

## Competing in HPLC Olympics - Comparison of Modern LC Columns

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**R**everse-phase HPLC, the technique of choice to solve multiple separation problems, has its acknowledged limitations. One of them is the lack of retention of highly polar compounds on conventional stationary phases. Traditionally, mobile phase additives, such as ion-pairing reagents, have been successfully employed for separation of these compounds. Practical limitations of ion-pairing chromatography include artifacts when using gradient elution, incompatibility with MS, ELSD, preparative chromatography, and more complex mobile phase preparations.

Alternately **HILIC** (**H**ydrophilic **I**nteraction **L**iquid **C**hromatography) mode of separation is convenient when very polar analytes need to be analyzed when conventional reverse-phase chromatography fails to produce significant retention. HILIC mode usually requires high concentration of acetonitrile (up to 99%) in the mobile phase to produce retention. This becomes a limitation for many polar analytes due to limited solubility of very polar molecules in high organic mobile phase.

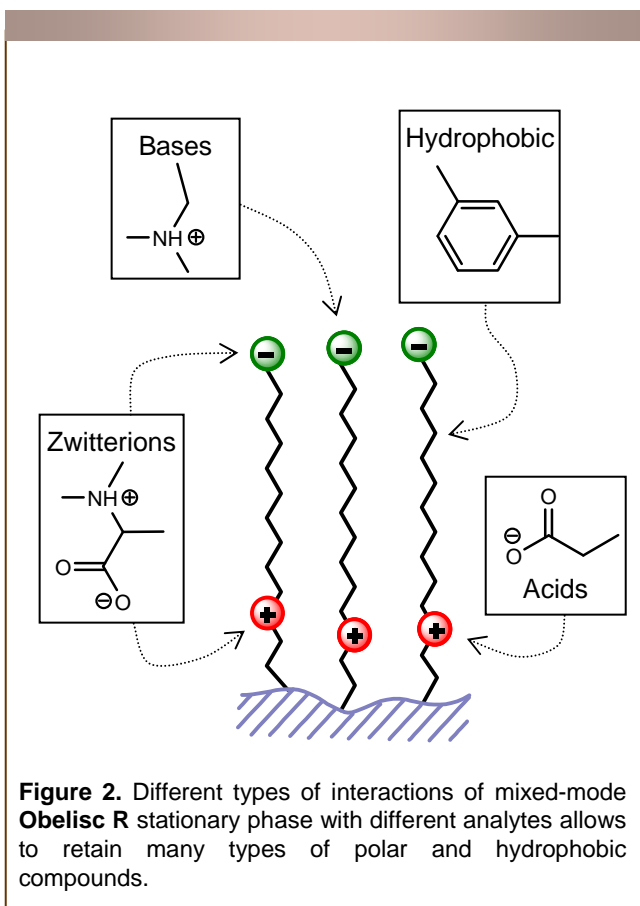
Another limitation is the inability of HILIC column to retain non very-polar or hydrophobic molecule. A mixture which contain polar and non-polar compounds would requires more than one HPLC method (HILIC+RP) to be resolved.

Technique	Organic concentration required	Hydrophobic compounds retained	Polar compounds solubility issue
HILIC	HIGH	NO	YES
Reverse Phase - AQ	LOW	YES	NO
Mixed - mode	ANY	YES	NO

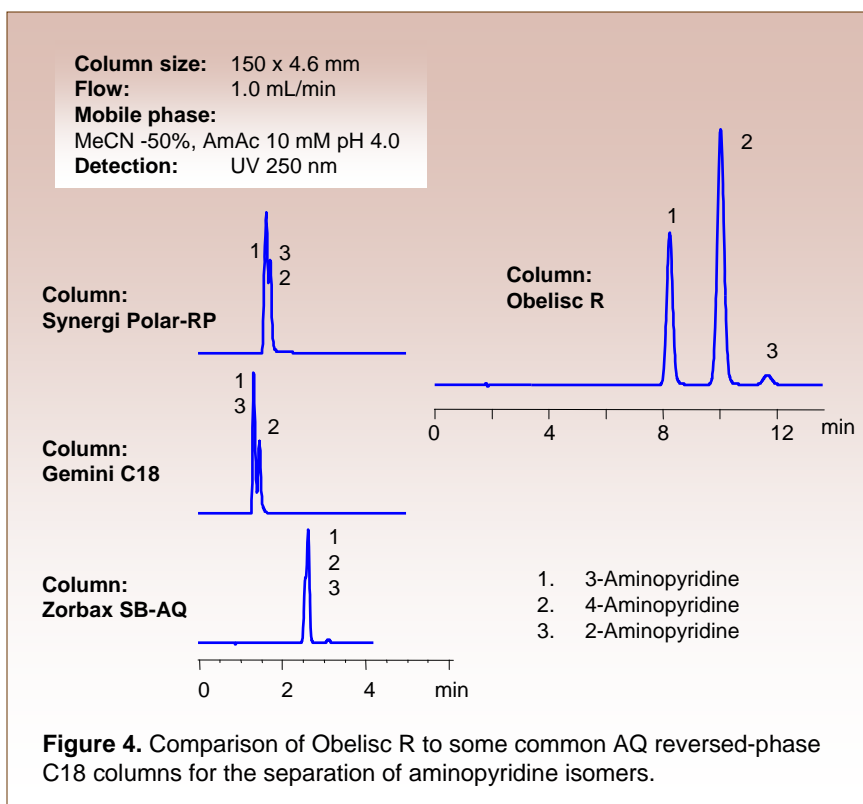
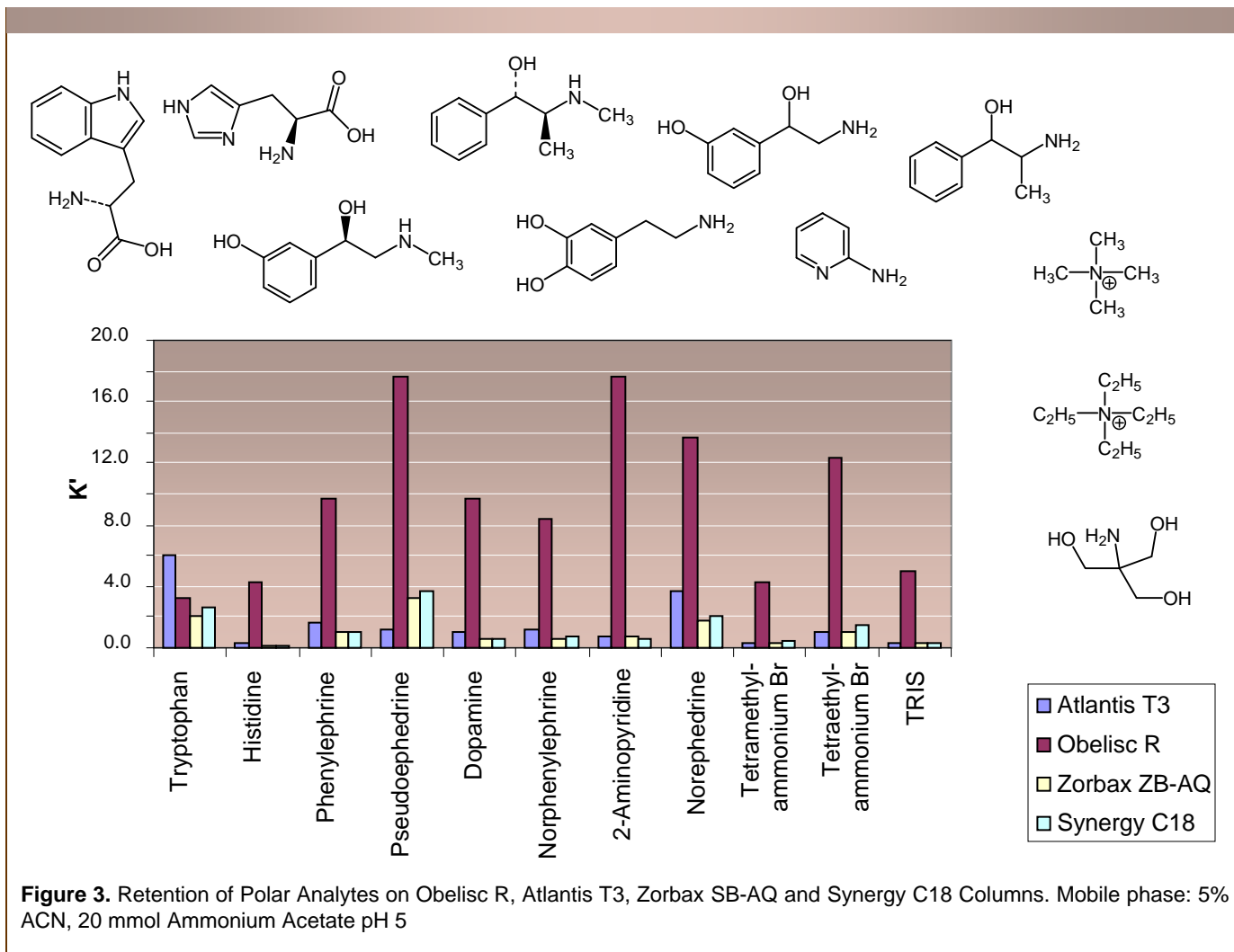
**Figure 1.** Three different techniques to analyze polar compounds

Yet another approach can be to use specially designed RP columns, commonly call AQ type columns. These columns, while retaining hydrophobic compounds, also offers extended retention of polar compounds, and are typically capable of resisting dewetting in “zero-organic” mobile phase (Fig. 1)

This letter compares retention characteristics of most common AQ columns with SIELC’s own Obelisc R columns that are based on mixed-mode technology. Obelisc R stationary phase combines three functional groups bound together to the silica surface: negatively charged, positively charged, and neutral hydrophobic. All three groups (Fig. 2) allow to generate multiple interactions with neutral, polar, hydrophobic, and hydrophilic molecules, and provide extended retention characteristics when compared with other AQ type columns (Fig. 3)



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

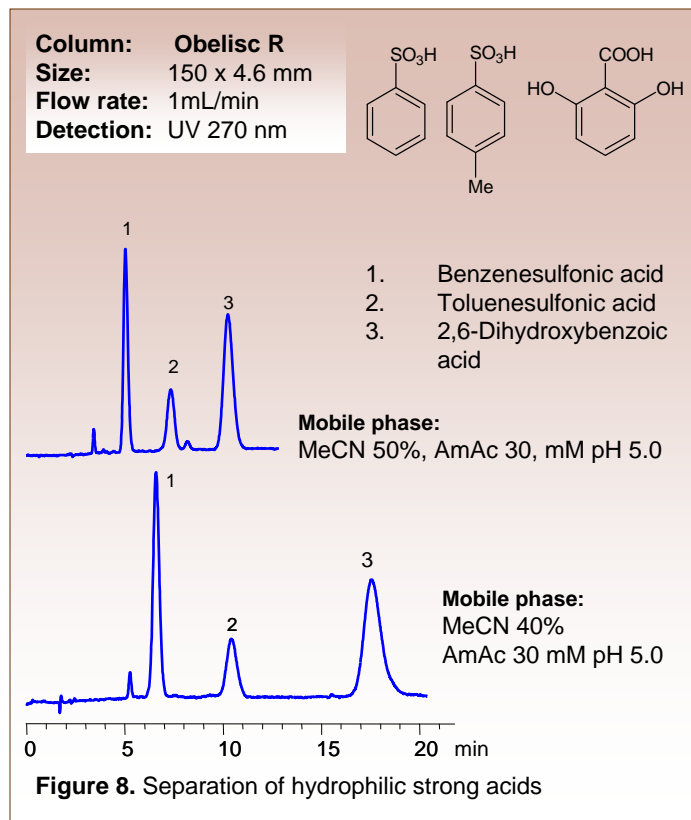
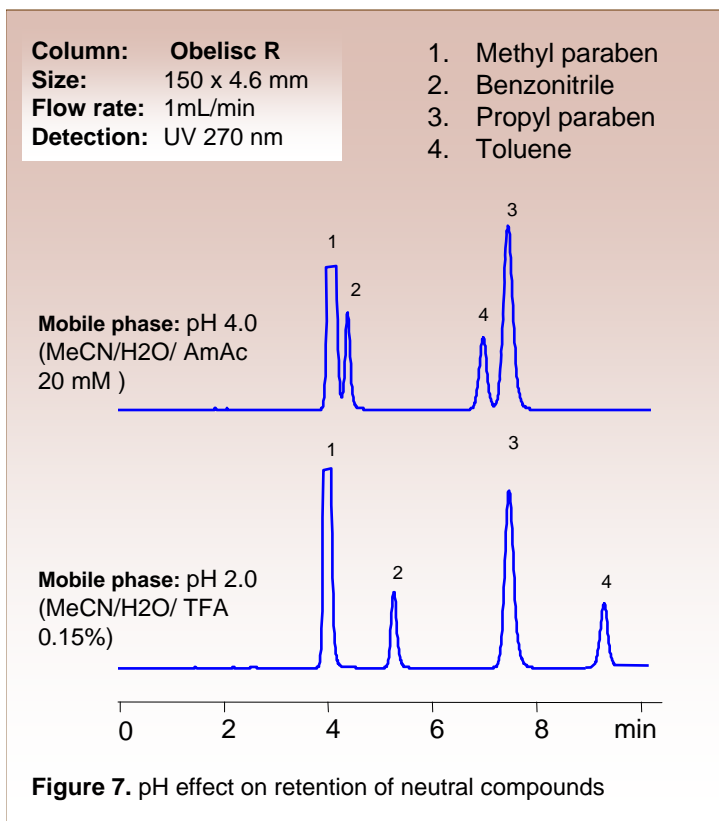
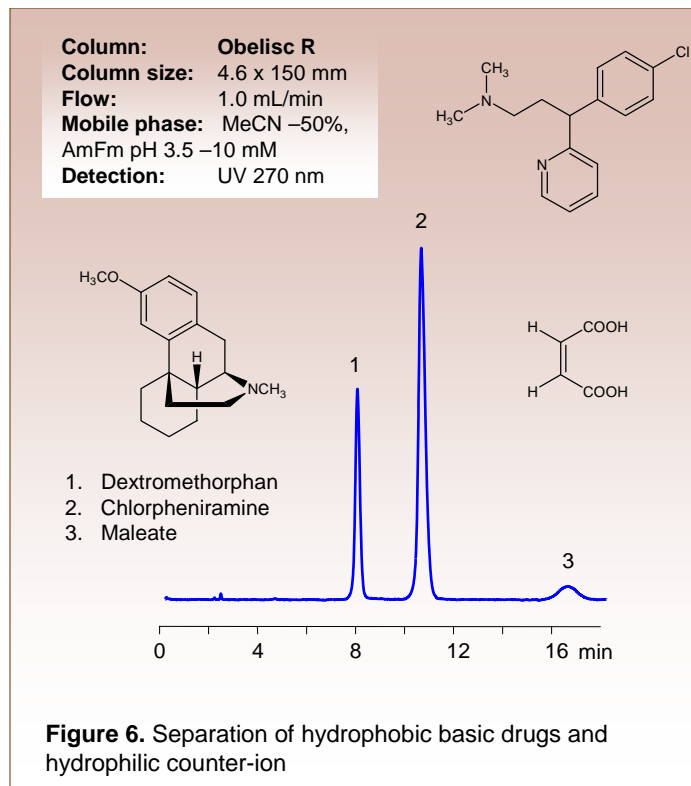
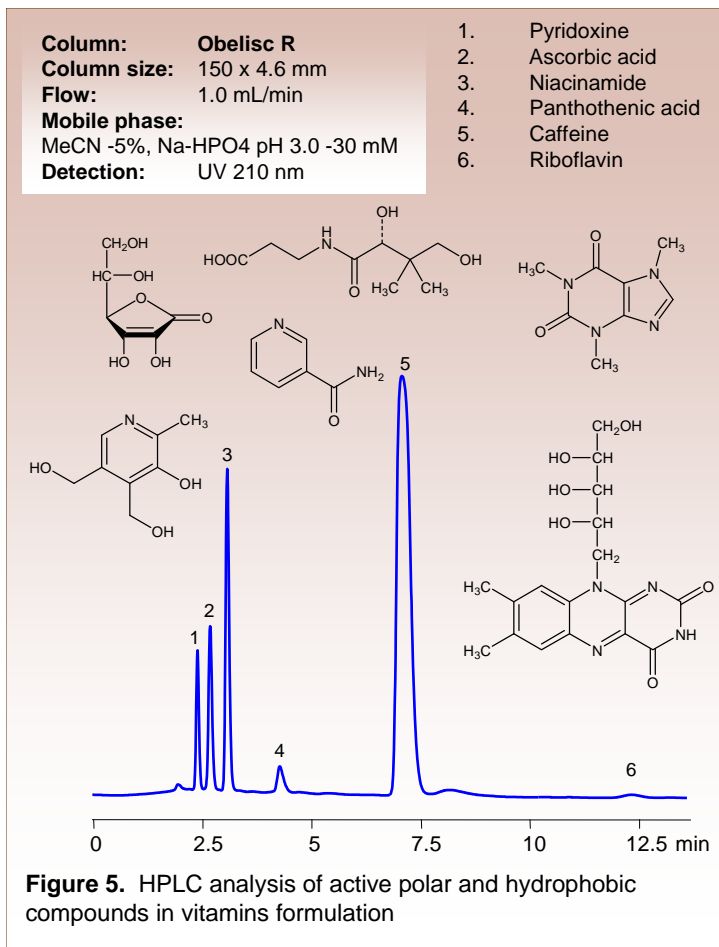


Mixed mode stationary phase can be used in wide range of organic concentrations and different acidic and neutral buffer systems, as opposed to HILIC mode of separation, where some typical low UV compatible buffers (such as sodium or potassium phosphates) have limited application due to poor solubility of these buffers in high organic systems.

Different classes of molecules can be successfully analyzed with mixed-mode approach (Fig 4-8).

It's interesting to note that pH and buffer concentration can effect selectivity of the mixed-mode stationery phase toward not only charged molecules, but neutral molecule as well (Fig. 7).

Simple mobile phase-compatible with low UV and/or MS and low buffer concentration are typical for this technology.



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