OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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# APPLICATION NOTE

## Simulated-Monolith™ Polymerics, Compared with Non-Porous Polymerics. Isocratic Run in ACN.

The certified sample 48270-U is also run in isocratic mode in ACN.

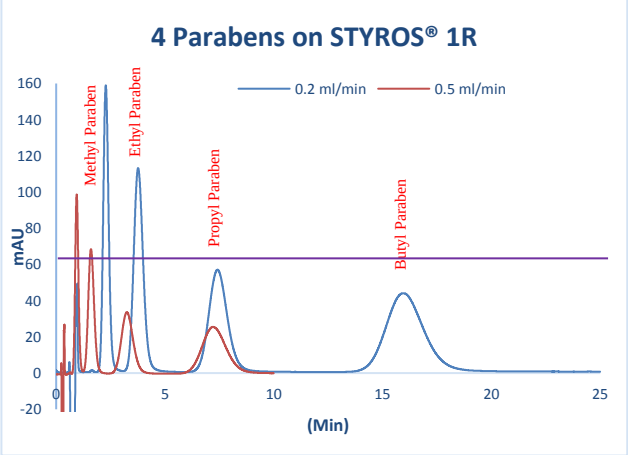


Table 1. Operating parameters.

| | |
|---|---|
| **HPLC System.** | Agilent 1290 with thermostatted column compartment and binary pump. |
| **Columns** | STYROS® 1R 2.1 X 50 mm (0.173 ml volume) |
| **Mobile phase.** | A: DI H20, 0.1 % TFA<br>B: 95:5 ACN: H2O 0.1% TFA |
| **Flow rates** | 0.2 ml/min (347 cm/hr of linear velocity on an empty column)<br>0.5 ml/min (867 cm/hr of linear velocity on an empty column) |
| **Isocratic:** | 30 % B |
| **Temperature** | 30°C |
| **Detection** | 254 nm |
| **Injection volume** | 20 µl |
| **Pressure Drop** | 30 bars at 0.2 ml/min, 77 bar at 0.5 ml/min. |
| **Sample:** | Certified diagnostic test 48270-U Supelco |

The back pressure is 77 bar at 0.5 ml/min. That includes the system. It equates to > 900 cm/hr of linear velocity in a packed column.
The separation is still baseline. Most importantly is the lack of deterioration of the column from leaching. There is no restriction on avoiding pure H2O or similar issues.

These columns are used during the automated digestion and mapping of proteins with immobilized enzymes with the line of StyrosZyme® columns from OraChrom.
They need to withstand the high pH when using Trypsin for digestion. Silica cannot.
The capacity of the column is also optimal to trap all the resulting peptide digests.

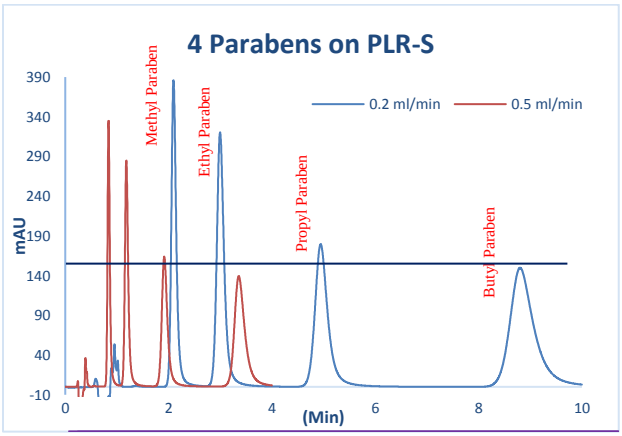


**Table 2. Operating parameters.**

| | |
|---|---|
| **HPLC System.** | Agilent 1290 with thermostatted column compartment and binary pump. |
| **Columns** | PLRP-S 100A 3uM 2.1 X 50 mm (0.173 ml volume) |
| **Mobile phase.** | A: DI H20, 0.1 % TFA<br>B: 95:5 ACN: H2O 0.1% TFA |
| **Flow rates** | 0.2 ml/min (347 cm/hr of linear velocity on an empty column)<br>0.5 ml/min (867 cm/hr of linear velocity on an empty column) |
| **Isocratic:** | 40 % B |
| **Temperature** | 30°C |
| **Detection** | 254 nm |
| **Injection volume** | 20 µl |
| **Pressure Drop** | 100 bars at 0.2 ml/min, 244 bar at 0.5 ml/min. |
| **Sample:** | Certified diagnostic test 48270-U Supelco |

The pressure increases drastically in the case of PLRP-S to 320 bar at 0.5 ml/min.
The resulting peaks are sharper with tailing that is indicative of the slow diffusion of analytes from the pores.
The difference is more than 3-fold increase in pressure that is important during industrial processing.

Unlike monolith, Simulated-Monolith™ is not prone to the "wall effects" and leaching that monolithic media suffer from.

Inc.