Where has my efficiency gone? Impacts of extracolumn peak broadening on performance, part 3: Tubing and detectors

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Where has my efficiency gone? Impacts of extra-column peak broadening on performance, Part III – Tubing and detectors

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[teaser]

Dispersion (broadening, or spreading) of analyte zones (peaks) outside of chromatography columns can seriously erode the resolution provided by good columns. In this installment we focus on the contributions of dispersion in connecting tubing and detectors to the total level of extra-column dispersion in a LC system.

[main text]

In the installments of “LC Troubleshooting” from the last two months we reviewed the basic concepts of extra-column dispersion (ECD) [1], how the level of ECD associated with a particular instrument can impact the apparent quality of a separation (for example, as measured by resolution), and discussed dispersion associated with the injection step in some detail [2].

In this month’s installment we continue our discussion of details associated with the contributions of specific system components. Figure 1 illustrates the contributions of different system components to the total observed variance, starting with the injection step and ending with the detection step. In principle the total observed peak variance is simply the sum of the variances contributed by each of the system components. This is only rigorously correct if the dispersion in each element of the system is independent of the others [3], but under most conditions, Equation 1 is accurate enough to guide method development and system optimization [4]. In this installment of “LC Troubleshooting” we continue by discussing dispersion in connecting tubing and detectors.

$$\sigma^2_{observed} = \sigma^2_{injection} + \sigma^2_{tubing,pre-col} + \sigma^2_{column} + \sigma^2_{tubing,post-col} + \sigma^2_{detection} \quad \text{Equation 1}$$

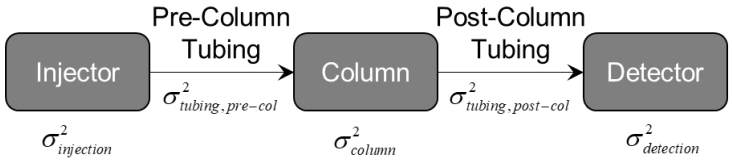

Figure 1. Illustration of the contributions of several LC system components to the total variance of peaks observed at the detector, $\sigma^2_{observed}$. We note that Equation 1 is valid using either time- or volume-based variances.

Dispersion in Connecting Tubing

It is a well known fact that increasing the length and inner diameter of connecting capillary in a chromatographic instrument increases dispersion. This dispersion is the result of the parabolic flow profile that is established in an open capillary under laminar flow conditions. Under these conditions the fluid in the center of the tube moves at twice the average velocity, whereas the fluid near the wall is stagnant (that is, stuck to the wall). Estimating the contribution of this dispersion that occurs in connecting capillaries to the total extra-column band broadening in a LC system is however not straightforward. The two limiting conditions for which analytical solutions exist are: 1) dispersion in a short, straight capillary at high flow rates (with negligible radial equilibration between velocity streams); and 2) dispersion in very long capillaries at low flow rates (that is, where full radial equilibration between velocity streams occurs by diffusion). In the first case, the dispersion contribution of the capillary is given by the Atwood-Golay equation [5]:

$$\sigma^2_{V,tub,AG} = \frac{\pi^2}{48} \cdot L^2_{tub} d^4_{tub} \quad (2)$$

where L_{tub} and d_{tub} are the length and diameter of the capillary. In the second case, the dispersion is given by the Taylor-Aris equation, where F is the the flow rate through the capillary and D_m is diffusion coefficient of the analyte in the mobile phase [6]

$$\sigma^2_{V,tub,TA} = \frac{\pi}{384} \cdot \frac{F \cdot L_{tub} \cdot d^4_{tub}}{D_m} \quad (3)$$

Most conditions used for practical LC methods lie between the extreme conditions where Equations 2 and 3 are valid, and thus require that we consider a partial radial equilibration of the analyte across the tubing diameter (that is, across different mobile phase velocity streams). For

this purpose we can used either a Giddings-style coupling of the previous expressions (Equation 4) [7], or an exponential model based on numerical simulations (Equation 5) [8]:

$$\sigma^2_{v,tub} = \frac{\pi^2 \cdot L^2_{tub} \cdot d^4_{tub}}{48 + 384 \cdot \pi \cdot L_{tub} \cdot \frac{D_m}{F}} \quad (4)$$

$$\sigma^2_{V,tub} = \sigma^2_{V,tub,TA} \cdot \left[1 - \frac{1}{\alpha \cdot L_{tub}} \cdot (1 - e^{-\alpha \cdot L_{tub}})\right] \quad (5)$$

with α given by $\alpha = 15{\cdot}04{\cdot}\pi{\cdot}D_m/F$. In practice, however, Equations 4 and 5 both seem to overestimate the degree of dispersion due to these capillaries, in comparison to experimental observations, especially at high flow rates [7]. One possible explanation for this observation comes from the fact that these equations are in fact only valid for a straight tube, which is seldom used in real instruments where the capillaries are coiled and/or bent to fit in different instrument components such as the injection valve, mobile phase preheaters, the column, and detector inlet. As a result of this coiling, centripetal forces acting on the liquid cause secondary radial flow effects in the liquid. Beyond a critical flow rate, these radial flow effects are pronounced enough to enhance radial mixing and thus the equilibration of the analyte across the radius of the tube. This in turn relaxes the axial dispersion that normally results from parabolic flow profile. Nevertheless, Equations 2-5 make it clear that using shorter and narrower capillaries reduces contribution of these tubes to the total extra-column dispersion. This is especially true when using gradient elution, where the volumes of peaks exiting the column are typically much smaller than they are when using isocratic elution. Figure 2 shows that even for a relatively short (140 mm) piece of tubing between column outlet and the detector inlet, the diameter of this tube can have a dramatic effect on peak width observed at the detector [3]. When considering the use of narrow capillaries however, one must be cautious about the pressure required to push the mobile phase through these tubes at the desired flow rate (ΔP_{tub}), as this increases with the inverse fourth power of the column diameter as shown in Equation 6 (where η is the dynamic viscosity of the mobile phase):

$$\Delta P_{tub} = 128 \cdot \frac{\eta \cdot L_{tub} \cdot F}{\pi \cdot d^4_{tub}} \quad (6)$$

For example, changing from a commonly used 120 µm i.d. stainless steel capillary to a 75µm i.d. capillary comes at the cost of a 6.5-fold higher pressure drop.

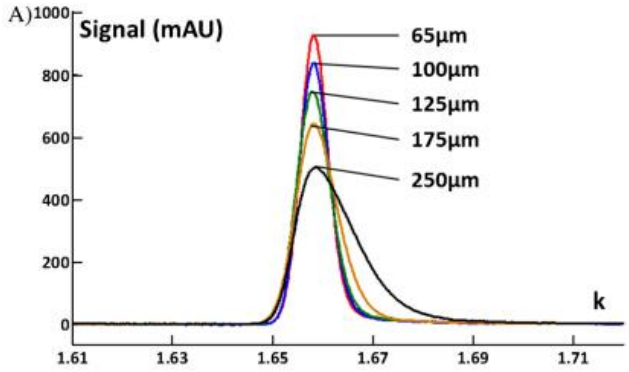

Figure 2. Example of the effect of post-column tubing diameter (L_{tub} = 140 mm in each case) on peak width and height in the case where gradient elution is used. The x-axis is effective retention factor ($[t_R\text{-}t_m]/t_m$). The column used was 50 mm x 2.1 mm i.d. (1.3 µm particles), the analyte was benzophenone, and the flow rate was 0.6 mL/min. Adapted from ref. [4]. Other conditions are described in this reference.

Dispersion in Detectors

Due to the widespread use of UV absorbance detectors in LC, most investigations into the contributions of detectors to extra-column dispersion have been focussed on UV detectors, although most fundamental aspects can also be applied on other types of detectors with flow-through detection cells. In a first, rough, approximation, these flow cells behave as an open-tubular flow path that is either circular or rectangular in cross section. As a result, the equations that govern dispersion in circular tubes can be used to estimate their contributions to extra-column dispersion. However, the entire fluidic path in a detector module is more than the detection cell itself, and often involves additional in- and outlet tubing, sharp turns, and/or changes in the cross sectional area or shape of channels. Nevertheless, in the literature the volumetric variance of dispersion attributed to the detector has often been related to the geometrical detection cell volume (V_{cell}) using an empirical relationship similar to that of the injection volume contribution [2]

$$\sigma^2_{V,detector} = \frac{V^2_{cell}}{\theta_{detector}} \quad (7)$$

The proportionality between cell volume and dispersion is not surprising as Equations 2-5 show that a longer and/or broader flow path results in more dispersion. Thus, there is a tradeoff between dispersion and sensitivity during flow cell design, as longer flow cells result in higher sensitivity [9], and broader cell design reduces signal noise. This is illustrated in Figure 3, where narrower peaks are obtained using the flow cell with the smaller volume, however this comes at the cost of

reduced sensitivity (that is, lower peak height) and a much higher baseline noise level. Innovations in the fluidic and optical characteristics of these cells over the past decades have enabled improvements in sensitivity (higher) and noise (lower), while maintaining or decreasing flow cell volume.

Recent studies have shown that Equation 7 provides poor estimates of the actual contributions of flow-through cells to the total extra-column dispersion [10,11]. As was discussed for the injection step [2], if a rectangular plug of analyte would move through the detector flow cell in a perfect plug flow without mixing, $\theta_{detector}$ would be 12. On the other hand, if the flow cell would behave as a "perfect mixer", and $\theta_{detector}$ would be 1. However, if there are zones in the flow cell that are poorly swept due to flaws in the cell design, then values of $\theta_{detector}$ below 1 can be observed [10]. In addition, another consequence of the similarities between dispersion in a flow-through cell and dispersion in a capillary is that dispersion in the flow cell is expected to be flow rate dependent. Using a custom detector that allowed continuous variation of the cell path length, Dasgupta et al. also showed that for short cell path lengths, the in- and outlet connections to the illuminated part of the flow cell make up the majority of the measured dispersion [10]. To a first approximation, the dispersion at higher flow rates, typically above 0.5 mL/min, from modern low volume flow cells can be estimated using Equation 7 with $\theta_{detector}$ = 0.5-0.8 [11]. Given the fact that the dispersion of a flow cell is significantly affected by its internal design, some instrument vendors no longer report the geometrical cell volume, and instead report the expected contribution of the flow cell to peak widths [11].

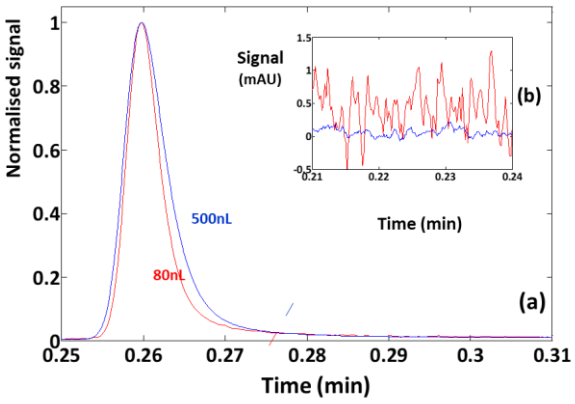

Figure 3. Comparison of peak width and baseline noise for UV flow cells with different volumes. Inset (b) shows the pre-peak region where the baseline noise is obviously much worse for the smaller flow cell. Note

that the peak height before normalization were about 360 and 560 mAU for the 80 and 500 nL flow cells, respectively. Adapted from ref. [3].

In discussions on dispersion associated with detectors it is important to note that settings associated with the electronic components of the detector (for example, sampling frequency, and detector rise time or time constant) can also affect peak width and shape. Although strictly speaking these do not contribute to extra-column dispersion in the same way as the other factors we have discussed (for example, detector cell volume), the effects of these settings can influence the observed peak variance and be confused with other contributions. Readers interested in learning more about this topic are referred to prior “LC Troubleshooting” articles [12], and some recent journal articles on the topic [13,14].

Finally, even though mass spectrometric (MS) detection is being used in more and more laboratories, there are far fewer studies of the contributions of MS detectors to extra-column dispersion than there are for UV detectors [15,16]. In the studies that have been done, however, it was found that when optimized MS settings are used, the ionization source and MS detector itself had little impact on observed peak widths. In fact, it was found that the tubing connecting the LC instrument to the MS was the most critical contributor to the extra-column dispersion for these hyphenated systems.

Summary

In this installment of “LC Troubleshooting” we have continued our discussion of details associated with the contributions of specific LC system components to extra-column dispsersion, this time focusing on connecting tubing and detectors. In the next installment in this series, we will discuss how the impact of extra-column dispersion can be different under isocratic and gradient elution conditions, and discuss the impact of post-column flow splitting on the total dispersion observed at the detector.

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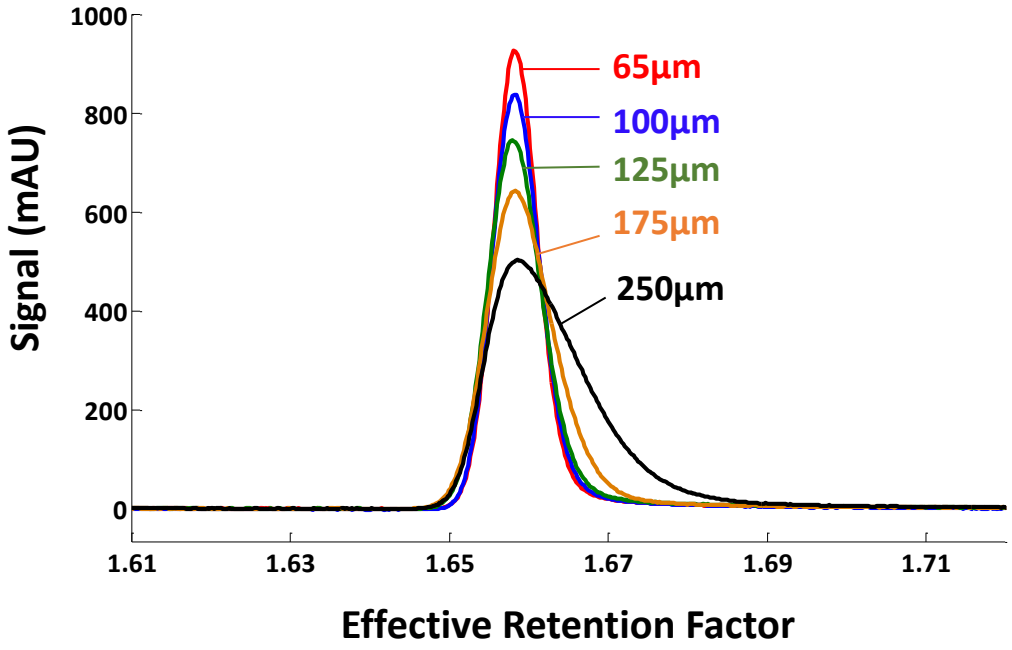
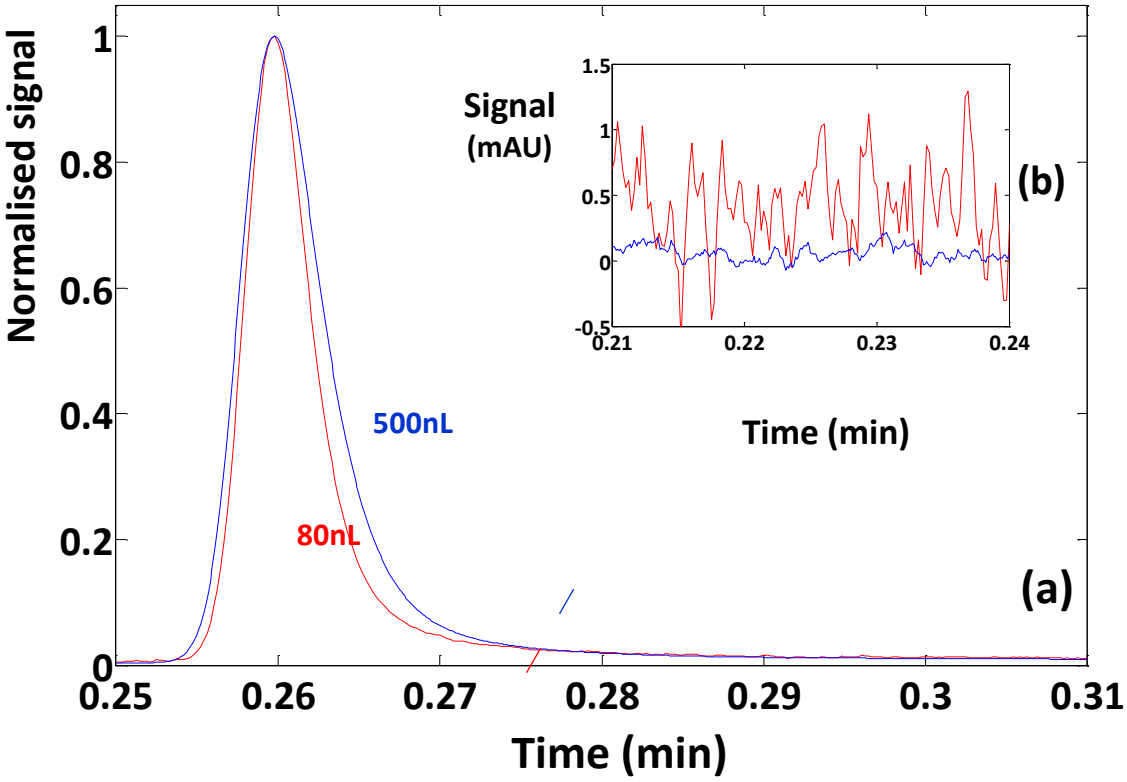

1000
800
600
400
200
0
Signal (mAU)
65µm
100µm
125µm
175µm
250µm
1.61
1.63
1.65
1.67
1.69
1.71
Effective Retention Factor


Normalised signal
1
0.8
0.6
0.4
0.2
0
0.25
0.26
0.27
0.28
0.29
0.3
0.31
Time (min)
500nL
80nL
(a)
Signal
(mAU)
1.5
1
0.5
0
-0.5
0.21
0.22
0.23
0.24
Time (min)
(b)