Extracolumn Dispersion Part 1:

THE WHAT AND WHY OF EXTRACOLUMN DISPERSION AND HOW TO REDUCE IT

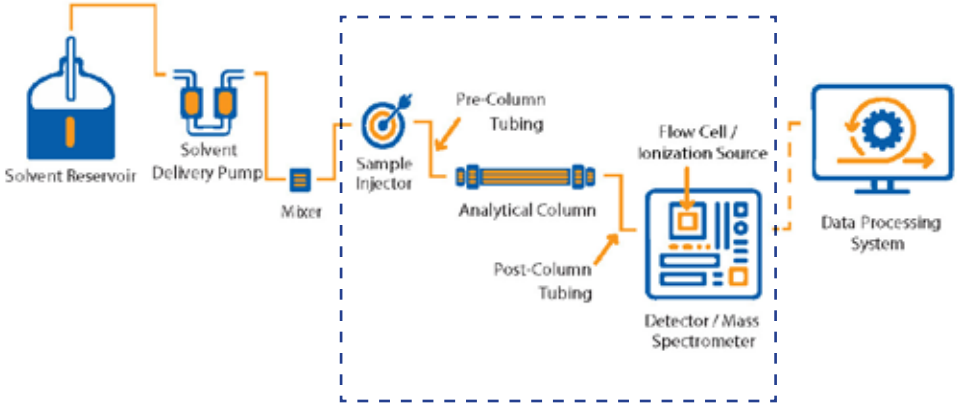
Band broadening or extracolumn dispersion (ECD) is what happens to an injection of sample as it makes its way from the injector to the UHPLC column to the detector. As the UHPLC column ID is reduced, the more critical it is to minimize the effects of ECD in order to observe the true efficiency of the UHPLC column. A comprehensive series of articles was recently published in LCGC North America [1-4] as well as a review article from on ECD [5]. The reader is referred to those for a more in-depth discussion of the topics. Additionally, a video describing extracolumn volume (ECV) is available: https://halocolumns.com/videos/halo-extra-column-volume/. Tom Jupille from LC Resources has commented that extracolumn volume is the cause while extracolumn dispersion is the effect. There is a relationship between ECD and ECV and so to convert from ECV to ECD, use equation 1.

$$ECD = \left(\frac{EVC}{4}\right)^2 \qquad (1)$$

ECD is measured in μL^2 while ECV is measured in µL. While it is easier to visualize ECV, it is more useful to know ECD since this value is an additive portion of the total dispersion in the system, which includes the column. See equation in Figure 1.

Figure 1. Equation for the total dispersion and the sources of dispersion in a UHPLC system.
The sources of dispersion include the injection volume, the pre-column tubing, the column, the post-column tubing, and the detector flow cell (all of the items surrounded by the dashed black line) in Figure 1.

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



WAYS TO ESTIMATE ECD

The following table lists the multiple ways to estimate ECD along with their advantages and disadvantages [5].

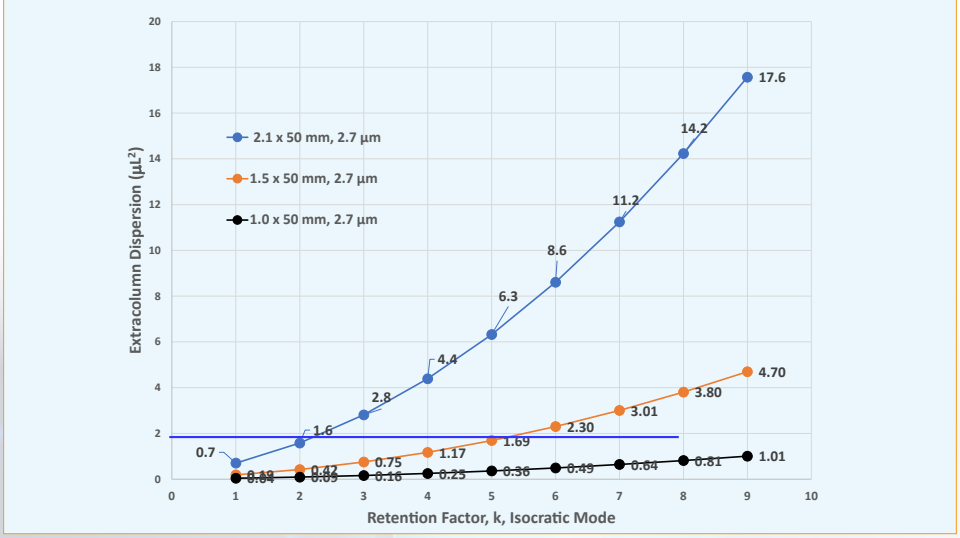
Table 1. Ways to Estimate ECD and their advantages and disadvantages

Method	Advantages	Disadvantage
No column	Fast, easy	Prone to errors
Linear extrapolation	No additivity issues since column remains in place	Extrapolates data outside of the experimental range; assumes that N is constant for all analytes used
Suppression-of-individual-instrument-component contributions	Identify which component(s) are most impactful	Need smaller volume components to replace existing components
2 point on column detection	Enables estimate of ECD without making changes to the system	Difficult to do with stainless steel columns
Web-based dispersion calculator (6) https://www.multidlc.org/dispersion_calculator/	Easy, gives visual plot of dispersion	Need to know information about separation (k; N) and details of how instrument is plumbed

IMPORTANCE OF ECD

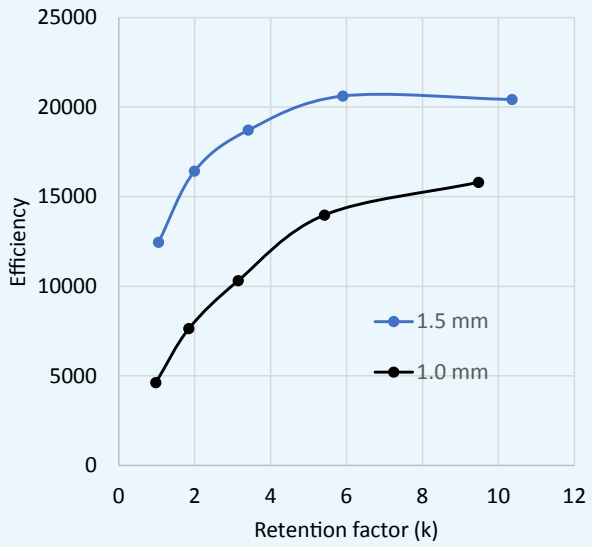
How do you know if your separation is subject to the effects of ECD? One way to know is to estimate the ECD. A good value for one column/system may be a poor value for another column/system. It depends on the particle size, column dimension, and method conditions (retention factor). See Figure 2.

Figure 2. Maximum Extracolumn Dispersion (μL²) for 90%Theoretical Resolution as Function of Retention Factor, k, for 50 mm Columns in 2.1, 1.5, and 1.0 mm IDs



As the column ID is reduced, the impact of ECD increases. As retention factor (k) increases, the maximum amount of dispersion for 90% resolution increases, which means that in order to overcome the ECD on a small ID column with short length, a relatively high retention factor is needed.

Figure 3. Plots of efficiency vs. retention factor for 1.5 and 1.0 mm ID columns.



Another way to know if your system has too much ECD is to reduce the ECD in your LC system and look for improvements in efficiency or peak width. If you observe that efficiency is increasing as the retention factor is increasing, this is a sign that your system has too much ECD for the column that you are using. See Figure 3.

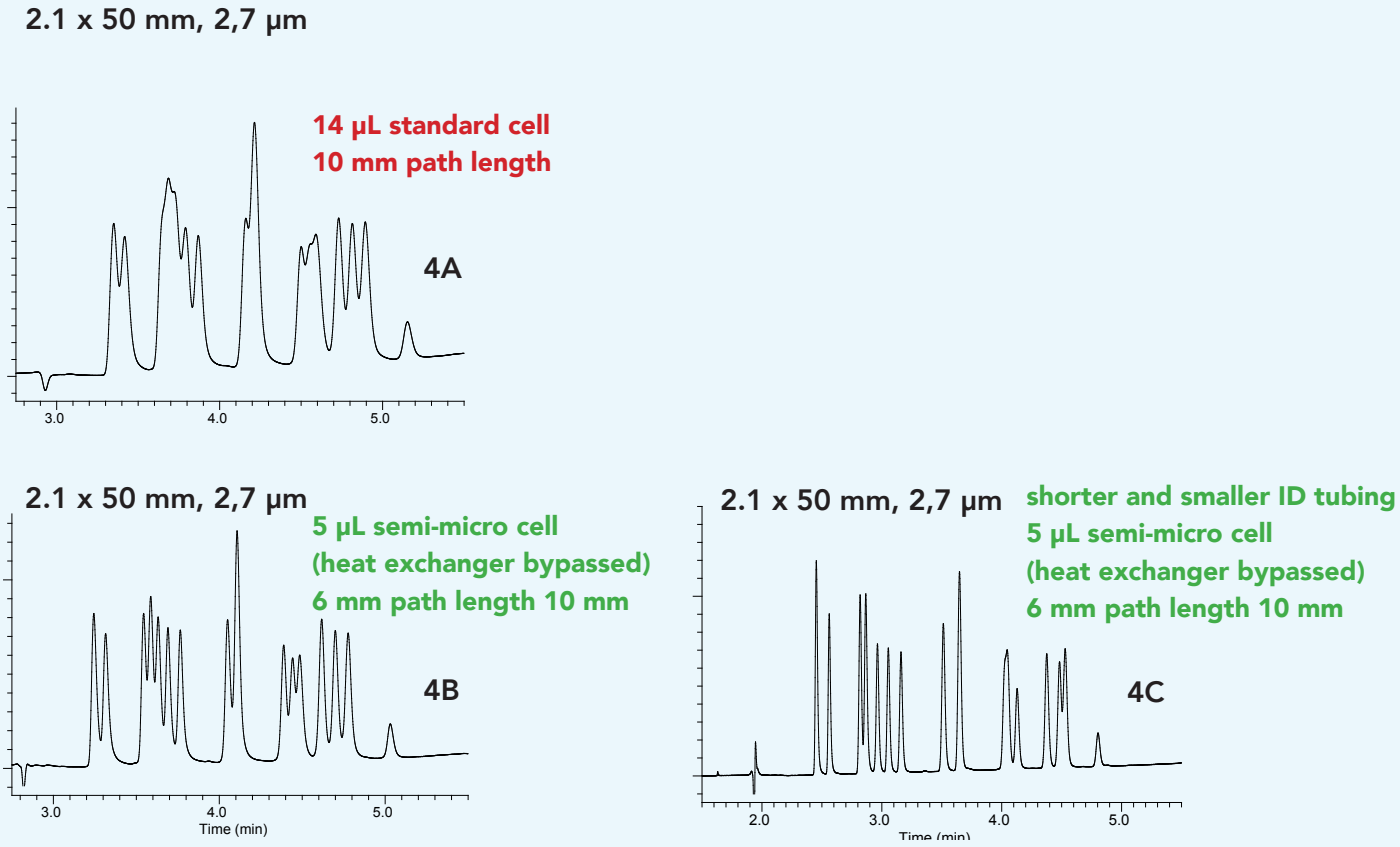
Table 2 lists ways to reduce ECD and improve the observed column performance.

Table 2. Recommendations for Instrument and Method Parameters That Affect ECD and Maximize Column Performance

Instrument or Method Parameter	Recommendations
Injection Volume	• Keep injection volume to a minimum according to desired sensitivity, S/N ratio and loading
Sample Solvent Composition	• Keep sample solvent strength equal to or less than initial mobile phase if possible. • If 100% organic must be used as sample solvent, use minimum injection volume possible.
Pre-column Tubing Volume	• Use minimum lengths of 100 µm ID tubing or smaller. Different tubing IDs in sample flow path can cause additional dispersion.
Heat Exchanger Volume	• Some instruments have column compartments with different heat exchanger volume options. • Use smallest volume consistent with flow rates, especially for 2.1 mm ID columns and smaller.
Post-column Tubing Volume	• Use minimum lengths of 100 µm ID tubing or smaller. Different tubing IDs in sample flow path can produce additional dispersion. • Note 50 or 75 µm ID tubing may provide better results at the expense of higher backpressure.
Detector Flow Cell Volume	• Use flow cell volumes 1-5 µL for most 2.1 and 3 mm ID columns and 1 µL flow cells for 1.5 mm ID columns. • Larger cell volumes (10-15 µL) can often be used for 4.6 mm ID columns in longer lengths.
Detector Data Rate	• Set data rate so that 20-40 pts are sampled across the narrowest peak. There is a compromise between minimum peak width (highest N) and S/N level as a function of data rate (Hz).
Detector Response Time	• For some instruments response time is inversely dependent on the data rate setting. • Maximum allowable response time is proportional to retention time and √(% broadening), i.e., loss in N and Rs, and inversely proportional √N. • Start with fastest response time and increase until just before no loss in N and noise is acceptable.

An example of excess ECD is demonstrated in Figure 4A. If an analyst was looking to identify which component has the greatest impact on ECD or which should be optimized, the answer would be the detector. In Figure 4B the improvement of only reducing the volume of the detector flow cell is demonstrated while in 4C the improvement of a smaller volume detector flow cell coupled with shorter, smaller ID tubing is demonstrated.

Figure 4A. Too much ECD in system so peaks are broad and coeluted. **Figure 4B.** Switching to a reduced volume detector flow cell improves the separation of cannabinoids using a HALO 90 Å C18, 2.7 µm, 2.1 x 50 mm column. **Figure 4C.** Additional improvements to the cannabinoid separation are made when the small volume flow cell is coupled with shorter, smaller ID tubing.



CONCLUSIONS

Remember that the smaller the column ID, the more susceptible the column is to ECD. In order to obtain the optimum performance from your HALO® columns, it is essential to reduce the ECD in your system if possible. This will ensure that you see the best resolution that HALO® columns are capable of delivering.

REFERENCE

1. 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.
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5. G. Desmet, K. Broeckhoven, TrAC Trends Anal. Chem. 119 (2019) 115619.
6. https://www.multidlc.org/dispersion_calculator/