

# TIPS FOR OPTIMIZING YOUR UHPLC SENSITIVITY AND THROUGHPUT

Nothing beats UHPLC for resolution, sensitivity, and baseline robustness. In fact, new UV and MS technologies now allow you to detect and quantify impurities down to **0.001%** of the main compound. Now *that's* confidence!

UHPLC operation and troubleshooting are very similar to HPLC; however, **UHPLC requires more scrupulous chromatographic hygiene.** This poster provides practical pointers to help you avoid the most common pitfalls.



## Accurate results start with the best sample prep

Analytical HPLC samples can contain an array of interferences that can affect the efficiency and performance of your HPLC method. Once you enter the world of UHPLC, the cleanliness of your sample becomes even more critical.

Agilent sample prep products and techniques accommodate a wide range of clean-up requirements, sample volumes, and workflows. Our diverse portfolio can help you increase column performance and lifetime, reduce backpressure build-up, and minimize mass spectrometer ion suppression/quantification.

New high-quality Agilent Captiva syringe filters arrive ready to use – and are tested and certified to be free of UV-absorbing substances at typical HPLC wavelengths with water, methanol, and acetonitrile. So you can be confident that extractables or other contaminants will not damage the integrity of your samples. Our Syringe Filter Online Selection Guide makes it fast and easy to choose the best syringe filter for your application. Try it now at: [www.agilent.com/chem/SyringeFilters](http://www.agilent.com/chem/SyringeFilters)

The chart below will help you choose the right sample prep products, based on your sample's purity level and the types of matrix interferences that need to be removed.

### Suggested sample preparation products

Technique	Supported Liquid Extraction (SLE)	Precipitation/Filtration	"Smart" Filtration	Solid Phase Extraction
Interference	Dilute and shoot	Chem Elut	Captiva	Captiva ND Lipids
Particulates	No	Yes	Yes	Yes
Proteins	No	Partial	No	Yes
Lipids	No	No	No	Yes
Oligomeric Surfactants	No	No	No	Yes
Salts	No	Yes	No	Yes



## Optimize your system for UHPLC columns

Agilent's 1200 Infinity Series takes speed and sensitivity to new heights for both HPLC and UHPLC applications. Now you can push flow rates with longer, narrower columns for improved separation power – or leverage the efficiency of small particles in shorter columns for maximum speed.

UHPLC helps conserve solvent, and is especially suitable for MS detectors. When using UHPLC columns, keep these key points in mind:

- For best results with high pressures (>600 bar), choose a narrow (2.1 mm or 3.0 mm id) column.
- Get optimal UV sensitivity using the 60 mm high sensitivity,  $\alpha = 4 \mu\text{L}$  Max-Light Cartridge Cell (G4212-60007).
- Use smaller (0.12 mm id) red tubing, and minimize tubing lengths between connections.
- Watch your system pressure trace to catch problems early. Change your filter when the pressure increases by 10%.
- Avoid large injection volumes.** Generally, injection volumes should be <5  $\mu\text{L}$  if your sample is dissolved in a "strong" solvent.
- Use a micro-heat exchanger, as opposed to the built-in heat exchanger. For UV detection, the data collection rate should be set at a minimum of 40 Hz.

### Recommended heat exchangers for 1200 Infinity Systems

Heat Exchanger	Part No.
High temperature heat exchanger (1.6 $\mu\text{L}$ , 0.12 mm id "R")	G1316-80002
High temperature heat exchanger (1.6 $\mu\text{L}$ , 0.12 mm id "L")	G1316-80003
Heat exchanger/cooler (1.5 $\mu\text{L}$ , 0.12 mm id)	G1316-80004*
Carrier for heat exchanger	G1316-89200*

\*Order these together with the heat exchanger  
\*\* 1200 Infinity Thermostatted Column Compartment G1316C already includes low dispersion heat exchangers

### Recommended stainless steel capillaries for 1260 Infinity Systems

From	To	Material	id (mm)	Length (mm)	Fitting Left	Fitting Right	Part No.
Pump	Autosampler	SS	0.17	900	Swagelok pre-swaged	Swagelok	G1329-87300
Pump	Autosampler	SS	0.17	700	Swagelok pre-swaged	Swagelok	G1312-87304
Pump	Autosampler	SS	0.17	500	Swagelok pre-swaged	Swagelok	G1312-87305
Pump	Autosampler	SS	0.17	400	Swagelok pre-swaged	Swagelok	G1312-87303
Autosampler	TCC	SS	0.17	180	Swagelok pre-swaged	Swagelok	G1313-87305
Autosampler	TCC	SS	0.12	180	Swagelok pre-swaged	Swagelok	G1313-87304
Thermostatted Autosampler	TCC	SS	0.17	380	Swagelok pre-swaged	Swagelok	01090-87306
Thermostatted Autosampler	TCC	SS	0.12	280	Swagelok pre-swaged	Swagelok	01090-87610
Manual Injector	TCC	SS	0.17	500	Swagelok	Swagelok	G1328-87600
TCC Outlet	Column Inlet	SS	0.17	90	Swagelok	Swagelok	G1316-87300
TCC Outlet	Column Inlet	SS	0.12	105	Swagelok pre-swaged	Swagelok	01090-87611
Column Outlet	DAD	SS	0.17	380	Swagelok pre-swaged	Swagelok	G1315-87311
Column Outlet	DAD	SS	0.12	150	Swagelok pre-swaged	Swagelok	G1315-87312
Female adapter for connecting long columns		SS	0.17	150	Swagelok		G1315-87303
Column Outlet	VWD	PEEK	0.17	600	Fingertight (not included)	Fingertight (not included)	5062-8522

### Estimated tubing volume

Color	id (mm)	id (in)	$\mu\text{L}/100 \text{ mm}$
Green	0.17	0.007	2.5
Red	0.12	0.005	1.3



## Use solvents and buffers properly with high-efficiency columns

High-efficiency columns have oversized frits on each end to contain their smaller particles. However, these frits can filter and trap particulates, causing pressure increases. **To prevent this from happening, you must keep your system free of contaminants.**

Agilent recommends that you *only* use Certified HPLC/MS grade solvents for UHPLC. Check with your solvent provider for certification on the following:

- Low solvent and metal impurities, to reduce interference with minute or unknown samples.
- Low trace metal specifications – no more than 5 ppb.
- Positive and negative mode specifications.
- LC/MS and QC testing. The more QC testing, the better the solvent!

### Tips for solvent and buffer usage:

- Use rugged stainless steel solvent filters instead of glass for high-pressure work.
- Buffers increase your chance of clogging. If you must use a buffer, use glass filters to minimize bacterial growth, and replace every 24 to 48 hours.
- Always mix modifiers carefully, using common consistencies (see chart below).
- Narrow columns require less solvent per analysis. Be sure to dump out old buffers regularly, and keep solvents as fresh as possible.

### UV cutoffs for common mobile phase modifiers

	v/v	pK <sub>a</sub> at 25 °C	Max. pH Range	UV Cutoff (nm)
Trifluoroacetic acid (TFA)	0.1%	0.3		210
	0.05%	0.3		210
	0.01%	0.3		210
Phosphate, pK <sub>1</sub>		2.1	1.1 to 3.1	<200
Phosphate, pK <sub>2</sub>		7.2	6.2 to 8.2	<200
Phosphate, pK <sub>3</sub>		12.3	11.3 to 13.3	<200
Citrate, pK <sub>1</sub>		3.1	2.1 to 4.1	230
Citrate, pK <sub>2</sub>		4.7	3.7 to 5.7	230
Citrate, pK <sub>3</sub>		6.4	5.4 to 7.4	230
Carbonate, pK <sub>1</sub>		6.1	5.1 to 7.1	<200
Carbonate, pK <sub>2</sub>		10.3	9.3 to 11.3	<200
Formate		3.8	2.8 to 4.8	210 (10 mM)
Acetic Acid (HAC)	1.0%	4.8	3.8 to 5.8	210
Acetate		4.8	3.8 to 5.8	210 (10 mM)
Ammonia		9.2	8.2 to 10.2	200 (10 mM)
Borate		9.2	8.2 to 10.2	n/a
Triethylamine (TEA)		10.8	9.8 to 11.8	<200
TRIS-HCl		8	7 to 9	212 (20 mM)

Shaded area indicates buffers that are more commonly used in LC/MS applications

### Common pH mobile phase modifiers for LC/MS

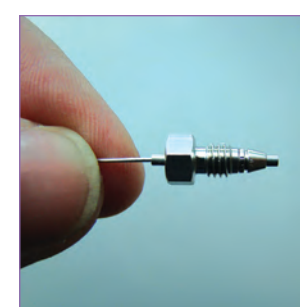
Concentrated Reagent	Formula Weight <sup>1</sup>	Density	Approx. Strength <sup>2</sup>	Molarity (M)	Normality (N)	Volume (mL) Required to Make 1000 mL Solution <sup>3</sup>	
						1 M	1 N
Acetic Acid (CH <sub>3</sub> COOH)	60.052	1.05	99.8%	17.4	17.4	57.5	57.5
Formic Acid (HCOOH)	46.026	1.13	90%	23.6	23.6	42.5	42.5
Ammonium Hydroxide (NH <sub>4</sub> OH)	35.046	0.90	56.6% <sup>4</sup>	14.5	14.5	69	69

<sup>1</sup> Based on Atomic Weight Table (32 °C = 12)  
<sup>2</sup> Representative value, w/w %  
<sup>3</sup> Rounded to nearest 0.5 mL  
<sup>4</sup> Equivalent to 28.0% w/w NH<sub>3</sub>

### Ensure your fittings are made correctly

**How to make a proper fitting**  
**IMPORTANT:** Be sure to use flat-cut stainless steel tubing that is free from burrs.

- Slide fitting at least 3/16 in from the flat end of the non-swaged tubing, supplied by Agilent. (Refer to stainless steel capillaries table for non-swaged tubing options.)
- Insert the assembly into the receiving port, pushing the tubing into the female port until it bottoms out.
- Finger-tighten the nut into the port until snug.
- Using a wrench, tighten the fitting to between 9 and 11 in-lbs (1.0-1.2 Nm). This will be about ¼ turn beyond fingertight.



An example of a "perfect" standard fitting.



## Look to Poroshell 120 columns for UHPLC

Poroshell 120 columns are stable to 600 bar, and are available in 8 bonded phases and one bare silica HILIC for method development flexibility.

### Agilent Poroshell 120 (2.7 $\mu\text{m}$ )

Size (mm)	EC-C18	EC-C8	Phenyl-Hexyl	SB-C18	SB-C8	SB-Aq	Bonus-RP	HILIC	EC-CN
4.6 x 150	693975-902	693975-906	693975-912	683975-902	683975-906	683975-914	693968-901	693975-901	693975-905
4.6 x 100	695975-902	695975-906	695975-912	685975-902	685975-906	685975-914	695968-901	695975-901	695975-905
4.6 x 75	697975-902	697975-906		687975-902					
4.6 x 50	699975-902	699975-906	699975-912	689975-902	689975-906	689975-914	699968-901	699975-901	699975-905
4.6 x 30	691975-902	691975-906		681975-902					
3.0 x 150	693975-302	693975-306	693975-312	683975-302	683975-306	683975-314	693968-301	693975-301	693975-305
3.0 x 100	695975-302	695975-306	695975-312	685975-302	685975-306	685975-314	695968-301	695975-301	695975-305
3.0 x 75	697975-302	697975-306		687975-302					
3.0 x 50	699975-302	699975-306	699975-312	689975-302	689975-306	689975-314	699968-301	699975-301	699975-305
3.0 x 30	691975-302	691975-306		681975-302					
2.1 x 150	683775-902	693775-906	693775-912	683775-902	683775-906	683775-914	693768-901	693775-901	693775-905
2.1 x 100	695775-902	695775-906	695775-912	685775-902	685775-906	685775-914	695768-901	695775-901	695775-905
2.1 x 75	697775-902	697775-906		687775-902					
2.1 x 50	699775-902	699775-906	699775-912	689775-902	689775-906	689775-914	699768-901	699775-901	699775-905
2.1 x 30	691775-902	691775-906		681775-902					

### Agilent Poroshell 120 Fast Guards for UHPLC

Size (mm)	EC-C18	EC-C8	SB-C18	Phenyl-Hexyl
4.6 x 5	820750-911	820750-913	820750-912	820750-914
3.0 x 5	823750-911	823750-913	823750-912	823750-914
2.1 x 5	821725-911	821725-913	821725-912	821725-914



## Conduct preventive maintenance

Supplement your preventive maintenance efforts with an annual Agilent Preventive Maintenance Agreement, proven to increase uptime, reduce repairs, and cut repair costs compared to other PM sources.

### Check and replace consumables

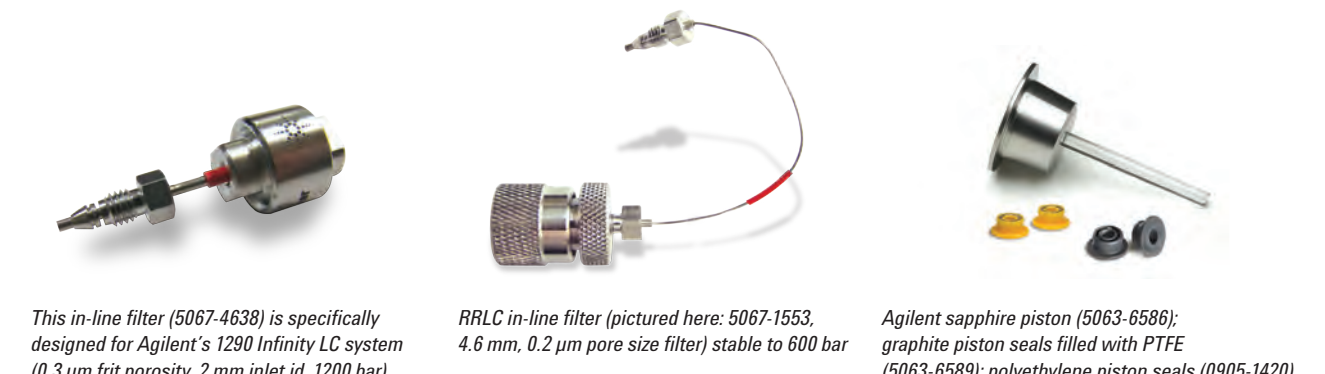
Replace the following often for dirty samples and high-volume usage:

- Pump piston and seals
- Autosampler needle, needle seats, and rotor seals
- In-line filters
- Use calibration capillary assay (G1312-67500) to test and calibrate your pump-head

### Use seal wash on your pump

The 1260 Infinity LC has two modular binary pump options – one with a seal wash and one without. If you have the seal wash, be sure to use it, as it helps clean the pump seal and reduce degradation. **If you don't have the seal wash option, we recommend that you purchase it and add it to your pump.**

Agilent's Seal Wash Kit for binary pumps (G1312-68711) includes 4 wash seal gaskets, 4 pump seals, 2 peristaltic pumps (pump cassette and motor), 4 seal keepers, 4 support ring assemblies, seal insert tool, and silicone tubing.



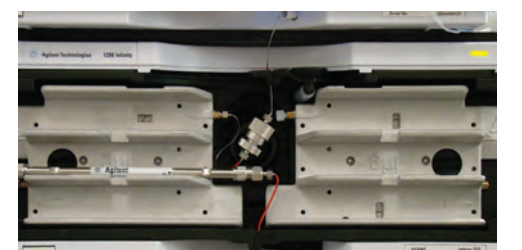
This in-line filter (5067-4630) is specifically designed for Agilent's 1200 Infinity LC system (0.3  $\mu\text{m}$  frit porosity, 2 mm inlet id, 1200 bar)

RPLC in-line filter (pictured here: 5067-1553, 4.6 mm, 0.2  $\mu\text{m}$  pore size filter) stable to 600 bar

Agilent sapphire piston (5063-6580), graphite piston seals filled with PTFE (5063-6589), polyethylene piston seals (0905-1420)

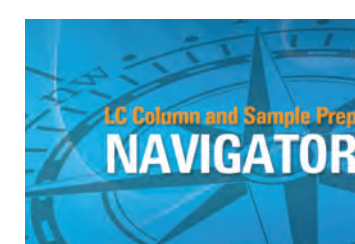
Use an in-line filter to keep solvent particulates from entering your analytical column.

For 600 bar instruments, including the 1260 Infinity LC, Agilent recommends an in-line filter (p/n 5067-1553).



### Remember to cover your instrument with an Agilent service agreement.

With service center operations in 65 countries, a global dispatch system, and call centers ready to assist with Agilent and non-Agilent instruments, Agilent provides the personalized support you need for greater efficiency, productivity, and confidence.



### Agilent Columns Navigator

Not sure what column will work best for you? Use the Agilent Columns Navigator for help selecting the right LC column for your method.

[www.agilent.com/chem/navigator](http://www.agilent.com/chem/navigator)

### CrossLab Supplies Online Interactive Selection Tool

With the CrossLab Selection Tool, it is quick and simple to identify the specific supplies you need. Answer just a few questions, and you will find the HPLC supplies for your particular applications.

[www.agilent.com/chem/SelectCrossLab](http://www.agilent.com/chem/SelectCrossLab)



You can find a complete listing of UHPLC parts and supplies in Agilent's 1200 Infinity Series Supplies Catalog (Pub number 5990-6511EN). Visit [www.agilent.com/chem/getguides](http://www.agilent.com/chem/getguides)

