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APPLICATION NOTE

Simulated-MonolithTM Polymerics, Compared with Non-Porous Polymerics.

In an unambiguous experiment, we have used the certified diagnostic test 58278 from Supelco to compare side by side the Simulated-MonolithTM made exclusively by OraChrom Inc. with PLRP-S 3uM made in GB.

4 small molecules were tested at > 350 and > 900 cm/hr of linear velocities.

Although the high pressure required with the non-porous polymeric is primarily to force the small molecules in the 100 Å diffusive pores for their separation, such high pressures are no longer required when using the Simulated-MonolithTM with convective pores.

The column back pressure of 19 bar at 0.2~ml/min for STYROS® at 0.2 ml/min of flow rate is to be compared with 98 bar with PLRP-S at a similar flow rate under similar conditions.

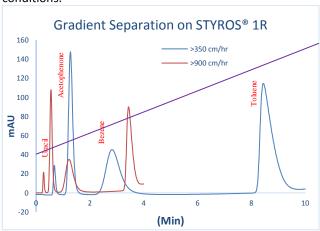


Table 1. Operating parameters.

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HPLC System.	Agilent 1260 with thermostatted column compartment and
	quaternary pump.
Columns	STYROS® 1R 2.1 X 50 mm (0.173 ml volume)
Mobile phase.	A: H20, 0.075 % TFA
	B: 5% H20 in ACN, 0.075 % TFA
Flow rates	0.2 ml/min (347 cm/hr of linear velocity on an empty
	column)
	0.5 ml/min (867 cm/hr of linear velocity on an empty
	column)
Gradient	40 to 90 % B in 10 min or
	40 to 90 % B in 4 min
Temperature	30°C
Detection	254 nm
Injection volume	6 μl
Pressure Drop	19 bars at 0.2 ml/min, 46 bar at 0.5 ml/min.
Sample:	Certified diagnostic test 58278 Supelco

The pressure increases drastically to 240 bar at 0.5 ml/min without resulting in additional increased performance with the PLRP-S column.

The back pressure is 46 bars for the STYROS® column at such flow rate.

These numbers are to be compared with 100 cm/hr that soft gel operates in vaccines and other biopharmaceuticals.

The leaching occurs even at low flow rates.

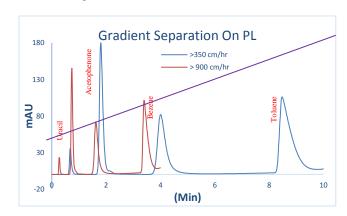


Table 2. Operating parameters.

HPLC System.	Agilent 1260 with thermostatted column compartment and
	quaternary pump.
Columns	PLRP-S 100A 3uM 2.1 X 50 mm (0.173 ml volume)
Mobile phase.	A: H20, 0.075 % TFA
	B: 5% H20 in ACN, 0.075 % TFA
Flow rates	0.2 ml/min (347 cm/hr of linear velocity on an empty
	column)
	0.5 ml/min (867 cm/hr of linear velocity on an empty
	column)
Gradient	40 to 90 % B in 10 min or
	40 to 90 % B in 4 min
Temperature	30°C
Detection	254 nm
Injection volume	6 μ1
Pressure Drop	98 bars at 0.2 ml/min, 240 bar at 0.5 ml/min.
Sample:	Certified diagnostic test 58278 Supelco

Unlike monolith, Simulated-Monolith $^{\text{TM}}$ is not prone to the "wall effects" and leaching that monolithic media suffer from.

