HALO®

TECHNICAL REPORT

TITLE: OPTIMIZING HPLC SAMPLE LOADING CAPACITY BY LEVERAGING PH TO IMPROVE RESULTS USING HALO® PCS AND HALO® ELEVATE IN THE STUDY.

MARKET SEGMENT: PHARMACEUTICAL

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INTRODUCTION

Sample load is an important factor in high-pressure liquid chromatography (HPLC) because it directly affects peak resolution, separation efficiency, and data accuracy. Loading too much sample can overload the column stationary phase, causing peak broadening, retention time shifts, and reduced column lifetime. In severe cases, overload can also increase the risk of column blockage and pressure buildup over time.

Sample loading capacity can be increased in several ways, including optimizing mobile phase conditions, selecting packing materials with appropriate characteristics, and adjusting the internal diameter of the column to support larger injection volumes or higher analyte mass on column.

This report explains practical ways to increase HPLC sample loading capacity while preserving chromatographic performance. It focuses on sample solvent selection, stationary phase chemistry, mobile phase conditions, pH control, internal column diameter, and puts all of these parameters together with a semi-preparative column scale-up example.

SAMPLE LOADING/RISK OF COLUMN OVERLOADING

In HPLC, sample load refers to the total mass or volume introduced onto the column. It can be described as either concentration load or volume load. Concentration load is evaluated by holding injection volume constant while changing the analyte mass. Volume load is evaluated by changing the injection volume while keeping analyte concentration constant.

Every HPLC column has a maximum loading capacity, and exceeding this limit can lead to column overload. Overloading a column can cause peak broadening, inconsistent retention times, and an increased risk of column plugging. For example, Figure 1 shows 1-Cl-4-nitrobenzene injected at increasing injection volumes. Peak shape clearly worsens as injection volume increases.

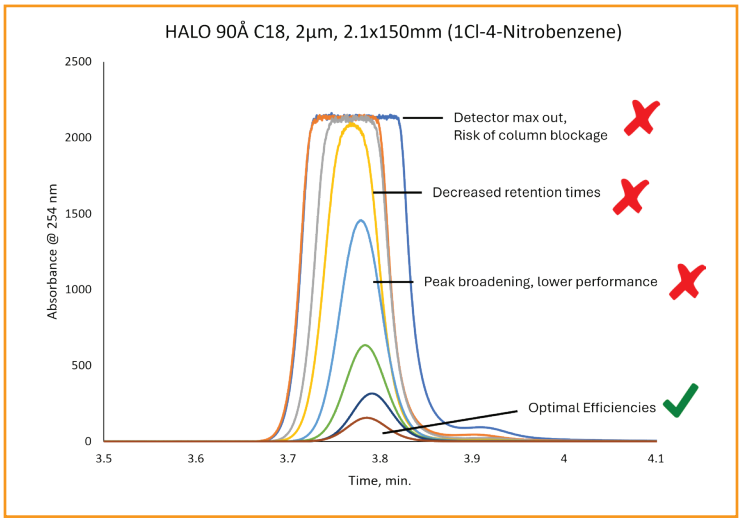


Figure 1. 1-Cl-4-Nitrobenzene injected at increasing injection volumes. Overloading your sample on a column will lead to peak broadening, inconsistent retention times, and a higher risk of plugging up the column.

BEST PRACTICES FOR OPTIMIZING SAMPLE LOAD:

Before injecting the sample on column there are a few things to consider for optimizing sample load. For example, try to ensure that the sample solvent is as weak or weaker than the initial mobile phase being used for analysis. Using too strong a solvent or too large a volume of too strong a sample solvent can cause peak shape and efficiency problems. Sample solvent mixtures that have different viscosities than the initial mobile phase can cause peak shape issues as well.

It is also important to consider the characteristics of the column packing material. Fully porous particles (FPP) will demonstrate a higher loading capacity than superficially-

KEY WORDS:

HPLC, sample loading capacity, column overloading, high-pH chromatography, HALO®ELEVATE, HALO® PCS, impurity analysis, column scale-up

porous particles (SPP) of the same column dimension due to the larger surface area. There are also now mixed-mode column chemistries that will improve the loading capacity of analytes due to its positive charge surface features, such as the HALO® PCS column line. For instance, figure 2 represents a separation of a peptide and a small impurity under acidic conditions (formic acid). The peak shape for these analytes is significantly improved due to the positive charge ligand on the surface when compared to a more traditional/uncharged C18 column, allowing for baseline resolution of the small impurity.

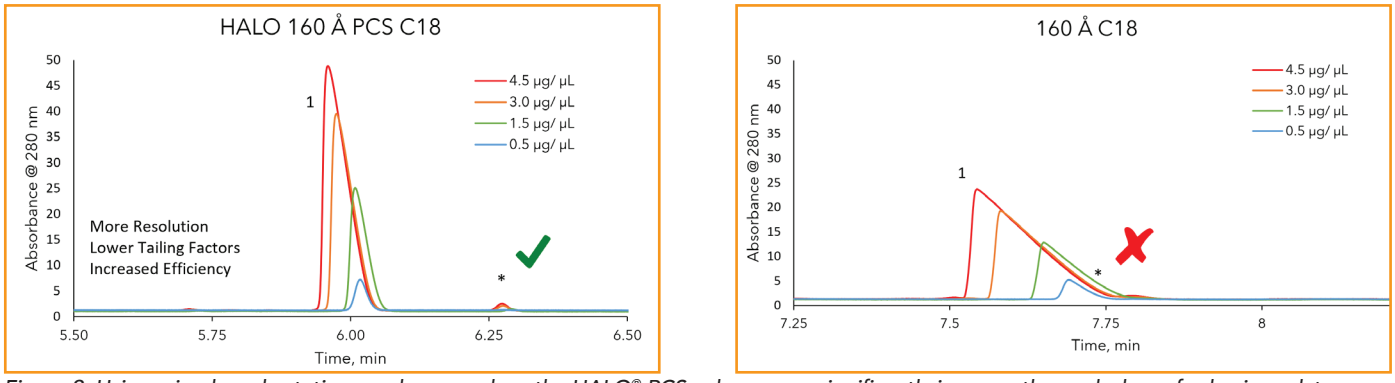
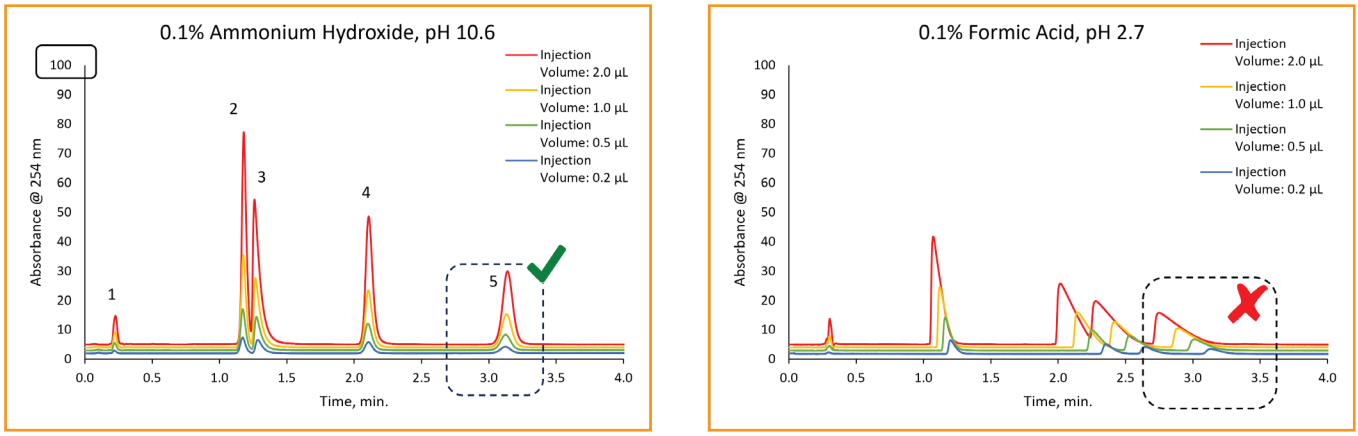


Figure 2. Using mixed-mode stationary phases such as the HALO® PCS columns can significantly improve the peak shape for basic analytes (including peptides, as shown above: S5Y Sequence) under low ionic strength mobile phase conditions

Adjusting the mobile phase parameters is a great way to optimize sample load, especially for basic compounds. There are several other 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. At low pH, basic compounds become positively charged which allow for unwanted interactions between the stationary phase/ silanols on the silica surface. The PCS column chemistry is designed to improve the peak shape for basic analytes while using low ionic strength mobile phases such as formic acid.

Under high pH conditions, basic molecules become deprotonated, allowing for an increase in retention (less polar) and significant improvement in chromatographic peak shape. This allows for much higher sample loading capacities compared to low pH conditions as seen in figure 3.



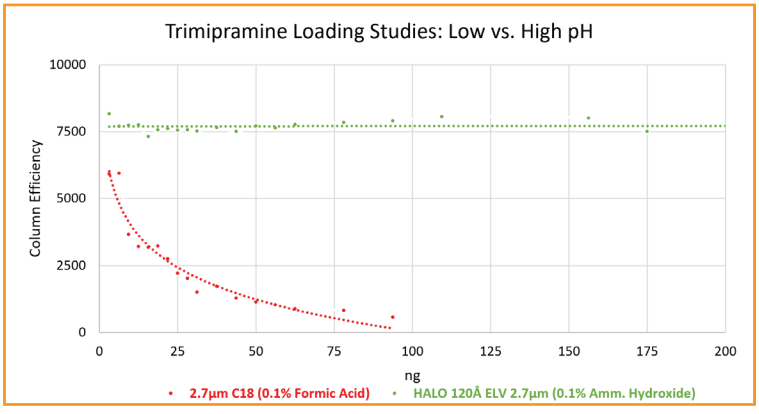


Figure 3. Trimipramine, peak 5, a common tricyclic antidepressant observed under low and high pH conditions at different sample loads on column.

Built upon proven 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 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.

As the internal diameter of a column increases, the total loading capacity is increasing also, allowing a larger amount of sample to be injected on column. One advantage in doing so is to analyze small impurities within a sample. It is important to note that in order to maintain the same chromatographic performance after increasing the internal diameter, the flow rate and injection volume must be adjusted as well. In order to do so, both the flow rate and internal diameter must be multiplied by the square of the ratio of the new diameter to the old diameter. $(d_2/d_1)^2$

CASE STUDY: ITRACONAZOLE AND RELATED IMPURITIES

Itraconazole is a prescription triazole antifungal medication. It acts as a weak base and is used to treat a wide variety of serious fungal and yeast infections.[1] Below demonstrates a separation of itraconazole under low pH and high pH testing conditions on a 2.1mm ID column dimension and HALO® ELEVATE C18 stationary phase. Peak shape is significantly improved under alkaline conditions which the HALO® ELEVATE column is designed for as described previously. Sample solvent was matched to the starting conditions of the method, in this case 60/40 water/ acetonitrile.

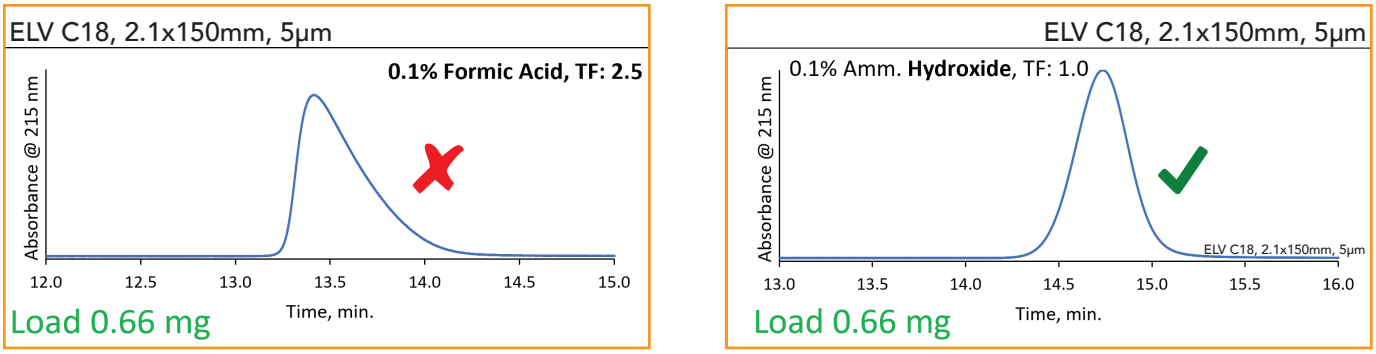


Figure 4. Comparison of itraconazole at low pH (0.1% formic acid) vs. high pH (0.1% ammonium hydroxide) on a 2.1x150mm, 5µm HALO® ELEVATE C18

Sample load is increased by increasing the internal diameter of the column from a 2.1mm to a 4.6 mm ID. By doing so, several impurities within the sample are observed. The separation is then scaled up to a 10 mm ID semi-prep column, allowing higher sensitivity for the impurities.

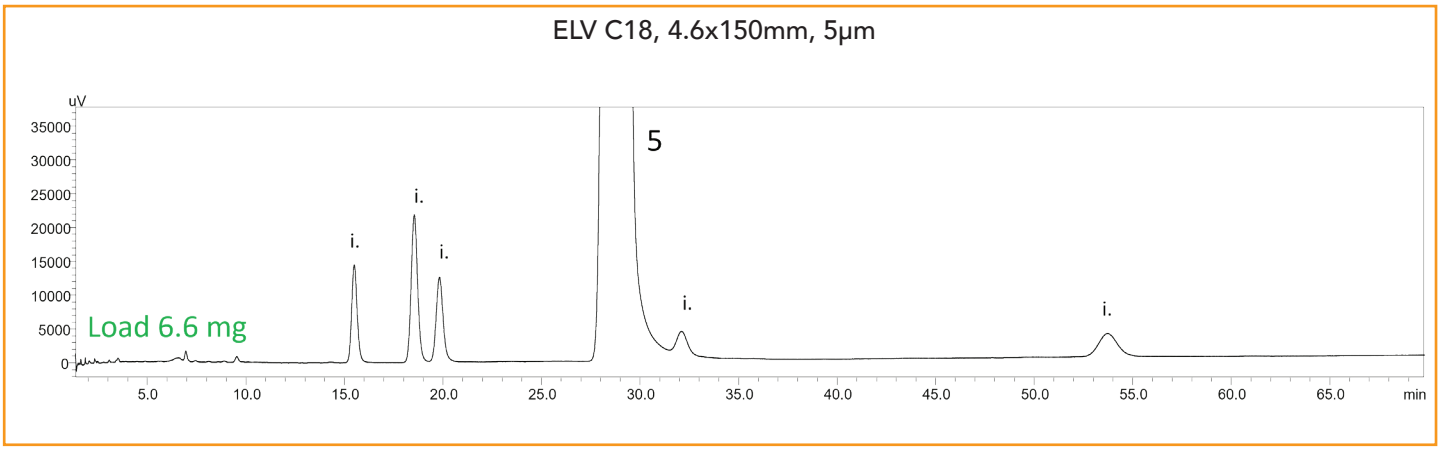


Figure 5. Itraconazole loaded at 6.6 mg on column on an ELEVATE 4.6x150mm, 5µm column. Impurities are now being observed within the chromatogram.

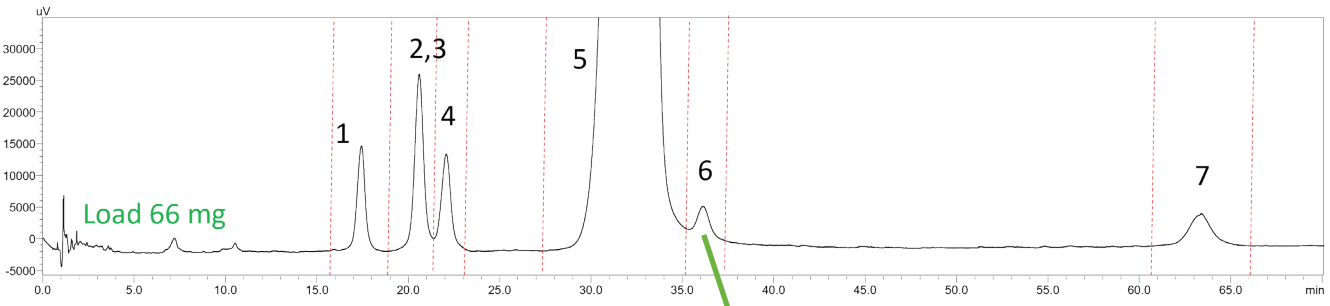
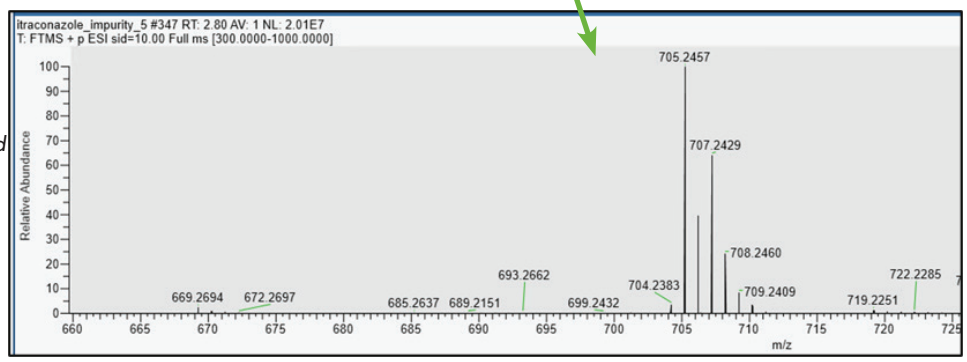


Figure 6. Itraconazole loaded at 66 mg on column on an ELEVATE C18 10x150mm, 5µm column. Impurities are fraction collected, dried down, and analyzed on a mass spectrometer.



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

PEAK IDENTITIES

1. 4-Triazolyl isomer
2. Propyl analog
3. Isopropyl analog
4. Epimer
5. Itraconazole
6. n-Butyl isomer
7. Didioxolanyl analog

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.

CONCLUSION:

Increasing sample loading capacity requires balancing the amount of material introduced onto the column with the chromatographic performance needed for reliable resolution, peak shape, and retention time reproducibility. Column overload can compromise a method including peak broadening, retention time shifts, and increased risk of plugging, so loading studies should be used to define practical limits before scaling. Loading capacity can be improved by optimizing the sample solvent, mobile phase strength, pH, ionic strength, and stationary phase chemistry. For basic analytes, high-pH operation and charged surface technologies such as HALO® PCS and HALO® ELEVATE can reduce unwanted silanol interactions and improve peak shape, enabling higher loads without sacrificing separation quality. Increasing the internal column diameter and moving toward semi-preparative conditions provides a direct path to greater sample capacity when flow rate and injection volume are scaled appropriately. Together, these strategies allow semi-prep chromatography to increase throughput and support impurity isolation while maintaining the selectivity and robustness established during analytical method development.

REFERENCES:

1. Mayo Clinic. (2026, June 1). Itraconazole (oral route) – Side effects & dosage. Mayo Clinic. https://www.mayoclinic.org/drugs-supplements/itraconazole-oral-route/description/drg-20071421