Practical Impact of Dispersion on Fast Chromatographic Separations

Dispersion is often a criterion which is important to consider when investing in analytical equipment. Column formats should be appropriate for the volume of the system in order to obtain reasonable chromatographic performance from the stationary phase. However, in practical terms, do we need to be concerned about attaining such minimal dispersion volumes to obtain the best chromatography, particularly for fast, ballistic methods?

Introduction

As a peak band migrates through a chromatographic system, the analyte possesses a parabolic flow, where the band is distorted by friction against the tubing walls, and other forces. These components can be divided into two sections; extra column, and intra column broadening. The intra column forces occur in the porous particles, whilst extra column broadening is apportioned to the liquid chromatography (LC) system. The dispersive effects attributed to the components of the LC system can have a detrimental impact on the chromatographic performance. These elements include the injection port, loop, tubing, valves, fittings, detector and data collection rate (Figure 1), which applies to both isocratic and gradient systems. The dispersion is observed to greater detriment in isocratic separations with a constant mobile phase on later eluting peaks compared to the early eluting compound.

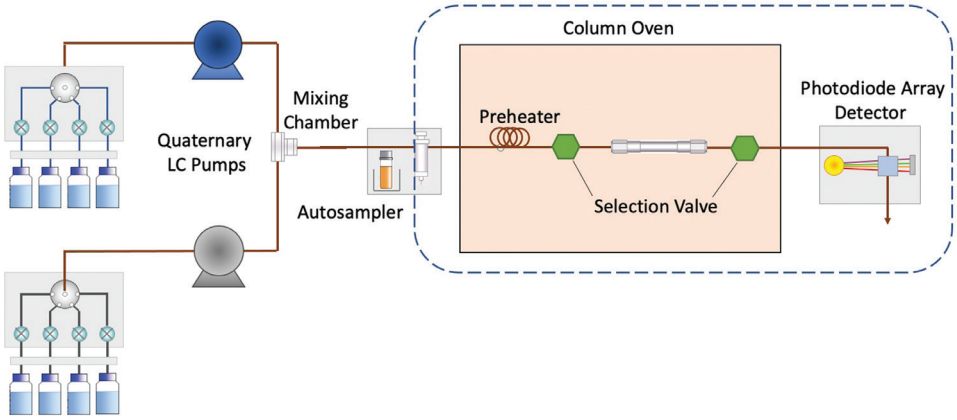

Figure 1: Schematic flow path of an LC system, with the components measured in dispersion encircled (blue dashed line).

The conventional HPLC with totally porous particle, 5 µm, 150 or 250 x 4.6 mm column formats are typically unaffected by dispersive effects as the peak variance caused by the column is greater than the variance caused by the LC extra-column components [1]. However, with the advancement in technology, including narrower bore columns, smaller particle sizes, and higher-pressure LC systems, the discussion regarding dispersion needs to be readdressed. Characteristically, conventional HPLC systems are not optimised where wider bore tubing is used, thus dispersion is measured at greater than 20 µL (Table 1). Nowadays, there are more LC system options available, where there are both UHPLC and UHPLC-Like systems available (please refer to Table 1 for differences). UHPLC systems are designed for greater throughput, performance and speed, and as such, requires the minimum possible extra column band broadening (<10 µL). The UHPLC-Like system offers greater capacity and performance than conventional HPLC, but without the added challenges associated with UHPLC analyses, thus the system can have a dispersion value of <15 µL.

The impact of dispersion can cause a loss of chromatographic performance, increased peak width, thus loss of resolution, which could prove detrimental to critical pairs in fast separations. However, to what level does the dispersion need to be optimised to? This article will provide a means of estimating the extra column band broadening and evaluate the impact of different parameters on the theoretical % efficiency yield.

Experimental

In order to assess dispersion and system volume, the following experimental parameters were applied: Water and methanol were proportionated using the pump to dispense 51:49 (v/v) respectively. The flow rate was set to 0.1 mL/min with a Zero Dead Volume (ZDV) union installed in place of a column. The sampling frequency was set to 40 Hz to adequately describe the peak and data collected at 254 nm (8 nm) reference 360 (100). A sample of 1% acetone in water / methanol (51:49 v/v) was prepared and chromatographed nine times after sufficient equilibration of the system. The injection volume was 0.5 µL and the column oven controlled at 40 °C [3].

LC separations were performed on a Shimadzu Nexera XS UHPLC system equipped with binary pumps (LC-40D XS) and proportionating valves, degassers (DGU-405), autosampler with cooling capabilities (SIL-40C XS), column oven (CTO-40C), and photodiode array detector (UHPLC PDA or HPLC PDA). The system controller was integrated into the binary pump. A 20 µL 3-dimensional micro-reactor mixer was installed.

*Table 1: Comparison of UHPLC, UHPLC-Like and HPLC systems. *Other vendor information obtained from Reference [2]*

	Conventional HPLC	UHPLC Like	UHPLC
			
Example	Prominence i-Series Nexera Lite	Nexera i-Series Nexera XR	Nexera XS Nexera X3
Pressure Limit	<440 bar	<660 bar	>1000 bar
Dispersion	>20 µL	<15 µL	<10 µL
Typical Column ID	4.6 mm	3.0 mm	2.1-3.0 mm
Other Vendor Examples*	Waters Alliance HPLC (Standard config. 45 µL) Agilent 1200 HPLC (30 µL)	Waters UPLC Classic (Switching valve, 16 µL) Agilent Infinity I 1290 (Standard config. 14 µL) Thermo Vanquish (Standard config. 16 µL)	Waters Acquity I-Class (Standard config. with fixed loop 4-6 µL)

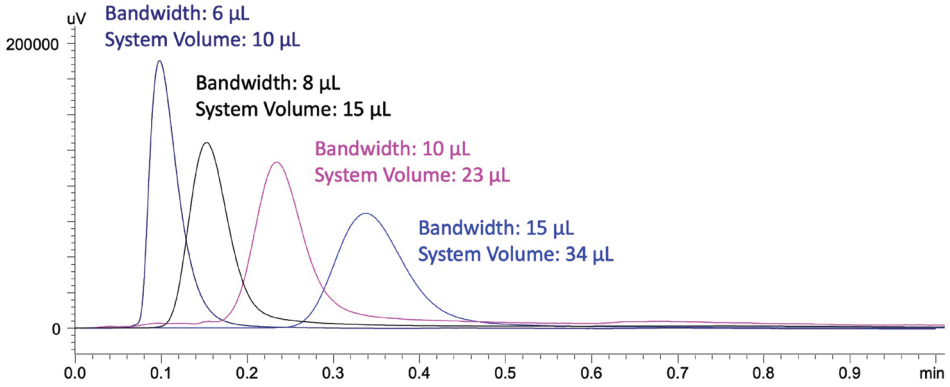

Figure 2: Comparison of the 1% acetone peak measured for dispersion on a UHPLC with variable volume injector. Extra column volume was added to simulate the effect of dispersion.

Impact of Dispersion

Dispersion can be measured by injecting a sample of acetone onto a ZDV union to quantify the extra column band broadening effect. The method conditions are in the experimental section, with an example of instruments with different system volumes and bandwidths in Figure 2.

To determine the instrument bandwidth, the retention time (t_R) and efficiency (N) at half height 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, µL/min*). Nine repeat measurements should be recorded to generate a statistically significant result.

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

$$Instrument\ Bandwidth = 4\sigma \quad \text{Eq. 2}$$

$$System\ Volume = t_R \times F \quad \text{Eq. 3}$$

What influences the impact of dispersion?

Retention factor, *k*

The speed the peak band migrates through the system can be highly influential for the effect of dispersion. The % efficiency yield was compared for a 50 x 2.1 mm column format, with 1.7 µm particle size, with retention factors, *k*, of 2, 5 and 10 (the optimum region for retention factors as described by the Purnell Fundamental Resolution Equation). The dispersion regions for UHPLC, UHPLC-Like and HPLC systems were also denoted on the graph (Figure 3(a)). The 50 x 2.1 mm column format with the 1.7 µm particle is suitable for UHPLC analyses and is the harshest condition to assess, therefore, any other column will have better performance. The largest value of *k* assessed (*k* = 10) achieved >90% efficiency yield for dispersion <13 µL. A *k* of 5 could achieve above 90% of the efficiency yield at the smallest dispersion values (<7 µL). The minimum retention factor of 2 was below 80% thus indicating that greater retention factors should be applied to achieve adequate chromatographic performance. In comparison, a HPLC column format (150 x 4.6 mm, 5 µm particle), with a *k* of 2 was mostly unaffected by the extra column band broadening, where the % efficiency yield was greater than 90% up to maximum 40 µL of dispersion assessed.

Column dimensions

The effect of dispersion is magnified for column formats with smaller column volumes, as the ratio between the two parameters is changed (Figure 3(b) and (c)). Thus, as expected, the longer the column length or wider the internal diameter, the less affected by dispersion. The retention factor was maintained at 5 and the particle size was standardised on 1.7 µm. The results indicate that the internal diameter has the greatest impact, where the 4.6 mm ID achieved greater than 90% efficiency yield below 30 µL dispersion. The 3.0 mm ID is a good compromise for reduced flow rate, solvent consumption and faster runs, for UHPLC and UHPLC-Like type systems. The 2.1 mm ID requires the LC system to be optimised by reducing the volume of tubing, the ID of tubing, optimising the flow cell, and potentially removing column switching valves.

Particle Size

The more efficient the column, the more the dispersion volume can have a negative impact (Figure 3(d)). The graph illustrates this, where the smallest 1.7 µm requires the most optimised UHPLC systems, and the 3 and 5 µm achieve a greater degree of the expected efficiency using systems up to UHPLC-Like. Again, the retention factor was standardised at 5, and the column format was set to 50 x 2.1 mm, therefore, if the internal diameter or column length was increased, this would also offer additional support for improving the efficiency yield. Totally porous particles were applied in this theoretical study, where superficially porous particles have a different particle structure which alters their diffusion pathway, thus decreasing the reduced plate height.

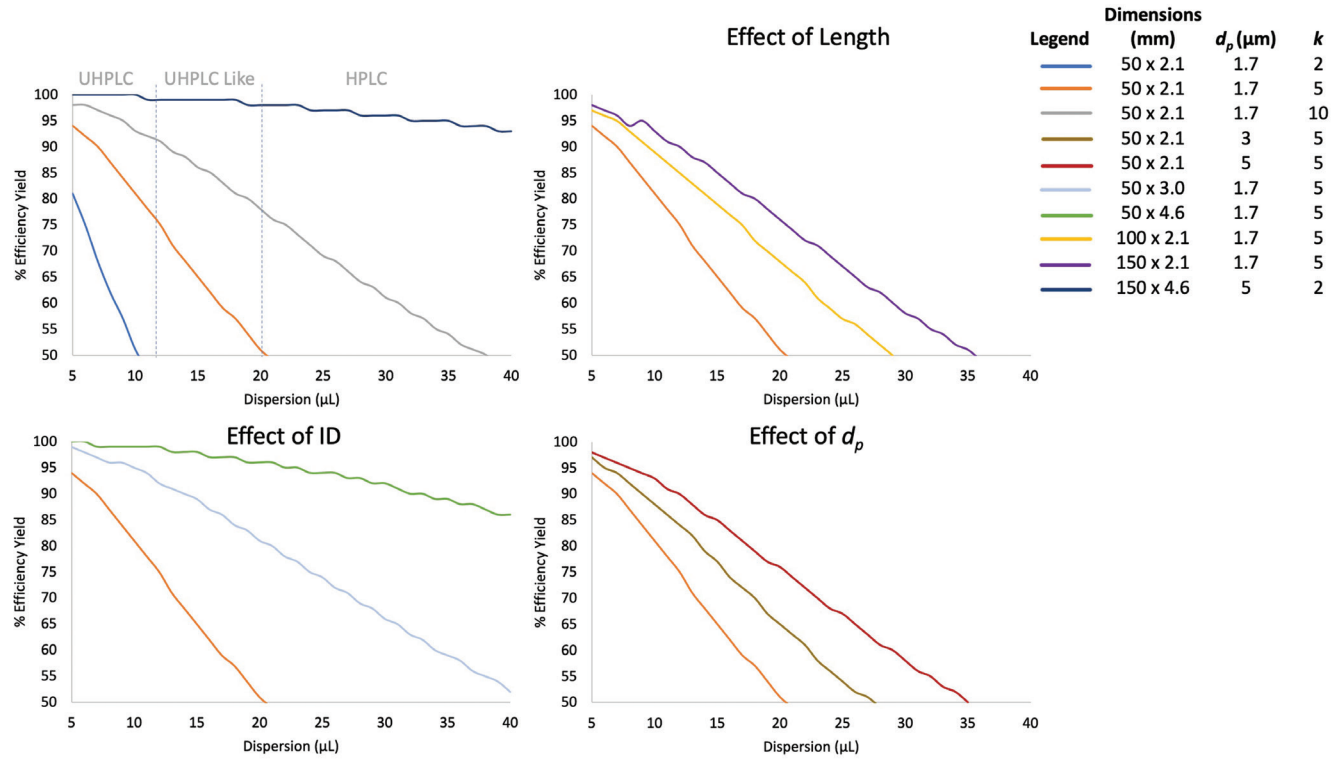

Figure 3: Establishing the effect of dispersion on the theoretical % efficiency yield expected for different totally porous particle column formats. (a) Effect of k, column 50 x 2.1 mm, 1.7 µm, k = 2, 5 or 10, and comparison of UHPLC vs HPLC, column 150 x 4.6 mm, 5 µm, k = 2, (b) Effect of column length, column 50 x 2.1 mm, 100 x 2.1 mm, 150 x 2.1 mm, 1.7 µm, k = 5, (c) Effect of column internal diameter, column 50 x 2.1 mm, 50 x 3.0 mm, 50 x 4.6 mm, 1.7 µm, k = 5 (d) Effect of particle size, column 50 x 2.1 mm, 1.7 µm, 3 µm or 5 µm, k = 5.

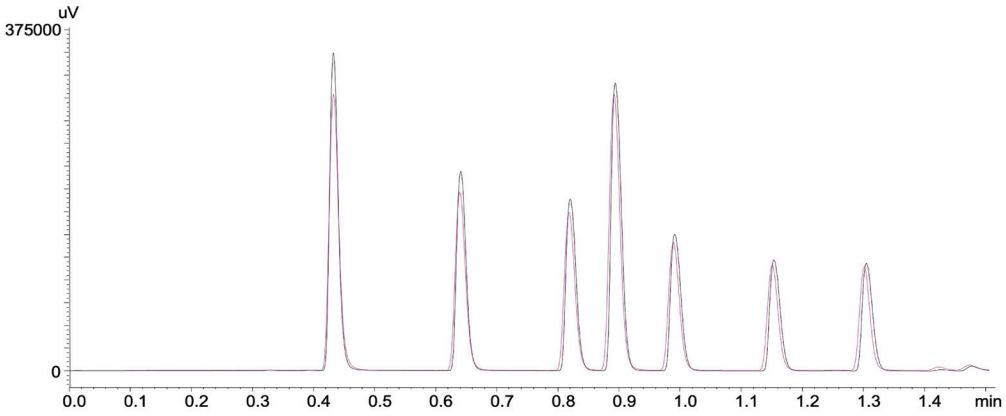

Figure 4: Overlaid chromatograms of alkylphenones chromatographed on a 1.5 minute gradient using an 6 µL dispersion system (black trace) and a 15 µL dispersion LC system (pink trace).

The results illustrate the importance of matching the column format to the instrument in order to obtain reasonable efficiencies. The Nexera XS with a variable volume injector and SPD-M30A capillary flow cell has a bandwidth of 6 µL as standard, but further optimisation with loop injector is also available. However, when chasing an ever-lower dispersion volume, there are consequences which may prove detrimental to the robustness of the system. Fixed loop injectors have the potential for greater carryover which can impede on accurate and reproducible quantitative data. The use of narrower bore tubing also can have potential issues. Stainless steel tubing can be manufactured by different pathways, however, there is a tolerance for the tubing where the narrower the tubing, the greater the impact of errors, therefore a reproducible source of tubing is a necessity. In addition, extra narrow bore tubing will increase the inherent back pressure of the LC system, excluding the column. A change in pressure can cause pressure sensitive peaks from an established method to move which can change the resolution between analytes. Finally, narrow bore tubing is prone to blockages, particularly if the sample matrixes is dirty or even from the mobile phase preparation.

Although it is possible to reduce the extra column band broadening using fixed loops and narrow bore tubing, from a practical point of view, does the dispersion need to be reduced to such extremes as ~5 µL, with its added complications? A series of alkylphenones were chromatographed using a ballistic gradient on a UHPLC C18 column (50 x 2.1 mm, 1.7 µm, Figure 4). The seven peaks were assessed on an LC system with 6 µL extra column band broadening and volume added to increase dispersion to 15 µL. The results denote there is an improvement in efficiency. The peak width at 10% height saw a 7.9% decrease between the UHPLC-Like and UHPLC conditions, whilst the peak capacity saw an increase of 8.4%. However, this is not as significant as might be expected. Ballistic gradients are usually performed at flow rates greater than the optimal linear velocity. Although 1.7 µm particle sizes have a flatter C-term in the van Deemter [4], which provides a wider optimal linear velocity range, the flow rate can still have a greater impact than the dispersive effects. Therefore, although in theory the dispersion can be reduced significantly, other factors play a more crucial role in affecting efficiency, therefore the bandwidth improvements are negligible.

Concluding Remarks

A simple and quick test can easily provide information regarding both the extra column band broadening, referred to as dispersion, as well as the system volume. This information gives crucial information regarding suitable column formats for that system, which can save buying expensive columns. For example, a UHPLC column format can never achieve its full chromatographic potential on a HPLC configuration, therefore, an appropriately optimised system should be employed.

The 2.1 mm column internal diameter is most suited to systems with dispersion of less than 12 µL, which can be achieved on UHPLC or UHPLC-Like systems which have been optimised. 3.0 mm column formats are most appropriate on UHPLC-Like instruments. The % efficiency yield of a 4.6 mm ID is largely unaffected by dispersion of less than 40 µL, even with retention factors of 2, thus HPLC systems are most compatible.

Although the 2.1 mm column format does require minimal extra column band broadening, there are other factors which can impact on obtaining reasonable efficiencies, such as operating at optimal flow rates. This has a bigger impact on the expected yields than the effect of reducing the dispersion. Therefore, although it is imperative to operate using minimal bandwidth, the practical implications intimate that there are other factors which may play a larger role, thus diminishing the impact of minimising the dispersion excessively.

Most laboratories aspire for robustness in combination with performance. Excellent dispersion can be achieved on the variable volume Nexera XS system with fantastic performance, however, if deemed necessary, it is possible to push it even further by introducing fixed loop autosamplers and extra narrow tubing. Nevertheless, with these changes, it can negatively impact on the ruggedness of any LC system. These considerations should be thought through thoroughly in combination with the purpose of the instrument, and column formats to truly attain the desired results.

References

[1.] J.W. Dolan, Extracolumn Effects, LCGC North America, 2008, 26, 1092-1098

[2.] M.W. Dong, UHPLC, Part 1: Perspectives and Instrumental Features, LCGC North America 2017, 35, 374-381

[3.] https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Supelco/General_Information/t408143.pdf (accessed 23/10/2020)

[4.] U.D. Neue, HPLC Columns: Theory, Technology and Practice, 1997, 1st ed. Wiley VCH