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CURRENT ISSUE

SMALL MOLECULE

Extracolumn Dispersion Part 2:

IMPACT TO ISOCRATIC AND GRADIENT UHPLC METHODS

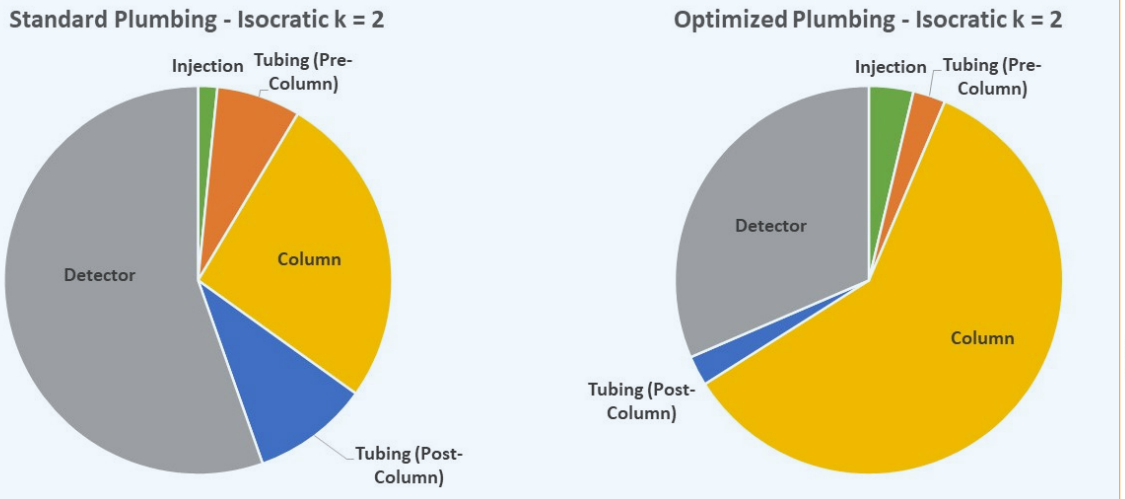
In part 1 of this series, extracolumn dispersion (ECD) was introduced, its importance was discussed, and ways to measure and reduce it were described. In part 2, the impact of ECD on isocratic and gradient methods will be investigated with the use of dispersion plots so that one can clearly see the contributions of each term to the total system dispersion. The plots are generated from the Web-based dispersion calculator [1] referenced in the recent series of articles published in LCGC North America [2-5]. Equation 1 shows all of the contributions to ECD:

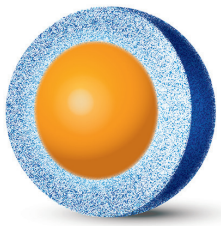
$$\sigma^2_{observed} = \sigma^2_{injection} + \sigma^2_{tubing,\ pre-col} + \sigma^2_{column} + \sigma^2_{tubing,\ post-col} + \sigma^2_{detection} \qquad (1)$$

The Dispersion Calculator includes input fields for all of these terms, the method conditions, and UHPLC column details. Readers are encouraged to try the Dispersion Calculator for themselves so they can compare the "before" and "after" for whichever method conditions and instrument configurations are of interest. In all cases, the objective is to maximize the pie slice that corresponds to the column (yellow) so that the true efficiency of the column is observed or the maximum resolution is achieved.

ISOCRATIC UHPLC METHODS

Figure 1. Comparison of total dispersion using an isocratic method for standard configuration vs. optimized configuration for a UHPLC.





For isocratic UHPLC methods, both the pre- and post-column components contribute to the ECD. In the example in Figure 1 above, one can see the contributions to total dispersion for an isocratic method when standard plumbing is used compared to optimized plumbing. Tables 1 and 2 provide the method conditions and instrument parameters, respectively.

Table 1. Method and instrument conditions common to both standard and optimized UHPLC systems.

Common to Both Standard and Optimized Systems	
Column	HALO 90 Å C18, 2.7 µm, 1.5 x 100 mm
Retention Factor (k)	2
Flow rate (mL/min)	0.2
Injection Volume (µL)	0.5
Diffusion Coefficient (m^2/s)	1.4×10^{-9}
Mobile Phase	50/50 water/ACN
Temperature (°C)	35
Mobile Phase Dynamic Viscosity (cP)	0.663
Needle seat diameter (µm)	75
Needle seat length (mm)	50

Table 2. Instrument parameters for standard and optimized UHPLCs.

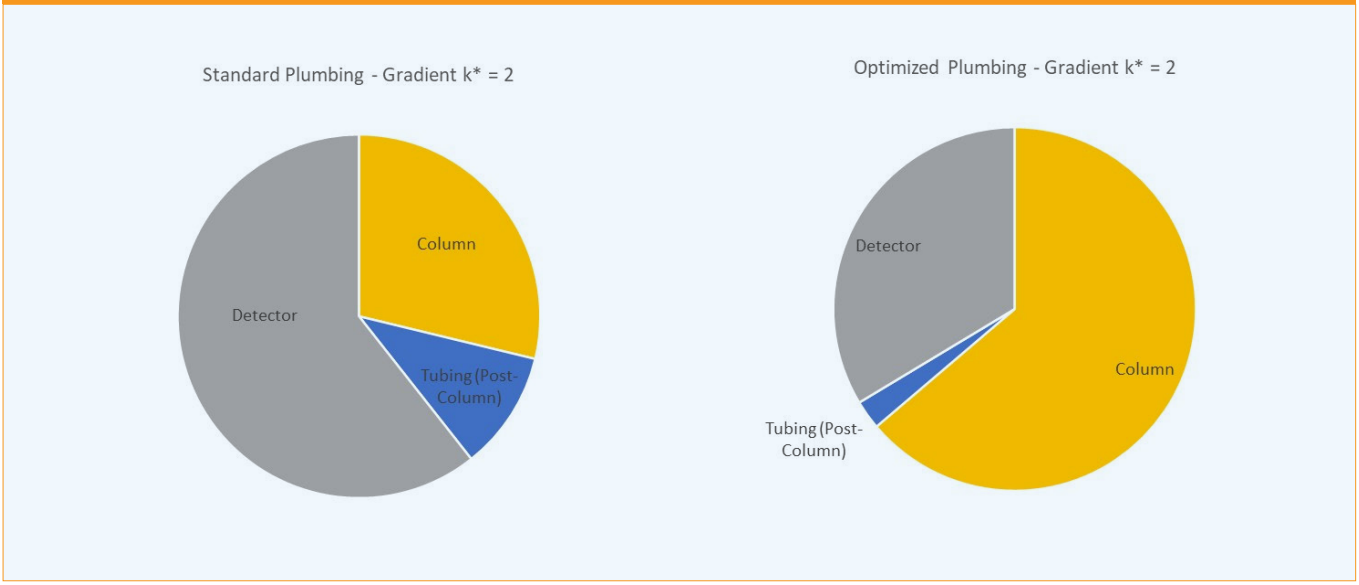
Instrument Parameter	Standard UHPLC	Optimized UHPLC
Pre-Column Tubing Volume (µL)	100 µm x 600 mm 4.7	75 µm x 350 mm 1.5
Post-Column Tubing Volume (µL)	100 µm x 800 mm 6.3	60 µm x 707 mm 2.0
Detector Flow Cell (µL)	2	1

Notice how the contribution to dispersion is dominated by the detector (gray) for the standard plumbing condition. When modifications to the system are made, the contribution from the column increases while the areas of the other pie slices all decrease. MarvelXact™ connectors of various volumes from IDEX are available via halocolumns.com for reducing ECD.

GRADIENT UHPLC METHODS

For gradient UHPLC Methods, the ECD is from the post-column tubing and the detector. The reason for this is the focusing that happens when lower solvent strength samples are injected under gradient conditions. In Figure 2, one can see the contributions to dispersion for standard plumbing vs. optimized plumbing using gradient conditions.

Figure 2. Comparison of total dispersion using a gradient method for standard configuration vs. optimized configuration for a UHPLC.



The same column and instrument parameters were used for the dispersion calculation for the gradient example. The only exception is that a 1 µL detector flow cell was used for both. Similar to the example for an isocratic condition, the detector (gray) dominates the dispersion for the standard plumbing configuration. When shorter ID tubing from the column to detector is swapped in, the contribution from the column (yellow) is maximized, which would then translate to reduced width peaks that are more intense. See the example below in Figure 3 using common over the counter cough and cold medicines.

Figure 3. Comparison of standard to optimized UHPLC configuration for a HALO 90 Å C18, 2.7 µm, 1.5 x 150 mm column. Peak identities in order are phenylephrine, acetaminophen, caffeine, doxylamine, guaifenesin, aspirin, salicylic acid, and dextromethorphan.

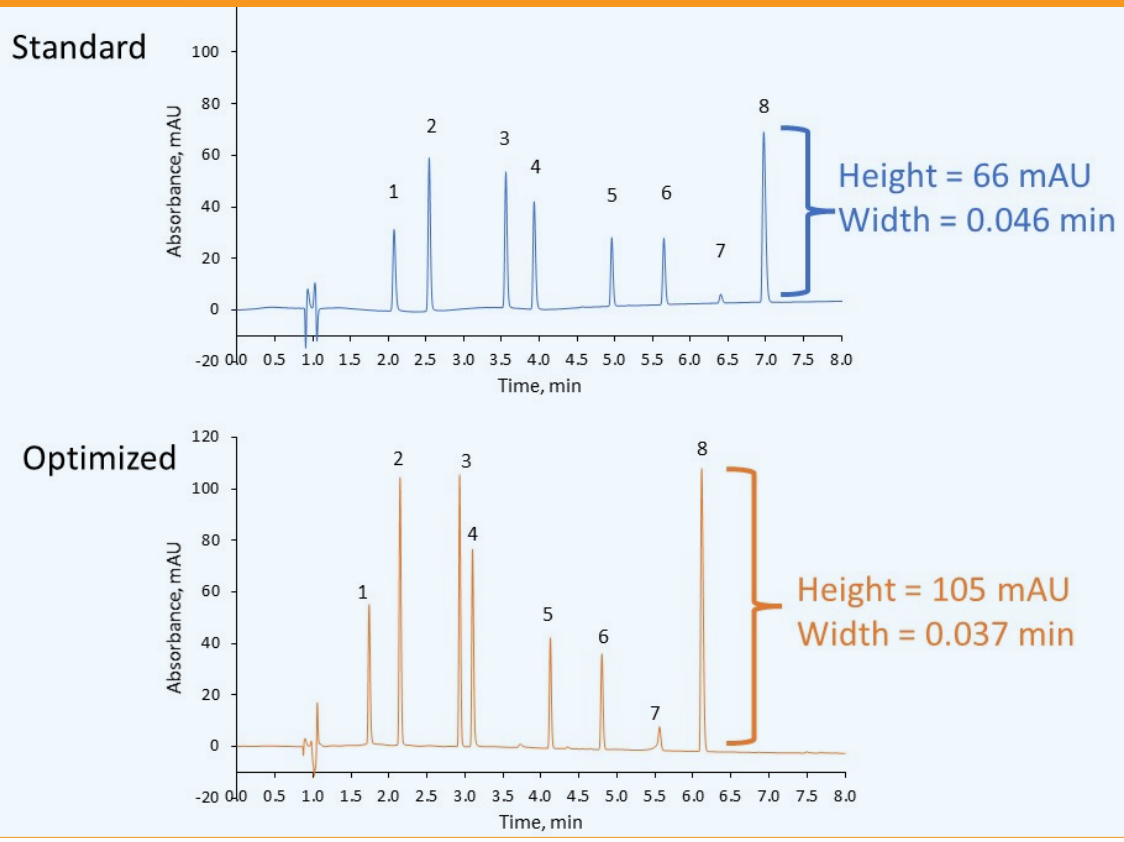


Table 3. Instrument Parameters for Standard and Optimized Configurations

	STANDARD	OPTIMIZED
Mixer	100 µL	20 µL
Tubing from injector to column	0.1 mm x 800 mm 6.3 µL	75 µm x 350 mm 1.5 µL
Tubing from column to Detector	0.1 mm x 509 mm 4 µL	60 µm x 707 mm 2 µL
Flow cell (µL)	1	1
ECD (μL^2)	14	2

Table 3 shows the comparison of the UHPLC instrument configuration going from standard to optimized. Reduction of the ECD from 14 to 2 μL^2 improves the observed performance of the HALO 1.5 mm ID column so that peak widths are reduced and peak intensities are increased.

CONCLUSIONS

The contributions to ECD are different for isocratic vs. gradient UHPLC methods. Only the volume after the column is impactful for gradient separations while both pre- and post- column contributions impact isocratic separations. In order to maximize the observed performance from the column, it is essential to reduce the volume in the system. Overall, there must be a compromise between what pressure can be accommodated and what sensitivity is desired when reducing UHPLC system ECD.

REFERENCE

1. https://www.multidlc.org/dispersion_calculator/
2. D.R. Stoll, K. Broeckhoven, Where Has My Efficiency Gone? Impacts of Extracolumn Peak Broadening on Performance, Part I: Basic Concepts, LCGC North America. 39 (2021) 159–166.
3. D.R. Stoll, K. Broeckhoven, Where Has My Efficiency Gone? Impacts of Extracolumn Peak Broadening on Performance, Part II: Sample Injection, LCGC North America. 39 (2021) 208–213.
4. D.R. Stoll, K. Broeckhoven, Where Has My Efficiency Gone? Impacts of Extracolumn Peak Broadening on Performance, Part III: Tubing and Detectors, LCGC North America. 39 (2021) 252–257.
5. D.R. Stoll, T.J. Lauer, K. Broeckhoven, Where Has My Efficiency Gone? Impacts of Extracolumn Peak Broadening on Performance, Part IV: Gradient Elution, Flow Splitting, and a Holistic View, LCGC North America. 39 (2021) 308–314.