

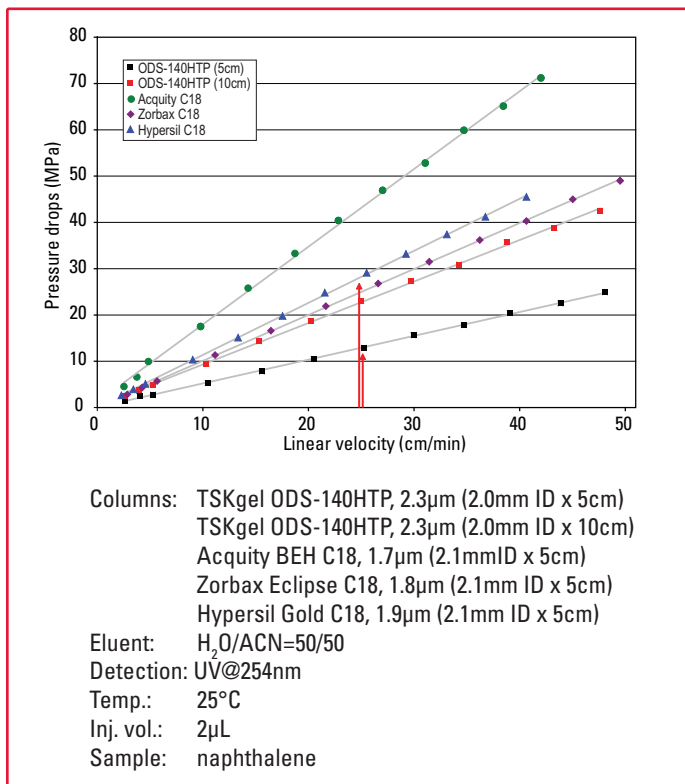
TSKgel ODS-140HTP, 2.3 μ m: Ultra High Performance Reversed Phase Columns for High Throughput Analysis

TSKgel
APPLICATION NOTE

Introduction

Short analyses time and high resolution are in great demand from R&D and QC departments within the pharmaceutical industry. Sub-two micron ODS reversed phase columns have recently been introduced to meet these requirements, but these columns require an ultra-high pressure HPLC system to achieve optimum performance. TSKgel ODS-140HTP, 2.3 μ m columns from Tosoh Bioscience have been developed to offer a combination of short analyses time and high resolution separations that can be run at modest pressures, making these columns compatible with conventional HPLC instrumentation. The polylayer bonding chemistry of these columns results in highly efficient and physically stable columns when operated at high linear velocities. In addition, TSKgel ODS-140HTP, 2.3 μ m columns can be efficiently operated at pressures not exceeding 9000psi in UPLC® and other ultra-high pressure HPLC systems, as well as in traditional HPLC systems.

Figure 1. Linear velocity vs. pressure drop for TSKgel ODS-140HTP, 2.3 μ m, 5cm and 10cm columns and competitive sub-2 μ m, 5cm columns



Results

TSKgel ODS-140HTP, 2.3 μ m columns operate at lower pressure than competitive sub-2 μ m columns. This is illustrated in *Figure 1*. The pressure drop of a 5cm TSKgel ODS-140HTP, 2.3 μ m column at 25cm/min is more than 50% lower than that for smaller particle size competitive columns. Not surprisingly, the pressure drop over a 10cm TSKgel ODS-140HTP, 2.3 μ m column was still lower than any of the competitive 5cm columns.

To demonstrate the physical stability of the TSKgel ODS-140HTP, 2.3 μ m columns, they were subjected to more than 1000 multi-step gradient cycles at high flow rate. *Figure 2* shows that the number of theoretical plates remained basically unchanged for a TSKgel ODS-140HTP column during 1110 gradient cycles consisting of five minute step gradients from 10% to 50% and from 50% to 100% methanol at 0.6mL/min. During each cycle, pressure fluctuated between 30 and 60MPa as a result of changes in mobile phase viscosity.

Figure 2. Physical stability of TSKgel ODS-140HTP column during 1110 gradient cycles

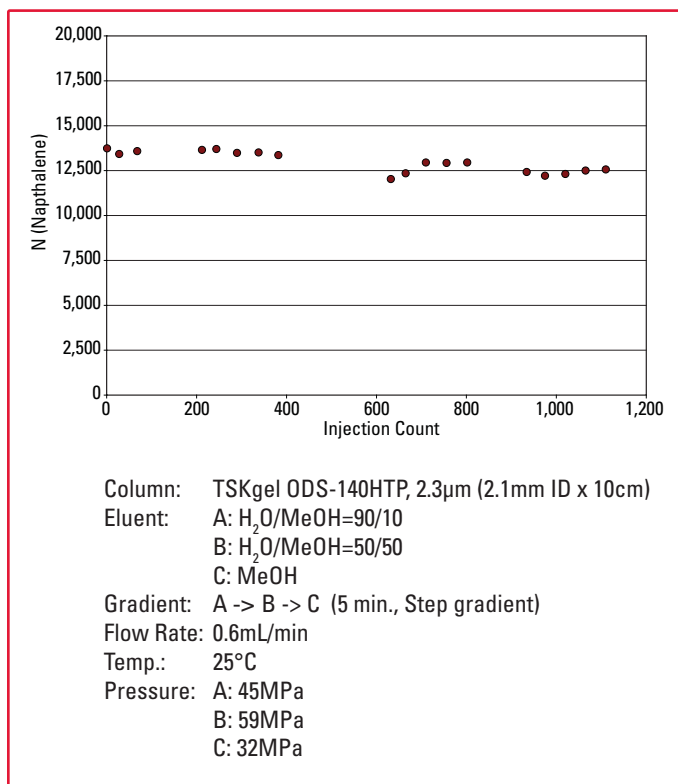


Figure 3. TSKgel ODS-140HTP column performance after the first step gradient cycle and after 1110 cycles

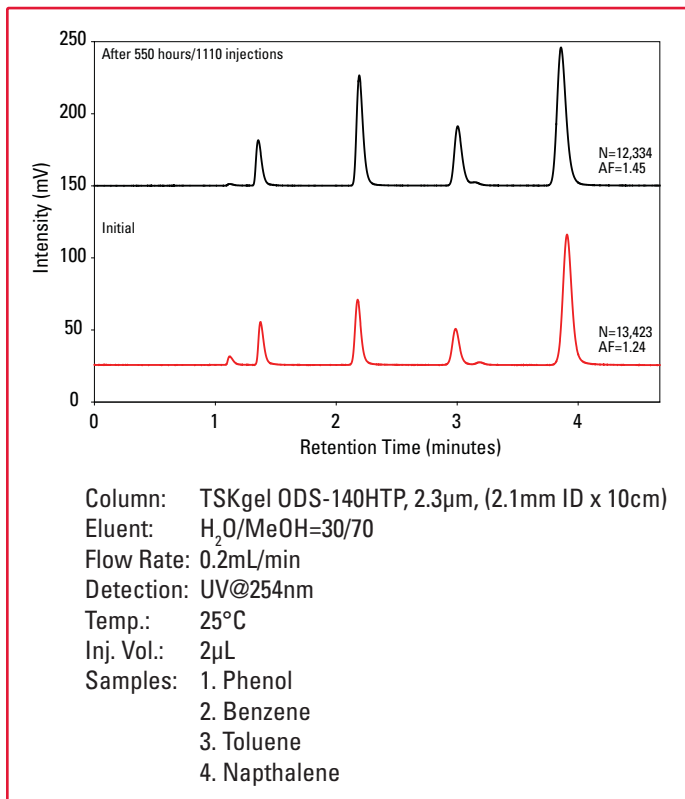
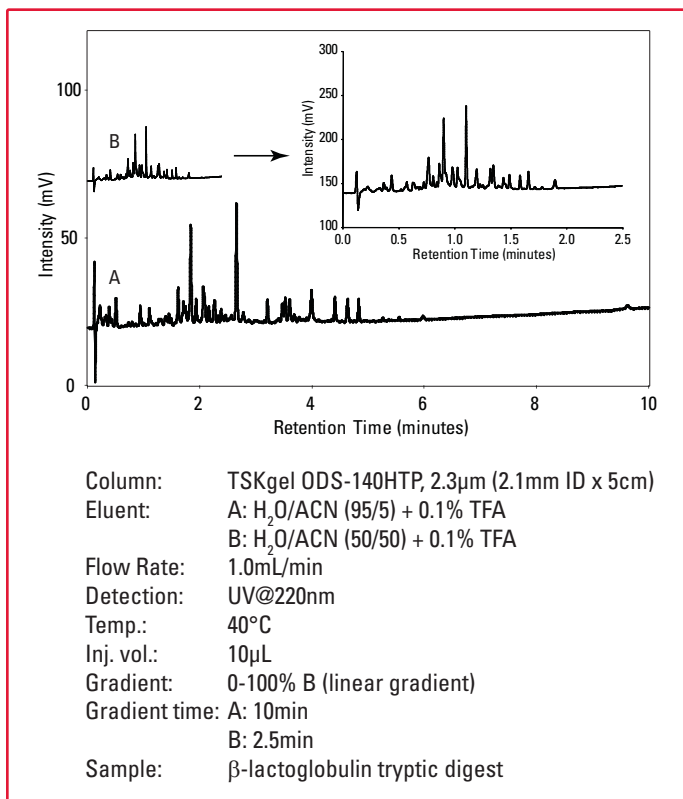


Figure 3 shows injections of test solutes after the first step gradient cycle and after 1110 cycles. The results clearly demonstrate the durability of the TSKgel ODS-140HTP, 2.3 μ m columns when operated at high flow rate and high pressure.

Figure 4 illustrates that high resolution of a peptide digest from beta-lactoglobulin can be obtained within minutes on a 2.1mm ID x 5cm TSKgel ODS-140HTP, 2.3 μ m column at a back pressure of about 35MPa (the highest pressure at about 15% acetonitrile in the gradient), i.e. still achievable in traditional HPLC systems. As expected, peak capacity improved when using a longer gradient time.

Figure 4. Separation of β -lactoglobulin tryptic digest using a short TSKgel ODS-140HTP column



Conclusion

TSKgel ODS-140HTP, 2.3 μ m reversed phase HPLC columns from Tosoh Bioscience provide high resolution and short analyses times at moderate pressures, enabling high throughput separations. These columns were designed to be used with either UPLC® (up to 9000psi) or in conventional HPLC systems.

For use in high throughput applications, including drug discovery, pharmacokinetics and peptide digest separations, TSKgel ODS-140HTP, 2.3 μ m columns offer excellent resolution.



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