

Accela Autosampler

Hardware Manual

60357-97000 Revision G

March 2011





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Software versions: Xcalibur 2.0.x or later data system, ChromQuest 4.2 or later data system, Thermo Foundation 1.0.x or later and Xcalibur 2.1.x or later

Revision history: Revision A, September 2006; Revision B, January 2007; Revision C, March 2008; Revision D, February 2009; Revision E, October 2009; Revision F, April 2010; Revision G, March 2011

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Changes that you make to your system might 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 Autosampler

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

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

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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.

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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.



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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/
<a href=

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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:



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CAUTION Symbol	CAUTION	VORSICHT	ATTENTION	PRECAUCION	AVVERTENZA
A	Electric Shock: This instrument uses high voltages that can cause personal injury. Before servicing, shut down the instrument and disconnect the instrument from line power. Keep the top cover on while operating the instrument. Do not remove protective covers from PCBs.	Elektroschock: In diesem Gerät werden Hochspannungen verwendet, die Verletzungen verursachen können. Vor Wartungsarbeiten muß das Gerät abgeschaltet und vom Netz getrennt werden. Betreiben Sie Wartungsarbeiten nicht mit abgenommenem Deckel. Nehmen Sie die Schutzabdeckung von Leiterplatten nicht ab.	Choc électrique: L'instrument utilise des tensions capables d'infliger des blessures corporelles. L'instrument doit être arrêté et débranché de la source de courant avant tout intervention. Ne pas utiliser l'instrument sans son couvercle. Ne pas enlever les étuis protecteurs des cartes de circuits imprimés.	Descarga eléctrica: Este instrumento utiliza altas tensiones, capaces de producir lesiones personales. Antes de dar servicio de mantenimiento al instrumento, éste debera apagarse y desconectarse de la línea de alimentacion eléctrica. No opere el instrumento sin sus cubiertas exteriores quitadas. No remueva las cubiertas protectoras de las tarjetas de circuito impreso.	Shock da folgorazione. L'apparecchio è alimentato da corrente ad alta tensione che puo provocare lesioni fisiche. Prima di effettuare qualsiasi intervento di manutenzione occorre spegnere ed isolare l'apparecchio dalla linea elettrica. Non attivare lo strumento senza lo schermo superiore. Non togliere i coperchi a protezione dalle schede di circuito stampato (PCB).
	Chemical: This instrument might contain hazardous chemicals. Wear gloves when handling toxic, carcinogenic, mutagenic, or corrosive or irritant chemicals. Use approved containers and proper procedures to dispose waste oil.	Chemikalien: Dieses Gerät kann gefährliche Chemikalien enthalten. Tragen Sie Schutzhandschuhe beim Umgang mit toxischen, karzinogenen, mutagenen oder ätzenden/reizenden Chemikalien. Entsorgen Sie verbrauchtes Öl entsprechend den Vorschriften in den vorgeschriebenen Behältern.	Chimique: Des produits chimiques dangereux peuvent se trouver dans l'instrument. Portez des gants pour manipuler tous produits chimiques toxiques, cancérigènes, mutagènes, ou corrosifs/irritants. Utiliser des récipients et des procédures homologuées pour se débarrasser des déchets d'huile.	Química: El instrumento puede contener productos quimicos peligrosos. Utilice guantes al manejar productos quimicos tóxicos, carcinogenos, mutagenos o corrosivos/irritantes. Utilice recipientes y procedimientos aprobados para deshacerse del aceite usado.	Prodotti chimici. Possibile presenza di sostanze chimiche pericolose nell'apparecchio. Indossare dei guanti per maneggiare prodotti chimici tossici, cancerogeni, mutageni, o corrosivi/irritanti. Utilizzare contenitori aprovo e seguire la procedura indicata per lo smaltimento dei residui di olio.
	Heat: Before servicing the instrument, allow any heated components to cool.	Hitze: Warten Sie erhitzte Komponenten erst nachdem diese sich abgekühlt haben.	Haute Temperature: Permettre aux composants chauffés de refroidir avant tout intervention.	Altas temperaturas: Permita que lop componentes se enfríen, ante de efectuar servicio de mantenimiento.	Calore. Attendere che i componenti riscaldati si raffreddino prima di effetturare l'intervento di manutenzione.
	Fire: Use care when operating the system in the presence of flammable gases.	Feuer: Beachten Sie die einschlägigen Vorsichtsmaßnahmen, wenn Sie das System in Gegenwart von entzündbaren Gasen betreiben.	Incendie: Agir avec précaution lors de l'utilisation du système en présence de gaz inflammables.	Fuego: Tenga cuidado al operar el sistema en presencia de gases inflamables.	Incendio. Adottare le dovute precauzion quando si usa il sistema in presenza di gas infiammabili.
	Eye Hazard: Eye damage could occur from splattered chemicals or flying particles. Wear safety glasses when handling chemicals or servicing the instrument.	Verletzungsgefahr der Augen: Verspritzte Chemikalien oder kleine Partikel können Augenverletzungen verursachen. Tragen Sie beim Umgang mit Chemikalien oder bei der Wartung des Gerätes eine Schutzbrille.	Danger pour les yeux: Des projections chimiques, liquides, ou solides peuvent être dangereuses pour les yeux. Porter des lunettes de protection lors de toute manipulation de produit chimique ou pour toute intervention sur l'instrument.	Peligro par los ojos: Las salicaduras de productos químicos o particulas que salten bruscamente pueden causar lesiones en los ojos. Utilice anteojos protectores al mnipular productos químicos o al darle servicio de mantenimiento al instrumento.	Pericolo per la vista. Gli schizzi di prodotti chimici o delle particelle present nell'aria potrebbero causare danni alla vista. Indossare occhiali protettivi quando si maneggiano prodotti chimici o si effettuano interventi di manutenzione sull'apparecchio.
<u>^</u>	General Hazard: A hazard is present that is not included in the above categories. Also, this symbol appears on the instrument to refer the user to instructions in this manual.	Allgemeine Gefahr: Es besteht eine weitere Gefahr, die nicht in den vorstehenden Kategorien beschrieben ist. Dieses Symbol wird im Handbuch auBerdem dazu verwendet, um den Benutzer auf Anweisungen hinzuweisen.	Danger général: Indique la présence d'un risque n'appartenant pas aux catégories citées plus haut. Ce symbole figure également sur l'instrument pour renvoyer l'utilisateur aux instructions du présent manuel.	Peligro general: Significa que existe un peligro no incluido en las categorias anteriores. Este simbolo también se utiliza en el instrumento par referir al usuario a las instrucciones contenidas en este manual.	Pericolo generico. Pericolo non compreso tra le precedenti categorie. Questo simbolo è utilizzato inoltre sull'apparecchio per segnalare all'utente di consultare le istruzioni descritte nel presente manuale.
	When the safety of a procedure is questionable, contact your local Technical Support organization for Thermo Fisher Scientific San Jose Products.	Wenn Sie sich über die Sicherheit eines Verfahrens im unklaren sind, setzen Sie sich, bevor Sie fortfahren, mit Ihrer lokalen technischen Unterstützungsorganisation für Thermo Fisher Scientific San Jose Produkte in Verbindung.	Si la sûreté d'une procédure est incertaine, avant de continuer, contacter le plus proche Service Clientèle pour les produits de Thermo Fisher Scientific San Jose.	Cuando la certidumbre acerca de un procedimiento sea dudosa, antes de proseguir, pongase en contacto con la Oficina de Asistencia Tecnica local para los productos de Thermo Fisher Scientific San Jose.	Quando e in dubbio la misura di sicurezza per una procedura, prima di continuare, s prega di mettersi in contatto con il Servizio di Assistenza Tecnica locale per prodotti di Thermo Fisher Scientific San Jose.

CAUTION Symbol	CAUTION	危険警告	危險警告
4	Electric Shock: This instrument uses high voltages that can cause personal injury. Before servicing, shut down the instrument and disconnect the instrument from line power. Keep the top cover on while operating the instrument. Do not remove protective covers from PCBs.	電撃:この計測器は高電圧を使用し、人体に危害を与える可能性があります。 保守・修理は、必ず操業を停止し、電源を切ってから実施して下さい。上部カ バーを外したままで計測器を使用しないで下さい。ブリント配線 板の保護カバーは外さないで下さい。	電擊:儀器設備使用會造成人身傷害的高伏電壓。在維修之前, 必須先關儀器設備並切除電源。務必要在頂蓋蓋上的情況下操作 儀器。請勿拆除PCB保護蓋。
	Chemical: This instrument might contain hazardous chemicals. Wear gloves when handling toxic, carcinogenic, mutagenic, or corrosive or irritant chemicals. Use approved containers and proper procedures to dispose waste oil.	化学物質: 危険な化学物質が計測器中に存在している可能性があります。毒性、 発がん性、突然変異性、腐食・刺激性などのある薬品を取り扱う際は、手袋を 着用して下さい。廃油の処分には、規定の容器と手順を使用して下さい。	化學品:儀器設備中可能存在有危險性的化學物品。接觸毒性 致癌、誘變或腐蝕/刺激性化學品時,請配帶手套。處置廢油 時,請使用經過許可的容器和程序。
	Heat: Before servicing the instrument, allow any heated components to cool.	熱:熱くなった部品は冷えるのを待ってから保守・修理を行って下さい。	高温:請先等高温零件冷卻之後再進行維修。
	Fire: Use care when operating the system in the presence of flammable gases.	火災 : 可燃性のガスが存在する場所でシステムを操作する場合は、充分な注意 を払って下さい。	火災:在有易燃氣體的場地操作該系統時,請務必小心謹慎。
	Eye Hazard: Eye damage could occur from splattered chemicals or flying particles. Wear safety glasses when handling chemicals or servicing the instrument.	眼に対する危険: 化学物質や微粒子が飛散して眼を傷つける危険性があります。化学物質の取り扱い、あるいは計測器の保守・修理に際しては防護眼鏡を着用して下さい。	眼睛傷害危險:飛濺的化學品或顆粒可能造成眼睛傷害。處理化學品或維修儀器設備時請佩戴安全眼鏡。
<u>^</u>	General Hazard: A hazard is present that is not included in the above categories. Also, this symbol appears on the instrument to refer the user to instructions in this manual.	一般的な危険:この標識は上記以外のタイプの危険が存在することを示します。また、計測器にこの標識がついている場合は、本マニュアル中の指示を参照して下さい。	一般性危險:說明未包括在上述類別中的其他危險。此外,儀器設備上使用這個標誌,以指示用戶本使用手册中的說明。
	When the safety of a procedure is questionable, contact your local Technical Support organization for Thermo Fisher Scientific San Jose Products.	安全を確保する手順がよくわからない時は、作業を一時中止し、お近く のサーモエレクトロンサンローゼプロダクトのテクニカールサポートセ ンターごご連絡ください。	如对安全程序有疑问,请在操作之前与当地的菲尼根技术服务中心联系。

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Preface

This manual describes the features, installation, and maintenance of the Accela[™] Autosampler. This manual also describes portions of the Xcalibur[™] or ChromQuest[™] data systems required to perform diagnostics and maintenance procedures for the autosampler.

Contents

- Related Documentation
- Finding the Manuals
- Accessing the Data System Help for the Autosampler
- Safety and Special Notices
- Special Precautions
- Good Laboratory Practices
- Contacting Us

Related Documentation

In addition to this guide, Thermo Fisher Scientific provides the following documentation as PDF files for the Accela family of LC instruments:

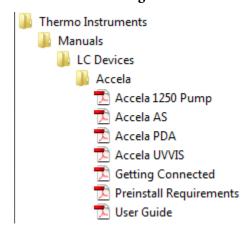
- Accela Preinstallation Requirements Guide
- Accela Getting Connected Guide
- Accela User Guide for LC Devices (formerly known as the Accela User Guide for Xcalibur 2.1.x)
- Accela User Guide for the ChromQuest Data System
- Help from within the data system

Finding the Manuals

For the ChromQuest data system, you can find the manuals on the ChromQuest software installation CD.

For Thermo Scientific mass spectrometry applications, such as the Xcalibur data system, you can find the manuals (provided as PDF files) on the data system computer.

- To access the manuals from LC Devices 2.1.0 or 2.2.0, choose **Start > All Programs > Xcalibur > Manuals > LC Devices > Accela**.
- To access the manuals for LC Devices 2.2.1 or later, from the data system computer, choose **Start > All Programs > Thermo Instruments > Manuals > LC Devices > Accela**.



Accessing the Data System Help for the Autosampler

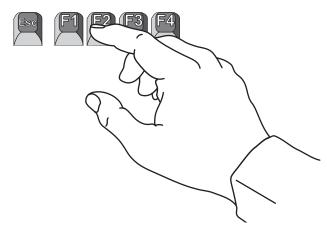
Your data system provides context-sensitive Help for these autosampler topics:

- Instrument configuration parameters for the autosampler
- Injection cycle and temperature control parameters for an acquisition method (instrument method in the Xcalibur data system or method in the ChromQuest data system)
- Calibration options for the autosampler
- Direct controls for the autosampler

❖ To open context-sensitive Help

Do one of the following:

• Press F1.



- Click the **Help** button, if available.
- For the ChromQuest data system, click the **Help** icon, ?

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.



CAUTION Alerts you to the presence of high voltage and to the potential injury that could occur from electrical shock were you to come in contact with a specific instrument area or component. It also tells you how to avoid contact with the high-voltage areas in your instrument.



CAUTION Alerts you to the presence of hot surfaces and to the potential injury that could occur were you to come in contact with a specific instrument area.



CAUTION Highlights a heavy lifting hazard.

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.

Special Precautions

Follow these precautions when handling the autosampler:

- Loading the Autosampler Tray Compartment
- Connecting the Waste Line
- Lifting and Carrying the Autosampler
- Avoiding Contact with Hot Surfaces

Loading the Autosampler Tray Compartment

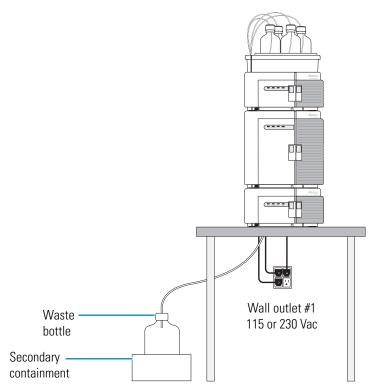
Do not place tall objects taller than 4.6 cm (1.8 in.) in the tray compartment. Placing tall objects in the tray compartment can damage the autosampler arm as it moves within the tray compartment to the sample vial and well locations specified in the injection sequence.



CAUTION Placing tall objects in the tray compartment can damage the autosampler's XYZ arm.

Connecting the Waste Line

Ensure that the waste line connected to the drainage manifold is routed to a waste bottle as shown below.



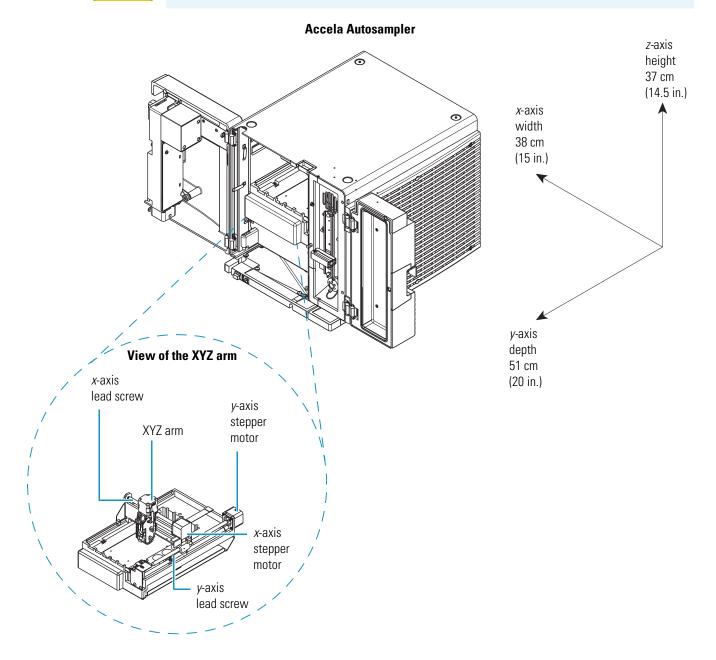
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Lifting and Carrying the Autosampler

The autosampler, at 30 kg (66 lbs) and with a height, width, and depth of $37 \times 38 \times 51$ cm (14.5 × 15 × 20 in.), is too heavy and too bulky for one person alone to handle safely. In addition, any jarring or uneven movement can cause misalignment of the autosampler's XYZ arm.



CAUTION For your safety and to avoid instrument damage, take care when handling the autosampler. Lifting or moving the autosampler requires a team effort.



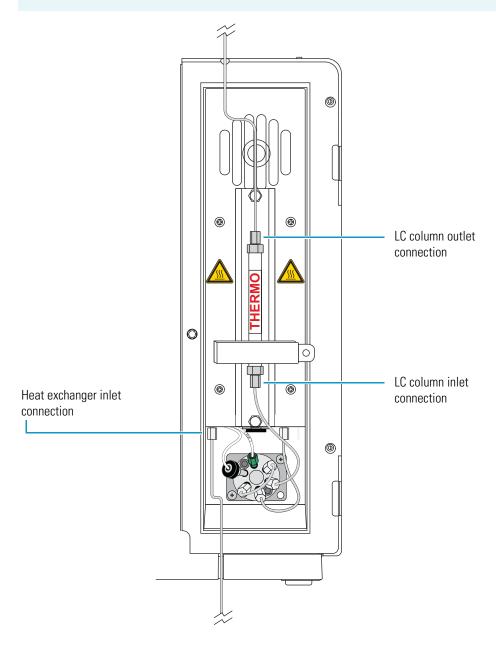
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Avoiding Contact with Hot Surfaces

The autosampler has two controlled temperature zones: the tray compartment and the column oven compartment. Because the column oven can reach temperatures as high as 95 °C (203 °F), ensure that the oven is at room temperature before installing or removing the LC column and before connecting the pump outlet to the inlet of the autosampler heat exchanger.



CAUTION To prevent personal injury, before installing the LC column or connecting the pump to the autosampler, make sure that the column oven compartment is at room temperature.



Good Laboratory Practices

To obtain optimal performance from your LC system and to prevent personal injury or injury to the environment, do the following:

- Keep good records.
- Read the manufacturers' Material Safety Data Sheets (MSDSs) for the chemicals being used in your laboratory.
- Remove particulate matter from your samples before you inject them into the liquid chromatograph.
- Use HPLC-grade solvents.
- Connect the drainage tubes from the pump, autosampler, and detector to an appropriate waste receptacle. Dispose of solvents as specified by local regulations.

Keep Good Records

To help identify and isolate problems with either your equipment or your methodology, for best results, keep good records of all system conditions (for example, % RSDs on retention times and peak areas, and peak shape and resolution). At a minimum, keep a chromatogram of a typical sample and standard mixture, well documented with system conditions, for future reference. Careful comparison of retention times, peak shapes, peak sensitivity, and baseline noise can provide valuable clues to identifying and solving future problems.

Chemical Toxicity

Although the large volume of toxic and flammable solvents used and stored in laboratories can be quite dangerous, do not ignore the potential hazards posed by your samples. Take special care to read and follow all precautions that ensure proper ventilation, storage, handling, and disposal of both solvents and samples. Become familiar with the toxicity data and potential hazards associated with all chemicals by referring to the manufacturers' Material Safety Data Sheets.

Sample Preparation

Always consider the solubility of your sample in the solvent/mobile phase. Sample precipitation can plug the column, tubing, or flow cell causing flow restriction. This obstruction can result in irreparable damage to the system. You can remove particulate matter by filtering the samples through 0.45 or 0.2 μm (or less) filters.

Solvent Requirements

Many chemical manufacturers provide a line of high-purity or HPLC-grade reagents that are free of chemical impurities. Typically, HPLC-grade solvents do not require filtration. You can significantly prolong the life and effectiveness of the inlet filters, check valves and seals, injector, and column by routine filtration of other types of solvents through a 0.45 or 0.2 μ m (or less) fluorocarbon filter before placing them in the solvent reservoir bottles.

Choose a mobile phase that is compatible with the sample and column you have selected for your separation. Remember that some solvents are corrosive to stainless steel.

Solvent Disposal

Make sure you have a solvent waste container or other kind of drain system available at or below the benchtop level. Most solvents have special disposal requirements and should not be disposed of directly down a drain. Follow all governmental regulations when disposing of any chemical.

High-Pressure Systems and Leaks

LC systems operate at high pressures. Because liquids are not highly compressible, they do not store much energy. Accordingly, the high pressures in an LC system pose little immediate danger. However, correct any leak that occurs as soon as possible. Always wear eye and skin protection when operating or maintaining an LC system. Always shut down the system and return it to atmospheric pressure before attempting any maintenance.

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.

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Click the link below.



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Introduction

The Accela Autosampler is a member of the Accela family of ultra-high-performance liquid chromatography instruments. The Accela Autosampler (see Figure 1) holds up to 200 standard vials, three 96-well plates, or three 384-well plates; contains a built-in column oven (5 to 95 °C); provides tray/sample temperature control (0 to 60 °C); and is capable of performing automated sample preparation routines.

Contents

- Autosampler Components
- Status LEDs
- Injection Modes
- Injection Sequence
- Autosampler Operation
- Automated Sample Preparation
- Specifications

Figure 1. Accela Autosampler



Autosampler Components

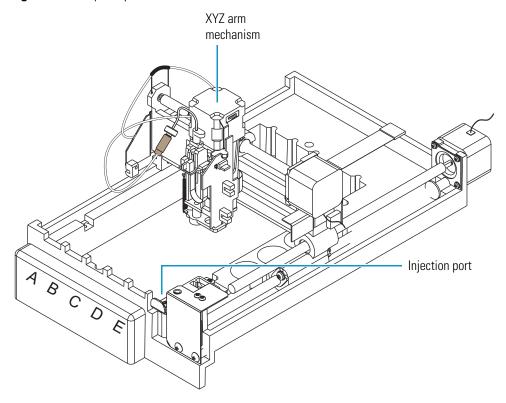
The main components of the Accela Autosampler are as follows:

- Tray Compartment
- Injection System
- Column Oven and Tray Compartment Heater/Cooler

Tray Compartment

Two types of sample trays are supplied with the Accela Autosampler: a conventional sample tray and a microwell carrier. The tray compartment can hold up to five conventional sample trays or one microwell carrier. The tray compartment also holds up to four 16 mL capacity reservoir vials that can hold solvent, reagent, or diluent. The reservoir vials are located behind the wash station and are designated RV1, RV2, RV3, and RV4. Figure 2 shows an empty tray compartment.

Figure 2. Tray compartment



From the left to the right side of the tray compartment, the five conventional sample trays are designated A, B, C, D, and E. Each conventional 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 microplates or up to three high-density microplates. From the front to the back of the tray compartment, the microplates are designated A, B, and C.

The low-density microplate contains 96 wells in an 8×12 configuration, designated A1 through H12, for a total capacity of 288 samples. Each well can hold up to 250 μ L of sample.

The high-density microplate contains 384 wells in a 16×24 configuration, designated A1 through P24, for a total capacity of 1152 samples. Each well can hold up to 50 μ L of sample.

The tray compartment door contains a magnetic switch. The magnet is located in the door and the switch is attached to the chassis. When you open the door, the switch signals the autosampler that the door is open. If you turn on the verification option for the door position when you configure the autosampler, the XYZ arm automatically moves to the back of the tray compartment when you open the door so that you can 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 injection is complete and the sequence pauses. Closing the autosampler door allows the sequence to resume.

Injection System

Figure 3 shows the injection system of the Accela Autosampler. The major components of the injection system are as follows:

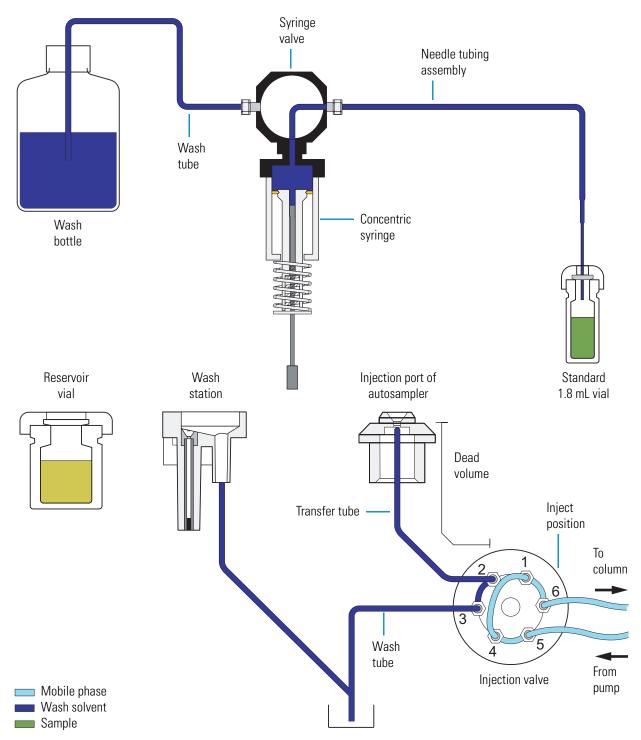
- Wash Bottle and Wash Bottle Tube
- XYZ Arm Mechanism
- Needle Tubing Assembly and Needle Assembly
- Syringe Valve
- Syringe
- Wash Station
- Injection Port of the Autosampler and Transfer Tube
- Injection Valve
- Sample Loop

You can order the replaceable components of the injection system by Thermo Scientific part number. "Consumables" on page 126 lists the part numbers.



CAUTION Because the XYZ arm moves to the sample position, do not place objects taller than 4.5 cm (1.8 in.) in the tray compartment.

Figure 3. Accela Autosampler injection components



Wash Bottle and Wash Bottle Tube

The wash bottle rests in the solvent platform on the top of the Accela stack. The wash bottle tube connects the wash bottle to the syringe valve. Both of the direct commands, Flush (from bottle) and Wash Needle (from bottle), draw solvent from the wash bottle. If you let the wash bottle run dry, the syringe barrel and the wash bottle tube fill with air. When this happens, the autosampler cannot draw sample into the needle tubing during an injection sequence.

IMPORTANT Check the volume of solvent in the wash bottle before performing a sequence of injections. Letting the wash bottle run dry compromises the performance of the autosampler.

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 lowers the needle along the z axis to the requested needle height. The syringe plunger descends, drawing sample into the needle tubing. The XYZ arm mechanism moves back to the home position, above the injection port of the autosampler, and then lowers the needle into the injection port. The syringe plunger ascends, expelling the sample from the needle tubing.

Do not move the XYZ arm manually. Instead, use the commands and options listed in Table 1 to control the position of the XYZ arm. For information on using the data system commands, see "Using the Xcalibur Direct Control Commands" on page 141 or "Using the ChromQuest Direct Commands" on page 143.

Table 1. Commands and options that control the position of the XYZ arm

Action	Data system	Command or option
Make the XYZ arm automatically move to the back of the tray compartment when	Xcalibur	Select the Verify Door Is Closed check box when you specify the instrument configuration options.
you open the tray door.	ChromQuest	Select the Verify Door Is Closed check box when you specify the instrument configuration options.
Move the XYZ arm to the back of the tray compartment.	Xcalibur	Use the Position Arm to Access Tray direct command.
	ChromQuest	Use the Position Arm to Access Tray direct control command.
Move the XYZ arm to its home position above the injection port and behind the	Xcalibur	Use the Set Arm to Home Position direct command.
wash station.	ChromQuest	Use the Go to Home direct control command.
Move the XYZ arm to the center front of	Xcalibur	Use the Remove Needle direct command.
the tray compartment, allowing easy access to the needle.	ChromQuest	Use the Needle Removal direct control command.

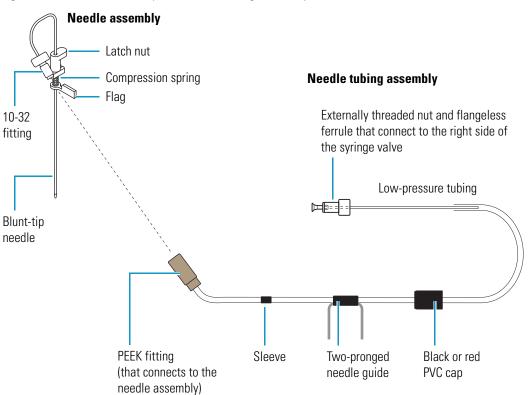
Needle Tubing Assembly and Needle Assembly

Figure 4 shows the needle assembly and the needle tubing assembly.

The needle assembly consists of a blunt-tip needle, a latch nut, a flag, a compression spring, and an externally threaded fitting that connects to the needle tube assembly.

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.

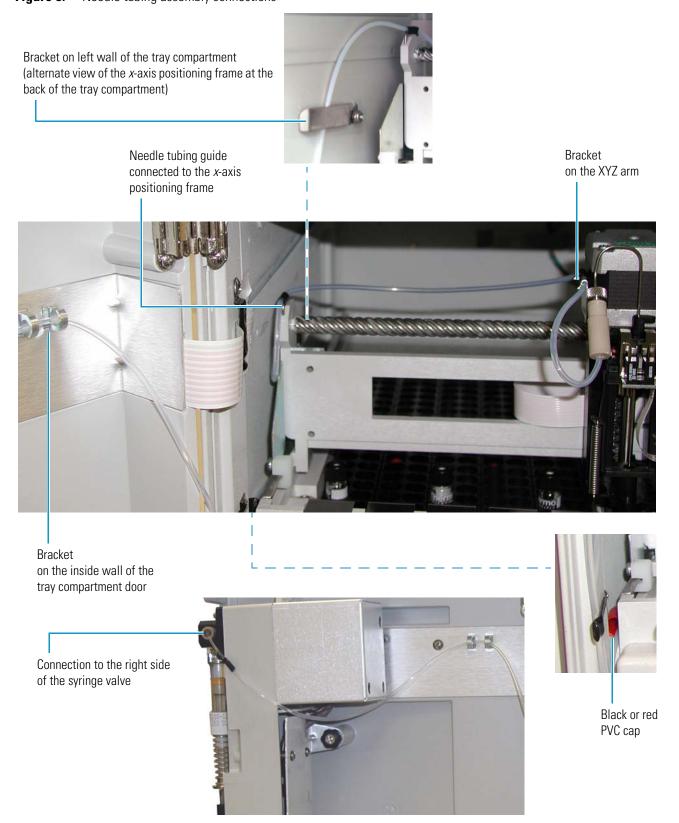
Figure 4. Needle assembly and needle tubing assembly



The needle mount on the XYZ arm holds the needle assembly. To prevent the needle tubing from interfering with the movement of the XYZ arm, the following items secure the needle tubing to the autosampler (see Figure 5):

- A bracket on the XYZ arm
- A guide on the *x*-axis positioning frame
- A bracket on the left wall of the tray compartment
- A PVC cap that holds the tubing below the metal runner for the x-axis positioning frame
- A bracket on the inner wall of the tray compartment door

Figure 5. Needle tubing assembly connections



1 Introduction

Autosampler Components

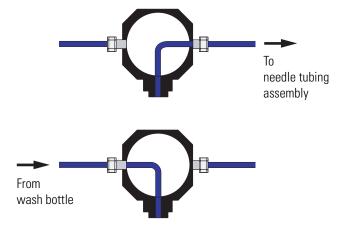
The standard needle tubing assembly holds approximately 560 μL of liquid. During an injection, the syringe draws sample into the needle tubing, not into the syringe barrel. If your autosampler is configured with a 2500 μL standard syringe, and you want to make partial loop injections greater than 500 μL or full loop injections greater than 150 μL , you must add additional needle tubing to your autosampler.

The tubing extension, which the autosampler needs to make large volume injections, holds up to 1 mL of liquid. Adding this additional tubing to your autosampler allows the withdrawal of up to 1500 μ L from a sample location or a reservoir vial. To attach this additional tubing to your autosampler, detach the needle tubing assembly from the syringe valve. Use the Teflon connector to attach the additional tubing to the needle tubing assembly. Attach the other end of the additional tubing to the syringe valve.

Syringe Valve

The syringe valve is a 2-position rotary valve (see Figure 6). In the wash bottle position, the downward movement of the syringe plunger draws wash solvent from the wash bottle into the syringe barrel. In the needle position, the downward movement of the syringe plunger draws liquid from a sample vial or a reservoir vial into the needle tubing. As the syringe plunger moves upward, it pushes liquid out of the needle tubing.

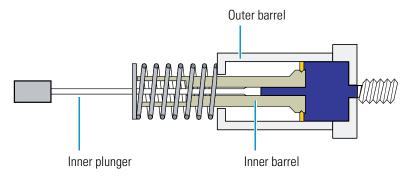
Figure 6. Two-position rotary syringe valve



Syringe

The Accela Autosampler uses a syringe with two concentric barrels to perform liquid transfers (see Figure 7). The autosampler uses the inner barrel plunger to draw and deliver sample amounts equal to or less than the nominal size of the syringe and the outer barrel plunger to draw and expel volumes up to $560 \, \mu L$.

Figure 7. Dual concentric syringe



The inner plunger wears more rapidly than the rest of the syringe. The lifespan of the inner plunger is approximately 50000 injections. Replace the inner plunger if you notice liquid leaking from the bottom of the syringe. For ordering information, see page 126.

Dual concentric syringes are available in 100, 250, and 500 μL sizes. In addition, a 2500 μL standard syringe is available. The size of the syringe determines the injection volume range. Table 2 lists the minimum and maximum injection volumes for each syringe size.

Table 2. Allowable injection volumes based on the syringe size

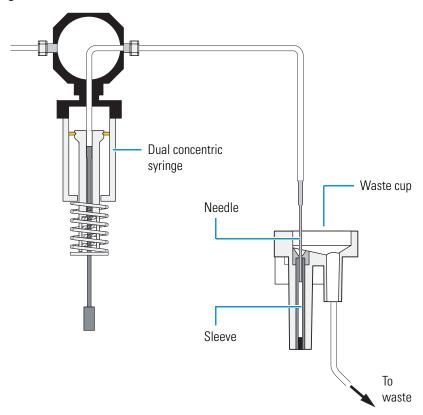
	In	jection volume (µL)		
Syringe size (µL)	Minimum	Maximum (No waste)	Maximum (Partial loop)	Maximum (Full loop)
100	0.1	20	20	20
250	0.1	100	100	100
500	0.1	250	250	250
2500	0.1	1000	1000	1000 ^a

^a Requires an extension to the standard needle tubing

Wash Station

The wash station is a waste cup containing an inner sleeve slightly larger than the needle (see Figure 8). Low-pressure tubing connects the waste cup to the waste bottle. To wash the outside of the needle, the XYZ arm moves the needle to the wash station and lowers the needle into the inner sleeve of the waste cup. The syringe draws solvent from the wash bottle or requested reservoir vial, and then expels the solvent through the needle. As it fills the waste cup, the expelled solvent flows up over the exterior surface of the needle, and then out the waste tubing to the waste bottle.

Figure 8. Wash station



Injection Port of the Autosampler and Transfer Tube

A transfer tube connects the injection port (see Figure 9) to the injection valve (see Figure 10). The transfer tubing has an inner diameter of 0.012 in. One end of the tubing has a flanged stainless steel fitting that connects to the injection port. The other end of the tubing has a PEEKTM fitting that connects to port 2 of the injection valve (see Figure 11).

IMPORTANT

- 1. The label attached to the transfer tube assembly specifies the internal volume of the tubing. Because the autosampler uses this value for its injection algorithm, you must type this value in the Dead Volume box when you configure the Accela Autosampler. If you do not enter the value listed on the label, your data might be compromised.
- 2. You can constrict the transfer tube by overtightening the fitting to port 2 of the injection valve. Lack of injection precision is a symptom of a constricted transfer tube.

Figure 9. Injection port of the Accela Autosampler

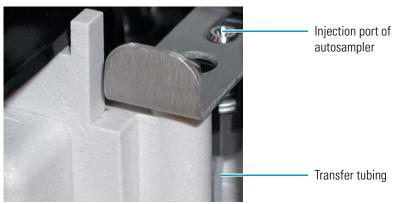
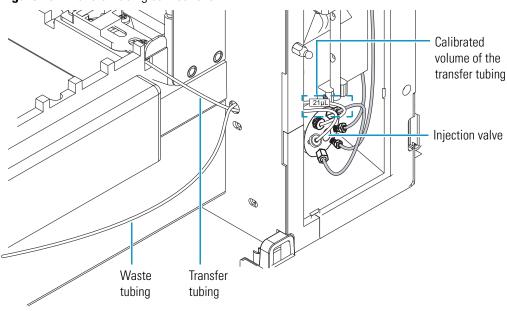


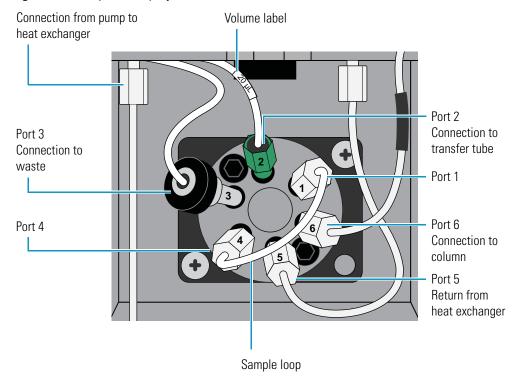
Figure 10. Transfer tubing connections



Injection Valve

The injection valve is a six-port, two-position valve that introduces sample onto the column by way of the sample loop (see Figure 11).

Figure 11. Six-port rotary injection valve



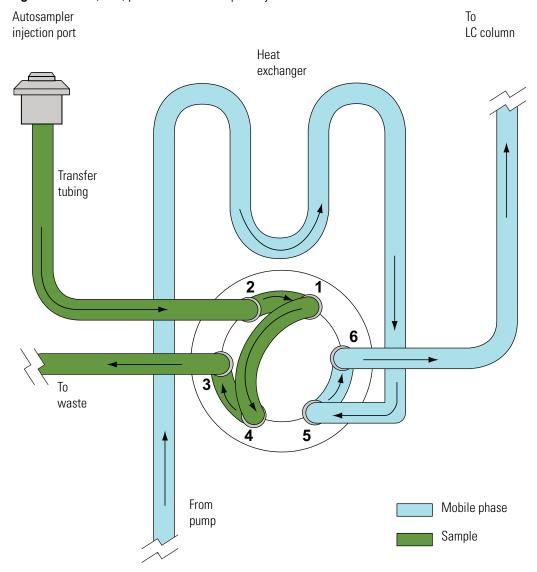
There are two positions for the injection valve: fill (load) and inject. Three passages are open in each position.

In the fill (load) position (see Figure 12), the three passages connect the following ports:

- The injection port of the injection valve (port 2) is connected to the front of the sample loop (port 1).
- The back of the sample loop (port 4) is connected to the waste line (port 3).
- The temperature-equilibrated mobile phase that has passed through the heat exchanger (port 5) is connected to the LC column (port 6).

To isolate the sample loop from the mobile phase stream, the autosampler rotates the valve to the load position (see Figure 12). While the mobile phase stream bypasses the loop, the syringe pushes sample through the transfer tubing and into the front of the sample loop. As the sample loop fills with sample, excess solution passes out of the back of the sample loop to waste.

Figure 12. Fill (load) position for the six-port injection valve



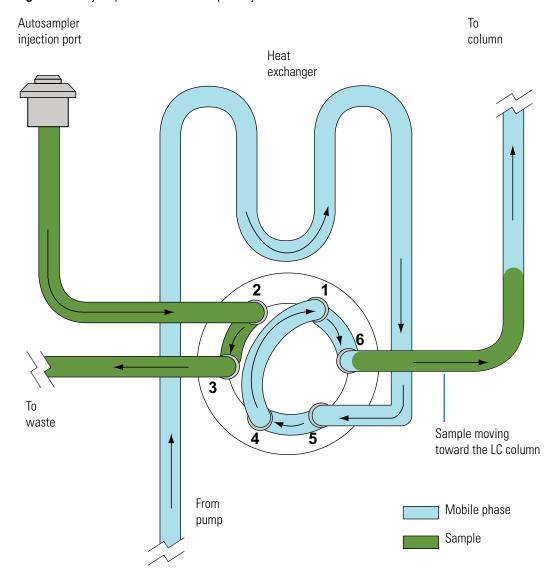
1 Introduction

In the inject position (see Figure 13), the three passages connect the following ports:

- The solvent return from the heat exchanger (port 5) is connected to the back of the loop (port 4).
- The front of the sample loop (port 1) is connected to the column inlet (port 6).
- The injection port of the injection valve (port 2) is connected to the waste line (port 3).

To put the sample loop into the path of the mobile phase stream, the autosampler rotates the valve to the inject position (see Figure 13). The mobile phase stream enters the sample loop from the back, backflushing the contents of the sample loop onto the column. Excess sample left in the transfer tube passes directly to waste. To provide ample rinsing of the sample loop with mobile phase, the injection valve remains in the inject position during the entire run.

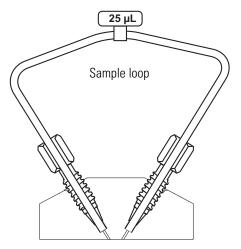
Figure 13. Inject position for the six-port injection valve



Sample Loop

The sample loop is a stainless steel tube with end fittings that holds the sample prior to its introduction onto the column. The sample loop is an interchangeable part swaged onto ports 1 and 4 of the six-port injection valve (see Figure 14).

Figure 14. Schematic of the sample loop attached to the injection valve



Thermo Fisher Scientific ships the Accela Autosampler with a 25 μ L sample loop. Sample loops are available in nominal sizes ranging from 5 to 1000 μ L. The accuracy of the sample loop volume is \pm 20%. This means that the actual volume of a 25 μ L sample loop is between 20 to 30 μ L. For ordering information, see "Consumables" on page 126.

Column Oven and Tray Compartment Heater/Cooler

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

Between the pump outlet and the autosampler injection valve, the mobile phase passes through a heat exchanger that is located behind the column oven (see Figure 75 on page 96). As the mobile phase passes through the heat exchanger, it equilibrates to the temperature of the column oven. The heat exchanger consists of a length of 0.004 in. ID tubing that adds only 3 μ L of gradient delay volume to the system (see Figure 75 on page 96).

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.

1 Introduction Status LEDs

Status LEDs

Four status light-emitting diodes (LEDs) labeled Power, Communication, Run, and Temperature are located on the front of the autosampler's tray compartment door (see Figure 15). Table 3 lists the states of the LEDs.

Figure 15. Status LEDs



Note To control the temperature of the column oven compartment and the tray compartment, select the Wait for Temperature Ready check box when you add the Accela Autosampler to the data system instrument configuration.

Table 3. Status LED states

LED	State	Meaning	
Power	Green	The autosampler power is on.	
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 ^a	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.	

^a The Temp LED remains green if you do not select the Wait for Temperature Ready check box when you add the Accela Autosampler to the instrument configuration.

Injection Modes

The Accela Autosampler can perform the following injection modes:

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

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

In the no waste injection mode, the autosampler 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.

IMPORTANT For no waste injections, do the following:

- Use a sample loop that is at least 5 μ L larger than the injection volume. Because the accuracy of the nominal size is \pm 20%, use an estimate of 80% for the actual size. For example, use 20 μ L as an estimate for the actual volume of a 25 μ L loop, and inject no more than 15 μ L 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 μ L of flush solvent and 3 μ L of air into the sample loop (see Figure 16), regardless of the requested injection volume.
- Inject at least 1.0 µL of sample.

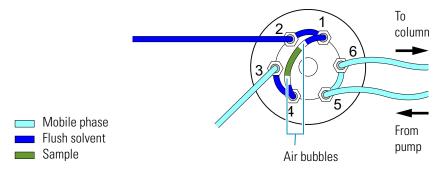
Approximately 0.25 μL of the sample is lost as it travels from the injection port through the transfer tubing and into the injection valve. Because of this loss, inject at least 1.0 μL of sample with the no waste injection mode.

The quantity of lost sample depends on the syringe rate. Decreasing the syringe rate decreases the sample loss. Thermo Fisher Scientific recommends a syringe rate no greater than 4 μ L/s for the no waste injection mode.

In addition to the sample, the no waste injection mode loads approximately 2 μL of flush solvent and 3 μL of air into the sample loop (see Figure 16). Because these values are independent of the injection volume, the chemistry of the flush solution can affect your chromatographic results. For best chromatographic results, consider matching the chemistry of the sample matrix, the flush solution, and the mobile phase.

1 Introduction Injection Modes

Figure 16. No waste injection mode – load position



Note The two 1.5 μ L air bubbles isolate the sample from the flush solvent.

Partial Loop Injection

In the partial loop injection mode, the autosampler withdraws $22~\mu L$ of excess sample from the vial in addition to the requested injection volume. The autosampler expels approximately one-half of the excess volume to waste before it meters the center of the sample plug into the front of the sample loop. It expels the second portion of excess sample to waste after the mobile phase stream backflushes the contents of the sample loop onto the column.

Because the autosampler meters sample into the sample loop, the partial loop injection mode is less precise but more accurate than the full loop injection mode. You can inject variable amounts of sample, ranging from a minimum of 0.1 μL to a practical maximum of one-half the volume of your sample loop. The laminar flow of fluid within the stainless steel sample loop causes this maximum volume limitation.

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 \pm 20% (which means that the actual volume of the standard 25 μL sample loop is between 20 μL and 30 μL), for best results, limit the maximum injection volume to 10 μL for the standard sample loop.

Full Loop Injection

In the full loop injection mode, the autosampler withdraws a sample volume sufficient to overfill the loop (according to the equation given on page 19). Because the actual injection volume is determined by the size of the loop, not the metering action of the stepper motor, a full loop injection is very reproducible. However, because the full loop injection mode completely fills the sample loop, you cannot inject variable amounts of sample.

Use the full loop injection mode when you want maximum precision and have unlimited sample. However, if you want to change the injection volume, you must replace the attached sample loop with one of a different size. The available sample loop sizes are listed in "Consumables" on page 126.

Note You enter the size of the sample loop when you configure the Accela Autosampler.

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

Amount withdrawn = $3 \times IV + DV + 7.5 \mu L$

Where:

IV = requested injection volume

DV = Volume of the transfer tube + volume of the injection port of the autosampler and the rotor slot (1.6 μ L)

This equation is valid until the volume exceeds the maximum capacity of the syringe. If you request a 100 μ L full loop injection and your autosampler has a 250 μ L syringe, the autosampler withdraws 265 μ L of sample solution from the vial, which is the maximum capacity of the syringe. A sample volume equal to the actual volume of the sample loop is injected onto the column. Table 4 lists the maximum capacity for each syringe.

Table 4. Maximum volume withdrawn for full loop injections

Nominal syringe size	Maximum volume withdrawn
Concentric 100 μL	101 μL
Concentric 250 μL	265 μL
Concentric 500 μL	535 μL
Standard 2500 μL	1544 μL ^a

 $^{^{\}text{a}}\,$ The data system limits the maximum amount withdrawn to 1544 $\mu L.$

1 Introduction Injection Sequence

Injection Sequence

The primary sequence of events for the full loop injection mode is as follows:

- 1. Getting ready for an injection
- 2. Drawing sample from the vial
- 3. Loading sample into the loop
- 4. Injecting sample
- 5. Post-injection events

The full loop injection mode draws enough sample to overfill both the transfer tube and the sample loop. The no waste injection mode, however, draws only the injection volume requested, and the partial loop injection mode draws only the injection amount requested plus an additional $22~\mu L$.

As a result, unlike the full loop mode, both the partial loop and no waste injection modes require additional solvent to push the sample all the way through the transfer tubing to the injection valve before the sample is loaded into the loop. The syringe draws this extra transport solvent from the wash bottle and uses it to compensate for the volume held by the injection port of the autosampler, the transfer tube, and the rotor slot of the injection valve. Before the sample is loaded into the loop, the syringe uses the transport solvent to push the sample to the injection valve.

The primary sequence of events for both the partial loop injection mode and the no waste injection mode is as follows:

- 1. Getting Ready for an Injection
- 2. Drawing Transport Solvent (Partial Loop and No Waste Injections)
- 3. Drawing Sample
- 4. Pushing Transport Solvent (Partial Loop and No Waste Injections)
- 5. Loading Sample into the Sample Loop
- 6. Injecting Sample onto the Column
- 7. Post-Injection Events

Table 5 outlines the sequential events that occur during an injection.

 Table 5.
 Injection sequence

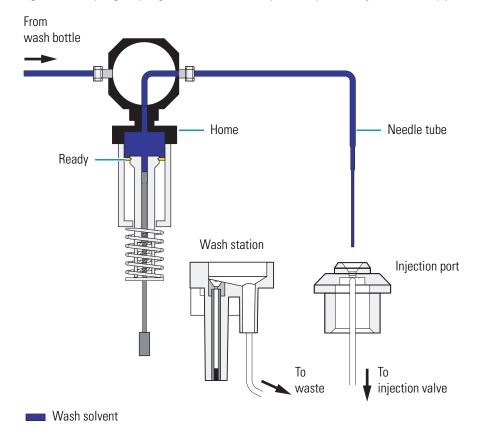
#	Full loop	#	Partial loop	No waste
1	Get ready for injection	1	Get ready for injection	Get ready for injection
			Switch injector to inject position	Switch injector to inject position
	N/A	2	Draw transport solvent	Draw transport solvent
			Switch syringe valve to wash solvent position	Switch syringe valve to wash solvent position
	Switch injector to fill position		Draw wash solvent (transport volume = total dead volume + 7.5 μL)	Draw wash solvent (transport volume = total dead volume)
2	Draw Sample	3	Draw Sample	Draw Sample
	Move XYZ arm to vial		Move XYZ arm to vial	Move XYZ arm to vial
	Switch syringe valve to needle position		Switch syringe valve to needle position	Switch syringe valve to needle position
	Draw 3 μL air bubble		Draw 3 μL air bubble	Draw 1.5 μL air bubble
	Draw sample [(3 × IV) + DV + 7.5 μL]		Draw sample equal to Inject Volume (IV) = 22 μL	Draw sample equal to Inject Volume (IV)
	Draw 3 μL air bubble		Draw 3 μL air bubble	Draw 1.5 μL air bubble
	N/A	4	Push Transport Solvent	Push Transport Solvent
	Move XYZ arm to injection port of autosampler		Move XYZ arm to injection port of autosampler	Move XYZ arm to injection port of autosampler
			Push the transport volume into the transfer tube	Push the transport volume (minus 1 µL) into the transfer tube
3	Load Sample into Loop	5	Load Sample into Loop	Load Sample into Loop
			Switch the injector to fill position	Switch the injector to fill position
	Overfill the sample loop		Meter the sample plug into the loop	Meter the sample plug + air bubbles + 2 μL wash solvent into the loop
4	Switch the injector to inject position	6	Switch the injector to inject position	Switch the injector to inject position
5	Post-Injection Events	7	Post-Injection Events	Post-Injection Events
	Perform optional flush/wash		Perform optional flush/wash	Perform optional flush/wash
	Home syringe		Home syringe	Home syringe
	Prepare for next injection		Prepare for next injection	Prepare for next injection

Getting Ready for an Injection

Before an injection sequence begins, the autosampler initializes the syringe plunger to the Ready position (see Figure 17).

- If the syringe plunger is in the Home Side of Ready position, the autosampler switches the syringe valve to the solvent bottle position, and then returns the syringe plunger downward to the Ready position at flush speed.
- If the syringe plunger is below the Ready position, the autosampler switches the syringe valve to the needle position, the XYZ arm moves the needle to the wash station, and the syringe plunger moves upward to the Ready position at flush speed.

Figure 17. Syringe, syringe valve, and needle position prior to injection (Ready position)



Drawing Transport Solvent (Partial Loop and No Waste Injections)

To draw transport solvent, the syringe valve switches to the wash bottle position. The amount of solvent that the syringe draws from the wash bottle, referred to as the transport volume, depends on both the injection mode and the configured value for the dead volume.

IMPORTANT A transfer tube connects the injection port of the autosampler to port 2 of the injection valve. The transfer tube is labeled with its factory calibrated volume. When you configure the autosampler, you must type the label value in the Dead Volume box of the Accela Configuration dialog box.

In the no waste injection mode, the syringe draws only enough solvent to compensate for the total dead volume of the system according to the following equation:

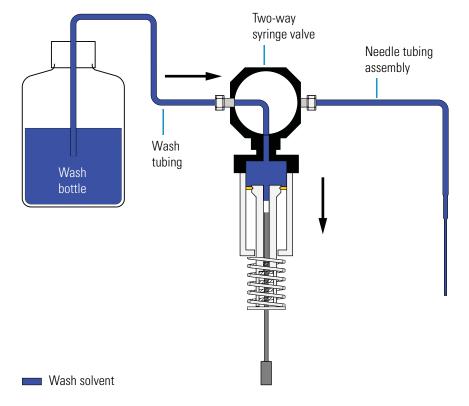
Transport solvent volume = Total dead volume

Where:

Total Dead Volume = Transfer tube volume + volume of the injection port of the autosampler and the rotor slot of the injection valve $(1.6 \,\mu\text{L})$

In the partial loop injection mode, the syringe draws enough solvent from the wash bottle to compensate for the total dead volume of the system plus an additional 7.5 μ L of solvent (see Figure 18).

Figure 18. Drawing wash solvent from the wash bottle



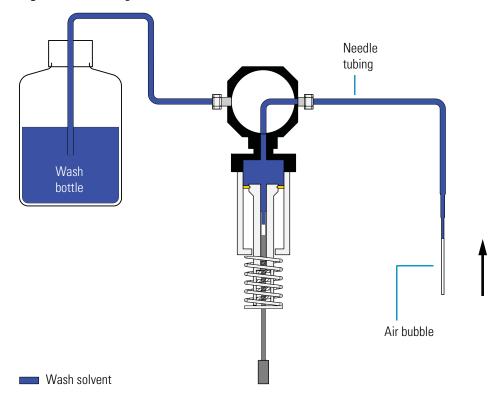
Drawing Sample

The autosampler draws sample from the sample vial or well as follows:

Note For the full loop injection mode, the injection valve rotates to the load position. For both the no waste injection mode and the partial loop injection mode, the injection valve remains in the inject position.

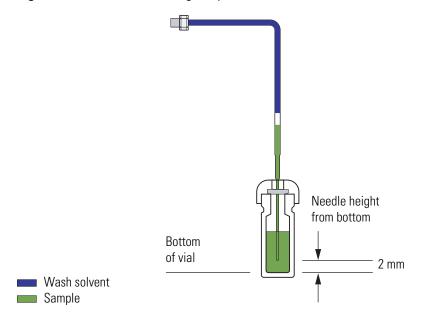
- 1. The XYZ arm moves to the sample vial.
- 2. The syringe valve switches to the needle position.
- 3. The inner syringe plunger descends, drawing an air bubble into the tip of the needle. Depending on the injection mode, the autosampler draws the following volume of air (see Figure 19):
 - For partial loop and full loop injections, the syringe draws 3 μL of air.
 - For no waste injections, the syringe draws 1.5 μ L of air.

Figure 19. Drawing the first air bubble



4. The XYZ arm lowers the needle tip into the sample vial to the depth requested (see Figure 20).

Figure 20. Needle withdrawing sample from a standard vial



Note The autosampler lowers the needle tip to the position requested in the Needle Height From Bottom box of the Accela Autosampler page of the Instrument Setup dialog box. The allowable range is 0.1 to 18 mm from the bottom of the vial.

- 5. The inner syringe plunger descends further, drawing sample at syringe sample speed. The volume of sample that the autosampler draws from the vial depends on the requested injection volume and the injection mode as follows:
 - For no waste injections, the autosampler draws only the requested injection volume.
 - For partial loop injections, the autosampler draws the requested injection volume plus an additional 22 μ L.
- 6. The XYZ arm raises the needle out of the sample vial (see Figure 21). The inner syringe plunger descends further, drawing a second air bubble into the needle. The sample plug is now isolated between the two air bubbles.

Wash bottle

Wash solvent Sample

Figure 21. Drawing a second air bubble, which isolates the sample plug

Pushing Transport Solvent (Partial Loop and No Waste Injections)

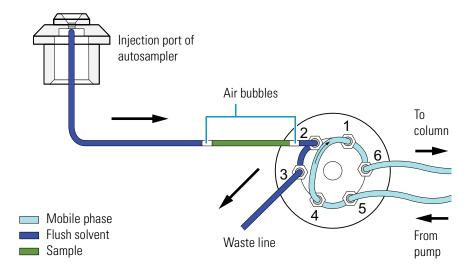
The XYZ arm moves to the home position, and then lowers the needle into the injection port of the autosampler. The inner syringe plunger moves upward, pushing the sample plug followed by the transport solvent into the transfer tube.

The actions that occur during the pushing transport solvent step depend on the injection mode.

Pushing Transport Solvent Step for No Waste Injections

The needle expels 1 μ L less than the transport volume into the transfer tube. The sample plug falls just short of the entry to the injection port (port 2) of the injection valve (see Figure 22).

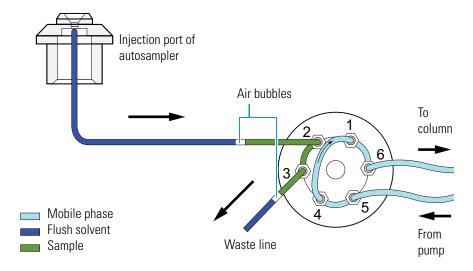
Figure 22. No Waste injection—Pushing transport solvent step



Pushing Transport Solvent Step for Partial Loop Injections

The needle expels the transport volume, which is equal to the dead volume between the injection port of the autosampler and the injection valve plus an excess of 7.5 μ L, into the transfer tube. This pushes 4.5 μ L of the sample plug plus the first 3 μ L bubble through the injection valve and out to waste (see Figure 23).

Figure 23. Partial Loop Injection—Pushing transport solvent step



1 Introduction Injection Sequence

Loading Sample into the Sample Loop

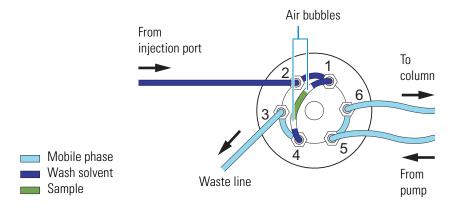
The injection valve switches to the fill position for both the partial loop injection mode and the no waste injection modes. For a full loop injection, the injection valve switches to the fill position at the beginning of the injection sequence. The syringe then pushes the sample into the sample loop. The Accela Autosampler issues a momentary Gradient Start signal.

The actions that occur during the sample loading step depend on the injection mode.

Sample Loading for No Waste Injections

The syringe meters the requested sample volume plus 5 μL into the sample loop. This pushes the sample plug, the two bracketing air bubbles, and approximately 2 μL of wash solvent into the sample loop (see Figure 24).

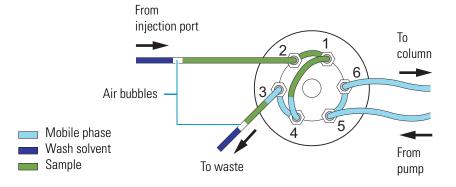
Figure 24. No Waste injection—Loading the sample loop



Sample Loading for Partial Loop Injections

The syringe meters the requested sample volume into the sample loop. This leaves approximately 16 μL of excess sample plus the second 3 μL air bubble in the transfer line (see Figure 25).

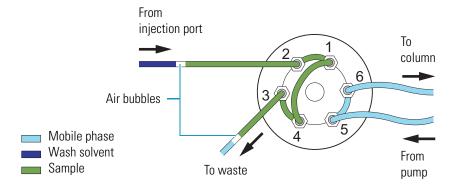
Figure 25. Partial Loop injection—Loading the sample loop



Sample Loading for Full Loop Injections

The needle expels the sample into the sample loop. As the sample loop becomes overfilled, excess sample exits through the waste line (see Figure 26).

Figure 26. Full Loop injection—Loading the sample loop

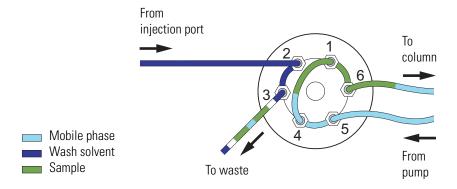


Injecting Sample onto the Column

The autosampler backflushes the contents of the sample loop onto the column as follows:

- 1. The injection valve switches to the inject position.
- 2. Mobile phase enters the sample loop from the back, sweeping the contents of the sample loop onto the column (see Figure 27).
- 3. The autosampler issues a momentary Inject Out signal.

Figure 27. Partial Loop injection—Injecting sample



1 Introduction Autosampler Operation

Post-Injection Events

At the end of an injection, the autosampler performs the following operations:

- 1. The autosampler performs an optional flush, wash, or both as specified in the instrument method.
 - **Flush** (optional): During an internal flush, the autosampler lowers the needle into the injection port, and then dispenses a specified volume of solvent through the needle and transfer line out to waste. You can select the solvent wash bottle or a reservoir vial as the flush solvent source. The internal flush removes excess or residual sample from the injection flow path. It does not flush the sample loop.
 - Wash (optional): During an external wash, the autosampler lowers the needle into
 the wash station, and then dispenses a specified volume of solvent through the needle.
 As the wash station overfills, the wash solvent flows up over the exterior of the needle,
 and then out to waste. You can select the solvent wash bottle or a reservoir vial as the
 wash solvent source.
- 2. The autosampler moves the syringe plunger to the Ready position, and then switches the syringe valve to point to the wash bottle.
- 3. The autosampler prepares for the next injection by moving the XYZ arm to the Home position, located above the injection port of the autosampler.

Autosampler Operation

You have full control of the autosampler from the Xcalibur and ChromQuest data systems and limited control of the autosampler from other Thermo Scientific mass spectrometry applications. The power switch is the only manual control. The data system Help, the *Accela User Guide for LC Devices*, and the *Accela User Guide for the ChromQuest Data System* provide information on how to operate the autosampler.

Note You can create instrument methods to control the Accela Autosampler from several Thermo Scientific mass spectrometry applications; however, you can calibrate the Accela Autosampler only from the ChromQuest or Xcalibur data system.

The Help and the manuals (provided as PDF files) include information on how to do the following:

- Calibrate the well bottom position for custom vials or wellplates (see "Calibrating the Accela Autosampler" on page 48).
- Perform direct commands to flush the syringe, move the XYZ arm (see "XYZ Arm Mechanism" on page 5), inject sample, and so on.
- Create an instrument method for data acquisition.

For information on accessing the manuals, see "Related Documentation" on page xiii.

Automated Sample Preparation

With the automated sample preparation feature, you can build a pretreatment routine that the autosampler performs before it performs an injection. The pretreatment routine can consist of multiple tasks including transferring liquid between sample vials, transferring liquid from one of the four reservoir vials to a sample vial, mixing, and more.

The autosampler performs each task by drawing liquid into or expelling liquid from the needle tubing. It uses the inner plunger of the concentric syringe for liquid transfers that require a high degree of precision, such as a transfer of liquid between sample vials. It uses the outer plunger of the concentric syringe for liquid transfers that require less precision, such as sample mixing, which is performed using an aspirate and expunge technique.

For a complete list of sample preparation tasks that the autosampler can perform and a set of rules for creating sample preparation routines, refer to the Help provided with the data system.

Specifications

These are specifications for the Accela Autosampler.

Sample capacity:	 200 vials in five removable trays 288 samples in three 96-well microplates 1152 samples in three 384-well microplates
Standard vial:	• 1.8 mL volume
Injection precision:	• ≤ 1.0% RSD at 5 μL and up
Partial loop injection volume:	 10 μL with standard 25 μL sample loop and 250 μL syringe Up to 1000 μL injection volume with optional loops and syringes
Fixed loop (full loop) injection volume:	 25 μL with standard loop and 250 μL syringe Up to 1000 μL injection volume with optional loops and syringes
Sample carryover:	 ≤ 0.1% 6 mL flush volumes, user-specified for internal and external needle wash cycles
Minimum sample volume:	• 1 μL can be injected from 5 μL
Needle height control:	Programmable in 0.1 mm incrementsActive vial bottom search selectable on/off
Column oven:	• Programmable in 1 °C increments from 5 to 95 °C
Tray temperature control:	 Programmable in 1 °C increments from 0 to 60 °C Typical vial temperature from 4 to 50 °C
Reagent vials:	 Four 16 mL vials can be specified for flush/wash or dilution functions
Remote inputs:	Pump ready, inject hold
Remote outputs:	 Pump stop, autosampler ready, inject, gradient start, four timed events
Ambient environment:	• 10 to 40 °C, 5 to 95% relative humidity, non-condensing
Dimensions:	• $37 \times 36 \times 50 \text{ cm } (h \times w \times d)$
	• $14.5 \times 15 \times 20$ in. $(h \times w \times d)$
Weight:	• 30 kg (66 lbs) See "Lifting and Carrying the Autosampler" on page xviii.
Power requirements:	• 100/120/220/240 Vac nominal; 50/60 Hz, 550 VA
Product certification:	• CSA, TÜV/GS, FCC (EMI), VDE (EMI)

Installation

To install the Accela Autosampler as part of an Accela UHPLC system, use the "Installation Checklist" on page 34 and follow the procedures in this chapter. Make a copy of the checklist and fill it out when the installation is complete. Include the completed checklist in your maintenance records.

"Changing the Sample Loop" on page 49 describes how to change the sample loop, a piece of stainless steel tubing with two end fittings connected to the injection valve. For full loop (fixed loop) injections, the sample loop size determines the injection volume.

Note Install the Accela Autosampler in the sequence presented in the Installation Checklist.

Contents

- Software Compatibility
- Installation Checklist
- Unpacking and Inspecting the Instrument
- Making Initial Instrument Preparations
- Making the Back Panel Connections
- Connecting the Solvent Lines
- Powering On the Autosampler
- Calibrating the Accela Autosampler
- Changing the Sample Loop

Software Compatibility

You have full control of the Accela Autosampler from the ChromQuest chromatography data system and the Xcalibur mass spectrometry application and limited control from several other Thermo Scientific mass spectrometry applications. The following data systems provide compatible firmware and a firmware upgrade utility (see "Downloading Firmware to the Autosampler" on page 133):

- For the ChromQuest 4.2 data system, you can find the Accela device drivers on the Accela Device Driver Add-on CD.
- For the ChromQuest 5.0 data system, you can find the Accela device drivers on the ChromQuest CD.

The following installation summarizes the steps that you must complete to properly install the

 For the Xcalibur data system, you can find the Accela device drivers on the LC Devices DVD.

Installation Checklist

Acce	ela Autosampler.
	Unpack and inspect your instrument (page 35).
	Read the safety notices (front of manual).
	Make the initial instrument preparations (page 35).
	Make the initial back panel connections (page 36).
	Power on the autosampler for the first time (page 47).
	Install the Xcalibur 2.0 (or later) data system or the ChromQuest 4.2 (or later) data system and connect remote communication outputs, as required for external devices that are not controlled by the data system.
	Add the Accela Autosampler to the data system instrument configuration. For instructions on specifying the configuration options for the Accela Autosampler, refer to the Accela User Guide for LC Devices, the Accela User Guide for the ChromQuest Data System, or the Help provided by the data system.
Thi	s Accela Autosampler was installed by:
(Na	ime) (Date)

Unpacking and Inspecting the Instrument

Carefully remove the autosampler from the shipping container and inspect both the autosampler and packing for any signs of damage. If you find any damage, save the shipping materials and immediately contact the shipping company.

The shipping container contains the Accela Autosampler and an accessory kit. Carefully check to make sure you received all the items listed on the packing list. Ensure that the needle tubing assembly is attached to the XYZ arm and the right side of the syringe valve, a 250 μL dual-concentric syringe is attached to the syringe drive mount, and a 25 μL sample loop is attached to the injection valve.

If any items are missing, contact your Thermo Fisher Scientific representative immediately.

To connect the autosampler to the other instruments in the Accela stack, you must have the Accela System Kit (see "Accela System Kit" on page 129). This kit is provided with orders for instruments sold together with either the Xcalibur or the ChromQuest data system or as a separate option.

Making Initial Instrument Preparations

Place the autosampler on a benchtop. Allow at least 15 cm (6 in.) of space between the back panel of the autosampler and any wall or obstruction. This provides clearance for the back-panel connectors and cool air flow.



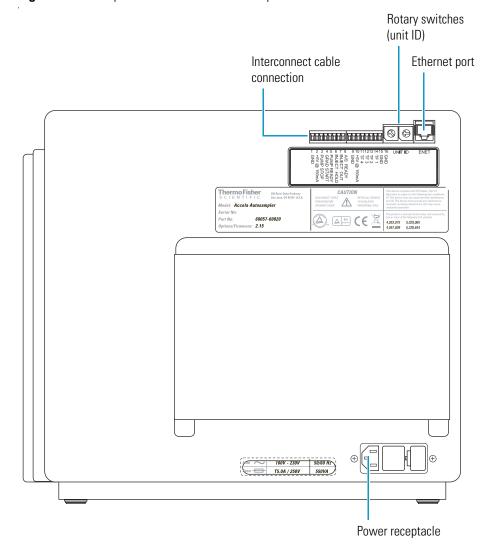
Installing the autosampler requires these tools:

- 5/16 in. open-end wrench
- Narrow flat-head screwdriver

Making the Back Panel Connections

To operate the Accela Autosampler, connect the power, Ethernet, and system interconnect cable to the back panel (see Figure 28) of the autosampler, and check the unit ID setting.

Figure 28. Back panel of the Accela Autosampler



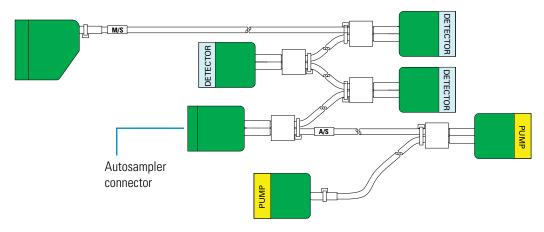
To make the back panel connections, follow these procedures:

- Connecting the System Interconnect Cable
- Connecting the Ethernet Cable
- Checking the Unit ID Settings
- Connecting the Autosampler to Line Power

Connecting the System Interconnect Cable

The system interconnect cable that synchronizes the timing of the Accela devices is in the Accela System Kit. The interconnect cable has seven combicon connectors: three have a blue DETECTOR label; two have a yellow PUMP label; one has a small A/S tag on its adjacent cable; and one has a small M/S tag on its adjacent cable (see Figure 29).

Figure 29. System interconnect cable with seven combicon connectors

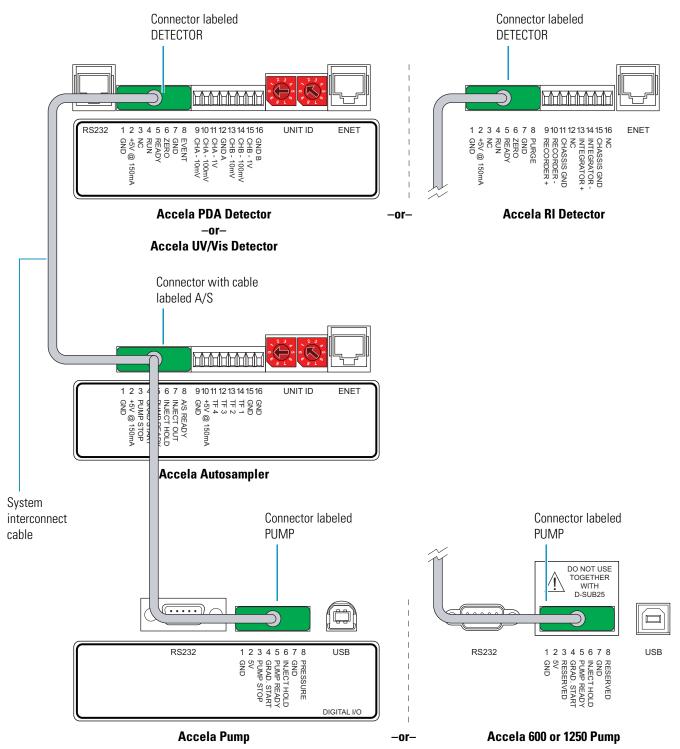


❖ To connect the interconnect cable

- 1. Plug the AS connector into the left 8-pin socket on the back panel of the Accela Autosampler (see Figure 30).
- 2. Attach one of the pump connectors to the Accela pump (Accela Pump, Accela 600 Pump, or Accela 1250 Pump).
- 3. If your LC system contains an Accela detector, attach one of the detector connectors to it.

Refer to the *Accela Getting Connected Guide* for instructions on how to connect the system interconnect cable to a Thermo Scientific MS detector.

Figure 30. System interconnect cable connections



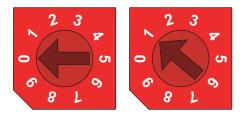
Connecting the Ethernet Cable

Connect the Ethernet port on the back panel of the Accela Autosampler to the Ethernet switch using the 7 ft length, CAT-5 Ethernet cable with ferrite supplied in the accessory kit.

Checking the Unit ID Settings

The Accela Autosampler is shipped with the unit ID preset using the two rotary switches located on the back panel (see Figure 31). The unit ID must correspond with the stack number that you specify in configuring the Accela Autosampler.

Figure 31. Rotary switches set to 01



The Communication page (see Figure 84 on page 123) of the Accela Autosampler Configuration dialog box in the Thermo Foundation Instrument Configuration application contains one box for the unit ID value. The default is 1, and the range is 1 to 99. The Accela AS Configuration dialog box (see Figure 85 on page 124) in the ChromQuest data system contains two boxes for the unit ID. The default value is 01 and the range is 01 to 99. The value of 0 or 00, respectively, is reserved for special service functions.

Connecting the Autosampler to Line Power

The Accela Autosampler is shipped with one of these power cords.

Destination	Plug type	Voltage rating	Current rating	P/N
United Kingdom	BS 1363	250 Vac	5 A	6003-0810
Switzerland	SEV 1011	250 Vac	10 A	6003-0620
China and Europe	CEE 7/7	250 Vac	10 A	6003-0330
United States and Canada	NEMA 5-15P	125 Vac	10 A	6003-0160

Local codes in your area might require installing another type of plug and receptacle. The Thermo Fisher Scientific field engineer for your country will provide the appropriate power plugs.

To connect the autosampler to line power

- 1. Ensure that the power switch on the front of the unit is in the Off position (released or out position).
- 2. Check the fuses (see "Replacing a Fuse" on page 97).
- 3. Connect the plug to the power receptacle (see Figure 28 on page 36) on the back panel of the autosampler.
- 4. Connect the other end of the power cord to line power.

When you are ready to power on the autosampler, go to "Powering On the Autosampler" on page 47.

Connecting the Solvent Lines

Before you can operate the LC system, connect the wash bottle that is contained in the solvent platform to the left side of the syringe valve by using the wash bottle tubing, connect the needle tubing to the right side of the syringe valve, and connect the autosampler to the pump.

To connect the solvent lines, follow these procedures:

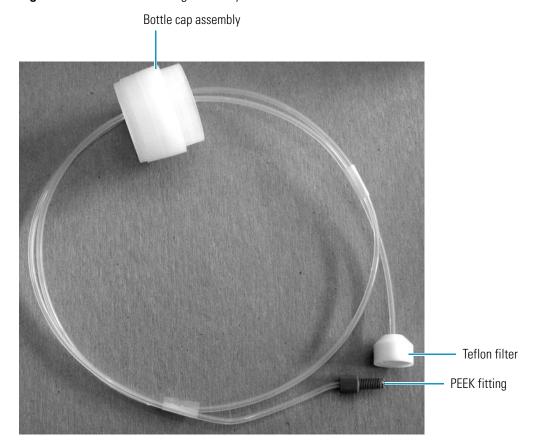
- Connecting the Wash Bottle
- Connecting the Needle Tubing
- Connecting the Autosampler to the Pump

IMPORTANT If the wash bottle tubing runs dry during a sequence of injections, the autosampler makes "blank" injections.

Connecting the Wash Bottle

The Wash Bottle Kit supplied in the Accela System Kit contains a 1 liter bottle and a tubing assembly (see Figure 32).

Figure 32. Wash bottle tubing assembly



❖ To connect the wash bottle

1. Fill the wash bottle with an appropriate solvent, and then place it in the solvent platform on the top of the system stack.

Note The solvent platform holds the 1 L wash bottle and four 1 L solvent reservoir bottles. In case of breakage, the solvent platform can hold up to 7 L and acts as a secondary container for the liquid in the solvent bottles.

- 2. Insert the Teflon filter into the bottom of the bottle and screw on the cap.
- 3. Connect the PEEK fitting to the left side of the syringe valve (see Figure 33).

Wash tubing Syringe Needle tubing fitting valve fitting

Figure 33. Wash tubing and needle tubing connections

Connecting the Needle Tubing

The Accela Autosampler is shipped with the needle tubing assembly connected and routed through the appropriate brackets. The internally threaded fitting of the needle tubing assembly is connected to the needle assembly and the externally threaded fitting is connected to the syringe valve. The needle tubing is routed through a bracket on the XYZ arm, a bracket on the left wall of the tray compartment, and a bracket on the tray compartment door. In addition, the two-pronged needle tubing guide is connected to the *x*-axis positioning frame, and the PVC cap (red or black) is pushed below the metal runner for the *x*-axis positioning frame.

Before you operate the autosampler, make sure that the needle tubing is routed through the appropriate brackets.



CAUTION The needle tubing is easily damaged. Before you operate the autosampler, make sure that the needle tubing is routed through the support brackets, that the needle tubing guide is connected to the *x*-axis positioning frame, and that the PVC cap is pushed below the metal runner for the *x*-axis positioning frame.

Connecting the Autosampler to the Pump

After you set up the Accela stack on the laboratory benchtop, connect the pump outlet to the autosampler inlet (heat exchanger inlet).



CAUTION To prevent personal injury, before connecting the pump to the autosampler, make sure that the column oven compartment is at room temperature.



CAUTION To prevent personal injury caused by skin contact with hazardous solvents, ensure that the pump is off before you connect it to the autosampler.

❖ To connect the autosampler heat exchanger to the Accela pump outlet

- 1. Connect a length of high-pressure tubing to the Accela pump outlet as follows:
 - a. Using a three-piece compression fitting with a 0.45 in. nut (see Figure 34), connect a length of 0.005 in. ID \times 1/16 in. OD tubing to the pump outlet.

The tubing length depends on the pump model:

- For the Accela Pump, connect a 20 cm (8 in.) length of precut tubing to the outlet of the dynamic mixer.
- For the Accela 600 Pump or the Accela 1250 Pump, connect a 26 cm (10 in.) length of precut tubing to the outlet of the placeholder module or the optional dynamic mixer.

Tip To identify the tubing ID, the precut tubing has a color-coded band:

• Red = 0.005 in.

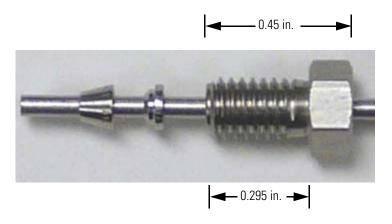
• Black = 0.007 in.

- Blue = 0.010 in.
- Yellow = 0.020 in.

Figure 34 shows the 0.45 in. length nut and the front and back ferrules for 1/16 in. OD tubing.

The 0.45 in. nut has a longer threaded portion than the 0.53 in. nut (0.295 in. > 0.225 in.). The longer threaded portion makes the 0.45 in. nut suitable for use with the deep ports of the dynamic mixer and the placeholder module.

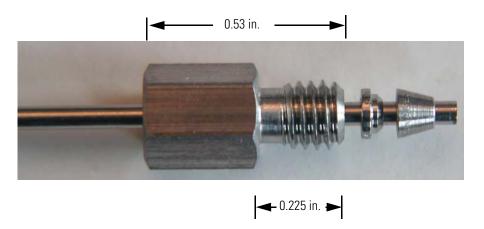
Figure 34. High-pressure fitting with a front and back ferrule and a 0.45 in. nut



- b. Using a 0.25 in. open-ended wrench, tighten the nut.
- 2. Route the tubing through the access slot in the autosampler drip tray.
- 3. Connect the other end of the tubing to the heat exchanger inlet as follows:
 - a. Using a three-piece compression fitting with a 0.53 in. nut, connect the other end of the tubing to the heat exchanger inlet.

Figure 35 shows the 0.53 in. length nut and the front and back ferrules for 1/16 in. OD tubing.

Figure 35. High-pressure fitting with a front and back ferrule and a 0.53 in. nut



b. Using a 0.25 in. open-ended wrench, tighten the nut.

Figure 36 through Figure 38 show the high-pressure connection between the autosampler and the pump.

Figure 36. Connection between the last version of the Accela Pump and the autosampler

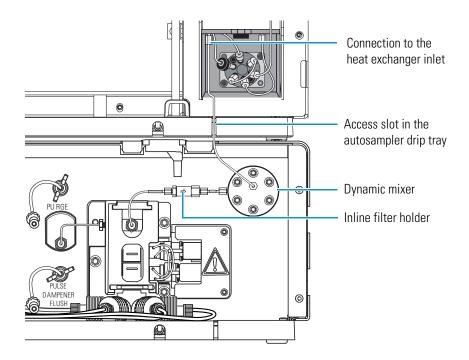


Figure 37. Connection between the Accela 600 Pump or the Accela 1250 Pump and the autosampler

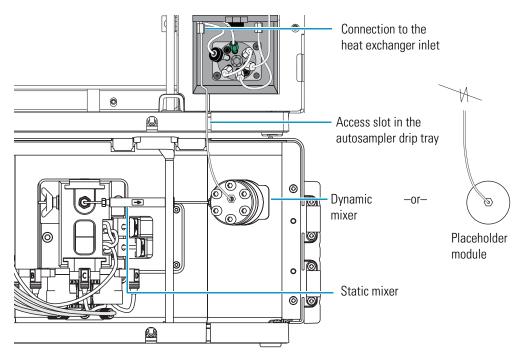
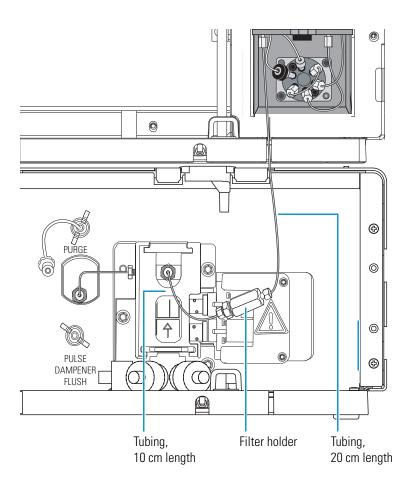


Figure 38. Connection between the autosampler and an earlier version of the Accela Pump without a dynamic mixer



Powering On the Autosampler

After installing solvent lines and connecting the autosampler to line power, you can power on the autosampler.

To turn on the power to the autosampler

- 1. Ensure that the power switch at the front of the unit is in the Off position (released or out position).
- 2. Attach the power cord to the power entry module on the back panel of the autosampler and connect it to the power source.
- 3. Turn the power on by pushing the power button in to engage it.
- 4. Make sure that the power indicator briefly lights amber and then turns solid green. If it does not light at all, see Chapter 6, "Routine Troubleshooting." If it stays amber or flashes amber or green, turn the power off for several seconds, and then turn it back on again. Call your Thermo Fisher Scientific service representative if you require assistance.

These events occur during the autosampler startup sequence:

- 1. The Power LED turns solid green.
- 2. If the autosampler is communicating with the data system computer, the Com LED turns from amber to green.
- 3. The Run LED and Temperature LED turn solid green.
- 4. The autosampler initializes the syringe, injection valve, and XYZ arm positions as follows:
 - a. Switches the two-way syringe valve to the flush bottle position.
 - b. Switches the injection valve to the inject position.
 - c. Moves the syringe to the home (empty) position.
 - d. Moves the syringe to the ready position.
 - e. Switches the two-way syringe valve to the needle position.
- 5. If the autosampler is communicating with the data system computer, the Com LED turns from amber to solid green.
- 6. Every time you close and reopen the data system, the autosampler initializes its hardware components (see step 4).

Calibrating the Accela Autosampler

The Accela Autosampler is factory calibrated. The data system provides calibration options for the following:

- Column oven temperature
- Tray compartment temperature
- XYZ arm position
- Depth of custom vials or microplate wells

Only a Thermo Fisher Scientific service representative can perform the first three calibration options.

Before you use any of the three custom tray options, perform the calibration that determines the z-axis distance that the needle must travel to reach the bottom of your custom vial or well plate.

To access the well bottom distance calibration option from the Xcalibur data system



1. From the Roadmap view, click the **Instrument Setup** icon.

The Instrument Setup window appears.

2. Click the **Accela AS** icon in the viewbar.

The Instrument Setup view for the Accela Autosampler appears.

3. From the Accela AS menu, choose **Calibration**.

The Calibration dialog box appears.

4. Select the **Well Bottom Distance** option.

To access the well bottom distance calibration option from the ChromQuest data system

- 1. From the Instrument window, choose **Control** > **Instrument Status**.
- 2. Click the **Accela AS** tab.
- 3. Click **Diagnostics**.
- 4. Click the **Calibration** tab.
- 5. Select the **Bottom Distance Calibration** option.

For instructions on how to perform this calibration, refer to the data system Help.





Changing the Sample Loop

Several sample loop sizes are available for the Accela Autosampler (see Table 6).

Table 6. Sample loops

Description	Part number
5 μL	00109-99-00023
10 μL	00109-99-00024
20 μL	00109-99-00025
25 μL	00109-99-00026
50 μL	00109-99-00027
100 μL	00109-99-00028
500 μL	00109-99-00029
1000 μL	00109-99-00030

To change the sample loop

- 1. Make sure that the autosampler is in Idle mode.
- 2. Using an open-end wrench, loosen the fittings at ports 1 and 4, and gently pull out the two ends of the sample loop. If you want to reuse the sample loop, label the end fittings with the port number (1 or 4) that the swaged fitting was connected to.
- 3. Insert the ends of the new sample loop in the same orientation as the old one.
- 4. Firmly press one end of the sample loop into port 1 of the injection valve as you hand-tighten the nut.
- 5. Firmly press the other end of the sample loop into port 4 of the injection valve as you hand-tighten the nut.
- 6. Using a 1/4 in. open-end wrench, gently tighten the fittings by an additional 3/4 turn.

Tip To connect tubing with a two-piece stainless steel fitting to a receiving port, press the tubing against the bottom of the port as you hand-tighten the fitting. Then tighten the fitting by an additional 3/4 turn with a wrench. Once you use the wrench to tighten the fitting, the ferrule portion of the two-piece fitting is permanently connected to the tubing.

To connect tubing with a pre-swaged fitting to a receiving port, press the tubing against the bottom of the port as you hand-tighten the fitting. Then tighten the fitting by an additional 1/4 to 1/2 turn with a wrench.

Sample Loading

Before you load your samples into the autosampler, make sure that your samples are completely soluble in the mobile phase and that you have filtered your samples and solvents through a $0.5~\mu m$ filter. These techniques minimize sample precipitation in the lines and remove particulate matter that could obstruct the flow through the autosampler injector or the column. In addition, make sure that the vial caps are securely fastened to the vials.

After you finish preparing your samples, you are ready to load them into the Accela Autosampler. The Accela AS Accessory Kit (see "Accela AS Accessory Kit" on page 128) contains trays that hold standard vials, inserts that hold 100 μ L vials, and carrier plates that hold 96- and 384-microwell plates.

Contents

- Standard Tray Type
- Carrier Trays for Microplates
- Important Precautions for Sample Loading
- Recommended Vials, Microplates, and Microplate Covers

Standard Tray Type

The Accela Autosampler Accessory Kit contains five standard trays. Trays A, B, C, D, and E are arranged in the autosampler from left to right. Each tray holds 40 standard, 1.8 mL vials arranged in two rows of 20. The accessory kit also contains a supply of standard, 1.8 mL vials, silicone and Teflon septa, and screw-top vial caps. You can order additional vials from Thermo Fisher Scientific.

The part numbers of the replaceable components in the accessory kit are listed in "Accela AS Accessory Kit" on page 128.

IMPORTANT If you are using screw cap vials, the caps must be firmly tightened. If the caps are not firmly tightened, the needle might push the septum into the bottom of the vial.

To install the standard tray type, follow these procedures:

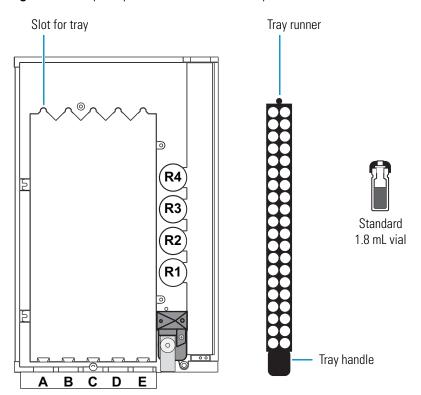
- Installing the Standard Tray Type
- Moving the XYZ Arm to the Back of the Tray Compartment

Installing the Standard Tray Type

❖ To install the sample trays into the tray compartment of the autosampler

- 1. Open the left door of the autosampler.
 - If you selected the Verify Door Is Closed check box when you configured the autosampler, the XYZ arm moves to the back of the tray compartment.
 - If the XYZ arm does not move to the back of the tray compartment, perform the appropriate direct command from the data system.
- 2. Hold the tray handle, tilting the back end of the tray down.
- 3. Insert the tray runner into the slot at the back of the tray compartment (see Figure 39).
- 4. Lower the front of the tray into place and press down firmly to seat the tray.

Figure 39. Tray compartment and standard tray



IMPORTANT Be sure that each tray is properly seated. If a tray is not completely seated, the autosampler will not operate correctly.

IMPORTANT To trigger the vial sensor, you must position custom vials in the tray so that the top of the vial reaches the minimum height of 1.55 in. Custom vials that fall below this minimum height do not trigger the vial sensor. When an acquisition sequence incurs a vial that is below the minimum height, the sequence halts and the following message appears: Vial Not Found.

Note When you open the autosampler door, the XYZ arm automatically moves to the back of the autosampler, allowing you to add samples to the tray during a run. After the door is closed, the arm returns to its position and resumes the operation that was in progress before the door was opened. You can enable this door interlock feature in the Configuration menu for the autosampler.

The accessory kit also contains a tray adapter for $100~\mu L$ vials and a tray adapter plate for microcentrifuge tubes.

❖ To use the tray adapter

Insert the tray adapter into the standard tray, and then secure it with the 1/4 in. Phillips head screw from the accessory kit.

Moving the XYZ Arm to the Back of the Tray Compartment

If you did not select the Verify Door Is Closed check box when you configured the autosampler, the XYZ arm does not move to the back of the tray compartment when you open the tray compartment door. From the data system, use a direct command to move the XYZ arm.

❖ To move the XYZ arm to the back of the tray compartment

- For the Xcalibur data system, open the Direct Control dialog box for the autosampler (see "Using the Xcalibur Direct Control Commands" on page 141). Select Position Arm to Access Tray, and then click Apply.
- For the ChromQuest data system, open the Direct Controls page for the autosampler (see "Using the ChromQuest Direct Commands" on page 143). Select **Position Arm to Access Tray**, and then click **Submit**.

Carrier Trays for Microplates

In addition to the standard tray option, the accessory kit contains a short microwell plate carrier and a tall, solid microwell plate carrier. These carrier trays hold up to three microplates. To use PCR plates, you can order a cooling adapter from Thermo Fisher Scientific.

Use the carrier trays as follows:

- For either 1 mL or 2 mL deep well plates, use the short microwell carrier.
- For PCR plates, use the short microwell carrier in combination with the cooling adapter.
- For standard depth 96- or 384-well plates, use the tall, solid microwell carrier.

Note If your samples require low temperature cooling, use the cooling adapter and the PCR plates instead of the standard 96-well microplates.

Figure 40 and Figure 41 show the short microwell plate carrier and the tall, solid microwell plate carrier, respectively.

Figure 40. Short microwell plate carrier

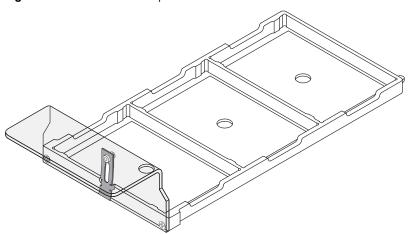
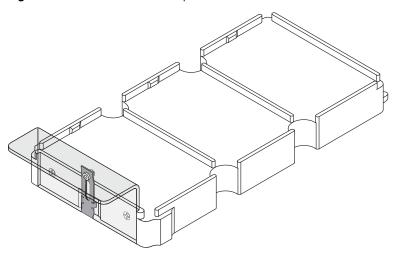


Figure 41. Tall, solid microwell plate carrier

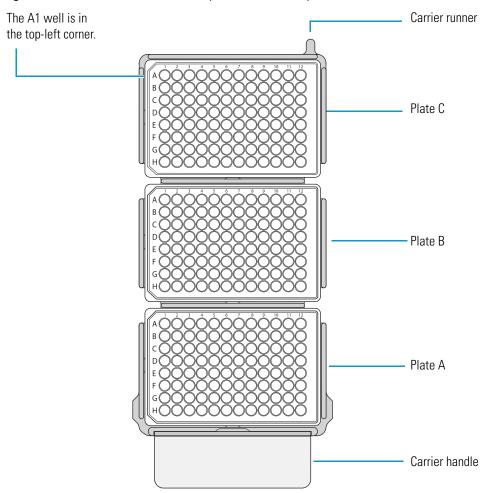


❖ To install the microplates onto the carrier tray

- If this is an option in your data system, determine the microplate orientation.
 With the Xcalibur and ChromQuest data systems, you can select the A1 top-left or A1 bottom-right orientation.
- 2. Hold the plates in the appropriate orientation as you snap them into the carrier slots.

Figure 42 shows a carrier with three 96-well plates in the A1 top-left orientation.

Figure 42. Carrier with three 96-well plates in the A1 top-left orientation



❖ To load the carrier tray into the autosampler tray compartment

1. Holding the carrier handle, insert the carrier runner into the slot in the back of the tray compartment tub.

Tip Use two hands to hold the tall, solid microwell carrier, as it weighs approximately 2.6 kg (5.8 lbs).

2. Lower the front of the carrier tray into place.

Important Precautions for Sample Loading

Because the needle mechanism moves to the vial or well during an injection sequence, punctures the vial septum or microwell plate cover, and then draws sample from a specified depth, follow these precautions when you load samples into the autosampler tray compartment:

- Check the height of custom trays before you place them in the tray compartment.
 - Objects taller than 4.5 cm (1.8 in.) can stall the autosampler arm. You must check the height of custom trays before you place them in the tray compartment.
- Use only recommended vials, vial caps, microplates, and microplate covers.
 - Thermo Fisher Scientific has tested the vials, vial caps, microplates, and microplate covers listed in the next section, "Recommended Vials, Microplates, and Microplate Covers," for use with the Accela Autosampler. Because the tip of the needle is blunt, the durometer (material hardness) of the vial cap septum is extremely important, as the needle might not properly pierce septa that are not recommended.
- Ensure that you screw on the vial caps tightly.
 - If the vial cap is loose, the needle can dislodge the septum during an injection. The needle can also dislodge the septum if it pierces the edge of the septum, rather than its center. If you suspect the needle is out of alignment, call your local Thermo Fisher Scientific service engineer to calibrate its alignment.
- Calibrate the depth of custom vials or microwell plates.
 - If you are using one of the three custom tray configurations, you must perform a calibration that determines the distance between the tip of the needle and the bottom of your vials or wells. Performing this calibration ensures that the needle tip descends to the height specified in your instrument method as it withdraws sample from the vial or well. Perform this calibration whenever you change the custom tray type configuration or use a different type of custom vial or microplate.

For instructions on how to perform bottom distance calibration, refer to the data system Help for the autosampler or the Accela user guide or getting started guide for your data system.

Recommended Vials, Microplates, and Microplate Covers

Thermo Fisher Scientific has tested and recommends the following vials, microplates, and microplate covers for use in your Accela Autosampler.

Recommended Vials

Table 7 lists the recommended sample vials.

Table 7. Approved vials

Description	Qty/pkg	Part number
1.8 mL vial with septa and screw cap	100	A4954-010
1.8 mL amber vial with septa and screw cap	100	A4965-010
Shell vial with cap	100	A4948-010
Shell vial with 100 μL insert and cap	100	60053-62008
1.8 mL vial with cross-slit septa and screw cap	100	A5400-010

Recommended Microplates

The microplate carriers hold three low-density 96-well or three high-density 384-well microplates.

Low-Density Microplates

All 96-well microplates that meet the dimensions of the Society for Biomolecular Screening and that have a maximum height of 0.80 cm (0.32 in.) are suitable. Polypropylene is the preferred material when working with organic samples.

Table 8 lists the 96-well microplates that Thermo Fisher Scientific approves for use with the Accela Autosampler. The supplier is SUN-SRi, a subsidiary of Thermo Fisher Scientific Inc.

Table 8. Recommended 96-well microplates

Description	Qty/pkg	Part number
96-well, U-bottom, Polypropylene, working well volume 250 μ L	10	500 833
	100	500 811
96-well, V-bottom, Polypropylene, working well volume 190 μL	10	500 835
	100	500 813

High-Density Microplates

All 384-well microplates that meet the dimensions of the Society for Biomolecular Screening and that have a maximum height of 0.32 in. are suitable. Polypropylene is the preferred material when working with organic samples.

Table 9 lists the 384-well microplates that Thermo Fisher Scientific approves for use with the Accela Autosampler.

Table 9. Recommended 384-well microplates

Description	Qty/pkg	Part number
384-well, U-bottom, Polypropylene,	10	500 841
working well volume 90 μL	100	500 819

Recommended Microplate Covers

Table 10 lists the covers for 96-well microplates that Thermo Fisher Scientific approves for use with the Accela Autosampler.

Table 10. Recommended 96-well microplate covers

Description	Qty/pkg	Source	Supplier catalog number
Micromat II/PS Round Pink Material: Teflon-coated silicone rubber	10/pkg	Thermo Fisher Scientific	400 079
Micromat CLR 96-well PS 7 mm Material: clear silicone rubber	10/pkg	Thermo Fisher Scientific	300 005
Clear heat-seal film	100/pkg	Thermo Fisher Scientific Matrix Technologies Inc.™	AB-0685
VIEWseal™ dry film adhesive-backed tape	100/pkg	Greiner Bio-One	676070

Table 11 lists the 384-well microplate covers that Thermo Fisher Scientific approves for use with the Accela Autosampler.

Table 11. Recommended 384-well microplate covers

Description	Qty/pkg	Source	Supplier catalog number
Clear heat-seal film	100/pkg	Thermo Fisher Scientific Matrix Technologies	AB-0685
VIEWseal™ dry film adhesive backed tape	100/pkg	Greiner Bio-One	676070

For more information about the microplates, heat-seal covers, and sealing equipment supplied by Thermo Fisher Scientific, click the following icon:



CAUTION Avoid using the following covers with your Accela Autosampler:

- Parafilm "M", manufactured by American National Can, or similar material
- POWERseal[™], manufactured by Greiner Bio One, or similar material
- Adhesive sealing tapes

Parafilm "M" and POWERseal covers leave small pieces of material on the needle that eventually clog the injection port. Adhesive sealing tapes leave an adhesive film on the needle causing system contamination. The adhesive builds up on the needle and in the injection port, and eventually the needle becomes completely clogged.



Routine Maintenance

This chapter contains information on the maintenance record keeping options provided by your Thermo Scientific data system, the routine maintenance procedures necessary to optimize the performance of the autosampler, and the suggested maintenance schedule.

Contents

- Maintenance Schedule
- Replacing the Rotor in the Valco Injection Valve
- Replacing the Inlet Filter for the Wash Bottle Solvent Line
- Replacing the Transfer Tubing
- Maintaining the Syringe Drive Assembly
- Troubleshooting a Blockage in the Injection System
- Replacing the Needle Tubing Assembly
- Replacing the Needle Assembly
- Changing the Syringe or Replacing the Inner Plunger
- Clearing a Plugged Heat Exchanger
- Replacing a Fuse
- Maintenance Cycles

For your own safety and to protect the autosampler from damage, follow the safety precautions in this manual when performing maintenance on the autosampler.



CAUTION To avoid electrical shock, do not remove the top cover or remove parts beyond the descriptions in the operation portions or in the maintenance topics of this manual.

Maintenance Schedule

The Accela Autosampler requires only a few simple maintenance procedures to keep it in optimum working condition. Table 12 contains the maintenance schedule for the autosampler. A Thermo Fisher Scientific service representative or other qualified person must perform the last procedure, the alignment of the XYZ arm.

To make the maintenance of your autosampler easier, the data system keeps track of how many injections, injection valve cycles, needle cycles, and syringe cycles the autosampler has performed. The data system alerts you to perform maintenance when the number of cycles exceeds a preset value. For information on setting up the tracking system, see "Maintenance Cycles" on page 98.

Note You are responsible for maintaining your autosampler. Routine maintenance does not fall under Thermo Fisher Scientific warranty; however, planned maintenance contracts are generally available. Contact your local representative if you are interested in purchasing a planned maintenance contract.

Table 12. Maintenance procedures and schedule

Frequency	Procedure	Performed by
Monthly	Checking the solvent tubing connections for leaks	User
Monthly	Running a standard sample	User
Every 15 000 to 20 000 injections	Replacing the rotor seal (Rheodyne™ injection valve)	User
	Replacing the rotor (Valco $^{\text{\tiny TM}}$ injection valve)	
Semiannually	Replacing the flush solvent inlet filter	User
As necessary	Replacing the injection port	User
As necessary	Clearing a plugged line or needle	User
As necessary	Changing the syringe	User
As necessary	Changing the sample loop	User
As necessary	Replacing a fuse	User
Semiannually	Cleaning and lubricating the syringe drive mechanism	User
Annually	Cleaning and lubricating the XYZ arm	Field service
Annually	Verifying the XYZ arm alignment	Field service

Note Table 12 lists recommended maintenance intervals based on operating the autosampler under typical conditions. Operating the autosampler under certain harsh conditions (corrosive solvents, extreme temperatures, and so on) might require more frequent maintenance than indicated in the table.

Replacing the Rotor in the Valco Injection Valve

The rotor forms a high-pressure seal with the stator. Replace the rotor when you notice a leak between either the stator and rotor or the adjacent ports of the injection valve, or when you notice a decrease in injection precision.

This section describes how to replace the rotor for the autosampler's Valco injection valve. For information on replacing the rotor seal for the Rheodyne injection valve that shipped with earlier versions of the autosampler, see "Replacing the Rotor Seal in the Rheodyne Valve" on page 137.



CAUTION Before you remove the injection valve from the autosampler, turn off the power to the autosampler and unplug the autosampler from line power.



Replacing the rotor requires these tools.

Tool	Use
1/4 in. open-end wrench	To remove the tubing from injection valve ports 5 and 6
#2 Phillips screwdriver	To disconnect the injection valve from the autosampler
9/64 in. hex wrench	To disassemble the injection valve

IMPORTANT Because component cleanliness affects the useful life of the injection valve, replace the rotor in a clean environment.

To replace the rotor, follow these steps:

- 1. Removing the Injection Valve from the Autosampler
- 2. Disassembling the Injection Valve
- 3. Cleaning the Stator
- 4. Installing a New Rotor
- 5. Reinstalling the Injection Valve

Removing the Injection Valve from the Autosampler

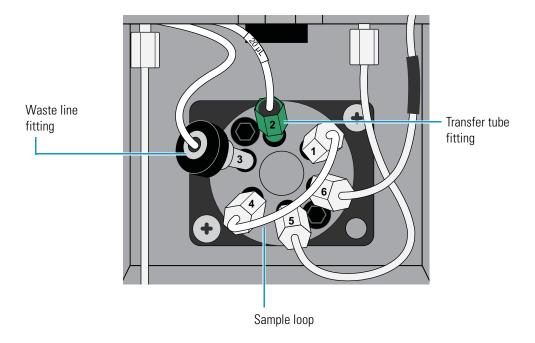
Before you remove the injection valve from the autosampler, prepare a clean environment where you can disassemble the valve.

❖ To remove the injection valve from the autosampler

- 1. Disconnect the solvent lines (see Figure 43) connected to the valve as follows:
 - Disconnect the waste tubing from port 3 of the injection valve.
 - Disconnect the transfer tubing from port 2 of the injection valve.
 - Using a 1/4 in. open-end wrench, disconnect the 1/16 in. OD high-pressure tubing from ports 5 and 6.

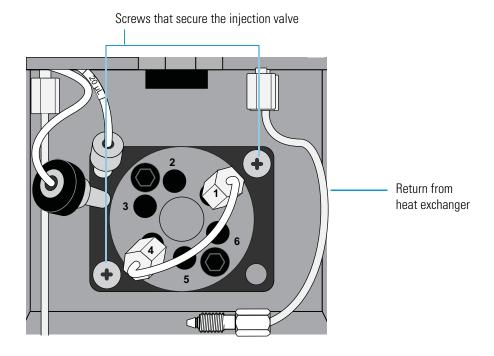
IMPORTANT You do not need to remove the sample loop from the injection valve to disassemble the injection valve. Once the sample loop has been swaged onto the injection valve, the fittings are no longer interchangeable. If you do remove the sample loop from the injection valve, take note of which fitting was connected to port 1 and which fitting was connected to port 4. When you reinstall the sample loop, connect the fittings to their respective ports.

Figure 43. Injection valve solvent line connections



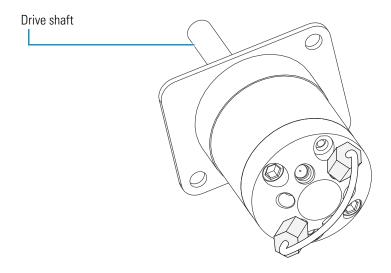
2. Using a #2 Phillips screwdriver, remove the two screws that secure the injection valve to the autosampler (see Figure 44).

Figure 44. Injection valve secured to the autosampler with two screws



- 3. Slide the injection valve forward out of the autosampler.
 - Figure 45 shows the drive shaft on the back side of the injection valve.

Figure 45. Injection valve with a view of the drive shaft on the back side of the valve



Disassembling the Injection Valve

Leave the sample loop connected to the stator as you disassemble the injection valve.

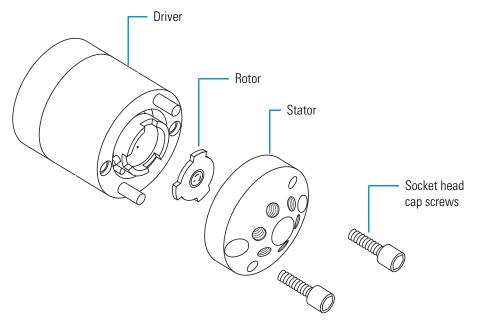
❖ To disassemble the injection valve

1. Using a 9/64 in. L-hex wrench, remove the two socket head cap screws that secure the stator to the driver (see Figure 46).



CAUTION The polished (sealing) surface of the stator contains six ports that excess handling can easily damage. As you remove the stator from the injection valve, avoid touching this polished surface, and never place the polished surface face down on a hard surface.

Figure 46. Injection valve (exploded view)



- 2. Gently pry the rotor away from the driver (see Figure 46).
- 3. Examine the sealing surface of the rotor for scratches. If scratches are visible, replace the rotor.

Cleaning the Stator

Before you replace the rotor, clean the stator.

To clean the stator

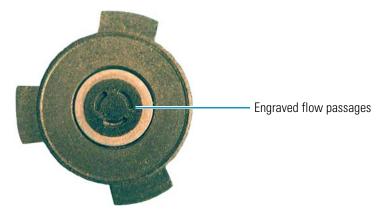
- 1. Inspect the stator to determine if it requires cleaning.
- 2. If the stator is dirty, swab it with HPLC-grade methanol. If more stringent cleaning is required, use a sonicator.
- 3. Inspect the remaining valve components for contamination. Clean them as necessary.

IMPORTANT If the stator is scratched, replace the valve. Scratches can damage the rotor and cause cross-port leaks.

Installing a New Rotor

The rotor has two sides. The side containing the engraved flow passages (see Figure 47) faces the stator.

Figure 47. Engraved flow passages in the Valco rotor



To install a new rotor

1. Replace the rotor in the driver, making sure that the rotor sealing surface with its engraved flow passages is facing out.

The pattern is asymmetrical to prevent improper placement.

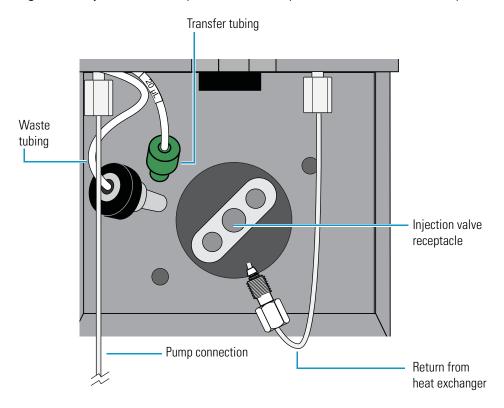
- 2. Replace the stator.
- 3. Insert the two socket head screws.
 - a. Using a 9/64 in. L-Hex wrench, tighten each screw until you feel resistance (approximately fingertight).
 - b. Tighten each screw by 1/8 turn, and then tighten each screw again, until the stator is secured to the driver. Take care not to overtighten the screws.

Reinstalling the Injection Valve

❖ To reinstall the injection valve

1. Align the drive shaft (see Figure 45) and the two prongs of the coupling unit with the three holes in the injection valve receptacle located in the lower portion of the column oven compartment. Then insert the injection valve into the receptacle (see Figure 48).

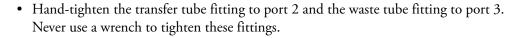
Figure 48. Injection valve receptacle in the lower portion of the column oven compartment



- 2. Align the holes in the mounting plate of the injection valve with the small holes next to the injection valve receptacle.
- 3. Using the two screws that you removed in step 1 on page 64, secure the injection valve to the autosampler with a #2 Phillips screwdriver.

4. Reconnect the solvent lines to the injection valve as follows (see Figure 48):







CAUTION Never use a wrench to tighten the transfer tube fitting. Over-tightening the fitting can do the following:

- Restrict the end of the tubing, which leads to less sample reaching the sample loop and results in smaller than expected peak areas or blank injections. A restriction in the end of the tubing can also cause the syringe to make a grinding sound during an injection sequence.
- Strip the threads on the fitting, which leads to solvent leaks.
- Hand-tighten the waste tube fitting to port 3.
- Press the end of the return tubing from the heat exchanger to the bottom of port 5 as you hand-tighten the high-pressure fitting. Then use a wrench to tighten the fitting by an additional 1/4 turn.
- Using high-pressure tubing and two high-pressure fittings, reconnect the LC column to port 6 of the injection valve.

Note To reconnect tubing with a pre-swaged stainless steel fitting to a valve port, hand-tighten the fitting as you press the tubing against the bottom of the port, and then use a wrench to tighten the fitting by an additional 1/4 to 1/2 turn.

To connect tubing with a two-piece stainless steel fitting to a mating port, press the tubing against the bottom of the port as you hand-tighten the fitting. Then tighten the fitting by an additional 3/4 turn with a wrench. Once you use the wrench to tighten the fitting, the ferrule portion of the two-piece fitting is permanently connected to the tubing.

• If you removed the sample loop, connect the appropriate ends of the sample loop to ports 1 and 4.

2 Transfer tube low-pressure fitting (hand-tighten only) 1 Sample loop fitting (tighten with 1/4 in. wrench) 3 Waste-tube low-pressure fitting (hand-tighten only) LC column connection 6 high-pressure fitting (tighten with 1/4 in. wrench) 4 Sample loop fitting Return from heat exchanger 5 (tighten with 1/4 in. connection wrench) high-pressure fitting

Figure 49. Schematic of the tubing connections to the injection valve

5. Turn on the pump flow and check for leaks.

Replacing the Inlet Filter for the Wash Bottle Solvent Line

Properly filtering the flush solvent extends the life of the injection valve and the LC column.

To replace the inlet filter

- 1. Be sure the autosampler is in Idle mode.
- 2. Remove the flush-solvent bottle cap and lift out the inlet tubing.
- 3. Remove the old inlet filter.



CAUTION Be sure to follow all of the safety precautions for each solvent, as prescribed by the MSDS.

- 4. Install a new filter, and then put the line back into the bottle. Tighten the solvent cap.
- 5. Flush the inlet filter and lines with an appropriate volume of solvent.

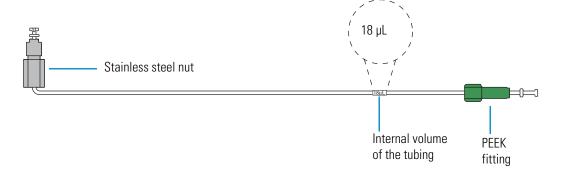
Replacing the Transfer Tubing

The transfer tubing that connects the injection port of the autosampler to port 2 of the injection valve can become worn, damaged, or plugged with use.

Replace the transfer tubing if it becomes plugged or if your injections become less reproducible (which can indicate of a worn or damaged transfer tube). In addition, replace the transfer tubing when you replace the needle.

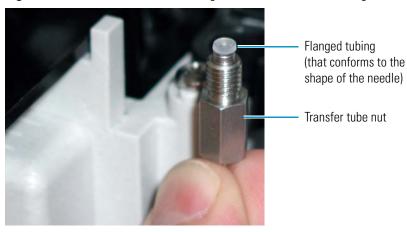
The transfer tubing assembly consists of two fittings and a 9 in. length of 0.012 in. ID tubing (see Figure 50). The stainless steel nut connects to the autosampler injection port and the green PEEK fitting connects to port 2 of the injection valve. The label near the PEEK fitting end of the tubing specifies the internal volume of the tubing.

Figure 50. Transfer tubing assembly



Tip Because the flanged end of the transfer tubing (see Figure 51) conforms to the shape of the needle, it is good practice to replace the transfer tubing when you replace the needle. The reverse is not true. The tip of the needle does not conform to the shape of the transfer tube fitting, so you do not need to replace the needle when you replace the transfer tubing.

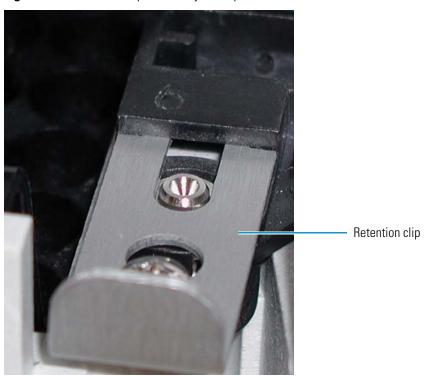
Figure 51. Transfer tube nut and flanged end of the transfer tubing



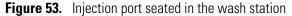
To replace the transfer tubing

1. To remove the aluminum retention clip (see Figure 52) from the wash station housing, pull it forward and then upward.

Figure 52. Retention clip for the injection port



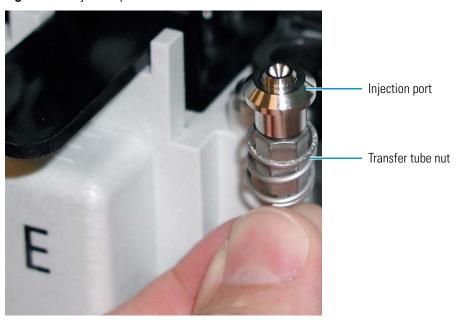
2. Pull the injection port out from the wash station (see Figure 53).





3. Unscrew the transfer tube nut from the injection port fitting (see Figure 54) and from port 2 of the injection valve, and then pull the tubing to the left through the hole in the wall between the tray compartment and the oven compartment (see Figure 10 on page 11). Discard the used transfer tube.

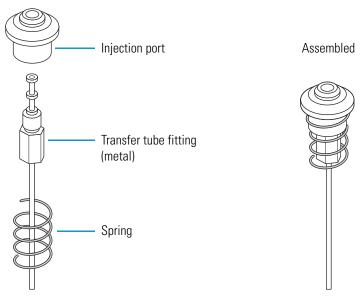
Figure 54. Injection port connected to the transfer tube nut



- 4. Screw the injection port onto the nut of the new transfer tubing.
- 5. Set the injection port into the spring (see Figure 55). Then insert it into the port in front of the wash station.

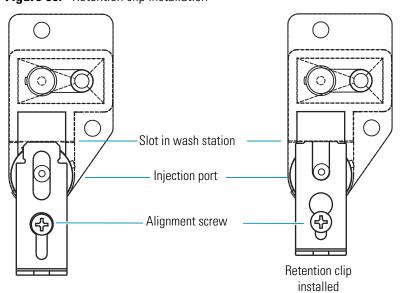
Replacing the Transfer Tubing

Figure 55. Transfer tubing, spring, and injection port



- 6. Reinstall the retention clip (see Figure 56) as follows:
 - Align the retention clip with the slot located in front of the wash station.
 - Using the retention clip to push the injection port down, align the circular cutout in the retention clip over the alignment screw.
 - Insert the end of the retention clip into the wash station slot as far as it will go.

Figure 56. Retention clip installation



7. Route the other end of the transfer tube through the hole in the wall between the tray and column oven compartments (see Figure 10 on page 11).



8. Taking care to avoid overtightening, hand-tighten the fitting to port 2 of the injection valve. Never use a wrench to tighten this fitting.



CAUTION Never use a wrench to tighten the transfer tube fitting. Overtightening constricts the transfer tube, causing poor injection precision or blank injections. Eventually, the damaged transfer tube becomes completely plugged.

IMPORTANT You must modify the instrument configuration by entering the new value for the internal volume of the transfer tubing. For instructions on updating the instrument configuration for the autosampler, refer to the data system Help.

Modify the instrument configuration for the internal volume of the new transfer tube.
 For information on specifying the volume of the transfer tubing, refer to the data system Help.

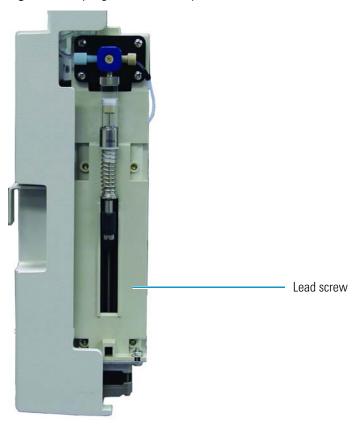
Maintaining the Syringe Drive Assembly

If you have an older version of the Accela Autosampler with an uncoated, stainless steel lead screw, as a part of routine maintenance, lubricate the lead screw once every six months or as needed. If the syringe drive skips steps and does not return to the home position after you perform a Flush Syringe direct command, lubricate the lead screw before you continue operation.

Note The syringe drive assembly in the current version of the Accela Autosampler does not require routine maintenance. The lead screw is coated with black Teflon and the drive nut is made of plastic, so there is minimal friction between these moving parts.

Figure 57 shows the uncoated, stainless steel lead screw in an older version of the Accela Autosampler.

Figure 57. Syringe drive assembly with an uncoated, stainless steel lead screw





Lubricating the lead screw requires these tools and materials:

Tools and materials

Flat-head screwdriver

Tri-Flow[™] lubricant (For ordering information, see Lubricant in the list of "Consumables" on page 126.)

❖ To clean and lubricate the lead screw of the syringe drive assembly

- 1. Remove the syringe drive assembly from the autosampler as follows:
 - a. Remove the wash bottle from the solvent platform and place it on the countertop next to the Accela LC stack.
 - b. Disconnect the needle tubing from the right side of the syringe valve.
 - c. Disconnect the wash tubing from the left side of the syringe valve.

- d. Remove the two thumbscrews that connect the syringe drive assembly to the inner side of the tray compartment door (see Figure 58).
- e. To disconnect the cable that is routed through the back of the syringe drive assembly and connected to the P2 receptacle on the inner side of the tray compartment door, press the connector tab (see Figure 59) and pull the connector out of the receptacle.

Figure 58. Syringe drive assembly disconnected from the inner side of the door

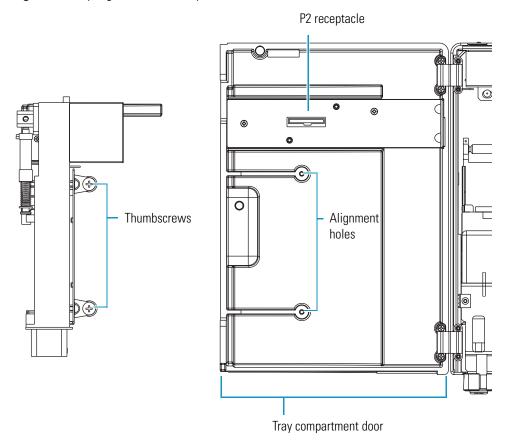
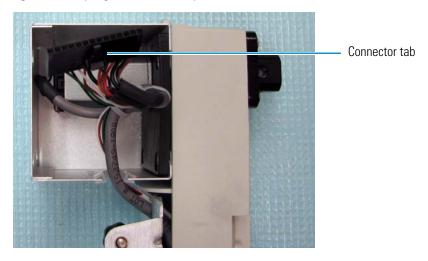


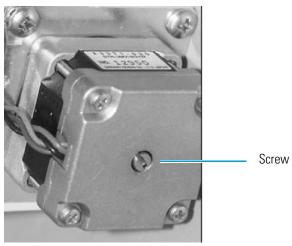
Figure 59. Syringe drive assembly cable



Note You can lubricate the lead screw without removing the base plate from the back of the syringe drive assembly.

- 2. Lubricate the lead screw as follows:
 - a. Through the cutout in the front of the syringe drive assembly, apply 2 to 3 drops of Tri-Flow lubricant to the top part of the lead screw.
 - b. Using a flat head screwdriver, turn the screw at the bottom of the motor (see Figure 60) counterclockwise until the arm is at the top position.

Figure 60. Screw at the bottom of the syringe drive motor



- c. Apply lubricant to the bottom part of the lead screw.
- 3. Reconnect the syringe drive assembly to the autosampler as follows:
 - a. Plug the syringe drive assembly cable into the P2 receptacle on the inner side of the tray compartment door.
 - b. Realign the syringe drive assembly with the alignment holes on the inner side of the tray compartment door (see Figure 58 on page 77).
 - c. Using the two thumbscrews that you removed in step 1d on page 77, secure the syringe drive assembly to the door.
- 4. Set the syringe to its home position (see "Setting the Syringe to the Home Position" on page 95).
- 5. To ensure that the lead screw has been properly lubricated, set up the autosampler as follows and watch the movement of the syringe as the autosampler flushes the syringe:
 - a. Fill the wash bottle with HPLC-grade water.

b. Perform a Flush Syringe direct command using these values:

Parameter	Value
Reservoir	Flush Bottle (Xcalibur data system or Tune window) Bottle (ChromQuest data system)
Volume (μL)	6000
Flush Speed (µL/sec)	100

If the lead screw has been properly lubricated, it does not stall as the autosampler flushes water through the system at a speed of $100 \,\mu\text{L/sec}$.

6. Refill the wash bottle with an appropriate solvent mixture for your applications.

Troubleshooting a Blockage in the Injection System

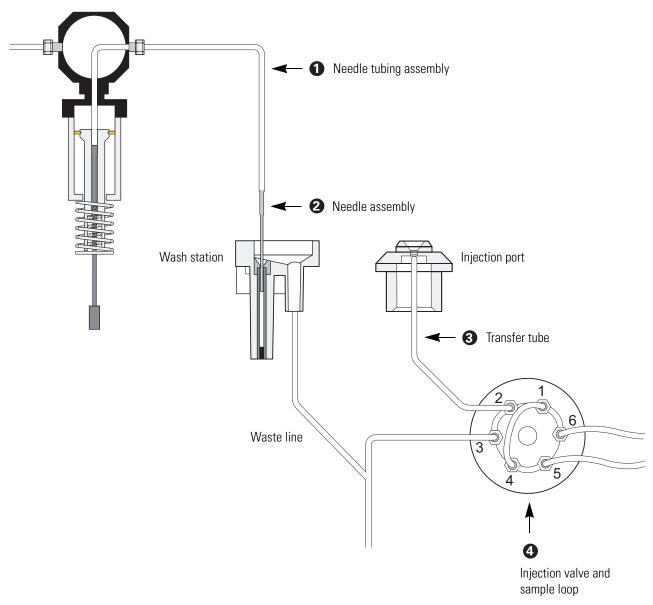
A blocked tubing or a plugged needle can cause the syringe drive to stall. A stalled syringe drive makes a grinding sound because the syringe motor must overcome a greater force to move the plunger.

If the injection system has a plug (as indicated by an abnormally loud grinding sound made by the syringe drive), determine where the plug is located by systematically checking the following sections of the injection system (see Figure 61):

- Section 1: Needle tubing assembly
- Section 2: Needle assembly
- Section 3: Transfer tubing
- Section 4: Injection valve and sample loop

Tip The transfer tube, needle, and small ports in the stator of the injection valve are the most commonly plugged components of the injection system. A damaged syringe valve can also reduce the solvent flow and cause a grinding sound.

Figure 61. Possible locations of a plug



To find and clear a plug, follow these steps until you find the blockage:

- 1. Testing the Needle Tubing and the Syringe Valve for Blockage
- 2. Testing the Transfer Tube for Blockage
- 3. Testing the Transfer Tube for Blockage
- 4. Cleaning the Injection Valve or the Sample Loop

Testing the Needle Tubing and the Syringe Valve for Blockage

To test the needle tubing for blockage

- 1. Move the XYZ arm to the front-center of the tray compartment (see "Setting the Needle to the Removal Position" on page 85).
- 2. Unscrew the needle tubing assembly from the needle assembly (see Figure 4 on page 6) and place the needle tubing fitting into a beaker.
- 3. As you perform a **Wash Needle** direct command with a 2000 μ L volume, observe the solvent flow and listen for a grinding sound from the syringe drive.
- 4. Depending on whether solvent flows freely out the end of the needle tubing and you hear a grinding sound from the syringe drive, do one of the following:
 - If the wash solvent flows out of the needle tubing at a normal rate and you do not hear a grinding sound, the needle tubing is not the source of blockage. Skip step 5 and step 6 and go to the next procedure to test the needle for blockage.

-or-

- If the flow appears to be constricted, go to step 5.
- 5. Verify that the syringe valve is not the cause of the constricted flow:
 - a. Unscrew the needle tubing from the right side of the syringe valve.
 - b. Perform a **Wash Needle** direct command using a 2000 μ L volume, and observe the solvent flow out of the right side of the syringe valve.
 - c. Depending on whether solvent flows freely out of the right side of the syringe valve, do one of the following:
 - If solvent flows out of the right side of the syringe valve, replace the needle tubing, which was the source of the blockage.
 - If solvent does not flow out of the right side of the syringe valve, replace the syringe valve.
- 6. After you replace the syringe valve or the needle tubing, home the position of the syringe (see "Setting the Syringe to the Home Position" on page 95).

If the needle tubing was not the blockage source, go to the next procedure, "Testing the Needle for Blockage."

Testing the Needle for Blockage

To test the needle for blockage

- 1. Remove the needle from the needle mount on the XYZ arm by pulling the latch nut of the needle assembly forward, and then pulling the needle up out of the needle mount.
- 2. Reconnect the needle tubing to the needle.
- 3. Holding the needle over the beaker, perform a **Wash Needle** direct command using a $2000~\mu L$ volume. Watch how freely solvent flows from the end of the needle, and listen for a grinding sound from the syringe drive.
- 4. Depending on whether solvent flows freely out of the end of the needle and you hear a grinding sound from the syringe drive, do one of the following:
 - If the solvent flow is constricted, the needle is constricted.

-or-

- If solvent flows out of the end of the needle at a normal rate and you do not hear a
 grinding sound from the syringe drive, the needle is not the source of blockage.
 Go to step 6.
- 5. To remove the needle blockage, run a clean wire through the needle or replace the needle as described in "Replacing the Needle Tubing Assembly" on page 85. After you replace the needle assembly, set the syringe to its home position.
- 6. If the needle is not the source of blockage, reconnect the injection system as follows:
 - a. Disconnect the needle from the needle tubing.
 - b. Move the XYZ arm to the front-center of the tray compartment (see "Setting the Needle to the Removal Position" on page 85).
 - c. Insert the needle into the needle mount on the XYZ arm, and then push the latch nut backward.
 - d. Reconnect the needle tubing to the needle, and then ensure that the needle tubing is routed through the appropriate brackets (see "Replacing the Needle Tubing Assembly" on page 85).

If the needle was not the blockage source, go to the next procedure, "Testing the Transfer Tube for Blockage."

Testing the Transfer Tube for Blockage

❖ To test the transfer tube for blockage

- 1. Disconnect the transfer tube from port 2 of the injection valve (see Figure 61 on page 80).
- 2. Place the free end of the transfer tube into a beaker to collect solvent.
- 3. As you perform a **Flush Syringe** direct command, listen for an abnormally loud grinding sound.
 - If the syringe drive does not stall, then the plug is in the injection valve or the sample loop. Reconnect the transfer tube to port 2 of the injection valve and go to the next procedure, "Cleaning the Injection Valve or the Sample Loop."
 - If the solvent flow is constricted through the transfer tube, replace the transfer tube (see "Replacing the Transfer Tubing" on page 71).
- 4. After you replace the transfer tube, home the position of the syringe (see "Setting the Syringe to the Home Position" on page 95).

If the transfer tube was not the blockage source, go to the next procedure, "Cleaning the Injection Valve or the Sample Loop."

Cleaning the Injection Valve or the Sample Loop

If the blockage was not in the needle tubing assembly or the transfer tube, it must be in either the injection valve or the sample loop. Clear the blockage, and then set the syringe to the home position.



CAUTION Do not clean the injection valve with a cleaning wire, which can damage the valve.

❖ To clear the blockage from the sample loop

- 1. Remove the sample loop from the valve.
- 2. Clear blockage from the sample loop by pushing a wire through it. Or, connect a new sample loop to the valve.
- 3. After you reconnect the sample loop, home the position of the syringe.

❖ To clear the blockage from the injection valve

- 1. Remove the sample loop from the valve (see "Changing the Sample Loop" on page 49).
- 2. Remove the valve from the autosampler (see "Removing the Injection Valve from the Autosampler" on page 64).

3. Disassemble the valve (see "Disassembling the Injection Valve" on page 66).



CAUTION The polished (sealing) surface of the stator contains six ports that excess handling can easily damage. As you remove the stator from the injection valve, avoid touching this polished surface, and never place the polished surface face down on a hard surface.

- 4. Place the stator (with the front face down) and the rotor (Valco injection valve) or rotor seal (Rheodyne injection valve) side-by-side in a beaker. Fill the beaker with HPLC-grade methanol or acetonitrile, and then place the beaker in an ultrasonic bath and sonicate for approximately 10 minutes.
- 5. Reassemble the valve (see "Installing a New Rotor" on page 67).
- 6. Reinstall the valve (see "Reinstalling the Injection Valve" on page 68).
- 7. After you clear the source of the blockage, home the position of the syringe (see "Setting the Syringe to the Home Position" on page 95).

Replacing the Needle Tubing Assembly

The needle tubing assembly connects the solvent path between the needle and the syringe valve.



CAUTION The needle tubing is easily damaged. Before you operate the autosampler, take care to route the needle tubing through the support brackets.

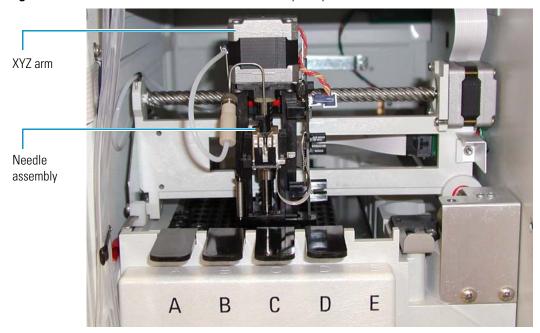
To replace the needle tubing assembly, follow these steps:

- 1. Setting the Needle to the Removal Position
- 2. Removing the Needle Tubing Assembly
- 3. Installing the Needle Tubing Assembly
- **4.** Setting the XYZ Arm to the Home Position

Setting the Needle to the Removal Position

Setting the needle to the removal position moves the XYZ arm to the front-center of the tray compartment and lowers the needle one inch (see Figure 62).

Figure 62. XYZ arm set to the front center of the tray compartment



❖ To set the needle to the removal position

Do the following:

- For the Xcalibur data system, open the Direct Control dialog box for the autosampler (see "Using the Xcalibur Direct Control Commands" on page 141). Select Needle Removal, and then click Apply.
- For the ChromQuest data system, open the Direct Controls page for the autosampler (see "Using the ChromQuest Direct Commands" on page 143). Select **Position Arm to Replace Needle**, and then click **Submit**.

To replace the needle tubing assembly, go to the next procedure, "Removing the Needle Tubing Assembly."

Removing the Needle Tubing Assembly

To remove the needle tubing assembly

- 1. Move the XYZ arm to the front center of the tray compartment (see "Setting the Needle to the Removal Position" on page 85).
- 2. Unscrew the needle tubing from the needle assembly.
- 3. Remove the needle tubing from the bracket on the XYZ arm (see Figure 64 on page 88).
- 4. Pull the two-pronged needle tubing guide out of the *x*-axis positioning frame (see Figure 65 on page 88).
- 5. Move the XYZ arm to the back of the tray compartment (see "Moving the XYZ Arm to the Back of the Tray Compartment" on page 53).
- 6. Remove the needle tubing from the bracket on the left wall of the tray compartment (see Figure 66 on page 89).
- 7. Remove the needle tubing from the bracket on the inside of the tray compartment door (see Figure 68 on page 90).
- 8. Pull the tubing out of the tray compartment. A black or red PVC cap keeps the tubing positioned below the metal runner for the *x*-axis positioning frame.
- 9. Unscrew the needle tubing from the right side of the two-way syringe valve (see Figure 69 on page 90).

Go to the next procedure, "Installing the Needle Tubing Assembly."

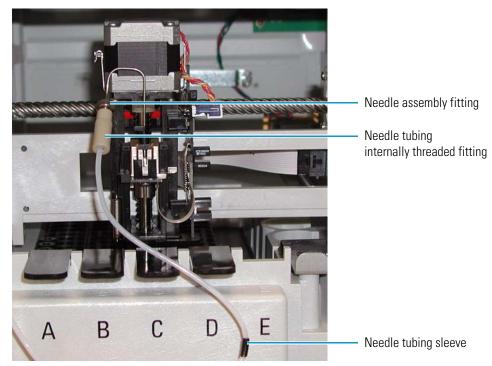
Installing the Needle Tubing Assembly

❖ To install a new needle tubing assembly

- 1. Connect the internally-threaded fitting of the needle tubing assembly to the needle assembly fitting (see Figure 63).
- 2. Verify that the length of tubing between the internally threaded fitting and the black sleeve is approximately 16.5 cm (6.5 in.) in length (see Figure 63).

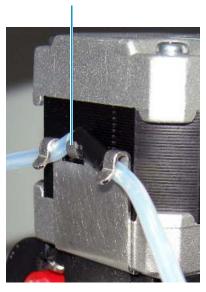
Tip When the needle tubing assembly is connected to the needle assembly, the distance between the needle tubing fitting and the letter E on the tray compartment is approximately 16.5 cm (6.5 in.).

Figure 63. Appropriate length of tubing between the needle tubing fitting and the needle tubing sleeve



3. Route the needle tubing through the bracket on the XYZ arm. Seat the black sleeve in the middle of the bracket (see Figure 64).

Figure 64. Needle tubing routed through the bracket on the XYZ arm Bracket on the XYZ arm



4. Insert the prongs of the needle tubing guide into the alignment holes in the *x*-axis positioning frame (see Figure 65).

Figure 65. Needle tubing guide inserted into the alignment holes in the *x*-axis frame



- 5. Move the XYZ arm to the back of the tray compartment (see "Moving the XYZ Arm to the Back of the Tray Compartment" on page 53).
- 6. Route the needle tubing through the bracket on the left wall of the tray compartment (see Figure 66).

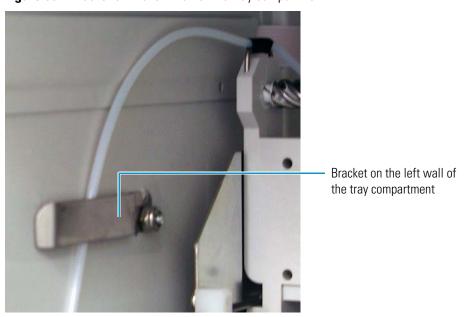
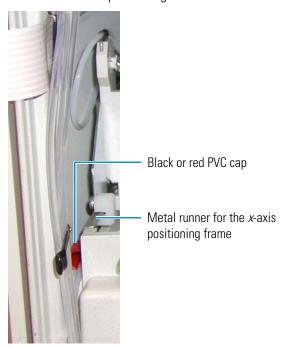


Figure 66. Bracket on the left wall of the tray compartment

7. Push the black or red PVC cap (that is attached to the needle tubing assembly) below the metal runner for the *x*-axis positioning frame (see Figure 67).

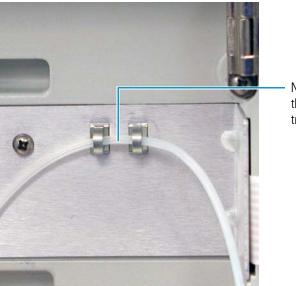
Figure 67. Black or red PVC cap that keeps the needle tubing below the metal runner of the *x*-axis positioning frame



Replacing the Needle Tubing Assembly

8. Route the needle tubing through the bracket on the inside of the tray compartment door (see Figure 68).

Figure 68. Bracket on the inside of the tray compartment door

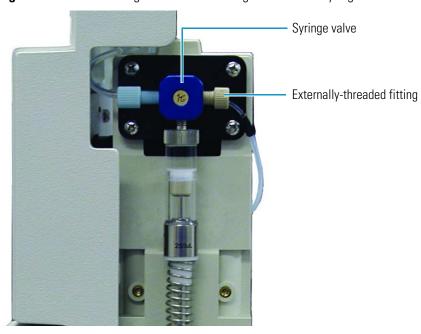


Needle tubing threaded through the bracket on inner side of the tray compartment door

Note The bracket on the tray compartment door of your autosampler might look different from the bracket shown in Figure 68.

9. Connect the externally threaded fitting of the needle tubing fitting to the right side of the syringe valve (see Figure 69).

Figure 69. Needle tubing connected to the right side of the syringe valve



Go to the next procedure, "Setting the XYZ Arm to the Home Position."

Setting the XYZ Arm to the Home Position

To set the XYZ arm to the home position, use the appropriate command from the data system.

❖ To set the XYZ arm to the home position

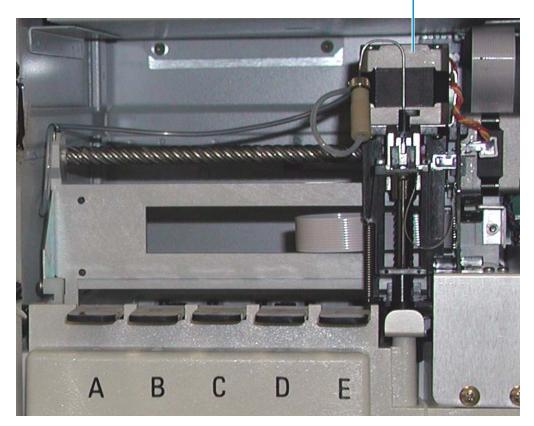
Do the following:

- For the Xcalibur data system, open the Direct Control dialog box for the autosampler (see "Using the Xcalibur Direct Control Commands" on page 141). Select **Set Arm to Home Position**, and then click **Apply**.
- For the ChromQuest data system, open the Direct Controls page for the autosampler (see "Using the ChromQuest Direct Commands" on page 143). Select **GoTo Home**, and then click **Submit**.

The home position of the XYZ arm is directly behind the wash station (see Figure 70).

Figure 70. XYZ arm in the home position

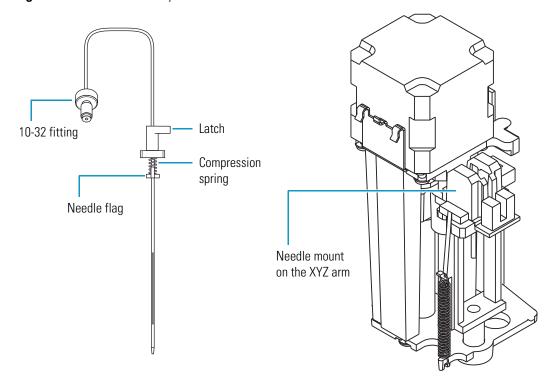
XYZ arm in the home position



Replacing the Needle Assembly

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 71). It slides into the needle mount on the XYZ arm and is secured with the latch.

Figure 71. Needle assembly



❖ To replace the needle assembly

- 1. Move the XYZ arm to the front-center of the tray compartment (see "Setting the Needle to the Removal Position" on page 85).
- 2. Remove the needle assembly 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 and out of the needle mount on the XYZ arm.
- 3. Install a new needle assembly as follows:
 - a. Slide the needle into the needle mount on the XYZ arm.
 - b. Turn the latch to the right.
 - c. Attach the needle tubing assembly to the needle assembly.
- 4. Home the position of the XYZ arm (see "Setting the XYZ Arm to the Home Position" on page 91).

Changing the Syringe or Replacing the Inner Plunger

You must change the syringe if a different size is required for your application. For the dual-concentric syringe, the lifespan of the inner plunger is approximately 50000 injections. If the inner plunger of the 250 µL syringe wears out—that is, if fluid leaks out of the bottom of the syringe, you must replace the plunger. For the inner plunger part number, see page 126.

To replace the syringe or the syringe plunger, follow these steps:

- 1. Setting the Syringe to the Removal Position
- 2. Removing the Syringe from the Autosampler
- 3. Replacing the Inner Plunger of the Syringe
- 4. Installing the Syringe
- 5. Setting the Syringe to the Home Position

Setting the Syringe to the Removal Position

❖ To set the syringe to the removal position

Do the following:

- For the Xcalibur data system, open the Direct Control dialog box for the autosampler (see "Using the Xcalibur Direct Control Commands" on page 141). Select Set Syringe to Removal Position, and then click Apply.
- For the ChromQuest data system, open the Direct Controls page for the autosampler (see "Using the ChromQuest Direct Commands" on page 143). Select **Position**Syringe for Removal, and then click Submit.

The inner plunger of the dual-concentric syringe and the syringe drive move downward.

Removing the Syringe from the Autosampler

❖ To remove the syringe

- 1. Set the syringe to the removal position (see the previous procedure, Setting the Syringe to the Removal Position).
- 2. Undo the screw that connects the syringe plunger to the syringe drive (see Figure 72).
- 3. Loosen and remove the syringe by turning the knurled top of the syringe counterclockwise (see Figure 72).

Knurled nut

Screw that connects the inner plunger to the syringe drive

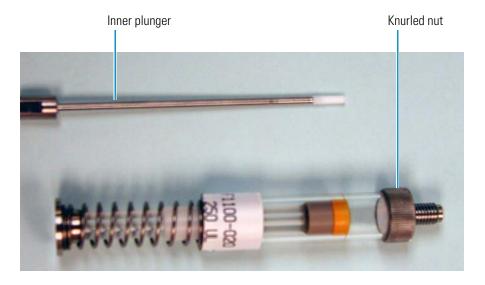
Figure 72. Syringe mounted to the syringe drive assembly

Replacing the Inner Plunger of the Syringe

To replace the inner plunger

- 1. Remove the syringe from the autosampler (see "To set the syringe to the removal position" on page 93).
- 2. Pull the worn plunger out of the bottom of the syringe (see Figure 73).
- 3. Replace the worn plunger with a new replacement inner plunger.

Figure 73. Inner plunger removed from the dual-concentric syringe



Installing the Syringe

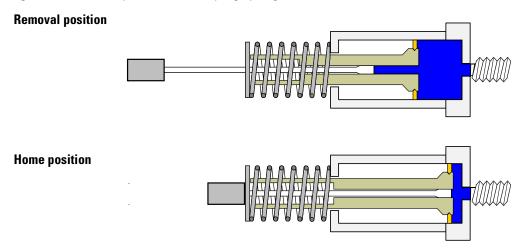
To reinstall the syringe

- 1. Screw the top of the syringe into the syringe valve.
- 2. Reconnect the bottom of the inner plunger to the syringe drive.
- 3. Home the position of the syringe plungers (see the next procedure, Setting the Syringe to the Home Position).

Setting the Syringe to the Home Position

Figure 74 shows the relative positions of the syringe plungers in the syringe removal position and the home position.

Figure 74. Relative positions of the syringe plungers



To set the syringe to the home position

Do the following:

- For the Xcalibur data system, open the Direct Control dialog box for the autosampler (see "Using the Xcalibur Direct Control Commands" on page 141). Select Set Syringe to Home Position, and then click Apply.
- For the ChromQuest data system, open the Direct Controls page for the autosampler (see "Using the ChromQuest Direct Commands" on page 143). Select **Position**Syringe to Home, and then click Submit.

The inner plunger moves upward until the nut on the bottom of the plunger meets the washer on the bottom of the syringe. Then both plungers move upward until they are 1.5 mm (0.06 in.) below the top of the outer syringe barrel.

IMPORTANT If you change the size of the syringe, you must change the syringe type parameter for the configuration of the Accela Autosampler.

Clearing a Plugged Heat Exchanger

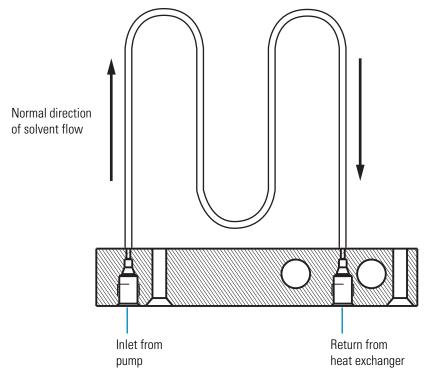
The heat exchanger is a piece of 0.004 in. ID × 1/32 in. OD stainless steel tubing located behind the column oven plate (see Figure 75).

To clear a plug from the heat exchanger, backflush the tubing by reversing the solvent flow.

❖ To backflush the heat exchanger tubing

- 1. Using a 1/4 in. open-end wrench, detach the tubing connected to both the inlet and the outlet of the heat exchanger.
- 2. Connect the tubing from the pump to the port on the right side of the heat exchanger.
- 3. Set the flow rate from the pump to 1 mL/min.
- 4. After you clear the plug, reconnect the pump to the inlet of the heat exchanger and reconnect the outlet of the heat exchanger to port 5 of the injection valve.

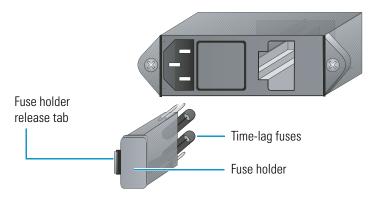
Figure 75. Schematic of heat exchanger



Replacing a Fuse

Instrument power is supplied by two 5.0 A, 250 V, TL, 5 OD \times 20 mm length fuses. The fuses are housed in the fuse compartment on the lower right corner as you are facing the back panel of the autosampler (see Figure 76). If an electrical component in your autosampler stops working, first check for a blown fuse.

Figure 76. Power entry module





CAUTION Disconnect the power cord before you replace fuses.

❖ To replace a fuse

- 1. Turn off the autosampler and disconnect its power cord.
- 2. Use a flat blade to press down on the plastic tab at the top edge of the fuse compartment and pull out the fuse holder.
- 3. Inspect each fuse for a burned or broken wire (which indicates a blown fuse).
- 4. Pull the blown fuse out of the holder and discard it. Place the new fuse (5.0 A, 250 V, TL, 5×20 mm) into the holder with the metal end visible.



CAUTION When you are replacing fuses, use only fuses of the type and current rating specified. Do not use repaired fuses and do not short-circuit the fuse holder.

- 5. Snap the fuse holder back into place.
- 6. Reconnect the power cord and turn on the autosampler.

If the replacement fuse fails in a short time, you have a serious electrical problem. Leave the blown fuse in place and contact your Thermo Fisher Scientific service representative.

Maintenance Cycles

The rotor (Valco injection valve) or rotor seal (Rheodyne injection valve), injection needle, and inner syringe plunger wear with use. From the data system, you can set the scheduled maintenance time (SMT) for these autosampler components.

To set the maintenance cycles, follow the procedure for your data system:

- Setting the Maintenance Cycles from the Xcalibur Data System
- Setting the Maintenance Cycles from the ChromQuest Data System

Setting the Maintenance Cycles from the Xcalibur Data System

Each page of the Maintenance Information dialog box contains a Usage Limit box, a Current Counter readback, a Set New Limit button, and a Zero Counter button. Use each page to set a new usage limit, view the current counter readback, or zero the current counter reading.

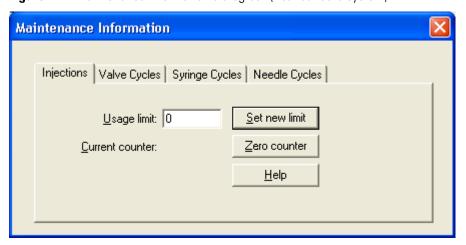
If the counter exceeds the usage limit, the Accela Autosampler cannot start a run until you perform the scheduled maintenance, or you select the Enable Maintenance Log check box of the Accela Autosampler Configuration dialog box.

❖ To set the scheduled maintenance limits from the Xcalibur data system

1. In the Instrument Setup window for the Accela AS, choose Accela AS > Maintenance.

The Maintenance Information dialog box appears (see Figure 77).

Figure 77. Maintenance Information dialog box (Xcalibur data system)



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,	I Jenen	ding on th	าค บรวดค ไบ	mit voli want :	to set onen	the appropriate page.
∠.	Depen	unig on u	ic usage iii	mil you want	to set, open	the appropriate page.

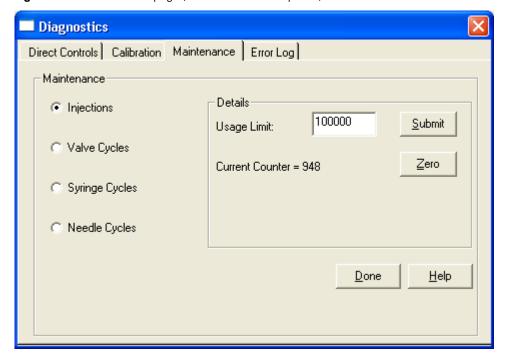
Page	Usage limit				
Injections	Maximum number of autosampler injections				
Valve Cycles	Maximum number of times that the autosampler can switch the valve between the fill and inject positions				
Needle Cycles	Maximum number of times that the autosampler can insert the needle into a vial septum, the injection port, or the wash station				
Syringe Cycles	Maximum number of times that the autosampler can return the syringe to the Ready position				

- 3. To change the usage limit, type the new value in the Usage Limit box, and then click **Set New Limit**.
- 4. To rezero the counter, click **Zero Counter**.

Setting the Maintenance Cycles from the ChromQuest Data System

The Maintenance page in the ChromQuest data system contains four options: Injections, Valve Cycles, Syringe Cycles, and Needle Cycles. After you select an option, its corresponding Details box appears on the right side of the Maintenance page. You can use each Details box to set a usage limit, view the current counter readback, or zero the current counter reading (see Figure 78).

Figure 78. Maintenance page (ChromQuest data system)



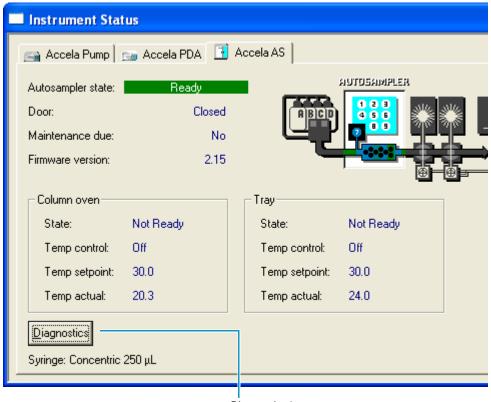
If the counter exceeds the usage limit, the Accela Autosampler cannot start a run until you perform the scheduled maintenance or you clear the Enable Maintenance Log check box of the Accela Autosampler Configuration dialog box.

❖ To set up the maintenance cycles from the ChromQuest data system

- In the online Instrument window, choose Control > Instrument Status.
 The Instrument Status window appears.
- 2. Click the **Accela AS** tab.

The Accela AS status page appears (see Figure 79).

Figure 79. Accela AS status page (ChromQuest data system)



Diagnostics button

3. Click **Diagnostics**.

The Diagnostics dialog box appears.

4. Click the **Maintenance** tab.

The Maintenance page appears (see Figure 78 on page 99).

- 5. Select the option that you want to set:
 - To set the usage limit for the number of user-defined injections, select the **Injections** option.
 - To set the usage limit for the number of user-defined valve cycles (from Fill to Inject), select the **Valve Cycles** option.
 - To set the usage limit for the number of user-defined syringe cycles where the syringe is put in the Ready position, select the **Syringe Cycles** option.
 - To set the usage limit for the number of user-defined needle cycles where the needle is inserted into a septum, injection port, or wash station, select the **Needle Cycles** option.
- 6. To change the usage limit, type the new value in the Usage Limit box, and then click **Submit**.

Diagnostics

Use the Accela Autosampler diagnostics to test the major electronic circuits within the instrument and to indicate whether the circuits pass or fail the tests. If the instrument electronics fail, the diagnostics can often locate the problem. However, to distinguish a hardware failure from an electronic failure, someone who is familiar with system operation and basic hardware theory must run the diagnostics.

You can use the diagnostics to test many of the Accela Autosampler components. The Thermo Fisher Scientific service philosophy is to troubleshoot the problem to the lowest part, assembly, PCB, or module listed in Appendix A, "Consumables and Service Parts." You can replace the parts listed in "Consumables" on page 126, and a Thermo Fisher Scientific field service engineer can replace the parts listed in "Service Parts" on page 127.

Contents

- Power Up Diagnostics
- Error Log in the Xcalibur Data System
- Error Log in the ChromQuest Data System
- Autosampler Validation Information

Power Up Diagnostics

Accela Autosampler power up diagnostics run automatically when you turn on the autosampler and take about 2 minutes to run. Most of the time required for the power up diagnostics is occupied testing the RAM.

If there is a memory error of a fatal type, the right three lights of the Status LEDs turn amber. If this happens, contact Thermo Fisher Scientific Technical Support. The CPU PCB probably needs to be replaced.

If diagnostics detect any other type of error, the Run Status LED illuminates amber and the autosampler records the error in its persistent memory log. You can access this error log through the data system.

5 Diagnostics

Power Up Diagnostics

Table 13 lists the startup diagnostics error messages. The left column lists the error messages that your Thermo Scientific field service engineer can view from HyperTerminal™ (or equivalent application). The right column lists the error messages that might appear in the autosampler error log.

When the startup test passes, the error log displays this message: No error. No other events occur.

Table 13. Autosampler startup error messages (Sheet 1 of 2)

HyperTerminal error messages	Error log messages
EC_SDIAGS_BAD_Z_FLAG	The z-axis home flag failed.
EC_SDIAGS_BAD_S_FLAG	The s-axis home flag failed.
EC_SDIAGS_BAD_X_FLAG_INT	The x-axis interrupt failed.
EC_SDIAGS_BAD_Y_FLAG_INT	The y-axis interrupt failed.
EC_SDIAGS_BAD_Z_FLAG_INT	The z-axis interrupt failed.
EC_SDIAGS_BAD_S_FLAG_INT	The s-axis interrupt failed.
EC_SDIAGS_BAD_1_MOTION_TIMER	Motion Timer A failed.
EC_SDIAGS_BAD_2_MOTION_TIMER	Motion Timer B failed.
EC_SDIAGS_BAD_3_MOTION_TIMER	Motion Timer C failed.
EC_SDIAGS_BAD_4_MOTION_TIMER	Motion Timer D failed.
EC_SDIAGS_BAD_Z_BOTTOM_FLAG	The z-bottom flag failed.
EC_SDIAGS_TIMER_SETUP_ERROR	Occurs if an error status is returned by the disable interrupt call.
EC_SDIAGS_NO_INJ_CABLE	The injector motor cable is not connected.
EC_SDIAGS_BAD_INJ_POS	The injection valve did not change position.
EC_SDIAGS_BAD_SYRINGE_VALVE_INT	The syringe valve interrupt did not occur.
EC_SDIAGS_BAD_FLUSH_VALVE_INT	The flush value interrupt did not occur.
EC_SDIAGS_BAD_CAPACITOR	The capacitor did not charge to the acceptable level.
EC_SDIAGS_X_MOTOR_NOT_PRESENT	The x-axis motor was not detected.
EC_SDIAGS_Y_MOTOR_NOT_PRESENT	The y-axis motor was not detected.
EC_SDIAGS_Z_MOTOR_NOT_PRESENT	The z-axis motor was not detected.
EC_SDIAGS_S_MOTOR_NOT_PRESENT	The s-axis motor was not detected.
EC_SDIAGS_XZ_CONNECTOR_NOT_ CONNECTED	The cable that connects the <i>x</i> - and <i>z</i> -axis motors is not connected.
EC_SDIAGS_BAD_REG_VALUE	One of the memory registers is not set correctly.

Table 13. Autosampler startup error messages (Sheet 2 of 2)

HyperTerminal error messages	Error log messages
EC_SDIAGS_MOTOR_ERROR	The motor diagnostic bits did not change to the correct state.
EC_SDIAGS_ISR_TIMEOUT	The interrupt did not occur before the interrupt timeout did.
EC_SDIAGS_METAL_OUTOF_RANGE	The oven or tray metal readings were not within the specified low and high.
EC_SDIAGS_OVEN_AIR_OUTOF_RANGE	The oven air readings were not within the specified low and high.
EC_SDIAGS_24V_OUTOF_RANGE	The oven or tray 24 V readings were not within the specified low and high.
EC_SDIAGS_12V_OUTOF_RANGE	The oven or tray 12 V readings were not within the specified low and high.
EC_SDIAGS_OVEN_MIRROR_OUTOF_RANGE	The readings for the oven mirror were not within the specified low and high.
EC_SDIAGS_UNKNOWN_DEVICE	A call was made to the temperature diagnostics with an unknown device.
EC_SDIAGS_VIAL_MIRROR_OUTOF_RANGE	The readings for the tray mirror were not within the specified low and high.
EC_SDIAGS_HOME_ERROR	A move to the home flag did not set the home flag bit correctly.

Error Log in the Xcalibur Data System

The error log in the Xcalibur data system lists the diagnostic error messages. You can access the error log through the status page of the Information View. The Information View is normally displayed on the left side of the Home Page window and contains two pages: Status and Acquisition Queue. If the Home Page window does not display this view, it has been turned off.

To open the Information View

From the Home Page window, choose **View > Info View** or in the toolbar, click turn the Information View on and off. See Figure 80.

Status | Acquisition Queue | ⊡- Run Manager Ready To Download Sequence: Sample Name: Working On: Position: Raw File: Inst. Method: - Accela AS Ready to Downlo Turn Device On Turn Device into Standby Turn Device Off AS Status Oven Tray Error log Target injection Vial position: A:A1 Injection volume (µL): 5.00 AS state: Waiting for download Door state: Close Maintenance due: No Firmware version: 2.15

Figure 80. Status page of the Information View with the Accela AS selected in the directory tree

To access the error log for the Accela Autosampler

- 1. Click the **Accela AS directory** in the Status window to activate its status pages.
- 2. Click the Error log tab to open the Error Log page (see Figure 81).

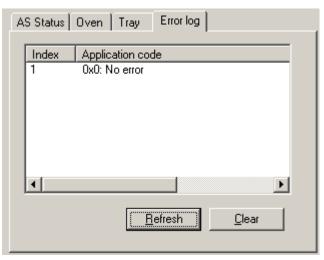


Figure 81. Error log for the Accela Autosampler

- 3. Click **Refresh** to display the list of errors.
- 4. After reviewing the error list, click **Clear** to erase the list.

Error Log in the ChromQuest Data System

The error log in the ChromQuest data system lists the diagnostic error messages.

❖ To open the error log

- 1. From the Instrument window, choose **Control > Instrument Status** to display the Instrument Status window.
- 2. Click the **Accela AS** tab to display the Accela AS page of the Instrument Status window.
- 3. Click **Diagnostics** to display the Diagnostics dialog box.
- 4. Click the **Error log** tab to display the error log for the Accela Autosampler.
 - To view the error log, click **Refresh**.
 - To clear the error log, click **Clear**.

Autosampler Validation Information

The Xcalibur data system keeps track of validation information. Use the Validation Information dialog box to enter the date when the validation occurred and the name of the person who performed the validation.

To open the Validation Information dialog box

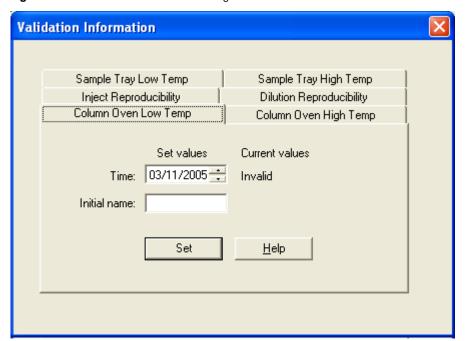
From the Instrument Setup window, choose **Accela AS > Validation**.

The Validation Information dialog box has the following pages:

- Sample Tray Low Temp
- Sample Tray High Temp
- Column Oven Low Temp
- Column Oven High Temp
- Inject Reproducibility
- Dilution Reproducibility

Use these pages to enter the calendar dates when the validation occurred and the initials of the person who performed the validation. See Figure 82.

Figure 82. Validation Information dialog box



Routine Troubleshooting

This chapter provides information to help you troubleshoot possible autosampler problems.

When troubleshooting, consider the following:

- Did the system fail when you were running samples?
- Did problems occur after you performed maintenance on the instrument?
- Did you change the system configuration, cables, or peripherals just before the problem occurred?

If the answer is yes to the first question above, the issue might be a hardware failure.

If the answer is yes to one of the latter two questions above, the issue is probably mechanical, not electrical. Verify that alignment, configurations, and cable connections are correct.

If, after reviewing the troubleshooting table included in this chapter, you would like additional assistance in troubleshooting a particular problem, contact Thermo Fisher Scientific Technical Support or your service representative.

Contents

- General Chromatography Troubleshooting
- Autosampler-Specific Troubleshooting
- Common Operator Errors
- Determining the Transfer Tubing Volume

General Chromatography Troubleshooting

Table 14 lists general chromatographic problems. For more information about chromatographic troubleshooting, refer to any HPLC troubleshooting reference book, or contact technical support or your service representative.

Table 14. General chromatography troubleshooting table (Sheet 1 of 2)

Symptom	Remedy
No flow	Check the mobile phase connections.Check for leaks.Refer to the hardware manual for your pump.
High backpressure	 Check the flow rate and system/column specifications. Check for tubing or column blockage. Refer to the hardware manual for your pump.
Unstable baseline or drift	System/column not equilibrated; allow more time.Refer to the detector troubleshooting guide.
Baseline noise	Check for air bubbles in system, degas solvents.Check for system/solvent contamination.Check the hardware manual for your pump.
No peaks	 Check the detector and data system computer connections. See "Autosampler-Specific Troubleshooting" on page 111. Check sample retention with chromatographic conditions.
Contamination/ghost peaks	 Clean the system and column. See "Autosampler-Specific Troubleshooting" on page 111. Refer to the hardware manual for your pump.
Poor peak shape	 Check the system for leaks. Check fittings and tubing lengths. Check column performance. See "Autosampler-Specific Troubleshooting" on page 111. Refer to the hardware manual for your pump. Refer to the hardware manual for your detector.
Poor retention time reproducibility	 Check the system for leaks and bubbles. System/column not equilibrated; allow more time. Check column performance. Check the hardware manual for your pump. See "Autosampler-Specific Troubleshooting" on page 111.

Table 14. General chromatography troubleshooting table (Sheet 2 of 2)

Symptom	Remedy
Poor peak area reproducibility	 Check column performance. See "Autosampler-Specific Troubleshooting" on page 111.
Non-integrated peaks or too many peaks	 Refer to the hardware manual for each of the modules in your LC system. Refer to the data system manual for information on setting peak identification and integration.
No instrument or device control	 Check the cable connections. Check the system configuration. Refer to the hardware manuals for each of the modules in your LC system.

Autosampler-Specific Troubleshooting

This section focuses on problems that might occur with the autosampler portion of your HPLC system. It contains the following topics:

- Chromatography Problems
- Hardware Problems

Chromatography Problems

Table 15 lists potential chromatography problems associated with the autosampler.

Table 15. Chromatography problems (Sheet 1 of 4)

Symptom		ssible problem	Rei	medy
Baseline drift when the injection valve changes from inject to fill	a.	Lack of thermal stability in the column oven compartment, detector, or mobile phase	a.	Eliminate drafts around the column compartment. Wait until the column oven has reached thermal equilibrium. If the problem persists, contact your Thermo Fisher Scientific service representative.
2. Shifting retention times	a.	Temperature variations in the lab	a.	Stabilize the temperature.
	b.	Column heater problems	b.	Contact your Thermo Fisher Scientific service representative.
3. Sensitivity changes	a.	Inconsistent sample injection volumes	a.	Check for a partially blocked injection valve and observe the pressure. If the problem persists, see "Replacing the Rotor in the Valco Injection Valve" on page 63.
	b.	Leaks between the injection valve and the column causing inconsistent sample volume injection	b.	Tighten or change the fitting.
	c.	Air bubbles aspirated into the lines along with sample	c.	Replenish/prepare fresh sample and repeat the injection.
				Decrease the syringe speed.
	d.	Incomplete sample mixing during sample preparation	d.	Thaw frozen samples completely before sample preparation to prevent sample "layering."
	e.	Sample adsorption to the walls of the injection valve or sample loop	e.	Change the mobile phase composition.
	f.	Sample solvent incompatible with the mobile phase	f.	Change the solvent. Use a different mobile phase if possible.
	g.	Sample insoluble in solvent	g.	Be sure the sample solvent has a lower elution strength than the mobile phase.
	h.	Air in syringe/sample lines	h.	Flush sample lines.

Table 15. Chromatography problems (Sheet 2 of 4)

Symptom	Pos	ssible problem	Remedy		
4. Irregular peak shapes	a.	The volume of sample injected exceeds the column's capacity.	a.	Decrease the volume injected or dilute the sample.	
	b.	The strength of the solvent used to dilute the sample exceeds the initial concentration of the mobile phase.	b.	If appropriate for your application ensure that the flush solvent is of the same composition as the solvent used to dissolve the sample and the its organic content never exceeds the initial composition of the mobile phase.	
	c.	Degradation of sample components	c.	Prepare fresh sample and repeat the injection.	
5. Band broadening	a.	Fitting problem at the injection valve-column connection	a.	Inspect for damage, mismatch, or incorrect assembly.	
	Ь.	Tubing ID too large	b.	Use 0.010 in. ID tubing from the injection valve to the column and from the column to the detector.	
6. Smaller than expected peak heights	a.	Small injection due to incorrect size syringe in configuration	a.	Check the syringe size in the autosampler device driver configuration.	
	b.	Incorrect injection volume for the injection mode	b.	Check the injection volume value is the file. For partial loop injections limit the maximum injection volume to half the sample loop size. For no waste injections, limit the maximum injection volume to 5 µ less than the sample loop size.	
	C.	Incorrect value for the transfer tubing	c.	Check the dead volume value in the configuration. The dead volume for the transfer tubing is specified on it attached label. See Figure 10 on page 11.	
	d.	Air in lines	d.	Perform a Needle Wash direct command to remove air from the wash bottle tubing and the syringe	
	e.	Incorrectly adjusted syringe drive mechanism	e.	Contact your Thermo Fisher Scientific service representative.	

Table 15. Chromatography problems (Sheet 3 of 4)

Symptom		ssible problem	Rer	nedy
Peaks during a blank injection (from a previous injection)	a.	Sample carryover due to residual sample in the sample loop	a.	Increase flush volume. Increase injection volume, add a blank vial between sample vials, or use a stronger flush solvent.
	Ь.	Sample carryover due to incorrectly assembled sample loop fittings (adding dead volume that can contain residual sample)	Ь.	Check and redo connections.
	c.	Sample carryover due to a contaminated needle guide	c.	Clean the needle guide.
	d.	Sample too concentrated	d.	Dilute the sample.
8. No peaks	a.	Empty wash bottle or excessive air in the wash tubing	a.	Fill the wash bottle with solvent. Remove air from the wash tubing and the syringe.
	b.	Plugged or bent needle	b.	Clean or replace the needle. Tighten the fittings and the syringe.
	c.	Leaking syringe valve fitting at the wash tubing connection	c.	Tighten the fitting.
	d.	Plugged or leaking tubing	d.	Replace faulty tubing.
9. Lack of precision, accuracy, or both on a sample of	a.	Air leak	a.	Tighten all fittings and repeat the run.
known content-random error	Ь.	Worn out syringe	b.	Replace the syringe (see "Changing the Syringe or Replacing the Inner Plunger" on page 93).
	c. Y	Worn out rotor (Valco injection valve)	c.	Replace the rotor (see "Replacing the Rotor in the Valco Injection Valve" on page 63)
	d. `	Worn out rotor seal (Rheodyne injection valve)		Replace the rotor seal (see "Replacing the Rotor Seal in the Rheodyne Valve" on page 137)

Table 15. Chromatography problems (Sheet 4 of 4)

Symptom		ossible problem		Remedy		
10. Irreproducibility of peak areas	a.	Plugged or bent needle	a.	Clean/replace needle (see "Replacing the Needle Assembly" on page 92). Tighten fittings and syringe.		
	b.	Leaking flush valve fitting	b.	Tighten fitting.		
	c.	Plugged or leaking tubing	c.	Replace faulty tubing.		
	d.	Sample viscosity too low or too high	d.	Change viscosity setting.		
	e.	Wash solvent viscosity too low or too high	e.	Change wash solvent or flush speed.		

Hardware Problems

Table 16 lists the potential hardware problems associated with the autosampler.

Table 16. Hardware problems associated with the Accela Autosampler (Sheet 1 of 4)

Symptom		Possible problem Reme		medy
Startup				
1. Nothing works when	a.	Power cord unplugged or faulty	a.	Plug in or replace the power cord.
instrument is turned on.	b.	Blown fuse	b.	Replace the fuse (see "Replacing a Fuse" on page 97).
	c.	Defective power switch, driver, transformer	c.	Contact your Thermo Fisher Scientific service representative.
Communications				
2. No Ethernet communication	a.	Incorrect computer NIC settings	a.	Check the address. Use only TCP/IP protocol.
	b.	Ethernet hardware problem	Ь.	Check the Ethernet switch and Ethernet cables.
3. External devices are not triggered.	a.	Incorrect timed event settings in the acquisition method	a.	Check the timed event settings in the acquisition method.
	b.	Interface equipment problem	b.	See "Connecting the System Interconnect Cable" on page 37 or refer to the <i>Accela Getting Connected Guide</i> for information on contact closure connections.
Vials				
4. Instrument cannot locate vial.	a.	Incorrect vial position entered into sample sequence	a.	Check the sample sequence.
	b.	Sample incorrectly placed in sample tray	b.	Check the tray arrangement.
	c.	XYZ arm misaligned	c.	Recalibrate the XYZ arm. Refer to the data system Help.
	d.	XYZ arm movement restricted	d.	Remove restriction and home arm. See "Important Precautions for Sample Loading" on page 56.
	e.	Loose left-hand guide bracket	e.	Ensure that the bracket is tightly attached and level.
5. Vials do not fit in tray.	a.	Incorrect vials	a.	Check that the vial diameter is 11 to 12 mm (0.43 to 0.47 in.).

Table 16. Hardware problems associated with the Accela Autosampler (Sheet 2 of 4)

Symptom	Pos	ssible problem	Rei	medy
6. Torn septum	a.	Bent needle	a.	Replace the needle (see "Replacing the Needle Tubing Assembly" on page 85).
7. Septum pushed into vial	a.	Incorrect vial caps	a.	Use only recommended vials and vial caps. See "Recommended Vials, Microplates, and Microplate Covers" on page 57.
	Ь.	Loose vial caps	b.	Ensure that the vial caps are fully tightened.
Injection valve				
8. Injection valve will not switch.	a.	Slipping injector drive	a.	Contact your Thermo Fisher Scientific service representative.
9. Injection valve continues to rotate until fault is generated.	a.	Loose or faulty sensor cable	a.	Contact your Thermo Fisher Scientific service representative.
10. Injection valve does not rotate to the proper position.	a.	Valve requires excessive torque.	a.	Contact your Thermo Fisher Scientific service representative.
11. Motor fails to stop after syringe is fully returned.	a.	Possible defective sensor	a.	Contact your Thermo Fisher Scientific service representative.
12. Sample loop will not fill.	a.	Blocked injection valve	a.	Follow "Troubleshooting a Blockage in the Injection System" on page 79 to isolate blockage. DO NOT clean valve with a cleaning wire or you could damage the valve.
13. Valve seal wears rapidly.	a.	Too much valve seal pressure against valve	a.	Decrease LC system pressure.
	b.	Particulate matter in valve	Ь.	Use an inline filter before the injection valve.
	c.	Inadequately filtered sample	c.	Filter with a 0.5 m filter before injection.
	d.	Buffer crystallization	d.	Do not allow buffers to stand in the system. Run a flush sequence when the system is not in use.

6 Routine Troubleshooting

Autosampler-Specific Troubleshooting

Table 16. Hardware problems associated with the Accela Autosampler (Sheet 3 of 4)

Symptom	Pos	ssible problem	Rei	medy
Needle				
14. Needle bent or broken	a.	Incorrect vials	a.	See the list of recommended replacement parts in Appendix A, "Consumables and Service Parts."
	b.	Alignment problem	b.	Contact your Thermo Fisher Scientific service representative.
	c.	Arm sticking	c.	Contact your Thermo Fisher Scientific service representative.
15. Needle plugging	a.	Multiple/incorrect septum	a.	Verify a single septum of the type recommended (see "Recommended Vials, Microplates, and Microplate Covers" on page 57).
	b.	Worn needle	b.	Replace the needle (see "Replacing the Needle Assembly" on page 92).
Needle Tubing				
16. Blockage in the needle tubing	a.	Crimped needle tubing	a.	Replace the needle tubing (see "Replacing the Needle Tubing Assembly" on page 85).
Syringe				
17. Loud buzzing from syringe drive	a.	Plugged lines or needle	a.	Clear lines or needle (see "Troubleshooting a Blockage in the Injection System" on page 79).
	b.	Defective limit switch	b.	Contact your Thermo Fisher Scientific service representative.
Transfer tube				
18. Lack of injection precision and accuracy, lack of precision or blank injections	a.	Constricted or plugged transfer tube	a.	Replace the damaged transfer tube. Avoid overtightening the fitting to port 2 of the injection valve (see "Replacing the Transfer Tubing" on page 71).
Leaks				
19. Leakage during loop filling	a.	Blocked loop or waste line, or valve not deflected far enough	a.	Flush or replace the sample loop; check and flush the waste line.

Table 16. Hardware problems associated with the Accela Autosampler (Sheet 4 of 4)

Symptom	Possible problem	Remedy
20. Cross-port leakage	a. Worn rotor seal (Rheodyne injection valve) or rotor (Valco injection valve	a. Replace the rotor seal (see "Replacing the Rotor Seal in the Rheodyne Valve" on page 137). Replace the rotor (see "Replacing the Rotor in the Valco Injection Valve" on page 63).
21. Liquid on vial caps	a. Leaky syringe valve	a. Replace the syringe valve.
Other	b. Leaky injection valve	b. Replace the rotor seal (see "Replacing the Rotor Seal in the Rheodyne Valve" on page 137). Replace the rotor (see "Replacing the Rotor in the Valco Injection Valve" on page 63).
22. Contaminated sample	a. Dirty vials	a. Replace vials.
·	b. Improper septum made of silico or other rubber-like material	b. Use the recommended septum (see "Recommended Vials, Microplates, and Microplate Covers" on page 57).
23. Elevated system pressures	Block between column and autosampler	a. Check for blockage and tubing restrictions.

Common Operator Errors

Operator errors can compromise the performance of the Accela Autosampler.

These are the most common operator errors:

- Letting the wash bottle run dry
- Routing the needle tubing incorrectly
- Selecting an incorrect syringe type in the Accela Configuration Autosampler dialog box
- Requesting an inappropriate injection volume for the sample loop size and the injection mode
- Entering an incorrect dead volume for the transfer tubing
- Overtightening the transfer tube fitting to port 2 or the wash tube fitting to port 3 of the autosampler injection valve

Insufficient Wash Solvent

Allowing the wash bottle to run dry disrupts the ability of the syringe to draw liquid into the needle tubing, which consequently results in blank injections. Fill the wash bottle with solvent, and remove air from the wash line tubing before running a sequence of injections.

Damaged Needle Tubing

Because the needle tubing is easily damaged, routing the needle tubing correctly is critical to the performance of the Accela Autosampler. Take care to avoid kinking the tubing as you route it through the bracket on the XYZ arm.

Incorrect Syringe Type

Because the autosampler cannot detect the syringe type connected to the syringe drive assembly, confirm that the syringe type in the Accela Configuration Autosampler dialog box matches the autosampler syringe.

The injection volume algorithm used by the autosampler depends on the syringe type that you select when you configure the data system. The distance that the inner syringe plunger must descend to draw the specified injection volume increases as the syringe size decreases. For example, to draw 10 μL of sample, the inner plunger of a 100 μL syringe must descend farther than the inner plunger of a 250 μL syringe. If you replace the 250 μL syringe with a 100 μL syringe, but neglect to modify the data system configuration, the autosampler injects a smaller than expected injection volume because the syringe plunger does not descend far enough to draw the requested injection volume.

Inappropriate Injection Volume

For best results, follow these rules:

- For no waste injections, limit the maximum injection volume to 5 μL less than the sample loop volume. Limit the minimum injection volume to 1 μL of sample.
- For partial loop injections, limit the maximum injection volume to 40 percent of the nominal sample loop volume.

Incorrect Dead Volume

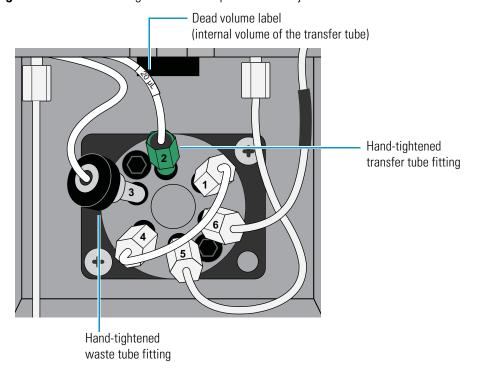
The dead volume of the 0.012 in. ID transfer tubing is specified on the label attached to the tubing. Entering an incorrect dead volume when you specify the configuration options for the autosampler can result in lower than expected peak areas or blank injections.

Overtightened Transfer Tube or Wash Tube Fittings

Avoid overtightening the low-pressure fittings to the injection valve (see Figure 83):

- Overtightening the transfer tube fitting constricts the transfer tube, causing a lack of injection precision. Eventually, the constricted transfer tube becomes plugged.
- Overtightening the waste tube fitting constricts the waste tube, increasing the backpressure in the injection system.

Figure 83. Transfer tubing connection to port 2 of the injection valve



Determining the Transfer Tubing Volume

The Dead volume configuration parameter is critical for the no waste injection mode. Specifying an incorrect dead volume value can result in lower than expected peak areas or blank injections. For best results, the specified dead volume must equal the actual volume of the transfer tubing that connects the autosampler injection port to port 2 of the injection valve.

❖ To determine the volume of the transfer tubing

1. From the Xcalibur or ChromQuest data system, set up an acquisition method for an analyte that you know the chromatographic conditions for.

Use chromatographic conditions that result in a relatively short retention time for the analyte (to minimize the run time). In the method, select the **No Waste Injection** option.

You can also use the autosampler test mix (0.5 percent toluene in methanol) that comes with the autosampler. With the chromatographic conditions listed in Table 17, toluene elutes at approximately two minutes.

Table 17. Chromatographic conditions for toluene

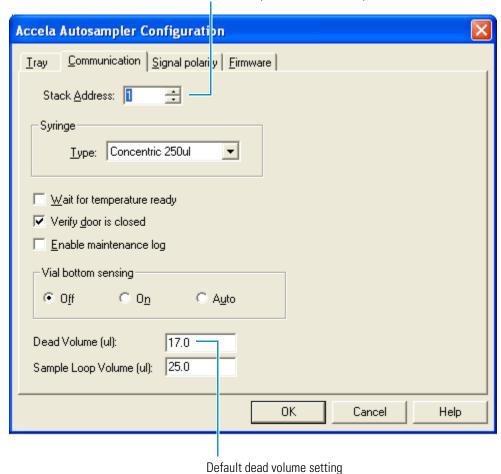
Parameter	Setting
Mobile phase	80:20 methanol/water
Flow rate	1 mL/min
Column	100 × 4.6 mm, C-18 column, 5 μm particle size, or equivalent
Temperature	Ambient
Wavelength	260 nm, 1 nm bandwidth for the PDA detector

2. Change the value for the Dead Volume autosampler configuration parameter. Start with a value of **15**.

Figure 84 shows the Communication page of the Accela Autosampler Configuration dialog box for Thermo Foundation Instrument Configuration, and Figure 85 shows the Accela AS Configuration dialog box in the ChromQuest data system. The default dead volume value is 17.0. The manufacturer determines the volume of the tubing used to produce each lot of transfer tubes (see Figure 83 on page 121) and places a label on each tube.

Figure 84. Communication page (Thermo Foundation Instrument Configuration)

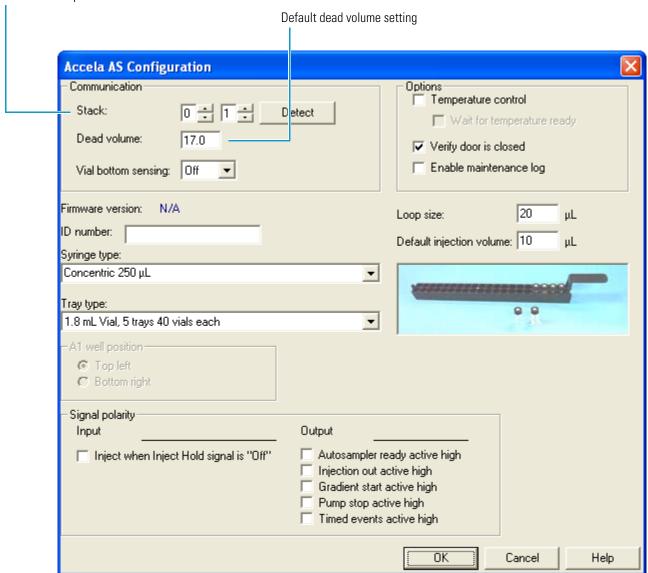
The Stack Address value must match the unit ID setting on the back panel of the autosampler.



Thermo Scientific

Figure 85. Accela AS Configuration dialog box (ChromQuest data system)

The Stack value must match the unit ID setting on the back panel of the autosampler.



- 3. From the Xcalibur or ChromQuest data system, inject 1 μL of sample using the no waste injection mode.
- 4. Repeat step 2 and step 3, increasing the dead volume by 1 μL each time until you have data points from 15 to 23 μL .
- 5. Plot the peak area versus dead volume. Check for an increase in peak area until it reaches a plateau.

Due to injection variability, the plateau might not be flat. Select the dead volume where the maximum is first reached.

Consumables and Service Parts

A consumable item is an item that is expected to wear with use or that comes in contact with system fluids or samples. As a result, you must periodically replace a consumable item. A service part is a subassembly of the autosampler. Normally, Thermo Fisher Scientific service representatives or other qualified personnel replace a service part. This chapter provides a comprehensive listing of consumables and Accela Autosampler service parts that you can order and replace.

This chapter also includes an itemized list of the parts in the Accela System Kit. This kit contains the hardware required to interconnect the system modules and the instrument device drivers required to operate the modules. The Accela System Kit is not packaged with the Accela Autosampler. You must order it separately or as part of a system bundled with either the Xcalibur or ChromQuest data system.

Note To control the Accela Autosampler, you must have the Xcalibur or ChromQuest data system and the Accela device drivers.

- For the Xcalibur data system, the Accela device drivers are provided on the LC Devices software CD.
- For the ChromQuest 4.2 data system, the Accela device drivers are provided on the Accela Add-On Device Drivers CD.
- For the ChromQuest 5.0 data system, the Accela drivers are provided on the ChromQuest data system CD.

Contents

- Consumables
- Service Parts
- Accela AS Accessory Kit
- Accela System Kit

Consumables

These parts are consumables for the Accela Autosampler. The sample loops are compatible with the Valco injection valve. Nut, compression, long, 10-32, 1/16 in. OD tube00101-07-00001 Plunger, replacement, concentric, 100 µL F1123-010 Plunger, replacement, concentric, 250 µL F1123-020 Plunger, replacement, concentric, 500 uL F1123-030 Syringe, dual concentric, $100~\mu L$ F1100-010 Syringe, standard, 2500 µL (with needle tubing extension) 60053-62002

Consumables (continued)	
Tubing assembly, 2.5 mL syringe	60053-60005
Vial kit, 1.8 mL w/250 μL insert, 100 per pack	60053-62017
Vial kit, 2 mL, 100 count, includes caps and septa (2 each)	A4954-010

Service Parts

Normally, Thermo Fisher Scientific service representatives or other qualified personnel replace
these service parts:
Assembly, arm
Assembly, cooler
Assembly, cooler fan F1110-010
Assembly, door left
Assembly, door right
Assembly, oven
Assembly, oven fan
Assembly, oven sensor
Assembly, tray sensor
Base, pod, z-axis
Cable, injector sensor
Cable, door LED
Cable, temperature control
Cable, assembly, DC oven motor
Cable, assembly temperature control
Cable, A/C input power
Cable, A/C to options PS F1080-010
Cable, backplane power
Cable, Oven DC power
Cable, cooler DC power
Cable, injector power
Column clamp
Drive, injector
Drive, syringe
Enclosure for the autosampler chassis
Gasket, oven door
Kit, Autosampler Calibration
Kit, adjustable arm lifting stop bracket
PCB, Arm Motor
PCB, CPU spare
PCB, Interconnect F9040-010
PCB, LED
PCB, Temperature ControlF9020-010
Power supply, main
Power supply, option
Switch, door
Wash station

Accela AS Accessory Kit

The Accela AS Accessory Kit includes consumables (such as vials, fittings, and test mixtures) as well as durable accessories (such as microplates, tray adapters, tools, and a power cord). The Accela AS Accessory Kit, P/N 60357-62001, contains the following items: Clip, microwell handle F1072-010 Connector, plug, 8-position, 3.8 mm pitch, minicombicon (2 each)......00004-02511 Screw, cross-recessed, 100 degree, flat countersunk head, type 1, Screw, pan head, $4-40 \times 1/4$ in. length, stainless steel, Test mixture, pkg., 1 mL, 5 count, dilution, for performance qualification A5135-010 Tray adapter plate for microcentrifuge tubes (5 each) F1008-020

Accela System Kit

The Accela System Kit, P/N 60057-60060 revision F, contains the cables, tubing, fittings, and
so on used to interconnect the system modules.
5-port Ethernet switch, 5 port, 10/100 Base-T, 100 - 240 V00825-01-00024
Cable, Ethernet with two ferrites
Cable, system interconnect cable, 7-connector
Ferrule, 0.188 in. length, for 1/16 in. OD tubing,
stainless steel (quantity 12)
Ferrule, Valco zero dead volume for 1/16 in. OD tubing,
10 000 psi rating (quantity 12)
Filter cartridge, UHP, 0.5 µm particle size, Teflon
Marker letter A, clip on for 1/8 in. OD tubing
Marker letter B, clip on for 1/8 in. OD tubing
Marker letter C, clip on for 1/8 in. OD tubing
Marker letter D, clip on for 1/8 in. OD tubing
Nut, compression, 0.45 in. length, for 1/16 in. OD tubing,
10-32 thread, stainless steel (quantity 12)
Solvent bottle, 1 liter (quantity 4)
Solvent bottle caps (outer portion) (quantity 4)
Solvent bottle cap adapter (inner portion) (quantity 4
Solvent bottle label set
Solvent bottle holder, solvent platform
Tubing, stainless steel, 0.005 in. ID \times 1/16 in. OD, 10 cm length 00301-01-00008
Tubing, stainless steel, 0.005 in. ID × 1/16 in. OD, 20 cm length
(to connect the Accela Pump outlet to the inlet
of the Accela Autosampler heat exchanger)
Tubing, stainless steel, 0.005 in. ID × 1/16 in. OD, 26 cm length
Tubing, stainless steel, 0.005 in. ID × 1/16 in. OD, 60 cm length
Vial kit, clear vials with 1.8 mL capacity, black screw-on caps,
100 vials and caps per package (quantity 2)
Instrument control software, LC Devices
(for use with the Xcalibur data system and
several other Thermo Scientific mass spectrometry applications) 60257-62005
Instrument control software,
Thermo pumps add-on for the ChromQuest 5.0 data systemCHROM-93034
Instrument control software,
Accela detectors add-on for the ChromQuest 5.0 data systemCHROM-98036
Instrument control software,
Accela Open Autosampler add-on
for the ChromQuest 5.0 data system
Instrument control software, data system independent
for the Accela Open Autosampler
(PAL loader and object manager)

Firmware Updates

Periodically, upgrades to the Accela Autosampler firmware might become available. In anticipation of future upgrades, both the Xcalibur and ChromQuest data systems come with the Accela Firmware Upgrade utility, which you can use to download firmware files to your Accela instrument modules. Firmware upgrade files have a .bin extension.

For the Xcalibur 2.0.7 or earlier data system and the Thermo Foundation application, the current firmware files and the Accela Firmware Upgrade utility are automatically installed to your personal computer during the installation of the data system.

For the ChromQuest data system, you can access the utility by choosing **Start > Programs > Chromatography > Accela Firmware Upgrade Utility**.

Contents

- Preparing the Autosampler for a Firmware Download
- Downloading Firmware to the Autosampler

Preparing the Autosampler for a Firmware Download

❖ To prepare the autosampler for a firmware download

1. Make sure you have the Accela AS firmware files that you intend to download and the Accela Firmware Upgrade Utility on your computer.

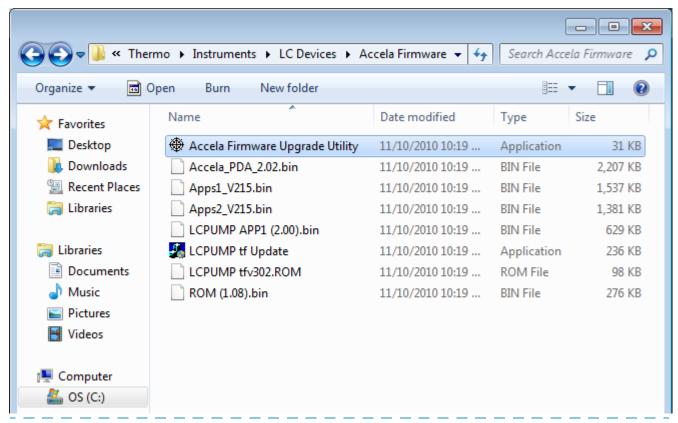
For the Xcalibur 2.0.7 or earlier data system, these files reside in the following folder:

drive:\Xcalibur\system\Accela Firmware

For the Thermo Foundation 1.0 or later application, these files reside in the following folder (see Figure 86):

drive:\Thermo\Instruments\LC Devices\Accela Firmware

Figure 86. Accela Firmware folder installed with the Accela devices on the LC Devices CD



2. Make a note of the current settings of the rotary switches on the back panel of the Accela Autosampler.

At the conclusion of this procedure, you return the switches to these settings.

- 3. Make sure that your autosampler is connected to the computer through the standard Ethernet connection.
- 4. Turn off the power to the Accela Autosampler.
- 5. Use a small, flat-head screwdriver to set the rotary switches on the back panel of the autosampler to 00.
- 6. Turn the autosampler power on.

The Communication LED blinks amber to confirm that the Accela Autosampler is configured for downloading the firmware.

Downloading Firmware to the Autosampler

To download firmware to the Accela Autosampler

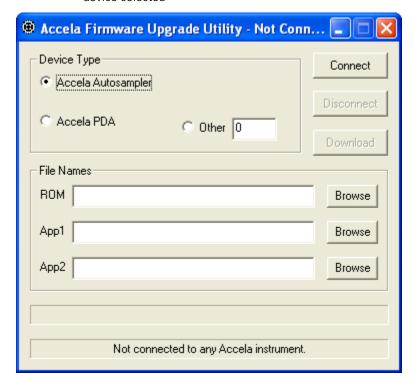
- 1. Start the Accela Firmware Upgrade Utility by doing one of the following:
 - For the Xcalibur data system, browse to the following directory, and then double-click Accela Firmware Upgrade Utility.exe file.

drive:\Xcalibur\system\Accela Firmware

 For the ChromQuest data system, from the computer desktop, choose Start > Programs > Chromatography > Accela Firmware Upgrade Utility.

The Accela Firmware Upgrade Utility window opens (see Figure 87).

Figure 87. Accela Firmware Upgrade Utility window, showing the Accela Autosampler device selected





CAUTION Avoid interrupting the firmware download process. After you click Connect, do not turn off the power to the Accela Autosampler Plus or close the Accela Firmware Upgrade Utility window until the download process is finished. If you terminate the connection during the download process or lose power to the autosampler, contact your local Thermo Fisher Scientific representative. The firmware board will need to be reprogrammed.

- 2. In the Accela Firmware Upgrade Utility window, initiate communication between the utility and the Accela Autosampler:
 - a. Under Device Type, select the **Accela Autosampler** option (see Figure 87).
 - b. Click **Connect**.

The status display at the bottom of the Accela Firmware Upgrade Utility window indicates that a connection has been made.

- 3. Under File Names, select the appropriate firmware files for the Accela Autosampler:
 - a. For the ROM box, click **Browse** and select **ROM(108).bin**.

The file name appears in the ROM box.

b. For the App1 box, click **Browse** and select **Apps1_V2.15.bin**.

The file name appears in the App1 box.

c. For the App2 box, click **Browse** and select **Apps2_V2.15.bin**.

The file name appears in the App2 box.

IMPORTANT You must select a *ROM* file, an *APP1*, and an *APP2* file. If you do not download these files together, your Accela AS will not work properly when you return it to normal operation.

4. After you select the appropriate firmware files, click **Download** and wait for the download to finish.

The utility notifies you when the download is complete.

Note When the Download Completed Successfully message appears, you can safely turn off the power to the autosampler and close the Accela Firmware Upgrade Utility.

- 5. Return the autosampler to normal operation after you finish upgrading the firmware as follows:
 - a. Close the Accela Firmware Upgrade Utility to terminate the connection with the Accela Autosampler.
 - b. Turn off the power to the Accela Autosampler.

IMPORTANT Make sure to turn the Accela Autosampler power off before adjusting the rotary switches.

c. Set the rotary switches back to their original settings.

IMPORTANT The Accela Autosampler will not connect to the Xcalibur data system when the rotary switch address is set to 00.

d. Turn on the Accela Autosampler power to resume normal operation.

Quick Reference Guides

This appendix contains the following two-page quick reference guides:

- Replacing the Rotor Seal in the Rheodyne Valve
- Replacing the Rotor in the Valco Valve
- Using the Xcalibur Direct Control Commands
- Using the ChromQuest Direct Commands



Replacing the rotor in the Valco injection valve or the rotor seal in a Rheodyne injection valve requires these tools and materials:

Tools and materials	Part number
Rotor seal for the Rheodyne injection valve	00109-99-00021
Rotor seal for the Valco injection valve	00110-03-00019
9/64 in. L-hex wrench	N/A
#2 Phillips screwdriver	N/A
1/4 in. open-end wrench	N/A

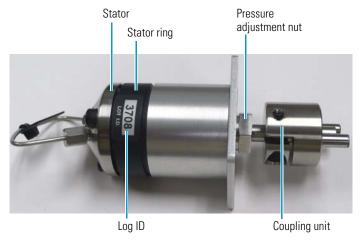
Replacing the Rotor Seal in the Rheodyne Valve

Rheodyne valves shipped with earlier versions of the autosampler.

To replace the rotor seal, follow these steps:

- Step 1: Removing the Injection Valve
- Step 2: Disassembling the Injection Valve
- Step 3: Installing a New Rotor Seal
- Step 4: Reassembling the Injection Valve
- Step 5: Reinstalling the Injection Valve

The rotor seal forms a high-pressure seal between the stator and the rotor. Replace the rotor seal when leaking occurs between the stator and the stator ring or when you notice a decrease in injection precision.



The lot ID sticker on the stator ring identifies the week and year (*WWYY*) of the manufacture date.

Each valve has a pressure adjustment nut that is factory adjusted to the optimal pressure setting. Depending on the manufacture date, you can identify the optimal pressure setting as follows:

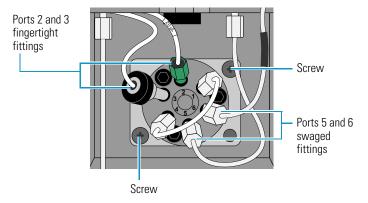
- For valves manufactured on week 38 of 2009 or later, the pressure adjustment nut is fixed at the optimal pressure setting.
- For injection valves manufactured from week 37 of 2008 to week 37 of 2009, the alignment of the factory-applied, red mark on the valve body and the dimple on the pressure adjustment nut corresponds to the optimal pressure setting for the valve.
- For injection valves manufactured before week 37 of 2008, the factory-applied red marks do not correspond to the optimal pressure setting. You must make your own marks.

Manufacture date	Alignment markings	Adjustable pressure nut
Initial release to week 36 of 2008	No	Yes
Week 37 of 2008 to week 37 of 2009	Yes	Yes
Week 38 of 2009 or later	No	No

Step 1: Removing the Injection Valve

To remove the injection valve from the autosampler

- 1. Disconnect the fingertight fittings from ports 2 and 3.
- 2. Using a 1/4 in. open-end wrench, loosen and remove the swaged fittings from ports 5 and 6.
- 3. Using a #2 Phillips screwdriver, loosen and remove the two screws that secure the valve to the autosampler, and then pull the valve out of the autosampler.

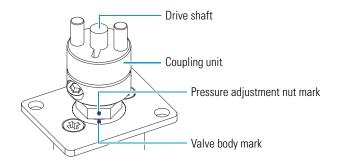


Step 2: Disassembling the Injection Valve

❖ To disassemble the injection valve

- 1. Determine the manufacture date of the valve.
- 2. For valves manufactured before week 37 of 2008, make reference marks on the pressure adjustment nut and the valve body to identify the current alignment.

After you replace the rotor seal and reassemble the valve, check the alignment of these marks to ensure that the position of the pressure adjustment nut is unchanged.

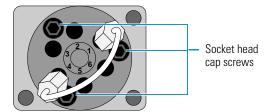




CAUTION Do not remove the coupling unit from the drive shaft of the injection valve. If you do remove the coupling unit, return the valve to Thermo Fisher Scientific for realignment.



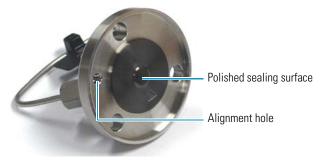
3. Using a 9/64 in. hex wrench, remove the three socket head cap screws that secure the stator to the valve.





CAUTION The polished (sealing) surface of the stator contains six easily damaged ports. Avoid touching this polished surface as you remove the stator from the injection valve. Do not place the polished surface face down on a hard surface.

4. Pull the stator off the valve. Place it on its side on a clean surface.



- 5. If the polished surface of the stator is scratched, replace the valve.
- 6. Pull the stator ring off the valve.
- 7. Pull the rotor seal off the three pins on the drive shaft.
- 8. Inspect the valve components for contamination. Clean as necessary.

Step 3: Installing a New Rotor Seal

❖ To install a new rotor seal

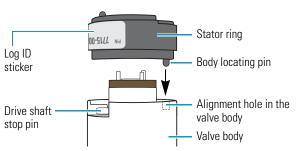
Hold the rotor seal with the engraved flow passages facing away from the drive shaft and mount the rotor seal onto the drive shaft's three alignment pins. The pins align with the rotor seal in only one way.



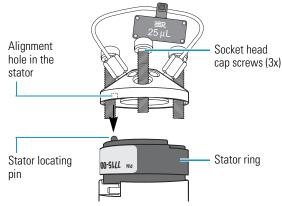
Step 4: Reassembling the Injection Valve

To reassemble the injection valve

- 1. Ensure that the drive shaft stop pin is positioned within the slot in the valve body.
- 2. To mount the stator ring onto the valve body, align the body locating pin with the alignment hole in the valve body. Then insert the body locating pin into the alignment hole.



3. To mount the stator onto the stator ring, hold the stator with its polished surface facing the stator ring. Align the alignment hole in the stator with the stator locating pin on the stator ring. Then insert the locating pin into the alignment hole.

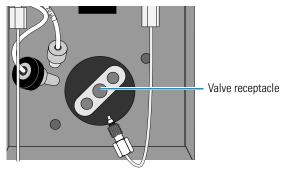


- 4. Insert the three socket head cap screws into the stator.
- 5. Using a 9/64 in. L-hex wrench, tighten each screw a little at a time, keeping the stator surface parallel to the stator ring surface.
- 6. If you have an adjustable torque wrench with a 1/4 in. shaft and a hex driver attachment, evenly torque the three socket head cap screws to 20 inch-pounds.
- 7. For valves manufactured before week 38 of 2009, ensure that the position of the pressure alignment nut is unchanged.

Step 5: Reinstalling the Injection Valve

To reinstall the injection valve

1. Align the holes in the valve plate with the two small holes to the left and right of the valve receptacle, and then insert the valve drive shaft into the valve receptacle.



- 2. Using a #2 Phillips screwdriver, tighten the screws that secure the valve to the autosampler.
- 3. Reconnect the transfer tubing to port 2 and the waste tubing to port 3. Hand-tighten the fittings.
- 4. Reconnect the heat exchanger outlet to port 5. Hand-tighten the fitting, and then tighten the fitting an additional 90 degrees (1/4 turn) with a 1/4 in. open-end wrench.
- 5. Start the solvent flow from the LC pump, and check for leaks.

Replacing the Rotor in the Valco Valve

The rotor forms a high-pressure seal with the stator. Replace the rotor when you notice a leak between either the stator and the rotor or the adjacent ports of the injection valve, or when you notice a decrease in injection precision.

Contents

- Step 1: Removing the Injection Valve
- Step 2: Disassembling the Injection Valve
- Step 3: Cleaning the Stator
- Step 4: Installing the Rotor
- Step 5: Reinstalling the Injection Valve

Replacing the rotor seal requires these tools.

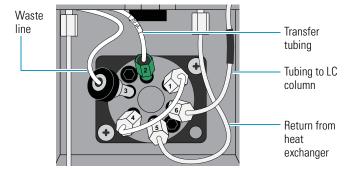
Tool	Use
1/4 in. open-end wrench	To remove the tubing from injection valve ports 5 and 6
9/64 in. hex wrench	To disassemble the injection valve
#2 Phillips screwdriver	To disconnect the valve from the autosampler

IMPORTANT Because component cleanliness affects the useful life of the injection valve, replace the rotor seal in a clean environment.

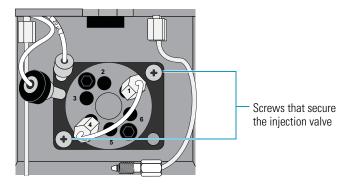
Step 1: Removing the Injection Valve

To remove the injection valve

- 1. Disconnect the waste tubing from port 3 of the injection valve.
- 2. Disconnect the transfer tubing from port 2 of the injection valve.
- 3. Using a 1/4 in. open-end wrench, disconnect the 1/16 in. ID high-pressure tubing from ports 5 and 6.



4. Using a #2 Phillips screwdriver, remove the two screws that secure the injection valve to the autosampler.



5. Slide the injection valve out of the autosampler.

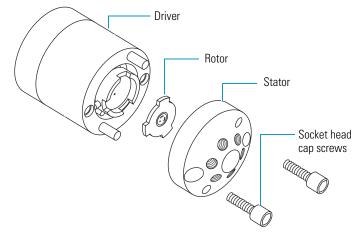
Step 2: Disassembling the Injection Valve

To disassemble the injection valve

1. Using a 9/64 in. L-hex wrench, remove the two socket head cap screws that secure the stator to the stator ring and the valve body.



CAUTION The polished (sealing) surface of the stator contains six ports that excess handling can easily damage. As you remove the stator from the injection valve, avoid touching this polished surface, and never place the polished surface face down on a hard surface.



- 2. Gently pry the rotor seal away from the driver.
- 3. Examine the sealing surface of the rotor seal for scratches. If scratches are visible, replace the rotor seal.



Step 3: Cleaning the Stator

❖ To clean the stator

- 1. Inspect the stator to determine if it requires cleaning.
- 2. If the stator is dirty, swab it with HPLC-grade methanol. If more stringent cleaning is required, use a sonication bath.
- 3. Inspect the remaining valve components for contamination. Clean as necessary.

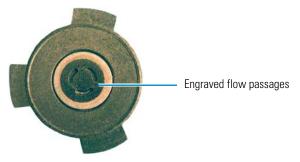
IMPORTANT If the stator is scratched, replace it. Scratches can damage the rotor seal and cause cross-port leaks.

Step 4: Installing the Rotor

❖ To install a new rotor

1. Replace the rotor in the driver, making sure that the rotor sealing surface with its engraved flow passages is facing out.

The pattern is asymmetrical to prevent improper placement.

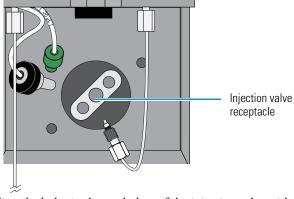


- 2. Replace the stator.
- 3. Insert the two socket head screws.
- 4. Alternating between the screws, slowly tighten the screws until both are snug. Do not overtighten them.

Step 5: Reinstalling the Injection Valve

❖ To reinstall the injection valve

1. Align the drive shaft and the two prongs of the coupling unit with the three holes in the injection valve port located in the lower portion of the column oven compartment. Then insert the injection valve into the receptacle.



- 2. Align the holes in the steel plate of the injection valve with the small holes next to the injection valve receptacle.
- 3. Using the two screws that you removed in step 4 on page 139, secure the injection valve to the autosampler.

- 4. Reconnect the solvent lines as follows:
 - Hand-tighten the transfer tube fitting to port 2 and the waste tube fitting to port 3. Never use a wrench to tighten these fittings.



CAUTION Never use a wrench to tighten the transfer tube fitting. Over-tightening the fitting can do the following:



- Restrict the end of the tubing, which leads to less sample reaching the sample loop and results in smaller than expected peak areas or blank injections. A restriction in the end of the tubing can also cause the syringe to make a grinding sound during an injection sequence.
- Strip the threads on the fitting, which leads to solvent leaks.
- Hand-tighten the waste tube fitting to port 3.
- Press the end of the return tubing from the heat exchanger to the bottom of port 5 as you hand-tighten the high-pressure fitting. Then use a wrench to tighten the fitting by an additional 1/4 turn.
- Using high-pressure tubing and two high-pressure fittings, reconnect the LC column to port 6 of the injection valve.

Note To reconnect tubing with a pre-swaged stainless steel fitting to a valve port, hand-tighten the fitting as you press the tubing against the bottom of the port, and then use a wrench to tighten the fitting by an additional 1/4 to 1/2 turn.

To connect tubing with a two-piece stainless steel fitting to a mating port, press the tubing against the bottom of the port as you hand-tighten the fitting. Then tighten the fitting by an additional 3/4 turn with a wrench. Once you use the wrench to tighten the fitting, the ferrule portion of the two-piece fitting is permanently connected to the tubing.

5. Turn on the solvent flow from the LC pump and check for leaks.

Using the Xcalibur Direct Control Commands

The On/Off switch is the only manual control provided with the Accela Autosampler. To perform tasks, such as moving the XYZ arm to the back of the tray compartment, use the direct control commands in the Instrument Setup window or the tune application for your Thermo Scientific mass spectrometer.

Direct Control Commands

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.
Flush syringe at Fill position	Flushes the needle tubing, the interior of the needle, and the sample loop with flush solvent.
Wash needle	Washes the exterior of the needle with solvent.
Needle removal	Sets the needle to the needle removal position.
	CAUTION. To avoid damaging the needle, execute this command before you remove the needle.
Set oven temperature	Sets the temperature of the column oven compartment without downloading an instrument method. The allowable temperature range is 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 allowable temperature range is 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. The available parameters are Vial, Volume, and Injection Mode.
	 The allowable vial or well entries depend on the tray configuration: For conventional trays, the allowable entries are A:01 to E:40. For 96-well plates, the allowable entries are A:A1 to C:H12. For 384-well plates, the allowable entries are A:A1 to C:P24.
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.



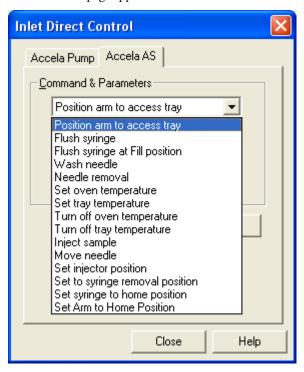
Applying a Direct Command

You can access the direct control commands from the Xcalibur 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.



❖ To open the Direct Control dialog box from the Xcalibur Roadmap view

1. Click the **Instrument Setup** icon.

The Instrument Setup window appears. The view bar on the left side of the window displays the configured instruments.

- 2. In the view bar, click the **Accela AS** icon. The Instrument Setup window for the autosampler appears.
- 3. From the menu bar, choose Accela AS > Direct Control. The Direct Control dialog box appears.



To apply a direct command

1. In the Commands list, select a command.

If the command requires additional parameters, these parameters appear below the Commands list. Make the appropriate entries and selections.

2. To execute the command, click **Apply**.

Using the ChromQuest Direct Commands

The On/Off switch is the only manual control provided with the Accela Autosampler. To perform tasks, such as moving the XYZ arm to the back of the tray compartment, use the direct commands.

Direct Commands

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.
Flush With Injection In Fill Position	Flushes the needle tubing, the interior of the needle, and the sample loop with flush solvent.
Set Oven Temperature	Sets the temperature of the column oven compartment without downloading an instrument method. The allowable temperature range is 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 allowable temperature range is 0 to 60 °C.
Turn Off Column Oven	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.
Temperature Control Upload	Determines if the autosampler has the optional temperature control feature.
Wash Needle	Washes the exterior of the needle with solvent.
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.
Initialize Hardware	Performs the following sequence of operations:
	 Switches the two-way syringe valve to the flush bottle position. Homes the syringe. Sets the syringe plunger back to the ready position. Switches the two-way syringe valve back to the needle position. Homes the syringe. After the autosampler initializes, it goes into the Waiting for Download state.
Position Arm to	Sets the needle to the needle removal position.
Replace Needle	CAUTION . To avoid damaging the needle, execute this command before you remove the needle.
Position Syringe to Home	Sets the syringe to its home position.
Position Syringe for Removal	Sets the syringe to its removal position.
Go to Home	Moves the XYZ arm to its home position, which is just above the injection port.

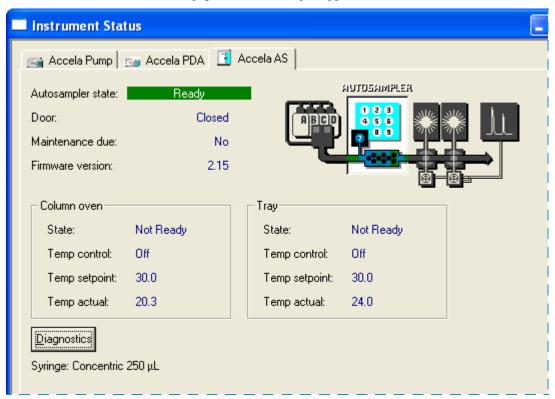


Submitting a Direct Command

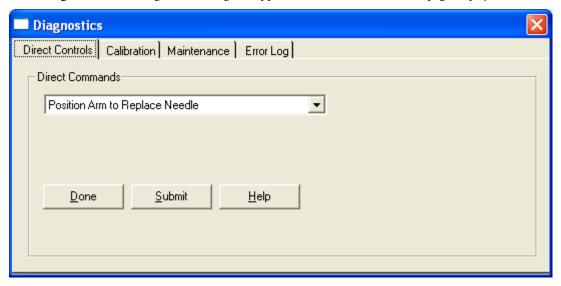
You can access the direct control commands from the Instrument Status window.

❖ To submit a direct command

- 1. In the online Instrument window, choose **Control > Instrument Status**. The Instrument Status window appears.
- 2. Click the **Accela AS** tab. The status page for the autosampler appears.



3. Click Diagnostics. The Diagnostics dialog box appears with the Direct Controls page displayed.



- 4. In the Direct Commands list, select a command.
 - If the command requires additional parameters, these parameters appear below the Commands list.
- 5. Make the appropriate entries and selections.
- 6. To execute the command, click **Submit**.

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