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.

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	Bond Elut SPE
Particulates	No	No	Yes	Yes	Yes
Proteins	No	Partial	Yes	Yes	Yes
Lipids	No	No	No	Yes	Yes
Oligomeric Surfactants	No	No	No	Yes	Yes
Salts	No	Yes	No	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.
- The standard flow cell for a 1290 Infinity LC is the 10 mm, σ_v = 1.0 µL Max-Light Cartridge Cell (G4212-60008).
- Get optimal UV sensitivity using the 60 mm high sensitivity, σ_v = 4 µL Max-Light Cartridge Cell (G4212-60007) preferable for 3.0 and 4.6 ID columns.
- 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 µL 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 µL, 0.12 mm ID "R")	G1316-80002
High temperature heat exchanger (1.6 µL, 0.12 mm ID "L")	G1316-80003
Heat exchanger/cooler (1.5 µL, 0.12 mm ID)	G1316-80004*
Carrier for heat exchanger (TCC SL)	G1316-83200*

**Order these together with the heat exchanger*

Recommended stainless steel capillaries for 1290 Infinity Systems

(For use with 2.1 mm and 3.0 mm ID columns)

From	To	ID (mm)	Length (mm)	Fittings	Part No.
Autosampler	TCC	0.12	340	Non-swaged	5067-4659
TCC	DAD	0.12	220	Non-swaged	5067-4660
Valve	HX1-HX2	0.12	90	Pre-swaged	5067-4649
Valve	Heat Exchanger	0.12	150	Pre-swaged	5067-4735
Column	Valve (short)	0.12	150	Non-swaged	5067-4650
Column	Valve (short)	0.12	170	Non-swaged	5067-4736
Column	Valve (long)	0.12	280	Non-swaged	5067-4651
Valve	Valve	0.12	120	Pre-swaged	5067-4652
Valve	Valve	0.12	150	Pre-swaged	5067-4737
Valve	Detector	0.12	200	Pre-swaged	5067-4653
Valve	Detector	0.12	200	1200 bar VHP fitting	5067-4746

Estimated tubing volume

Color	ID (mm)	ID (in)	µL/100 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 undersized 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_a at 25 °C	Max. pH Range	UV Cutoff (nm)
Trifluoracetic acid (TFA)	0.1%	0.3		210
	0.05%	0.3		210
	0.01%	0.3		210
Phosphate, pK_1		2.1	1.1 to 3.1	<200
Phosphate, pK_2		7.2	6.2 to 8.2	<200
Phosphate, pK_3		12.3	11.3 to 13.3	<200
Citrate, pK_1		3.1	2.1 to 4.1	230
Citrate, pK_2		4.7	3.7 to 5.7	230
Citrate, pK_3		6.4	5.4 to 7.4	230
Carbonate, pK_1		6.1	5.1 to 7.1	<200
Carbonate, pK_2		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[1]	Density	Approx. Strength[2]	Molarity (M)	Normality (N)	Volume (mL) Required to Make 1000 mL Solution[3]	
						1 M	1 N
Acetic Acid (CH_3COOH)	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_4OH)	35.046	0.90	56.6%[4]	14.5	14.5	69	69

1. Based on Atomic Weight Table (32 °C = 12)
2. Representative value, w/w %
3. Rounded to nearest 0.5 mL
4. Equivalent to 28.0% w/w NH_3

Filter solvents and samples with care

Remember, filtering can increase the chance of contamination. Always use a filter membrane that is compatible with your solvent.

Filter/solvent compatibility guidelines

Type	Qty.	Compatibility Notes	Part No.
Regenerated cellulose filter membranes 47/46	100/pk	• Use with aqueous or organic solvents • Very low nonspecific protein binding membrane • OK with acetonitrile, methanol, formic acid, isopropyl alcohol, methylene chloride	3150-0576
Nylon filter membranes 47/46	100/pk	• Good for aqueous and organic samples within a pH of 3-10 • OK with acetonitrile and methanol	9301-0895
PTFE filter membranes 47/46	10/pk	• Good for aggressive samples • Generally good chemical compatibility • OK with acetonitrile, methanol, formic acid, isopropyl alcohol, methylene chloride	9150-0509

Agilent solvent filter and degasser (3150-0577)

Look to ZORBAX Rapid Resolution High Definition (RRHD) columns for UHPLC

ZORBAX RRHD columns are stable to 1200 bar, and are available in an expanding family of phases for reliable method scalability and transfer.

	Eclipse Plus C18	Eclipse Plus C8	Eclipse XDB-C18	Extend-C18
RRHD 2.1 x 150 mm, 1.8 µm	959759-902	959759-906	981759-902	759700-902
RRHD 2.1 x 100 mm, 1.8 µm	959758-902	959758-906	981758-902	758700-902
RRHD 2.1 x 50 mm, 1.8 µm	959757-902	959757-906	981757-902	757700-902
RRHD 3.0 x 150 mm, 1.8 µm	959759-302	959759-306	981759-302	
RRHD 3.0 x 100 mm, 1.8 µm	959758-302	959758-306	981758-302	758700-302
RRHD 3.0 x 50 mm, 1.8 µm	959757-302	959757-306	981757-302	757700-302

	StableBond SB-C18	StableBond SB-C8	StableBond SB-Phenyl	StableBond SB-CN
RRHD 2.1 x 150 mm, 1.8 µm	859700-902	859700-906	859700-912	859700-905
RRHD 2.1 x 100 mm, 1.8 µm	858700-902	858700-906	858700-912	858700-905
RRHD 2.1 x 50 mm, 1.8 µm	857700-902	857700-906	857700-912	857700-905
RRHD 3.0 x 150 mm, 1.8 µm	859700-302	859700-306		
RRHD 3.0 x 100 mm, 1.8 µm	858700-302	858700-306	858700-312	858700-305
RRHD 3.0 x 50 mm, 1.8 µm	857700-302	857700-306	857700-312	857700-305

Conduct preventive maintenance

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

Check and replace consumables

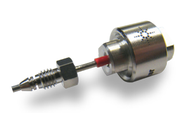
Replace the following often for dirty samples and high-volume usage:

- Pump piston and seals
- Autosampler needle, needle seats, and rotor seals
- In-line filters

Use seal wash on your pump

Your 1290 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 inline filter (5067-4638) is specifically designed for Agilent's 1290 Infinity LC system (0.3 µm frit porosity, 2 mm inlet ID, 1200 bar)

Agilent sapphire piston (5063-6586); graphite piston seals filled with PTFE (5063-6589); polyethylene piston seals (0905-1420)

Use new removable fittings that are designed for ultra high-pressure

Agilent's 1200 bar removable fitting (for 1/16 in OD capillaries) consists of a stainless steel screw, an internal stainless steel ferrule, and a front ferrule in PEEK. The fitting can be used throughout the flow path, but because it can be reused without losing tightness, it is especially suitable for the connection between the heat exchanger and column.

Description	Part No.	Picture
Very high-pressure (VHP) fitting for UHPLC	5067-4733 (short) 5067-4738 (long) 5067-4739 (extra long)	

This new and improved fitting replaces the standard stainless steel Swagelok fitting, which was not removable. If your heat exchanger uses the non-removable Swagelok fitting, you should change the heat exchanger when switching to another manufacturers' column.

How to make a proper fitting

IMPORTANT: Be sure to use flat-cut stainless steel tubing that is free from burrs.

1. 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.)
2. Insert the assembly into the receiving port, pushing the tubing into the female port until it bottoms out.
3. Finger-tighten the nut into the port until snug.
4. 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 finger tight.

An example of a "perfect" standard fitting – Agilent's new 1200 bar removable fittings are best for high-pressure work

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.

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**

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