EFFICIENT LC METHOD DEVELOPMENT
with Agilent InfinityLab Poroshell 120 columns

STEP 1 Select particle size

UHPLC — We recommend
- Complex separations
- High-throughput samples

InfinityLab Poroshell 120 1.9 µm
- Fast performance
- More runs per day
- High sensitivity
- Best resolution
- Solvent savings

UHPLC/HPLC — We recommend
- Multi-purpose analysis

InfinityLab Poroshell 120 2.7 µm
- Faster runs on UHPLC and existing HPLC systems
- For UHPLC: Higher performance, more samples analyzed per day, and solvent savings

HPLC — We recommend
- Legacy methods

InfinityLab Poroshell 120 4 µm
- Faster performance with legacy methods
- More samples analyzed per day

STEP 2 Select column chemistry

BEST ALL AROUND

Column	Pore Size	Temp Limit	pH Range
InfinityLab Poroshell 120 EC-C18 — 1.9 µm, 2.7 µm, 4 µm			
InfinityLab Poroshell 120 EC-C8 — 1.9 µm, 2.7 µm, 4 µm	120 Å	60 °C	2.0-8.0
InfinityLab Poroshell 120 Phenyl-Hexyl — 1.9 µm, 2.7 µm, 4 µm			

LOW-pH MOBILE PHASES

Column	Pore Size	Temp Limit	pH Range
InfinityLab Poroshell 120 SB-C18, 2.7 µm	120 Å	90 °C	1.0-8.0
InfinityLab Poroshell 120 SB-C8, 2.7 µm	120 Å	80 °C	1.0-8.0

HIGH-pH MOBILE PHASES

Column	Pore Size	Temp Limit	pH Range
InfinityLab Poroshell HPH-C18 — 1.9 µm, 2.7 µm, 4 µm	100 Å	60 °C	3.0-11.0
InfinityLab Poroshell HPH-C8 — 2.7 µm, 4 µm			

ALTERNATIVE SELECTIVITY

Column	Pore Size	Temp Limit	pH Range
InfinityLab Poroshell 120 Bonus-RP — 2.7 µm	120 Å	60 °C	2.0-8.0
InfinityLab Poroshell 120 PFP — 1.9 µm, 2.7 µm, 4 µm			

POLAR ANALYTES

Column	Pore Size	Temp Limit	pH Range
InfinityLab Poroshell 120 SB-Aq — 2.7 µm	120 Å	80 °C	1.0-8.0
InfinityLab Poroshell 120 HILIC — 1.9 µm, 2.7 µm, 4 µm	120 Å	60 °C	0.0-8.0
InfinityLab Poroshell 120 EC-CN — 2.7 µm	120 Å	60 °C	2.0-8.0

STEP 3 Use this general protocol to develop fast, robust LC methods

1 Test initial conditions: 10-95% gradient with ACN plus 0.1% formic acid (low pH) at 30 °C.

2 Adjust slope (% organic over time) to incorporate the first and last peak. Optimize for analytes of interest.

3 If ACN gives poor peak shape or retention, change the organic modifier to MeOH.

4 Adjust column temperature (up to allowable limits).

5 Try alternate column chemistries to vary retention and selectivity.

Suggested columns to use as a starting point
- **Acidic compounds:** InfinityLab Poroshell 120 SB-Aq or SB-C18
- **Acidic/neutral compounds:** InfinityLab Poroshell 120 EC-C18
- **Basic compounds:** InfinityLab Poroshell HPH-C18
- **Polar compounds:** InfinityLab Poroshell 120 HILIC

Columns with Alternate Selectivity
PFP, Phenyl Hexyl, Bonus-RP, SB-CN, SB-Aq

For help with choosing a different selectivity, visit
www.hplccolumns.org

► Troubleshooting Chromatographic Issues

Poor peak shape, tailing, and poor resolution
- Avoid overloading the column; test different injection volumes/concentrations.
- Check for loose connections and leaks. **TIP:** Use InfinityLab QuickConnect fittings for leak-free connections.
- Basic compounds: Try a high pH mobile phase to reduce analyte interactions.
- Acidic compounds: Use ion pairing reagents to minimize analyte interactions.
- Increase the separation temperature.

Inconsistent results from run to run
- Ensure adequate column equilibration.
- Check lamp signal quality. **TIP:** Track lamp performance with an InfinityLab lamp with RFID.
- Run a standard test mixture to rule out instrument contributions to inconsistency.
- Be sure that your injection solvent is compatible with method starting conditions.

Ghost peaks
- Confirm mobile phase identity and purity. Perform a blank run to check for carryover; if carryover persists, change the needle seat and rotor seal.
- Make sure the flow cell is clean. **TIP:** Track usage with InfinityLab MaxLight flow cells with RFID.
- Know the properties of all injected components—even those not UV visible.
- Wash all components off the column with an appropriate % organic.
- Increase wash duration at the end of the run.
- Ensure the needle wash solvent strength is appropriate for sample components.
- Use an InfinityLab column ID tag to track column history; if necessary, try a new column.

Accelerate your analytical method development workflow
The Agilent InfinityLab LC Method Development Solution gives you automated access to hundreds of unique separation conditions—without changing a single column or solvent bottle.

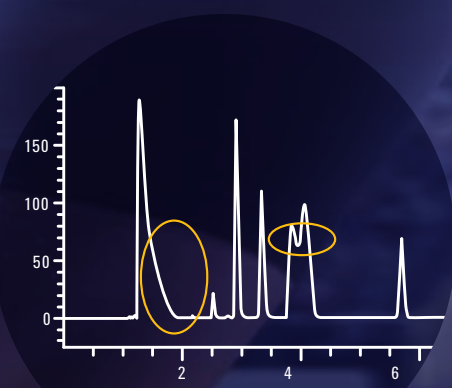For more information, visit **www.agilent.com/chem/welivelcmetdev**

Helpful tools and links
LC Column Navigator Tool: Get recommendations for a new column, based on method parameters. **www.agilent.com/chem/navigator**
Method Validation Kits: Convenient, cost-effective kits put columns from three separate lots right at your fingertips. **www.agilent.com/chem/method_validation_kits**
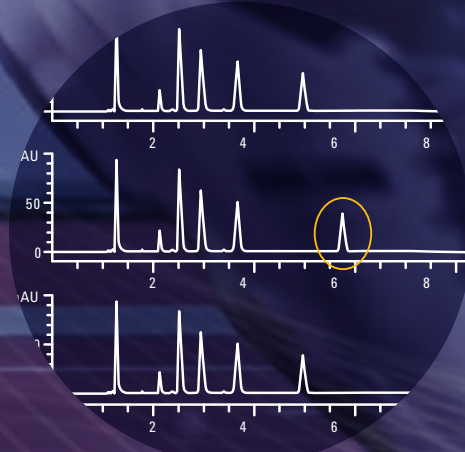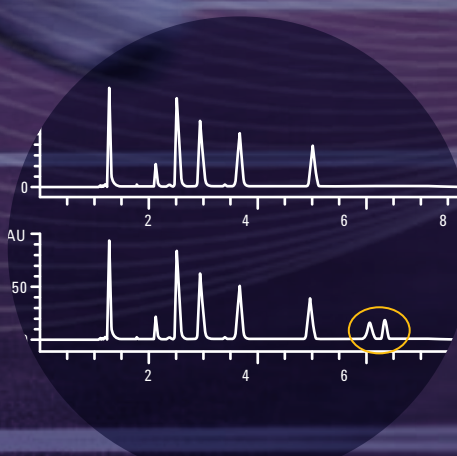Find the right parts and supplies for your LC system. **www.agilent.com/chem/agilentresources**

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