Kromasil® Eternity™

Application note

Improving selectivity, resolution and purification productivity using pH

Reversed phase chromatography based on traditional silica is the workhorse for the separation and purification of samples in the analytical, drug discovery and development laboratories. The pH range where these traditional stationary phases operate is between 2 and 8. Scientists regularly come across applications where selectivity, resolution as well as loading onto the column are challenging at the above mentioned pH range. Therefore, researchers seek an alternative stationary phase material that resists and has a consistent performance at a wider pH window to effectively and efficiently analyze challenging mixtures as well as isolate compounds outside the common pH range.

Organosilane enforced silica based materials with high mechanical and chemical stability at a wider range of pH constitute an important alternative for scientists to use in separation and purification of samples. This application note illustrates the utility of such enforced phases using pH as a tool. This work also shows that consistent packing of stable materials across different column sizes allows for straight forward scale-up.

Analysis of an alkaloid mixture at low pH

The chromatographic analysis of a sample containing lidocaine, papaverine, noscapine, and diphenhydramine was carried out at low pH using a Kromasil EternityXT 5 µm C18, 4.6 x 150 mm column. The mobile phase composition was acetonitrile/water/formic acid [30/70/0.1] at a flow rate of 1.0 mL/min, temperature 30°C and the run was monitored using a UV detector at 220 nm. The chromatographic result is shown in Figure 1.

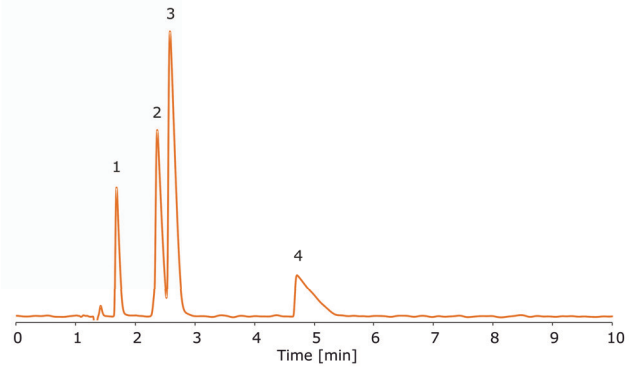

Figure 1: *Analysis of a sample at low pH*

As seen in the figure, these bases exit the column quickly. The low retention times are due to the nature of the compounds in the sample combined with the low pH conditions resulting in ionized substances that elute fast, compromising selectivity and resolution. Also, if the scientist would need to purify any of the compounds in this mixture, the loading per run would be low due to the low resolution between the peaks, resulting in low productivity and yield per run, contributing to low efficiency in the laboratory.

Substances: 1: Lidocaine, 2: Papaverine, 3: Noscapine, 4: Diphenhydramine
Column: Kromasil EternityXT 5 µm C18, 4.6 x150 mm
Mobile phase: acetonitrile/water/formic acid [30/70/0.1]
Flow rate: 1.0 mL/min
Temperature: 30°C
Detection: UV @ 220 nm

Analysis of an alkaloid mixture at high pH

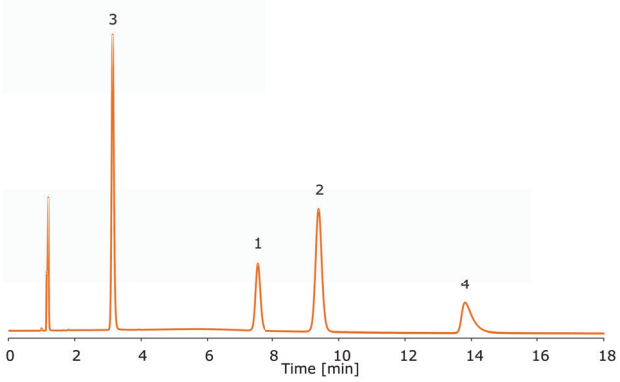

Figure 2: *Analysis of a sample at high pH*

Substances: 1: Lidocaine, 2: Papaverine, 3: Noscapine, 4: Diphenhydramine
Column: Kromasil EternityXT 5 µm C18, 4.6 x150 mm
Mobile phase: acetonitrile/10 mM ammonium carbonate, pH of 10.5 [50/50]
Flow rate: 1.0 mL/min **Temperature**: 30°C
Detection: UV @ 220 nm

Figure 2 shows the result for the same experiment carried out under the same conditions with the exception of the mobile phase composition. This chromatographic result was obtained with a mobile phase composition of acetonitrile/10 mM ammonium carbonate, pH of 10.5 [50/50]. Under the new conditions, the basic compounds in the sample turn neutral and they are further retained in the column. As a consequence of the interaction between the compounds in neutral state and the stationary phase, the chromatographic result of Figure 2 shows significant baseline resolution. In addition, there is an improvement of peak shape and, for this particular case, selectivity reversal. So in the event that the scientist needs to purify any of these peaks, it is possible to load more sample at one time onto the column and consequently isolate more product per run compared to low pH. Thus, this would result in faster purification turnaround.

Scalability built-in

Chromatography is a unique technique where scale-up can be straight forward as long as the material has good mechanical and chemical stability and the columns are packed consistently. Figure 3 shows the chromatograms for the separation of a sample containing dimethyl phtalate, toluene, biphenyl and phenanthrene using three Kromasil EternityXT 5 µm C18 columns of the same length and increasing internal diameters. Briefly, the internal diameters of the columns were 4.6, 10 and 21.2 mm ID, for the figure's top, middle and bottom results, respectively. The experiments were run under the same mobile phase conditions of acetonitrile/water [70/30], at a temperature of 25°C and monitored using a UV detector at 254 nm. It is to note that the flow rates and injection volumes were scaled according to the column dimensions. As seen in the figure, the retention times and the peak shapes are maintained independent of the column size. This result is noteworthy as the difficulty of packing columns increases with column diameter.

In conclusion, the experiments presented here illustrate the utility of Kromasil EternityXT columns for challenging mixtures where the compounds can have better retention when exposed to high pH eluent. Further, the Kromasil EternityXT analytical and preparative columns results indicate that the column packing is such that they deliver excellent matching retention times between column sizes. This is significant for when scientists need to carry out efficient scale-up and offer isolated material on a timely basis for structure elucidation, impurity characterization and trial studies at various stages of drug development in the pharmaceutical and natural products industries.

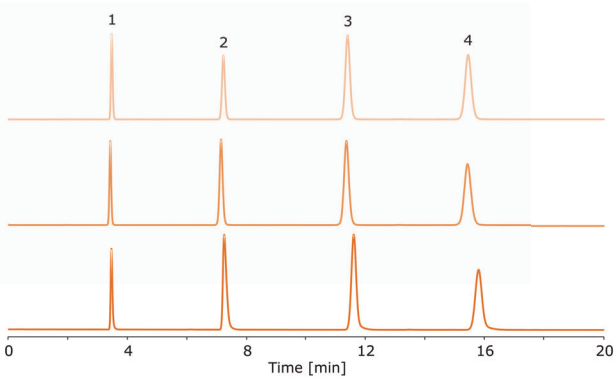

Figure 3: *Scale-up comparison with Kromasil EternityXT 5 µm C18 columns*

Substances: 1: dimethyl phtalate, 2: toluene, 3: biphenyl, 4: phenanthrene
Stationary phase: Kromasil EternityXT 5 µm C18
Column length: 150 mm
Column diameter: 4.6 (top), 10 (middle) and 21.2 mm (bottom), respectively.
Mobile phase: acetonitrile/water [30/70]
Temperature: 25°C **Detection**: UV @ 254 nm