

THE NEW HALO 1.5

HALO®

A BETTER PATH TO SEPARATIONS



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NEW DIMENSION • INCREASED SENSITIVITY • SOLVENT SAVINGS

TAKING SEPARATIONS TO A NEW DIMENSION

“I want more **sensitivity** with my conventional UHPLC system...”

“I need an **easy to use** microflow solution...”

“How can I get **more performance** from my LC and LCMS systems?”

“We have a goal to **reduce solvent consumption**...”

INNOVATION YOU CAN TRUST - PERFORMANCE YOU CAN RELY ON

THE NEW HALO® 1.5 – DEFINING A NEW DIMENSION IN CHROMATOGRAPHY

The adoption of UHPLC instrumentation pushed LC separations to a new level of speed and resolution. In the years since, chromatographers continue to push separation limits in response to increasing challenges. How can I improve my separation in a faster time, or with less solvent consumption, increased sensitivity, or all of the above? While the specific drivers may be different, the overall goal is the same – ‘I need more, but without sacrificing anything’.

In 2006 Advanced Materials Technology broke from convention by introducing Fused-Core® particle technology to the market. These little HALO® particles delivered high-speed, high-performance separations without the consequence of high back pressures allowing scientists to adapt their HPLC systems to rival the UHPLC systems.

Now in 2022 AMT breaks new ground again with a 1.5 mm internal diameter HALO® column to push the boundaries of adopted UHPLC systems. Founded on all of the benefits of Fused-Core® particles, the HALO® 1.5 delivers increased sensitivity and reduced solvent consumption, allowing scientists to experience the benefits of capillary columns without the pains of specialized microflow systems.



DELIVERING MORE PERFORMANCE

- ✓ **More Sensitivity** from conventional UHPLC Systems
- ✓ **Higher Ionization Efficiencies** from LCMS systems
- ✓ **Reduced Solvent Consumption** compared to 2.1 mm ID columns
- ✓ **Easy to Implement** microflow solution

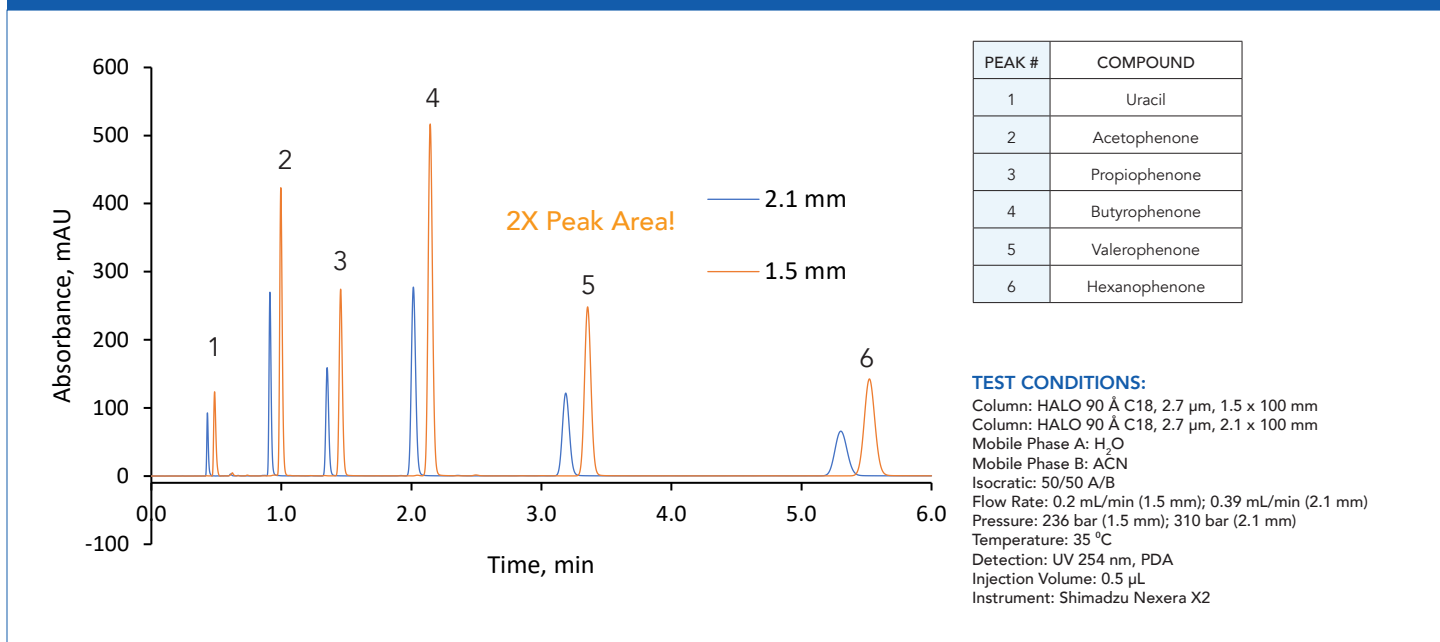
MORE SENSITIVITY

"I want **more sensitivity** with my conventional UHPLC system"

Through newly designed specialized manufactured fluidic hardware, the new HALO® 1.5 delivers increased signal response in comparison to 2.1 mm ID columns and demonstrates higher efficiencies compared to 1 mm ID columns on optimized UHPLC systems.

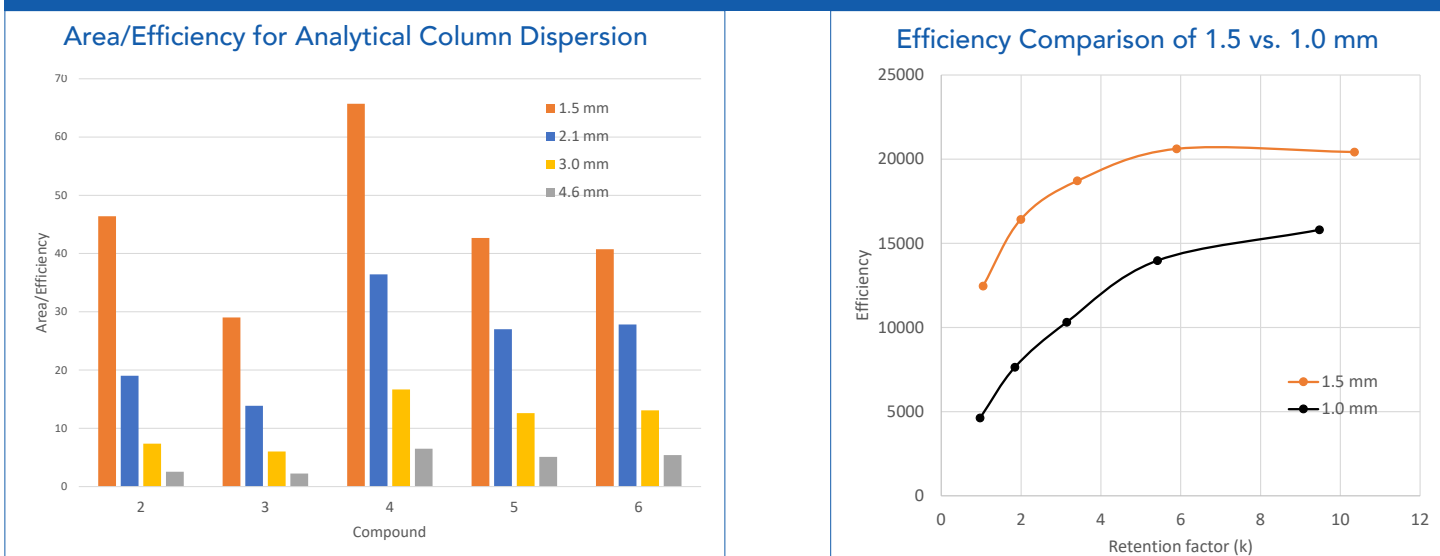
ISOCRATIC SEPARATION OF ALKYLPHENONES SHOWS AVERAGE OF 2X GREATER AREA USING 1.5 MM COLUMN

Using a 1.5 mm ID column compared to a 2.1 mm ID column, the peak areas are doubled and the sensitivity is improved using the same injection volume on both columns.



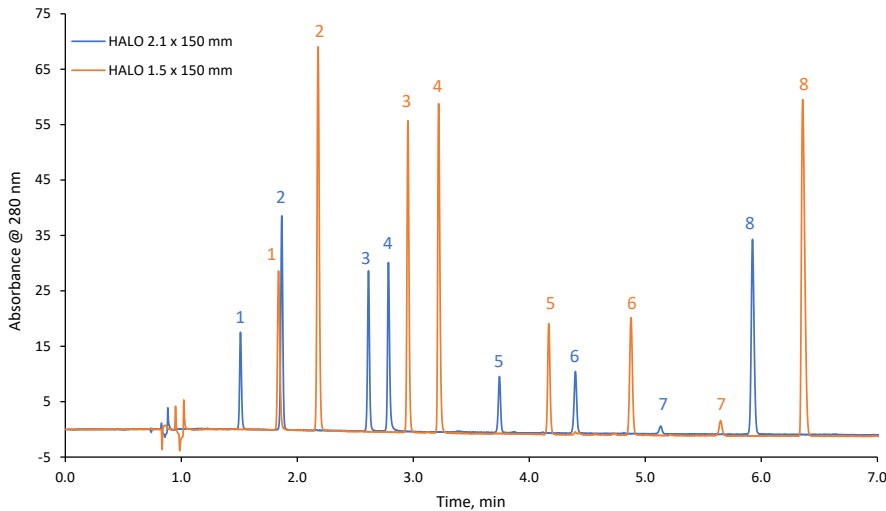
DEMONSTRATION OF IMPROVED EFFICIENCY WITH HALO® 1.5

A mixture of alkylphenones was run on 1.0, 1.5, 2.1, 3.0, and 4.6 mm ID columns. The area/efficiency of the 1.5 mm ID column outperforms all of the analytical ID columns. While the 1.0 mm ID should perform better than the 1.5 mm ID, even on an optimized UHPLC system the dispersion is too great for a 1.0 mm ID column, thus causing lower efficiencies at all retention factors.



GRADIENT SEPARATION OF OTC COUGH AND COLD MEDICINES

With extracolumn dispersion minimized, the 1.5 mm ID column shows taller peaks compared to 2.1 mm ID column providing greatest benefit for minor components.



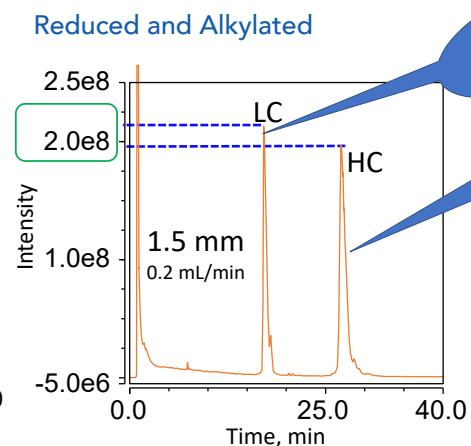
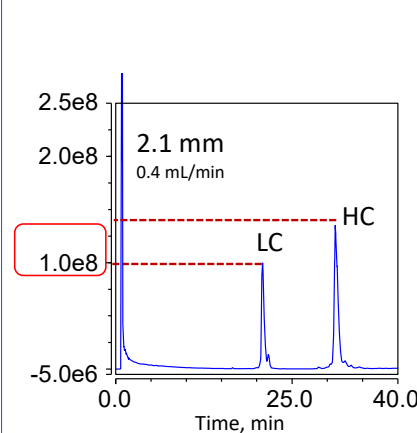
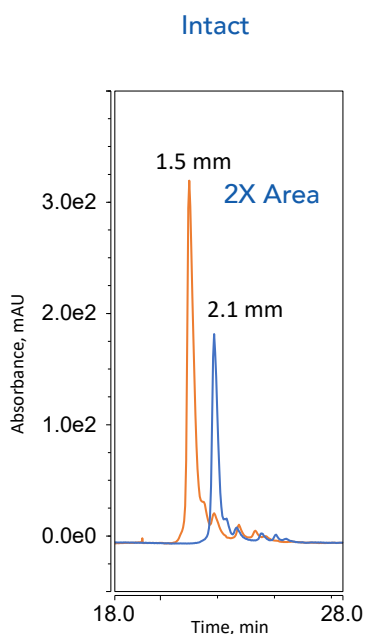
PEAK #	COMPOUND
1	Phenylephrine
2	Acetaminophen
3	Caffeine
4	Doxylamine
5	Guafenesin
6	Aspirin
7	Salicylic Acid
8	Dextromethorphan

TEST CONDITIONS:

Mobile Phase A: Water/0.15% TFA
 Mobile Phase B: ACN/0.1% TFA
 Gradient: 5-50 %B in 8 min
 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
 Injection Volume: 0.5 µL
 Detection: UV 280 nm, PDA
 Instrument: Shimadzu Nexera X2

1.5 MM ID BENEFITS FOR INTACT AND REDUCED AND ALKYLATED MAB CHARACTERIZATION

The HALO 1000 Å Diphenyl in 1.5 mm ID shows double the area for intact trastuzumab compared to the same separation run on a 2.1 mm ID HALO 1000 Å Diphenyl column. Greater than double the area is observed when used for the separation of reduced and alkylated trastuzumab using MS detection.



TEST CONDITIONS:

Column: HALO 1000 Å Diphenyl, 2.7 µm, 1.5 x 150 mm
 Column: HALO 1000 Å Diphenyl, 2.7 µm, 2.1 x 150 mm
 Mobile Phase A: Water/0.1% DFA
 Mobile Phase 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 Trastuzumab
 Sample Solvent: 100 mM Ammonium Bicarbonate
 LC System: Shimadzu Nexera X2
 MS System: ThermoFisher Q Exactive

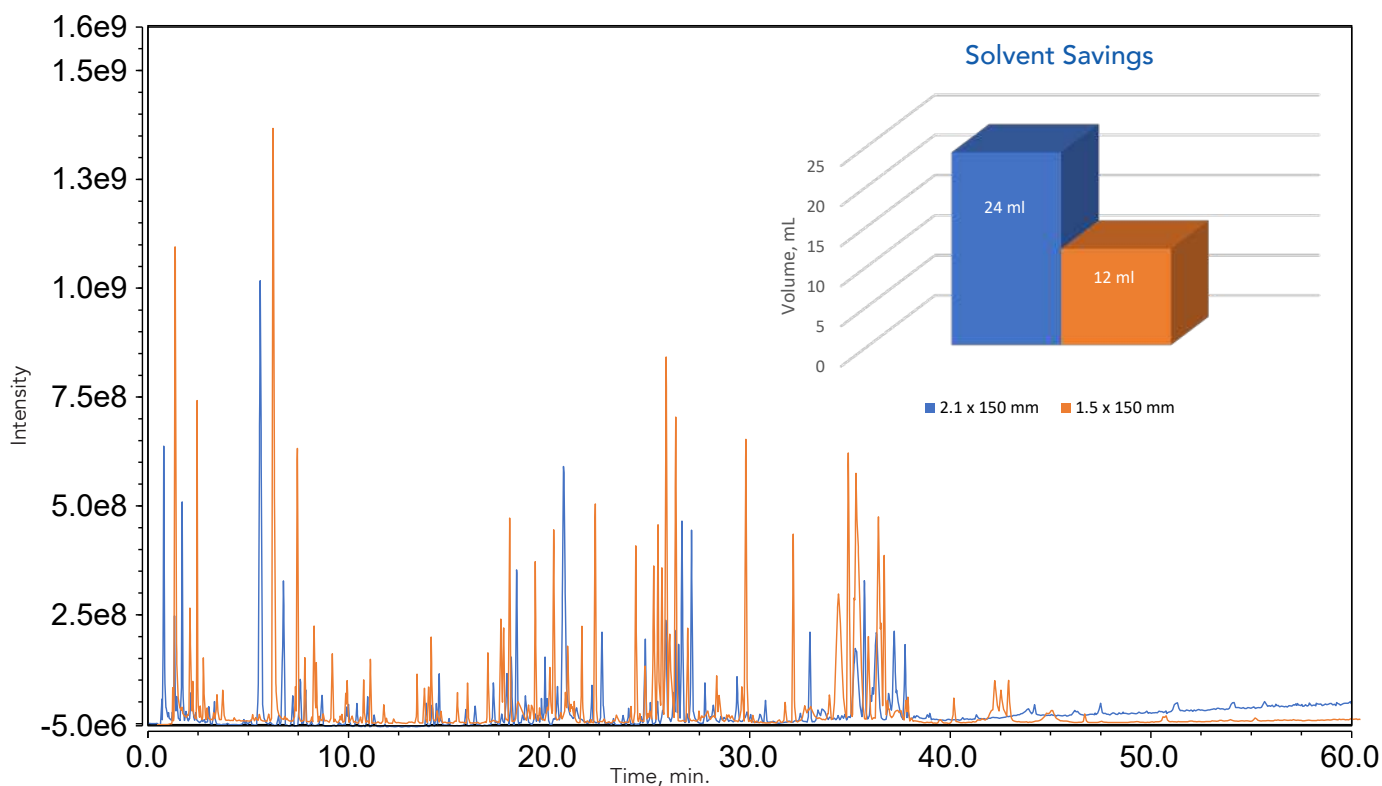
SOLVENT REDUCTION

The costs associated with solvents continue to grow from both a purchase price perspective and price of waste removal. In step with efforts to decrease the amount of environmental impact generated from hazardous chemicals, companies are seeking solutions to go **greener**. A decrease in column ID is

PEPTIDE MAP SOLVENT SAVINGS USING HALO 160 Å ES-C18 IN 1.5 MM ID



When a 1.5 mm ID column is used in comparison to a 2.1 mm ID column for a peptide map of trastuzumab, 50% of the solvent is saved since the 1.5 mm ID column is run at 0.2 mL/min compared to 0.4 mL/min for the 2.1 mm ID column. For a 60 minute analysis, only 12 mL of solvent is used vs. 24 mL of solvent for the 2.1 mm ID column. Not only is solvent saved, but the cost of waste disposal is also reduced.



TEST CONDITIONS:

Column: HALO 160 Å ES-C18, 2.7 μ m, 1.5 x 150 mm
Column: HALO 160 Å ES-C18, 2.7 μ m, 2.1 x 150 mm
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 μ L 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

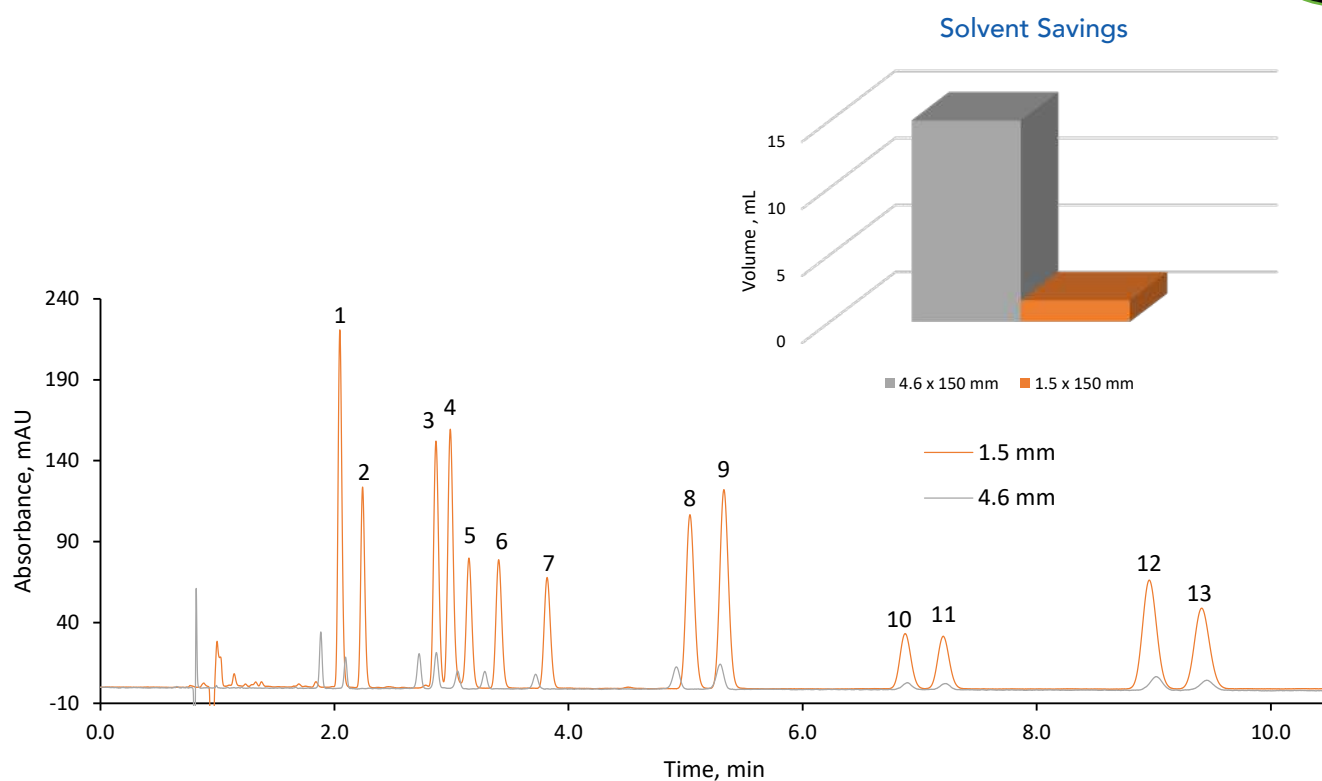
**"We have a goal to
reduce solvent consumption"**

optimized with lower flow rates resulting in an overall solvent savings. Moving to the HALO® 1.5 helps analysts and companies reduce their solvent consumption.

An added benefit using the HALO® 1.5 is increased sensitivity without sacrificing speed!

>9X REDUCTION IN SOLVENT GOING FROM A 4.6 MM ID COLUMN TO A 1.5 MM ID COLUMN

An even larger amount of solvent is saved when a separation is moved from a 4.6 mm ID column to a 1.5 mm ID column. In this isocratic separation of 13 cannabinoids, note the sensitivity increase with the 1.5 mm ID column compared to a 4.6 mm ID column.



TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm
 Mobile Phase A: Water/ 0.1% Formic Acid
 Mobile Phase B: Acetonitrile/ 0.1% Formic Acid
 Isocratic: 75 %B
 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

PEAK IDENTITIES:

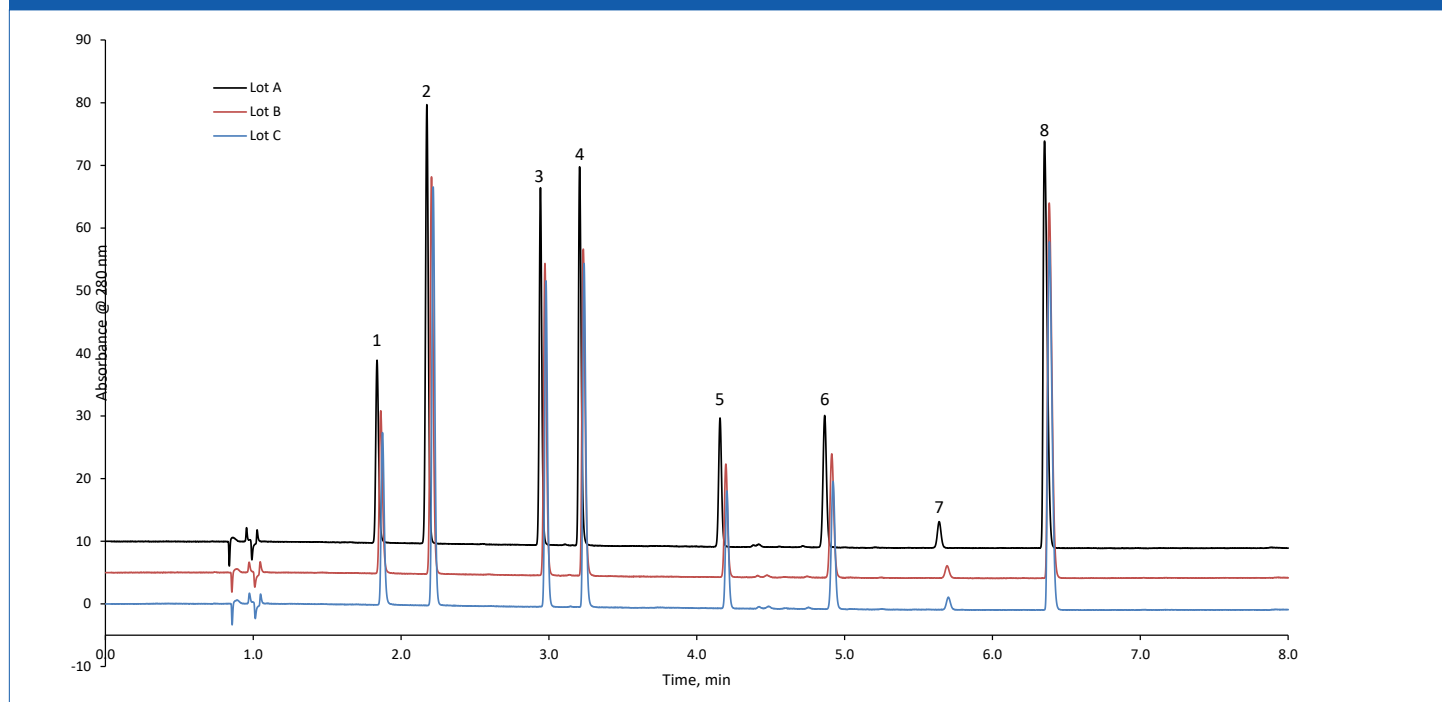
- 1. CBDVA
- 2. CBDV
- 3. CBDA
- 4. CBGA
- 5. CBG
- 6. CBD
- 7. THCV
- 8. THCVA
- 9. CBN
- 10. 9-THC
- 11. 8-THC
- 12. CBC
- 13. THCA

STABILITY

The new HALO® 1.5 meets the same rigorous quality standards of all HALO® products. Multi lot testing on the new hardware design and a QA report on every column produced from our ISO certified facility results in reliability you can depend on, column to column, analysis to analysis for the lifetime of the method.

EXCELLENT LOT TO LOT REPRODUCIBILITY WITH THE 1.5 MM ID HARDWARE

Separation of OTC cough and cold medicines using three different lots of the 1.5 mm ID column hardware shows confidence in reproducibility.

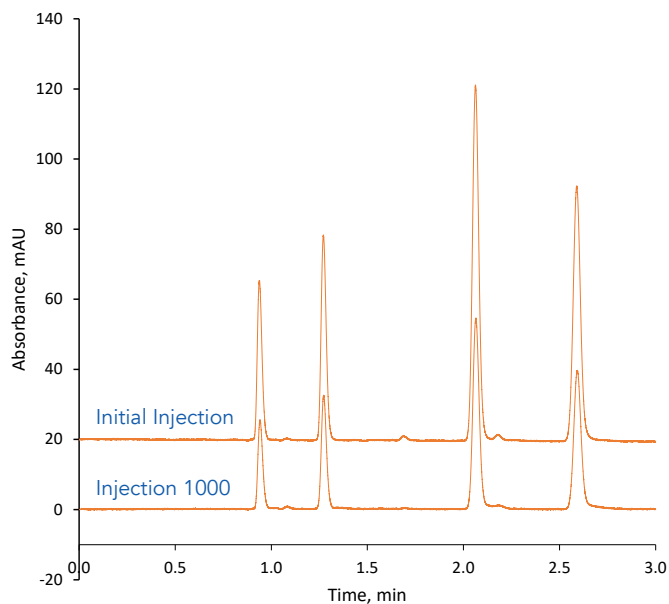


Test conditions for the above chromatogram are the same as p.3 top figure.



STABILITY DEMONSTRATION WITH HALO 1000 Å DIPHENYL

The HALO 1000 Å Diphenyl in 1.5 mm ID column hardware was tested at 600 bar for 1000 injections. No loss in efficiency or retention was observed over the course of the experiment.

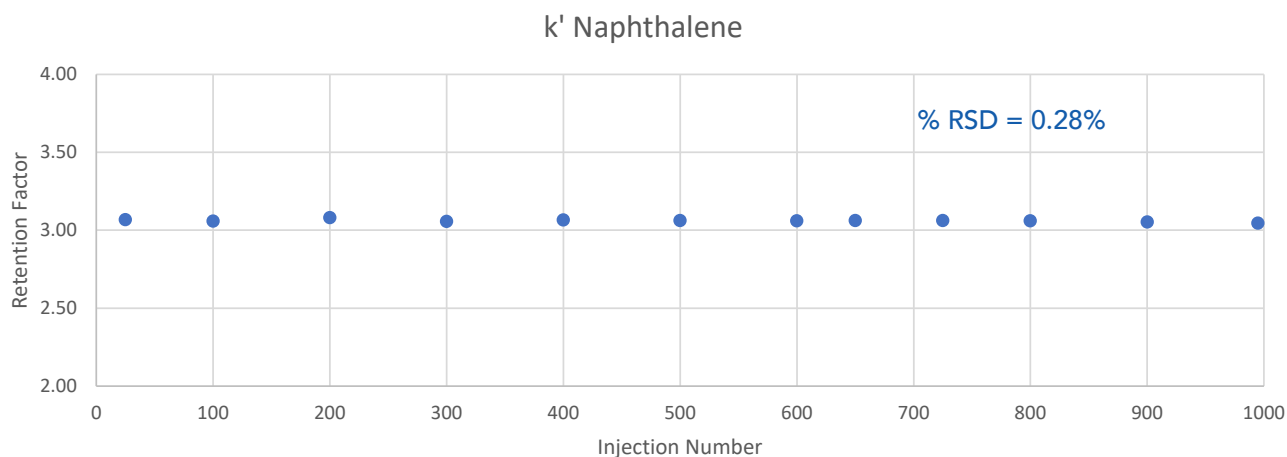


TEST CONDITIONS:

Column: HALO 1000 Å Diphenyl, 2.7 μm , 1.5 x 150 mm
Mobile Phase A: Water
B: Acetonitrile
Isocratic: 25 %B
Flow Rate: 0.4 mL/min
Back Pressure: 600 bar
Temperature: 30 °C
Detection: 254 nm, PDA
Injection Volume: 0.2 μL
Sample Solvent: 60/40 ACN/ Water
Data Rate: 200 Hz
Response Time: 0.005 sec.
Flow Cell: 1 μL
LC System: Shimadzu Nexera X2

600 BAR STABILITY USING HALO 90 Å C18

A HALO 90 Å C18 1.5 mm ID column was run for 1000 injections to demonstrate the stability of the new 1.5 mm ID hardware. The retention factor of naphthalene was stable across all of the injections.



TEST CONDITIONS:

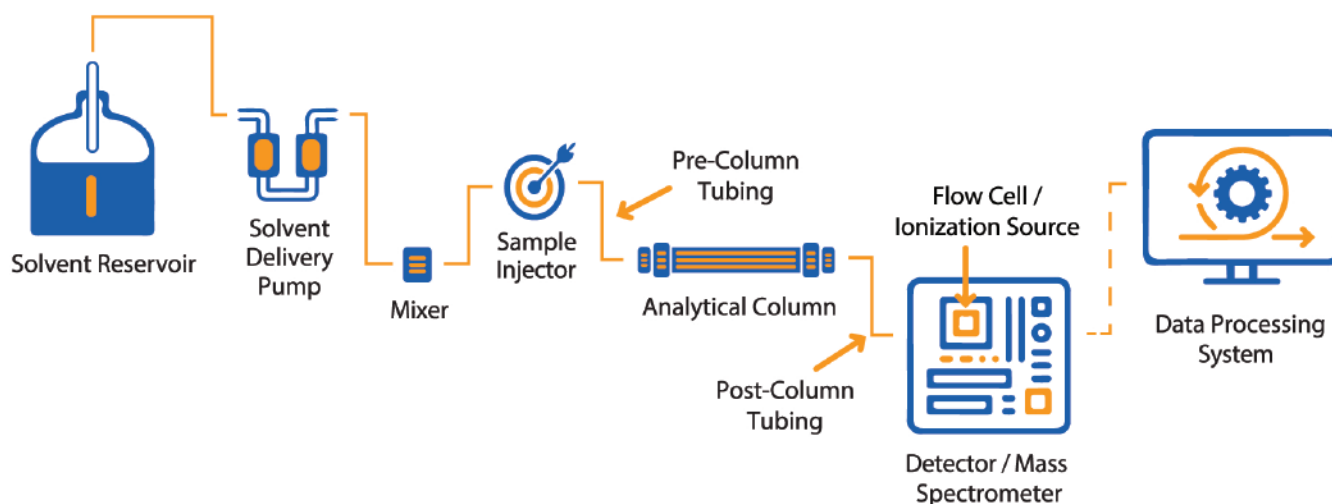
Column: HALO 90 Å C18, 2.7 μm , 1.5 x 150 mm
Mobile Phase A: Water
B: Acetonitrile
Isocratic: 60 %B
Flow Rate: 0.6 mL/min
Back Pressure: ~600 bar
Temperature: 30 °C
Detection: 254 nm, PDA

Injection Volume: 0.2 μL
Sample Solvent: 60/40 ACN/ Water
Data Rate: 200 Hz
Response Time: 0.005 sec.
Flow Cell: 1 μL
LC System: Shimadzu Nexera X2

SYSTEM OPTIMIZATION

Success with smaller ID columns requires attention to optimizing the LC system hardware for best performance. While manufacturers of UHPLC systems in general have already reduced system volumes, consideration should be taken to items like tubing and flow cells which lead to extra column dispersion.

Most critical when changing a gradient method from a current column to the new 1.5 mm ID column is the system dwell volume and extra column dispersion. The dispersion occurs post column in the tubing leading to the detector and within the detector itself. Under isocratic conditions, the dispersion comes from both pre- and post-column tubing as well as injection volume and detector.

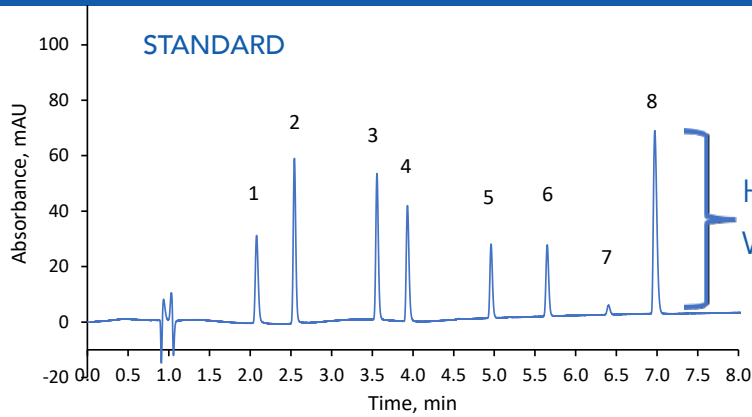


EXAMPLE DEMONSTRATING REDUCTION OF UHPLC VOLUMES

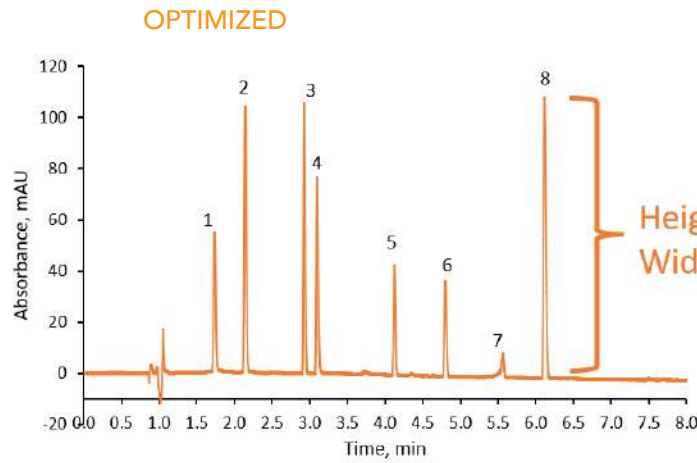
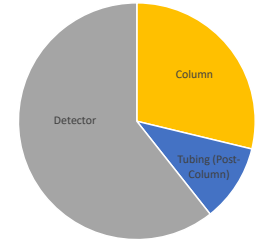
COMPONENT	STANDARD UHPLC SYSTEM	OPTIMIZED UHPLC SYSTEM
Mixer (μL)	100	20
Pre Column Tubing Volume (μL)	0.1 mm x 800 mm 6.3	75 μm x 350 mm 1.5
Post Column Tubing Volume (μL)	0.1 mm x 509 mm 4	60 μm x 707 mm 2
Flow Cell (PDA) Volume (μL)	1	1
Extra Column Dispersion (μL ²)	14	2

COMPARISON OF STANDARD TO OPTIMIZED SYSTEM CONFIGURATION FOR OTC COUGH AND COLD MEDICINES

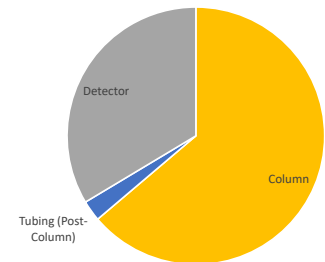
When the UHPLC is optimized, peak heights are taller and peak widths are smaller, leading to improved sensitivity. Notice how the pie wedges for the post-column tubing and the detector are reduced in size when the UHPLC system is optimized for this gradient separation.



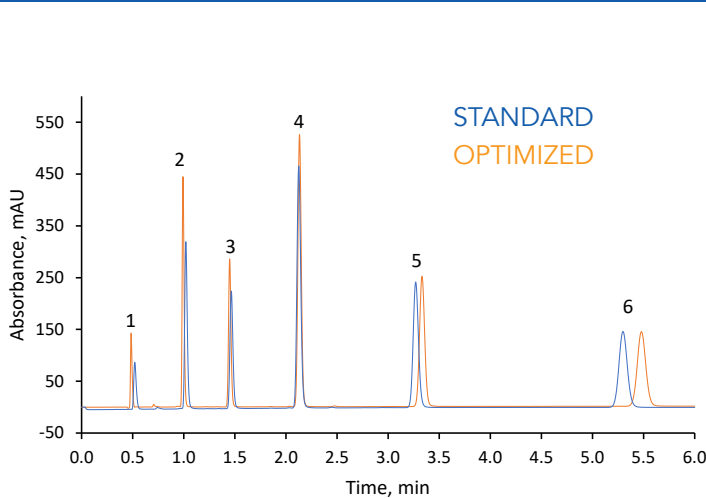
Standard Plumbing - Gradient $k^* = 2$



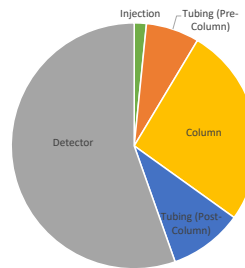
Optimized Plumbing - Gradient $k^* = 2$



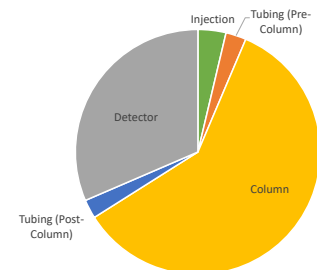
Using isocratic conditions, the extracolumn dispersion comes from both pre- and post-column tubing, the injection volume, and the detector. In this comparison the peaks are taller and the peak efficiencies are increased with the optimized UHPLC system.



Standard Plumbing - Isocratic $k = 2$



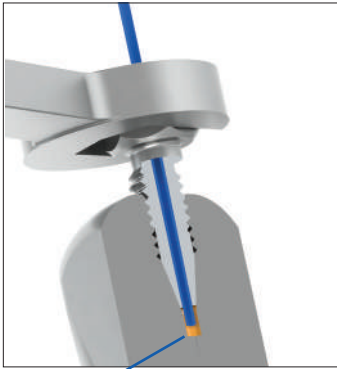
Optimized Plumbing - Isocratic $k = 2$



ENSURE A PERFECT CONNECTION WITH MarvelXACT™

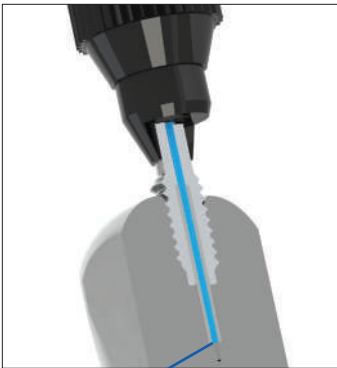
MarvelXACT™ connection systems have been designed to eliminate the risk of under- or over-tightening with a patented torque limiting mechanism. This unique feature emits a haptic “click” feedback when it reaches the optimum torque, assuring a perfect installation every time. MarvelXACT™ incorporates advanced MarvelX™ Sealing Technology to deliver precise face sealing (sealing at the port bottom), which eliminates additional internal volume, and minimizes carryover risk, peak tailing, and peak broadening.

MarvelXACT™ VS. CONVENTIONAL CONED FITTINGS



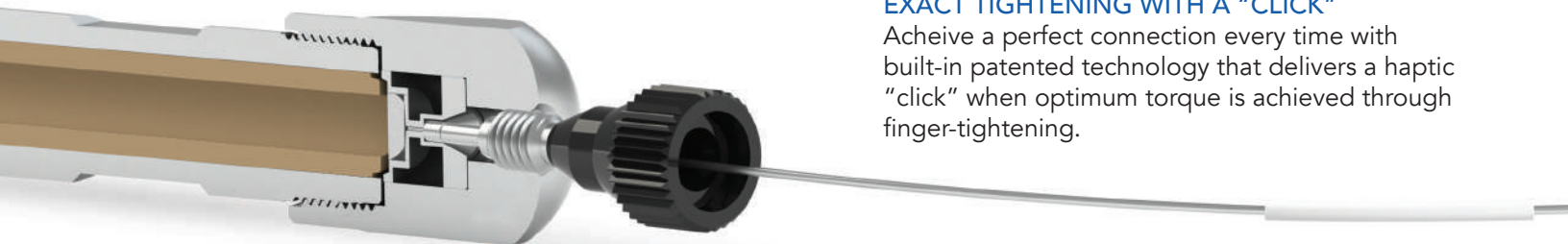
EXTRA INTERNAL VOLUME

Conventional coned fittings require a ferrule in conjunction with a fitting for proper sealing. They depend on tools, to improve sealing performance, which significantly increases probability of extra internal volume and poor chromatography results. The mechanical tightening increases wear leading to higher replacement costs.



ZERO DEAD VOLUME

MarvelXACT™ fittings do not depend on ferrules. They seal with hand tightening at the bottom of the port, which significantly reduces required torque and enables many more connects and disconnects reducing wear and increasing product life. An enhanced proprietary tip design also ensures zero dead volume (ZDV) and better chromatography results.



EXACT TIGHTENING WITH A “CLICK”

Achieve a perfect connection every time with built-in patented technology that delivers a haptic “click” when optimum torque is achieved through finger-tightening.

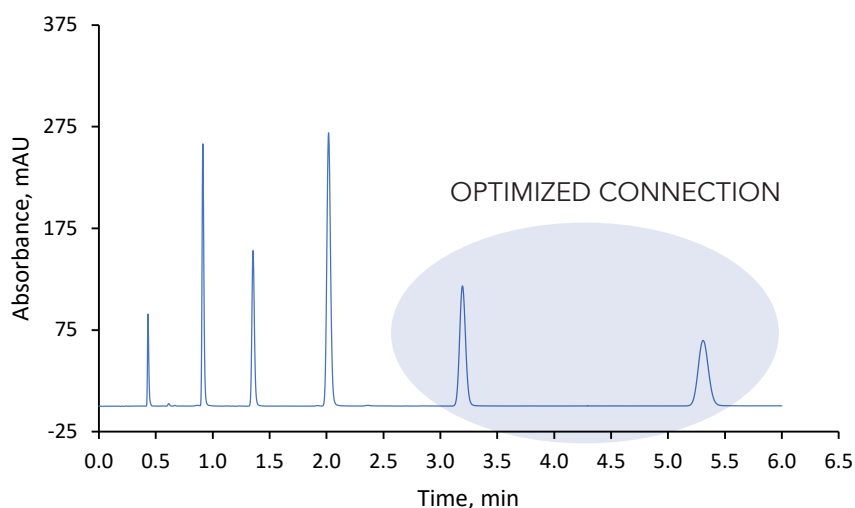
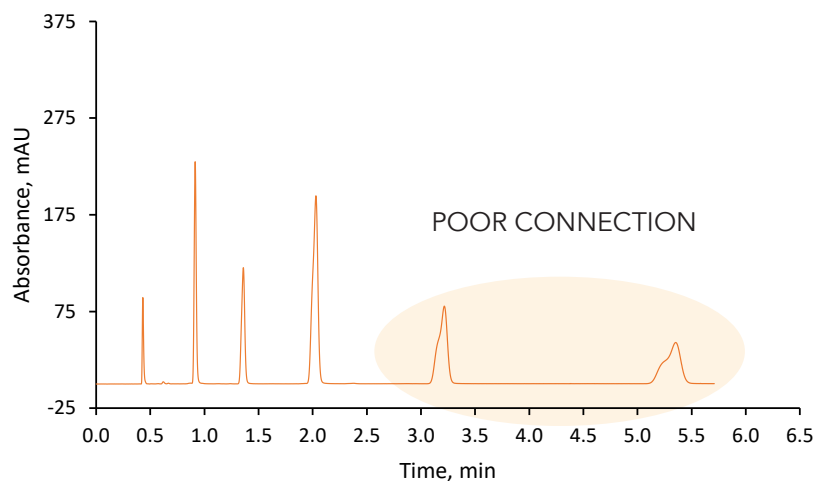


FLEXIBLE TUBING

1/32" OD tubing prevents kinking and allows considerable flexibility to route throughout the instrument.

CONNECTION CONSEQUENCES

When a poor connection is made to the injector, distorted peak shape and leaks may occur. In this example, the connecting tubing was not fully seated into the injector port which caused a slow leak and distorted peak shape, especially for the later eluting peaks.



FINGER-TIGHT TO UHPLC

MarvelXACT™ is truly a finger-tight connection system that has a patented torque-limiting mechanism for exact tightening every time, and seals up to 19000 psi (~1310 barr) for routine use.

SMALL & ACCESSIBLE

Fittings are small enough to fit in tight spaces, yet allow for finger-tightening at UHPLC pressures.

ROBUST TIP

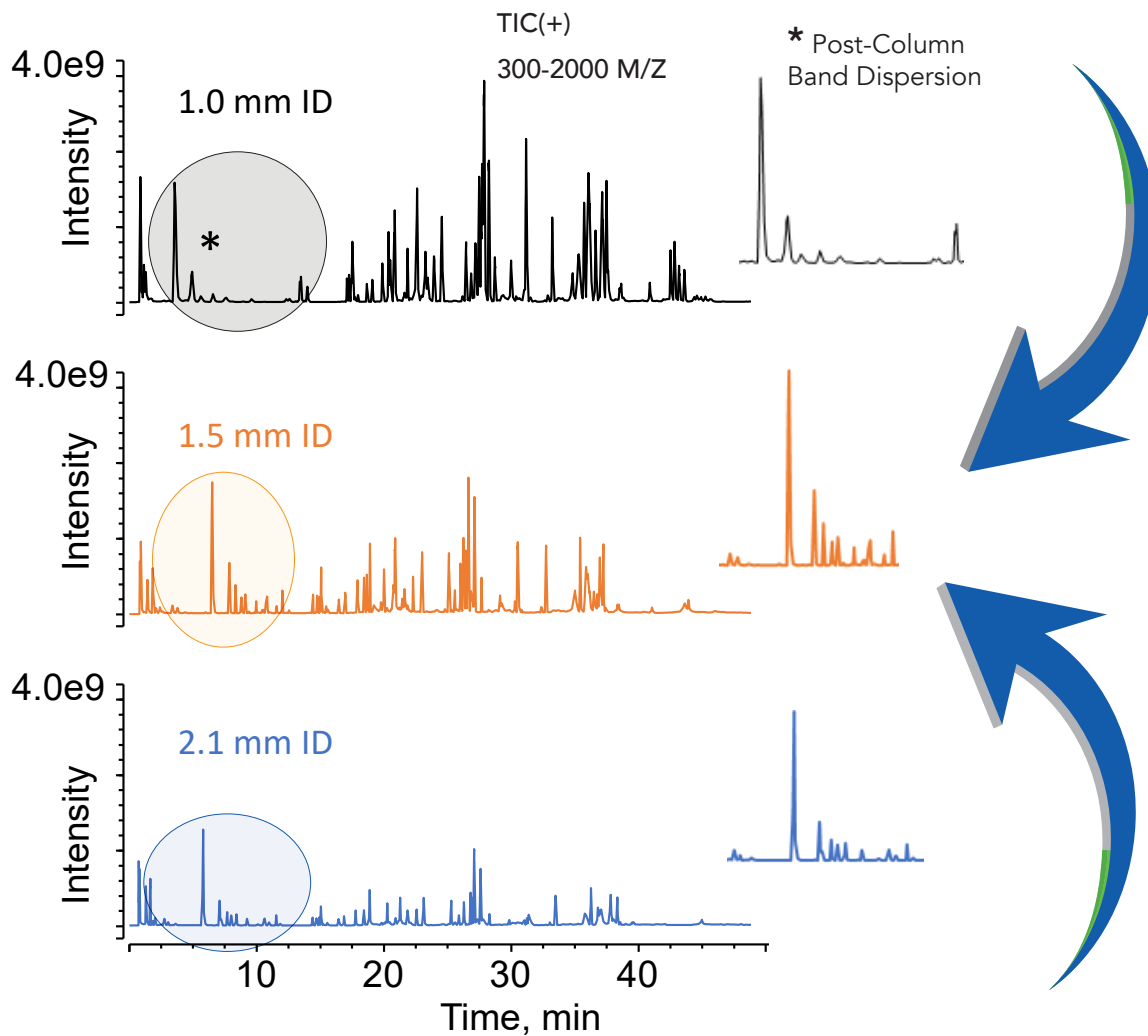
Enable robust structure, superior re-usability, and minimizes chances of tip damage from connecting and disconnecting.



EXTRA COLUMN DISPERSION

IMPACT OF EXTRACOLUMN DISPERSION ON DIFFERENT ID COLUMNS

The 1.0 mm ID column shows broad peak shape due to the amount of extracolumn dispersion going from the column to the source of the MS. The 1.5 mm ID performs better than both the 2.1 mm ID (in terms of peak height) and the 1.0 mm ID (in terms of peak width).



TEST CONDITIONS:

Column: HALO 160 Å ES-C18, 2.7 μm , 1.5 x 150 mm
Column: HALO 160 Å ES-C18, 2.7 μm , 2.1 x 150 mm
Column: HALO 160 Å ES-C18, 2.7 μm , 1.0 x 150 mm
Mobile Phase A: Water/0.1% DFA
B: Acetonitrile/0.1% DFA
Gradient: 2-50 %B in 60 min
Flow Rate: 0.1 mL/min for 1.5 mm ID
0.2 mL/min for 1.5 mm ID
0.4 mL/min for 2.1 mm ID

Back Pressure: 265 bar (1.0 mm)
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 TEST CONDITIONS:

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

CONVERTING TO THE NEW 1.5

WHY SHOULD WE CARE ABOUT EXTRACOLUMN DISPERSION?

The analyte bands or peaks naturally broaden as they move through the connecting tubing in a UHPLC system. The smaller the column ID, the more this broadening could impact the results of the column. If the true efficiency of the column is the goal, then the extracolumn dispersion must be reduced. The extracolumn dispersion comes from the injector, the pre-column tubing, the heat exchanger, the post-column tubing, and the detector. For isocratic separations, all of these impact the observed efficiency. For gradient separations, only the post-column tubing and the detector impact the observed efficiency. In order to maximize what efficiency the column is capable of delivering, the extracolumn volume needs to be reduced as much as possible by using shorter length, smaller ID tubing. However, changing to smaller ID tubing will increase the pressure of the system so a compromise must be made in order to still be able to run at the flow rates needed for optimum column performance.

EQUATION FOR SCALING FLOW RATE

$$F_2 = F_1 \times \frac{(\pi R_2)^2}{(\pi R_1)^2} = F_1 \times \frac{(R_2)^2}{(R_1)^2} = F_1 \times \frac{(D_2)^2}{(D_1)^2}$$

Where F = flow rate

R = radius

D = diameter

1 = original column

2 = column being changed to

		COLUMN IDS				
		4.6	3.0	2.1	1.5	1.0
FLOW RATES (mL/min)	0.96	0.41	0.20	0.10	0.045	
	1.44	0.61	0.30	0.15	0.068	
	1.92	0.82	0.40	0.20	0.091	
	2.40	1.02	0.50	0.26	0.113	
	2.88	1.22	0.60	0.31	0.136	

EXTRACOLUMN DISPERSION REFERENCES

1. D.R. Stoll, K. Broeckhoven, LCGC North America. 39, Issue 4 (2021) 159–166.
2. G. Desmet, K. Broeckhoven, TrAC Trends Anal. Chem. 119 (2019) 115619.
3. K. Broeckhoven, J. De Vos, and G. Desmet, LCGC Europe 30 (2017) 618– 625.

HOW TO SELECT THE BEST CONNECTORS FOR THE BEST 1.5 MM ID PERFORMANCE

- In general, use the shortest length and smallest ID tubing that will work for your system
- Are you running isocratic or gradient?
 - If isocratic, then pre-column tubing is most impactful - reduce length and ID of tubing before the column
 - If gradient, then post-column tubing is most impactful reduce the length and ID of tubing after the column
- Does your system have a heat exchanger?
 - If so, consider bypassing it as long as you are able to operate your method without it
- Is there a smaller volume flow cell available for your system?
 - If so, consider switching to it

STEPS FOR SELECTING CONNECTING TUBING

1. Measure the length in mm from the injector to the column and from the column to the detector or MS source
2. Select ID tubing and length that will fit according to the table below being mindful of your system back pressure limits:

L(mm)	ID (µm)	volume (uL)	
150	25	0.07	Red
	50	0.29	Orange
	75	0.66	Green
	100	1.18	Green

L(mm)	ID (µm)	volume (uL)	
350	25	0.17	Red
	50	0.69	Red
	75	1.55	Green
	100	2.75	Green

L(mm)	ID (µm)	volume (uL)	
600	25	0.29	Red
	50	1.18	Red
	75	2.65	Orange
	100	4.71	Green

Green = minimal extra back pressure (<15 bar)
 Orange = may generate too much back pressure (>40 bar)
 Red = not recommended (>100 bar)



ISO 9001:2015 certified QMS



HALO® 1.5 PRODUCT OFFERINGS

1.5 MM COLUMN SIZES AND PHASES

ANALYTICAL COLUMNS

BONDED PHASE	PORE SIZE	DESCRIPTION	PART NUMBER
C18	90 Å	HALO 90 A C18, 2.7 µm, 1.5 x 50 mm	9281X-402
		HALO 90 A C18, 2.7 µm, 1.5 x 100 mm	9281X-602
		HALO 90 A C18, 2.7 µm, 1.5 x 150 mm	9281X-702
ES-C18	160 Å	HALO 160 A ES-C18, 2.7 µm, 1.5 x 50 mm	9212X-402
		HALO 160 A ES-C18, 2.7 µm, 1.5 x 100 mm	9212X-602
		HALO 160 A ES-C18, 2.7 µm, 1.5 x 150 mm	9212X-702
C4	1000 Å	HALO 1000 A C4, 2.7 µm, 1.5 x 50 mm	9271X-414
		HALO 1000 A C4, 2.7 µm, 1.5 x 100 mm	9271X-614
		HALO 1000 A C4, 2.7 µm, 1.5 x 150 mm	9271X-714
Diphenyl	1000 Å	HALO 1000 A Diphenyl, 2.7 µm, 1.5 x 50 mm	9271X-426
		HALO 1000 A Diphenyl, 2.7 µm, 1.5 x 100 mm	9271X-626
		HALO 1000 A Diphenyl, 2.7 µm, 1.5 x 150 mm	9271X-726

CONNECTION TUBING OPTIONS

DESCRIPTION	VOLUME	PART NUMBER
MarvelXACT™ PLS 25µm x 150mm	75 nl	PL7025150
MarvelXACT™ PLS 25µm x 350mm	170 nl	PL7025350
MarvelXACT™ PLS 25µm x 600mm	295 nl	PL7025600
MarvelXACT™ PLS 50µm x 150mm	295 nl	PL7050150
MarvelXACT™ PLS 50µm x 350mm	685 nl	PL7050350
MarvelXACT™ PLS 50µm x 600mm	1178 nl	PL7050600
MarvelXACT™ PLS 75µm x 150mm	665 nl	PL7075150
MarvelXACT™ PLS 75µm x 350mm	1545 nl	PL7075350
MarvelXACT™ PLS 75µm x 600mm	2650 nl	PL7075600
MarvelXACT™ PLS 100µm x 150mm	1178 nl	PL7100150
MarvelXACT™ PLS 100µm x 350mm	2750 nl	PL7100350
MarvelXACT™ PLS 100µm x 600mm	4710 nl	PL7100600
MarvelXACT™ PEEKsil™ 25µm ID x 150mm	75 nl	PS7025150
MarvelXACT™ PEEKsil™ 25µm ID x 350mm	170 nl	PS7025350
MarvelXACT™ PEEKsil™ 25µm ID x 600mm	295 nl	PS7025600
MarvelXACT™ PEEKsil™ 50µm ID x 150mm	295 nl	PS7050150
MarvelXACT™ PEEKsil™ 50µm ID x 350mm	685 nl	PS7050350
MarvelXACT™ PEEKsil™ 50µm ID x 600mm	1178 nl	PS7050600
MarvelXACT™ PEEKsil™ 75µm ID x 150mm	665 nl	PS7075150
MarvelXACT™ PEEKsil™ 75µm ID x 350mm	1545 nl	PS7075350
MarvelXACT™ PEEKsil™ 75µm ID x 600mm	2650 nl	PS7075600
MarvelXACT™ PEEKsil™ 100µm ID x 150mm	1178 nl	PS7100150
MarvelXACT™ PEEKsil™ 100µm ID x 350mm	2750 nl	PS7100350
MarvelXACT™ PEEKsil™ 100µm ID x 600mm	4710 nl	PS7100600



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