SIELC

Obelisc

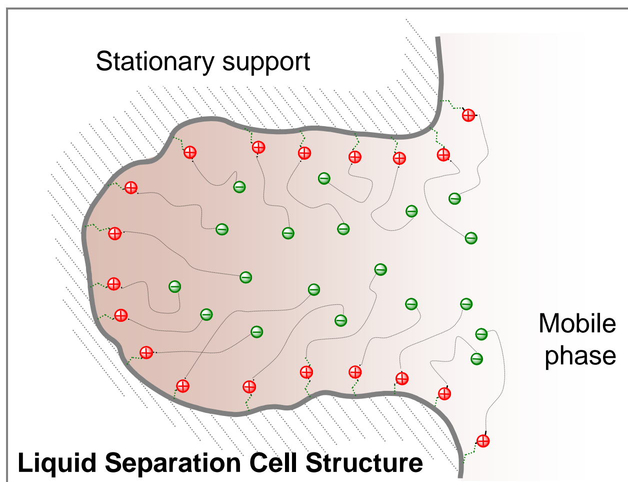
LC Columns with Liquid Separation Cell Technology

Obelisc™ HPLC Columns - Liquid Separation Cell Technology

Introduction

Obelisc™ HPLC columns are the latest, innovative columns from SIELC Technologies, the inventors of Primesep®. With multiple patents pending, Obelisc columns are the first commercially available columns with Liquid Separation Cell technology (LiSC™).

LiSC technology is based on a new chemical modification of the silica gel pores that creates a liquid separation cell with its own charge characteristics, ionic strength, and hydrophobic properties. Like living cells which exist in equilibrium with the outside environment, liquid separation cells exist in constant equilibrium with the mobile phase.



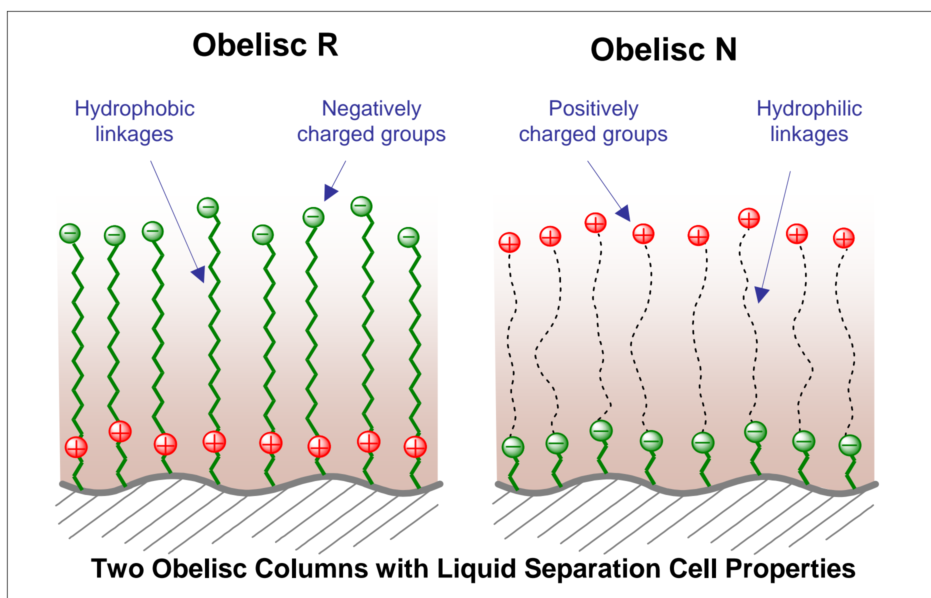
Liquid Separation Cell Structure

Similarities to a living cell

- The cell internal environment is very different from the external environment (mobile phase);
- The pore walls form the dimensions and structure of the liquid separation cell;
- The cell internal environment has stationary (bound) and exchangeable (mobile) components;
- The pH and ionic strength inside the cell differs from outside the cell;
- The pore opening acts as a membrane channel for the analytes;
- The cell responds to changes in external environment with physical and chemical changes
- These changes include conformational changes in the long hydrophobic and hydrophilic chains, degree of ionization, charge distribution and counter-ion properties.

Three main characteristics of the cell make it different from traditional chromatography materials		
Significantly higher ionic-strength in the cell vs. mobile phase ion strength, providing quick mass transfer of charged analytes in and out of the cell even with low concentration of buffer in the mobile phase	**T**he stationary phase occupies the entire volume of the cell, not just the walls as in other stationary phases, providing high capacity of the phase	**P**ositive and negative charges of the cell ligand separated by a long organic chain allow both positive and negative charges to simultaneously participate in electrostatic interaction.

Two columns based on LiSC technology, Obelisc R and Obelisc N, separate polar and non-polar compounds using multiple separation mechanisms.



Two Obelisc Columns with Liquid Separation Cell Properties

This cost effective duo replaces multiple HPLC columns such as reversed-phase (RP), AQ-type reversed-phase, polar-embedded group RP columns, normal-phase, cation-exchange, anion-exchange, ion-exclusion, and HILIC (Hydrophilic Interaction LIquid Chromatography) columns.

Method development is simplified by using only two Obelisc columns and a few simple mass spec or low UV (<220 nm) compatible mobile phases.

Obelisc R, with reversed-phase characteristics, and Obelisc N, with normal-phase characteristics, differ in the type and proximity of their charged groups and the hydrophobicity of their long chains. Obelisc R has cationic groups close to the silica surface separated from anionic groups by a hydrophobic chain. Obelisc N has anions close to the surface separated from cationic groups by a hydrophilic chain.

Obelisc R and Obelisc N Columns - A Powerful Duo for HPLC

- Two columns able to retain and separate all types of small molecules and their mixtures
- Fast method development with only two columns
- Simple mobile phase selection
- Multiple separation modes (RP, NP, HILIC, IE)
- Mass spec, ELSD, preparative and low UV (<220 nm) compatible low concentration buffers.
- Adjustable selectivity in all modes based on organic content, pH, and buffer strength

Applying Obelisc liquid separation cells to chromatographic separations provides very different results from all other chromatographic columns. Charge and chain conformation are tunable properties that allow dramatic changes in separation selectivity. Method development now returns to an experimental science rather than trial-and-error screening of columns.

Figure 1 shows the separation of a mixture with similar mobile phases on Obelisc R and N. Peak order is reversed just by changing acetonitrile content in the mobile phase. The hydrophobic propyl paraben retains much longer on Obelisc R due to the column's reversed-phase characteristics. The polar compounds, dopamine and DOPA, retain much longer in normal-phase mode on Obelisc N.

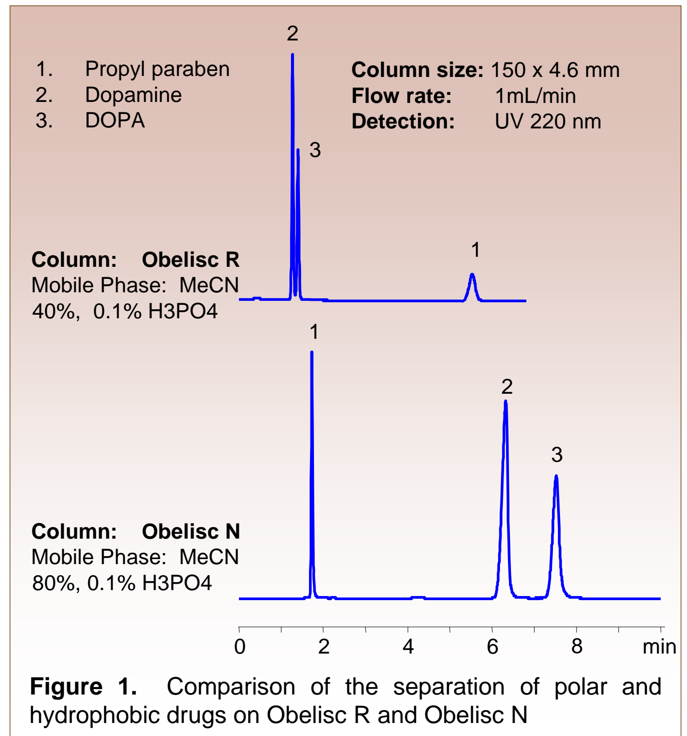


Figure 1. Comparison of the separation of polar and hydrophobic drugs on Obelisc R and Obelisc N

Obelisc R

Obelisc R has reversed-phase character and can be used in traditional, reversed-phase type applications. Due to the presence of ionic groups and a long hydrophobic chain, Obelisc R offers additional retention and tuning that is not available with traditional reversed-phase columns. Typical mobile phases used with Obelisc columns are based acetonitrile, water, and the mass spec compatible buffers ammonium formate (pH 3) and ammonium acetate (pH 5). If it is necessary to detect in low UV (<220 nm) then phosphate buffer is recommended.

Figure 2 shows the tuning of Obelisc R by changing acetonitrile concentration and buffer pH.

Figure 3 is a comparison of Obelisc R and Zorbax SB-AQ in a polar drug separation. AQ-type columns are designed to work in low organic mobile phases to analyze polar compounds in reversed-phase chromatography. The seven drugs are retained and resolved on Obelisc R, but there is a lack of retention and resolution on the AQ column even with low organic content in the mobile phase.

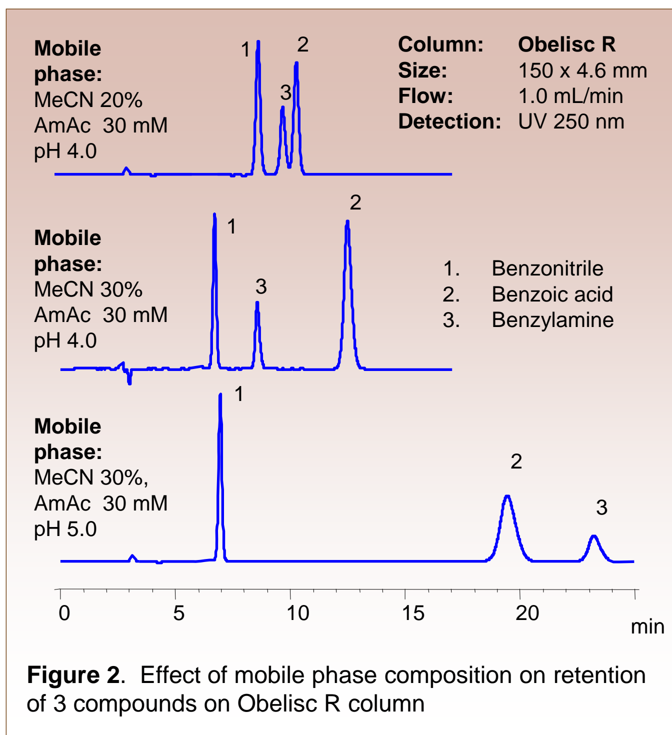


Figure 2. Effect of mobile phase composition on retention of 3 compounds on Obelisc R column

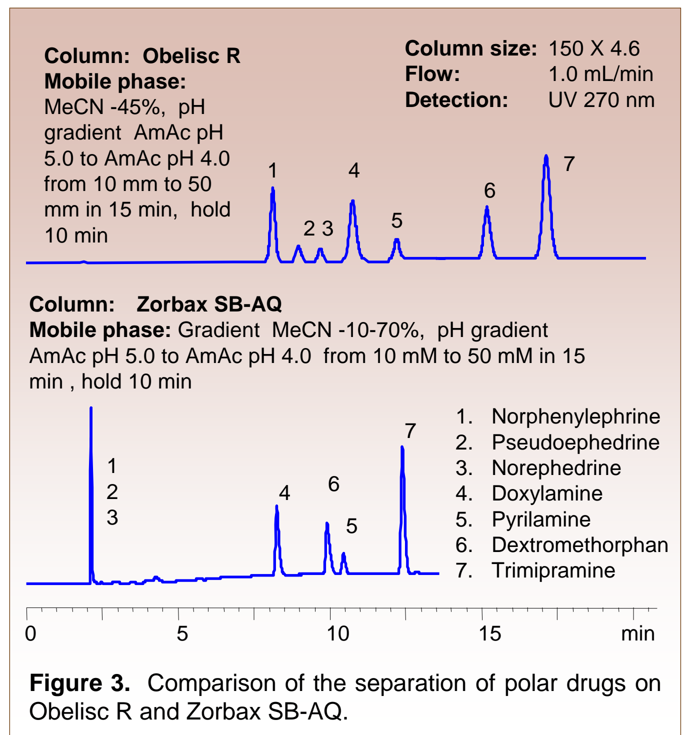


Figure 3. Comparison of the separation of polar drugs on Obelisc R and Zorbax SB-AQ.

Figure 4 shows the separation of a complex mixture of acids, bases, amino acids, and neutral compounds on Obelisc R. Note that the most hydrophobic compound, toluene (peak 6) elutes prior to the more polar 2,6-lutidine, benzylamine and benzoic acid. In Figure 5 the very polar hydrophilic herbicides, paraquat and diquat, are resolved in RP conditions with baseline separation with ammonium formate buffers.

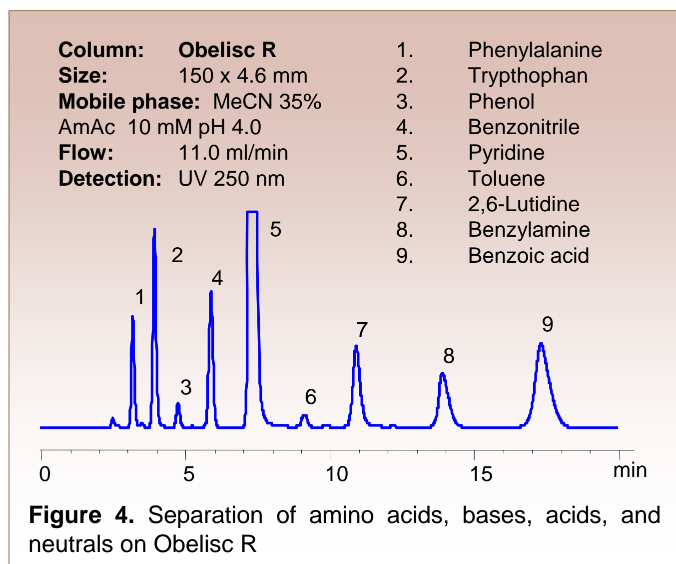


Figure 4. Separation of amino acids, bases, acids, and neutrals on Obelisc R

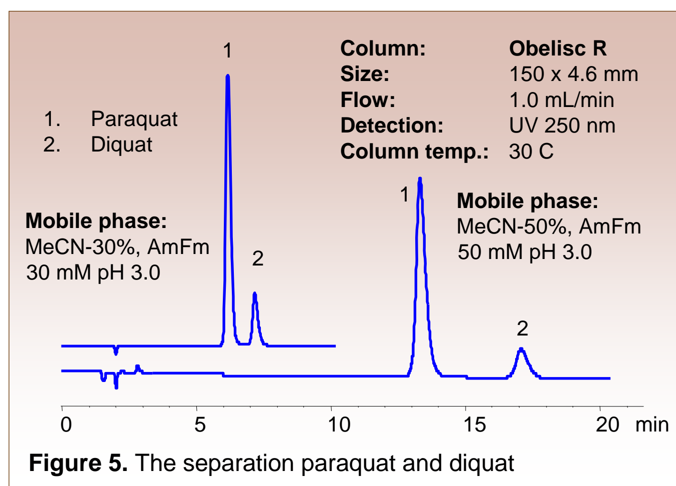


Figure 5. The separation paraquat and diquat

The separation of polar isomers is a difficult task on traditional C18 phases. The added polar interactions available on Obelisc R provides the necessary selectivity. Figure 6 compares the separation of aminopyridines on Obelisc R and some common C18 phases.

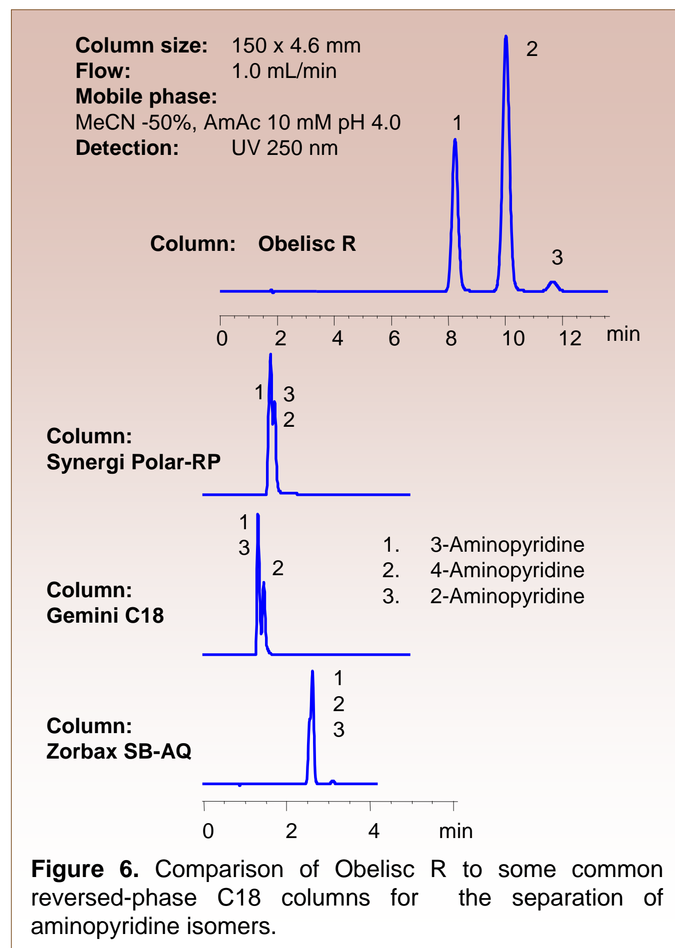


Figure 6. Comparison of Obelisc R to some common reversed-phase C18 columns for the separation of aminopyridine isomers.

Obelisc R offers a large improvement in the retention of polar compounds over traditional reversed-phase columns.

In Figure 7 capacity factors (k') for 10 polar compounds are plotted for Obelisc R and two common reversed-phase columns. In all cases Obelisc R has the most retention--up to a 10 times more retention.

The two reversed-phase columns show little appreciable difference from each other.

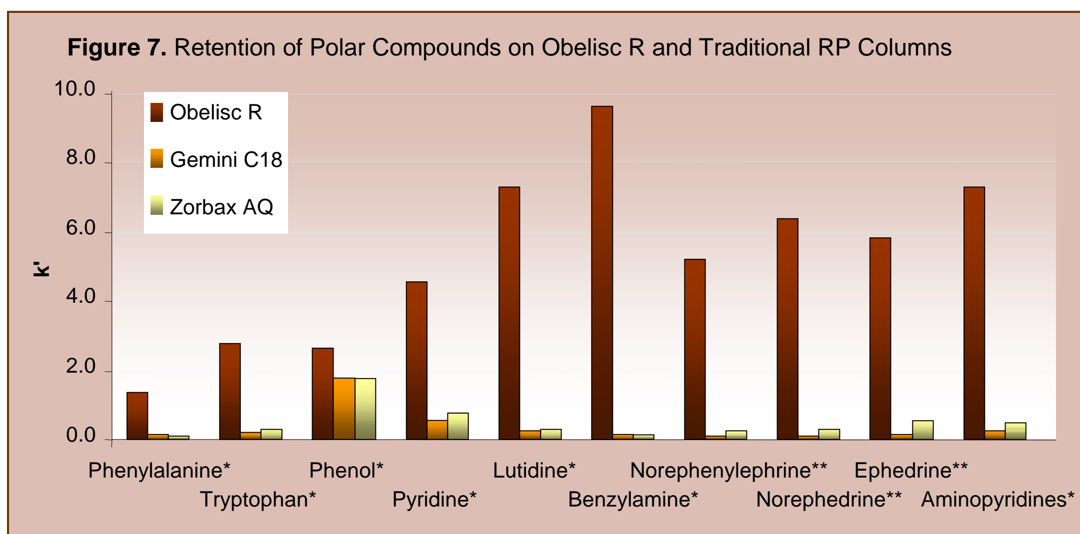


Figure 7. Retention of Polar Compounds on Obelisc R and Traditional RP Columns

* **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

Obelisc N has very polar characteristics and works well for polar and charged analytes. In ion-exchange mode, charged analytes interact with oppositely charged groups on the stationary phase.

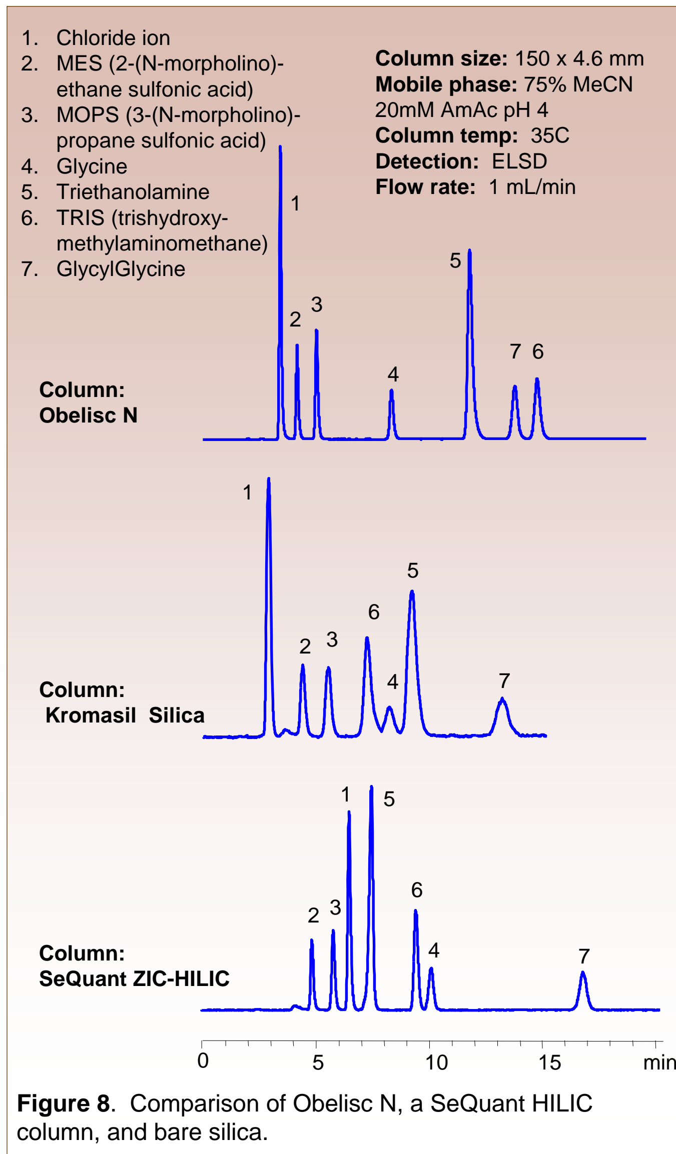


Figure 8. Comparison of Obelisc N, a SeQuant HILIC column, and bare silica.

Obelisc N offers both positively and negatively charged groups to interact with positively or negatively charged analytes.

In traditional HILIC mode, a charged or neutral polar analyte interacts with a water layer on the polar stationary phase surface. On Obelisc N the charges are greatly separated and independently accessibility which results in different selectivity compared to traditional HILIC and silica columns (Figure 8).

Figure 9 shows the effect of pH and organic content on a separation of sugars, amino acids, and carboxylic acids. Figure 10 is an example of sugars in HILIC mode.

Mobile phase composition changes the conformation of the long hydrophilic chain. This affects the properties of the liquid separation cell and changes separation selectivity. Figure 11 shows the effect of buffer concentration on the resolution of isomeric aminobutyric acids. By simply changing buffer concentration baseline separation is achieved.

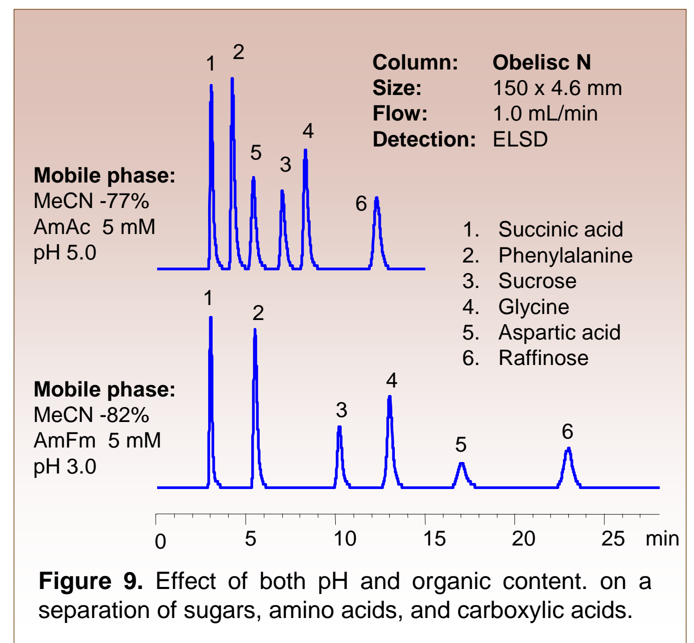


Figure 9. Effect of both pH and organic content. on a separation of sugars, amino acids, and carboxylic acids.

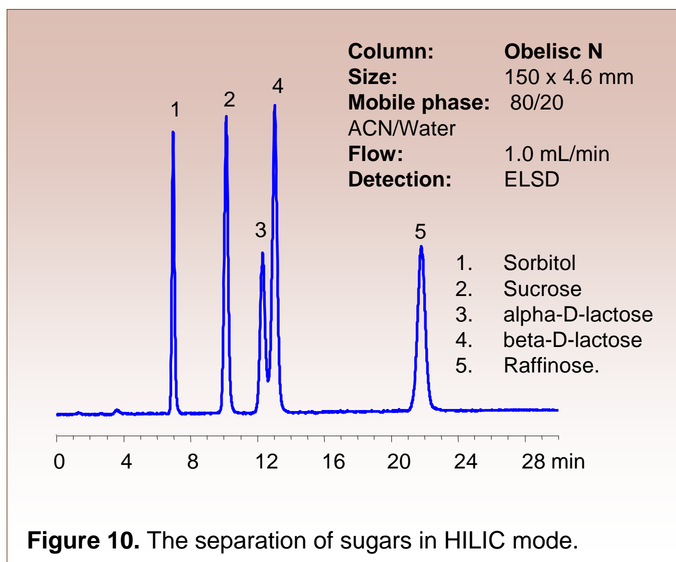


Figure 10. The separation of sugars in HILIC mode.

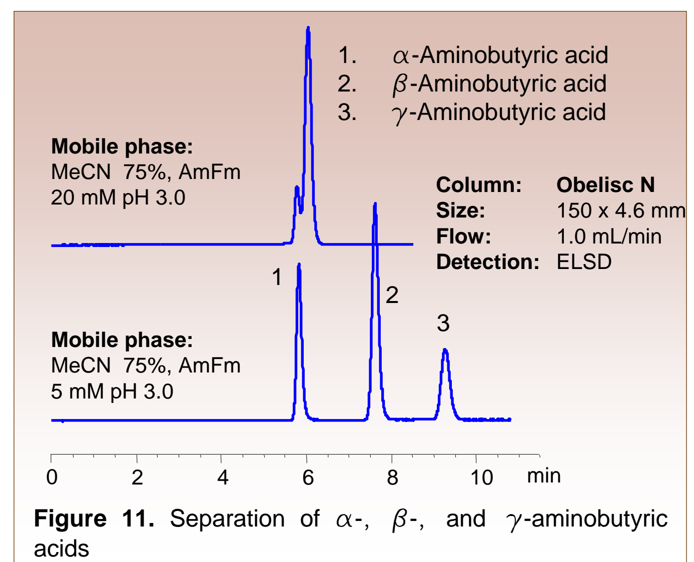


Figure 11. Separation of α-, β-, and γ-aminobutyric acids

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

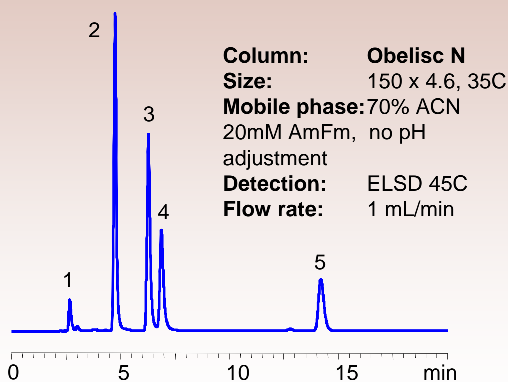
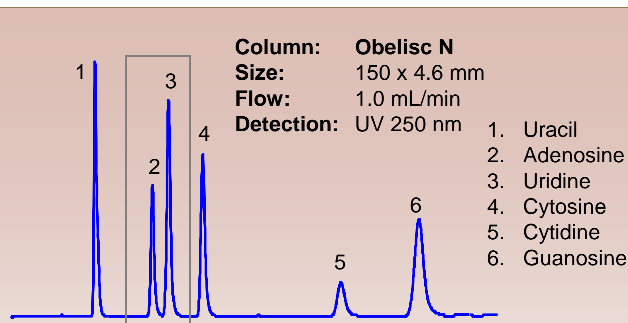


Figure 12. The separation of glyphosate reaction intermediates and impurities.



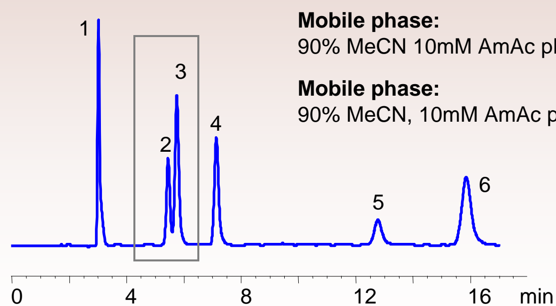


Figure 13. Separation of nucleic bases at pH 4 and 5.

Figure 12 shows the separation of polar glyphosate reaction intermediates.

Figure 13 is a separation of nucleic bases, which demonstrates how selectivity can be tuned by mobile phase pH. pH 5 resolves peaks 2 and 3 which were not resolved at pH 4.

Figure 14 shows an ion chromatography separation of anions and cations with ELSD detection.

Typical mobile phases used with Obelisc N columns are based acetonitrile, water, and the mass spec compatible buffers ammonium formate (pH 3) and ammonium acetate (pH 5). If it is necessary to detect in low UV (<220 nm) then phosphate buffer is recommended.

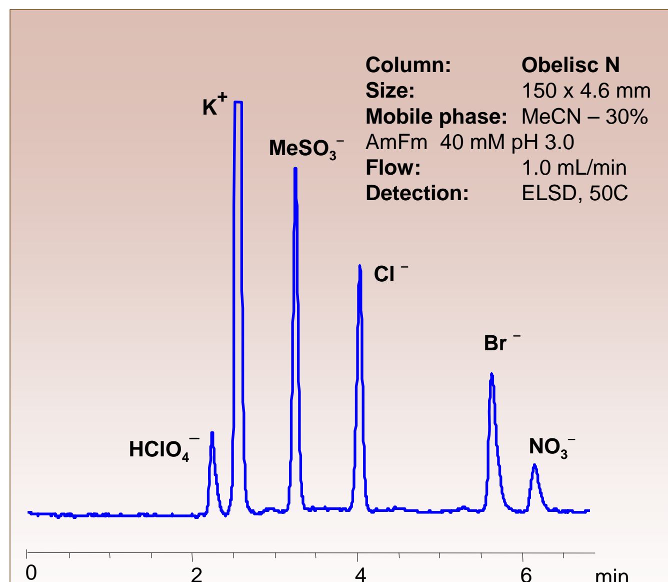
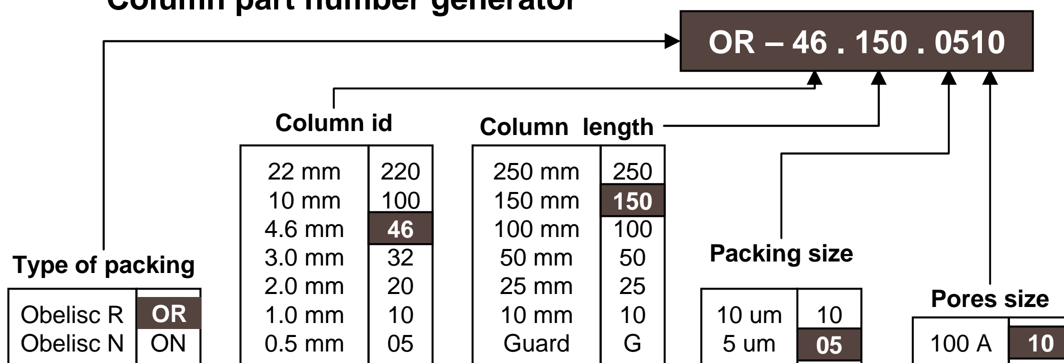


Figure 14. The separation of potassium, perchlorate, methanesulfonic, chloride, bromide, and nitrate ions on Obelisc N with ELSD detection.

Obelisc columns are available in lengths of 10, 25, 50, 100, 150, and 250 mm and inner diameters of 0.5, 1, 2, 3, 4.6, 10, and 22 mm. Obelisc phases are based on spherical silica particles with 100Å pores and particle sizes of 5 and 10 µm. Other column dimensions available upon request. Guard columns are integrated with a male connector and do not require holders.

Column part number generator



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formerly Allsep Technologies

For decades liquid chromatography stationary phase design has been dominated by the goal to eliminate multiple, or “unwanted”, interactions and to obtain a simple and predictable retention mechanism. Unfortunately, the simplification of the retention process limits the ability to control elution order of the analytes and the scope of available applications this system can be used for. As a response to this limitation, hundreds of different reverse-phase columns were introduced in the last years to cover a variety of analytical situations.

In contrast, PrimesepTM stationary phases were intentionally designed with two major interactions offered on the same column. Both interactions are independently adjustable with mobile-phase composition producing unlimited states of the stationary phase. The hydrophobic interaction is controlled by the amount of organic modifier in the mobile phase (as in any reverse-phase column), while the ion-exchange interaction is controlled by the ion-strength and pH of the mobile phase (as in other ion-exchange columns). This unique property allows using one stationary phase for numerous applications, including analyses of polar and non-polar, ionizable and neutral, organic and inorganic compounds. The behavior of PrimesepTM columns is predictable and reproducible. The method development process is simple and versatile.

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