shown in Figure 156.

Figure 156. Qual Browser window, displaying a chromatogram cell and a spectrum cell



Determining Peak Areas

In a chromatogram, the area of an isolated peak is directly proportional to the concentration of the analyte.

❖ To make a peak area determination using the Qual Browser application

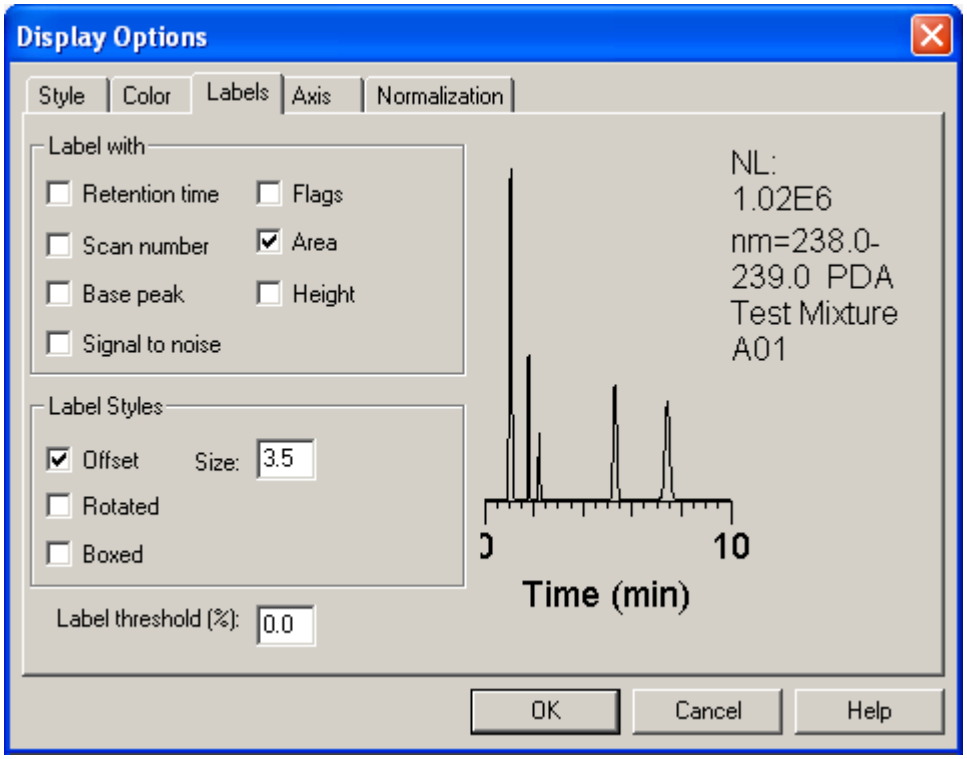
1. Open the Qual Browser application.
2. Open the data file (.raw) of interest (see "Opening a Raw Data File in Qual Browser" on page 219).
3. Ensure that the chromatogram cell is the active cell, as indicated by a gray border.
4. Select the chromatogram that you want to integrate as follows:
   a. Right-click the chromatogram and from the shortcut menu choose **Ranges**.

      The Chromatogram ranges dialog box appears.
   b. In the Detector list, select the detector type:
      - To integrate a scan wavelength, select **PDA**.
      - To integrate a discrete wavelength channel, select **UV**.
   c. Ensure that **Avalon** is selected as the Peak Algorithm.
   d. Select the appropriate wavelength:
      - To display the chromatogram for a scan wavelength, select **Wavelength Range** from the Plot Type list, and then type a value for a wavelength within your scan range in the Range box.
      - To display the chromatogram for a discrete wavelength channel, select **Channel A**, **B**, or **C** from the Plot Type list.
5. Turn on peak detection by right-clicking the chromatogram cell and choosing **Peak Detection > Toggle Detection in This Plot f**rom the shortcut menu.
6. To display numerical values for areas of the chromatographic peaks, do the following:
   a. Right-click the chromatogram cell and choose **Display Options**.

      The Display Options dialog box appears.
   b. Click the **Labels** tab.

      The Labels page appears (see Figure 157).

Figure 157. Labels page in the Display Options dialog box



c. Select the **Area** check box.

d. Click **OK** to return to the chromatogram cell.

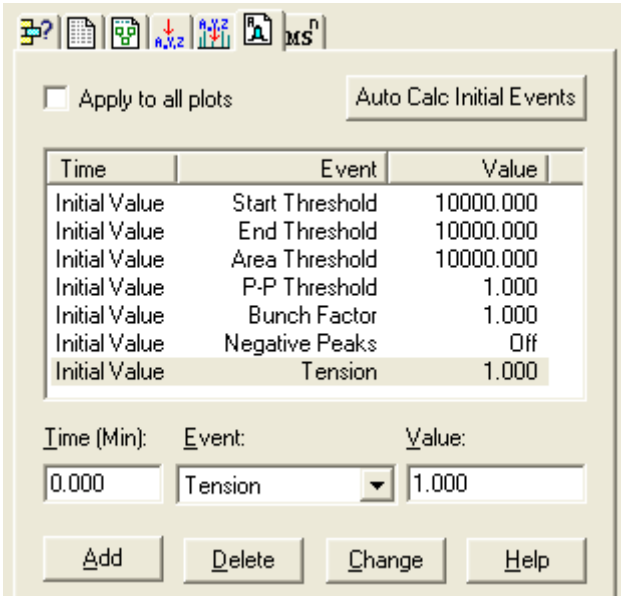
7. To set the integration parameters, do the following:

   a. Right-click the chromatogram and choose **Peak Detection > Settings** from the shortcut menu.

      The Avalon Peak Detection Settings page appears on the left side of the window (see Figure 158).

      Note The Avalon Peak Detection Settings page appears only when Avalon is selected as the peak algorithm in the Chromatogram Ranges dialog box.

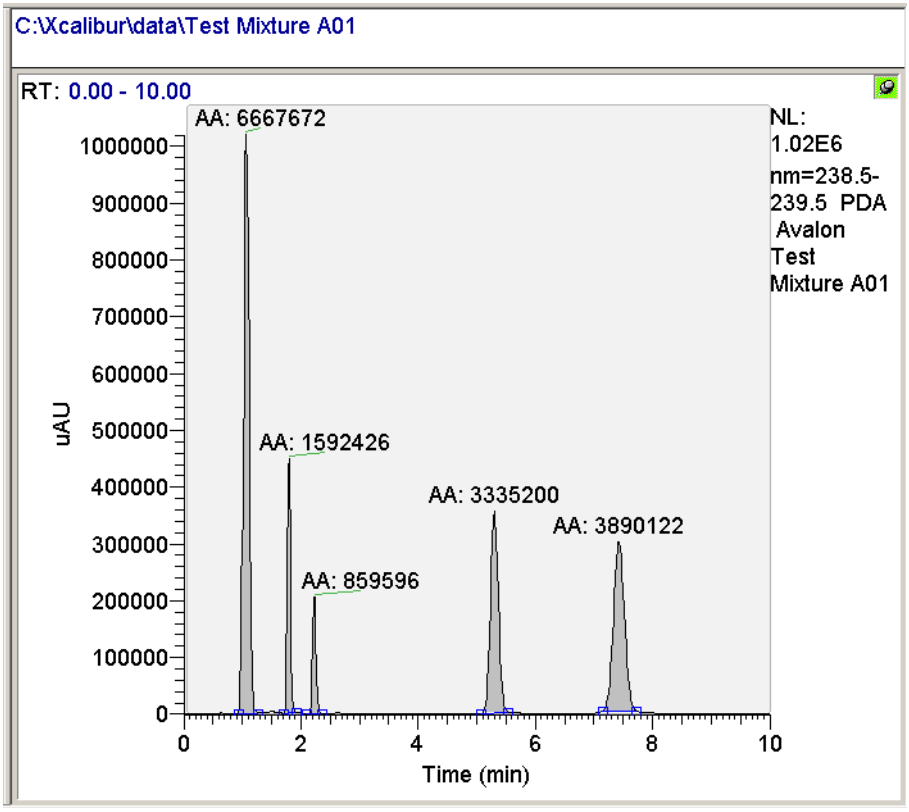
Figure 158. Avalon Peak Detection Settings page



b. Click **Auto Calc Initial Events** to force the Avalon peak integration algorithm to determine the "best" values for the following initial events: Start Threshold, End Threshold, Peak Threshold, P-P Threshold, Bunch Factor, Negative Peaks, and Tension.

Figure 159 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 159. Avalon Peak Detection Settings page displaying a chromatogram acquired with the PDA detector



Calculating the Purity of the Chromatographic Peaks

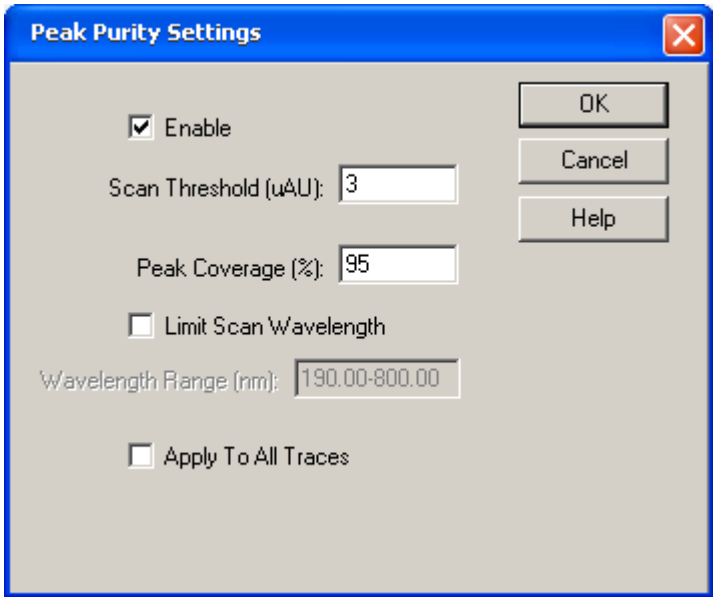
The data system can calculate the spectral purity of your chromatographic peaks by comparing the similarity of the spectra across the peak to a spectrum from the peak apex. The calculation is affected by the integration of the scan chromatogram and by the scan threshold, peak coverage, and scan wavelengths that you set in the Peak Purity Settings dialog box.

❖ To display the purity values for the integrated peaks

1. Select a chromatogram for a scan wavelength (see "Displaying Scan Wavelength Chromatograms" on page 233).
2. Set the integration parameters for the chromatogram (see "Determining Peak Areas" on page 251).
3. Right-click the chromatogram cell and choose **Peak Purity** from the shortcut menu.

   The Peak Purity Settings dialog box appears (see Figure 160).

Figure 160. Peak Purity Settings dialog box



4. Select the **Enable** check box.
5. In the Scan Threshold (µAU) box, type an appropriate scan threshold.

   The scan threshold limits the portion of the peak included in the analysis to spectral slices that have a lambda max above the scan threshold. You can set this limit to eliminate noise from the analysis. The limits for this box are 0 to 2 000 000 µAU.
6. In the Peak Coverage % box, type an appropriate value for your application. The limits for this parameter are 1 to 100% coverage.

   At a setting of 100%, the data system compares all the spectral slices that fall within the beginning and ending tick marks of the integrated peak. To limit the peak purity calculation to a specific range of wavelengths in the scan, select the Limit Scan Wavelength check box, and then enter a wavelength range in the Wavelength Range box.
7. Click **OK** to close the Peak Purity Settings dialog box, and then view the effect of your peak purity settings.

   The following figures show the effect of various settings on peak purity:

   - Figure 161 shows the effect of the scan wavelength on peak purity.
   - A comparison of Figure 162 and Figure 163 on page 257 demonstrates the effect of integration on peak purity.
   - Figure 164 on page 258 shows the effect of the scan threshold setting on peak purity.
   - Figure 165 on page 258 shows the effect of the peak coverage setting on peak purity.

Figure 161. Comparison of peak purity results for two different scan wavelengths

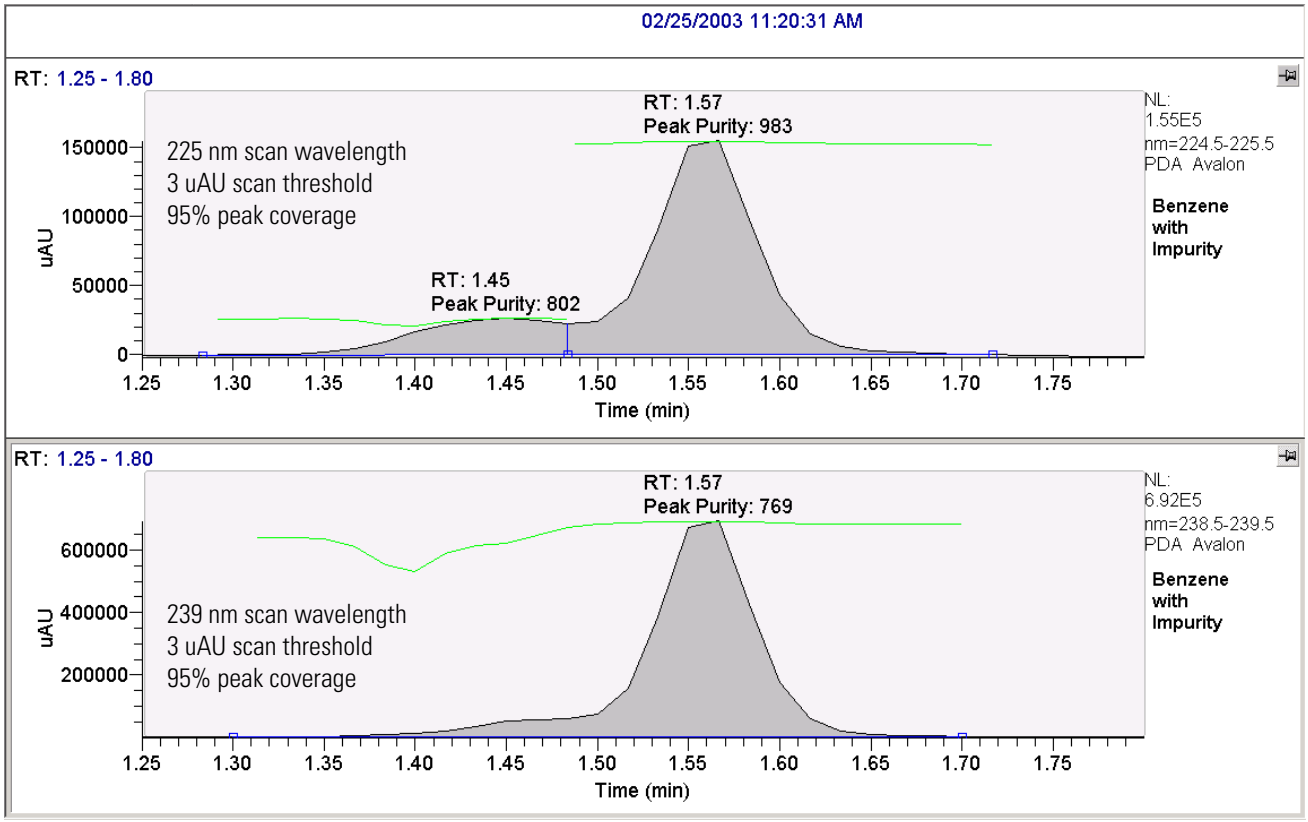


Figure 162. Chromatogram for the 225 nm scan wavelength with default integration parameters

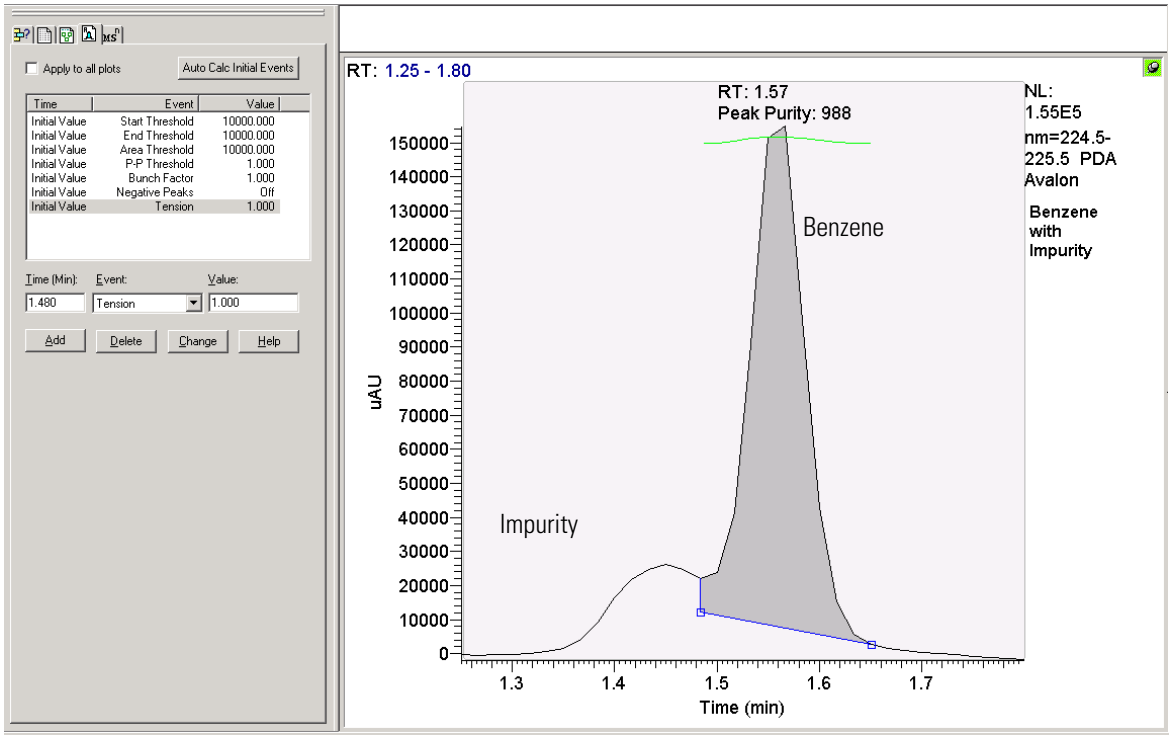


Figure 163. Chromatogram for the 225 nm scan wavelength with Auto calc integration parameters

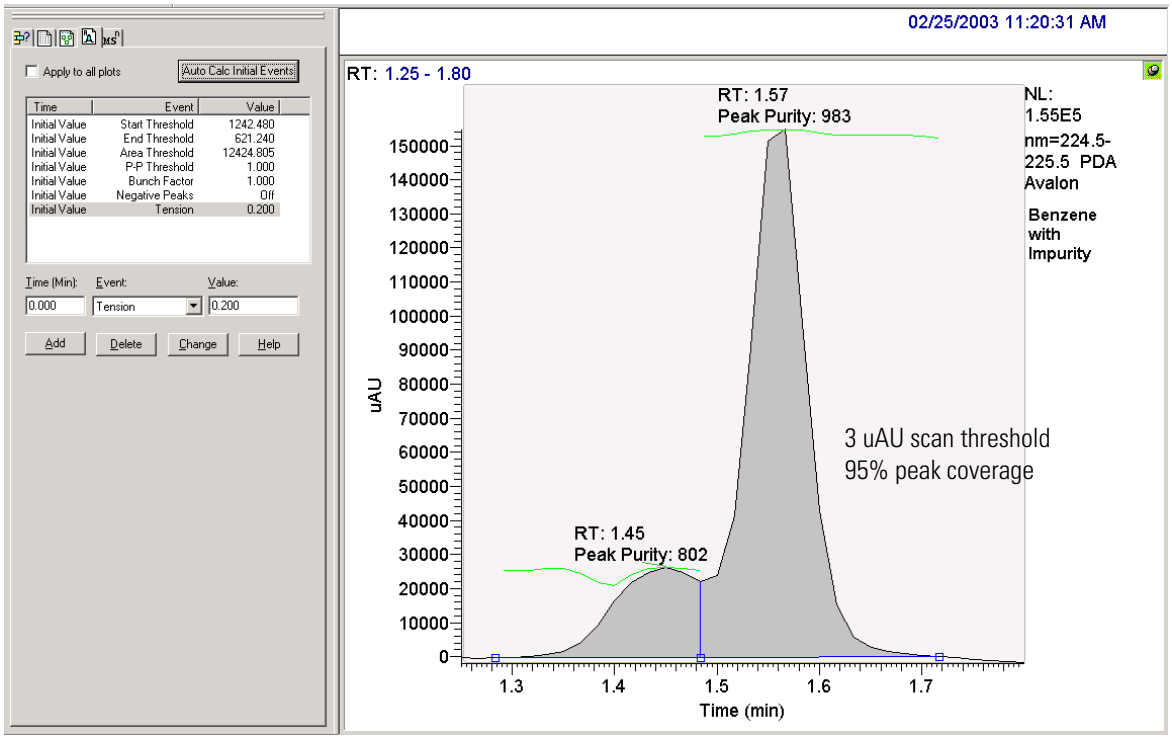


Figure 164. Effect of scan threshold on peak purity calculation

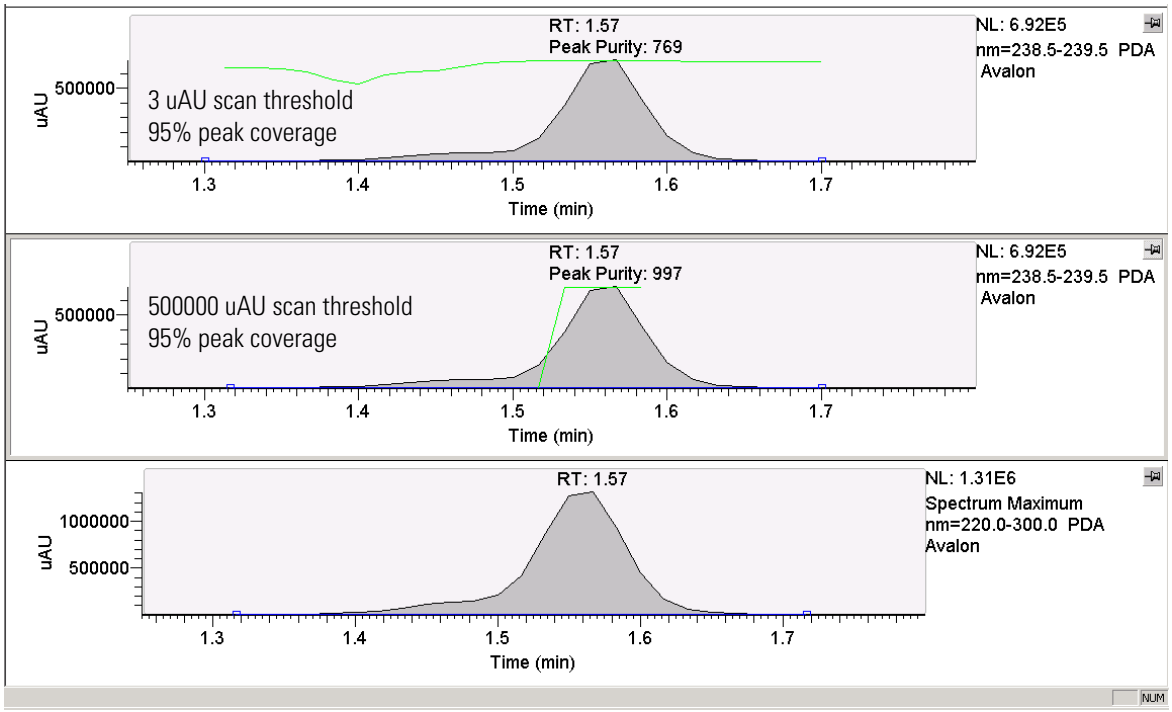
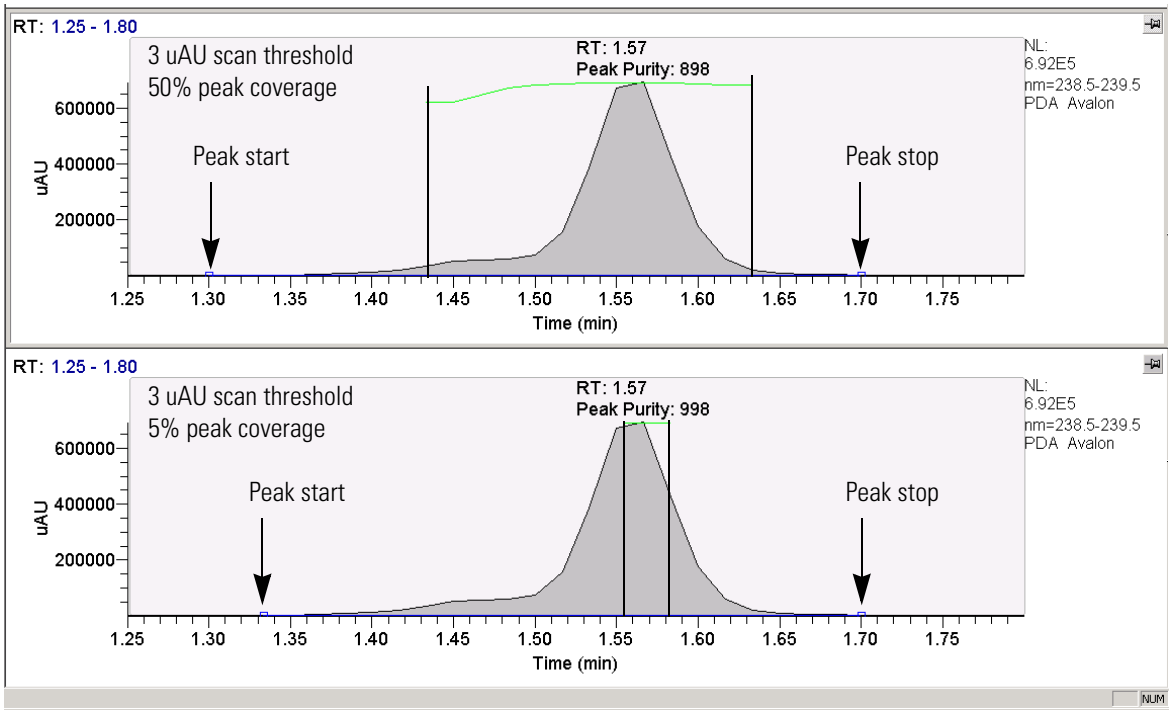


Figure 165. Effect of peak coverage on peak purity calculations



PDA Detector Performance Check and Calibration

This chapter describes the performance verification and calibration procedures for the PDA detector.

Contents

The PDA detector is factory tested for linearity, noise, and drift. Because of the sensitivity of its optical bench, adjust the light throughput to the diode array and recalibrate the PDA detector after you install it, and each time you move it, change its flow cell, or replace either of the lamps.You must also adjust the light throughput to the diode array if you change the configuration setting for the diode array scan rate.

Verifying the Performance of the PDA Detector

Before you perform either calibration procedure, turn on both lamps (see "Turning the Lamps On or Off" on page 167), let the temperature of the detector stabilize for approximately one hour, and adjust the light throughput to the diode array detector.

To adjust the light throughput to the diode array, follow these procedures:

- Creating a Display Method
- Adjusting the Light Throughput to the Diode Array

Creating a Display Method

Methods that display a plot of integrated light intensity versus diode number have a .spda file extension.

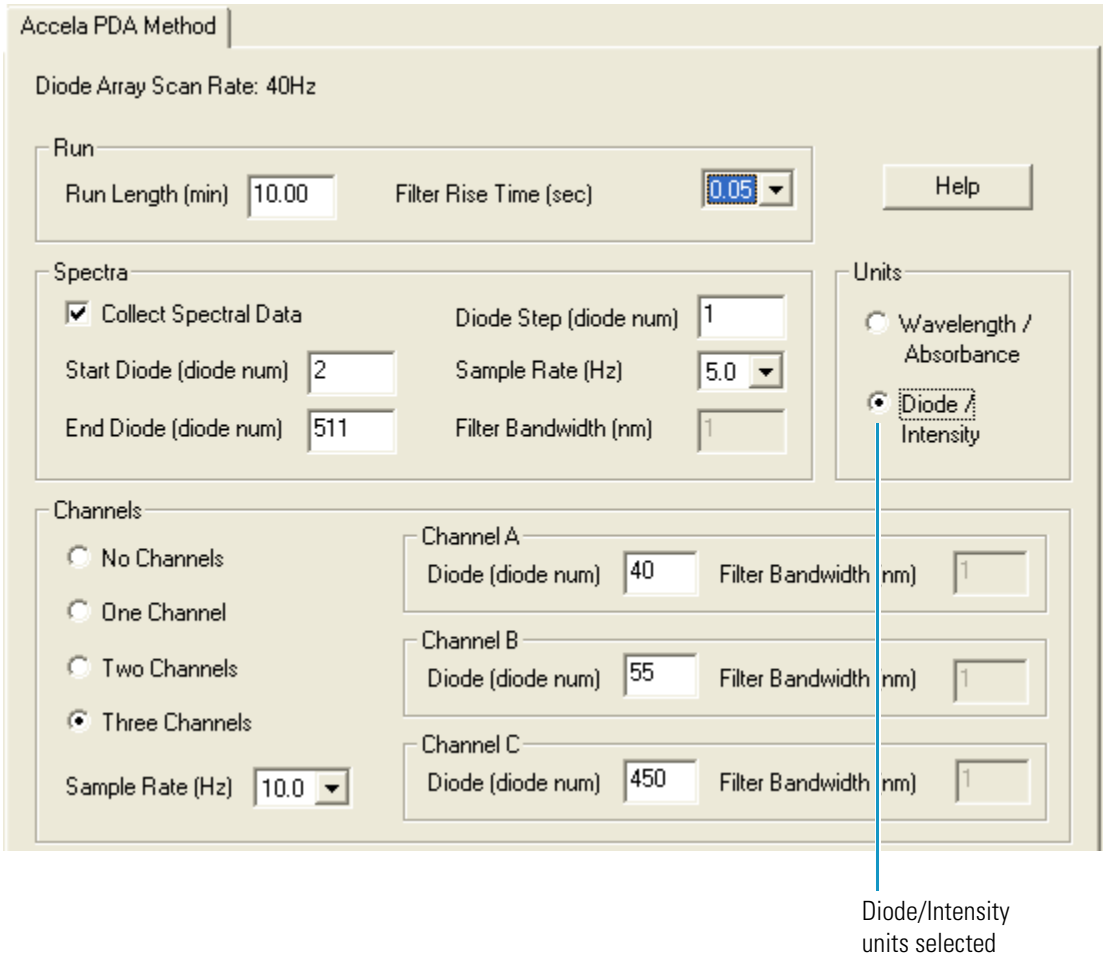
❖ **To create a display method for the PDA detector**

1. In the view bar of the Instrument Setup window, click the **Accela PDA** button.

   The view for the PDA detector appears.

2. In the Units area, select the **Diode/Intensity** option (see Figure 166).

Figure 166. Accela PDA Method page with the Diode/Intensity option selected



3. Verify that the following parameters are specified on the Accela PDA Method page in the Spectra area:
   - Start Diode (diode num) = **2**
   - End Diode (diode num) = **511**
   - Diode Step (diode num) = **1**

Note For a display method, the following parameters are not downloaded to the PDA detector: run length, filter rise time, and sample rate.

4. Choose **File > Save** to save the display method.

   An error message appears (see Figure 167).

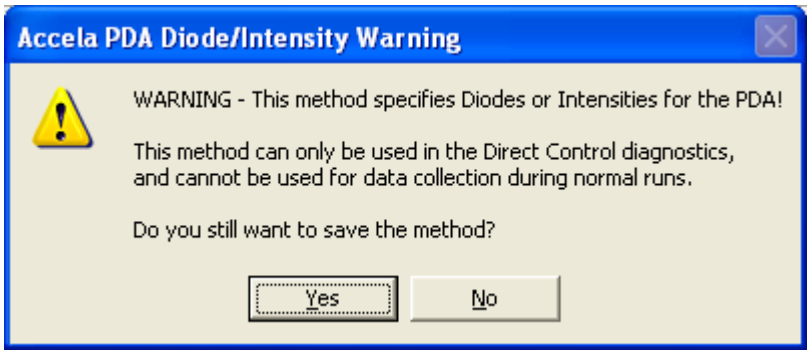
   Figure 167. Error message that appears when you attempt to save a display method

5. Click **OK**.

6. From the menu bar, choose **Accela PDA > Direct Control**.

   The Accela PDA Diode/Intensity Warning dialog box appears (see Figure 168).

   Figure 168. Accela PDA Diode/Intensity Warning dialog box



7. Click **Yes**.

   The Save PDA Display Method dialog box appears.

8. Save the display method.

   The data system saves the method with a .spda file extension.

   Note The .spda file extension is a special file extension used for all method files based on Diode/Intensity units. When you create a file with an .spda file extension, you can only load and use it in the Direct Control dialog box.

   After you save the method, the Direct Control dialog box appears with the Display page open.

9. To adjust light throughput to the diode array, go to "Adjusting the Light Throughput to the Diode Array."

Adjusting the Light Throughput to the Diode Array

The PDA Detector has two attenuators that control the light throughput from the lamps. Manually adjusting the position of the attenuators increases or decreases the amount of light falling onto the array.

Tip The integrated light intensity viewed on the Display page is a function of the light throughput to the diode array and the diode array scan rate. When you change the diode array scan rate, you must adjust the light throughput.

- If you increase the diode array scan rate (for example, from 20 to 80 Hz), you must increase the light throughput to achieve the same intensity counts.
- If you reduce the diode array scan rate (for example, from 80 to 20 Hz), check the intensity counts, and if necessary reduce the light throughput to avoid saturating the array. When the array is saturated, the response from the PDA detector is a flat baseline.

Adjusting the position of the attenuator tabs changes the light throughput to the diode array: up increases the light throughput and down decreases the light throughput.

Adjust the light throughput to the diode array whenever you do the following:

- Observe an increase in the detector noise level.
- Move the detector.
- Replace either lamp or the flowcell.
- Change the configuration setting for the diode array scan rate.

The first time that you adjust the attenuators, you must create a display method that records light intensities. You can identify display methods by their .spda file extension. After creating the method for adjusting the attenuators, save it with a descriptive name, such as diagnostics.spda, and store it for future use to simplify future adjustments of the attenuators.

Note Before you adjust the attenuators, replace the column with a flow restrictor, and set the pump to deliver HPLC-grade water at a flow rate of 1 mL/min through the flowcell.

To adjust the light output from the lamps, follow these procedures in order:

1. "Preparing the LC System for an Attenuator Adjustment" on page 263
2. "Adjusting the Attenuators" on page 264
3. "Adjusting the Attenuators" on page 264

Preparing the LC System for an Attenuator Adjustment

❖ To prepare the LC system for an attenuator adjustment

1. Ensure that the lamps are on.

   You can view the lamp status from the Configuration page of the Direct Control dialog box or from the PDA detector view of the Status page of the Information view. For information about the Configuration page, see "Configuration Page" on page 175.

2. Replace the LC column with a flow restrictor.

3. Start pumping HPLC-grade water at a flow rate of 1 mL/min through the flowcell.

Go to the next procedure, "Accessing the Attenuators."

Accessing the Attenuators

❖ To access the attenuators

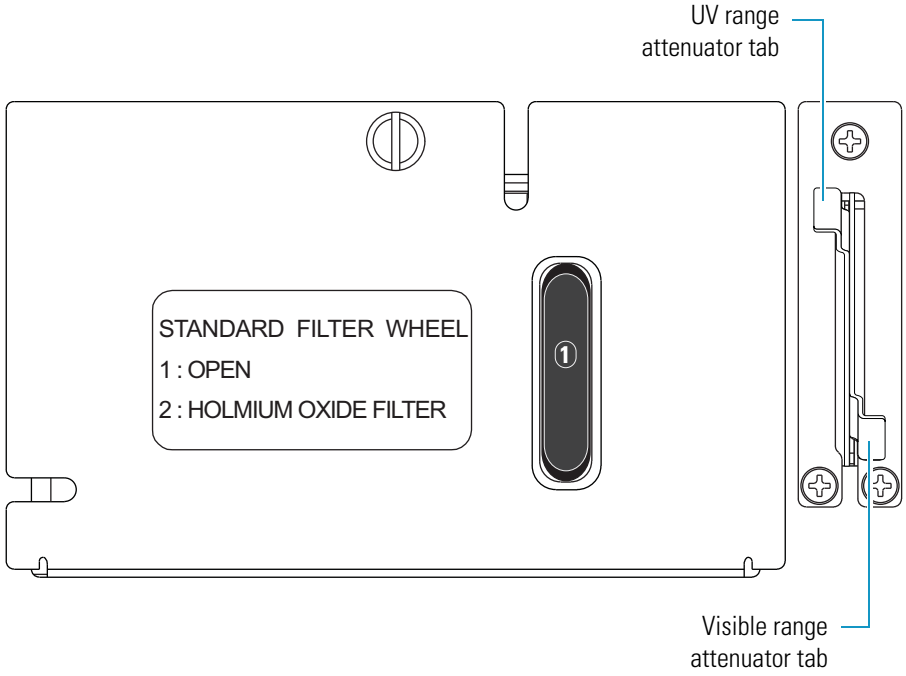
Open the front doors of the detector.

Note For the discontinued Accela PDA Detector, you must remove the flowcell cover to access the attenuator tabs.

The attenuators are located on the right side of the front panel. Two black tabs are attached to the attenuators for manual adjustments (see Figure 169).

The left tab controls the light throughput from the deuterium lamp (UV region) and the right tab controls the light throughput from the tungsten lamp (Visible region). Moving an attenuator tab up increases the light throughput, and moving the tab down decreases the light throughput to the diode array.

Figure 169. Attenuator tabs for the Accela PDA (80 Hz) Detector



Go to the next procedure, "Adjusting the Attenuators" on page 264.

Adjusting the Attenuators

❖ To adjust the attenuators

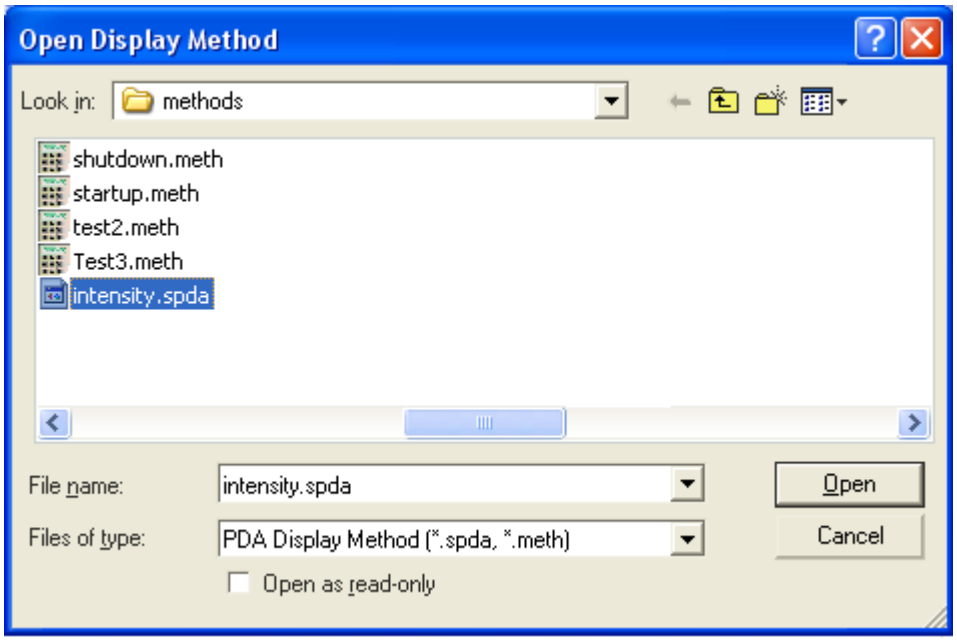
1. If you have not already done so, create a display method to view the light intensity from the lamps (see "Creating a Display Method" on page 259).
2. Load the display method to the detector as follows:
   a. In the Direct Control dialog box for the PDA detector, click the **Display** tab.

      The Display page appears.

   b. In the Control area, click **Load Method**.

      The Open Display Method dialog box appears (see Figure 170).

Figure 170. Open Display Method dialog box for the PDA detector



c. Choose your display method file (.spda).

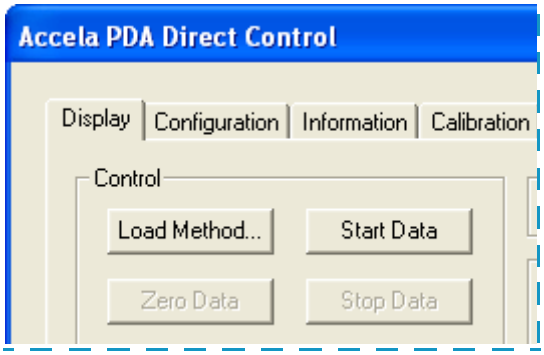
d. Click **Open**.

The file name of the display method appears in the Current Method readback on the Display page (see Figure 172 on page 266).

3. Start the data stream and adjust the attenuators as follows:

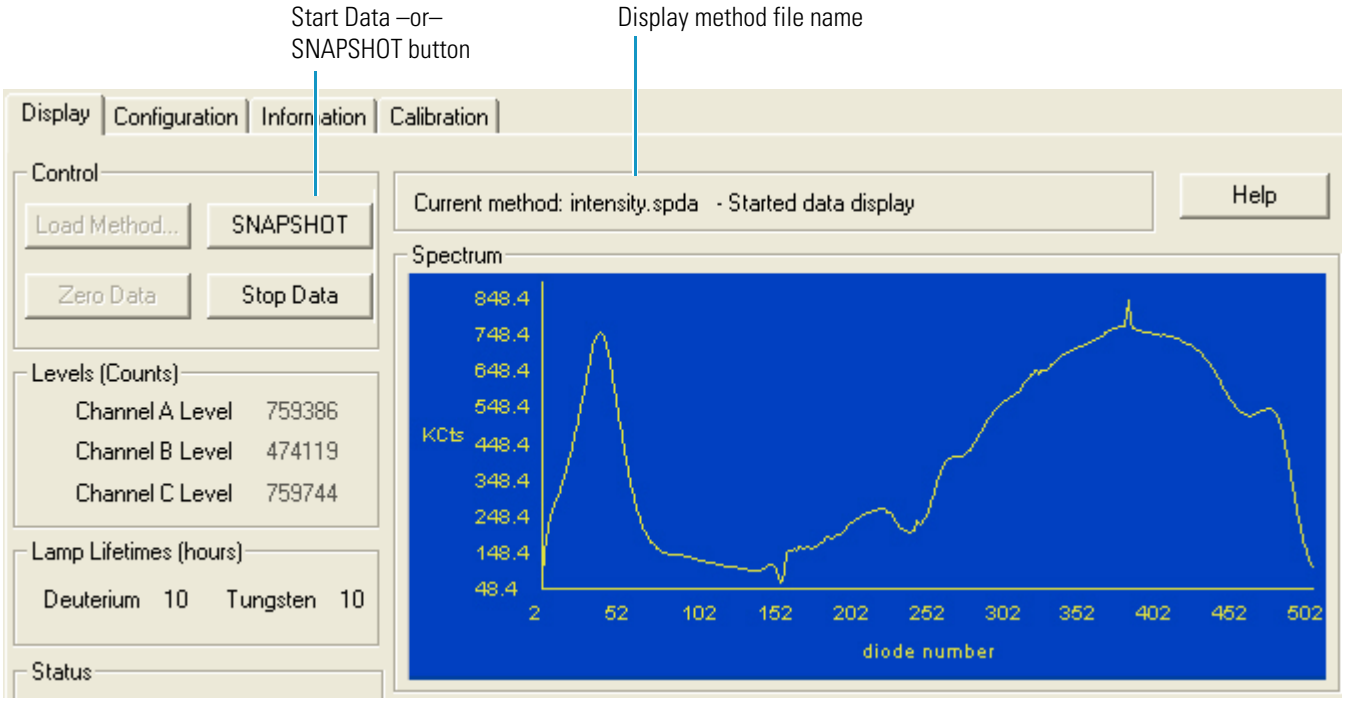
a. In the Control area (see Figure 171), click **Start Data**.

Figure 171. Start Data button on the Display page



The spectrum of light intensities appears in the top window (see Figure 172). For the UV region, the diode of maximum intensity is between diode 10 and diode 50. For the Visible region, the diode of maximum intensity is between diode 400 and diode 500. Ignore the spike at approximately diode number 380. This spike is due to the deuterium lamp.

Figure 172. Display page for the PDA detector with an intensity spectrum



b. Adjust the attenuators (see Figure 169 on page 264) as follows:

- Adjust the attenuator with the left tab on the PDA (UV range attenuation) to achieve a maximum value from 400 000 to 900 000 intensity counts in the region between diode number 10 and diode number 40.
- Adjust the attenuator with the right tab (Visible range attenuation) to achieve a maximum value from 400 000 to 900 000 intensity counts in the region between diode number 400 and diode number 500.

4. To record the light intensities, do the following:

- To save a picture of the scan to the Clipboard, press ALT+PRINT SCREEN. Paste this picture into a text editor such as Microsoft™ Word. Keep this scan for future comparisons to see if there is degradation in light intensity. Date the printout and add it to your maintenance records.
- To save the data to a file, click **SNAPSHOT**.

  The data system saves a comma-separated values file named **PDASnapshot.csv** to the following folder on the data system computer:

  C:\Xcalibur\data

  A date and time stamp are appended to the file name. The format of the date and time stamp is MMDDYYHHMMSS. MM is the month. DD is the day of the month. YY is the last two digits of the year. HH is the hour in military time. MM are minutes. SS are seconds. You can open this file with the Excel application.

For a wavelength/absorbance method, the file contains the absorbance values for the spectral scan and the discrete channel wavelengths at the moment that you clicked Snapshot.

For an intensity/diode method, the file contains the intensity values for the scan of the diode array and the intensity values for up to three individual diodes.

5. After you finish adjusting the attenuators, replace the flowcell access cover, close the front doors of the detector, and replace the flow restrictor with your LC column.

Calibrating the PDA Detector

For information about calibrating the PDA detector, see these topics:

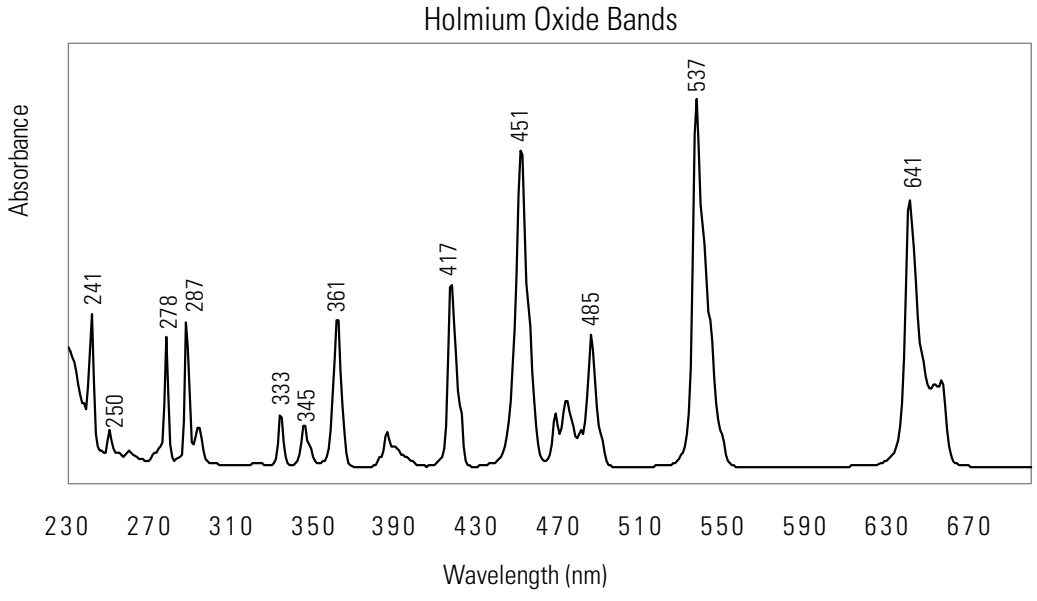
- Calibration Page for the PDA Detector
- Performing a Dark Current Calibration
- Performing a Wavelength Calibration
- Creating and Editing a Custom Wavelength Calibration List

To calibrate the wavelength accuracy of the optical bench, the PDA detector uses a holmium oxide reference solution, one of the four calibration files provided with the Xcalibur data system, or your own custom wavelength calibration file.

IMPORTANT Before you perform a wavelength calibration, verify that the diode array is not saturated (see "Adjusting the Light Throughput to the Diode Array" on page 262).

Figure 173 shows the holmium oxide spectrum.

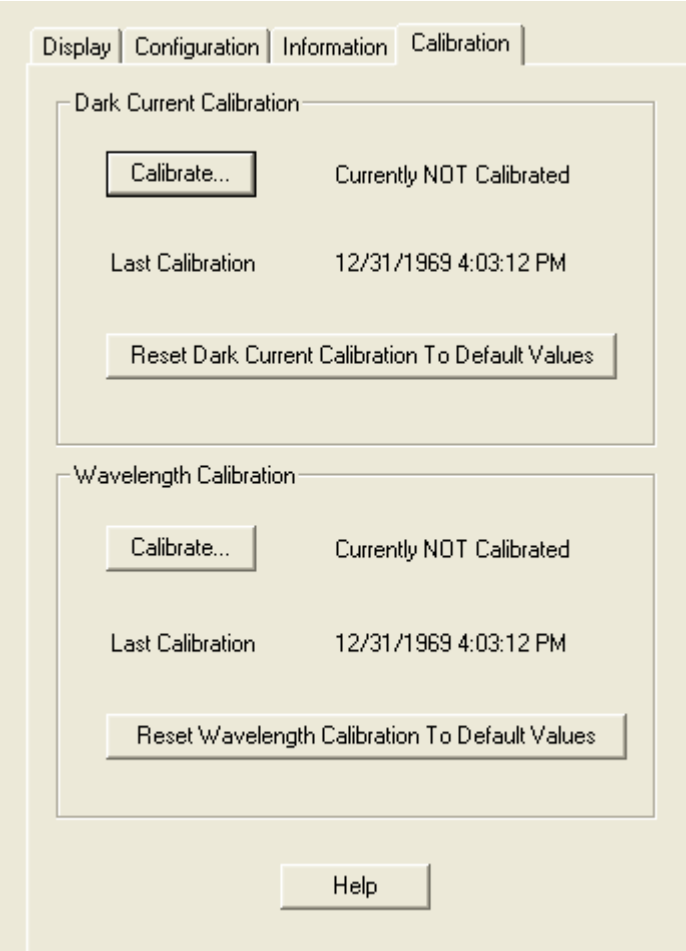
Figure 173. Holmium oxide spectrum



Calibration Page for the PDA Detector

Use the Calibration page (see Figure 174) to perform the dark current and wavelength accuracy calibrations. Perform these calibrations on a regular basis and as part of the operation verification procedure.

Figure 174. Calibration page, showing that the detector is not calibrated



Before you perform a wavelength calibration, verify that the diode array is not saturated (see "Adjusting the Light Throughput to the Diode Array" on page 262).

❖ To open the Calibration page

1. From the PDA detector view, choose **Accela PDA > Direct Controls**.

   The Direct Control dialog box appears.

2. Click the **Calibration** tab.

Note The diode array used in the PDA detector has a background count level at each diode even when both lamps are off. This background count level is called the dark current. This background or dark current must be subtracted from the intensity counts before the intensity values are converted to absorbance units (AU). After you perform a dark current calibration, the PDA detector corrects the spectrum intensity values for the dark current.

❖ To start a dark current calibration

1. Turn on both lamps and wait one hour for the lamps to equilibrate (see "Turning the Lamps On or Off" on page 167).
2. In the Dark Current Calibration area, click **Calibrate**.

   The Dark Current Calibration wizard appears.
3. Follow the instructions provided by the wizard. For more information, click **Help** at the bottom of the Dark Current Calibration dialog box.

❖ To start a wavelength calibration

1. Disconnect the LC column from the system, and then pump HPLC-grade methanol through the flowcell to remove any contaminants.
2. Turn on both lamps and wait one hour for the lamps to equilibrate (see "Turning the Lamps On or Off" on page 167).
3. Verify that the array is not saturated ("Adjusting the Light Throughput to the Diode Array" on page 262).
4. In the Wavelength Calibration area, click **Calibrate**.

   The Wavelength Calibration wizard appears.
5. Follow the instructions provided by the wizard. For more information, click **Help** at the bottom of the Wavelength Calibration dialog box.

❖ To reset the wavelength or dark current calibration to the default values

- To reset the dark current calibration values, in the Dark Current Calibration area, click **Reset Dark Current Calibration To Default Values**.
- To reset the wavelength calibration values, in the Wavelength Calibration area, click **Reset Wavelength Calibration To Default Values**.

Calibration Page Parameters

Table 51 describes the parameters on the Calibration page for the PDA detector.

Table 51. Calibration page parameters for the PDA detector

Parameter	Description
Dark Current Calibration	
Calibrate	Starts the dark current calibration.
Last Calibration	Indicates whether or not the PDA detector is currently calibrated. If the detector has not been calibrated, the readback is Not Calibrated. If the detector has been calibrated, the date and time of the last calibration are displayed.
Reset Dark Current Calibration To Default Values	Reloads the default instrument dark current calibration values. When you perform a dark current calibration, the Xcalibur data system compares the results to the last saved calibration file. This can cause successive calibrations to deviate from the default. Occasionally, you must reset to the default calibration values before performing a new dark current calibration to get calibration values as close to the default as possible.
Wavelength Calibration	
Calibrate	Starts the wavelength calibration.
Last Calibration [Wavelength]	Indicates whether the PDA detector is calibrated. If the detector has not been calibrated, this readback displays Not Calibrated. If the detector has been calibrated, this readback displays the date and time of the last calibration.
Reset Wavelength Calibration To Default Values	Reloads the default instrument wavelength calibration values. When you perform a wavelength calibration, the Xcalibur data system compares the results to the last saved calibration file. This can cause successive calibrations to deviate from the default. Occasionally, you must reset to the default calibration values before performing a new wavelength calibration to get calibration values as close as possible to the default.

Performing a Dark Current Calibration

The function of the array calibration is to measure and correct for the dark current produced by the diodes of the photodiode array. The dark current is the small amount of background signal that is produced by the diodes of the array even when both lamps are turned off. Typical dark current values for the Accela PDA (80 Hz) Detector range from 2000 to 4000 counts.

The environmental conditions of your laboratory can cause the dark current of the diode array to increase over time. For best results, perform an array calibration (dark current) after any of the following events occur:

- After 100 hours of use or monthly, whichever comes first
- Whenever a 5 °C change in the operating environment occurs
- After you move the detector
- After you replace the lamp
- After you download a new firmware file

Because the dark current produced by the diodes rises as the temperature within the detector rises, warm up the lamps for one hour before you perform a dark current calibration. Warming up the lamps for one hour allows the detector to equilibrate to its normal operating temperature.

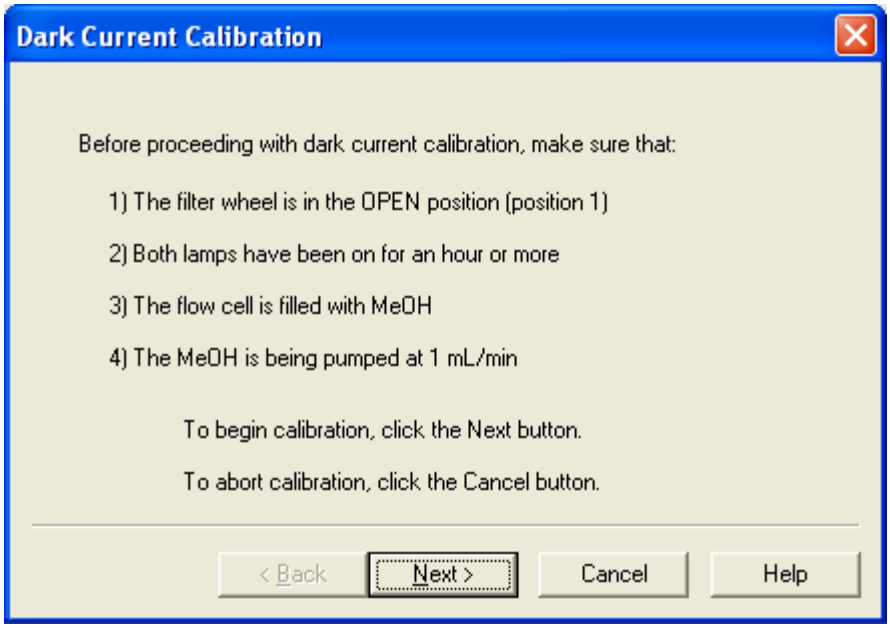
The lamps turn off during the dark current calibration routine. After the dark current calibration routine finishes, the lamps turn back on.

❖ To perform the dark current calibration

1. Pump methanol through the flow cell at 1 mL/min.
2. Turn on both lamps and wait one hour for the lamps to equilibrate.
3. Open the Calibration page for the PDA detector.
4. To start the wizard for the dark current calibration, click **Calibrate** in the Dark Current Calibration area.

   The preconditions page appears (see Figure 175).

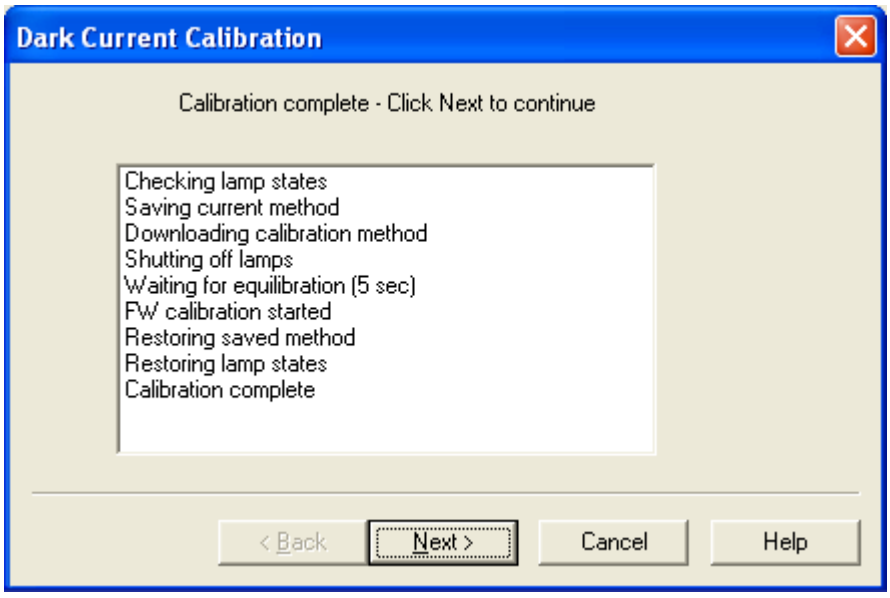
Figure 175. Preconditions page



5. Read the list of preconditions, and determine if the PDA detector is ready:
   - If the detector meets all the preconditions, click **Next** to proceed with the calibration.

     The status page appears.
   - If the detector does not meet all the preconditions, click **Cancel** to exit the wizard. Then prepare the PDA detector for calibration and begin this wizard again.
6. Observe the status pane as the calibration proceeds (see Figure 176).

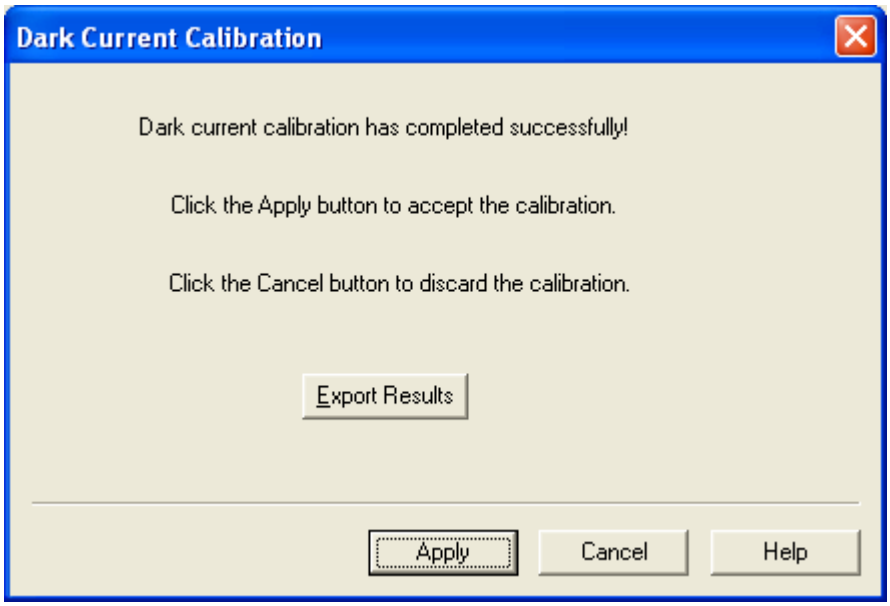
Figure 176. Calibration status page



7. When the calibration is complete, click **Next**.

   The next page of the wizard appears (see Figure 177). You can export the results of the calibration from this page.

   Figure 177. Export results page

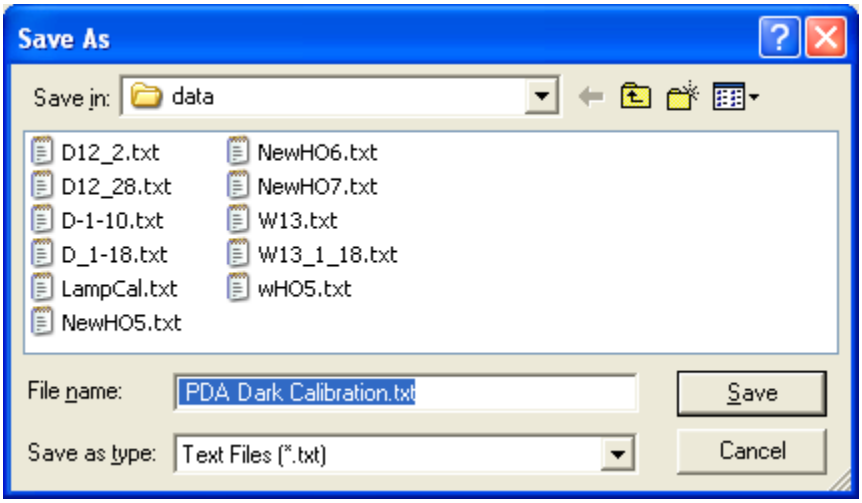


8. (Optional) To print a record of the dark current calibration, do the following:

   a. Click **Export Results**.

      The Save As dialog box appears (see Figure 178).

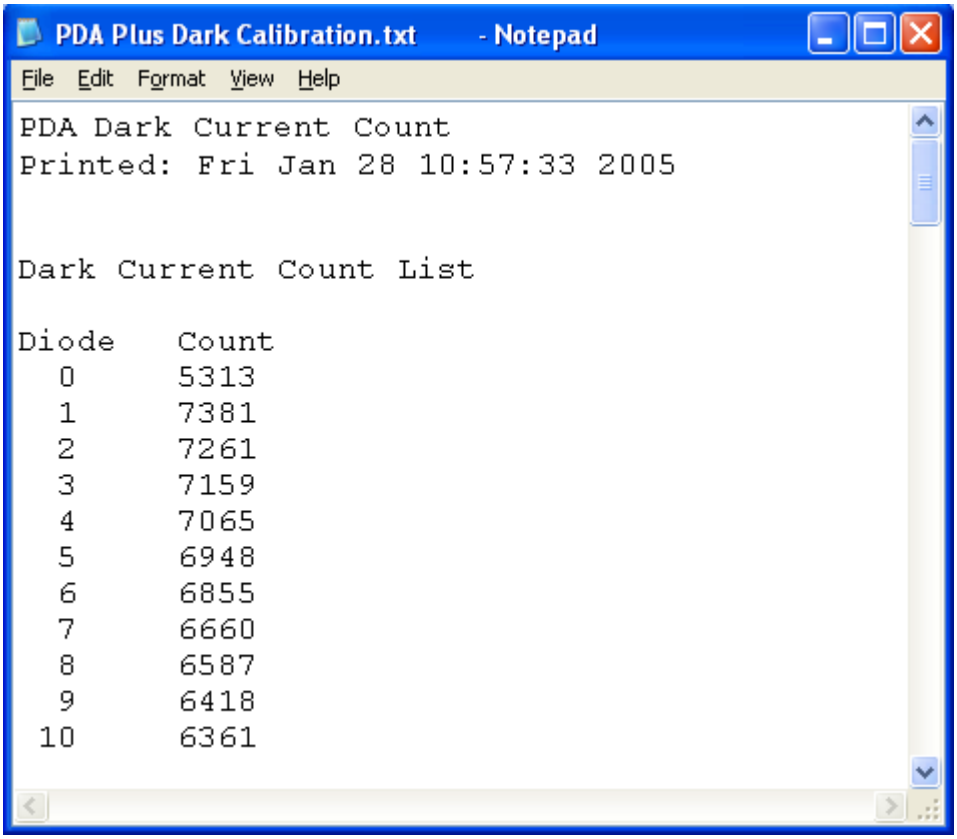
      Figure 178. Save As dialog box, showing the file extension for a text file



   b. Type a name in the File Name box, and then click **Save**.

      Once you have saved the file with a name of your choice, you can view or print the contents of the file using any text editing program (see Figure 179).

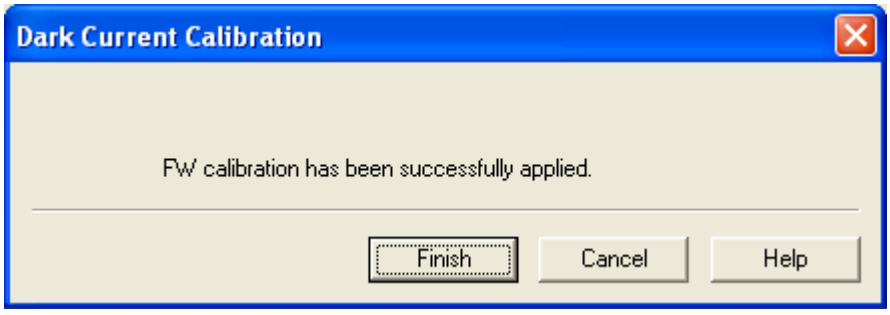
Figure 179. PDA dark current calibration text file



9. Click **Apply** to download the calibration results to the detector.

   The wizard proceeds to the success confirmation page (see Figure 180).

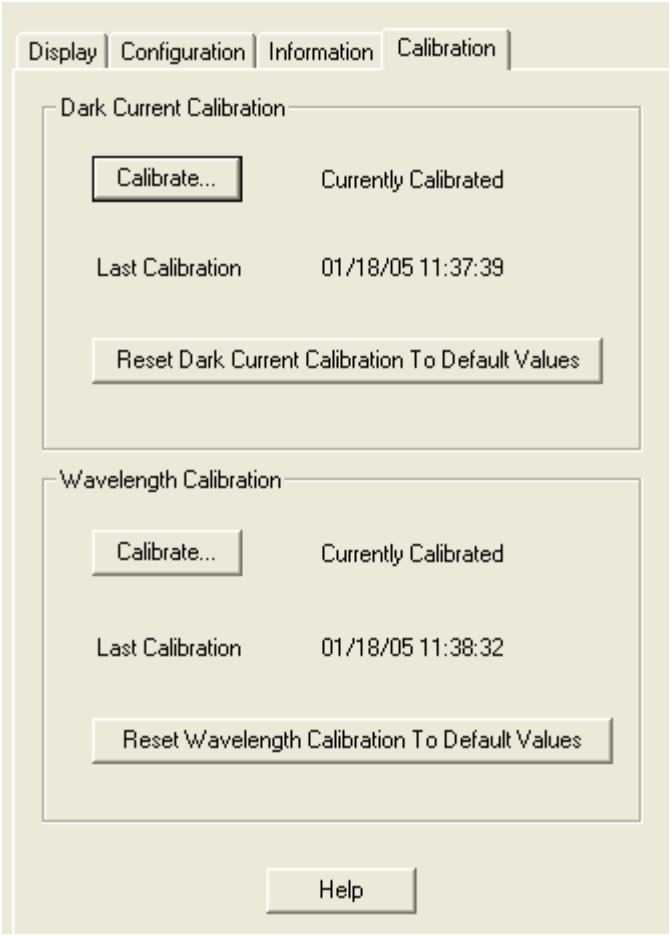
   Figure 180. Success confirmation page



10. Click **Finish** to close the wizard.

    The calibration is saved and the date and time of calibration are displayed in the Dark Current Calibration area of the Calibration page as the Last Calibration (see Figure 181).

Figure 181. Calibration page, showing the Currently Calibrated status



Performing a Wavelength Calibration

The alignment of the spectrum on the diode array depends on the physical alignment of various components of the optical bench. The alignment can become offset if the detector is sharply jolted, in shipping, for example. Such bumps and jars can slightly change the wavelength of light reaching the photodiode array. The automated wavelength calibration procedure determines the detector's wavelength accuracy and adjusts the detector's wavelength algorithm to correct for any misalignment.

❖ To perform a wavelength calibration

1. Pump HPLC-grade methanol at 1 mL/min through the flowcell.
2. Turn on both lamps and wait one hour for the lamps to equilibrate as follows:
   a. From the PDA detector view, choose **Accela PDA > Direct Controls**.

      The Direct Control dialog box appears.

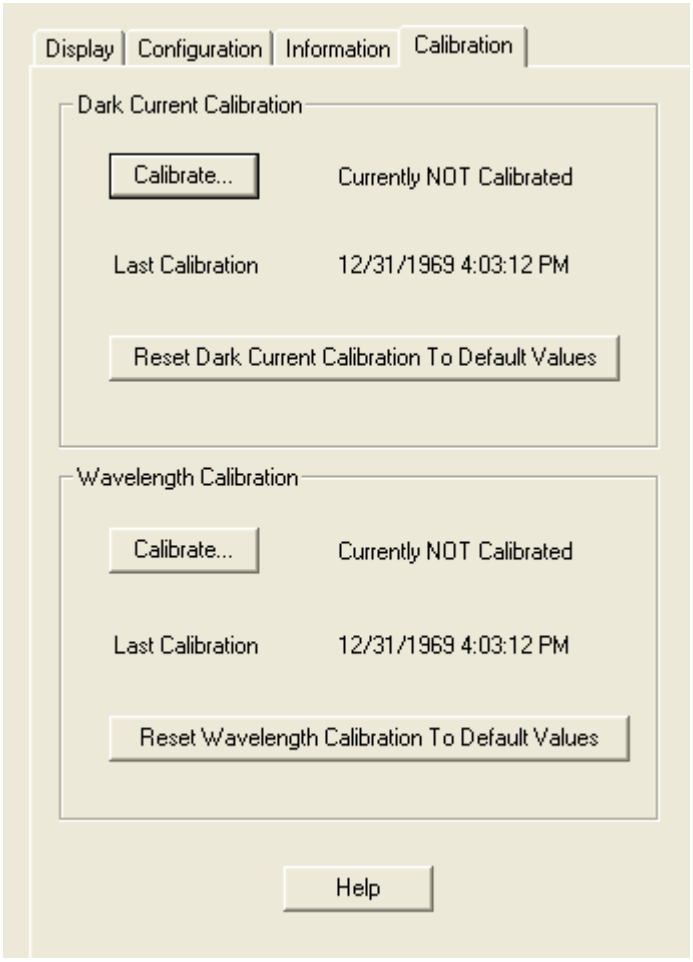
   b. Click the **Configuration** tab.

      The Configuration page appears.

   c. In the Deuterium Lamp area, click **Lamp On** to turn on the deuterium lamp.
   d. In the Tungsten Lamp area, click **Lamp On** to turn on the tungsten lamp.
3. Start the Wavelength Calibration wizard as follows:
   a. Click the **Calibration** tab.

      The Calibration page appears (see Figure 182).

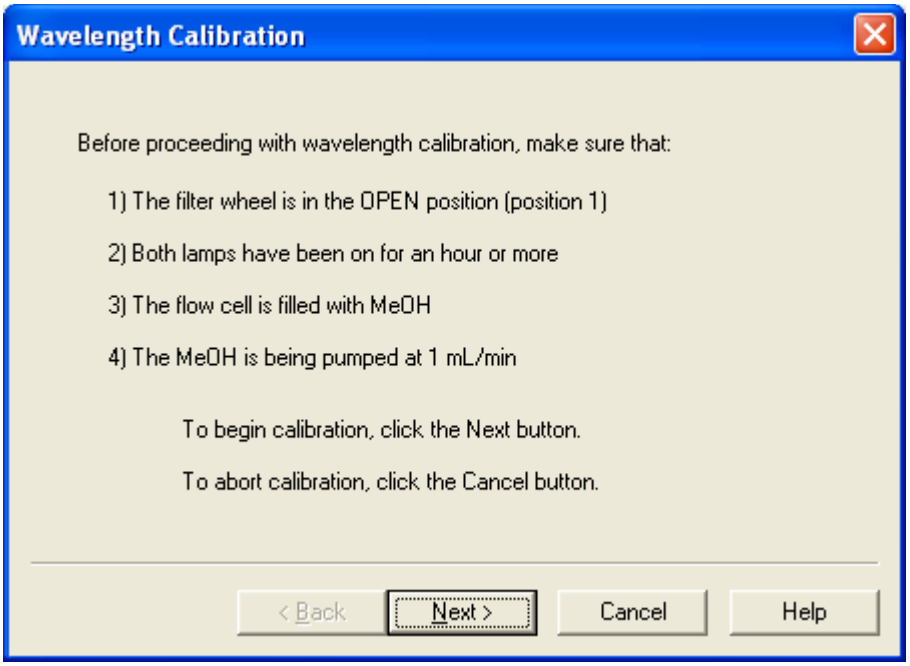
Figure 182. Calibration page for the PDA detector



b. In the Wavelength Calibration area, click **Calibrate**.

Page 1 of the wizard appears. This page lists the preconditions required to perform a wavelength calibration (see Figure 183).

Figure 183. Preconditions page



c. Read the preconditions, and determine if the detector meets these preconditions:

- If the detector meets all of the preconditions, click **Next** to proceed to the next page of the wizard where you are prompted to select a wavelength file.
- If the detector does not meet all the preconditions, click **Cancel** to exit the wizard and prepare the PDA detector for calibration.

Note On any page of the wavelength calibration wizard, you can click Cancel to exit the procedure.

4. Do the following:

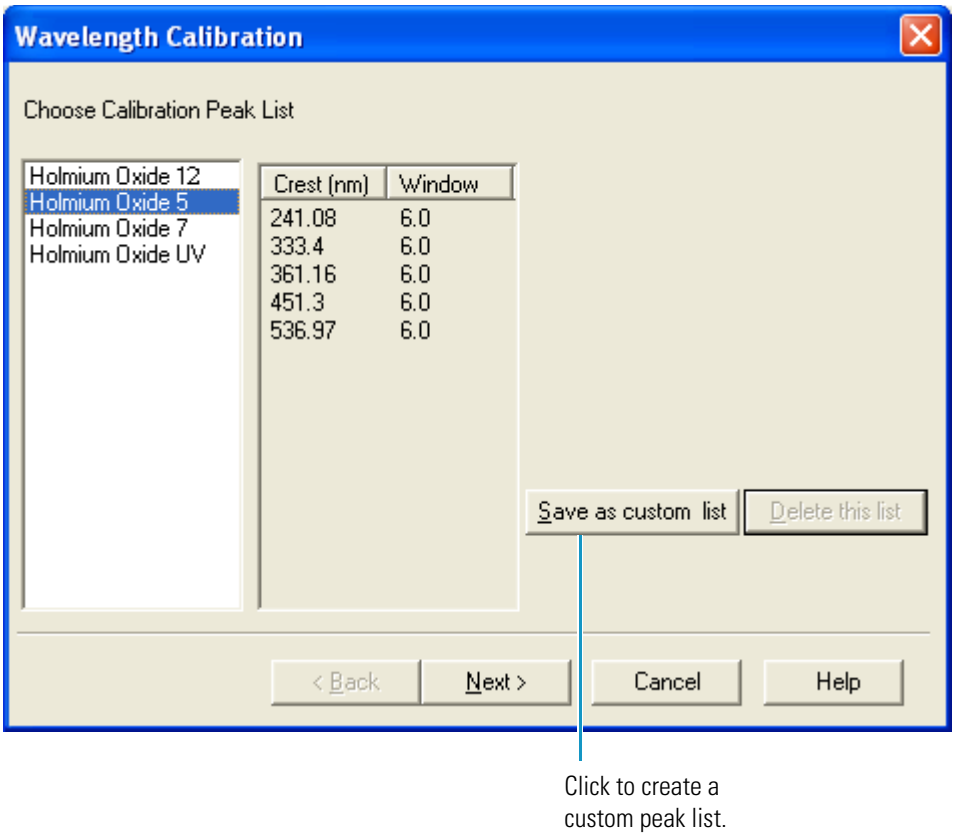
   a. Select a peak set from the list.

      The peak set should span the wavelengths you use under normal operating conditions.

      Figure 184 shows the selection of the Holmium Oxide 5 peak set. This wavelength list instructs the program to calibrate the detector at each of the five wavelengths shown.

Note The data system has four calibration files to select from. For example, the Holmium Oxide UV file contains five wavelengths in the UV region, while the other files use sets of wavelengths from both the UV and visible wavelength regions. The holmium oxide absorbance maxima are selected from a spectrum published in "Holmium Oxide Solution Wavelength Standard from 240 to 640 nm - SRM 2034 (NIST Special Publication 260-54)."

Figure 184. Choose Calibration Peak List page



Note For information about creating a custom peak list, see "Creating and Editing a Custom Wavelength Calibration List" on page 285.

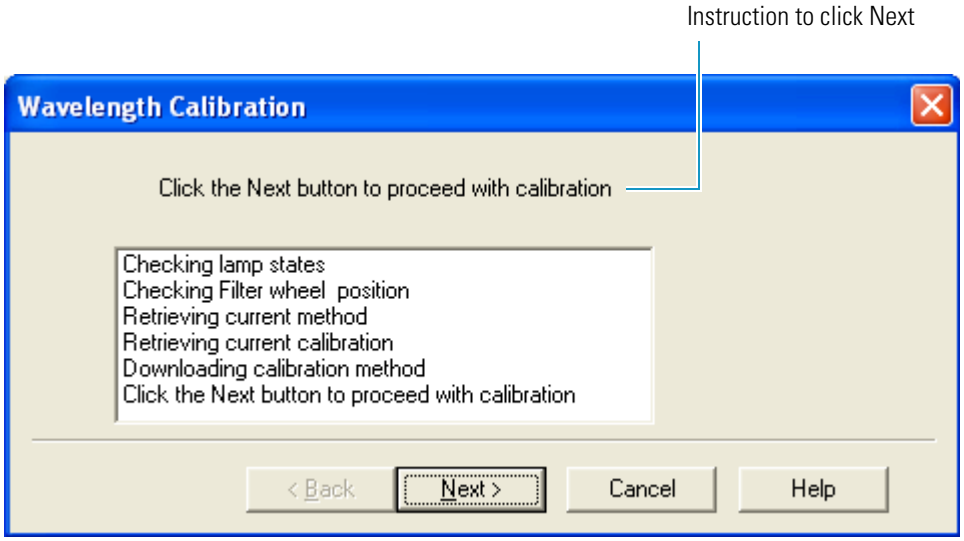
b. Click **Next.**

The next page of the wizard appears (see Figure 185).

5. Do the following:

a. Observe the status pane that tells you the wavelength file is being downloaded (see Figure 185).

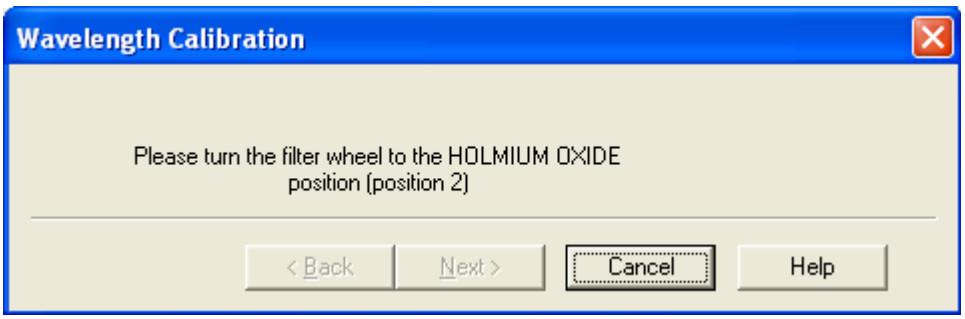
Figure 185. Download status for the selected peak list page



b. After you see the message: Click the Next button to proceed with the wavelength calibration, click **Next**.

The next page of the Wavelength Calibration wizard appears (see Figure 186). This page instructs you to place the filter wheel in position 2.

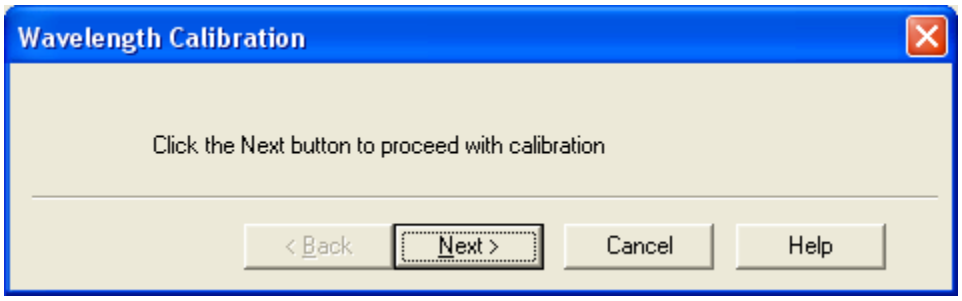
Figure 186. Request to rotate the filter wheel to position 2 page



6. Rotate the Holmium Oxide filter wheel to position 2 as directed.

After you turn the wheel, the Next button becomes available (see Figure 187).

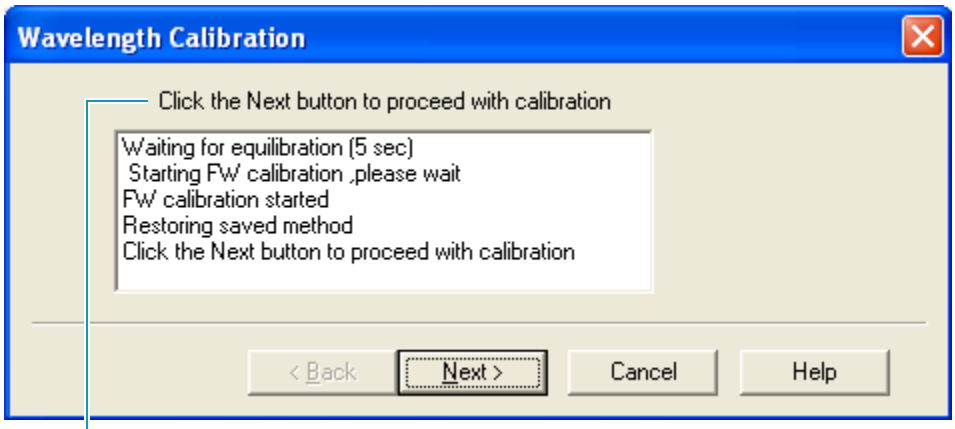
Figure 187. Available Next button



7. Click **Next**.

   The calibration status page of the wizard appears (see Figure 188).

Figure 188. Calibration status page



8. Do the following:

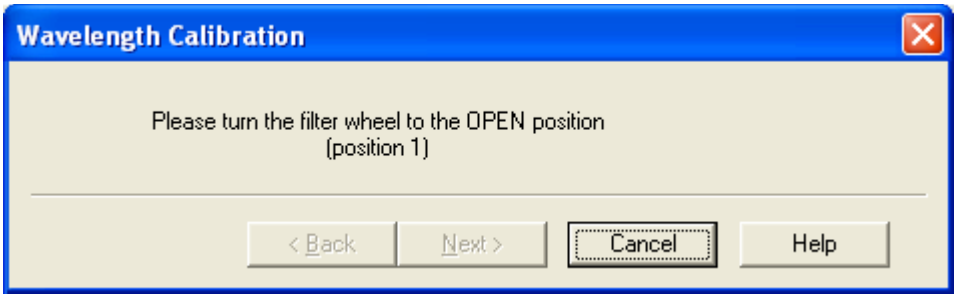
   a. Observe the status pane (see Figure 188).

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

   b. When you see the message: Click the Next button to proceed with calibration, click **Next**.

      The next page of the wizard appears (see Figure 189).

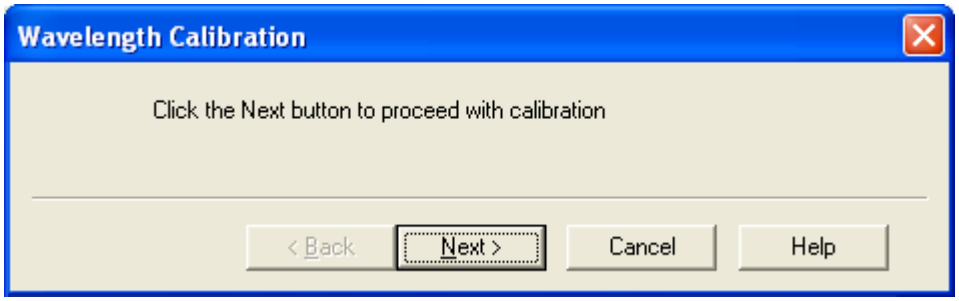
Figure 189. Request to rotate the filter wheel to position 1 page



9. Rotate the wheel back to position 1 (Open) as instructed.

   After you rotate the wheel, the Next button becomes available (see Figure 190).

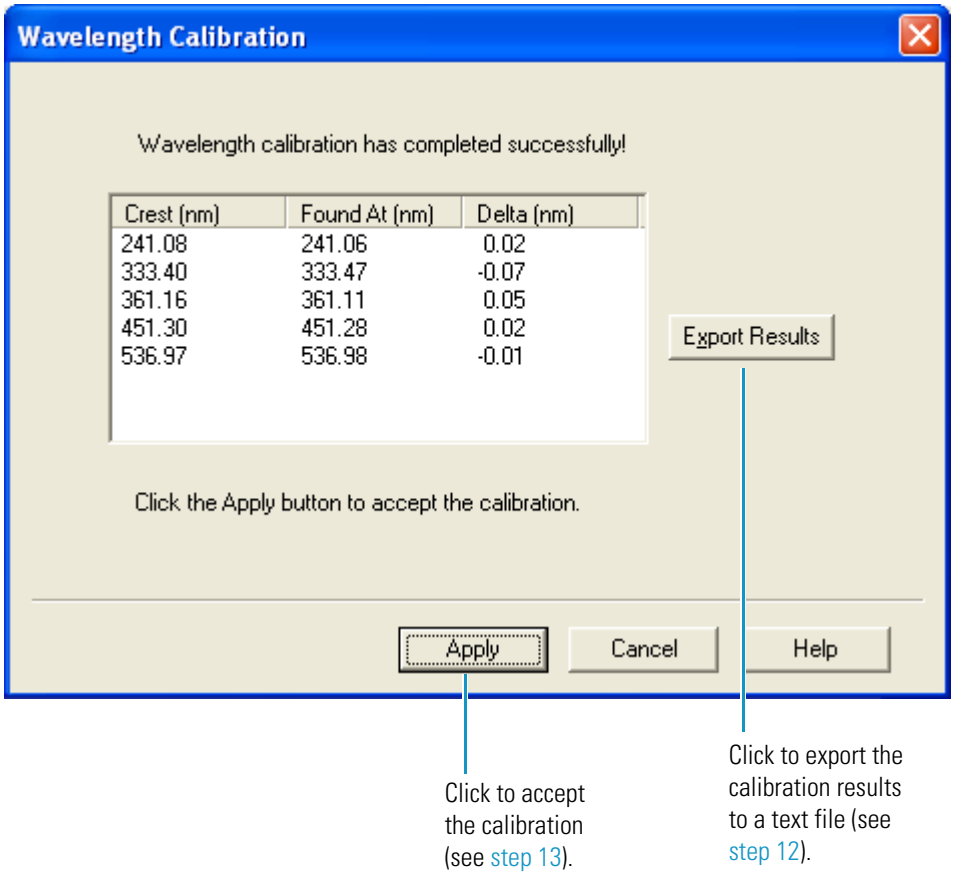
   Figure 190. Available Next button



10. Click **Next** to proceed.

    The results page for the wavelength calibration appears (see Figure 191).

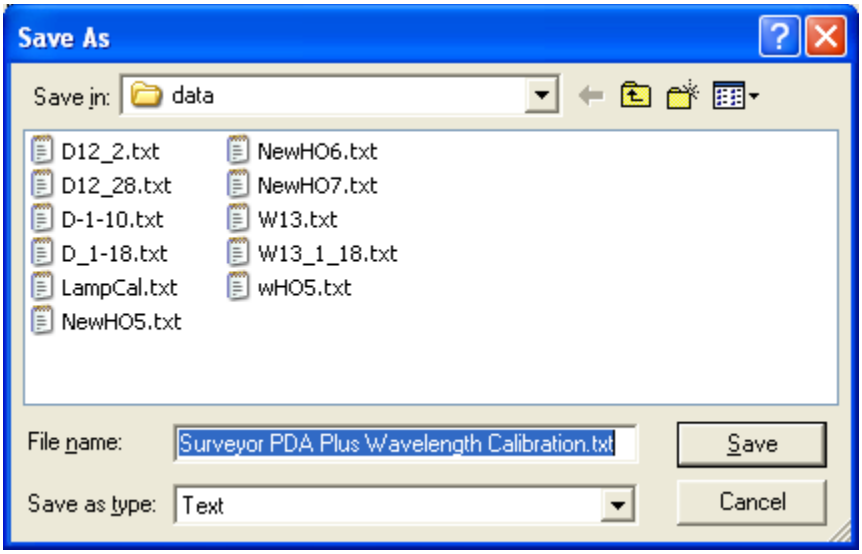
Figure 191. Delta values page



11. Verify that the delta values are within ±1 nm:
    - If the Delta values are acceptable, go to step 12.
    - If the delta values are not within the range of ±1 nm, do not export the results. Complete the calibration procedure. Then, repeat the wavelength calibration. After applying a second calibration, if the Delta values are still not within the range of ±1 nm, call your Thermo Fisher Scientific service representative for assistance.
12. (Optional) To print a report of the calibration results, do the following:
    a. Click **Export Results** to print the results to a file.

       The Save As dialog box appears (see Figure 192).

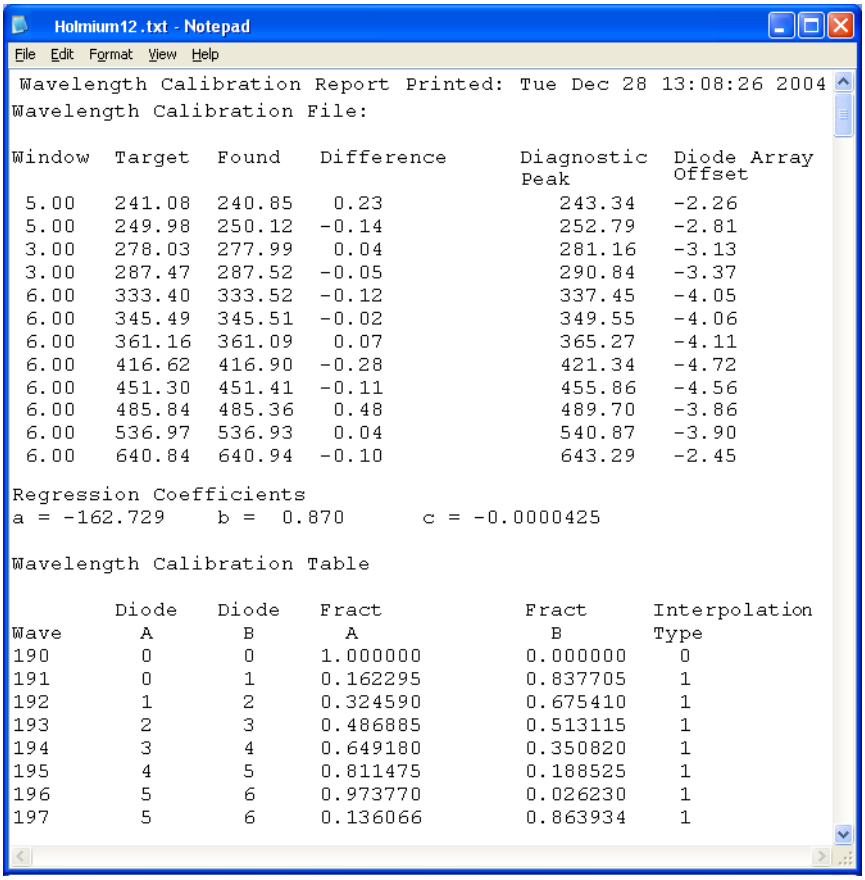
Figure 192. Save As dialog box



b. Type a name in the File Name box, and then click **Save**.

After you save the file with a name of your choice, you can view or print the contents of the file using any text editing program (see Figure 193).

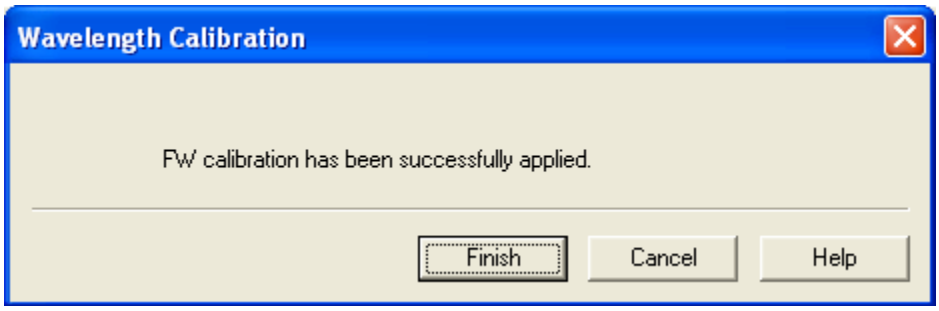
Figure 193. Wavelength Calibration file, viewed in Microsoft Notepad



13. To apply the calibration results to the detector, click **Apply** on the Delta Values page (see Figure 191 on page 283).

    The success confirmation page appears (see Figure 194).

    Figure 194. Success confirmation page for a wavelength calibration



14. Click **Finish** to complete the wavelength calibration.

    The calibration is saved. The date and time of the calibration are displayed in the Wavelength Calibration area of the Calibration page (see Figure 182 on page 277).

Creating and Editing a Custom Wavelength Calibration List

Use the Custom List Name dialog box to name a custom wavelength calibration list (WCL file).

❖ To open the Custom List Name dialog box and the Edit Peak List area

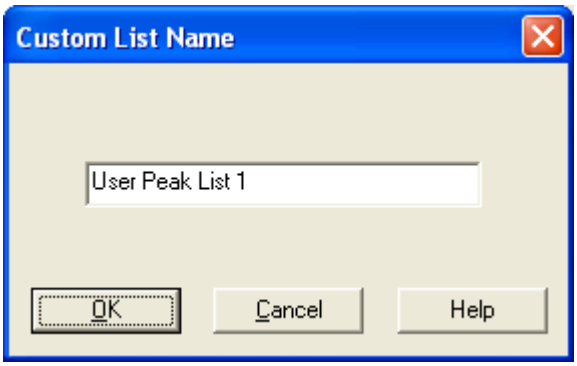
1. Start the Wavelength Calibration wizard (see "Performing a Wavelength Calibration" on page 276).
2. Verify that the PDA detector meets the preconditions for a wavelength calibration and click **Next**.

   The wavelength list page appears (see Figure 184 on page 279).

3. Click **Save As Custom List**.

   The Custom List Name dialog box appears (see Figure 195).

Figure 195. Custom List Name dialog box



4. Type a name for the list in the box, and then click **OK** to add the name to the Choose Calibration Peak List and close the dialog box.

   The name of the custom list appears in the Choose Calibration Peak List box.

5. In the Choose Calibration Peak List box, click the custom list name.

   The wavelength list becomes available.

6. In the wavelength list, click a wavelength that you want to edit.

   The Edit Peak List area appears to the right of the wavelength list.

❖ To create a custom wavelength calibration list

1. Start the Wavelength Calibration wizard (see "Performing a Wavelength Calibration" on page 276).
2. Click **Save As Custom List**.

   The Custom List Name dialog box appears.

3. In the box, type a name.
4. Click **OK**.

   The name of the new wavelength calibration list appears in the Choose Calibration Peak List box.

❖ To edit the wavelength calibration list

1. Click the **Crest/Window** pane.

   The Edit Peak List area appears.

2. To add a wavelength, type a wavelength in the Peak Crest box and a window in the Peak Window box, and then click **Add**.
3. To delete a wavelength, select the wavelength, and then click **Delete**.

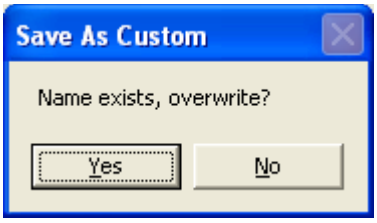
4. Click **Save As Custom List** to save the edited list.

   The Custom List Name dialog box appears.

5. Type the name of the custom list in the box.

   The Save As Custom dialog box appears (see Figure 196).

   Figure 196. Save As Custom dialog box



6. Click **Yes** to save the wavelength calibration list.

Edit Peak List Area

Table 52 describes the parameters in the Edit Peak List area on the Choose Calibration Peak list page of the Wavelength Calibration wizard.

Table 52. Edit Peak List area of the Wavelength Calibration wizard for the PDA detector

Parameter	Description
Peak Crest	Specifies a spectral band. The holmium oxide wheel contains a solution of holmium oxide in perchloric acid. Range: 190 to 800 nm
Peak Window	Specifies the search window for the peak crest. Start wavelength = peak crest – peak window/2 End wavelength = peak crest + peak window/2 Range: 0.1 to 611.0
Buttons	
Modify	Modifies the selected spectral band.
Add	Adds a spectral band.
Delete	Deletes a spectral band.

Autosampler Calibration and Record Keeping

This chapter describes the calibration and record keeping procedures for the autosampler.

Contents

The autosampler does not require calibration upon arrival at its shipping destination. However, if you use custom vials or custom microplates, you must perform the Well Bottom Distance calibration, which determines the actual depth of a vial or microplate well (see "Well Bottom Distance Calibration" on page 321). If problems occur with the column oven control, the tray temperature control, or the arm positioning, contact a Thermo Fisher Scientific service representative. This appendix includes calibration procedures for these items; however, to calibrate the autosampler's temperature zones and XYZ arm position, you must have the kit that contains the necessary test fixtures.

Calibrating the Autosampler

There are four calibration options for the autosampler:

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

The column oven and oven compartment temperature calibrations and the arm calibration are typically performed by a Thermo Fisher Scientific field service representative. To perform these calibrations yourself, you must have the service kit that contains the temperature test fixtures. In addition, to calibrate the temperature sensors for the autosampler's temperature-controlled zones, your Thermo Scientific application must be capable of providing feedback for these sensors.

Note Typically, a Thermo Fisher Scientific field service representative calibrates the controlled temperature zones and the XYZ arm position. To perform the temperature calibrations yourself, order the Field Service Calibration Kit (P/N 60053-62001). To calibrate the XYZ arm, order the LED light fixture and the non-reflective target port fixture.

If you choose to use custom vials or custom microwell plates, you must perform the Well Bottom Distance calibration, which determines the depth of the custom vial or microplate well. Perform this calibration you select a new custom tray configuration and each time you use a new type of custom vial or custom microwell plate.

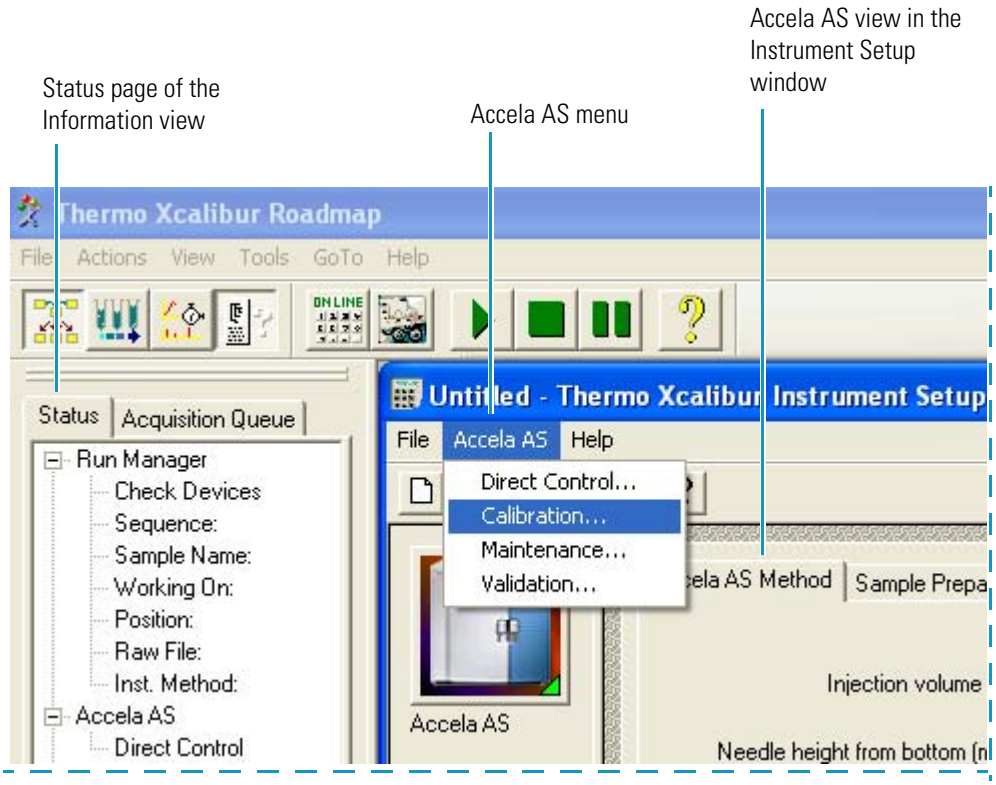
Note If you are controlling the autosampler from a Thermo Scientific data system other than the Xcalibur data system, refer to the Help provided with your data system for information about accessing the Calibration dialog box for the autosampler.

❖ To open the Calibration dialog box for the autosampler

1. Open the view for your autosampler.

   Figure 197 shows the autosampler view of the Thermo Xcalibur Instrument Setup window to the right of the Status page of the Information view.

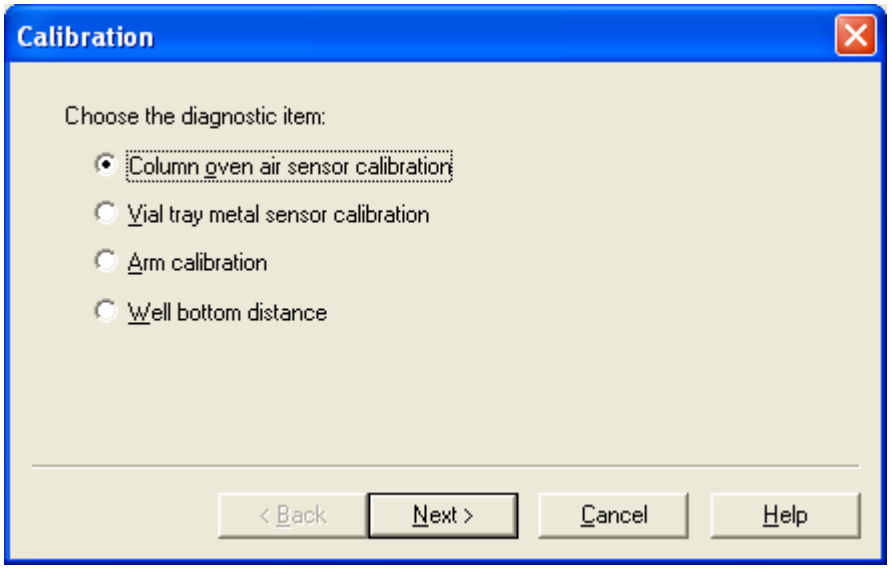
Figure 197. Accela AS menu in the Accela AS view



2. In the menu bar, choose **Accela AS > Calibration**.

   The Calibration dialog box appears (see Figure 198).

Figure 198. Calibration dialog box for the autosampler



Column Oven Air Sensor Calibration

To calibrate the column oven temperature, you must have the oven sensor test fixture provided in the Field Service Calibration Kit (P/N 60053-62001) for the autosampler.

CAUTION Because the column oven compartment can reach temperatures as high as 95 °C (203 °F), ensure that the column oven is off and that the column oven compartment is at room temperature before you install the oven sensor test fixture.

To calibrate the column oven temperature, follow these procedures in order:

1. Preparing to Calibrate the Column Oven Temperature
2. Specifying the Internal Set Target for the Column Oven Calibration Temperature
3. Adjusting the Temperature Calibration for the Column Oven

Preparing to Calibrate the Column Oven Temperature

❖ **To set up the system for a temperature calibration**

1. Open the status view for the autosampler's oven temperature.

   To open the Oven page from the Xcalibur data system, do the following:

   a. If the Information view is closed, click the **Information View** button () to open the view.

      The Information view opens with the Status page displayed.

   b. Verify that the status reads Ready to Download.

      For information about checking the status, see "Checking the Status of the LC Devices" on page 137.

   c. Click **Accela AS** in the directory tree.

      The status pages for the autosampler appear at the bottom of the Status page.

   d. Click the **Oven** tab.

      The Oven status page appears.

2. On the Oven page, check the column oven status.
3. If the column oven is on and set to a high temperature, turn the column oven off, open the column oven door, and wait for the column oven to cool to room temperature.

   For information about using the direct controls to turn the column oven on or off, see "Controlling the Tray and Oven Compartment Temperatures" on page 198.

4. Install the oven sensor test fixture (869C thermometer or equivalent) as follows:
   a. If it is not already open, open the column oven door.
   b. Loosen the top thumbscrew that holds the column clamp.
   c. With the sensor facing down, slide the metal cable protector under the right side of the clamp.
   d. Verify that the sensor is between the upper and lower column clamps and that it is not touching any metal.
   e. Tighten the thumbscrew on the sensor, route the cable of the sensor so as not to interfere with the door, and then close the column oven door.
5. Open the Calibration dialog box for the autosampler. For information about accessing the Calibration dialog box from the Xcalibur data system, see "Calibrating the Autosampler" on page 290.

Specifying the Internal Set Target for the Column Oven Calibration Temperature

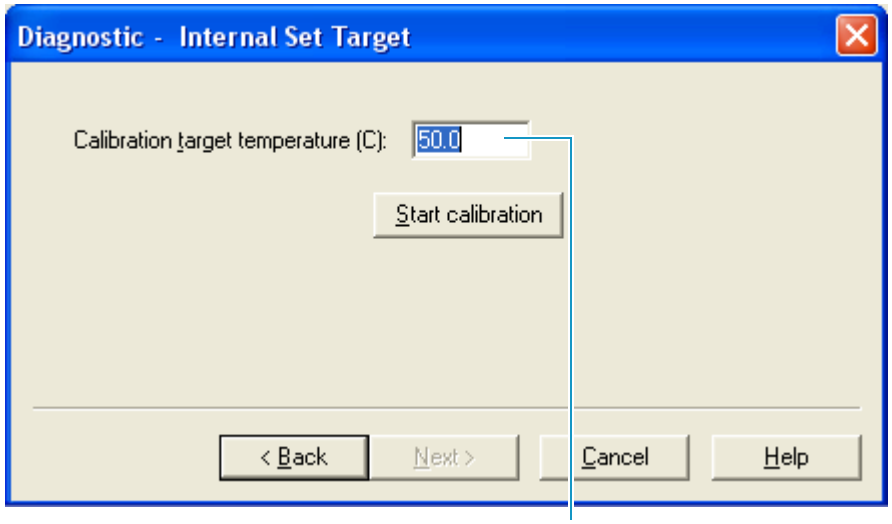
Use the Internal Set Target page of the Column Oven Air Sensor Calibration wizard to specify the calibration temperature.

❖ To specify the internal set target for the calibration temperature

1. Set up the system to perform the column oven calibration (see "Preparing to Calibrate the Column Oven Temperature" on page 292).
2. Select the **Column Oven Air Sensor Calibration** option, and then click **Next**.

   The Diagnostic - Internal Set Target page of the Column Oven Air Sensor Calibration wizard appears (see Figure 199). The default calibration temperature is 50.0 °C.

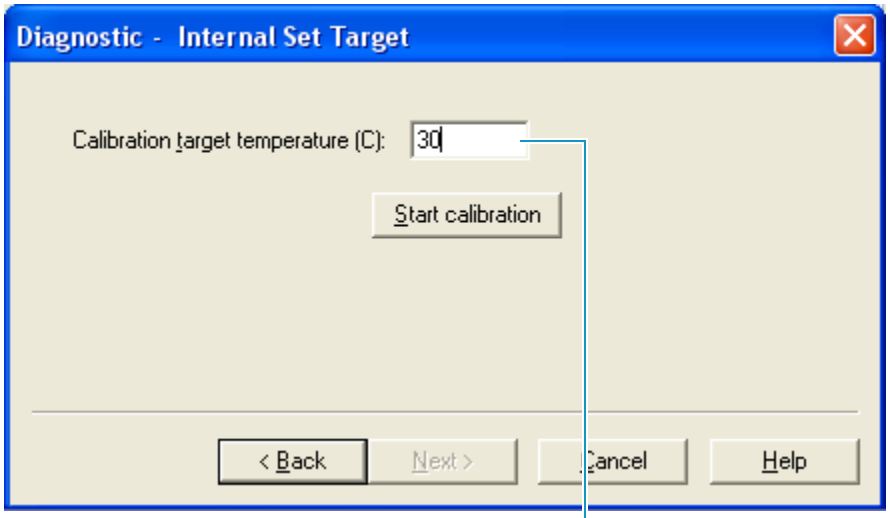
Figure 199. Diagnostic - Internal Set Target page of the Column Oven Air Sensor Calibration wizard with the default temperature setting of 50.0 °C



The default temperature for the calibration of the column oven temperature is 50.0 °C.

3. In the Calibration Target Temperature (C) box, type **30** (see Figure 200).

Figure 200. Diagnostic - Internal Set Target page of the Column Oven Air Sensor Calibration wizard with the user setting of 30 °C



User setting. The range is 5 to 95 °C.

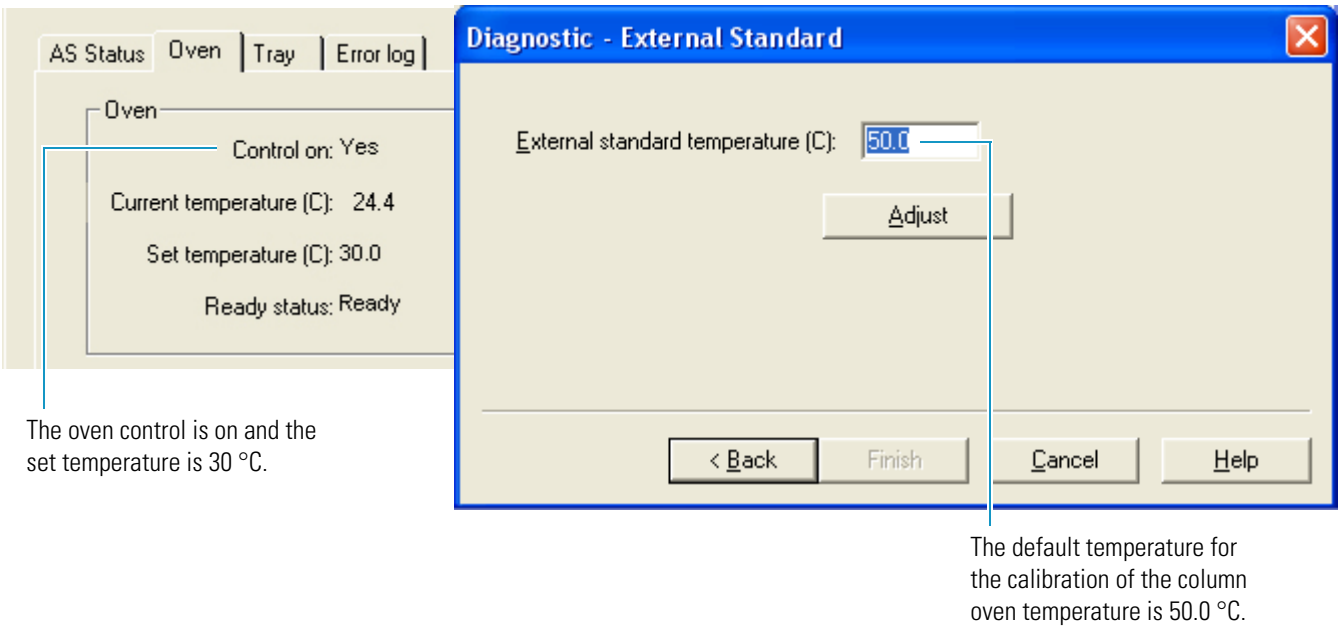
4. Click **Start Calibration**.

   The data system turns on the column oven and downloads the target temperature. The Next button becomes available.

5. Click **Next**.

The Diagnostic - External Standard page of the Column Oven Air Sensor Calibration wizard appears (see Figure 201). The default calibration temperature for the external standard is 50 °C.

Figure 201. Oven page and the Diagnostic - External Standard page of the Column Oven Air Sensor Calibration wizard



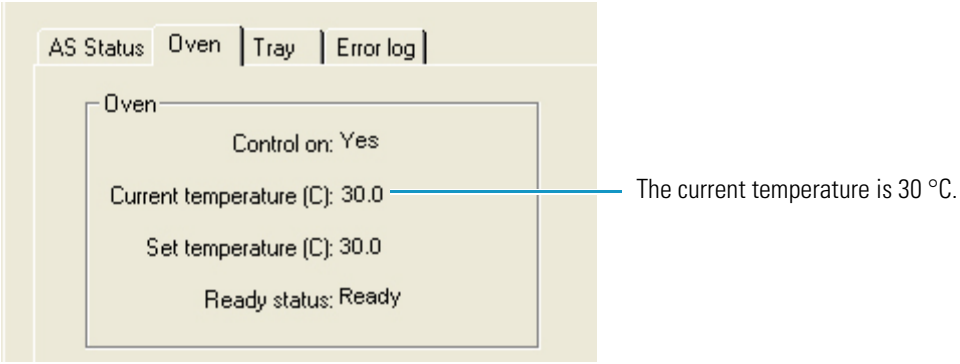
Adjusting the Temperature Calibration for the Column Oven

❖ **To adjust the temperature calibration for the column oven**

1. Specify the calibration temperature and start the Column Oven Air Sensor Calibration wizard (see "Specifying the Internal Set Target for the Column Oven Calibration Temperature" on page 293).
2. Wait for the current temperature readback on the Oven page to reach 30.0 °C (see Figure 202).

   The Current temperature readback displays the temperature recorded by the autosampler's internal temperature sensor.

Figure 202. Oven page, showing that the current temperature has reached the set temperature



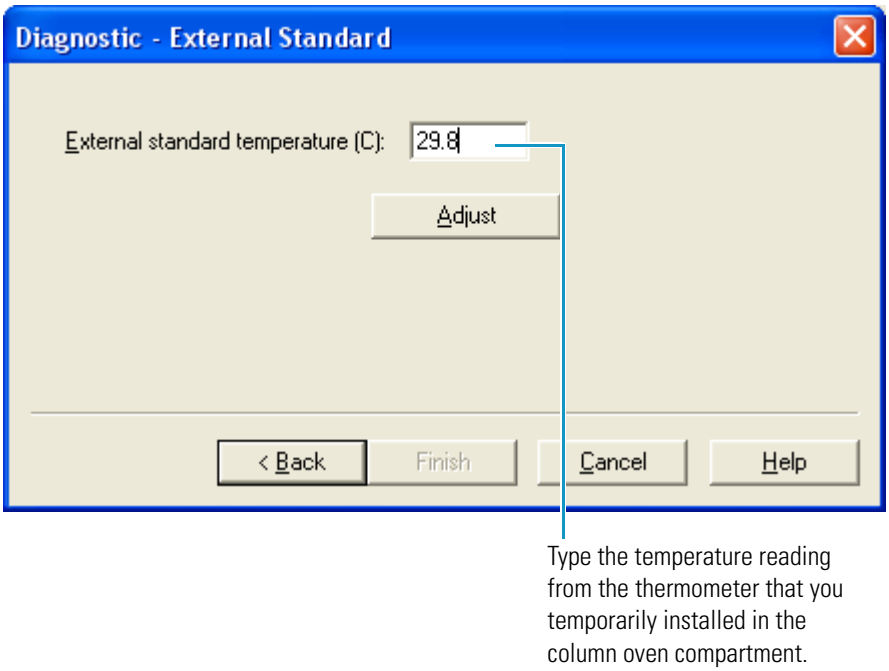
3. When the current temperature readback on the Oven page (autosampler view on the Status page of the Information view) reaches exactly 30.0 °C (see Figure 202), do the following:

   a. Type the reading from the 869C thermometer in the External Standard Temperature (C) box (see Figure 203).

      For example, if the thermometer reading is 29.8 °C, type 29.8 in the External Standard Temperature (C) box. The range is 0 to 110.0 °C.

      Note The external standard is the thermometer.

      Figure 203. Diagnostic - External Standard page of the Column Oven Air Sensor Calibration wizard

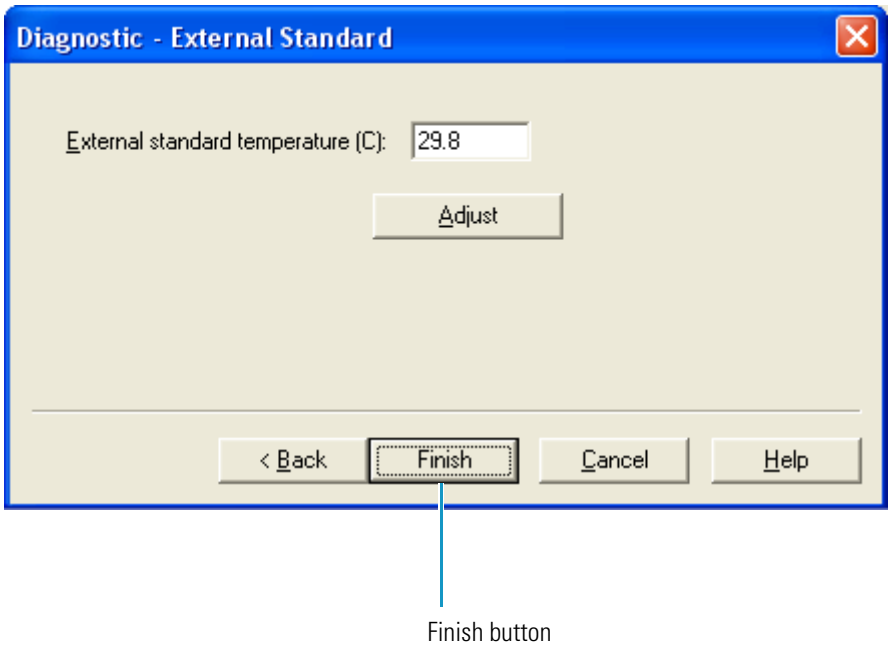


   b. Verify that the current temperature on the Oven page is still displaying 30.0 °C.

   c. On the Diagnostic - External Set Target page (see Figure 203), click **Adjust**.

The data system downloads the temperature calibration to the autosampler and the Finish button becomes available (see Figure 204).

Figure 204. Diagnostic - External Standard page of the Column Oven Air Sensor Calibration wizard



d. Repeat step 3a through step 3c until the readings from the 869C thermometer and the current temperature readout agree within ±0.2 °C.

4. When the temperatures on the 869C thermometer and the Current Temperature readback are within ±0.2 °C of each other, click **Finish** (see Figure 204).

5. On the Oven page, verify that the Current Temperature readback is stable at 30.0 °C with a maximum temperature drift of ±0.2 °C.

6. After you verify the stability of the column oven temperature, turn off the column oven using the autosampler direct controls and remove the temperature probe.

   For information about the autosampler direct controls, see "Controlling the Tray and Oven Compartment Temperatures" on page 198.

Vial Tray Metal Sensor Calibration

Use the Vial Tray Metal Temperature Sensor Calibration wizard and an external temperature sensor to calibrate the vial tray temperature control.

To perform the vial tray metal sensor calibration procedure, you must have the following items provided in the Field Service Calibration Kit (P/N 60053-62001):

- Calibrated Omega 869C RTD thermometer or equivalent
- A/S vial tray sensor

To calibrate the column oven temperature, follow these procedures in order:

1. Preparing to Calibrate the Tray Compartment Temperature
2. Specifying the Internal Set Target for the Tray Compartment Temperature
3. Adjusting the Tray Temperature Calibration

Preparing to Calibrate the Tray Compartment Temperature

❖ **To set up the system to calibrate the temperature of the tray compartment**

1. Install the tray temperature sensor as follows:
   a. Open the door to the tray compartment.
   b. Install the tray temperature sensor into location E of the tray compartment.

      The tray temperature sensor is a standard tray with a temperature sensor cemented into a middle vial location of the tray.
   c. Route the cable of the sensor through the notch at the top on the tray compartment so that it does not interfere with the door closure.
   d. Close the door to the tray compartment.
2. Open the status page for the tray temperature.

   To open the Tray page from the Xcalibur data system, do the following:
   a. If the Information view is closed, click the **Information View** button () to open the view.

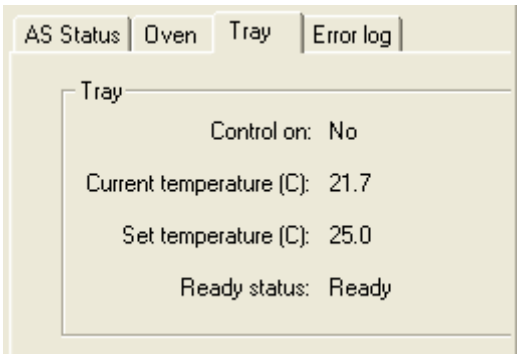
      The Information view opens with the Status page displayed.
   b. Verify that the status reads Ready to Download. For more information, see "Checking the Status of the LC Devices" on page 137.
   c. Click the listing for the autosampler in the directory tree.

      The status pages for the autosampler appear at the bottom of the Status window.

3. Click the **Tray** tab.

   The Tray page appears (see Figure 205).

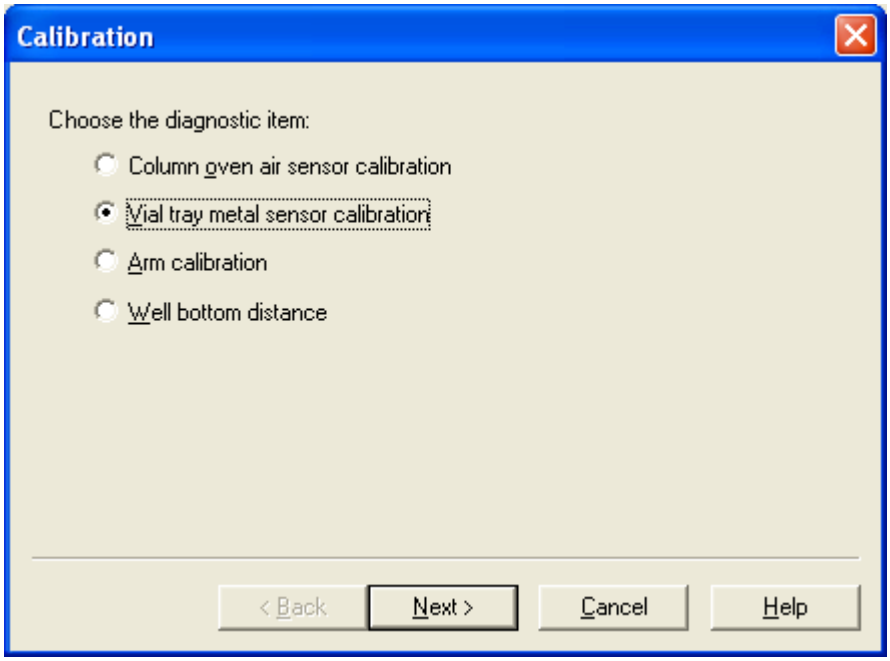
   Figure 205. Tray page



4. Open the Calibration dialog box for the autosampler (see "Calibrating the Autosampler" on page 290).

5. Select the **Vial Tray Metal Sensor Calibration** option (see Figure 206).

   Figure 206. Calibration dialog box



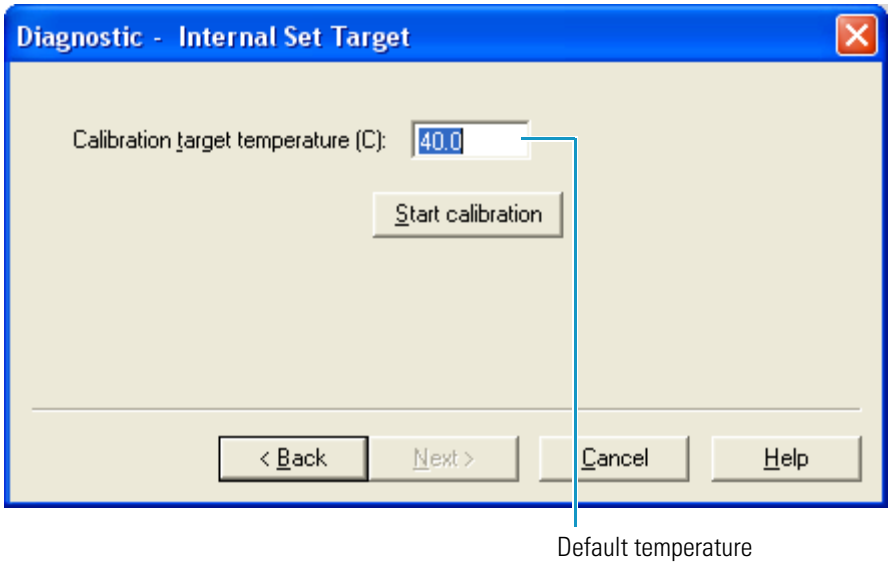
6. Click **Next**.

   The Diagnostic - Internal Set Target page of the Vial Tray Metal Sensor Calibration wizard appears (see Figure 207). The default external standard temperature is 40.0 °C.

Specifying the Internal Set Target for the Tray Compartment Temperature

Use the Diagnostic – Internal Set Target page (see Figure 207) of the Vial Tray Metal Sensor Calibration wizard to specify the calibration temperature.

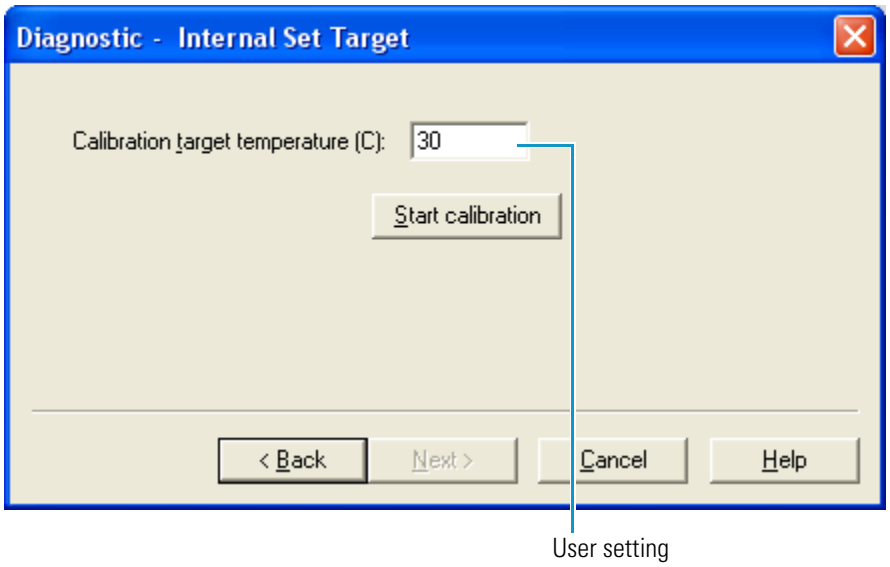
Figure 207. Diagnostic - Internal Set Target page of the Vial Tray Metal Sensor Calibration wizard



❖ **To specify the target calibration temperature for the tray compartment and start the calibration**

1. Prepare the system for the temperature calibration (see "Preparing to Calibrate the Tray Compartment Temperature" on page 298).
2. In the Calibration Target Temperature box, type **30.0**, and then click **Start Calibration** (see Figure 208).

Figure 208. Diagnostic -Internal Set Target page of the Vial Tray Metal Sensor Calibration wizard

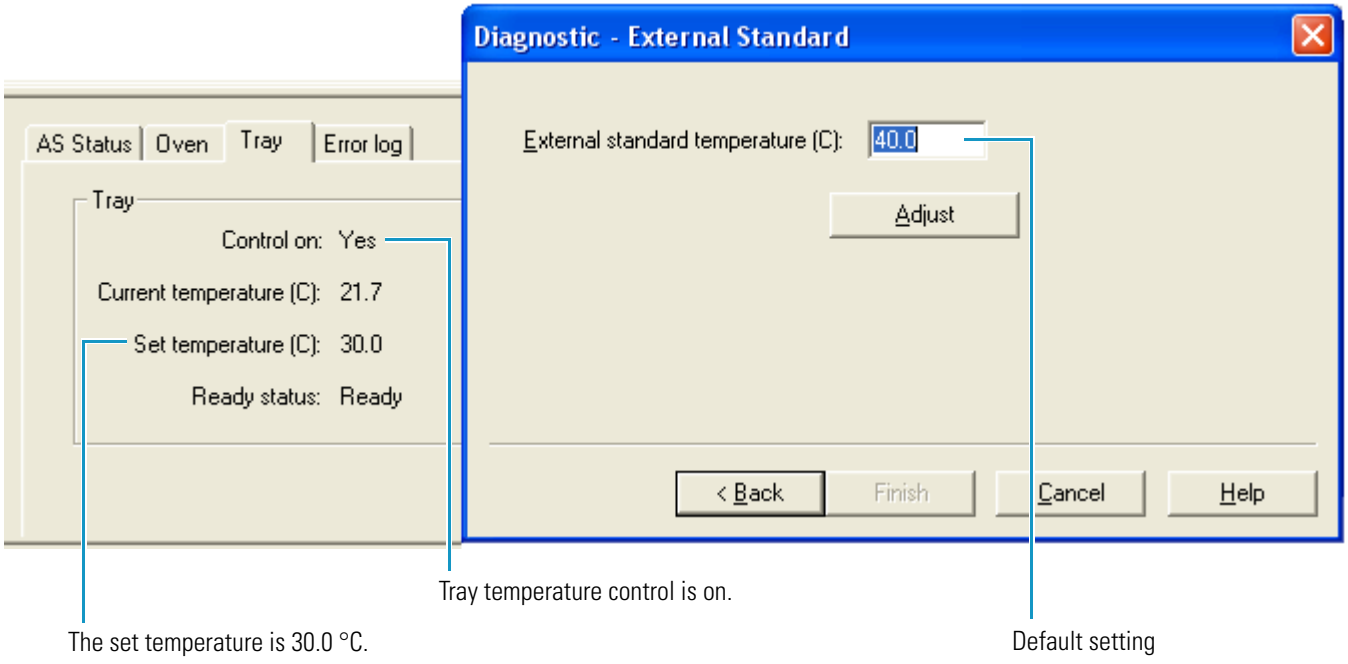


The data system turns on the tray temperature control and downloads the set temperature of 30.0 °C. The Next button becomes available.

3. Click **Next**.

The Diagnostic - External Standard page appears (see Figure 209). The default external standard temperature is 40.0 °C. The range is 0 to 110.0 °C.

Figure 209. Tray page and the Diagnostic - External Standard page of the Vial Tray Metal Sensor Calibration wizard



Adjusting the Tray Temperature Calibration

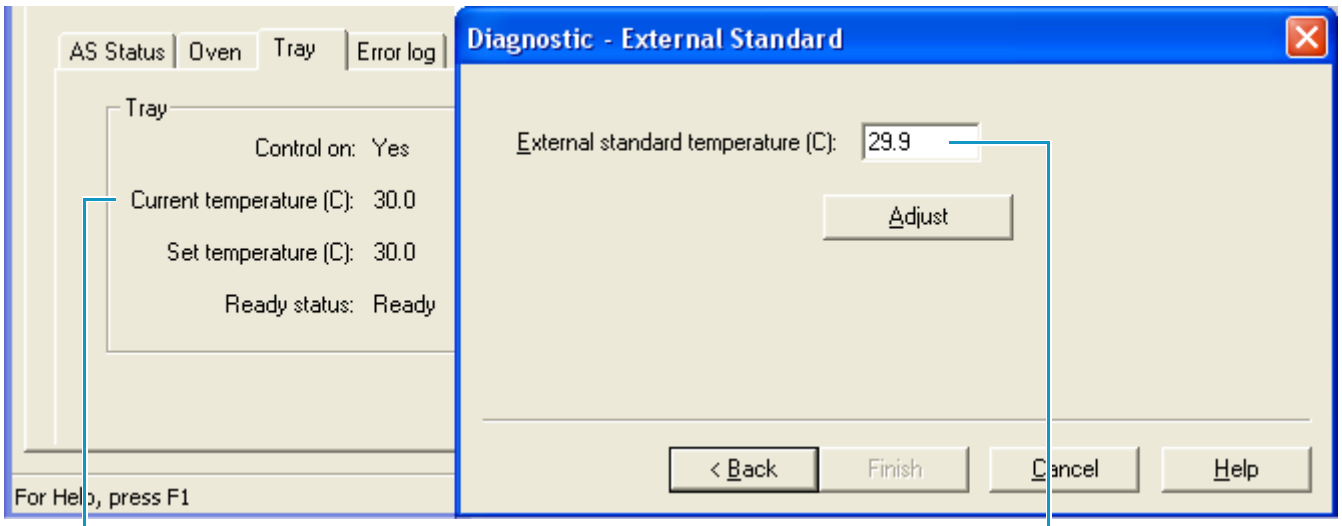
Use the Diagnostic – External Standard page of the Vial Tray Metal Sensor Calibration wizard, the status readout on the Tray page, and the external temperature sensor to adjust the temperature calibration of the tray compartment.

❖ To adjust the calibration temperature of the tray compartment

1. When the current temperature readback on the Tray page reaches exactly 30.0 °C (see Figure 210), do the following:

   a. In the External Standard Temperature (C) box (see Figure 210) on the External Set Target page, type the reading from the thermometer.

Figure 210. Tray page (status view) and the Diagnostic - External Standard page of the Vial Tray Metal Sensor Calibration wizard

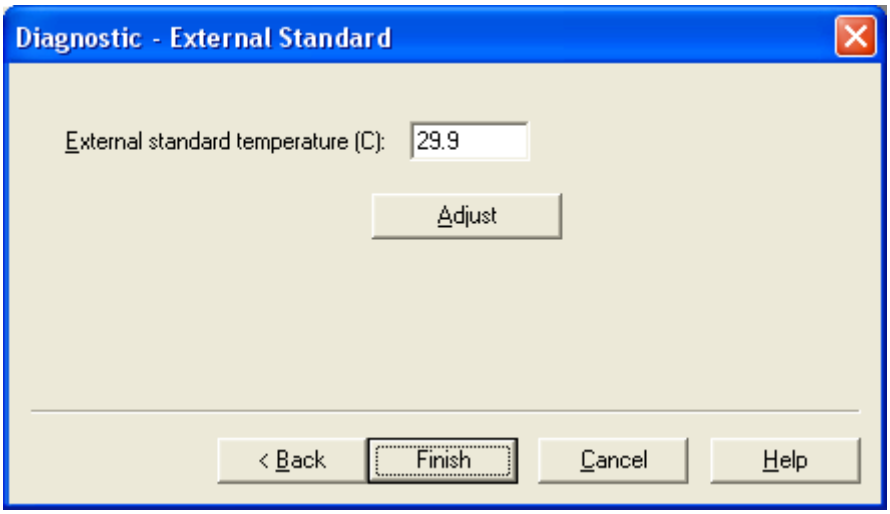


   b. On the Tray page, verify that the Current Temperature readback still displays 30.0 °C.

   c. On the Diagnostic - External Standard page (see Figure 210), click **Adjust**.

   The data system downloads the calibration to the autosampler and the Finish button becomes available (see Figure 211).

Figure 211. Diagnostic - External Standard page of the Vial Tray Metal Sensor Calibration wizard with an available Finish button



d. Repeat step 1a through step 1c until the temperatures on the thermometer and the current temperature readout (see Figure 210) agree within ± 0.2 °C.

2. When the temperatures on the 869C thermometer and the Current Temperature readout are within ±0.2 °C of each other, click **Finish** at the bottom of the Diagnostic - External Set Target page.

3. On the Tray status page, verify that the Current Temperature readout is stable at 30.0 °C with a maximum temperature drift of ± 0.2 °C.

4. After you have verified the stability of the oven tray temperature, remove the temperature probe.

Arm Calibration

During an injection, the XYZ arm moves between the specified vial or well location and the injection port. When you move the autosampler, you can jar the XYZ arm, causing it to lose alignment.

Performing the arm calibration procedure requires you to visually align the XYZ arm over the injection port. To make this visual alignment, you must replace the needle with an LED light fixture that shines a small beam of red light onto the port and replace the stainless steel injection port with a non-reflective target fixture. These test fixtures do not come with the autosampler; you must order them from Thermo Fisher Scientific.

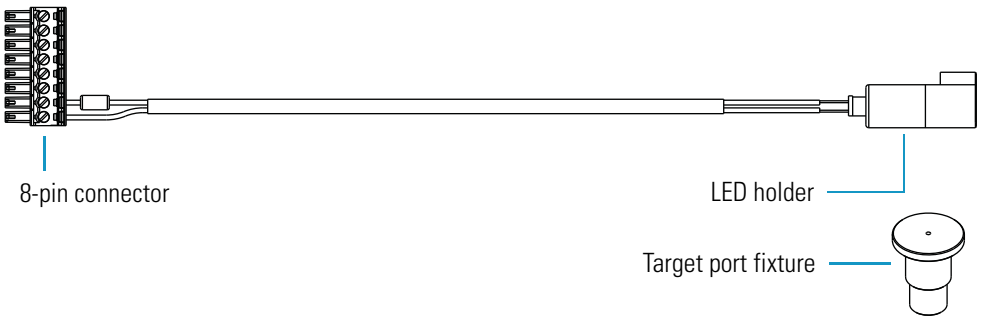
After following the instructions in the Arm Calibration wizard to adjust the XYZ arm alignment, test the alignment by making a set of injections from one or two 384-well microplates. The microplate carrier and the 384-well microwell plates are provided in the Autosampler Accessory Kit.

To calibrate the XY position of the XYZ arm, you must have these items:

- LED light fixture (P/N 60357-60021)
- Target port fixture (P/N 60357-20021)
- Microplate carrier
- One or two 384-well microtitre plates
- Masking tape

Figure 212 shows the LED light and the target port fixtures.

Figure 212. Arm calibration fixtures available by special order



To calibrate XY position of the XYZ arm and verify the calibration, follow these procedures:

1. Modifying the Autosampler Instrument Configuration
2. Starting the Arm Calibration Wizard
3. Moving the Arm to the Needle Removal Position
4. Installing the LED Light Fixture and the Target Port Fixture
5. Moving the XYZ Arm to the Home Position
6. Aligning the Light Beam with the Target
7. Applying the Arm Calibration Setting
8. Checking the Arm Calibration Offset Values
9. Testing the Alignment of the XYZ Arm

Modifying the Autosampler Instrument Configuration

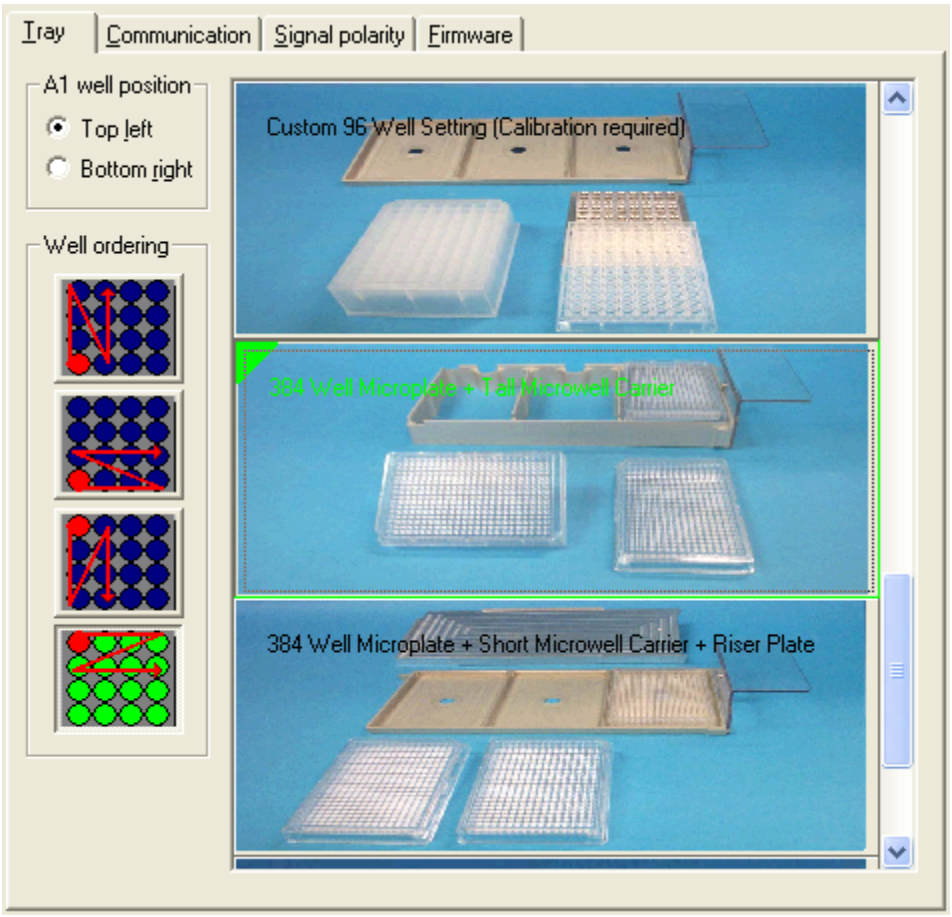
To calibrate the arm position, turn off the feature that prevents control of the autosampler when the tray compartment door is open.

❖ To modify the instrument configuration for the autosampler

1. Open the Accela Autosampler Configuration dialog box.

   The dialog box opens with the Tray page displayed (see Figure 213).

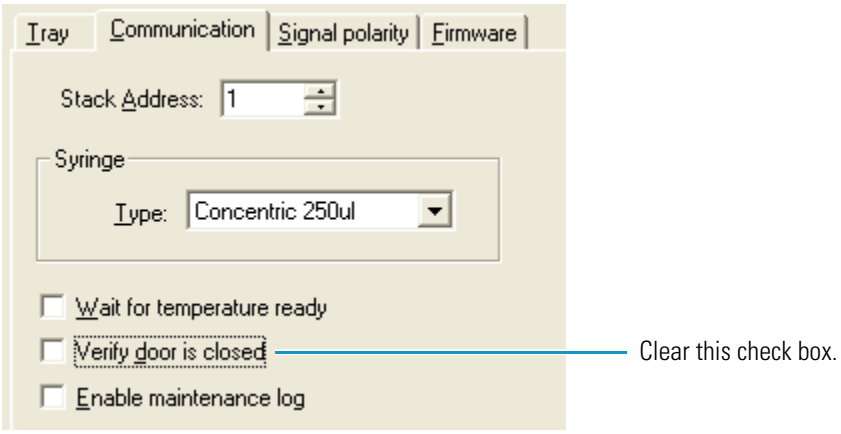
Figure 213. Tray page with the 384-well microplate tray type selected



2. Select the 384-well plate tray type.
3. In the A1 Well Position area, select the **Top Left** option.
4. Click the **Communication** tab.

   The Communication page appears.
5. Clear the **Verify Door Is Closed** check box (see Figure 214).

Figure 214. Communication page with the Verify Door Is Closed check box cleared



When the Verify Door Is Closed check box is selected, the data system prevents you from starting an injection sequence or using some of the direct control commands for the autosampler when the tray compartment door is open.

6. Click **OK** at the bottom of the dialog box to accept the settings and close the dialog box.
7. Click **Done** to close the Thermo Foundation Instrument Configuration window.

Go to the next procedure, "Starting the Arm Calibration Wizard."

Starting the Arm Calibration Wizard

Use the Arm Calibration wizard to adjust the home position of the XYZ arm.

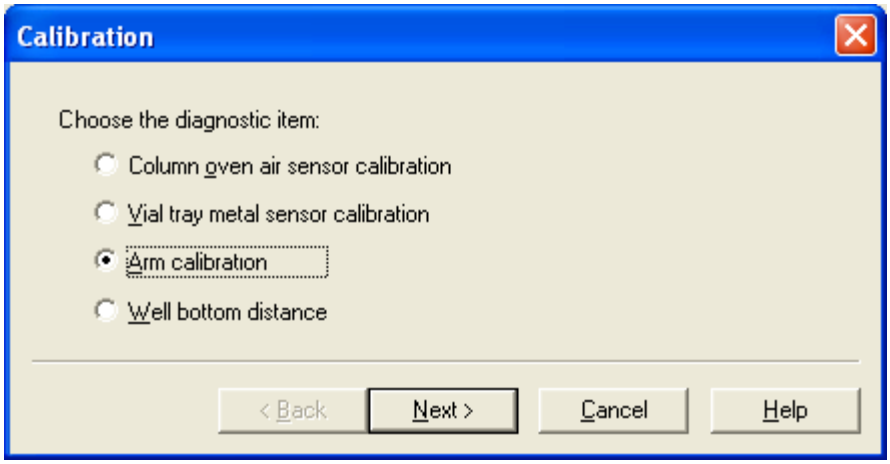
For information about the LED light and non-reflective needle port fixtures that you must have to perform the Arm Calibration wizard, see "Arm Calibration" on page 304.

❖ To start the Arm Calibration wizard

1. If you have not already done so, modify the autosampler instrument configuration as described in "Modifying the Autosampler Instrument Configuration" on page 305.
2. Open the view for the autosampler.
3. From the Accela AS menu, choose **Calibration**.

   The Calibration dialog box appears (see Figure 215).
4. Select the **Arm Calibration** option.

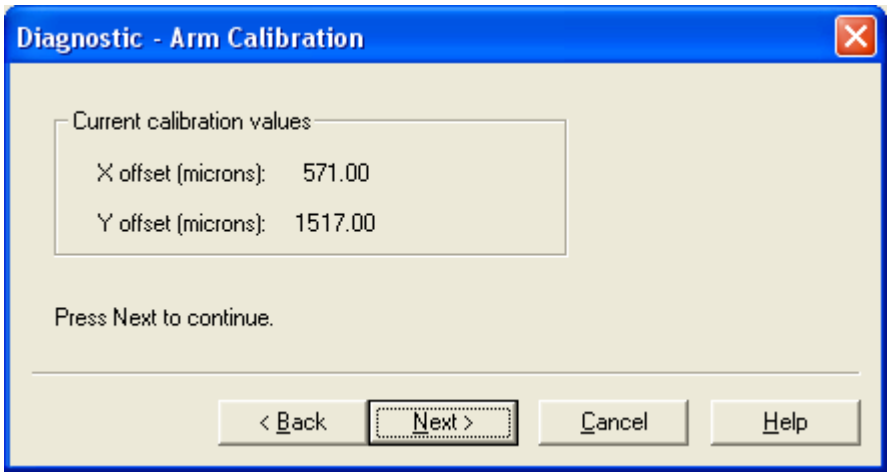
Figure 215. Calibration dialog box with the Arm Calibration option selected



The Arm Calibration wizard appears (see Figure 216).

The Current Calibration Values area displays the current calibration values for the *x*-axis and *y*-axis position of the XYZ arm over the autosampler injection port.

Figure 216. Current Calibration Values page



Note The user interface specifies the *x*- and *y*-axis offsets in microns, which is another term for micrometers.

1000 micrometers = 0.04 inches

The diameter of the hole in the center of the target port fixture is 0.047 inches.

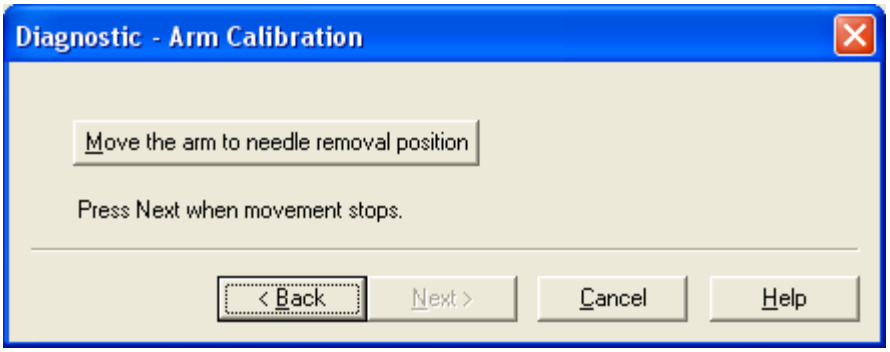
5. Click **Next** to proceed to the next page of the wizard.

Go to the next procedure, "Moving the Arm to the Needle Removal Position."

Moving the Arm to the Needle Removal Position

Use this page of the Arm Calibration wizard to move the arm to the needle removal position (see Figure 217).

Figure 217. Move the Arm to Needle Removal Position button page

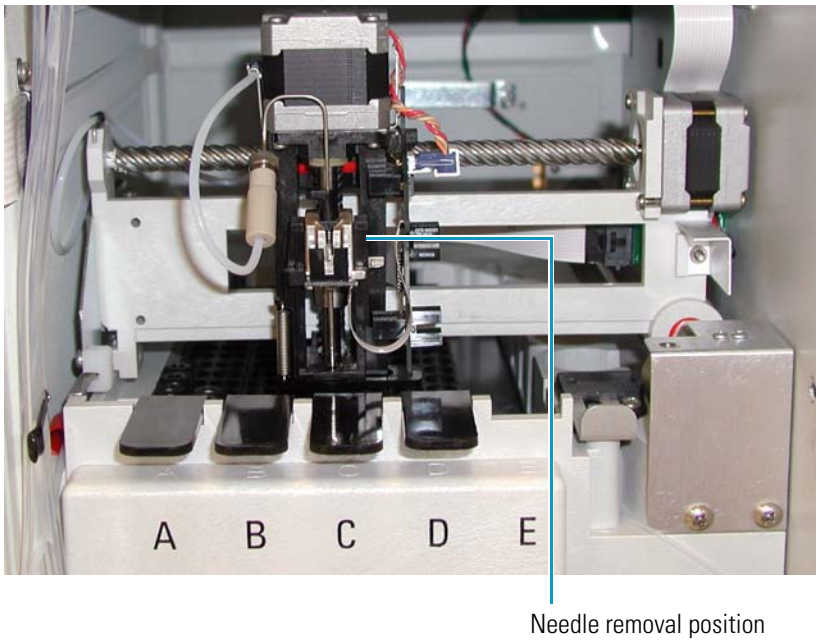


❖ To move the XYZ arm to the needle removal position

1. Click **Move the Arm to Needle Removal Position**.

   The XYZ arm moves to the center front of the tray compartment and the needle holder moves down 1 inch (see Figure 218).

   Figure 218. XYZ arm in the needle removal position (Accela Autosampler)



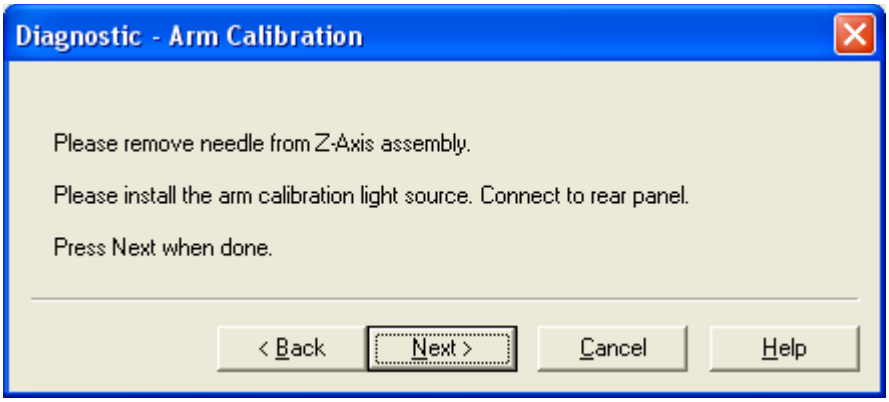
2. Click **Next** to proceed to the next page of the wizard.

Go to the next procedure, "Installing the LED Light Fixture and the Target Port Fixture."

Installing the LED Light Fixture and the Target Port Fixture

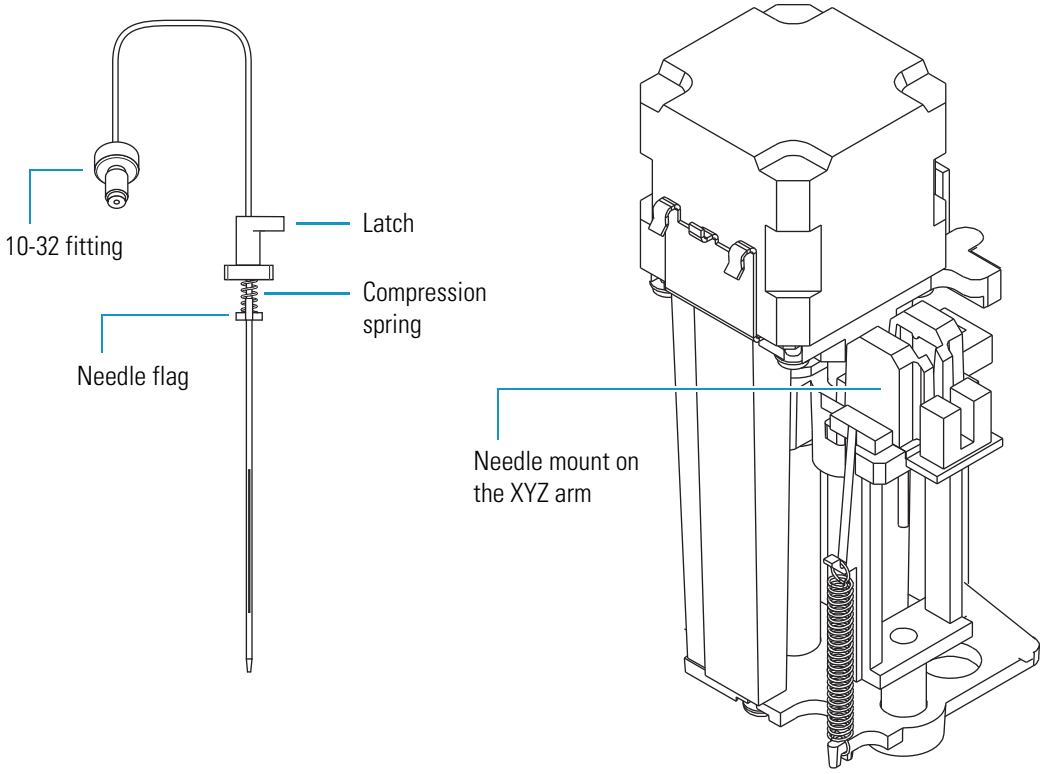
This page of the Arm Calibration wizard instructs you to remove the needle and install the calibration light source (see Figure 219).

Figure 219. Request to install the LED light fixture page



The needle assembly is a welded piece of 0.012 in. ID stainless steel tubing with an externally threaded fitting, a needle flag, a latch, and a compression spring (see Figure 220). It slides into the needle mount on the XYZ arm and is secured with the latch. The needle tubing assembly has an internally threaded fitting that connects to the 10-32 externally threaded fitting of the needle assembly.

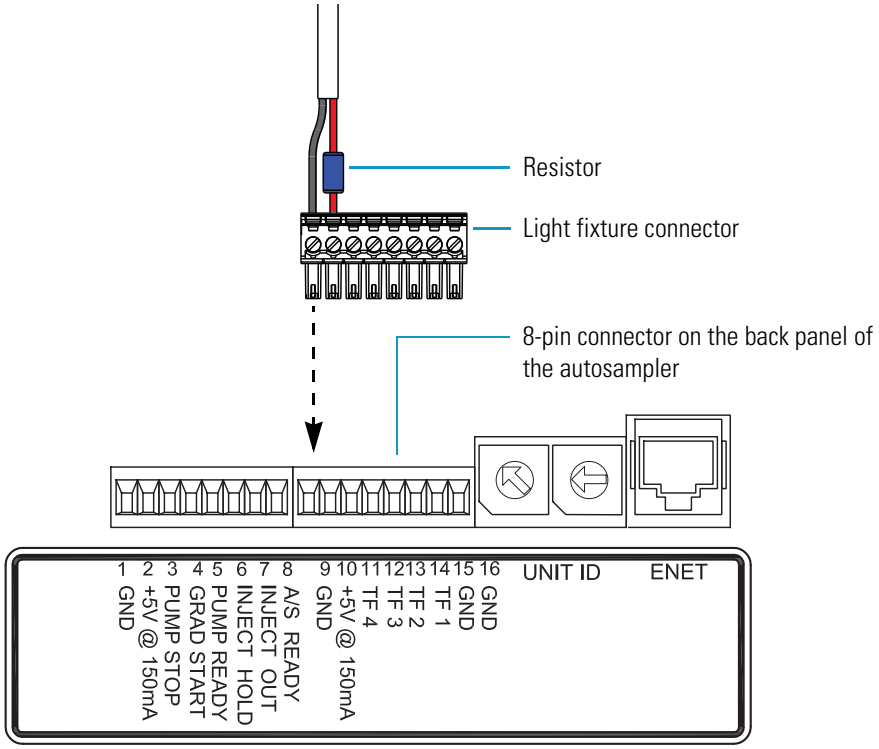
Figure 220. Needle assembly



❖ To install the light fixture and the target port fixture

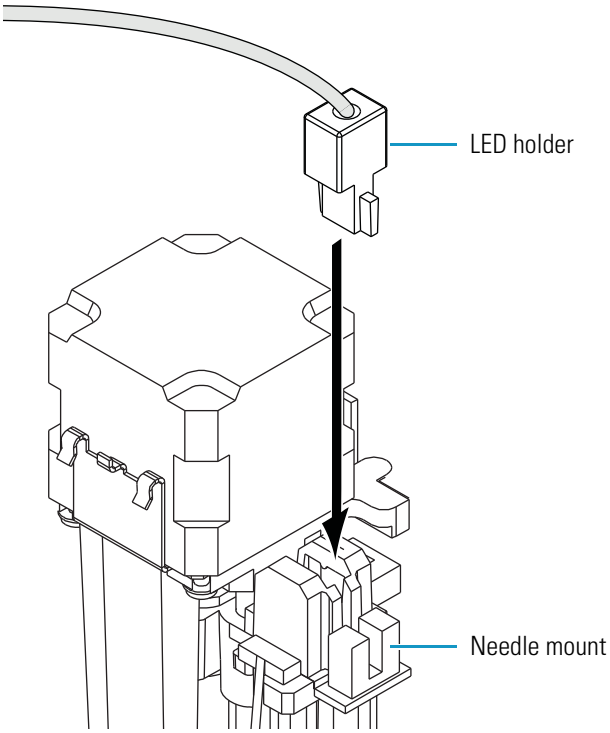
1. Remove the needle assembly from the needle mount as follows:
   a. Unscrew the needle tubing assembly from the needle assembly.
   b. Pull the latch nut of the needle assembly forward.
   c. Pull the needle up from the needle mount on the XYZ arm.
2. Install the LED light fixture as follows:
   a. Connect the 8-pin connector of the light fixture cable to the timed events connector on the back panel of the autosampler (see Figure 221). The red wire with the resistor connects to the +5V autosampler pin and the black wire connects to the autosampler ground pin.

Figure 221. Cable connection for the light fixture



   b. Verify that the LED is brightly lit.
   c. Insert the LED holder into the needle mount (see Figure 222).

Figure 222. Inserting the LED holder into the needle mount



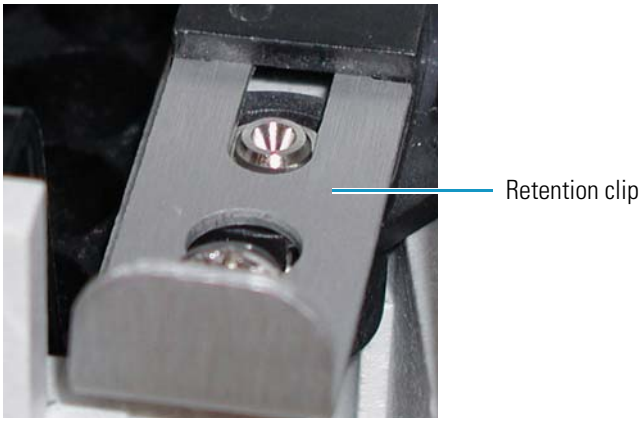
d. Push the LED holder down until it meets resistance from the needle mount.

e. Make sure that the light fixture cable is routed so that it does not obstruct the movement of the XYZ arm.

3. Install the target port fixture as follows:

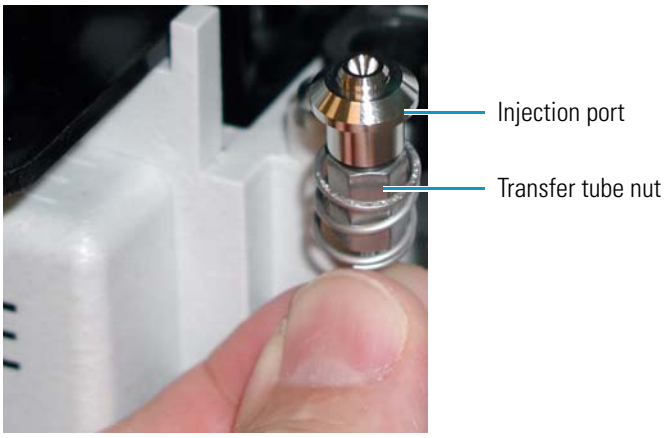
a. Remove the aluminum retention clip (see Figure 223) from the wash station housing, pull it forward, and then upward.

Figure 223. Retention clip for the autosampler injection port



b. Pull the injection port out from the wash station (see Figure 224).

Figure 224. Autosampler injection port connected to the transfer tube nut



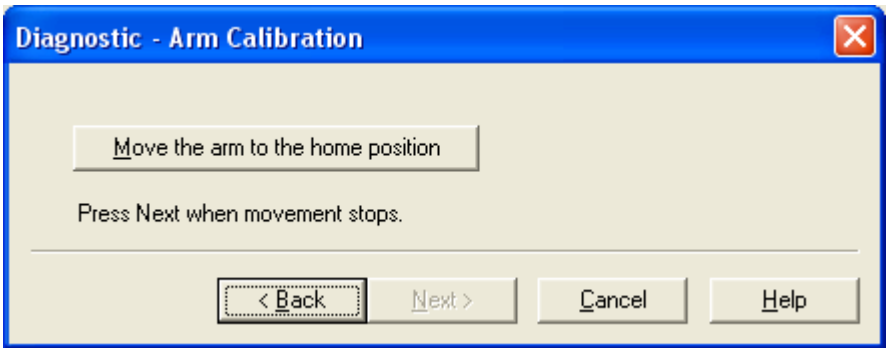
4. Insert the target port fixture (see Figure 212 on page 304) into the position previously held by the injection port.
5. Click **Next** to proceed to the next page of the wizard.

Go to the next procedure, "Moving the XYZ Arm to the Home Position."

Moving the XYZ Arm to the Home Position

Use this page of the Arm Calibration wizard (see Figure 225) to move the XYZ arm to the home position. In the home position, the XYZ arm is aligned over the autosampler injection port.

Figure 225. Move the Arm to the Home Position button page



❖ To move the XYZ arm to the home position

1. Click **Move the Arm to the Home Position**.

   The XYZ arm moves to the home position. The Next button becomes available.
2. Click **Next** to proceed to the next page of the Arm Calibration wizard.

Go to the next procedure, "Aligning the Light Beam with the Target."

Aligning the Light Beam with the Target

Use this page of the Arm Calibration wizard (see Figure 226) to adjust the fine position of the XYZ arm.

Two lead screws and two stepper motors control the *x*-axis and *y*-axis position of the XYZ arm (see Figure 227).

The Left and Right buttons move the XYZ arm along the *x* axis. The Forward and Backward buttons move the XYZ arm along the *y* axis.

Figure 226. Alignment buttons page

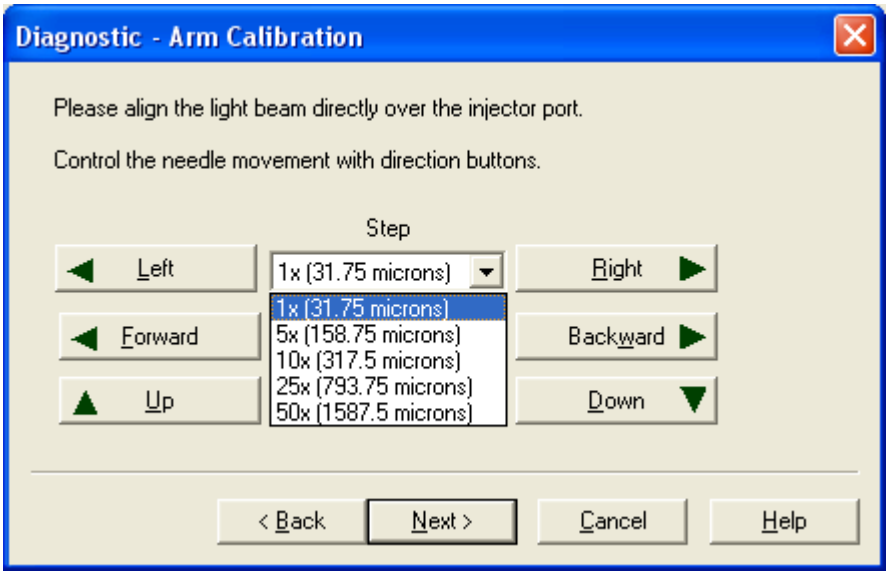
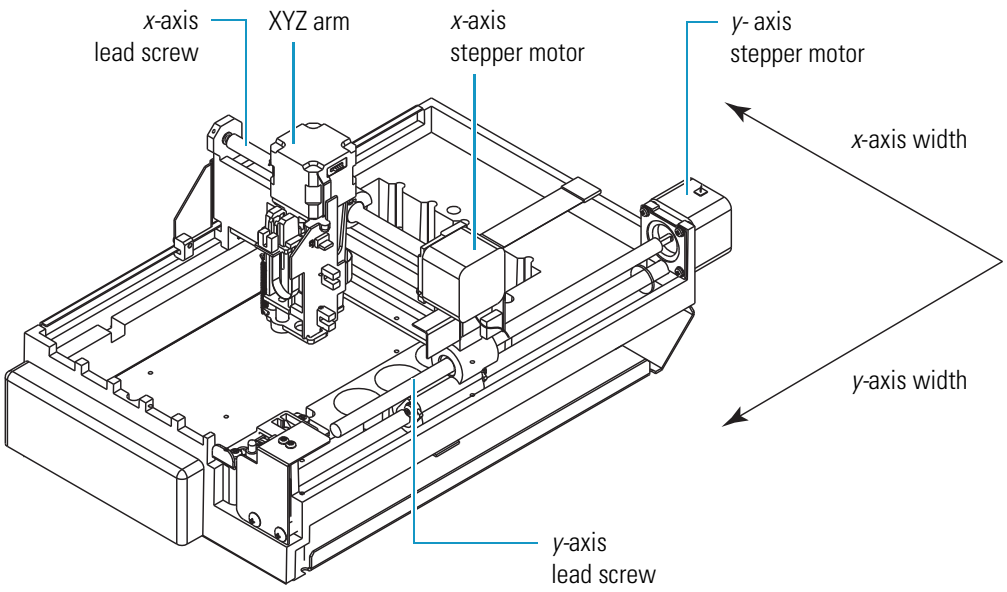


Figure 227. XYZ arm of the Accela Autosampler



❖ **To align the light beam over the hole in the target port fixture**

1. In the Step list of the Arm Calibration wizard, select **25x (793.75 microns)**.

   This selection moves the arm 793.75 micrometers (0.03125 inches) each time you click Left, Right, Forward, or Backward.

   1 micrometer = 3.937×10^{-5} inches

2. Click **Left**, **Right**, **Forward**, and **Backward** to align the position of the light beam with the hole in the center of the target port fixture.

3. In the Step list, select a smaller step size, and continue aligning the position of the light beam.

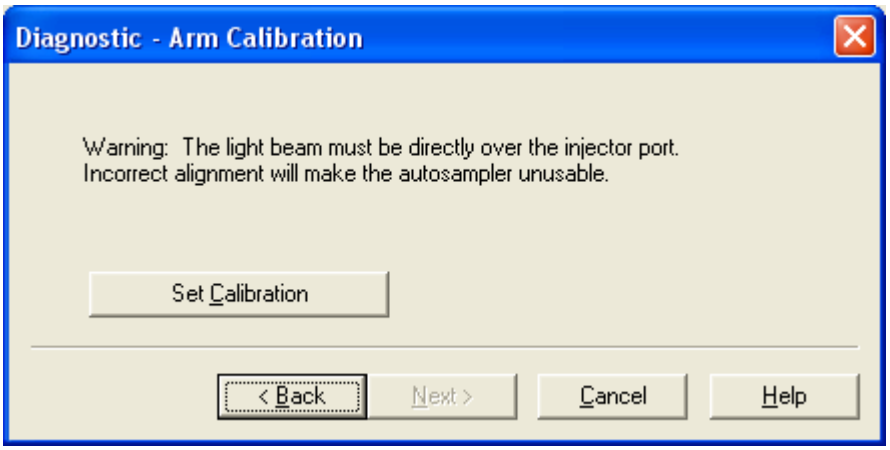
4. After you align the light beam with the hole in the center of the target port fixture, click **Next** to proceed to the next page of the wizard.

Go to the next procedure, "Applying the Arm Calibration Setting."

Applying the Arm Calibration Setting

Use this page of the Arm Calibration wizard (see Figure 228) to download the new arm calibration settings.

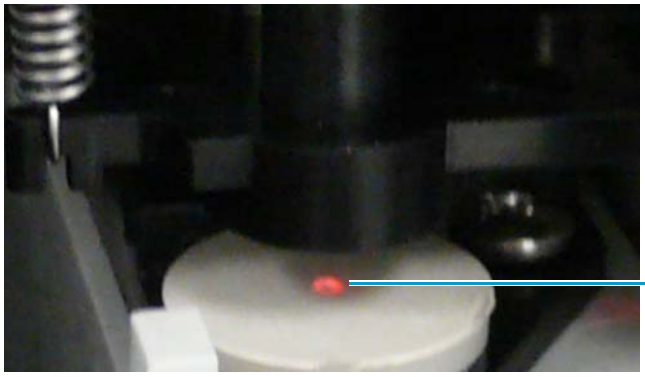
Figure 228. Set Calibration button page



❖ **To apply the current calibration settings for the arm position**

1. Ensure that the light beam is centered over the hole in the target port fixture (see Figure 229).

Figure 229. LED light beam centered over the hole in the target port fixture



2. Click **Set Calibration**.

   The Next button becomes available.

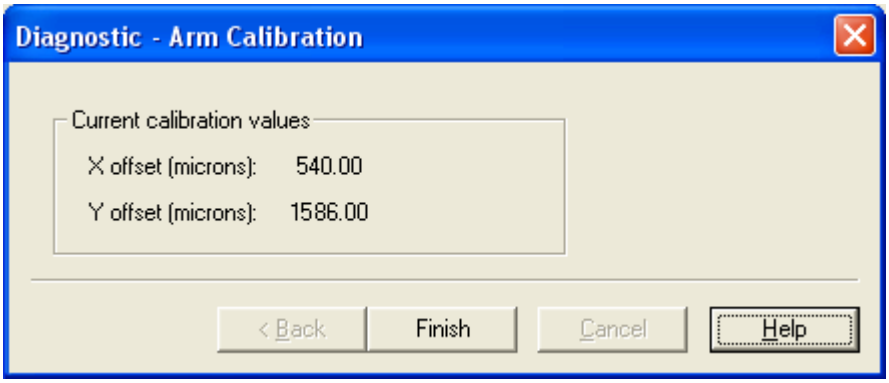
3. Click **Next** to proceed to the final page of the wizard.

Go to the next procedure, "Checking the Arm Calibration Offset Values."

Checking the Arm Calibration Offset Values

Use this page of the Arm Calibration wizard to check the calibration values and close the wizard (see Figure 230).

Figure 230. Final page of the Arm Calibration wizard with the Finish button



❖ To verify the calibration setting

1. In the Current Calibration Values area, verify the following:
   - The X Offset readback displays a value from +900 to –900 µm.
   - The Y Offset readback displays a value from 1500 to 2500 µm.

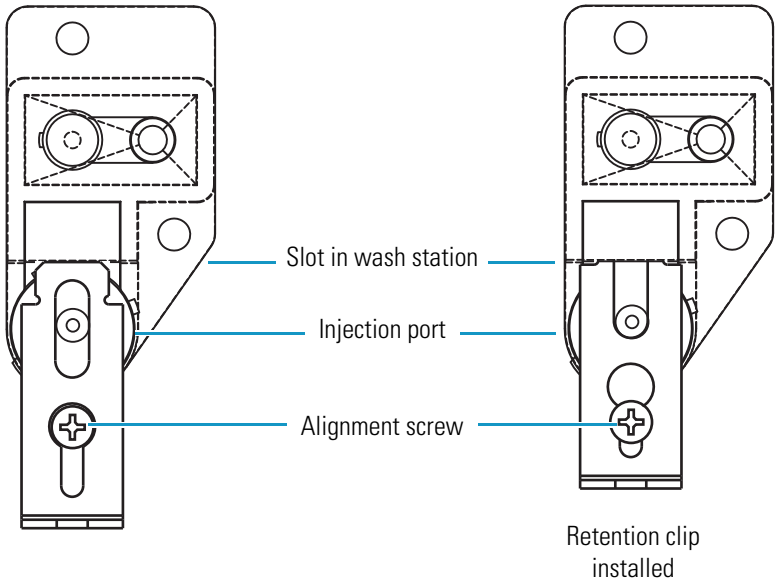
Tip 1000 microns = 1000 micrometers = 0.04 inches

The diameter of the hole in the center of the target port fixture is 0.047 inches.

2. Click **Finish** to accept the settings and close the Arm Calibration wizard.
3. Remove the LED and target port calibration fixtures.
4. Reinstall the injection port as follows:
   a. Insert the injection port into the port in front of the wash station.
   b. Align the retention clip with the slot located in front of the wash station.
   c. Using the retention clip to push the injection port down, align the circular cutout in the retention clip over the alignment screw.
   d. Insert the end of the retention clip into the wash station slot as far as it will go.

   Figure 231 shows the installation of the retention clip.

   Figure 231. Retention clip installation



5. Reinstall the needle assembly as follows:
   a. Remount the syringe drive assembly.
   b. Slide the needle into the needle mount on the XYZ arm.
   c. Turn the latch to the right.
   d. Reconnect the needle tubing assembly to the needle assembly.

Leave the autosampler view of the Instrument Setup view open and go to the next procedure, "Testing the Alignment of the XYZ Arm."

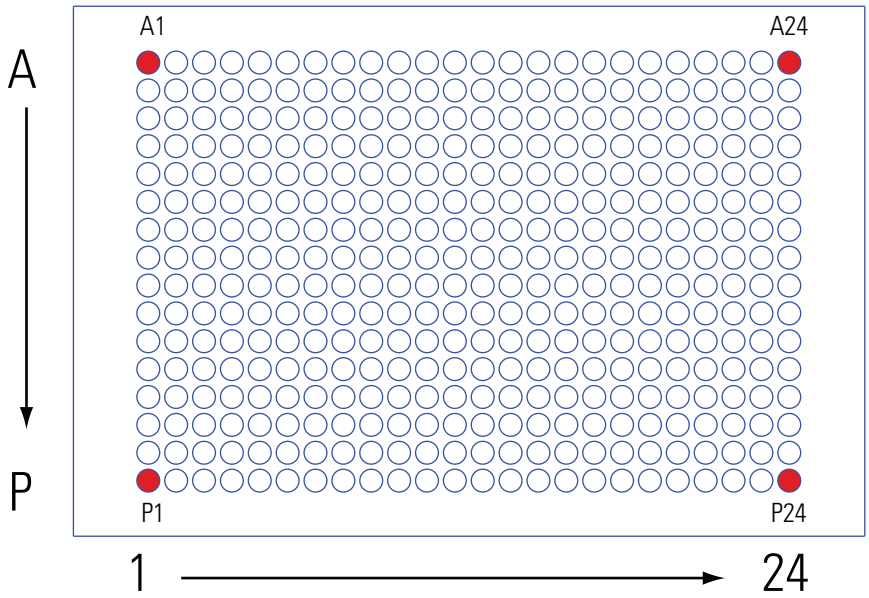
Testing the Alignment of the XYZ Arm

To test the alignment of the XYZ arm, make injections from the four corners of two 384-well microplates.

❖ To test the alignment of the XYZ arm

1. Cover columns 1, 2, 23, and 24 of two 384-well microplates with tape (see Figure 232).

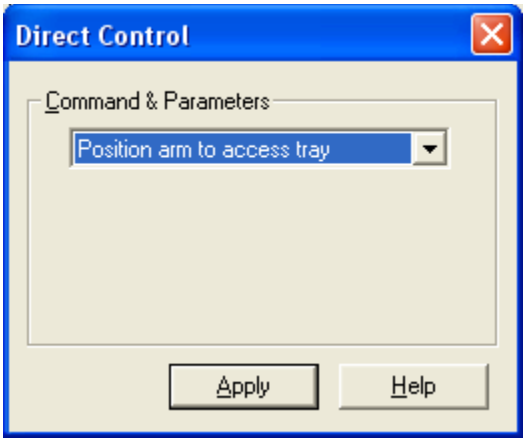
   Figure 232. 384-well microplate positions



2. Place the microplates in each end of the microwell carrier, leaving the middle position empty.
3. Load the microwell carrier into the tray compartment of the autosampler as follows:
   a. From the menu bar, choose **Accela AS > Direct Control**.

      The Direct Control dialog box appears (see Figure 233).

Figure 233. Direct Control dialog box for the autosampler



b. Select **Position Arm to Access Tray**, and then click **Apply**.

The XYZ arm moves to the back of the tray compartment.

c. Insert the microwell carrier into the tray compartment.

d. Verify that the microwell carrier sits flat and is properly installed.

4. Send the XYZ arm to the home position as follows:

a. In the Direct Control dialog box, select **Go to Home Position**.

b. Click **Apply**.

The XYZ arm moves to the home position, which is above the injection port.

5. Make an injection from each corner of the microplate as follows:

a. In the Direct Control dialog box, select **Inject Sample**.

The sample injection parameters appear (see Figure 234).

Figure 234. Direct Control dialog box with the Inject Sample command selected

b. In the Vial box, type **C:A1**.

c. Click **Apply** and wait for the injection to finish.

   The XYZ arm moves to the location of the A:1 well of the (C) microplate at the back of the tray compartment, and then lowers the needle through the masking tape and into the well.

d. In the Vial box, type **C: A24**.

e. Click **Apply** and wait for the injection to finish.

f. In the Vial box, type **C: P1**.

g. Click **Apply** and wait for the injection to finish.

h. In the Vial box, type **C: P24**.

i. Click **Apply** and wait for the injection to finish.

6. Remove the microplate and check the location of the injections as follows:

   a. In the Direct Control dialog box, select **Position Arm to Access Tray**.

      The XYZ arm moves to the back of the tray compartment.

   b. Remove the tray from the tray compartment, and inspect the holes in the tape. Verify that the holes are at or near the center of the microplate wells. Each hole must be at least 0.05 cm (0.02 inches) from the edge of the well.

7. If the results are not satisfactory, repeat the arm calibration, and then repeat the alignment test with the second (A) microplate, located at the front of the tray compartment.

Well Bottom Distance Calibration

The autosampler supports custom vials and well plates in addition to the standard 1.8 mL vials, 96-well plates, and 384-well plates. However, to use custom vials or well plates, you must first determine how far the autosampler needle must travel to reach the bottom of the vials or wells.

Use the Well Bottom Distance wizard to determine the distance that the needle must travel to reach the bottom of a vial or well. The XYZ arm uses this value when you select one of the custom tray configurations.

IMPORTANT Because the autosampler stores only one value for the custom well bottom distance, you must perform a well bottom distance calibration each time you select a new custom tray type configuration and each time you use a different type of custom vial or custom microwell plate.

To perform a well bottom distance calibration, follow these procedures in order:

1. Starting the Well Bottom Distance Wizard
2. Selecting the Calibration Method for the Well Bottom Distance
3. Depending on the selected calibration method, follow one of these procedures:
   - Manually Entering the Well Bottom Distance

   –or–

   - Using the Needle Sensor to Determine the Well Bottom Distance

Starting the Well Bottom Distance Wizard

❖ **To start the Well Bottom Distance wizard**

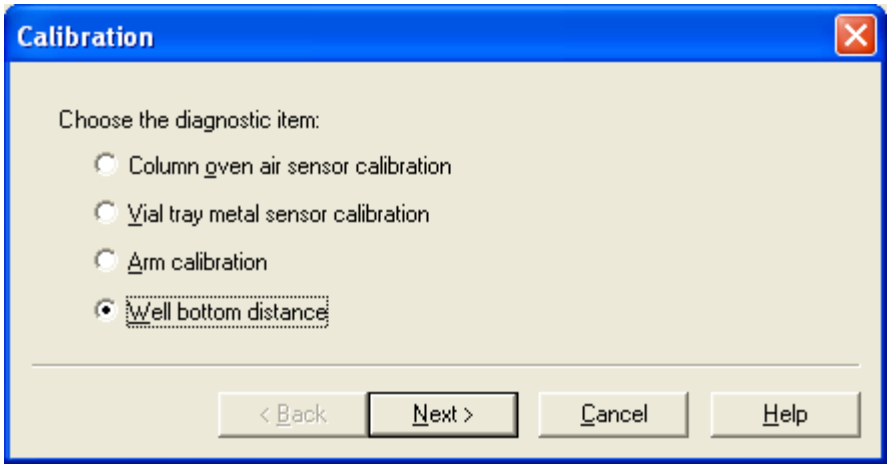
1. Open the status view for the autosampler.

   For information about opening the status view from the Xcalibur data system, see "Checking the Status of the LC Devices" on page 137.

2. Verify that the autosampler status reads Ready to Download.
3. Open the view for the autosampler.
4. From the Accela AS menu, choose **Calibration**.

   The Calibration dialog box appears (see Figure 235).

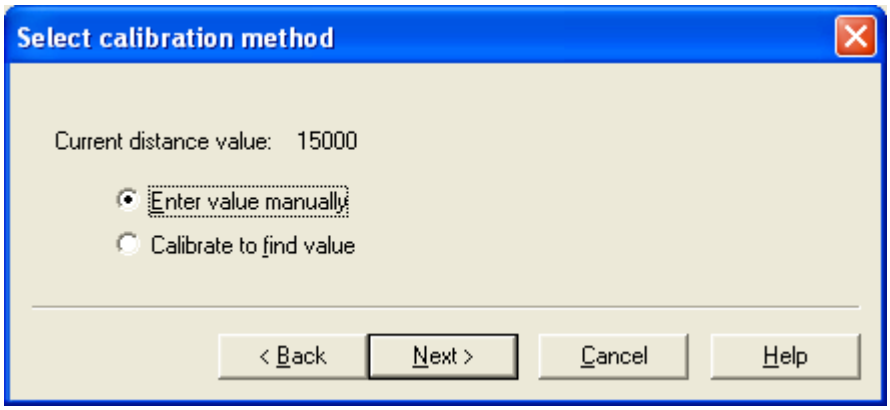
Figure 235. Calibration dialog box with the Well Bottom Distance option selected



5. Select the **Well Bottom Distance** option, and then click **Next**.

   The Select Calibration Method page appears (see Figure 236).

   Figure 236. Select Calibration Method page



Go to the next procedure, "Selecting the Calibration Method for the Well Bottom Distance" on page 322.

Selecting the Calibration Method for the Well Bottom Distance

Use the Select Calibration Method page of the Well Bottom Distance wizard to select a calibration method. You can manually enter a bottom distance value for your custom vials or microplates, or you can use the autosampler's needle sensor feature to determine the correct distance value.

❖ **To select the calibration method for the well bottom distance**

Do one of the following:

- Select the **Enter Value Manually** option if you want to enter a previously determined value for the well bottom distance. Click **Next**. Then go to the next procedure, "Manually Entering the Well Bottom Distance."

–or–

- Select the **Calibrate to Find Value** option to perform an active calibration. Click **Next**. Then go to "Using the Needle Sensor to Determine the Well Bottom Distance" on page 324.

Note The autosampler stores only one well bottom distance value for custom tray configurations. The number at the top of this dialog box is the current value for the distance.

Manually Entering the Well Bottom Distance

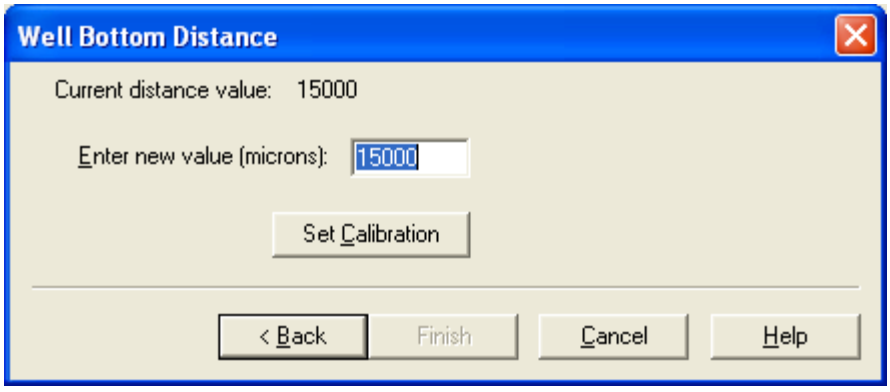
❖ **To manually enter a new value for the well bottom distance**

1. In the Enter New Value (microns) box, type the new value in micrometers (see Figure 237).

   The range is 15 000 to 46 990 micrometers (15 to 46.9 mm).

   Note The user interface specifies the bottom distance in microns, which is another term for micrometers.

   Figure 237. Manual calibration page



2. Click **Set Calibration**.

   The new bottom distance value (in microns) appears at the top of the dialog box and the Finish button becomes available.

   1 micron = 1 micrometer

3. Click **Finish** to close the Well Bottom Distance dialog box.

Using the Needle Sensor to Determine the Well Bottom Distance

The autosampler has a needle sensor that can determine the bottom distance of custom vials or microplate wells.

❖ To determine the well bottom distance with the autosampler's needle sensor

1. Remove the cap or lid from your vial or well plate.

   IMPORTANT Remove vial caps or well plate lids before performing an active well bottom distance calibration. As the needle pierces a vial cap or a well plate lid, the spring in the needle mechanism is compressed, which can cause premature activation of the needle sensor.

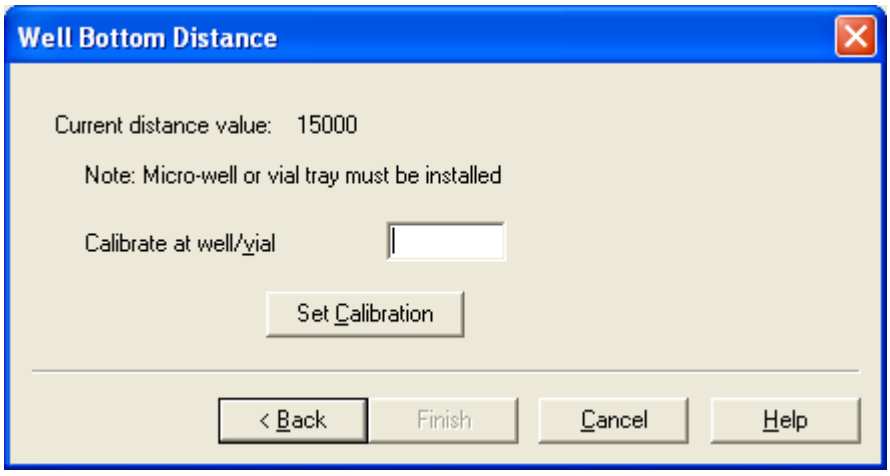
   IMPORTANT Before placing a custom tray into the autosampler tray compartment, check the height limitations for 96-well and 384-well plates listed in the hardware manual for your autosampler. Tall objects will stall the autosampler arm.

2. Place the vial into a tray or the well plate into a carrier, and then place the tray or carrier into the tray compartment of the autosampler.

3. In the Calibrate at Well/Vial position box (see Figure 238), type a vial or well location.

   Note If you are calibrating the bottom distance of a vial, verify that you have placed a vial in the selected location.

   Figure 238. Calibrate to find page



4. Click **Set Calibration** to activate the autosampler.

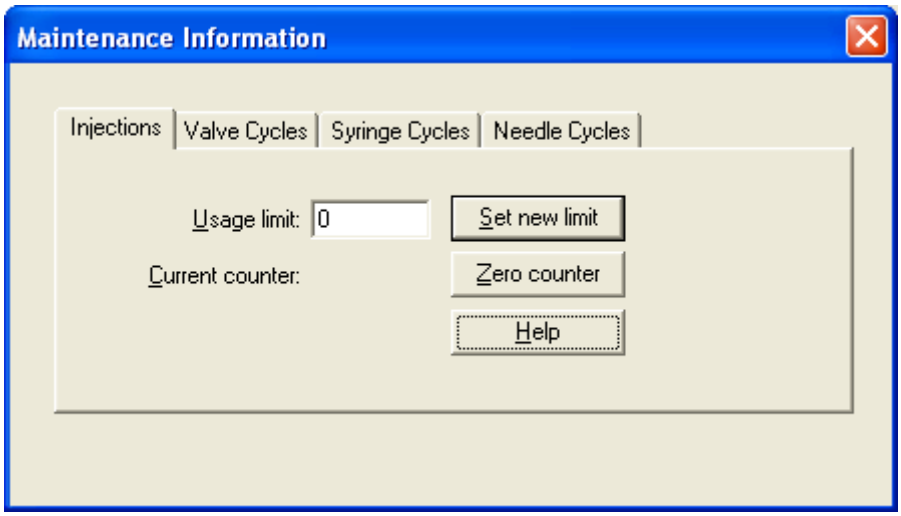
   After the XYZ arm moves to the selected location, the needle mechanism descends until it detects the bottom of the vial or well. After it detects the bottom, the XYZ arm moves to the home position. In the Well Bottom Distance wizard, the new bottom distance calibration value appears at the top of the page.

5. Click **Finish** to accept the calibration.

Autosampler Maintenance Information

Use the Maintenance Information dialog box (see Figure 239) to set the scheduled maintenance time (SMT) for the autosampler maintenance items: Injections, Valve Cycles, Needle Cycles, and Syringe Cycles.

Figure 239. Maintenance Information dialog box



❖ To open the Maintenance Information dialog box

1. Open the view for your autosampler.

   The Method page for your autosampler appears.

2. In the menu bar, choose **Accela AS > Maintenance**.

❖ To set a new usage limit

1. Click the tab for the page that contains the maintenance item that you want to change the usage limit for.
2. In the Usage Limit box, type the usage limit.
3. Click **Set New Limit**.

❖ To reset the usage counter after performing the scheduled maintenance task

1. Click the tab for the scheduled maintenance task.
2. Click **Zero Counter**.

Note If you selected the Enable Maintenance Log check box on the Communication page of the Accela Autosampler dialog box when you configured the Accela Autosampler, you can set the scheduled maintenance times (see "Communication Page" on page 50).

Maintenance Information Parameters

Table 53 describes the parameters on the Maintenance Information pages.

When anyone of these counters on the Maintenance Information pages exceeds the user-specified usage limit, the Maintenance Due readback displays Yes and the data system prevents the autosampler from making injections.

To enable the autosampler, you must zero the counter or change the usage limit. If the usage limit is based on a scheduled maintenance plan, perform the scheduled maintenance.

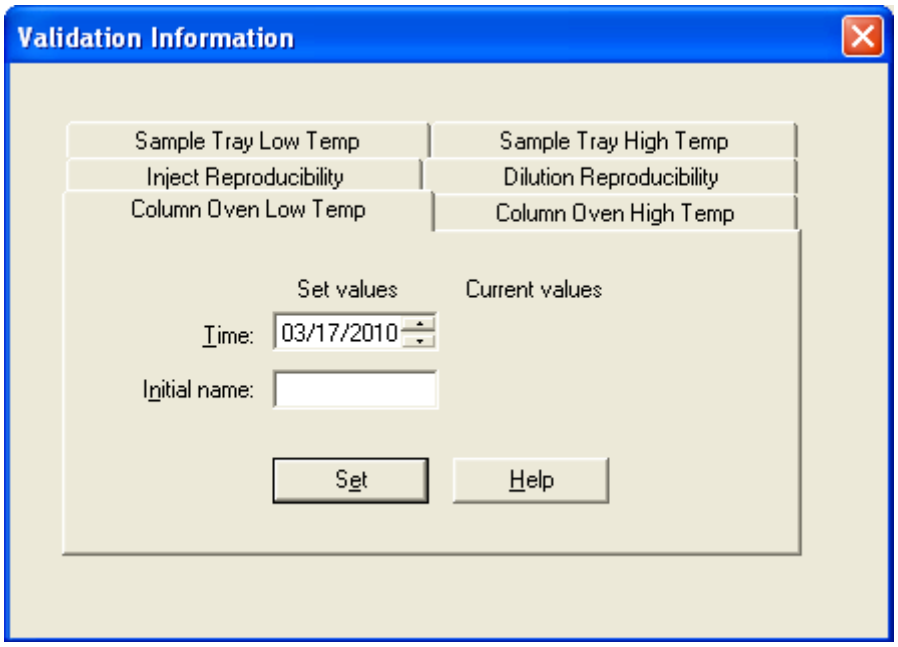
Table 53. Maintenance Information dialog box pages and parameters

Page or Parameter	Description
Page	
Injections	Use this page to set the scheduled maintenance time for the number of user-defined injections (excluding any cancelled injections).
Valve Cycles	Use this page to set the scheduled maintenance time for the number of user-defined valve cycles for the injection valve (from Fill to Inject).
Needle Cycles	Use this page to set the scheduled maintenance time (SMT) for the number of user-defined needle cycles when the needle is inserted into a septum, injection port, or wash station.
Syringe Cycles	Use this page to set the scheduled maintenance time (SMT) for the number of user-defined syringe cycles where the syringe is put in the Ready position.
Parameter	
Usage Limit	Use this box to specify the new usage limit for the selected maintenance item.
	Note When a counter exceeds the usage limit, the autosampler cannot start a run until you reset the counter to zero, change the usage limit, or clear the Enable Maintenance Log check box on the Communication page of the Accela Autosampler Configuration dialog box (see "Communication Page" on page 50).
Set New Limit	Downloads the new usage limit to the autosampler.
Current Counter and Zero Counter	Displays the current count. When the counter exceeds the usage limit (scheduled maintenance time), the autosampler cannot start a run until you perform the scheduled maintenance or clear the Enable Maintenance Log check box on the Communication page of the Autosampler Configuration dialog box.
Zero Counter	Zeros the counter for the selected maintenance item.

Autosampler Validation Information

Use the Validation Information dialog box (see Figure 240) to enter the validation date for the indicated parameter and the name of the person who performed the validation.

Figure 240. Validation Information dialog box



❖ To open the Validation Information dialog box

1. Open the view for your autosampler.

   The Method page for your autosampler appears.

2. In the menu bar, choose **Accela AS > Validation**.

   The Validation Information dialog box appears.

❖ To specify your user name and the validation date

1. In the Time box, type or select the validation date.
2. In the Initial Name box, type your initials.
3. Click **Set**.

Validation Information Parameters

Table 54 describes the parameters on the Validation Information dialog box.

Table 54. Validation Information dialog box parameters

Parameter	Description
Time	To change the date that the validation was performed, click the arrows to increase or decrease the value, and then click Set.
Initial Name	To change the name of the person who performed the validation, type the new value in the Initial Name box, and then click Set.
Current Values	This readback displays the current time and initial name.
Button	
Set	Enter the appropriate values in the Time box and the Initial Name box, and then click Set to set the values.

Making a Single Injection from the Tune Window

If you are using the Accela LC system as an inlet to a Thermo Scientific mass spectrometer, you can use the direct control dialog boxes available from the Tune window to make a single injection and acquire data.

For information about setting up the LC/MS system, refer to the Getting Connected Guide for your mass spectrometer. For information about acquiring raw data files from the tune program for your mass spectrometer, refer to the Getting Started Guide for your mass spectrometer.

Note You can use the PDA Direct Control dialog box to perform diagnostic tests such as checking the performance of the deuterium lamp. To acquire PDA data for a single injection, you must run a single row sequence from the Sequence window.

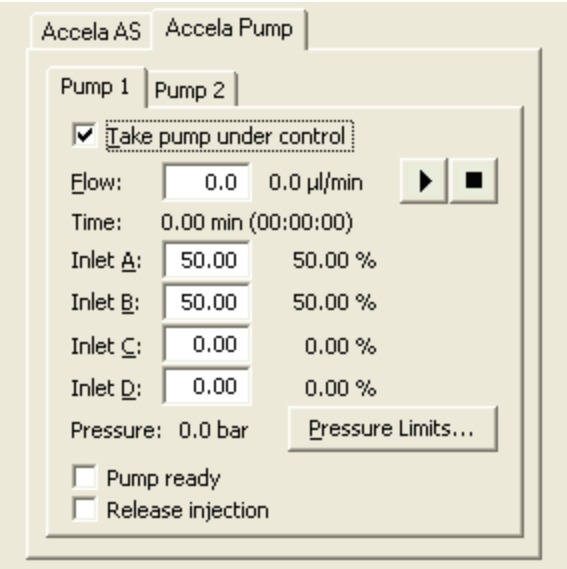
❖ To inject a single sample from the Tune window

1. Prepare the LC system for operation. See Chapter 5, "Daily Operation."
2. From the Tune window for your Thermo Scientific mass spectrometer, choose **Setup > Inlet Direct Control**.

   The Inlet Direct Control dialog box that contains tabbed pages for each configured LC device appears.
3. Start the solvent flow from the pump as follows:
   a. Click the **Accela Pump**, **Accela 600 Pump**, or **Accela 1250 Pump** tab.

      The Accela pump page appears (see Figure 241).

Figure 241. Accela Pump page of the Inlet Direct Control dialog box



b. Select the **Take Pump Under Control** check box.

c. Type the appropriate flow rate in the Flow box.

d. Type the appropriate solvent proportions in the Inlet boxes.

e. Select the **Pump Ready** check box.

f. Click ▶ (Start) to start the solvent flow.

4. Start the injection as follows:

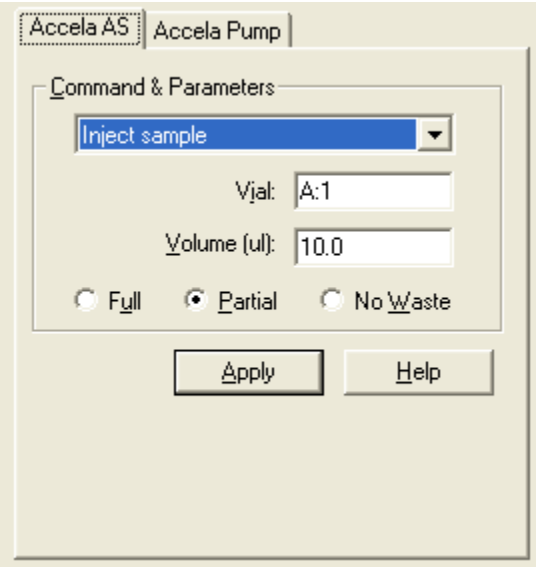
a. In the Inlet Direct Control dialog box, click the **Accela AS** tab.

The Accela AS page appears (see Figure 242).

b. In the list of commands, select **Inject Sample**.

The Inject sample parameters appear (see Figure 242).

Figure 242. Accela AS page of the Direct Inlet Control dialog box



c. In the Vial box, type the location of the sample. Ensure that you have placed a sample in this location.

   For information about the vial and well notation, see "Vial and Well Notation" on page 5.

d. In the Volume (µL) box, type the volume that you want to inject.

e. Select the injection mode: **Full**, **Partial**, or **No Waste**. For more information, see "Injection Modes" on page 17.

f. Click **Apply**.

   The autosampler loads the sample into the sample loop connected to the injection valve as follows:

   The XYZ arm of the autosampler moves to the sample location, and then lowers the needle into the sample vial. The syringe plunger descends, drawing sample into the needle tubing. The XYZ arm moves back to the injection port, and then lowers the needle into the injection port. The syringe plunger ascends, pushing sample into the injection port, through the transfer tubing, and into the sample loop of the injection valve.

5. On the Accela pump page, select the **Release Injection** check box.

   The injection valve switches to the inject position, allowing mobile phase to backflush the sample out of the sample loop and into the LC column.

Index

Numerics

A

B

C

D

E

F

G

H

I

L

M

N

O

P

Q

R

S

T

U

V

W

X

Z