New Mixed-Mode HILIC - Advances in Retention Control and Selectivity

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The liquid chromatography process involves interaction of analytes with a stationary phase. **HILIC** (**H**ydrophilic **I**nteraction **LI**quid **C**hromatography) mode of separation is convenient when very polar analytes need to be analyzed, but conventional reverse-phase chromatography fails to produce significant retention. HILIC mode requires very high concentration of acetonitrile (up to 99%) in the mobile phase to produce retention.

Several types of columns are typically used for HILIC separation mode. Bare silica gel is the most common, but the least convenient. Silica gel problems include silica dissolution in aqueous mobile phases at close to neutral pHs, poor peak shapes of basic molecules, and very low retention of acidic compounds even in high organic concentration.

Other common HILIC phases are built around surface modified bonding of very polar functional groups with a stationary support.

One such phase (SeQuant® ZIC-HILIC) includes a $^{+}N(Me)_2CH_2CH_2SO_3^{-}$ ligand. This ligand is net neutral because it includes two oppositely charged functional groups. Close proximity of these two charges preclude their independent interaction with analytes. We have recently discovered that by making a ligand with an increased space between two oppositely charged groups, the electrostatic interaction starts to produce a significant effect on retention of positively and negatively charged molecules. This phenomena was implemented in a new mixed-mode HPLC HILIC stationary phase Obelisc™ N (Fig 1). A significantly increased retention was observed with all types of mobile phases.

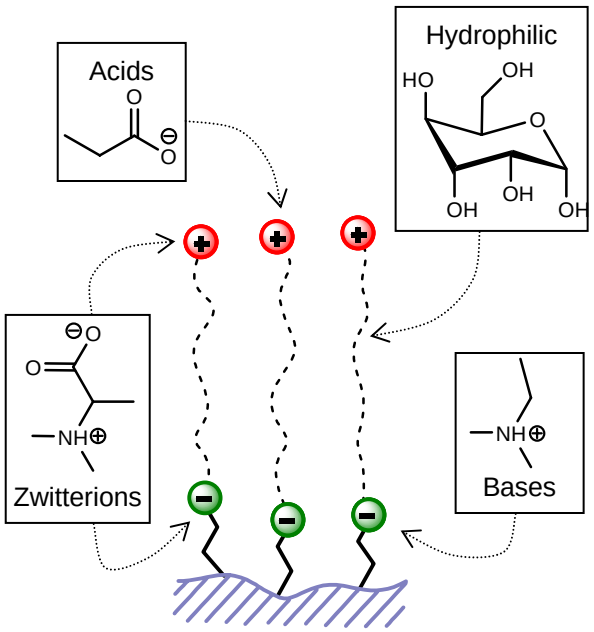

Figure 1. Different types of interactions of Obelisc N stationary phase with different analytes allow to retain many types of charged and hydrophilic compounds.

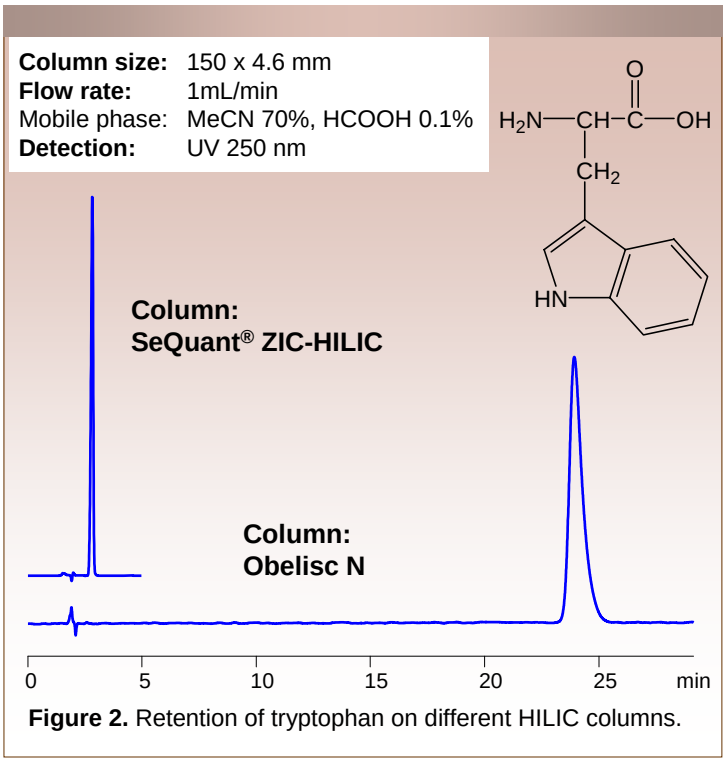

Figure 2. Retention of tryptophan on different HILIC columns.

For example, amino acids can be retained with reduced concentration of acetonitrile (Fig. 2). While traditional HILIC columns retain molecules very poorly at less than 70% organic in the mobile phase, Obelisc N can be operated in wide organic concentration ranges from 0 organic to over 99% (Fig. 3).

Acidic compounds, such as p-toluenesulfonic acid, or basic compounds, such as epinephrine, retained 2-5 times more on Obelisc N column compared with other HILIC separation methods (Fig. 4, 5).

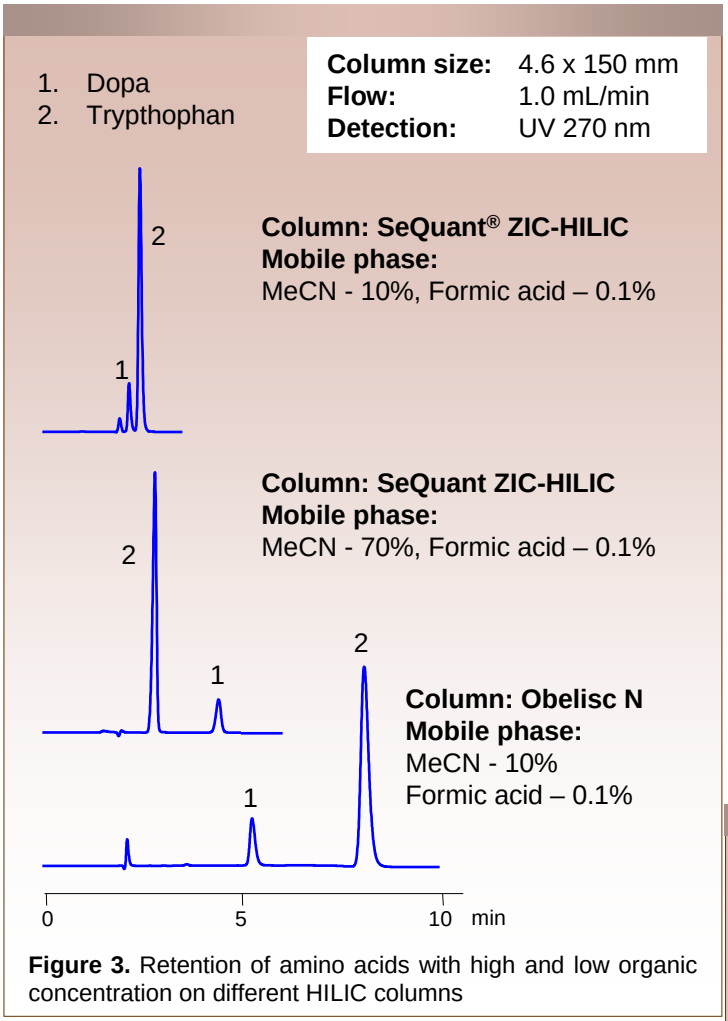

Figure 3. Retention of amino acids with high and low organic concentration on different HILIC columns

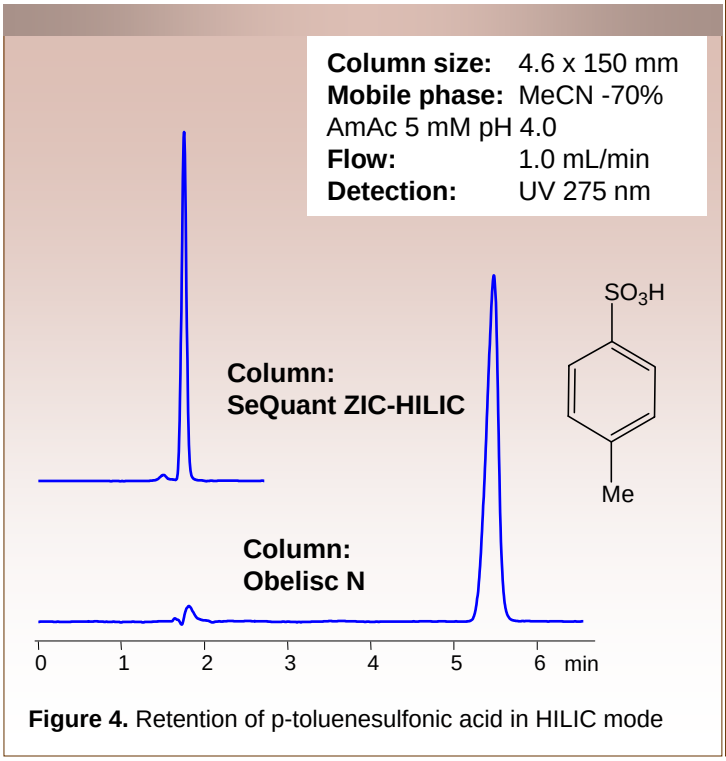

Figure 4. Retention of p-toluenesulfonic acid in HILIC mode

These unique properties offer several benefits. For example, a sample can be dissolved in a diluent with high water concentration, since often the polar compounds are poorly soluble in MeCN.

Changing concentration of acetonitrile without losing retention allows to adjust selectivity of separation, producing at times a reverse elution order (Fig. 5).

pH of the mobile phase and concentration of the buffer are two additional parameters that can be used to adjust selectivity of the analytes.

MS-friendly buffer systems are used for all separations. Low concentration of buffer, typically within 5-50 mM range, allows to obtain high sensitivity of MS signal which can be further increase by choosing different organic modifier concentrations.

HILIC column can not be operated in MeOH-based mobile phases, since they produce very little retention even at high MeOH concentration. Obelisc N columns, in contrast, show significant retention due to increased polar/ionic interaction which is more independent of type of organic modifier (Fig. 6).

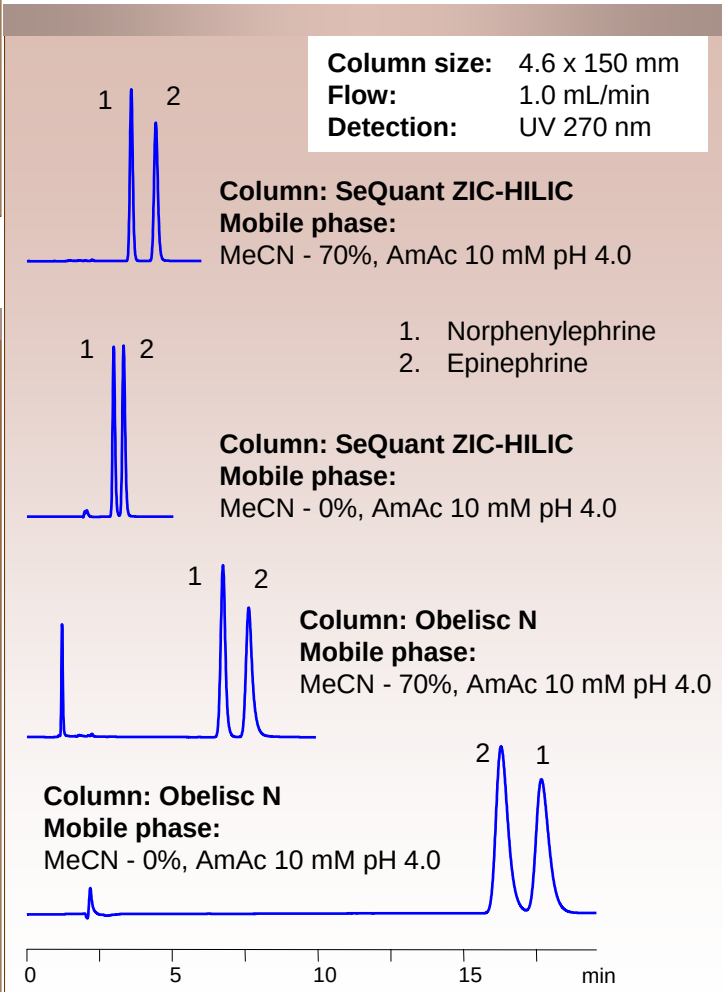

Figure 5. Retention of norphenylephrine and epinephrine on different HILIC columns

Use of methanol instead of acetonitrile is required when a method utilizes a nitrogen specific detectors.

Only Obelisc N mixed-mode column allows to use methanol in HILIC separation .

Several other separation examples of different polar compounds of neutral and charged nature are demonstrated (Fig 7-9). See even more examples at http://www.hilic.com.

1. Trypthophan
2. Dopa

Column size: 4.6 x 150 mm
Mobile phase: MeOH -70% Formic acid -0.1%
Flow: 1.0 mL/min
Detection: UV 270 nm

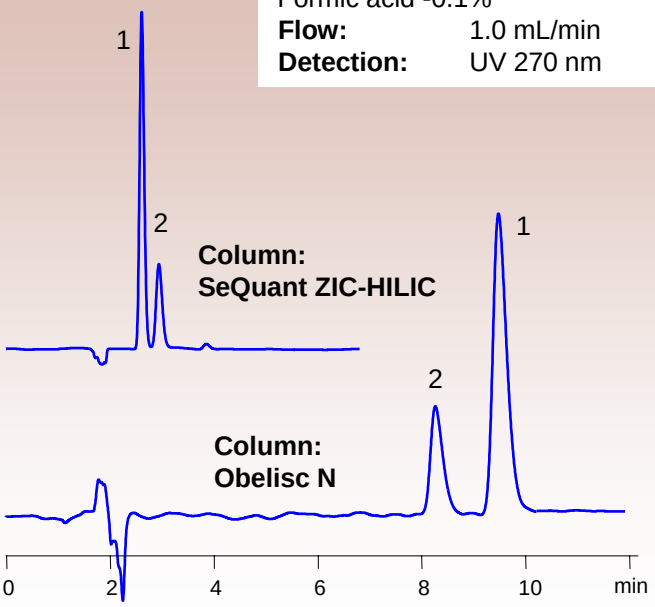

Figure 6. Retention of tryptophan and DOPA on different HILIC columns with MeOH based mobile phase.

1. Sorbitol
2. Sucrose
3. alpha-D-lactose
4. beta-D-lactose
5. Raffinose.

Column: **Obelisc N**
Size: 150 x 4.6 mm
Mobile phase: 80/20 ACN/Water
Flow: 1.0 mL/min
Detection: ELSD

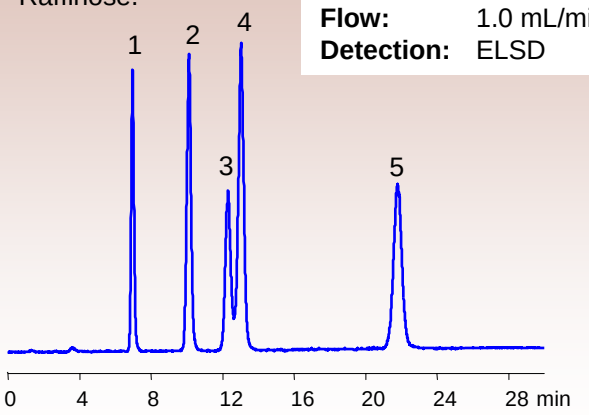

Figure 8. The separation of sugars in HILIC mode.

Mobile phase: MeCN -77% AmAc 5 mM pH 5.0

Column: **Obelisc N**
Size: 150 x 4.6 mm
Flow: 1.0 mL/min
Detection: ELSD

1. Succinic acid
2. Phenylalanine
3. Sucrose
4. Glycine
5. Aspartic acid
6. Raffinose

Mobile phase: MeCN -82% AmFm 5 mM pH 3.0

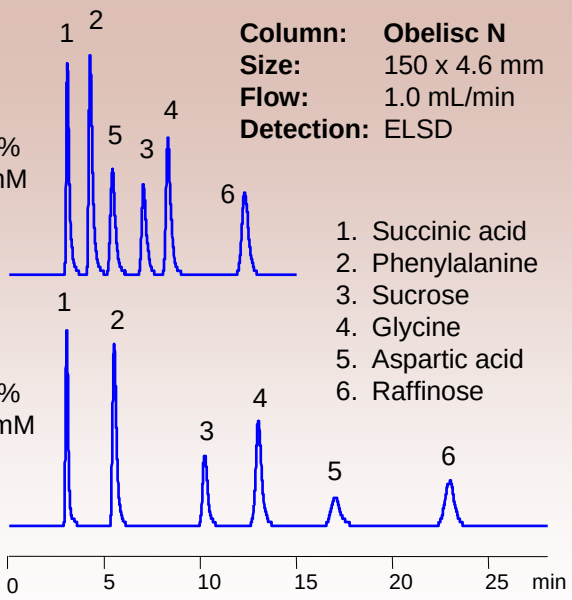

Figure 7. Effect of both pH and organic content. on a separation of sugars, amino acids, and carboxylic acids.

1. Glyphosate impurity
2. IDA (iminodiacetic acid)
3. PMIDA (N-phosphonomethyl)iminodiacetic acid
4. Glyphosate
5. DEA (diethanolamine)

Column: **Obelisc N**
Size: 150 x 4.6 mm
Mobile phase: 70% ACN 20mM AmFm, no pH adjustment
Detection: ELSD 45C
Flow rate: 1 mL/min

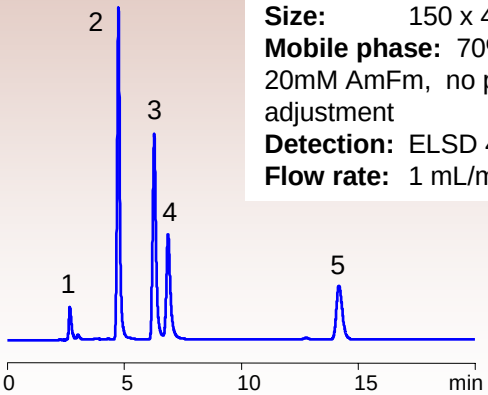

Figure 9. The separation of glyphosate reaction intermediates and impurities.

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