White Paper

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Higher Efficiency and Sample Loading Capacity Yield Greater Return on Investment with InfinityLab Poroshell 120 Preparative LC Columns

Authors

Lakshmi Subbarao,
Megan Kenny, and Linda Lloyd
Agilent Technologies, Inc.

Introduction

The benefits of analytical columns packed with superficially porous particles (SPPs) are well known.[1] A superficially porous particle consists of a solid silica core that is covered by a porous shell. This creates a shorter diffusion path for an analyte, providing chromatographic speed and efficiency advantages over totally porous particles (TPPs). Recently, Agilent introduced the InfinityLab Poroshell 120 preparative LC column, the first preparative column packed with 4 µm SPP particles. These columns bring the SPP advantages to a larger scale, while still using standard preparative HPLC instrumentation.

The value of SPP-packed columns stands out in high-throughput environments, where speed is critical. At high flow rates, the performance of TPP columns declines dramatically, whereas SPP columns maintain their performance. The objectives of this study are two-fold: to compare the loadability of SPP and TPP preparative columns at high flow rates (typical in discovery laboratories), and to understand how this influences the return on investment.

Experimental

The experimental conditions can be found in Table 1. All data were acquired using an Agilent 1260 Infinity II Preparative LC system. All standards were acquired from Sigma-Aldrich (St. Louis, MO, U.S.). LC-grade solvents were acquired from Burdick and Jackson.

Mass loadability is commonly used as a metric to assess a column's purification performance. The loadability is determined by increasing the mass of sample on the column until the compound of interest can no longer be separated from impurities. Loadability depends on various factors, such as polarity of an analyte, column packing material, linearity velocity of mobile phase, injection volume, retention, selectivity, and sample matrix.

To focus on the contribution from column packing material, steps were taken to minimize the impact of these other factors.

- **Polarity of analyte:** Selection of a neutral probe (toluene). Uracil was also added to the standard solutions to serve as a void volume marker.
- **Linear velocity of mobile phase:** Both columns were run at the same linear velocity. The Poroshell column was run at 1.5 times its optimal flow rate, based on a previous van Deemter study.[2] The flow rate of the TPP column was adjusted so that the retention time of uracil (unretained peak) matched the Poroshell column.
- **Injection volume:** A conservative injection volume (400 µL) was chosen to avoid overloading the TPP column, which has a smaller internal diameter (id) than the Poroshell column.

Table 1. Experimental parameters.

Parameter	TPP column	InfinityLab Poroshell 120
Analyte	Toluene in 50/50 acetonitrile/dimethyl sulfoxide	
Column	TPP C18, 19 × 150 mm, 5 µm	Agilent InfinityLab Poroshell 120 SB-C18, 21.2 × 150 mm, 4 µm (p/n 670150-90)
Flow Rate	30 mL/min	37.5 mL/min
Mobile Phase	64/36 acetonitrile/water	60/40 acetonitrile/water
Injection Volume	400 µL	400 µL
Wavelength	254 nm	254 nm

- **Retention and selectivity:** The retention time of toluene (retained peak) was similar on both columns. The isocratic mobile phase composition was adjusted so that the retention time of toluene matched that of the Poroshell column. Selectivity concerns were negated by only analyzing one compound.
- **Sample matrix:** All standards were prepared in 50/50 acetonitrile/dimethyl sulfoxide for both columns.

Standard solutions of varying concentrations of toluene (Table 2) were injected onto both columns. Table 2 also lists the corresponding mass on column for each standard solution. The mass on column is calculated using Equation 1.

Mass on column = toluene standard concentration × injection volume (mL)

Equation 1. Mass on column calculation.

Table 2. Concentration of toluene standards.

Toluene Standard Concentration (mg/mL)	25	50	100	150	200	250	300	350
Mass On Column (mg)	10	20	40	60	80	100	120	140

Results and discussion

Figure 1 is an overlay of the injections for each respective column.

The peak width at 10% peak height for each standard was plotted against the mass on column. The plots for each column can be found in Figure 2.

At the lowest levels (10 mg on column), both columns produce peaks of similar width, confirming that the injection volume is suitable and does not overload either column, despite the differences in column volumes. At higher mass loads, the TPP peaks are consistently broader than the Poroshell column. At a peak width of ~10 seconds (green box in Figure 2), the Poroshell column can accommodate 60 mg on column, compared to 20 mg on the TPP column. This allows for almost a 300% increased mass load on the InfinityLab Poroshell 120 column.

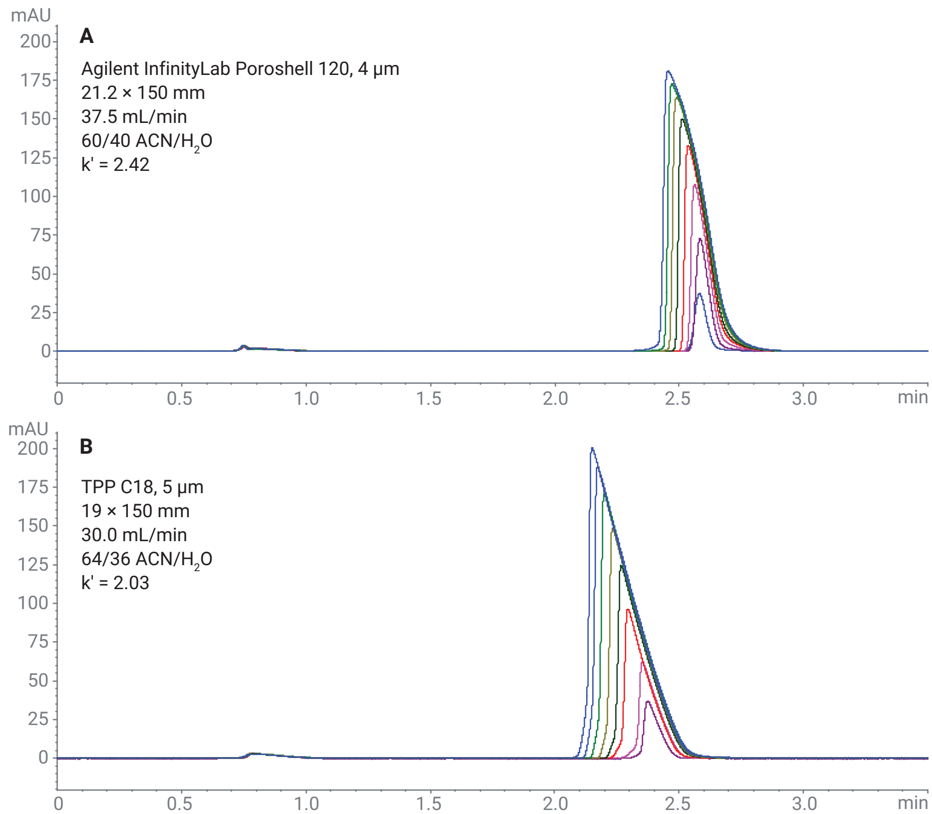

Figure 1. Injection overlay of toluene standards, from 25 to 350 mg/mL.

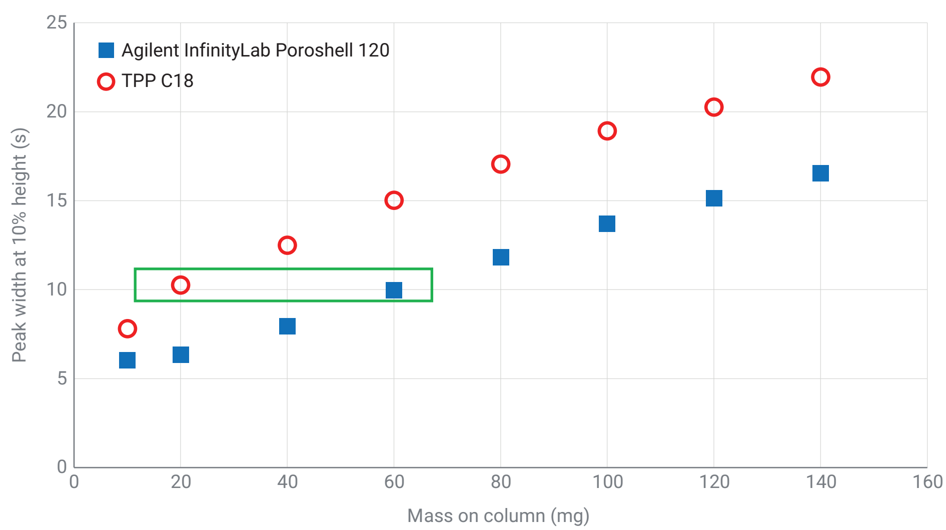

Figure 2. Plot of peak width at 10% peak height versus mass on column.

Economic implications

To better understand the financial impact of the Poroshell column's increased mass load, consider a purification campaign of 1,000 samples. Some general operating costs are listed in Table 3.

Using these general costs, the consumable costs per injection were calculated for each column and can be found in Table 4.

Table 5 shows a summary of the purification costs per campaign for each column. The purification cost per sample using a TPP column is $111, whereas the cost using the Poroshell column is $39, which is a 64% savings. By being able to load three times as much material onto the Poroshell column, each sample can be purified in a third of as many injections as the TPP column. This reduces the total run time by a third, cutting the other costs (scientist/overhead) by 67% as well.

A new phase must be evaluated before it can be incorporated into the purification workflow. Table 6 lists the costs associated with evaluating a Poroshell column. The cost to change is offset by incorporating the evaluation columns into the purification workflow. The cost to change would only impact the first campaign and not subsequent ones.

Table 3. General operating costs for a purification laboratory.

General Operation Costs	Lab overhead	$125/hour
	Scientist cost	$150/hour
	Acetonitrile, HPLC Grade	$115/Liter
	Water, HPLC Grade	$29/Liter
	Waste disposal	$50/Liter

Table 4. Costs per injection for consumables.

		TPP Column	InfinityLab Poroshell 120
Column Cost	Length	150 mm	150 mm
	Internal diameter	19 mm	21.2 mm
	Price per column	$3,408	$3,120
	Lifetime	500 injections	500 injections
	Column cost per injection	$6.82/injection	$6.24/injection
Mobile Phase Cost	Analysis time	3.5 min	3.5 min
	Flow rate	30 mL/min	37.5 mL/min
	Mobile phase consumed per injection	105 mL	131.25 mL
	Mobile phase composition (ACN/H_2O)	64/36	60/40
	Solvent cost and disposal	$133.88/Liter	$130.44/Liter
	Mobile phase cost per injection	$14.06/injection	$17.12/injection
	Consumables cost per injection (column cost + mobile phase cost)	$20.87/injection	$23.36/injection

Table 5. Purification costs based on a campaign of 1,000 injections.

		TPP Column	InfinityLab Poroshell 120
Total Purification Cost Per Campaign (1,000 Samples)	Number of samples	1,000	1,000
	Number of injections per sample	3	1
	Number of injections per campaign	3,000	1,000
	Total time (analysis time x number of injections)	175 hours	58 hours
	Total lab overhead (lab overhead x total time)	$21,875	$7,292
	Total scientist cost (scientist cost x total time)	$26,250	$8,750
	Total consumables cost (consumables cost x no of injections)	$62,621	$23,361
	Total cost per campaign	$110,746	$39,402
	Purification cost per sample	$111/sample	$39/sample
	Cost savings	**$71,344 or $71/sample**	
	% savings	**64%**	

Table 6. Costs associated with evaluating and changing to an InfinityLab Poroshell 120 column.

Cost of Change	Evaluation time	20 hours
	Evaluation cost (scientist and overhead cost × evaluation time)	$5,500
	Cost of three Agilent Poroshell columns	$9,360
	Total cost of change	$14,860
	Using evaluation columns for campaign (column cost × 1,000 injections)	$–7,800
	Net cost to change	$7,060

Return on investment

Factoring in the conversion costs, the Poroshell column has the potential to save over $62,000 with an ROI of 728% (Table 7).

Economic implications: 21.2 mm id InfinityLab Poroshell 120 versus 30 mm id TPP column

Rather than making multiple injections on a smaller column to purify a given sample, many practitioners would select a column with a larger id so that only one injection is required. Table 8 is a summary of the cost comparison when using a 30 mm id TPP column. In addition to the increased price associated with a larger id column, more solvent is consumed which also increases mobile phase costs.

Table 9 is a comparison of the campaign costs for the 30 mm id TPP and InfinityLab Poroshell 120 columns. The reduction in time as well as overhead/scientist costs are negated by the increase in consumable costs. The Poroshell column still provides a significant savings of 42%.

The ROI when switching to Poroshell from a 30 mm id TPP column is 233% (Table 10).

Table 7. Return on investment (when compared to a 19 mm id TPP column).

		TPP Column	InfinityLab Poroshell 120
Return on Investment	Total cost per campaign + net cost of change	$110,746	$48,022
	Agilent column saves		$62,724
	ROI% (savings/net cost to change)		**728%**

Table 8. Consumable costs using a 30 mm id TPP column.

		TPP Column	InfinityLab Poroshell 120
Column Cost	Length	150 mm	150 mm
	Internal diameter	30 mm	21.2 mm
	Price per column	$8,454	$3,120
	Lifetime	500 injections	500 injections
	Column cost per injection	$16.91/injection	$6.24/injection
Mobile Phase Cost	Analysis time	3.5 min	3.5 min
	Flow rate	75 mL/min	37.5 mL/min
	Mobile phase consumed per injection	262.5 mL	131.25 mL
	Mobile phase composition (ACN/H_2O)	64/36	60/40
	Solvent cost and disposal	$133.88/Liter	$130.44/Liter
	Mobile phase cost per injection	$35.14/injection	$17.12/injection
	Consumables cost per injection (column cost + mobile phase cost)	$52.05/injection	$23.36/injection

Table 9. Purification costs when using a 30 mm id TPP column.

		TPP Column	InfinityLab Poroshell 120
Total Purification Cost Per Campaign (1,000 Samples)	Number of samples	1,000	1,000
	Number of injections per sample	1	1
	Number of injections per campaign	1,000	1,000
	Total time (analysis time × number of injections)	58 hours	58 hours
	Total lab overhead (lab overhead × total time)	$7,292	$7,292
	Total scientist cost (scientist cost × total time)	$8,750	$8,750
	Total consumables cost (consumables cost × no. of injections)	$52,052	$23,361
	Total cost per campaign	$68,094	$39,402
	Purification cost per sample	$68/sample	$39/sample
	Cost savings	**$28,692 or $29/sample**	
	% Savings	**42%**	

Table 10. Return on investment (when compared to 30 mm id TPP column).

		TPP Column	InfinityLab Poroshell 120
Return on Investment	Total cost per campaign + net cost of change	$68,094	$48,022
	Agilent column saves		$20,072
	ROI% (savings/net cost to change)		**233%**

Conclusion

The InfinityLab Poroshell 120 preparative LC column, packed with 4 µm superficially porous Poroshell particles, delivers almost a 300% increase in sample load when compared to a traditional totally porous particle column. This has significant implications for the value of this column over traditional TPP columns when compared to a column of similar size (19 mm id) or a larger one (30 mm id). InfinityLab Poroshell 120 Preparative columns deliver a 64% cost saving and over seven times the return on investment per 1,000 sample purification campaign when compared to a similar sized totally porous particle column. Comparing to a 30 mm id TPP column, the InfinityLab Poroshell 120 Preparative columns deliver 42% cost savings and approximately twice the return on investment.

References

1. Gratzfeld-Huesgen, A.; Naegele, E. Maximizing Efficiency Using Agilent InfinityLab Poroshell 120 Columns. *Agilent Technologies application note*, publication number 5990-5602EN, **2016**.
2. Subbarao, L. Developing Fast Purification Methods for Natural Products Using an Agilent InfinityLab Poroshell 120 SB-C18 Preparative LC Column. *Agilent Technologies application note*, publication number 5994-3518EN, **2021**.

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