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Dispersion

Protocols for LC Instruments
2020

What is Dispersion?

Dispersion is a measure of analyte band broadening between the injector and detector, excluding the contribution from the column. As a sample is injected into the flow path, the analyte band begins to spread by both intra- and extra-column effects. The intra-column effects are dictated by the efficiency of the packed column bed. A poorly packed column will result in a large degree of band spreading, resulting in wide, inefficient peaks. The extra-column band broadening is a result of the injector, tubing, valves and detector in the LC system. As such, dispersion is one of the fundamental tests which should be performed to characterise an LC system.

Historically for HPLC analysis, the intra-column effects had the greatest impact on band spreading. However, with the changing trend towards UHPLC, narrow bore ID and smaller particle sizes, the impact of extra-column band broadening is far more noticeable and detrimental to the separation. Therefore, it is important to experimentally measure the LC's dispersion.

How is Dispersion Measured?

Dispersion can be measured by injecting a sample of acetone onto a zero dwell volume (ZDV) union to measure the extra column band broadening effect. The method conditions are described below in *Figure 1*, with an example of two systems with different system volumes and bandwidths.

To determine the instrument bandwidth, the retention time (t_R) and efficiency at half height (N) should be recorded. These can be inserted into *Eq. 1* to calculate sigma (σ), which in turn can calculate instrument bandwidth (4σ, *Eq. 2*). Consequently, the system volume can also be determined by this test by multiplying the average retention time of acetone by the flow rate (*Eq. 3*).

Method Conditions	
Sample:	1% Acetone in water / methanol (51:49 v/v)
Mobile Phase:	Water / methanol (51:49 v/v)
Flow Rate:	0.1 mL/min
Column:	Zero dwell volume (ZDV) union
Injection Vol.:	0.5 µL
Oven Temp.:	40 °C
Run Time:	1.5 mins
Number Inj.:	9
Sampling Frequency:	12.5 Hz

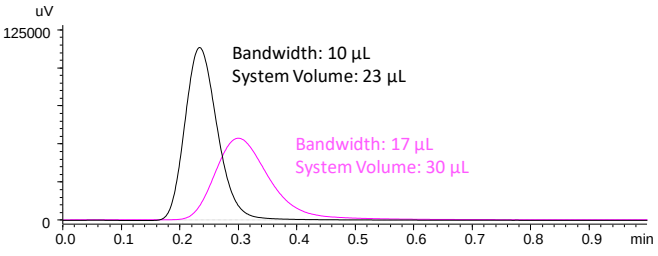

Figure 1 Operating conditions for system dispersion and example chromatograms of two different systems.

$$\sigma = \frac{t_R \times F}{\sqrt{N}} \qquad Eq.\ 1$$

$$Instrument\ Bandwidth = 4\sigma \qquad Eq.\ 2$$

$$System\ Volume = t_R \times F \qquad Eq.\ 3$$

Why Should Dispersion be Measured?

As previously described, dispersion impacts on the efficiency of a separation, where it can lead to analyte band spreading. The expected % efficiency yield for different column formats with 1.7 µm packing material were compared over a range of dispersion values. The 10 and 17 µL dispersion calculated in *Figure 1* were marked on the plots to illustrate the impact on the efficiency yield. For the different column lengths with constant ID, the 17 µL dispersion saw all three columns below 90% of the expected efficiency for that column. At 10 µL, the 100 and 150 mm column lengths achieved at least 90%, whilst the 50 mm achieved just over 80% of the expected efficiency which may still be deemed acceptable for applications.

When the column length was kept constant and the ID changed, it was noted that the 50 x 4.6 mm was the only column to achieve greater than 90%, whilst the 50 x 3.0 mm achieved greater than 85% with dispersion of 17 µL and the 2.1 mm ID achieved approximately 60% of the expected efficiency. Both the 50 x 3.0 and 50 x 4.6 mm columns were greater than 90% with the lower dispersion system, whilst the 50 x 2.1 mm possessed greater than 80% of the expected efficiency. Both of these examples illustrate the need to match the column parameters to the instrument capabilities.

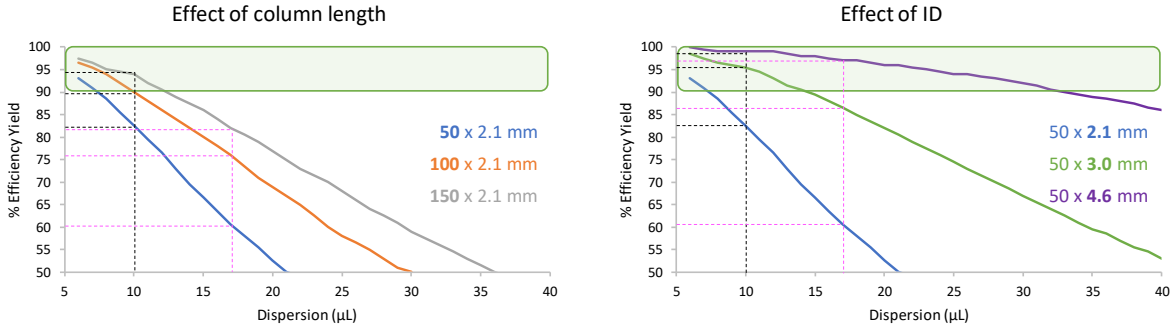

Figure 2 Theoretical *comparison of different column formats packed with 1.7 µm material on the effect of dispersion versus % efficiency yield. The expected efficiency yield for bandwidths of 10 and 17 µL (black and pink trace, respectively) were added to illustrate the necessity for the right instrument for the right column.*

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The system volume can be used to correctly calculate retention factor (*k, Eq. 4*). This enables an accurate comparison between different systems. This is particularly beneficial for small column volumes (i.e. 50 x 2.1 mm column formats) or for systems which have significant system volumes.

$$k = \frac{(t_R - t_{ext}) - (t_0 - t_{ext})}{(t_0 - t_{ext})} \qquad \textit{Eq. 4}$$

Can Dispersion be Improved?

There are a few options available in order to improve dispersion. However, it is important to discuss why the dispersion needs to be reduced. For example, it may not be necessary to reduce the dispersion markedly for HPLC analyses which typically use >100 mm length and >3.0 mm column ID and >3 µm particle sizes. If the requirement is to use narrow bore columns or perform ultrafast analyses, then it may be prudent to reduce the bandwidth to approximately ~12 µL.
If it is necessary to improve the dispersion:

- Reduce the length of excessive tubing
- Trade blue 0.010" tubing for red 0.005" tubing
- Are column switches and valves installed? Are these essential?
- Is a low volume dispersion kit available?
- What is the flow cell design and volume?

As with most aspects of chromatography, there is a compromise. For example, by reducing the ID of the tubing, the pressure can increase substantially which may be problematic for certain stationary phases as well as causing changes in selectivity.

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