



Waters LC Microscale Support Layer 3.0.0 for Agilent CDS

Release Notes

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1 General Information

1.1 Copyright notice

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1.3 About these release notes

This document contains information about the features and functions introduced, changed, or removed with this version of the product. It contains a list of major feature changes as well as descriptions of known defects, fixed defects, and observed product behaviors (concessions) that Waters deems of importance to customers. This document does not include:

- An exhaustive list of all changes to this version of the product.
- Descriptions of defects that were not known to Waters at the time of the product's release.
- Descriptions of defects that cannot be reproduced by Waters.
- Information about customer requests for enhancements to the product.
- Specific details of changes to proprietary aspects of product components, features, computational algorithms, and software code.

The changes listed in this document are relative to the immediately previous version of the product. If you are updating an older version of the product, review the release notes for all the intervening versions to ensure that you understand the cumulative impact of the product changes.

Carefully review the information in this document before you install the product. If you have questions about how installing this product might affect your environment or if you need more information about this product, contact your Waters representative.

1.4 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.

1.5 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 34 Maple Street Milford, MA 01757 USA From the USA or Canada, phone 800-252-4752 or fax 508-872-1990.

1.5.1 Updated information

Refer the Waters website (www.waters.com) and click **Support > Support Documents and Downloads** for updates to this document.

2 Glossary

Term/Abbreviation	Definition
ACQUITY UPLC MClass, M-Class systems	Brand name for ACQUITY UPLC systems with lowest dispersion binary solvent delivery.

Term/Abbreviation	Definition
AIC	Agilent Instrument Controller
ASM	ACQUITY UPLC M-Class Auxiliary Solvent Manager
CDS	Chromatography Data System
ChemStation	Brand name for this Legacy third party CDS, which controls Waters LC's and controls Agilent's LCs and MS, with the current version.
CM-Aux	Auxiliary Column Manager, it can be configured with TVM.
Console	The console is a software application that provides a convenient way to configure settings, monitor performance, run diagnostic tests, and maintain the system and its modules. It replaces the keypads and small screen displays traditionally found on the front of instruments.
Defect	A discrepancy between a computed, observed, or measured value or condition and the true, specified or theoretically correct value or condition.
DP	Driver pack
DP 2022 R1	Driver Pack 2022 Release 1 (Microscale), Driver Pack 2022 Release 1 (Analytical)
Dual Pump Trapping	M-Class with 2D technology has streamlined 2D-LC separations with a highly intuitive menu-driven method setup, standardized separation chemistries, and intelligent valve operation. Expanding on the use of a novel 2D reversed-phase/reversed-phase (RP/RP) approach, where the first dimension runs at pH 10 and the second at pH 2, grants the ability to robustly increase sample throughput with MS compatible eluents.
ICS	Instrument Control Software

Term/Abbreviation	Definition
Load ahead	<p>During acquisition, the CDS sends the instrument Sample Manager ICS the vial position of the next injection so that the sample manager can pick the sample up while the previous injection is still completing. This functionality reduces cycle time.</p> <p>Note: The load ahead option for ACQUITY, engaging load ahead with ACQUITY UPLC M-Class systems for flow rates below 1.0 microliter/minute. Engaging load ahead at nano flow rates can disrupt gradient formation resulting in adverse effects on column lifetimes.</p>
Localization	<p>A supported CDS may support other languages than English. At minimum the Waters Driver Pack in English must be supported with localized versions of the supported CDS.</p>
.NET Framework	<p>.NET Framework is a software development framework from Microsoft. It provides a controlled programming environment where software can be developed, installed, and executed on Windows-based operating systems.</p>
OpenLab	<p>Brand name for this third party CDS, which controls Waters LC's and controls Agilent's LCs and MS, with the current version.</p>
PDA	<p>Photodiode array detector – for example, an eλ-PDA</p>
Sample Trapping	<p>Sample Trapping occurs when analytes of interest are captured on a trapping column, constituting the first dimension of the analysis, and then transferred to a second column for an analytical separation (constituting the second dimension).</p>
Single Pump Trapping	<p>Single Pump Trapping is eluted from the trap onto the column as a focused band.</p>
SO	<p>Sample organizer used with rotary-tray based sample managers such as H/I-Class and ACQUITY Systems such as ACQUITY UPLC I-Class Series Systems, ACQUITY UPLC H-Class Series Systems, and ACQUITY UPLC M-Class Systems.</p>

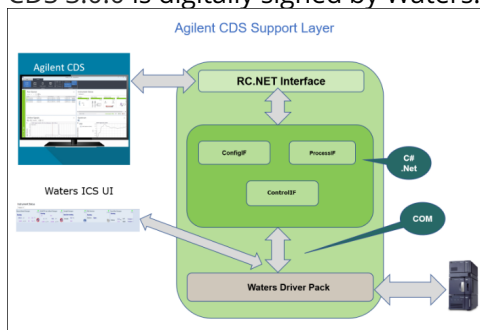
Term/Abbreviation	Definition
SRS	Software Requirements Specification
Trap Column	<p>M-Class trap columns are designed for optimal performance for nano to micro scale liquid chromatographic separation for biomarker discovery, protein and peptide identification and characterization. All M-Class columns are designed for 15 kpsi (1,000 bar) operating pressure and handle pressure fluctuations extremely well during injections and thus provide superior longevity.</p> <p>The 2G trap columns are designed to minimize peak broadening while maximizing chromatographic efficiency. They can be used in a variety of 1D (low pH) nano flow applications. For 2D applications where the first dimension utilizes high pH, Waters recommends the use of 2D trap columns. This specific offering contains Waters Symmetry 100 Å pore, C18, 5 µm particles. Symmetry silica particles offer on average the highest retention for peptides and are thus most suitable for trapping applications.</p> <p>The 2G V/M trap columns connect with a V-detail to the main valve and a smaller M-detail fitting to a T-piece or other suitable connection point in the LC system. Due to their design, 2G trap columns are suitable for bi-directional flow.</p>
TUV	Tunable Ultraviolet Detector

Term/Abbreviation	Definition
TVM	<p>The Trap Valve Manager (TVM) is equipped with two (2) 6-port, 2-position low dispersion valves. The valves are commanded through user-defined methods and can operate independently of each other. The TVM can be configured for:</p> <ul style="list-style-type: none"> • Direct injection • Single-pump trapping • Dual-pump trapping <p>The TVM will support two (2) trapping columns with internal diameters ranging from 50 µm to 500 µm and lengths up to 50 mm. The TVM will support one (1) analytical column within a separate thermally controlled environment ranging from 5 °C above ambient to 65 °C. The analytical column compartment of the Heating and Trapping Module will support one (1) column with internal diameters ranging from 75 µm to 1 mm and lengths up to 250 mm. The analytical column compartment is located within an arm that is rotated from the center of the system outward to interface more closely with the MS source.</p>
UPLC	Ultra-Performance Liquid Chromatography
Verify Files	<p>Verify Files contains information about the exact version of the CDS, drivers, and options that are installed on the workstation. It also contains checksum values for all program files related to installation and is used to confirm that there were no issues with installation. It is an important documentation for customers in regulated industries.</p>
Waters Support Layer for Agilent CDS	<p>A software component, comprising the Waters Driver Support Layer (an Agilent OpenLab CDS DDK implementation) and the DDK/OIP Transport framework, that allows the Agilent OpenLab CDS software to enable and control Waters UPLC instruments.</p>
µBSM	Microscale Binary Solvent Manager
µSM	Microscale Solvent Manager

Term/Abbreviation	Definition
μSM-FL	Sample manager with fixed-loop design. μSM-FL denotes a fixed-loop M-Class that can reach a maximum back pressure of 15,000 psi. This micro sample manager is available only with ACQUITY UPLC M-Class Series Systems. A biocompatible wetted path version supports up to 15,000-psi operational back pressures. Only one sample manager can be configured in a system.

3 Waters Support Layer for Agilent CDS 3.0.0 - Overview

These release notes explain the details for deployment and installation of the Support Layer designed for control of microscale. The Support Layer is designed to work with Agilent OpenLab software. Waters Support Layer for Agilent CDS 3.0.0 is digitally signed by Waters.



4 Related documents

Ensure that the necessary software is installed as described in the installation and configuration guide. See the *Waters LC Support Layer for Agilent CDS Installation and Configuration Guide* (715008538) for the installation and configuration procedures.

Refer to the following documents on www.waters.com:

- *Waters Driver Pack 2022 Release 1 Installation and Configuration Guide* (715006278 v08)
- *Waters Driver Pack 2022 Release 1 (for Microscale systems) Release Notes* (715007518 v00)
- *Certificate of Structural Integrity - Waters™ Driver Pack 2022 Release 1 (Microscale)* (716009824 Revision A)
- If not available with system (as a USB): Download a copy of Waters Driver Pack 2022 Release 1 (Microscale) (667006778) from www.waters.com
- If not available with system (as a USB): *Download a copy of Waters Support Layer for Agilent CDS* from www.waters.com

Note: The user documentation for Agilent CDS is included with the installer package of the appropriate CDS and can be found in the “Doc” folders in the applicable language.

5 Compliance recommendations

Any time you install, change, or uninstall software or system modules in a regulated environment, Waters recommends that you follow your organization's approved standard operating procedures.

A risk-based review may assist you in a regulated environment to evaluate changes detailed in the release notes. Using company SOPs, determine if any documentation updates or re-qualification of the system modules, chromatographic system, or chromatographic data system (CDS) are required.

Recommendation:

- Run the Verify Files utility, and then review the resulting file for an entry that states "No installation changes were detected".
- Run the Agilent SVT tool and verify that the result is "Pass".

5.1 Software re-qualification options

To verify that the new files are loaded properly during the software installation, perform a software installation qualification.

5.2 What's new in 3.0.0 release

This release provides the following new features:

- Waters Driver Pack 2022 Release 1 (Microscale) (667006778) is supported.
- Multiple pumps of the same type (2 x microBSMs) and different types (microBSM and ASM) are supported.
- Supports multiple detectors.
- Single injection/manual injection outside the sequence, using the injection volume specified in the LC method, is supported.
- M-Class for direct injection, single pump trapping, and dual pump trapping is supported.
- Supports the ability to support micro-scale and analytical scale LC systems (that use different drivers), using a switch utility, when users need to swap systems.

Note:

Waters Support Layer for Agilent CDS supports the following topologies on Agilent OpenLab:

- Standalone Workstation
- Client/Server

6 Features not supported by Waters Support Layer for Agilent CDS 3.0.0

The functionality that is not currently available with Waters Support Layer for Agilent CDS 3.0.0:

Known issue	Comments
Load Ahead with overlapped injection is not supported in Microscale.	Reason: Due to the need to switch the valve in the middle of operation. A valve switch on M-Class results in ramping the flow to zero which would not work during an acquisition.
Microscale does not support localization.	N/A
Dual pump trap and elute with detector in M-Class is not supported.	N/A
M-Class supports only PC name as the system name, which will be populated automatically in the configuration window. You must not update the default system name.	N/A
OpenLab ChemStation: in a method with PDA detector 3D mode enabled, where no 2D channels were enabled at the same time, 3D data will not be captured, and after the acquisition is complete and during the data analysis, a "No Signals Available" message appears.	You must enable at least one 2D channel in a method along with the 3D mode.

7 Features not supported by ACQUITY instruments

This section lists all known issues and limitations that you may encounter using the ACQUITY instruments with Agilent OpenLab software.

- The method upload function is not supported. You can still select the Upload from Instrument option.
- When upgrading to a newer version of the driver, it is necessary to reconfigure existing ACQUITY instrument configurations in Agilent OpenLab software. To do so, rescan the modules via Scan and close the driver configuration window with OK. Reconfiguration is required because the data stored in Agilent OpenLab software configuration for the ACQUITY instruments has been changed.
- After configuration or reconfiguration of a Waters instrument, it is necessary to reboot the workstation/AIC.
- After changing an existing instrument configuration by adding/deleting or changing any module, you need to reboot the PC. When using a Client/Server system, you must reboot the AIC as well.
- Some auxiliary traces and instrument curves are not supported. Use the UPLC ACQUITY Console Detector Node to view those traces and curves.
- The following signals are not available outside of the run. In this case, the latest received data point is displayed in the Online Plot:
 - PDA Ch1 to Ch8
 - PDA Spectrum
- Only psi is supported as a pressure unit.

-
- It is necessary to set up the Sample Organizer via ACQUITY Console and not in the Agilent OpenLab software interface. Use only ACQUITY Console to set up the plates. To assign the Sample Organizer to the Sample Manager, follow these steps:
 1. Open the ACQUITY Console from the **Instrument Status** window.
 2. Select the **Sample Manager** on the left-hand side.
 3. Open the **Sample Organizer Configuration** window via the main menu by selecting **Configure > Sample Organizer**.
 4. To close the window, select the serial number of the **Sample Organizer** and click **OK**.
 - Two detectors using 3D mode are allowed per instrument. Acquisition with 3D acquisition activated for each detector is possible. However, in Data Analysis, the spectrum result is available for only one detector.
Note: EZChrom supports only one 3D detector.
 - The automatically generated 2475 FLR Max Plot signal is not available in Online Plot.
 - All modules of the same type (for example, μ BSM) run in the same ACQUITY core process, regardless of whether they are running in different instrument configurations. If one module stops working due to an underlying ACQUITY core driver issue, it will affect all other instruments using the same driver. In this case, restart the AIC or workstation.
Note:
 - It is possible to configure and operate up to four ACQUITY systems on one AIC.
 - ACQUITY and Alliance instruments can coexist.

For a complete list of defects, refer to [Known issues in this release](#).

8 Driver compatibility

The release of Waters Support Layer for Agilent CDS 3.0.0 provides support for acquisition control of Waters ACQUITY M-Class. It requires the Waters Driver Pack 2022 Release 1 (Microscale).

9 System and software requirements

This version of the Waters Support Layer for Agilent CDS 3.0.0 supports the following versions of Agilent OpenLab software:

- OpenLab CDS – 2.6 and 2.7 (tested by Waters)
- OpenLab EZChrom – A.04.09 and A.04.10 (tested by Waters)
- OpenLab ChemStation – C.01.09, C.01.10, and LTS 01.11 (tested by Waters)

Refer to the Waters website (www.waters.com) for the latest information regarding compatibility with future versions of Agilent OpenLab software.

For support of subsequent OpenLab CDS versions, check [SubscribeNet](#).

Note: If you want to reuse an instrument created with driver versions prior to 3.0.0, delete the instrument and recreate it with identical settings. This is required to reuse existing methods and sequences.

You can reuse all methods, sequences, and data, provided you perform the above steps.

This procedure applies to methods, sequences, and data created with Waters Alliance driver versions prior to 3.0.0.

9.1 Waters Support Layer for Agilent CDS 3.0.0 software requirements

Waters Support Layer for Agilent CDS 3.0.0 is compatible with the following operating systems:

9.1.1 Workstation

- Windows 10, 64-bit, Enterprise and Professional Operating Systems

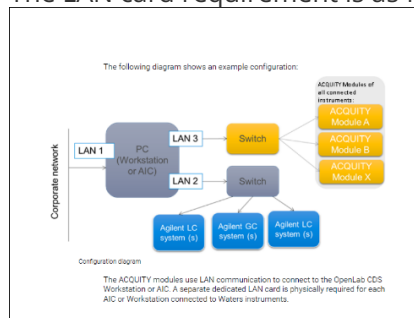
Note: Waters Support Layer for Agilent CDS 3.0.0 can be configured only on the Client and AIC.

9.1.2 Client/Server system

- Windows Server 2012 R2
- Windows Server 2016
- Windows Server 2019
- Windows 10

You must enable the .NET Framework 3.5 feature or the installation cannot proceed. See *Configuring Microsoft .NET Framework 3.5* in the *Waters LC Support Layer for Agilent CDS Installation and Configuration Guide* (715008538).

The LAN card requirement is as follows:



The Waters Support Layer for Agilent CDS 3.0.0 requires Waters Driver Pack 2022 Release 1 and Agilent OpenLab software (refer to the Agilent OpenLab software installation guides).

9.2 Supported Waters LC instrument modules and firmware

Waters Support Layer for Agilent CDS 3.0.0, which is installed with Waters DP 2022 R1, supports the Waters LC modules listed in the following table.

The following firmware versions are tested by Waters, and Waters expects no incompatibilities if you follow the guidelines. Refer to the Waters website (www.waters.com) for additional firmware compatibility guidelines.

Notes:

- Multiple modules of the same type of detector are not supported within the same instrument.
- For Checksum, ICS versions, and Firmware versions, refer to the *Waters Driver Pack 2022 Release 1 (for Microscale systems) Release Notes* (715007518 v00).

Waters recommends that you use the following firmware revisions with Waters Support Layer for Agilent CDS 3.0.0:

ICS/Driver or Module	Supported systems	Product status	Part number
μBSM	M-Class Systems	Current Product	186016003 - Binary Solvent Manager
ASM	M-Class Systems	Current Product	186016022- Auxiliary Solvent Manager
μFL	M-Class Systems	Current Product	186016012- Microscale μSM-Fixed Loop
μFL	M-Class Systems	Current Product	186016016 - Microscale TVM
Detectors			
TUV	ACQUITY UPLC, H/I/M-Class Systems	Discontinued	186015028 - ACQUITY TUV
	ACQUITY UPLC, H/I/M-Class Systems	Current Product	186015031 - ACQUITY TUV TC
	ACQUITY Premier Systems	Current Product	186018008 - PREMIER TUV
PDA	ACQUITY UPLC, H/I/M-Class Systems	Discontinued	186015026 - ACQUITY PDA
	ACQUITY UPLC, H/I/M-Class Systems	Discontinued	186015030 - ACQUITY PDA
	ACQUITY UPLC, H/I/M-Class Systems	Current Product	186015032 - ACQUITY PDA TC
	ACQUITY UPLC, H/I/M-Class Systems	Current Product	186015033 - ACQUITY PDA eLambda TC
	ACQUITY Premier Systems	Current Product	186018009 - PREMIER PDA eLambda

ICS/Driver or Module	Supported systems	Product status	Part number
	H/I-Class Systems	Current Product	186015053 - ACQUITY PDA TS

9.3 Supported vial and plate formats

For the available vial and well plate configurations, see the ACQUITY UPLC Sample Manager Overview and Maintenance Guide on the ACQUITY UPLC System Documentation CD and the online Help.

10 Waters Support Layer for Agilent CDS 3.0.0 for ACQUITY M-Class Microscale System Support

The ACQUITY UPLC M-Class system features ultra-low system and delay volume, which allows maximized peak capacity and resolution for sample-limited analyses that require high-capacity separations. The system is ideal as an LC inlet for highly sensitive MS-based assays. The ACQUITY UPLC M-Class delivers nano, capillary, and micro-scale separations, in a flow rate ranging from 200 nL/min to 100 µL/min for columns with internal diameter ≤1 mm. ACQUITY UPLC M-Class can optimally drive sub-2-micron separations with an upper pressure envelope of 15,000 psi (1034 bar). ACQUITY UPLC M-Class systems are available in several configurations:

- Direct Inject
- Trap/elute single-pump trapping with µBSM
- Trap/elute dual-pump trapping with µBSM and ASM
- Trap/elute dual-pump trapping with two (2) µBSMs

Additionally, ACQUITY UPLC M-Class has non-reactive wetted surfaces throughout to maintain sample integrity. Column trapping or trap/elute is the use of an on-line column for sample trapping and a separate analytical column for chromatographic resolution.

The operational details are listed below:

1. Analytes in solution are injected and retained on a trapping column while unwanted solutes are washed to waste through a two-position valve.
2. After washing the trapping column, the valve state changes, and gradient elution begins, causing the analytes to elute from the trapping column and refocus on the analytical column for gradient elution before detection.
3. The packing in the trapping column is a higher capacity reverse-phase (RP) support with lesser affinity for the analytes than the RP packing material in the analytical column.
4. Column trapping is used at micro-scale because you can load a large sample volume on a trapping column, remove solutes that may impact MS sensitivity, and not disturb the equilibration of the analytical column.
5. Trap/elute can be performed with one (1) µBSM, one (1) µBSM, and one (1) ASM, or two (2) µBSMs.

11 Acquisition control with Agilent OpenLab software

This section discusses acquisition control of Waters ACQUITY M-Class and compares the acquisition control of Waters chromatographs with that of other systems already supported by Agilent OpenLab software.

11.1 Waters ACQUITY Console

The ACQUITY UPLC Console is a separate application that supports diagnostic functions for the configured ACQUITY UPLC M-Class systems. The application provides a convenient way to configure settings, monitor performance, run diagnostic tests, and maintain the system and its modules. It replaces the keypads and small screen displays traditionally found on the front of system instruments. The console functions independently of the data applications, and the direct control functions are disabled when an acquisition is in progress.

You can launch the ACQUITY UPLC Console using the right-hand menu option. Right-click "Launch Console" from the Direct Device Control.

To apply best practices, review the following items before you use ACQUITY UPLC M-Class system:

- System or instrument module preferences, to set preferences such as the behavior of the sample manager when a vial is missing.
- Configure instrument modules, including μ SM-FL (with/without Sample Organizer)
 - Configure "Plate Setup"
 - Volumes of Loops and Needles for Sample Manager
- Module Diagnostics
- Help

All functions available from the Direct Device Control are accessible from the console as well, provided acquisition is not in progress. These include:

Solvent Manager Status

- System back pressure and delta back pressure (pressure change as a rolling average over 1.0 minutes)
- Composition: %A, %B
- Flow rate
- Stop Flow

Sample Manager Status

- Sample Compartment Temperature

Trap Valve Manager Status (optional)

- Column Temperature
- Valve position

PDA or TUV Detector (optional)

- Wavelength Setting
- Absorbance Units

11.1.1 Best practices for improving system performance

Below are some suggestions and best practices on how to achieve more reproducible chromatography.

Autozeroing the flow transducers

The flow transducers should be autozeroed under the following conditions:

- Changes in lab temperature are observed and consistently differ from when the transducers were last autozeroed.
- Seasonally, when the exterior temperature significantly changes.
- When a significant change is made to the mobile phase composition being used (for example, changing from a mobile phase containing formic acid to one containing ammonium formate or from acetonitrile to methanol).

Note: When refreshing mobile phase, an autozero is not required.

Autozero with breaking fluidic connections

The most reliable and consistent way to autozero the flow transducers is to break the fluidic connection at the pump outlet and insert a pin plug according to the following instructions. Due to the complexity of 2D system configurations and dual-pump trapping, this is the only recommended way to successfully autozero the flow transducers for these system configurations.

1. Break the fluidic connection (if already made) from the exit of the μ BSM and remove the tubing.
2. Prime the A and B lines of μ BSM for 5 minutes.
3. Set the μ BSM to flow at 25 μ L/min 50:50 A/B for 15 minutes.
Note: Place an absorbent material below the outlet of the pump to capture the flowing solvent.
4. Stop flow.
5. Wait at least 5 minutes and monitor measured flow rate for stability.
6. Pin plug the outlet of the μ BSM.
7. Again, wait at least 5 minutes and monitor measured flow rate for stability.
8. Run the Autozero flow transducers diagnostic, from the Troubleshooting menu of each pump within the console.
9. Reconnect tubing and ensure that there are no leaks.
Refer to the *ACQUITY UPLC M-Class System Guide* (715003588 Revision C) and the *M-Class 75- and 300- μ m Scale Tubing Upgrade Kit with ZenFit Connection Technology Installation Instructions* (715005458 Revision A).

Autozeroing without breaking fluidic connections

For a single-pump trapping system configuration, the flow transducers can be autozeroed without breaking any fluidic connections. If autozeroing the flow transducers according to the following instructions, it is critical that the system is fully depressurized before the autozero diagnostic is run.

1. Prime the A and B lines of μ BSM for 5 minutes.
2. Set the μ BSM to flow in trapping mode at 15-25 μ L/min (depending on the total back pressure of the system) 50:50 A/B for 15-20 minutes.
Note: Ensure that this does not exceed the maximum pressure of the column you are using.
3. Reduce the μ BSM flow rate to 5 μ L/min and flow for 5 minutes.
4. Set the μ BSM to flow in trapping mode at 0 μ L/min 50:50 A/B. Monitor the measured flow rates for stability.
5. Wait at least 5 minutes without disturbing the system.
6. Set the μ BSM to flow in analytical mode at 0 μ L/min 50:50 A/B. Monitor the measured flow rates for stability.
7. Again, wait at least 5 minutes without disturbing the system.
8. Run the **Autozero flow transducers** diagnostic, from the **Troubleshooting** menu of each pump within the console.

Flow ramping

Requirement: When using 75 μ m columns in a configuration, or in any trapping configuration, you must utilize flow ramping. Otherwise, when valves are switched, the sudden pressure change could cause back flow in the column or

damage to the column bed. This will impact the chromatographic reproducibility and performance. In some cases, a rapid pressure change on the column can fracture the fritting, which could allow particles to enter and block the emitter or valves and cause the system to over pressure.

To turn on flow ramping:

1. From the Inlet Method Parameters page, click **Advanced**.
2. In the Specify flow rate behavior when a valve changes position field, select **Flow rate is ramped to zero, valve position changes, flow rate is ramped**.

Flow rate on the B flow controller

For the most reproducible chromatography, the B side flow controller should operate at flow rates greater than 10 nL/min.

To determine the flow rate of the B flow controller:

1. Multiply the analytical/trapping flow rate by %B composition in the gradient table. If the calculated B flow rate is less than 10 nL/min, you must increase the %B composition in the gradient table.
Recommendation: Set the composition of solvent B $\geq 3.0\%$ for the analytical portion of the chromatography. However, this value may need to be increased based on the total flow rate of the system.

Initial gradient hold

When using a 75 μm column, after a gradient starts, the B side flow controller is typically running at its lowest flow rate in the entered gradient. Because of this, it can take time to fully stabilize the B flow controller, so it consistently delivers B mobile phase. To achieve the most reproducible chromatography, insert an isocratic hold at the beginning of your gradient table. The system should pump a volume between 75-150 nL of mobile phase B. The hold time required depends on multiple factors, including but not limited to:

- System configuration
- Initial flow rate and composition
- Total back pressure
- Changes in the ambient temperature
- Current method

Example: For a gradient running at 300 nL/min, with the initial condition of 95% A and 5% B, you should insert an isocratic hold, to pump a volume between 75-150 nL of mobile phase B, at 95/5 for between 5 and 10 minutes.

Recommendation: The isocratic hold time required can vary from system to system and should be investigated and optimized during method development phase.

Trapping flow rate and trapping composition

When trapping with columns that have a diameter $\leq 300 \mu\text{m}$, the trapping flow rate should be set to a value less than 10 $\mu\text{L}/\text{min}$. The best chromatographic reproducibility is observed when the trapping flow rate is set to 5 $\mu\text{L}/\text{min}$.

Recommendation: The optimum trapping flow rate and conditions should be investigated and optimized during the method development phase.

See **Flow rate on the B flow controller** for recommendations on the minimum percent B recommended for use when trapping.

Re-equilibration of column at the end of analysis

For the most reproducible chromatography, particularly when using flow ramps, the column pressure must return to a stable and consistent value during the system re-equilibration step after the end of the analytical gradient.

Recommendation: The length of the isocratic hold/re-equilibration step should be investigated and optimized during method development phase.

System equilibration after zero flow

Powering off the μ BSM, or setting a flow rate of 0 μ L/min, can cause the pump heads to retract. This process can aspirate mobile phase back into the flow controllers. Priming the pumps effectively primes the pump heads and the lines up to the vent valve, but it does not clear the solvent in the solvent lines from the vent valve to the flow controller. At flow rates <500 nL/min, it can take several injections (up to seven) to sufficiently equilibrate the solvent lines. If the system is configured in such a way that the flow from the μ BSM can be diverted to waste, either directly or through a trap column, the system can be equilibrated quickly to avoid a number of blank injections.

1. Equilibrate the system.

See also: *To equilibrate the system* for detailed instructions on how to equilibrate the system.

2. After equilibrating the system sufficiently, acquire 1 to 2 blanks before collecting data from samples.

Note: To avoid re-equilibrating the system often, the LC flow should be left on at a 50/50 reduced flow rate of 200 nL/min, if possible. If the flow must be turned off, the re-equilibration step can be shortened if the flow rate is gradually stepped down, rather than set immediately to a value of 0 μ L/min.

Equilibrating the system

To equilibrate a system, perform the following steps:

1. Prime lines A and B for at least 2 minutes.
2. Configure the TVM valve positions to divert the flow from the μ BSM to waste either through a trap column or waste tube.
3. Set the μ BSM to flow at a composition of 50:50 A/B at a flow rate such as 15-20 μ L/min.
Note: The set flow rate is dependent on the total system back pressure and should be adjusted so that it does not exceed the maximum pressure of the column.
4. Run the flow under these conditions for 15-20 minutes.
5. Change the composition to match the initial composition of the gradient that will be run but leave the flow rate at 15-20 μ L/min. Allow it to run at this flow for at least 5 minutes to clear the system lines and/or trap of the 50:50 mobile phase.
6. Set the flow rate to the initial analytical flow rate that will be used in the selected inlet method that will be run and allow the system to equilibrate until the system pressure reaches a stable value.

Re-equilibration between methods

When changing methods during acquisition, use two blanks between the methods to ensure that the system is at its initial conditions prior to acquiring sample data.

Temperature sensitivity

The flow controllers in the μ BSM can be affected by large or rapid changes (>1.5 $^{\circ}$ C/hr) in the room temperature. A large temperature change in the lab can cause the gradient to be generated in a non-reproducible manner that will affect chromatographic reproducibility. Methods that utilize nanoscale flow rates (<500 nL/min) are more susceptible to

changes in lab temperature. Some changes in lab temperature can be overcome by running the system at a higher flow rate (for example, increasing from 0.3 µL/min to 0.5 µL/min). When increasing the flow rate, it may be necessary to increase the column temperature or use a shorter column to ensure that the system does not over pressure at the higher flow rates.

Note: If the changes in lab temperature are too large/rapid, it may be necessary to relocate the system to a more temperature-stable area of the lab or contact the lab facilities manager to improve lab temperature stability.

11.2 Event log workspace

The Event Log Workspace contains logs of system events, including errors, warnings, and messages. This information for the LC System is also available in the ACQUITY Console Software Logs, where detailed Help topics describe the event and might be useful in troubleshooting and performing system diagnostics. Right-click the instrument status panel and then select "**Launch Console**" to open the console. In the left-hand pane, select "**Logs**" to access the event log workspace.

12 Known issues in this release

This section lists the known issues and solutions for this release. The numbers identify issues that Waters and Agilent personnel monitor within a system change request tracking tool.

For the Waters DP 2022 R1 there are no known issues; however, refer to Waters documentation for reported issues and to Agilent documentation for known issues with Agilent OpenLab software.

Defect reference	Description	Module affected	Workaround
INFASLR-20	<p>In the method report, there is no indication that certain SM fields are disabled.</p> <p>The following fields show Automatic in the method report though they are disabled in the method.</p> <p>Full Loop Overfill Factor: Automatic</p> <p>Syringe Draw Rate: Automatic</p> <p>Needle Placement: Automatic</p> <p>Pre-Aspirate Air Gap: Automatic</p> <p>Post-Aspirate Air Gap: Automatic</p>	Sample Manager Method report	N/A

Defect reference	Description	Module affected	Workaround
<p>INFASLR-24</p> <p>INFASLR-30</p>	<p>Starting the instrument in ChemStation directly after reconfiguration results in unresponsive ChemStation at the start of the run.</p> <p>ACQUITY: Cannot disconnect smoothly from the AIC after reconfiguration.</p>	<p>All the modules - Configuration</p>	<p>Reconfiguring an existing instrument system requires a PC reboot.</p> <ol style="list-style-type: none"> 1. Reconfigure a system. 2. Reboot the PC. 3. Launch the instrument from CDS.
<p>INFASLR-89</p>	<p>Method validation error for PDA spectrum settings does not prevent UI from being closed.</p> <p>Selecting specific settings for 3D spectrum of PDA causes CDS to display a Waters DP error message, and multiple clicks are required to close this error message.</p>	<p>PDA module - Method Editor</p>	<p>Click OK in the message box until the pop-up stops appearing. When the message box pop-up stops, ensure that the range is given correctly, and then save the method.</p>
<p>INFASLR-92</p>	<p>Sample Manager does not show correct value for set temperature.</p>	<p>SM module - Status UI</p>	<p>Sample Manager temperature values are not displayed properly in the Status UI. To see the correct values, view the tooltip.</p>
<p>INFASLR-95</p>	<p>Units for RI Temperature Flow Cell in the Method Audit Trail is missing.</p>	<p>RI module - Audit Trail</p>	<p>N/A</p>
<p>INFASLR-383</p>	<p>Download method does not reset the flow to "0" for all the pumps in multi-pump scenario.</p>	<p>Pumps</p>	<p>N/A</p>
<p>INFASLR-623</p>	<p>Dual Detector support: FLR detector check box is not selected in the LC Method with default method, but it should be selected.</p>	<p>Detectors</p>	<p>Manually select/clear and save the method.</p>

Defect reference	Description	Module affected	Workaround
INFASLR-638	Method with non-zero flow rate: With current flow rate "0" in the status panel, when user downloads method with a non-zero flow rate, then the flow rate is applied to one of the pumps, but the flow rate must not be applied.	Pumps	N/A
INFASLR-640	CDS validation is missing, acquisition can be run when priming is in progress.	Pumps and Sample Manager	Wait until priming is completed.
INFASLR-735	The "Injection Volume" field occasionally changes the alignment.	Alliance HPLC and ACQUITY	N/A
INFASLR-745	Direct control changes of DP detectors are not logged in the activity log.		N/A
INFASLR-758	Method import of a different configuration is not supported: A method resolution dialog appears while loading a method with different configuration. However, method is saved without resolved parameters.	Alliance HPLC and ACQUITY	Do not use the method resolution dialog. Instead create a new method.
INFASLR-761	The "Injection Volume" is not updated with a valid range in the method editor. You must specify a valid value.	ACQUITY - Method	The instrument will throw an error if the injection value is out of range. In such a scenario, the user must reset the instrument from the status panel.
INFASLR-762	The field "Run Time" is not checked for valid entry.	Method Editor	The value is not validated during editing, but it will be validated while saving the method.
INFASLR-763	You cannot customize the installation path in the installer.	Installer	Do not change the path; proceed with the default path.
INFASLR-766	The instrument status UI displays only one wavelength in dual mode.	Status Panel	Refer to ACQUITY console to view both wavelength details.

Defect reference	Description	Module affected	Workaround
INFASLR-825	ACQUITY setup 2.4 cannot detect the Waters Agilent CDS Support Layer Driver, so it can be installed on top.	Installer	Uninstall ACQUITY 2.4 and reinstall Waters Support Layer for Agilent CDS 3.0.0.
INFASLR-1014	The TVM valve does not move to position 1 from position 2 when the user runs the acquisition.	Trap Valve Manager	<p>Reset the TVM from the CDS Instrument Status panel or launch the Console and perform the following steps:</p> <ul style="list-style-type: none"> • Click System > Control > Set flow. • In the Set Flow dialog, select Position 1 for the TVM position. • Click OK.

13 Issues fixed in 3.0.0 release

Defect reference	Description	Affected module
CRI-3115	ACQUITY's Instrument Status screen must be redesigned.	StatusUI

Defect reference	Description	Affected module
INFASLR-11	<p>Supports acquisition of configuration dependent diagnostic channels. The auxiliary signal data that is dependent on the Sample Manager configuration will not be saved as part of an acquisition data that can be viewed during data processing.</p> <p>ACQ-FTN: Sample Organizer Temperature, Preheater Temperature, and Column Temperature</p> <p>ACQ-rFTN: Sample Pressure, Column Temperature, Sample Organizer Temperature, and Preheater Temperature</p> <p>ACQ-SM: Column Temperature, Sample Organizer Temperature, and Preheater Temperature</p> <p>ACQ-nSM: Column Temperature, Preheater Temperature, and Sample Organizer Temperature</p>	Acquisition, monitor signal
INFASLR-67	Running a method with PDA Lamp Off does not switch into run.	Acquisition
INFASLR-130	WDHCPServerSvc increases Private Bytes during long sequence.	Acquisition
INFASLR-676	M-Class-Different Type-Dual Pump Trapping: When users run acquisition with sequence, TVM goes to Error status as Communication failure with Sample Manager	Acquisition
INFASLR-681	Support Layer Driver files are not listed in the SVT report for EZChrom	SVT report
INFASLR-685	M-Class Dual Pump Trapping: Unable to perform acquisition in ChemStation	Acquisition

Defect reference	Description	Affected module
INFASLR-700	Update the driver title in the SVT report.	SVT Report, Installer
INFASLR-711	SVT report to include missing files and Agilent Single Quadrapole LC/MS entry to list in the Configuration window.	SVT Report
INFASLR-723	Unsaved changes dialog appears when user tries to submit single run after saving the method in OpenLabCDS and Chemstation.	Method Editor
INFASLR-798	Injection Volume Input does not work correctly	Method Editor
INFASLR-824	When users run long sequence with 2D and 3D alternate method with 500 injections, sequence is not completed and stays in the Equilibrating method status.	Acquisition
INFASLR-867	Acquity FLR instrument sometimes provides two spectrums with the same time stamp at the beginning. Waters driver filters out the first spectrum in such scenarios. Hence, the spectrum graph displays a missing spectrum at the end of the graph. You can ignore this gap, if auto-zero is disabled when gain is 1 and enabled when gain is 20 while running an acquisition.	Acquisition
INFASLR-1013	The method report does not match the method parameters. If the PDA is set to display eight channels with different wavelengths, the method report displays incorrect wavelength values for channels seven and eight.	Method Editor

14 Antivirus considerations

Some real-time virus scanners mistake normal data acquisition and instrument control for virus activity, and thus interfere with proper operations. Full-system scans and live updates can be network-intensive, disk-intensive, and CPU-intensive, and they can also interfere with normal data acquisition. Schedule scans and updates for idle times when data acquisition does not occur.

Certain antivirus program features like "intrusion prevention" and "tamper protection" can also interfere with normal operation. Disable them.

Exclude C:\Program Files (x86)\Waters Instruments and C:\Program Files\Waters Instruments from virus scanning.