Best Practices for Using an Agilent LC System

Technical Note

This technical note describes best practices for using an Agilent LC system.

Contents

Agilent Technologies

Housekeeping

How to deal with solvents

- Use clean bottles only.
  - Use borosilicate glass bottle only.
  - Rinse bottle with desired solvent before refilling it.
  - Bottles can get contaminated with detergents form the dishwasher.
- Exchange water-based solvents daily.
  - Algae growth may block the degasser or filters.
  - Precipitation of insoluble salts may block filters or capillaries.
- Select solvent volume to be used up within 1 – 2 days.
- Use only HPLC-grade solvents and water filtered through 0.2 µm filters.
  - Residues or contaminations may block filters or capillaries.
- Label bottles correctly with bottle content, and filling date / expiry date.
- Use solvent inlet filters to protect the system from incoming particles.
- Reduce risk of algae growth: use brown bottles for aqueous solvents, avoid direct sunlight or wrap the bottles in aluminium foil.

CAUTION

Contaminated seal wash solvent

➔ Do not recycle seal wash solvent to avoid contamination

➔ Weekly exchange seal wash solvent

➔ Use of Seal Wash Bottle Head Kit (5067-6131) is strongly recommended

Extra measures with Acetonitrile (ACN)

- Filter ACN using a 0.45 µm nylon filter.

NOTE

Filtering through nylon filters is not recommended for High Sensitivity LCMS.

- Fill ACN in brown bottles and keep amount to minimum to prevent photochemical reactions and oxidation.

NOTE

Add 5 – 10 % water to ACN, especially for LCMS applications when 0.1 % formic acid is present (if possible).

- Flush the system monthly with warm water (60 – 70 °C (140 – 158 °F)) - 1 L at 2 mL/min to dissolve traces of ACN reaction products.

How to prepare samples

CAUTION

Possible sample precipitation

➔ Take care that the sample is complete soluble in both, the used sample solvent and the mobile phase at starting conditions.

➔ Match the sample solvent matches and the proposed mobile phase as closely as possible to prevent precipitation.

- Filter, decant, or centrifuge sample to separate from insoluble solid.
- Take care that the sample solvent is free of particles.

Daily / Weekly tasks

Daily tasks

- Replace solvents and solvent bottles for mobile phases based on water/buffer.
- Replace solvents and solvent bottles for organic mobile phase latest every second day.
- Check presence of seal wash solvent.
- Purge each channel with fresh solvent at 2.5 – 3 mL/min for 5 min.
- Equilibrate your system with composition of your application for 15 min. Use conditioning for 1290 systems.

Weekly tasks

- Change seal wash solvent (10 % / 90 % isopropanol/water) and bottle.
- Flush all channels with water at 2.5 – 3 mL/min for 5 min to remove salt deposits if buffer applications were used.
- Inspect solvent filters for dirt or blockages. Clean or exchange if no flow is coming out of the solvent line when removed from the degasser inlet.

Power up / Shut-down the system

Power-up the system

Power up the pump

- Use new or different mobile phase (as required).
- Purge each channel with 2.5 – 3 mL/min for 5 min. Open the purge valve (1260) or use the purge command (1290).
- Equilibrate your system with composition of your application for 15 min. Use conditioning for 1290 systems.

Power up the sampler

- Purge the autosampler daily, and before and after sample analysis, especially if you are using buffers.
  - Set flow to required value of your application and close the purge valve.
  - Pump for approximately 10 min.
- Use fresh needle wash and/or needle seat backflush solvents like methanol or acetonitrile and water mixtures without buffer.
- Ensure that the vials contain enough sample solution for all injections.

Power up the detector

- Warmup the lamp for at least 1 h.
- For RI detectors only: flush the reference and sample side with fresh solvent used for the current application.

Shut-down the system

NOTE Use 50\50 Methanol\Water or 2-propanol\Water without additions to store system.

Long-term storage of the column

- Flush the column with appropriate solvent found in the column manual.
- Remove and seal column, and store according to good laboratory practice if needed.

Long-term shut-down of the system

- Flush system with water to remove buffer.
- Remove all samples from the sampler and store according to good laboratory practice.
- Use recommended solvents to store the system.
- Power off the system.

Recommendations for degassers

CAUTION

Condensation of vapor inside the degasser

If an internal or high performance degasser is used with low boiling solvents, the solvent vapors can condensate inside the degasser chamber when the vacuum pump is turned off.

➔ Purge all solvent channels with 2-propanol and let the degasser pump for two more hours.

- Check compatibility of solvent with degasser and application
  - Use internal or high performance degassers for standard applications
  - Use the standard degasser (G1322A or G7122A) for RI applications
  - Use the standard degasser for high volatile solvents with vapor pressure below 100 mbar at room temperatur.
- Use the **Evacuation Mode** if degassing performance of internal degassers is not optimal. Access it in the degasser control from the instrument control screen in the Agilent LabAdvisor.

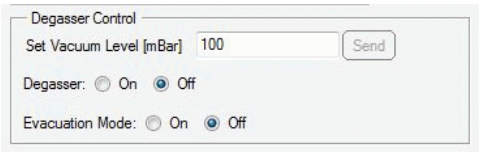

Figure 1 Degasser Control for internal degassers in Agilent LabAdvisor

NOTE

Follow the instructions prompted on the screen when starting the **Evacuation Mode**.

Recommendations for pumps

- Check pumps performance on regular basis.
- Perform preventive maintenance in the recommended usage interval.
- Prepare the pump as recommended like described in the power up section to ensure optimal performance and best life time.
- Use the seal wash function as recommended to ensure optimal performance and best life time, see below.

Recommendations for Pumps with MCGV

Select channels for Multi-Channel Gradient Valve (MCGV)

- Use lower channels (A and/or D) for buffer solutions.
- Regularly flush all MCGV channels with 200 mL of warm water to remove possible salt deposits.
- Check compatibility of buffers and organic solvents to avoid precipitation in the MCGVs mixing chamber.

NOTE When mixing incompatible solvents, salts can precipitate at the point of mixing blocking the downstream flow path and damaging parts.

Seal Wash (usage mandatory when installed)

Seal Wash (G4204A, G4220A, all 1260 Pumps)

CAUTION

Contaminated seal wash solvent

➔ Do not recycle seal wash solvent to avoid contamination

➔ Weekly exchange seal wash solvent

➔ Use of Seal Wash Bottle Head Kit (5067-6131) is strongly recommended

Using the seal wash function is strrongly recommended when using water or water based solvents like buffer, other non-volatile solvents or additives that could deposit on pistons and seals. The seal wash function regularly cleans these parts automatically.

Benefits of Seal Wash Operation:

- Removal of particles, salt crystals and other non-volatile residues from the pistons and seals, which have the potential to damage the piston and piston seals
- Lubrication of seal/piston interface
- Cooling of pistons

Seal Wash Dialog in your CDS

The dialog can be found under the control screen, it is recommended to use the settings displayed in Figure 2 on page 10.

Be aware that:

- The seal wash settings are NOT method parameters
- The seal wash has to be turned on again manually after:
  - An ERROR has been cleared
  - Power on

Seal Wash Operation:

- PERIODIC operation, for example 0.5 min every 7 min
  - Setting can be changed in the Control screen, see Figure 2 on page 10.

    The settings are available via the context menu, see Figure 4 on page 10.
  - Typical solvent flow is 0.7 mL/min what corresponds to an approximate consumption of 3 mL/h of or 0.5 L/week at constant operation
- Use 10 % 2- Propanol in water
  - 100 % 2- Propanol for normal phase applications
- Position wash solvent bottle above and waste bottle below instrument
  - *DO NOT* recycle the seal wash solvent
  - Usage of the Seal Wash Bottle Head Kit is recommended (Agilent Part Number: 5067-6131)

- Exchange solvent weekly
  - *Do not* refill seal wash solvent, always use properly cleaned, fresh bottles
- Test Peristaltic Pump
  - Touch the peristaltic pump with you finger to check that the pump is working, or confirm seal wash solvent flow by looking seal wash solvent dropping out of the tubing

Table 1 Seal wash dialog and operation

G4204A, G4220A	all 1260 Pumps

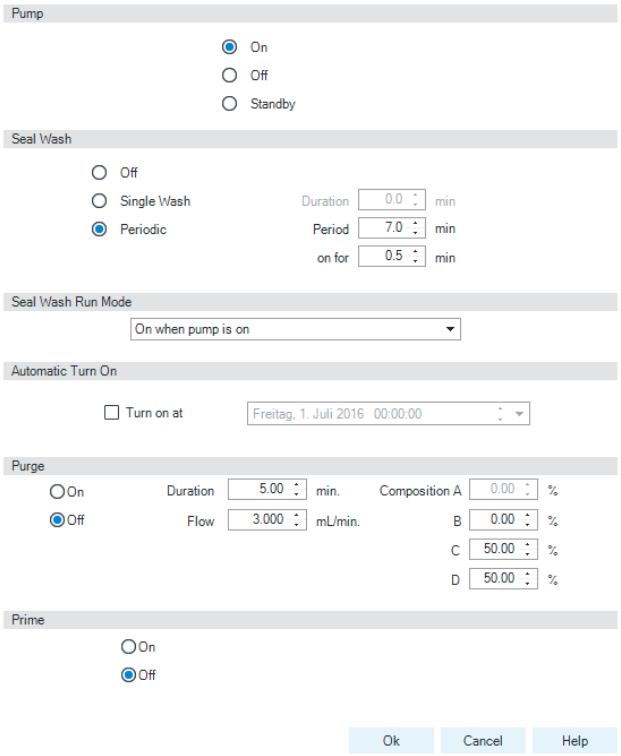

Figure 2 Seal wash settings (1290 Infinity Pumps)

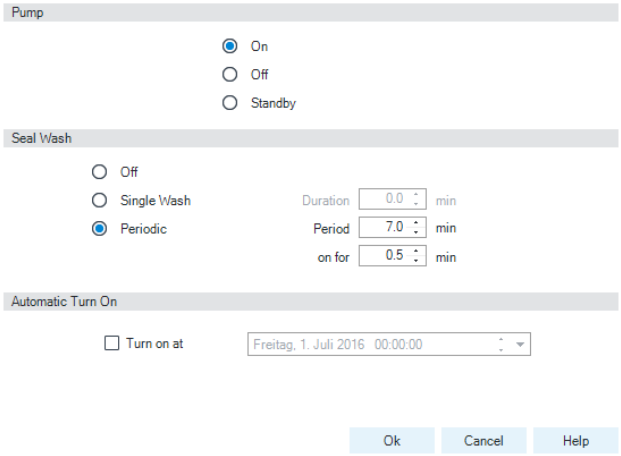

Figure 3 Seal wash settings (all 1260 pumps)

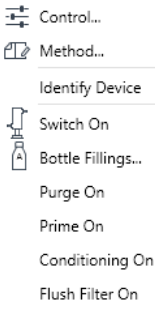

Figure 4 Context Menu (1290 Infinity Pumps)

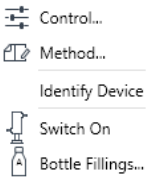

Figure 5 Context Menu (all 1260 pumps)

Seal Wash (G7104A, G7120A)

CAUTION

Contaminated seal wash solvent

➔ Do not recycle seal wash solvent to avoid contamination

➔ Weekly exchange seal wash solvent

➔ Use of Seal Wash Bottle Head Kit (5067-6131) is strongly recommended

The seal wash pump is turned on when the analytic pump is moving solvent to remove deposits from pistons and seals. The seal wash function regularly cleans these parts automatically.

The seal wash sensor will constantly check the performance of the seal wash system and warn the user in case an anomaly has been detected.

Seal Wash Operation:

- The seal wash interval is set to 30 s on every 7 min.
- The flow is set to 500 µL/min.
- The integrity of the seal wash system is checked at regular intervals
- Typical solvent usage is about 1 L per week
- Use 10 % isopropanol in water
  - 100 % isopropanol for normal phase applications
- Position wash solvent bottle above and waste bottle below instrument
  - *DO NOT* refill the seal wash solvent, always use properly cleaned, fresh bottles
  - Usage of the Seal Wash Bottle Head Kit is recommended (Agilent Part Number: 5067-6131)
- Exchange solvent weekly
  - *DO NOT* recycle seal wash solvent bottles

- The EMF symbol will turn yellow once the pumps seal wash sensor detects an irregularity
  - Change the seal wash solvent and trigger the Seal Wash Prime function from the context menu (see Figure 6 on page 12)

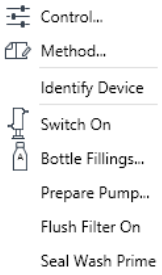

Figure 6 Context Menu

  - Check seal wash tubing and filter for kinks, leaks or blockages
  - Check for blockages of the waste tubings, make sure that the solvent waste can drain off freely:

    If solvent waste is building up in the tube, the sensor can't perform correctly
  - Check the peristaltic pump for function
    - Touch the peristaltic pump with you finger to check that the pump is working, or
    - Confirm seal wash solvent flow by looking seal wash solvent dropping out of the tubing

Recommendations for samplers

- Purge the autosampler after sample analysis.
  - Remove buffer with HPLC grade water.
  - Remove contaminating substances with a strong solvent, for example pure acetonitrile.
  - Toggle the injection valve between Mainpass and Bypass while purging.
- Always use fresh wash solvent for the needle or seat wash function.
  - Remove buffer with HPLC grade water.
  - Remove contaminating substances with a strong solvent, for example pure acetonitrile.
- Place the wash solvent reservoir for needle wash (optional: needle seat flush) into the solvent cabinet.
  - Use an appropriate solvent based on the sample and mobile phase properties.

NOTE The composition of the needle-wash solvent should be the most solubilizing compatible solvent (your strongest diluent). Selecting it is part of the method development. A mixture of 50 % up to 100 % organic solvent in distilled water is a good choice for many applications.

- Check the drainage routing of the washport outlet into a waste container.
- Fill each vial with enough sample solution for all injections.
  - Use Agilent recommended vials only.
  - Do not overfill the vials, that is to say fill each vial up to 90 % only.
  - Use pre-slitted septa when drawing large volumes or multiple times from the same vial.
- Filter, decant, or centrifuge sample to separate from insoluble solid.

NOTE Sample solvent should be free of particles.

- Take care that the sample solvents match the proposed mobile phase as closely as possible.

Recommendations for pumps and samplers with optional inline filter

Optional inline filter for pumps and samplers (1290 Infinity and 1290 Infinity II)

In most cases, the lifetime limiting factor for UHPLC columns is high backpressure. Particulate matter in the sample is collecting on the inlet frit of the column which causes an increase in the backpressure until the pressure limit of the system is reached. Using the inline filter is recommended to prevent blockages of the column frit, that is when sample preparation does not allow for filtering or the sample may form precipitate.

The following modules can be equipped with an additional inline filter:

- Pumps (G7104A, G4204A):

  Inline Filter Assembly (5067-5407)

- All Agilent Technologies autosamaplers:

  1290 Infinity II Inline Filter Kit (5067-6189)

This inline filter with a nominal filter pore size of 0.3 µm protects the UHPLC column effectively from clogging by particulate matter from samples or from the UHPLC system.

Advantages of the inline filter:

- Very small internal volume
  - Delay volume with rigid capillary 1.3 µL
  - Delay volume with flexible capillary 1.6 µL
- Specified for working at high pressures (max. operating pressure 1300 bar)

Installing the inline filter into the G4204A or G7104A is recommended to protect the downstream system from blockages in the following cases:

- When solvent combinations are used that can form precipitation after mixing,
- When running applications with buffers or additives when columns with small particle sizes are used

General hints for effective usage of the inline filter:

- Filter solvents before usage
- Follow best practices
- Back-flush the filter in the pump weekly (start Flush Filter from the context menu)
- Exchange the filter frit (Frit 0.3 µm for inline filter, 5/pk (5023-0271)) every 1000 injections or when the back-pressure rises by 15 %

NOTE

See *Technote G7167-90130* for further reference.

Recommendations for columns

- Use columns only in the marked direction.
- Always use suitable fittings for your specific column.
  - Different vendor columns require different fitting dimensions.
  - Using an unappropriate fitting may result in peak dispersion or even terminal damage to the column.
  - Agilent recommends using A-Line fittings to overcome fitting incompatibilities when using different vendor columns.
- Always adhere to operating and application limits, as put forth in the column user guide.
- Equilibrate the column with 10 – 20 column volumes before use.
  - It is advisable to do an intermediate flush with a mobile phase of the correct composition without additives before equilibrating to the final solvent with additives.
- The use of a guard column is recommended to protect your column and increase its lifetime.

NOTE Long-term storage of columns should always be in the appropriate storing solvent, for more details on the column in use, see the User Guide inserted in the column package.

Recommendations for detectors

CAUTION

Frequent lamp on/off

Reduced lifetime of the lamp

➔ Avoid unnecessarily switching on/off the lamp.

NOTE

There is a safety period/wait time before a lamp can be re-ignited after it has been turned off.

- Warm-up the lamp at least 1 h.
- Keep environment and ambient temperature stable.
  - Do not expose the detector to direct sun light.
  - Do not expose the detector to too much air current from the HVAC.
- Install pressure relieve valve when connecting a second detector after the Max-light cartridge cell.
- Use the recommended waste lines for each detector type. Avoid pinching the waste tube after the cell outlet.
- Ensure that the detector flow cell is bubble free.
- For RI detectors only: flush the reference and sample side with fresh solvent used for the current application.
- Flush the flow cell after use.
  - Use HPLC grade water to remove salts.
  - Use isopropanol to remove organic solvents.
- Before removing an unused flowcell for storage fill it with isopropanol to prevent algae growth.

Additional Information for 1290 Infinity and 1290 Infinity II Pumps

The Agilent 1290 Infinity and 1290 Infinity II Pumps are equipped with automatic purge valves. This enables a variety of additional functions not available in Agilent Pumps with manual purge valve. It is possible to prepare the pump (set paramaters and start the functions **Purge**, **Condition**, or **Prime**) with the software.

Purge

Use the Purge function to:

- Fill the system with fresh or different solvent.
  - Ensure that the new solvent is miscible with the previous solvent.
  - Prevent damage to the degasser or pump by using an intermediate step with a co-miscible solvent, if necessary.
- Remove air bubbles in tubes and pump heads.
  - After the pump in use for the first time.
  - After the pump has been idle for a few hours or more (air may have diffused into the solvent lines).

As soon as the duration time of the purge ends the module automatically switches to analytical conditions again.

Condition

If micro air bubbles persist in the pump head the overall pump performance may look correct but the pump will perform extra work and accuracy/precision are negatively affected. To remove the air efficiently the Condition function can be used. During Conditioning, the pump is delivering flow into the system (column) and the last used method settings, like flow, composition and max pressure are used.

Use a reasonable flow rate (for example 1.5 mL/min), composition setting (for example A: 50 % B: 50 %) and back pressure (>200 bar) to ensure efficient air bubble removal from all heads.

If applicable generate an extra conditioning method at the beginning of the sequence.

Condition the pump if you see:

- Excessive pressure ripple.
- Excessive composition ripple (baseline noise/mixing noise – noise level changes with the composition), when you are sure that the solvent type is correctly set, and there is no evidence of out of specification leakage in the pump.

Conditioning may be necessary:

- Daily when starting the pump
- After a long period of standby
- After running out of solvent
- After service or repair

CAUTION

Filling empty solvent lines

Damage to the seal

➔ Use a syringe to fill empty solvent lines.

➔ Do not use the **Prime** procedure to fill empty solvent lines.

Prime

The Prime function is helpful if air has entered the pump heads. The module draws solvent at high speed with all pump drives simultaneously and dispenses it into the waste position of the automatic purge valve. This is done 20 times before the process comes to an end.

Use the Prime function to:

- Fill pump heads when completely dry.
- Free a potentially blocked valve.

The described functions can be triggered from the driver interface:

- 1290 Infinity

NOTE

For parameter settings, see Figure 2 on page 10.

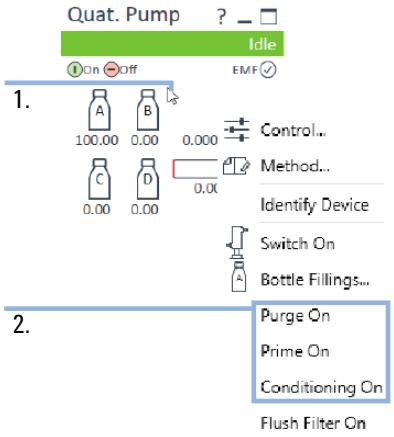

Figure 7 Prepare the pump (1290 Infinity).

1.	Right click on the module dashboard
2.	Select the appropriate function to start the procedure

The user-optimized Prepare Pump context menu replaces the classical menu:

- 1290 Infinity II Pumps

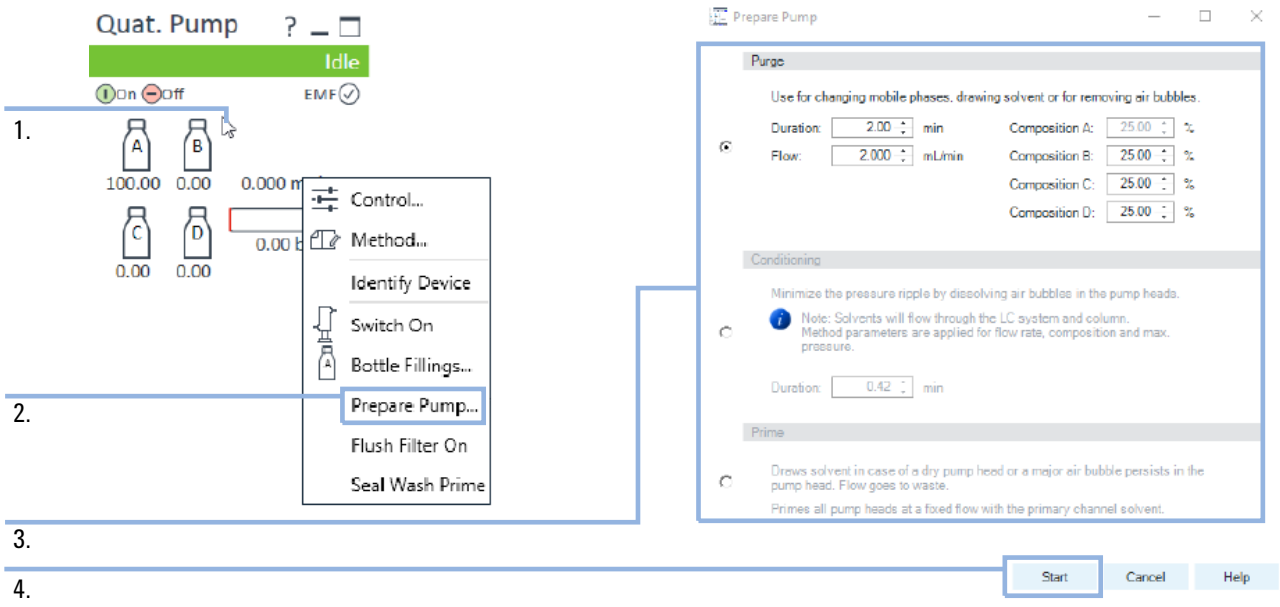

Figure 8 Prepare the pump (1290 Infinity II pumps)

1.	Right click on the module dashboard
2.	Select **Prepare Pump...**
3.	Select the procedure and fill in adequate parameters
4.	Click **Start** to run the selected procedure

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