

Waters™

Alliance iS HPLC Systems User Guide

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1 General information

1.1 Copyright notice

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1.2 About Alliance iS Systems documentation

Alliance iS HPLC systems have extensive online documentation. You can access the documentation by browsing www.waters.com or clicking the Help button from the system touchscreen.

From the Waters Help Center (<https://help.waters.com/help/en.html>), you can search content for terms or phrases or click **I need help with a product** to browse to the Product Support page (<https://help.waters.com/help/en/product-support.html>). From this page, you can search content or click **Alliance iS HPLC System** to access the Alliance iS HPLC System Support page (<https://help.waters.com/help/en/product-support/alliance-is-system-support.html>). From this page, you can search content or open a specific document.

Note: The Empower CDS has online documentation that includes user guides and Help accessed from the user interface.

1.2.1 Searching for information

A search box appears at the top of the pages in the Waters Help Center, including the Alliance iS System Support page (<https://help.waters.com/help/en/product-support/alliance-is-system-support.html>). Using this box, you can find the specific information you need by conducting searches of varying complexity.

There are two types of plain-text search terms: single words and phrases. You must enclose phrases in quotation marks, as in "data acquisition". The Boolean AND operator is implied. The search function looks at topic titles and for specific keywords.

If your search yields no results, or results that do not provide the information you seek, try searching for different terms or removing quotation marks from phrases.

If there are too many results, try using the exclusionary Boolean OR or NOT operators.

1.3 Trademarks

Alliance™ is a trademark of Waters Corporation.

eConnect™ is a trademark of Waters Corporation.

Empower™ is a trademark of Waters Corporation.

MaxPeak™ is a trademark of Waters Corporation.

MP35N® is a registered trademark of SPS Technologies, Inc.

TaperSlit™ is a trademark of Waters Corporation.

Waters™ is a trademark of Waters Corporation.


Waters Quality Parts™ is a trademark of Waters Corporation.

All other trademarks are property of their respective owners.

1.4 Safety considerations

Some reagents and samples used with Waters instruments and devices can pose chemical, biological, or radiological hazards (or any combination thereof). You must know the potentially hazardous effects of all substances you work with. Always follow good laboratory practices and consult your organization's standard operating procedures as well as your local requirements for safety.

1.4.1 Safety hazard symbol notice

The  symbol indicates a potential hazard. Consult the documentation for important information about the hazard and the appropriate measures to prevent and control the hazard.

1.4.2 Electrical power safety notice

Do not position the device so that it is difficult to disconnect the power cord.

1.4.3 Equipment misuse notice

If equipment is used in a manner not specified by its manufacturer, the protection provided by the equipment may be impaired.

1.4.4 Safety advisories









Consult the "Safety advisories" appendix in this publication for a comprehensive list of warning advisories and notices.












1.5 Operating the device

When operating the device, follow standard quality-control (QC) procedures and the guidelines presented in this section.

1.5.1 Applicable symbols

The following symbols can be present on the device, system, or packaging.

Symbol	Definition
	Manufacturer
	Date of manufacture
	Confirms that a manufactured product complies with all applicable European Community directives
	UK Conformity Assessed marking confirms that a manufactured product is in conformity with the applicable requirements for products sold within Great Britain
	Australia EMC compliant
	Confirms that a manufactured product complies with all applicable United States and Canadian safety requirements
	Confirms that a manufactured product complies with all applicable United States and Canadian safety requirements
	Environmentally friendly use period (China RoHS): indicates the number of years from the date of manufacture until the product, or components within the product, are likely to be discarded or degrade into the environment

Symbol	Definition
	ACT (Accountability, Consistency, and Transparency) is an environmental impact factor label that provides third-party verification of the sustainable impacts of a life science lab product, its operations, and its end of life.
	Consult instructions for use
	Alternating current
	Electrical and electronic equipment with this symbol may contain hazardous substances and should not be disposed of as general waste. For compliance with Waste Electrical and Electronic Equipment legislation, contact Waters Corporation for the correct disposal and recycling instructions.
	For indoor use only
	No pushing
	Do not connect to an LC system
	Indicates the maximum load you can place on that item (for example, 10kg)
	Indicates that the part can be cleaned in an ultrasonic bath
	Serial number
	Part number, catalog number

1.5.2 Audience and purpose

This guide is intended for use only by professionally trained and qualified laboratory personnel who operate and maintain Waters products.

1.5.3 Intended use of the system

The Alliance iS HPLC systems perform liquid chromatography to separate, identify, and quantify components of a liquid mixture. It supports column particle sizes down to 2.7 µm and pressures up to 12,000 psi for routine laboratory analyses. The Alliance iS HPLC system comes standard with UV absorbance detection in the form of a TUV or a PDA detector.

Note: The Alliance iS Column Heater Cooler (CHC) is an AC-powered device that houses the fluidics column used to perform HPLC (High Pressure Liquid Chromatography) separations for the Alliance iS HPLC systems. The CHC assembly can provide temperature to the column from 4 °C to 90 °C. If the LC column is equipped with a passive NFC tag (13.56 MHz), it is read when the front door of the CHC is closed. The antenna circuitry is normally not active until the door closure service event. Data from the tag is stored in the system.

1.5.4 Information on the Alliance iS Bio HPLC System

The Alliance iS Bio HPLC System is a bio-inert HPLC solution designed to address the specific challenges faced by quality control labs running biopharmaceutical applications. The system's flow path is designed with biocompatible materials of construction and Waters MaxPeak High Performance Surfaces (HPS) technology.

1.5.5 Calibrating

To calibrate LC systems, adopt acceptable calibration methods using at least five standards to generate a standard curve. The concentration range for standards must include the entire range of QC samples, typical specimens, and atypical specimens.

1.5.6 Quality control

Routinely run three QC samples that represent subnormal, normal, and above-normal levels of a compound. If sample trays are the same or very similar, vary the location of the QC samples in the trays. Ensure that QC sample results fall within an acceptable range, and evaluate precision from day to day and run to run. Data collected when QC samples are out of range might not be valid. Do not report these data until you are certain that the instrument performs satisfactorily.

1.6 EMC considerations

1.6.1 FCC radiation emissions notice

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

1.6.2 Near field communications (NFC)/RFID aspects

The Alliance iS Column Heater Cooler (CHC) is used with Alliance iS HPLC systems. The CHC can be equipped with NFC/RFID technology. The national approvals associated with this RF feature are associated only with the CHC and not with other sections of the system or the system in its entirety. The 13.56-MHz NFC/RFID reader is located on the door of the CHC. It performs a read cycle when the door is closed. The duration of the read cycle is less than one second. It is inactive until the next door open/door close event. The power is less than 2 W.

1.6.3 ISM classification: ISM group 1 class A

This classification was assigned in accordance with CISPR 11 Industrial Scientific and Medical (ISM) instruments requirements.

Group 1 products apply to intentionally generated and/or used conductively coupled radio-frequency energy that is necessary for the internal functioning of the equipment.

Class A products are suitable for use in all establishments other than residential locations and those directly connected to a low-voltage power supply network supplying a building for domestic purposes.

There may be potential difficulties in ensuring electromagnetic compatibility in other environments due to conducted and radiated disturbances.

This equipment complies with the emission and immunity requirements described in the relevant parts of IEC/EN 61326: Electrical equipment for measurement, control, and laboratory use — EMC requirements.

1.6.4 Canada spectrum management emissions notice




This class A digital product apparatus complies with Canadian ICES-001.

Cet appareil numérique de la classe A est conforme à la norme NMB-001.

1.6.5 Other country-specific EMC considerations

The following country-specific considerations apply to the use of the Alliance iS Column Heater Cooler (CHC).

Brazil	Este equipamento não tem direito à proteção contra interferência prejudicial e não pode causar interferência em sistemas devidamente autorizados. Para maiores informações, consulte o site da ANATEL – www.anatel.gov.br
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Korea	
Taiwan	<p>取得審驗證明之低功率射頻器材，非經核准，公司、商號或使用者均不得擅自變更頻率、加大功率或變更原設計之特性及功能。低功率射頻器材之使用不得影響飛航安全及干擾合法通信；經發現有干擾現象時，應立即停用，並改善至無干擾時方得繼續使用。前述合法通信，指依電信管理法規定作業之無線電通信。低功率射頻器材須忍受合法通信或工業、科學及醫療用電波輻射性電機設備之干擾。</p>
Thailand	<div data-bbox="436 827 1130 1520" style="border: 1px solid black; padding: 10px;">  <p>เครื่องวิทยุคมนาคมนี้ ได้รับยกเว้น ไม่ต้องได้รับใบอนุญาตให้มี ใช้ซึ่งเครื่องวิทยุคมนาคม หรือตั้งสถานีวิทยุคมนาคมตามประกาศ กสทช. เรื่อง เครื่องวิทยุคมนาคม และสถานีวิทยุคมนาคมที่ได้รับยกเว้นไม่ต้องได้รับใบอนุญาต วิทยุคมนาคมตามพระราชบัญญัติวิทยุคมนาคม พ.ศ. 2498</p>  <p>nabp. โทรคมนาคม กำกับดูแลเพื่อประชาชน Call Center 1200 (InSW5)</p> </div> <p>เครื่องโทรคมนาคมและอุปกรณ์นี้มีความสอดคล้องตามมาตรฐานหรือข้อกำหนดทางเทคนิคของ กสทช.</p>

1.7 Additional resources

Waters provides the following additional resources to ensure your continued success with our products.

Knowledge base: Obtain quick answers to your troubleshooting questions. Access support articles on Waters instrumentation, informatics, and chemistry.

eLearning courses: Learn anytime, anywhere, and at your own pace with eLearning courses.

Customer education: Waters Educational services team is the leading training organization empowering scientists to maximize their skills in UPLC, HPLC, LC-MS, and data management.

Application notes: View our online digital library of Application notes for advanced analytical technologies including chromatography, mass spectrometry, columns and sample preparation, and data management software, demonstrating impactful scientific and operational benefits.

How-to video library: View/download the latest product how-to videos.

Graphical parts locator: Identify and order parts using an interactive graphical navigator. Access maintenance procedures and reference documents.

Product selection tools and resources: A collection of wizards that help you pick the correct chemistry consumables to meet your separation requirements, including vials, plates, filters, column selectivity charts, and more.

1.8 Contacting Waters

Contact Waters with technical questions regarding the use, transportation, removal, or disposal of any Waters product. You can reach us through the Internet, telephone, fax, or conventional mail.

Contact method	Information
www.waters.com	The Waters website includes contact information for Waters locations worldwide.
iRequest	iRequest is a secure Web service form that allows you to request support and service for Waters instruments and software or to schedule a planned service activity. These types of support and services may be included as part of your maintenance plan or support plan. You may be charged for the requested service if you do not have appropriate plan coverage for your product. Note: In areas managed by authorized distributors, iRequest may not be available. Contact your local distributor for more information.
Local office contact information	For worldwide locations, telephone, fax, and conventional mail information is available at the Local Offices website.

Contact method	Information
Corporate contact information	Waters Corporation 34 Maple Street Milford, MA 01757 USA From the USA or Canada, phone 800-252-4752.

1.9 Customer comments

We seriously consider every customer comment we receive. Help us better understand what you expect from our documentation so that we can continuously improve its accuracy and usability. To report any errors that you encounter in this document or to suggest ideas for otherwise improving it, reach us at tech_comm@waters.com.

2 Safety advisories

The following sections present the safety advisories for the Alliance iS HPLC System.

2.1 Warning symbols

Warning symbols alert you to the risk of death, injury, or seriously adverse physiological reactions associated with the misuse of an instrument or device. Heed all warnings when you install, repair, or operate any Waters instrument or device. Waters accepts no liability in cases of injury or property damage resulting from the failure of individuals to comply with any safety precaution when installing, repairing, or operating any of its instruments or devices.

The following symbols warn of risks that can arise when you operate or maintain a Waters instrument or device or component of an instrument or device. When one of these symbols appears in a manual's narrative sections or procedures, an accompanying statement identifies the applicable risk and explains how to avoid it.



Warning: (General risk of danger. When this symbol appears on an instrument, consult the instrument's user documentation for important safety-related information before you use the instrument.)



Warning: (Risk of burn injury from contacting hot surfaces.)



Warning: (Risk of electric shock.)



Warning: (Risk of fire.)



Warning: (Risk of sharp-point puncture injury.)



Warning: (Risk of hand crush injury.)



Warning: (Risk of injury caused by moving machinery.)



Warning: (Risk of exposure to ultraviolet radiation.)



Warning: (Risk of contacting corrosive substances.)



Warning: (Risk of exposure to a toxic substance.)



Warning: (Risk of personal exposure to laser radiation.)



Warning: (Risk of exposure to biological agents that can pose a serious health threat.)



Warning: (Risk of tipping.)



Warning: (Risk of explosion.)



Warning: (Risk of high-pressure gas release.)

2.2 Notices

Notice advisories appear where an instrument, device, or component can be subject to use or misuse that can damage it or compromise a sample's integrity. The exclamation point symbol and its associated statement alert you to such risk.



Notice: To avoid damaging the case of the instrument or device, do not clean it with abrasives or solvents.

2.3 Bottles Prohibited symbol

The Bottles Prohibited symbol alerts you to the risk of equipment damage caused by solvent spills.



Prohibited: To avoid equipment damage caused by spilled solvent, do not place reservoir bottles directly atop an instrument or device or on its front ledge. Instead, place the bottles in the bottle tray, which serves as secondary containment in the event of spills.

2.4 Required protection

The Use Eye Protection and Wear Protective Gloves symbols alert you to the requirement for personal protective equipment. Select appropriate protective equipment according to your organization's standard operating procedures.



Requirement: Use eye protection when performing this procedure.



Requirement: Wear clean, chemical-resistant, powder-free gloves when performing this procedure.

2.5 Warnings that apply to all Waters instruments and devices

When operating this device, follow standard quality-control procedures and the equipment guidelines in this section.



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



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



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



Avvertenza: Qualsiasi modifica o alterazione apportata a questa unità e non espressamente autorizzata dai responsabili per la conformità fa decadere il diritto all'utilizzo dell'apparecchiatura da parte dell'utente.



Advertencia: Cualquier cambio o modificación efectuado en esta unidad que no haya sido expresamente aprobado por la parte responsable del cumplimiento puede anular la autorización del usuario para utilizar el equipo.



警告： 未经有关法规认证部门明确允许对本设备进行的改变或改装，可能会使使用者丧失操作该设备的合法性。



警告： 未經有關法規認證部門允許對本設備進行的改變或修改，可能會使使用者喪失操作該設備的權利。



경고: 규정 준수를 책임지는 당사자의 명백한 승인 없이 이 장치를 개조 또는 변경할 경우, 이 장치를 운용할 수 있는 사용자 권한의 효력을 상실할 수 있습니다.



警告: 規制機関から明確な承認を受けずに本装置の変更や改造を行うと、本装置のユーザーとしての承認が無効になる可能性があります。



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

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



Avertissement : Manipulez les tubes en polymère sous pression avec précaution:

- Portez systématiquement des lunettes de protection à proximité de tubes en polymère sous pression.
- Éteignez toute flamme se trouvant à proximité de l'instrument.
- Évitez d'utiliser des tubes sévèrement déformés ou endommagés.
- N'exposez pas les tuyaux non métalliques au tétrahydrofurane, ou THF, ou à de l'acide nitrique ou sulfurique concentré.
- Sachez que le chlorure de méthylène et le diméthylesulfoxyde entraînent le gonflement des tuyaux non métalliques, ce qui réduit considérablement leur pression de rupture.



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

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



Avvertenza: Fare attenzione quando si utilizzano tubi in materiale polimerico sotto pressione:

- Indossare sempre occhiali da lavoro protettivi nei pressi di tubi di polimero pressurizzati.
- Spegnerne tutte le fiamme vive nell'ambiente circostante.
- Non utilizzare tubi eccessivamente logorati o piegati.
- Non utilizzare tubi non metallici con tetraidrofurano (THF) o acido solforico o nitrico concentrati.
- Tenere presente che il cloruro di metilene e il dimetilsolfossido provocano rigonfiamento nei tubi non metallici, riducendo notevolmente la resistenza alla rottura dei tubi stessi.



Advertencia: Se recomienda precaución cuando se trabaje con tubos de polímero sometidos a presión:

- El usuario deberá protegerse siempre los ojos cuando trabaje cerca de tubos de polímero sometidos a presión.
- Apagar cualquier llama que pueda estar encendida en las proximidades.
- No se debe trabajar con tubos que se hayan doblado o sometido a altas presiones.
- Es necesario utilizar tubos de metal cuando se trabaje con tetrahidrofurano (THF) o ácidos nítrico o sulfúrico concentrados.
- Hay que tener en cuenta que el diclorometano y el dimetilsulfóxido dilatan los tubos no metálicos, lo que reduce la presión de ruptura de los tubos.



警告： 当有压力的情况下使用聚合物管线时，小心注意以下几点：

- 当接近有压力的聚合物管线时一定要戴防护眼镜。
- 熄灭附近所有的火焰。
- 不要使用已经被压瘪或严重弯曲的管线。
- 不要在非金属管线中使用四氢呋喃或浓硝酸或浓硫酸。
- 要了解使用二氯甲烷及二甲基亚砷会导致非金属管线膨胀，大大降低管线的耐压能力。



警告： 當在有壓力的情況下使用聚合物管線時，小心注意以下幾點。

- 當接近有壓力的聚合物管線時一定要戴防護眼鏡。
- 熄滅附近所有的火焰。
- 不要使用已經被壓扁或嚴重彎曲管線。
- 不要在非金屬管線中使用四氫呋喃或濃硝酸或濃硫酸。
- 要了解使用二氯甲烷及二甲基亞砷會導致非金屬管線膨脹，大大降低管線的耐壓能力。



경고: 가압 폴리머 튜브로 작업할 경우에는 주의하십시오.

- 가압 폴리머 튜브 근처에서는 항상 보호 안경을 착용하십시오.
- 근처의 화기를 모두 끄십시오.
- 심하게 변형되거나 꼬인 튜브는 사용하지 마십시오.
- 비금속(Nonmetallic) 튜브를 테트라히드로푸란(Tetrahydrofuran: THF) 또는 농축 질산 또는 황산과 함께 사용하지 마십시오.
- 염화 메틸렌(Methylene chloride) 및 디메틸설폭사이드(Dimethyl sulfoxide)는 비금속 튜브를 부풀려 튜브의 파열 압력을 크게 감소시킬 수 있으므로 유의하십시오.



警告: 圧力のかかったポリマーチューブを扱うときは、注意してください。

- 加圧されたポリマーチューブの付近では、必ず保護メガネを着用してください。
- 近くにある火を消してください。
- 著しく変形した、または折れ曲がったチューブは使用しないでください。
- 非金属チューブには、テトラヒドロフラン (THF) や高濃度の硝酸または硫酸などを流さないでください。
- 塩化メチレンやジメチルスルホキシドは、非金属チューブの膨張を引き起こす場合があります、その場合、チューブは極めて低い圧力で破裂します。

This warning applies to Waters instruments fitted with nonmetallic tubing or operated with flammable solvents.



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



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



Warnung: Der Benutzer wird darauf aufmerksam gemacht, dass bei unsachgemäßer Verwendung des Gerätes die eingebauten Sicherheitseinrichtungen unter Umständen nicht ordnungsgemäß funktionieren.



Avvertenza: Si rende noto all'utente che l'eventuale utilizzo dell'apparecchiatura secondo modalità non previste dal produttore può compromettere la protezione offerta dall'apparecchiatura.



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



警告： 使用者必须非常清楚如果设备不是按照制造厂商指定的方式使用，那么该设备所提供的保护将被削弱。



警告： 使用者必須非常清楚如果設備不是按照製造廠商指定的方式使用，那麼該設備所提供的保護將被消弱。



경고: 제조업체가 명시하지 않은 방식으로 장비를 사용할 경우 장비가 제공하는 보호 수단이 제대로 작동하지 않을 수 있다는 점을 사용자에게 반드시 인식시켜야 합니다.



警告: ユーザーは、製造元により指定されていない方法で機器を使用すると、機器が提供している保証が無効になる可能性があることに注意して下さい。

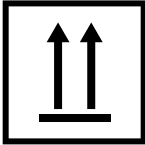



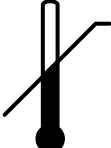
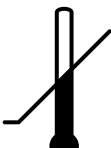
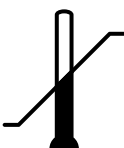
2.6 Electrical symbols

The following electrical symbols and their associated statements can appear in instrument manuals and on an instrument's front or rear panels.

Symbol	Description
	Electrical power on
	Electrical power off
	Standby
	Direct current
	Alternating current
	Alternating current (three phase)
	Safety ground
	Frame or chassis terminal connection
	Fuse
	Functional ground
	Input
	Output
	Indicates that the device or assembly is susceptible to damage from electrostatic discharge (ESD)

2.7 Handling symbols

The following handling symbols and their associated statements can appear on labels affixed to the packaging in which instruments, devices, and component parts are shipped.

Symbol	Description
	Keep upright!
	Keep dry!
	Fragile!
	Use no hooks!
	Upper limit of temperature
	Lower limit of temperature
	Temperature limitation

3 System overview

The Alliance iS HPLC System is the first-ever purpose-built HPLC system designed for Waters Empower Chromatography Data System (CDS), focused on improving outcomes for QC laboratories.

With specifically engineered capabilities, this system helps you reduce inefficiencies and complexity, improve method transfer and migration success rates, and ensure timely results by way of:

- Intuitively preventing many common errors
- Providing fast, simple guidance at the point of need
- Boosting productivity and capacity through effective resource utilization and reliable operation
- Driving workflow efficiencies and quality improvements
- Improving data integrity

The Alliance iS Bio HPLC System is designed specifically for biopharmaceutical QC labs. The flow path is designed using biocompatible materials like MP35N and titanium (Ti), along with bio-inert MaxPeak HPS Technology, ensuring resilient and durable performance for even the most challenging biopharmaceutical applications. Combined with MaxPeak HPS Technology, the Alliance iS Bio HPLC System offers:

- Reduction in non-specific adsorption
- Corrosion-resistant materials
- Error reduction as standard
- Intuitively simple guidance at the point of need
- Faster, more reproducible results
- Improved data integrity

3.1 System features

Key system features include an intuitive touchscreen and smart column capabilities.

The Alliance iS system offers a range of new and unique features:

- A system made for the Waters Empower Software Suite (Empower) (see [Empower features \(Page 55\)](#))
- An easy-to-use, advanced touchscreen integrated with Empower software (see [Touchscreen features \(Page 50\)](#))
- System status lighting via the touchscreen

- Color-coded solvent tubing clips to maintain organization and traceability
- The Intelligent Method Translator App (iMTA) (see [Intelligent Method Translator \(Page 56\)](#))
- eConnect HPLC columns (see [eConnect technology \(Page 50\)](#))
- waters_connect System Monitoring via the waters_connect Cloud Platform
- Smart on-board technology linked to the new Waters Help Center
- Corrosion-Resistant and Bio-Inert (Alliance iS Bio HPLC System only)

3.1.1 Flow-through-needle injector

The FTN mechanism facilitates the transfer of LC methods and improves injection accuracy.

The sample manager's FTN mechanism aspirates a sample and holds it in the sample needle, prepared to inject the sample onto the column. The needle serves as part of the injection flow path when the sample is pushed onto the column.

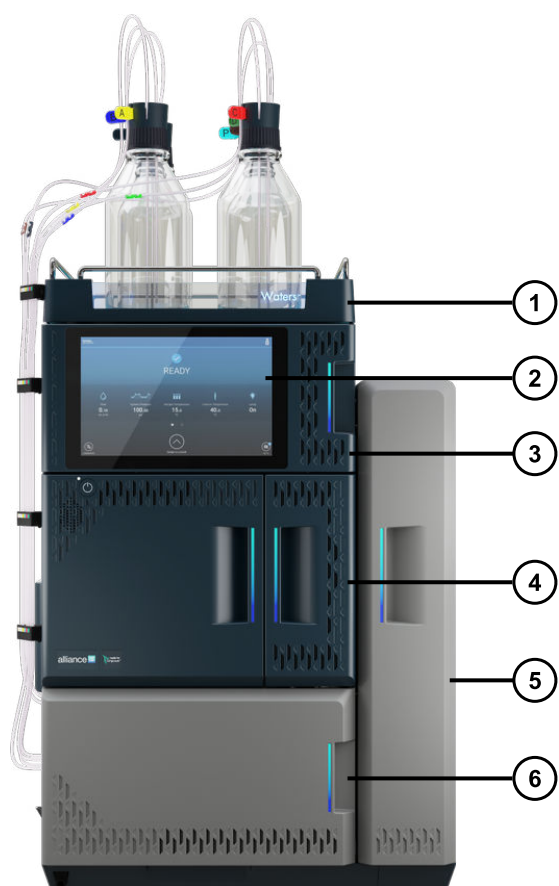
The FTN mechanism decreases cycle time for small-volume injections and does not require learning new injection modes. Mobile phase gradients pass through the needle during injection, ensuring complete sample recovery.

3.2 System components

The system's core modules are a sample manager, pump, and column compartment.

The following figure depicts the system that includes core modules and a detector.

Figure 3–1: Alliance iS system components



- ① Bottle tray
- ② Touchscreen
- ③ Detector
- ④ Sample manager
- ⑤ Column compartment
- ⑥ Pump

The system includes specifically these core modules:

Note: The area inside the system's chassis that houses the sample manager and pump is referred to as the Integrated Fluidics Module (IFM) assembly.

- Sample manager: flow-through-needle (SM-FTN) (See [Sample manager features \(Page 43\)](#).)
- Pump: quaternary solvent manager (QSM) (See [Pump features \(Page 49\)](#).)
- Column compartment: column heater/cooler (CHC) (See [Column heater/cooler features \(Page 49\)](#).)

In addition to the core modules, the system includes a tunable ultraviolet (TUV) or a photodiode array (PDA) detector. See [TUV detector features \(Page 27\)](#) or [PDA detector features \(Page 34\)](#).

3.2.1 TUV detector features

The detector operates as an integral part of a Waters chromatography system.

The Alliance iS tunable ultraviolet (TUV) optical detector is a two-channel, ultraviolet/visible (UV/Vis) absorbance detector. The detector is controlled by Empower and offers an analytical flow cell with a volume of 16.3 µL and a path length of 10 mm.

The flow cell design reflects Waters' TaperSlit technology.

The detector features:

- Improved equilibration and warm-up time
- Enhanced sensitivity using the patented TaperSlit flow cell, which channels light through the cell for better energy throughput
- Lamp optimization for the best performance over the lifetime of a deuterium lamp
- Low baseline noise performance (<5 µAU)
- Enhanced as well as new functionality to mitigate the affects of changes in ambient temperature (see the following capabilities table)
- Flexible sampling rates from 1 to 160 Hz for normal and fast LC separations
- Independent optimization of sampling rates and filter time constants gives you the ability to tune your detector to your separation goals

Table 3–1: TUV detector capabilities

Capability	Description
Two modes of operation	Single- or Dual-Wavelength (See Single-Wavelength mode (Page 32) or Dual-Wavelength mode (Page 33) .)
Automatic, second-order filter	Supports standard absorbance, UV/Vis functionality and spectrum scan feature
Full diagnostic capability	Supports built-in diagnostic tools to optimize functionality and performance

Table 3–1: TUV detector capabilities (continued)

Capability	Description
Thermal wander management (enhanced) and active thermal control (new)	Reduce performance issues due to changes in ambient temperature (See TUV detector thermal wander management and active thermal control (Page 34) .)
For optional cuvette cell: Restriction: You must remove the detector's flow cell before inserting a cuvette cell.	
Cuvette qualification	Facilitates qualification of the detector by insertion of a standard in a cuvette. This feature is supported by Waters qualification kits available for purchase (optional cuvette sold separately).
Cuvette sample analysis	Allows recording of the spectrum of any sample placed in the cuvette

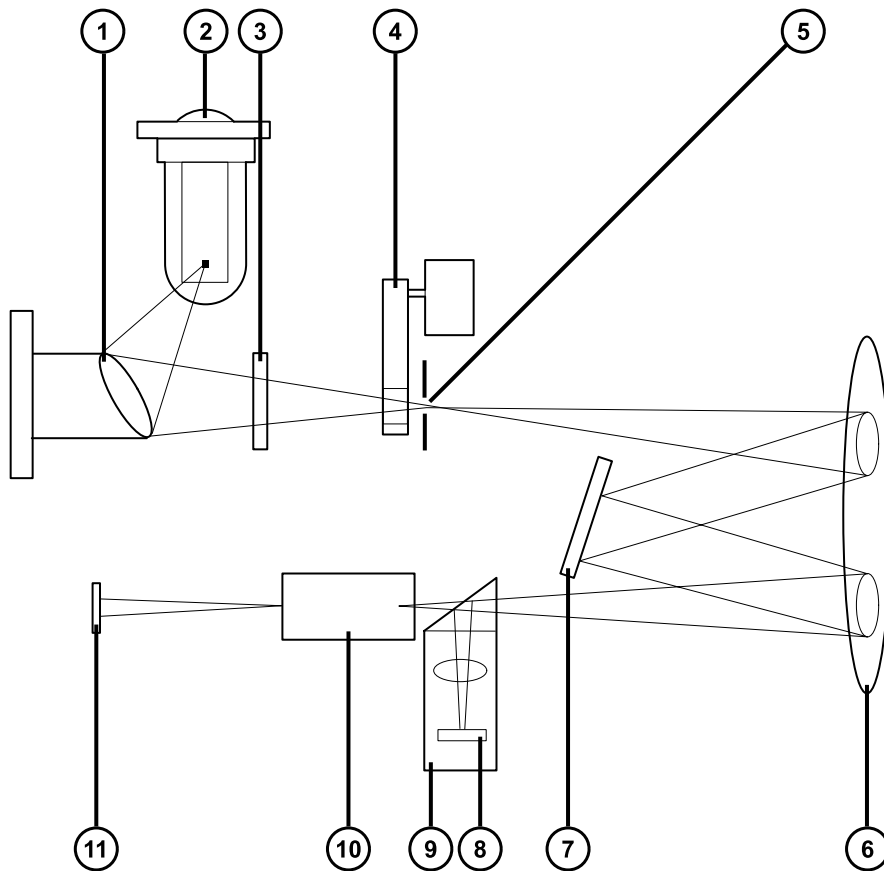
3.2.1.1 TUV detector optics

The detector optics are based on a Fastie-Ebert monochromator.

The following figure shows the components that make up the detector optics assembly.

Note: In addition to what is shown in the figure, detector optics also include a shutter, wavelength calibration filter, and second-order filter.

Figure 3–2: Detector optics assembly



- ① Mirror
- ② High-brightness deuterium (D2) lamp
- ③ Window
- ④ Filter wheel
- ⑤ Slit
- ⑥ Mirror
- ⑦ Grating
- ⑧ Reference photodiode
- ⑨ Beam splitter
- ⑩ TaperSlit flow cell

⑪ Sample photodiode

3.2.1.1.1 TUV optics assembly light path

The TUV detector employs an extremely efficient design for exceptionally high light throughput.

The TUV detector operates as follows:

1. A mirror collects light from the lamp and focuses it through the filter wheel and onto the entrance slit. Another mirror directs light toward the grating, while a different portion of this mirror focuses dispersed light of a particular wavelength band, determined by the grating angle, onto the entrance of the flow cell. Light exits the flow cell to the sample photodiode.
2. The beam splitter, located just ahead of the flow cell, diverts a portion of the light to a reference photodiode.
3. When you specify a new wavelength through the system's touchscreen (or through the Empower software), the detector rotates the grating to the appropriate position.
4. The currents from the photodiodes are integrated and digitized for processing by the signal processing electronics and output to a computer or integrator.

3.2.1.1.2 Filtering noise

To minimize noise, the detector provides a Hamming filter.

The detector's Hamming filter is a digital finite-impulse-response filter that creates peak height degradation and enhances the filtering of high-frequency noise.

The behavior of the filter depends on the filter time constant you select. The filter programming options in the Method Editor are No Filter, Slow, Normal, Fast, and Custom. When selecting Slow, Normal, or Fast, you do not need to specify a value. The filter constant is determined by the sampling rate. When selecting Custom, you can specify a value but it will be rounded up or down to a value based on the sampling rate. Selecting No Filter or Custom and specifying a value of "0.0" disables all filtering.

The filter time constant adjusts the time window over which the data is filtered, thus controlling the degree of baseline smoothing and the impact on peak height degradation. Optimizing this parameter in the method ensures that the highest signal-to-noise ratios are achieved for a particular application.

Faster time constant settings produce these effects:

- Narrow peaks with minimal peak distortion and time delay
- Very small peaks become harder to discriminate from baseline noise
- Less baseline noise is removed

Slower time constant settings produce these effects:

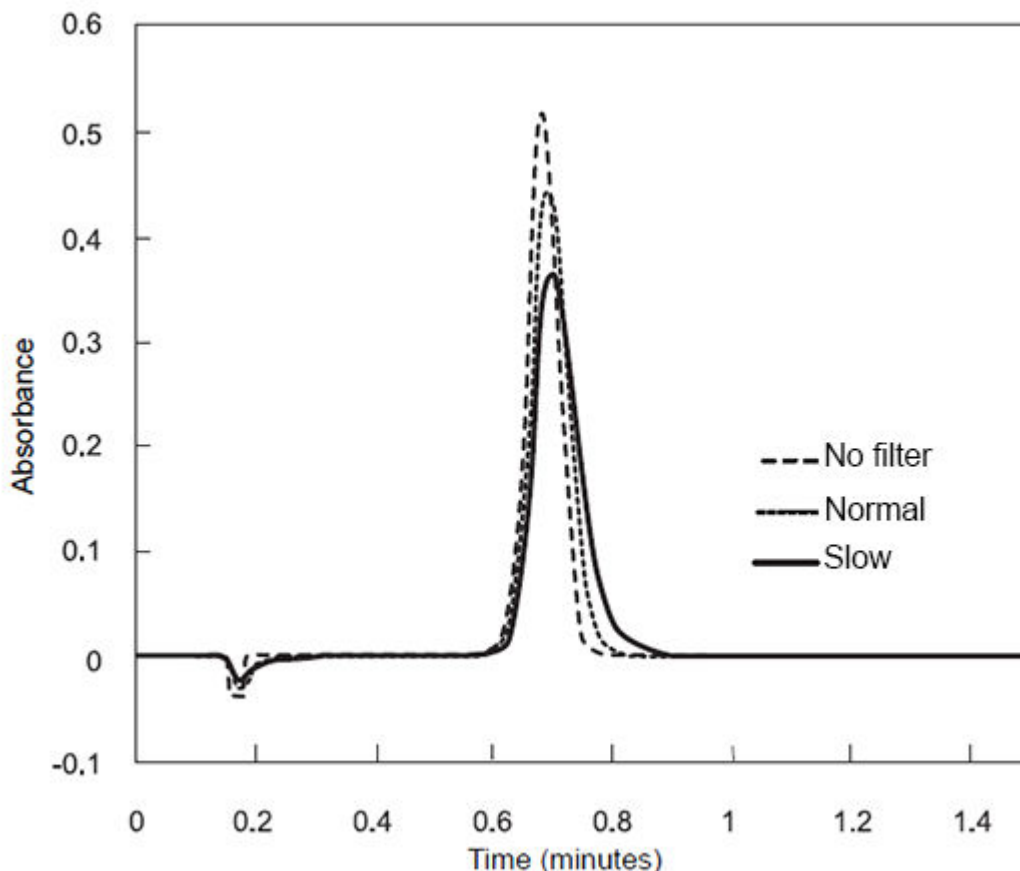
- Greatly decreased baseline noise
- Shortened and broadened peaks

Tip: Although the peak shape shows some distortion and the signal output is delayed with different time constants, the peak area remains the same.

At each sampling rate, the software includes fast or normal filtering constants that are appropriate for high-speed or high-sensitivity applications, respectively.

The following figure shows the relationship between increased filter time constant and absorbance.

Figure 3–3: Filter time constant comparison



3.2.1.2 TUV detector wavelength verification and test

If you run the detector continuously, Waters recommends that you perform wavelength verification weekly.

The detector's deuterium arc lamp and integral erbium filter exhibit peaks in the transmission spectrum at known wavelengths. Upon startup, the detector verifies calibration by comparing the locations of these peaks with expected wavelengths based on calibration data stored in the detector's memory. If the results of this verification differ from the stored calibration by more than 1.0 nm, the detector displays a `Wavelength Verification Failure` message. When required, the detector verifies, rather than recalibrates, on startup to avoid errors arising from residual materials left in the flow cell.

You can initiate a manual wavelength calibration at any time. A manual calibration replaces the previous calibration data with new data.

The verification and calibration algorithms are virtually identical. However, the verification algorithm can issue an error message indicating that actual data do not match stored data where the calibration algorithm replaces the stored data with the new.

The detector wavelength verification procedures establish an approximate Home position using a grating homing sensor. When Home is established, the detector locates and references the 656.1-nm peak in the deuterium lamp emission spectrum.

The integral erbium filter moves into the common light path ahead of the flow cell entrance slit, enabling the detector to locate three additional spectral features at these wavelengths:

- 256.7 nm (UV)
- 379.0 nm
- 521.5 nm

The verification tests for the detector require five minutes of lamp warmup time.

3.2.1.3 Operational modes

Detector capabilities include two modes of operation.

The detector operates in Single- or Dual-Wavelength mode and allows spectrum scanning using a flow cell or optional cuvette.

(See the *Empower online Information System* for control information in this software environment.)

3.2.1.3.1 Single-Wavelength mode

Single-Wavelength mode is the detector's default mode of operation.

The detector supports monitoring of a single wavelength from 190 nm to 700 nm, settable in 1-nm increments on channel A.

In this mode, the detector automatically engages the optical, second-order filter for wavelengths 370 nm and above and removes it for wavelengths under 370 nm. This filter blocks unwanted UV light from striking the diffraction grating and interfering with absorbance detection above 370 nm.

When using the detector in this mode, you can configure the additional parameters listed in the following table and set up an Events table for the detector.

Table 3–2: Single-Wavelength mode configurable parameters

Parameter	Description
Lamp	Set the detector's lamp to On or Off
Data rate (Hz)	Specify a sampling frequency up to 160 Hz (single channel, 2 Hz for dual channel)

Table 3–2: Single-Wavelength mode configurable parameters (continued)

Parameter	Description
Wavelength A (nm)	Specify a wavelength for channel A, from 190 nm to 700 nm, settable in 1-nm increments Note: If the detector is in Dual-Wavelength mode, the value of Wavelength B also appears.
Filter time constant (s)	Program a filter time. Options are No Filter, Slow, Normal, Fast, and Custom (see Filtering noise (Page 30) for detailed information about this parameter, these setting options, and the different effects produced).
Autozero (two options): <ul style="list-style-type: none"> • Autozero on Inject Start • Autozero on Wavelength Change 	Set when to autozero (resets the detector offsets)

3.2.1.3.2 Dual-Wavelength mode

In Dual-Wavelength mode, the detector can monitor two wavelengths, one on channel A and one on channel B.

Because the sampling frequency is reduced to 1 or 2 Hz, use of Dual-Wavelength mode is limited to more standard chromatography where peak widths span at least 20 seconds to enable full characterization of a peak.

In this mode, the following conditions apply:

- If both selected wavelengths are greater than 370 nm, the detector applies the second-order filter to block unwanted UV light.
- If both selected wavelengths are less than or equal to 370 nm, the detector removes the second-order filter.
- If the selected wavelengths bracket the 370-nm threshold, the detector does not apply the second-order filter and issues an alarm message that any data collected for the wavelength above 370 nm can contain inaccuracies because of possible UV light interference (UV effects).

3.2.1.4 Cuvette operations

The detector's cuvette option is used to measure the absorbance spectrum of a sample in a cuvette.

Note: This section pertains only to use of the optional cuvette.

To generate and store a spectrum:

1. Acquire a Blank scan, which measures the absorbance of the contents of the cuvette over the desired wavelength range.
2. Acquire a sample (Absorbance) scan, which measures the absorbance of the analyte dissolved in solution.

Result: The detector subtracts the Blank scan from the sample scan to create a sample spectrum.

3.2.1.5 TUV detector thermal wander management and active thermal control

These built-in detector capabilities reduce risk to performance due to changes in ambient temperature.

Thermal wander management (enhanced) – To decouple baseline performance from ambient temperature changes, the detector uses insulation, fans, heater, baffling, and active thermal control.

Active thermal control (new) – To ensure baseline stability in the presence of ambient temperature changes, the detector actively controls the temperature of the optics bench.

3.2.2 PDA detector features

The detector operates as an integral part of a Waters chromatography system.

The Alliance iS photodiode array (PDA) detector is an optical detector capable of simultaneous 2D and 3D operation. The detector is controlled by Empower and offers an analytical flow cell with a volume of 8.4 μL and a path length of 10 mm.

The flow cell design reflects Waters TaperSlit technology.

The detector features:

- Improved equilibration and warm-up time
- Enhanced sensitivity using the patented TaperSlit flow cell, which channels light through the cell for better energy throughput
- Actuator-controlled variable slit
- Lamp, flow cell, and optical filter ID
- Cuvette options
- Precision grating alignment
- Thermal isolation and control
- Home position confirmation
- Lamp optimization for the best performance over the lifetime of a deuterium lamp
- Low baseline noise performance ($<10 \mu\text{AU}$)

- Enhanced and new functionality to mitigate the effects of changes in ambient temperature (see the following capabilities table)
- Flexible sampling rates from 1 to 160 Hz for normal and fast LC separations
- Independent optimization of sampling rates and filter time constants gives you the ability to tune your detector to your separation goals

Table 3–3: PDA detector capabilities

Capability	Description
Automatic, second-order filter	Supports standard absorbance, UV/Vis functionality, and the spectrum scan feature
Full diagnostic capability	Supports built-in diagnostic tools to optimize functionality and performance
Thermal wander management (enhanced) and active thermal control (new)	Reduces performance issues due to changes in ambient temperature (See PDA detector thermal wander management and active thermal control (Page 39) .)
Cuvette qualification	Facilitates qualification of the detector by insertion of a standard in a cuvette. This feature is supported by Waters qualification kits available for purchase (optional cuvette sold separately).
Cuvette sample analysis	Allows recording of the spectrum of any sample placed in the cuvette

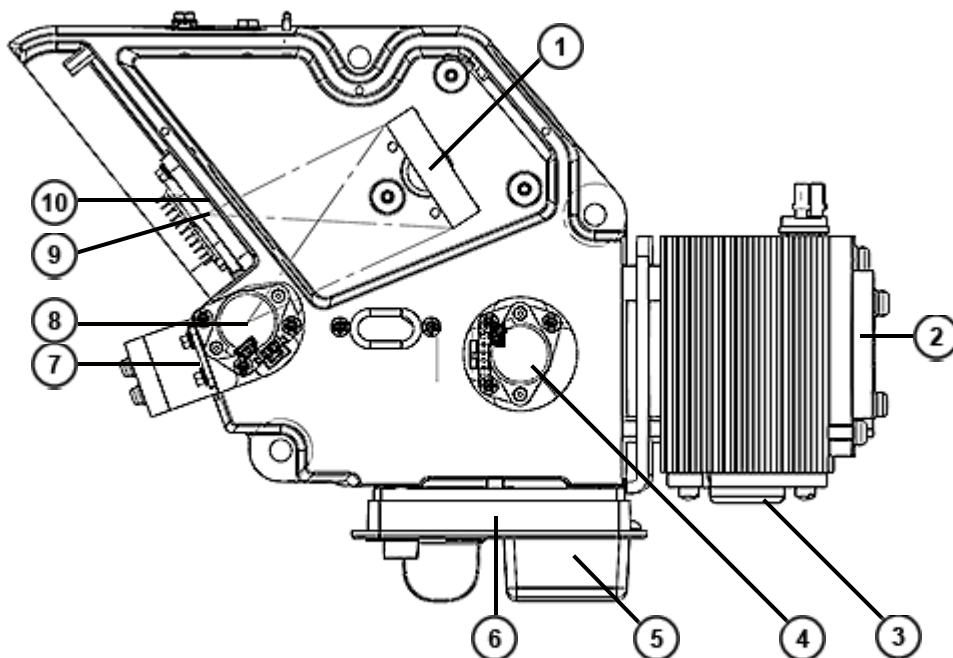
3.2.2.1 PDA detector optics

The PDA detector optics bench passes light through the solution under study and then separates it for analysis.

The detector is an ultraviolet/visible light (UV/Vis) spectrophotometer. With an array of 518 photodiodes, the detector operates within a range of 190 to 800 nm.

The figure below illustrates the light path through the optics assembly of the detector.

Figure 3–4: Optics assembly light path



- ① Grating – Disperses light into bands of wavelengths and focuses them onto the plane of the photodiode array.
- ② M1 mirror – Focuses light from the deuterium source lamp.
- ③ Lamp – Deuterium source lamp.
- ④ Filter flag/shutter – Flag positions for measuring open (sample) and blocked (dark) beam energies and a third for wavelength verification.
- ⑤ Cuvette holder - Keeps the cuvette stable and correctly aligned in the detector and allows light to pass through the sample for precise analysis.
- ⑥ Flow cell – Houses the segment of the flow path (containing eluent and sample) through which the polychromatic light beam passes.
- ⑦ Spectrograph mirror and mask – The mirror focuses light transmitted through the flow cell onto the slit at the entrance to the spectrographic portion of the optics. The mirror mask defines the size of the beam at the grating.

- ⑧ Variable slit - Determines the resolution and amount of light striking the PDA sensor. Smaller slit widths result in a smaller, sharper image that yields improved resolution, but with lower light throughput. Larger slit widths have higher light throughput, which can improve sensitivity and noise at the expense of lower resolution. The width of the slit is variable, but the default value is 50 μm .
- ⑨ Photodiode array (PDA) sensor - A linear array of 518 photodiodes, 50 μm in width, that measures the intensity of the dispersed light as a function of position on the sensor. Calibration enables the position data to be converted to wavelength to yield spectral data.
- ⑩ Order filter – Reduces the contribution of second-order diffraction of UV light (less than 370 nm) to the light intensity observed at visible wavelengths (greater than 345 nm).

3.2.2.1.1 PDA optics assembly light path

The detector employs an extremely efficient design for exceptionally high light throughput.

The detector operates as follows:

1. The light emitted by the deuterium lamp is refocused by an elliptical mirror into the flow cell.
2. The light beam then expands to fill the grating, which separates the beam into its component wavelengths and then refocuses those onto the PDA sensor.

3.2.2.1.2 Filtering noise

To minimize noise, the detector provides a Hamming filter.

The detector's Hamming filter is a digital finite-impulse-response filter that creates peak height degradation and enhances the filtering of high-frequency noise.

The behavior of the filter depends on the filter time constant you select. The filter programming options in the Method Editor are No Filter, Slow, Normal, Fast, and Custom. When selecting Slow, Normal, or Fast, you do not need to specify a value. The filter constant is determined by the sampling rate. When selecting Custom, you can specify a value but it will be rounded up or down to a value based on the sampling rate. Selecting No Filter or Custom and specifying a value of "0.0" disables all filtering.

The filter time constant adjusts the time window over which the data is filtered, thus controlling the degree of baseline smoothing and the impact on peak height degradation. Optimizing this parameter in the method ensures that the highest signal-to-noise ratios are achieved for a particular application.

Faster time constant settings produce these effects:

- Narrow peaks with minimal peak distortion and time delay
- Very small peaks become harder to discriminate from baseline noise
- Less baseline noise is removed

Slower time constant settings produce these effects:

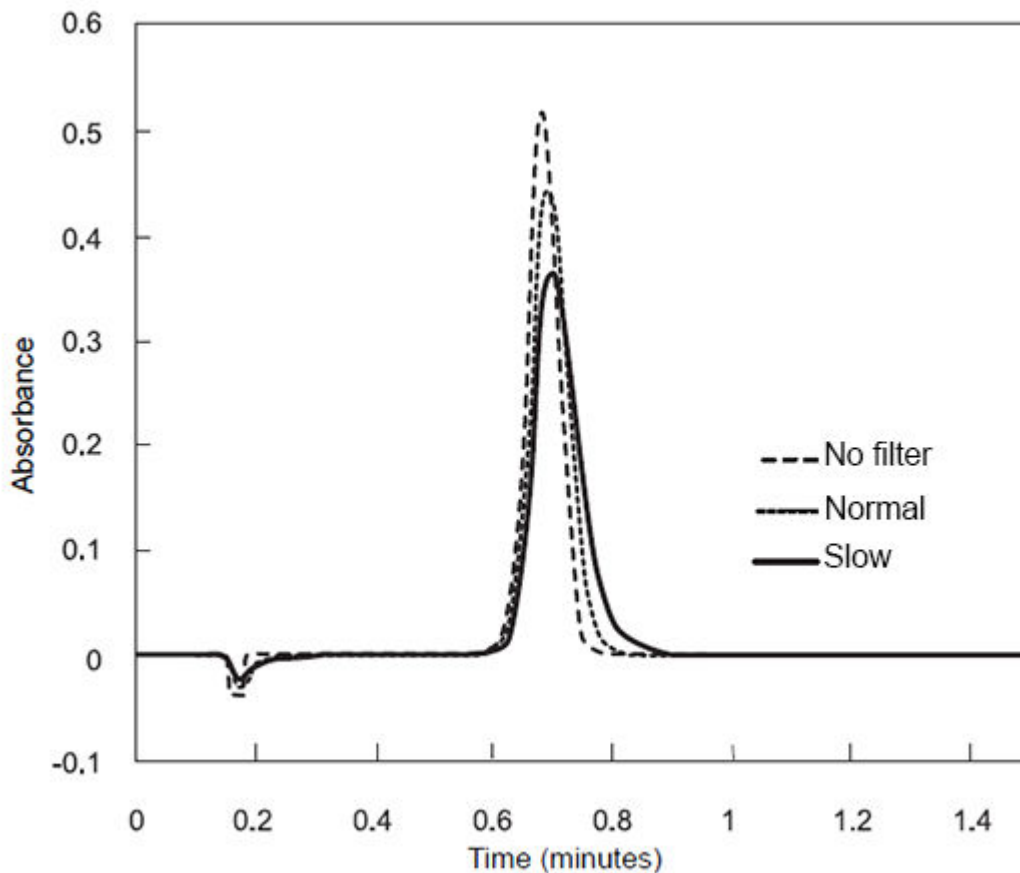
- Greatly decreased baseline noise
- Shortened and broadened peaks

Tip: Although the peak shape shows some distortion and the signal output is delayed with different time constants, the peak area remains the same.

At each sampling rate, the software includes fast or normal filtering constants that are appropriate for high-speed or high-sensitivity applications, respectively.

The following figure shows the relationship between increased filter time constant and absorbance.

Figure 3–5: Filter time constant comparison



3.2.2.2 PDA detector wavelength verification and test

If you run the detector continuously, Waters recommends that you perform wavelength verification weekly.

The PDA detector's calibration is verified using two spectral peaks from the deuterium lamp and three absorbance peaks from its integrated erbium filter. Upon startup, the detector verifies calibration by comparing the locations of these peaks with expected wavelengths based on calibration data stored in the detector's memory. If the results of this verification differ from the

stored calibration by more than 1.0 nm, the detector displays a `Wavelength Verification Failure` message.

Wavelength verification typically requires a clean optical path through the flow cell to ensure that the signal is transmitted to the sensor. A flow cell that has been idle for some time may have bubbles or contaminants present that can obstruct the light path and interfere with wavelength verification. For safety purposes, flow is not initiated on instrument startup; if wavelength verification fails at startup, it is recommended that you rerun the verification workflow with the flow cell flushed and with the flow equilibrated.

In the event that subsequent verification fails, the PDA can be recalibrated. Note that recalibration can invalidate spectral library matching and the peak purity analyses performed with a prior calibration.

3.2.2.3 Cuvette operations

The detector's cuvette option is used to measure the absorbance spectrum of a sample in a cuvette.

Note: This section pertains only to use of the optional cuvette.

To generate and store a spectrum:

1. Acquire a Blank scan, which measures the absorbance of the contents of the cuvette over the desired wavelength range.
2. Acquire a sample (Absorbance) scan, which measures the absorbance of the analyte dissolved in solution.

Result: The detector subtracts the Blank scan from the sample scan to create a sample spectrum.

3.2.2.4 PDA detector thermal wander management and active thermal control

These built-in detector capabilities reduce risk to performance due to changes in ambient temperature.

Thermal wander management (enhanced) – To decouple baseline performance from ambient temperature changes, the detector uses insulation, fans, heater, baffling, isolated thermal zones, and active thermal control.

Active thermal control (new) – To ensure baseline stability in the presence of ambient temperature changes, the detector actively controls the temperature of the optics bench and lamp.

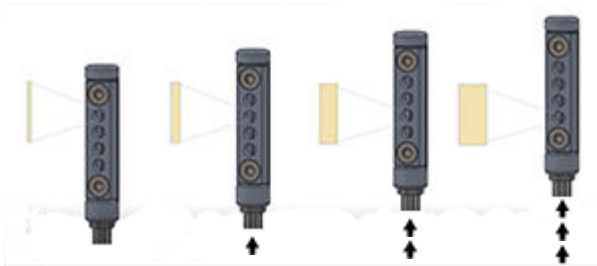
3.2.2.5 Variable slit width

Variable slit width allows the user to balance resolution and throughput to best fit their analysis.

This Alliance iS PDA detector features an actuator-controlled variable slit. The slit width sizes are:

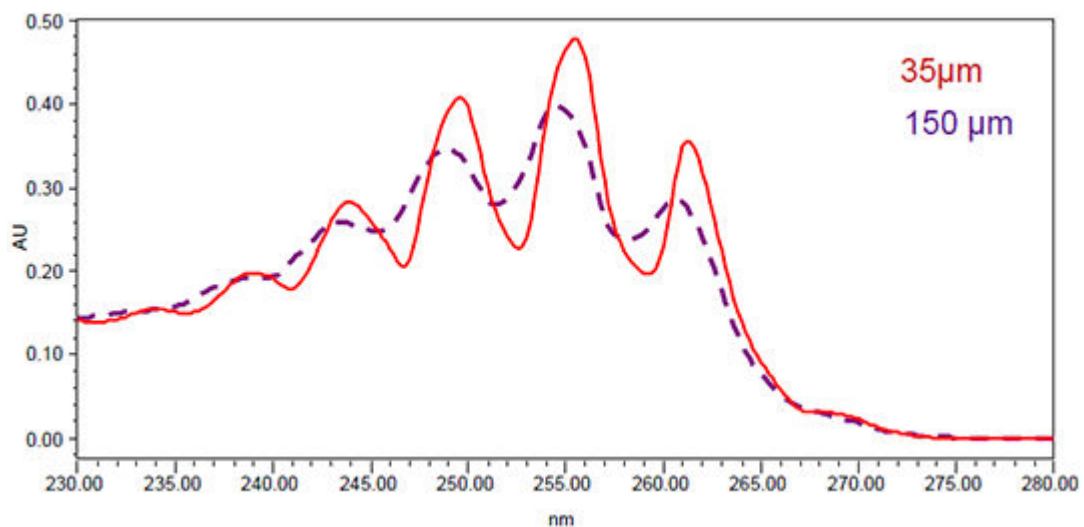
- 35 μm
- 50 μm (default)
- 100 μm
- 150 μm

Figure 3–6: Alliance iS PDA variable slits



A small slit provides the sharpest spectral peak resolution while a large slit provides the best signal with respect to noise for improved sensitivity.

Figure 3–7: Impact of slit width on resolution of benzene



The slits are designed to deliver energy levels proportional to their areas.

3.2.2.6 Verifying the PDA detector's calibration

Verify the detector calibration after removing and replacing a flow cell or if verification fails on system startup.

To verify the PDA detector's calibration, prime the system and run flow for ten minutes to flush the flow cell with solvent, and ensure that it is fully wetted. A 90:10 water/acetonitrile mixture is recommended at 0.5 mL/min. Warm up the deuterium lamp for a minimum of five minutes, and ensure that it is in the "ON" state prior to verification.

If buffers were recently used in the flow cell, Waters recommends that you first flush with 10 mL of HPLC-grade water, followed by 10 mL of a low-surface-tension solvent such as methanol (provided it is miscible with the previous mobile phase).

To verify detector calibration:

1. From the touchscreen, tap **Maintain > Verify Calibration**. Follow the on-screen prompts to complete the verification process. When verification is complete, the screen displays `Verify Calibration Passed`.
2. Tap **RE-VERIFY** to run the verification process again, or click **DONE**.

3.2.2.7 Calibrating the PDA detector

Calibrate the detector if wavelength verification fails with a properly flushed flow cell.

To perform detector calibration, prime the system and run flow for 10 minutes to flush the flow cell with solvent and ensure that it is fully wetted. A 90:10 water/acetonitrile mixture is recommended at 0.5 mL/min. The lamp should be warmed up for a minimum of 5 minutes and in the "ON" state prior to calibration.

If buffers have been used recently in the flow cell, it is recommended that you first flush with 10 mL of HPLC-grade water, followed by 10 mL of a low-surface-tension solvent such as methanol (provided it is miscible with the previous mobile phase).

3.2.2.7.1 Erbium calibration

Erbium calibration uses the on-unit erbium filter to recalibrate the instrument. Erbium calibration is most commonly done after replacement of an optical component.

You can perform an erbium calibration for the PDA detector from the system touchscreen.

Important:

- A prior mercury calibration is a prerequisite to performing the erbium calibration.
- Impurities in the flow cell can affect wavelength calibration. Ensure that the flow cell is clean before you perform calibration.
- This procedure can adversely affect spectral library matching and peak purity analyses.
 1. From the touchscreen, tap **HEALTH > Troubleshoot**.
 2. Tap the detector icon.
 3. Tap **Erbium Calibration**, follow the on-screen prompts, and then tap **START** to begin the calibration process.
The screen displays the calibration values.
 4. Tap **DONE**.

3.2.2.7.2 Mercury calibration

Mercury calibration uses a mercury-argon spectral calibration lamp to calibrate or recalibrate the instrument. Mercury calibration is typically performed at the factory but can be performed by qualified Waters field service engineers if necessary. Note that mercury recalibration should only

be performed if erbium calibration fails or the prior mercury calibration parameters were wiped or corrupted.

A Waters field service engineer can perform a mercury calibration for the PDA detector from the system touchscreen.

Important:

- Mercury calibration is to be performed only by a Waters field service engineer.
- Impurities in the flow cell can affect wavelength calibration. Ensure that the flow cell is clean before you perform calibration.
- This procedure can adversely affect spectral library matching and peak purity calculations.

3.2.2.8 Testing the PDA detector for noise and drift

A dirty flow cell or a flow cell with bubbles can cause excessive noise and drift.

You can run a noise and drift test on the PDA detector from the system touchscreen.

1. From the touchscreen, tap **HEALTH > Troubleshoot**.
2. Tap the detector icon.
3. Tap **Noise and Drift Test**, follow the on-screen prompts, and then tap **START** to begin the test.

The screen reads `Noise and Drift Test Completed` and displays the test results.

4. Tap **DONE**.

3.2.2.9 2D and 3D acquisition

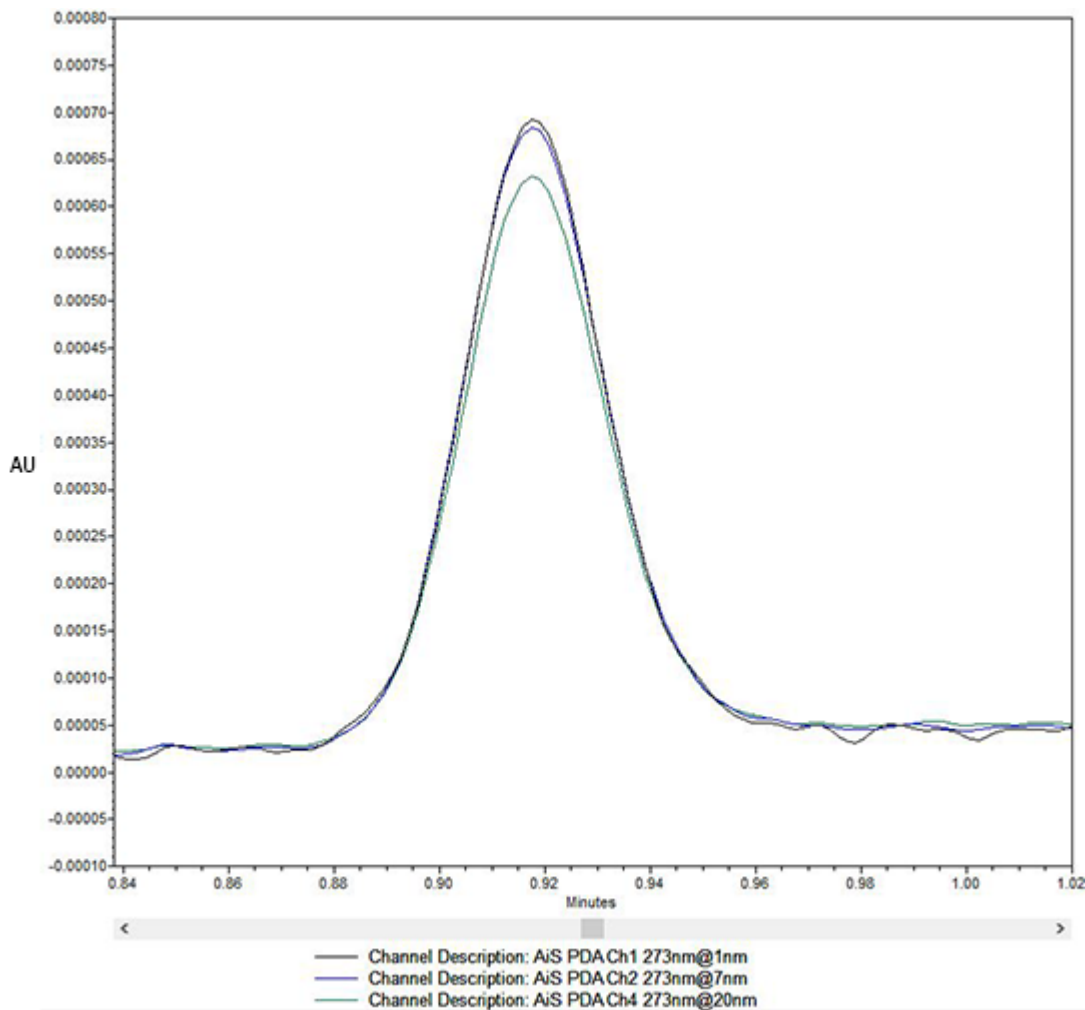
The Alliance iS system PDA detector is capable of both 2D and 3D acquisition.

The PDA detector can simultaneously collect two types of data channels: spectra (3D) and chromatograms (2D). For best results in library matching and peak-purity analysis, set the 3D resolution to 1 nm.

For chromatograms (2D data), select a resolution to optimize signal amplitude, baseline noise, and linear dynamic range. When the monitoring wavelength for an analyte corresponds to a peak's lambda max, increasing bandwidth tends to decrease peak height while reducing baseline noise and linear dynamic range.

Tip: A resolution of 4 nm is effective for many analytes.

Figure 3–8: Resolution comparison for caffeine



3.2.3 Sample manager features

The sample manager uses a direct injection mechanism to inject samples drawn from vials and plates onto a chromatographic column..

The Alliance iS Sample Manager maintains sample temperature, aspirates precise samples, and then injects them into the mobile phase using a flow-through-needle (FTN) design. The needle becomes part of the fluidics path when the sample is injected onto the column. The mobile phase cleanses the needle during the run, ensuring complete sample recovery and minimizing carryover. Additionally, the sample positioning mechanism controls the location of the sample plates relative to the sample needle, reducing extra system volume.

The standard configuration supports sample injection volumes of 0.1 to 100 μL . Optional extension loops can extend the injection volume up to 2000 μL . The sample temperature is regulated within a range of 4 $^{\circ}\text{C}$ (39.2 $^{\circ}\text{F}$) to 40 $^{\circ}\text{C}$ (104 $^{\circ}\text{F}$).

Note: A 100- μL extension loop is included in the standard configuration.

The sample manager features:

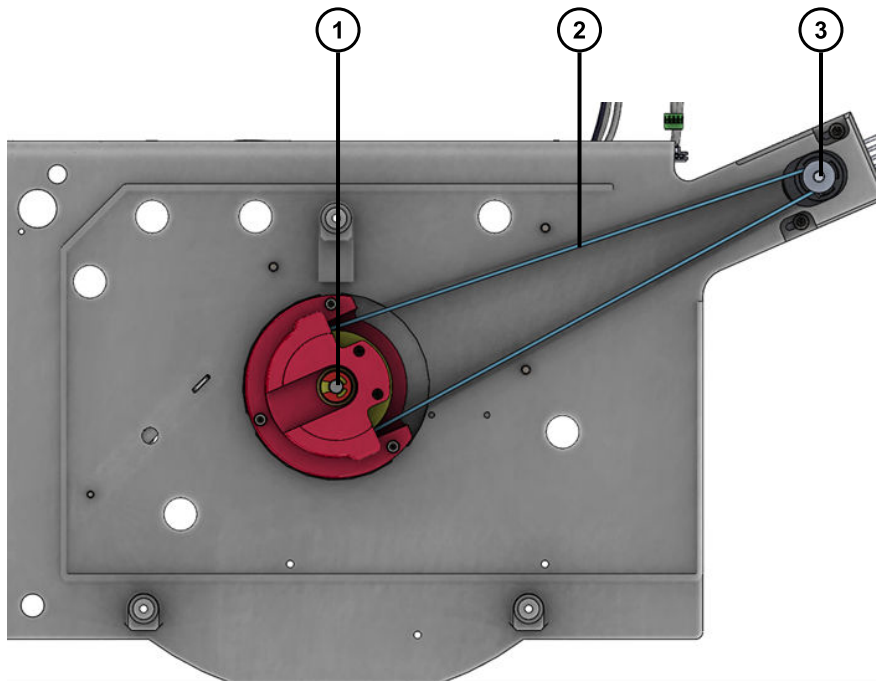
- Three-plate sample capacity
- Precise sample metering
- Accurate sample temperature control
- Exceptional carryover performance (0.002% maximum)
- Consistent solvent consumption
- Puncture robustness

3.2.3.1 Needle positioning mechanism

A dual-axes needle positioning mechanism draws the samples from the vials in the sample plates that the sample manager will inject onto a chromatographic column.

To line up the needle with the vials in the wells of the sample plates inside the sample compartment, the needle positioning mechanism's two rotary axes control the orientation of the sample plates and the relative position of the sample needle carriage. Both rotary axes rotate a shaft using a belt and motor. The needle carriage rotates approximately 90° away from the inject port while the sample plates are on a continuous 360° rotary axis.

Figure 3–9: Needle positioning mechanism's dual-rotary axes, located below the sample compartment

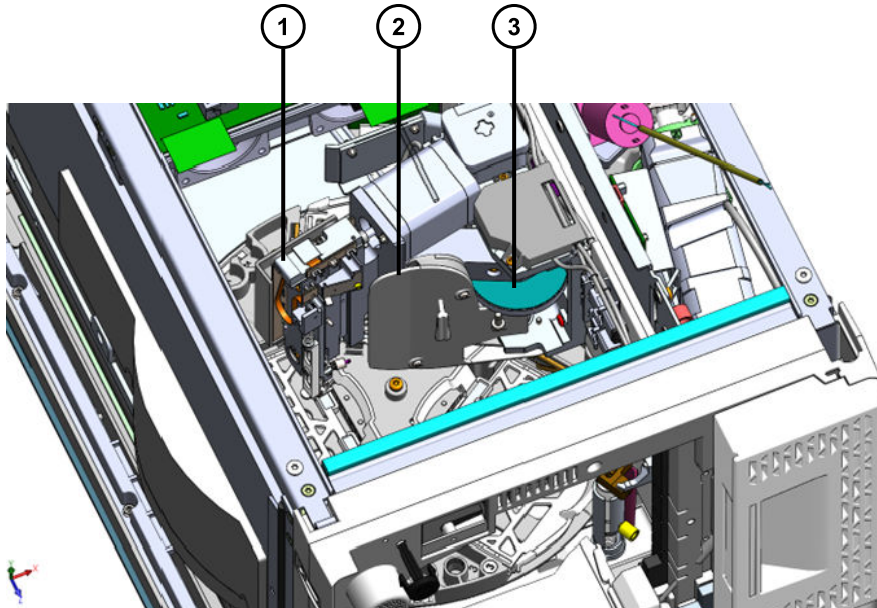


① Platter rotary axis

② Belt

- ③ Motor axis

Figure 3–10: Needle carriage's rotary axis, located inside the sample compartment



- ① Needle carriage
- ② Needle cartridge
- ③ Needle carriage rotary axis

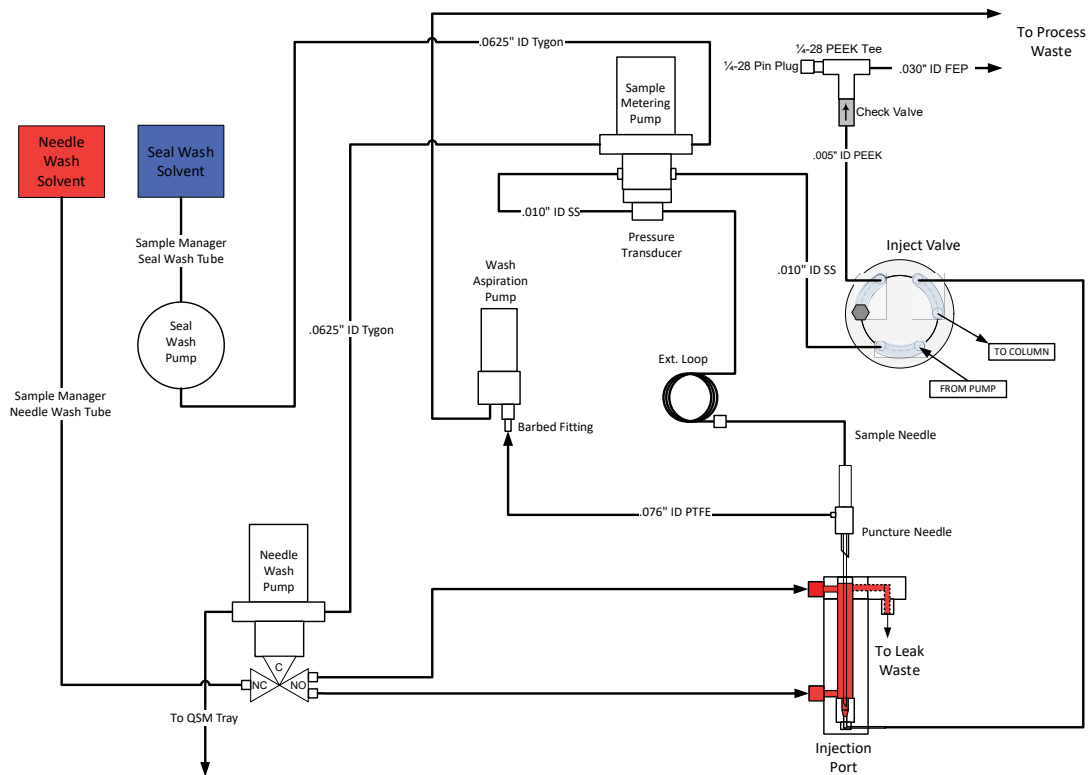
3.2.3.2 Injection system

The injection flow path includes the assemblies required to aspirate a sample and deliver it to the column.

The injection process involves the needle, optional extension loop, sample metering pump, injection valve, and injection/wash port.

Note: For a multi-draw system configuration, an optional multi-draw valve is available.

Figure 3–11: Sample manager flow path for single-draw aspiration and injection



3.2.3.3 Injection mechanics

The sample needle goes through a specific sequence for each injection.

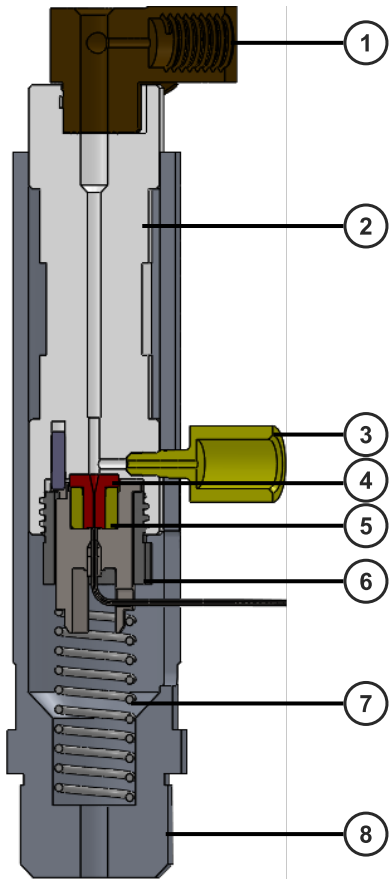
During an injection, the following sequence takes place:

1. The needle moves to the sample vial and aspirates sample from it.
2. The needle carriage inserts the needle into the injection/wash port.

Note: The entire injection port assembly rests on a spring and is guided inside a metal housing.

3. As the needle is inserted into the injection/wash port, it presses against the seat and forms a high-pressure seal.
4. The injection valve turns to start the injection.
5. The wash pump washes the outside of the needle during the injection.

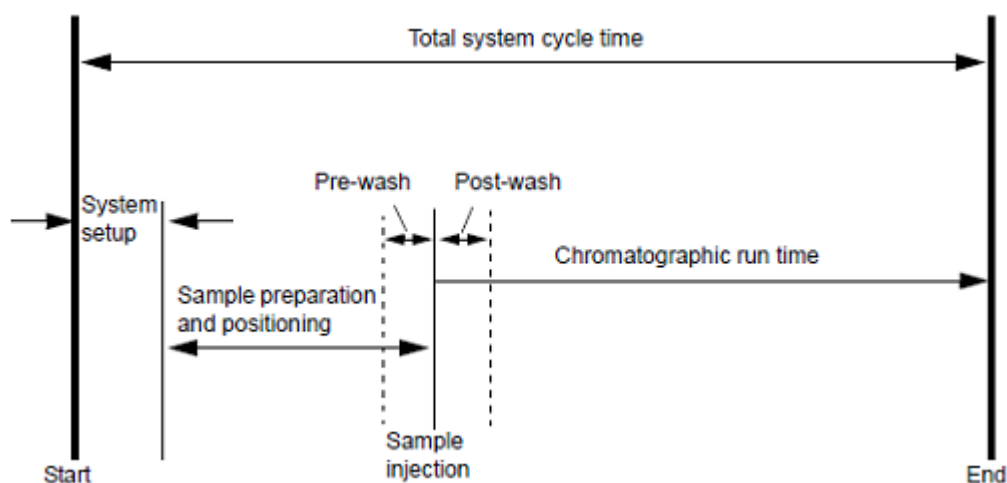
Figure 3-12: Needle seat assembly, cross view



- ① Angled wash manifold
- ② Sample needle support sleeve
- ③ Fitting
- ④ Seat
- ⑤ Seat port assembly
- ⑥ Seal cup locking nut
- ⑦ Compression spring
- ⑧ Needle wash housing

The following figure shows standard injection mode cycle time.

Figure 3–13: Standard injection mode cycle time definition



3.2.3.3.1 Wash system

The wash sequence does not allow wash solvent to enter the sample stream.

The wash system cleans the exterior of the sample needle while it is inside the injection/wash port.

3.2.3.3.2 Priming modes

The sample manager has three priming modes available.

- Needle Wash Solvent – where the wash solvent flows through the needle wash pump
- Sample Metering Pump – uses the solvent manager to prime the sample metering pump (the sample metering pump is downstream of the solvent manager pumps)
- Seal Wash Solvent – prime occurs in the sample manager area of the IFM rather than the pump area

3.2.3.3.3 Thermal system

The thermal system maintains the temperature specified for the sample compartment (settable temperature range 4 to 40 °C in 0.1 °C increments).

Tips:

- The sample manager's fans stop circulating air whenever the sample compartment door is open.
- When the device is controlling temperature, the sample tray rotates slowly to help maintain a uniform temperature across the plates.

3.2.4 Pump features

The system uses a low-pressure mixing pump.

The Alliance iS Quaternary Solvent Manager (QSM) can blend up to four degassed solvents (A, B, C, and D). A gradient proportioning valve (GPV) is used to dynamically blend solvents in any specified combination, producing repeatable and reproducible gradient segments regardless of solvent compressibility and system back pressure. Integrated degasser chambers (one per solvent line) automatically remove dissolved gases from up to four elution solvents.

The pump features:

- Automated, continuous compressibility compensation for accurate and precise delivery at pressures up to 12,000 psi
- Leak sensors to identify and manage solvent leaks during unattended operation
- Programmable flow rates that range from 0.001 to 10.000 mL/min, in 0.001-mL increments

3.2.4.1 Pressure flow envelope

The pump module comprises a single pump and a proportioning valve.

The pump provides solvent flow at analytical flow rates up to 5 mL/min at 12,000 psi and linear change to 4,000 psi at 10 mL/min.

3.2.5 Column heater/cooler features

This module manages and maintains column temperature for your system.

The Alliance iS Column Heater/Cooler (CHC) is a compartment in the system that controls the thermal environment of the column using a combination conductive heater and cooler. When the compartment temperature is set, either directly from the console or within a method, a command is sent to the CHC that switches the compartment heating/cooling engine on or off. The CHC continues to heat or cool until the compartment achieves the specified temperature set point.

The CHC features:

- Integrated passive preheating
- Temperature setting range of 4 °C (39.2 °F) to 90 °C (194 °F)
- Column eConnect technology
- Column clips for easy column removal and replacement
- Tool-free fittings (TFFs)

Supported column specifications:

- Length: 300 mm (maximum)
- ID: 8.0 mm (maximum)
- Guard or in-line filter: 30 mm (maximum)

3.2.5.1 Column heater/cooler operation

This module is a combination conductive heater and cooler.

When the column compartment temperature is set, either directly from the console or within a method, a command is sent to the CHC to switch the compartment heater/cooler engine on or off. On the basis of feedback from the compartment thermistor, the thermoelectric device continues to heat, or cool, until the compartment achieves the specified temperature set point.

Recommendation: When sample and column temperature are important to an application, in addition to specifying explicit temperature set points in the method, specify appropriate temperature limits. Together, these settings ensure that system operation occurs only within the defined limits and that any occurrence of an unacceptable deviation from the set points is flagged by an error message acknowledging the variance.

3.2.5.2 Column configuration

Although not included as part of your system, columns are available for purchase on the Waters website.

The CHC column compartment accommodates a single LC column, with maximum dimensions of 8.0 mm ID and 300 mm length, and a single pre-column consumable, with maximum dimensions of 8.0 mm ID and 30 mm length.

3.2.5.3 eConnect technology

eConnect smart column capabilities ensure complete column traceability and facilitate post-run troubleshooting.

Waters' new eConnect technology is a standard feature of the CHC that can be utilized by purchasing eConnect-enabled versions of Waters chromatographic columns (eConnect HPLC Columns). Ready-to-use from the box, eConnect columns are securely fitted with a near-field communication (NFC)-enabled eConnect device tag during manufacture, ensuring that they are always automatically identified, verified, and tracked by the system.

After you install an eConnect-tagged column in the CHC and shut the component's door, the tag is detected automatically. Then, the tag's eConnect technology interacts with a compatible version of Empower system software to read the column's unique device identifiers, display this information on the system's touchscreen, and log that data for each injection.

3.2.6 Touchscreen features

The Alliance iS HPLC System's touchscreen allows the user to perform many tasks and saves time by minimizing moving back and forth between the system and the Empower workstation. The navigation panel on the left-hand side of the touchscreen provides buttons for accessing views for performing specific tasks. The following table lists the views.

Table 3–4: Touchscreen views/buttons

View	Description
Home (Page 51)	Displays real-time status information.
Setup (Page 52)	Prepares the system to start up or shut down. Manages solvents.
Plots (Page 52)	Displays available plots.
Maintain (Page 53)	Provides component replacement and calibration procedures.
Health (Page 53)	Provides procedures for troubleshooting, resolving, and reporting issues.
System (Page 54)	Provides actions for configuring the system, creating or reviewing logs, performing administrator tasks, viewing performance counters, configuring leak sensors, and viewing the About screen.
Commands (Page 54)	Provides actions with immediate effects, such as toggling the lamp on/off and resetting the system.

The following table describes the controls at the top of the touchscreen window.

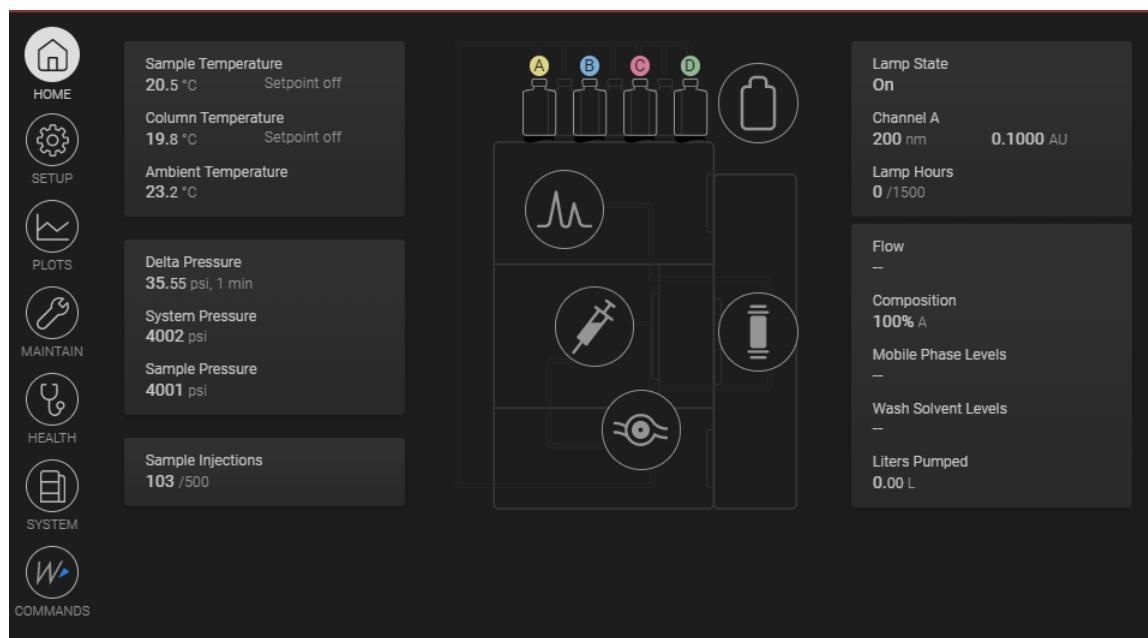
Table 3–5: Additional touchscreen controls

Control	Description
System status	IDLE, RUNNING, or ERROR
Preferences	Provides access to the following settings: Display and Themes, Instrument Name, Lock Screen, and User Note.

3.2.6.1 Touchscreen Home view

The Home view displays the real-time status of the system. The following figure shows the Home view.

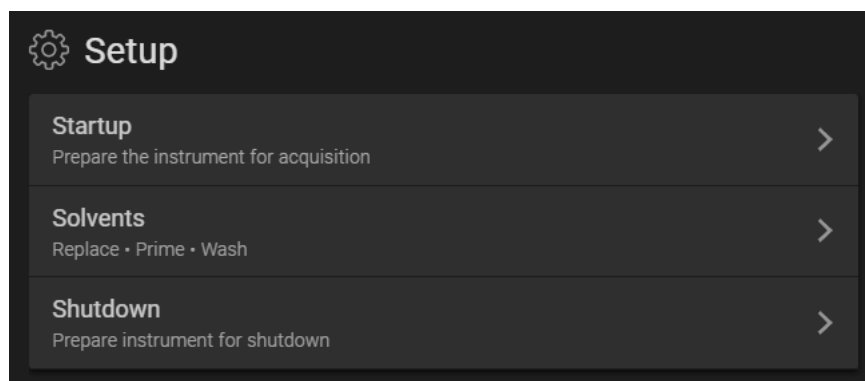
Figure 3–14: Home view



3.2.6.2 Touchscreen Setup view

The Setup view provides actions used to prepare the system for data acquisition. The following figure shows the Setup view.

Figure 3–15: Setup view (main)



See:

- [Priming a dry solvent manager via the touchscreen \(Page 64\)](#)
- [Equilibrating the Alliance iS HPLC System \(Page 81\)](#)
- [Preparing to shut down the Alliance iS HPLC System \(Page 87\)](#)

3.2.6.3 Touchscreen Plots view

The Alliance iS HPLC System produces data plots continuously for display on the touchscreen. You can configure up to four diagnostic plots for up to 96 hours. The following table describes the available plots.

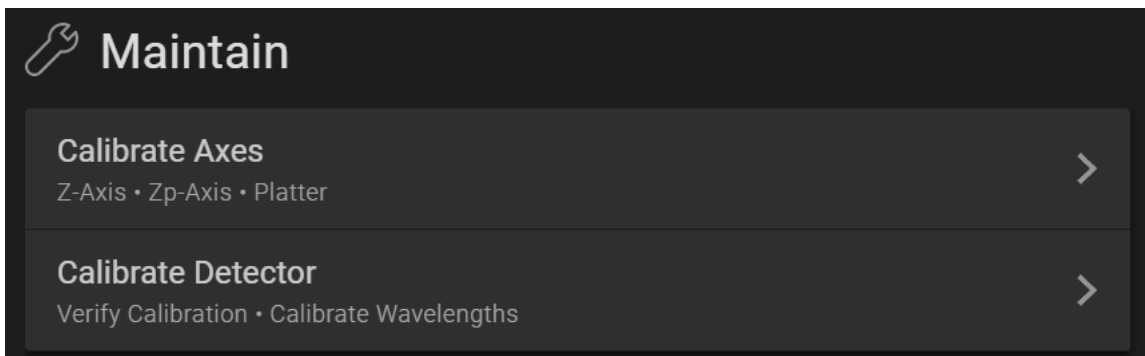
Table 3–6: Data plots produced by the system

Plot	Description
Sample manager diagnostics	Provides sample and ambient temperatures in °C and sample pressure in psi or a user-selected unit.
Column module diagnostics	Displays channels such as Column Temperature.
Detector diagnostics	Displays channels such as absorbance and wavelength (TUV only).
Pump diagnostics	Displays channels such as System Pressure and Flow and Composition.

3.2.6.4 Touchscreen Maintain view

The Maintain view provides workflows used to replace components and calibrate the system. The following figure shows the Maintain view.

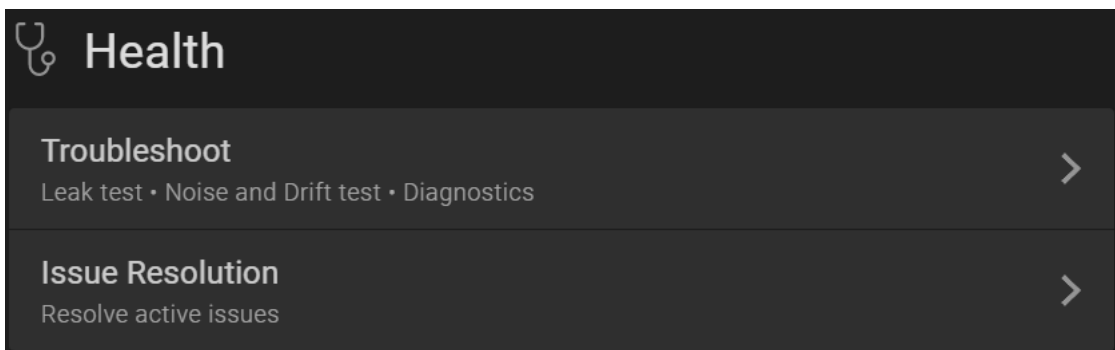
Figure 3–16: Maintain view (main)



3.2.6.5 Touchscreen Health view

The Health view presents workflows used to troubleshoot issues that may be present in the system. The following figure shows the Health view.

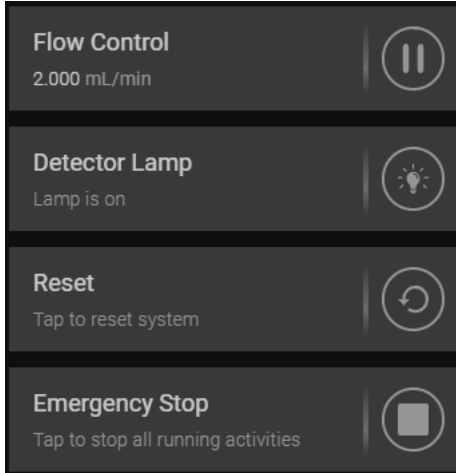
Figure 3–17: Health view (main)



3.2.6.6 Touchscreen Commands view

The Commands view provides actions that have immediate effects. The following figure shows the Commands view.

Figure 3–18: Commands view



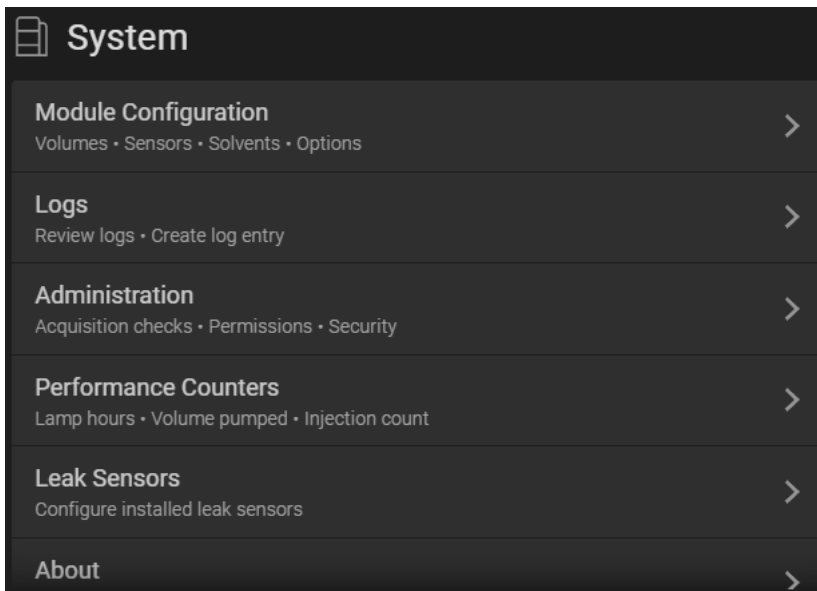
See:

- [Equilibrating the Alliance iS HPLC System \(Page 81\)](#)

3.2.6.7 Touchscreen System view

The System view provides miscellaneous actions used to configure the system and provide system-level information. The following figure shows the System view.

Figure 3–19: System screen (main)

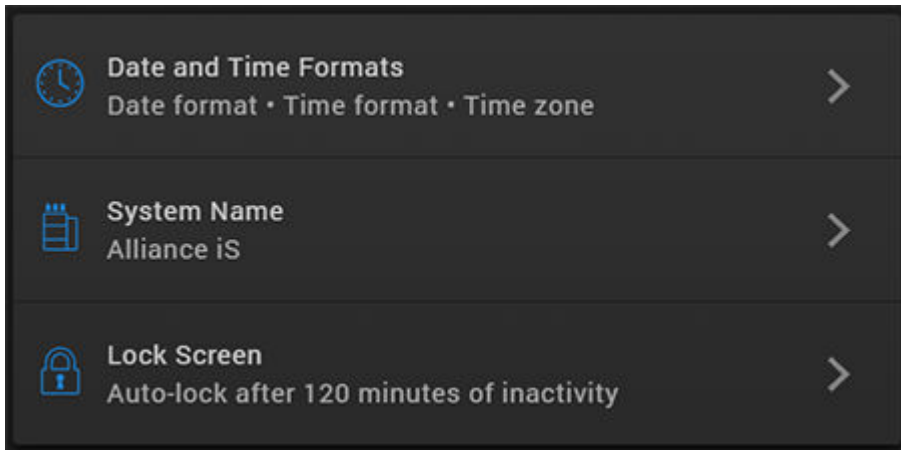


3.2.6.8 Touchscreen Preferences view



From the home screen, tap the preferences icon to access the Preferences view. The Preferences view provides settings that affect the touchscreen user interface but not the operation of the system. The following figure shows the Preferences view. **LOCK** and **DONE** buttons are below the preferences.

Figure 3–20: Preferences view



3.2.6.9 Touchscreen language selection

You can select the system display language from the touchscreen.

Note: This process affects display language on the touchscreen only and is independent of language selection within Empower.

1. Tap the **LANGUAGE** button in the lower-left corner of the system lock screen.
2. Tap to select the appropriate language. From top to bottom, the options are English, Simplified Chinese, Japanese, and Korean.

3.2.7 Empower features

The Empower CDS provides features that are specific to the Alliance iS HPLC System. For more information, see the following topics, [Intended use of the system \(Page 11\)](#), and the *Empower online Information System*.

3.2.7.1 Empower Alliance iS HPLC System features

Empower provides the following features for the system:

- The System Audit Trail includes actions performed at the system.
- The system sends column usage information to Empower, which stores the information in the column history tables.
- Empower sends information about the current sample and system suitability status to the system for display on the touchscreen.
- Users can request sample validation checks by the system prior to submission and execution. Resulting issues appear in the Message Center.

3.2.7.2 Alliance iS HPLC System console

The Alliance iS HPLC System console is accessible through the Empower control panel. For convenience, the console provides some of the information that appears on the touchscreen's [Home view \(Page 51\)](#) at the Empower workstation.

3.2.7.3 Intelligent Method Translator

The Intelligent Method Translator app (iMTA) converts non-Alliance iS HPLC System methods into Alliance iS HPLC System methods. The method translation process maps parameters stored in Empower instrument methods to the system's instrument settings. Translated instrument methods are viewable in Empower's Instrument Method Editor.

For more information on the Intelligent Method Translator app, see the *Intelligent Method Translator App User's Guide* (715008502).

4 System preparation

This section helps you prepare your Waters system for use. Proper setup is critical to successful operation of the system.

4.1 Powering-on the system

The power button is located on the front door of the sample manager.

After the system is plugged in, the power button LED blinks.

To power-on the system:

1. Push the power button located on the front door of the sample manager.
The power button LED will light up steadily and the system will power-on. When the power-on process completes, the Idle screen appears.

Figure 4–1: Alliance iS power button



2. Sign in to the Alliance iS HPLC System. See [Signing in and out of the Alliance iS HPLC System \(Page 78\)](#).

4.2 Powering-off the system

The power button is located on the front door of the sample manager.

To power-off the system:

Push the power button located on the front door of the sample manager.
The system will power-off.

Figure 4–2: Alliance iS power button



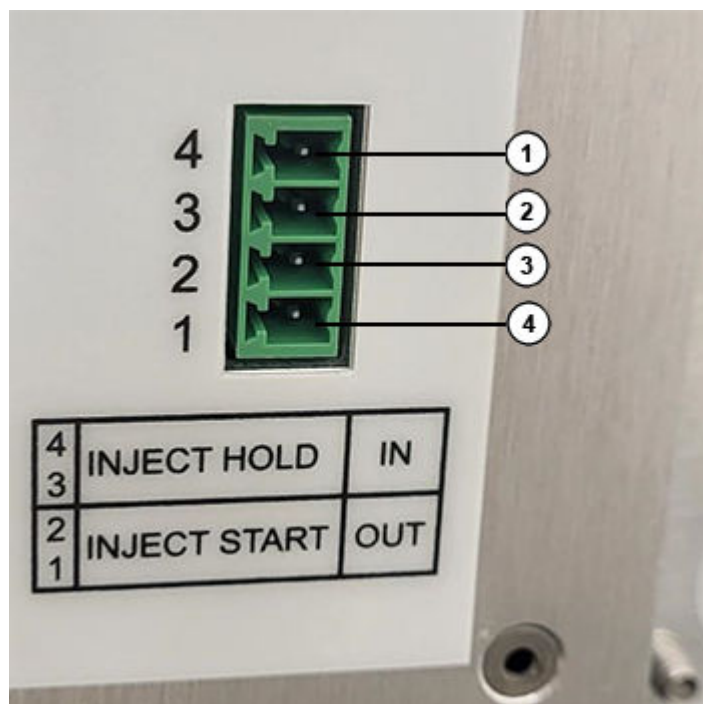
4.3 I/O signal connector

The I/O signal connector provides a means for the system to communicate with external LC components.

Note: The I/O signal connector was implemented with Alliance iS HPLC system version 1.1. This information applies to systems starting at version 1.1.

The IFM's rear panel includes a removable connector that holds the screw terminals for I/O signals. This connector is keyed so that it can receive a signal cable inserted only one way.

Figure 4–3: Alliance iS I/O signal connector



- ① Inject hold
- ② Inject hold
- ③ Inject start
- ④ Inject start

Table 4–1: Alliance iS analog/event connections

Signal connections	Description
Inject hold	Reserved for future use.
Inject start	An output that triggers other LC components to start at the injection time of the Alliance iS HPLC System.

4.4 Installing the column

Install the column in the CHC prior to running samples.

Fittings and column clips are designed to be intuitive when installing a column in the Alliance iS Column Heater Cooler (CHC).

Note: Power-on the system before installing the column to ensure proper RF column identification when the CHC door is closed. See [Powering-on the system \(Page 57\)](#).

1. Open the column compartment door.
2. Move the lower column clip as necessary to match the size of the column.
3. Remove the plugs from the inlet and outlet ends of the column.
4. Orient the column so that the outlet faces up (see the arrow on the column) and the inlet faces down.
5. Screw the fittings on the column compartment tubing finger-tight to the column inlet and outlet.
6. Insert the column into the upper and lower clips so that the clip grasps the exposed threads on the fitting.

Figure 4–4: Column seated in column clip



7. Close the column compartment door.

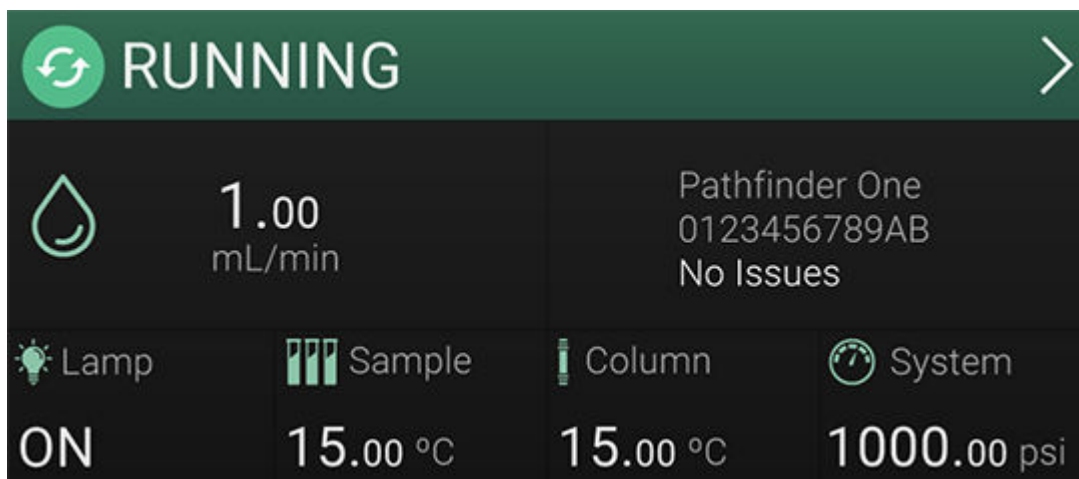
4.5 Opening the console from Empower software

After powering-on the system, open the console from Empower.

You can access the console from the Empower system status panel.

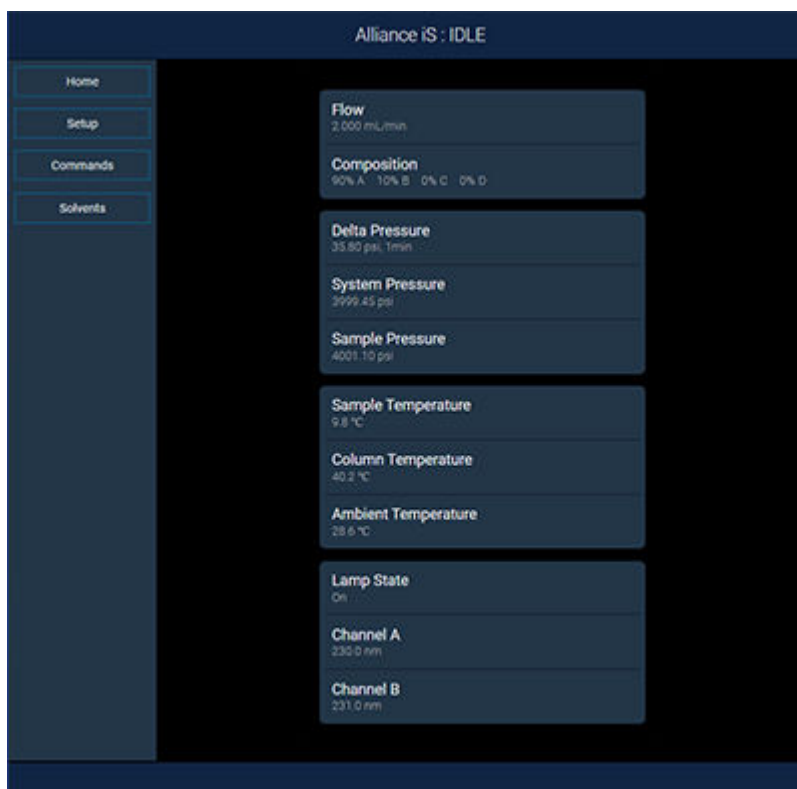
1. From the Empower Run Samples menu, click the arrow in the upper right-hand corner of the system control panel.

Figure 4–5: Launching the system console



2. From the console, you can access configuration and detailed statuses of all parts of the system.

Figure 4–6: System console



4.6 Priming the system

After powering-on the system, you must prime it before the system is ready for use.

Requirement: You must prime the system after starting it, as well as after changing the mobile phase, after changing the sample needle, and after the system is idle for four hours or more.

Requirement: You must install a column before priming the system. See [Installing the column \(Page 59\)](#).

Recommendation: If you are introducing new solvents, prime them at 4 mL/min for seven minutes. Alternatively, prime the solvents at 4 mL/min for three minutes. Ensure that sufficient quantities of solvent are available for priming.

There are multiple ways to prime the system from the touchscreen:

- Tap **SETUP > Startup** to prime all solvents, prime the needle wash and seal wash, and specify the solvent composition, flow rate, column and sample temperatures, and needle characterization for your next system startup.
- Tap **SETUP > Solvents > Prime Mobile Phase Solvents** to prime the pump.
- Tap **HOME**, tap the solvent bottle icon, tap a mobile phase condition card, and then tap **Prime Solvent** to prime an individual mobile phase.
- Tap **SETUP > Solvents > Prime Sample Metering Pump** to prime the sample metering pump.

Tip: From the touchscreen, you can select the **Setup > Startup** feature to prime all solvents, prime the needle wash and seal wash, and specify the solvent composition, flow rate, column and sample temperatures, and needle characterization for your next system startup. For details, see the touchscreen.

4.6.1 Priming the seal-wash system

Priming the seal-wash system is part of the system startup workflow on the touchscreen.

Prime the seal wash in the Alliance iS QSM to fill the tubing paths with solvent.

Tip: When primed, the seal-wash system is used to lubricate the plungers and flush away solvent and any precipitated salts that were dragged past the plunger seals from the high-pressure side of the piston chambers.

Prime the seal-wash system in all of the following situations:

- After using buffered mobile phase
- When the pump is inactive for a few hours or longer
- When the pump is dry



Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.



Warning: To avoid personal contamination with biologically hazardous or toxic compounds, wear clean, chemical-resistant, powder-free gloves when performing this procedure.



Warning: To avoid eye injury, use eye protection when performing this procedure.



Notice: To avoid damaging the seats and seals of solenoid valves in the solvent path, do not use a nonvolatile buffer as the seal wash solvent.



Notice: To avoid clogging system tubing, ensure that the seal-wash solvent is compatible with the mobile phase conditions.



Notice: To avoid contaminating system components, do not recycle seal wash.

Tip: The seal-wash system is self-priming. You cannot prime it with a syringe under normal plumbed conditions.

Recommendations:

- Use seal wash that is fully soluble with all chromatographic solvents and that contains at least 10% organic solvent. This concentration prevents microbial growth and ensures that the seal wash can solubilize the mobile phase.
- Before priming the seal-wash system, ensure that the volume of seal wash is adequate for priming.
- Titanium is subject to corrosion in anhydrous methanol, which you can avoid by adding a small amount of water (~3 %). Slight corrosion is possible when ammonia is >10 %. If using an Alliance iS Bio HPLC system, you can alternatively remove the titanium filter sinkers (the system loses its first line of protection from particulates) or replace them with stainless steel sinkers instead if your analysis is unaffected by biocompatibility considerations.

Required tools and materials

- Chemical-resistant, powder-free gloves
- Protective eyewear
- Seal wash solution
- Tubing adapter (startup kit)

To prime the seal wash system:

1. Ensure that the seal wash inlet tubing is immersed in the wash solvent.
2. On the touchscreen, tap **HOME**, tap the solvent bottle icon, tap the seal wash condition card, and then tap **Prime Solvent** to prime the seal wash.

Note: You can also prime the seal wash as part of the system startup process.

3. On the touchscreen, tap **Setup > Startup**.
4. Follow the remaining on-screen prompts to finish the system startup process.

4.6.2 Priming the pump

Priming the pump is part of the system startup workflow on the touchscreen.

Priming prepares a new system for use or for a change in reservoirs or solvents. It also prepares a system for restarting after it is idle for more than four hours. During priming, the vent valve moves to the vent position, allowing flow to go to waste. The flow rate during priming is 10 mL/min.

Recommendation: Ensure that all solvents in solvent reservoirs A, B, C, and D are full and miscible.



Notice: To prevent salts from precipitating in the system, introduce an intermediate solvent, such as water, when changing from buffers to high-organic-content solvents. Consult the solvent miscibility tables in the Solvent Considerations section of the system guide.

Ensure that the solvent reservoirs contain sufficient solvent for adequate priming and subsequent operation of the system, and that the waste container can hold all the used solvent. For example, at 10 mL/min, priming for two minutes uses about 20 mL of each solvent.



Warning: To avoid spills, empty the waste container at regular intervals.

Requirement: Prime all solvent lines with solvent to ensure that the degasser and the gradient proportioning valve function properly.

4.6.2.1 Priming a dry pump via the touchscreen

Priming the pump is part of the system startup workflow on the touchscreen.

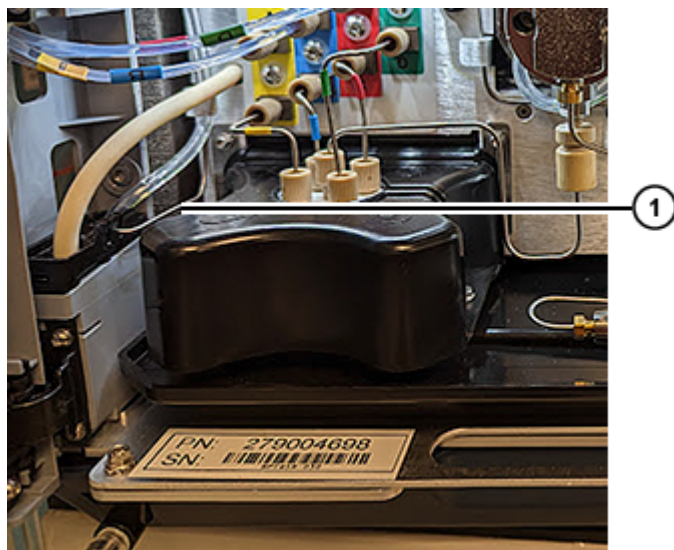
To prime a dry pump via the touchscreen:

1. Open the pump's front door.

Note: The pump's door is the system's bottom door.

2. Locate the 0.062-inch vent valve solvent waste line located on the left-hand side of the pump bay, nested in the left bezel. Leave the 0.062-inch vent valve solvent waste line submerged into the process waste for now.

Figure 4–7: Location of solvent vent tubing



① Solvent vent tubing

3. On the touchscreen, tap **Setup > Solvents > Prime Mobile Phase Solvents** and follow the on-screen prompts.
4. From the Prime Solvent by Solvent Line screen, tap **Prime Solvent A, Prime Solvent B, Prime Solvent C**, and/or **Prime Solvent D**.
5. Follow the remainder of the on-screen prompts to finish the priming process.
6. While the priming is in process, lift the 0.062-inch vent valve waste tube from the process waste manifold, exposing the end. You should see a steady flow of solvent after five minutes. Direct any flow into the top of the process waste cover (manifold) to prevent spills. If there is no flow, monitor solvent inlet lines A, B, C, and D to determine if they are filled with solvent.

Tip: When solvent flows from the vent tubing continuously, the path is primed.

Requirement: Ensure that enough solvent remains in the solvent reservoirs to supply subsequent methods.

4.6.3 Priming the sample manager

Priming the sample manager is part of the system startup workflow on the touchscreen.

Priming fills the wash system with wash solvent or the injection pathway with mobile phase. You prime the system to accomplish these tasks:

- Preparing a new sample manager for operation
- Preparing a sample manager for operation after it has been idle for more than 24 hours
- Changing the wash solvent
- Removing bubbles from the lines

Ensure that the wash solvent is correctly composed, that it is of LC-MS grade, and that it is miscible with other solvents used in the system. Use filters in all solvent reservoirs, and ensure that the volumes of solvents suffice for priming.

Note: You can prime the seal wash or needle wash by tapping the respective condition card on the system status dashboard. You also can prime the seal wash, needle wash, and sample metering pump as part of the system startup workflow. Tap **Setup > Startup** and follow the remainder of the on-screen prompts to finish the system startup process.

To prime the sample metering pump and wash solvent:

1. On the touchscreen, tap **Setup > Solvents > Prime Sample Metering Pump** and follow the on-screen prompts..
2. Follow the remainder of the on-screen prompts to finish the system startup process.

4.7 Choosing extension loops

An extension loop affects injection volume and system pressure. Be sure to select the correct loop for your application.

Extension loops, which increase the volume of sample that can be drawn and held for injection, are an optional part of the injection system. You install them between the needle and the pressure transducer.

Table 4–2: The following extension loops are available to use in the sample manager

Loop size ^a
50 µL
100 µL - Standard

a. The loop sizes shown are the maximum injection size the loop supports. For instance, the 100-µL loop supports injection sizes up to 100 µL.

4.8 Installing and replacing extension loops

Add an extension loop to the sample manager to add additional injection volume for larger samples. Replace an extension loop as needed to compensate for a different total injection volume.



Warning: To avoid personal contamination with biologically hazardous or toxic compounds, wear clean, chemical-resistant, powder-free gloves when performing this procedure.



Warning: To avoid eye injury, use eye protection when performing this procedure.

Required tools and materials

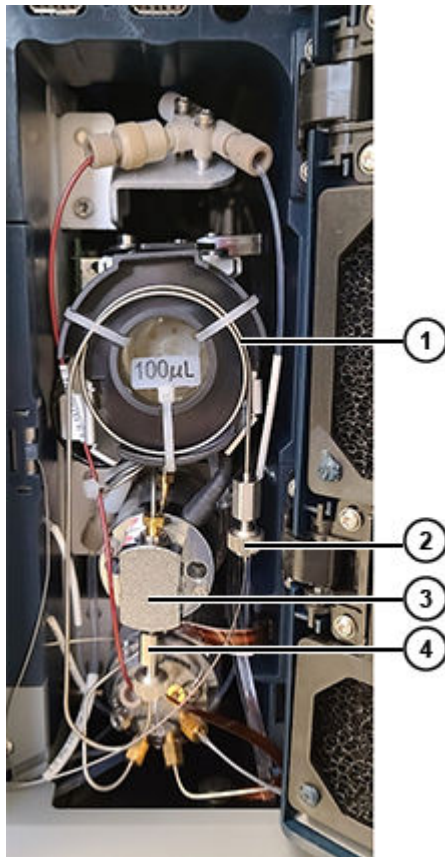
- Extension loop kit
- Chemical-resistant, powder-free gloves
- Protective eyewear

4.8.1 Installing an extension loop in a single-valve system

In a single-valve system, the extension loop is installed between the pressure transducer and the sample needle.

1. If system flow is running, stop the flow. On the touchscreen, tap **COMMANDS**, and then tap the pause button next to **Flow is On**.
2. Open the sample manager fluidics door.

Figure 4–8: Single-valve system



- ① Extension loop with holder

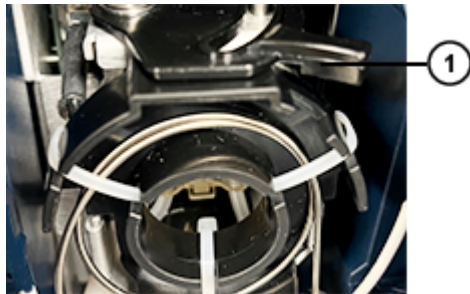
② Sample needle tool-free fitting

③ Pressure transducer

④ TFF adapter fitting

3. Disconnect the tool-free fitting (TFF) ② that is attached to the extension loop and the sample needle. There is no need to remove the TFF adapter fitting ④ from the pressure transducer.

Figure 4–9: Extension loop latch



① Extension loop latch

4. Push the extension loop latch ① backward to release the extension loop holder and remove it from the sample manager.
5. Install a different size extension loop between the TFF adapter fitting ④ and the sample needle. There is no need to remove the TFF adapter fitting ④ from the pressure transducer.
6. Push the extension loop latch forward to secure the extension loop holder in the sample manager.
7. On the touchscreen, tap **System > Module configuration** and follow the on-screen prompts to specify the extension loop volume configuration.
8. On the touchscreen, tap **Setup > Startup** and follow the on-screen prompts to prime and prepare the system for use.

4.9 Modifying needle and extension loop configuration parameters

The needle and extension loop must be properly configured on the touchscreen in order to avoid errors or system performance issues.

To modify the needle or extension-loop volume setting:

1. On the touchscreen, tap **System** > **Module configuration**, and then tap the sample



manager button .

2. Follow the remaining on-screen prompts to select the correct extension loop size configuration.

4.10 Choosing the needle-placement setting

If the needle is placed too high, it may not draw enough sample. If the needle is placed too low, it elevates the risk of introducing debris or precipitates into the system fluidics.

Needle placement is the vertical distance from the tip of the sample needle to the bottom of the sample vial. The default setting for the needle placement prevents the needle from reaching the bottom of the vial.

! **Notice:** To avoid damaging the needle, follow the guidelines in this section, ensure that the needle is calibrated, and use the appropriate needle-placement setting for your sample plates or vials.

You can change the default needle-placement setting in the software in two places: on the **Dilution** tab of the Sample Manager instrument method editor and in the Advanced Settings dialog box.

Table 4–3: Default needle-placement settings

Plate type	Default
48-vial	4.0 mm (automatic)
All other plates	2.0 mm


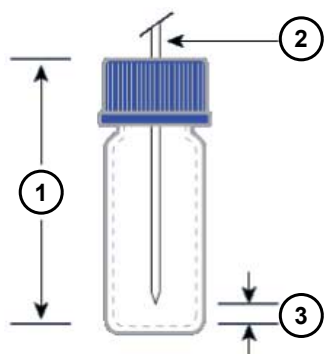
Note: The default needle-placement values listed in the table above represent the dimension specified by  in the figure below.

Figure 4–10: Sample needle in vial



- ① Vial depth
- ② Sample needle
- ③ Distance from tip of sample needle to bottom of sample vial

4.11 Creating a new plate type

Create standard or custom sample plates to use in sample set methods. Set the plate types correctly in Empower to avoid bending the needles.

Refer to the *Empower online Information System* for instructions on creating a new plate type.

4.11.1 Creating a new plate type using an existing plate type as a template

Starting with an existing plate type as a template can save time compared with creating an all new plate type.

Refer to the *Empower online Information System* for instructions on creating a new plate type using an existing plate type as a template.

4.12 Advanced settings

The system has a number of user-configurable settings suitable for the advanced user.

This section covers a number of advanced settings available with the Alliance iS HPLC System.

4.12.1 Choosing a draw rate for the sample syringe

If the chosen draw rate is too high, it can cause a "Drawing sample rate excessive" message.

You can change draw rate settings in the instrument method editor.

4.12.2 Recovering maximum sample from vials

Improper system settings can negatively affect how much sample is drawn from the vial.

The system has an optional vial bottom sensing feature. When it is enabled, the system will probe the bottom of the vial and then back off slightly so that the needle is very close to the bottom.

The default ANSI plate (48 vials) definition for the 2-mL Maximum Recovery Vials can leave some sample in the vial. If you must recover the maximum amount of sample possible, turn on the vial bottom sensing function.

See also: For information about enabling the vial bottom sensing feature, see [Setting up a method \(Page 97\)](#).

See also: Waters Sample Vials and Accessories brochure on www.waters.com.

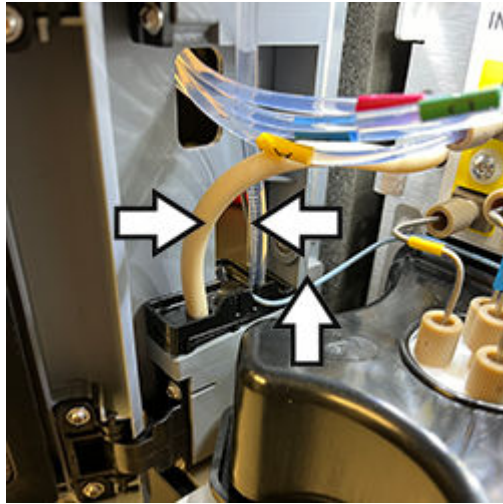
4.13 Separating condensate waste from LC waste

Separating condensate waste from LC waste allows the user to dispose of chemical waste separately.

Alliance iS systems are shipped with the condensate waste and LC waste routed to a single waste port on the front of the system.

1. Open the pump compartment door.
2. Remove the waste line tubing (see arrows) from the drain cup.

Figure 4–11: Tubing in pump compartment drain cup



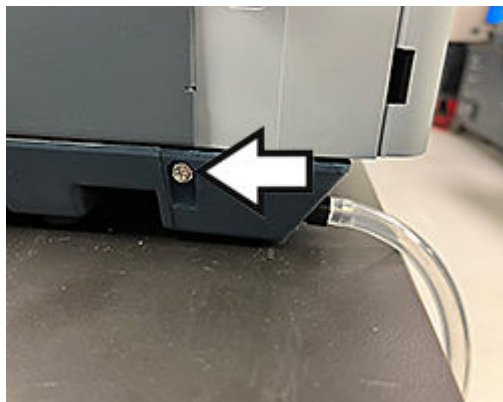
3. Lift the drain cup up.

Figure 4–12: Lifting the drain cup up



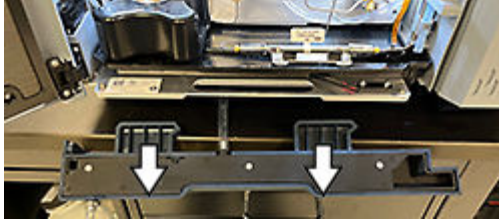
4. Remove the screw (see arrow) on the left-hand side that secures the drain tray.

Figure 4–13: Drain tray screw



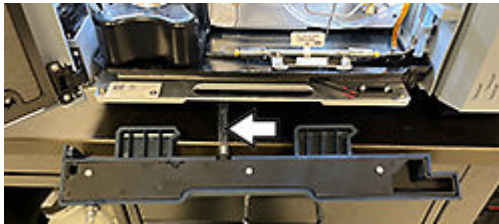
5. Pull the drain tray forward.

Figure 4–14: Pulling the drain tray forward



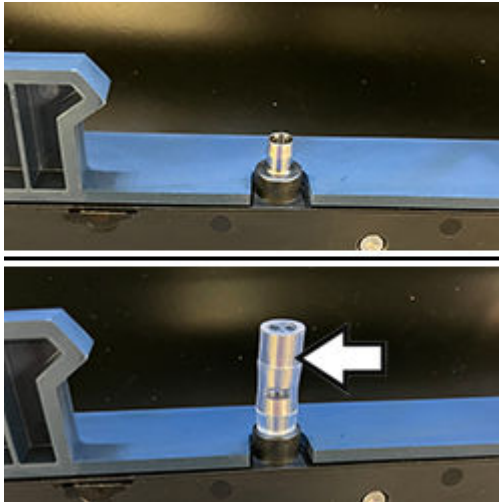
6. Remove the tubing from the drain tray.

Figure 4–15: Removing the tubing from the drain tray



7. Install the plug (see arrow) onto the drain tray barbed fitting.

Figure 4–16: Installing the drain tray plug



8. Pull the drain tray tubing out of the back of the system and reroute it around the outside of the system to the front.

9. Push the drain tray back in.

Figure 4–17: Pushing the drain tray in



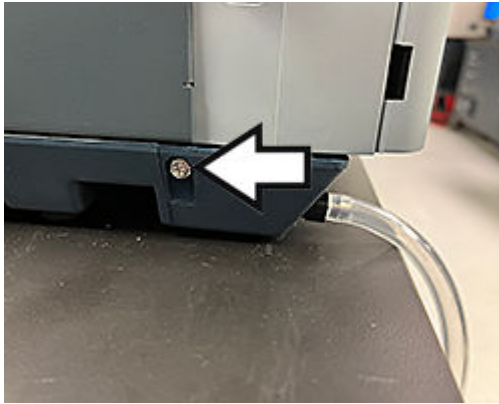
10. Reinstall the drain cup.

Figure 4–18: Installing the drain cup



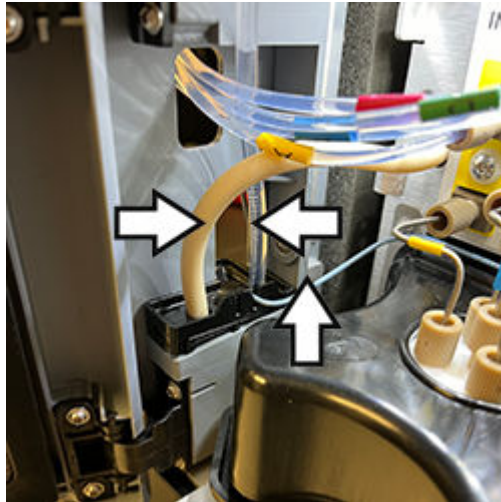
11. Install the screw (see arrow) on the left-hand side that secures the drain tray.

Figure 4–19: Drain tray screw



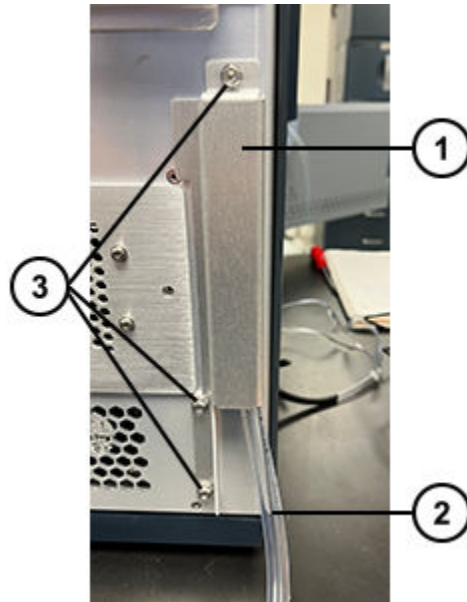
12. Place the waste line tubing (see arrows) back into the drain cup.

Figure 4–20: Tubing in pump compartment drain cup



13. If the condensate waste line is not long enough to reach the waste container:
 - a. Remove the three screws and the cover on the back of the system that covers the condensate waste line.

Figure 4–21:



- ① Condensate waste line cover
- ② Condensate waste line
- ③ Screws

- b. Remove the condensate waste line (see arrow) from the SHC (sample heater cooler) drain cup.

Figure 4–22: Disconnecting condensate waste line



- c. Make and install a new line long enough to connect to waste and connect it to the back of the system.
- d. Reinstall the three screws and the cover on the back of the system that covers the condensate waste line.
- e. Connect the new line to a dedicated condensate waste container. To avoid trapping air in the line, ensure that the tube is straight.

5 Method management

The Alliance iS HPLC System can run instrument methods in Empower projects that are translated by the Intelligent Method Translator (iMTA) app. See [Intelligent Method Translator \(iMTA\) \(Page 56\)](#), which specifies the systems whose instrument methods are translatable.

5.1 Transferring methods

The Alliance iS HPLC System can produce results comparable to those achieved on many other HPLC systems. However, when moving a regulated method from one system to another, typically no modifications to the instrument method can be made. For example, the column type (diameter) specified for a transferred method cannot be changed. Even with the same column type, results may not always be identical. If there is a significant difference in retention time, the dwell volume can be compensated by adjusting the gradient start relative to the injection. Extra-column effects can also be modified.

See the *Dwell Volume and Extra-Column Volume: What Are They and How Do They Impact Method Transfer* white paper (720005723EN) on www.waters.com.

5.2 Measuring dwell volume

When transferring a gradient LC method, you can produce comparable retention times by measuring dwell volume on both systems. The dwell volume is the system volume between the point where the gradient is formed and the column inlet.

You can measure dwell volume using the midpoint of a 0–100% gradient. To do so, run a gradient between two identical solvents, A and B, spiking the B solvent with a marker. Perform the measurement after configuring the system without the column for the instrument method being transferred, [replacing the column \(Page 159\)](#) with a low-volume restrictor to ensure proper pump function.

See also: “Measuring system volume for methods transfer” in the *Empower online Information System*.

6 Daily routine analysis

The Alliance iS HPLC System helps you to efficiently perform daily routine analyses by providing advanced hardware capabilities, an intuitive touchscreen, and connectivity with the Empower CDS. This section provides the instructions.

6.1 Signing in and out of the Alliance iS HPLC System

You can unlock the system at the kiosk, or lock it without powering-down, by signing in or out.

To sign in to the system and then lock and sign out:

1. Observe the Idle screen, which shows that the system is locked.
2. Swipe upward on the touchscreen. The [Home view \(Page 51\)](#) appears.
3. Perform work until you are ready to sign out.
4. Tap the **Preferences** button in the upper-right corner of the touchscreen.
5. Tap the **Lock** button at the bottom of the Preferences screen.
6. Tap the **SIGN OUT** button.
The notification "SIGN OUT displays the lock page. You will be signed out in *n* seconds" appears. Upon completion, the system is locked and transitions to the IDLE state.

6.2 Starting the hardware and software

When the Alliance iS HPLC System hardware is running, start the Empower software.

To start the hardware and software:

1. Unlock or power-on the Alliance iS HPLC System. (If the system is powered-down, see [Powering-on the system \(Page 57\)](#).)
2. From the touchscreen, tap the **Commands** button in the left-hand pane.
The [Commands view \(Page 54\)](#) appears.
3. Ensure that **Detector Lamp** status is `Lamp is on` or `Lamp is warming`.
If the status is `Lamp is off`, tap the bulb button and hold it as the timer counts down.
Then wait for the status to progress from `Lamp is warming` to `Lamp is on`.

Note: Allow 30-60 minutes for the detector lamp to warm up. For this reason, using the **Detector Lamp** command to turn on the lamp is preferable to turning it on during the setup process.

4. Start Empower at the workstation.
5. Open Run Samples.
The Empower control panel starts automatically in the status pane.
6. From the control panel, start the Alliance iS HPLC System console by clicking the right-arrow in the upper-right corner.
From the console, you can access configuration, diagnostics, and detailed statuses for all parts of the system.
7. If necessary, open the Empower project needed for the analysis.

6.3 Setting up solvents

Mobile phase solvents and the seal wash, needle wash, and purge solvents are set up before running equilibration.



Warning: To avoid personal contamination with biologically hazardous or toxic compounds, wear clean, chemical-resistant, powder-free gloves when performing this procedure.



Warning: To avoid eye injury, use eye protection when performing this procedure.

Required tools and materials

- Chemical-resistant, powder-free gloves
- Protective eyewear
- Clean solvent bottles

To set up solvents:

1. Prepare solvents in the clean bottles as required for the method.

Note: There can be up to four mobile phase solvent lines, identified by the system as A, B, C, and D. Pay particular attention to how the solvents are lettered in the project because their identifiers determine which solvent line to connect to each bottle. The seal wash, needle wash, and purge solvents have dedicated lines, but it is possible for a method to specify filling a bottle with the same preparation as a mobile phase bottle.

If necessary, obtain the solvent specifications for the method from the SOP or the Empower project.

2. Rotate each of the solvent bottles:
 - a. Remove the cap and solvent line tube from the bottle that is being replaced.
 - b. Remove that bottle from the tray.

- c. Pass the tube whose identification tag corresponds to the solvent through the opening in the replacement cap and install the cap on the bottle.
- d. Place that bottle on the tray as shown in the following figure.

Figure 6–1: Connected solvent bottles



3. From the touchscreen, set up initial priming of the solvent lines as described in [Equilibrating the Alliance iS HPLC system \(Page 81\)](#).

Note: If it is necessary to prime solvent lines outside of the equilibration workflow, return to the Home screen and select the appropriate workflow on the Setup > Solvents screen.

See:

- [Priming the seal-wash system \(Page 62\)](#)
- [Priming the pump \(Page 64\)](#)
- [Priming the sample manager \(Page 65\)](#)
- [Solvent considerations \(Page 166\)](#)
- [Replacing solvent bottle filters \(Page 101\)](#)

6.4 Installing or replacing the column

Install or replace the column required for the method after placing the filled solvent bottles on the tray so that old mobile phase cannot pass through the new column.

To install the column, follow the instructions in [Installing the column \(Page 59\)](#).

To replace the column, follow the instructions in [Replacing the column \(Page 159\)](#).

6.5 Equilibrating the Alliance iS HPLC system

Equilibration prepares the system for accurate data acquisition. Run the equilibration workflow when the system is idle for four hours or more, or after changing the mobile phase or sample needle.

To equilibrate the system:

1. From the touchscreen, click **Commands** to see the [Commands view \(Page 54\)](#).
2. Ensure that the **Detector Lamp** status is `Lamp is on`. Wait as long as necessary.

Note: Allow 30-60 minutes for the detector lamp to warm up. For this reason, using the **Detector Lamp** command to turn on the lamp is preferable to turning it on during the following setup process.

3. When the lamp status changes to `Lamp is on`, return to the [Home view \(Page 51\)](#) and tap **Setup** to see the [Setup view \(Page 52\)](#).
4. Tap **Startup** and follow the directions on the screen. Actions include:
 - a. Priming the mobile phase
 - b. Priming wash solvents
 - c. Setting sample temperatures and checkpoints
 - d. Switching on the detector lamp
 - e. Setting flow rate, composition, and duration
5. Review the settings on the Summary screen and tap **Start** to proceed with equilibration. The message `System Startup In progress` appears on the Status screen while equilibration is running. When equilibration ends, the system is ready for routine work.

6.6 Preparing and loading samples

The sample manager holds three ANSI/SBS-standard plates or trays that are loaded through the sample compartment door. Specific ANSI standard well plates, vial trays, vials, and cap mats or sealing caps are approved and required for use with the system. Incorrect loading of the plates or trays can result in an error.



Warning: To avoid personal contamination with biologically hazardous or toxic compounds, wear clean, chemical-resistant, powder-free gloves when performing this procedure.



Warning: To avoid eye injury, use eye protection when performing this procedure.

Required tools and materials

- Chemical-resistant, powder-free gloves
- Protective eyewear

To prepare and load samples:

1. Prepare the samples as specified for the method.
2. Fill the sample plates or trays as specified for the method. Waters vials are preferred.
See [Observing vial and plate recommendations \(Page 98\)](#).

Tip: Vial positions V1 through V12, located on the right-hand and left-hand sides of the sample tray, accommodate 4-mL vials. Contact Waters for inserts that allow you to use 2-mL vials in these positions.

3. Open the sample manager compartment door to access the platter as shown in the figure.

Figure 6–2: Sample manager platter



4. Press the plate/tray selector switch in the lower-left corner of the sample manager as needed to select position 1, 2, or 3.

Exception: If you press the selector switch while a diagnostic function is running, the sample manager is priming, or the sample needle is accessing the sample tray, making an injection, or being cleaned, the sample manager compartment light blinks continuously and the plate position does not change. The switch operates again after the sample manager completes the task in progress.

5. Pull out the frame for the selected position by grasping its handle.
6. Load the plate or tray onto the extended frame. The tray should sit flat.

Tip: For trays, “A” represents the row and “1” represents the vial position.

Figure 6–3: Trays loaded on the sample platter

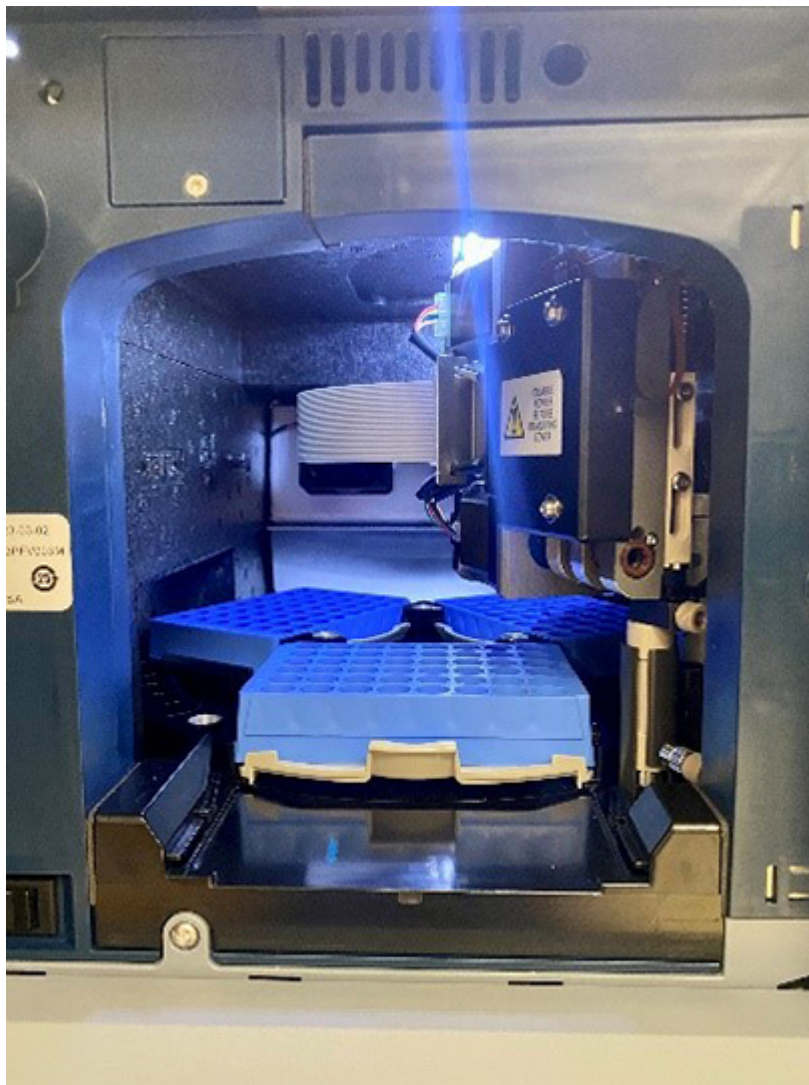
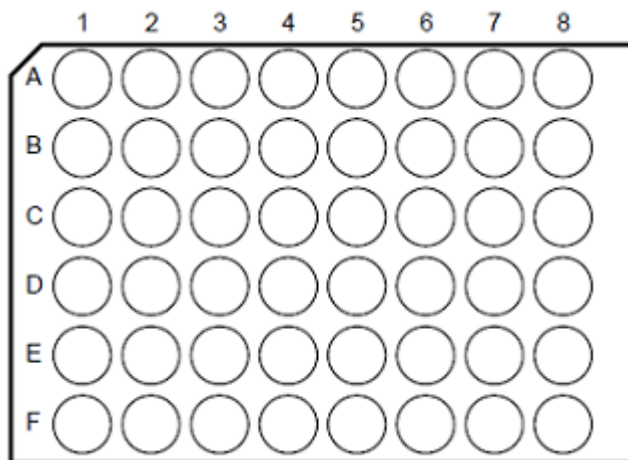


Figure 6–4: Sample plate vial positions



7. Slide the plate or tray backward until it clicks into place.

! **Notice:** To avoid damaging the sample needle, the sample plates must be positioned correctly, and the sample tray must be fully engaged.

8. Repeat the previous steps for each plate or tray.

9. Close the sample compartment door.

6.7 Verifying system status and health

Verify system status and health before you start to acquire data, periodically during the day, and whenever problems arise.

6.7.1 Data acquisition checks

Lab managers can set up pre-run and run-time data acquisition checks for minimizing common errors. The touchscreen provides the interface for setting the checks. From the Home screen, the path to the workflows is **System > Administration > Acquisition Checks**. The Acquisition Checks screen offers **Pre-Run Checks** and **Run-Time Checks**.

Note: This feature works with Empower 3.8.0 and later.

Data acquisition can proceed when all active pre-run checks pass. Checks can include:

- **Column must be installed:** Checks whether a column is installed in the column heater, but only if using a Waters column with a readable tag.
- **Column must match method:** Checks whether the installed column is appropriate for the selected method, but only if using a Waters column with a readable tag.
- **No pending preventative maintenance:** Checks the date set by **Preventative Maintenance > Set Preventative Maintenance**.

- **System is qualified:** Checks the date set by **Administration > System Qualification > Set the System Qualification**. This date must be supported by valid documentation and test results and is typically updated when a Waters engineer re-qualifies the system. Data acquisition cannot proceed if qualification is expired.
- **Mobile phase is not expired:** Checks the date for each of the mobile phase solvents (A, B, C, D) used by the method.
- **Sample plates must be installed**
- **Sample plates must match method**
- **All vials present**

Data acquisition halts when run-time checks detect selected issues while sample sets are running. Checks can include:

- **Mobile phase is low:** When any mobile phase solvent bottle is less than 10% full.
- **Wash solvent is low:** When any wash solvent bottle is less than 10% full.
- **Leak is detected:** Always enabled. Checking of the pump, column, detector, or Sample Manager leak sensors is controlled by **System > Leak Sensors**.
- **Vial is missing:** Always enabled. Checking for vials in the locations specified for the sample set is automatic.

6.7.2 Monitoring from the touchscreen

The touchscreen provides information on system state and conditions. For example, the status bar at the top of the window shows whether the Alliance iS HPLC System is currently running samples. When the system is on and not running, the dashboard shows Idle status and the screen color is blue. When the system is running, the dashboard shows Running status and the screen color is green. Red signifies error status.

The touchscreen's [Home view \(Page 51\)](#) shows current system conditions such as temperatures, pressures, and injection counts.

The touchscreen's [Health view \(Page 53\)](#) provides some troubleshooting tools.

6.7.3 Monitoring from the Empower control panel

You can monitor the Alliance iS HPLC System from the control panel of the Empower CDS, which appears at the bottom of the Run Samples window and can also be launched through the QuickStart menu. The control panel displays key system conditions such as state, temperature, and pressure.

Note: The values on the Empower control panel are read-only.

6.7.4 Monitoring from the Alliance iS HPLC System console

The Alliance iS HPLC System console is accessed from the Empower control panel. The console displays the current (or most recent) values of parameters, including temperatures and pressures, as follows:

Note: You cannot change settings through the console. Settings are changed at the touchscreen or by loading a method.

Figure 6–5: System console



Flow	2.000 mL/min
Composition	100% A 0% B 0% C 0% D
Delta Pressure	39.62 psi, 1min
System Pressure	4002.66 psi
Sample Pressure	3998.70 psi
Sample Temperature	20.3 °C
Column Temperature	19.9 °C
Ambient Temperature	21.4 °C
Lamp State	On
Channel A	200.0 nm
Channel B	230.0 nm

6.7.5 Acquiring data

To acquire chromatographic data from the Alliance iS system, prepare and run the sample set in Empower. See the Data Acquisition, Acquiring Data section in the *Empower online Information System*.

6.8 Reviewing the results

Review sample set results in Empower. See the Data Analysis section in the *Empower online Information System*.

6.9 Printing the report

Generate and print the report for sample set data in Empower. See the Report Generation section in the *Empower online Information System*.

6.10 Preparing to shut down the Alliance iS HPLC System

Note: See [Powering-off the system \(Page 58\)](#) for more information related to shutdown.

1. From the touchscreen, tap **SETUP** to see the [Setup view \(Page 52\)](#).
2. Tap **Shutdown** and follow the onscreen instructions to complete the next steps. Actions include:
 - a. Setting the sample temperature and set point
 - b. Setting the column temperature and set point
 - c. Setting flow rate and composition
 - d. Setting the lamp state
3. Review the settings on the Summary screen and tap **Start** to proceed.
4. Tap **Done**.
Shutdown continues to run in the background unless an error occurs.

6.10.1 Shutting down for less than 24 hours

For short-term idle times (less than 24 hours), maintain the solvent flow to preserve flow cell cleanliness.

If a few hours will pass before the next injection, reduce the flow rate in the interim to a few tenths of a mL/min to conserve solvent. Keep the detector operating and the column heater at operating temperature during this period.

To shut down the system for less than 24 hours:

1. Continue to pump the initial mobile phase mixture through the column. Doing so prevents accumulation of contaminants in the flow cell and maintains the column equilibrium necessary for good retention time reproducibility.
2. To lengthen lamp life, extinguish the detector lamp by tapping **COMMANDS > UV Detector Lamp**.

`Power off Lamp` appears on the screen.

6.10.2 Shutting down for more than 24 hours

Failure to flush the system/detector before shutdown can result in a plugged flow cell.

To shut down the detector for more than 24 hours:

1. Extinguish the detector lamp by tapping **COMMANDS > UV Detector Lamp**.
`Power off Lamp` appears on the screen.
2. Remove buffer salts and additives by flushing with water.
3. Flush the column and flow cell with 100% pure organic solvent.

7 Performance optimization

Review the following subjects related to optimizing the performance of the Alliance iS HPLC System:

- [General guidelines \(Page 88\)](#)
- [Preventing leaks \(Page 90\)](#)
- [Setting up a method \(Page 97\)](#)
- [Sample chamber considerations \(Page 98\)](#)
- [Observing vial and plate recommendations \(Page 98\)](#)
- [Cycle time between injections \(Page 99\)](#)
- [Maximizing column lifetime \(Page 99\)](#)

7.1 General guidelines

When performing an HPLC analysis, follow these general recommendations.

- Use high-quality (HPLC- or MS-grade) solvents, buffers, and additives.
- Use high-quality (HPLC- or MS-grade) water.
- Always use solvent filters on tubing in solvent bottles.
- Filter buffers with a 0.45- μ m filter membrane.
- Keep concentrated stock solutions to use when preparing working solutions, and, to maximize shelf life, refrigerate them when not using them.
- Do not add fresh buffer to old (a practice known as "topping off"). Doing so can promote microbial growth.
- Keep all solvent lines primed.
- Flush buffers from the system when they are not in use, taking care to avoid using solvents that can precipitate or otherwise react.
- Use 10%-20% organic solvent in water as a storage solvent if you expect the system to remain idle longer than 24 hours.
- Keep the seal wash line primed.
- Monitor the level in the waste vessel to ensure that it can accommodate all the expected waste.

For information about preventing and eliminating contamination, see *Controlling Contamination in LC/MS Systems (715001307)* on the Waters website (www.waters.com).

7.1.1 Carryover

Carryover is observed in chromatographic systems when a previously injected analyte appears as a peak in the chromatogram of subsequent samples.

Carryover tends to occur when a small amount of analyte remains in the system after a sample is injected. You can measure carryover by observing analyte peaks that appear when you run a blank sample immediately after an analytical sample.

Waters specifies sample carryover on the Alliance iS System at 0.002% maximum.

A common cause of carryover is inadequate washing of the system, specifically the sample needle. Choosing an appropriate wash solvent can minimize carryover for a particular analysis (see [Purge and wash solvent guidelines \(Page 174\)](#)). The wash solvent must be strong enough to dissolve any remaining sample on the needle, and the wash duration must be long enough to remove the residue from the system.

Method conditions also affect carryover. Too short a hold-time at the final conditions of a gradient, especially if the gradient is steep, can fail to remove all analytes from the system or the column. It is important to completely flush the system and re-equilibrate the column before proceeding to a subsequent analysis.

When trying to minimize carryover, the hydrophobicity and solubility of samples as well as cleanliness during sample preparation are additional factors to consider, as is contamination from sample preparation tools.

Tip:

- Test your sample in the wash solvent to ensure that it does not cause either the analyte or the matrix to precipitate.

7.1.1.1 Reducing carryover

Failure to follow specified guidelines can lead to unwanted carryover between injections.

In a chromatographic system, any substance that creates unwanted peaks or excessive background noise is contamination. Carryover, a specific type of contamination, occurs when sample material remaining in the system after an injection appears as peaks in subsequent injections, compromising quantification. To optimize system performance, carryover must be minimized and held to an acceptable level (often below the limits of detection).

Note: Carryover can occur from column interactions or in the system. You can identify column carryover by performing a double gradient on the column. If carryover is observed in the second gradient, Waters recommends washing the column in a strong solvent.

Carryover can result from incorrectly installed tubing, fittings, or other hardware or by ineffective wash solvents. Take these actions to reduce carryover:

- Restrict extension loop usage to one system.
- Ensure that all tubing connections are properly seated. Before tightening the fitting compression screws, tubing must seat properly (without internal gaps) inside all connection

ports. Poorly seated connections create reservoirs of unnecessary space that retain sample, increasing carryover. (See [Preventing leaks \(Page 90\)](#).)

- Inspect the needle guide for sample residue or debris, which can cause carryover. If necessary, clean or replace the guide.
- Avoid plate or vial sealing systems that use sticky substances, which can cause carryover.
- If you suspect sample interaction with the needle material, increase the strength of the wash solvent, or increase the wash time.
- When selecting wash solvents, follow these [Purge and wash solvent guidelines \(Page 174\)](#).

See also: For more information about controlling contamination in chromatographic systems, see *Controlling Contamination in LC/MS Systems (715001307)* on the Waters website (www.waters.com).

7.2 Preventing leaks

Preventing leaks during an analysis ensures adequate flow pressure in the system and the integrity of the sample.

Leaks can occur at any tubing connection, gasket, or seal, but they are most common at tubing connections. Low-pressure leaks (on the intake side of the solvent manager's pump) cause solvent loss and air introduction during the intake cycle. Leaks at high-pressure fittings (downstream of the check valves) can release solvent but do not introduce air.

To prevent leaks, follow Waters' recommendations for the proper tightening of system fittings. Note that different techniques apply to re-tightening fittings and installing them for the first time.

7.2.1 Installation recommendations for fittings

Reduce the risk of tubing connection leaks by following Waters recommendations. Also, ensure that appropriate and properly tightened fittings are used as described.

Three types of fitting assemblies are used within the system:

- Polyetheretherketone (PEEK), polymer-based
- Stainless steel (SST), gold-plated
- Tool-free fittings (TFF)



Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.



Warning: To avoid personal contamination with biologically hazardous materials, wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Required tools and materials

- Chemical-resistant, powder-free gloves
- Protective eyewear
- 1/4-inch open-end wrench, for tightening or loosening SST (gold-plated) fittings with two-piece ferrules
- Permanent marker

When connecting tubing, heed the following recommendations for installing and tightening fittings:

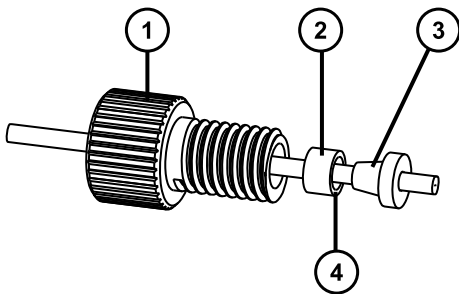
- Before tightening compression screws, ensure that the tubing is fully bottomed in its connection port.
- For easier accessibility, use long compression screws to attach tubes to the injector and vent valve.
- Whenever you loosen fittings during maintenance, examine for cracks, stripped threads, and deformations.
- Whenever you loosen or replace fittings during maintenance, perform the solvent manager leak test (see your system's online Help).
- Except for those that are tool-free, do not reuse SST fittings more than six times.

7.2.1.1 Short or long 1/4-28 fitting with flangeless ferrule and stainless steel lock ring

Tighten the fitting finger-tight.

Important: Short fittings are intended for use with 1/16-inch OD tubing. Long fittings are intended for use with 1/8-inch OD tubing.

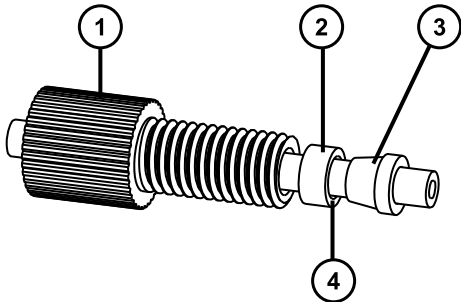
Figure 7–1: Short 1/4-28 fitting with flangeless ferrule and SST lock ring, first use or reinstallation



- ① Compression screw
- ② Lock ring
- ③ Ferrule

- ④ End of lock ring with larger ID

Figure 7-2: Long 1/4-28 fitting with flangeless ferrule and SST lock ring, first use or reinstallation



- ① Compression screw
- ② Lock ring
- ③ Ferrule
- ④ End of lock ring with larger ID

7.2.1.2 High-pressure pin plug

This fitting type is used to plug an unused port or, in some instances, to pressure-check the system.

Tighten the fitting finger-tight, plus approximately a 1/6-turn using a wrench.

Figure 7-3: High-pressure pin plug, first use or reinstallation

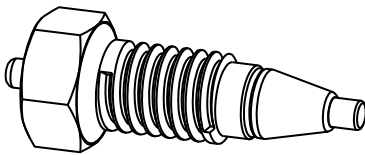
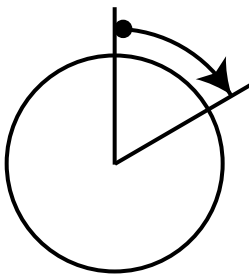


Figure 7-4: High-pressure pin plug tightening, first use or reinstallation



7.2.1.3 Metallic fitting with short or long flats and two-piece metallic ferrule (V-detail)

The procedure for tightening metallic fittings differs for new versus reused fittings.

In order to avoid leaks, see the following recommendations in this guide:

- See [Preventing leaks \(Page 90\)](#) and [Installation recommendations for fittings \(Page 90\)](#).
- For detailed instructions about assembling new fittings, see [Assembling new metallic fittings \(Page 95\)](#).

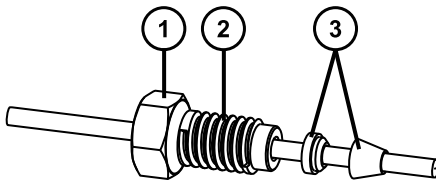
! **Notice:** Before tightening compression screws, ensure that the tubing is fully bottomed in its connection port.

! **Notice:** Different techniques apply to installing new fittings and re-tightening previously used fittings.

First use

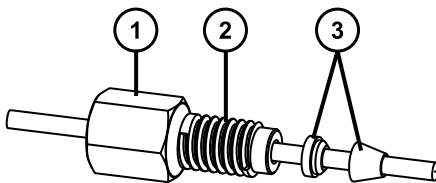
! **Notice:** Tighten the fitting finger-tight plus an additional 3/4-turn using a 1/4-inch open-end wrench.

Figure 7–5: Metallic fitting with short flats and two-piece metallic ferrule, first use



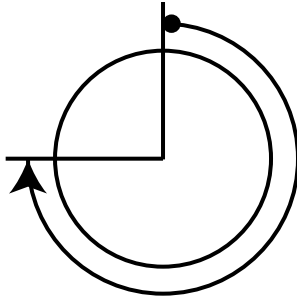
- ① Short flats
- ② Compression screw
- ③ Two-piece metallic ferrule

Figure 7–6: Metallic fitting with long flats and two-piece metallic ferrule, first use



- ① Long flats
- ② Compression screw
- ③ Two-piece ferrule

Figure 7–7: Metallic fitting with short or long flats and two-piece metallic ferrule, first-use tightening

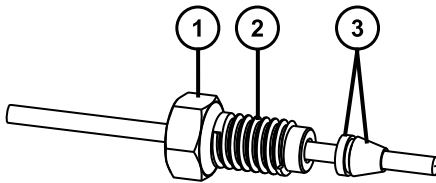


Reinstallation

! **Notice:** For best performance, only reinstall this connection into the same port from which it was removed.

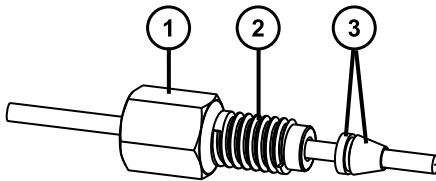
Tighten the fitting finger-tight plus as much as an additional 1/6-turn using a 1/4-inch open-end wrench.

Figure 7–8: Metallic fitting with short flats and two-piece metallic ferrule, reinstallation



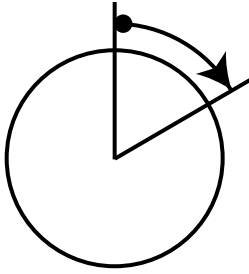
- ① Short flats
- ② Compression screw
- ③ Two-piece metallic ferrule

Figure 7–9: Metallic fitting with long flats and two-piece metallic ferrule, reinstallation



- ① Long flats
- ② Compression screw
- ③ Two-piece ferrule

Figure 7–10: Metallic fitting with short or long flats and two-piece metallic ferrule, reinstallation tightening



7.2.1.3.1 Assembling new metallic fittings

To ensure proper assembly, you must mark new metallic fittings before tightening them.



Warning: To avoid eye injury, use eye protection when performing this procedure.



Notice: To prevent contaminating system components, wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Required tools and materials

- Chemical-resistant, powder-free gloves
- Protective eyewear
- 1/4-inch open-end wrench, for metallic fittings with two-piece ferrules
- Permanent marker

To assemble a new metallic fitting:

1. Insert the end of a tube into the hexagonal end of the compression screw.
2. Insert the tube into the larger end of the ferrule.
3. Insert the tube into the connection port.
4. Rotate the compression screw clockwise into the connection port until the screw is finger-tight.



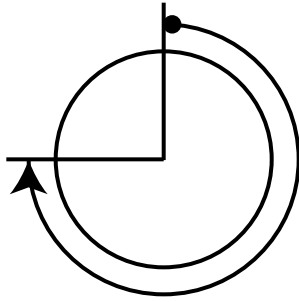
Notice: Before tightening compression screws, ensure that the tubing is fully bottomed in its connection port.

5. Using the permanent marker, mark the compression screw at the 12-o'clock position.
6. Using the permanent marker, mark the connection port at the 9-o'clock position.
7. Ensure that the tubing makes contact with the bottom of the connection port, and then use the 1/4-inch open-end wrench to rotate the compression screw clockwise 3/4-turn until the two marks line up.



Notice: Before tightening compression screws, ensure that the tubing is fully bottomed in its connection port.

Figure 7–11: New fitting, first-use tightening



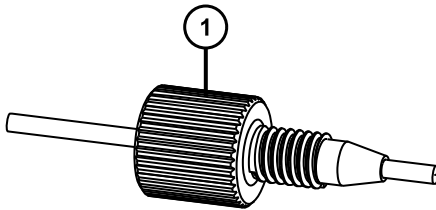
7.2.1.4 One-piece PEEK fitting

Tighten the fitting finger-tight.

Tip: Using the aluminum flangeless nut extender (included in the system startup kit) will help achieve proper tightness of this fitting.

! **Notice:** Before tightening compression screws, ensure that the tubing is fully bottomed in its connection port.

Figure 7–12: One-piece PEEK fitting, first use or reinstallation



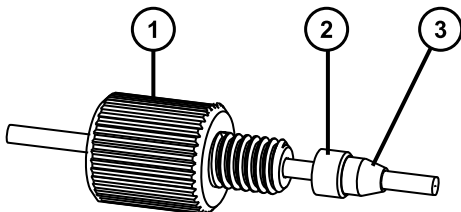
① Compression screw

7.2.1.5 PEEK fitting with PEEK ferrule and stainless steel lock ring

Tighten the fitting finger-tight.

! **Notice:** Before tightening compression screws, ensure that the tubing is fully bottomed in its connection port.

Figure 7–13: PEEK fitting with PEEK ferrule and stainless steel lock ring, first use or reinstallation



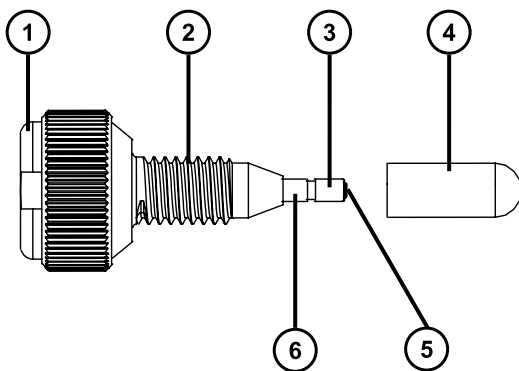
① Compression screw

- ② Lock ring
- ③ Ferrule

7.2.1.6 Tool-free fitting

Tighten the fitting finger-tight.

Figure 7–14: TFF, first use or reinstallation



- ① Retainer cap
- ② Compression screw
- ③ Fitting collar
- ④ Protective cap
- ⑤ Face seal gasket
- ⑥ Welded tubing assembly

7.3 Setting up a method

You create a new method using the system's Empower software.

(See [Method management \(Page 77\)](#) for information on measuring dwell volume and transferring methods.)

From the Empower startup window:

1. Click **Browse Projects**.
2. From the main menu, click **File > New Method**, and then click **Instrument Method**, **Processing Method**, or **Method Set**.

3. Specify the method settings.

7.4 Sample chamber considerations

When the sample manager door is open, there is a possibility of injury. Exercise caution.



Warning: To avoid puncture wounds, keep hands and loose clothing clear of the needle assembly mechanism while it is moving. Note that the sample manager interior light flashes whenever the sample compartment door is open and the needle assembly mechanism is about to move.

7.5 Observing vial and plate recommendations

Incorrect selection of sample vials and plates can lead to issues with system function and performance.

Waters recommends that you observe these usage guidelines for sample vials and plates in the sample manager:

- Vials
 - Use only Waters-certified vials.
 - Ensure that vial holders conform to ANSI/SBS standards.
- Plates
 - Use only Waters-approved plates and cap mats.
 - When selecting a new plate supplier, especially for 384-well plates, measure the plate size to ensure compatibility with Waters' specifications for the sample manager.
 - To avoid warping plates, do not centrifuge them.
 - Be aware that plates containing samples with high concentrations of organic solvent can give inconsistent results at or above room temperature due to solvent evaporation.
- Covers
 - Use cap mats on sample plates whenever possible.
 - Use pre-slit cap mats/seals and vial caps. Use of non-pre-slit cap mats and vial caps can cause clogging in the wash drainage lines.
 - To prevent sample spillage or needle damage, use only Waters-approved covers on the sample vials.

See also: *Waters Sample Vials and Accessories Brochure* (720001818EN), or visit https://www.waters.com/nextgen/us/en/products/vials--plates--and-certified-containers.html?icid=hm-fea_00512 for information on plates and vials.

7.6 Cycle time between injections

Sample draw rate affects cycle time.

You can reduce cycle time by setting an appropriate sample draw rate that allows maximum throughput and performance.

7.7 Maximizing LC column lifetime

For maximum column lifetime, follow the manufacturer's recommendations at all times.

Important: To greatly improve column lifetime and performance, Waters recommends that you obtain and follow the manufacturer's guidelines and operational ranges for column temperature, mobile phase pH, and buffer additives.

! **Caution:** When operating the Alliance iS Bio HPLC System above pH 10, ensure that you are using the optional high pH kit. For further assistance, contact Waters.

8 Maintenance

This chapter provides maintenance procedures for the Alliance iS HPLC System that users or Waters field service personnel can perform.

8.1 Viewing Alliance iS HPLC System information

From the touchscreen, tap **System** > **About**. From the About screen you can select **HARDWARE** or **SOFTWARE** information.

8.2 Safety and handling



Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.



Warning: To avoid electric shock, do not remove protective panels from the device. The components within are not user-serviceable.



Notice: To avoid damaging electrical components and circuitry, do not disconnect an electrical assembly while electrical power is applied to a module. To completely interrupt power, set the on/off switch to the "off" position, and then disconnect the power cord from the ac source. Wait 10 seconds before disconnecting an assembly.

8.3 Configuring maintenance warnings

Warnings for preventive maintenance and system qualification dates are configurable.

The Alliance iS HPLC System can be configured to warn users that deadlines for preventive maintenance or system qualification are approaching.

The lab manager can set the deadline and a reminder for preventive maintenance through the touchscreen UI. Tap **System** > **Administration** > **Preventative Maintenance** > **Set Preventative Maintenance**.

Similarly, the lab manager can set the annual deadline and a reminder for system qualification. Tap **System** > **Administration** > **System Qualifications** > **Set the System Qualification**.

8.4 Ordering spare parts

To ensure that your system operates as designed, use only Waters Quality Parts. Visit <https://www.waters.com/nextgen/nl/en/c/promo/spare-parts.html> for information about Waters Quality Parts and how to order them.

8.5 Cleaning the exterior of the equipment



Warning: To avoid electric shock:

- Ensure that the electrical power to the equipment is interrupted.
- When cleaning the surface of the equipment, apply water to a cloth, and then wipe the instrument or device. Do not spray or otherwise apply water directly onto any equipment surface.



Warning: To avoid personal injury, use eye and hand protection during the cleaning process.

Required tools and materials

- Chemical-resistant, powder-free gloves
- Protective eyewear

To clean the exterior of the equipment:

- Clean surfaces of the equipment using only a clean, soft, lint-free paper towel or clean cloth dampened with water.

8.6 Replacing solvent bottle filters

Replace solvent bottle filters when unexpected results caused by contamination are observed.

Solvent filters are critical clean parts that protect your system from contamination. A clogged solvent bottle filter can cause weak or intermittent loss of prime, a bad gradient profile, retention time shifts, and broad peaks. A contaminated solvent bottle filter can cause a contamination peak.

Required tools and materials

- Chemical-resistant, powder-free gloves
- Protective eyewear
- Solvent bottle filters, 6-pack (700013884)

To replace the solvent bottle filters:

1. Remove the filtered end of the solvent tubing from the solvent bottle.
2. Remove the old filter from the short piece of fluoropolymer tubing.

Note: Do not remove the solvent tubing from the solvent bottle cap.

3. Insert the new filter into the fluoropolymer tubing, pushing until it contacts the solvent tubing.

Notes:

- Titanium solvent filters are identified by "Ti" on the top surface of the filter.
 - Titanium is subject to corrosion in anhydrous methanol, which you can avoid by adding a small amount of water (approximately 3%). Slight corrosion is possible when ammonia is >10%. If using an Alliance iS Bio HPLC system, you can alternatively remove the titanium filter sinkers (the system loses its first line of protection from particulates) or replace them with stainless steel sinkers if your analysis is unaffected by biocompatibility considerations.
4. Insert the filtered end of the solvent tubing into the solvent bottle.
 5. Shake the solvent tubing to remove any air from the filter.
 6. Submerge the entire filter in the solvent.
 7. Prime the pump. See [Priming the pump \(Page 64\)](#).

8.7 Pump maintenance procedures

This section provides maintenance procedures for the Alliance iS HPLC System pump that users or Waters field service personnel can perform. Procedures include:

- Servicing the pump compartment air filter
- Replacing the pump leak sensor
- Replacing the pump mixer
- Replacing the in-line filter cartridge on the primary check valve
- Replacing the accumulator check valve

8.7.1 Pump maintenance schedule

The pump has a recommended maintenance schedule.

Users can perform the following routine pump maintenance procedures.

Maintenance procedure	Frequency
Replacing solvent bottle filters (Page 101)	As needed; during scheduled routine maintenance

Maintenance procedure	Frequency
Servicing the pump compartment air filter (Page 103)	As needed; during scheduled routine maintenance
Replacing the pump leak sensor (Page 104)	As needed
Replacing the pump mixer (Page 107)	As needed; during scheduled routine maintenance
Replacing the in-line filter cartridge on the primary check valve (Page 108)	As needed; during scheduled routine maintenance
Replacing the accumulator check valve (Page 114)	As needed; during scheduled routine maintenance

8.7.2 Servicing the pump compartment air filter

You can clean or replace the pump compartment air filter.

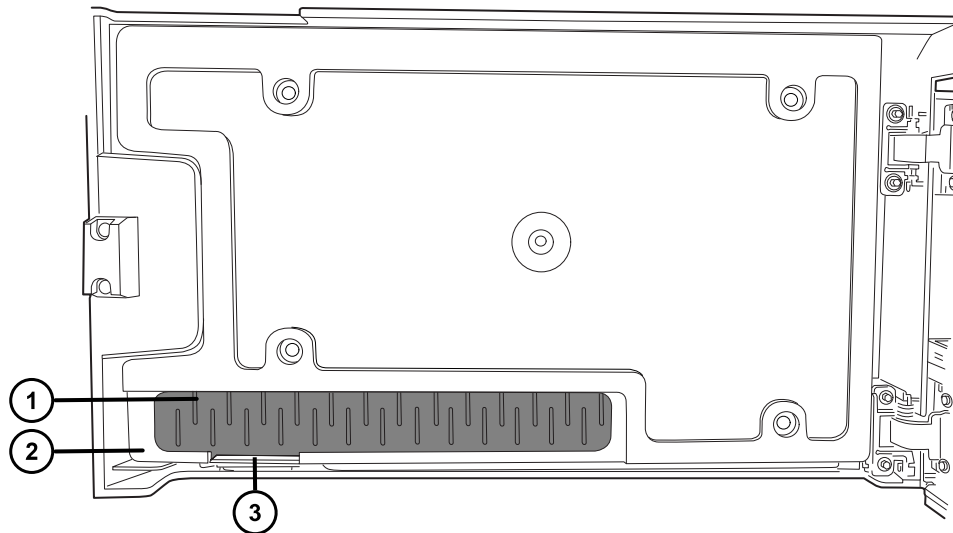
Required tools and materials

- Mild detergent and water
- Air filter (if replacing)

To service the air filter:

1. Open the pump compartment door.
2. Compress the air filter upward above the frame relief area. Then pinch the filter and pry it away from the frame.

Figure 8–1: Pump compartment air filter



- ① Air filter
- ② Air filter frame
- ③ Frame relief area

3. Do one of the following:
 - Clean the air filter using a mild detergent and water, and then dry the filter.
 - Discard the old air filter.
4. Compress the air filter slightly and reattach inside the frame.
5. Close the pump compartment door.

8.7.3 Replacing the pump leak sensor

Users or Waters field service personnel can replace the pump leak sensor.



Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.



Warning: To avoid personal contamination with biologically hazardous or toxic compounds, wear clean, chemical-resistant, powder-free gloves when performing this procedure.



Warning: To avoid eye injury, use eye protection when performing this procedure.



Requirement: Wear clean, chemical-resistant, powder-free gloves when performing this procedure.

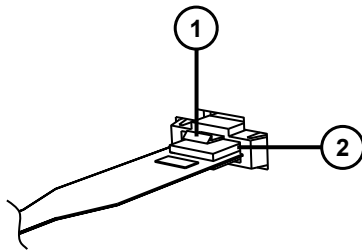
Required tools and materials

- Chemical-resistant, powder-free gloves
- Protective eyewear
- Replacement leak sensor

To replace the leak sensor:

1. Open the pump compartment door.
2. Press down on the leak sensor connector tab and remove the connector from the receptacle.

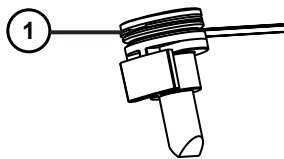
Figure 8–2: Leak sensor connector



- ① Tab
- ② Leak sensor connector

3. Grasp the leak sensor by its serrations and pull upward to remove it from its reservoir.

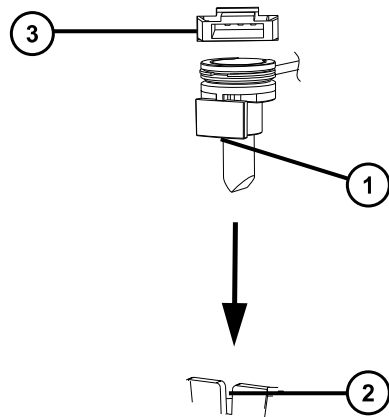
Figure 8–3: Leak sensor serrations



- ① Serrations

4. Unpack the new leak sensor.
5. Align the leak sensor's T-bar with the slot in the front of the pump tray, and then slide the leak sensor into the slot.

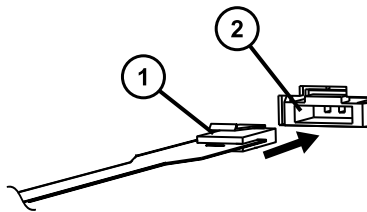
Figure 8–4: Aligning T-bar with slot



- ① T-bar
- ② Slot in leak sensor reservoir
- ③ Leak sensor port on receptacle

6. Connect the leak sensor connector to the front of the device.

Figure 8–5: Attaching leak sensor connector



- ① Leak sensor connector
- ② Leak sensor receptacle on front of pump tray plate

7. Close the pump compartment door.

8. From the touchscreen [Commands view \(Page 54\)](#), tap **Reset**.

9. From the touchscreen [System view \(Page 54\)](#), tap **Leak Sensors** and then enable the **QSM Leak Sensor**.

8.7.4 Replacing the pump mixer

Users or Waters field service personnel can replace the pump mixer.



Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.



Notice: To avoid contaminating system components, wear clean, chemical-resistant, powder-free gloves when performing this procedure.

Required tools and materials

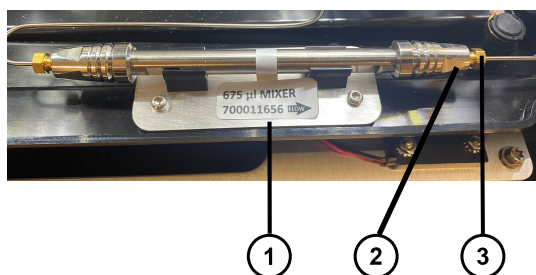
- Chemical-resistant, powder-free gloves
- 1/4-inch open-end wrench
- 3/8-inch open-end wrench
- Replacement mixer

Note: There is an optional 690- μ L diffusion bonded mixer kit that offers significantly better mixing performance than the traditional 675- μ L or 680- μ L mixers, resulting in lower compositional noise and a quieter baseline in select applications. For information on installing the 690- μ L diffusion bonded mixer, see the *Ti Diffusion Bonded Mixer - 690 μ L Kit Installation Guide* (715009251).

To replace the mixer:

1. Flush the pump with nonhazardous solvent.
2. Stop the solvent flow.
3. Open the pump compartment door.
4. Remove the mixer from the mixer clip.
5. Using the 3/8-inch open-end wrench to hold the mixer in place, disconnect the outlet compression fitting using the 1/4-inch open-end wrench.

Figure 8–6: Location of the outlet compression fitting for the mixer



- ① Mixer
- ② Wrench flats

③ Outlet compression fitting

- Using the 3/8-inch open-end wrench to hold the mixer, disconnect the inlet compression fitting using the 1/4-inch wrench.

Figure 8–7: Location of the inlet compression fitting for the mixer



①

②

③

① Inlet compression fitting

② Wrench flats

③ Mixer

- Unpack the replacement mixer.

Note: Ensure that the arrow on the mixer is pointing from left to right.

- Reattach the compression fittings to the mixer and tighten them finger-tight, plus as much as an additional 1/6-turn for existing fittings or 1/2-turn for new fittings.
- Insert the body of the mixer into the clips.
- Close the pump compartment door.
- From the touchscreen [Commands view \(Page 54\)](#), tap **Reset**.

8.7.5 Replacing the in-line filter cartridge on the primary check valve

Users or Waters field service personnel can replace the in-line filter cartridge on the pump primary check valve.



Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.



Notice: To avoid contaminating system components, wear clean, chemical-resistant, powder-free gloves when performing this procedure.

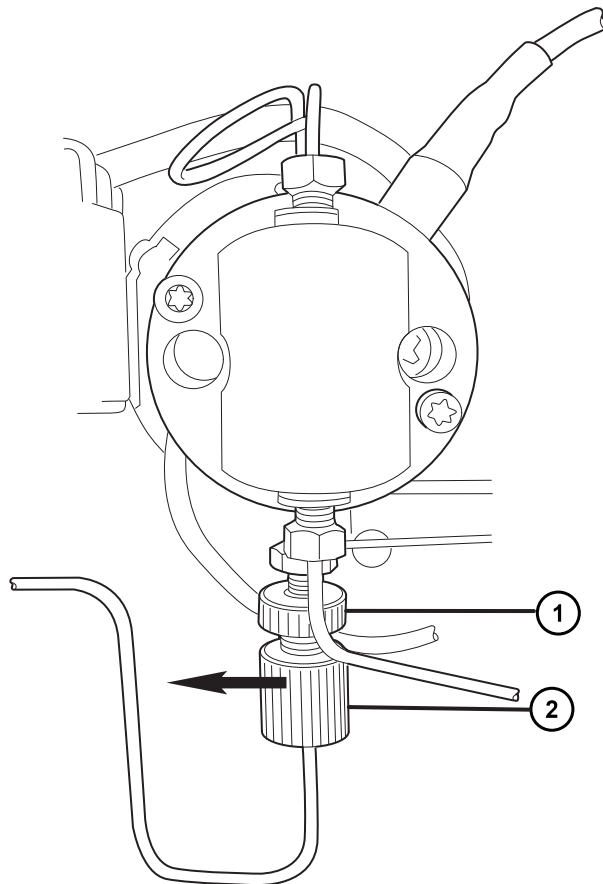
Required tools and materials

- Chemical-resistant, powder-free gloves
- Replacement in-line filter cartridge

To replace the in-line filter cartridge on the primary check valve:

1. Flush the pump with nonhazardous solvent.
2. [Power-off the system \(Page 58\)](#).
3. Open the pump compartment door.
4. Hold the ferrule holder fitting while unscrewing and remove the cap nut from the fitting.

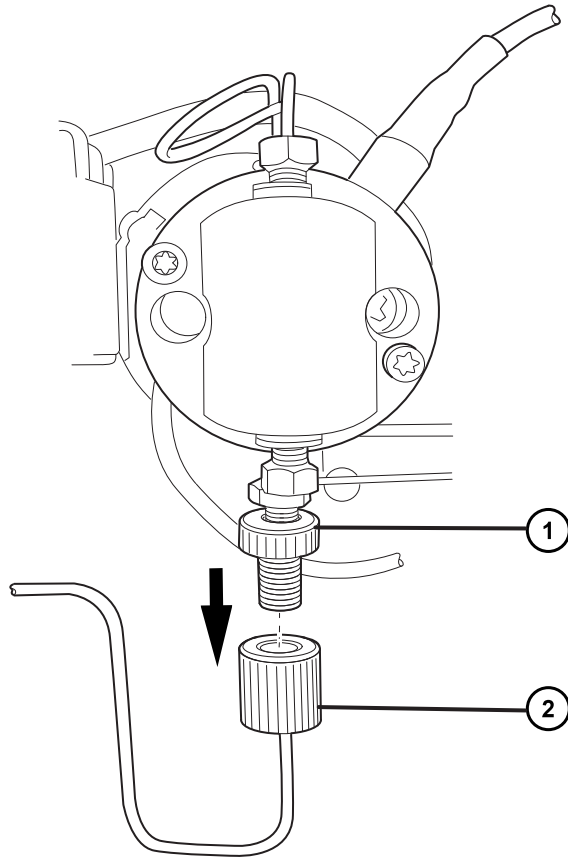
Figure 8–8: Unscrewing the cap nut and ferrule holder



① Ferrule holder fitting

② Cap nut

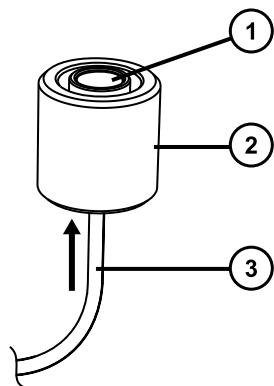
Figure 8–9: Removing the cap nut from the ferrule holder



- ① Ferrule holder fitting
- ② Cap nut

5. Slide the cap nut down the tube and disengage the filter from the ferrule holder fitting.

Figure 8–10: Pulling the cap nut from the tube

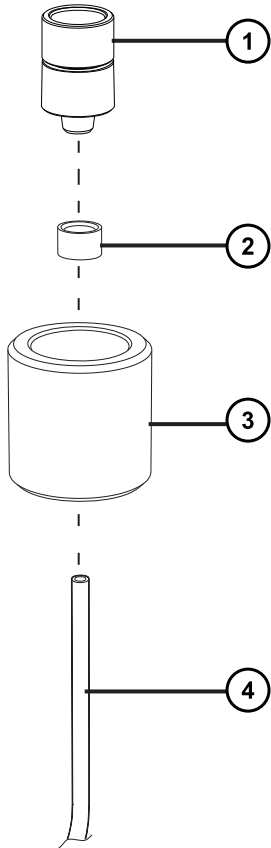


- ① In-line filter cartridge

② Cap nut

③ Tube

Figure 8–11: In-line filter cartridge, locking ring, and cap nut



① In-line filter cartridge

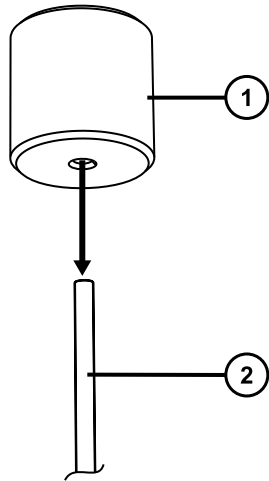
② Metal locking ring

③ Cap nut

④ Tube

6. Slide the metal locking ring downward off the in-line filter cartridge and slide the cartridge off the tube.
7. Inspect the filter cartridge to determine whether it is constructed of stainless steel or titanium (Ti), and ensure that you have the correct replacement cartridge. No marking indicates a filter cartridge constructed of stainless steel, and "Ti" indicates a filter cartridge constructed of titanium.
8. Slide the cap nut over the end of the tube.

Figure 8–12: Sliding cap nut on tube

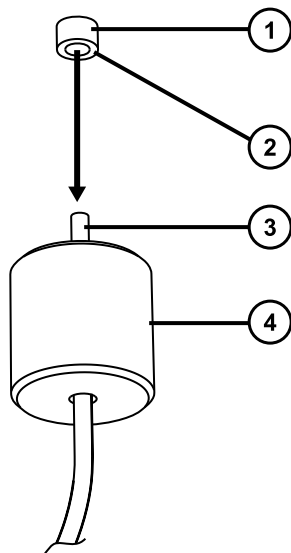


① Cap nut

② Tube

9. Slide the metal locking ring onto the tube, ensuring that the thicker end of the metal locking ring faces toward the cap nut.

Figure 8–13: Sliding metal locking ring on tube



① Metal locking ring

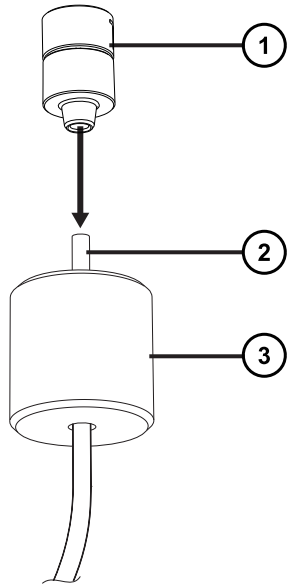
② Thicker end of metal locking ring facing toward cap nut

③ Tube

④ Cap nut

10. Unpack the new in-line filter cartridge.
11. Place the new in-line filter cartridge onto the end of the tube.

Figure 8–14: Placing in-line filter cartridge onto end of tube



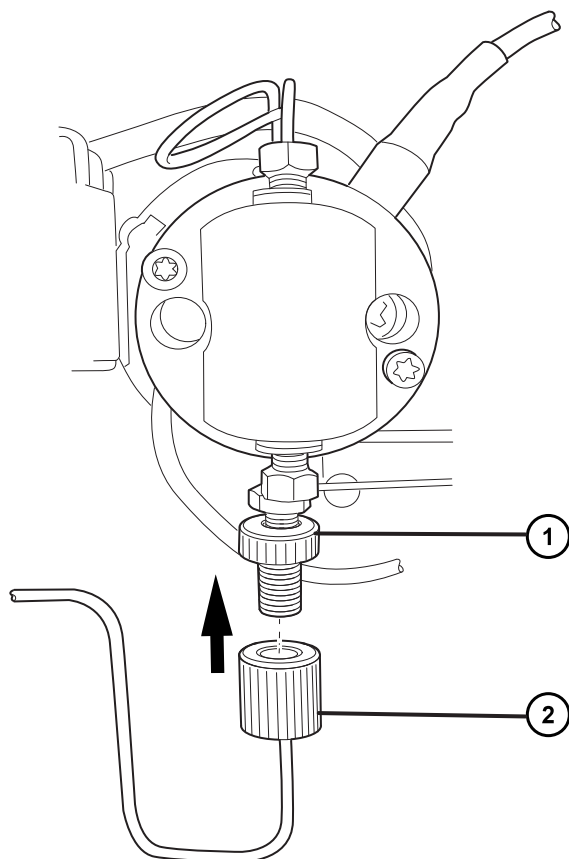
① In-line filter cartridge

② Tube

③ Cap nut

12. Verify that the cartridge is bottomed out in the tube, and then slide the metal locking ring onto the boss on the bottom of the in-line filter cartridge while making sure the tube stays bottomed out.
13. Insert the in-line filter cartridge with tubing into the ferrule holder fitting, finger-tighten the cap nut until it bottoms out, and then tighten it 1/4-turn.

Figure 8–15: Installing the cap nut on the ferrule holder fitting



① Ferrule holder fitting

② Cap nut

14. Close the pump compartment door.
15. [Power-on the system \(Page 57\)](#).
16. [Prime the pump \(Page 64\)](#).

8.7.6 Replacing the accumulator check valve

Users or Waters field service personnel can replace the pump accumulator check valve.



Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.



Warning: To avoid eye injury, use eye protection when performing this procedure.

! **Notice:** To avoid contaminating system components, wear clean, chemical-resistant, powder-free gloves when performing this procedure.

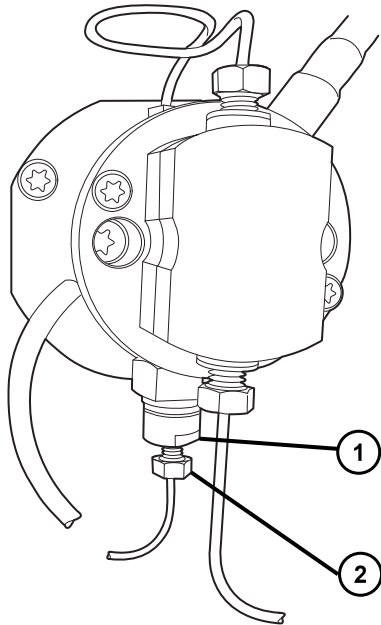
Required tools and materials

- Chemical-resistant, powder-free gloves
- Protective eyewear
- 1/4-inch open-end wrench
- 5/16-inch open-end wrench
- 1/2-inch open-end wrench
- Replacement accumulator check valve assembly

To replace the accumulator check valve:

1. Flush the pump with nonhazardous solvent.
2. Power-off the solvent manager.
3. Open the pump compartment door.
4. Using the 5/16-inch open-end wrench to hold the check valve in place, disconnect the compression fitting by using the 1/4-inch open-end wrench.

Figure 8–16: Compression fitting on the check valve

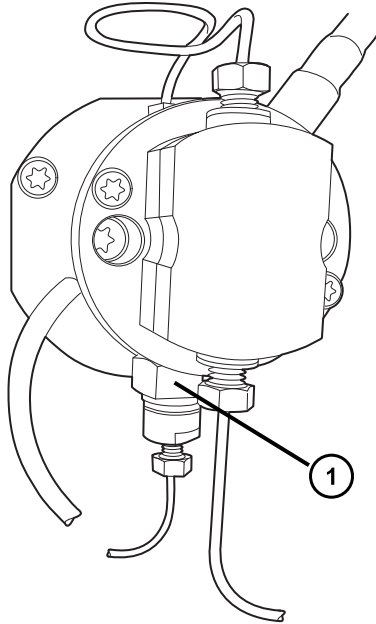


- ① Place the 5/16-inch open-end wrench here
- ② Compression fitting

5. Use the 1/2-inch open-end wrench to loosen the check valve, and then remove the check valve assembly from the pump head.

! **Notice:** When you remove the valve assembly, ensure that the PEEK washer, which is normally on the top face of the check valve, does not remain in the pump head.

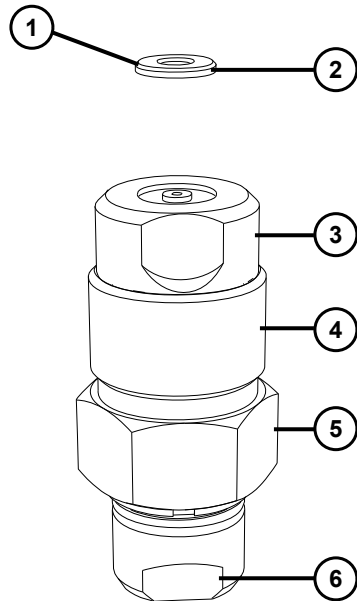
Figure 8–17: Check valve assembly on the accumulator pump head



- ① Place the 1/2-inch open-end wrench here

6. Unpack the new check valve.
7. Ensure that the new PEEK washer is inserted into the new check valve so that its chamfered edge faces away from the check valve.

Figure 8–18: Accumulator check valve



- ① Chamfered edge
- ② PEEK washer
- ③ Check valve
- ④ Check valve housing
- ⑤ 1/2-inch hex nut
- ⑥ 5/16-inch open-end wrench flat

8. Insert the check valve assembly into the pump head, tighten the check valve nut with your fingers to the extent possible, and then use the 1/2-inch wrench to tighten the nut an additional 1/8-turn.
9. Using the 5/16-inch open-end wrench to hold the check valve in place, reattach the compression fitting to the check valve.
10. Tighten the compression fitting with your fingers to the extent possible, and then use the 1/4-inch wrench to tighten the fitting as much as an additional 1/6-turn for an existing fitting, or as much as 1/2-turn for a new fitting.
11. Close the pump compartment door.
12. Power-on the solvent manager.
13. Prime the solvent manager (see [Priming the pump \(Page 64\)](#)).

8.8 Sample manager maintenance procedures

This section provides maintenance procedures for the Alliance iS HPLC System sample manager that users or Waters field service personnel can perform.

The procedures include:

- Replacing the leak sensor
- Calibrating the needle z axis
- Replacing the needle seal and seal port tubing
- Replacing the needle

8.8.1 Sample manager maintenance schedule

The sample manager has a recommended maintenance schedule.

Users can perform the following routine sample manager maintenance procedures.

Maintenance procedure	Frequency
Replace the sample manager leak sensor (Page 118)	As needed
Calibrate the needle z axis (Page 121)	After a needle change or as needed
Replace the needle seal and seal port tubing (Page 121)	During scheduled routine maintenance or as needed
Replace the needle (Page 132)	During scheduled routine maintenance or as needed

8.8.2 Replacing the sample manager leak sensor

Users or Waters field service personnel can replace the sample manager's leak sensors.

The sample manager has lower and upper leak sensors. The leak sensors monitor for fluid leaks at the column heater and sample manager and stop system flow when either sensor detects approximately 1.5 mL of accumulated fluid. Faulty leak sensors can result in undetected fluid spills.

The procedures for replacing the leak sensors are identical.



Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.



Warning: To avoid personal contamination with biologically hazardous materials, wear clean, chemical-resistant, powder-free gloves when performing this procedure.



Warning: To avoid eye injury, use eye protection when performing this procedure.



Notice: To avoid damaging electrical components and circuitry, do not disconnect an electrical assembly while electrical power is applied to a module. To completely interrupt power, set the on/off switch to the "off" position, and then disconnect the power cord from the ac source. Wait 10 seconds before disconnecting an assembly.

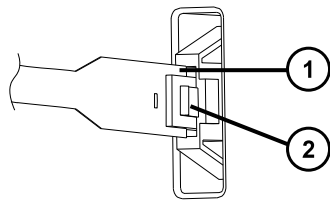
Required tools and materials

- Chemical-resistant, powder-free gloves
- Protective eyewear
- Replacement leak sensor

To replace the leak sensor:

1. Power-off the system (Page 58).
2. Open the sample manager compartment door.
3. Press down on the tab to detach the leak sensor connector from the front of the device.

Figure 8–19: Leak sensor connector

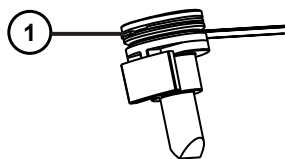


① Connector

② Tab

4. Grasp the leak sensor by its serrations and pull upward on it to remove it from its reservoir.

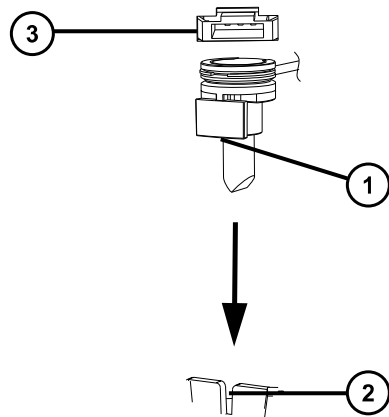
Figure 8–20: Leak sensor serrations



① Serrations

5. Unpack the new leak sensor.
6. Align the leak sensor T-bar with the slot in the side of the leak sensor reservoir and slide the leak sensor into place.

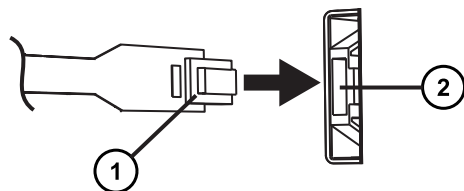
Figure 8–21: Aligning the leak sensor's T-bar with slot



- ① Leak sensor T-bar
- ② Slot in leak sensor reservoir
- ③ Leak sensor port on front of device

7. Connect the leak sensor connector to the front of the sample manager.

Figure 8–22: Attaching leak sensor connector



- ① Leak sensor connector
- ② Leak sensor port on front of device

8. Close the sample manager compartment door.

9. [Power-on the system \(Page 57\)](#).

10. From the touchscreen [Commands view \(Page 54\)](#), tap **Reset**.

11. From the touchscreen [System view \(Page 54\)](#), tap **Leak Sensors**, and then enable the **SM Leak Sensor**.

8.8.3 Calibrating the needle z axis

Users or Waters field service personnel can calibrate the sample manager needle's z axis.

You must calibrate the needle before you use the sample manager for the first time and whenever you replace the sample needle. Failing to calibrate the needle can damage it. The calibration procedure is identical for all needles.

To calibrate the needle z axis, do the following:

1. From the touchscreen, tap **Maintain > Calibrate needle Z axis**.
2. Follow the directions on the screen.

8.8.4 Replacing the needle seal and seal port tubing

To prevent disruptions to your workflow, replace the needle seal and seal port tubing annually during the prescribed preventive maintenance (PM) schedule or any time the seal appears dirty, contaminated, or clogged. Also replace the seal any other time it is necessary to replace the needle.

Replacing the needle seal and the seal port tubing involves removing the following:

- Wash station assembly
- Locking nut that houses the needle seal
- Existing needle seal
- Existing seal port tubing (holds the locking nut) and attached to the wash station assembly



Warning: To avoid personal contamination with biologically hazardous or toxic compounds, wear clean, chemical-resistant, powder-free gloves when performing this procedure.



Warning: To avoid eye injury, use eye protection when performing this procedure.

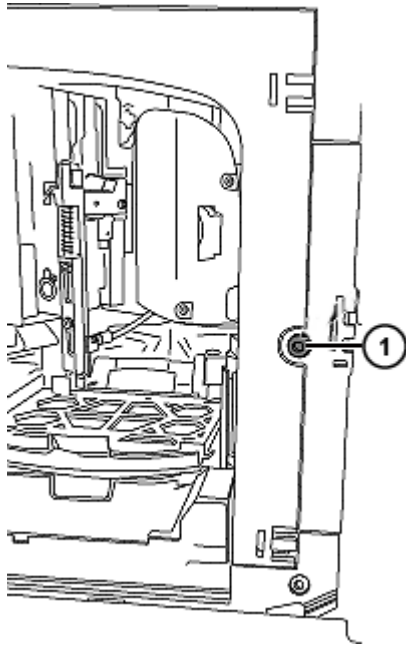
Required tools and materials

- Seal kit
- Seal port tubing (Waters part name: Assy, Seat Port, SST, 0.007 ID)
- Chemical-resistant, powder-free gloves
- Protective eyewear
- 7/16-inch open-end wrench (procedure requires two wrenches)
- T10 TORX driver
- T20 TORX driver

To replace the needle seal:

1. Ensure that the system is powered-on.
2. Open the sample compartment door and the fluidics compartment door.
3. Remove any sample plates from the sample compartment.
4. Move the sample needle into service position:
 - a. Tap **MAINTAIN > Service > Prepare to Replace the Needle**.
5. Using the T20 TORX driver remove the captive screw that secures the access panel, and then remove the panel.

Figure 8–23: Captive screw on access panel



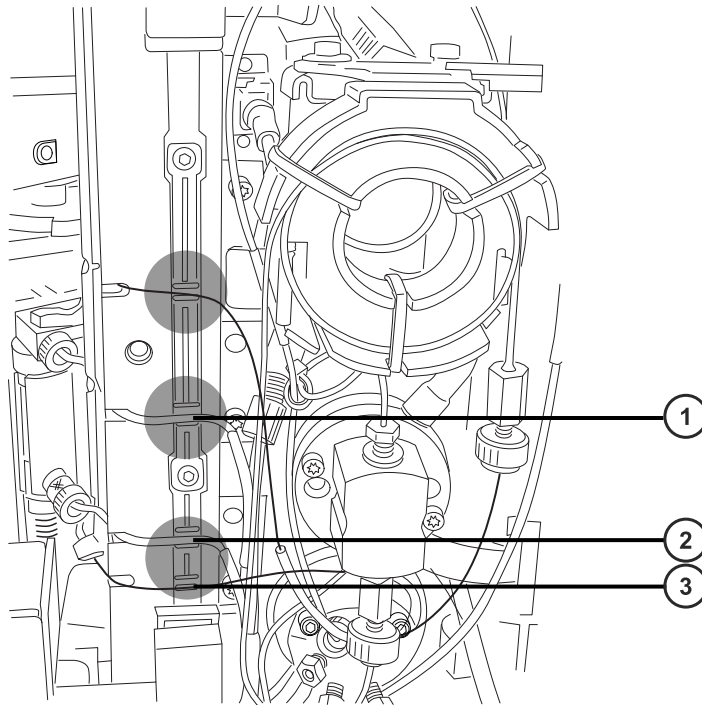
① Captive screw

6. To lift the wash station assembly out of the wash station holder:

Note: You will not remove the wash station assembly (rather, you will temporarily detach it from the holder).

- a. Locate the wash tubing and seal port tubing secured in the bottom three clips. Release the tubing from the three clips.

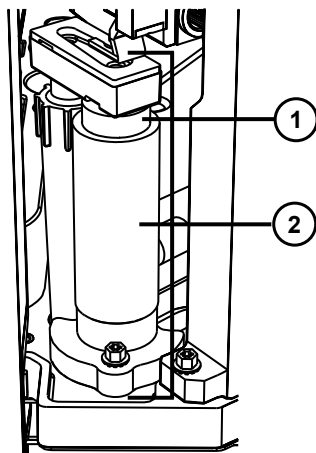
Figure 8–24: Wash tubing and seal port tubing location in clips



- ① First wash tubing in clip
- ② Second wash tubing in clip
- ③ Seal port tubing in clip

b. Push down on the wash station assembly, and then rotate it clockwise to temporarily remove it from the wash station holder.

Figure 8–25: Release the wash station assembly from the wash station holder

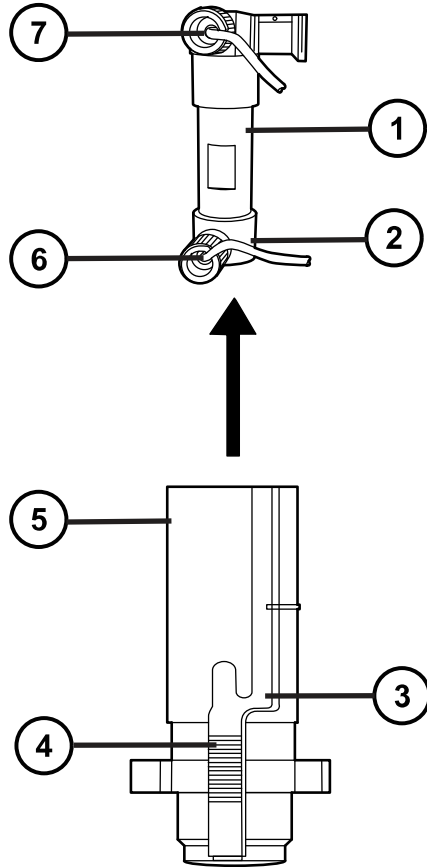


- ① Wash station assembly (inside the wash station holder)

② Wash station holder

7. Lift the wash station assembly out of the wash station holder.

Figure 8–26: Releasing the wash station assembly from the wash station holder



① Support sleeve

② Locking nut location (nut not visible)

③ Slot

④ Spring

⑤ Wash station holder

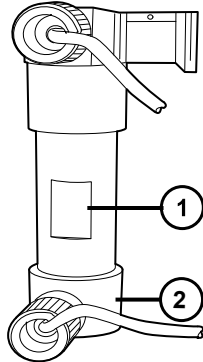
⑥ Seal port tubing in PEEK fitting (image shows tubing shorter than it actually appears)

⑦ Seal port tubing in PEEK fitting (image shows tubing shorter than it actually appears)

Requirement: Ensure that the locking nut remains seated inside the spring.

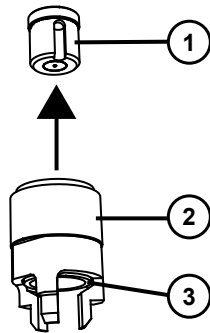
8. Locate the locking nut attached to the bottom of the wash station assembly that houses the needle seal. To access the needle seal, remove the locking nut using two 7/16-inch open-end wrenches to ensure proper grip.

Figure 8–27: Wrench placement locations to remove the locking nut



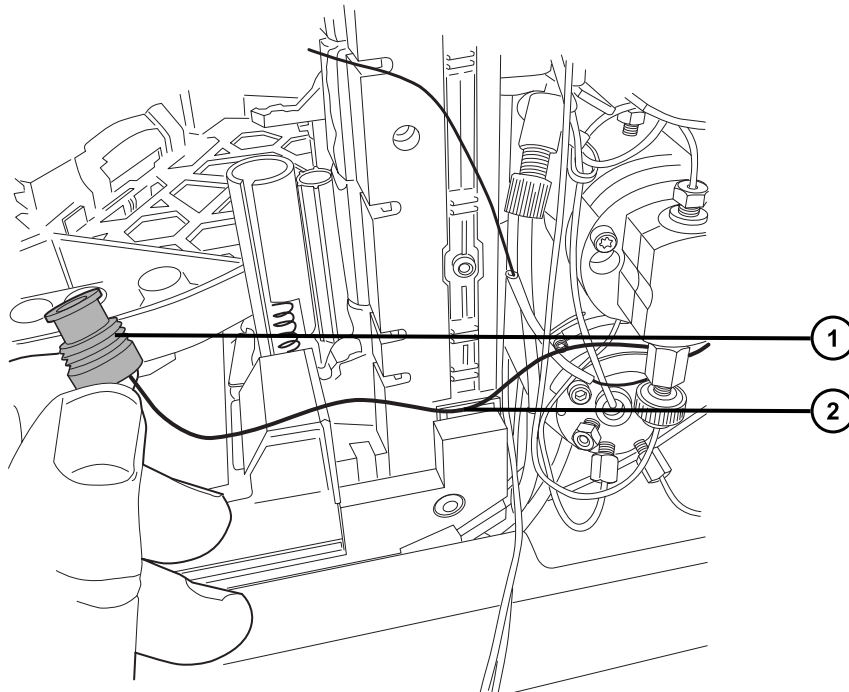
- 1 Place one 7/16-inch open-end wrench here on the support sleeve
 - 2 Place the other 7/16-inch open-end wrench here near the locking nut (not visible)
9. Lift the outer edge (or lip) of the locking nut. Tilt the locking nut to remove the needle seal, and then discard.

Figure 8–28: Removing the needle seal from the locking nut



- 1 Seal
 - 2 Locking nut (actual nut updated and shows no prongs)
 - 3 Seal port
10. Waters recommends replacing the seal port tubing when you replace the needle seal. To remove the seal port tubing:
 - a. Unscrew the tool-free fitting attached to the seal port tubing, and then remove one end of the seal port tubing from port 1 in the inject valve.
 - b. Thread the seal port tubing through the locking nut, and then remove it.

Figure 8–29: Seal port tubing removal



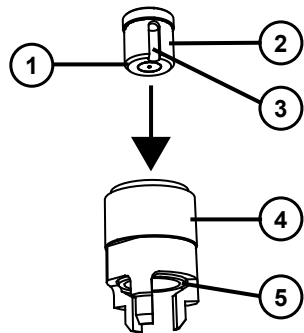
- ① Locking nut
- ② Seal port tubing

11. Insert the replacement seal into the locking nut. The seal is keyed, ensuring its correct installation, as shown in the following two figures.

! **Notice:** To prevent contaminating system components, wear clean, chemical-resistant, powder-free gloves, and work on a clean surface when replacing the seal.

Note: The following image shows prongs, but the actual locking nut does not have prongs.

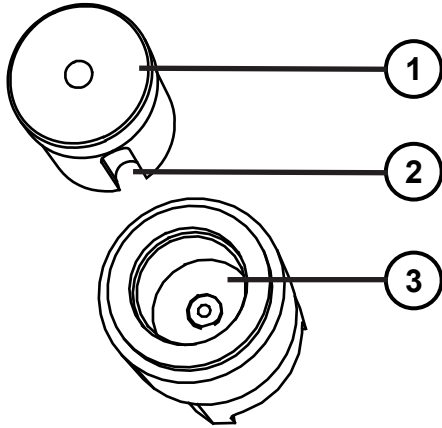
Figure 8–30: Inserting replacement seal into the locking nut



- ① Smaller-diameter end

- ② Seal
- ③ Notch
- ④ Locking nut
- ⑤ Seal port

Figure 8–31: Seal notch location



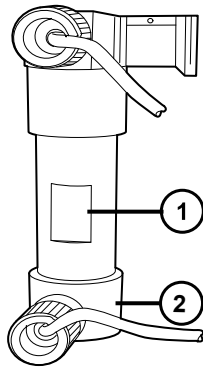
- ① Seal
- ② Notch
- ③ Seal cup

12. Finger-tighten the locking nut into the bottom of the wash station assembly.
13. Place the two 7/16-inch open-end wrenches on the washing station support sleeve, and then tighten.



Notice: To avoid damaging the seal port tube, do not excessively twist the tube.

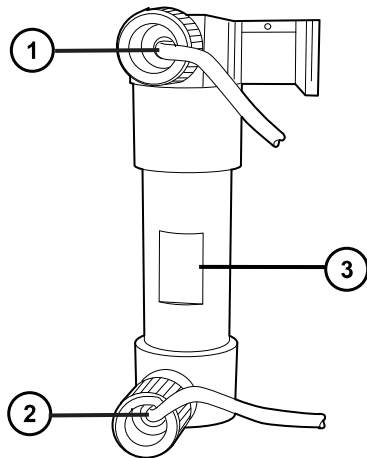
Figure 8–32: Wrench placement locations



- ① Place one 7/16-inch open-end wrench here on the support sleeve
- ② Place the other 7/16-inch open-end wrench here

14. Ensure that the seal port tubing remains in line with the PEEK fitting in the support sleeve.

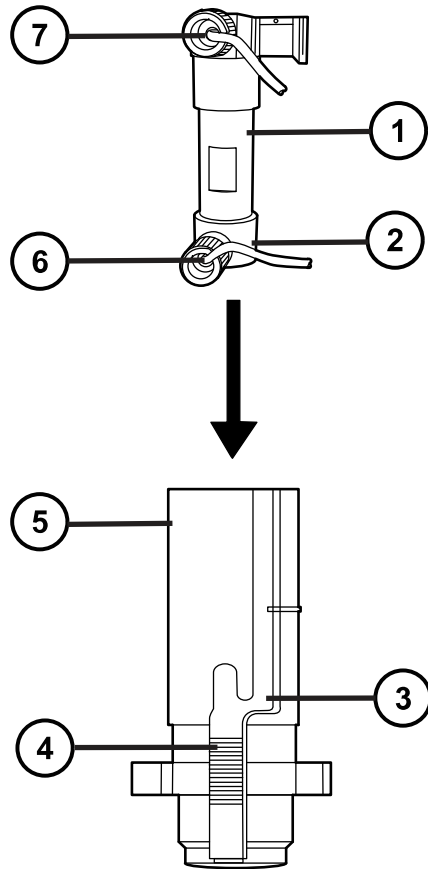
Figure 8–33: Seal port tubing in PEEK fitting



- ① Seal port tubing in top PEEK fitting
- ② Seal port tubing in bottom PEEK fitting
- ③ Support sleeve

15. Slide the seal port tubing into the slot on the side of the housing.

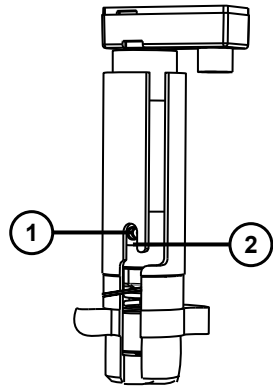
Figure 8–34: Sliding seal port tubing into the slot



- ① Support sleeve
- ② Locking nut location (nut not visible)
- ③ Slot
- ④ Spring
- ⑤ Wash station holder
- ⑥ Seal port tubing in PEEK fitting (image shows tubing shorter than it actually appears)
- ⑦ Seal port tubing in PEEK fitting (image shows tubing shorter than it actually appears)

16. Slide the support sleeve into the housing, ensuring that the fitting hole on the support sleeve aligns with the slot on the housing.

Figure 8–35: Inserting support sleeve into the housing



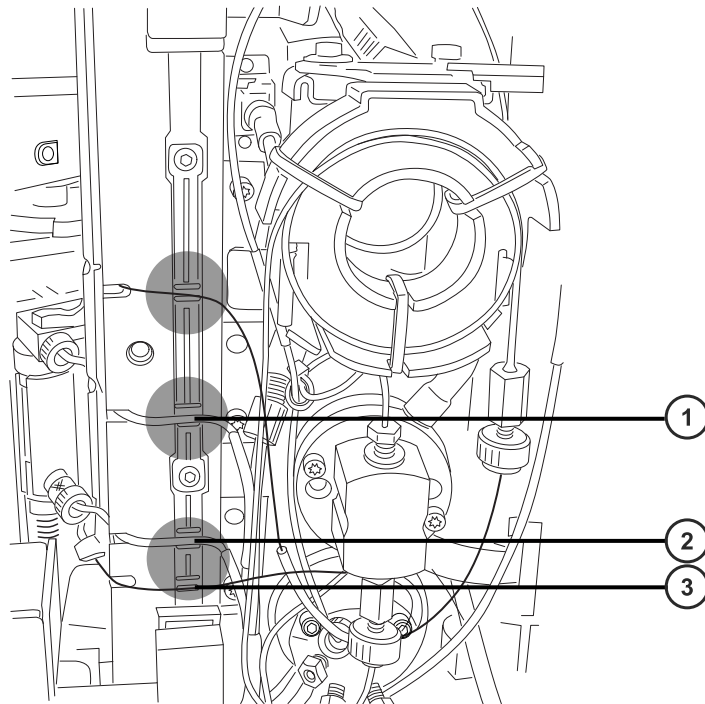
① Fitting hole

② Slot

17. Reinsert the tubing in the clips on the side of the sample compartment. For example, route the wash tubing and seal port tubing through the clips.

Requirement: The tubing is secured to the wall and must not interfere with operation of the sample tray or the vertical motion of the wash port.

Figure 8–36: Replace the wash tubing and the seal port tubing in the clips on the sample compartment wall



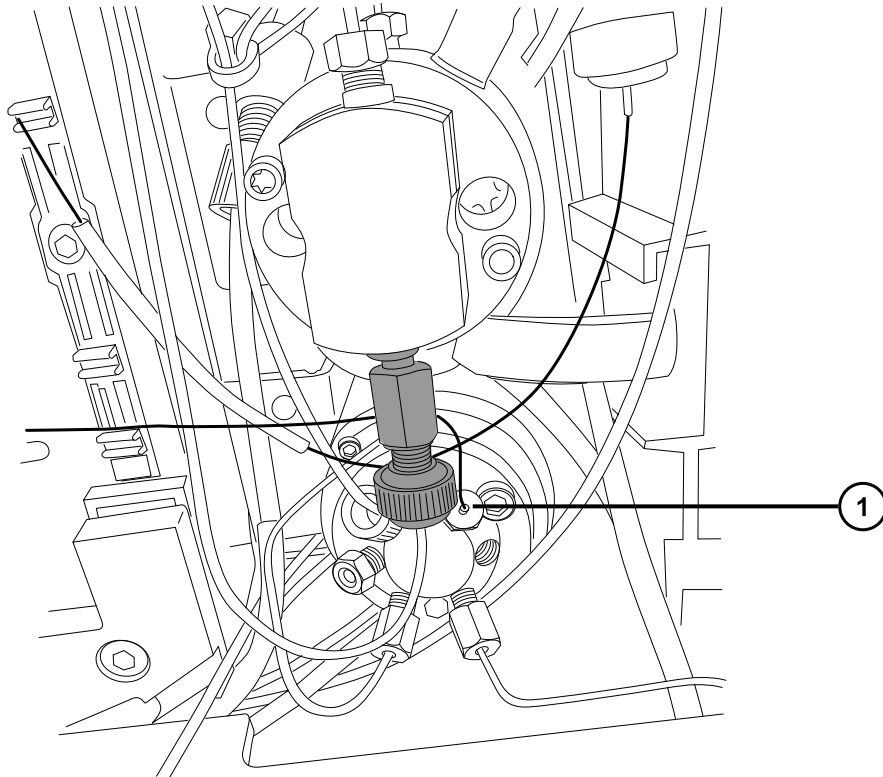
① First wash tubing in clip

② Second wash tubing in clip

③ Seal port tubing in clip

18. Screw the seal port tubing end into port 1 of the injection valve, and then use the 1/4-inch open-end wrench to tighten the fitting 1/4-turn beyond finger-tight.

Figure 8–37: Attach the seal port tubing to the injection valve



① Port 1 on injection valve

19. Reinstall the access panel, and then use the T20 TORX driver to tighten the single screw that secures the access panel to the front of the unit.

Requirement: Ensure that the seal port tubing and sample needle tubing are routed through the gap in the access panel and that they do not cross each other.

20. Close the sample compartment door and the fluidics compartment door.
21. Tap **COMMANDS > Reset** to turn the motors back on and home the needle carriage.
22. Complete the needle seal readiness test to verify that the needle seal works properly.

8.8.5 Replacing the sample needle

Replace the needle annually during prescribed preventive maintenance (PM) or any time the needle appears damaged or bent.

Recommendation: Waters Technical Service recommends that you replace the needle seal each time you replace the needle. See [Replacing the needle seal and seal port tubing \(Page 121\)](#) after you complete this procedure.



Warning: To avoid personal contamination with biologically hazardous or toxic compounds, wear clean, chemical-resistant, powder-free gloves when performing this procedure.



Warning: To avoid eye injury, use eye protection when performing this procedure.

Required tools and materials

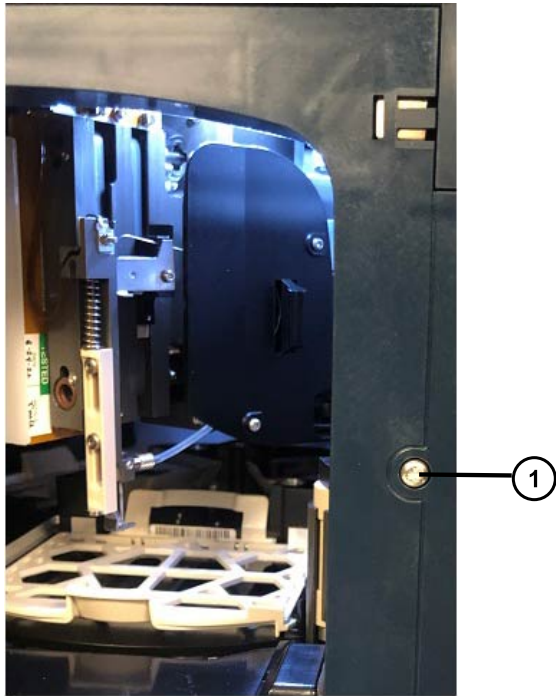
- Needle assembly, also known as sample needle cartridge (700013880)
- Chemical-resistant, powder-free gloves
- Protective eyewear
- T20 TORX driver

To replace the sample needle:

! **Caution:** Ensure that you install the needle cartridge as described in the following procedure. The needle comes assembled in the needle cartridge and can be broken easily if it is mishandled.

1. Ensure that the system is powered-on.
2. Remove any sample plates from the sample compartment.
3. Move the sample needle into service position:
 - a. Tap **MAINTAIN > Service > Prepare to Replace the Needle**.
4. Open the sample compartment door and the fluidics compartment door.
5. Using the T20 TORX driver, loosen the captive screw that secures the access panel, and then remove the panel.

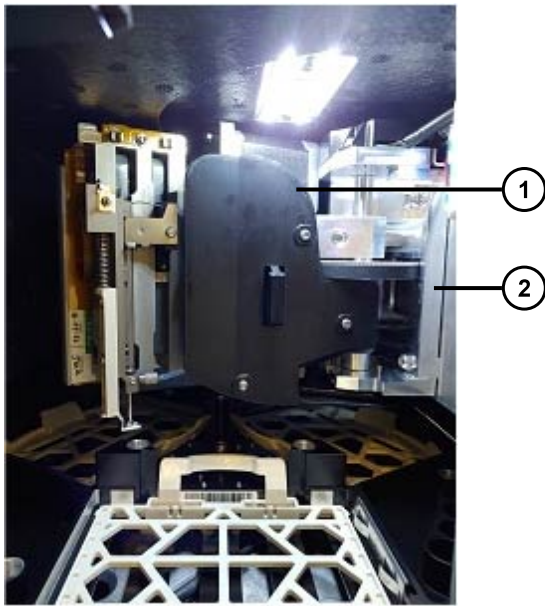
Figure 8–38: Captive screw on access panel



① Captive screw

6. Locate the needle cartridge in the sample compartment. The needle cartridge houses the needle and helps to secure it in place.

Figure 8–39: Needle cartridge location in the sample compartment

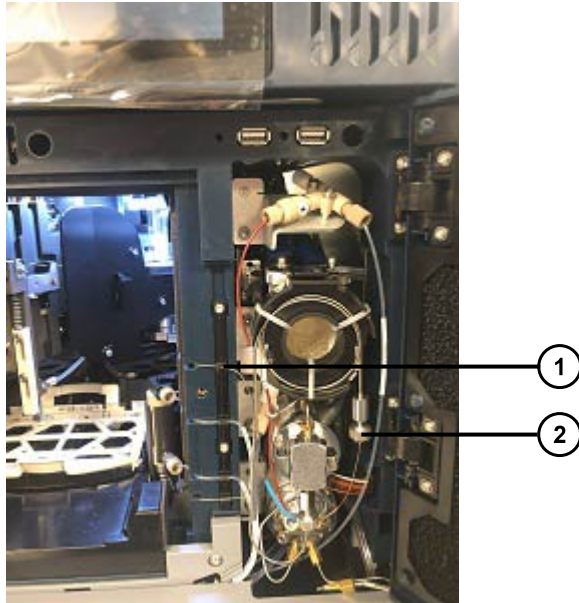


① Needle cartridge

② Sample compartment area

7. From the fluidics compartment, unscrew the tool-free fitting that connects the sample loop to the end of the needle. Once it is unscrewed, remove the needle tubing from the top clip where the needle crosses the fluidics compartment to the sample compartment.

Figure 8–40: Tool-free fitting and clip

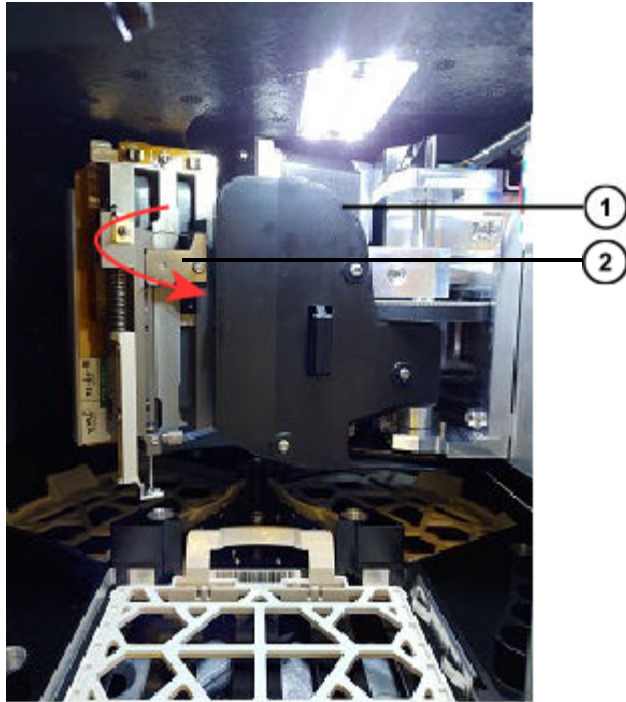


① Top clip

② Tool-free fitting

8. Turn the rotating latch on the needle carriage counterclockwise to release the needle cartridge.

Figure 8–41: Rotating latch on the needle carriage (red arrow indicates counterclockwise direction to open)

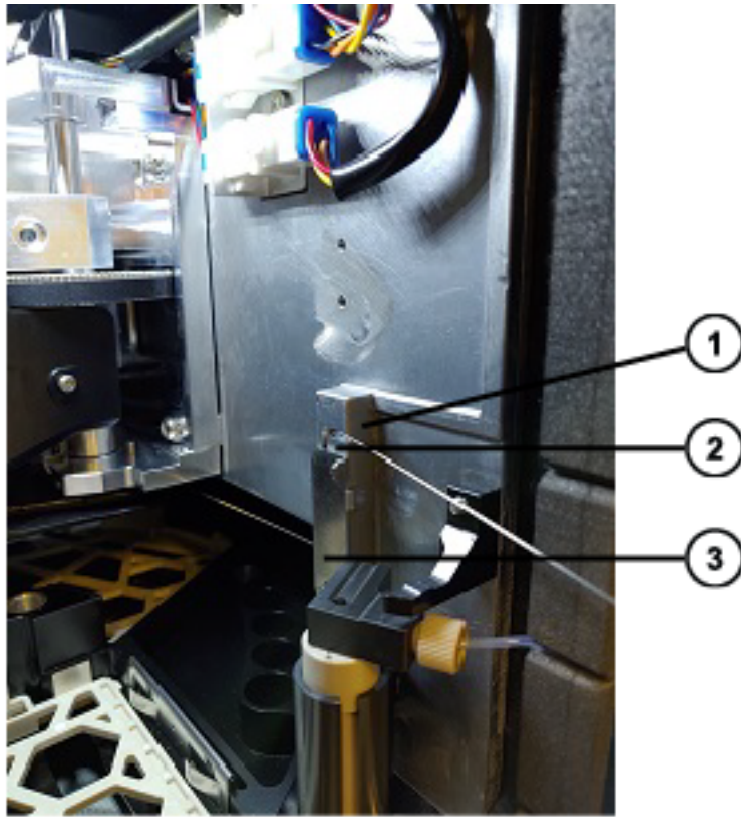


① Needle carriage

② Rotating latch

9. Locate the block with a small notch that secures the sample needle on the right-hand side of the sample compartment wall. Open the rotating latch clockwise, and then remove the step-shape portion of the sample needle from the notch.

Figure 8–42: Second rotating latch and notch on the sample compartment wall



- ① Block on sample compartment wall
- ② Step-shape portion of the sample needle in the small notch
- ③ Second rotating latch on compartment wall

10. Push the sample needle forward and then lift it out of the puncture needle assembly at the bottom of the needle carriage area. Then remove the needle cartridge from the sample compartment.



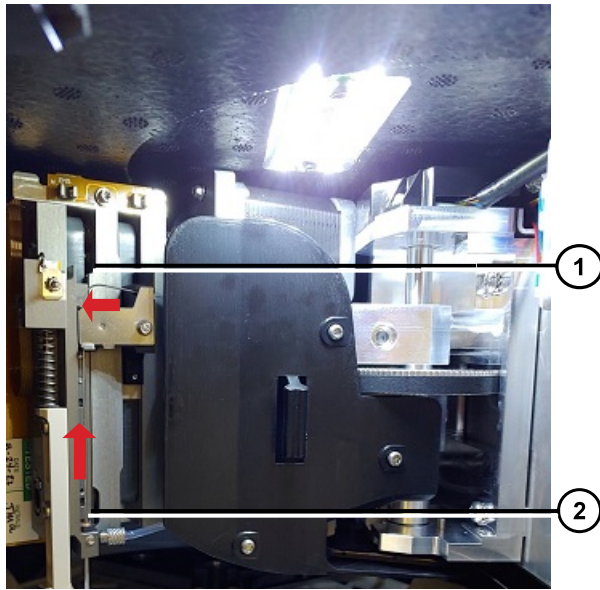
Warning: To avoid puncture injuries, handle sample needles, syringes, fused silica lines, and borosilicate tips with extreme care.



Notice: To avoid damage to the end of the needle, do not touch or press the end of the sample needle.

Note: The puncture needle assembly consists of the puncture needle, vespel, and PEEK housing.

Figure 8–43: Removing sample needle from the puncture needle assembly



- ① Sample needle
- ② Puncture needle assembly

11. Obtain the replacement sample needle. Remove the protective sleeve from the needle tip.
12. Locate the magnet in the recessed section of the needle carriage arm. Mount the needle cartridge onto the magnet.



Warning: To avoid puncture injuries, handle sample needles, syringes, fused silica lines, and borosilicate tips with extreme care.



Notice: To avoid damage to the end of the needle, do not touch or press the end of the sample needle.

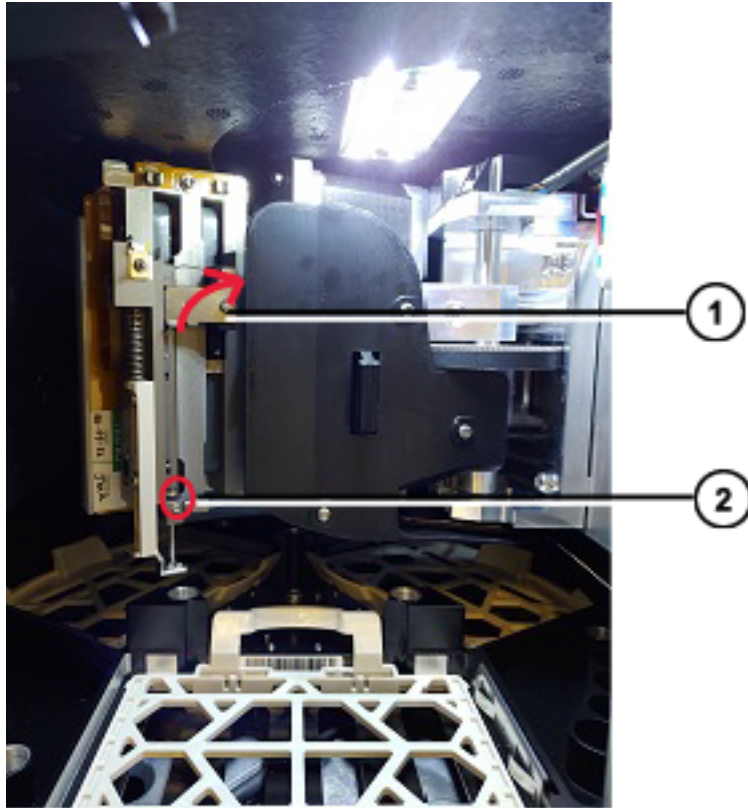
Figure 8–44: Magnet location in needle carriage arm



- ① Needle carriage arm
- ② Magnet in the recessed section

13. To install the sample needle into the needle carriage assembly:
 - a. Slide the sample needle into the vespel guide at the bottom of the puncture needle assembly as shown in the following figure.
 - b. Insert the ferrule at the top of the needle into the needle retainer at the top.
 - c. After placing the ferrule into the needle retainer, place the tubing into the notch located above the latch.
 - d. Close the rotating latch by turning clockwise.

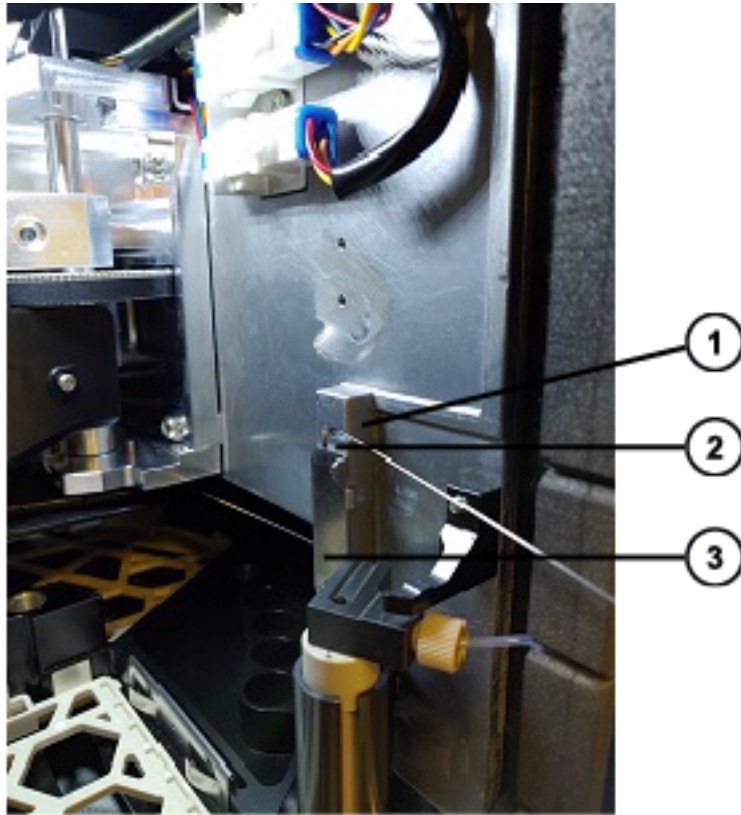
Figure 8–45: Install the sample needle into the needle carriage assembly



- ① Rotating latch (ferrule is behind the rotating latch)
- ② Vespel guide

14. Route the needle tubing through the notch in the small block on the sample compartment wall. Then turn the rotating latch counterclockwise to secure the needle tubing in the notch.

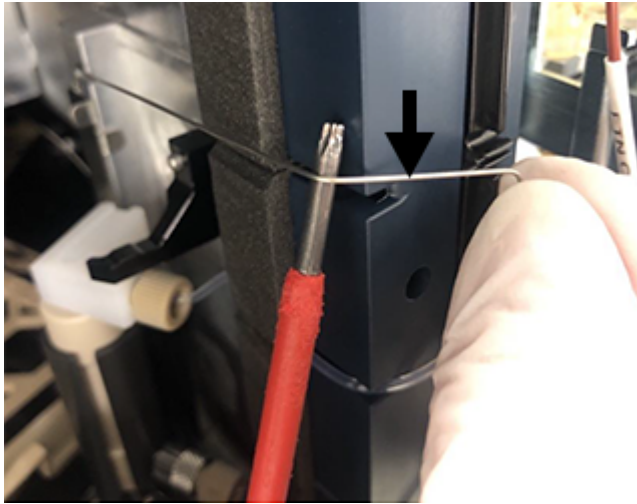
Figure 8–46: Secure the needle tubing in the notch (second rotating latch on compartment wall)



- ① Small block on sample compartment wall
- ② Needle tubing in notch
- ③ Rotating latch on sample compartment wall

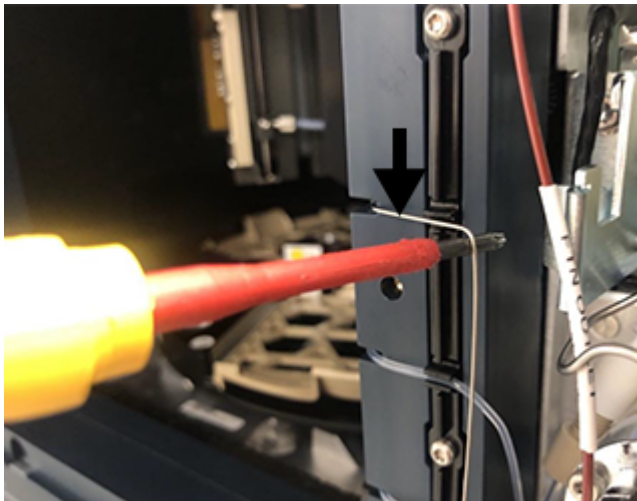
15. Bend the needle tubing (arrow) to the right around the bezel with the T20 TORX driver.

Figure 8–47: Bending the needle tubing around the compartment bezel



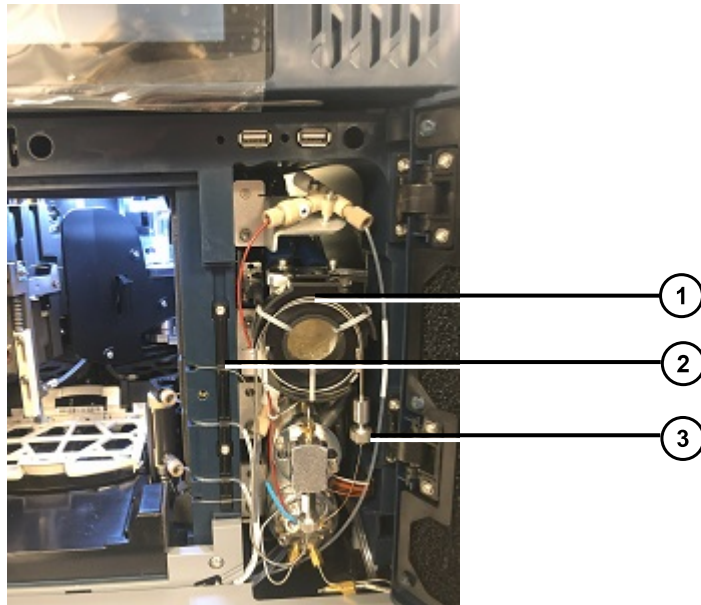
16. Bend the needle tubing (arrow) down as shown with the T20 TORX driver.

Figure 8–48: Bending the needle tubing down



17. Connect the sample needle to the extension loop using the tool-free fitting. Then secure the needle tubing in the top clip.

Figure 8–49: Reconnecting the sample needle to the extension loop

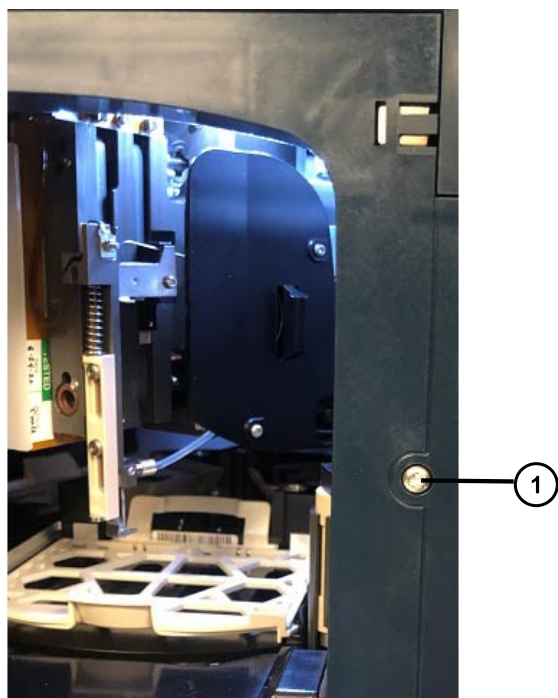


- ① Extension loop
- ② Needle tubing in top clip
- ③ Tool-free fitting

18. Reinstall the access panel and align the tabs at the top of the access panel with the slots in the bezel. Then install the access panel and use the T20 TORX driver to tighten the one captive screw that secures the panel to the front of the system.

Requirement: Ensure that the needle tubing is routed in the top clip as indicated in the previous step.

Figure 8–50: Captive screw on access panel



- ① Captive screw

19. Close the sample compartment door and the fluidics compartment door.

Recommendation: Waters recommends that you replace the needle seal each time you replace the needle.

20. Calibrate the needle:

- a. Tap **MAINTAIN > Calibrate Axes > Calibrate Z-Axis** and follow the on-screen prompts until **Z-Axis Calibration Passed** appears on the screen. Tap **DONE**.
- b. Tap **MAINTAIN > Calibrate Axes > Calibrate Zp-Axis** and follow the on-screen prompts until **Zp-Axis Calibration Passed** appears on the screen. Tap **DONE**.
- c. Tap **MAINTAIN > Calibrate Axes > Calibrate B θ -Axis** and follow the on-screen prompts until **B θ -Axis Calibration Passed** appears on the screen. Tap **NEXT > HOME > DONE**.

8.9 Detector maintenance procedures

This section provides the maintenance procedures for the Alliance iS HPLC System TUV and PDA detectors that users or Waters field service personnel can perform.

The procedures include:

- Replacing the leak sensor
- Replacing the flow cell sensor
- Replacing the lamp

8.9.1 Replacing the detector leak sensor

A leak sensor in the drip tray continuously monitors the detector for leaks.



Warning: To avoid personal contamination with biologically hazardous or toxic compounds, wear clean, chemical-resistant, powder-free gloves when performing this procedure.

The detector's leak sensor stops system flow when it detects accumulated, leaked liquid in its reservoir. When the sensor detects a leak, the system's touchscreen displays an alarm message.

Required tools and materials

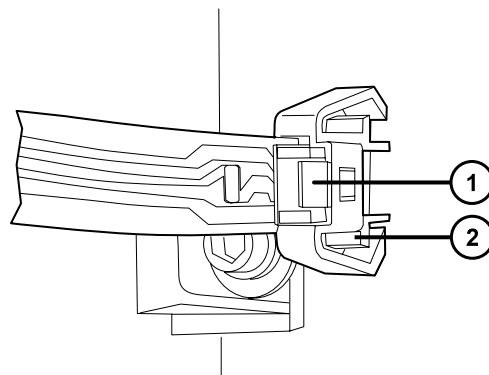
- Chemical-resistant, powder-free gloves
- Replacement leak sensor

To replace the detector's leak sensor:

1. Open the detector's door, gently pulling its right-hand edge toward you.
2. Remove the old leak sensor:
 - a. To detach the leak sensor connector from the front of the instrument, press down on the release tab.

Note: The following figure is for representation only. Your hardware may differ slightly from that shown.

Figure 8–51: Detaching the leak sensor



① Release tab

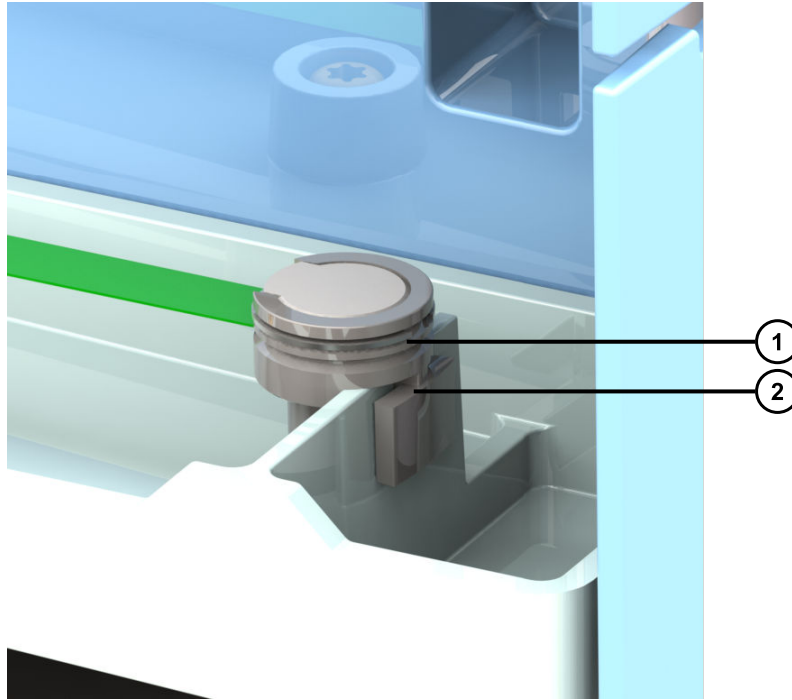
② Leak sensor connector

- b. To remove the leak sensor from the reservoir, grasp it by the serrations around its top and pull upward on it (see Figure "Installed leak sensor, rear view").

3. Install the new leak sensor:

- a. Holding the leak sensor by its serrations, align its T-bar with the slot in the side of the reservoir and slide it into place (see Figure "Installed leak sensor, rear view").

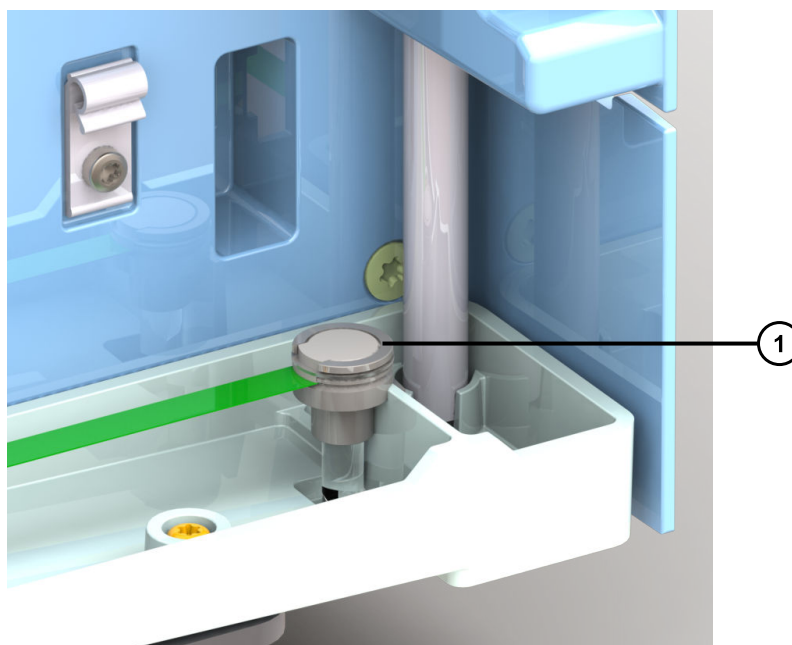
Figure 8–52: Installed leak sensor, rear view



① Serrations

② T-bar engaged with reservoir slot

Figure 8–53: Installed leak sensor, front view



① Leak sensor

- b. Plug the leak sensor connector into the front of the instrument.
4. Close the detector's door.
5. From the system's touchscreen [Commands view \(Page 54\)](#), tap **Reset**.

8.9.2 Replacing the TUV detector's flow cell

To prevent system errors, replace the TUV detector's flow cell any time it appears dirty, contaminated, or clogged.

See also: [Controlling Contamination in LC/MS Systems \(715001307\)](#).

Required tools and materials

- Chemical-resistant, powder-free gloves
- 1/4-inch flat-blade screwdriver
- HPLC-grade methanol
- HPLC-grade water
- Replacement flow cell

To replace the TUV detector's flow cell:

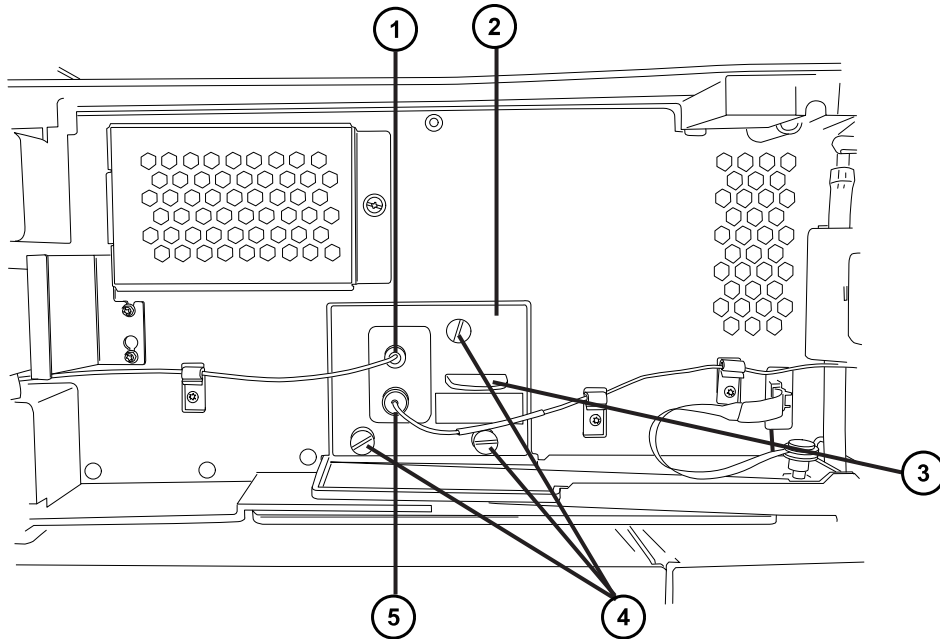


Notice:

- To avoid contaminating the flow cell, wear clean, chemical-resistant, powder-free gloves when handling, removing, or replacing it.
- To avoid damaging the flow cell, handle it with care. Do not disassemble the flow cell.

1. Open the TUV detector's door, gently pulling its right-hand edge toward you.
2. Disconnect the TUV detector's inlet and outlet tubing.

Figure 8–54: Locating the flow cell (TUV detector door open view)



- ① Outlet tubing
- ② Flow cell assembly
- ③ Flow cell handle
- ④ Thumbscrews (3)
- ⑤ Inlet tubing

3. Remove and then, prior to storing, flush the flow cell:

- a. Using the screwdriver, loosen the three thumbscrews on the flow cell assembly's front plate.
- b. Remove the flow cell by grasping its handle and gently pulling the assembly toward you.
- c. Before putting it into storage, Waters recommends that you flush the old flow cell. Select a solvent compatible with the samples and mobile phases that you have been using. If you have been using buffers, flush the cell with 10 mL of HPLC-grade water and then flush with 10 mL of a low-surface-tension solvent, such as methanol.

Requirement: Ensure that the solvent used is miscible with the previous mobile phase.

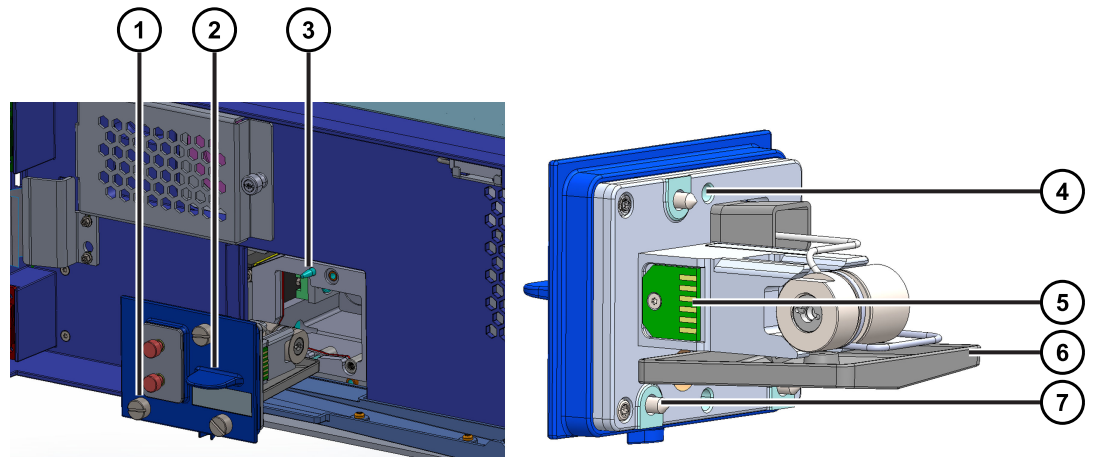
4. Unpack and inspect the new flow cell, ensuring that the flow cell type is correct for your application.

Note: When replacing the flow cell, replace the flow cell inlet tubing with the tubing included with the new flow cell.

5. Install the new flow cell:
 - a. Align the flow cell assembly with the front of the opening, and then insert it slowly so that its two alignment receptacles (on the rear face of the front part of the cell flange) engage the pins in the cell compartment.

Tip: As you insert the cell, its drip tray helps ensure proper alignment of the compartment's pins and the cell flange's receptacles.

Figure 8–55: Installing the TUV detector's flow cell assembly



- ① Thumbscrew (3)
- ② Flow cell handle (label underneath will differ depending on cell type)
- ③ Compartment alignment pin (2)
- ④ Cell flange alignment receptacle (2)

- ⑤ Flow cell ID chip
- ⑥ Drip tray
- ⑦ Thumbscrew (3, rear view)

- b. Continue inserting the flow cell until the three thumbscrews align with their holes in the bulkhead.
 - c. Hand tighten the thumbscrews, and then verify that the screws are secure using the screwdriver.
6. Connect the inlet tubing to the main column connection and flow cell inlet, and then connect the outlet tubing to the flow cell outlet.
 7. Ensure that the flow cell is filled with degassed, transparent solvent (acetonitrile or water) that is free of air bubbles.
 8. Power-cycle the system.
 9. After the system is powered-on, on its touchscreen, tap **Maintain > Calibrate Detector > Verify Calibration**.

Note: If verification fails, troubleshoot and reattempt verification. If verification still fails, tap **Maintain > Calibrate Detector > Calibrate Wavelengths**.

8.9.3 Replacing the PDA detector's flow cell

To prevent system errors, replace the PDA detector's flow cell any time it appears dirty, contaminated, or clogged.

See also: *Controlling Contamination in LC/MS Systems (715001307).*

Required tools and materials

- Chemical-resistant, powder-free gloves
- 1/4-inch flat-blade screwdriver
- HPLC-grade methanol
- HPLC-grade water
- Replacement flow cell

To replace the PDA detector's flow cell:

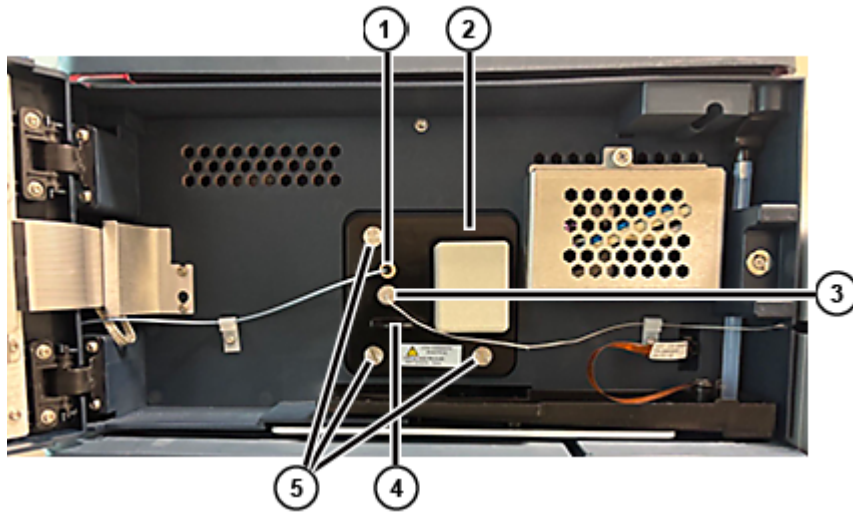


Notice:

- To avoid contaminating the flow cell, wear clean, chemical-resistant, powder-free gloves when handling, removing, or replacing it.
- To avoid damaging the flow cell, handle it with care. Do not disassemble the flow cell.

1. Open the PDA detector's door, gently pulling its right-hand edge toward you.
2. Disconnect the PDA detector's inlet and outlet tubing.

Figure 8–56: Locating the flow cell (PDA detector door open view)



- ① Outlet tubing
- ② Flow cell assembly
- ③ Inlet tubing
- ④ Flow cell handle
- ⑤ Thumbscrews (3)

3. Remove and then, prior to storing, flush the flow cell:
 - a. Using the screwdriver, loosen the three thumbscrews on the flow cell assembly's front plate.
 - b. Remove the flow cell by grasping its handle and gently pulling the assembly toward you.
 - c. Before putting it into storage, Waters recommends that you flush the old flow cell. Select a solvent compatible with the samples and mobile phases that you have been

using. If you have been using buffers, flush the cell with 10 mL of HPLC-grade water and then flush with 10 mL of a low-surface-tension solvent, such as methanol.

Requirement: Ensure that the solvent used is miscible with the previous mobile phase.

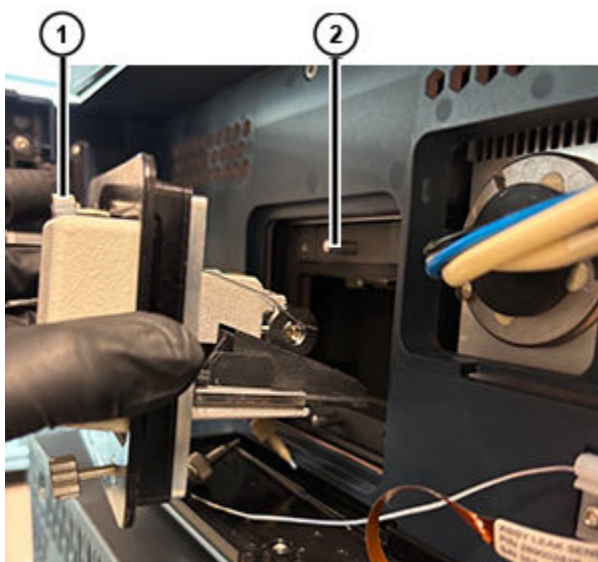
4. Unpack and inspect the new flow cell, ensuring that the flow cell type is correct for your application.

Note: When replacing the flow cell, replace the flow cell inlet tubing with the tubing included with the new flow cell.

5. Install the new flow cell:
 - a. Align the flow cell assembly with the front of the opening, and then insert it slowly so that its two alignment receptacles (on the rear face of the front part of the cell flange) engage the pins in the cell compartment.

Tip: As you insert the cell, its drip tray helps ensure proper alignment of the compartment's pins and the cell flange's receptacles.

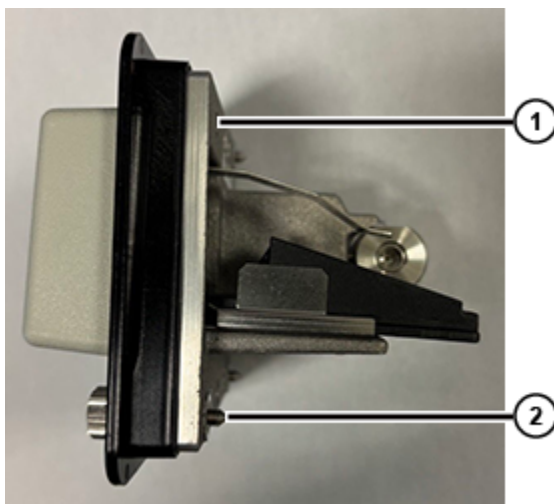
Figure 8–57: Installing the PDA detector's flow cell assembly



- ① Thumbscrew (3)
- ② Compartment alignment pin (2)

- b. Continue inserting the flow cell until the three thumbscrews align with their holes in the bulkhead.

Figure 8–58: Aligning the detector's flow cell assembly



- ① Cell flange alignment receptacle (2)
- ② Thumbscrew (3)

- c. Hand tighten the thumbscrews, and then verify that the screws are secure using the screwdriver.
6. Connect the inlet tubing to the main column connection and flow cell inlet, and then connect the outlet tubing to the flow cell outlet.
7. Ensure that the flow cell is filled with degassed, transparent solvent (acetonitrile or water) that is free of air bubbles.
8. Power-cycle the system.
9. After the system is powered-on, on its touchscreen, tap **Maintain > Verify Calibration**.

Note: If verification fails, troubleshoot and reattempt verification. If verification still fails, perform an erbium calibration. See [Erbium calibration \(Page 41\)](#).

8.9.4 Replacing the TUV detector's lamp

Replace the TUV detector's lamp annually during the prescribed preventive maintenance (PM) or any time it repeatedly fails to light or when the detector fails to calibrate. The system automatically senses the lamp upon installation, and its serial number and installation date are automatically recorded in the Lamp Change Record table.

Note: Waters warrants 2000 hours of lamp life, or one year from date of purchase, whichever comes first.



Warning: To prevent burn injuries, allow the lamp to cool for 30 minutes before removing it. The lamp housing gets extremely hot during operation.



Warning: To avoid eye injury from ultraviolet radiation exposure:

- Power-off the detector before changing the lamp.
- Wear eye protection that filters ultraviolet light.
- Keep the lamp in the housing during operation.

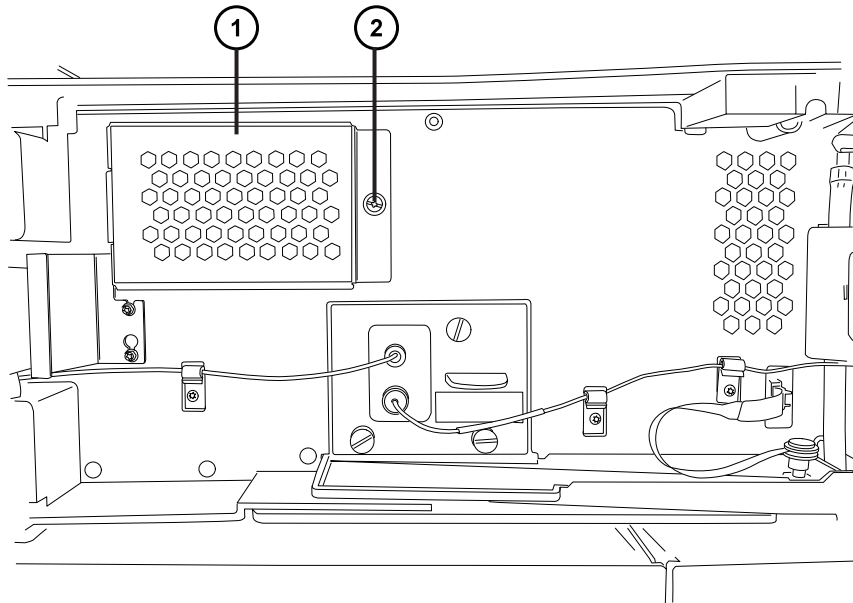
To replace the lamp:



Caution: Ensure that you allow the lamp to cool for at least 30 minutes before starting this procedure.

1. Power-off the system, and then disconnect the power cord.
2. Open the TUV detector's door.
3. Locate the lamp assembly cover (see the following figure).
4. To remove the lamp assembly cover, use a Phillips screwdriver to remove the one Phillips-head screw (see the following figure).
5. Lift the lamp assembly cover off.

Figure 8–59: Lamp assembly cover

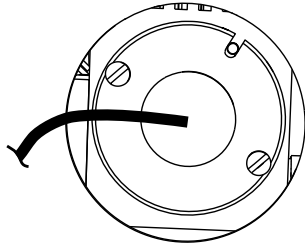


① Lamp assembly cover

② Phillips-head screw

6. Disconnect the power cord to the lamp.
7. Loosen the two captive screws in the lamp base.

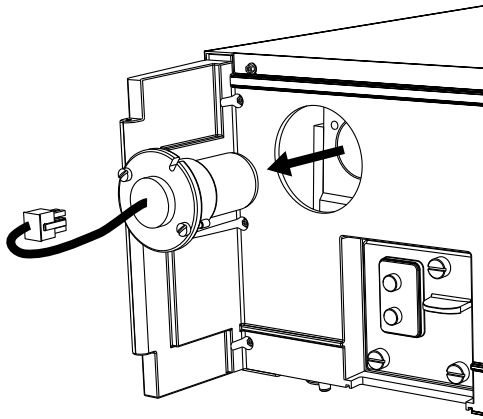
Figure 8–60: Lamp assembly base captive screws



8. Lift out the lamp assembly from the lamp housing.

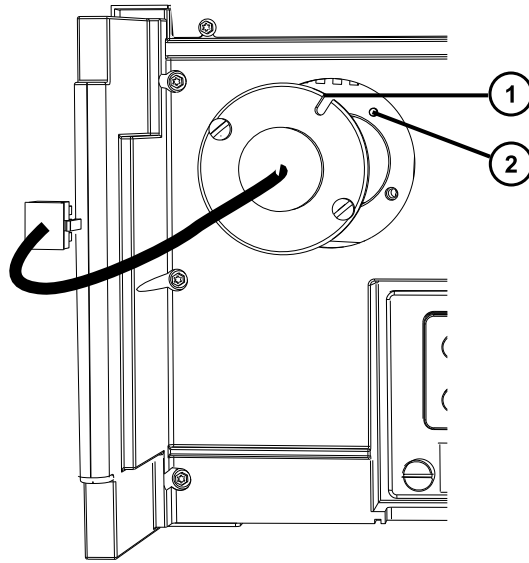
! **Notice:** To prevent shattering the glass, use care when disposing of the lamp because the lamp gas is under slight negative pressure.

Figure 8–61: Removing the lamp assembly



9. Position the new lamp so the cut-out located on the lamp base is at the 1 o'clock position, in line with the alignment pin on the lamp housing.

Figure 8–62: Aligning the lamp



- ① Cut-out on lamp base at 1 o'clock position
- ② Alignment pin on lamp housing

10. Gently push the lamp forward until it bottoms into position.
11. Tighten the two captive screws.
12. Reconnect the lamp's power connector.
13. Reinstall the lamp assembly cover using the one Phillips-head screw.
14. When ready to resume operation of the TUV detector, reconnect the power cord, and then power-on the system.

8.9.5 Replacing the PDA detector's detector lamp

Replace the PDA detector's lamp annually during the prescribed preventive maintenance (PM) or any time it repeatedly fails to light or when the detector fails to calibrate. The system automatically senses the lamp upon installation, and its serial number and installation date are automatically recorded in the Lamp Change Record table.

Note: Waters warrants 2000 hours of lamp life, or one year from date of purchase, whichever comes first.



Warning: To prevent burn injuries, allow the lamp to cool for 30 minutes before removing it. The lamp housing gets extremely hot during operation.



Warning: To avoid eye injury from ultraviolet radiation exposure:

- Power-off the detector before changing the lamp.
- Wear eye protection that filters ultraviolet light.
- Keep the lamp in the housing during operation.

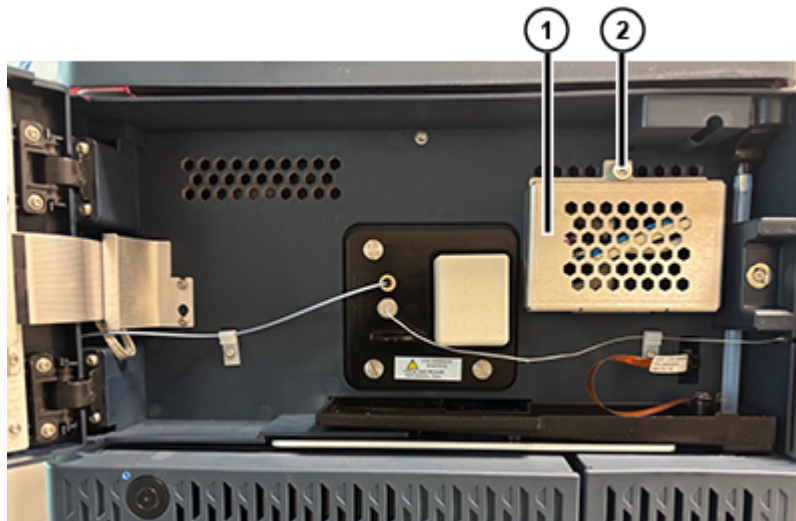
To replace the lamp:



Caution: Ensure that you allow the lamp to cool for at least 30 minutes before starting this procedure.

1. Power-off the system, and then disconnect the power cord.
2. Open the PDA detector's door.
3. Locate the lamp assembly cover (see the following figure).
4. To remove the lamp assembly cover, use a Phillips screwdriver to remove the one Phillips-head screw (see the following figure).
5. Lift the lamp assembly cover off.

Figure 8–63: Lamp assembly cover

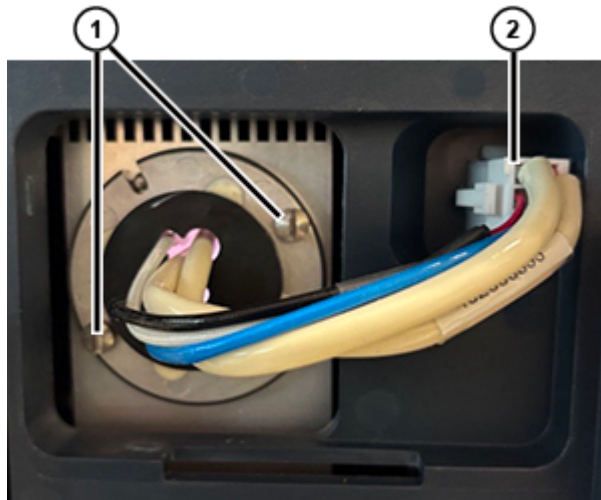


① Lamp assembly cover

② Phillips-head screw

6. Disconnect the power cable to the lamp.
7. Loosen the two captive screws in the lamp base.

Figure 8–64: Lamp assembly base captive screws



① Captive screws

② Lamp cable

8. Lift out the lamp assembly from the lamp housing.

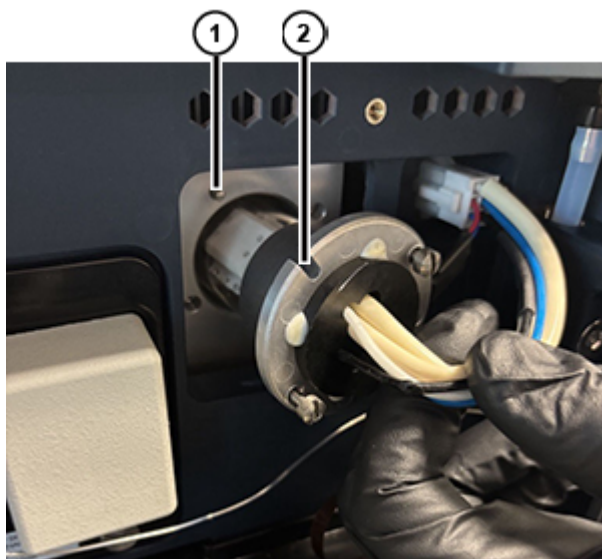
! **Notice:** To prevent shattering the glass, use care when disposing of the lamp because the lamp gas is under slight negative pressure.

Figure 8–65: Removing the lamp assembly



9. Position the new lamp so the cut-out located on the lamp base is at the 11 o'clock position, in line with the alignment pin on the lamp housing.

Figure 8–66: Aligning the lamp



- ① Cut-out on lamp base at 11 o'clock position
- ② Alignment pin on lamp housing

10. Gently push the lamp forward until it bottoms into position.
11. Tighten the two captive screws.
12. Reconnect the lamp's power connector.
13. Reinstall the lamp assembly cover using the one Phillips-head screw.
14. When ready to resume operation of the PDA detector, reconnect the power cord, and then power-on the system.

8.10 Column heater maintenance procedures

This section provides maintenance procedures for the Alliance iS HPLC System column heater that users or Waters field service personnel can perform.

The procedures include:

- Replacing the column
- Replacing the column leak sensor

8.10.1 Column heater maintenance schedule

The column heater has a recommended maintenance schedule.

Users can perform the following routine column heater maintenance procedures.

Maintenance procedure	Frequency
Replace the solvent filters (Page 101)	During scheduled routine maintenance or as needed
Replace the column (Page 159)	During scheduled routine maintenance or as needed
Replace the column leak sensor (Page 162)	During scheduled routine maintenance or as needed

8.10.2 Replacing the column

Waters eConnect tag-enabled columns use near-field communication (NFC) technology that provides an automated solution for identifying and tracing HPLC columns and their usage history. To ensure high-quality chromatographic data, replace the column annually, or any time you notice peak shape problems or loss of resolution.



Warning: To avoid personal contamination with biologically hazardous or toxic compounds, wear clean, chemical-resistant, powder-free gloves when performing this procedure.



Warning: To prevent burn injuries, allow sufficient time for the column to cool before opening the compartment door. The column, compartment, tubing, fittings, and door liner can be hot.

To remove the existing column:

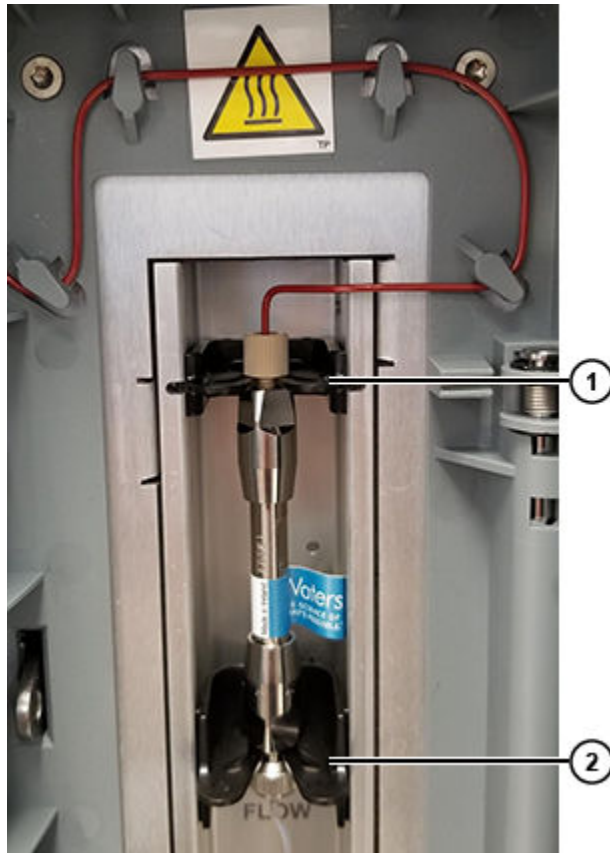
If installing a Waters-branded column, this procedure requires no tools. You can easily replace it using the column clips and fittings.

1. Open the column compartment door.

Note: Before removing the column, verify that the compartment temperature is sufficiently cool.

2. Remove the column from the two black clips that secure it in place:
 - a. Locate the fitting on the bottom of the column, and then pull the bottom of the column out of the black clip.
 - b. Next, remove the upper part of the column. Holding the bottom of the column in one hand, locate the fitting on the top and use your other hand to pull the top part of the column out of the black clip.

Figure 8–67: Removing the column from the black clips

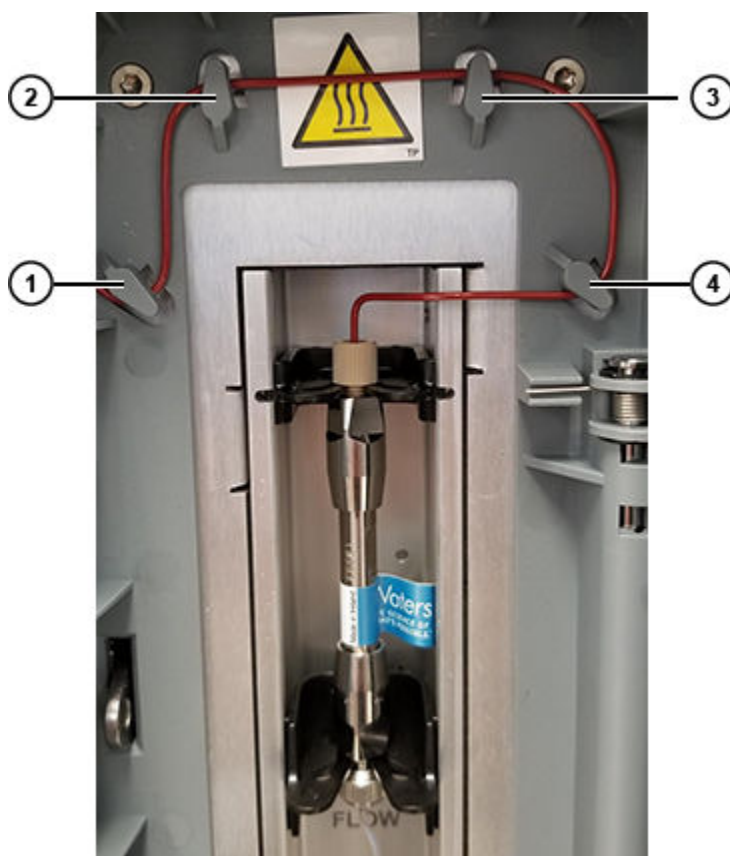


- ① Top black clip
- ② Bottom black clip

c. Locate the fasteners that secure the tubing at the top of the column compartment, and then remove the tubing from fasteners 2 through 4 only.

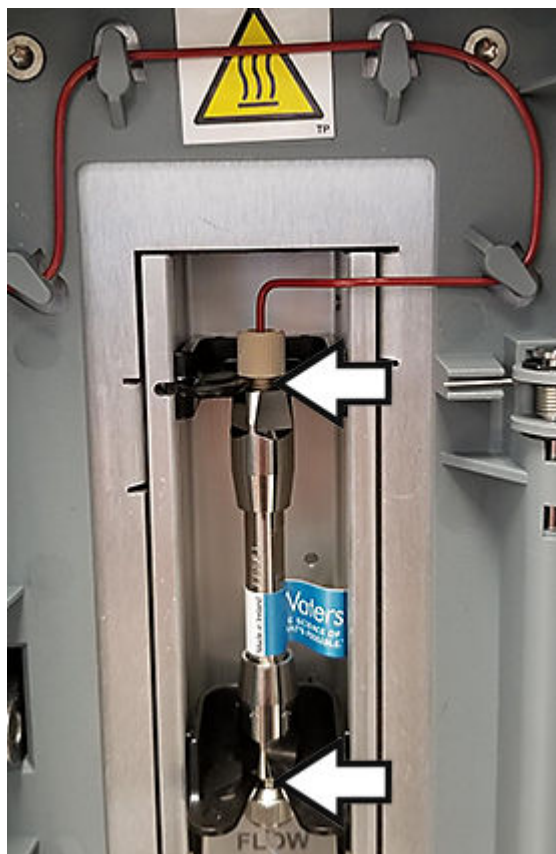
Tip: You will not remove the tubing from fastener 1 because it secures the tubing to another module and should remain connected.

Figure 8–68: Four fasteners that secure the tubing



- d. Unscrew the fitting located on the bottom of the column and set it aside to install the new column.
- e. Unscrew the fitting located on the top of the column and set it aside to install the new column. Follow the remaining steps to install the new column.
3. Remove the protective plugs from the top and the bottom of the new column, and then place them in the column shipping carton for future use.
4. Orient the column so that the outlet faces up (see the arrow on the column) and the inlet faces down.
5. Screw the column inlet and column outlet fittings you set aside earlier onto the column finger-tight.
6. If necessary, adjust the lower column clip to match the size of the new column.
7. Install the tubing by routing it into fasteners 2 through 4 located at the top of the column compartment.
8. Insert the column into the upper and lower black clips, ensuring that each black clip grasps the exposed threads on the fitting.

Figure 8–69: Installing the replacement column



9. Close the column compartment door.

Note: Verify that the tubing is situated inside the compartment before you close the column compartment door.

8.10.3 Replacing the column heater leak sensor

Users or Waters field service personnel can replace the column heater sensor.



Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.



Warning: To avoid personal contamination with biologically hazardous or toxic compounds, wear clean, chemical-resistant, powder-free gloves when performing this procedure.



Warning: To avoid eye injury, use eye protection when performing this procedure.



Requirement: Wear clean, chemical-resistant, powder-free gloves when performing this procedure.

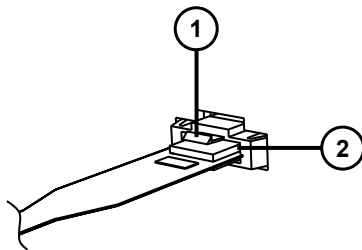
Required tools and materials

- Chemical-resistant, powder-free gloves
- Protective eyewear
- Replacement leak sensor

To replace the leak sensor:

1. Open the column compartment door.
2. Press down on the tab to detach the leak sensor connector from the front of the device.

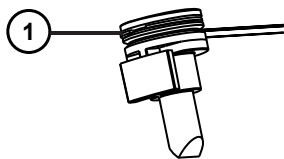
Figure 8–70: Leak sensor connector



- ① Tab
- ② Leak sensor connector

3. Grasp the leak sensor by its serrations and pull upward on it to remove it from its reservoir.

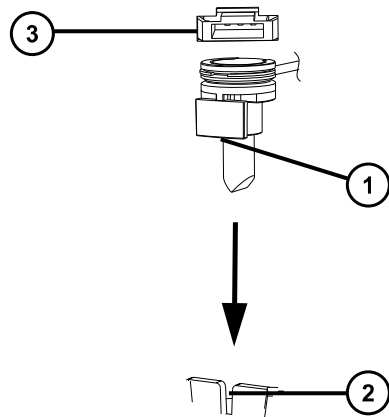
Figure 8–71: Leak sensor serrations



- ① Serrations

4. Unpack the new leak sensor.
5. Align the leak sensor's T-bar with the slot in the side of the leak sensor reservoir, and then slide the leak sensor into place.

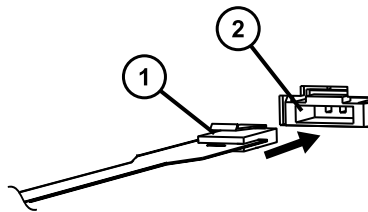
Figure 8–72: Aligning T-bar with slot



- ① T-bar
- ② Slot in leak sensor reservoir
- ③ Leak sensor port on front of device

6. Connect the leak sensor connector to the front of the device.

Figure 8–73: Attaching leak sensor connector



- ① Leak sensor connector
- ② Leak sensor port on front of device

- 7. Close the column compartment door.
- 8. From the touchscreen [Commands view \(Page 54\)](#), tap **Reset**.
- 9. From the touchscreen [System view \(Page 54\)](#), tap **Leak Sensors** and then enable the **QSM Leak Sensor**.

9 Disposal protocols

Disposal of system components is performed either by Waters personnel or by the customer per local jurisdiction.

9.1 Description of constituent materials

For detailed descriptions of Waters materials, see [Safety Data Sheets \(www.waters.com/SDS\)](http://www.waters.com/SDS) on waters.com.

9.2 Disposal of system components

Disposal of system components is performed either by Waters personnel or by the customer per local jurisdiction.

10 Solvent considerations



Warning: Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials. Consult the Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.

Review the following subjects related to the solvent considerations necessary when operating the Alliance iS HPLC System:

- [Preventing contamination \(Page 166\)](#)
- [Solvent quality \(Page 166\)](#)
- [Solvent preparation \(Page 167\)](#)
- [Solvent recommendations \(Page 168\)](#)
- [Common solvent properties \(Page 176\)](#)
- [Solvent miscibility \(Page 177\)](#)
- [Solvent stabilizers \(Page 179\)](#)
- [Solvent viscosity \(Page 179\)](#)
- [Wavelength selection \(Page 179\)](#)

10.1 Preventing contamination

Explore the Waters website for resources on controlling contamination.

For information about preventing and eliminating contamination, see *Controlling Contamination in LC/MS Systems* (715001307) on the Waters website (www.waters.com).

10.2 Solvent quality

For the best possible results, use MS-grade solvents.

The minimum requirement for solvents is HPLC-grade. Filter solvents through an appropriate membrane filter.

Recommendation: To ensure that the filter is appropriate for the solvents used, heed the recommendations of the filter's manufacturer or vendor.

10.2.1 Clean solvents

Waters stresses the importance of always using clean solvents in your system.

Clean solvents ensure reproducible results and permit system operation with minimal need for maintenance.

Dirty solvents can cause baseline detector noise and drift, and they can clog solvent reservoir filters, inlet filters, and capillary lines.

10.2.2 Buffered solvents

When using a buffer, choose good quality reagents, filtering them through a 0.2- μ m membrane filter.

Recommendation: To discourage microbial growth, replace 100% mobile aqueous phase daily.

Adjust the pH of aqueous buffers. Filter them to remove insoluble material, and then blend them with appropriate organic modifiers. After you use a buffer, flush it from the pump by wet priming using at least five system volumes of HPLC-grade distilled or deionized water.

! **Caution:** When operating the Alliance iS Bio HPLC System above pH 10, ensure that you are using the optional high pH kit. For further assistance, contact Waters.

For shutdowns of more than a day's duration, flush the pump with a 20% methanol (MeOH)/water solution to prevent microbial growth.

See also: For information on preventing contamination, refer to *Controlling Contamination in LC/MS Systems* (715001307) on the Waters website (www.waters.com).

10.2.3 Water

Use water only from a high-quality water purification system.

! **Notice:** Using 100% water can cause microbial growth. Waters recommends changing 100% water solutions daily. Adding a small amount (~10%) of an organic solvent prevents microbial growth.

If the water system does not deliver filtered water, filter the water through a 0.2- μ m membrane filter.

10.3 Solvent preparation

Proper solvent preparation, primarily filtration, can prevent many pumping problems.

Recommendation: Store mobile phases in borosilicate glass reservoirs type 1, class A² or type 3.3³. Use high-quality, brown-tinted glassware to inhibit microbial growth. Use aluminum foil or Waters caps to cover the reservoirs.

10.4 Solvent recommendations

Review important information on the solvents recommended and not recommended for your system.

To determine whether you can use solvents not addressed by the following subjects without adversely affecting component or system performance, contact Waters ([Contacting Waters \(Page 14\)](#)).

- [General solvent guidelines \(Page 168\)](#)
- [Wash solvent guidelines \(Page 174\)](#)

10.4.1 General solvent guidelines

Always observe Waters' general recommendations related to solvents.

- To inhibit microbial growth, use high-quality, brown-tinted glassware.
- Filter solvents through a 0.2- μ m filter, or use pre-filtered solvents. Small particles can permanently block a system's capillary lines. Filtering solvents also improves check valve performance.

10.4.1.1 Recommended solvents

Consult this list of solvents Waters recommends for use in your system.

Waters recommends the following solvents for use in your system:

- Acetonitrile (ACN)
- Isopropanol (IPA)
- Methanol (MeOH)

Note: Titanium is subject to corrosion in anhydrous methanol, which you can avoid by adding a small amount of water (approximately 3%). Slight corrosion is possible when ammonia is >10%.


- Water

10.4.1.2 Solvents to avoid


Consult this list of solvents to avoid using in your system.

Avoid the following substances:

- Solvents that contain halogens:
 - Bromine
 - Chlorine

- Fluorine
- Iodine
-  **Warning:** Peroxide contaminants in THF can spontaneously and destructively explode when you partially or completely evaporate the THF.

Compounds that form peroxides, such as ultraviolet (UV)-grade ethers, non-stabilized THF, dioxane, and diisopropylether (If you must use compounds that form peroxides, be sure to filter them through dry aluminum oxide, to adsorb formed peroxides. Do not allow them to remain on a system for more than 24 hours.)

- Solutions that contain strong concentrations (greater than 0.1% wt) of complexing agents like EDTA
-  **Notice:** The standard pH operating range for your system is 1.0 to 13.0. Operating the system below pH 1.0 or above pH 13.0 for more than brief periods can result in increased wear on system components not included in preventive maintenance kits and the need for more frequent routine preventive maintenance.

Strong acids and strong bases

- For strong acids, unless using them as cleaning agents, use them only in weak concentration. Avoid using acids as mobile phases when their pH is less than 1.0.
- For strong bases, use them only in weak concentration. Avoid using bases as mobile phases when their pH is greater than 10.0 (or greater than pH 12.0 when using the high pH tubing kit).
- When operating the Alliance iS Bio HPLC System above pH 10, ensure that you are using the optional high pH kit. For further assistance, contact Waters.

10.4.1.2.1 Material limitations for strong acids and bases

Strong acids and bases can negatively affect certain types of tubing, fittings, and component materials.

- Polyetheretherketone (PEEK) can be degraded in strong mineral acids, such as nitric and sulfuric acids, especially in the presence of halogens and halogen-containing chemicals.
- Silica capillaries and their polyimide coatings start to degrade at pH 8 and greater.
- Stainless steel (SST) can be attacked by certain acids below pH 2.3, especially in the presence of halogens and halogen-containing chemicals and strong mineral acids like nitric and sulfuric acids. SST can also experience degradation when used with organic acids in organic solvents.
- Quartz flow cells can have degraded lifetimes in the presence of pH greater than 12.
- Polyimide used in the needle seat is stable in a pH range between 1 and 10 and in most organic solvents. It degrades in the presence of concentrated mineral acids like sulfuric

acid and in the presence of glacial acetic acid. It is degraded by elevated basic conditions, especially in the presence of ammonia or ammonium salts or acetates.

- To maintain optimal performance, it is recommended that you periodically inspect and, if necessary, replace all relevant components. Additionally, when operating the Alliance iS Bio HPLC System above pH 10, ensure that you are using the optional high pH kit. For further assistance, contact Waters.

10.4.1.3 System solvent recommendations

Follow these Waters system-level recommendations when choosing solvents.




Warning: To avoid personal contamination with biologically hazardous, toxic, or corrosive materials, you must understand the hazards associated with their handling. Guidelines prescribing the proper use and handling of such materials appear in the latest edition of the National Research Council's publication, *Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards*.

To avoid injury when working with hazardous materials, consult the Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials and follow good laboratory practices.

For recommended system cleaning and flushing procedures, contact Waters.

See also: The cleaning procedures in *Controlling Contamination in LC/MS Systems* (715001307) on the Waters website (www.waters.com).

Waters makes the following general recommendations for your system:

-  **Warning:** Peroxide contaminants in THF can spontaneously and destructively explode when you partially or completely evaporate the THF.

Important: If you use any of the following solvents, you must install a hexane/THF compatibility kit (see [Waters hexane/THF compatibility kits \(Page 173\)](#)). When using hexane or THF, minimize the use of PEEK components by replacing PEEK tubing with SST or MP35N tubes.

You can use the following solvents as the mobile phase in your system:

- Acetone
- Ethyl acetate
- Hexane
- THF

Note: As with many nonaqueous solvents, however, the solvents in the preceding list can shorten system and component life compared with equipment running typical reverse-phase solvents.

- When using non-stabilized THF, ensure that your solvent is freshly prepared. Previously opened bottles contain peroxide contaminants, which cause detector baseline drift.

Important: Methanesulfonic acid is not recommended for use in this system.

- You can use the following solvents in weak dilutions (less than 10% vol.) as sample diluents:
 - Chloroform
 - Halogenated solvents
 - Methylene chloride
 - Toluene
- Because they serve as a substrate for microbial colonies, aqueous solvents must not remain in a shut-down system. Microbes can clog system filters and capillary lines. To prevent their proliferation, add a minimum of 10% of an organic solvent, such as ACN or MeOH.

Note: Titanium is subject to corrosion in anhydrous methanol, which you can avoid by adding a small amount of water (approximately 3%). Slight corrosion is possible when ammonia is >10%. If using an Alliance iS Bio HPLC system, you can alternatively remove the titanium filter sinkers (the system loses its first line of protection from particulates) or replace them with stainless steel sinkers if your analysis is unaffected by biocompatibility considerations.

- To determine whether a specific method is suitable for use with your systems' components, contact your Waters sales representative or local technical support organization.

10.4.1.3.1 Integrated Fluidics Module solvent recommendations

To optimize overall performance without adversely affecting the sample manager and pump hardware, follow certain solvent recommendations.

The Integrated Fluidics Module (IFM) assembly refers to the area in the Alliance iS System's chassis that houses the sample manager and pump.

For detailed recommendations related to the components of the IFM, see:

- [Sample manager solvent recommendations \(Page 171\)](#)
- [Pump solvent recommendations \(Page 172\)](#)

10.4.1.3.2 Sample manager solvent recommendations

Follow these solvent recommendations specific to the sample manager.

- Do not use buffers as needle wash solvents. You can use acids and bases.
- Typical organic sample diluents such as DMF and DMSO are supported.

10.4.1.3.3 Pump solvent recommendations

Follow these solvent recommendations specific to the pump.

- **!** **Notice:** To avoid damaging and clogging components in the wash and purge flow path, Waters recommends not using nonvolatile buffers or additives as wash solvents.

IPA or organic solvents are effective seal wash solvents for normal-phase separations that employ mobile phases of intermediate polarity (such as hexane or THF). When you use nonvolatile buffers and salts, decrease the seal wash solvent interval (see [Wash solvent recommendation \(Page 175\)](#) for more information about the use of nonvolatile buffer solutions).

- The seal wash system must never run dry, particularly during separations that use a polar mobile phase.
- Ensure that the mobile phase is completely soluble in and compatible with all of the solvents in use on the system (see [Wash solvent guidelines \(Page 174\)](#)).
- For reverse-phase applications, use aqueous seal wash solutions with a weak organic component (for example, 1:9 MeOH/water).

10.4.1.3.4 Detector solvent recommendation

Follow this solvent recommendation specific to the detector.

To transport a flow cell while temperatures are below 5 °C, fill it with alcohol.

10.4.1.4 Other solvents

This list comprises solvents you can use in your system after installing a Waters compatibility kit.

Note: Without the appropriate compatibility kit, these solvents can shorten equipment life. If you routinely use the solvents on this list, Waters recommends that you install the Waters hexane/tetrahydrofuran (THF) compatibility kit applicable to your system.

See: [Waters hexane/THF compatibility kits \(Page 173\)](#)

- Acetone
- Ethyl acetate
- Hexane
- THF

See also: [System solvent recommendations \(Page 170\)](#)

When you change solvents, consider solvent polarity. When switching between polar and nonpolar solvents, flush the system with a miscible and compatible solvent, such as IPA.

10.4.1.4.1 Waters hexane/THF compatibility kits


A Waters hexane/THF compatibility kit can be installed in relevant systems.

Waters' hexane/THF compatibility kits are designed for users who must run their system with certain solvents (see [Other solvents \(Page 172\)](#)) or combinations of these solvents at high concentrations and high pressure.

Note: For the part number of the hexane/THF compatibility kit applicable to your system, contact Waters (see [Contacting Waters \(Page 14\)](#)).

10.4.1.5 Additives/modifiers

Consult this list of additives and modifiers and their specific quantities for use in your system.

- Acetic acid, $\leq 0.3\%$ vol.
- Ammonium acetate, ≤ 50 mM
- Ammonium bicarbonate, ≤ 10 mM
- Ammonium hydroxide, ≤ 50 mM
- Ethylenediaminetetraacetic acid (EDTA), $\leq 0.1\%$ wt.
- Formic acid, $\leq 0.2\%$ vol.
-  **Warning:** To avoid damaging the system components, and to prevent contact with corrosive material that can be present on the components that hexafluoroisopropanol (HFIP) damages, do not use HFIP in wash solvents.

HFIP for oligonucleotide applications, 1% to 4% aqueous solutions

- Heptafluorobutyric acid, $\leq 0.1\%$ vol.
- Phosphate buffer, ≤ 10 mM
- Triethylamine (TEA), $\leq 0.1\%$ vol.
- Trifluoroacetic acid (TFA), $\leq 0.1\%$ vol.

10.4.1.6 Sample diluents

Consult this list of sample diluents for use in your system.

- ACN
- ACN/water mixtures
- Chloroform
- Dimethyl sulfoxide (DMSO)
- Dimethylformamide (DMF)
- IPA
- Isooctane
- MeOH

- MeOH/water mixtures
- Methylene chloride
- Water

10.4.1.7 Cleaning agents

You may use these cleaning agents in the system.

Note: Cleaning agents require short (less than 30 minutes) contact time when flushing instruments.

- Formic acid ($\leq 30\%$)
- Phosphoric acid ($\leq 30\%$)
- Sodium hydroxide ($\leq 1M$)

See also: The cleaning procedures in *Controlling Contamination in LC/MS Systems* (715001307) on the Waters website (www.waters.com).

10.4.2 Wash solvent guidelines

To reduce the risk of carryover, follow these wash solvent guidelines.

Wash solvents clean the sample needle between injections and remove traces of the previous sample. They are commonly stronger than an application's isocratic mobile phase and are often as strong as—or even stronger than—the final mobile phase conditions for a gradient separation. Because they are not injected onto a column, determining an appropriate wash solvent requires, for all practical purposes, no chromatographic considerations.

For best performance, follow these guidelines when selecting wash solvents. Otherwise, you can increase the risk of carryover. However, these guidelines do not prohibit all other solvent combinations, which you can run with lower performance expectations or by manipulating injection parameters.

! **Notice:** To avoid damaging and clogging components in the wash and purge flow path, Waters recommends not using nonvolatile buffers or additives as wash solvents.

See also: [Wash solvent recommendation \(Page 175\)](#) for details on the Waters recommendation regarding nonvolatile buffer solutions.

- **Important:** Wash solvents must be compatible and miscible with both the application's mobile phase and the sample components. They must also be fully soluble with the mobile phase and sample and should not cause precipitation.

Use wash solvents based on the sample and mobile phase chemistries of your application.

- Wash solvents must be strong enough to easily dissolve the sample and thereafter maintain solubility.
- For buffered aqueous, reverse-phase chromatographic conditions, wash solvent with a high concentration of organic solvent is typically used, such as 80 to 100% ACN or MeOH with the remainder water.

Note: Titanium is subject to corrosion in anhydrous methanol, which you can avoid by adding a small amount of water (approximately 3%). Slight corrosion is possible when ammonia is >10%. If using an Alliance iS Bio HPLC system, you can alternatively remove the titanium filter sinkers (the system loses its first line of protection from particulates) or replace them with stainless steel sinkers if your analysis is unaffected by biocompatibility considerations.

10.4.2.1 Wash solvent recommendation

To maintain system condition and overall performance, follow this wash solvent recommendation.

The use of nonvolatile (solid salt) buffer solutions in wash solvents is permitted in limited concentration only. Generally, however, it is not recommended.

Solid, salt-based buffer solutions can dry and leave a salt residue that can scratch sealing surfaces, clog tubing, and damage the wash pump. Some applications control pH to promote sample solubility, which manifests in better peak shape and narrower peaks upon elution. Better sample solubility in the mobile phase also results in the sample components being less likely to adhere onto surfaces such as the stationary phase or the needle. If pH control is important to separation performance, consider it when determining the composition of wash solvent. For example, if acid (low pH) is required to keep the sample in solution during the separation, it is likely to be a required component of the wash solvent, necessary to dissolve the sample adhering to the needle's surface and to rinse the wash station.

! **Caution:** When operating the Alliance iS Bio HPLC System above pH 10, ensure that you are using the optional high pH kit. For further assistance, contact Waters.

10.4.2.2 Optional uses for wash solvents

You have the option to utilize wash solvents for these additional purposes.

- By default, the system washes the exterior of the sample needle after an injection, but you can choose to also utilize wash solvents in an optional procedure that cleans the exterior of the needle before or after an injection.

See also: [Wash system \(Page 48\)](#) for further description of the default needle cleaning process.

- To ascertain proper flow through the waste tubing and to confirm that the wash system is operating properly, you can choose to prime the wash system with wash solvents.

10.5 Common solvent properties

Consult this table for the properties of some common chromatographic solvents.

Table 10–1: Properties of common solvents

Solvent	Vapor pressure mm Hg (Torr)	Boiling point (°C)	Flash point (°C)
ACN	88.8 at 25 °C	81.6	6
Acetone	184.5 at 20 °C	56.29	-20
<i>n</i> -Butyl acetate	7.8 at 20 °C	126.11	22
<i>n</i> -Butyl alcohol	4.4 at 20 °C	117.5	37
<i>n</i> -Butyl chloride	80.1 at 20 °C	78.44	-9
Chlorobenzene	8.8 at 20 °C	131.69	28
Chloroform	158.4 at 20 °C	61.15	n/a
Cyclohexane	77.5 at 20 °C	80.72	-20
Cyclopentane	400 at 20 °C	49.26	-7
Dimethylacetamide (DMA)	1.3 at 25 °C	166.1	70
DMF	2.7 at 20 °C	153.0	58
DMSO	0.6 at 25 °C	189.0	88
<i>o</i> -Dichlorobenzene	1.2 at 20 °C	180.48	66
Dichloromethane	350 at 20 °C	39.75	n/a
1, 4-Dioxane	29 at 20 °C	101.32	12
Ethyl acetate	73 at 20 °C	77.11	-4
Ethyl alcohol	43.9 at 20 °C	78.32	15
Ethyl ether	442 at 20 °C	34.55	-45
Ethylene dichloride	83.35 at 20 °C	83.48	13
Heptane	35.5 at 20 °C	98.43	-4
Hexane	124 at 20 °C	68.7	-22
IPA	32.4 at 20 °C	82.26	12
Iso-octane	41 at 20 °C	99.24	-12
Isobutyl alcohol	8.8 at 20 °C	107.7	28
Isopropyl myristate	<1 at 20 °C	182.6	164
MeOH	97 at 20 °C	64.7	11
Methyl <i>t</i> -butyl ether	240 at 20 °C	55.2	-28
Methyl ethyl ketone	74 at 20 °C	79.64	-9

Table 10–1: Properties of common solvents (continued)

Solvent	Vapor pressure mm Hg (Torr)	Boiling point (°C)	Flash point (°C)
Methyl isobutyl ketone	16 at 20 °C	117.4	18
<i>N</i> -Methylpyrrolidone	0.33 at 25 °C	202.0	86
Pentane	420 at 20 °C	36.07	-49
<i>n</i> -Propyl alcohol	15 at 20 °C	97.2	23
Propylene carbonate	n/a	241.7	135
Pyridine	18 at 25 °C	115.25	20
TEA	57 at 25 °C	89.5	-9
TFA	97.5 at 20 °C	71.8	-3
THF	142 at 20 °C	66.0	-14
Toluene	28.5 at 20 °C	110.62	4
1,2,4-Trichlorobenzene	1 at 20 °C	213.5	106
Water	17.54 at 20 °C	100.0	n/a
<i>o</i> -Xylene	6 at 20 °C	144.41	17

10.6 Solvent miscibility

Before you change solvents, consult this table to determine solvent miscibility.

Be aware of the following effects and considerations:

- Temperature affects solvent miscibility. If you are running a high-temperature application, consider the effect of the higher temperature on solvent solubility.
- Buffers dissolved in water can precipitate when mixed with organic solvents.
- You can make changes involving two miscible solvents directly. Changes involving two solvents that are not totally miscible (for example, from chloroform to water) require an intermediate solvent like *n*-propanol.
- When you switch from a strong buffer to an organic solvent, thoroughly flush the system with distilled water before you add the organic solvent.

Note: λ cutoff is the wavelength at which the absorbance of the solvent equals 1 AU.

Table 10–2: Solvent miscibility

Solvent	Polarity index	Viscosity cP, 20 °C (at 1 atm)	Boiling point °C (at 1 atm)	Miscibility number (M)	λ cutoff (nm)
ACN	6.2	0.37	81.6	11, 17	190
Acetic acid	6.2	1.26	117.9	14	n/a
Acetone	5.4	0.32	56.3	15, 17	330
Benzyl alcohol	5.5	5.80	205.5	13	n/a
DMF	6.4	0.90	153.0	12	n/a
DMSO	6.5	2.24	189.0	9	n/a
Ethanol	5.2	1.20	78.3	14	210
<i>n</i> -Hexane	0.0	0.313	68.7	29	n/a
MeOH	6.6	0.60	64.7	12	210
Methoxyethan ol	5.7	1.72	124.6	13	n/a
1-Propanol	4.3	2.30	97.2	15	210
2-Propanol	4.3	2.35	117.7	15	n/a
THF	4.2	0.55	66.0	17	220
Triethylamine	1.8	0.38	89.5	26	n/a
Water	9.0	1.00	100.0	n/a	n/a

10.6.1 Using miscibility numbers

Use miscibility numbers to predict the miscibility of a liquid with a standard solvent.

To predict the miscibility of two liquids, subtract the smaller miscibility number (M-number) value from the larger M-number value:

- If the difference between two M-numbers is 15 or less, the two liquids are miscible, in all proportions, at 15 °C.
- A difference of 16 indicates a critical solution temperature from 25 to 75 °C, with 50 °C as the optimal temperature.
- If the difference is 17 or greater, the liquids are immiscible, or their critical solution temperature is above 75 °C.

Some solvents prove immiscible with solvents at both ends of the lipophilicity scale. These solvents receive a dual M-number:

- The first number, always lower than 16, indicates the degree of miscibility with highly lipophilic solvents.
- The second number applies to the opposite end of the scale. A large difference between these two numbers indicates a limited range of miscibility.

For example, some fluorocarbons are immiscible with all the standard solvents and have M-numbers of 0 and 32. Two liquids with dual M-numbers are usually miscible with each other.

A liquid is classified in the M-number system by testing for miscibility with a sequence of standard solvents. A correction term of 15 units is then either added or subtracted from the cutoff point for miscibility.

10.7 Solvent stabilizers

Solvent stabilizers are added to slow or stop solvent degradation.



Warning: Certain solvents degrade, or become unstable, over time. Highly unstable solvents present a potential explosion hazard.



Notice: Do not leave solvents containing stabilizers, such as THF with butylated hydroxytoluene (BHT), to dry in the system's flow path. A dry flow path, including the detector flow cell, becomes contaminated with residual stabilizer, and a substantial cleaning effort is needed to restore the flow path to its initial condition.

10.8 Solvent viscosity

In gradient chromatography, the viscosity changes that occur as the solvents are mixed in varying proportion can affect pressure changes during the run.

Although, generally, viscosity is not a consideration when you use a single solvent or when under low pressure, if you do not know the extent to which pressure changes affect the analysis, monitor the pressure during the run.

10.9 Wavelength selection

Consult these tables to obtain wavelength cutoff values for common solvents and mixed mobile phases.

- [Wavelength cutoffs for common solvents \(Page 180\)](#)
- [Wavelength cutoffs for mixed mobile phases \(Page 180\)](#)

10.9.1 Wavelength cutoffs for common solvents

Consult this table to obtain the wavelength cutoff values for common solvents.

Notes:

- λ (or UV) cutoff is the wavelength at which the absorbance of the solvent equals 1 AU.
- Operating at a wavelength near or below the cutoff increases baseline noise because of solvent absorbance.

Table 10–3: Wavelength cutoffs for common solvents

Solvent	λ cutoff (nm)
ACN	190
Acetone	330
Diethylamine	275
Ethanol	210
IPA	205
Isopropyl ether	220
MeOH	205
<i>n</i> -Propanol	210
THF	230

10.9.2 Wavelength cutoffs for mixed mobile phases

Consult this table to obtain the approximate wavelength cutoff values for solvents, buffers, detergents, and mobile phases.

The solvent concentrations represented are those most commonly used. If you want to use a different concentration, you can determine approximate absorbance using Beer's law, because absorbance is proportional to concentration.

Notes:

- λ (or UV) cutoff is the wavelength at which the absorbance of the solvent equals 1 AU.
- Operating at a wavelength near or below the cutoff increases baseline noise because of solvent absorbance.

Table 10–4: Wavelength cutoffs for various mobile phases

Mobile phase	λ cutoff (nm)
Acetic acid, 1%	230
Ammonium acetate, 10 mM	205
Ammonium bicarbonate, 10 mM	190

Table 10–4: Wavelength cutoffs for various mobile phases (continued)

Mobile phase	λ cutoff (nm)
3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 0.1%	215
Diammonium phosphate, 50 mM	205
Disodium EDTA, 1 mM	190
4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 10 mM, pH 7.6	225
Hydrochloric acid, 0.1%	190
2-(<i>N</i> -Morpholino)ethanesulfonic acid (MES), 10 mM, pH 6.0	215
4-Octylphenol polyethoxylate (Triton X-100), 0.1%	240
Polyoxyethylene (35) lauryl ether (Brij 35), 0.1%	190
Potassium phosphate, dibasic, 10 mM	190
Potassium phosphate, monobasic, 10 mM	190
Sodium acetate, 10 mM	205
Sodium chloride, 1 M	207
Sodium citrate, 10 mM	225
Sodium dodecyl sulfate, 0.1%	190
Sodium formate, 10 mM	200
Triethylamine, 1%	235
Trifluoroacetic acid, 0.1%	190
Tris(hydroxymethyl)aminomethane hydrochloride (Tris HCl), 20 mM, pH 7.0	202
Tris HCl, 20 mM, pH 8.0	212
Waters Paired-Ion Chromatography (PIC) Reagent A, 1 vial/liter	200
Waters PIC Reagent B-6, 1 vial/L	225
Waters PIC Reagent B-6, low UV, 1 vial/L	190
Waters PIC Reagent D-4, 1 vial/L	190

10.9.2.1 Mobile phase absorbance

Consult this table to obtain the absorbances at several wavelengths for frequently used mobile phases.

To reduce baseline noise, choose the mobile phase carefully.

The best mobile phase for your application is one that is transparent at the chosen detection wavelengths. With such a mobile phase, ensure that any absorbance is caused only by the sample. Absorbance by the mobile phase also reduces the linear dynamic range of the detector by the amount of absorbance the Autozero function cancels. Wavelength, pH, and concentration of the mobile phase affect its absorbance. Examples of several mobile phases are given in the following table, where the absorbances are based on a 10-mm path length.

Note: When running normal-phase solvents, install the Waters hexane/THF conversion kit applicable to your system (see [Waters hexane/THF compatibility kits \(Page 173\)](#)).



Caution: When operating the Alliance iS Bio HPLC System above pH 10, ensure that you are using the optional high pH kit. For further assistance, contact Waters.

Table 10–5: Mobile phase absorbance measured against air or water

Mobile phase	Absorbance (AU) at specified wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
Solvents										
ACN	0.05	0.03	0.02	0.01	0.01	<0.01	n/a	n/a	n/a	n/a
IPA	1.80	0.68	0.34	0.24	0.19	0.08	0.04	0.03	0.02	0.02
MeOH (degassed)	1.91	0.76	0.35	0.21	0.15	0.06	0.02	<0.01	n/a	n/a
MeOH (not degassed)	2.06	1.00	0.53	0.37	0.24	0.11	0.05	0.02	<0.01	n/a
Unstablized THF (fresh)	2.44	2.57	2.31	1.80	1.54	0.94	0.42	0.21	0.09	0.05
Unstablized THF (old)	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	2.5	1.45
Acids and bases										
Acetic acid, 1%	2.61	2.63	2.61	2.43	2.17	0.87	0.14	0.01	<0.01	n/a

Table 10–5: Mobile phase absorbance measured against air or water (continued)

Mobile phase	Absorbance (AU) at specified wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
Diammonium phosphate, 50 mM	1.85	0.67	0.15	0.02	<0.01	n/a	n/a	n/a	n/a	n/a
Disodium EDTA, 1 mM	0.11	0.07	0.06	0.04	0.03	0.03	0.02	0.02	0.02	0.02
Hydrochloric acid, 0.1%	0.11	0.02	<0.01	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Phosphoric acid, 0.1%	<0.01	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Triethylamine, 1%	2.33	2.42	2.50	2.45	2.37	1.96	0.50	0.12	0.04	<0.01
Trifluoroacetic acid, 0.1%	1.20	0.78	0.54	0.34	0.22	0.06	<0.02	<0.01	n/a	n/a
Buffers and salts										
Ammonium acetate, 10 mM	1.88	0.94	0.53	0.29	0.15	0.02	<0.01	n/a	n/a	n/a
Ammonium bicarbonate, 10 mM	0.41	0.10	0.01	<0.01	n/a	n/a	n/a	n/a	n/a	n/a
HEPES, 10 mM, pH 7.6	2.45	2.50	2.37	2.08	1.50	0.29	0.03	<0.01	n/a	n/a
MES, 10 mM, pH 6.0	2.42	2.38	1.89	0.90	0.45	0.06	<0.01	n/a	n/a	n/a
Potassium phosphate, dibasic (K ₂ HPO ₄), 10 mM	0.53	0.16	0.05	0.01	<0.01	n/a	n/a	n/a	n/a	n/a
Potassium phosphate, monobasic (KH ₂ PO ₄), 10 mM	0.03	<0.01	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Sodium acetate, 10 mM	1.85	0.96	0.52	0.30	0.15	0.03	<0.01	n/a	n/a	n/a
Sodium chloride, 1 M	2.00	1.67	0.40	0.10	<0.01	n/a	n/a	n/a	n/a	n/a
Sodium citrate, 10 mM	2.48	2.84	2.31	2.02	1.49	0.54	0.12	0.03	0.02	0.01
Sodium formate, 10 mM	1.00	0.73	0.53	0.33	0.20	0.03	<0.01	n/a	n/a	n/a
Sodium phosphate, 100 mM, pH 6.8	1.99	0.75	0.19	0.06	0.02	0.01	0.01	0.01	0.01	<0.01
Tris HCl, 20 mM, pH 7.0	1.40	0.77	0.28	0.10	0.04	<0.01	n/a	n/a	n/a	n/a
Tris HCl, 20 mM, pH 8.0	1.80	1.90	1.11	0.43	0.13	<0.01	n/a	n/a	n/a	n/a
Surfactants										

Table 10–5: Mobile phase absorbance measured against air or water (continued)

Mobile phase	Absorbance (AU) at specified wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
Brij 35, 1%	0.06	0.03	0.02	0.02	0.02	0.01	<0.01	n/a	n/a	n/a
CHAPS, 0.1%	2.40	2.32	1.48	0.80	0.40	0.08	0.04	0.02	0.02	0.01
Polyoxyethylene sorbitan monolaurate (Tween 20), 0.1%	0.21	0.14	0.11	0.10	0.09	0.06	0.05	0.04	0.04	0.03
Sodiumdodecyl sulfate (SDS), 0.1%	0.02	0.01	<0.01	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Triton X-100, 0.1%	2.48	2.50	2.43	2.42	2.37	2.37	0.50	0.25	0.67	1.42
Waters PIC reagents										
PIC A, 1 vial/L	0.67	0.29	0.13	0.05	0.03	0.02	0.02	0.02	0.02	<0.01
PIC B6, 1 vial/L	2.46	2.50	2.42	2.25	1.83	0.63	0.07	<0.01	n/a	n/a
PIC B6, low UV, 1 vial/L	0.01	<0.01	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
PIC D4, 1 vial/L	0.03	0.03	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.01

11 Specifications

For all specifications related to your system (operating specifications) and its modules (performance specifications), see the *Alliance iS HPLC System Specification Sheet* (720007867EN) or the *Alliance iS Bio HPLC System Specification Sheet* (720008262EN) on the Waters website (www.waters.com). Reproducibility of specifications depends on the conditions in individual laboratories.

For additional information about your system's specifications, refer to the *Alliance iS HPLC Systems Site Preparation Guide* (715008415), also on the Waters website, or contact Waters (see [Contacting Waters \(Page 14\)](#)).