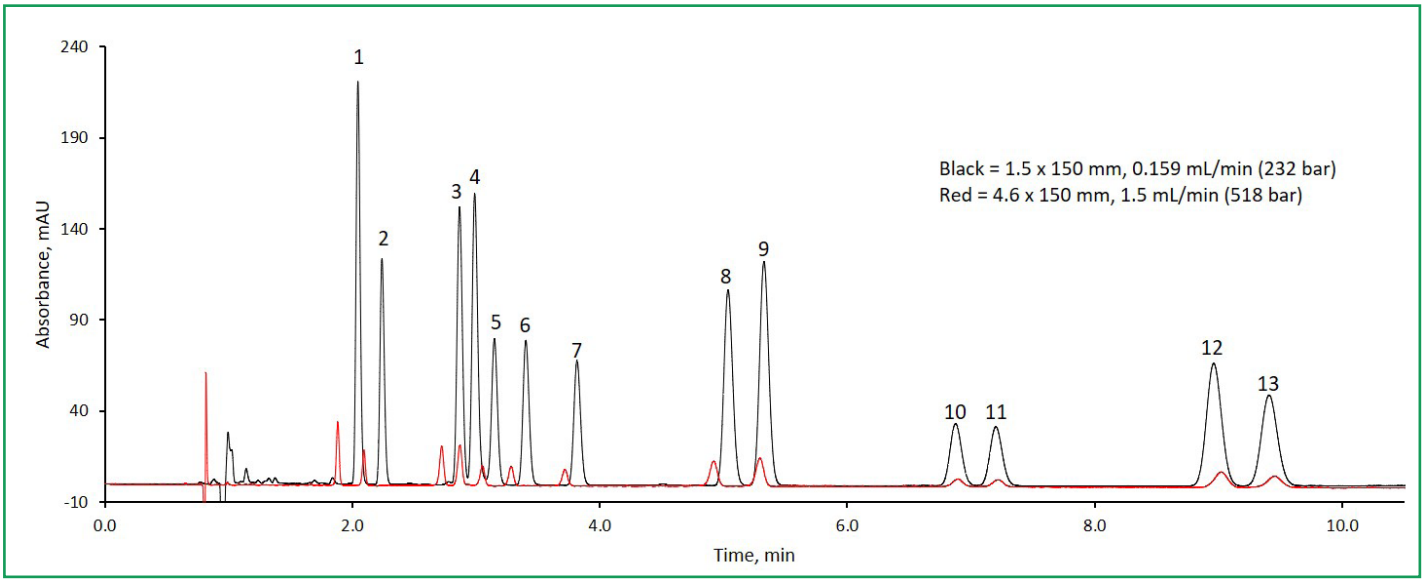
HALO®

CANNABIS

Sensitivity and Solvent Savings using a 1.5 mm ID Column with Cannabinoids

283-CN



PEAK IDENTITIES

1. CBDVA
2. CBDV
3. CBDA
4. CBGA
5. CBG
6. CBD
7. THCV
8. THCVA
9. CBN
10. 9-THC
11. 8-THC
12. CBC
13. THCA

TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 1.5 x 150 mm
Part Number: 9281X-702
Column: HALO 90 Å C18, 2.7 µm, 4.6 x 150mm
Mobile Phase A: Water/ 0.1% Formic Acid
Mobile Phase B: Acetonitrile/ 0.1% Formic Acid
Isocratic: 75% B
Flow Rate: 0.159 mL/min (1.5x150)
Flow Rate: 1.5 mL/min (4.6x150)
Temperature: 30 °C
Detection: UV 228 nm, PDA
Injection Volume: 0.5 µL
Sample Solvent: 75/25 ACN/ Water
Data Rate: 100 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

A separation of cannabinoids is performed on a HALO 90 Å C18 column. Switching from a 4.6 mm ID to a 1.5 mm ID column diameter increases overall sensitivity along with significantly reducing solvent consumption. The extra column volume has been reduced by optimizing the pre/post-column tubing as well as the flow cell. This makes the 1.5 mm ID column an ideal candidate for increased sensitivity without the investment into a specialized low flow HPLC system.

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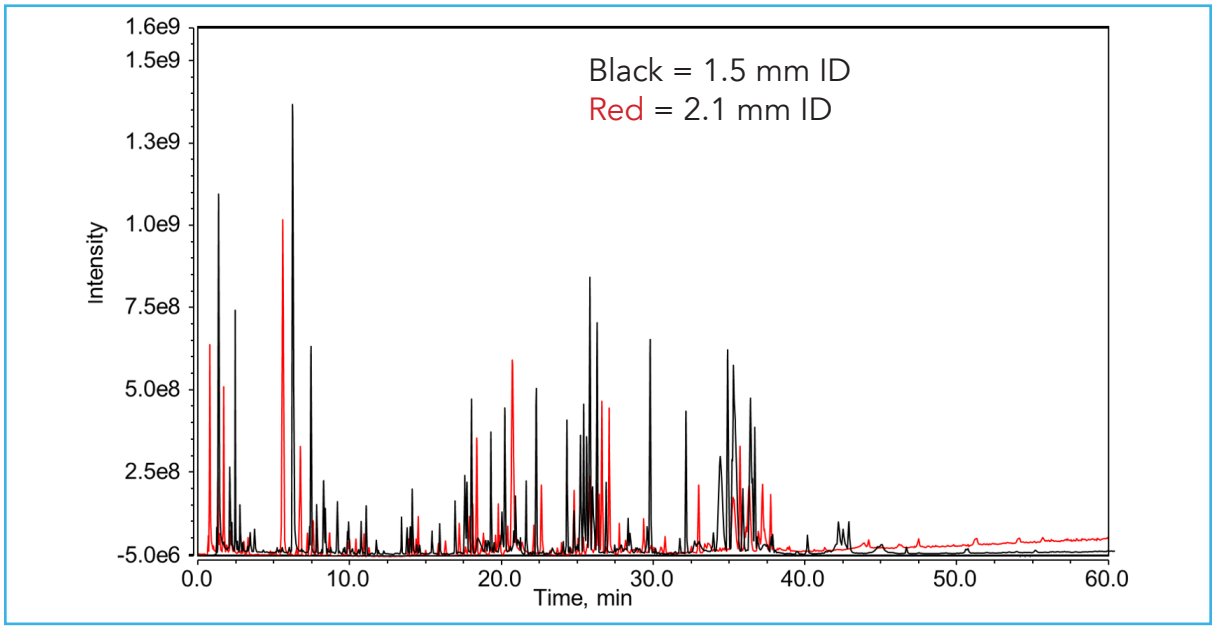
BIOPHARMACEUTICALS

Increased Sensitivity and Solvent Savings of Trastuzumab Tryptic Digest using a 1.5 mm ID Column

284-BIO



TEST CONDITIONS:

Column: HALO 160 Å ES-C18, 2.7 µm, 1.5 x 150 mm
Part Number: 9212X-702
Column: HALO 160 Å ES-C18, 2.7 µm, 2.1 x 150 mm
Mobile Phase A: Water/0.1% DFA
B: Acetonitrile/0.1% DFA

Gradient:

Time (min)	%B
0.0	2
60.0	50

Flow Rate: 0.2 mL/min for 1.5 mm ID
0.4 mL/min for 2.1 mm ID
Back Pressure: 310 bar (1.5 mm)
444 bar (2.1 mm)
Temperature: 60 °C
Detection: ESI +
Injection Volume: 2 µL of 1.25 mg/mL trastuzumab tryptic digest
Sample Solvent: 1.5 M guanidine HCl/0.5% formic acid
LC System: Shimadzu Nexera X2
MS System: ThermoFisher Q Exactive

MS CONDITIONS:

Spray Voltage (kV): 3.8
Capillary temperature: 320 °C
Sheath gas: 35
Aux gas: 10
RF lens: 50

A separation of Trastuzumab tryptic digest is performed on a HALO 160 Å ES-C18 column using a ThermoFisher Q Exactive. By switching from a 2.1 mm ID to a 1.5 mm ID column there is an increase in overall sensitivity along with a significant reduction in solvent consumption highlighted with a long analysis time, such as with a peptide map. Extra column volume was reduced by optimizing the tubing from the column outlet to the MS source. The use of a 1.5 mm ID column delivers an increase in sensitivity and reduces solvent usage without having to invest into a specialized micro flow HPLC system.

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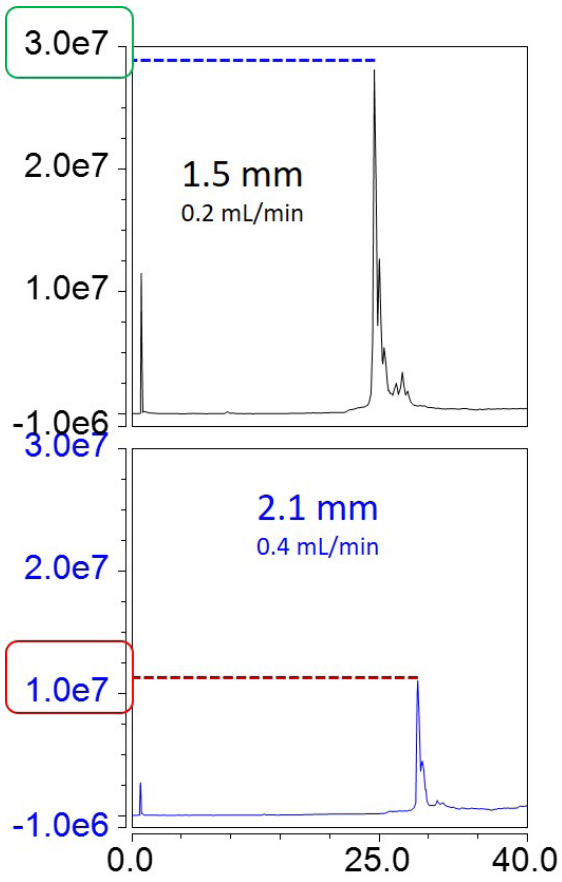
BIOPHARMACEUTICALS

Increased Sensitivity of Intact Trastuzumab Using a 1.5 mm ID Column

285-BIO



TEST CONDITIONS:

Column: HALO 1000 Å Diphenyl, 2.7 µm, 1.5 x 150 mm
Part Number: 9212X-702
Column: HALO 1000 Å Diphenyl, 2.7 µm, 2.1 x 150 mm
Mobile Phase A: Water/0.1% DFA
B: 50% Acetonitrile/50% n-propanol/0.1% DFA
Gradient: 27-36 %B in 40 min
Flow Rate: 0.2 mL/min for 1.5 mm ID
0.4 mL/min for 2.1 mm ID
Back Pressure: 252 bar (1.5 mm)
272 bar (2.1 mm)
Temperature: 60 °C
Injection Volume: 3 µL of 1.0 mg/mL trastuzumab
Sample Solvent: 100 mM ammonium bicarbonate
LC System: Shimadzu Nexera X2
MS System: ThermoFisher Q Exactive

MS CONDITIONS:

Spray Voltage (kV): 3.8
Capillary temperature: 320 °C
Sheath gas: 35
Aux gas: 10
RF lens: 50

A separation of intact Trastuzumab was performed on a HALO 1000 Å Diphenyl column. The switch from a 2.1 mm ID column to a 1.5 mm ID allows for an increase in sensitivity and reduces overall solvent consumption. In this example both peak intensity and area are increased. This sensitivity was acheived by optimizing the post-column tubing. The 1.5 mm ID column is ideal for achieving more performance from a UHPLC system saving on the investment of a specialized low flow HPLC.

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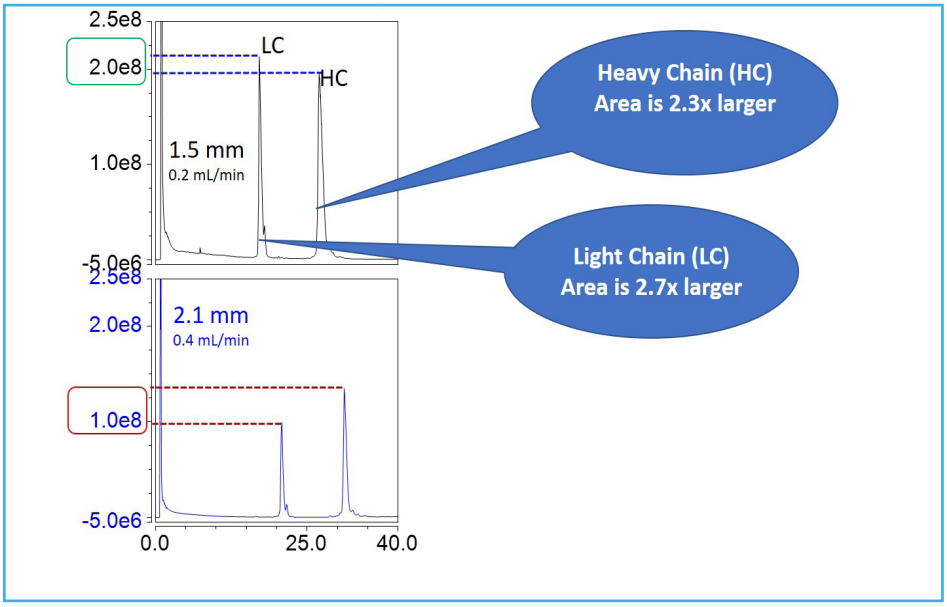
BIOPHARMACEUTICALS

Demonstration of Increased Sensitivity with Reduced Trastuzumab Using a 1.5 mm ID Column

286-BIO



PEAK IDENTITIES

LC = Light Chain
HC = Heavy Chain

TEST CONDITIONS:

Column: HALO 1000 Å Diphenyl, 2.7 µm, 1.5 x 150 mm
Part Number: 9212X-702
Column: HALO 1000 Å Diphenyl, 2.7 µm, 2.1 x 150 mm
Mobile Phase A: Water/0.1% DFA
B: 50% Acetonitrile/50% n-propanol/0.1% DFA

Gradient:

Time (min)	%B
0.0	27
40.0	36
40.1	27
45.0	27

Flow Rate: 0.2 mL/min for 1.5 mm ID
0.4 mL/min for 2.1 mm ID
Back Pressure: 252 bar (1.5 mm)
272 bar (2.1 mm)
Temperature: 60 °C
Injection Volume: 3 µL of 1.0 mg/mL reduced and alkylated trastuzumab
Sample Solvent: Water/0.1% TFA
LC System: Shimadzu Nexera X2
MS System: ThermoFisher Q Exactive

MS CONDITIONS:

Spray Voltage (kV): 3.8
Capillary temperature: 320 °C
Sheath gas: 35
Aux gas: 10
RF lens: 50

A separation of reduced and alkylated Trastuzumab is performed on a HALO 1000 Å Diphenyl column. Switching to a 1.5 mm ID column from the 2.1 mm ID provides a significant increase in sensitivity for the reduced and alkylated Trastuzumab along with a reduced flow rate. This increase in sensitivity can be achieved by using a 1.5 mm ID column in conjunction with optimized tubing post-column which provides a cheaper route for increased sensitivity without the investment into a specialized HPLC system.

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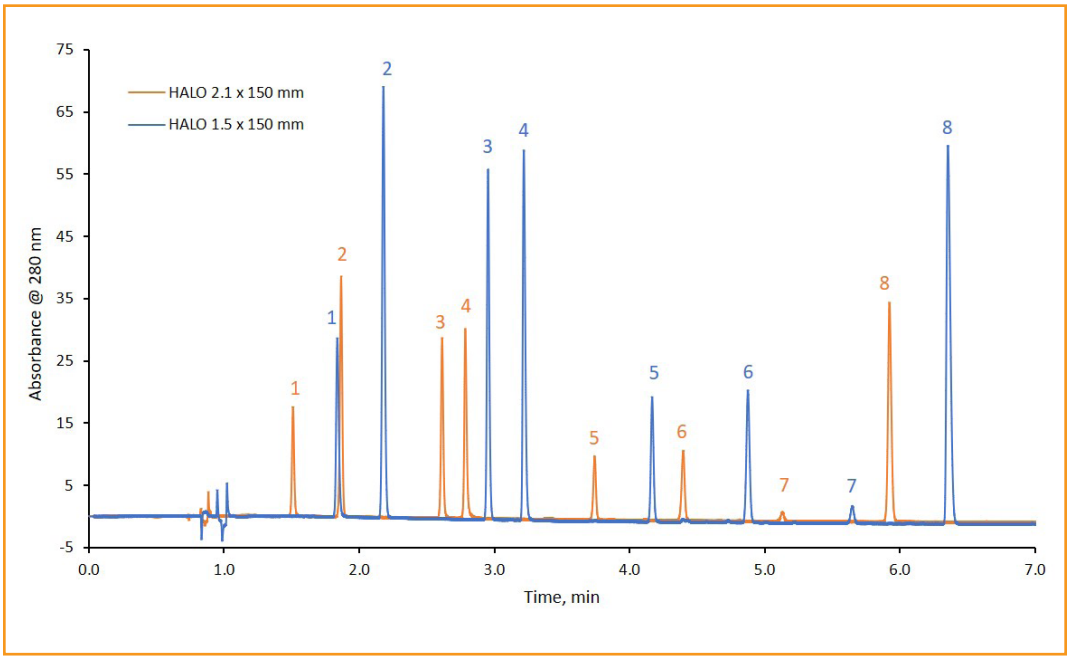
PHARMACEUTICALS

Increased Sensitivity in Small Molecule Applications with Cough & Cold Medications

287-P



PEAK IDENTITIES

1. Phenylephrine
2. Acetaminophen
3. Caffeine
4. Doxylamine
5. Guiafenesin
6. Aspirin
7. Salicylic Acid
8. Dextromethorphan

TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 1.5 x 150 mm
Part Number: 9281X-702
Column: HALO 90 Å C18, 2.7 µm, 2.1 x 150 mm
Mobile Phase A: Water/ 0.15% TFA
Mobile Phase B: ACN/ 0.1% TFA

Gradient:

Time(min)	%B
0.0	5
8.0	50
9.0	100
9.5	5
13.0	5

Flow Rate: 0.2 mL/min for 1.5 mm
0.4 mL/min for 2.1 mm
Pressure: 425 bar/1.5 mm
470 bar/2.1 mm
Temperature: 35 °C
Detection: UV 280 nm, PDA
Injection Volume: 0.5 µL
Data Rate: 100Hz
Response Time: 0.025 sec.
Flow Cell: 1 µL
Instrument: Shimadzu Nexera X2

A separation of eight different small molecules commonly found in cough and cold medicines is performed on a HALO 90 Å C18 column. The comparison shown on the chromatogram illustrates the overall increase in sensitivity when switching from a larger ID column to the smaller 1.5 mm ID column. Extra column volume was reduced by optimizing the post-column tubing. This increase in sensitivity is seen in peak height and area counts, making the switch to a 1.5 mm column ideal for those trying to get more sensitivity out of their UHPLC system without the investment into a micro flow HPLC system.

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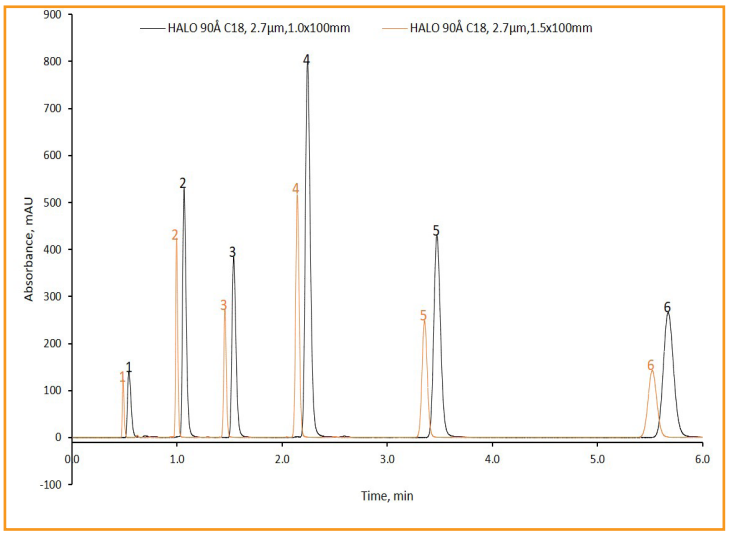
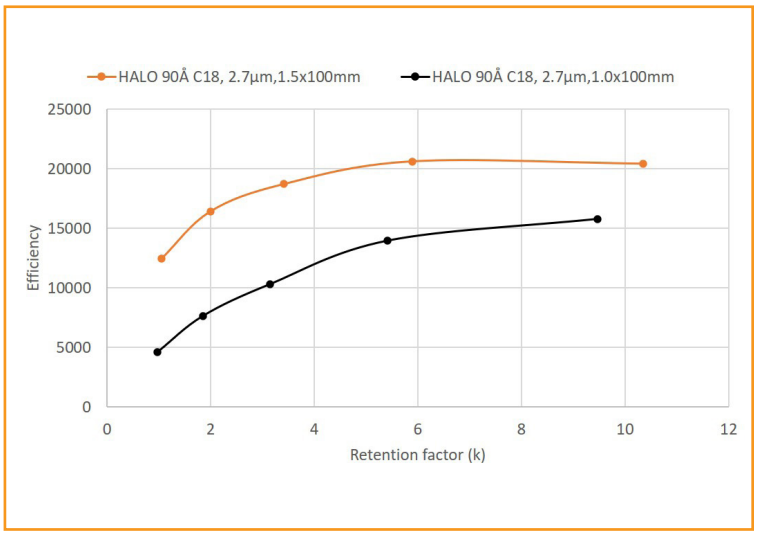
PHARMACEUTICALS

Efficiency of 1.5 mm ID Columns Demonstrated Using Alkylphenones

288-P





TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 1.5 x 100 mm
Part Number: 9281X-602
Column: HALO 90 Å C18, 2.7 µm, 1.0 x 100 mm
Part Number: 92811-602
Mobile Phase A: Water
Mobile Phase B: ACN
Isocratic: 50/50 Water/ACN
Flow Rate: 0.20 mL/min (1.5 mm)
0.09 mL/min (1.0 mm)
Pressure: 236 bar (1.5 mm)
193 bar (1.0 mm)
Temperature: 35 °C
Injection Volume: 0.5 µL
Detection: UV 254 nm, PDA
Instrument: Shimadzu Nexera X2

PEAK IDENTITIES

1. Uracil
2. Acetophenone
3. Propiophenone
4. Butyrophenone
5. Valerophenone
6. Hexanophenone

A separation of alkylphenones was performed on a HALO 90 Å C18 column. The 1.5 mm ID column has increased plate efficiency compared to the 1.0 mm ID column. While the 1.0 mm ID column has increased area compared to the 1.5 mm, this area increase is in width and not completely in peak height. In order to reap the benefits of a 1.0 mm ID column a specialized micro flow HPLC system is needed. The 1.5 mm ID column can give an increase in sensitivity and efficiency without the investment into a specialized system.

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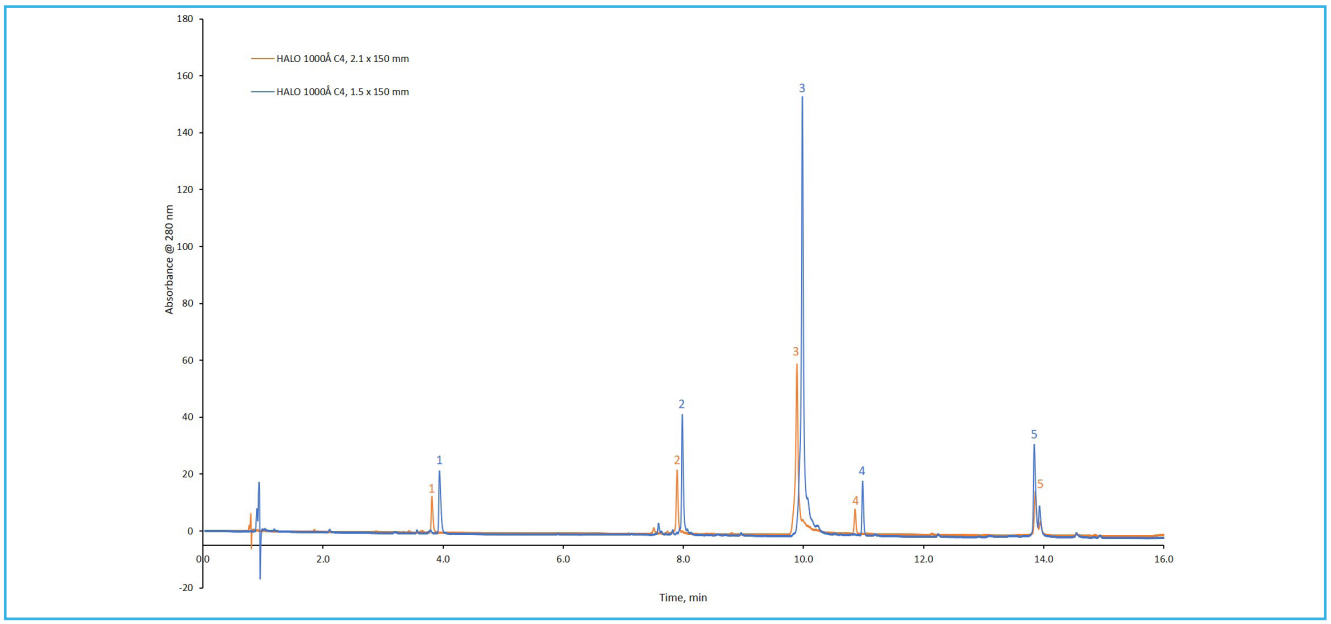
BIOPHARMACEUTICALS

Sensitivity Increase of Mixed Proteins Through the Use of a 1.5 mm ID column

289-BIO



TEST CONDITIONS:

Column: HALO 1000 Å C4, 2.7 µm, 1.5 x 150 mm
Part Number: 9271X-714
Column: HALO 1000 Å C4, 2.7 µm, 2.1 x 150 mm
Mobile Phase A: Water/ 0.1% TFA
Mobile Phase B: 80/20 ACN/Water / 0.1% TFA
Gradient:

Time (min)	%B
0.00	24
15.00	57
16.00	100
17.00	100
18.00	24

Flow Rate: 0.2 mL/min for 1.5 mm
0.4 mL/min for 2.1 mm
Pressure: 228 bar/1.5 mm
264 bar/2.1 mm
Temperature: 80 °C
Detection: UV 280 nm, PDA
Injection Volume: 2.0 µL
Sample Solvent: Water
Data Rate: 40 Hz
Response Time: 0.050 sec.
Flow Cell: 1µL
Instrument: Shimadzu Nexera X2

PEAK IDENTITIES

1. Ribonuclease A
2. Lysozyme
3. SiluLite Sigma mAb
4. Alpha-lactalbumin
5. Enolase

A mix of proteins was separated using a HALO 1000 Å C4 column. The switch from a 2.1 mm to a 1.5 mm ID column gives a significant overall increase in sensitivity while maintaining similar test conditions. Optimization of the post-column tubing reduced the extra column volumes for this experiment. The 1.5 mm ID column can deliver an increase in sensitivity for separations without the investment of a specialized micro flow HPLC system.

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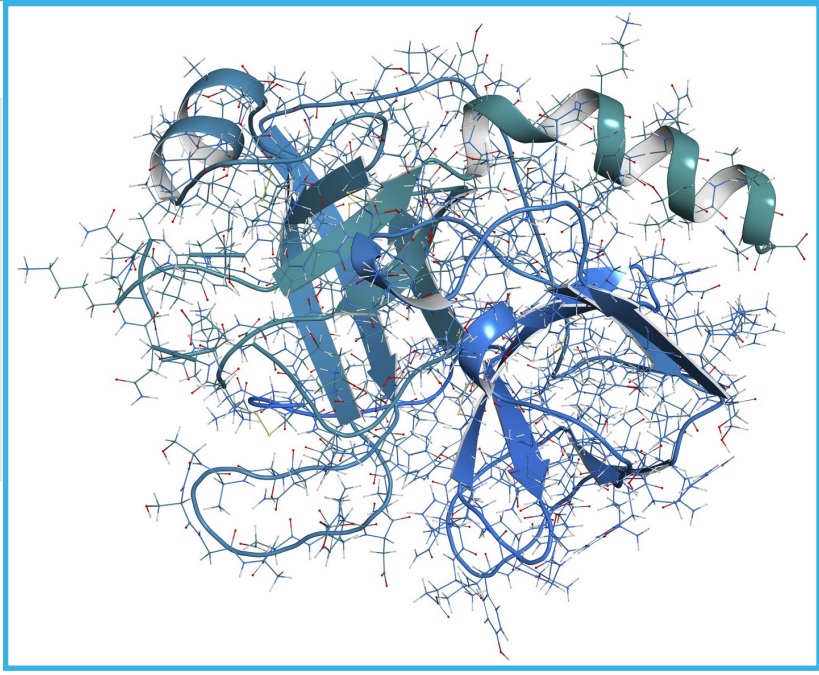
TECHNICAL REPORT

TITLE: **ENHANCING THE SENSITIVITY OF TOP-DOWN AND BOTTOM-UP PROTEOMIC APPLICATIONS USING THE NEW HALO 1.5 MM ID COLUMN**

MARKET SEGMENT: BIOCLASS



AUTHOR:

Andrew Harron Ph.D., Application Scientist

ABSTRACT

Decreasing the internal diameter of a column can increase detector response, lower solvent consumption, and bring multiple benefits to the overall analysis workflow. This has fueled the development of capillary columns which provide very high performance, but require specialized instruments in order to realize that performance. Here we present the HALO® 1.5 mm ID column, which shows enhanced sensitivity and solvent savings on a conventional UHPLC system. The only modification required to realize this performance is the reduction of extra column volume, which can easily be done by the consumer.

INTRODUCTION

The introduction of smaller diameter columns in HPLC has been an ongoing evolution in the field for a number of decades. Many advances in sensitivity and solvent savings have been realized by switching from a 4.6 mm internal diameter (ID), to a 2.1 mm ID. Typically, scaling down to lower ID column can reduce the flow rate and solvent volume needed to reach the same optimal linear velocity, without increasing run time. In addition, sensitivity gains in the detector can be realized as well, as UV and MS, two of the most widely used detectors in LC, and both are concentration dependent. Therefore, the maximum signal, or peak height for the analyte of interest, is directly related to the maximum concentration of the analyte while it is being analyzed. Thus, when the same sample volume is injected on both a larger ID and a smaller ID column, there is a net increase of sample concentration on the smaller ID column, leading to a greater response and increased peak height. In addition, in the case of electrospray LC-MS applications, higher ionization efficiency is typically found at lower flow rates, allowing for better desolvation, thus increasing ion production and transfer, and therefore ion current. The greater concentration of the sample on the column, coupled with the lower flow rate can enhance the overall sensitivity of the detector.[1-3]

However, there are factors that must be considered when changing to a smaller ID column for analysis, and paramount among them is band broadening due to extra column volume (ECV). ECV, is comprised of the volumes of mobile phase, excluding the column packing, that accumulate as the analyte traverses along the pathway from the injector to the detector. The efficiency of a column separation can be greatly affected by ECV, and can vary from particle size and column dimension, however in general, smaller diameter columns show more of an effect than larger diameter columns. This is problematic for analytes with low retention, often analyzed in high throughput applications. There are ways to minimize the ECV by incorporating minor adjustments to the system, for example optimizing the LC system by replacing the tubing from the column to the detector with low volume tubing, will help to minimize the dispersion.[1-3]

Upon minimization of ECV, the benefits that are offered with a smaller diameter column are increase in signal response, and a decrease in mobile phase usage, benefits that can be extremely useful in proteomics and biobased applications, as long run times are often required, and lack of sensitivity can be a challenge. Here, we present the HALO® 1.5 mm ID column for proteomics analysis, including both top down and bottom-up approaches, demonstrating gains in sensitivity and solvent savings compared to a 2.1 mm ID column.[2-3]

KEY WORDS:

Top-down, Bottom-up, solvent savings, increased sensitivity, HALO 160 Å ES-C18, HALO 1000 Å Diphenyl

EXPERIMENTAL PEPTIDE MAPPING

Column: HALO 160 Å ES-C18, 2.7 µm, 1.5 x 150 mm
Part Number: 9212X-702
Column: HALO 160 Å ES-C18, 2.7 µm, 2.1 x 150 mm
Part Number: 92122-702
Mobile Phase A: Water/0.1% DFA B: Acetonitrile/0.1% DFA
Gradient: 2-50 %B in 60 min
Flow Rate: 0.2 mL/min for 1.5 mm ID
0.4 mL/min for 2.1 mm ID
Back Pressure: 310 bar (1.5 mm) 444 bar (2.1 mm)
Temperature: 60 °C
Detection: ESI +
Injection Volume: 2 µL of 1.25 mg/mL Trastuzumab Tryptic Digest
Sample Solvent: 1.5 M Guanidine HCl/0.5% Formic Acid

All solvents used were MS grade. Methanol, acetonitrile, mobile phase additives, and individual standards were obtained from MilliporeSigma (St. Louis, MO), unless specified otherwise.

TRYPSIN DIGESTION OF TRASTUZUMAB

Trastuzumab was denatured and alkylated using 50 mM Tris-HCl (pH 7.8)/1.5M Guanidine-HCl, and 2-iodoacetamide (Sigma Aldrich). Trypsin (Promega) was added in a ratio of 1:30 (w:w; Trypsin:mAb) followed by an incubation at 37 °C overnight. The reaction was quenched by 0.5% Formic Acid and analyzed by LCMS.

Samples were analyzed on a Shimadzu Nexera X2 (Shimadzu Scientific Instruments, USA). Mass spectra were acquired using a Thermo QE Orbitrap mass spectrometer (Bremen, Germany) using a heated electrospray (HESI-II) probe on the Ion Max source.

MS CONDITIONS

Voltage	3.8 kV
Aux gas	10 arbitrary units
Sheath gas	35 arbitrary units
Sweep gas	0 arbitrary units
Rf lens	50 v
Heater temp	225 °C
Capillary temp	325 °C

EXPERIMENTAL REDUCED AND ALKYLATED AND INTACT ANALYSIS OF TRASTUZUMAB

Column: HALO 1000 Å Diphenyl, 2.7 µm, 1.5 x 150 mm
Part Number: 9212X-702
Column: HALO 1000 Å Diphenyl, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-726
Mobile Phase A: Water/0.1% DFA B: 50% Acetonitrile/50% n-propanol/0.1% DFA
Gradient: 27-36 %B in 40 min
Flow Rate: 0.2 mL/min for 1.5 mm ID
0.4 mL/min for 2.1 mm ID
Back Pressure: 252 bar (1.5 mm) 272 bar (2.1 mm)
Temperature: 60 °C
Detection: PDA, 220 nm
Injection Volume: 3 µL of 1.0 mg/mL Reduced and Alkylated Trastuzumab
Sample Solvent: Water/0.1% TFA

Samples were analyzed on a Shimadzu Nexera X2 (Shimadzu Scientific Instruments, USA). Mass spectra were acquired using a Thermo QE Orbitrap mass spectrometer (Bremen, Germany) using a heated electrospray (HESI-II) probe on the Ion Max source.

MS CONDITIONS

Voltage	3.8 kV
Aux gas	10 arbitrary units
Sheath gas	35 arbitrary units
Sweep gas	0 arbitrary units
Rf lens	50 v
Heater temp	225 °C
Capillary temp	320 °C

RESULTS-PEPTIDE MAPPING

Peptide mapping, a bottom-up technique, is typically used for confirmation of a monoclonal antibody (mAb) and to monitor post translational modifications (PTMs), such as oxidation or deamidation. This method can provide site-specific information regarding PTMs, which is imperative to test for during production, processing or storage.[4] Multiple phases are available in the 1.5 mm ID, and for peptide mapping the HALO® ES-C18 was selected for stability and robust nature of the phase.

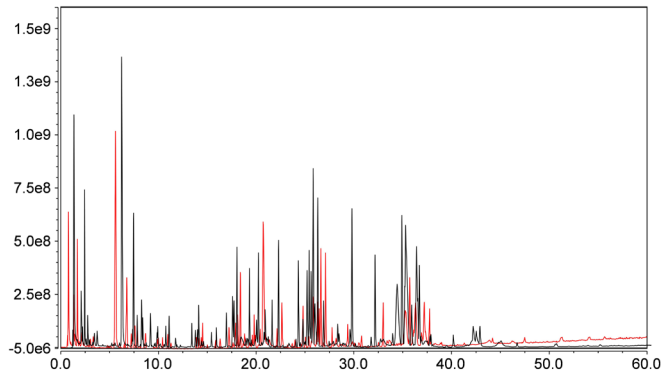


Figure 1. Trastuzumab Tryptic Digest by LCMS

Figure 1 shows a Tryptic Digest of Trastuzumab on the HALO 160 Å ES-C18, 2.7 µm, 1.5 x 150 mm (black trace) and the HALO 160 Å ES-C18, 2.7 µm, 2.1 x 150 mm (red trace). As is clearly visible the 1.5 mm ID column has higher intensity peaks and larger areas than the 2.1 mm ID column. In addition, as this is a 60-minute run, solvent quantity becomes an issue and needs to be considered. Figure 2 shows the amount of solvent that can be saved over the course on a single run (A) and a total batch consisting of 100 injections (B), showing a savings of 2:1 for the 1.5 compared with the 2.1.

Over the course of an entire batch, the 1.5 mm ID column can not only provide higher sensitivity, but also requires half the solvent compared to the 2.1 mm ID column.

Extrapolated over time, this can provide significant cost saving measures to the consumer, as the volatility in the consumable market is very difficult to budget for. This can be especially beneficial in mAb development, for example during QA analysis and monitoring for PTMs, prior to product release.

Figure 2. Solvent savings between a single injection (A) and a a simulated batch of 100 injections (B)

RESULTS-REDUCED AND ALKYLATED TRASTUZUMAB

A typical workflow for bottom-up proteomics also includes the reduction of disulfide bonds to limit solvent accessible cystine residues. It is used for identification as it is very difficult to identify disulfide bonds with just database searching.4 The reduced and alkylated trastuzumab was investigated on the HALO 1000 Å Diphenyl, 2.7 µm, 1.5 x 150 and the HALO 1000 Å Diphenyl, 2.7 µm, 2.1 x 150 mm. Figure 3 shows, the light and heavy chain of the mAb separated by the Diphenyl phase. The overlayed results, of the light chain (LC) and heavy chain (HC), on the 1.5 mm (blue trace) and the 2.1 mm (red trace), clearly show the intensity gains provided by the smaller ID column, which makes the 1.5 mm an ideal choice for alkylation experiments, and overall bottom-up approaches.

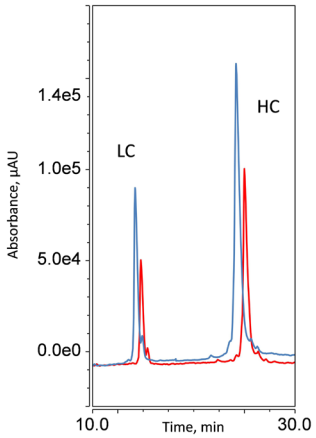


Figure 3.UV of reduced and alkylated trastuzumab showing the light chain (LC) and heavy chain (HC) on a 1.5mm ID column (Blue) and a 2.1mm ID column (red)

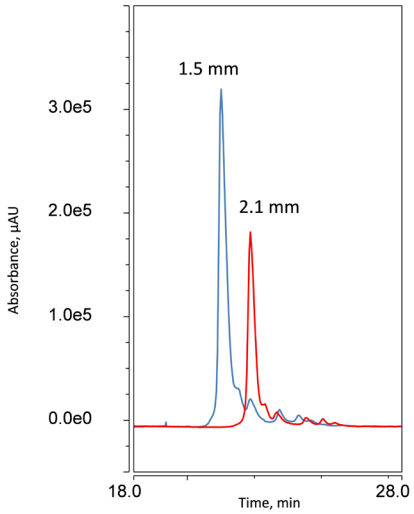


Figure 4.UV of Intact trastuzumab on a 1.5mm ID column (Blue) and a 2.1mm ID column (red)

RESULTS-INTACT TRASTUZUMAB

Intact mass analysis, or top-down analysis, is determination of a protein's total molecular weight without digestion or fragmentation and is especially useful for matching amino acid sequences and physicochemical analysis.[4] The intact analysis of Trastuzumab was investigated on the HALO 1000 Å Diphenyl, 2.7 µm, 1.5 x 150 mm and the HALO 1000 Å Diphenyl, 2.7 µm, 2.1 x 150 mm. Figure 4 shows the pattern of higher sensitivity and solvent savings continued on the intact protein. With the 1.5 mm ID column (blue trace) providing a 3x increase in area and peak height compared to the 2.1 mm ID column (above).

This trend is also seen in figure 5, which shows the LCMS of the intact protein, the reduced and alkylated protein exposing the light and heavy chain, and the digested protein. The 1.5 mm column (black trace) shows an increase of 3x in ionization efficiency, compared with the 2.1 mm column (Blue trace).

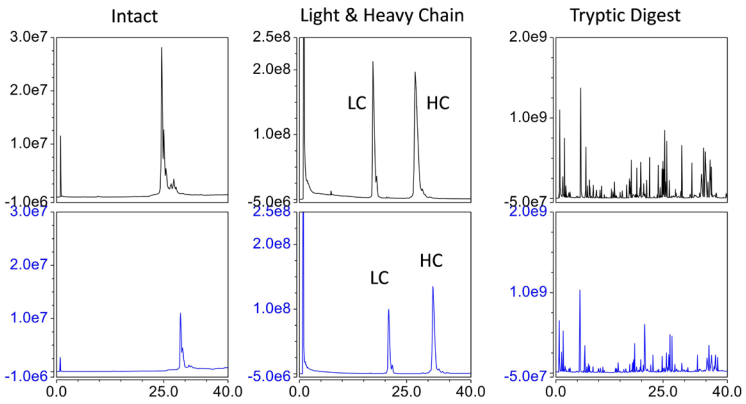


Figure 5. LCMS of the intact protein, the reduced and alkylated protein exposing the light and heavy chain, and the digested protein. The 1.5mm column (black trace) and 2.1 mm column (Blue trace)

CONCLUSION:

Trastuzumab was analyzed in a top-down and bottom-up approach using both the HALO® Diphenyl and ES-C18 phase incorporating the new 1.5 mm ID column and compared to the 2.1 mm ID column. In all cases the 1.5 mm ID column showed enhanced sensitivity compared to the 2.1 mm ID, and provided increased solvent savings to the customer. These results, combined with the characteristic HALO® quality, reliability, and robustness, make the 1.5 mm ID column a welcome addition to the HALO® family of products, and an ideal tool for proteomic analysis.

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HALO®

TECHNICAL REPORT

TITLE: IMPROVING SMALL MOLECULE ANALYSIS: INCREASING THE SENSITIVITY WHILE REDUCING SOLVENT CONSUMPTION BY USING 1.5 MM ID COLUMNS

MARKET SEGMENT: PHARMACEUTICAL

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ABSTRACT

The new HALO® 1.5 mm ID columns offer an alternative to analytical and narrow bore columns for greater separation performance with the benefits of increased sensitivity, reduced solvent consumption/reduced waste disposal costs, and ease of implementation with standard UHPLC systems compared to larger ID (2.1 mm and greater) and smaller ID (1 mm and less) columns. Examples will be shown to demonstrate the benefits of this novel column dimension which is used for superficially porous particles for the first time.

INTRODUCTION

Chromatographers are familiar with the benefits of reducing column ID: increased sensitivity, reduced solvent consumption therefore reduced waste disposal costs/overhead expenses, and reduced sample consumption. However, there are challenges that must be overcome to realize these benefits, including the need for specialized pumps that are capable of accurately delivering reduced flows. With the new HALO® 1.5 mm ID columns, existing UHPLC systems with low dispersion can be used instead of having to purchase specialized HPLC systems. The HALO® 1.5 mm ID columns offer increased sensitivity compared to larger ID columns (2.1 mm ID and greater). These columns also offer solvent savings since they are run at approximately half the flow rate of 2.1 mm ID columns, which means solvent savings of 50%. Finally, the HALO® 1.5 mm column hardware is robust and rugged, similar to analytical bore columns which enables long column lifetimes.

One aspect of switching to smaller ID columns is the impact of extracolumn dispersion[1-4], which is the broadening or spreading of an analyte band outside of the column. When too much extracolumn dispersion is present, the true performance of the column becomes masked causing reduced resolution and intensity (broad, short peaks). In order to successfully implement the HALO® 1.5 mm ID columns with UHPLC systems, the extracolumn dispersion should be minimized by using shorter, smaller ID connecting tubing, reduced injection volumes, and smaller volume flow cells for UV detection. All of these changes must be balanced in order to be able to operate at reasonable flow rates without overpressurizing the system and with the desired sensitivity.

KEY WORDS:

sensitivity, solvent savings, HALO 90 Å C18, microflow, extracolumn dispersion, 1.5 mm

EXPERIMENTAL:

The examples in this report will demonstrate increased sensitivity and reduced solvent use for a homologous series of alkylphenones, a mix of over the counter cough and cold medicines, and cannabinoid standards. All of the experiments were run using a Shimadzu Nexera (Columbia, MD) that had been optimized with an IDEX MarvelXACT™ PEEK-lined stainless steel connector (Northbrook, IL) from the injector to the column of 75 µm x 350 mm. The low dispersion flow cell was used in place of the semi-micro flow cell. All solvents used were MS grade. Acetonitrile, mobile phase additives, and individual standards were obtained from MilliporeSigma (St. Louis, MO).

Alkylphenones:

Column: HALO 90 Å C18, 2.7 µm, 1.5 x 100 mm
Part Number: 9281X-602
Column: HALO 90 Å C18, 2.7 µm, 2.1 x 100 mm
Part Number: 92812-602
Mobile Phase A: Water; B: Acetonitrile
Isocratic: 50/50 A/B
Flow Rate: 0.2 mL/min (1.5 mm); 0.39 mL/min (2.1 mm)
Back Pressure: 236 bar (1.5 mm); 310 bar (2.1 mm)
Temperature: 35 °C
Detection: UV 280 nm, PDA
Injection Volume: 0.5 µL
Sample Solvent: 84/16 water/methanol

Cough and Cold Medicines:

Column: HALO 90 Å C18, 2.7 µm, 1.5 x 150 mm
Part Number: 9281X-702
Column: HALO 90 Å C18, 2.7 µm, 2.1 x 150 mm
Part Number: 92812-702
Mobile Phase A: Water/0.15% TFA; B: Acetonitrile/0.1% TFA
Gradient: 5-50 %B in 8 min
Flow Rate: 0.2 mL/min (1.5 mm); 0.4 mL/min (2.1 mm)
Back Pressure: 425 bar (1.5 mm); 470 bar (2.1 mm)
Temperature: 35 °C
Detection: UV 280 nm, PDA
Injection Volume: 0.5 µL
Sample Solvent: 84/16 water/methanol

Cannabinoids:

Column: HALO 90 Å C18, 2.7 µm, 1.5 x 150 mm
Part Number: 9281X-702
Column: HALO 90 Å C18, 2.7 µm, 4.6 x 150 mm
Part Number: 92814-702
Mobile Phase A: Water/0.1% Formic Acid; B: Acetonitrile/0.1% Formic Acid
Isocratic: 75 %B
Flow Rate: 0.159 mL/min (1.5 mm); 1.5 mL/min (4.6 mm)
Back Pressure: 232 bar (1.5 mm); 518 bar (4.6 mm)
Temperature: 30 °C
Detection: UV 228 nm, PDA
Injection Volume: 0.5 µL
Sample Solvent: 75/25 ACN/ Water

RESULTS (ALKYLPHENONES):

In order to investigate the impact of column ID on the area obtained, a series of alkylphenones was run under isocratic conditions using 1.5 mm and 2.1 mm ID HALO 90 Å C18 columns. The comparison of the two columns is shown in Figure 1.

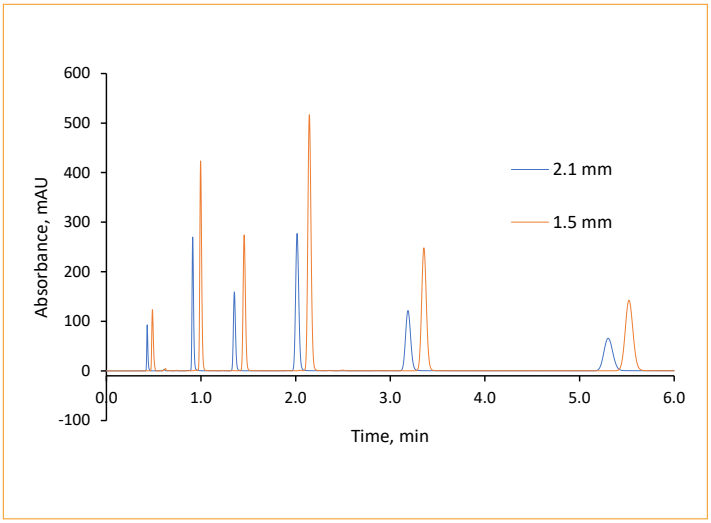

Figure 1. Isocratic separation of alkylphenones comparing 2.1 mm to 1.5 mm ID. Peak identities (in order) are uracil, acetophenone, propiophenone, butyrophenone, valerophenone, and hexanophenone.

The areas obtained using the 1.5 mm ID column are double those using the 2.1 mm ID column. The same injection volume was used for both columns, which demonstrates that a small volume injected on the 1.5 mm ID results in a taller peak. See Figure 2.

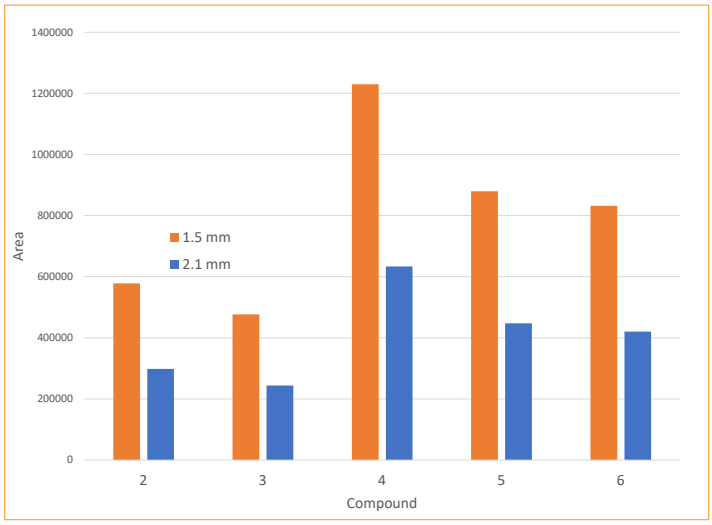

Figure 2. Graph showing an increase of two times the areas with the 1.5 mm ID HALO column compared to the 2.1 mm ID column. Peak identities same as Figure 1.

The same separation was run on a 1.0 mm ID HALO 90 Å C18 column. However, the plates were lower compared to the 1.5 mm ID column at every retention factor value, but significantly lower at retention factors less than 4. See Figure 3.

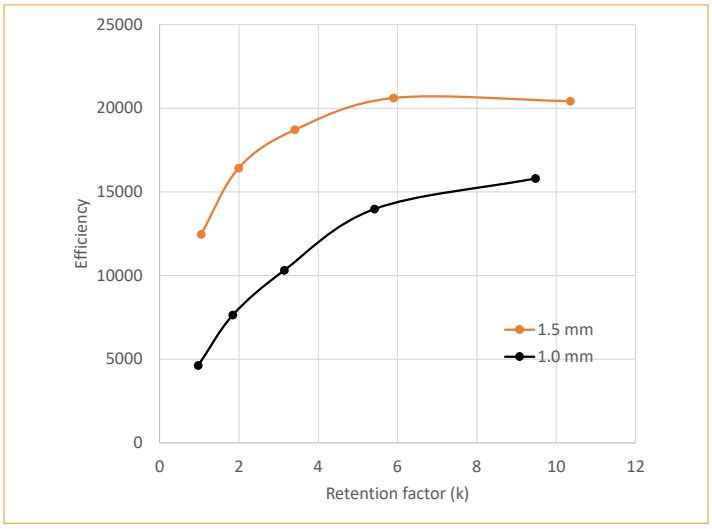

Figure 3. Effect of retention factor on efficiency using 1.5 mm and 1.0 mm HALO 90 Å C18 columns.

As previously mentioned the impact of extracolumn dispersion is increased as the column ID is decreased. The 1.0 mm ID column is more susceptible to the impact of extracolumn dispersion thus explaining the cause of the reduced efficiency. In order to observe the true performance of the 1.0 mm ID column, a lower dispersion HPLC system would need to be used.

RESULTS (COUGH AND COLD MEDICINES):

A gradient separation of common over the counter cough and cold medicines was run to further demonstrate the advantages of using a 1.5 mm ID HALO 90 Å C18 column over a 2.1 mm ID HALO 90 Å C18 column. Analysis of these compounds is commonly performed. Note the sensitivity improvement aids separations where minor components are present, such as for salicylic acid (peak 7), which is a decomposition product of aspirin (peak 6).

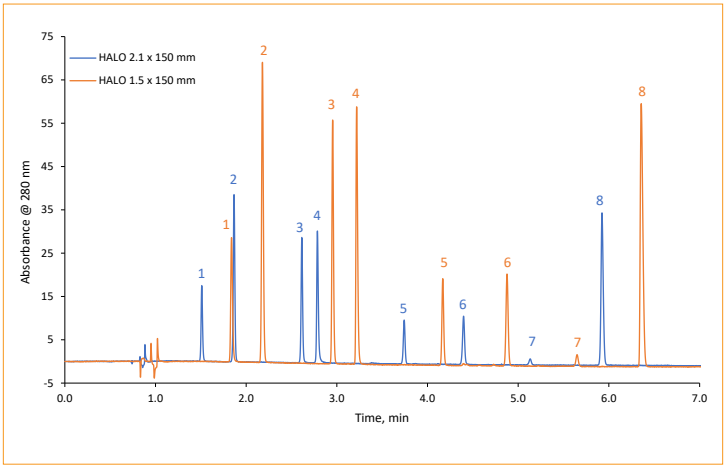

Figure 4. Comparison of a gradient separation of common over the counter cough and cold medicines run on 1.5 mm and 2.1 mm columns. Peak identities (in order) are phenylephrine, acetaminophen, caffeine, doxylamine, guaifenesin, aspirin, salicylic acid, and dextromethorphan.

Similar to the isocratic separation of alkylphenones, the peak areas for the cough and cold medicines were doubled with the 1.5 mm ID column compared to the 2.1 mm ID column along with two times the savings in solvent consumption.

RESULTS (CANNABINOIDS):

In order to demonstrate the impact of sensitivity and solvent savings, a 1.5 mm ID HALO 90 Å C18 column was run compared to a 4.6 mm ID HALO 90 Å C18 column using a mixture of 13 cannabinoids. The flow rate was scaled on the 1.5 mm ID column so that the same linear velocity was used for both columns. The separation is shown in Figure 5.

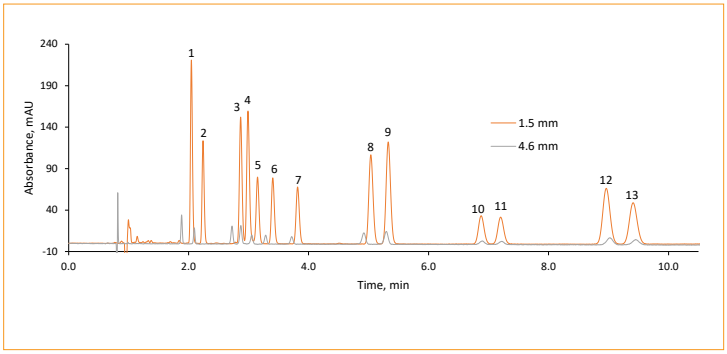

Figure 5. Comparison of an isocratic separation of 13 cannabinoids run on 1.5 mm and 4.6 mm ID HALO 90 Å C18 columns. Peak identities (in order) are CBDVA, CBDV, CBDA, CBGA, CBG, CBD, THCV, THCVA, CBN, 9-THC, 8-THC, CBC, and THCA.

By reducing the column ID from a 4.6 mm ID to a 1.5 mm ID, the solvent consumption is reduced from 15 mL/injection to 1.59 mL/injection, which is a reduction of 9.4 times. See Figure 6.

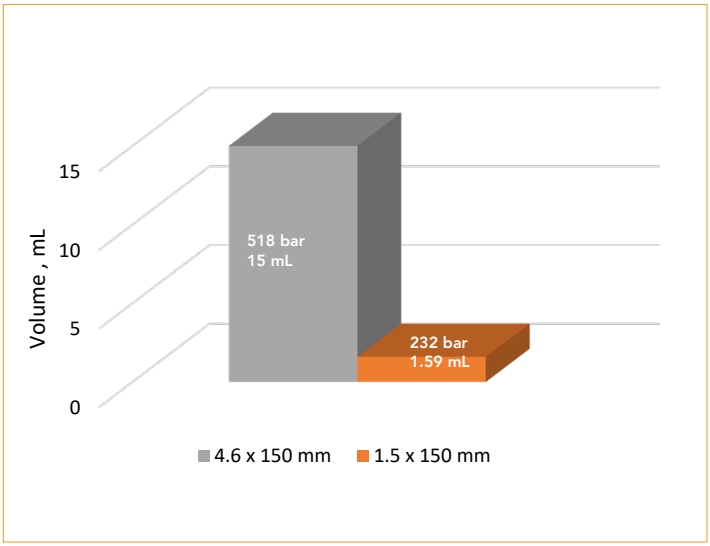

Figure 6. Comparison of the solvent used per injection for a 4.6 mm ID compared to a 1.5 mm ID column.

CONCLUSION:

The new HALO® 1.5 mm ID columns offer a reliable microflow column solution which enables increased sensitivity, reduced solvent consumption/waste disposal, and reduced sample consumption for a variety of applications. Bridging a gap between a 2.1 mm ID and a 1 mm ID with the ability to be used on standard UHPLC systems provides a balance of ease of use from an analytical system with implementation to that of standard analytical columns and the increased sensitivity provided by a specialized micro flow system. The robust column design, manufactured in Advanced Materials Technology's ISO accredited facility provides quality assurance measures resulting with long column lifetimes and excellent reproducibility.

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