Analysis of quaternary amines (paraquat and diquat) in MS compatible reversed - phase conditions

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Abstract

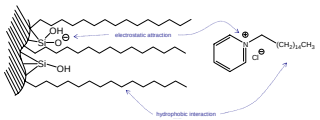
Quaternary amines are difficult compounds to analyze in reversed-phase (RP) conditions. For example, quaternary amines such as paraquat and diquat are important herbicides and their analysis in the environment is a common problem. This problem is twofold. First, many quaternary amines are very polar, and as result are difficult to retain under RP chromatography conditions. Second is the asymmetrical peak shape that results from strong ionic interaction of the quaternary amines with the stationary phase support.

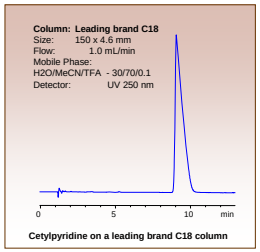
SIELC Technologies has developed Primesep® B and Obelisc™ R HPLC columns. These columns are the latest advances over C18 columns by incorporation of positive charges on Primesep B and both positive and negative charges on Obelisc R columns. This evolution not only eliminates the interaction of amines with the underlying silica support, but also further expands the selectivity possible in chromatographic separations.

To our knowledge, Obelisc R is the first separation media that allows the analysis of multi-charged quaternary amines under simple MS-compatible reversed-phase conditions. Typical mobile phases are based on ammonium formate (pH 3.0) buffer and acetonitrile to control selectivity and the degree of hydrophobic interaction. Symmetrical peaks, high column efficiency, and high selectivity characterize the HPLC methods developed on Obelisc R columns. Quaternary amines as well as other amines can be successfully retained and analyzed using Obelisc R columns in MS friendly reversed-phase conditions.

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

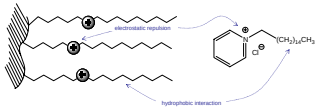
Cetylpyridine is a quaternary amine that retains by reversed-phase interaction on silica-based C18 columns. Hydrophobic interactions are obtained between the C18 chains on the stationary phase and the long chain on cetylpyridine. In addition the basic amine on cetylpyridine electrostatically interacts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

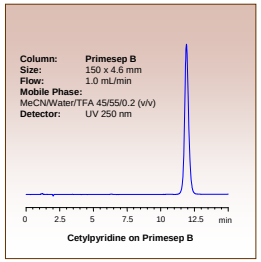




Cetylpyridine on a leading brand C18 column

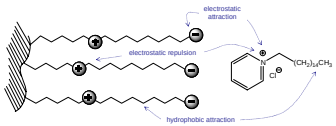
Primesep B has the same hydrophobic interactions, but Primesep B also contains an embedded cation on the stationary phase which shields the underlying silica surface by electrostatic repulsion. The end result is reversed-phase retention with symmetrical peak shape

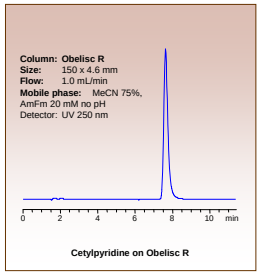




Cetylpyridine on Primesep B

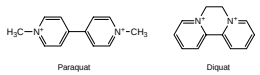
The final evolution is the addition of multiple charges to the hydrophobic chain in Obelisc R. Obelisc R contains both positive and negative charges on the hydrophobic chain. The silica surface shielding and hydrophobic interactions are similar to Primesep B, but additional electrostatic attraction is obtained. Obelisc R requires more acetonitrile in the mobile phase compared to the silica-based C18 and Primesep 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.





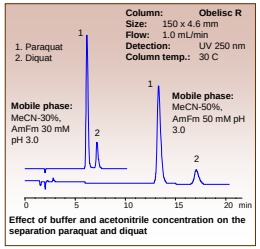
Cetylpyridine on Obelisc R

Paraquat and Diquat on Obelisc R



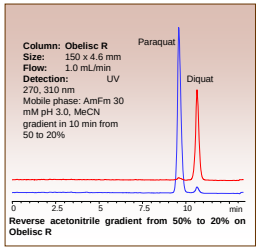
Paraquat and diquat are difficult to retain on reversed-phase columns and often elute in the column void. Obelisc R retains and separates paraquat and diquat with mass spec compatible mobile phases based on acetonitrile and ammonium formate. No ion pair reagents are required due to the ionic groups present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

Unlike separations on reversed-phase C18 columns, 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.



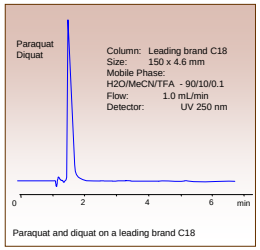
Effect of buffer and acetonitrile concentration on the separation paraquat and diquat

Applying a reversed gradient of acetonitrile further stresses the opposite effect of acetonitrile on retention. In this example all conditions are constant except for a decreasing concentration gradient of acetonitrile from 50% to 20%.



Reverse acetonitrile gradient from 50% to 20% on Obelisc R

Paraquat and diquat are difficult to retain on reversed-phase C18 columns. In this example paraquat retains slightly on a C18 column, but without resolution.

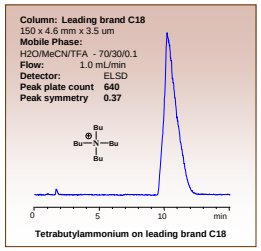


Paraquat and diquat on a leading brand C18

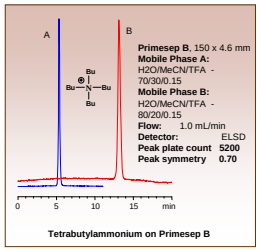
Quaternary Amines on Primesep B

On leading brand C18 column poor peak shape and low efficiency is obtained.

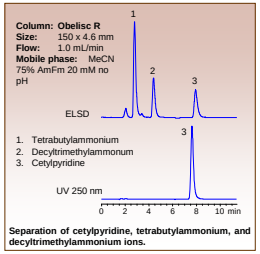
Quaternary amines are difficult to separate with good peak shape. Tetrabutylammonium is retained on Primesep B with good peak shape and high efficiency. On leading brand C18 column poor peak shape with low efficiency is obtained.



Tetrabutylammonium on leading brand C18



Tetrabutylammonium on Primesep B

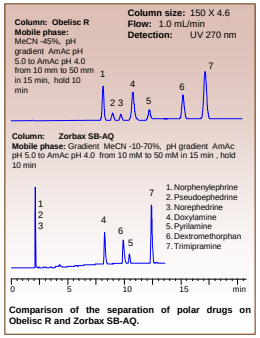


Separation of cetylpyridine, tetrabutylammonium, and decyltrimethylammonium ions.

A mixture of cetylpyridine, tetrabutylammonium, and decyltrimethylammonium ions is shown on Obelisc R with mass spec compatible conditions. Cetylpyridine has UV absorbance, but ELSD is required to detect all three compounds.

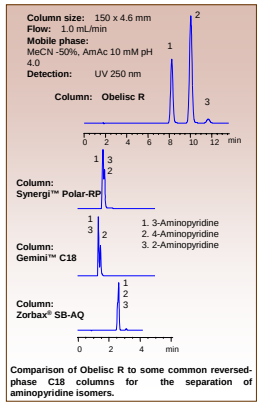
Primary, Secondary and Tertiary Amines on Obelisc R

Many pharmaceuticals are polar and contain amine functional groups, whether they are 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.



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

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.



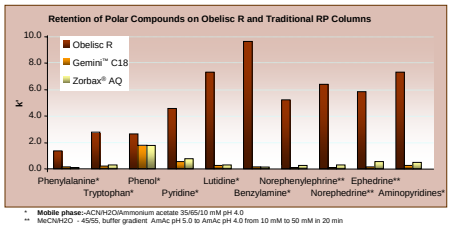
Comparison of Obelisc R to some common reversed-phase C18 columns for the separation of aminopyridine isomers.

Polar compounds retention on Obelisc R

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

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 10 times more retention.

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



Conclusion

Obelisc R and Primesep B columns separate paraquat, diquat, and other amines with good peak shape using MS compatible mobile phases. The latest advance over C18 columns is the incorporation of positive charges on Primesep B and both positive and negative charges on Obelisc R columns. This evolution not only eliminates the interaction of amines with the underlying silica support, but also further expands the selectivity possible in chromatographic separations.

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