

# Universal Silanol Solution

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## Abstract

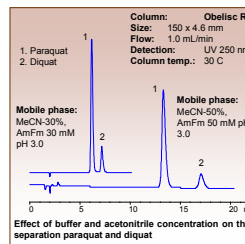
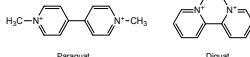
Silanol interaction is a common problem in the reversed-phase (RP) analysis of basic molecules on silica-based columns. A suppression of this interaction by using high purity silica gel, endcapping, and mobile phase modifiers are common practices to improve peak shape and separation efficiency. From another perspective silanol interaction provides additional selectivity for reversed-phase method development and can benefit many types of separations if left intact. However it is difficult to control the amount of residual silanol groups on the silica surface to make reproducible RP columns with high silanol activity. Also the column performance will be very dependent on the reproducibility of the silica production process itself.

An initial evolution of RP columns was the incorporation of positively charged functional groups on the hydrophobic chain as in Primsep® B. This positive charge close to the silica surface shields the silanols and improves peak shape and column reproducibility. Primsep B also provides many more options to tune selectivity and perform separations not previously possible.

The latest advance is incorporation of both positively and negatively charged groups on Obelisc™ R and N columns. This evolution not only solves the silanol problem, but also further expands the reach of chromatography. Additional polar interactions are available between the analyte and stationary phase to expand the selectivity available with only two HPLC columns.

## Obelisc R Applications

### Paraquat and Diquat on Obelisc R

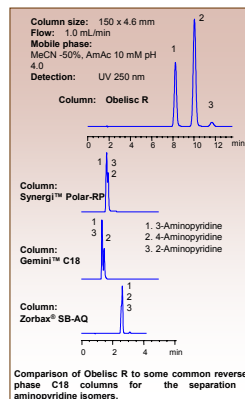
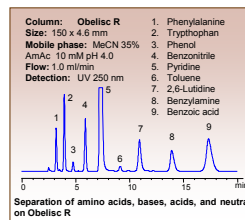
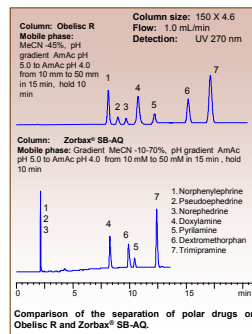


Paraquat and diquat are difficult to retain on reversed-phase columns and often elute in the column void. Obelisc R retains and separates paraquat and diquat with mass spec compatible mobile phases based on acetonitrile and ammonium formate. No ion pair reagents are required due to the ionic groups present on the hydrophobic chain.

Unlike a separation on a reversed-phase C18 column, the retention time of both peaks increases with increasing acetonitrile concentration in the mobile phase. This effect shows the impact of additional ionic interaction with the stationary phase.

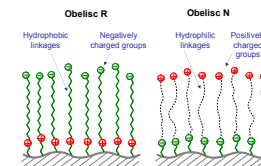
Many pharmaceuticals are polar and contain amines, whether primary, secondary, tertiary or quaternary amines. Obelisc R separates a mixture of seven amine-containing pharmaceuticals with mass spec compatible conditions. The same mixture on a Zorbax® SB-AQ shows resolution of the more hydrophobic compounds, but no retention for the more polar compounds.

The versatility of Obelisc R is demonstrated by the baseline separation of amino acids, bases, acids and neutrals. Obelisc's alternative selectivity to C18 columns is shown by the elution of toluene before the more polar 2,6-lutidine, benzylamine, and benzoic acid.



Aminopyridine isomers are polar compounds that differ only in the location of a primary amine on the pyridine ring. Obelisc R retains and separates aminopyridines with a mass spec compatible mobile phase. Common reversed-phase columns show little retention and no resolution of these compounds due to their lack of electrostatic interactions.

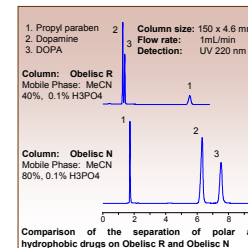
## Universal Silanol Solution for Normal-Phase Separations -- Obelisc N



Normal-phase separations often have the same tailing and low efficiency problems as reversed-phase separations. Silica columns rely on a polar silanol surface to retain polar analytes by electrostatic interactions. As in reversed-phase columns, the amount and type of exposed silanols varies with the production process.

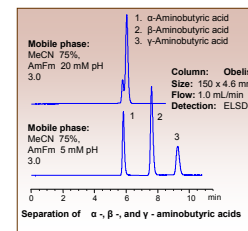
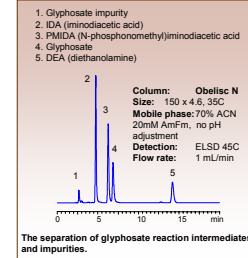
Obelisc N for normal phase, is the complementary column to Obelisc R. Obelisc N contains both positively and negatively charged functional groups, but their location is opposite to Obelisc R. The negative charge is located close to the silica surface and the positive charge is located at the end of the chain. Another difference is that the chain is hydrophilic on Obelisc N whereas Obelisc R has a hydrophobic chain.

The selectivity of Obelisc N and R is complementary as shown in a separation of a neutral, propyl paraben, and polar amines, dopamine and DOPA. On Obelisc N propyl paraben elutes first with resolution of dopamine and DOPA. On Obelisc R propyl paraben elutes last.



## Obelisc N Applications

The very polar glyphosate and its reaction intermediates and impurities are separated on Obelisc N with a mass spec compatible mobile phase. In this example evaporative light scattering detection (ELSD) was used to detect the non-UV active compounds.



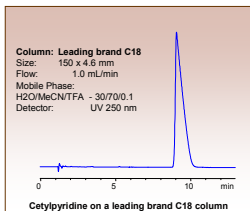
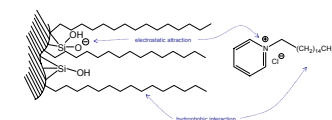
$\alpha$ -,  $\beta$ -, and  $\gamma$ -aminobutyric acids are very polar isomers that are resolved on Obelisc N. The separation is dependent on buffer concentration as shown by changing buffer concentration from 20 mM to 5 mM.

## Conclusion

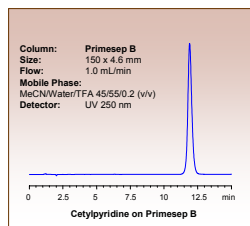
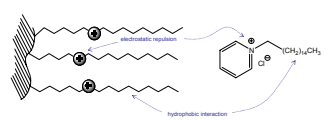
Through stationary phase evolution the silanol problem is solved. The latest evolution is incorporation of both positively and negatively charged groups on Obelisc R and N columns which both shield and/or neutralize the silica surface. The addition of these charged groups provides different selectivity from common reversed-phase and normal-phase columns which can be easily tuned to separate complex mixtures.

## Evolution of stationary phases from Silica-based C18, to Primsep B, to Obelisc R

Cetylpyridine is a quaternary amine that retains by reversed-phase mode on silica-based C18 columns. Hydrophobic interactions are obtained between the C18 chains on the stationary phase and the long chain on cetylpyridine. In addition the basic amine on cetylpyridine electrostatically interacts with the polar active silanols on the silica surface which causes peak tailing.



Primsep B has the same hydrophobic interactions, but Primsep B also contains an embedded cation on the stationary phase which shields the underlying silica surface by electrostatic repulsion. The end result is reversed-phase retention with symmetrical peak shape.



The final evolution is the addition of multiple charges to the hydrophobic chain in Obelisc R. Obelisc R contains both positive and negative charges on the hydrophobic chain. The silica surface shielding and hydrophobic interactions are similar to Primsep B, but additional electrostatic attraction is obtained with cetylpyridine. Obelisc R requires more acetonitrile in the mobile phase compared to the silica-based C18 and Primsep B to elute cetylpyridine. The end result is reversed-phase retention with symmetrical peak shape and the potential of additional selectivity due to the availability of both positive and negative charges.

