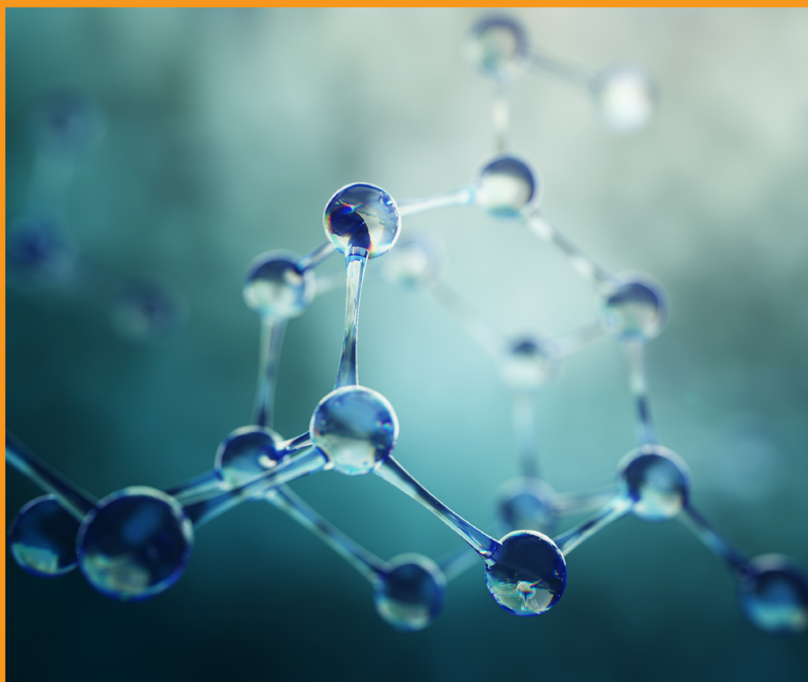
HALO®

TECHNICAL REPORT: AMT-TR12_20

TITLE: **HALO® RESOLUTION, SENSITIVITY AND STABILITY FOR METHOD SCREENING**

MARKET SEGMENT: PHARMACEUTICAL



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ABSTRACT

This technical note demonstrates the high resolving power offered by six columns from the HALO® reversed phase portfolio in the analytical scale 4.6 mm internal diameter (i.d.). Subsequently, the long-term stability is highlighted for the C18 phase at >8,000 column volumes (under acidic and buffered conditions) on a reduced column format (2.1 mm i.d.). This leads to a reduction of both solvent consumption and waste by up to 80%, while maintaining high resolution.

INTRODUCTION

Column screening studies targeting the separation of a critical pair are often performed with more than one column of a similar format in order to determine which column chemistry provides sufficient resolution. Ideally, the selected column is also tested for stability to determine the lifetime of the column and ensure reproducibility of the results throughout method development and validation stages of the final assay.

This technical note outlines the ability to resolve critical peak pairs with superior resolution and conduct rigorous column lifetime studies in acidic and buffered environments while saving solvent, time and money without sacrificing column performance.

KEY WORDS:

HALO®, method screening, stability, critical pair, small molecule, pharmaceutical

EXPERIMENTAL DATA:

All experiments were run on a Shimadzu Nexera HPLC instrument (Columbia, MD) using a UV diode array detector (1 µL flow cell) set at a wavelength of 254 nm, and LabSolutions software (Shimadzu).

For the resolution study: six different reversed phase stationary phases with the following properties were tested: 4.6×150 mm column format, 2.7 micron (µm), 90 Å superficially porous particle (SPP) packed columns. The stationary phases characterized in this study were as follows: C18, Biphenyl, AQ-C18, pentafluorophenyl (PFP), RP-Amide and Phenyl-Hexyl HALO® columns from Advanced Materials Technology, Inc. (Wilmington, DE). Separation conditions were as follows: mobile phase A: 0.05% trifluoroacetic acid (TFA) in water and mobile phase B: 0.05% TFA in acetonitrile; gradient conditions: 3-95% B from 0.0 to 11.7 min, held at 95% B to 12.0 min, return to initial conditions (3%B) from 12.0-12.5 min and held at 3% B for an additional two minutes. Injection volume: 3 µL, temperature 40 ºC, flowrate at 1.5 mL/min and UV wavelength set at 260 nm.

For the stability study a 2.1×150 mm column format, 2.7 micron (µm), 90 Å superficially porous particle packed C18 column was used from Advanced Materials Technology, Inc. (Wilmington, DE). The first 230 injections were performed with similar conditions as the resolution study except the concentration of the modifier in both mobile phase A and B was doubled to a concentration of 0.1% TFA and the flowrate was set to 0.3 mL/min. Injection volume: 3 µL, temperature 40 ºC, and UV wavelength set at 260 nm.

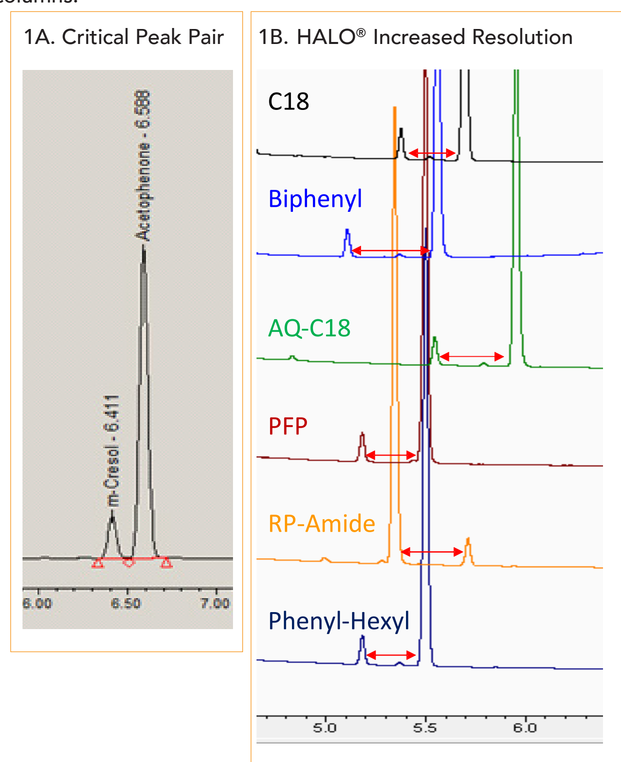
Subsequently 100 column volumes were eluted with 50/50 mobile phase A: 10 mM potassium phosphate buffer (pH 7.2) and mobile phase B: acetonitrile. Then, 230 injections were performed under the 10 mM potassium phosphate buffer separation conditions as follows: 3-60% B from 0-15.7 min and held at 50% until 24.75 min and returned to initial gradient conditions (3%B in 0.5 min) and held at 3% B for an additional two minutes. Injection volume: 3 µL, temperature 40 ºC, flowrate at 0.3 mL/min and UV wavelength set at 260 nm.

RESULTS:

Resolution and sensitivity

A representative critical peak pair in Figure 1 (A) illustrates the baseline separation of m-cresol and acetophenone achieved via a fully porous particle packed column. This same target peak pair was separated using the same fixed conditions on different selectivities within the HALO® reversed-phase range Figure 1 (B).

Figure 1. Critical peak pair easily resolved when employing HALO® columns.



All of the HALO® alternatives provided an increased distance between the two peaks denoted by the red markers; showcasing the high resolving power of each of the different selectivities. Furthermore, each of the HALO® columns differed in their interactions resulting in different retention times; proving to be ideal options to tune the selectivity of separations by tuning the selectivity of the column. This becomes paramount in situations when target analytes require a difference in retention times, such as when similar structured analytes from the sample matrix co-elute with the target peaks. Hence, there is a difference in interactions while maintaining high performance. For example, in the case of m-cresol and acetophenone, when compared to the C18 HALO® column the RP-Amide experienced the largest difference in selectivity with an elution order change.

An added benefit of the high resolving power is the ability to resolve and detect extra peaks that may be unknown impurities/degradants of the standards, artefacts of the mobile phase and/or the mobile phase additives. For example, low abundant species that can often be left un-detected, have been masked in the baseline noise of the separation or co-elute within the main peak.

Though the chromatogram 'looks busier' with extra peaks for the HALO® separations; this is a beneficial characteristic demonstrating the column's ability to improve the sensitivity without having to employ an expensive detector (e.g. mass spectrometry). This is critical because as many minor peaks

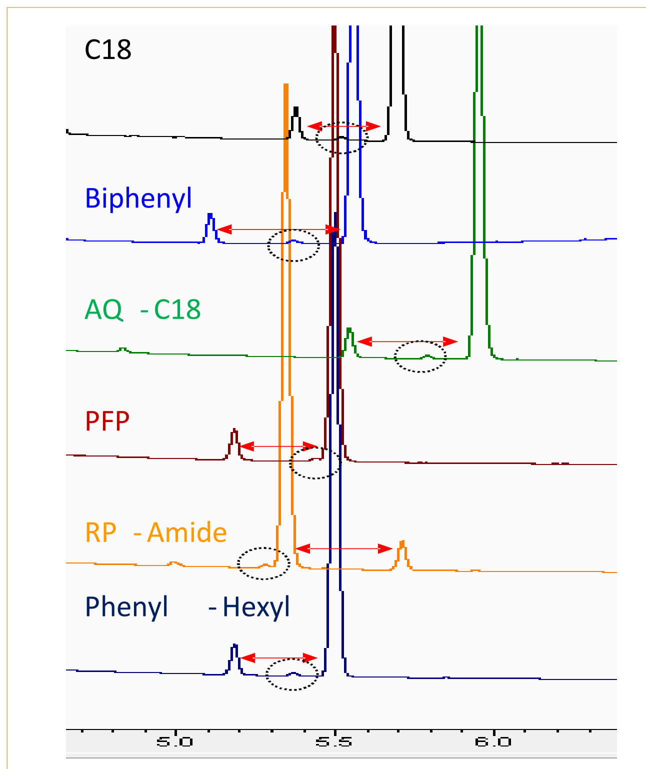
as possible need to be identified and resolved from the target/main peaks as it may complicate future drug development/manufacturing stages. For example, if they are compounds that interfere with the drug's stability, efficacy, or in the worst-case scenario have adverse effects, it is best to have gained the information during early development stages. Hence, the analytical method must employ the best tools available in terms of resolution, sensitivity and stability.

In Figure 2, the unknown peak is circled to highlight this advantage of improved sensitivity of low abundant peaks with SPP column technology. HALO® columns provide an alternative, cost-effective, plug and play strategy to improve sensitivity and resolution that does not require upgrading any hardware on the conventional 400 bar instrumentation.

Figure 2. Additional benefit of resolving and detecting low abundant peaks.

HALO® increased sensitivity

- Critical pair - well resolved
- Extra peak resolved



Stability

This study was performed with a HALO® C18 column and downscaled from 4.6 to a 2.1 mm internal diameter (i.d.) format to save on mobile phase consumption. Downscaling from a 4.6 mm to a 2.1 mm i.d. enabled the flowrate to be reduced from 1.5 mL/min to 0.3 mL/min hence only one fifth of the mobile phase consumption/waste generation was required. A total of 8,380 column volumes eluted in this study. Performing this on a 4.6 mm i.d. would have required >12 L of mobile phase whilst, conducting it on a 2.1 mm i.d. only 2.4 L was required.

The separation profiles were visually reproducible with a slight baseline shift. The chromatograms are illustrated in Figures 3 and 4 for the different mobile phase conditions and bracketing 100 and 110 injections, respectively.

Figure 3. 0.1% TFA conditions bracketing 100 injections (1,250 column volumes).

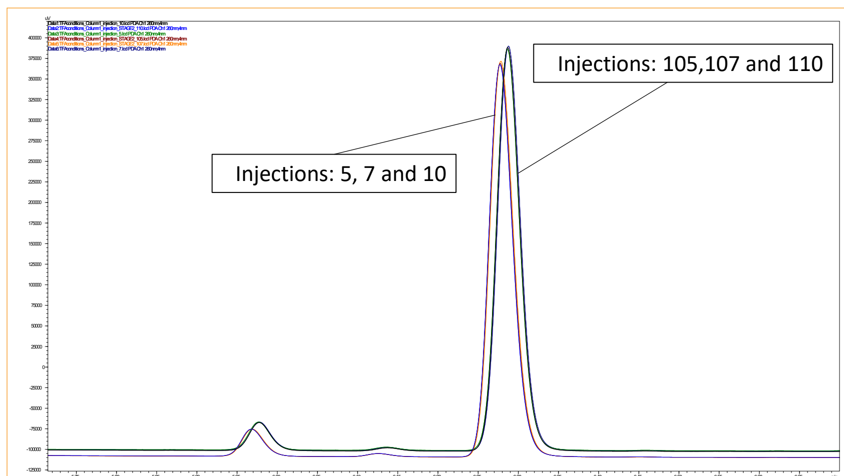
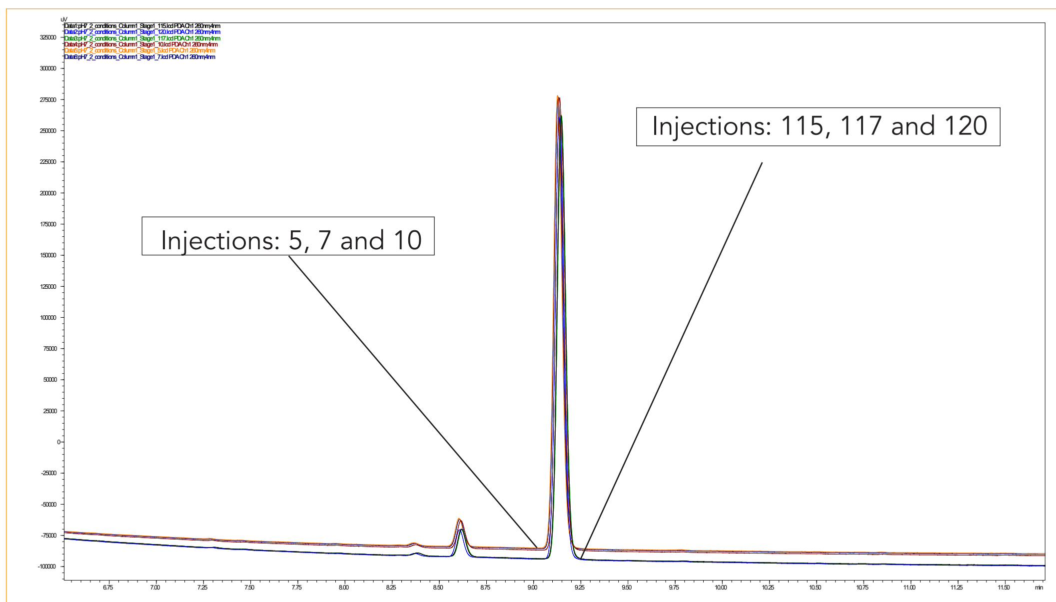


Figure 4. 10 mM potassium phosphate buffer conditions bracketing 110 injections (approximately 2,600 column volumes).



The reproducibility in terms of %RSD for key chromatography metrics are listed in Table 1; despite a baseline shift occurring, this did not have detrimental effect on the results. The retention time had a very small %RSD of ≤0.11, a critical figure of merit especially for applications where the retention time window for identifying a peak is very narrow.

Overall, the %RSD was kept below 5% for the peak area; the largest %RSD of 4.56 under the 0.1% TFA conditions and improved to 3.34 for the 10 mM phosphate buffer at pH 7.2 mobile phase conditions. The resolution deviation demonstrated the separation attributes of the column did not deviate significantly with a %RSD ≤1.9; associated to the column's retention, selectivity and column performance variability.

Table 1. %RSD reproducibility

	Retention time	Peak area	Resolution
	0.1% TFA mobile phase conditions		
m-cresol	0.09	3.89	1.90
acetophenone	0.11	4.56	1.70
	Mobile phase buffered at pH 7.2 conditions		
m-cresol	0.10	3.34	1.10
acetophenone	0.09	1.16	1.00

CONCLUSION:

This technical note outlined the ability for the HALO® reversed phase columns to provide superior resolution, sensitivity and stability advantages which are critical for analytical methods in the pharmaceutical industry. A critical peak pair could easily be separated relative to a fully porous particle packed column. Additionally, the ability to downscale stability assays by simply reducing the internal diameter from 4.6 to 2.1 mm i.d. saved 80% of the mobile phase consumption. The retention time %RSD did not deviate above 0.11 and the resolution 1.90; highlighting the column's attributes for developing analytical methods with superior column technology with over 8,000 column volumes that employed different mobile phase environments (0.1% TFA followed by 10 mM phosphate (pH 7.2) conditions).

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