PRACTICAL APPLICATION OF FUSED-CORE® COLUMNS WITH CONVENTIONAL HPLC INSTRUMENTATION:

Understanding the Importance of Extracolumn Volume

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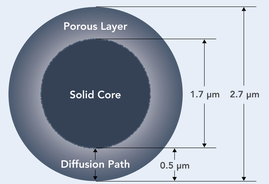
Objectives of the Study

- Determine the effects of extracolumn volume and dispersion on performance of HALO® Fused-Core® columns when using conventional instrumentation.
- Determine how much extracolumn volume and dispersion is allowed to achieve acceptable performance from various HALO column geometries
- Provide instrument configuration and parameter recommendations to chromatographers so that Fused-Core columns can deliver acceptable and optimum performance levels for both high throughput and high resolution separations

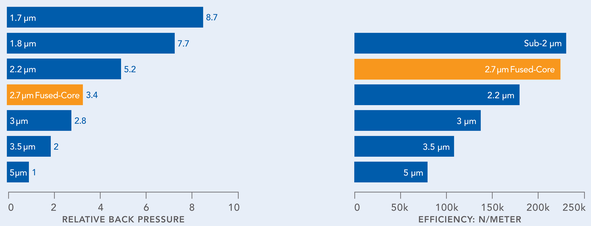
HALO Fused-Core Particle



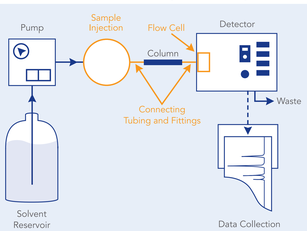
Interstitial porosity is 0.43 and total porosity 0.50

Specific surface area of porous layer is 220 m^2/gram

HALO Fused-Core Columns Exhibit UHPLC-like Performance at Conventional HPLC Pressure



Extracolumn dispersion: Experimental parameters



In this schematic of an HPLC system, the gold color indicates where extra column volume, ECV, is located.

- Injector
  - Injection volume
  - Sample solvent strength
  - Injector plumbing volume
- Tubing in Sample Path
  - Internal Diameter (ID)
  - Length
  - Pre-column vs. post column
- Detector
  - Flow cell volume and design
  - Response time (filtering)
  - Data rate

- ECV = total volume outside of the column that contributes to the observed peak volume
- High-efficiency and low-volume columns are more susceptible to efficiency losses due to band broadening from ECV and detector signal filtering
- Impact of extra-column dispersion varies inversely with column volume, peak volume, and retention

Estimating maximum acceptable extracolumn volume, w_{ec}

$$w_{ec} = 4 \times \left[\frac{V_m^2 \times (k+1)^2}{N_{theor}} \times \frac{1 - \left(\frac{N_{obs}}{N_{theor}} \right)}{\left(\frac{N_{obs}}{N_{theor}} \right)} \right]^{1/2}$$

- V_m in µL
- (N_{obs}/N_{theor}) is the percentage of theoretical plates as fraction

Maximum Acceptable ECV

- Lower for higher efficiency columns (N) (smaller d_p lower H/h)
- Lower for smaller volume columns (V_m)
- Higher for longer retention (k)

Example: Calculating maximum ECV to achieve 90% of resolution (81% of N) when k =3.

4.6 x 250 mm, 5 µm column
$N_{obs}/N_{theor} = 0.81$, $N_{theor} = 20{,}000$; V_m 2.5 mL
W_{ec} = 125 µL

3 x 50 mm, 2.7 µm HALO
V_m ~ 177 µL, h ~1.45, $N_{theor} = 12770$
W_{ec} = 12 µL

Most conventional HPLCs have $30 < w_{ec} < 100$ µL

Affect of sources of extracolumn dispersion on isocratic and gradient separations

Extracolumn dispersion before the column has much more effect on isocratic separations than gradient separations

- Injection volume
- Sample solvent composition
- Flow path in autosampler and valve
- Tubing from the autosampler to mobile phase preheater (if present)
- Precolumn heat exchanger
- Tubing from precolumn heat exchanger to column
- Volume due to any in-line filters, unions, guard columns, etc.

Extracolumn dispersion after the column effects both isocratic and gradient separations

- Tubing from the column to flow cell
- Flow cell volume and design
- Signal filtering due to detector response time

Experimental: Instrumentation and Configurations

Agilent 1100 Quaternary System

- Popular HPLC system in many laboratories
- Standard degasser
- Quaternary pump with low pressure mixing, delay volume ~1.4 mL
- Standard autosampler with variable volume injector
- Standard tubing ID (0.007") and lengths in sample flow path
- Variable wavelength UV-VIS detector
  - Flow cells
    - -1 µL, 5 µL and 14 µL
  - Response times
    - -0.0625, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 sec

Evaluation of 3 Instrument Configurations with Different ECVs

- ECVs chosen for acceptable performance with respective column sizes
  - Standard configuration
  - Low ECV configuration
  - Ultra-Low ECV configuration

Experimental: Column dimensions, test mixture and criterion selected

Columns

- 4.6 mm ID: 50, 100, and 150 mm
- 3.0 mm ID: 50 and 100 mm
- 2.1 mm ID: 50 mm

Column geometries selected based on (1) column volume, (2) greater likely use by more chromatographers and (3) historical sales data

Test Mixture

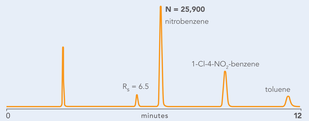
- Probe analytes chosen from among mono-substituted and di-substituted benzenes
  - uracil, benzyl alcohol, benzonitrile, nitrobenzene, anisole, 1-chloro-4-nitrobenzene, toluene
- k values from ~0.35 to ~3.5 vs. uracil void marker

Criterion

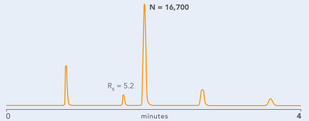
- Aim for $N_{obs} \geq 81\%$ of N_{theor} ($R_{obs} \geq 90\%$ of maximum theoretical R_s)
- Reduce ECV and decrease response times to improve N for smaller columns

HALO Fused-Core columns deliver significantly better performance when ECV is reduced

Conventional C18 4.6 x 250 mm, 5 µm, 1 mL/min



HALO C18 4.6 x 100 mm, 2.7 µm, 1 mL/min

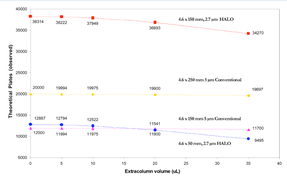


Standard Configured Agilent 1100
- ECV ~36 µL
- Standard flow cell; 14 µL 0.5 sec. response time
- Standard length and ID tubing (0.007" ID x 750 mm total)

HALO C18 4.6 x 100 mm 2.7 µm, 2 mL/min

Ultra-Low ECV Configured Agilent 1100
- ECV ~10 µL
- Semi-micro flow cell; 5 µL 0.5 sec. response time
- Reduced length and ID tubing (0.005" ID x 460 mm total)

Theoretical plates vs. ECV for conventional and HALO Fused-Core columns



How to improve resolving power with high efficiency, smaller volume columns

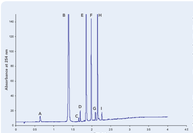
Reduce Extracolumn Dispersion

- Use smaller volume flow cell (≤ 5 µL)
- Use fastest practical data rate and shortest detector response time
  - Set detector response time to fastest setting that provides acceptable S/N. (< 0.2 seconds recommended for HALO columns.)
  - Set data rate to collect at least 20 data points across the narrowest peak of interest. (> 5 Hz recommended for HALO columns.)
- Reduce tubing ID and length between injector and flow cell
  - Connection from column to flow cell is more important than from autosampler to column
  - use a smaller volume pre-column heat exchanger (1.6 µL) (if necessary)
- Use smallest practical injection volume (repeatability)
- Choose sample solvent composition to be weaker than mobile phase, solubility permitting

Configuring your instrument for ultrafast and high resolution HPLC

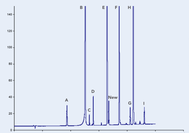
	Standard Configuration	Low Volume Configuration	Ultralow Volume Configuration
Total ECV	~36 µL	~17 µL	~12 µL
Flow Cell	Standard, 14 or 13 µL	Semi-micro, 5 µL	Micro, 1 or 2 µL
Tubing	0.007" ID, Standard Lengths	0.005" ID, Minimal lengths	0.005" ID, Minimal lengths
Heat Exchanger	3 µL, Standard	3 µL, Standard	1.6 µL, Micro
Acceptable Fused-Core Columns	4.6 mm ID columns > 50 mm lengths	All 4.6 mm ID columns and 3.0 mm ID columns ≥75 mm lengths	All 4.6, 3.0, and 2.1 mm ID columns

Ultrafast and high resolution separations with low ECV configured HPLC system



Column: 3 x 50 mm, 2.7 µm HALO C18
Flow Rate: 1.0 mL/min
Inj. Vol.: 1 µL
Col. Temp.: 40 °C
Gradient: 5–95% ACN/water (0.1% HCOOH) in 2 minutes, hold 1.5 min.
Instrument: Agilent 1100 configured with low ECV

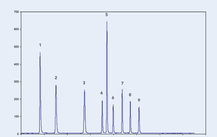
Ultrafast separation for screening columns and conditions during method development or for high sample throughput.



Column: 4.6 x 150 mm, 2.7 µm HALO C18
Flow Rate: 1.0 mL/min
Inj. Vol.: 5 µL
Col. Temp.: 40 °C
Gradient: 5–95% ACN/water (0.1% HCOOH) in 14 minutes
Instrument: Agilent 1100 configured with low ECV

High resolving power for impurity profiling

Ultra-fast gradient separation of 9 component sample in ~1 minute with HALO Fused-Core column on a low ECV configured HPLC system



HALO C18, 3.0 x 50 mm, 2.7 µm
Injection Volume: 0.9 µL
Flow Rate: 2.0 mL/min
Gradient 35–90% ACN/water in 0.5 min., injector program used
Column Temperature: 40 °C
VWD, semi-micro flow cell (5 µL)

Response Time: 0.0625 sec
Data Rate: 13.7 Hz
Detection: UV at 254 nm
Agilent 1100 QuaternaryLow ECV (17.5 µL)
Initial Pressure: 305 bar (4423 psi)

Sample: 1. acetanilide, 2. acetophenone, 3. propiophenone, 4. butyrophenone, 5. benzophenone, 6. valerophenone, 7. hexanonphenone, 8. heptanophenone, 9. octanophenone

Conclusions

Extracolumn dispersion in conventional HPLC systems can limit the benefits of columns that generate fast eluting, small volume peaks.

Achieving "UPLC-like" performance is possible with conventional HPLC equipment by reducing extracolumn band broadening and using an appropriate HALO Fused-Core column.