May 2007 Newsletter

New Mixed Mode Approach-Taking Control of Your Separations

By SIELC, Inc. Prospect Heights, IL, USA, www.sielc.com

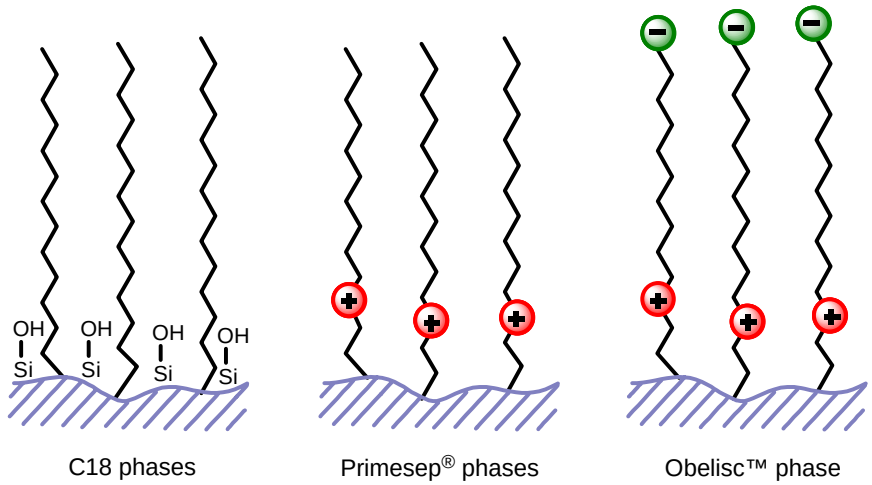


Figure 1. Evolution of mixed-mode stationary phase chemistry leads to the Obelisc separation platform, addressing difficult issues of traditional C18 chemistry: symmetrical peaks of basic compounds, retention of polar compounds, controllable selectivity, and low buffer concentration for both UV and MS applications.

The liquid chromatography process involves an interaction of analytes with a stationary phase. Traditional C18 or C8 chemistry provides hydrophobic interaction but never completely eliminates secondary ionic or polar interactions with silica sublayer. The degree and nature of these secondary interactions makes one C18 column different from another brand C18 column. In fact, every RP column is a mixed-mode column with poorly defined secondary interactions.

Primesep® RP phases are built around controllable secondary ionic interaction, eliminating non-controllable silica effects.

Now SIELC introduces a new generation of mixed-mode chemistry - Obelisc™ - built around zwitterionic hydrophobic stationary phase (Fig. 1).

Obelisc™ R is a column developed primarily for reversed-phase chromatography, with benefits of retention of polar molecules normally poorly retained on RP C18 columns. Having two oppositely-charged ions in a ligand allows to generate high ion-strength within stationary phase layer, which attract polar molecules by different type of polar interactions. At the same time, the hydrophobic nature of the stationary phase still retains well the molecules of hydrophobic nature commonly analyzed by C18 (Fig. 2).

Specially designed AQ C18 columns allow to work in low organic content mobile phase, but offer small improvement in retention of polar molecules compared to the Obelisc™R column (Fig. 3). Both acidic and basic polar molecules show significant improvement in degree of retention, typically exceeding 200%, compared with the best available AQ type phases. Figures 4-6 show the difference and the similarity of Obelisc R phase with some modern stationary phases.

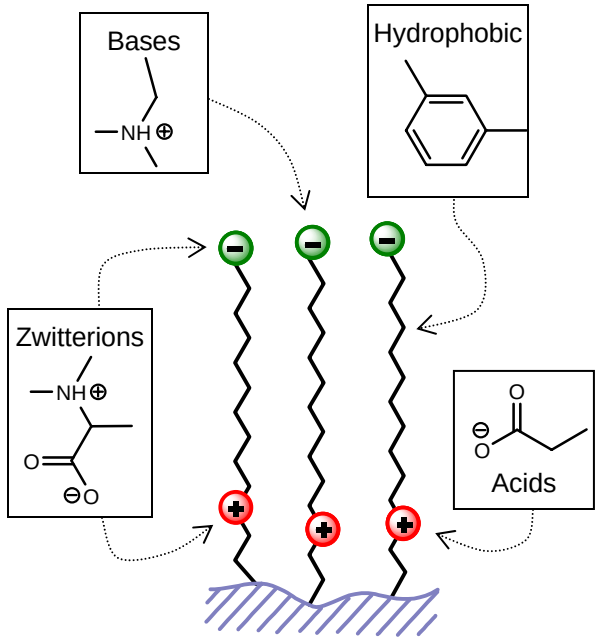
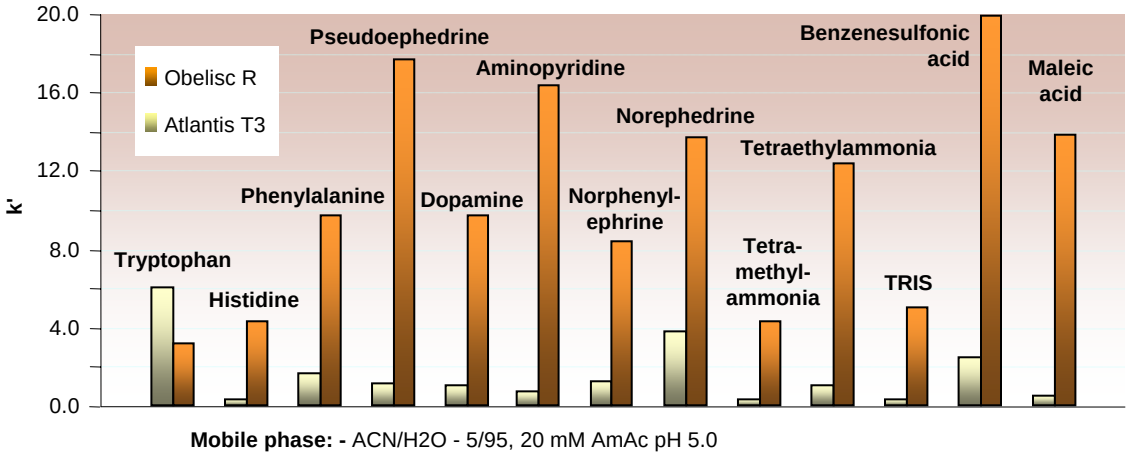


Figure 2. Different types of interactions of Obelisc R stationary phase with different analytes allows to retain many types of polar and hydrophobic compounds.



Mobile phase: - ACN/H2O - 5/95, 20 mM AmAc pH 5.0

Figure 3. Comparative retention of Polar Compounds at pH 5 - Obelisc R vs. Atlantis T3 (previously one of the leading column offered for retention of polar compounds).

The retention and selectivity difference of the Obelisc R column makes this stationary phase the first choice when complex mixtures of polar and non-polar molecules need to be analyzed, especially with MS, ELSD, or CAD detection setting.

Obelisc R can be operated with or without organic modifier. In zero-organic mobile phases, no dewetting is observed with Obelisc, but in many cases, higher concentration of organic modifier can be use with very polar compounds, either to simplify evaporation stage in preparative chromatography, increase sensitivity in LC-MS, or just to adjust selectivity of separation (Fig. 6-10). Additionally, due to the high ion strength of the stationary phase, the loading capacity of Obelisc R columns is much higher than that of C18 columns. This is a significant advantage in preparative chromatography or in the case of high sample loading (Fig. 11-12).

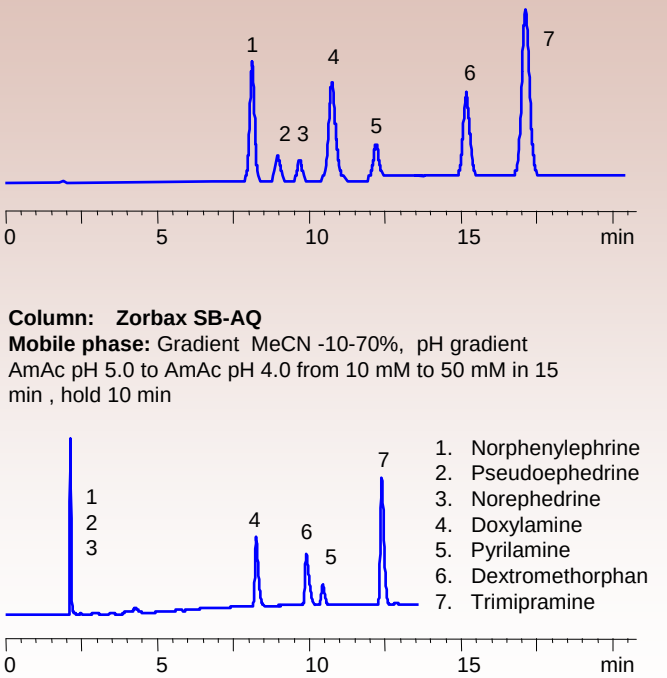


Figure 4. Comparison of the separation of polar drugs on Obelisc R and Zorbax SB-AQ.

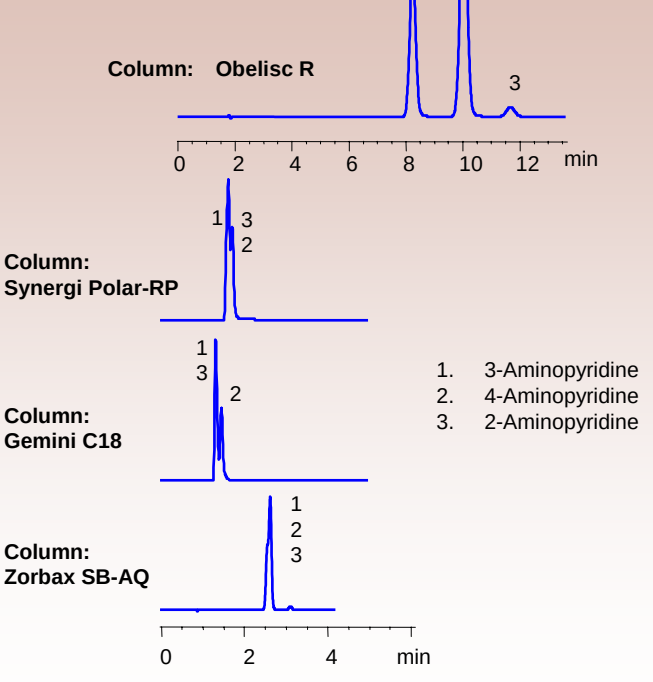


Figure 5. Comparison of Obelisc R to some common reversed-phase C18 columns for the separation of aminopyridine isomers.

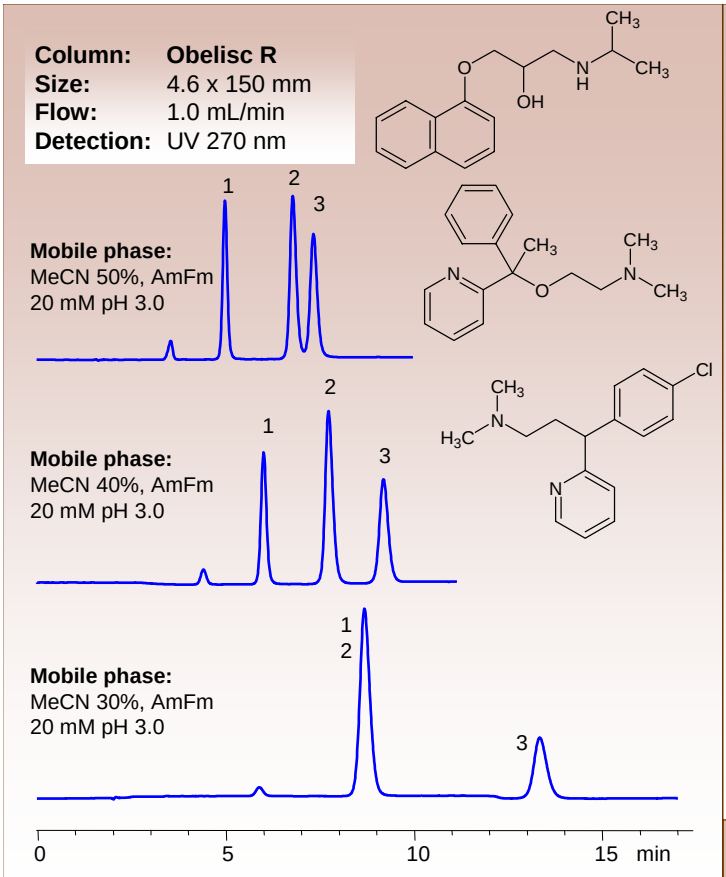


Figure 6. Separation of hydrophobic basic drugs

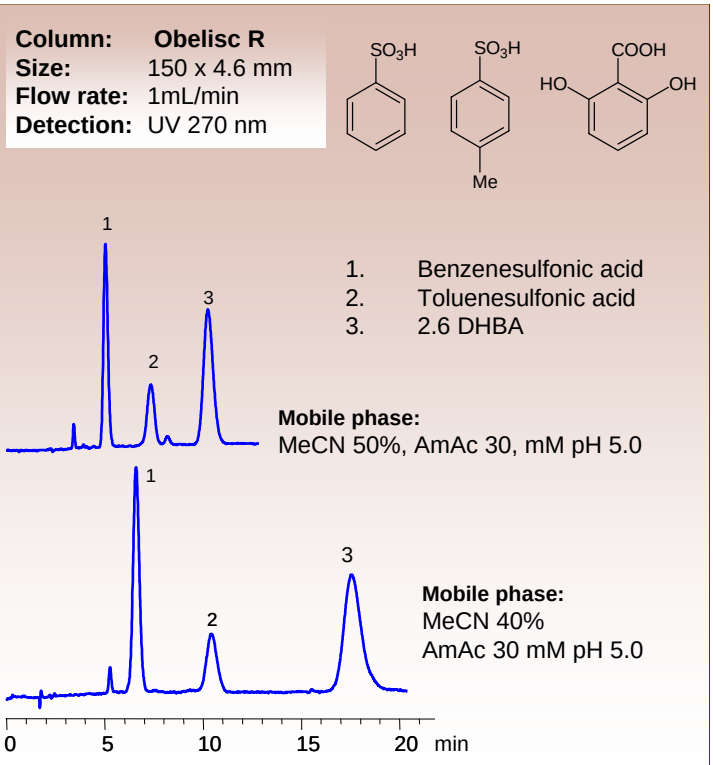


Figure 7. Separation of hydrophilic strong acids

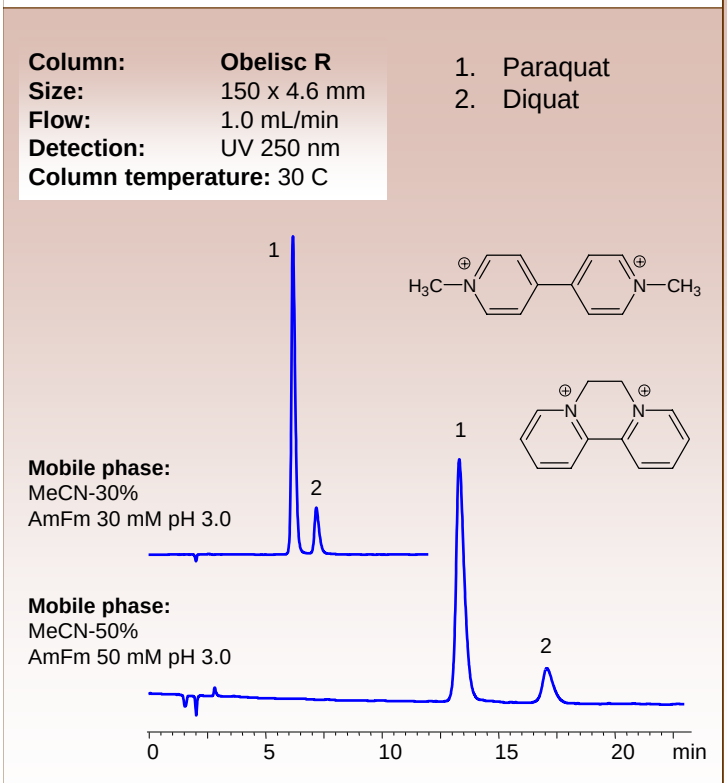


Figure 8. Separation of paraquat and diquat in MS compatible conditions

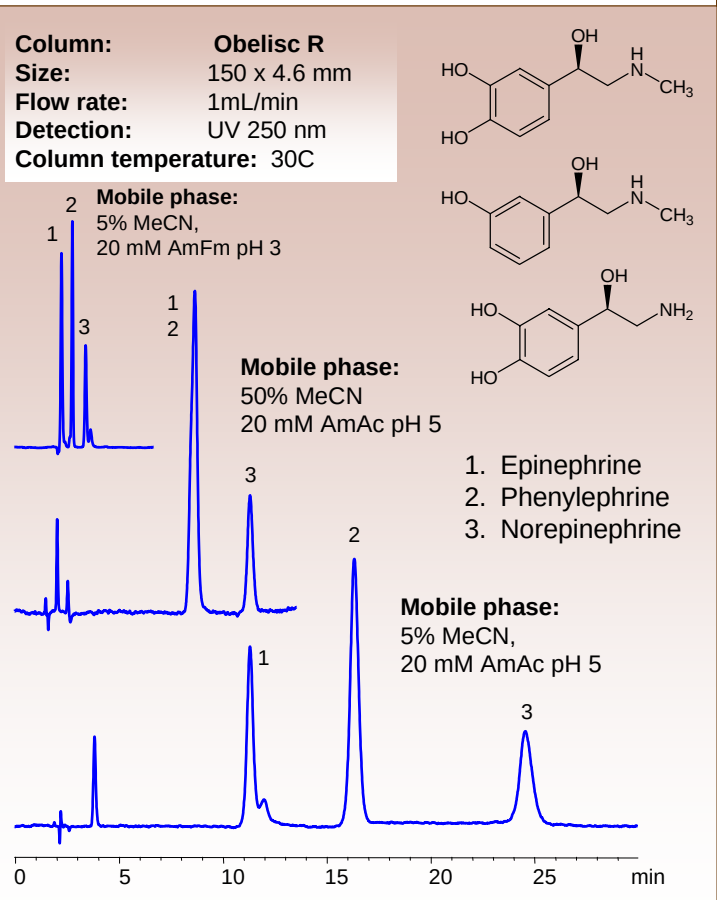
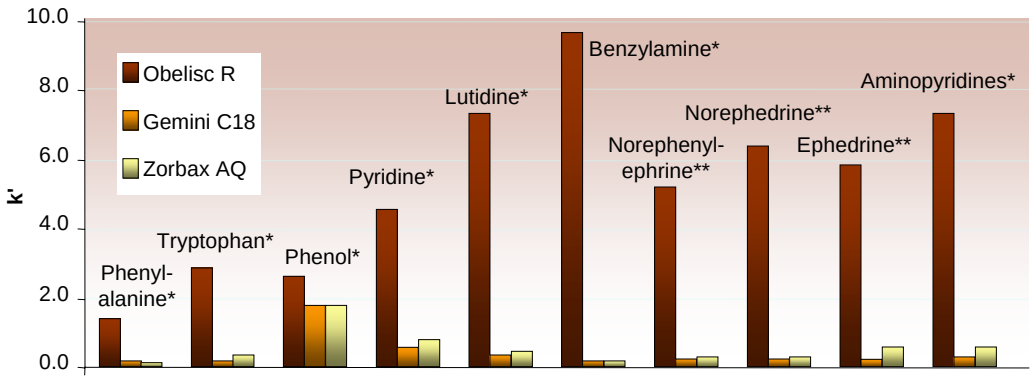


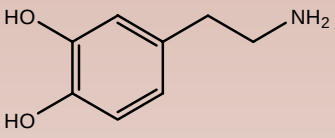
Figure 9. Separation of hydrophilic basic drugs



* **Mobile phase:-**ACN/H2O/Ammonium acetate 35/65/10 mM pH 4.0
** MeCN/H2O - 45/55, buffer gradient AmAc pH 5.0 to AmAc pH 4.0 from 10 mM to 50 mM in 20 min

Figure 10. Retention of Polar Compounds on Obelisc R and Traditional RP Columns

Column size: 150 x 4.6 mm
Flow: 1.0 ml/min
Detection: UV 300 nm
Sample: 50 mg/ml in H2O



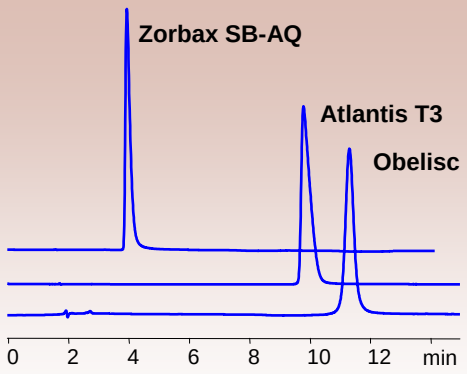
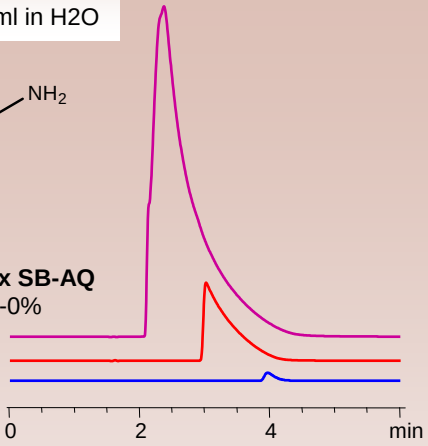


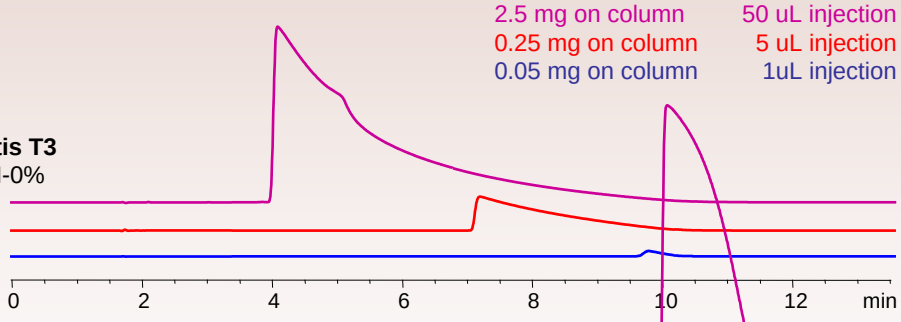
Figure 11. Analytical size injection of dopamine in 3 columns. Conditions for each column are identical to the loading study conditions.

Column: **Zorbax SB-AQ**
Mobile phase: MeCN-0%
AmFm 30 mM



2.5 mg on column 50 uL injection
0.25 mg on column 5 uL injection
0.05 mg on column 1uL injection

Column: **Atlantis T3**
Mobile phase: MeCN-0%
AmFm 30 mM



Column: **Obelisc R**
Mobile phase: MeCN-30%
AmFm 30 mM

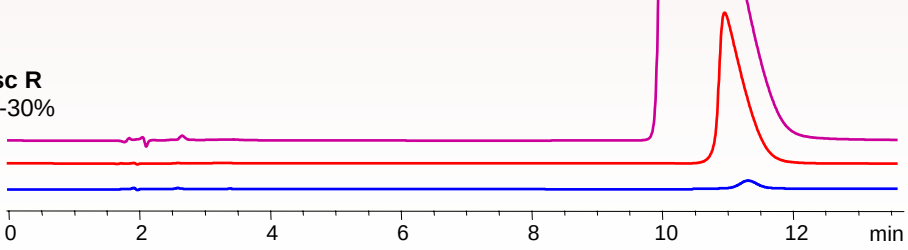


Figure 12. Comparative loading study of dopamine by three columns designed for retaining of polar compounds.

SIELC Technologies, Inc., 65 E. Palatine Rd, Suite 221, Prospect Heights, IL 60070 USA.
Ph. 847.229.2629, Fax 847.655.6079, mail@sielc.com, www.sielc.com