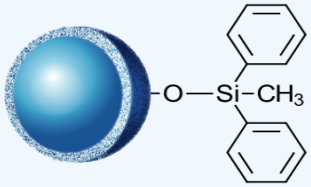
The HALO® BioClass Diphenyl Phase: Discussion and Best Uses

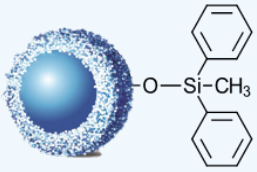
Advanced Materials Technology offers two diphenyl bondings on the well-known superficially porous particle. The new HALO 400 Å Diphenyl, 3.4 µm column and 1000 Å Diphenyl, 2.7 µm columns are primarily used for protein and monoclonal antibody analysis due to its larger pore size. The columns provide narrow peak shapes and better sample recoveries for large biomolecules that range from 2kDa and higher when compared to smaller pore sizes and fully porous particles. The new 3.4 µm superficially porous particle consists of a 3 µm core and a 0.2 µm shell with 400 Å pores while the 2.7 µm superficially porous particle consists of a 1.7 µm core and a 0.5 µm shell with 1000 Å pores. Figure 1 below shows a comparison of the two particles.

Figure 1. HALO® BioClass Diphenyl Options

HALO 400 Å Diphenyl, 3.4 µm Particle

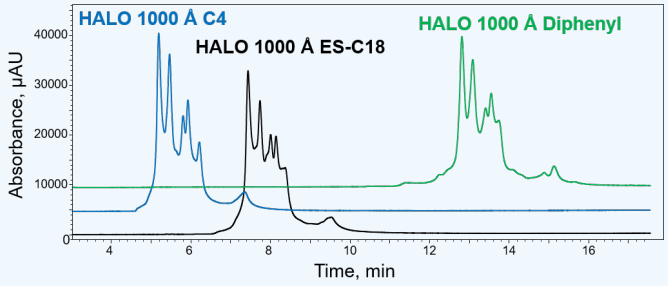


HALO 1000 Å Diphenyl, 2.7 µm Particle



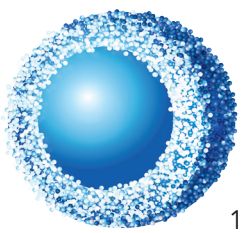
The diphenyl phase offers a unique selectivity to help separate complex samples such as IgG1 and IgG2 monoclonal antibodies. Figure 2 shows a comparison resolving denosumab isoforms (mAb used to help treat bone cancer) on three different 1000 Å bonded phases. The diphenyl phase is retained the longest. While there are minor differences for this IgG2 in this particular comparison, since biopharmaceutical production involves designing custom mAbs with particular characteristics, screening multiple bonded phases could reveal important differences. This is especially true when looking at protein variants.

Figure 2. IgG2 Comparison On HALO 1000 Å C4, ES-C18, And Diphenyl



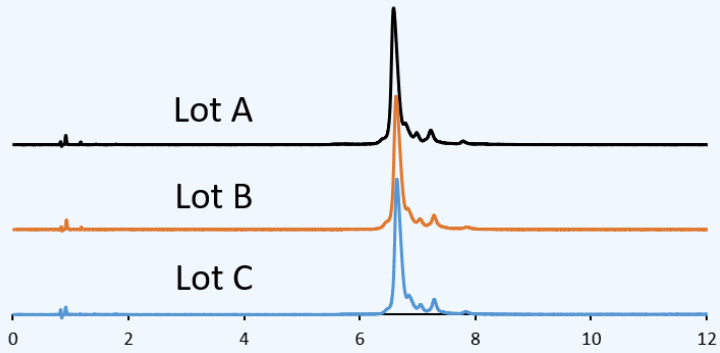
TEST CONDITIONS

Columns: HALO 1000 Å C4, 2.7 µm, 2.1 x 150 mm
HALO 1000 Å ES-C18, 2.7 µm, 2.1 x 150 mm
HALO 1000 Å Diphenyl, 2.7 µm, 2.1 x 150 mm
Flow Rate: 0.2 mL/min
Temperature: 80°C
Injection Volume: 2 µL of 2 mg/mL denosumab in water/0.1% TFA
Instrument: Shimadzu Nexera
Detection: PDA at 280 nm
Mobile Phase A: 2:10:88 n-propanol/ACN/H2O + 0.1% difluoroacetic acid (DFA)
Mobile Phase B: 70:20:10 n-propanol/ACN/H2O + 0.1% DFA
Gradient: 16-26% B in 20 min



Both 400 and 1000 Å Diphenyl phases show excellent lot to lot reproducibility in order to maintain reliable and repeatable results for the user. Tight manufacturing processes used by Advanced Materials Technology ensure that the highest quality column performance is achieved. For example, Figure 3 shows trastuzumab (mAb used to treat breast cancer) on three different lots of HALO 1000 Å Diphenyl. Resolution of minor components are repeatable along with the retention time of the IgG1 monoclonal antibody.

Figure 3. Lot-To-Lot Reproducibility Of HALO 1000 Å Diphenyl

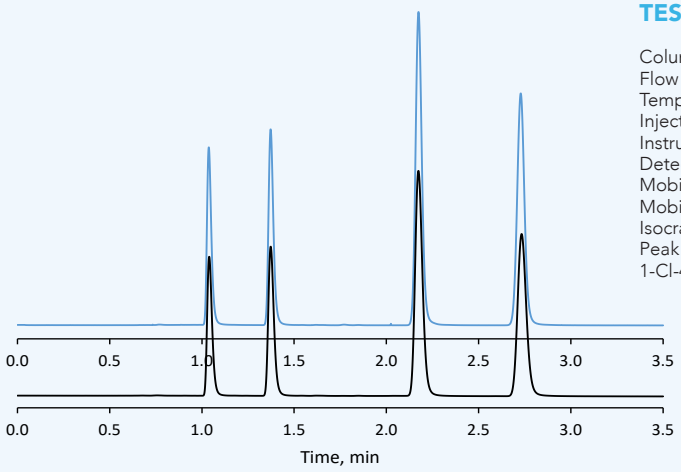


TEST CONDITIONS

Column: HALO 1000 Å Diphenyl, 2.7 µm, 2.1 x 150 mm
Flow Rate: 0.4 mL/min
Temperature: 80 °C
Injection Volume: 2 µL of 2 mg/mL trastuzumab in water/0.1% TFA
Instrument: Shimadzu Nexera
Detection: PDA at 280 nm
Mobile Phase A: water/0.1% TFA
Mobile Phase B: 80/20 ACN/ Water/0.085% TFA
Gradient: 40-47.5% B in 12 min

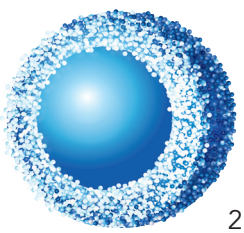
Both 400 Å and 1000 Å Diphenyl columns are also very stable allowing for long column lifetimes. Figure 4 shows the results of a high-pressure stability test using neutral compounds on a HALO 400 Å Diphenyl column. The column has experienced 10,000 column volumes at 600 bar and maintained its peak shape, retention, and held a consistent back pressure.

Figure 4. HALO 400 Å Diphenyl Stability



TEST CONDITIONS

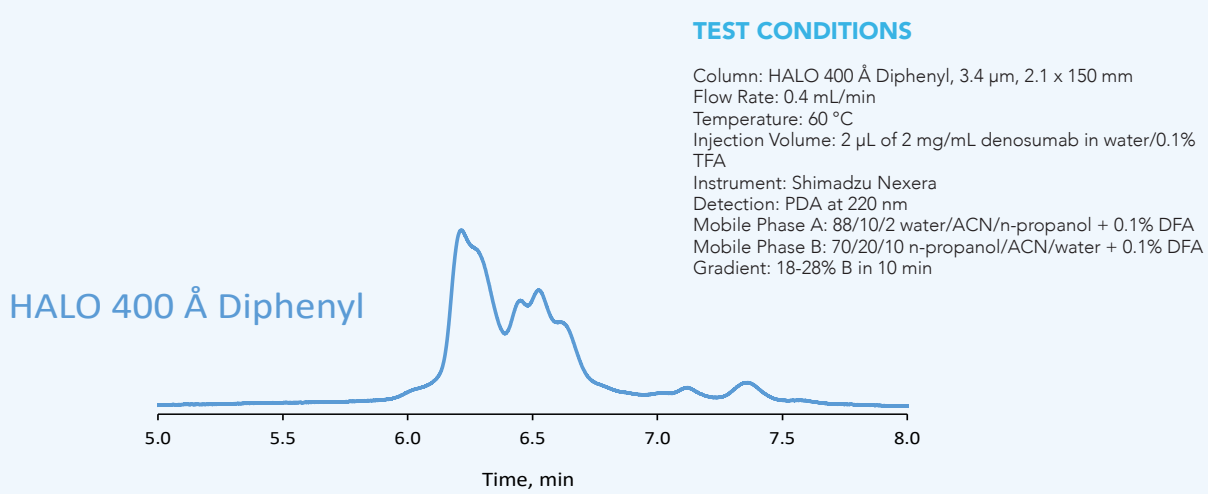
Column: HALO 400 Å Diphenyl, 3.4 µm, 2.1 x 150 mm
Flow Rate: 0.3 mL/min
Temperature: 30 °C
Injection Volume: 0.5 µL
Instrument: Shimadzu Nexera
Detection: PDA at 254 nm
Mobile Phase A: water
Mobile Phase B: ACN
Isocratic: 75/25 A/B
Peak Identities: uracil, phenol, propiophenone, 1-Cl-4-nitrobenzene (in elution order)



HALO 400 Å DIPHENYL: BEST USES

For analysts whose goal is high throughput and/or less emphasis on ultimate resolution yet looking for the critical quality attributes of their biopharmaceutical in the shortest amount of time, the larger particle size and thinner shell of the HALO 400 Å Diphenyl may be used to their advantage while developing release assay methods. The HALO 400 Å Diphenyl with 3.4 µm particle size will have lower overall back pressures when compared to the 1000 Å, 2.7 µm particle or a sub-2 µm fully porous particle. Figure 5 shows a fast analysis of denosumab in under 8 minutes on the HALO 400 Å Diphenyl column. Shorter run times allow reduced mobile phase consumption and higher throughput.

Figure 5. Fast Separation Of Denosumab On HALO 400 Å Diphenyl



If resolution is more important than speed, then the method may be adjusted accordingly. The 400 Å Diphenyl phase shows excellent resolution for monoclonal antibodies when compared to similar columns on the market. For example, denosumab was analyzed again on a HALO 400 Å Diphenyl column compared to a 300 Å fully porous diphenyl column. The HALO 400 Å column outperformed the 300 Å fully porous diphenyl column by providing much better resolution at 2.5x lower back pressure along with a shorter analysis time. See Figure 6.

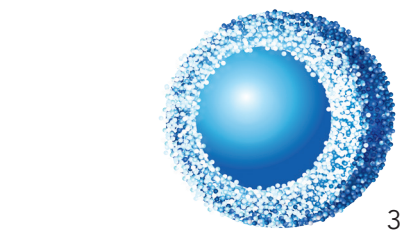
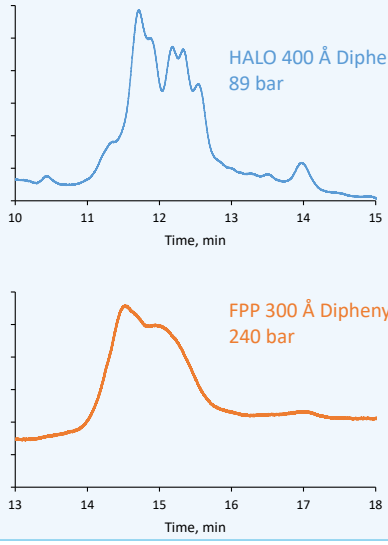


Figure 6. Increased Resolution And Lower Back Pressure with HALO 400 Å Diphenyl Compared To An FPP 300 Å Diphenyl



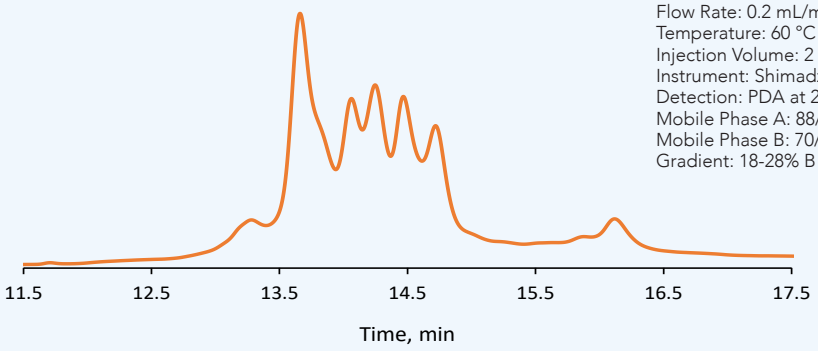
TEST CONDITIONS

Columns: HALO 400 Å Diphenyl, 3.4 µm, 2.1 x 150 mm
FPP 300 Å Diphenyl, 1.8 µm, 2.1 x 150 mm
Mobile Phase A: 88/10/2 water/ACN/n-propanol + 0.1% DFA
Mobile Phase B: 70/20/10 n-propanol/ACN/water + 0.1% DFA
Gradient: 18-28% B in 20 min
Flow Rate: 0.2 mL/min
Temperature: 60 °C
Injection Volume: 2 µL of 2 mg/mL denosumab in water/0.1% TFA
Instrument: Shimadzu Nexera
Detection: PDA at 220 nm

HALO 1000 Å DIPHENYL: BEST USES

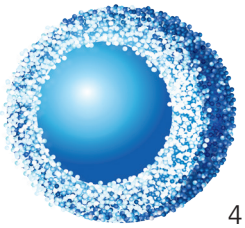
For the ultimate resolution of monoclonal antibodies or other large proteins, HALO 1000 Å Diphenyl phase is recommended. The large pores allow unrestricted access of mAbs to the bonded phase, while the higher surface area/thicker shell enables high resolution separations of various mAb isoforms. Figure 7 shows a high resolution separation of denosumab.

Figure 7. High Resolution Separation Of Denosumab On HALO 1000 Å Diphenyl Column



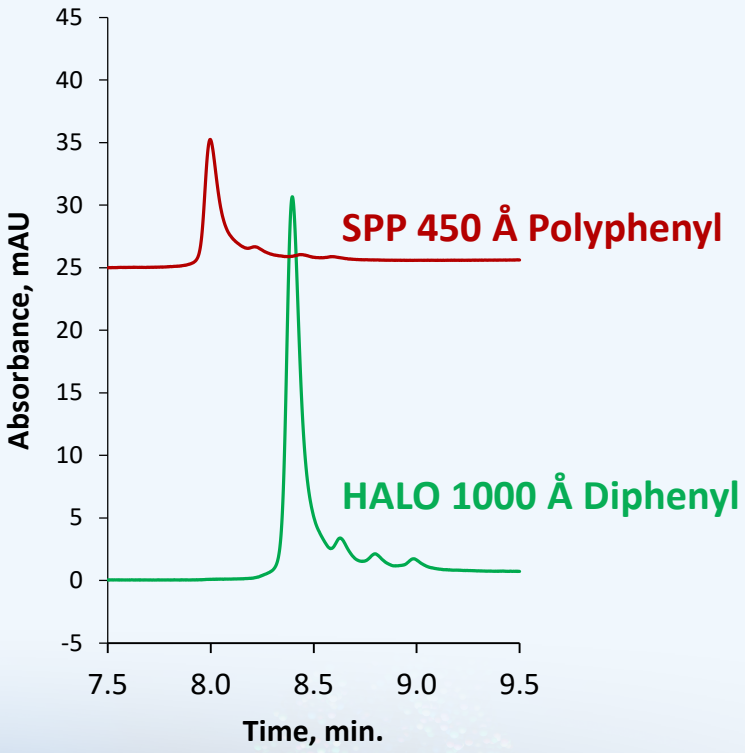
TEST CONDITIONS

Column: HALO 1000 Å Diphenyl, 2.7 µm, 2.1 x 150 mm
Flow Rate: 0.2 mL/min
Temperature: 60 °C
Injection Volume: 2 µL of 2 mg/mL denosumab in water/0.1% TFA
Instrument: Shimadzu Nexera
Detection: PDA at 220 nm
Mobile Phase A: 88/10/2 water/ACN/n-propanol + 0.1% DFA
Mobile Phase B: 70/20/10 n-propanol/ACN/water + 0.1% DFA
Gradient: 18-28% B in 20 min



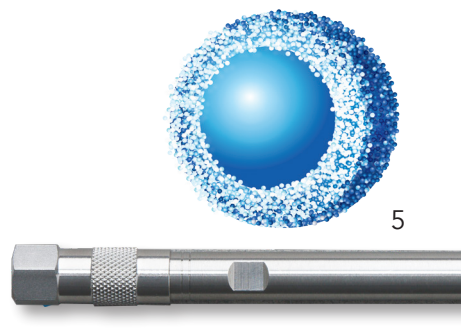
Both HALO 400 Å and 1000 Å Diphenyl columns are stable up to 90 °C for high temperature separations. It is generally accepted that sample recovery of proteins and mAbs improves as temperature increases, therefore temperatures of 60 °C or greater are often used to maximize sample recovery. However, temperature related artifacts have been observed when operating at these higher temperatures. Because of this, a temperature gradient is recommended and operating at lower temperature may prove desirable. A comparison of trastuzumab (Figure 8) at 40 °C on both a HALO 1000 Å Diphenyl column and a 450 Å SPP Polyphenyl column shows impressive protein recovery with the HALO® column. The HALO® Diphenyl column also demonstrates improved resolution, retention, and peak area compared to the competitor SPP column. This increased retention and resolution clearly demonstrate the benefit of unrestricted large pore access to the bonded phase and that high recoveries are possible with lower operating temperatures.

Figure 8. Comparison of HALO 1000 Å Diphenyl to Competitor Polyphenyl at 40 °C



TEST CONDITIONS

Columns: HALO 1000 Å Diphenyl, 2.7 µm, 2.1 x 150 mm
SPP 450 Å PolyPhenyl, 2.7 µm, 2.1 x 150 mm
Mobile Phase A: water/0.1% TFA
Mobile Phase B: ACN/0.1% TFA
Gradient: 30-45% B in 15 min
Flow Rate: 0.4 mL/min
Temperature: 40 °C
Injection Volume: 2 µL of 2 mg/mL trastuzumab in water/0.1% TFA
Instrument: Shimadzu Nexera
Detection: PDA at 280 nm



CONCLUSIONS

For biopharmaceutical separation scientists, the HALO 400 Å and 1000 Å Diphenyl columns are two beneficial additions to the protein chemist's separation toolbox for mAb release assays and characterization methods. The HALO® Diphenyl offers a unique selectivity compared to C4 and C18 and demonstrates excellent stability with good sample recovery while outperforming the competitors to deliver quality and performance – every time.