

How to Keep a Good Thing Going: Preventive and Routine Maintenance for Your HPLC System

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8 November 2022

Tasks in the HPLC Lab



Annual Tasks



- Major Preventative Maintenance (PM)
 - Pump – Clean pistons, replace piston seals, replace frit in purge valve
 - Tests: Leak rate test, pressure test
 - Autosampler – Clean transport rods, replace needle, replace needle seat, replace rotor seal
 - Tests: Pressure test, accuracy by mass or linearity test
 - Column compartment – Replace rotor seal in column switching valve
 - Tests: Heating and cooling speed and accuracy tests
 - Detector – Inspect flow cell for leaks, typically only replace lamp if intensity test fails
 - Tests: Holmium oxide test, intensity test, wavelength verification, dark current test, filter test

Semi-annual

- “Minor PM”
 - Check maintenance counters
 - Run module diagnostic tests in Lab Advisor
 - Replace glass or clean stainless steel solvent filter frits for mobile phase bottles
 - Check solvent vent and replace as needed
 - Change rough pump oil for LC/MS systems



Glass filter, solvent inlet, 20 µm pore size, 5041-2168



Stainless steel filter, solvent inlet, 10 µm pore size, 01018-60025



InfinityLab solvent bottle, clear, 1 L, 9301-6528



InfinityLab solvent bottle, amber, 1 L, 9301-6526



Quarterly

- Basic housekeeping
 - Inspect the system for leaks
 - Wipe down/clean the system
 - Replace frit in purge valve
 - Flush aqueous channels with IPA
 - Run module diagnostic test through Lab Advisor
 - Check your spares/consumables inventory and restock



Monthly

Focusing on data

- Review and update your Statistical Quality Control (SQC) charts
- Routine PC maintenance
- Backup data
- Check expiration dates on stored chemical stocks
- Check certification dates on lab devices, such as balances, pipettes, and thermometers



Instrumentation

- For LC/MS systems, clean the inner source

Weekly

Exchange aqueous mobile phase

- Take down existing bottle of aqueous mobile phase and discard
- Rinse clean the spare bottle with HPLC water at least twice
- Prepare aqueous mobile phase and filter, if using a buffer
- Refill aqueous mobile phase reservoir and place back on system



For LC/MS clean outer source

Daily

Calibrate

- Run at least one full calibration curve per day
 - Preferably, run a calibration curve with each sequence
- System suitability
- Check standards for comparison to column benchmark
- Run control samples
 - Preferably, run controls with each sequence

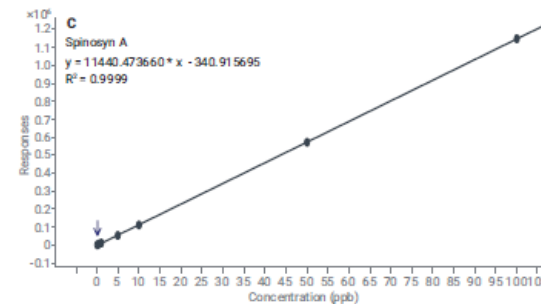
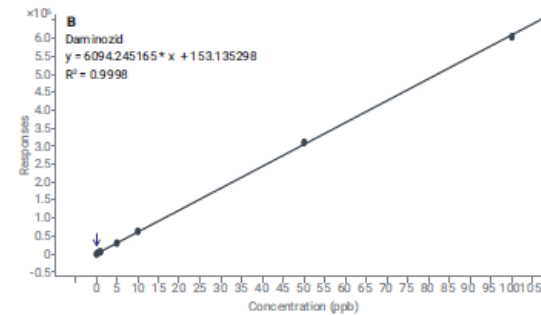
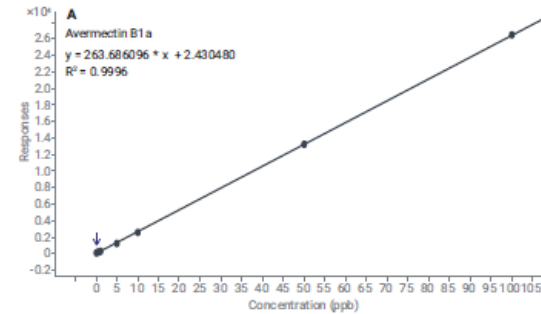


Figure 3. Example calibration curves, California list.

Performance-based Activities

Performance standard

- Injector performance
 - Carryover
 - Injecting blanks after the highest calibration standards will usually indicate if the method or system has an issue with carryover. If carryover was not initially present but instead increased with time, the issue may be due to the sample matrix. A strong flush can often correct carryover issues, if not, then replacing the needle, needle seat, and column are all potential next steps.
 - Precision
 - When injector precision decreases significantly, this usually indicates that the rotor seal on the injection valve needs to be replaced.



Needle assembly 1290 vialsampler,
G7129-87201



Needle seat, PEEK, 600 bar, 0.12 mm id,
G1367-87012



Needle assembly multisampler,
G4267-87201

Performance-based Activities

Performance standard

- Backpressure
 - System pressure increases with blockages
 - Blockages can include:
 - Clogged inline filter
 - Dirty guard column
 - Clogged column
 - Precipitates in the flow path
 - Precipitation in the flow path is most often seen in the needle seat due to sample solvent/mobile phase differences.



Infinity LC inline filter, 5067-6189

Easy-to-use hardware available in all chemistries

High-performance Agilent UHPLC guards are designed for use with fast LC columns. They connect directly to the column inlet; no extra hardware is needed.

Agilent UHPLC guards are available in all InfinityLab Poroshell 120 chemistries—giving you confidence that the guard column will not adversely affect your separations.

Turn to **Pages 128–131** to find out more.



Performance-based Activities

Performance standard

- Peak shape
 - Peaks tend to broaden and tail as a column is used. Materials, often matrix-related, build up on the column and create sites for secondary partitioning.
 - When peak symmetry increases beyond a threshold value that is reasonable for the application and scientist, the column should be cleaned
 - Columns are typically cleaned by backflushing with a strong organic solvent. This is done at a slow flow rate for an extended period



Resources for Support

- Tech support: www.agilent.com/chem/techsupport
- Agilent product catalogs: www.agilent.com/chem/catalog
 - InfinityLab LC Supplies catalog (5991-8031EN)
- Resource page: <http://www.agilent.com/chem/agilentresources>
 - Quick reference guides
 - Catalogs, column user guides
 - Online selection tools, how-to videos
- Agilent University: www.agilent.com/crosslab/university
- YouTube: Agilent Channel
- Your local FSE and specialists
- Agilent service contracts



Contact Agilent Chemistries and Supplies Technical Support



Available in the U.S. and Canada, 8-5 all time zones

1-800-227-9770 option 3, option 3:

Option 1 for GC and GC/MS columns and supplies

Option 2 for LC and LC/MS columns and supplies

Option 3 for sample preparation, filtration, and QuEChERS

Option 4 for spectroscopy supplies

Option 5 for chemical standards

Option 6 for Prozyme products



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