Improving Chromatography of Basic Compounds

UHPLC and LC-MS separation materials and methodologies have evolved over the years to meet the challenges of the growing complexity of the separations themselves. Stephanie Rosenberg, Director of Sales and Marketing, discusses how utilizing the new HALO® positively charged surface columns impact the performance of LC and LC-MS separations for basic compounds.

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LCGC: Why did AMT introduce a positively charged surface column?
STEPHANIE ROSENBERG: With the many stationary phases out there, it is normal to ask, "do we really need another one?" Many of the separations today utilize a standard reversed phase silica C18 column, and this is certainly a proven multi-purpose go-to column for a majority of applications to get the job done. However, there are times when an alternative is needed. Such is the case when basic analytes are involved. Basic compounds run on a C18 column often appear chromatographically as tailed peaks due to their interactions with unreacted silanols on the column packing surface, making integration and data interpretation difficult. This is particularly true when using low ionic strength mobile phase additives, such as formic acid. The positively charged surface eliminates this problem by effectively neutralizing the silanols right at the packing surface.

LCGC: Are there other ways to improve peak shape with basic compounds that do not involve a positively charged surface chemistry?
ROSENBERG: There are. Formic acid is desirable for its liquid chromatography-mass spectrometry (LC-MS) compatibility, but another way that peak shapes for basic compounds can be improved is with mobile phase additives, such as phosphate buffers, which can be used for UV separations. It is crucial to keep in mind that those buffers will render the method undesirable for mass spec since phosphate buffers are non-volatile and will contaminate the MS by accumulating around the ionization source.

Another way to improve the peak shape for basic compounds is to use trifluoroacetic acid (TFA) as a mobile phase additive. This is a well-known approach in bio-chromatography for LC-MS of large molecules but does have a drawback of reducing the MS signal, thus lowering the sensitivity of the analysis. The technique to choose depends on the type of detector you are using and the versatility of the method you want to develop. The PCS column we have developed is another tool in that chromatography toolbox.

LCGC: Outside of improved peak shape, are there other advantages to the HALO® PCS?
ROSENBERG: Yes, there are—one of which is higher loading capacities of basic compounds. Since the peak symmetry is well maintained on the HALO® PCS, a higher concentration of sample can be loaded on-column when compared to a standard C18. This is desirable when you are trying to find impurities around that base peak.

Another is alternate selectivity. With the HALO® PCS C18 in both a 90 A and 160 A pore size for small molecule and peptides respectively, they provide an alternate USP L1 selectivity, which chromatographers can take advantage of for method development.

Lastly, at the heart of HALO® products is the Fused-Core® particle technology which by its very design of a solid silica core delivers fast, efficient, and highly reproducible separations.

LCGC: You've discussed bases, but what about mixtures of bases with acids and neutrals?
ROSENBERG: When designing this product, it was important for our R&D team to develop the final embodiment to not have negative chromatography effects on acids and neutrals. The amount of positive charge placed on the packing surface has been engineered to provide good peak shapes for basic compounds without adversely affecting the peak shapes of acidic compounds. To demonstrate this, each lot is tested and our quality assurance test that is shipped with every column, is composed of a mix of acids, bases, and neutrals.

LCGC: Are there specific applications that benefit most with the HALO® PCS?
ROSENBERG: Peptide mapping is a good example. Peptides are complex. For starters, they are amphiprotic molecules which means they possess both amine and carboxylic functional groups where they can act as an acid or a base. Both functional groups ionize in the aqueous mobile phase, affecting their relative hydrophobicity which plays a key role in retention of reverse phase separations. Secondly, peptides also possess hydrophobic side chains, now introducing hydrophilic and hydrophobic characteristics. With so many factors at play, the stationary phase and analysis conditions, specifically the mobile phase pH, are important. We have demonstrated peptide interactions with our mid-pore (160 A) positively charged surface. Operating under weakly acidic mobile phase conditions, such as formic acid, provide narrower peak widths and improved tailing factors over other mid-pore C18 columns.

Basic drugs such as tricyclic antidepressant panels are another example. Drugs like Trimipramine, Amitriptyline, and Nortriptyline are known to demonstrate high tailing on traditional C18 columns.

Lastly, leaving the pharmaceutical sector, our applications lab obtained very good results on a HALO® PCS column for a pesticides panel run via LC-MS.

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LCGC: In what column dimensions are the HALO® PCS columns available?
ROSENBERG: The columns are available in the most popular column dimensions, including AMT's new 1.5 mm ID columns. These are most beneficial for high sensitivity ultra-high-performance liquid chromatography (UHPLC) and LC-MS applications, as well as helping scientists meet their green solvent saving initiatives by reducing solvent consumption. The solvent consumption for the HALO® 1.5 mm ID columns is 50% less than 2.1 mm ID columns.

LCGC: Where can someone learn more about the new HALO® PCS columns?
ROSENBERG: We welcome them to visit us on the web at halocolumns.com where they can access technical materials and find their local distributor. They can also reach out via phone or through our social media channels any time they want to talk chromatography with us!