

HPLC Tips: Caring for your UV detector flow cell

If you want the best LC data, you need a properly working UV flow cell that is matched to your application. The primary goal is to choose the flow cell length that provides the best sensitivity while maintaining chromatographic resolution.

Unfortunately, people often think about flow cells only when they troubleshoot problems with their HPLC system. Maintenance of your UV flow cell is simple and every user of the HPLC system can do it. To start, you should keep in mind four general preventive measures:



Agilent 1290 Infinity LC

- Many flow cells have quartz windows or fused silica components, so avoid using alkaline solutions with pH greater than 9.5, which can attack these components and impair optical performance.
- To prevent crystallization inside the flow cell, always flush it with water after running buffers or salt solutions, especially at high pHs.
- When your LC is unused overnight, make sure your flow cell contains at least 10% organic mobile phase to prevent algae growth.
- Some modern flow cells use coated or uncoated fused silica capillaries. You can adversely affect coated capillaries by turning on the UV lamp without solvent flow, so read the manufacturer's recommendations carefully.

Flow cell selection

It is very important to carefully select a proper flow cell for your HPLC system. You may need a high-pressure flow cell when several detectors – or a detector and a fraction collector – are connected in series. In addition, check that the

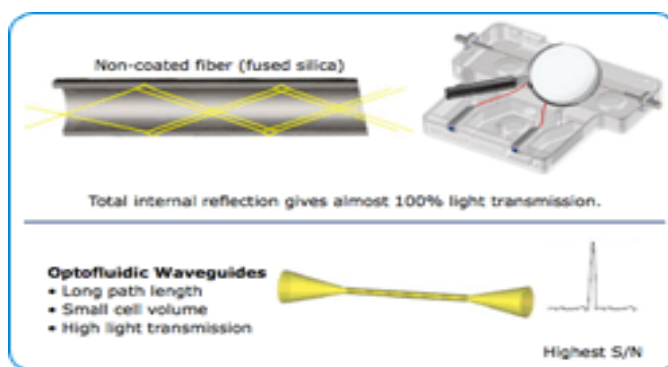
flow cell has the proper volume and optical path length to ensure the optimal resolution and sensitivity.

Many people assume that small-diameter columns always require very low-volume flow cells, but the column length, particle size and gradient slope all play a role. For example, a 4.6 mm column running steep gradients produces a smaller peak volume than a long 2.1 mm column operated under high-resolution conditions.

For guidelines on flow cell selection, use the [Agilent LC Method Translator](#). When you enter your current or proposed new conditions in the Advanced Mode tab, the Agilent Method Translator estimates the peak volume. To minimize any unnecessary dispersion that could reduce the apparent chromatographic resolution, your flow cell should be no larger than about 10% of the peak volume.

Note also that absolute cell volume is not always the best parameter to consider; the critical parameter is effective cell volume. For example, the new cell design in the [Agilent 1290 Infinity Diode Array Detector](#) relies on optofluidic waveguides, which use the principle of total internal reflection to achieve sensitivity without the cell dispersion effects that compromise resolution.

Figure 1. Flow cells that use optofluidic waveguides produce high sensitivity, even for peaks that elute in a very small volume. ([Click here](#) to see this image larger.)



Flushing the flow cell

If your system was shut down unexpectedly during an unattended sequence run, it is possible that the sample is still trapped in your flow cell. If the system was stopped overnight with salt buffer, it is important to start the system flush with a lower flow rate and watch the system pressure. A warm-water flush using the 60 °C setting on your column compartment is an effective way to clean your UV flow cell.

Mass spectrometer (MS) considerations

Dispersion in the UV flow cell can have significant effects on MS performance. If your chromatographic peak width is too broad in the MS trace, check the peak width without the upstream flow cell. You may want to consider an alternative

plumbing scheme, such as a tee before the UV flow cell to split to the MS source.

These tips should help you use and maintain your UV flow cell and keep it in top operating condition. Note that with the new Agilent Max-Light flow cell in the [Agilent 1290 Infinity LC System](#), the optofluidic cartridge design reduces many of the concerns presented here, while offering significant advantages in sensitivity and performance.

You can access additional HPLC tips and references from the [Agilent podcast Web site](#). When you link to the [podcast on flow cells](#), be sure to explore the Agilent LC Method Translator and the LC Maintenance Guide.

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