Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

Alliance iS HPLC System User Guide

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Version 00

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1 General information

1.1 Copyright notice

1.2 About Alliance iS documentation

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

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

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 System to access the Alliance iS 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.

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. Phrases must be enclosed by 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 on 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.

TaperSlit™ is a trademark of Waters Corporation.

THE SCIENCE OF WHAT'S POSSIBLE™ 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 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.

1.4.3 Electrical power safety notice

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

1.4.4 Equipment misuse notice

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

1.4.5 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

Symbol	Definition
	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
	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)
	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 Alliance iS HPLC system

The Alliance iS HPLC system serves QC departments that support the uninterrupted supply of safe and effective products. Specific features of the Alliance iS HPLC system are unlocked within the Empower CDS to help prevent common errors by up to 40% and reduce the associated risks of common and preventable errors associated with HPLC systems.

1.5.4 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.

1.5.5 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 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.

1.6.2 ISM classification: ISM group 1 class B

This classification was assigned in accordance with CISPR 11 Industrial Scientific and Medical (ISM) instrument 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 B products are suitable for use in both commercial and residential locations and can be directly connected to a low-voltage, power-supply network.

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.

1.7 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.
Corporate contact information	Waters Corporation Global Support Services 34 Maple Street Milford, MA 01757 USA From the USA or Canada, phone 800-252-4752 or fax 508-872-1990.

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

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

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 Specific warnings

Note: Under construction.

2.3 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.

2.4 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.

2.5 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.

2.6 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 Bedienungsbefugnis 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.
- Spegnere 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砜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。
- 不要使用已經被壓癟或嚴重彎曲管線。
- 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碸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 디메틸술폭시드(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、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。

2.7 Warnings that address the replacement of fuses

The following warnings pertain to instruments and devices equipped with user-replaceable fuses. Information describing fuse types and ratings sometimes, but not always, appears on the instrument or device.

Finding fuse types and ratings when that information appears on the instrument or device:

Warning: To protect against fire, replace fuses with those of the type and rating printed on panels adjacent to instrument fuse covers.

Avertissement : Pour éviter tout risque d’incendie, remplacez toujours les fusibles par d’autres du type et de la puissance indiqués sur le panneau à proximité du couvercle de la boîte à fusible de l’instrument.

Warnung: Zum Schutz gegen Feuer die Sicherungen nur mit Sicherungen ersetzen, deren Typ und Nennwert auf den Tafeln neben den Sicherungsabdeckungen des Geräts gedruckt sind.

Avvertenza: Per garantire protezione contro gli incendi, sostituire i fusibili con altri dello stesso tipo aventi le caratteristiche indicate sui pannelli adiacenti alla copertura fusibili dello strumento.

Advertencia: Para evitar incendios, sustituir los fusibles por otros del tipo y características impresos en los paneles adyacentes a las cubiertas de los fusibles del instrumento.

警告： 为了避免火灾，应更换与仪器保险丝盖旁边面板上印刷的类型和规格相同的保险丝。

警告： 為了避免火災，更換保險絲時，請使用與儀器保險絲蓋旁面板上所印刷之相同類型與規格的保險絲。

경고: 화재의 위험을 막으려면 기기 퓨즈 커버에 가까운 패널에 인쇄된 것과 동일한 타입 및 정격의 제품으로 퓨즈를 교체하십시오.

警告: 火災予防のために、ヒューズを交換する場合は、装置ヒューズカバーの隣のパネルに記載されている種類および定格のヒューズをご使用ください。

Finding fuse types and ratings when that information does not appear on the instrument or device:

Warning: To protect against fire, replace fuses with those of the type and rating indicated in the “Replacing fuses” section of the Maintenance Procedures chapter.

Avertissement : Pour éviter tout risque d’incendie, remplacez toujours les fusibles par d’autres du type et de la puissance indiqués dans la rubrique « Remplacement des fusibles » du chapitre traitant des procédures de maintenance.

Warnung: Zum Schutz gegen Feuer die Sicherungen nur mit Sicherungen ersetzen, deren Typ und Nennwert im Abschnitt "Sicherungen ersetzen" des Kapitels "Wartungsverfahren" angegeben sind.

Avvertenza: Per garantire protezione contro gli incendi, sostituire i fusibili con altri dello stesso tipo aventi le caratteristiche indicate nel paragrafo "Sostituzione dei fusibili" del capitolo "Procedure di manutenzione".

Advertencia: Para evitar incendios, sustituir los fusibles por otros del tipo y características indicados en la sección "Sustituir fusibles" del capítulo Procedimientos de mantenimiento.

警告： 为了避免火灾，应更换“维护步骤”一章的“更换保险丝”一节中介绍的相同类型和规格的保险丝。

警告： 為了避免火災，更換保險絲時，應使用「維護步驟」章節中「更換保險絲」所指定之相同類型與規格的保險絲。

경고: 화재의 위험을 막으려면 유지관리 절차 단원의 “퓨즈 교체” 절에 설명된 것과 동일한 타입 및 정격의 제품으로 퓨즈를 교체하십시오.

警告: 火災予防のために、ヒューズ交換ではメンテナンス項目の「ヒューズの交換」に記載されているタイプおよび定格のヒューズをご使用ください。

2.8 Electrical symbols

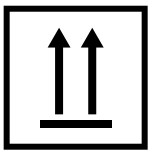
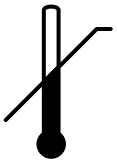
The following electrical symbols and their associated statements can appear in instrument manuals and on an instrument’s front or rear panels.

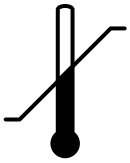
Symbol	Description
	Electrical power on
	Electrical power off
	Standby
	Direct current
	Alternating current
	Alternating current (three phase)
	Safety ground
	Frame or chassis terminal connection
	Fuse
	Functional ground
	Input
	Output

Symbol	Description
	Indicates that the device or assembly is susceptible to damage from electrostatic discharge (ESD)

2.9 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

Symbol	Description
	Temperature limitation

3 System overview

The Waters Alliance iS System is the next-gen LC solution for pharma QC departments.

To pharmaceutical customers who require a modern HPLC system that can easily replicate their existing methods, the Alliance iS System provides improved performance specifications. It delivers improved injection precision, lower analyte carryover, and increased back-pressure tolerance that matches or exceeds competitive offerings. It is an ideal and competitive system for transferring methods from any HPLC system.

The Alliance iS System, which provides true plug-and-play method compatibility for HPLC separations, can replicate established assays developed on older HPLC platforms. The system can also improve productivity by pairing with UHPLC column technology without requiring manual adjustment. Lastly, the system accepts UPLC methods that have undergone adjustment for larger particle sizes.

The Alliance iS System consists of a pump (QSM), an autosampler (SM-FTN), a CHC, and a detector (TUV) on Empower only.

3.1 Features of the system

The Alliance iS System performs validated assays using quaternary-based LC technology that adjusts for the differences between HPLC and UHPLC separations.

These are among the system's features and capabilities:

- QSM pump and SM-FTN design enhancements that minimize dispersion and reduce cycle time
- Plug-and-play method compatibility with HPLC
- *Direct programming of gradients, in units of pH and ionic strength, using Waters Auto•Blend Plus technology to vary composition, eliminating the need to manually prepare mobile phases*
- Automatic management of gradient start time and pre-injection steps, in parallel, minimizing cycle time and maximizing sample throughput
- Automatic counteraction of differences in system dwell volume without the need to alter gradient-table inputs
- Continuous needle cleansing during the run, minimizing carryover
- CHC options that ensure method repeatability from lab to lab
- Optical detection options that maximize HPLC performance

3.1.1 Flow-through-needle injector

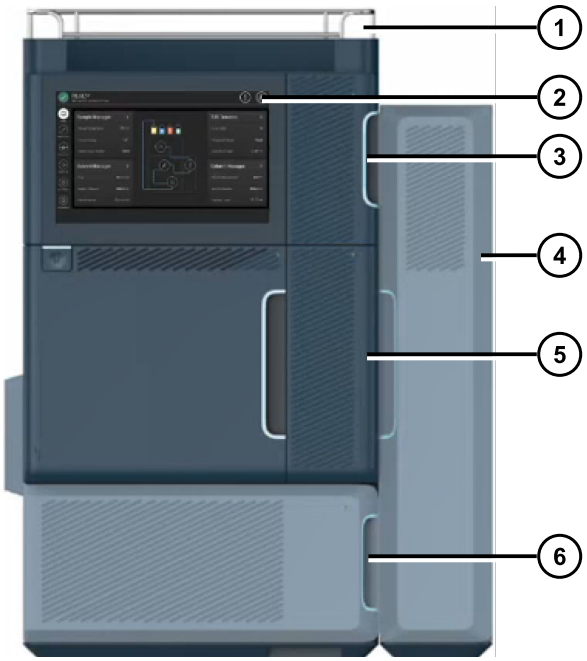
The sample manager's FTN mechanism aspirates a sample and holds it in the sample needle, prepared to inject the sample onto the column. The needle serves as part of the injection flow path when the sample is pushed onto the column.

The FTN mechanism facilitates the transfer of LC methods, improves injection accuracy, 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 following illustration depicts an Alliance iS System stack that includes core modules and a detector.

Figure 3–1: Alliance iS System stack



1. Bottle tray
2. Touchscreen
3. Detector
4. Column compartment
5. Autosampler

6 Pump

The system includes specifically these core modules:

- Autosampler: SM-FTN
- Column compartment: CHC
- Pump: QSM

In addition to the core modules, the system includes a detector (TUV).

3.2.1 Detector features

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

The Alliance iS TUV Detector is an optical, two-channel, ultraviolet/visible (UV/Vis) absorbance detector designed for HPLC applications. For the Alliance iS System, this detector is configured with Empower Chromatography Data Systems (CDS) software.

Table 3–1: TUV detector capabilities

Capability	Description
Programmability	Stores as many as five user-defined programs (or methods) consisting of as many as 50 programmable, timed events and two threshold events each
Two primary modes of operation	Single- or Dual-Wavelength
Automatic, UV filter	Supports spectrum scan, display, subtraction, storage, and playback, in addition to standard absorbance and UV/Vis functionality
Full diagnostic capability	Supports built-in diagnostic tools to optimize functionality and performance
Two contact closure outputs	The detector has two configurable switches, each of which accommodating a maximum of +30 Vdc, 1.2-A current carrying capacity, and 0.5-A current switching. The switches (SW1 and SW2) can trigger fraction collectors and other external devices. They can also activate according to time, absorbance threshold, or ratio criteria.
Active thermal control	
Thermal wander management	The detector's insulation, fans, and baffling are designed to mitigate the thermal instability caused by ambient temperature changes.

Table 3–1: TUV detector capabilities (continued)

Capability	Description
Median Baseline Filter (MBF)	A variation of the data mode, the MBF decreases the effects of gradient separations on the chromatographic baseline. It enhances the UV detector's baseline stability by decreasing its curvature, making the development of integration methods easier.
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. Waters qualification kits, available in cuvette form, support this feature., which allows the detector to serve as a spectrophotometer.
Cuvette sample analysis	Allows recording of the spectrum of any sample placed in the cuvette

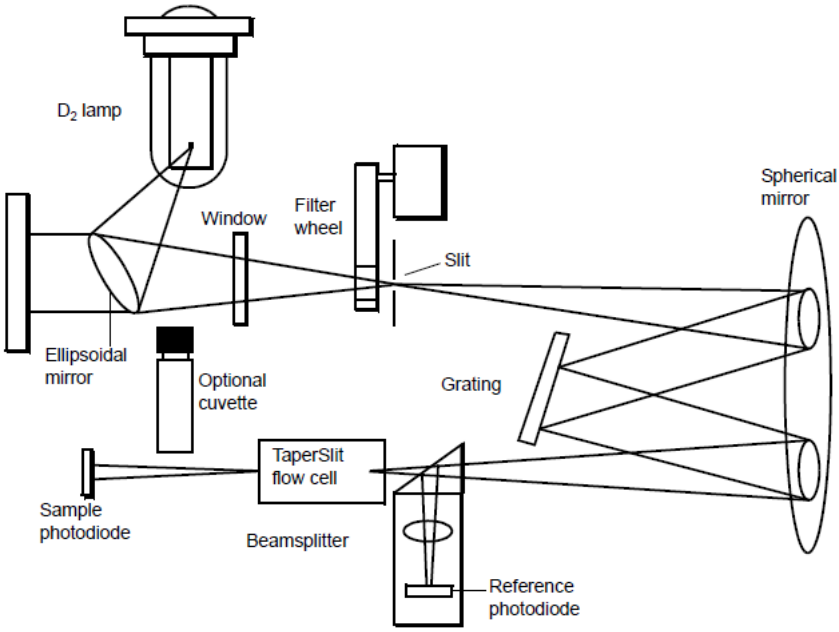
3.2.1.1 Detector optics

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

The TUV detector optics are based on a Fastie-Ebert monochromator and include these components as shown in the following diagram:

- High-brightness deuterium (D2) lamp
- Two mirrors: one off-axis, ellipsoidal mirror and one spherical mirror
- Filter wheel
- Shutter, wavelength calibration filter, and second-order filter
- Entrance slit
- Blazed, plane-holographic, diffraction grating
- Beamsplitter
- Sample and reference photodiodes
- Waters TaperSlit flow cell (its entrance is the exit slit of the monochromator)
- Optional cuvette cell

Figure 3–2: TUV detector optics assembly



3.2.1.1.1 Optics assembly light path

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

The detector employs an extremely efficient design for exceptionally high-light throughput. It operates as follows:

1. The ellipsoidal mirror collects light from the lamp and focuses it through the filter wheel and onto the entrance slit. The spherical mirror directs light toward the grating. A different portion of the spherical 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.

   Note: With the optional cuvette cell, light exits the flow cell and passes through the cuvette location to the sample photodiode.

2. The beamsplitter, 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 detector's front panel (or through Empower software), the detector rotates the grating to the appropriate position.

4. The preamplifier board integrates and digitizes the currents from the photodiodes for processing by the signal processing electronics and output to a computer or integrator.

3.2.1.1.2 Filtering noise

The detector provides a Hamming filter to minimize noise. The 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. You can program a filter time to be Fast, Slow, Normal, or Other. If you select Fast, Slow, or Normal, you do not need to specify a value. The filter constant is determined by the sampling rate. If you select Other, you can specify a value but it will be rounded up or down to a value based on the sampling rate. Selecting Off or Other 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.

Decreasing the time constant settings produces 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

Increasing the time constant settings produces these effects:

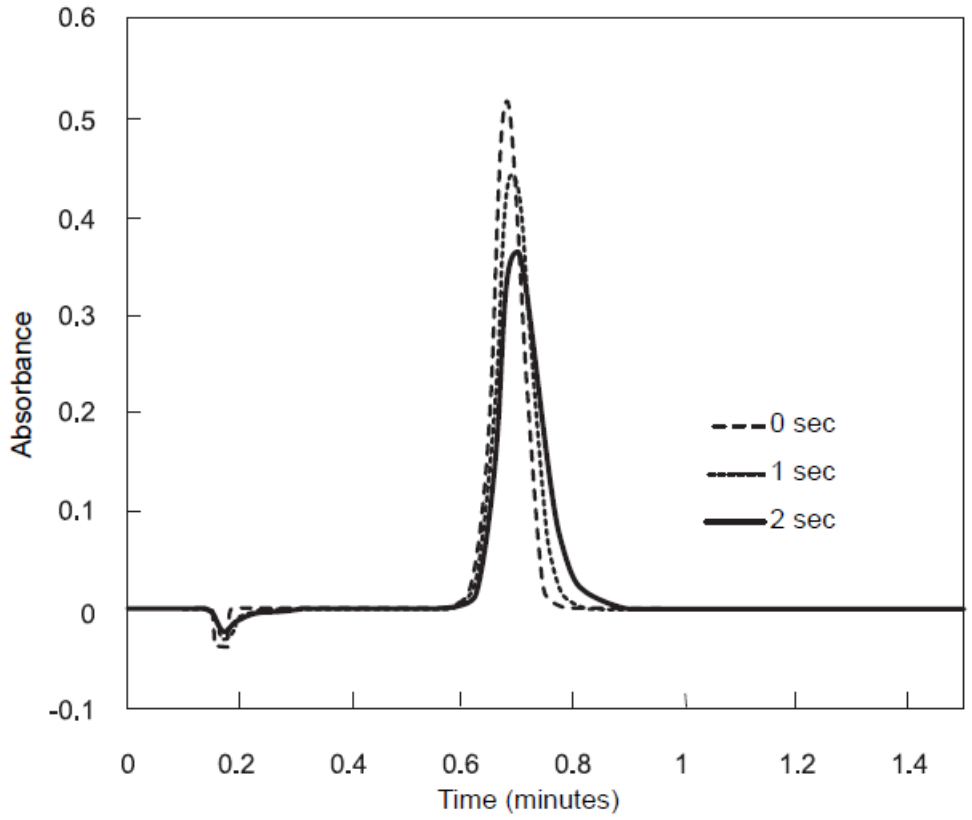
- Greatly decreased baseline noise
- Shortened and broadened peaks

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 Wavelength verification and test

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.

If you run the detector continuously, Waters recommends that you perform wavelength verification weekly by cycling power to the detector.

3.2.1.3 Operational modes

Detector capabilities include two primary modes of operation.

The detector operates in Single- or Dual-Wavelength mode, allows spectrum scanning using a flow cell or optional cuvette, and provides various analog output functions: Absorbance, Difference Plot, RatioPlot, and MaxPlot.

See: The Empower online Help for additional 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. While the detector is operating in this mode, you can configure the analog outputs for channel B, so you can use channel B to obtain additional information about the wavelength selected on channel A.

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

You can configure several additional parameters when using the detector in this mode.

3.2.1.3.1.1 Primary parameters

The primary parameters applicable to operating the detector in Single-Wavelength mode are described in the following table.

Table 3–2: Single-Wavelength mode, primary parameters

Parameter	Description
Wavelength (nm)	Specifies a wavelength for channel A, from 190 nm to 700 nm, settable in 1-nm increments
Sensitivity (AU)	Specifies the scaling factor for the analog-output channels and corresponds to the AU value where the analog outputs become saturated at full-scale values. AUFS vary, from 0.0001 to 4.000 AUFS.

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

Parameter	Description
	Tip: Changing the sensitivity setting affects the 2-V output.
Chart polarity (+ or –)	Reverses the polarity of the charted chromatogram. Select **+** for a normal chromatogram, or select **–** for an inverted chromatogram. This function changes the direction of the plot on the 2-V output.
Filter time constant (s)	Programs a filter time. Options are Fast, Slow, Normal, and Other (see "Filtering noise" for detailed information about this parameter, these setting options, and the different effects produced).
Analog rate	Specifies a frequency value as high as 80 Hz.

3.2.1.3.1.2 Secondary parameters

While in Single-Wavelength mode, you can access several pages of these secondary, or less frequently specified, parameters by pressing Next on the Absorbance (or Home) page:

- Absorbance offset (in mV)
- Autozero on inject
- Autozero on λ changes

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. The sampling frequency is reduced to 1 or 2 Hz, limiting the use of this mode to more standard chromatography where peak widths span at least 20 seconds to enable full characterization of a peak.

By running the detector in this mode, you can use the RatioPlot or MaxPlot functions to obtain additional information about an analyte. You can select any two wavelengths from 190 nm to 700 nm.

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 a warning message that any data collected for the wavelength

above 370 nm can contain inaccuracies because of possible UV light interference (second-order effects).

3.2.1.3.2.1 Chart-out function options

When operating in Dual-Wavelength mode, the detector offers additional function options for analog outputs to those offered in Single-Wavelength mode. The default operating function for Dual-Wavelength mode is Absorbance.

Table 3–3: Dual-Wavelength mode, additional functions for analog output

Function option	Description
Absorbance (A and B)	Standard LC operating function in which the current absorption is scaled and sent directly out the analog output. The scaling depends on the AU setting and the absorbance offset. The absorbance value is scaled for the 2-V analog output. If a setting of 1 AU/V is desired, you can set an AU of 2.0000 for either the A or B output channels that can be controlled independently, even in Single-Wavelength mode.
Difference Plot (A-B)	Plots the arithmetic difference in absorbance for the two monitored wavelengths
RatioPlot (A/B)	Produces the ratio of absorbance from two wavelengths. Theoretically, the ratio is constant for a pure chromatographic peak and variable for an impure peak, which results in a nonsquared response. Instead of a programmable AU, the detector provides minimum and maximum ratio values that scale the ratio plot proportionally. In addition, a configurable minimum absorbance threshold activates ratio output scaling only when it reaches the absorbance at both wavelengths.
MaxPlot	Results in the output of the larger of the two absorbance values scaled to the selected AU setting. Use this function when observing, with one data channel, multiple compounds that exhibit absorbances at two separate wavelengths.

3.2.1.3.2.1.1 RatioPlot function

The detector allows ratio plotting: comparing the absorbances of a compound or analyte at two wavelengths. The detector's RatioPlot function divides absorbances at two selected wavelengths

and plots the resulting ratio on a data system over one output channel (channel A). Use this function when detecting hidden components within individual peaks.

The RatioPlot of a spectrally homogeneous peak appears as a rectangular wave. The RatioPlot of an impure peak appears as a distorted wave.

To obtain a RatioPlot, you must operate the detector in Dual-Wavelength mode. The RatioPlot is output on the selected channel.

3.2.1.3.2.1.2 MaxPlot function

The detector's MaxPlot function monitors absorbance at two selected wavelengths and plots the maximum absorbance value for each sample component.

To obtain a MaxPlot, you must operate the detector in Dual-Wavelength mode. The MaxPlot, the greater of the two absorbance values, is output on the selected channel.

3.2.1.4 Spectrum scanning

When the detector is operating under the control of the CDS, the scanning function is disabled. To acquire spectra from either the flow cell or the cuvette, you can use the detector as a spectrophotometer when required.

When the detector is operating under the control of the CDS, the scanning function is disabled.

To acquire spectra from either the flow cell or the cuvette, you can use the detector as a spectrophotometer when required. The major difference between the detector and a double-beam spectrophotometer is that the detector uses only one flow cell or cuvette when required, rather than a sample and a reference pair. You can scan and store as many as three spectra (three reference or zero scans, or three sample scans) for playback or to compare with other spectra.

Recommendation: When using the optional cuvette cell, use a matched pair of cuvettes for the zero and sample scans.

The detector obtains an absorbance spectrum by performing two types of scans on the flow cell or using the cuvette:

- Zero scan — Characterizes the baseline absorbance spectrum of a solvent
- Sample scan — Subtracts the zero scan, so the results displayed or charted are of the sample only

To obtain a spectrum of a sample using the detector, run a zero scan first, followed by a sample scan. Typically, you run the zero scan using pure solvent. The sample scan is a scan of the analyte dissolved in that solvent.

Spectra can be simultaneously charted on the channel A output or acquired and stored in memory for later playback.

3.2.1.5 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 cell.

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

To generate and store a spectrum:

Note: Since the cuvette scan is acquired by measuring the absorbance from a light path that includes both the flow cell and the cuvette, the solvent conditions in the flow cell must be identical for both scans.

1. Acquire a zero scan, which measures the absorbance of the contents of the cuvette and flow cell over the desired wavelength range.
2. Acquire a sample (absorbance) scan, which measures the absorbance of the analyte dissolved in mobile phase.

The detector subtracts the zero scan from the sample scan to create a sample spectrum.

3.2.1.6 Thermal wander management

The detector's insulation, fans, and baffling are designed to mitigate thermal instability caused by ambient temperature changes.

3.2.1.7 Active thermal control

3.2.2 Pump features

The QSM is a low-pressure mixing, high-pressure pump that can process four degassed solvents simultaneously using a GPV to dynamically create a specified composition. The GPV produces predictable gradient segments regardless of solvent compressibility and system back pressure. Solvent selection and proportioning occur on the low-pressure (intake) side of the solvent delivery system, and solvents continue to blend under high pressure in each piston chamber.

Additional features include:

- An integrated degasser that operates at all flow rates, including the maximum flow rate, with an independent channel for each solvent.
- An automatic, programmable seal wash. The seal wash pump prevents the buildup of precipitates on the pump plungers by washing them with seal wash solvent at programmable intervals.
- A vent valve that automatically switches to waste for priming and rapid solvent changeover.

3.2.2.1 Pressure flow envelope

The QSM provides steady (pulse-free) solvent flow at analytical flow rates of up to 5 mL/min at 10,000 psi and linear change to 4000 psi at 10 mL/min.

3.2.2.2 Pressure transducer overview

The APT, which measures the pressure on the low-pressure side of the degasser system, is unaffected by altitude or barometric changes.

Table 3–4: APT specifications

Item	Specification
Units displayed	kPa, bar, psia
Sign of displayed unit	Positive
Theoretical maximum vacuum	0.0 psia
Operating range	0.00 to 1.54 psia
Typical value	0.70 to 1.20 psia

3.2.3 Sample manager features

The needle is part of the high-pressure sample flow path in the sample manager's flow-through-needle mechanism.

You can submit samples for analysis in the Alliance iS System by loading microtiter plates or vials onto the rotary sample tray of the SM-FTN. The sample manager then uses a direct-injection mechanism to inject samples drawn from plates and vials onto a chromatographic column.

Optional extension loops, installed between the sample needle and the injection valve, can increase the injection volume beyond that of the sample needle.

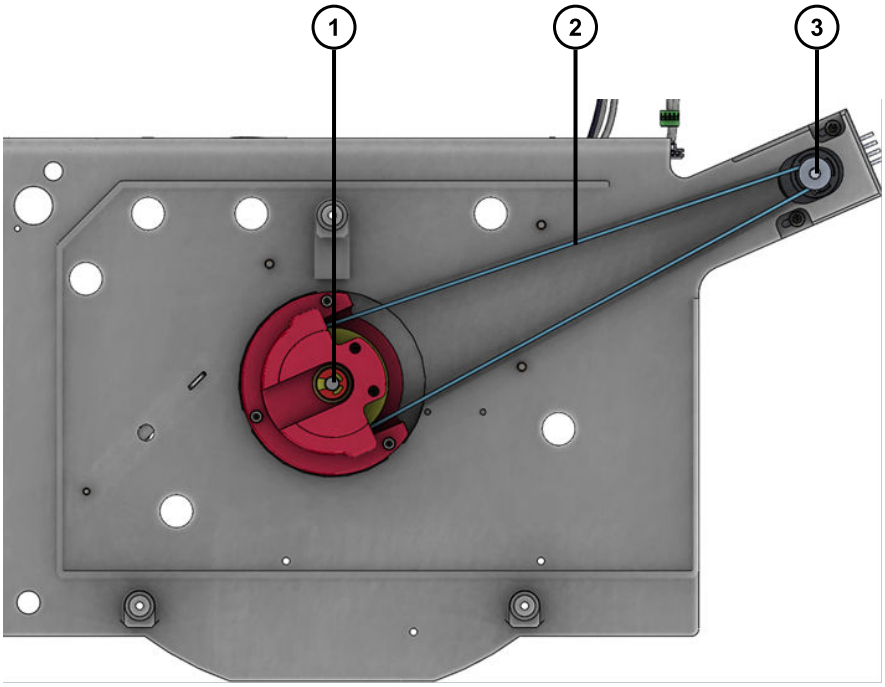
Note: The 100-µL extension loop is included in the standard configuration.

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.

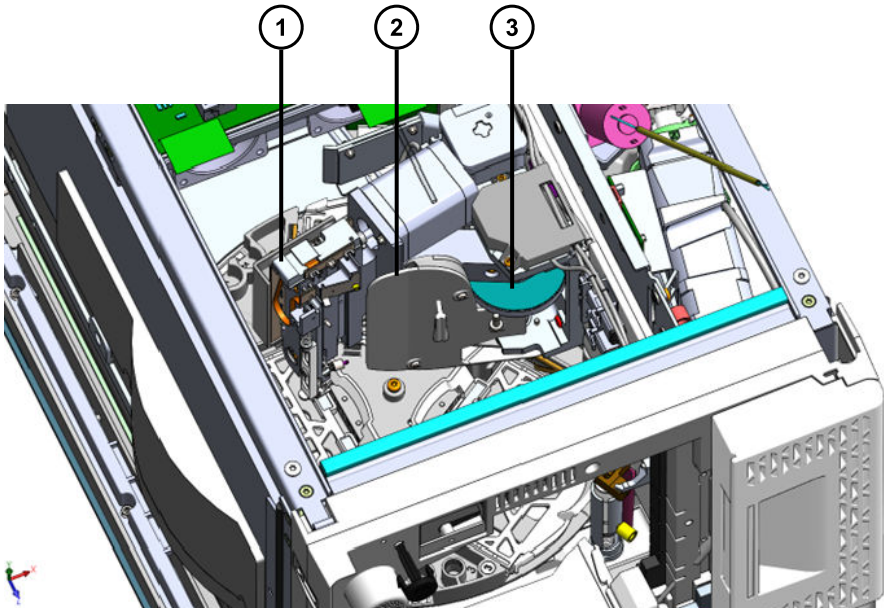
To line up the needle with the vials in the wells of the sample plates inside the sample compartment, the needle positioning mechanism’s two rotary axes control the orientation of the sample plates and the relative position of the sample needle carriage. Both rotary axes rotate a shaft using a belt and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

Figure 3–4: Needle positioning mechanism's dual-rotary axes, located below the sample compartment



1. Platter rotary axis
2. Belt
3. Motor axis

Figure 3–5: Needle carriage's rotary axis, located inside the sample compartment



1 Needle carriage

2 Needle cartridge

3 Needle carriage rotary axis

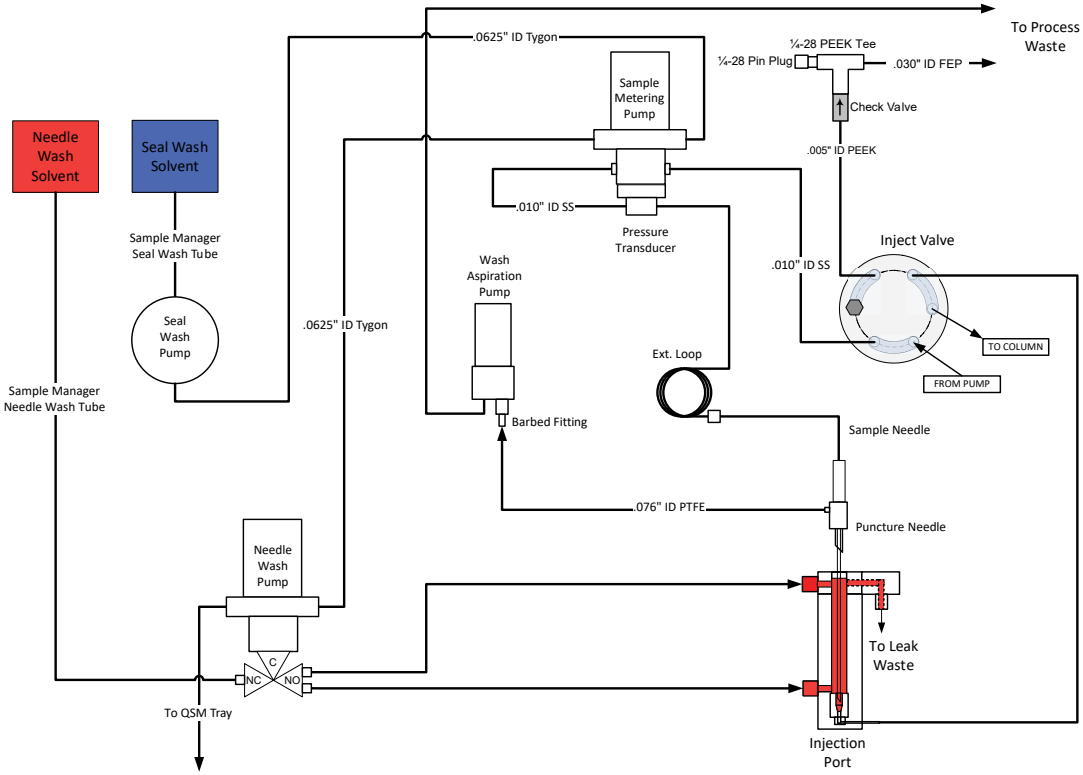
3.2.3.2 Injection system

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 3–6: SM-FTN flow path for single-draw aspiration and injection



3.2.3.3 Injection mechanics

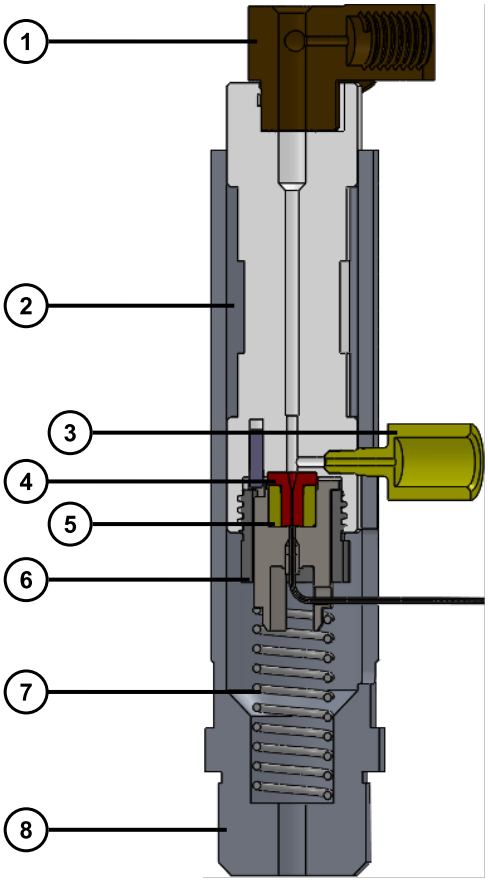
The sample needle goes through a specific sequence for each injection.

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.

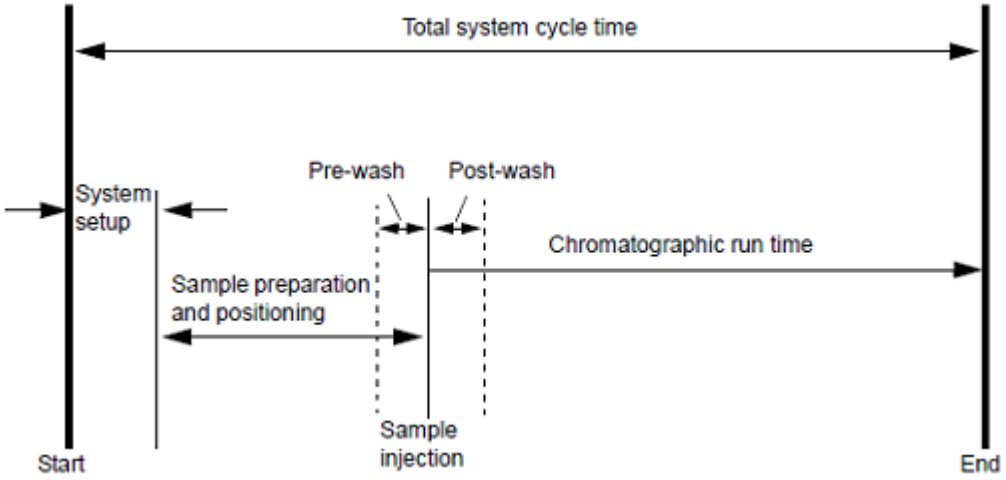
Figure 3–7: Needle seat assembly, cross view



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 3–8: 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 Column heater/cooler features

The CHC is the standard column heating/cooling module that 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 between 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 configurations include:

- Column length: 300 mm
- Column ID: 7.8 mm (maximum)
- Column guard or column in-line filter: 30 mm (maximum)

3.2.4.1 Column heater/cooler operation

The CHC module is 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 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.

3.2.4.2 Column configuration

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.

Note: Columns are not included as part of your system, but are available for purchase on the Waters website (www.waters.com).

3.2.4.3 eConnect technology

Waters' new eConnect technology is a standard feature of the CHC that can be utilized by purchasing eConnect-enabled versions of Waters chromatographic columns. These columns are fitted with eConnect tags during manufacture.

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 touch screen, and log that data for each injection.

3.2.5 Touchscreen features

Alliance iS's touchscreen allows the user to simply perform many tasks at the kiosk. It also saves time by minimizing the effort of moving back and forth between the instrument and the Empower system. The navigation panel on the left side of the touchscreen provides buttons for accessing views for performing specific tasks. The following table lists the views.

Note: Access to touchscreen views and features is permission-dependent.

Table 3–5: Touchscreen views/buttons

View	Description
Home (Page 48)	Displays real-time status information.
Setup (Page 48)	Prepares the system to start up or shut down. Manages solvents.
Plots (Page 50)	Displays available plots.
Maintain (Page 49)	Provides component replacement and calibration procedures.
Health (Page 50)	Provides procedures for troubleshooting, resolving, and reporting issues.
System (Page 51)	Provides actions for configuring the system, creating or reviewing logs, performing preventative maintenance, performing administrator tasks, removing or restoring power to the instrument, viewing performance counters, configuring leak sensors, and viewing the About screen.
Commands (Page 50)	Provides actions for turning off chromatography activity or power.

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

Table 3–6: Additional touchscreen controls

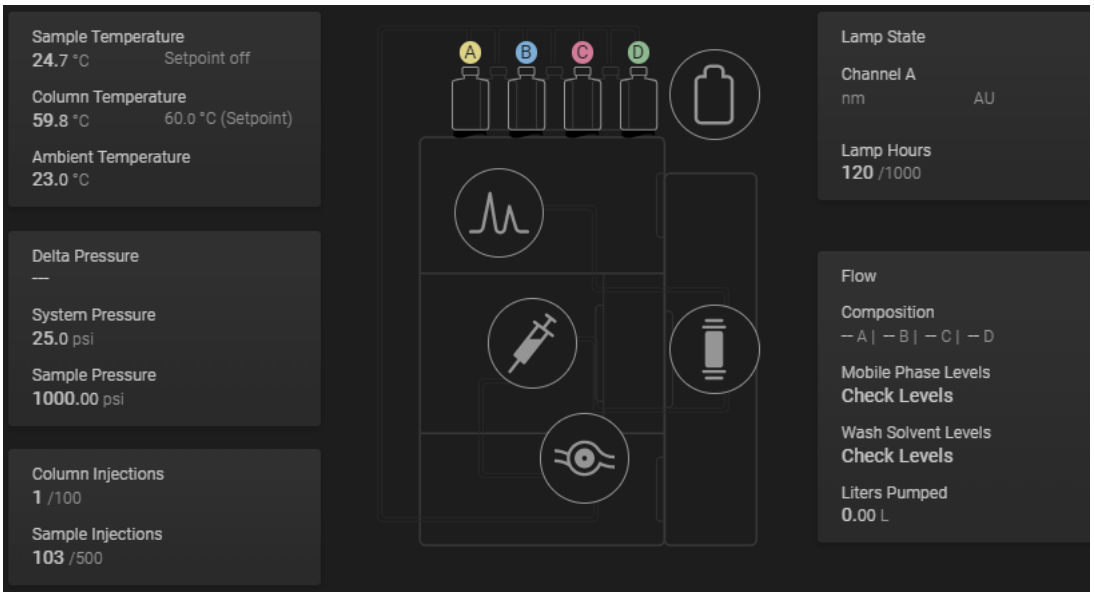
Control	Description
System status	One of: IDLE, RUNNING, or ERROR.
Preferences	Provides access to the following settings: Display and Themes, System Name, Lock Screen, Units and Numbers, Remote Access, and User Note.
Volume	Indicates whether system volume is on or muted.
Camera	If present, indicates that the system is controlled remotely by Empower.
Green checkmark	If present, saves the user selection.
< and >	If present, displays the previous or next screen in a task sequence.
Red X	If present, terminates the task in progress and displays the Home view.

3.2.5.1 Touchscreen Home view

The Home view mirrors the information provided by Empower's Alliance iS console by displaying the real-time activity and status of the instrument. The following figure shows the Home view.

Note: The Home view displays by default after you sign in to the kiosk. The initial information is from the previous session.

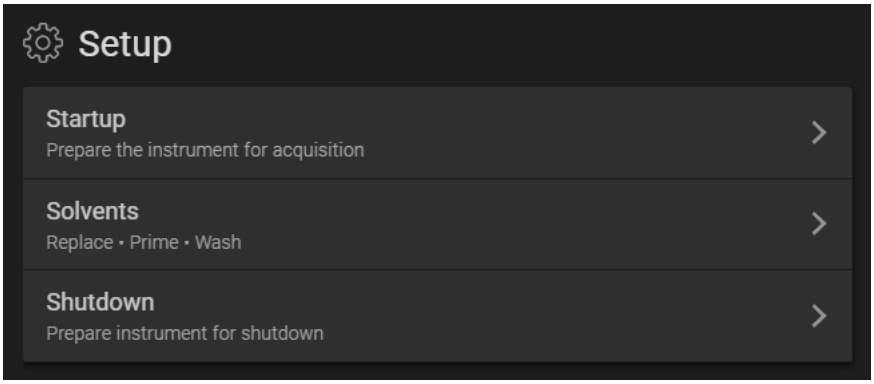
Figure 3–9: Home view



3.2.5.2 Touchscreen Setup view

The following figure shows the workflows provided by the Setup view.

Figure 3–10: Setup view (main)



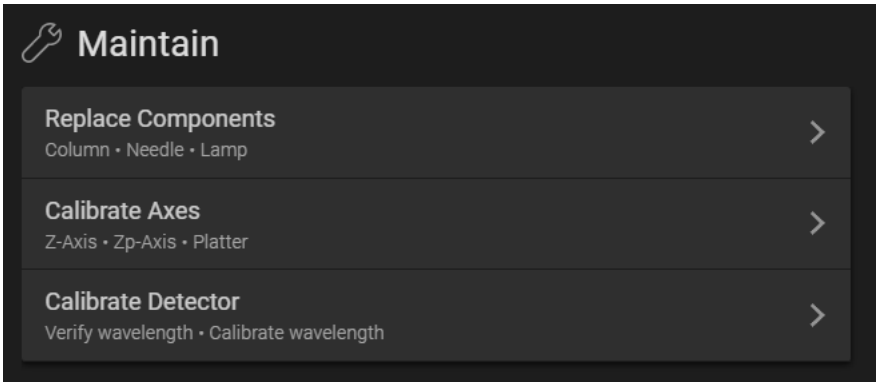
See:

- Priming a dry solvent manager via the touchscreen (Page 66)
- Equilibrating the Alliance iS system (Page 83)
- Preparing to shut down the Alliance iS system (Page 92)

3.2.5.3 Touchscreen Maintain view

The following figure shows the actions provided by the Maintain view.

Figure 3–11: Maintain view (main)



See:

- Moving the pump plunger backward (Page 136)
- Replacing the column (Page 192)
- Replacing the flow cell (Page 185)
- Replacing the lamp (Page 188)
- Replacing the needle (Page 159)
- Replacing the needle seal and return line (Page 169)

3.2.5.4 Touchscreen Plots view

Alliance iS produces data plots continuously for display on the touchscreen and by the console and Empower control panel. The following table describes the available plots.

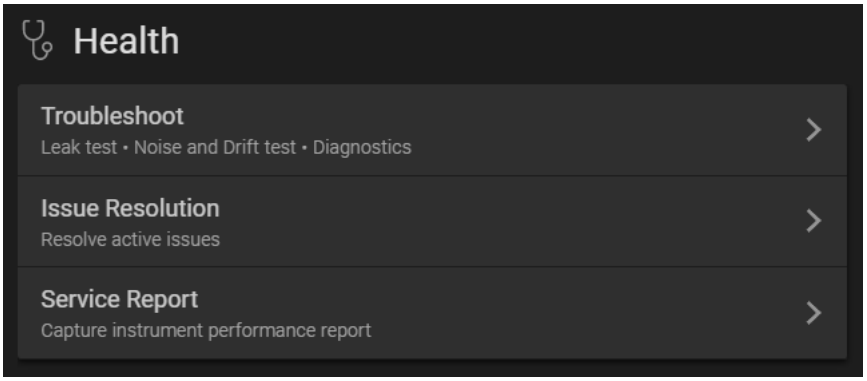
Table 3–7: Data plots produced by Alliance iS

Plot	Description
Autosampler diagnostics	Provides up to 96 hours of trending data for sample and ambient temperatures in ˚C and sample pressure in psi or a user-selected unit.
Column module diagnostic	Provides the column compartment temperature. Access is limited to Analysts, Managers, and Waters engineers.
Detector diagnostics	Provides absorbance channels A and B in AU. Access is limited to Analysts, Managers, and Waters engineers. **Note:** Channel B is available only when Dual mode is selected.
Pump diagnostics	Provides system, degasser, primary, and accumulator pressures in psi or a user-selected unit, solvents A, B, C, and D as percentages, flow as mL/min, and accumulator and primary leak rates in nL/min.

3.2.5.5 Touchscreen Health view

The following figure shows the actions provided by the Health view.

Figure 3–12: Health view (main)



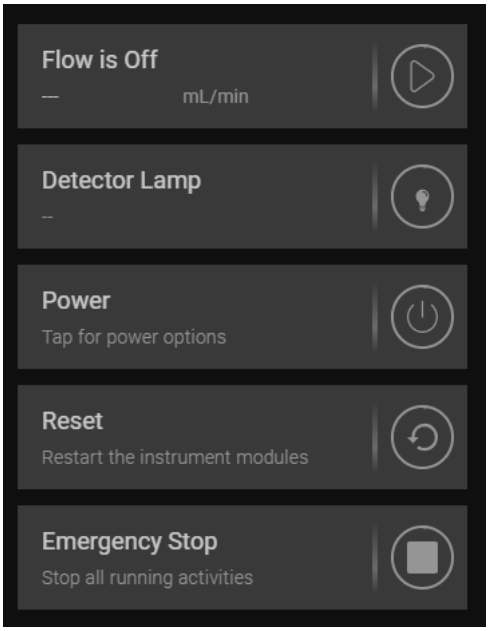
See:

- Running the needle seal readiness test (Page 204)
- Running the system leak test (Page 203)

3.2.5.6 Touchscreen Commands view

The following figure shows the actions provided by the Commands view.

Figure 3–13: Commands view



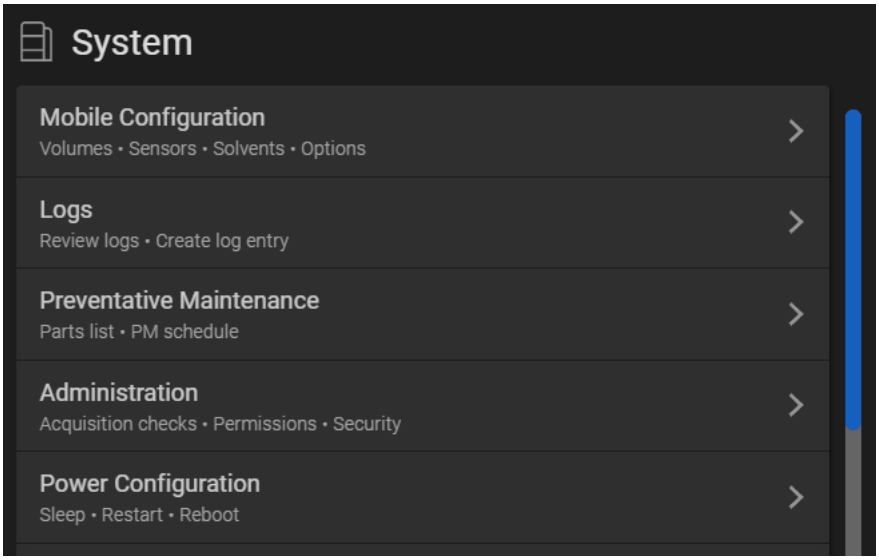
See:

- Equilibrating the Alliance iS system (Page 83)

3.2.5.7 Touchscreen System view

The following figure shows the actions provided by the System view.

Figure 3–14: System view (main)



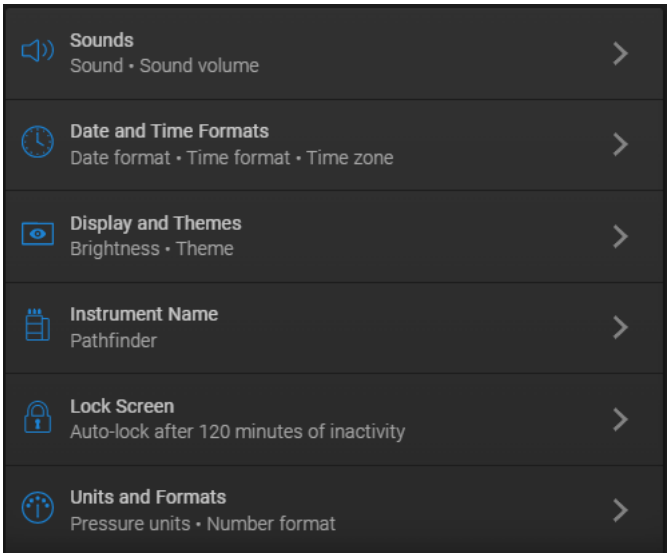
Note: Scroll down to select any of these options:

- Performance counters: Lamp hours, Volume pumped, Injection count
- Leak sensors: Configure installed leak sensors
- About: SW versions, HW versions, Product information

3.2.5.8 Touchscreen Preferences view

The following figure shows the actions provided by the Preferences view.

Figure 3–15: Touchscreen Preferences, main view



3.2.6 Empower features

The Empower CDS provides features that are specific to the Alliance iS system. For more information, see the following topics, Intended use of the Alliance iS HPLC system (Page 13), and the Empower online documentation.

3.2.6.1 Empower Alliance iS features

Empower provides the following features for the Alliance iS system:

- The System Audit Trail includes actions taken by Alliance iS.
- Alliance iS sends configuration information to Empower, which stores the information instead of eCord.
- Users can control the operation of the instrument.
- Users can request sample validation checks by Alliance iS prior to submission and execution. Resulting issues appear in the Message Center.

3.2.6.2 Alliance iS system console

The Alliance iS 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 48) at the Empower workstation.

See Monitoring from the Empower control panel (Page 90) and Monitoring from the Alliance iS system console (Page 90).

3.2.6.3 Intelligent Method Translator

The Intelligent Method Translator app (iMTA) converts non- Alliance iS methods into Alliance iS methods. The method translation process maps instrument method parameters stored in Empower projects to Alliance iS instrument settings. iMTA can translate methods that run on the following systems:

- Waters: Alliance HPLC, Arc HPLC, and ACQUITY Arc
- Agilent: Agilent1100 and Agilent1260

Waters' instrument modules whose parameters are mapped include Pump, Autosampler, Column Compartment, and Detector. Third-party mappings are specific to their instrument modules.

Translated projects are viewable in Empower's Method Editor, which is opened through the iMTA. For more information, see the online Help for the Intelligent Method Translator.

3.3 Auto Additions

The Auto Additions feature is designed to save time by setting up an injection with as many as up to ten different samples.

If you use Empower 3 to control the autosampler, you can use the Auto Additions function to make an injection composed of sample from as many as 10 vials, one of which is the sample vial. You can specify a delay time, to allow the sample to mix after sample from all vials is in the needle and extension loop.

Requirement: The total volume of an Auto Additions injection must be less than the sample loop volume.

See also: Empower 3 online Help for additional information about using the Auto additions function.

3.4 Selecting auto-dilution

Choose the auto-dilution option to dilute dissolved samples (containing no solids) using a solvent that the sample syringe delivers. You can specify an interval, to allow time for sample mixing.

To choose the dilution option:

1. In the instrument method editor, click the **ACQ-FTN** tab and then the **Dilution** tab.
2. Select the box to enable dilution.
3. Specify a needle height, purge-solvent volume, and a post-dilution delay interval.

4 Installation and configuration

This section helps you set up and configure your Waters system for use. Proper setup is critical to successful operation of the system.

4.1 Setting up the system

Proper setup is critical to correct operation and performance of the system.

To set up the system:

1. Prepare the site as specified in the Alliance iS HPLC System Site Preparation Guide.
2. Connect solvent bottles. See Setting up solvents (Page 81).
3. Connect all waste and exhaust connections. See Making waste connections (Page 58).
4. Confirm that the workstation meets the minimum requirements specified in the Empower 3.8.0 Installation, Configuration, and Upgrade Guide.
5. Install version 3.8.0 of Empower.
6. Install the performance test project as described in Performance optimization (Page 94).
7. Confirm proper communication between all system modules.
8. Confirm that no software error messages appear.

4.2 External connections

When the system is set up, you need to make additional external connections in order to operate it.

Make the following external connections when installing the system:

- Network connections (Page 56)
- Power connections (Page 56)
- Solvent bottles. See Setting up solvents (Page 81).
- Waste tubing connections. See Making waste connections (Page 58).

4.2.1 Network connections

A network cable must be connected to an external workstation in order to communicate with Empower.

Connect the network cable from the rear of the system to the acquisition client.

Figure 4–1: Connecting network cable

4.2.2 Power connections

A single cable provides power to the whole system.

Connect the power cable from the rear of the system to a suitable outlet.

Figure 4–2: Connecting power cable

Note: The power cables connecting the CHC and the detector to the rear of the Integrated Fluidics Module (IFM) assembly will be in place at the time the system is installed and must not be replaced.

4.2.3 Making solvent bottle connections

You will need to connect solvent bottles before the system can be used.

You will need to connect solvent bottles when installing the system. See Setting up solvents (Page 81).

4.2.4 Making waste connections

Proper waste connections ensure that drips and system waste are routed to approved waste collection containers.

To avoid solvent and sample spills, make waste connections from the appropriate system outlets to an approved waste container.

Figure 4–3: Connecting the system to a waste container

4.2.5 Installing the column

Install the column in the CHC prior to running samples.

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

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 and the inlet faces down.
5. Screw the tool-free 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 tool-free fitting.

Figure 4–4: Column seated in column clip

7. Close the column compartment door.

4.3 Powering-on the system

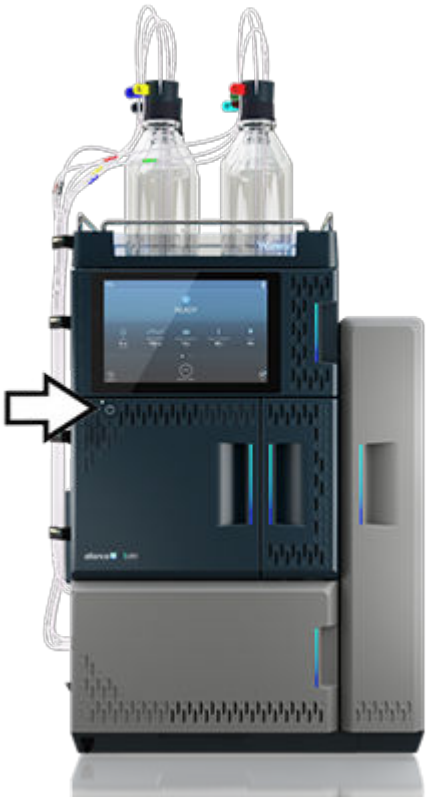
The Alliance iS system power button is located on the front door of the sample manager.

After the system is plugged in, the power button LED will blink.

To power-on the system:

Push the power button located on the front door of the sample manager.
The power button LED will light up solid and the system will power-on.

Figure 4–5: Alliance iS power button



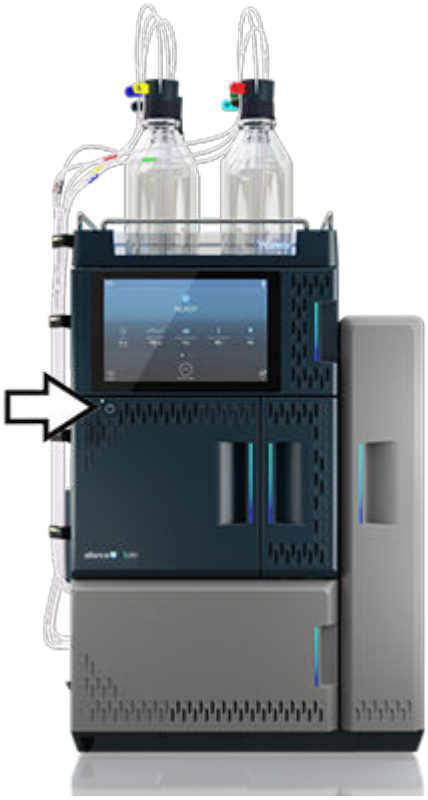
4.4 Powering-off the system

The Alliance iS system 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 4–6: Alliance iS power button



4.5 Opening the console from Empower software

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

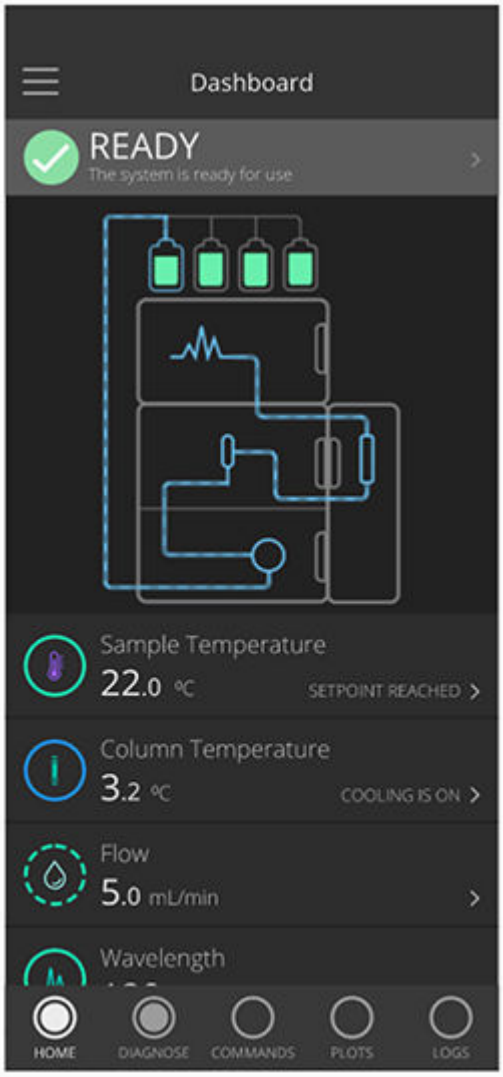
You can access the console from the Empower system status panel.

1. From Empower, click the arrow on the upper right-hand side of the system status panel.

Figure 4–7: Launching the system console

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

Figure 4–8: 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.

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.

Tip: In the console, you can select the **Setup** > **Startup** feature to prime all solvents, prime the needle wash and seal wash, and to 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:

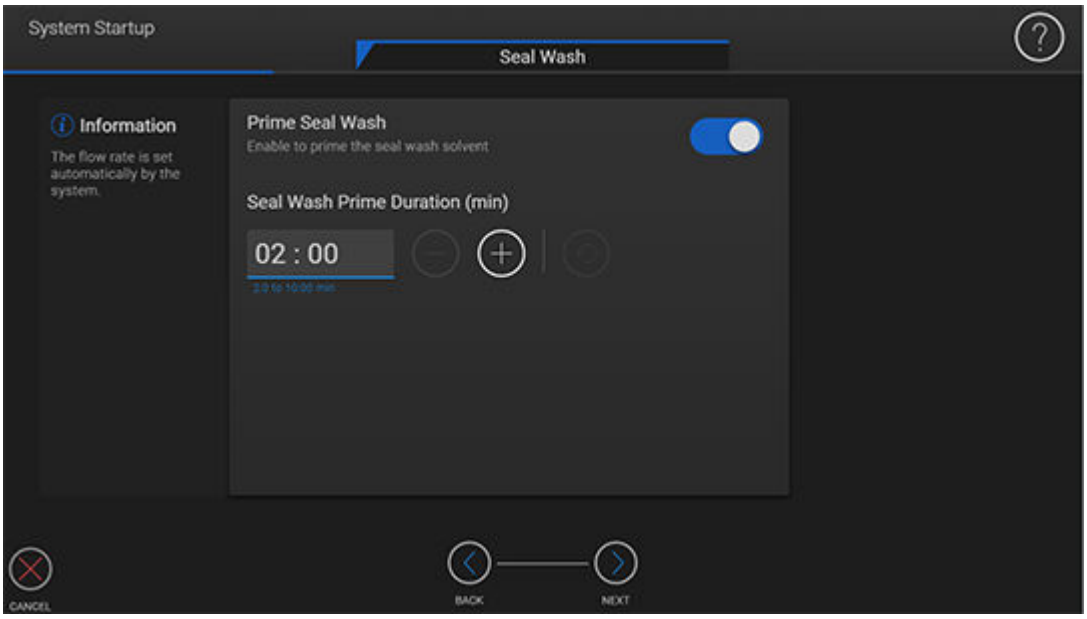
- Use seal wash that is fully soluble with all chromatographic solvents and that contains at least 10% organic solvent. This concentration prevents microbial growth and ensures that the seal wash can solubilize the mobile phase.
- Before priming the seal-wash system, ensure that the volume of seal wash is adequate for priming.

Required tools and materials

- Chemical-resistant, powder-free gloves
- Protective eyewear
- Clean 30-mL syringe (startup kit)
- 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 **Setup** > **Startup**. Follow the on-screen prompts to prime solvents and tap **Next**.
3. From the Seal Wash screen, tap the button to select Prime Seal Wash, and set the Seal Wash Prime Duration.



4. Follow the remainder of the on-screen prompts to finish the system startup process.

4.6.2 Priming the pump

After powering-on the system, prime the pump.

Priming prepares a new system for use and 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 without disturbing the column or sample manager with minimum back pressure, and directing flow to waste. The flow rate during priming is 10 mL/min.

During priming, the vent valve moves to the vent position, allowing flow without disturbing the column or sample manager with minimum back pressure, and directing flow to waste.

Tip: If you are priming a dry pump, using a syringe shortens the time required for priming.

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 gradient proportioning valve function properly.

4.6.2.1 Priming a dry pump via the touchscreen

You can prime a dry pump via the touchscreen.

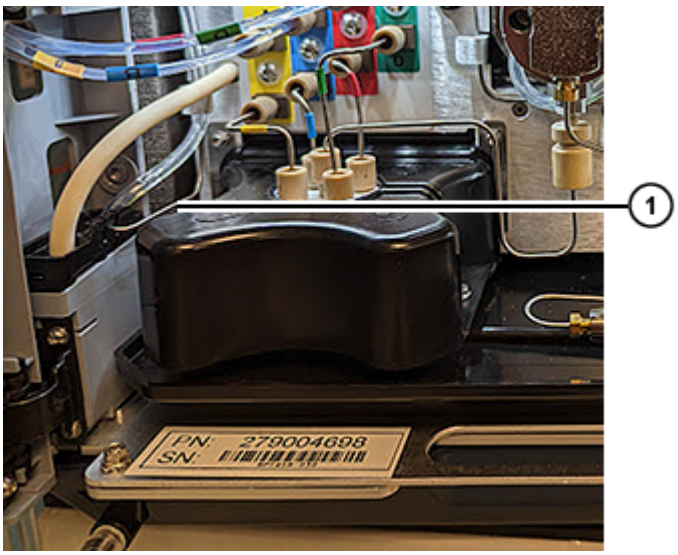
You can prime the pump via the touchscreen.

To prime a dry pump via the touchscreen:

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

   Open the pump’s front door.
2. Locate the .062 inch vent valve solvent waste line located on the left-hand side of the pump bay, nested in the left bezel. Leave the .062 inch vent valve solvent waste line submerged into the process waste for now.

Figure 4–9: Location of solvent vent tubing



1 Solvent vent tubing

3. On the touchscreen, tap **Setup** > **Startup** > **NEXT**.
4. From the Prime Solvents screen, select solvent line **A**, **B**, **C**, or **D**.
5. In the Priming Duration box, specify the number of minutes.

   Default: 2.0 minutes

   Recommendation: Prime the pump until a steady flow exits the vent tubing (typically four to seven minutes per solvent).
6. Follow the remainder of the on-screen prompts to finish the system startup process.
7. While the priming is occurring, lift the .062 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 the of 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. If there is still air in the tubing, you might want to prime with a syringe. See #unique_112.

   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

Prime the sample manager before use if it is new or has been sitting idle for more than 24 hours.

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.

To prime the sample syringe and wash solvent:

1. On the touchscreen, tap **Setup** > **Startup** > **NEXT** > **NEXT** > **NEXT**.
2. From the Needle Wash screen, tap the button to select Prime Needle Wash, and set the Needle Wash Prime Duration. Click **NEXT**.
3. From the Sample Metering Pump screen, tap the button to select Prime Sample Metering Pump.
4. Follow the remainder of the on-screen prompts to finish the system startup process.
5. **Table 4–1: Priming parameter values**

Parameter	Wash solvent	Purge solvent
Priming range	1 to 600 seconds	1 to 100 cycles
Default priming	15 seconds	5 cycles
Recommended priming: dry inlet tube	180 seconds	100-µL syringe: 60 cycles 250-µL syringe: 24 cycles 500-µL syringe: 12 cycles
Recommended priming: changing solvents	180 seconds	100-µL syringe: 50 cycles 250-µL syringe: 20 cycles 500-µL syringe: 10 cycles

Note: Each priming cycle requires approximately TBD seconds.

When the reported system status is “Idle", priming is finished.

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 injection valve port.

Note: Any injection greater than 80 µL requires the multidraw valve option.

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

Loop size
50 µL
100 µL - Standard
250 µL
1000 µL
2000 µL

Recommendations:

- The recommended maximum injection volume is 75% of the total volume of needle and sample loop.
- Changing the extension loop status (installing a loop, replacing the loop, or removing the loop) can result in increased carryover. To prevent carryover, install a new needle whenever you install, change, or remove the extension loop. See Reducing carryover.

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

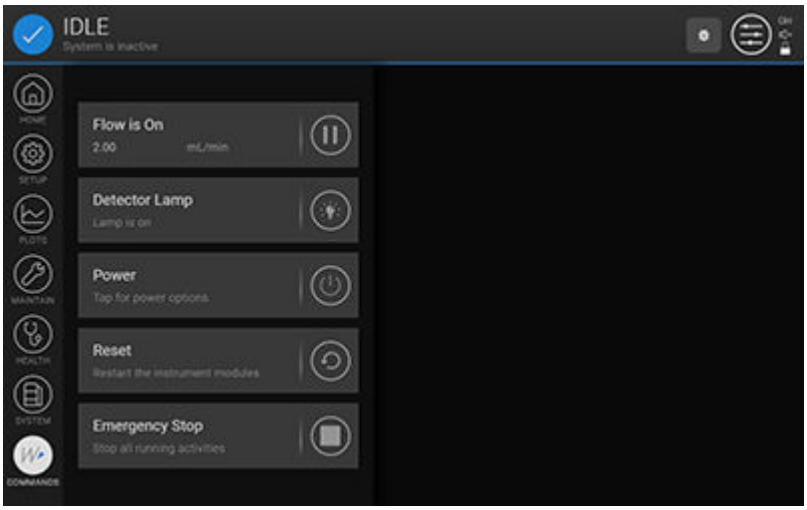
- Extension loop kit
- Chemical-resistant, powder-free gloves
- Protective eyewear

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.

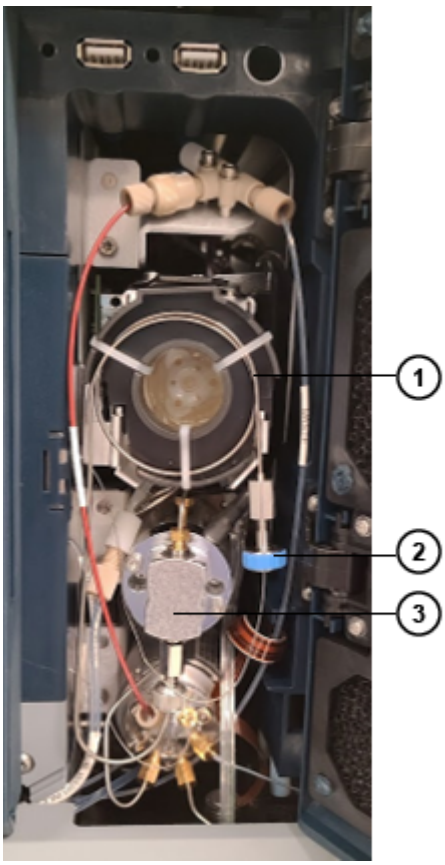
1. If system flow is running, stop the flow. On the touchscreen, tap **COMMANDS**, and then tap the pause button next to **Flow is On**.

   Figure 4–10: COMMANDS screen

2. Open the sample manager fluidics door.
3. Disconnect the tool-free fitting that is attached to the extension loop and to the sample needle.

Figure 4–11: Single-valve system



1. Extension loop with holder
2. Sample needle tool-free fitting
3. Pressure transducer

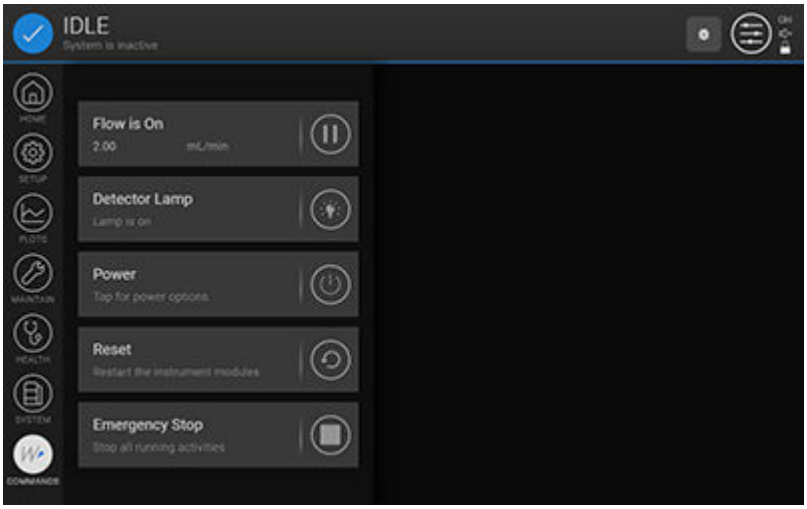
4. Install a different sized extension loop between the TFF adapter fitting and the sample needle.
5. See Modifying needle and extension loop configuration parameters (Page 73), for reporting the correct extension loop volume configuration in the touchscreen.
6. On the touchscreen, tap **Setup > Startup** and follow the remaining on-screen prompts to prime and prepare the system for use.

4.8.2 Installing an extension loop in a two-valve system

In a two-valve system, the extension loop is installed between the multidraw valve and the sample needle.

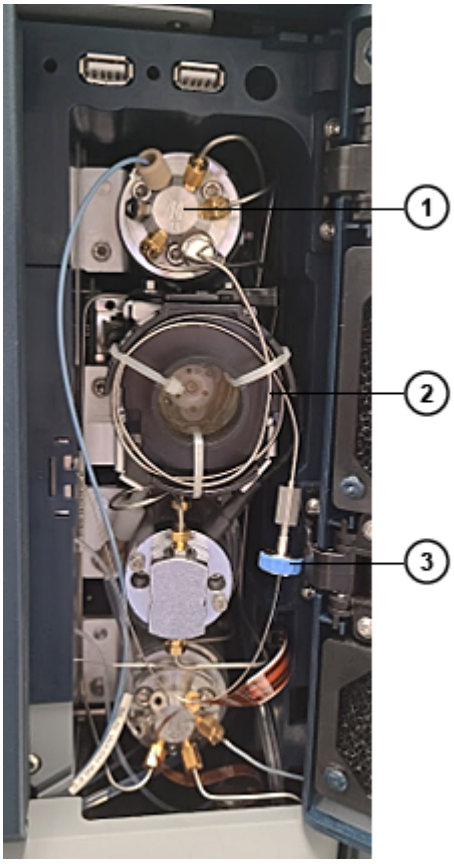
1. If system flow is running, stop the flow. On the touchscreen, tap **COMMANDS**, and then tap the pause button next to **Flow is On**.

Figure 4–12: COMMANDS screen



2. Open the sample manager fluidics door.
3. Disconnect the tool-free fitting that is attached to extension loop and to sample needle.

Figure 4–13: Multidraw valve system



1 Multidraw valve

(2) Extension loop with holder

(3) Sample needle tool-free fitting

4. Using the tool-free fittings, install the extension loop between the multidraw valve and the sample needle.
5. See Modifying needle and extension loop configuration parameters (Page 73), for reporting the correct extension loop volume configuration in the touchscreen.
6. On the touchscreen, tap **Setup > Startup** and follow the remaining 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 need to be properly configured in the touchscreen in order to avoid errors and/or system performance issues.

To modify the needle or extension-loop volume setting:

1. On the touchscreen, tap **System** > **Mobile configuration**, and then tap the sample manager button  .
2. Tap the **Extension Loop Installed** button, and then tap **50** or **100** as applicable. Tap **DONE**.

4.10 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.

The optimal draw rate for the syringe plunger depends on the volume and viscosity of the sample and the specified cycle time. The default draw rate for the 48-µL needle is 100 µL/min.

You can also specify the draw rate, in microliters per minutes, if desired.

Table 4–3: Maximum syringe draw rates

Solvent type	48-µL needle
50:50 acetonitrile/water	TBD µL/min
100% water	TBD µL/min
100% acetonitrile	TBD µL/min

Table 4–3: Maximum syringe draw rates (continued)

Solvent type	48-µL needle
100% dimethyl sulfoxide (DMSO)	TBD µL/min

Increasing the syringe draw rate reduces the time required to aspirate sample. Note that increasing the draw rate too much results in poor area and height reproducibility. When increasing the draw rate, be sure to confirm the continued acceptability of the method.

4.11 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.

See also: For information about calibrating the needle, see Calibrating the needle.

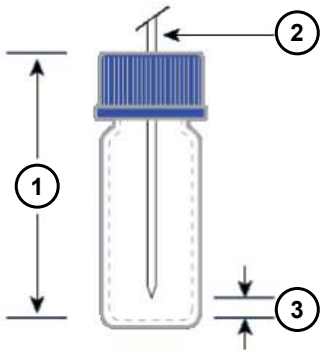
You can change the default needle-placement setting in the software in two places: on the **Dilution** tab of the Sample Manager instrument method editor and in the Advanced Settings dialog box.

Table 4–4: 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 (3) in the figure below.

Figure 4–14: Sample needle in vial



1 Vial depth

2 Sample needle

3 Distance from tip of sample needle to bottom of sample vial

4.12 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. The system will probe the bottom of the vial and then back off slightly so that the needle is very close to the bottom.

See also: For information about enabling the vial bottom sensing feature, see Setting up a method (Page 103).

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

4.13 Creating a new plate type

If the plate type is not set correctly in Empower, bent needles can result.

1. In Empower software, open the Configure System window.
2. From the Empower Configuration tree, select **Plate Types**.
3. From the menu, select **File** > **New** > **Plate Type**.
4. Under Plate Type, type a suitable name for the new plate type.

5. Type the dimensions of the new plate type.
6. Click **OK**.

   See also: The console online Help for additional information about plate type properties and dimensions.

4.13.1 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 to creating an all new plate type.

1. In Empower software, open the Configure System window.
2. From the Empower Configuration tree, select **Plate Types**.
3. Select the existing plate type you want to use as the template for the new plate type.
4. From the menu, select **File** > **Properties**.
5. Under Plate Type, type a suitable name for the new plate type.
6. Change the dimensions to fit the new plate type.
7. Click **OK**.

   Result: A copy of the plate definition is created with a new name and revised dimensions.

   Tip: The ANSI-48Tube0.65mLHolder plate type is defined in Empower software so that the needle does not use sample near the bottom of the vial, greatly increasing the residual volume. To reduce the residual volume, change the depth parameter to 28.5.

4.14 Loop-offline option

The loop-offline option specifies the amount of time the sample is flushed from the sample loop before the next injection.

Loop offline is the time at which the injection valve moves from the inject position to the load position during a run. This can occur during the run to remove dwell volume or to prepare for a load-ahead operation. Take the loop offline before the first gradient change reaches the injection valve, or after the gradient returns to the initial conditions. You can also specify a time.

Note: Dwell volume is significant only in systems that use extension loops larger than 50 µL.

1.
2. When it is enabled, you can either specify a time in minutes or select **Automatic**.

   Notes:

- The **Automatic** setting causes the Sample Manager to wait for three times the volume of the needle and loop combined to flow before the injection valve position is changed to Load, to ensure that the sample has flowed completely out of the needle and loop.
- If the time specified exceeds the runtime, the injection valve will not be moved before the start of the next injection.

4.15 Determining when to take the needle and extension loop offline

In order to flush the sample from the loop, it needs to be taken offline.

When the needle and extension loop are eliminated from the flow path, they contain the mobile phase composition. Solutes that deposit in the needle and extension loop because of poor solubility are not transferred to the column until the gradient composition dissolves the sample and flushes it onto the column. At that point, the solute's high-retention factor (k') causes it to elute from the column in one column volume. Choosing the correct time to take the needle and extension loop offline ensures that all of the sample is flushed from the loop.

If you are taking the needle and extension loop offline before the first gradient change, ensure that you first completely flush the sample onto the column. To do so, use solvent at the initial gradient composition. The volume of solvent must be at least five times the injection volume.

Apply this formula to calculate the extension time:

$$\text{Loop offline time (min)} = \frac{\text{Sample volume (µL)}}{\text{Flow rate (µL/min)}} \times 5$$

Example: For a flow rate of 500 µL per minute, with a 20-µl loop installed, the loop offline time should be at least 0.20 minutes.

$$\text{Loop offline time (min)} = \frac{20\,(\mu L)}{500(\mu L/min)} \times 5$$

$$\text{Loop offline time (min)} = .04 \times 5$$

$$\text{Loop offline time (min)} = 0.20\ \text{min}$$

If you are taking the needle and optional extension loop offline after the end of the gradient, ensure that they are completely filled with solvent of the initial gradient composition before taking them offline.

In addition, observe these considerations when determining the correct needle and loop offline time:

- If the wash solvent time is longer than the loop offline time, the needle is not taken offline until the needle wash is complete.
- The needle and extension-loop offline time must not occur if their contents are of a higher concentration than the initial gradient conditions.

Tip: A programmed gradient typically flows through all parts of the instrument that contact the sample. If you initiate the needle and extension-loop offline option before the gradient reaches its final conditions, the highly organic portion of the gradient does not pass through the needle. As a result, the gradient can fail to remove all sample from the needle, resulting in low sample recovery and an increased risk of carryover.

4.16 Choosing the loop-offline option

Proper use of the loop-offline option help minimize cycle times and prevent carryover during a series of injections.

To choose the loop-ofline option:

1. In the instrument method editor, click the **ACQ-FTN** tab, and then click the **General** tab.
2. Select the check boxes for the loop-offline option.
3. Specify an interval for the loop-offline option.

   Tip: A programmed gradient typically flows through all parts of the instrument that contact the sample. If you initiate the needle and extension-loop offline option before the gradient reaches its final conditions, the highly organic portion of the gradient does not pass through the needle. As a result, the gradient may fail to remove all sample from the needle, resulting in low sample recovery and an increased risk of carryover.

5 Method management

The Alliance iS system supports modifying methods through Empower's Method Editor and transferring methods developed for other Waters systems and some third-party systems through the Intelligent Method Translator (iMTA).

5.1 Measuring dwell volume

When transferring a gradient LC method, you must calculate the dwell volume of the two systems. The dwell volume is the system volume between the point where the gradient is formed and the column inlet. The dwell volume varies from system to system but is typically between 0.1 and 2.0 mL.

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 method being transferred, replacing the column (Page 192) with a low-volume restrictor, to ensure proper pump function.

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

5.2 Transferring methods

Alliance iS can execute LC methods that were developed for other systems. The column type (diameter) specified for a transferred method cannot be changed. Even with the same column type, there may be some variability in the chromatographic results. For example, if there is a significant difference in retention time, the dwell volume can be compensated. 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.

See Intelligent Method Translator (iMTA) (Page 53).

6 Daily routine analysis

6.1 Starting the hardware and software

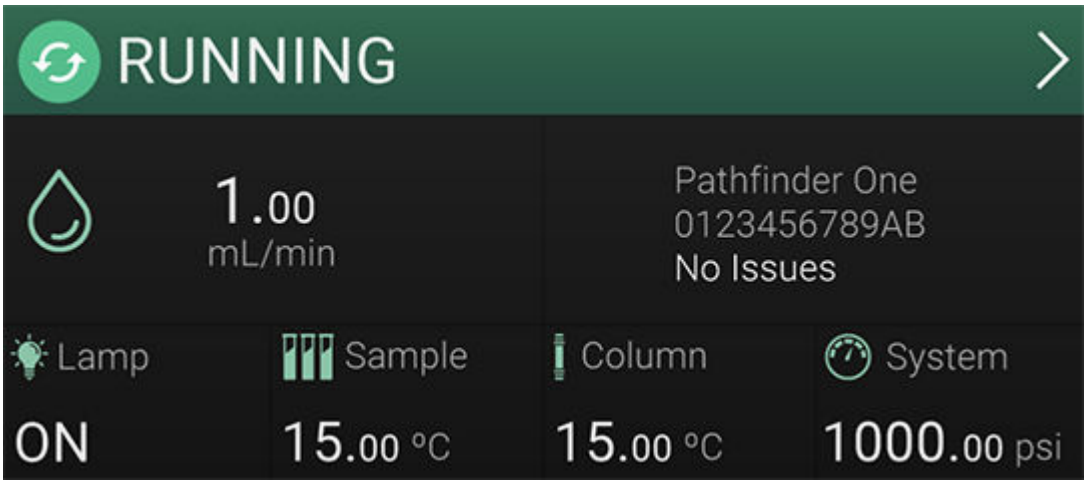
After waking up the Alliance iS system, start the Empower control panel and Alliance iS console if Empower is not already running.

Note: The Alliance iS system is assumed to be powered-up and asleep. System state is IDLE. If the system is powered-down, see Powering-on the system (Page 60).

To start the hardware and software:

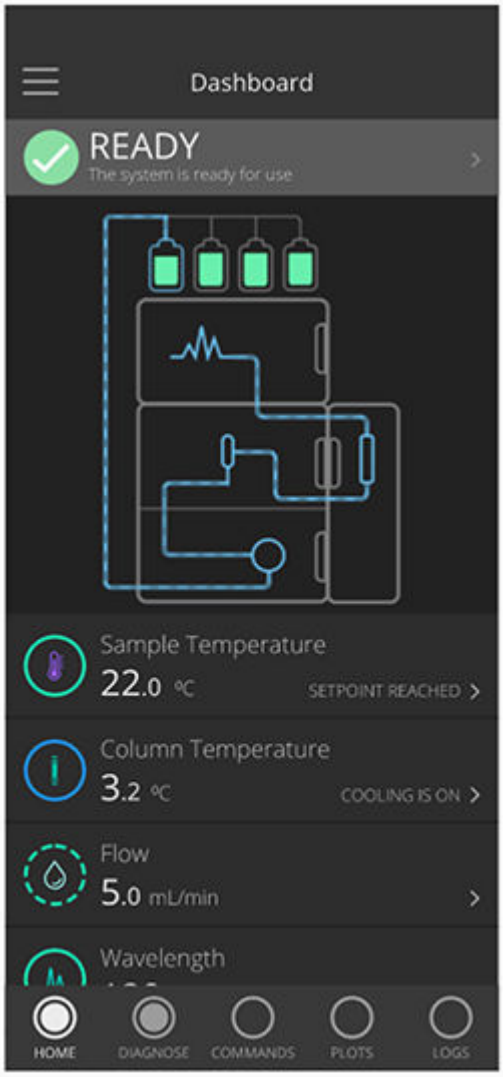
1. Tap the **Swipe to unlock** button on the Idle screen to advance to the Sign in to screen.
2. Tap the keypad to enter your four-digit password and then tap the checkmark.
   The Home view (Page 50) appears.
3. Tap the **Commands** button in the left-hand pane.
   The Commands view (Page 50) appears.
4. Ensure that **Detector Lamp** status is `Lamp is on` or `Lamp is warming`.
   If status is `Lamp is off`, tap the bulb button and hold for 3 seconds as the timer counts down. Then wait for 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 following setup process.
5. Start Empower at the workstation.
   The control panel starts automatically in the status pane.
   Figure 6–1: Control panel (to be updated)



6. From the control panel, start the Alliance iS console by clicking the right-arrow in the upper right corner.

Figure 6–2: Alliance iS console (to be updated)



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

7. Open the Empower project for the analysis.

6.2 Setting up solvents

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

To set up solvents:

1. Consult the solvent specifications for the open project at the Empower workstation.

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.

2. Prepare solvents as required for the method.
3. For each of the solvent bottles:
    a. If the bottle is on the bottle tray, remove the cap and solvent line tube. Otherwise remove just the cap.
    b. Place the bottle on a surface other than the bottle tray. This precaution prevents spillage on the system.
    c. Fill the bottle.
    d. Pass the tube whose identification tag corresponds to the solvent through the opening in the cap.
    e. Replace the cap and place the bottle on the tray as shown in the following figure.

Figure 6–3: Connected solvent bottles

Set up initial priming of the solvent lines as described in Equilibrating the Alliance iS system (Page 83). Use the appropriate workflow on the **Setup > Solvents** screen if it is necessary to prime solvent lines outside of the equilibration workflow.

See:

- Priming the seal-wash system (Page 64)
- Priming the QSM (Page 66)
- Solvent considerations (Page 208)
- Replacing the solvent bottle filters (Page 156)
- Replacing the optional solvent selection valve cartridge (Page 114)

6.3 Installing the column

Install the column after loading the solvent bottles so that old mobile phase cannot pass through the new column.
To install the column:

1. If necessary, remove the current column.
2. Install the column required for the current method by following the instructions in Installing the column (Page 58).

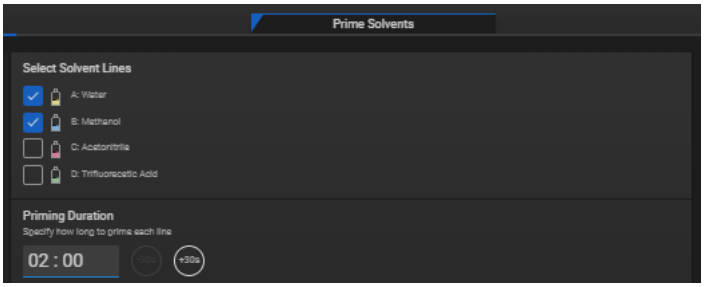
6.4 Equilibrating the Alliance iS system

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. On the touchscreen, click **Commands** to see the Commands view (Page 50).
2. Ensure that **Detector Lamp** status is `Lamp is on`.
   If status is `Lamp is off`, tap the bulb button and hold for 3 seconds as the timer counts down. Then wait for 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 following setup process.
3. When lamp status changes to `Lamp is on`, tap **Setup** to see the Setup view (Page 48).
4. Tap **Startup**, read the guidance on the Welcome screen, and then tap **Next** to advance to the Prime Solvents screen.

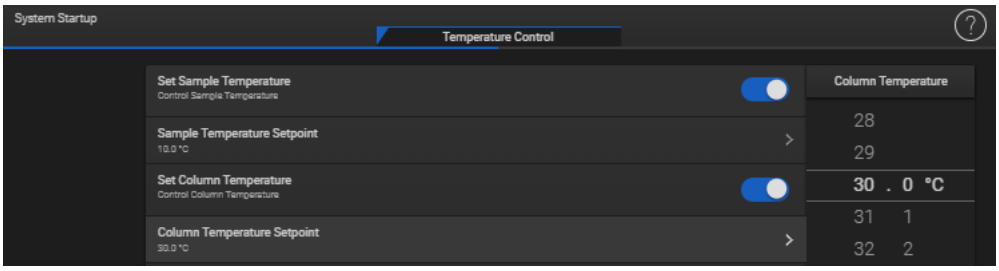
Figure 6–4: Prime Solvents screen



5. Select the solvent lines to be primed.
6. Increment or decrement **Priming Duration** in units of 30 seconds and tap **Next**.

   Note: You can also edit the minute or seconds fields manually.
7. If needed for the analysis, toggle-on **Prime Seal Wash**, enter **Seal Wash Prime Duration** in units of minutes and seconds, and tap **Next**.
8. If needed for the analysis, toggle-on **Prime Needle Wash**, enter **Needle Wash Prime Duration** in cycles, and tap **Next**.
9. If the sample metering pump is probably dry, enable **Prime Sample Metering Pump** and tap **Next** to advance to the Temperature Control screen.

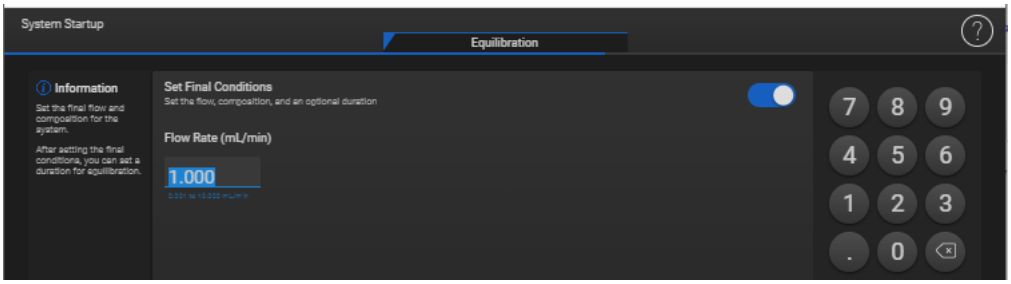
Figure 6–5: Temperature Control screen



10. Toggle-on **Set Sample Termperature**.
11. Set **Sample Temperature Setpoint** using the up- and down-arrow keys.
12. Toggle-on **Set Column Termperature**.
13. Set **Column Temperature Setpoint** using the up- and down-arrow keys. Then tap **Next** twice to advance to the first Equilibration screen.

    Note: The **Detector Lamp** screen is bypassed because the lamp is already on and should be warmed up.

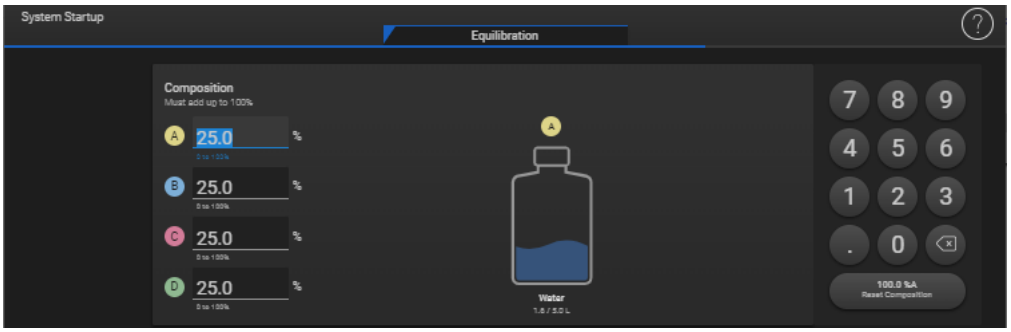
Figure 6–6: Equilibration Final Conditions and Flow Rate screen



14. Toggle-on **Set Final Conditions**.
15. Set **Flow Rate** using the keypad and tap **Next** to advance.

    Note: A minimum of five column volumes is recommended.

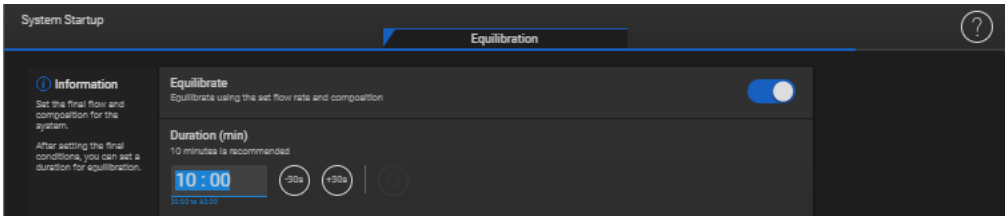
Figure 6–7: Equilibration Composition screen



16. Edit the **Composition** percentages using the keypad and tap **Next** to advance.

    Note: The resulting amounts must total 100%.

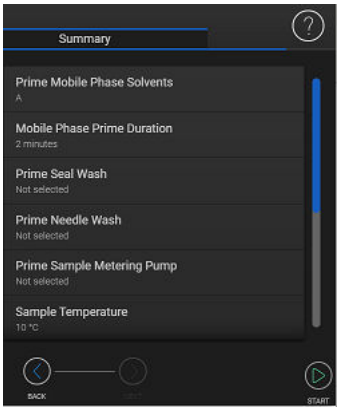
Figure 6–8: Equilibration pre-start screen



17. Toggle-on **Equilibrate** to enable equilibration.
18. Increment or decrement **Duration** in units of 30 seconds and tap **Next** to advance to the Summary screen.

    The recommended duration is 10 minutes.

Figure 6–9: Summary screen



19. Review the settings on the Summary screen and tap **Start**.
    The message `System Startup In progress` displays on the Status screen while equilibration is running.
20. When equilibration ends, examine the results to ensure that the system is ready for routine work.

    Note: The retention times of the test injections' peaks should be correct for today's analysis.

6.5 Preparing and loading samples

The sample manager holds two 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.

See Sample preparation equipment and Cleaning test sample glassware.

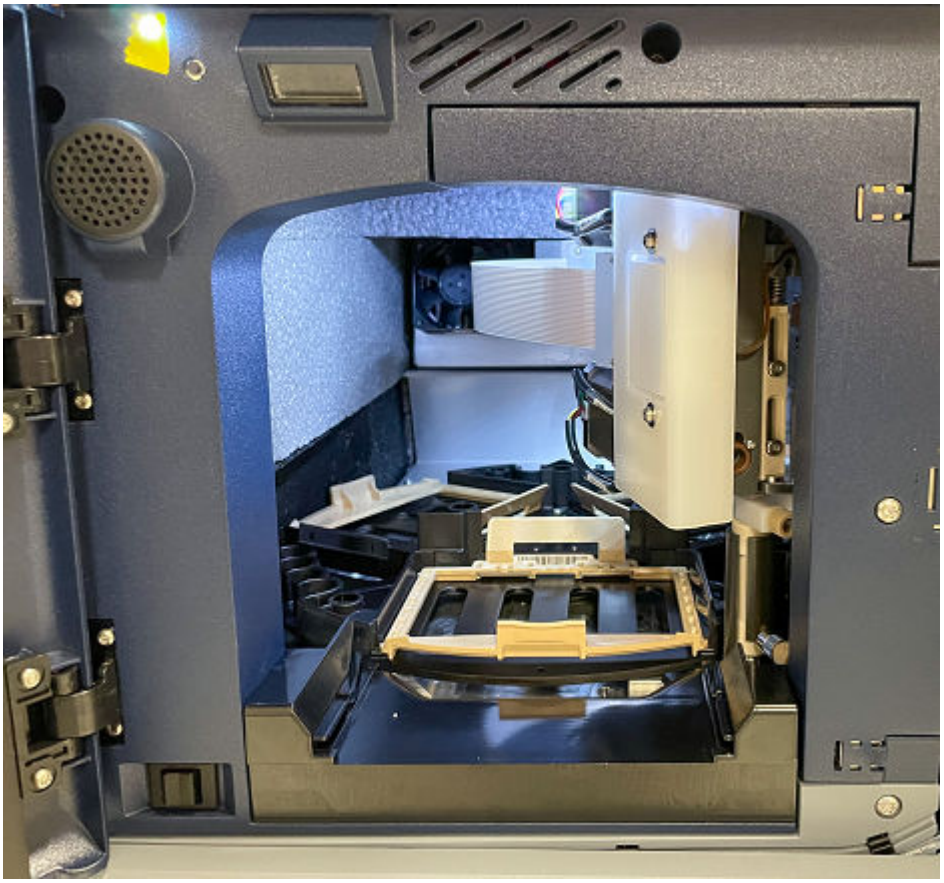
2. Fill the sample plates or trays as specified for the method. Waters vials are preferred.

   See Observing vial and plate recommendations (Page 104).

   Tip: Vial positions V1 through V4, 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 6–10: 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 device beeps once 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 so that its forward edge touches the handle.

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

Figure 6–11: Loading a tray onto the sample platter

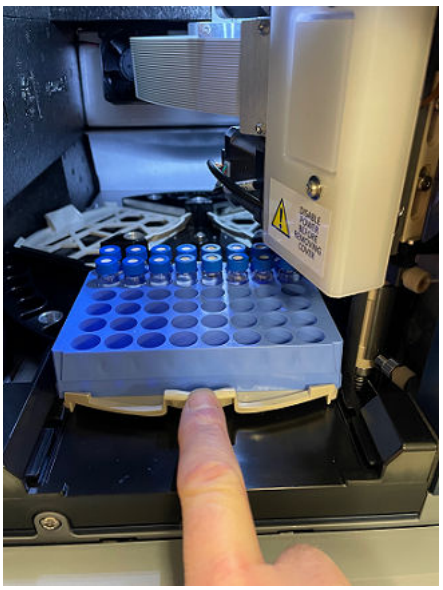
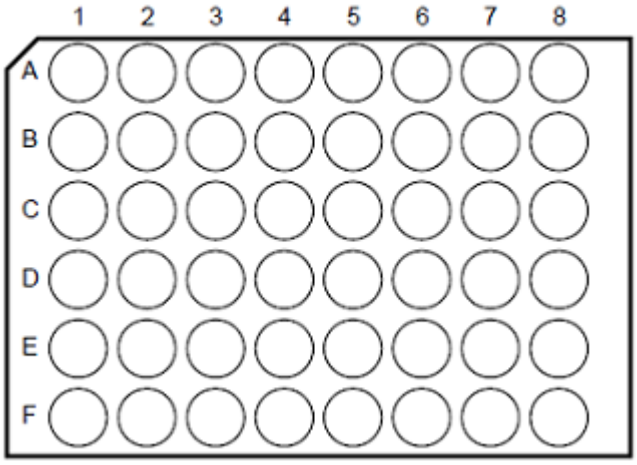


Figure 6–12: Sample plate vial positions



7. Slide the plate or tray backwards 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 above steps for each plate or tray.
9. Close the sample compartment door.

6.6 Checking system status and health

Check system status and health before starting to acquire data, periodically during the day, and should problems arise.

6.6.1 Data acquisition checks

Lab managers can set pre-run and run-time data acquisition checks for common errors that persist until it is necessary to change them. The touchscreen interface provides short workflows for setting the checks. The path to the workflows is **System** > **Administration** > **Acquisition Checks**.

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**

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, TUV detector, or autosampler 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.

6.6.2 Monitoring from the touchscreen

The status bar at the top of the touchscreen shows whether the Alliance iSsystem is currently running samples. When the system is on and not running, the dashboard shows Idle status and screen color is blue. When the system is running, the dashboard shows Running status and screen color is green.

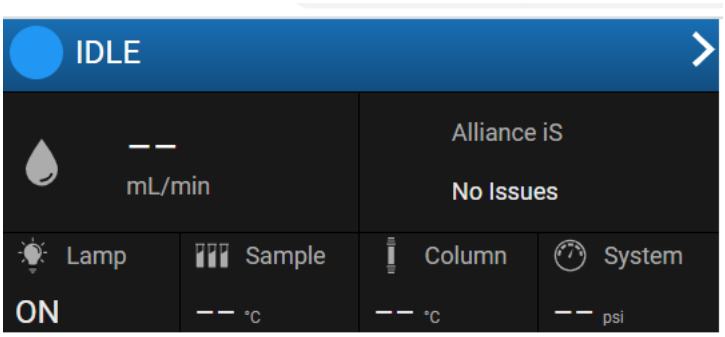
The touchscreen's Home view (Page 48) shows the current (or most recent) values of parameters such as temperatures, pressures, and injection counts.

The touchscreen's Health view (Page 50) provides tools for identifying problems and resolving identified issues. You can also capture and send a service report to Waters.

6.6.3 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 launch through QuickStart menu. The control panel displays the current (or most recent) values of several parameters as follows:

Figure 6–13: Empower control panel



6.6.4 Monitoring from the Alliance iS system console

The Alliance iSHPLC 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:

Figure 6–14: Alliance iS system console

6.7 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 documentation.

6.8 Reviewing the results

Review sample set results in Empower. See the Data Analysis section in the Empower online documentation.

6.9 Printing the report

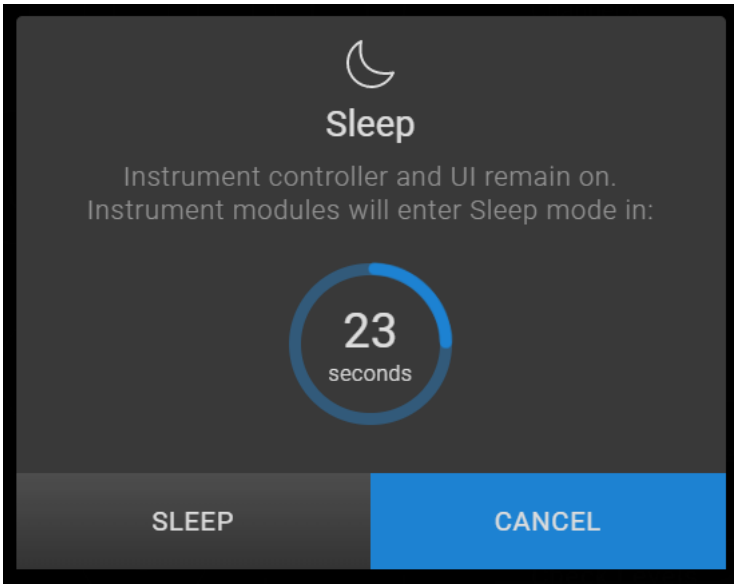
Generate and print the report for a sample set data in Empower. See the Report Generation section in the Empower online documentation.

6.10 Putting the Alliance iS system to sleep

The Alliance iSsystem can be put to sleep overnight. When asleep, the system operates at the lowest level of power consumption that still allows it to respond to the touchscreen or remote console.

To put the system to sleep, select **Commands** > **Power** > **Sleep** from the touchscreen. The following notification displays:

Figure 6–15: Sleep mode notification



6.11 Preparing to shut down the Alliance iS system

Note: See Powering-down the system (Page 60) for more information relating to shutdown.

1. On the touchscreen, tap **Setup** to see the Setup view (Page 48).
2. Tap **Shutdown**, read the guidance on the Welcome screen, and then tap **Next** to advance to the Sample Temperature screen.
3. Toggle-on **Set Sample Temperature**.
4. Increment or decrement **Sample Temperature Setpoint** in units of 30 degrees and tap **Next** to advance to the Column Temperature screen.
5. Toggle-on **Set Column Temperature**.
6. Increment or decrement **Column Temperature Setpoint** in units of 30 degrees and tap **Next** to advance to the Flow Control screen..
7. Toggle-on **Set Flow Rate**.
8. Set **Flow Rate** using the keypad and tap **Next** to advance to the Composition screen.

   Note: The workflow bypasses Composition if **Set Flow Rate** is toggled-off.
9. Edit the **Composition** percentages using the keypad and tap **Next** to advance to the Lamp Control screen.

   Note: The resulting amounts must total 100%.
10. Toggle the Lamp state and tap **Next** to advance to the Summary screen.
11. Review the settings on the Summary screen and tap **Start**.
    The message `Shutdown in Progress` displays on the Status screen while shutdown is running.

12. Tap **Done**.
    Shutdown continues to run in the background unless an error occurs.

7 Performance optimization

Review the following subjects related to your Alliance iS HPLC System:

7.1 General guidelines

The Alliance iS System provides the ability to replicate established HPLC methods by emulating a system's dwell volume and mixing behavior.

(See *Controlling Contamination in LC/MS Systems* (715001307) on the Waters website (www.waters.com.)

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-µm filter membrane.
- Keep concentrated stock solutions to use when preparing working solutions, and refrigerate it when not using it to maximize shelf life.
- 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.

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. Choosing an appropriate wash solvent can minimize carryover for a particular analysis. The wash solvent must be strong enough to dissolve any remaining sample, 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.

Tips:

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

See: Purge and wash solvent guidelines (Page 212) to help reduce the risk of carryover.

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 you tighten the fittings, tubing must seat properly (without internal gaps) inside all connection ports. Poorly seated connections create reservoirs of unnecessary space that retain sample, increasing carryover.
- 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 212).

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.

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 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 leak 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 versus installing them for the first time.

7.2.1 Installation recommendations for fittings

Three types of fitting assemblies are used within the system: PEEK (polymer-based), SST (gold-plated), and TFF (SST). When connecting tubing, heed the following recommendations for installing and tightening fittings.

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.

Recommendations:

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

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

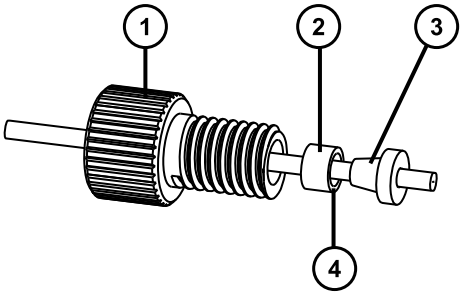
7.2.1.1 Short or long 1/4-28 fitting with flangeless ferrule and stainless steel lock ring

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

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.

Tighten the fitting finger-tight.

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



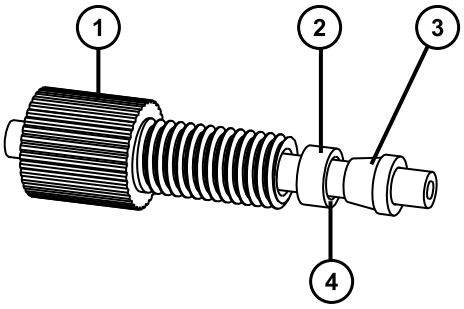
1 Compression screw

2 Lock ring

3 Ferrule

4 End of lock ring with larger inside diameter (ID)

Figure 7–2: Long 1/4-28 fitting with flangeless ferrule and stainless steel lock ring, first-use or reinstall



1 Compression screw

2 Lock ring

3 Ferrule

4 End of lock ring with larger inside diameter (ID)

7.2.1.2 High-pressure pin plug

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

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

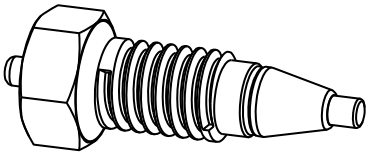
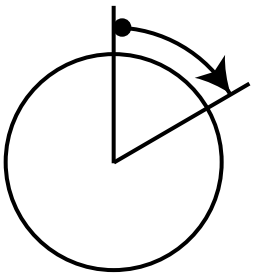


Figure 7–4: High-pressure pin plug tightening, first-use or reinstall



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

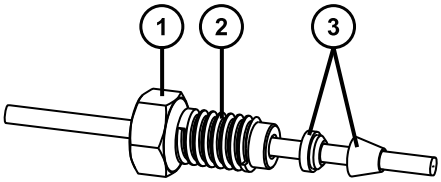
The procedure for tightening metallic fittings differs for new versus reused fittings.

First-use

Tighten the fitting finger-tight plus an additional 3/4-turn using a 1/4-inch open-end wrench. For detailed instructions about assembling new fittings, see the *Assembling new fittings* section of the *Alliance iS System Guide*.

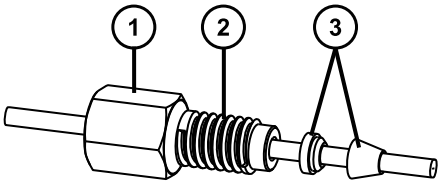
Tip: To prevent band spreading, before you tighten the compression screw, ensure that the tubing is fully bottomed in the connection port.

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



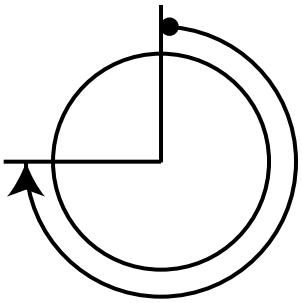
1. Short flats
2. Compression screw
3. Two-piece metallic ferrule

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

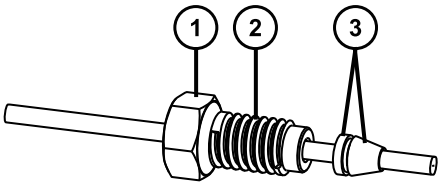
Figure 7–7: Metallic fitting with short or long flats and two-piece metallic ferrule, first-use tightening



Reinstall

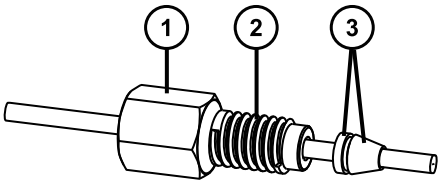
Tighten the fitting finger-tight plus as much as an additional 1/6-turn using a 1/4-inch open-end wrench.

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



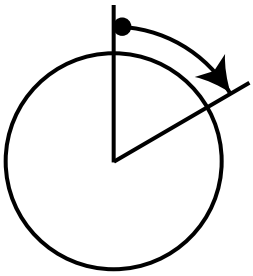
1. Short flats
2. Compression screw
3. Two-piece metallic ferrule

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



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, reinstall tightening



7.2.1.3.1 Assembling new metallic fittings

You must mark new metallic fittings before tightening them to ensure proper assembly.

You must mark new metallic fitting and tubing assemblies with ferrules and ensure that the two marks are aligned when you tighten 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

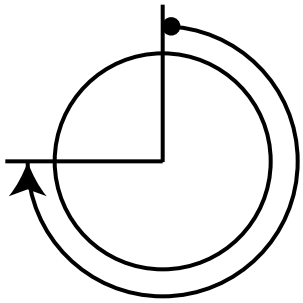
- Chemical-resistant, powder-free gloves
- Protective eyewear
- 1/4-inch open-end wrench, for metallic fittings with two-piece ferrules
- Permanent marker

To assemble new metallic fittings:

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.

   Tip: To prevent band spreading, before you tighten the compression screw, ensure that the tubing is fully bottomed in the 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.

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



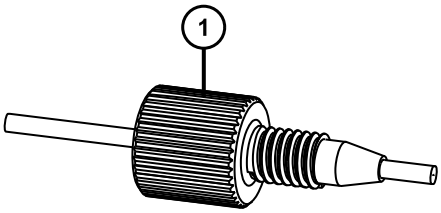
7.2.1.4 One-piece PEEK fitting

Tighten the fitting finger-tight.

Tips:

- Before tightening this fitting, ensure that the tubing is fully bottomed in the connection port.
- Using the aluminum flangeless nut extender (included in the system startup kit) will help achieve proper tightness of this fitting.

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

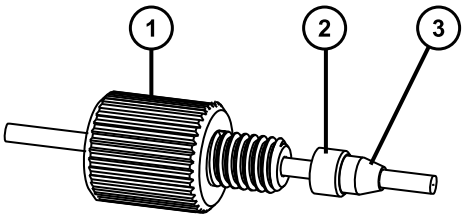


1 Compression screw

7.2.1.5 PEEK fitting with PEEK ferrule and stainless steel lock ring

Tighten the fitting finger-tight.

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



1 Compression screw

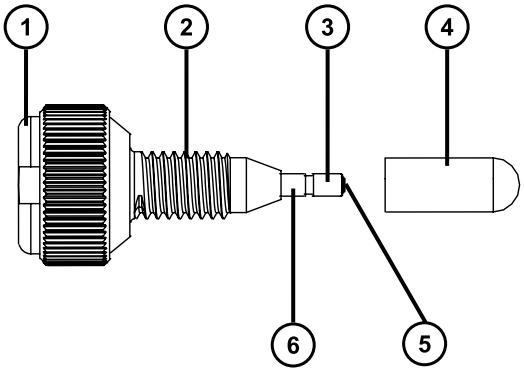
2 Lock ring

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 79) 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**, and **Method Set.**
3. Specify the method settings.

7.4 Sample chamber considerations

When the autosampler 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 beeps three times 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: For more information about plates and vials, see the *Waters Sample Vials and Accessories Brochure* (720001818EN) or visit the plate selector and vial selector on the Waters website.

7.6 Cycle time between injections

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

7.7 Reproducibility

The precision (area reproducibility) when using the SM-FTN is ≤1% RSD for injection volumes from 0.5 to 0.9 µL; ≤0.5% RSD for injection volumes from 1.0 to 4.9 µL; and ≤0.25% RSD for injection volumes of 5.0 µL or greater.

7.8 Maximizing LC column lifetime

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

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

8 Preventive Maintenance

8.1 Maintenance

This is the start of your concept.

8.2 Maintenance safety guidelines

When performing maintenance procedures, adhere to the following safety guidelines.

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.

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

8.3 View module information

The module information provides you with the serial number of the module, firmware checksum, and the firmware and component software versions. This information is for reference only.

Note: When using Empower, the module information also provides the client and LAC/E software versions.

To view module information:

1. On the main page, from the **Configure** menu, select **View module information** to open the Module Information dialog box.

   Alternative: You can also hover over the module image on the System page to view module information.

2. Click **OK**.

8.4 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.

8.5 Spare parts

To ensure that your system operates as designed, use only Waters Quality Parts. Visit www.waters.com/wqp for information about Waters Quality Parts, including how to order them.

8.6 Configuring maintenance warnings

Maintenance counters provide real-time usage status information that can help you determine when to schedule routine maintenance for specific components. You can set usage thresholds and maintenance warnings that alert you when a component reaches the designated threshold limit. By setting threshold limits and monitoring these usage counters regularly, you can minimize unexpected failures and unscheduled downtime during important work. For information on setting maintenance warnings, consult the ACQUITY UPLC Console online Help.

8.7 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.

8.8 Pump maintenance

The customer can perform the following pump maintenance tasks:

-

8.8.1 Recommended maintenance schedule for the pump

Maintenance procedure	Frequency
Replace solvent filters	During scheduled routine maintenance or as necessary
Clean the air filter in the door	As necessary
Replace the air filter in the door	During scheduled routine maintenance or as necessary
Replace the leak sensor	As necessary
Replace the mixer in path 1	During scheduled routine maintenance or as necessary
Replace the optional mixer in path 2	During scheduled routine maintenance or as necessary
Replace the vent valve cartridge	As necessary
Replace the optional solvent selection valve cartridge	As necessary
Replace the primary check valve	During scheduled routine maintenance or as necessary
Replace the in-line filter cartridge on the primary check valve	During scheduled routine maintenance or as necessary
Replace the accumulator check valve	During scheduled routine maintenance or as necessary
Replace the pump-head seals and plungers	During scheduled routine maintenance or as necessary

Maintenance procedure	Frequency
Replace the pump head and seal-wash housing	As necessary
Clean the device's exterior with a soft, lint-free cloth, or paper dampened with water	As necessary

8.8.2 Servicing the air filter in the door

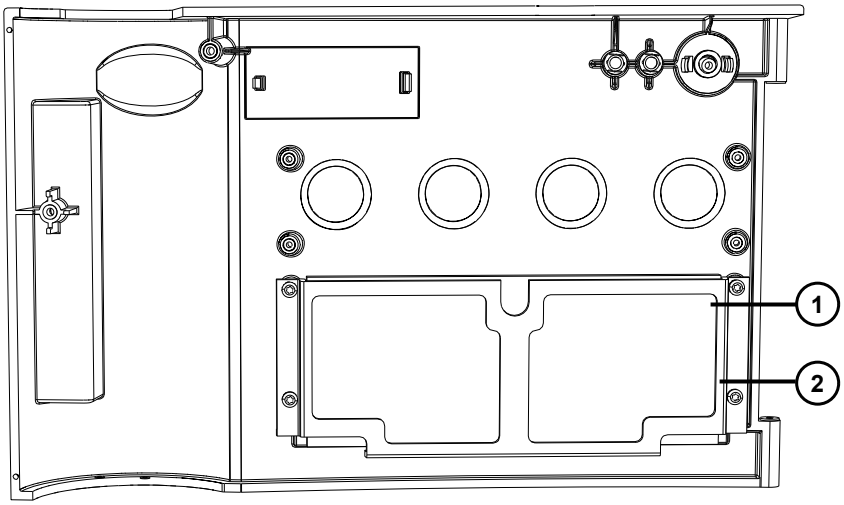
Required tools and materials

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

To service the air filter:

1. Open the bottom door.
2. Slide the air filter up and out of the frame inside the door.

Figure 8–1: Door air filter



1 Air filter

2 Air filter frame

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. Slide the air filter back into the frame.

8.8.3 Replacing the 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

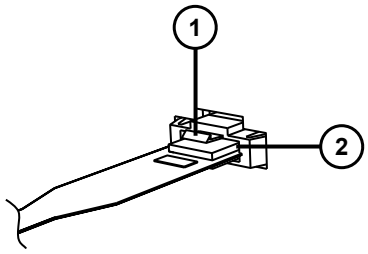
- Chemical-resistant, powder-free gloves
- Protective eyewear
- Leak sensor

To replace the leak sensor:

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.

1. Power-off the solvent manager.
2. Open the solvent manager’s door.
3. Press down on the tab to detach the leak sensor connector from the front of the device.

Figure 8–2: Leak sensor connector

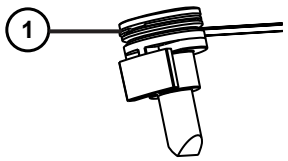


1 Tab

(2) Leak sensor connector

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

Figure 8–3: Leak sensor serrations



(1) Serrations

5. Unpack the new leak sensor.
6. 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 8–4: Aligning T-bar with slot

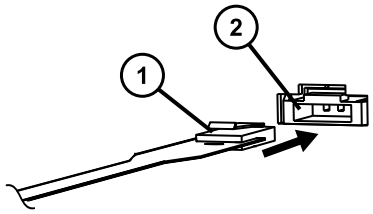
(1) T-bar

(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 device.

Figure 8–5: Attaching leak sensor connector



(1) Leak sensor connector

(2) Leak sensor port on front of device

8. Power-on the solvent manager.
9. In the console, select the solvent manager, and then click **Control** > **Reset QSM** to reset the solvent manager.

8.8.4 Replacing the Mixer

Provide context for your task here (optional).

Required tools and materials

-
-
-

To replace the mixer:

Enter your first step here.

8.8.5 Replacing the vent valve cartridge

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

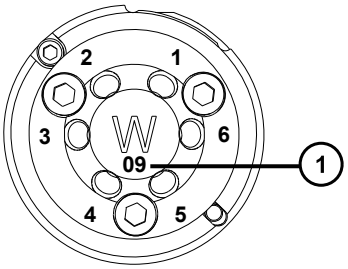
- Chemical-resistant, powder-free gloves
- Protective eyewear
- 1/4-inch open-end wrench
- 2-mm hex wrench
- Vent-valve cartridge

To replace the vent-valve cartridge:

1. In the Console, select the solvent manager, and then click **Interactive Display** > **Control**  .
2. Ensure that the vent valve is set to the "Vent" position.

   Tip: To change the setting to the "Vent" position, click the underlined vent-valve position and select **Vent**.
3. Open the solvent manager door.
4. Inspect the valve cartridge's construction material indicator, which indicates whether the cartridge is constructed of stainless steel or titanium, and ensure that the replacement cartridge has the same material indicator.

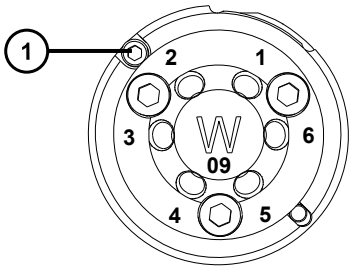
Figure 8–6: Location of construction material indicator



1 Valve cartridge construction material indicator

5. Use the 1/4-inch open-end wrench to remove the fittings attached to the vent-valve cartridge.
6. Use the 2-mm hex wrench to remove the hex screw at the 10-o'clock position on the vent-valve cartridge.

Figure 8–7: Hex screw on a generic vent-valve cartridge



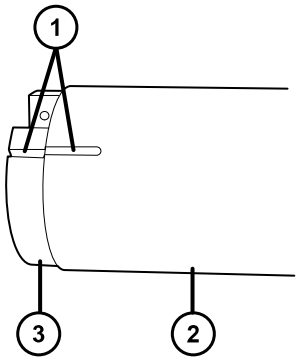
1 Hex screw

7. Remove the vent-valve cartridge from the vent-valve cartridge chamber by pulling the cartridge straight forward.
8. Unpack the replacement vent-valve cartridge.
9. Ensure that the groove in the cartridge housing aligns with the groove on the drive clamp.

Tip: If the grooves do not align, turn the drive clamp until they do.

Note: Avoid scratching the drive clamp or body.

Figure 8–8: Correct vent-valve cartridge and drive clamp groove alignment



1. Aligned grooves
2. Vent-valve cartridge housing
3. Drive clamp

10. Insert the new vent-valve cartridge into the vent-valve cartridge chamber.

    Requirements:

    - Orient the new cartridge exactly as the old one was oriented.
    - The vent-valve cartridge must slide fully into the vent-valve cartridge chamber. If it does not, report the problem to Waters Technical Service.

11. Insert the 2-mm hex screw at the 10-o'clock position on the vent-valve cartridge.

    Tip: Use the 2-mm hex wrench to tighten it.

12. Use the 1/4-inch open-end wrench to reattach all fittings, and then tighten them as much as 1/6-turn beyond finger-tight (for existing fittings) or 3/4-turn beyond finger-tight (for new fittings).
13. Prime the solvent manager (see Priming the solvent manager).

8.8.6 Replacing the optional solvent selection valve cartridge

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

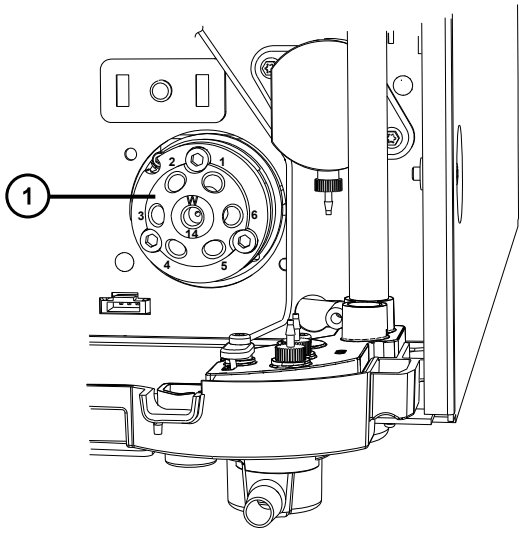
- Chemical-resistant, powder-free gloves
- Protective eyewear
- 2-mm hex wrench (startup kit)
- Solvent selection valve cartridge

To replace the optional solvent selection valve cartridge:

1. Open the solvent manager’s door.
2. Ensure that the solvent-selection valve is set to D_6.

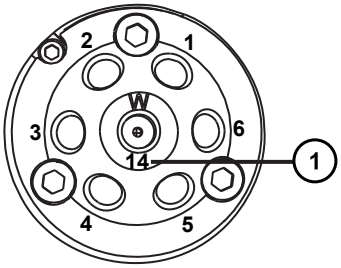
   Warning: To avoid injuries arising from contact with spilled solvent (the result of unintentional siphoning), move the solvent bottles to a location below the solvent manager.
3. Move the solvent bottles to a location below the solvent manager.
4. Inspect the valve cartridge's construction material indicator, which indicates whether the cartridge is constructed of stainless steel or titanium, and ensure that the replacement cartridge has the same material indicator.

Figure 8–9: Location of solvent selection valve



1 Solvent selection valve

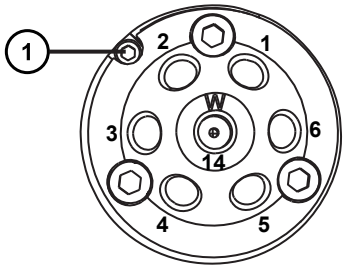
Figure 8–10: Location of construction material indicator



1 Valve cartridge construction material indicator.

5. Remove the finger-tight fittings attached to the solvent selection valve cartridge.
6. Use the 2-mm hex wrench to remove the hex screw at the 10 o'clock position on the cartridge.

Figure 8–11: Hex screw on solvent selection valve cartridge



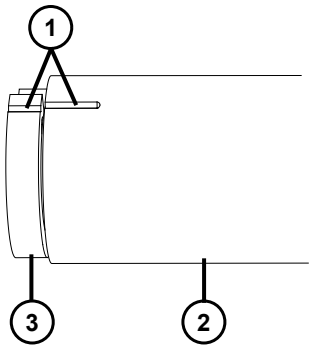
1 Hex screw

7. Remove the cartridge from the solvent selection valve assembly by pulling it straight forward.
8. Unpack the replacement cartridge.
9. Ensure that the groove in the cartridge housing aligns with the groove on the drive clamp.

Tip: If the grooves do not align, turn the drive clamp until they do.

Note: Avoid scratching the drive clamp or body.

Figure 8–12: Correct solvent selection valve cartridge and drive clamp groove alignment



1 Aligned grooves

2 Solvent selection valve cartridge housing

3 Drive clamp

10. Insert the new cartridge into the cartridge chamber.

    Requirements:

    - Orient the new cartridge exactly as the old one was oriented.
    - The cartridge must slide fully into the solvent selection valve assembly. If it does not, report the problem to Waters Technical Service.

11. Insert the 2-mm hex screw at the 10 o'clock position on the solvent selection valve cartridge, and then use the 2-mm hex wrench to tighten it.
12. Reattach all fittings and finger-tighten them.
13. Return the solvent bottles to their original locations.
14. Prime the solvent manager (see Priming the solvent manager).

    Requirement: Prime all six D lines.

8.8.7 Replacing the in-line filter cartridge on the 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

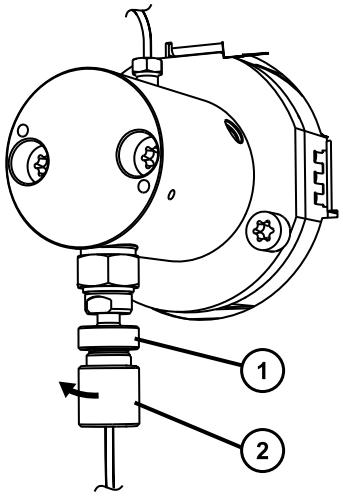
- Chemical-resistant, powder-free gloves
- In-line filter cartridge

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

1. **Requirement:** Wear clean, chemical-resistant, powder-free gloves when replacing the in-line filter cartridge on the primary check valve.

   Flush the solvent manager with nonhazardous solvent.
2. Power-off the solvent manager.
3. Open the solvent manager's door.
4. Unscrew and remove the cap nut from the ferrule holder fitting.

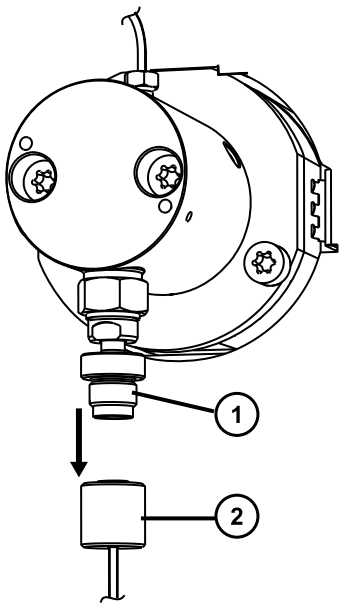
Figure 8–13: Unscrewing the cap nut from the ferrule holder fitting



1 Ferrule holder fitting

2 Cap nut

Figure 8–14: Removing cap nut from ferrule holder fitting

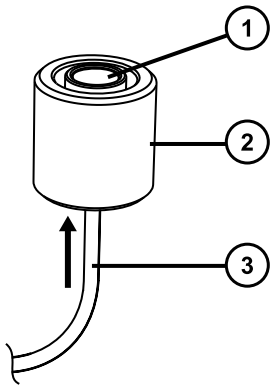


1 Ferrule holder fitting

2 Cap nut

5. Pull the cap nut off the tube to remove the in-line filter cartridge.

Figure 8–15: Pulling cap nut from tube

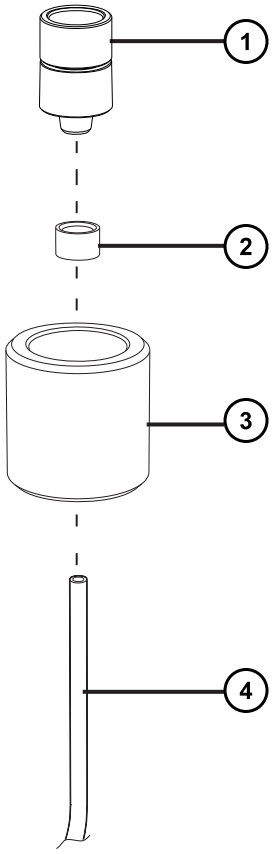


1 In-line filter cartridge

2 Cap nut

3 Tube

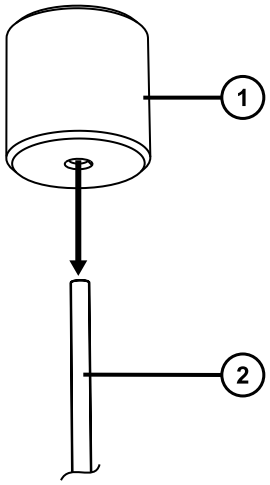
Figure 8–16: In-line filter cartridge, locking ring, and cap nut



1. In-line filter cartridge
2. Metal locking ring
3. Cap nut
4. Tube

6. Inspect the filter cartridge to determine whether it is constructed of stainless steel or titanium, 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.
7. Slide the cap nut over the end of the tube.

Figure 8–17: Sliding cap nut on tube

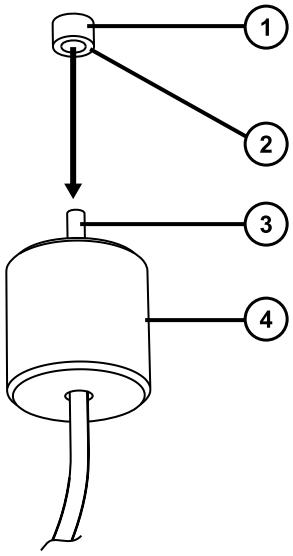


1 Cap nut

2 Tube

8. Slide the metal locking ring onto the tube, ensuring that the thicker end of the metal locking ring is facing toward the cap nut.

Figure 8–18: Sliding metal locking ring on tube



1 Metal locking ring

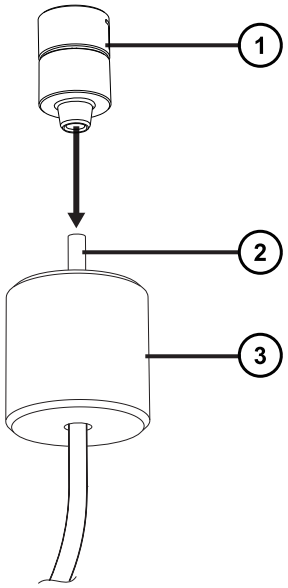
2 Thicker end of metal locking ring facing toward cap nut

3 Tube

4 Cap nut

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

Figure 8–19: Placing in-line filter cartridge onto end of tube



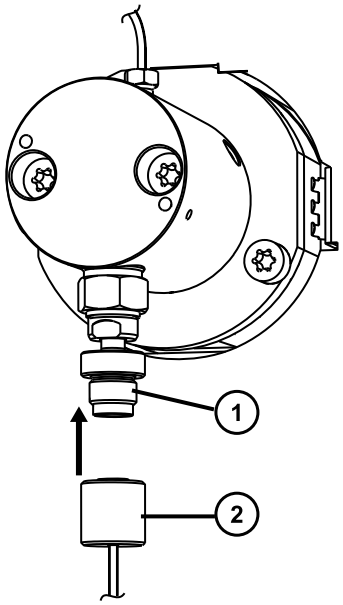
1 In-line filter cartridge

2 Tube

3 Cap nut

11. Insert the in-line filter cartridge with tubing into the ferrule holder fitting and finger-tighten the cap nut to the extent possible.

Figure 8–20: Installing cap nut on ferrule holder fitting



1. Ferrule holder fitting
2. Cap nut

12. Power-on the solvent manager.
13. Prime the solvent manager (see Priming the solvent manager).

8.8.8 Replacing the Check Valve Filter

Provide context for your task here (optional).

Required tools and materials

-
-
-

To replace the check valve filter:

Enter your first step here.

8.8.9 Replacing the 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.

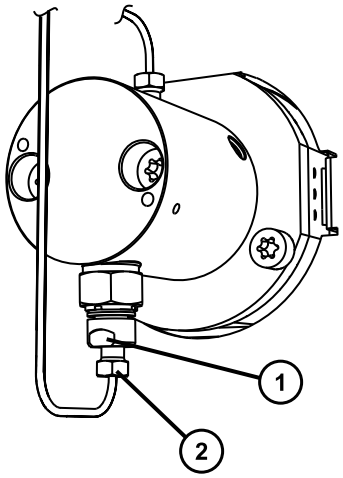
Required tools and materials

- 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
- Accumulator check valve assembly

To replace the accumulator check valve:

1. Flush the solvent manager with nonhazardous solvent.
2. Power-off the solvent manager.
3. Open the solvent manager’s 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 8–21: Compression fitting on the check valve



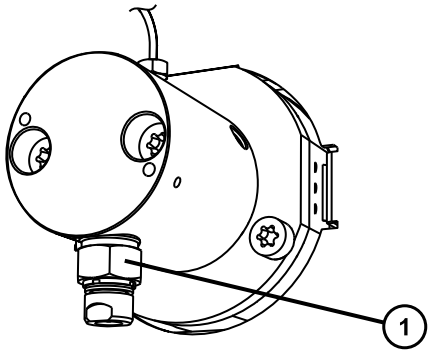
1 Place the 5/16-inch open-end wrench here

2 Compression fitting

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.

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.

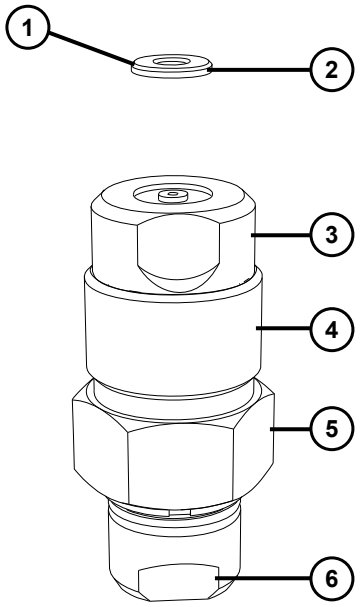
Figure 8–22: Check valve assembly on the accumulator pump head



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 8–23: 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. Power-on the solvent manager.
12. Prime the solvent manager (see Priming the solvent manager).

8.8.10 Replacing the accumulator check valve

A blocked or faulty check valve can cause the system pressure to drop to near zero during operation.

The check valve allows system fluid to flow in one direction, from the inlet to the outlet, and prevents fluid backflow. The ball inside the check valve can get stuck and cause a blockage. When this happens, the system pressure will drop to near zero during operation. To solve this issue, the check valve needs to be cleaned or replaced. To clean the check valve, remove it from the system and sonicate it in water, then methanol, and then water again. If sonication does not help, tap the check valve on the bench top a few times to try to get the ball inside to move freely. If the ball remains stuck, replace the check valve.

Required tools and materials

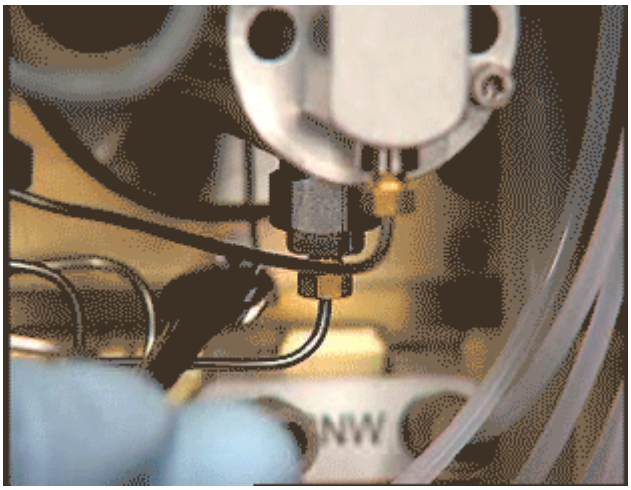
- Chemical-resistant, powder-free gloves
- 1/4-inch open-end wrench
- 5/16-inch open-end wrench
- 1/2-inch open-end wrench
- Accumulator check-valve assembly (70001064)

Before you begin, be sure to flush the system with nonhazardous solvent.

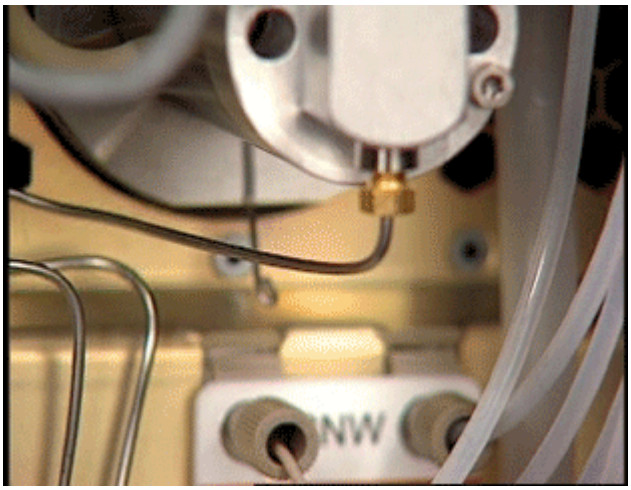
To replace the accumulator check valve:

1. Open the bottom door and locate the check valve.

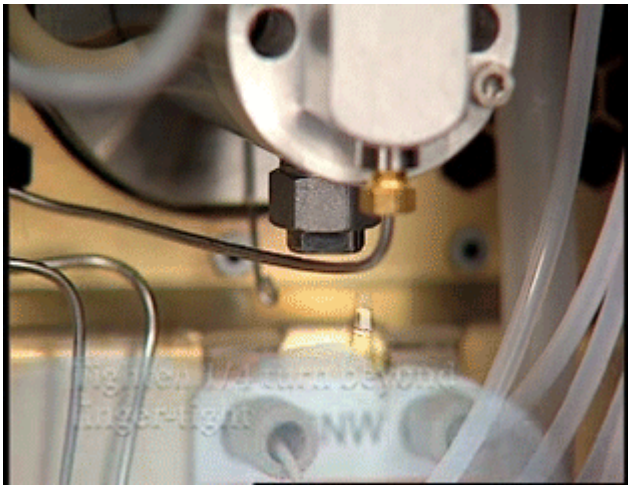
2. Remove the check valve.



3. Insert the new check-valve 1/8 turn past finger tight.



4. Reattach the inlet tubbing fitting 1/4 past finger tight.



5. Prime the quaternary pump.

8.8.11 Replacing the accumulator check valve

A blocked or faulty check valve can cause the system pressure to drop to near zero during operation.

The check valve allows system fluid to flow in one direction, from the inlet to the outlet, and prevents fluid backflow. The ball inside the check valve can get stuck and cause a blockage. When this happens, the system pressure will drop to near zero during operation. To solve this issue, the check valve needs to be cleaned or replaced. To clean the check valve, remove it from the system and sonicate it in water, then methanol, and then water again. If sonication does not help, tap the check valve on the bench top a few times to try to get the ball inside to move freely. If the ball remains stuck, replace the check valve.

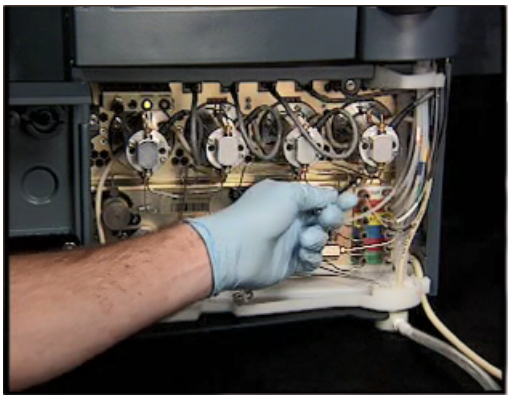
Required tools and materials

- Chemical-resistant, powder-free gloves
- 1/4-inch open-end wrench
- 5/16-inch open-end wrench
- 1/2-inch open-end wrench
- Accumulator check-valve assembly (part number 70001064)

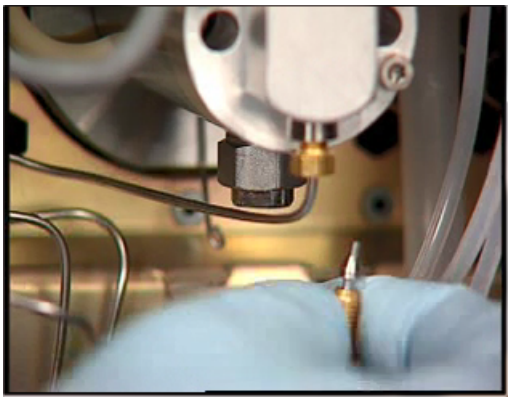
Before you begin, be sure to flush the system with nonhazardous solvent.

To replace the accumulator check valve:

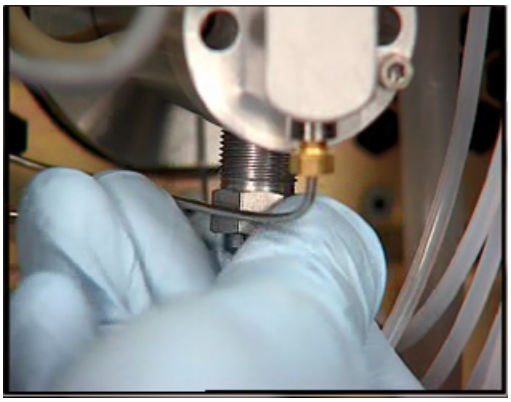
1. Open the bottom door and locate the check valve.



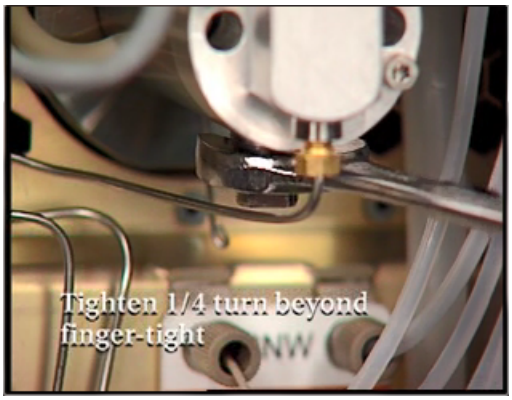
2. Remove the inlet tubbing.



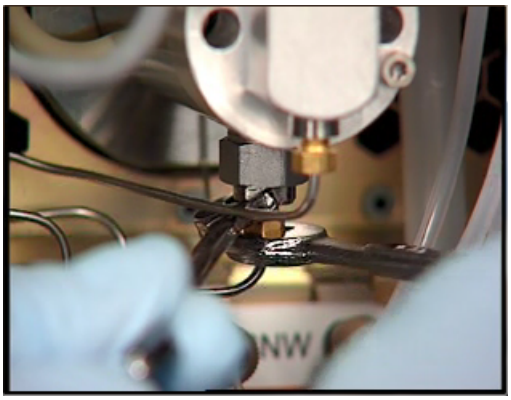
3. Remove the check valve.



4. Insert the new check-valve 1/4 turn past finger tight.



5. Reattach the inlet tubbing fitting 1/8 past finger tight.



6. Prime the quaternary pump.

8.8.12 Replacing the accumulator check valve - video

A blocked or faulty check valve can cause the system pressure to drop to near zero during operation.

The check valve allows system fluid to flow in one direction, from the inlet to the outlet, and prevents fluid backflow. The ball inside the check valve can get stuck and cause a blockage. When this happens, the system pressure will drop to near zero during operation. To solve this issue, the check valve needs to be cleaned or replaced. To clean the check valve, remove it from the system and sonicate it in water, then methanol, and then water again. If sonication does not help, tap the check valve on the bench top a few times to try to get the ball inside to move freely. If the ball remains stuck, replace the check valve.

https://www.youtube.com/embed/xFNXEPBa9Vs

Required tools and materials

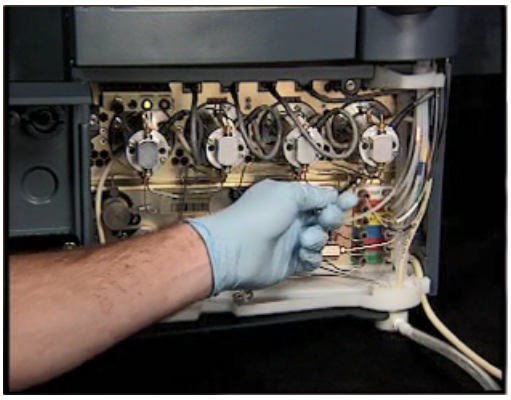
- Chemical-resistant, powder-free gloves
- 1/4-inch open-end wrench
- 5/16-inch open-end wrench

- 1/2-inch open-end wrench
- Accumulator check-valve assembly (70001064)

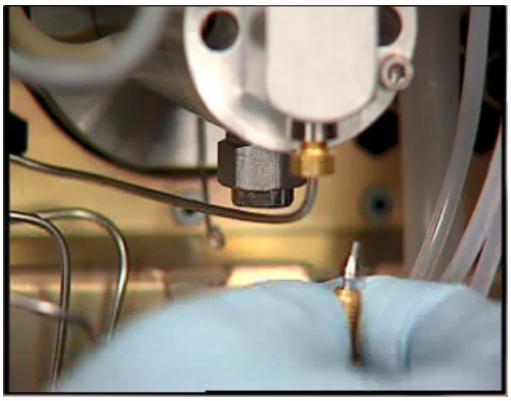
Before you begin, be sure to flush the system with nonhazardous solvent.

To replace the accumulator check valve:

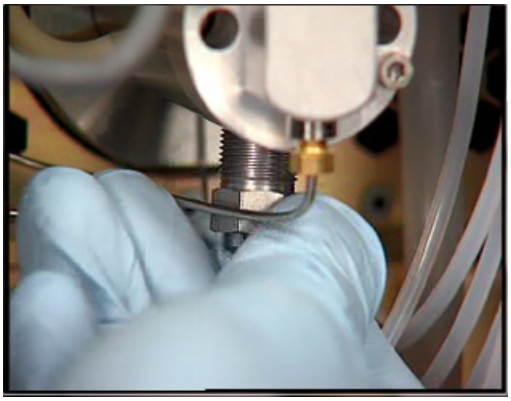
1. Open the bottom door and locate the check valve.



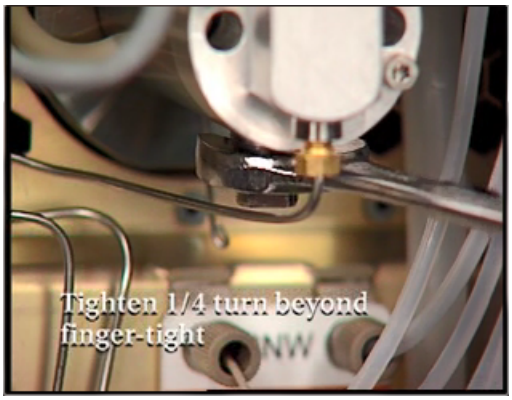
2. Remove the inlet tubbing.



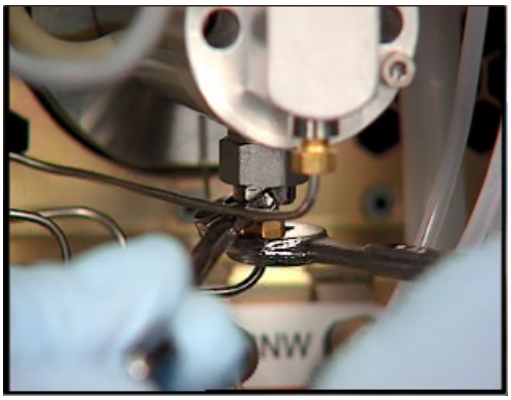
3. Remove the check valve.



4. Insert the new check-valve 1/4 turn past finger tight.



5. Reattach the inlet tubbing fitting 1/8 past finger tight.



6. Prime the quaternary pump.

8.8.13 Replacing the pump plungers and seals

The primary and actuator pump plungers and seals should be replaced annually to ensure reliable system performance, or when issues related to worn or damaged seals and plungers arise, such as: leak test failures, unstable pressure, and shifting retention times.

This procedure can be used to replace the plunger and seals for both the primary and actuator pumps.

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
- 1/4-inch open-end wrench
- T27 TORX driver
- Plunger removal tool
- Seal-removal tool
- Sharp tool, such as a dental pick
- Fluoropolymer O-ring
- Methanol
- Pump head (if replacing)
- Seal-wash housing (if replacing)
- Seal-wash seal
- Primary pump head rebuild kit (P/N 700011072)
- Accumulator pump head rebuild kit (P/N 700011073)

To replace the pump plunger and seals:

1. Flushing the quaternary pump with nonhazardous solvent.
2. Moving the pump-head plunger backward.
3. Removing the pump head.
4. Removing the pump-head plunger.
5. Removing the pump-head seals.
6. Installing the new pump-head seals.
7. Installing the new pump-head plunger.
8. Reinstalling the pump head.
9. Performing the quaternary pump leak test.

8.8.14 Replacing the primary pump head and accumulator pump head plunger and seals

Replace pump components annually in accordance with the Waters preventive maintenance (PM) schedule, or any time they appear damaged to prevent a disruption in your workflow.

Use these procedures to replace the following:

- Primary pump head plunger and seals
- Accumulator pump head plunger and seals

Replace the pump head components annually in accordance with the Waters preventive maintenance (PM) schedule, or any time they appear damaged.

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.

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

Note: If necessary, you can also replace the pump head and seal wash housing during this procedure.

Required tools and materials

- Chemical-resistant, powder-free gloves
- 5/16-inch open-end wrench (used for replacing the accumulator pump components only)
- 1/4-inch open-end wrench
- T27 TORX driver
- Plunger removal tool
- Seal-removal tool
- Sharp tool, such as a dental pick
- Needle-nose pliers

Required materials

- Fluoropolymer O-ring
- Methanol
- Plunger seal with back-up ring
- Pump head (if replacing)
- Seal wash housing (if replacing)
- Seal wash seal

Replacing the plunger and seals in the primary pump head and accumulator pump head involves these steps:

1. Flushing the solvent manager with nonhazardous solvent.
2. Moving the pump head plunger backward.
3. Removing the pump head.
4. Removing the pump head plunger.

5. Removing the pump head seals.
6. Installing the new pump head seals.
7. Installing the new pump head plunger.
8. Reinstalling the pump head.
9. Performing the leak test.

   Tip: If the leak test results are unsatisfactory, pressurize the seals to properly seat them. To do so, run the solvent manager at 58,605 kPa (586 bar, 8500 psi) for 30 minutes, or run the leak test until results are satisfactory.

8.8.14.1 Moving the pump plunger backward

Use the touchscreen UI to move the pump plunger backward before replacing pump components.

To move the pump plunger backward:

1. Flush the pump with nonhazardous solvent.
2. From the touchscreen, select the solvent manager, and then tap **Maintain** > **Replace Components**.
3. From the Replace Components page, scroll down and then tap **Pump Maintenance**.
4. Tap **Pump Head Maintenance**.
5. Select the plunger head you want to move backward.
6. Tap **Move Backward**, and then wait for the plunger to stop.

8.8.14.2 Removing the primary pump head or accumulator pump head

Replace pump components annually in accordance with the Waters preventive maintenance (PM) schedule, or any time they appear damaged to prevent a disruption in your workflow.

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

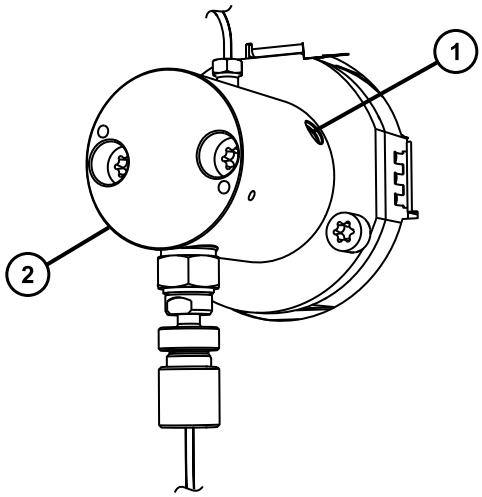
- Chemical-resistant, powder-free gloves
- Needle-nose pliers
- 1/4-inch open-end wrench
- T27 TORX driver

To remove the primary pump head or accumulator pump head:

1. **Requirement:** Wear clean, chemical-resistant, powder-free gloves when removing the primary pump head or accumulator pump head.

   Locate the seal wash tubing secured to the seal wash housing with barbed fittings. Use needle-nose pliers to remove the seal wash tubing.

   Figure 8–24: Location of seal wash tubing connections

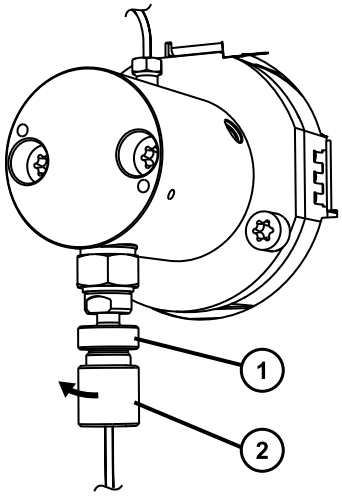
   

   (1) Location of seal wash tubing connection

   (2) Location of seal wash tubing connection

2. Follow these steps if replacing the primary pump head only. Proceed to step 3 if replacing the accumulator pump head.
   a. Loosen the cap nut on the inlet filter fitting, but do not remove it from the fitting.

Figure 8–25: Loosening cap nut on inlet filter fitting

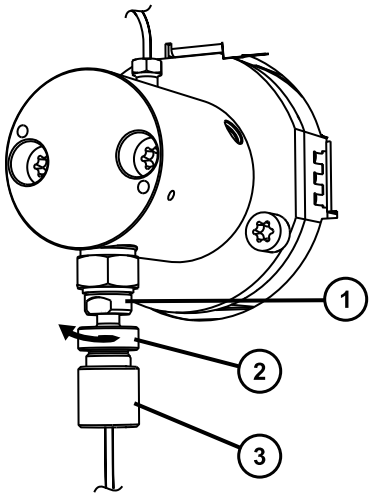


1 Inlet filter fitting

2 Cap nut

b. Unscrew and remove the inlet filter fitting and cap nut from the primary check valve.

Figure 8–26: Unscrewing inlet filter fitting and cap nut from primary check valve



1 Primary check valve

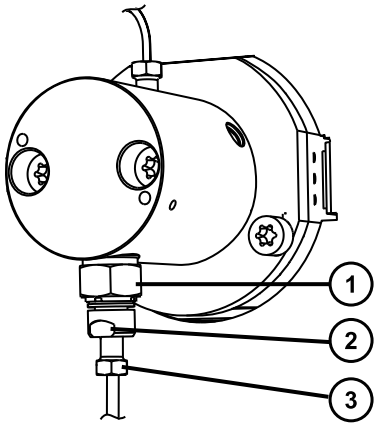
2 Inlet filter fitting

3 Cap nut

c. Disconnect the transducer from the accumulator check valve.

3. Follow these steps if replacing the accumulator pump head only. Proceed to step 4 to resume procedures for replacing the primary pump head.
    a. Using the 5/16-inch open-end wrench to hold the check valve cartridge in place, disconnect the tubing connection from the check valve using the 1/4-inch open-end wrench.

    Figure 8–27: Location of check valve tubing connection

    1 Check valve

    2 Place 5/16-inch open-end wrench here

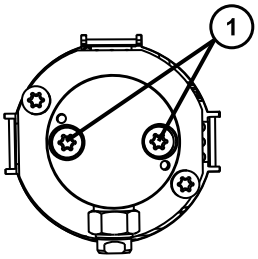
    3 Place 1/4-inch open-end wrench here

    b. Disconnect the transducer from the vent valve.

Important: The remaining steps are the same for the primary pump head or the accumulator pump head.

4. Using the T27 TORX driver, loosen the two head bolts 1/2-turn.

Figure 8–28: Head bolts on primary pump head

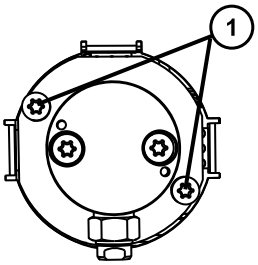


1 Head bolts

Notice: To avoid damaging the plunger, support the head from below as you remove it, and do not tilt the head when withdrawing it.

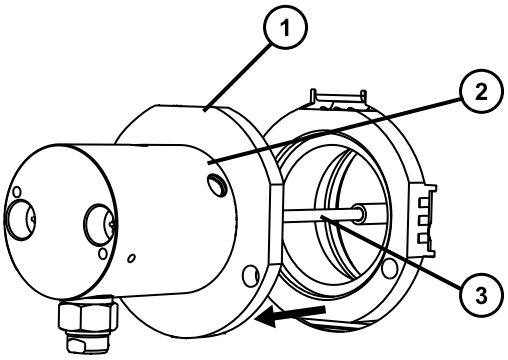
5. Using the T27 TORX driver, loosen and remove the two support plate bolts, and then gently pull the pump head and support plate off the actuator housing, ensuring that you do not to tilt the head during the extraction.

Figure 8–29: Bolts on primary pump head support plate



1 Bolts on support plate

Figure 8–30: Removing pump head and support plate from actuator housing



1 Support plate

2 Pump head

3 Plunger

6. Stand the pump head upright on a clean surface.

8.8.14.3 Removing the pump head plunger

Replace pump components annually in accordance with the Waters preventive maintenance (PM) schedule, or any time they appear damaged to prevent a disruption in your workflow.

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.

Recommendation: Replace the plunger seals when you replace the plunger.

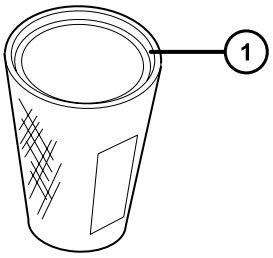
Required tools and materials

- Chemical-resistant, powder-free gloves
- Protective eyewear
- Plunger removal tool (recommended)

To remove the pump head plunger:

1. Locate the recessed side of the plunger removal tool.

   Figure 8–31: Recessed side of plunger removal tool

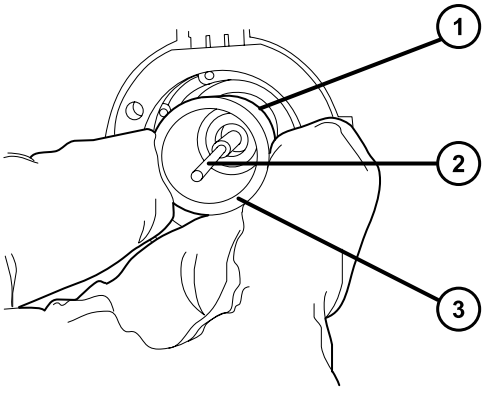
   

   1 Recessed side of plunger removal tool

2. Use the recessed side of the plunger tool to push the release collar inward, and then remove the plunger.

   Warning: To avoid finger or hand lacerations from sharp-edged surfaces, use care when installing or removing head-assembly components. Bending or twisting the sapphire piston shaft can cause it to fracture or splinter.

   Figure 8–32: Plunger removal tool on release collar

   1 Spring-loaded release collar

2 Plunger

3 Plunger removal tool

8.8.14.4 Removing the pump head seals

Replace pump components annually in accordance with the Waters preventive maintenance (PM) schedule, or any time they appear damaged to prevent a disruption in your workflow.

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.

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

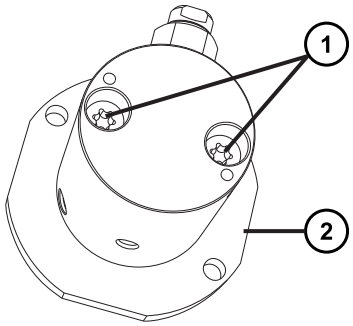
Required tools and materials

- Chemical-resistant, powder-free gloves
- T27 TORX driver
- Seal-extraction tool
- Sharp tool

To remove the pump head seals:

1. Stand the pump head upright on a clean surface.
2. Using the T27 TORX driver, completely loosen the two head bolts to release the support plate from the pump head.

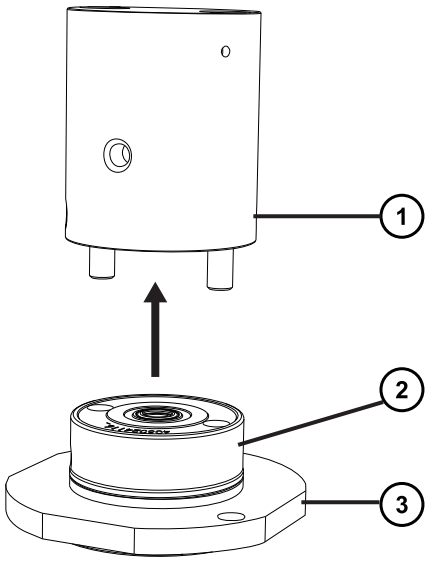
Figure 8–33: Releasing support plate from pump head (this image does not show the transducer)



1. Head bolts
2. Support plate

3. Lift the pump head from the support plate.

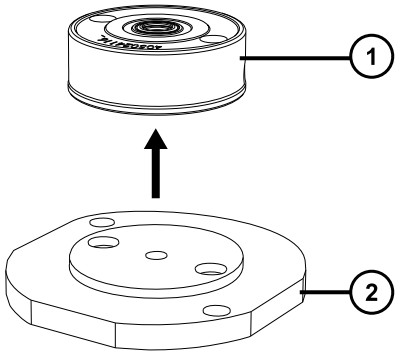
Figure 8–34: Lifting pump head from support plate



1. Pump head
2. Seal wash housing
3. Support plate

4. Lift the seal wash housing from the support plate.

Figure 8–35: Lifting seal wash housing from support plate

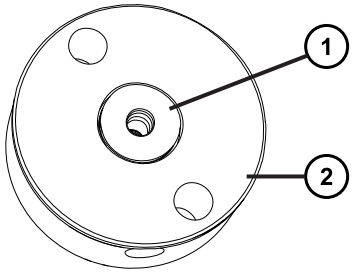


1 Seal wash housing

2 Support plate

5. Remove the old seal wash seal from the seal wash housing and discard the old seal.

Figure 8–36: Seal wash seal



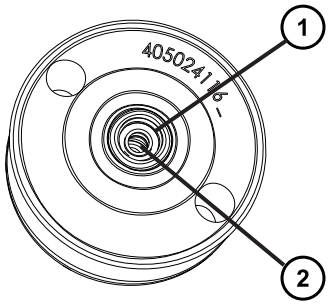
1 Seal wash seal

2 Seal wash housing

Notice: To avoid scratching any metal surfaces, use care when screwing the threaded end of the seal removal tool into the plunger seal.

6. Taking care not to scratch any surfaces, screw the threaded end of the seal extraction tool into the plunger seal on the reverse side of the seal wash housing, and then carefully withdraw the plunger seal and back-up ring.

Figure 8–37: Plunger seal location in seal wash housing



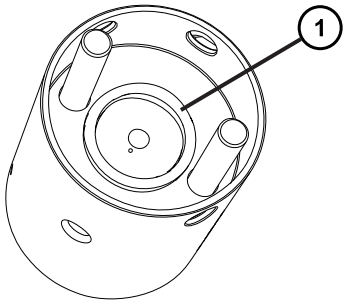
1 Plunger seal and back-up ring

2 Insert threaded end of seal extraction tool here

7. Use a dental pick or sharp tool to remove the O-ring.

Notice: To avoid scratching any metal surfaces, use care when using a sharp tool to remove the O-ring.

Figure 8–38: O-ring location in pump head



1 O-ring

8.8.14.5 Installing the new pump head seals

Replace pump components annually in accordance with the Waters preventive maintenance (PM) schedule, or any time they appear damaged to prevent a disruption in your workflow.

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.

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

Note: If necessary, you can also replace the seal wash housing during this procedure.

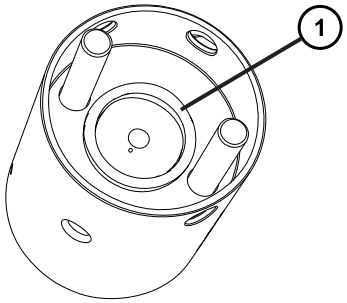
Required tools and materials

- Chemical-resistant, powder-free gloves
- T27 TORX driver
- Seal-extraction tool
- Methanol
- Fluoropolymer O-ring
- Plunger seal and plunger seal back-up ring
- Seal wash housing (if replacing)
- Seal wash seal

To install new pump head seals:

1. Lubricate the new O-ring with methanol and press the O-ring into its seat with your thumbs.

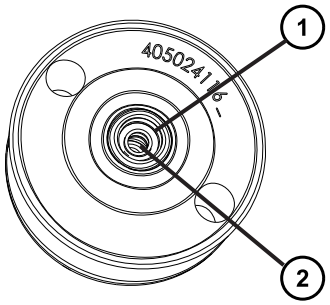
   Figure 8–39: O-ring installed in pump head

   1 O-ring

2. Lubricate the new plunger seal with methanol.
3. To install the pump head seals:
   a. Insert the smooth end of the seal extraction tool into the plunger seal back-up ring and place the back-up ring in the reverse side of the seal wash housing.
   b. Insert the smooth end of the seal extraction tool into the plunger seal and place the seal in the reverse side of the seal wash housing.

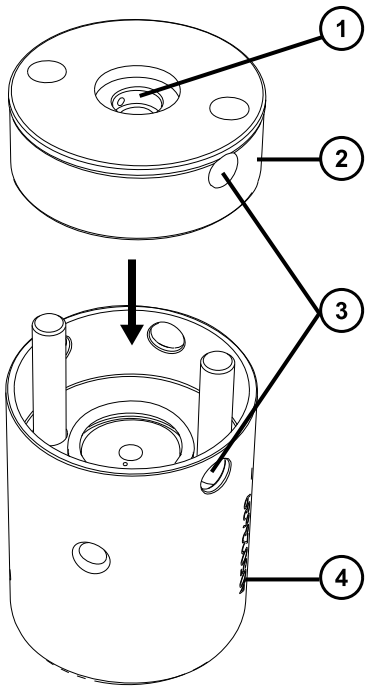
Figure 8–40: Plunger seal location in seal wash housing



1. Plunger seal and back-up ring
2. Insert threaded end of seal extraction tool here

4. With the seal wash seal cavity facing upward, orient the seal wash housing so that the holes on its side align with the holes on the side of the pump head, and then guide it into place.

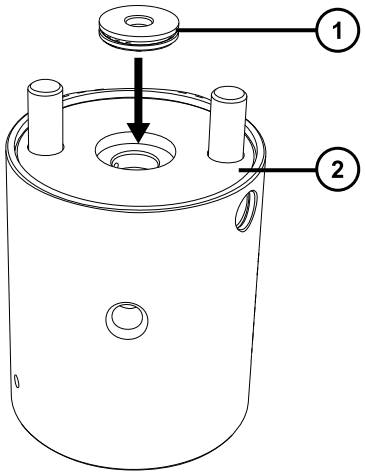
Figure 8–41: Installing seal wash housing in pump head



1. Seal wash seal cavity
2. Seal wash housing
3. Holes aligned
4. Pump head

5. Press the seal wash seal into the cavity gently with the soft side of your thumb.

Figure 8–42: Installing seal wash seal in seal wash housing

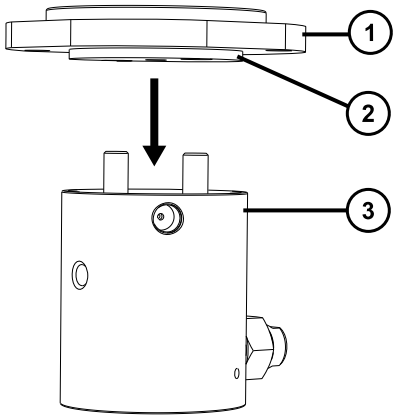


1 Seal wash seal

2 Seal wash housing

6. Place the support plate on top of the pump head, ensuring that the support plate's smaller boss is facing toward the pump head and the round side of the plate is oriented toward the bottom side of the pump head.

Figure 8–43: Support plate smaller boss facing toward pump head

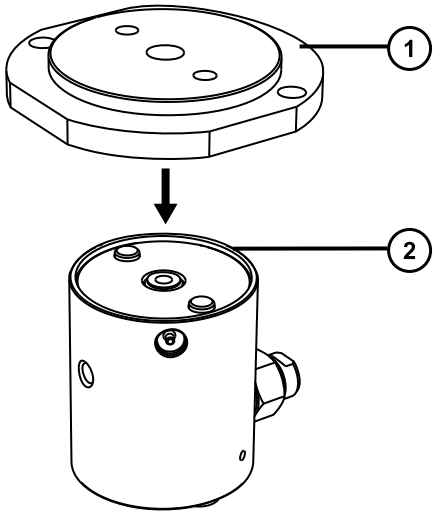


1 Support plate

2 Smaller boss on support plate

3 Pump head

Figure 8–44: Round side of support plate oriented toward bottom side of pump head

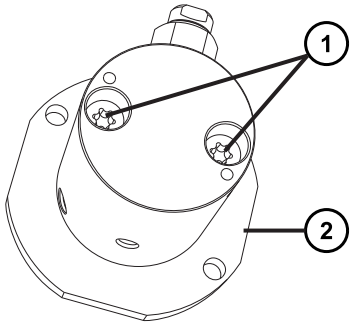


1 Round side of support plate

2 Bottom side of pump head

7. Holding the assembly together, use a T27 TORX driver to fully tighten the pump head screws, and then loosen 1/2 turn.

Figure 8–45: Securing support plate to pump head



1 Head bolts

2 Support plate

Recommendation: Replace the plunger whenever you replace the plunger seal.

8.8.14.6 Installing the new pump head plunger

Replace pump components annually in accordance with the Waters preventive maintenance (PM) schedule, or any time they appear damaged to prevent a disruption in your workflow.

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.

Recommendation: Replace the plunger when you replace the plunger seals.

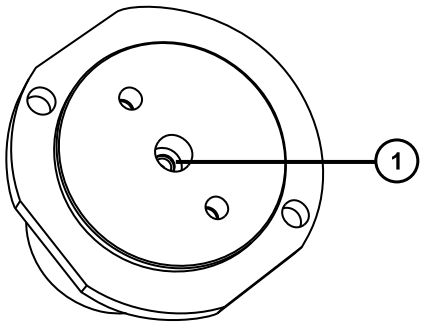
Required tools and materials

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

To install the new pump head plunger:

1. Flip the pump head assembly over, and then fill the seal cavity with methanol.

   Figure 8–46: Seal cavity location in pump head assembly

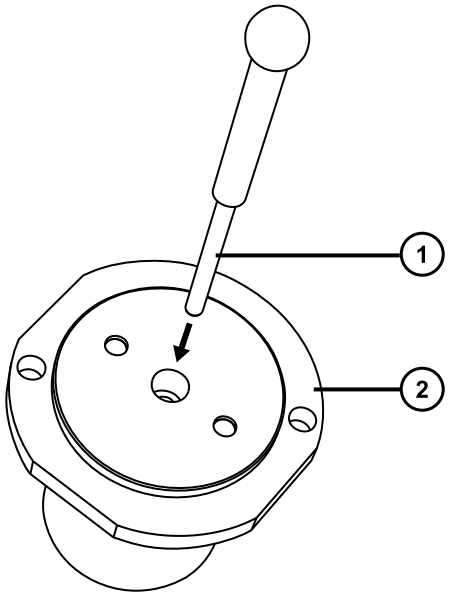
   

   1 Seal cavity

2. For this step, you must ensure that the sapphire part of the plunger does not touch any stainless steel surfaces in your lab (for example the wash housing, pump head, or a work surface like a lab bench). Using caution, position the plunger so that it is perpendicular to your work area, and then insert the sapphire part of the plunger into the seal cavity.

   Requirement: You must ensure that the sapphire part of the plunger does not touch stainless steel surfaces in your work space.

Figure 8–47: Inserting plunger (sapphire part) into pump head



1 Plunger (sapphire part)

2 Support plate

8.8.14.7 Reinstalling the primary pump head or accumulator pump head

Replace pump components annually in accordance with the Waters preventive maintenance (PM) schedule, or any time they appear damaged to prevent a disruption in your workflow.

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.

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

Note: If necessary, you can also replace the pump head during this procedure.

Required tools and materials

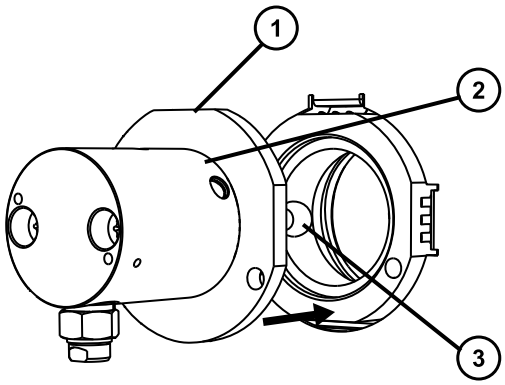
- Chemical-resistant, powder-free gloves
- 1/4-inch open-end wrench
- T27 TORX driver
- Pump head (if replacing)

To reinstall the pump head:

Notice: To avoid damaging the plunger, ensure that the pump head assembly is not tilted relative to the actuator housing when you position it on the mechanism.

1. Verify that the actuator piston is moved backward. If not, complete Moving the pump plunger backward (Page 136), and then continue to the next step.
2. Carefully slide the head assembly and plunger into the actuator piston, ensuring that you do not tilt the head.

Figure 8–48: Installing pump head, support plate, and plunger on the actuator piston



1 Support plate

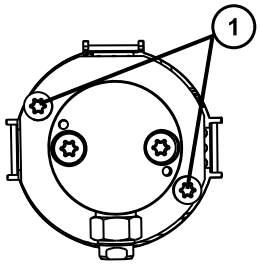
2 Pump head

3 Plunger

Notice: To avoid damaging the plunger, alternately tighten the support plate screws 1/4-turn so that they are uniformly torqued.

3. Hold the pump head assembly securely against the actuator housing, and then use the T27 TORX driver to tighten the support plate bolts securely.

Figure 8–49: Support plate bolts on pump head



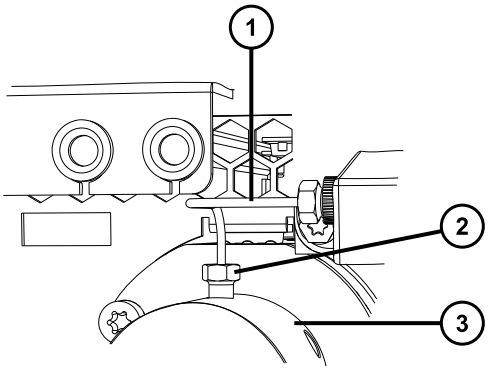
1 Support plate bolts

4. Reattach the outlet tubing fitting to the pump head:

a. Finger-tighten as tight as possible.

b. Use the 1/4-inch open-end wrench to tighten the fitting an additional 1/6-turn for existing fittings or 3/4-turn for new fittings.

Figure 8–50: Outlet tubing fitting on pump head



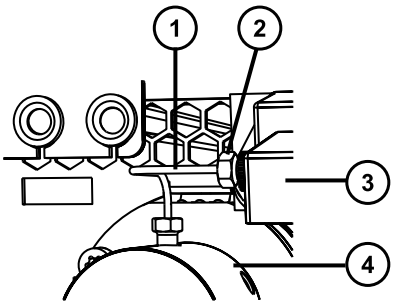
1 Outlet tubing

2 Outlet tubing fitting

3 Primary pump head

5. Using the 1/4-inch open-end wrench, tighten the fitting that secures the outlet tubing to the transducer.

Figure 8–51: Tightening fitting that secures outlet tubing to transducer



1 Outlet tubing

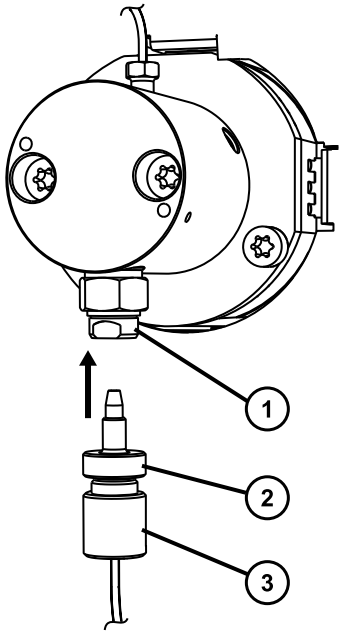
2 Outlet tubing fitting (tighten this fitting)

3 Transducer

4 Primary pump head

6. Insert the ferrule holder fitting into the primary check valve, ensuring that the tubing loops behind the check valve, and finger-tighten the ferrule holder fitting.

Figure 8–52: Inserting inlet filter fitting and cap nut into primary check valve

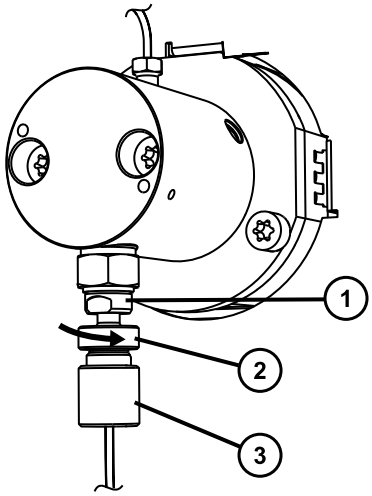


1 Primary check valve

2 Inlet filter fitting

3 Cap nut

Figure 8–53: Tightening inlet filter fitting and cap nut on primary check valve



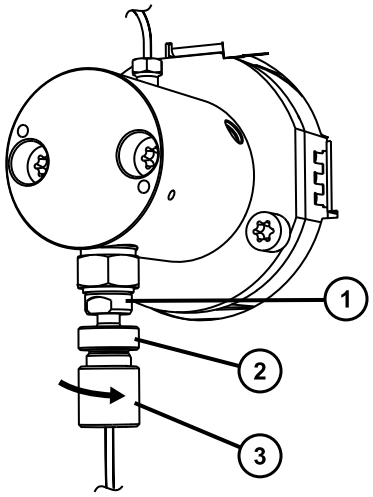
1 Primary check valve

2 Inlet filter fitting

3 Cap nut

7. Finger-tighten the cap nut as tight as possible.

Figure 8–54: Tightening cap nut on inlet filter fitting



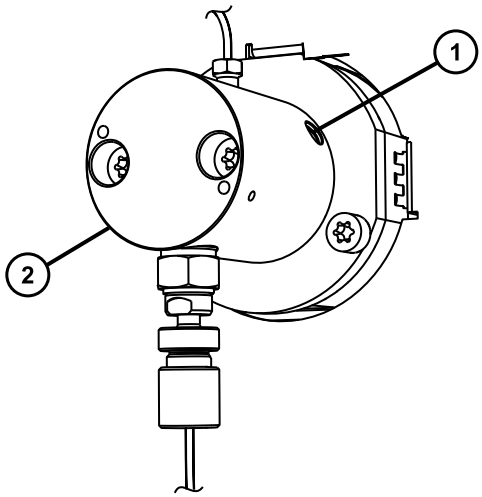
1 Primary check valve

2 Inlet filter fitting

3 Cap nut

8. Reinstall the seal wash tubing on the barbed fittings on the seal wash housing.

Figure 8–55: Location of seal wash tubing



1 Location of seal wash tubing

2 Location of seal wash tubing

8.8.15 Replacing the solvent bottle filters

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 to be observed.

Solvent bottle filters are critical clean parts made of stainless steel to protect your system from contamination.

Required tools and materials

- Chemical-resistant, powder-free gloves
- Protective eyewear
- Solvent bottle filter, 7/pk (P/N 700003616)

To replace the solvent bottle filters:

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

   Note: Do not remove the solvent tubing from the solvent bottle cap.
3. Insert the new solvent reservoir filter into the fluoropolymer tubing, pushing until it contacts the solvent tubing.

   Note: Titanium solvent reservoir filters are identified by "Ti" on the top surface of the filter.
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. Be sure to submerge the entire filter in the solvent.
7. Prime the solvent manager.

8.9 Autosampler maintenance

The customer can perform the following autosampler maintenance tasks:

-

8.9.1 Recommended autosampler routine maintenance schedule

Maintenance procedure	Frequency
Replace the leak sensor	As needed
Replace the seal	During scheduled routine maintenance or as needed

Maintenance procedure	Frequency
Replace the sample needle and needle guide	When its capacity or material compatibility doesn't suit your chromatographic needs, or as needed
Replace the sample syringe	During scheduled, routine maintenance, when its capacity doesn't suit your chromatographic needs, or as needed
Replace the wash syringe	During scheduled routine maintenance or as needed
Clean the injection port	As needed
Replace the injection valve cartridge	During scheduled routine maintenance or as needed
Clean the instrument with a soft, lint-free cloth, or paper dampened with water	As needed

8.9.2 Washing the needle's exterior

You can access the needle wash function through the console.

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

To wash the needle's exterior:

1. In the console, select the sample manager, and then click **Control** > **Wash Needle**.

   Alternative: In the sample manager control panel of the data application, right-click, and then click **Wash Needle**.

2. In the Needle Wash box, specify the wash duration, in seconds.

   Table 8–1: Needle wash parameter values

Solvent	Range	Default
Wash solvent	1 to 99 seconds	30 seconds

   Tip: The flow rate of the wash solvent is approximately 10 to 20 mL/min, with 90:10 water/acetonitrile. The wash flow rate varies with the viscosity of the solvent.

3. Click **OK**.

   Result: The needle wash begins. When it ends, the status returns to idle.

8.9.2.1 Stopping a needle wash routine before it finishes

You can interrupt an active needle wash routine through the console.

To stop a needle wash routine before it finishes:

In the sample manager information window, click **Control** > **Reset SM**.

Alternative: In the sample manager control panel of the data application, right-click, and then click **Reset SM**.

8.9.3 Calibrating the needle z axis

Callibrate the autosampler needle before using it for the first time.

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.

Required tools and materials

- Business card

To calibrate the needle, do the following:

1. Click **Maintain** > **Calibrate needle Z axis**.
2. Open the sample manager door.
3. Remove the plates from the trays.
4. Place the business card in the center of the tray.
5. Verify that the plates are removed and the business card is in place, and then click **Next**.
6. When ready, click **Move needle**.

   The needle moves to the initial position.
7. When the needle is in position, click **Next**.
8. Move the needle down by clicking the down arrow. Stop when the needle almost touches the card.
9. Select the **Needle is in position, almost touching the card** checkbox.
10. To save the calibration, click **Next**.
11. Optionally, print the calibration report, which contains the Z offset value.
12. Click **Finish**.

8.9.4 Replacing the needle

Replace the needle annually during the prescribed preventive maintenance (PM) schedule 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 return line > not return line (Page 169) 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

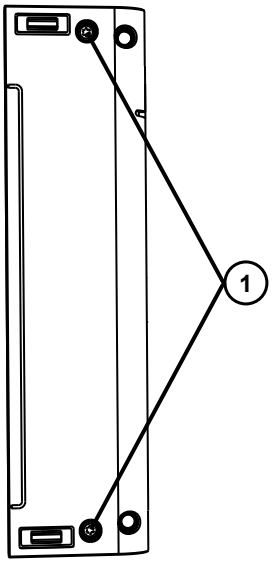
- Needle kit (replacement part)
- Chemical-resistant, powder-free gloves
- Protective eyewear
- T20 TORX driver

To replace the needle:

1. Power-on the sample manager.
2. Remove any sample plates from the sample compartment.
3. From the touchscreen, tap **Maintain** > **Replace Components > Replace Needle**.
4. Tap **NEXT**.
5. Open the sample compartment door and the fluidics compartment door.
6. Using the T20 TORX driver, loosen the captive screw that secures the access panel, and then remove the panel.

   Notice: Subsequent drafts will show the system with one screw as described in the caption.

Figure 8–56: Captive screw on access panel



1 Captive screw

7. Unscrew the needle from the finger-tight fitting, and then release it from the clip.

   Figure 8–57: Sample needle location

   Image will show the following:

   1 Sample needle

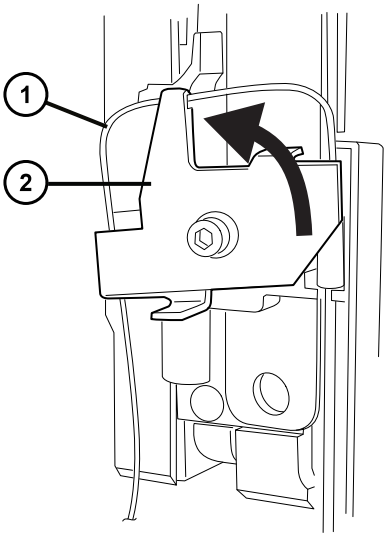
   2 Finger-tight fitting

   3 Clip

8. Locate the two needle latches (one on the needle carriage and one on the right side of the compartment). Push the needle latches back to release the needle mounting cylinder from its mounting cavity and the needle tubing from its notch.

   Notice: Subsequent drafts will show the system with two needle latches as described in the caption.

Figure 8–58: Two needle latches in closed position

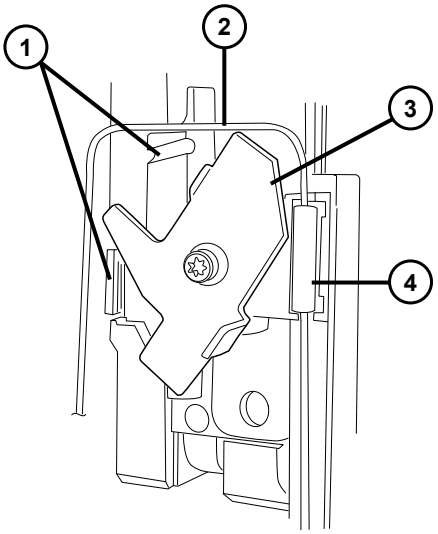


1 Needle tubing

2 Needle latches

Notice: Subsequent drafts will show the system with only the top notch as described in the caption.

Figure 8–59: Image to be updated (Needle latches/grooves are different) >>Needle latches in open position



1 Notch

2 Needle tubing

3 Latches

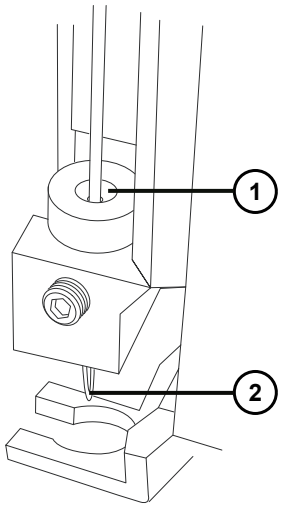
4 Ferrule

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.

9. Lift the needle tip from the piercing needle at the bottom of the needle mechanism, then and remove the needle assembly from the compartment.

Figure 8–60: Needle tip in piercing needle



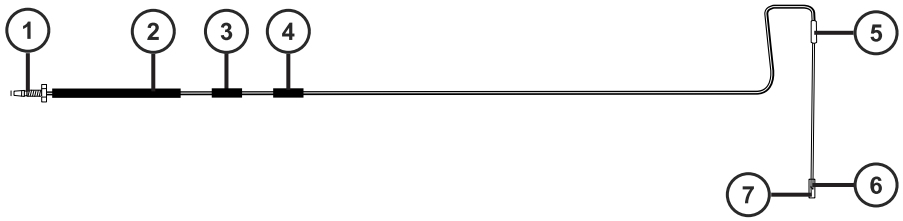
1 Piercing needle

2 Needle tip

10. Install the new needle guide conical side up and tighten the set screw.
11. Remove the protective sleeve from the needle tip.

Notice: Subsequent drafts will show the new needle.

Figure 8–61: Need new pic >> Sample needle assembly



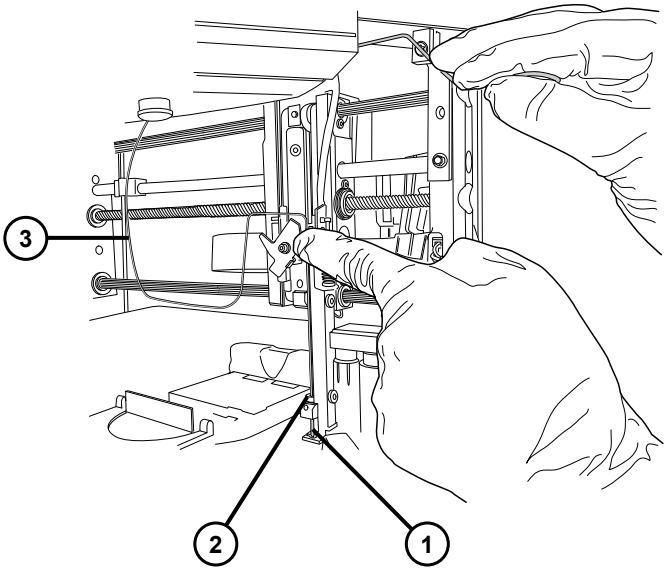
1. Fitting
2. ID sleeve
3. HPS identifier
4. Mounting sleeve
5. Needle mounting cylinder
6. Needle tip
7. Protective sleeve

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.

12. While holding the needle assembly in both hands to control its position within the sample compartment, insert the needle tip into the piercing needle at the bottom of the needle mechanism, forming a loop with the tubing that extends back into the sample compartment, as shown below.

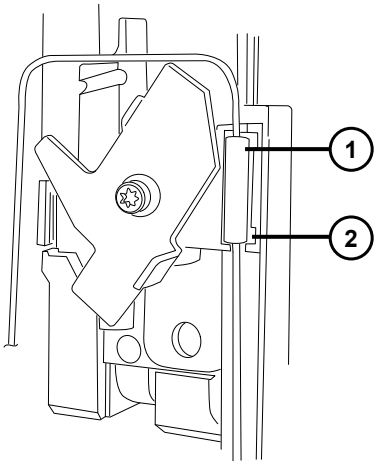
Figure 8–62: ***Once available, will replace with a new image and step for updated system >>Installing needle assembly in sample compartment



1. Needle tip
2. Piercing needle
3. Needle tubing loop at the back of the sample compartment

13. Insert the ferrule into the mounting cavity.

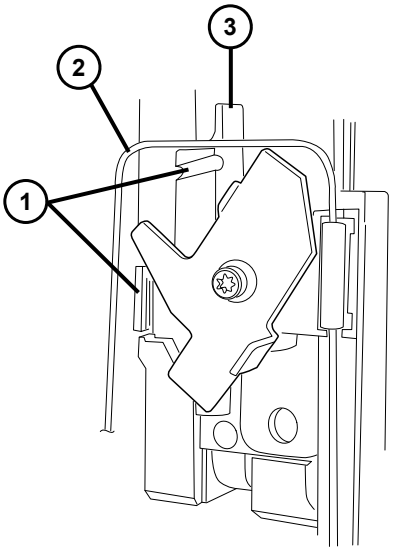
Figure 8–63: ***Once available, will replace with a new image and step for the updated system >>Ferrule in mounting cavity



1. Ferrule
2. Mounting cavity

14. Route the needle tubing through the two notches below the Z-flag.

Figure 8–64: ******Once available, will replace with a new image and step for the updated system

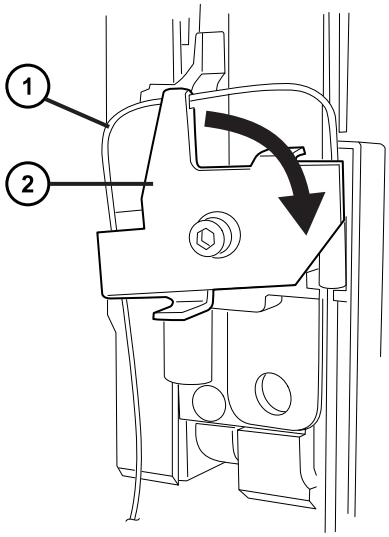


1. Notches
2. Needle tubing
3. Z-flag

15. Close the needle latch, as shown here, to secure the needle assembly.

> **Notice:** Subsequent drafts will show the system with one needle latch as described in the caption.

Figure 8–65: Needle latch in closed position

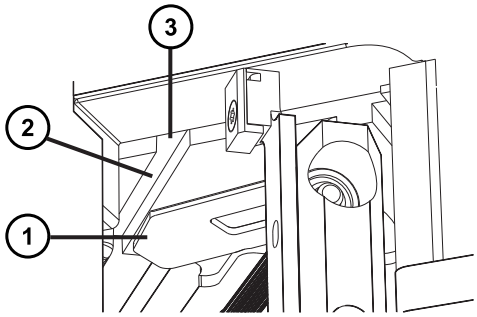


1 Needle tubing

2 Needle latch

16. ***Once available, will replace with a new image and step for the updated system >>** Ensure that the needle tubing is routed to the left-hand side of the needle carriage's rails and that it is secured in the guide channel on the roof of the compartment.

Figure 8–66: Location of needle tubing in piercing needle



1 Needle tubing guide cover

2 Location of needle tubing

3 Needle-tubing guide channel

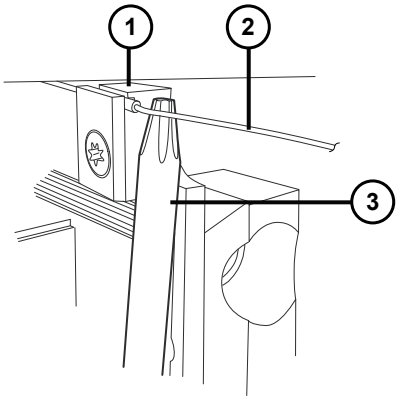
17. Secure the needle tubing in the (black) bracket on the side of the sample compartment. Lower the needle latch.
18. ***Once available, will replace with a new step for the updated system**Using the T10 TORX driver, tighten the screw on the needle tubing clamp.

Note: You might need to slightly rotate or twist the needle to ensure that it routes straight down the channel before tightening the screw on the needle tubing clamp.

19. ***Need new image and step for the updated system >>** At the needle tubing clamp, bend the needle to the right toward port 4 on the injection valve.

    Tip: Use a small, rounded tool (such as a T10 TORX driver) to aid in bending the tubing.

    Figure 8–67: Bending needle tubing at needle tubing clamp



    1 Needle tubing clamp

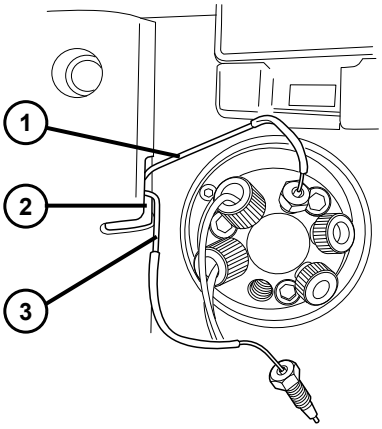
    2 Needle tubing

    3 Rounded tool

20. Reinstall the access panel and use the T20 TORX driver to tighten the two screws that secure the panel to the front of the unit.

    Requirement: Ensure that the seal port tube and the needle tubing are routed through the gap in the access panel.

    Figure 8–68: Tubing routed through access panel gap



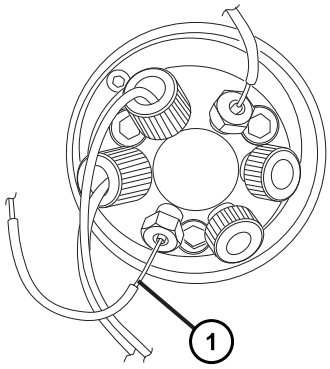
    1 Seal port tube

2 Access panel gap

3 Sample needle tubing

21. Using a rounded tool (such as the shaft of the T20 TORX driver), make a bend and connect the needle tubing to the extension loop port or, if the extension loop is not installed, to port 4 on the injection valve.

Figure 8–69: Needle tubing installed on injection valve



1 Needle tubing

22. Ensure that the needle tubing is fully inserted into port 4 on the injection valve, and then thread the fitting into the port.
23. While holding the needle tubing against the bottom of the port, finger-tighten the compression screw, and then use the 1/4-inch open-end wrench to tighten an additional 3/4-turn.

    Note: Failure to fully bottom the needle tubing and ferrule could cause carryover and poor chromatography.

    See also: If you are using an extension loop, to properly connect to port 4, refer to the appropriate procedure.
24. Close the sample compartment door and the fluidics compartment door.
25. Calibrate the needle.

    Recommendation: Replace the needle seal whenever you replace the needle.
26. You must complete the needle seal readiness test to verify that the needle seal works properly. Follow the steps outlined in Running the needle seal readiness test (Page 204).
27. Waters recommends that you replace the needle seal each time you replace the sample needle (see Replacing the needle seal and return line > not return line (Page 169).

8.9.5 Replacing the needle seal and return line > not return line

To prevent a disruption in your workflow, replace the needle seal and return line annually during the prescribed preventive maintenance (PM) schedule or any time the seal appears dirty, contaminated, or clogged.

From Thursday 3/2 GSS mtg >>

return line is ASSY, SEAT PORT, SST, .007 ID,

Replacing the needle seal and the return line involves removing the following:

- Wash station assembly
- Locking nut that houses the needle seal
- Existing needle seal
- Existing return line (attached to the wash station with the locking nut)

Note: Replace the needle seal each time you replace the sample needle.

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

- Replacement parts needed:
  - Seal kit
  - Return line
- 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:

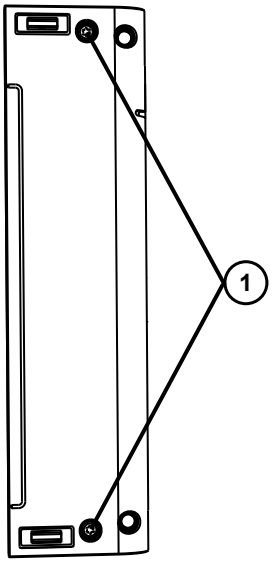
1. From the touchscreen, tap **Maintain** > **Replace components** > **Replace Needle Seal**.
2. Review the Welcome page for important information, and then Tap **NEXT**.
3. Review the important safety notices, and then tap **NEXT**.
4. Review the required preconditions, and then tap **START**.

Result: The needle carriage moves toward the back of the sample compartment.

5. Open the sample compartment door and the fluidics compartment door.
6. Using the T20 TORX driver, loosen the captive screw that secures the access panel, and then remove the panel.

Notice: Image to be updated when showing the one screw (see caption).

Figure 8–70: Captive screw on access panel

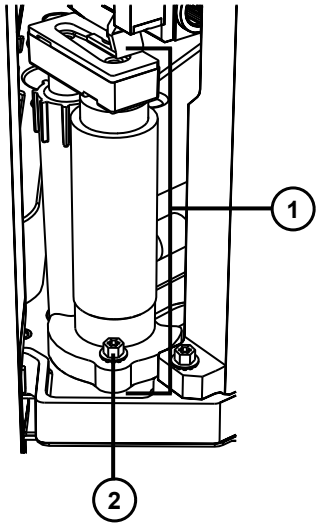


1 Captive screw

7. Remove the two pieces of tubing on the wash station, and then remove the tubing from the injector.

Notice: Image to be updated showing the updated tubing.

Figure 8–71: Location of tubing on the wash station



1. Wash station assembly
2. Wash station tubing
3. Injector

8. To remove the wash station assembly, push down on it, and then rotate and lift up to release. Remove the wash station.

> **Notice:** Image to be added showing the wash station and holder.

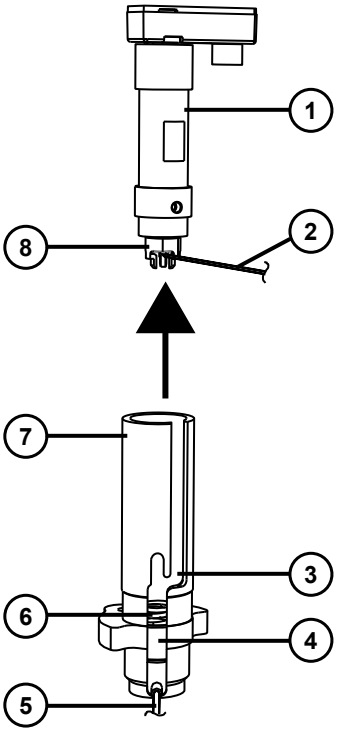
Figure 8–72: Remove the wash station from the holder

1. Wash station
2. Wash station holder

9. Slide the support sleeve from the wash station housing and guide the seal port tube through the slots.

> **Notice:** Image to be updated showing an updated image with no load cell/cable and an updated locking nut.

Figure 8–73: Sliding support sleeve out of the housing

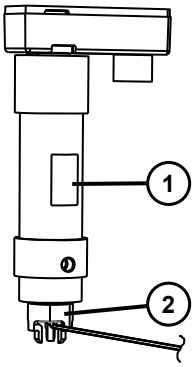


1. Support sleeve
2. Seal port tube
3. Slot
4. Spring cup
5. Load cell cable **(Not in Beta system)**
6. Spring
7. Housing
8. Locking nut

10. Locate the locking nut attached to the bottom of the wash station 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.

Notice: Image to be updated showing an updated locking nut.

Figure 8–74: Wrench placement locations to remove the locking nut

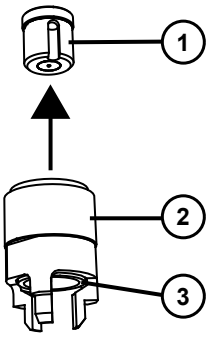


1. Place one 7/16-inch open-end wrench here on the support sleeve.
2. Place the other 7/16-inch open-end wrench here on the locking nut.

11. Lift the outer edge (or lip) of the locking nut. Tilt the locking nut to remove the seal from the seal port, and then discard the seal.

Notice: Image to be added when showing an updated locking nut and seal port.

Figure 8–75: Removing the seal from seal port



1. Seal
2. Locking nut
3. Seal port

12. Waters recommends replacing the return line when you replace the needle seal. To remove the return line:
    a. Remove one end of the return line from the inject valve.
    b. Unscrew the finger-tight fitting.
    c. Thread the return line through the locking nut, and then remove it.

Figure 8–76: Return line location in the locking nut >> insert image from Arc OMG

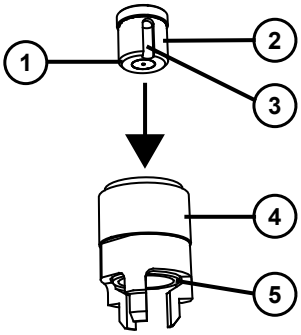
1. Return line
2. Locking nut

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

13. Insert the new seal into the seal port. The seal is keyed, ensuring its correct installation, as shown in the following image.

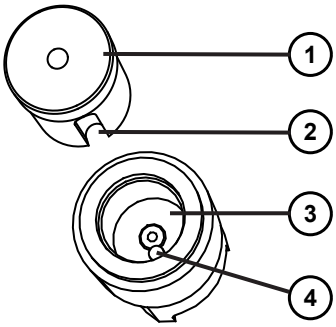
Notice: Image to be added showing an updated locking nut and seal port.

Figure 8–77: Inserting new seal into seal port



1. Smaller-diameter end
2. Seal
3. Notch
4. Locking nut
5. Seal port

Figure 8–78: Seal notch

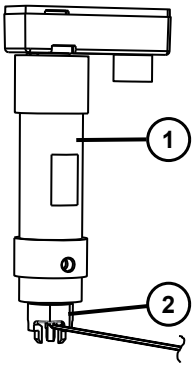


1. Seal
2. Notch
3. Seal cup
4. Pin (Subsequent drafts will not include this pin)

14. Finger-tighten the locking nut into the bottom of the wash station.

Notice: Image to be added showing an updated locking nut and seal port.

Figure 8–79: Locking nut on the washing station

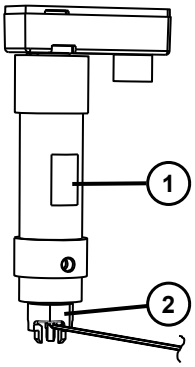


1. Washing station support sleeve
2. Locking nut

15. Place the two 7/16-inch open-end wrenches on the washing station support sleeve and tighten.

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

Figure 8–80: Wrench placement locations

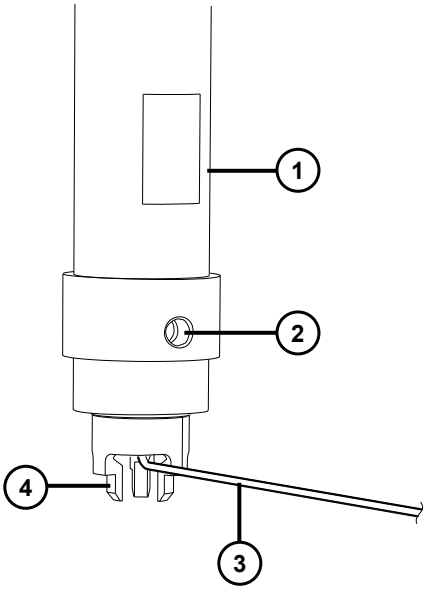


1. Place one 7/16-inch open-end wrench here
2. Place the other 7/16-inch open-end wrench here

16. Ensure that the seal port tube remains in line with the threaded hole in the support sleeve.

> **Notice:** Image to be updated showing an updated seal port tube, locking nut and seal port.

Figure 8–81: Seal port tube aligned with threaded hole

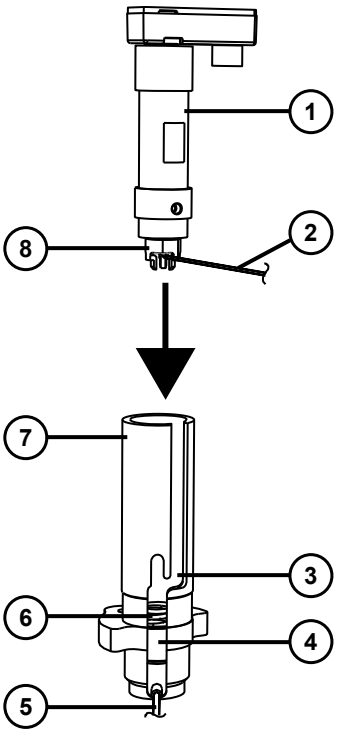


1. Support sleeve
2. Threaded hole
3. Seal port tube (not bent as shown, will update per Beta system)
4. Step in locking nut

17. Slide the seal port tube into the slot on the side of the housing.

> **Notice:** This image shows a load cell cable. Image to be updated showing no load cell cable and an updated locking nut and seal port. The seal port now has a groove cut into it for the tubing and also has the prongs on it. The nut no longer has the prongs for the spring.

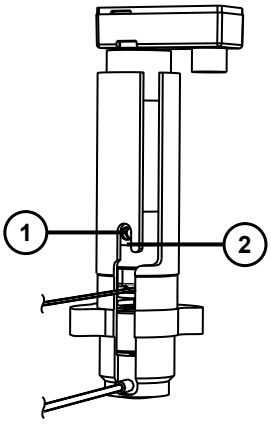
Figure 8–82: Sliding seal port tube into slot



1. Support sleeve
2. Seal port tube
3. Slot
4. Spring cup
5. Load cell cable **(Not in Beta system)**
6. Spring
7. Housing
8. Locking nut

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

Figure 8–83: Support sleeve in housing



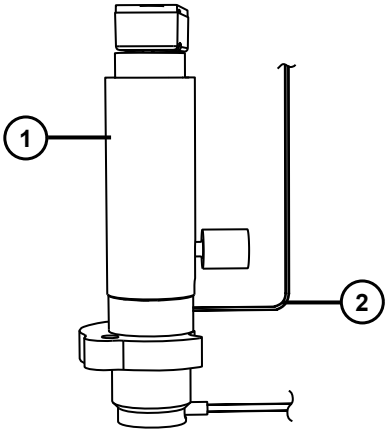
1 Fitting hole

2 Slot

19. Ensure that the seal port tube is bent upward at a 90-degree angle, and that it is approximately 2.5 cm (1.0 inch) from the housing.

    Note: To avoid binding the seal port tube, ensure that the tube does not contact either side of the inject/wash station assembly.

Figure 8–84: Seal port tube bent upward at right angle



1 Housing

2 Seal port tube bent upward

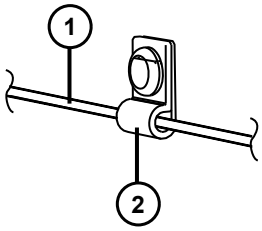
20. Adjust the amount of tubing in the sample compartment by sliding it in and out of the foam.

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

21. Route the wash tubing through the clip on the inside of the sample compartment.

Notice: Image to be added showing an updated clip.

Figure 8–85: Wash tubing secured by clip on sample compartment wall

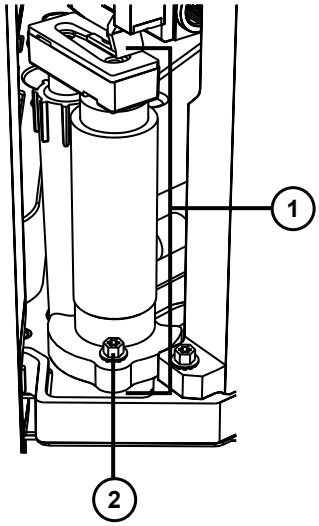


1 Wash tubing

2 Clip

22. Using your fingers, tighten the PEEK fitting that secures the wash station to the sample compartment floor.

Figure 8–86: Wash station assembly



1 Wash station

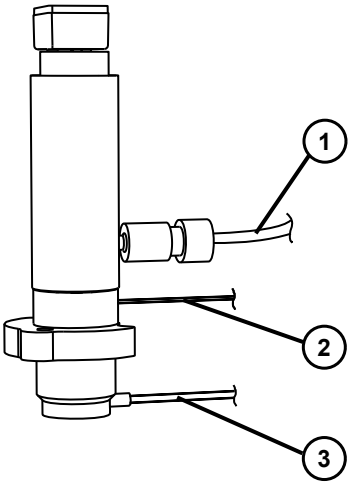
2 PEEK fitting

23. Using gentle radius bends, route the seal port tube up the right-side edge of the sample compartment, exiting behind the sample needle tubing and to the right.

Requirement: Ensure that the cable emerges from the injection port assembly without any tight bends.

Notice: This image shows a load cell cable. Image to be updated showing no load cell cable, and two connections to the wash station.

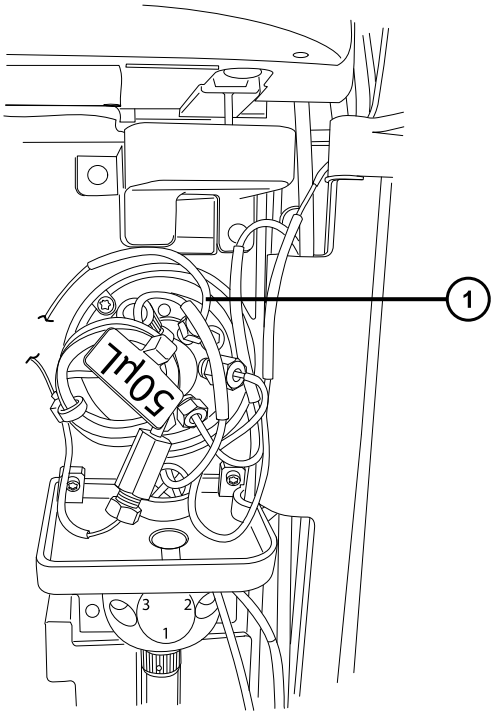
Figure 8–87: Tubing and cable locations



1. Wash tubing
2. Seal port tube
3. Load cell cable **(Not in Beta system)**

24. Screw the seal port's fitting 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 8–88: Seal port tube location on injection valve

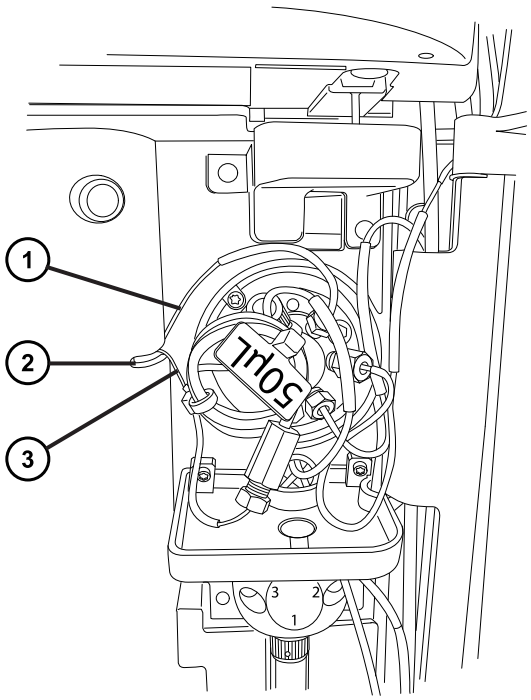


1 Seal port tube

25. Reinstall the access panel, and then use the T20 TORX driver to tighten the one screw to secure the panel to the front of the unit.

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

Figure 8–89: Tubing routed through access panel gap



1. Seal port tube
2. Access panel gap
3. Needle tubing

26. Close the sample compartment door and the fluidics compartment door.
27. You must complete the needle seal readiness test to verify that the needle seal works properly. Follow the steps outlined in Running the needle seal readiness test (Page 204).

8.9.6 Replacing the inject valve

Provide context for your task here (optional).

Enter your first step here.

8.9.7 Replacing the sample syringe

Provide context for your task here (optional).

Enter your first step here.

8.9.8 Cleaning the injection port

Provide context for your task here (optional).

Enter your first step here.

8.10 Detector maintenance

The customer can perform the following detector maintenance tasks:

•

8.10.1 Replacing the detector's leak sensor

The detector has a leak sensor in the drip tray that monitors the detector for leaks. The sensor stops system flow when it detects accumulated, leaked liquid in its reservoir. The kiosk displays an error message when the sensor detects a leak.

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

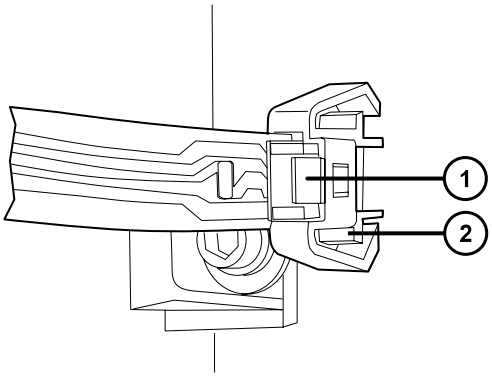
Required tools and materials

- Chemical-resistant, powder-free gloves
- Leak sensor

To replace the detector leak sensor:

1. Open the detector door, gently pulling its right-hand edge toward you.
2. Press down on the tab to detach the leak sensor connector from the front of the instrument.

Figure 8–90: Detaching the leak sensor

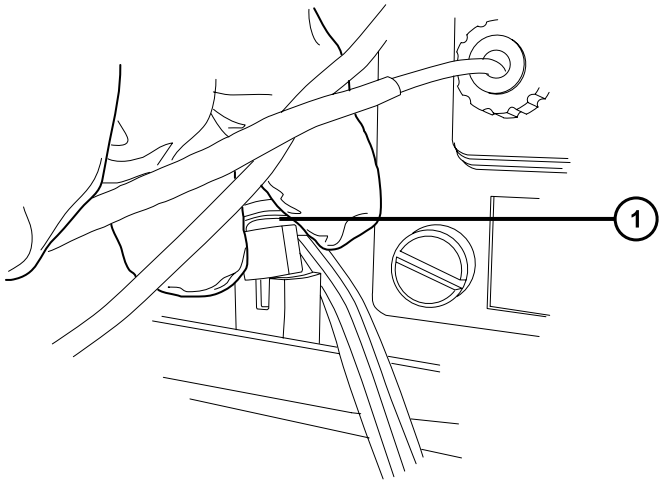


1 Press down on the tab to release the connector

2 Leak sensor connector

3. Remove the leak sensor from its reservoir by grasping it by its serrations and pulling upward on it.

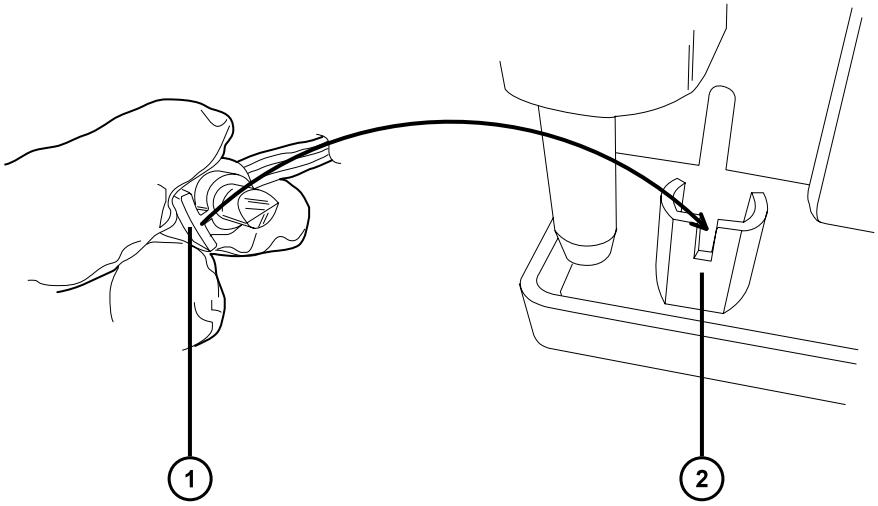
Figure 8–91: Removing the leak sensor



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.

Figure 8–92: Align the leak sensor T-bar with the slot on the leak sensor reservoir

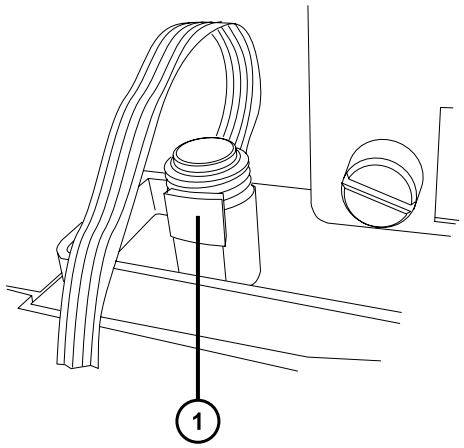


1 T-bar

2 Slot on the leak sensor reservoir

6. Slide the leak sensor into place.

Figure 8–93: Leak sensor installed in leak sensor reservoir



1 Leak sensor installed

7. Plug the leak sensor connector into the front of the instrument.
8. In the console, select your detector from the system tree.
9. In the detector information window, click **Control** > **Reset** to reset the detector.

8.10.2 Replacing the flow cell

To prevent system errors, replace the flow cell annually during the prescribed preventive maintenance (PM) schedule or any time it appears dirty, contaminated, or clogged.

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

Required tools and materials

- 1/4-inch flat-blade screwdriver
- Chemical-resistant, powder-free gloves

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.

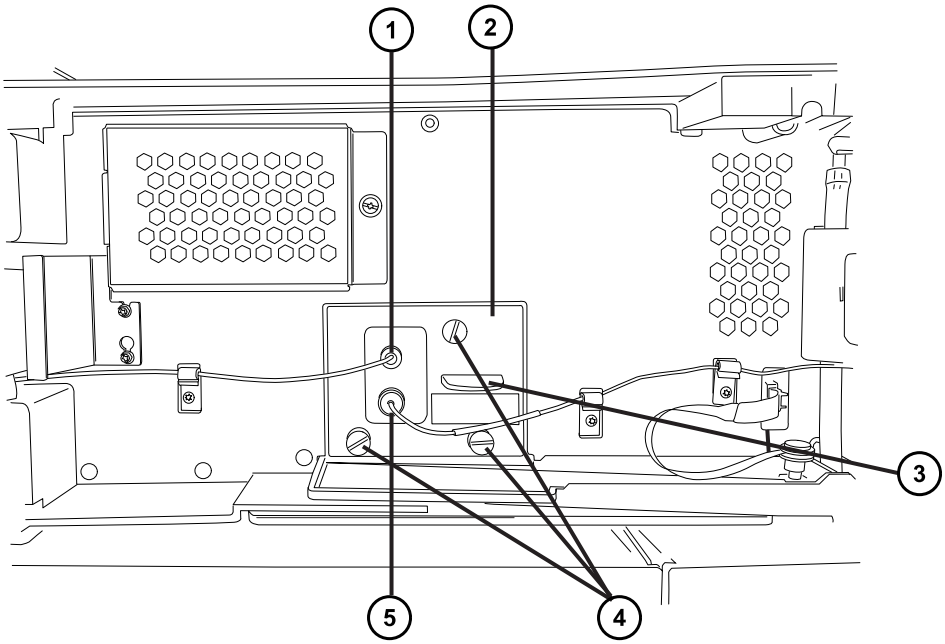
To replace the flow cell:

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.

1. Use the touchscreen to set the following preconditions:
   - Power-off the lamp.
   - Stop the solvent flow.
   - Place the detector in low-power state.

   From the touchscreen tap **Maintain** > **Replace Components** > **Replace Flow Cell**.
2. Tap **NEXT** until you view the Preconditions option, and then tap **START** to set the preconditions.
3. Open the detector door, gently pulling its right-hand edge toward you.
4. Disconnect the detector's inlet and outlet tubing.

Figure 8–94: Flow cell location in the detector (front door opened)



1. Outlet tubing
2. Flow cell assembly
3. Flow cell handle
4. Three thumb screws
5. Inlet tubing

5. Remove the flow cell:
   a. Using a 1/4-inch, flat-blade screwdriver, loosen the three thumbscrews on the flow cell assembly's front plate.
   b. Grasp the handle and gently pull the assembly toward you.

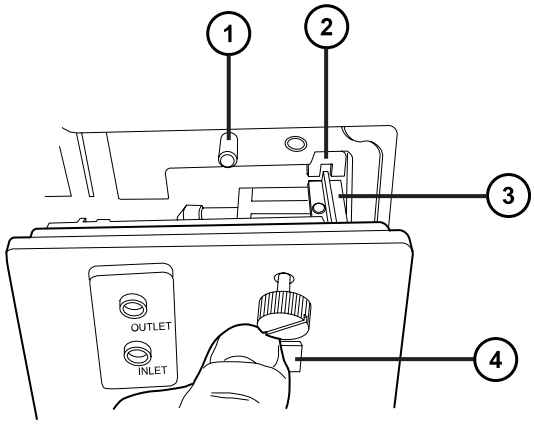
Note: To avoid damaging the capillary tubing, do not touch it.

6. 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.

7. Align the flow cell assembly with the front of the opening, and then insert it slowly so that the guides on the front part of the cell flange engage the rails in the sample cell compartment.

   Figure 8–95: Installing the flow cell assembly



1 Dowel pin

2 Rail

3 Guide

4 Flow cell handle

8. After the flange and rails are engaged, continue inserting the flow cell until the dowel pins on the instrument engage the corresponding holes on the cell holder.
9. Continue to insert the flow cell until the three thumbscrews align with their holes in the bulkhead.
10. Hand tighten the thumbscrews. Verify that the screws are secure using a screw driver.
11. Connect the inlet tubing to the main column connection and flow cell inlet, and then connect the outlet tubing to the flow cell outlet.
12. Before you power-on the detector, ensure that the flow cell is filled with degassed, transparent solvent (acetonitrile or water) and free of air bubbles.
13. Configure the new flow cell. From the touchscreen tap **Maintain** > **Replace Components** > **Replace Flow Cell**. Then tap **NEXT** and follow the onscreen instructions to configure the flow cell as required.

8.10.3 Replacing the lamp

Replace the lamp annually during the prescribed preventive maintenance (PM) schedule 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 into the Lamp Change Record table.

Note: Waters warrants 2000 hours of lamp life, or one year since 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.

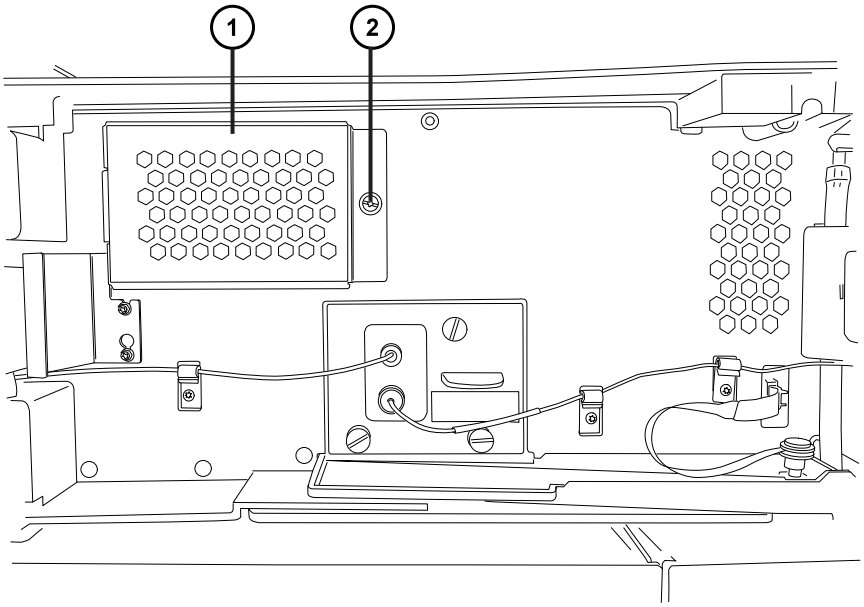
To replace the lamp:

Caution: When you start this procedure from the touchscreen, the software automatically powers-off the lamp (if not already powered-off). Ensure that you allow the lamp to cool for at least 30 minutes.

1. Use the touchscreen to set the following preconditions:
   - Power-off the lamp.
   - Stop the solvent flow.

   From the touchscreen tap **Maintain** > **Replace Components** > **Replace Lamp**.
2. Tap **NEXT** until you view the Preconditions option, and then tap **START** to set the preconditions.
3. Power-off the detector, and then disconnect the power cord.
4. Open the detector door.
5. Locate the lamp assembly cover (see the following figure).
6. To remove the lamp assembly cover, remove the one Philips head screw that attaches it to the detector (see the following figure).
7. Lift the lamp assembly cover off.

Figure 8–96: Lamp assembly cover

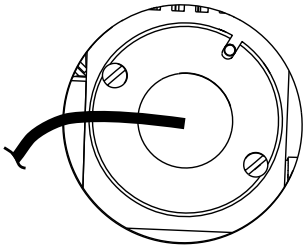


1 Lamp assembly cover

2 Phillips head screw

8. Disconnect power to the lamp, and then disconnect the cord.
9. Loosen the two captive screws in the lamp base.

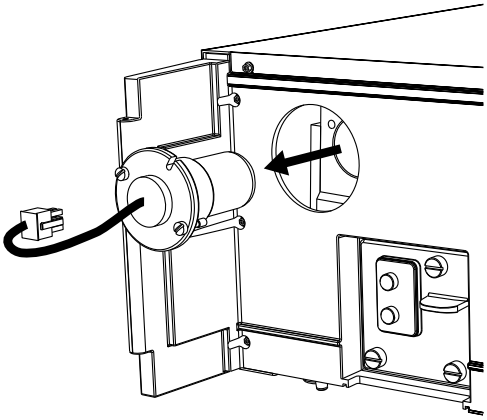
Figure 8–97: Lamp assembly base captive screws



10. Lift out the lamp assembly from the lamp housing.

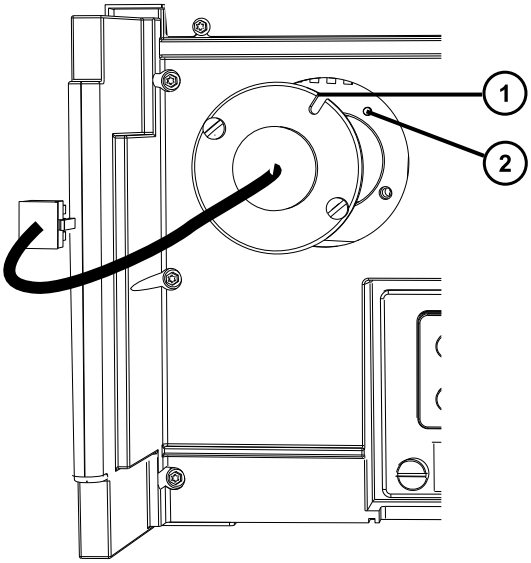
> **Notice:** To prevent shattering the glass, use care when disposing of the lamp because the lamp gas is under slight negative pressure.

Figure 8–98: Removing the lamp assembly



11. 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 8–99: Aligning the lamp



1. Cut-out on lamp base at 1 o’clock position
2. Alignment pin on lamp housing

12. Gently push the lamp forward until it bottoms into position.
13. Tighten the two captive screws.
14. Reconnect the lamp’s power connector.
15. Reinstall the lamp assembly cover using the one Philips head screw.
16. When ready to resume operation of the detector, reconnect the power cord and then power-on the unit.

8.11 Column heater maintenance

The customer can perform the following column heater maintenance tasks:

•

8.11.1 Recommended maintenance schedule for the column heater

Maintenance procedure	Frequency
Replace solvent filters	During scheduled routine maintenance or as necessary
Clean the air filter in the door	As necessary
Replace the air filter in the door	During scheduled routine maintenance or as necessary
Replace the leak sensor	As necessary
Replace the mixer in path 1	During scheduled routine maintenance or as necessary
Replace the optional mixer in path 2	During scheduled routine maintenance or as necessary
Replace the vent valve cartridge	As necessary
Replace the optional solvent selection valve cartridge	As necessary
Replace the primary check valve	During scheduled routine maintenance or as necessary
Replace the in-line filter cartridge on the primary check valve	During scheduled routine maintenance or as necessary
Replace the accumulator check valve	During scheduled routine maintenance or as necessary
Replace the pump-head seals and plungers	During scheduled routine maintenance or as necessary
Replace the pump head and seal-wash housing	As necessary
Clean the device's exterior with a soft, lint-free cloth, or paper dampened with water	As necessary

8.11.2 Replacing the column

To extend the life of a column, replace it annually during the prescribed preventive maintenance (PM) schedule or any time that you notice peak shape problems or loss of resolution.

If installing a Waters-branded column, this procedure requires no tools. You can easily replace it using the column clips and tool-free fittings. Replacing the column involves removing the following:

- Column that is installed
- Tubing
- Tool-free fittings

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.

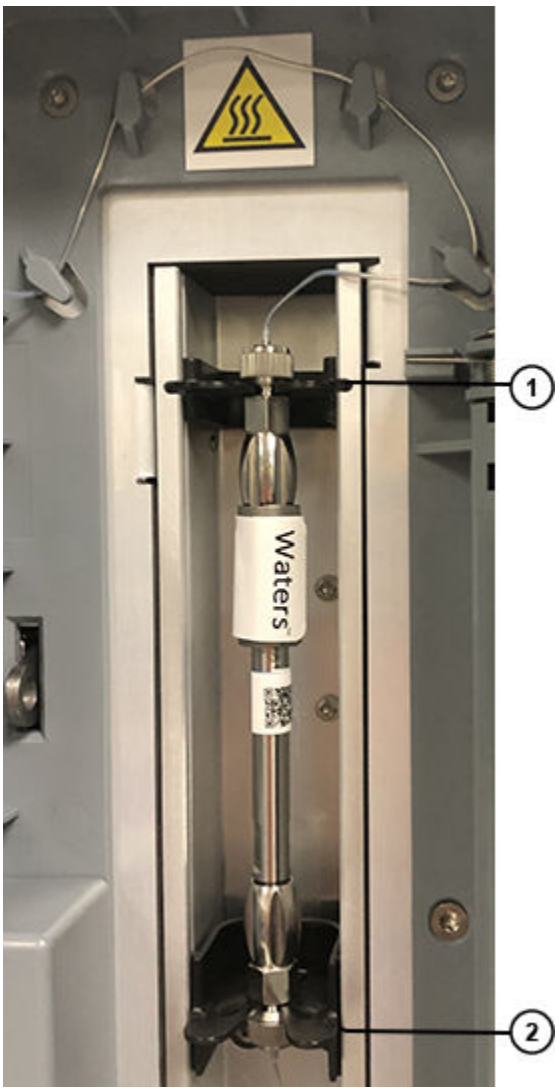
To remove the existing column:

Note: After you remove the column, determine if you will move it to storage to reuse at a later date. If moving to storage, you can flush the column from the touchscreen. Tap **Maintain** > **Replace components** > **Replace Column**. Then tap **NEXT** until you view the Flush Column option.

1. Open the column compartment door.
2. For safety, the system automatically shuts off column temperature and flow. However, you can use the touchscreen to verify that the compartment temperature is cool enough before you start to remove the column. From the touchscreen tap **Maintain** > **Replace components** > **Replace Column**. Then tap **NEXT** until you view the Preconditions option.

   Tip: A warning appears if the column compartment is too hot to proceed.
3. From Preconditions, verify that the column temperature is cool and the flow is stopped. Tap **NEXT**.
4. 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 of it and use your remaining hand to pull the top part of the column out of the black clip.

Figure 8–100: Removing the column from the black clips



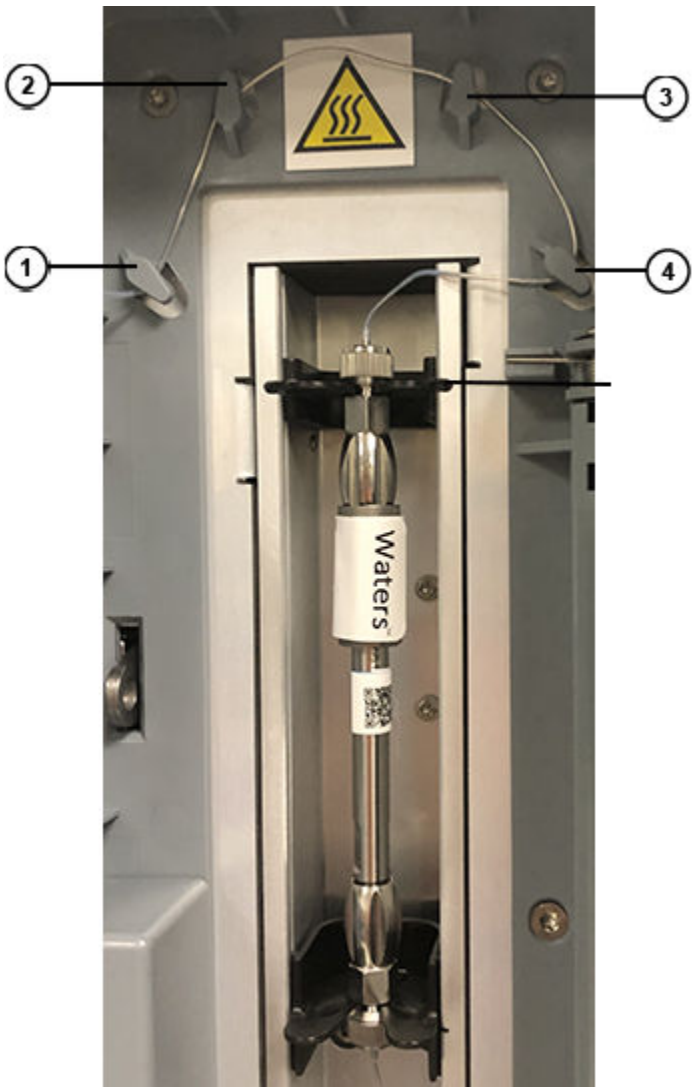
1 Top black clip

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 8–101: Four fasteners that secure the tubing



d. Unscrew the fitting located on the bottom of the column, and set aside to install the new column.

e. Unscrew the fitting located on the top of the column, and set aside to install the new column. Use the remaining steps to install the new column.

5. Remove the protective plugs from the top and the bottom of the new column, and then place in the column shipping carton for future use during storage.
6. Orient the column so that the outlet faces up (see the arrow on the column) and the inlet faces down.
7. Screw the column inlet and column outlet tool-free fittings that you set aside earlier onto the column finger tight.
8. If necessary, adjust the lower column clip to match the size of the new column.
9. Install the tubing by routing it into the fasteners 2 through 4 located at the top of the column compartment.

10. Insert the column into the upper and lower black clips, ensuring that each black clip grasps the exposed threads on the tool-free fitting.

    Figure 8–102: Installing the replacement column

11. Close the column compartment door.

    Note: Verify that the tubing is situated inside the compartment before you close the column compartment door.

12. Configure the new column. From the touchscreen tap **Maintain** > **Replace components** > **Replace Column**. Then tap **NEXT** and follow the onscreen instructions to configure the column as required.

8.11.3 Servicing the air filter

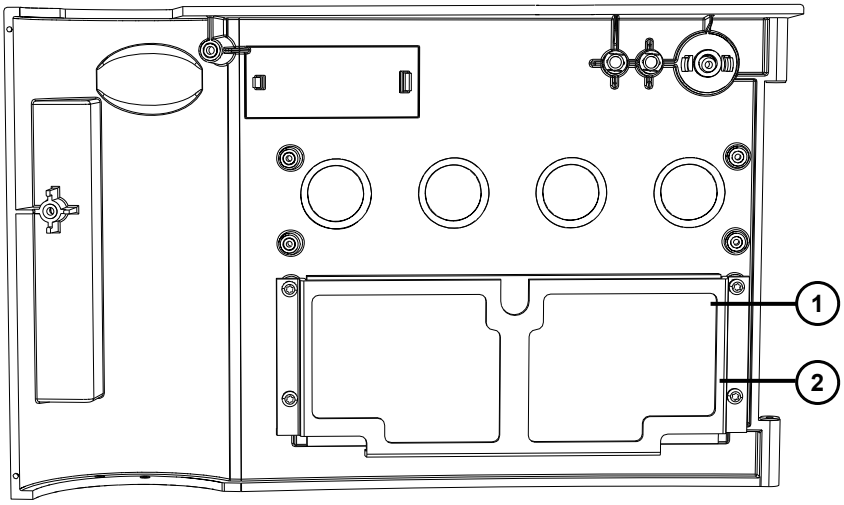
Required tools and materials

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

To service the air filter:

1. Open the bottom door.
2. Slide the air filter up and out of the frame inside the door.

Figure 8–103: Door air filter



1 Air filter

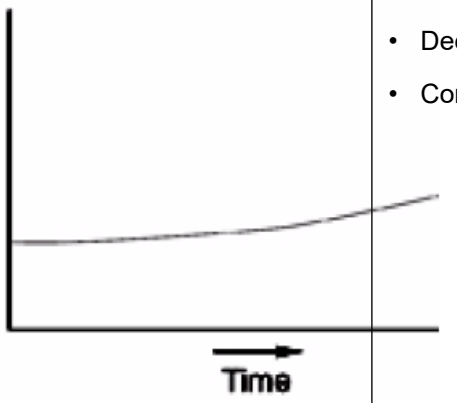
2 Air filter frame

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. Slide the air filter back into the frame.

9 Troubleshooting

9.1 Symptoms

Use this list of symptoms to get more information about unexpected or undesirable behaviors and how to fix them.

Symptom	Description	Possible Cause
Slow baseline drift	The baseline slowly drifts upward. 	• Ambient temperature • Contaminated solvent • Decreased UV energy • Contaminated column
Late-eluting peak	Peaks that used to elute at a particular retention time are now consistently eluting at a later retention time.	• Inappropriate gradient method • Incorrect sample preparation • Column issue • Faulty check valve (Page 129)

9.2 Resolving environmental problems

This section explains how to resolve problems caused by unstable environmental conditions.

This is the start of your concept.

9.3 Resolving solvent issues

This section explains how to resolve problems caused by incorrect or contaminated solvents.

This is the start of your concept.

9.4 Resolving sample issues

This section explains how to resolve problems caused by incorrect or contaminated samples.

This is the start of your concept.

9.5 Resolving quaternary pump problems

This section explains how to resolve problems caused by a malfunctioning quaternary pump.

This is the start of your concept.

9.5.1 Resolving pressure issues

This section contains topics that explain how to resolve low or no system pressure issues.

Replacing the accumulator check valve (Page 124) Describes how to replace the accumulator check valve (original OMG version).
Replacing the accumulator check valve (Page 127) Describes how to replace the accumulator check valve (multiple animations version).
Replacing the accumulator check valve (Page 129) Describes how to replace the accumulator check valve (one animation version).
Replacing the accumulator check valve - video (Page 131) Describes how to replace the accumulator check valve (embedded video version).

9.5.2 Resolving leak issues

This section contains topics that explain how to resolve a system leak.

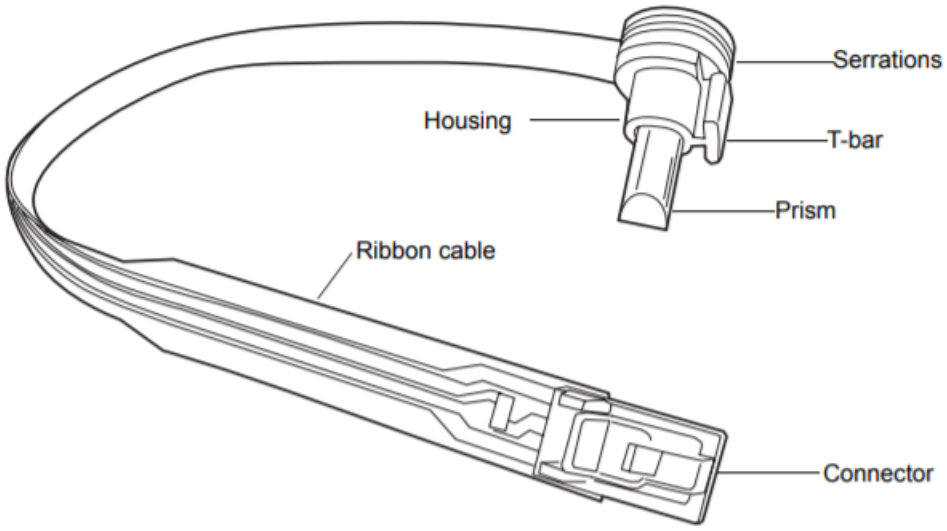
Overview (Page 199) Describes the leak sensor components.
Locating the source of the leak (Page 199) Describes troubleshooting procedure and common sources of leaks.
Drying the leak sensor (Page 200) Describes how to clean the leak sensor after the leak has been resolved.

How to perform a dynamic leak test (Page 200) Describes how to test the system for leaks.
Replacing the leak sensor (Page 110) Describes how to replace a faulty leak sensor.

9.5.2.1 Overview

Leak sensors in the drip trays continuously monitor the system for leaks. A leak sensor stops system flow when its optical sensor detects about 1.5 mL of accumulated, leaked liquid in its surrounding reservoir. At the same time, the system and the Empower Console displays an error message alerting you that a leak has developed.

Figure 9–1: Leak sensor components



You must clear a leak error before system flow can resume. Resolving a leak error involves:

- Locating the source of the leak
- Repairing the leak
- Removing the leak sensor from the drip tray
- Drying the leak sensor
- Drying the liquid in the leak sensor reservoir
- Reinstalling the leak sensor
- Resetting the system

9.5.2.2 Locating the source of the leak

Provide context for your task here (optional).

Enter your first step here.

9.5.2.3 Drying the leak sensor

Provide context for your task here (optional).

Enter your first step here.

9.5.2.4 How to perform a dynamic leak test

Performing a dynamic leak test:

1. Ensure that the solvent line is fully submerged in solvent in the solvent reservoir.
2. From Empower, open the Console.
3. Click **Quaternary pump** from the system tree.
4. Click **Maintain**.
5. Click **Leak Test**.
6. Select the appropriate solvent line, ensure that the prime option is selected.
7. Click **Start**.

   The test runs and displays the test results.
8. If the test fails, prime the system again and run the leak test one more time.

9.6 Responding to a leak sensor alarm

A leak sensor alarm may indicate an actual leak or a malfunction in the sensor itself. Investigate and remedy the source of the alarm immediately.

After approximately 1.5 mL of liquid accumulates in a leak-sensor reservoir, an alarm sounds indicating that the leak sensor detected a leak.

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.

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

Notice: To avoid scratching or otherwise damaging the leak sensor:

- Do not allow buffered solvents to accumulate and dry on it.
- Do not submerge it in a cleaning bath.

Required tools and materials

- Chemical-resistant, powder-free gloves
- Cotton swabs
- Nonabrasive, lint-free wipes

To respond to a leak sensor alarm:

1. In the console's Leak Sensors dialog box, confirm that the solvent manager's leak sensor detected a leak.

   Tip: When a leak is detected, a "Leak Detected" error message appears.

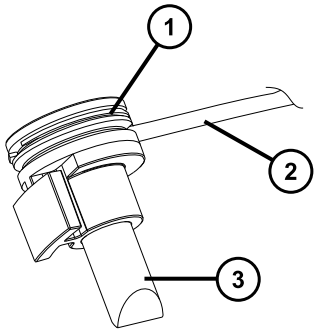
   Notice: To avoid damaging electrical components and circuitry, do not disconnect an electrical assembly while electrical power is applied to a module. To completely interrupt power, set the on/off switch to the "off" position, and then disconnect the power cord from the ac source. Wait 10 seconds before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

2. Power-off the solvent manager.
3. Open the solvent manager's door.
4. Locate the source of the leak and make the repairs necessary to stop it.

   Notice: To avoid damaging the leak sensor, do not grasp it by the ribbon cable.

5. Remove the leak sensor from its reservoir, grasping the sensor by its serrations, and pull upward.

Figure 9–2: Leak-sensor assembly



1 Serrations

2 Ribbon cable

③ Prism

Tip: If you cannot easily manipulate the leak sensor after removing it from its reservoir, detach the connector from the front of the device (see Replacing the leak sensor (Page 110)).

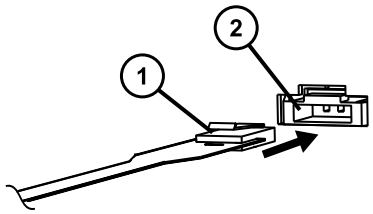
6. Use a nonabrasive, lint-free wipe to dry the leak-sensor prism.
7. Roll up a nonabrasive, lint-free wipe, and use it to absorb the liquid from the leak-sensor reservoir and its surrounding area.

Figure 9–3: Leak-sensor reservior

① Leak-sensor reservoir

8. With a cotton swab, absorb any remaining liquid from the corners of the leak-sensor reservoir and its surrounding area.
9. Align the leak sensor's T-bar with the slot in the side of the leak-sensor reservoir, and slide the leak sensor into place.
10. If you detached the connector from the front of the device, reattach it.

Figure 9–4: Attaching leak-sensor connector



① Leak sensor connector

② Leak sensor port on front of device

11. Power-on the solvent manager.
12. In the console, select the solvent manager, and then click **Control** > **Reset module** to reset the solvent manager.

9.7 Diagnostic tests

Prior to using the system, you can run a needle seal readiness test from the Maintain menu.

You can select these diagnostic tests from the autosampler's Maintain menu:

- Running the needle seal readiness test (Page 204), which confirms that there is a rise in pressure when flow is directed through the needle, the needle seal, and the static return

tubing. It indirectly confirms that there is no drop in system pressure when it registers a positive rise in pressure.

The Maintain menu also lists these functions:

- Replace components, which enables you to replace the sample syringe, needle, and needle seal.
- Calibrating the needle *z* axis, which calibrates the vertical position of the needle.
- Disabling motors, which you do before manually moving the sample tray and R-carriage.
- Parking the sample needle and injection valve, which you do before storing the system or replacing a needle or valve.

See also:

- For information on the solvent manager's leak test, the or the *ACQUITY Premier Quaternary Solvent Manager Overview and Maintenance Guide* (715007058).
- The console online Help for additional information about running diagnostic tests.

9.7.1 Running the system leak test

The System Leak Test evaluates the fluid-handling integrity of the system. Perform a leak test whenever you replace or loosen fittings during maintenance.

The test exposes leaks in:

- Check valves
- Tubes
- Fittings
- Plungers
- Plunger high-pressure seals
- Vent valve on the solvent manager

Symptoms of leaks can include visible drips, inconsistent retention times, or increased baseline noise. For best results:

- Run the test at your typical maximum operating pressure.
- Use only fresh, clean, degassed solvent.
- Prime the system before you run the test.

Recommendation: Do not perform the test until you condition the seals for at least 15 to 30 minutes at 9000 psi. The leak test can fail if the seals are not conditioned.

To perform the test:

1. From the touchscreen, tap **Health** > **Troubleshoot**.
2. From System Diagnostics, tap **System Leak Test**.

3. Review the Welcome page for important information, and then tap **NEXT**.
4. Select a solvent line to use for the leak test, and then tap **NEXT**.
5. From Setup, tap **Standard Leak Test** or **Custom Leak Test**.
6. Specify the target pressure, and then tap **NEXT**.
7. Review the Summary page, and then tap **START** to run the leak test.

9.7.2 Running the needle seal readiness test

The needle seal readiness test indicates whether or not there is a leak at the needle seal before you start an injection.

Requirement: You must run the test after you install a new needle seal (see Replacing the needle seal and return line > not return line (Page 169)).

1. From the touchscreen, tap **Health** > **Troubleshoot**.
2. Next, an interactive image illustrating the system components appears. From System Diagnostics, tap the needle icon to view Autosampler diagnostics.
3. Tap **Needle seal readiness test**.
4. Review the Welcome page for important information, and then tap **NEXT**.
5. From Setup, specify the flow rate, and then tap **NEXT**.

   Recommendation: Waters recommends that you specify a flow rate of 1.000 mL/min.
6. Review the Summary page, and then tap **START** to run the test.
   The test completes in approximately two minutes.

10 System upgrade

Note: Under construction.

10.1 Empower updates

To update Empower software, see the *Empower System Installation and Configuration Guide*.

10.2 Empower ICS updates

To update Empower ICS software:

1. Download the installation files from https://code.waters.com/confluence/x/2gEeTQ.

   Note: Access to this page is restricted. Contact Mike Jackson or Rick Earle if you lack permission.

2. If the Alliance iS ICS is already installed, uninstall Orion ICS Setup from Windows Apps & Features, which also removes the Orion ICS entry from Apps & Features.

   Note: It is unnecessary to disable IIS before installing Empower ICS.

3. Download and unzip the *OrionICS-0.1.0-release-INS-26254-20220810-alphadrop5.1.zip* file.

4. Install the new version by running the following command: OrionSystemSetup.exe

Note: If during the ICS installation an error indicates that the WatersNGINXInstrumentService service cannot start, refer to the work-around on https://code.waters.com/confluence/x/hQNHTQ.

10.3 Kiosk/console updates

Note: Under construction.

10.4 Firmware updates

Note: Under construction.

10.5 System release notes

Note: Under construction.

11 Disposal protocols

Disposal of system components is performed either by Waters personnel or by the customer per local jurisdiction.

11.1 Description of constituent materials

For detailed descriptions of Waters materials, see Safety Data Sheets on waters.com.

11.2 Disposal of system components

Disposal of system components is performed either by Waters personnel or by the customer per local jurisdiction.

12 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.

This section covers the solvent considerations necessary when operating the Alliance iS System. This information applies only to the Alliance iS System and components.

12.1 Preventing contamination

Explore the Waters website for resources on controlling contamination.

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

12.1.1 Clean solvents

Clean solvents ensure reproducible results and permit operation with minimal instrument maintenance.

Dirty solvents can cause baseline detector noise and drift, and they can clog solvent reservoir filters, inlet filters, and capillary lines.

12.1.2 Solvent quality

Use MS-grade solvents for the best possible results. The minimum requirement is HPLC-grade. Filter solvents through an appropriate membrane filter.

Recommendation: Heed the recommendations of the filter's manufacturer or vendor to ensure that the filter is appropriate for the solvents used.

12.1.3 Solvent preparation

Proper solvent preparation, primarily filtration, can prevent many pumping problems.

Recommendation: Store mobile phases in borosilicate glass reservoirs type 1, class A[2] or type 3.3[3]. Use high-quality, brown-tinted glassware to inhibit microbial growth. Use aluminum foil or Waters caps to cover the reservoirs.

12.1.4 Water

Use water only from a high-quality water purification system. If the water system does not deliver filtered water, filter the water through a 0.2-µm membrane filter.

Notice: Using 100% water can cause microbial growth. Waters recommends changing 100% water solutions daily. Adding a small amount of an organic solvent (~10%) prevents microbial growth.

12.1.4.1 Using buffers

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. When using a buffer, choose good quality reagents, filtering them through a 0.2-µm membrane filter.

For shutdowns of more than a day's duration, flush the pump with a 20% methanol/water solution to prevent microbial growth.

Important: Some buffers can be incompatible with mass spectrometers. Consult the documentation that accompanies your instrument for compatible buffers.

See also: For information on preventing contamination, refer to *Controlling Contamination in LC/MS Systems* (715001307) on the Waters website (www.waters.com).

12.1.4.1.1 Buffered solvents

When using a buffer, choose good quality reagents, filtering them through a 0.2-µm membrane filter.

Recommendation: To discourage microbial growth, replace 100% mobile aqueous phase daily.

See also: For information on preventing contamination, refer to *Controlling Contamination in LC/MS Systems* (715001307) on the Waters website (www.waters.com).

12.2 Solvent recommendations

This section provides information on wash and purge solvents and lists the solvents recommended for your system. Contact Waters Customer Service or Marketing to determine whether you can use solvents not addressed by the these lists without adversely affecting component or system performance.

12.2.1 General solvent guidelines

Always observe the following general solvent guidelines:

- Use high-quality, brown-tinted glassware to inhibit microbial growth.
- Filter solvents through a 0.2-µm filter, or use prefiltered solvents. Small particles can permanently block a system's capillary lines. Filtering solvents also improves check valve performance.

12.2.1.1 Recommended solvents

- ACN
- IPA
- Methanol
- Water

12.2.1.2 Other solvents

You can use the solvents listed below in your system.

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 a hexane/THF compatibility kit.

See: Hexane/THF compatibility kit (Page 210)

- Acetone
- Ethyl acetate
- Hexane
- THF

See also: System recommendations (Page 214)

When you change solvents, consider solvent polarity. When switching between polar and nonpolar solvents, flush the system with miscible and compatible solvents, such as IPA.

12.2.1.2.1 Hexane/THF compatibility kit

The Alliance iS System Hexane/THF Compatibility Kit (contact Waters for part number) can be installed in relevant systems. It is designed for users who must run their system with hexane, THF, acetone, ethyl acetate, or combinations of these solvents at high concentrations and high pressure.

12.2.1.3 Additives/modifiers

- ≤0.3% vol. acetic acid
- ≤50 mM ammonium acetate
- ≤10 mM ammonium bicarbonate
- ≤50 mM ammonium hydroxide
- ≤0.1% wt. EDTA

- ≤0.2% vol. formic acid
- ≤0.1% vol. heptafluorobutyric acid
- 

  Warning: To avoid damaging the system components, and to prevent contact with corrosive material that can be present on the components that HFIP damages, do not use HFIP in wash solvents.

  1% to 4% aqueous solutions of HFIP for oligonucleotide applications
- ≤10 mM phosphate buffer
- ≤0.1% vol. TEA
- ≤0.1% vol. TFA

12.2.1.4 Sample diluents

- ACN
- ACN/water mixtures
- Chloroform
- DMF
- DMSO
- IPA
- Isooctane
- Methanol
- Methanol/water mixtures
- Methylene chloride
- Water

12.2.1.5 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 (≤30%)
- Phosphoric acid (≤30%)
- Sodium hydroxide (≤1M)

See also: The cleaning procedures in *Controlling Contamination in LC/MS Systems* (715001307) on the Waters website (www.waters.com).

12.2.2 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 212) 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.

12.2.2.1 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.

12.2.2.2 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 45) 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.

12.3 Solvents to avoid

Avoid these solvents:

- Solvents that contain halogens: chlorine, fluorine, bromine, or 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 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 2.0 to 12.0. Operating the system below pH 2.0 or above pH 12.0 for more than brief periods can result in increased wear on system components not included in preventive maintenance kits and requires 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 2.0.
  - For strong bases, use them only in weak concentration. Avoid using bases as mobile phases when their pH is greater than 12.0.

12.3.1 Material limitations for strong acids and bases

- 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.
- 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 will start to degrade at pH 8 and higher.*
- Polyimide used in the needle seat is stable in a pH range between 1 and 10 and in most organic solvents. It will degrade in the presence of concentrated mineral acids like sulfuric acid and in the presence of glacial acetic acid. It will be degraded by elevated basic condition, especially in the presence of ammonia or ammonium salts or acetates.

12.4 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 Hexane/THF compability kit (Page 210)). When using hexane or THF, minimize the use of PEEK components by replacing PEEK tubing with SST 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.
- To determine whether a specific method is suitable for use with your systems' components, contact your Waters sales representative or local technical support organization.

12.4.1 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 215)
- Pump solvent recommendations (Page 216)

12.4.1.1 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.

12.4.1.2 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 212) 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 212)).
- For reverse-phase applications, use aqueous seal wash solutions with a weak organic component (for example, 1:9 MeOH/water).

12.4.2 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.

12.5 Common solvent properties

The following table lists the properties for some common chromatographic solvents.

Table 12–1: Properties of common solvents

Solvent	Vapor pressure mm Hg (Torr)	Boiling point (°C)	Flash point (°C)
Acetone	184.5 at 20 °C	56.29	-20
Acetonitrile	88.8 at 25 °C	81.6	6
n-butyl acetate	7.8 at 20 °C	126.11	22
n-butyl alcohol	4.4 at 20 °C	117.5	37
n-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	—
Cyclohexane	77.5 at 20 °C	80.72	-20

Table 12–1: Properties of common solvents (continued)

Solvent	Vapor pressure mm Hg (Torr)	Boiling point (°C)	Flash point (°C)
Cyclopentane	400 at 20 °C	49.26	-7
o-Dichlorobenzene	1.2 at 20 °C	180.48	66
Dichloromethane	350 at 20 °C	39.75	—
Dimethyl acetamide	1.3 at 25 °C	166.1	70
Dimethyl sulfoxide	0.6 at 25 °C	189.0	88
N, *N*-Dimethylformamide	2.7 at 20 °C	153.0	58
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
Iso-octane	41 at 20 °C	99.24	-12
Isobutyl alcohol	8.8 at 20 °C	107.7	28
Isopropyl alcohol	32.4 at 20 °C	82.26	12
Isopropyl myristate	<1 at 20 °C	182.6	164
Methanol	97 at 20 °C	64.7	11
Methyl *t*-butyl ether	240 at 20 °C	55.2	-28
Methyl ethyl ketone	74 at 20 °C	79.64	-9
Methyl isobutyl ketone	16 at 20 °C	117.4	18
N-Methylpyrrolidone	0.33 at 25 °C	202.0	86
Pentane	420 at 20 °C	36.07	-49
n-Propyl alcohol	15 at 20 °C	97.2	23
Propylene carbonate		241.7	135
Pyridine	18 at 25 °C	115.25	20
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
Triethylamine	57 at 25 °C	89.5	-9

Table 12–1: Properties of common solvents (continued)

Solvent	Vapor pressure mm Hg (Torr)	Boiling point (°C)	Flash point (°C)
Trifluoroacetic acid	97.5 at 20 °C	71.8	-3
Water	17.54 at 20 °C	100.0	—
o-xylene	6 at 20 °C	144.41	17

12.6 Solvent miscibility

Before you change solvents, refer to the following table to determine solvent miscibility. Be aware of these effects:

- 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.
- 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.
- 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 12–2: Solvent miscibility

Polarity index	Solvent	Viscosity cP, 20 °C (at 1 atm)	Boiling point °C (at 1 atm)	Miscibility number (M)	λ cutoff (nm)
6.2	ACN	0.37	81.6	11, 17	190
6.2	Acetic acid	1.26	117.9	14	—
5.4	Acetone	0.32	56.3	15, 17	330
0.0	N-hexane	0.313	68.7	29	—
4.2	THF	0.55	66.0	17	220
1.8	Triethylamine	0.38	89.5	26	—
4.3	1-propanol	2.30	97.2	15	210
4.3	2-propanol	2.35	117.7	15	—
5.2	Ethanol	1.20	78.3	14	210
5.5	Benzyl alcohol	5.80	205.5	13	—

Table 12–2: Solvent miscibility (continued)

Polarity index	Solvent	Viscosity cP, 20 °C (at 1 atm)	Boiling point °C (at 1 atm)	Miscibility number (M)	λ cutoff (nm)
5.7	Methoxyethanol	1.72	124.6	13	—
6.4	Dimaethylformamide	0.90	153.0	12	—
6.5	DMSO	2.24	189.0	9	—
6.6	Methanol	0.60	64.7	12	210
9.0	Water	1.00	100.0	—	—

12.6.1 Using miscibility numbers (M-numbers)

Use miscibility numbers (M-numbers) to predict the miscibility of a liquid with a standard solvent.

To predict the miscibility of two liquids, subtract the smaller 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.

12.7 Solvent stabilizers

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 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.

Solvent stabilizers are added to slow or stop solvent degradation.

12.8 Solvent viscosity

Generally, viscosity is not a consideration when you use a single solvent or when under low pressure. In gradient chromatography, however, the viscosity changes that occur as the solvents are mixed in varying proportion can effect pressure changes during the run.

If you do not know the extent to which pressure changes affect the analysis, monitor the pressure during the run.

12.9 Wavelength selection

The tables in this section provide UV cutoff values for these items:

- Common solvents
- Common mixed mobile phases

12.9.1 Ultraviolet cutoffs for common solvents

The following table shows the UV cutoff (the wavelength at which the absorbance of the solvent equals 1 AU) for some common chromatographic solvents. Operating at a wavelength near or below the cutoff increases baseline noise because of solvent absorbance.

Table 12–3: UV cutoff wavelengths for common chromatographic solvents

Solvent	UV cutoff (nm)
Acetone	330
Acetonitrile	190
Diethyl amine	275
Ethanol	210

Table 12–3: UV cutoff wavelengths for common chromatographic solvents (continued)

Solvent	UV cutoff (nm)
Isopropanol	205
Isopropyl ether	220
Methanol	205
n-Propanol	210
THF	230

12.9.2 Mixed mobile phases

The following table provides approximate wavelength cutoffs 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.

Note: λ cutoff is the wavelength at which the absorbance of the solvent equals 1 AU.

Table 12–4: Wavelength cutoffs for various mobile phases

Mobile phase	UV cutoff (nm)
Acetic acid, 1%	230
Ammonium acetate, 10 mM	205
Ammonium bicarbonate, 10 mM	190
CHAPS 0.1%	215
Diammonium phosphate, 50 mM	205
Disodium EDTA, 1 mM	190
HEPES, 10 mM, pH 7.6	225
Hydrochloric acid, 0.1%	190
MES, 10 mM, pH 6.0	215
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	190
Sodium formate, 10 mM	200

Table 12–4: Wavelength cutoffs for various mobile phases (continued)

Mobile phase	UV cutoff (nm)
TRIS HCl, 20 mM, pH 7.0	202
TRIS HCl, 20 mM, pH 8.0	212
Triethylamine, 1%	235
Trifluoroacetic acid, 0.1%	190
Triton X-100, 0.1%	240
Waters PIC Reagent A, 1 vial/liter	200
Waters PIC Reagent B-6, 1 vial/liter	225
Waters PIC Reagent B-6, low UV, 1 vial/liter	190
Waters PIC Reagent D-4, 1 vial/liter	190

12.9.3 Mobile phase absorbance

This following table lists 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 Alliance iS Hexane/THF Conversion Kit.

Table 12–5: 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										
Acetonitrile	0.05	0.03	0.02	0.01	0.01	<0.01	—	—	—	—
IPA	1.80	0.68	0.34	0.24	0.19	0.08	0.04	0.03	0.02	0.02
Methanol (degassed)	1.91	0.76	0.35	0.21	0.15	0.06	0.02	<0.01	—	—
Methanol (not degassed)	2.06	1.00	0.53	0.37	0.24	0.11	0.05	0.02	<0.01	—
Unstablized THF (fresh)	2.44	2.57	2.31	1.80	1.54	0.94	0.42	0.21	0.09	0.05

Table 12–5: 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
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	—
Diammonium phosphate, 50 mM	1.85	0.67	0.15	0.02	<0.01	—	—	—	—	—
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	—	—	—	—	—	—	—
Phosphoric acid, 0.1%	<0.01	—	—	—	—	—	—	—	—	—
Triethylamine, 1%	2.33	2.42	2.50	2.45	2.37	1.96	0.50	0.12	0.04	<0.01
Trifluoroacetic acid	1.20	0.78	0.54	0.34	0.22	0.06	<0.02	<0.01	—	—
Buffers and salts										
Ammonium acetate, 10 mM	1.88	0.94	0.53	0.29	0.15	0.02	<0.01	—	—	—
Ammonium bicarbonate, 10 mM	0.41	0.10	0.01	<0.01	—	—	—	—	—	—
HEPES, 10 mM, pH 7.6	2.45	2.50	2.37	2.08	1.50	0.29	0.03	<0.01	—	—
MES, 10 mM, pH 6.0	2.42	2.38	1.89	0.90	0.45	0.06	<0.01	—	—	—
Potassium phosphate, dibasic, (K_2HPO_4), 10 mM	0.53	0.16	0.05	0.01	<0.01	—	—	—	—	—
Potassium phosphate, monobasic (KH_2PO_4), 10 mM	0.03	<0.01	—	—	—	—	—	—	—	—
Sodium acetate, 10 mM	1.85	0.96	0.52	0.30	0.15	0.03	<0.01	—	—	—
Sodium chloride, 1 M	2.00	1.67	0.40	0.10	<0.01	—	—	—	—	—
Sodium citrate, 10 mM	2.48	2.84	2.31	2.02	1.49	0.54	0.12	0.03	0.02	0.01

Table 12–5: 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
Sodium formate, 10 mM	1.00	0.73	0.53	0.33	0.20	0.03	<0.01	—	—	—
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	—	—	—	—
Tris HCl, 20 mM, pH 8.0	1.80	1.90	1.11	0.43	0.13	<0.01	—	—	—	—
Waters PIC reagents										
BRI J 35, 1%	0.06	0.03	0.02	0.02	0.02	0.01	<0.01	—	—	—
CHAPS, 0.1%	2.40	2.32	1.48	0.80	0.40	0.08	0.04	0.02	0.02	0.01
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	—	—
PIC B6, low UV, 1 vial/L	0.01	<0.01	—	—	—	—	—	—	—	—
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
4-octylphenol polyethoxylate (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
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
SDS, 0.1%	0.02	0.01	<0.01	—	—	—	—	—	—	—

13 Specifications

The operating and performance specifications presented here depend on the conditions in individual laboratories. Refer to the *Alliance iS Site Preparation Guide*, or contact the Waters Technical Service organization for additional information about specifications.

Notes:

- If your system includes a TUV detector, consult the *TUV Overview and Maintenance Guide* for specifications.

13.1 System specifications

Refer to this table for general system specifications.

See also: For individual module physical, environmental, and input/output specifications, consult its overview and maintenance guide.

Table 13–1: Alliance iS System general specifications

Item	Specification
Dwell volume (total system)	≤1600 µL
Gradient delay volume	≤1100 µL
Integrated leak management	Leak sensors as standard and safe leak handling
Quantum synchronization	Injection synchronization between pump and injector enhances retention time reproducibility
Operating flow rate range	0.001 to 10.000 mL/min, in 0.001-mL increments
Maximum operating range	10,000 psi up to 5.000 mL/min, linearly decreasing to 4000 psi at 10mL/min
pH range [a]	1 to 13
Unattended operation	Leak sensors and safe leak handling, full 96-hour diagnostic data display through console software

Table 13–1: Alliance iS System general specifications (continued)

Item	Specification
Cycle time	≤30 seconds inject-to-inject

a. The standard pH operating range for the system is between 1 and 13. Operating the system at pH 1 or above pH 13 for more than brief periods can result in increased wear on system components not included in preventive maintenance kits and require more frequent routine preventive maintenance intervals. Specifically, the impact of pH on wetted materials in the system can have these effects:

- Polyetheretherketone (PEEK) can degrade in strong mineral acids such as nitric and sulfuric acids, especially in the presence of halogens and halogen-containing chemicals.
- Polyimide used in the needle seat is stable in a pH range between 1 and 10 and in most organic solvents. It does degrade in the presence of concentrated mineral acids, like sulfuric acid, and in the presence of glacial acetic acid. Also, it degrades by elevated basic condition, especially in the presence of ammonia or ammonium salts or acetates.
- Quartz flow cells can have degraded lifetimes in the presence of pH greater than 12.
- Silica capillaries and their polyimide coatings start to degrade at pH 8 and higher.
- Stainless steel (SST) can degrade when exposed to 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 degrade when used with organic acids in organic solvents.

13.1.1 Instrument control specifications

Table 13–2: Alliance iS instrument control specifications

Attribute	Specification
Informatics compatibility	Empower CDS
Communications	Ethernet or D-sub interconnect cable
Event input/output	Contact closure, TTL I/O, or both

13.1.2 Environmental specifications

Table 13–3: Alliance iS environmental specifications

Attribute	Specification
Usage location	For indoor use only
Acoustic noise, system	<65 dBA
Ambient operating temperature	4 to 40 °C
Operating temperature stability	ΔT ≤ ±2 °C/hr
Ambient operating humidity	20% to 80%, non-condensing
Operating altitude	≤ 2000 m
Ambient transportation and storage temperature	-30 °C to 60 °C

Table 13–3: Alliance iS environmental specifications (continued)

Attribute	Specification
Ambient transportation and storage humidity	20% to 85%, non-condensing

13.1.3 Electrical specifications

Note: The total system power is a function of the devices, modules, and instruments that it comprises. For further information on an individual component, refer to its specific operator's guide.

Table 13–4: Alliance iS System electrical specifications

Attribute	Specification
Power requirements	100 to 240 Vac +/- 10%
Line frequency	50–60 Hz
Power consumption	• CHC: 240 VA • SM-FTN: 400 VA • QSM: 200 VA • TUV Detector

13.1.4 Physical specifications

Table 13–5: Alliance iSphysical specifications

Attribute	Specification
Height	57.1 cm (22.5 inches)
Width	55.9 cm (22.0 inches)
Depth	62.8 cm (24.7 inches)
Weight	62.1 kg (137.0 lbs) [a]

a. Actual system weight will vary depending upon the solvent and number of solvent bottles in the solvent tray.

13.1.5 Center of gravity

13.1.6 Wetted materials of construction

13.2 Performance specifications

The tables in this section list the performance specifications for the Alliance iS System modules.