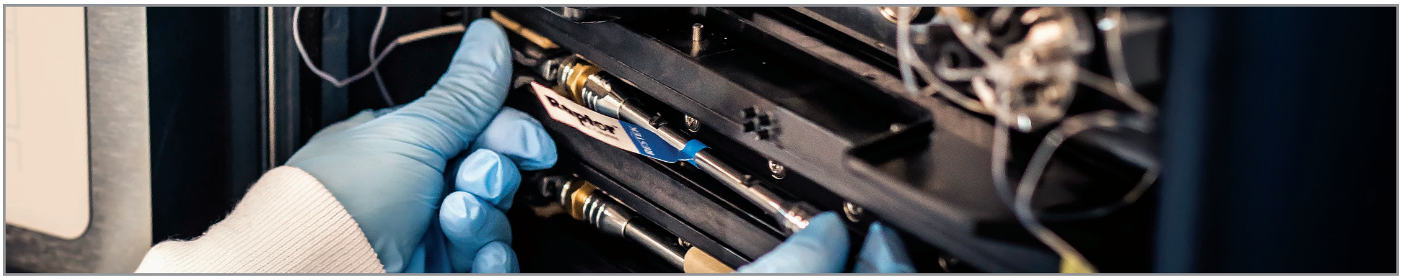


Routine LC Maintenance

Simple Steps to Preventing Unexpected Downtime

- Improve instrument performance.
- Prevent extended, unscheduled downtime.
- Increase data quality and consistency.

While it is easy to see the wisdom in the advice that "an ounce of prevention is worth a pound of cure" (Benjamin Franklin), making time for preventative measures is much harder to do. But, despite busy schedules and heavy workloads, one of the best ways to increase lab efficiency is to invest time in preventative LC maintenance. By developing a program of regularly scheduled events that ensure your instrument is running well, you will minimize unplanned downtime for unexpected repairs, improve component lifetime, and ensure more consistent performance. All of this adds up to a cost-effective strategy for protecting data quality and increasing overall lab productivity.

Routine LC maintenance programs should establish specific inspection, cleaning, and replacement schedules for the serviceable parts of your instrument. This includes mobile phases, pumps, autosamplers, columns/guards, and detectors. Specific protocols and recommendations will vary by manufacturer and model, so always consult your instrument manual when developing your procedures. In addition, consider the types of methods and samples you are running—more frequent service will be required for high-throughput testing of dirty samples than would be needed when fewer samples or cleaner matrices are being analyzed. In this article, we will explore each area of focus for routine LC maintenance and provide guidance on developing a targeted program that is effective for your lab.

Routine LC Maintenance Components

Mobile Phases

Many performance and troubleshooting issues can be traced back to mobile phase problems, primarily related to contaminants and leaks. Contaminants can be microbial or particulate and can ultimately cause incorrect and variable mobile phase ratios, system blockages, and high back pressure. To avoid this, use only fresh, filtered mobile phases that are made with HPLC-grade solvents, and clean or replace your solvent inlet filters regularly. Glass filters are inexpensive and environmentally friendly, so it is best practice to simply replace them regularly. Stainless-steel filters can either be replaced or cleaned by sonication in isopropanol (note that glass filters should not be sonicated because they can fracture and/or shed fragments). Always store prepared mobile phases in clean bottles and keep them capped to prevent contamination. Be sure to use proper bottles with secure caps; storing mobile phases in old solvent bottles topped with lab film or foil is not recommended because this allows evaporation of volatile solvents and additives, which can change the concentration of the solutions.

Because particulates can also contaminate the degasser, it is important to flush the degasser lines with isopropanol (use water first and then isopropanol if using a buffered mobile phase to prevent precipitation). The degasser tubing can become brittle over time and crack; this causes the unit to become very noisy, indicating that the tubing needs to be trimmed or replaced. Maintaining a detailed instrument logbook that tracks symptoms such as this will help you determine appropriate time intervals when setting up a routine LC maintenance schedule.

Leaks are another common mobile phase issue that can be minimized with a good routine maintenance plan. Small leaks can cause loss of sample, increased retention, and decreased signal while large leaks can cause the instrument to automatically shut down, which stops data collection and could mean you need to rerun the batch. To prevent leaks, include regular inspection of tubing and fittings for moisture and residue in your routine maintenance plan. Verify that the end fittings between the mobile phase tubing and the column are tightened and secured, but take care not to overtighten because this can damage the fitting. You may need to remove the fitting and inspect it for any signs of damage, or simply replace it at regular intervals. High-pressure fittings are more durable than standard fittings and are a useful alternative that can allow for longer intervals between maintenance events.

Your routine LC maintenance schedule should be supplemented by daily checks of mobile phase components and system plumbing. Before starting your analyses, check mobile phase clarity and preparation date, be sure the levels in the mobile phase and waste containers are adequate, and trace tubing lines and fittings with a dry lab tissue; this will help you spot small leaks that can be missed by visual inspection alone.

Pumps

Binary and quaternary pumps are another part of your system that should be included in any routine LC maintenance plan. Serviceable parts include the pistons, piston seals, inlet and outlet check valves, and the purge valve frit. Pistons and valves are the more durable parts of the pump and may only require replacement only once or twice a year. Simply wiping them with water:methanol (50:50) and inspecting them for wear when changing the piston seals is adequate, but be sure to keep replacement parts on hand so they are available when you need them.

In contrast, the piston seals and purge valve frits need more frequent attention. Piston seals will wear rapidly, especially if using high buffer concentration mobile phases, and often need to be changed every three to six months. If your system has a seal rinse option, check to ensure that there is an adequate supply of fresh seal rinse solution (typically 90:10 water:isopropyl alcohol) as part of your routine maintenance plan. For closed loop seal wash kits, inspect the level of seal rinse solution often and replace it regularly. Following these steps and making sure the seal rinse lines are properly primed will help maximize the lifetimes of in-pump consumables.

Purge valve frits can become dirty from collecting debris from pump seal wear and usually need to be replaced at the same time. Leaks at the pump head, inconsistent retention times, and unstable pressures can indicate the piston seals are breaking down and need to be changed while high back pressure or irregular peak shapes can point toward a clogged purge valve frit. A careful review of the instrument logbook can help you decide what service interval is best to set for your instrument. For Agilent instruments, you may find Restek's video on replacing a PTFE frit to be helpful.

In addition to following a good routine LC maintenance plan, you can further extend pump lifetime by storing it under favorable conditions. When the pump will not be in use, even if just overnight, first prepare it by flushing it with 90% water for about 15 minutes to remove any buffer salts, followed by 100% organic. Then, either turn the pump off or reduce the flow to 0.1 mL/min.

Autosampler

In order to prevent leaks, clogs, and carryover, injector valve rotor seals, stators, stator faces, needles, and needle seats should be inspected frequently and replaced as needed. When inspecting these parts, take particular care to examine the autosampler valve components (stator, stator face, etc.) for blockages or scratches. Generally speaking, rotor seals and needle seats will need to be replaced more frequently than stators and needles. As with other parts of the LC system, mobile phases with high concentrations of buffer salts can accelerate wear and their use may need to be offset by more frequent LC maintenance. One of the best ways to prevent autosampler problems is to filter or centrifuge samples prior to injection to remove particulates. Syringe filters and filter vials make this a simple task that is well worth the time because it helps prevent injection valve damage and back pressure increases due to blockages.

Columns and Guard Columns

As a regular part of LC maintenance, inspect the column connections (as well as all other system connections) for leaks, pinched tubing, and loose fittings. When replacing tubing, use short lengths of narrow tubing to minimize system volume, thereby reducing peak dispersion and loss of resolution. Always check the tubing ends to ensure a clean, square cut because irregular cuts can cause void volumes to form. Also, it is best practice to replace a fitting if it is leaking because it is likely damaged and further tightening can exacerbate the problem.

There are no serviceable parts in the column compartment itself, but turning the oven off when not in use will help lengthen the lifetime of both the column and the oven. Filtration is another way to extend both column lifetime and service intervals. Using a guard column or UltraShield UHPLC precolumn filter offers excellent protection from both particulates and contaminants. Be sure to keep a log of baseline pressure as increasing pressure is a good indication of a blockage; set the guard column replacement schedule based on the pressure trends you observe in the logbook.

When it is time to replace the guard column, check that your system pressure returns to normal. If it does not, it may be time to regenerate or replace the column. To regenerate an HPLC or UHPLC column, flush it in the direction shown on the column with a series of solvents. Use a minimum of 20 column volumes of each solvent and flush in the order shown in Table I. Our videos on column cleaning/regeneration and extending column lifetime offer other useful tips for column care.

Table I: Solvent flushing sequence for LC column regeneration.

Reversed-Phase Columns	Normal Phase Columns
1. Water:methanol (95:5 v/v)	1. Isopropyl alcohol
2. Methanol	2. *n*-Hexane
3. Isopropyl alcohol	3. Ethanol
4. *n*-Hexane	4. Original mobile phase
5. Isopropyl alcohol	
6. Methanol	
7. Water:methanol (95:5 v/v)	
8. Original mobile phase	

Once a new or regenerated LC column has been installed, be sure to follow all usage guidelines, including pH limits, temperature limits, and storage solvent. Maintaining a column log in addition to an instrument log is an excellent way to prevent problems when returning a column to service or preparing it for use in a different method.

Detector

Detectors are complex and have relatively few customer-serviceable parts. Regular inspection and cleaning protocols should carefully follow the instrument manual. Spikes or an increase in baseline noise may indicate a failing lamp, so keeping an extra on hand is recommended. Always consult your instrument manual before attempting installation.

Setting an Effective Schedule

The most fundamental element of an effective routine LC maintenance program is that it is performed on a regular basis following a planned schedule. To maximize effectiveness, minimize disruption, and avoid downtime for unexpected instrument failures, the schedule should be set considering instrument logbook history, sample matrices, number of samples run, and method details (e.g., use of buffers, additives, or derivatization reagents). Instruments used heavily for analyzing dirty samples under harsher method conditions will require more frequent maintenance than those used lightly to analyze relatively clean samples. Restek offers guidelines for Agilent 1100 and 1200, Shimadzu, Waters ACQUITY, and Waters Alliance systems, which are useful starting points for setting effective schedules that are tailored specifically to your laboratory.

Returning to Service with Performance Qualification

After any LC maintenance event, whether routine or nonroutine, it is essential to test the instrument prior to returning it to service. This is done through performance qualification (PQ) testing, which—while mandatory in regulated laboratories—is beneficial to unregulated labs as well. The purpose of performance qualification is to empirically prove that the overall system (hardware and software in combination) will perform as intended and meet predetermined specifications. Performance testing should be done following standard operating procedures that include tests and limits that will demonstrate the instrument is capable of meeting specifications relevant to its intended use.

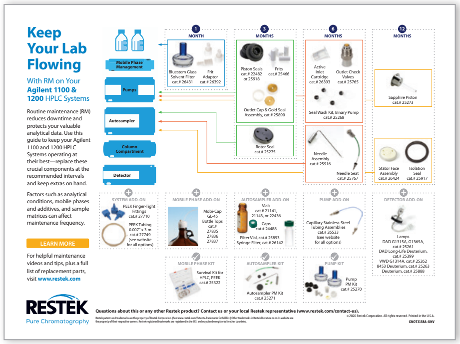
Common tests include leak checking, pump flow accuracy and stability, oven and autosampler temperature, wavelength accuracy, dwell volume and gradient accuracy, signal-to-noise ratio, linearity, accuracy, precision, and carryover. Labs should determine which tests and acceptance criteria are appropriate based on the methods that will be used for routine sample analysis. Following a maintenance event, first test that the affected module is working correctly and then evaluate the system as a whole. For example, after replacing pump seals, first assess pump flow precision and accuracy as well as mixing; then, perform quantitative and qualitative chromatographic assessments. Passing instruments can be returned to service, but root cause analysis, repair, and further PQ testing will be necessary for any instrument that fails.

Once an instrument has been cleared for use, there are a number of daily checks that should be used to verify readiness and performance capability. Keeping a careful log of performance testing results and daily checks will provide a valuable picture of what "good" looks like and will make it much easier to diagnose potential problems or identify when consumables need to be replaced. Daily protocols should be established by each lab, but often include recording system pressure and retention time checks in addition to the system suitability tests defined in each analytical method.

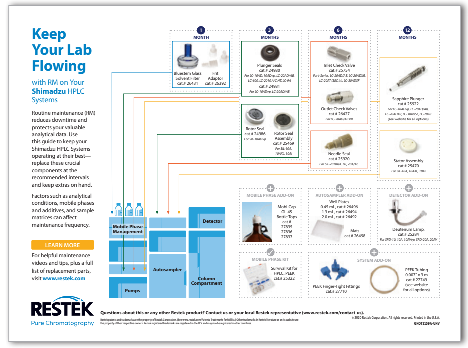
Summary

Investing time in regular LC maintenance is one of the best ways to ensure that your instrument is performing well and consistently delivering reliable results. By developing routine procedures based on your specific systems, samples, and methods, you can prevent lengthy downtime for troubleshooting and repairs as well as minimize the need to rerun batches lost due to QC failures or instrument shutdowns. With good mobile phase management; proper care of system components (pumps, autosamplers, columns/guards, and detectors); and relevant performance qualification testing; you can maximize the instrument lifetime, uptime, and protect data quality.

Keep your lab flowing with routine maintenance schedules from Restek.



Routine Maintenance for Agilent 1100 and 1200 HPLC Systems (GNOT3158A-UNV)



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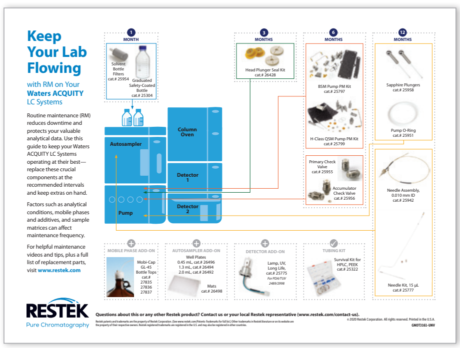
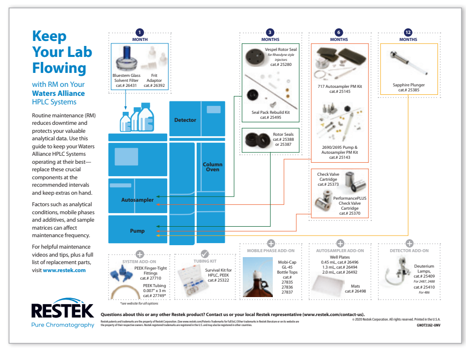


Routine Maintenance for Waters ACQUITY LC Systems (GNOT3161-UNV)



Routine Maintenance for Waters Alliance HPLC Systems (GNOT3162-UNV)

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