HALO® Elevate: Universal Column for Small Molecule Method Development

Introduction

Built upon proven Fused-Core® particle technology for speed and efficiency, the HALO® Elevate C18 incorporates surface modified organo-silane technology for alkaline resistance resulting in excellent stability in both high and low pH environments.

With a wide operational use range of pH 2-12, HALO® Elevate allows for robust method development, flexible to work the full range of operating conditions for separation selectivity of acids, bases, and neutral analytes.

Silica: Pros and Cons

Silica in the form of either particles or monoliths is the most commonly used support for the production of HPLC packings. High mechanical strength is a strong advantage for silica particles, allowing the formation of packed beds that are stable for long periods and high operating pressures. Silica-based columns also provide higher values of plates/ column efficiencies (N), compared to other support materials. Some other advantages of silica are…

- It can be bonded with different ligands allowing for selectivity differences (e.g. C8, C18, Phenyl, Cyano)

- Compatible with all organic solvents and water/ do not swell or shrink with a change of solvent

One disadvantage of silica is that it dissolves in mobile phases at pH > 8, which can result in a short lifetime for silica columns.

HALO® Elevate C18 Development

Many chromatographers are familiar with the difficulties of separating basic compounds on silica-based columns due to the increased peak broadening and tailing that may occur, however, it is important to note that silica-based columns are preferred due to faster equilibration times, along with much higher-pressure stability. (compared to polymer-based columns) One theory for why peak tailing occurs is due to the basic analyte interacting with the stationary phase and free silanols, which allow peak broadening as a result. This becomes significantly worse at higher sample loads; as more sample is loaded on the column, tailing factors increase. Another theory for peak tailing (especially when the compound is anionic) is mutual repulsion wherein the adsorption of a small amount of charge to the stationary phase surface results in repulsion of similarly charged analyte molecules that enter the zone of previously adsorbed analytes, thus leading to broadening of the zone. [2]

There are several ways to improve the peak shape of basic compounds such as increasing the ionic strength of the mobile phase (adding a salt/buffer), use of an ion pair agent, elevating the pH of the mobile phase, using a non-silica-based column, or even using an alternative stationary phase such as a charged surface material. For example, figure 1 below shows doxylamine, a common antihistamine run under acidic and basic conditions on a HALO® Elevate column. Not only is there a significant improvement in peak shape under alkaline conditions, but retention is also increased.

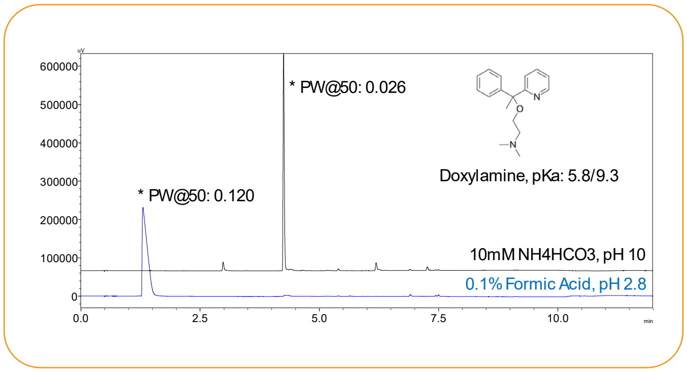


Figure 1: Doxylamine run under acidic and basic conditions using a HALO® Elevate C18 column

HALO® Elevate Column Robustness: Stability & Lot-to-Lot Reproducibility

The main objective during HALO® Elevate development was to make a material that is stable under alkaline conditions to improve the peak shape and retention for basic analytes. In order to achieve this goal, an organic/ inorganic surface modification was introduced protecting the silica from harsh alkaline conditions. This modification allows users to run in alkaline conditions for extended periods of time.
Figure 2 demonstrates both the benefits and the reproducibility of this modification, a separation of amitriptyline (3) and acenaphthene (2) over 500 injections run at pH 10, 60 °C.

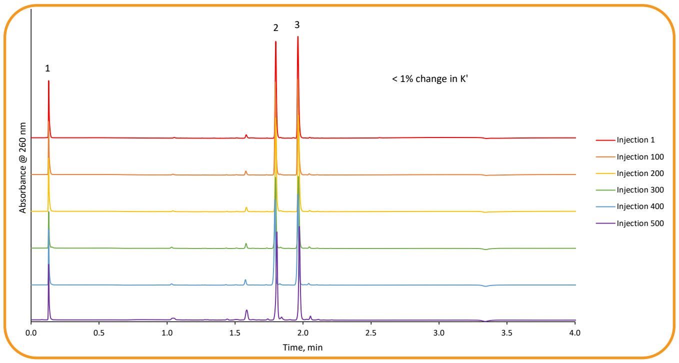


Figure 2. A stability run of a common tricyclic antidepressant (amitriptyline, 3) is achieved using a HALO® Elevate C18 column run at 60 °C, pH 10. Less than a 1% change in retention is achieved over 20,000 column volumes.

Along with excellent column stability, lot to lot repeatability is crucial for method development and cannot be underestimated. Figure 3 demonstrates six different lots of HALO® Elevate C18 tested using a mix of a neutral compound and 5 basic compounds. Run conditions include pH 10 at 60 °C. The average %RSD across all of the compounds was < 0.6%.

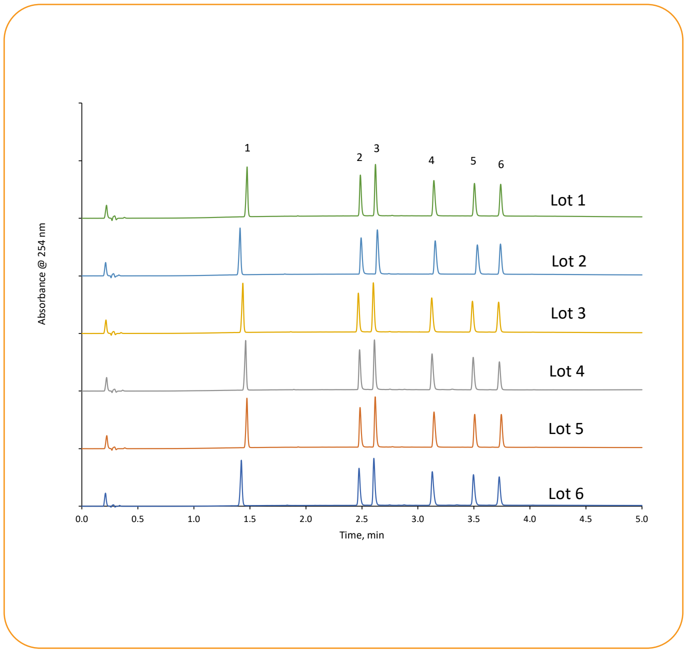


Figure 3. HALO® Elevate C18 Lot-to-lot reproducibility under alkaline conditions.

Improved Loading Capacity for Basic Analytes

At low pH, basic compounds become positively charged which allow for unwanted interactions between the stationary phase/ silanols on the silica surface. Under high pH conditions, basic molecules become deprotonated, increasing retention (becoming less polar) and significantly improving chromatographic peak shape. This allows for much higher sample loading capacities compared to low pH conditions as seen in figure 4.

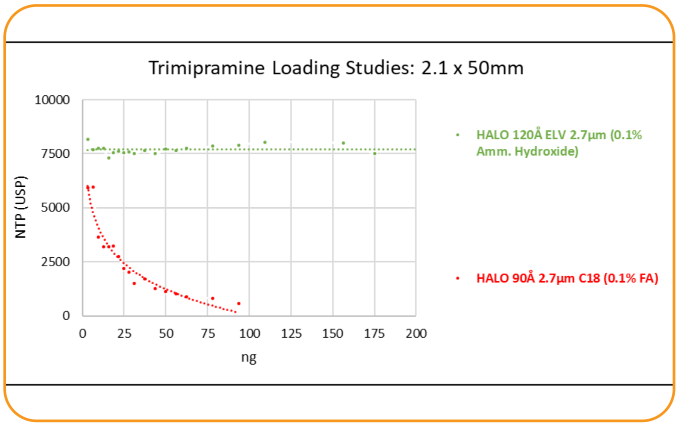


Figure 4. Trimipramine, a common tricyclic antidepressant observed under low and high pH conditions at different sample loads on column.

HALO® Elevate C18: Method Development using Wide pH Range

HALO® Elevate incorporates a wide pH compatibility from 2 to 12 which allows not only chromatographic separations to be performed under high pH conditions, but low pH options are also available. This versatility allows for easier method development along with achieving selectivity differences that may benefit a separation. For example, figure 5 demonstrates how pH of the mobile phase affects a chromatographic separation with an acid, base, and neutral analyte. Adequate retention is achieved for the acidic compound under low pH conditions while improved retention and peak shape is achieved for the basic analyte while under high pH conditions. It is beneficial to know the chemical characteristics during method development including pKa, to obtain the best results possible. Having the pH of your mobile phase too close to the compound's pKa value can result in poor peak shape and resolution due to the presence of ionized and non-ionized forms of the analyte.

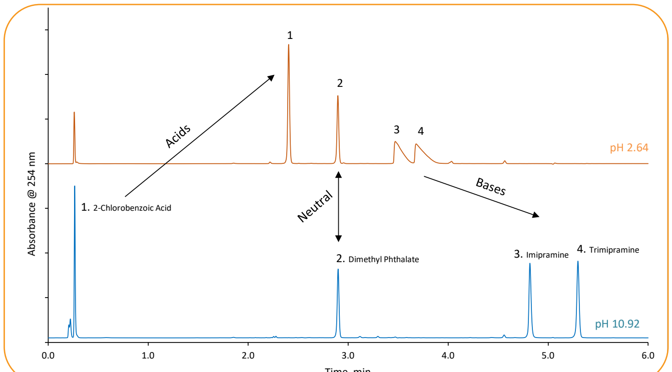


Figure 5: Acid, base, and neutrals separated under high and low pH conditions.

It is important to note that peak shape for basic analytes can be further improved under acidic conditions by adding an ion pair agent, such as trifluoroacetic acid. (TFA). For example, figure 6 shows a separation of procainamide and 4-aminobenzoic acid under acidic and basic conditions. The use of trifluoracetic acid allows for peak shape improvement under acidic conditions. While TFA gives sharp peaks and good retention, the ionization efficiency is poor when running LC/MS applications. With formic acid, the ionization efficiency is high, but the peak shapes are poor. The use of either formic acid/ammonium formate or difluroacetic acid are good options for a balanced result between good peak shape and good ionization efficiency.

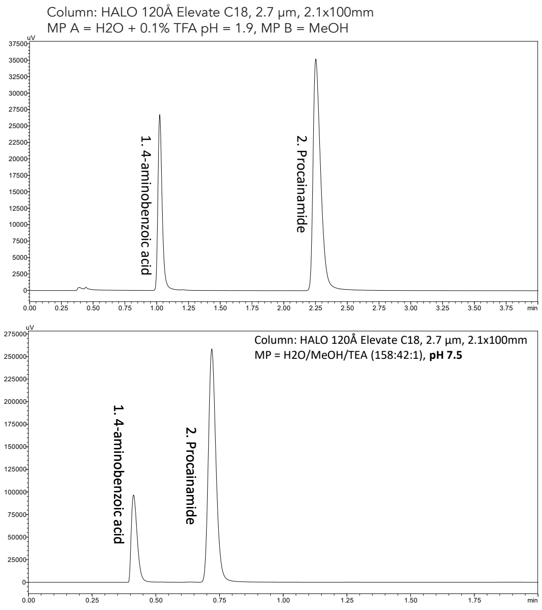
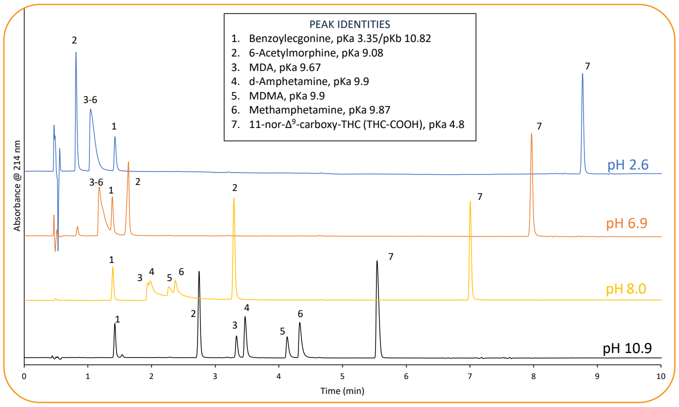


Figure 6. Acid and base separation under acidic and basic conditions.

Another example (figure 7) includes a mixture of abused drugs separated on a HALO® Elevate C18 column under various pH ranges. Selectivity is altered as the pH is changed which can be used as a tool during method development. Not only are retention times shifted, but an improvement in peak shape is observed for the basic analytes as pH is increased above the designated pKa. The acidic compound (THC-COOH) is more retained as the pH of the mobile phase is decreased, due to its acidic properties.


PEAK IDENTITIES

1. Benzoylecgonine, pKa 3.35/pKb 10.82
2. 6-Acetylmorphine, pKa 9.08
3. MDA, pKa 9.67
4. d-Amphetamine, pKa 9.9
5. MDMA, pKa 9.9
6. Methamphetamine, pKa 9.87
7. 11-nor-Δ^9-carboxy-THC (THC-COOH), pKa 4.8

Figure 7. Selectivity manipulation using wide pH range HALO® Elevate column

Conclusion

Whether you are separating acids, bases, or neutrals HALO® Elevate allows you to achieve excellent peak shape and column stability under a wide pH range due to its alkaline resistant properties. Adjusting the pH of your mobile phases is an excellent way to modify retention, improve peak shape, and achieve improved selectivity for your chromatographic separations.

References

1. Introduction to Modern Liquid Chromatography, 3rd Edition, L. R. Snyder, J. J. Kirkland, J. W. Dolan, 2010, John Wiley & Sons, Inc.

2. Anal. Chem. 2006, 78, 2532-2538: Overload for Ionized Solutes in Reversed-Phase High-Performance Liquid Chromatography David V. McCalley*

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