

Scaling Small Molecule Purification Methods for HPLC

Reversed-phase liquid chromatography (LC) is often associated with quantitative or analytical-scale analysis, but it is also useful as a preparative-scale tool for purification. For any purification project, there are three possible targets: purity, yield, and throughput. These targets are related to one another and are commonly represented as a triangle.

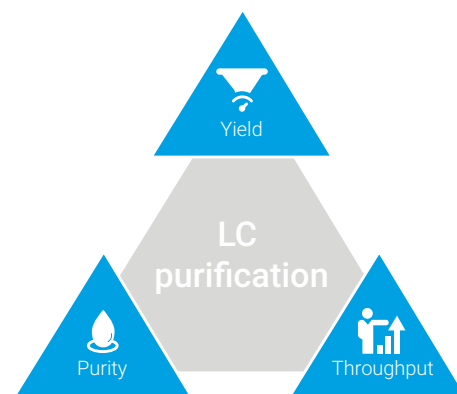
Two of these targets can be achieved at the expense of the third. You can clarify your path forward by prioritizing the objectives of your purification project before method development.

Tips for bulk purification

Bulk purifications prioritize purity and yield at the expense of throughput,¹ and these guidelines will assist you with method development.

- Your column only needs to resolve the target compound from impurities. All other peaks can coelute.
- Always verify that your sample is soluble in mobile phase solvents.
- Work out the method and loading conditions on the analytical system before scaling them to the preparative system.
- To account for dwell volume differences, remember to incorporate an isocratic hold where necessary.

¹The Tale of Two Samples: Understanding the Purification Workflow from Different Perspectives. 5994-4707EN



Bulk purification workflow best practices

1 Check sample solubility in the mobile phase. A sample that precipitates or “crashes” out of solution can leave a mess behind on a preparative LC instrument.

2 Screen stationary phases and mobile phase solvents. Stationary and mobile phases have the biggest impact on peak elution order, so screening columns and solvents is the foundation of a solid purification method. Several types of stationary phases are typically screened on an analytical system, and standard mobile phases (acetonitrile and methanol) are preferred for initial screening work. The pH of the mobile phase should also be considered.

When selecting analytical screening columns, you should also investigate the availability of preparative columns. Scaling to a preparative column of the same phase and length will give the most reproducible results. To improve the sample load of crude mixes with relatively low solubility, we recommend a column with the largest internal diameter (id) available. Table 1 lists stationary phases available in both analytical and preparative dimensions.

3 Optimize your separation. For bulk purification, the objective is to collect a high-purity fraction of the compound while maximizing yield. To find the mobile phase composition required for optimal resolution, use the slope of the gradient and account for delay volume.

Table 1. Column selection criteria

Objective	Phase	Applications
Throughput	InfinityLab Poroshell 120 SB-C18 InfinityLab Poroshell 120 HPH-C18	Low pH High pH
Alternate selectivity for throughput or yield	ZORBAX Eclipse Plus C18 ZORBAX Eclipse Plus C8 ZORBAX StableBond C18 ZORBAX StableBond C8 ZORBAX Eclipse Plus Phenyl-Hexyl	General purpose; high pH Lower retention of hydrophobic analytes vs. C18 Low pH Lower retention of hydrophobic analytes vs. C18 Alternate selectivity for aromatic compounds
Yield	InfinityLab Pursuit XRs C18 InfinityLab Pursuit XRs C8 InfinityLab Pursuit XRs Diphenyl	Maximum loadability; high pH Lower retention of hydrophobic analytes vs. C18 Maximum loadability; alternate selectivity for aromatic compounds

4 Determine the column's maximum sample load. Use the highest-concentration solution to make several injections of increasing volume. The highest injection volume where the peaks maintain separation can be scaled up and used for your purification method.

5 Scale your method to the preparative instrument. The injection volume and flow rate are geometrically scaled from the analytical method using equations 1 and 2. Select your preparative column dimensions based on sample loading requirements and instrumentation parameters as listed in Table 2.

Equation 1. Preparative column flow rate calculation assuming identical lengths of the analytical and preparative columns:

$$f_p = f_a \left(\frac{id_p}{id_a} \right)^2$$

f_a = flow rate of the analytical column
 id_p = internal diameter of the preparative column
 id_a = internal diameter of the analytical column

Equation 2. Preparative column injection volume calculation:

$$V_p = V_a \left(\frac{id_p}{id_a} \right)^2$$

V_a = injection volume on the analytical column
 id_p = internal diameter of the preparative column
 id_a = internal diameter of the analytical column

One factor that can impact scaling is the difference in instrument dwell time. Dwell time is the amount of time it takes for the mobile phase to travel from the mixing point in the pump to the column head. The solvent mixing technique, autosampler, tubing length, and ID can all impact the dwell time, which acts like an isocratic method.

When you scale an analytical gradient method to a preparative instrument, it's important to factor dwell time into the beginning of your gradient method. Failing to do so can cause differences between analytical and preparative retention times—and can even change your chromatographic separation.

6 Purify your target compound from your sample. Target compound collection is triggered by UV threshold and time window. This combination prevents accidental collection of another peak while minimizing the collection volume. You may need to perform several injections to collect the desired amount of product. If so, you can avoid retention time shift by allowing the column to flush from 8 to 10 column volumes before each injection. The purity of the combined fractions is confirmed using an analytical system.

Recommended flow rates and instrumentation are outlined for each dimension.

Table 2. Range of analytical to preparative Agilent instruments and column dimensions.

Column Internal Diameter	Analytical	Semipreparative and Preparative			
2.1 mm	0.4–0.6 mL/min				
3.0 mm	0.5–1 mL/min				
4.6 mm	1–2 mL/min				
9.4 or 10 mm		4–10 mL/min			
21.2 mm			18–42 mL/min		
30 mm				34–85 mL/min	
50 mm					94–236 mL/min
Purification range (mg)	1–15	7–70	30–300	64–640	180–1800
Instrumentation	1220/1260/1290 Infinity II analytical-scale LC purification systems 1260 Infinity II preparative LC systems 1290 Infinity II preparative LC systems				

Flow range extensions made possible by exchangeable pump heads

InfinityLab preparative LC columns portfolio



Selecting the right instrument for your application

Whether you're looking for a quality entry-level system or an ultra-high-performance biocompatible solution, Agilent has an HPLC or UHPLC configuration for you.



Agilent 1220/1260/1290 Infinity II Analytical-Scale LC Purification Systems

- Best for purification of multi-milligram quantities
- Recommended flow rates for purification workflows from 0.8–8 mL / min
- Recommended with analytical column dimensions 3.0–10 mm ID



Agilent 1260 Infinity II Preparative LC System

- An ideal workhorse for automated sample injection when gradient elution is required
- Flow rates up to 50 mL/min for purification
- For best results use InfinityLab 21 mm ID columns



Agilent 1290 Infinity II Preparative LC System

- Dynamic flow range up to 200 mL/min
- Seamless method transfer from rapid analytical scouting runs to gram-level purification on a single system
- Accommodates purification with up to 50 mm ID columns

Easy selection and ordering information

To order listed items from the Agilent online store, simply click the MyList header links. Then, enter the quantities for the products you need, click Add to Cart, and proceed to checkout. Your list will remain under Favorite Products for your use with future orders.

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MyList of analytical and preparative HPLC columns

Description	Part Number	
	Analytical (4.6 x 150 mm)	Preparative (21.2 x 150 mm)
ZORBAX StableBond C8, 5 µm	883975-906	585150-906
ZORBAX StableBond C18, 5 µm	883975-902	585150-902
ZORBAX Eclipse Plus C18, 5 µm	959993-902	595150-902
ZORBAX Eclipse Plus C8, 5 µm	959993-906	595150-906
ZORBAX Eclipse Plus Phenyl-Hexyl, 5 µm	959993-912	595150-912
Pursuit XRs 100Å C8, 5 µm	A6010150X046	INF6010250X212
Pursuit XRs 100Å C18, 5 µm	A6000150X046	INF6000150X212
Pursuit XRs 100Å Diphenyl, 5 µm	A6020150X046	INF6020150X212
InfinityLab Poroshell 120 SB-C18, 4 µm	683970-902	670150-902
InfinityLab Poroshell HPH-C18, 4 µm	693970-702	670150-702



MyList of analytical scale supplies

Description	Part Number
Delay and checkout calibrant, for Agilent purification systems	5190-8223
Captiva Premium Syringe Filter, polypropylene housing, nylon membrane, 25 mm diameter, 0.45 µm pore size, 100/pk	5190-5093
InfinityLab Quick Change inline filter assembly, for HPLC	5067-1602
InfinityLab Quick Change inline filter assembly, for UHPLC	5067-1603
Agilent InfinityLab Quick Connect fitting, (for connection on column inlet)	5067-5965
Agilent InfinityLab Quick Connect capillary, MP35N 0.12 x 105 mm (for Quick Connect fitting)	5500-1578
Agilent InfinityLab Quick Turn fitting, (for connection on column outlet)	5067-5966
Quick Turn capillary MP35N 0.12 x 280 mm, (for Quick Turn fitting)	5500-1596
Mounting tool for QuickTurn fittings	5043-0915
Capillary MP35N 0.12 x 90 mm SL/SL ns/ns, (for connecting guard and column)	5004-0018
A-line screw top vial, 2 mL, amber, write-on spot, 100/pk Vial size 12 x 32 mm (12 mm cap)	5190-9590
Screw cap, bonded, blue, PTFE/white silicone septa, 100/pk. Cap size 12 mm	5190-7021



MyList of preparative scale supplies

Description	Part Number
Preparative sample loop, stainless steel, 5 mL	5068-0351
Sample loop, stainless steel, 5 mL, 1/8 in, for 1290 Infinity II Series Preparative Open-Bed ALS/Collector G7158B	5068-0334
Sample loop, stainless steel, 10 mL, 1/8 in, for 1290 Infinity II Series Preparative Open-Bed ALS/Collector G7158B	5068-0335
Sample loop, stainless steel, 20 mL, 1/8 in, for 1290 Infinity II Series Preparative Open-Bed ALS/Collector G7158B	5068-0336
Multi draw loop kit, 5 mL, for G7157A, 1260 Infinity II Preparative Autosampler. Includes stainless steel loop 5 mL, plus stainless steel high-flow union	G7157-68711
InfinityLab long-life deuterium DAD/MWD lamp, with RFID tag	2140-0820
InfinityLab preparative flow cell, for MWD (G7165A) and DAD (G7115A), 0.3 mm path length	G7115-60002
Capillary stainless steel for flow cells 15 - 40 mL/min flow rates, with special fitting for flow cell connection	5500-1343
InfinityLab capillary kit, for flow rate range 15-40 mL/min or column with 21.2 mm ID. For 1290 Infinity II Purification Systems with UV detector.	5067-7016
InfinityLab capillary kit, for flow rate range 40-80 mL/min or column with 30 mm ID. For 1290 Infinity II Purification Systems with UV detector.	5067-7017
InfinityLab tubing kit, 50 mL/min, with RFID, for 1290 Infinity II preparative open-bed fraction collector	G9321-60952
Vial, screw top, clear, high recovery, 5 mL, for LC, 30/pk	5188-5369
Cap, 16 mm, screw for 6 mL vials, 100/pk	9301-1379
Septa, for 6 mL vials, 100/pk. Septum size: 16 mm (for 16 mm cap)	9301-1378
Vial, screw top, amber, write-on spot, certified, 2 mL, 100/pk	5182-0716
Vial, screw top, clear, write-on spot, certified, 2 mL, 100/pk	5182-0715
Cap, screw, blue, preslit PTFE/silicone, 100/pk	5183-2076
Glass Test Tubes, 25 x 150 mm, 55 mL, 100/pk	5190-9091
Glass Test Tubes, 30 x 150 mm, 85 mL, 100/pk	5190-9090
InfinityLab tube container, 10 tubes, 30 x 150 mm, 10 tubes, ambient for 1290 Infinity II Preparative Open-Bed Fraction Collector and Sampler/Collector	G9321-60015
InfinityLab tube container, 10 tubes, 25 x 150 mm, 18 tubes, ambient for 1290 Infinity II Preparative Open-Bed Fraction Collector and Sampler/Collector	G9321-60025
InfinityLab tube container, 10 tubes, 30 x 100 mm, 10 tubes, ambient for 1290 Infinity II Preparative Open-Bed Fraction Collector and Sampler/Collector	G9321-60058
InfinityLab tube container, 10 tubes, 25 x 100 mm, 18 tubes, ambient for 1290 Infinity II Preparative Open-Bed Fraction Collector and Sampler/Collector	G9321-60035
Glass Test Tubes, 30 x 100 mm, 45 mL, 100/pk	5042-6458
Glass Test Tubes, 25 x 100 mm, 35 mL, 100/pk	5042-6459

MyList of solvent and waste handling supplies

Description	Part Number
Solvent bottle glass filter, solvent inlet, 20 µm	5041-2168
InfinityLab solvent filtration flask, glass, 2 L	5191-6781
InfinityLab solvent bottle, clear, 125 mL	9301-6527
InfinityLab solvent bottle, clear, 500 mL	9301-6523
InfinityLab solvent bottle, clear, 1 L	9301-6528
Kit, including InfinityLab Stay Safe cap (S60 thread, 4 ports) and 10 L waste can. The cap must be used with a charcoal filter (5043-1193).	5043-1338
InfinityLab charcoal filter with time strip, 58 g	5043-1193
Bottle head assembly for 1260/1290 Infinity II Preparative LC system	G7161-60042
Solvent bottle, amber, 2 L	9301-6341
InfinityLab waste can, GL45, 6 L with Stay Safe cap	5043-1221

Purify your samples with maximum flexibility

For isolation and purification of your samples with exceptional purity and recovery, InfinityLab LC Purification Solutions offer high-performance instrumentation, columns, software, and services for analytical- to preparative-scale workflows. A comprehensive and scalable portfolio based on a single platform gives you the choice to tailor a system to meet your laboratory's current and future needs.

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