Advantage of Silica Monolithic HPLC Columns – Highly Reproducible Results with Onyx™

Michelle Korby and Dr. Krishna Kallury
Phenomenex Inc., Torrance, CA, USA

Introduction

Monoliths are rod-shaped continuous bed silica or polymeric materials, which offer an alternative to conventional particle-packed columns for analytical and preparative liquid chromatography.[1-3] Silica monoliths are more easily prepared in comparison with their polymeric counterparts and provide larger surface areas for small molecule separations. The main advantages of monoliths are their superior performance for fast separations with low pressure at high flow rates and favorable mass transfer efficiency. Most of the documented literature on silica-based monolith columns pertains to their preparation, influence of pore network properties on separation efficiency, applications in the pharmaceutical/environmental areas, and comparison of their performance characteristics with particulate C18 HPLC columns. In this note, we demonstrate the reproducibility of different batches of Onyx™ Monolithic C18 columns, an aspect sparsely addressed by others.

Materials and Methods

Four Onyx™ Monolithic C18, 100 x 4.6mm columns (serial numbers 040090-37 (batch #1), 040100-29 (batch #2), 040120-33 (batch #3), and 040420-20 (batch #4)) were obtained from Phenomenex, Torrance, California, USA. All solvents and chemical probes are from Sigma (St. Louis, Missouri). An Agilent 1100 HPLC instrument, equipped with a VWD, quaternary pump, in-line degasser and autosampler was used for all HPLC studies, employing HP ChemStation software (version A.09.01).

PEAK ASYMMETRY

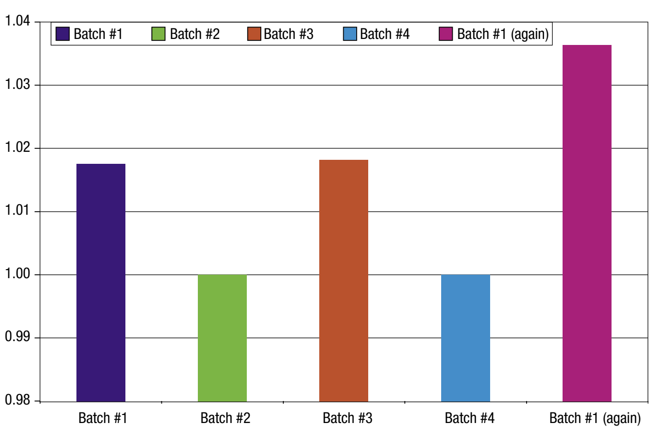
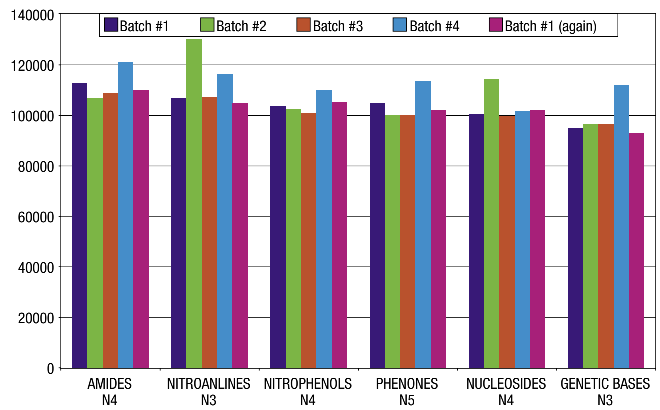


Figure 1: Peak asymmetry ratio comparison for ethylbenzene versus toluene (mobile phase: methanol/20mM potassium phosphate, pH 7.0 [80:20]; 1mL/min flow rate; column temperature 40ºC; detection wavelength 254nm)

EFFICIENCY (plates/meter) ACETONITRILE



EFFICIENCY (plates/meter) METHANOL

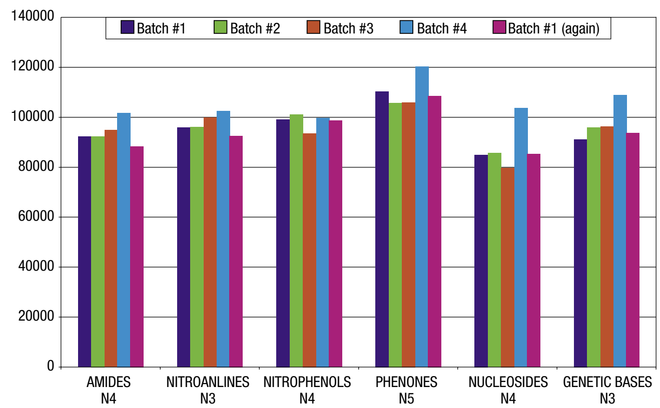


Figure 2: Comparison of Efficiencies for four batches of Onyx™ Monolithic C18 columns [mobile phase: acetonitrile/water (40:60) or methanol/water(60:40) for Nitroanilines, Nitrophenols, Amides and Phenones; 20mM ammonium acetate, pH 4.5/organic for Nucleobases and Nucleotides]

Results and Discussion

We used several parameters to establish the reproducibility of the four columns studied. These include peak asymmetry, plate counts per meter, retention factors for polar as well as hydrophobic probes, and uniformity of the sorbent surface for different types of physico-chemical interactions. In addition, we investigated run-to-run reproducibility on a single column (040090-37) by running a test of diverse probes initially on this first column, then running the test on the other three columns, then rerunning the same probes again on the first column.

In Figure 1, the reproducibility of the peak asymmetry ratio of ethylbenzene versus toluene is shown for all four Onyx™ Monolithic C18 columns. The RSD for this ratio for all four columns is 1.5%. Reproducibility of efficiencies (plate counts/

Figure 3:
Reproducibility of Aryl Alkyl Ketones in acetonitrile/water (60:40)

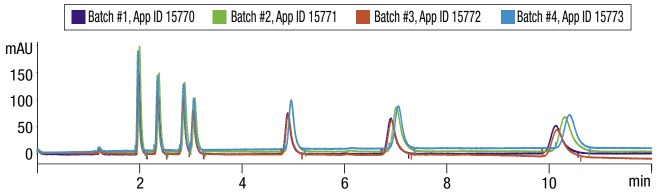


Figure 4:
Reproducibility of Nucleobases in methanol/ 20mM ammonium acetate, pH 4.5 (8:92)

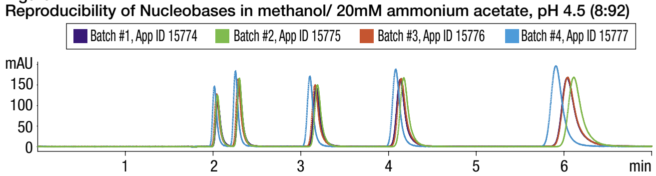
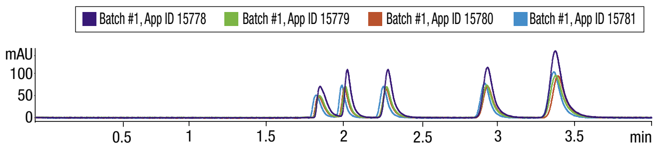


Figure 5:
Reproducibility of Nucleosides in acetonitrile/ 20mM ammonium acetate, pH 4.5 (5:95)

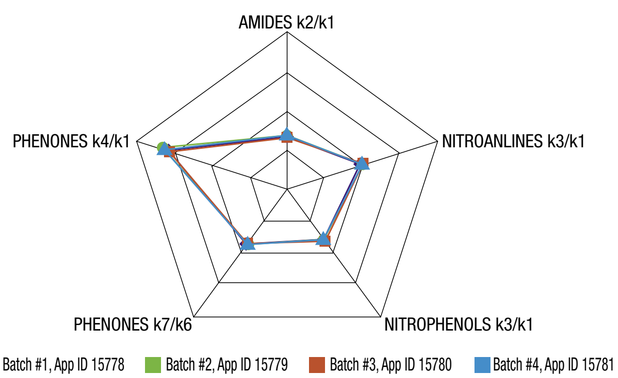


meter) for different classes of probes for two mobile phases, water/acetonitrile and water/methanol, is included in Figure 2. The RSDs are in the range 5-7%.

In Figures 3-5 are presented the chromatograms of three sets of probes (nucleobases, nucleosides and a series of homologous aryl alkyl ketones) on all four columns with different batches. The retention factors for all the probes are within 5% for all the columns. In Figure 6, the Tanaka plots are shown for columns from all four batches, with representative parameters for hydrogen bonding acceptors (amides), hydrogen bonding donors (nitrophenols), pi-pi interactions (benzophenone), and dipolar (nitroanilines) and hydrophobic (methylene selectivity) probes at each corner. It is clear from the similar shapes of the Tanaka graphs in Figure 6, that all these interactions are very reproducible, batch-to-batch, for both acetonitrile and methanol mobile phases.

Finally, the run-to-run reproducibility on the same column was evaluated for acidic, basic, neutral polar, and neutral hydrophobic probes. The data shown in Figure 7 shows less than 1% variation between the two runs.

Figure 6: Reproducibility of the chemical reactivity of the Onyx Monolithic C18 columns in methanol (below) and acetonitrile (above, right) illustrated in the form of Tanaka Plots for hydrophobic/pi-pi/dipolar/hydrogen bond donor/hydrogen bond acceptor interctions



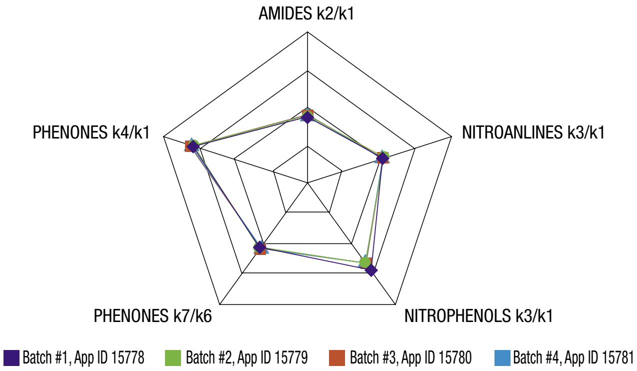
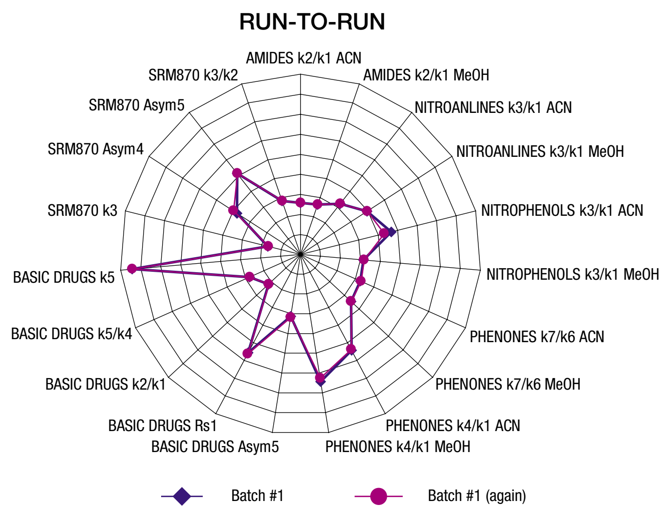


Figure 7: Run-to-run reproducibility of Onyx™ Monolithic C18 column (040090-37) for Amide, Nitroaniline, Nitrophenol, Aryl alkyl ketone, SRM870 and Basic Drug probes in acetonitrile/water (or buffer) mobile phases. The pink and purple lines represent the second and first runs, respectively, and show complete overlap to confirm reproducibility.



References

1. Y. Chu and C.F. Poole, J. Chromatogr. A, 2003, **1003**, 113-121.
2. H. Zeng, Y. Deng and J. Wu, J. Chromatogr. B, 2003, **788**, 331-337.
3. K. Cabrera, J. Sep. Sci. 2004, **27** 843-852.

If you would like more information on these columns, or any of the applications listed, please contact Phenomenex.

ORDERING INFORMATION

Order Number	Description
CHO-7643-TN	Onyx Monolithic C18 100x4.6mm

For a digital copy of this Technical Note, please visit www.Phenomenex.com/TechNotes/1025