Improved HPLC Method Development using Wide pH Range

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Introducing HALO® ELEVATE

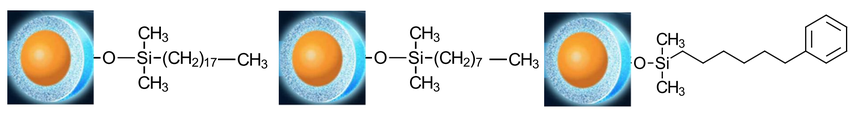
Built upon Fused-Core® particle technology for speed and efficiency, the HALO® ELEVATE column line incorporates surface modified organo-silane technology for alkaline resistance resulting in excellent stability in high pH environments.

With a wide operational use range of pH 2-12, HALO® ELEVATE allows for robust method development and improved separations for basic compounds that may present problems such as poor peak shapes, inadequate retention or limited load tolerance at low pH. Ideal for use with high pH mobile phases.

FEATURES OF HALO® ELEVATE

- THREE PHASE OFFERINGS
  - C18, C8, Phenyl-Hexyl
- EXCELLENT STABILITY for high pH, high temperature environments
- THREE PARTICLE SIZES: 2, 2.7, and 5µm
- ROBUST COLUMN OPTION to work the full range of operating conditions for separation selectivity of acids, bases, neutrals and zwitterions
- PROVEN HALO® FUSED-CORE® TECHNOLOGY for uniform column loading, quality separations, speed and ruggedness

Product Characteristics



C18

Ligand: dimethyloctadecyl
Pore Size: 120Å
USP Designation: L1
Particle Sizes: 2, 2.7, 5µm
Surface Area: 70, 75, 60m²/g
Carbon Load: 5.2, 5.6, 4.5%
PMax: 1000, 600, 600 bar
End Capped: Yes
Aqueous Comp. No
pH Range: 2-12
Temperature Maximum: 60°C

C8

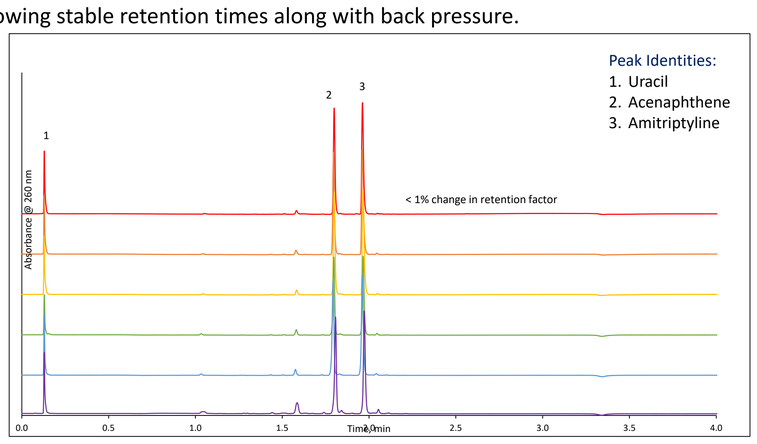
Ligand: dimethyloctyl
Pore Size: 120Å
USP Designation: L7
Particle Sizes: 2, 2.7, 5µm
Surface Area: 70, 75, 60m²/g
Carbon Load: 3.8, 4.2, 3.2%
P Max: 1000, 600, 600 bar
End Capped: Yes
Aqueous Comp. No
pH Range: 2-12
Temperature Maximum: 60°C

Phenyl-Hexyl

Ligand: dimethylphenylhexyl
Pore Size: 120Å
USP Designation: L11
Particle Sizes: 2, 2.7, 5µm
Surface Area: 70, 75, 60m²/g
Carbon Load: 4.5, 5.1, 4.0%
P Max: 1000, 600, 600 bar
End Capped: Yes
Aqueous Comp. Yes
pH Range: 2-12
Temperature Maximum: 60°C

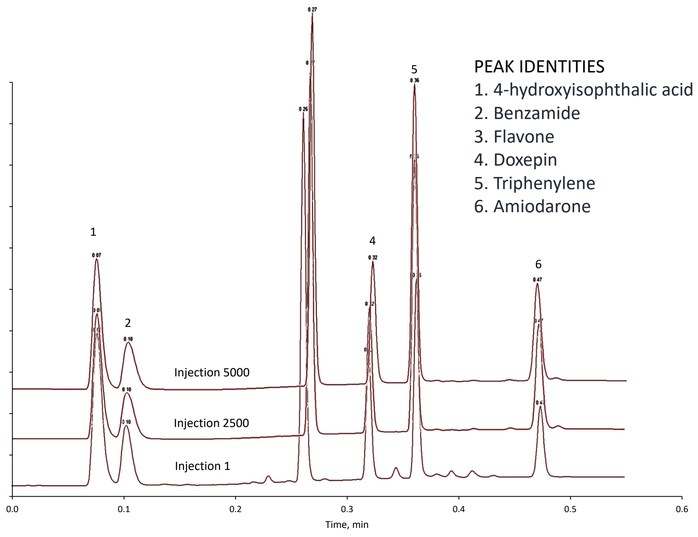
Stability at High pH/ High Temperature Conditions

High pH and high temperature stability are critical for extending column life and expanding method development options. While standard silica-based columns are typically limited to pH 2–8 and temperatures below 60 °C, specialized column technologies enable reliable operation at more extreme conditions, such as pH 2–12 and higher column oven temperatures. Below demonstrates the impressive stability of the HALO® ELEVATE column line running at high temperature (60°C), and high pH (10) for over 20,000 column volumes. Less than a 1% change in retention is achieved showing stable retention times along with back pressure.



- Less than a 1% change in retention is achieved over 20,000 column volumes.

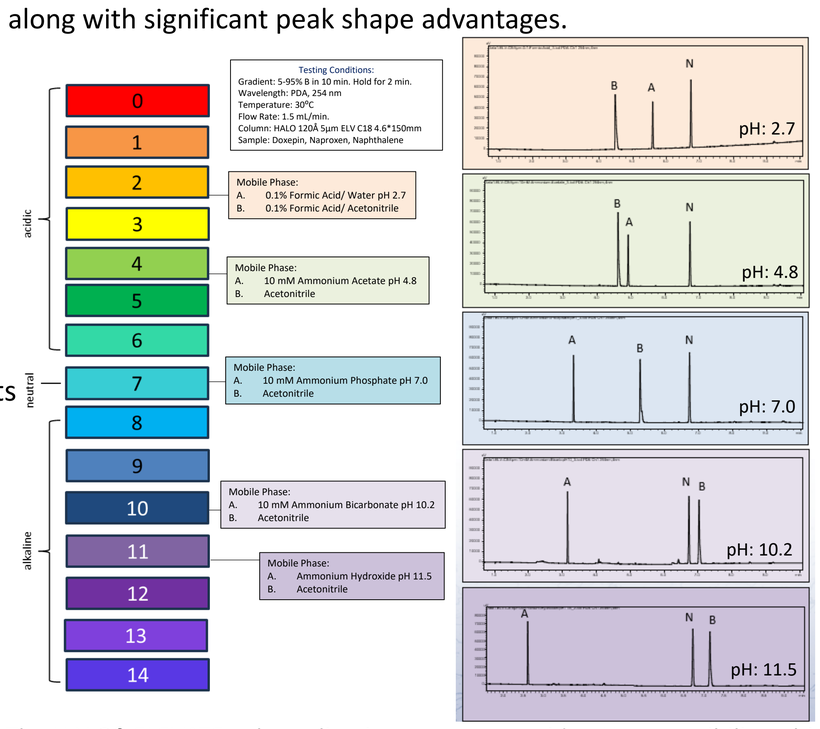
HALO 120 Å ELV C18, 2.7 µm, 1.5 x 50 mm, A: 0.1% NH4OH, pH:11
B: Acetonitrile; Gradient: 20-95% B in 0.25 min., 95-100% B in 0.2 min.,hold at 100% B for 0.1 min.
Flow Rate: 1.0 mL/min; Max Back Pressure: 550 bar; Temperature: 60 °C; Injection: 1.0 µL
Detection: UV Wavelength Range: 210-400 nm; MS Scan Range: 100-1250 Da
LC System: Waters Acquity H-Class
Data Courtesy of: Boehringer Ingelheim (Biberach, Germany)



- 1.5 mm ID HALO® ELEVATE C18 column run at 5 times the optimum flow rate demonstrated excellent reproducibility and stability for retention time, peak shape, and back pressure over the course of 5000 injections

Alternative Selectivity based on pH

Adjusting the pH of the HPLC mobile phase is a great way to alter selectivity, particularly for ionizable compounds such as acids and bases. Depending on the analyte of interest, changing the pH of your mobile phase can also give retention time improvements along with significant peak shape advantages.

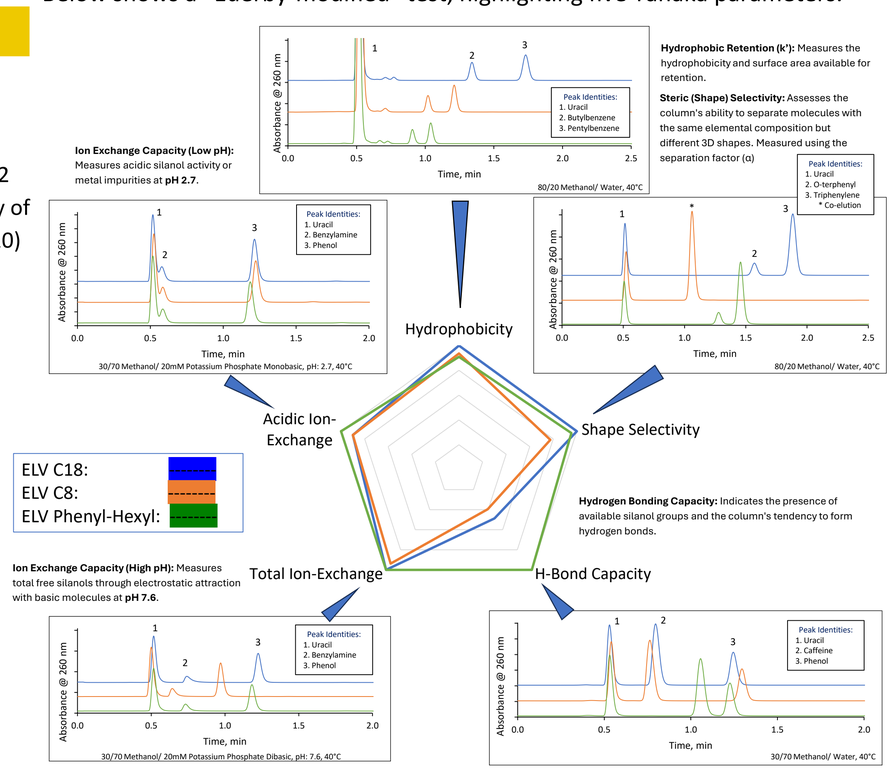


The HALO® ELEVATE column line incorporates a wide pH compatibility which allows not only peak shape advantages. chromatographic separations to be performed under low pH conditions, but high pH options are also available. (pH 2-12) This versatility allows for easier method development along with achieving selectivity differences that may benefit separation. This figure shows a mixture of an acid (Naproxen), base (Doxepin), and neutral (Naphthalene) analyte run under low, medium, and high pH conditions. Not only is HALO ELEVATE able to run at high pH (12), but it may also be run at low pH (as low as 2).

Alternate Selectivity via Column Stationary Phase

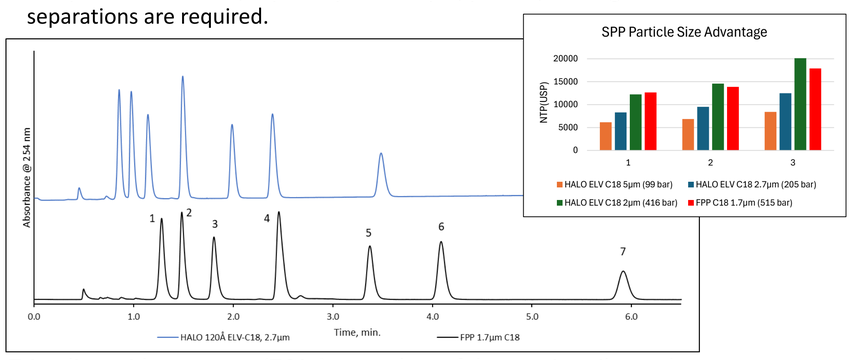
The HPLC Tanaka test is a standardized characterization protocol used to evaluate the selectivity and performance of reversed-phase (RP) columns. Developed by Nobuo Tanaka in 1989, it uses a set of specific chemical probes to measure how a stationary phase interacts with different types of molecules.

The results are typically visualized using a hexagonal radar plot (often called a "Tanaka Plot"), where each axis represents a different chromatographic property. Below shows a “Euerby-modified” test, highlighting five Tanaka parameters.



Advantage of Smaller Particle Sizes: SPP vs. FPP

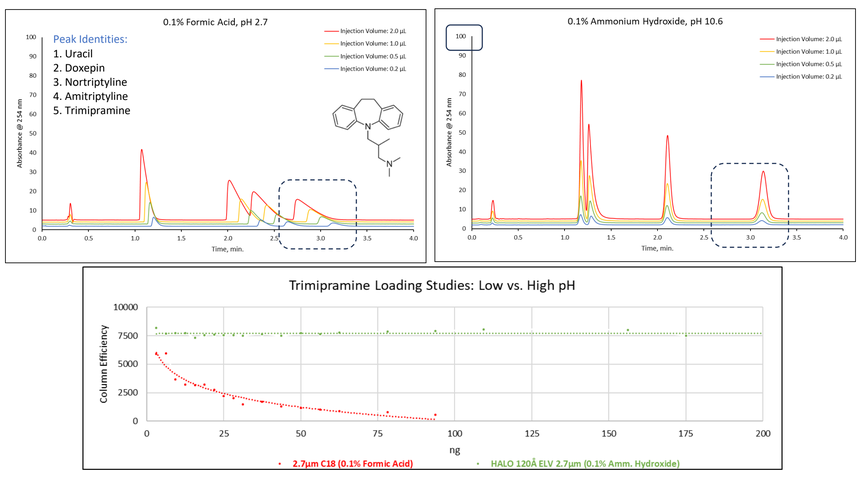
Using smaller particle sizes in High Performance Liquid Chromatography (HPLC) offers several key advantages that greatly enhance the efficiency, resolution, and overall performance of chromatographic separations. One of the most important benefits is the increase in separation efficiency. Smaller particles provide a greater surface area/unit volume for interactions between the stationary phase and analysis, which leads to narrower chromatographic peaks and improved separation of compounds. The increase in efficiency is reflected by a higher number of theoretical plates, a key measure of column performance in HPLC. Although these columns require higher operating pressures and low dispersion instrumentation, the improvements in speed and performance make them highly valuable for modern analytical laboratories, especially when rapid, precise, and high-resolution separations are required.



Tricyclic antidepressants (TCAs) are a class of drugs primarily used to manage depression. A separation of antidepressants is achieved under high pH conditions using a HALO® ELEVATE C18 column. As particle size becomes reduced, column efficiencies will increase along with an increase in column back pressure. Superficially porous particles provide an additional advantage over fully porous particles of similar sizes, allowing for higher column efficiencies and back pressure advantages due to the limited diffusional path provided by the solid silica core.

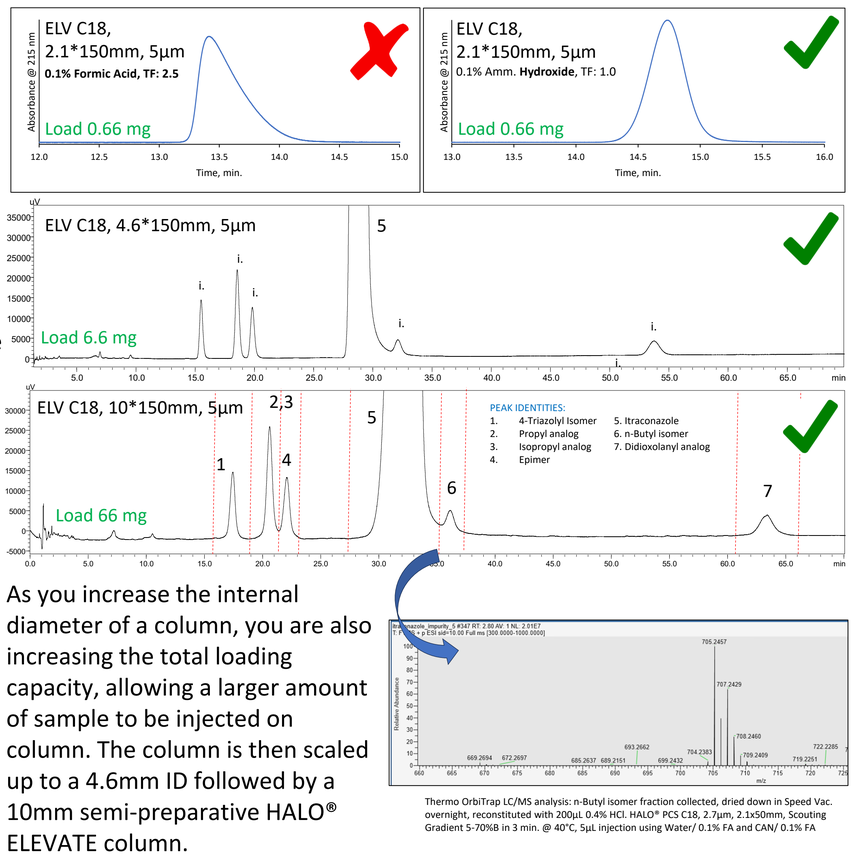
Loading Capacity

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/ efficiency. This allows for much higher sample loading capacities compared to low pH conditions as seen in the figure below. In general, you want to set the pH of your mobile phase 2 units above the analyte’s pKa value, allowing the base to become deprotonated (neutral). Below demonstrates trimipramine, a common tricyclic antidepressant (base) observed under low and high pH conditions at different sample loads on column.



Impurity Analysis via Semi-Prep.

Below demonstrates a separation of itraconazole and related impurities under low pH and high pH testing conditions. Peak shape is significantly improved under alkaline conditions, allowing for higher sensitivity for related impurities.



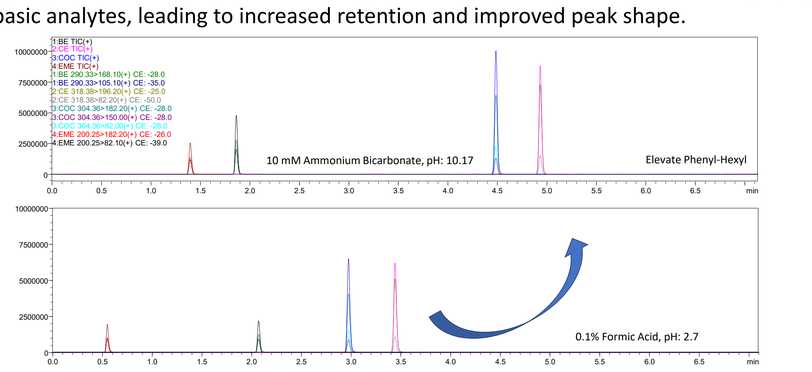
As you increase the internal diameter of a column, you are also increasing the total loading capacity, allowing a larger amount of sample to be injected on column. The column is then scaled up to a 4.6mm ID followed by a 10mm semi-preparative HALO® ELEVATE column.

Thermo OrbiTrap LC/MS analysis: n-Butyl isomer fraction collected, dried down in Speed Vac. overnight, reconstituted with 200µL 0.4% HCl. HALO® PCS C18, 2.7µm, 2.1x50mm, Scouting Gradient 5-70%B in 3 min. @ 40°C, 5µL injection using Water/ 0.1% FA and CAN/ 0.1% FA

One of the main advantages of preparative (prep) column chromatography is its ability to isolate and purify large quantities of target compounds for downstream use, such as active pharmaceutical ingredients or even small impurities. With the higher sample load, impurities are then fraction collected, dried down, and injected into a mass spectrometer to identify the impurities mass.

LC/MS/MS Application: Cocaine Metabolites

Low pH/acidic mobile phases are typically used to separate cocaine (base) and its metabolites because cocaine is protonated and leads to high ionization/ sensitivity in ESI(+). High pH mobile phase/basic mobile phases tend to lead to better chromatography for basic analytes, leading to increased retention and improved peak shape.



Summary

- The HALO® ELEVATE column line is a wide pH (2-12) compatible silica material used for acidic, basic, and neutral analytes. Available in C18, C8, and Phenyl-Hexyl. (2, 2.7, 5µm)
- Excellent stability is achieved under high pH (10) and high temperature (60°C) conditions, showing reproducible retention times along with stable back pressure.
- Sample loading is improved for basic compounds when high pH conditions are used, allowing for impurity analysis advantages.
- Using pH as method development tool for basic compounds can be very successful provided the pH is > pKa of the compounds.