Spark HOLLAND

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Dispersion in U(HPLC) flow paths

How to recognize and minimize dispersion and maximize sensitivity

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Introduction

Dispersion is a critical factor in Liquid Chromatography (LC) that can have a negative effect on the accuracy and quality of analytical results. In LC, dispersion refers to the broadening of a sample band as it moves through the chromatographic system, having an impact on the results. Dispersion mainly happens due to a phenomenon known as laminar flow. When a liquid is moving down a cylindrical tube, such as HPLC tubing, friction causes the fluid at the borders of the tube to flow slower than in the middle, resulting in a parabolic distribution (Figure 1a). This causes the sample to reach the detector somewhat diluted, which results in a broader peak.

In Ultra-High-Performance Liquid Chromatography (UHPLC), dispersion is especially significant. Figure 1b compares two different peaks, showing similar areas but different peak heights and widths. The blue peak demonstrates peak broadening, which negatively affects detection limits, sensitivity, and resolution. Peak height is used to determine peak dispersion, as a lower peak height indicates greater dispersion, leading to a loss in sensitivity. Sharper peaks, like the turquoise peak, are desirable because they are higher and easier to distinguish from background noise, improving performance, especially when measuring lower concentrations (O'Haver, 2018). The following work aims to understand and minimize dispersion in helping to improve efficiency, sensitivity, and accuracy, ensuring sharper peaks and better analytical outcomes.

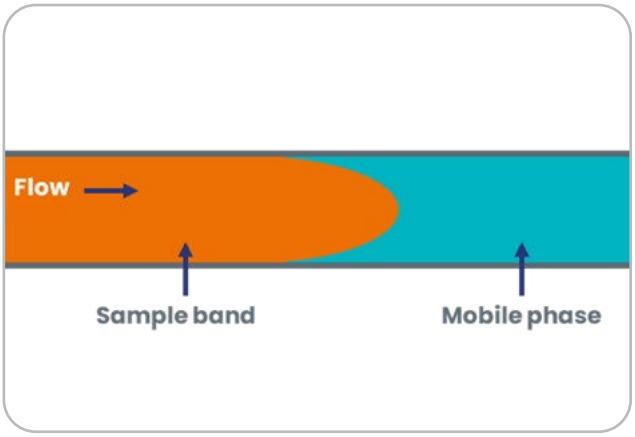

Figure 1a. Depiction of laminar flow, where liquid flows through a tube in a parabolic distribution, liquid on the edges flow slower than in the middle.

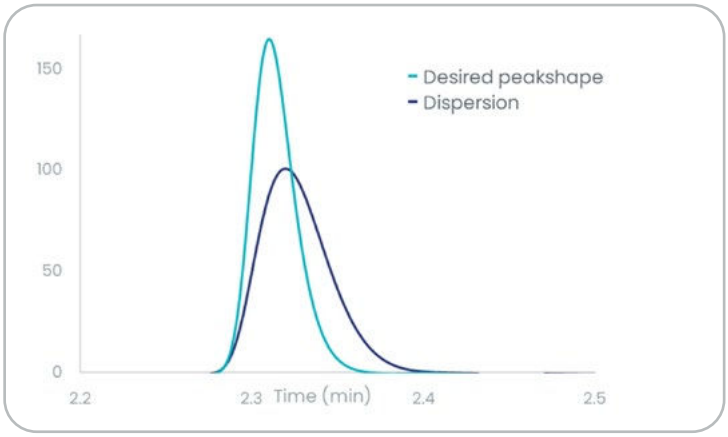

Figure 1b. Chromatogram showing the same sample with different peak shapes, caused by dispersion.

Influence on peak height		
Compound	**0.25 mm ID**	**0.13 mm ID**
Acetophenone	100%	152%
Butyrophenone	100%	123%
Valerophenone	100%	108%

Table 1. Influence of pre-column tubing ID on peak height, calculated by determining the efficiency:

$$\frac{\textit{Peak height 0.13 mm ID}}{\textit{Peak height 0.25 mm ID}} \times 100 = \textit{\% Efficiency}$$

Influence of flow path design on peak dispersion

To assess how different components of the flow path influence peak ispersion, a series of experiments were conducted. The findings provide insights into optimizing system performance by adjusting valve configurations and the internal diameter (ID) of the tubing within the flow path. All the tested parameters can influence dispersion, including variations in tubing volumes, pre- and post-column tubing ID, sample loop ID, and the different valves in the flow path: injection valve, diverter valve, and column selection valve (CSV). To test the different parts of the flow path either an isocratic or gradient elution was performed, with different phenones. Pre-column and valve experiments were performed using isocratic elution, while post-column experiments used gradient elution. The isocratic elution was used for pre-column determination to avoid correcting any influences within the column, unlike gradient elution. The reason being that in an isocratic elution, analytes move at a constant rate based on their affinity with the mobile and stationary phases. In gradient elution, as the organic content in the mobile phase increases, analytes transition from the stationary to the mobile phase, accelerating through the column. Moreover, to make the experimental setup uniform, a partial loop fill injection mode and a 3 µl injection volume were always used.

Pre-Column Tubing Inner Diameter

Choosing the appropriate tubing for a flow path is an important decision. This section focusses on how the tubing's ID affects pre-column dispersion. The tested sections included tubing from the pump to the autosampler and from the autosampler to the column. Both tubing lengths were identical, but their internal diameters differed: one had an ID of 0.25 mm, and the other 0.13 mm, leading to different total volumes. Results are shown in Figure 2a. As shown in Figure 2b, the peak height for the 0.13 mm tubing is clearly higher, showing how using a smaller ID tubing can have an influence on peak shape and dispersion, even if the peak areas are similar. This effect was particularly noticeable for early eluting compounds, as shown in Table 1.

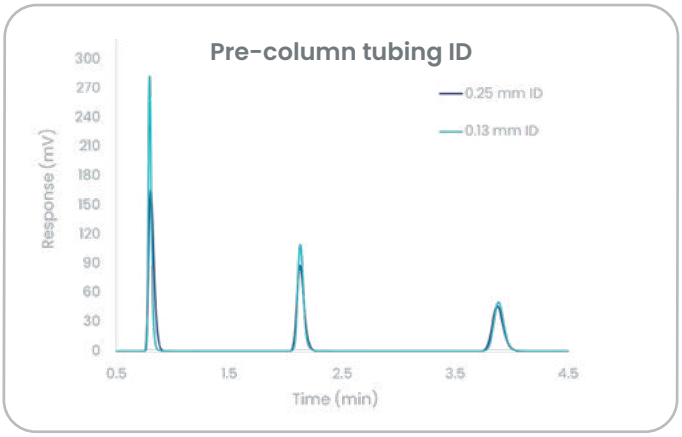

Figure 2. Peak formation after testing different pre-column tubing ID. Figure 2a. Overlay showing all three compounds tested.

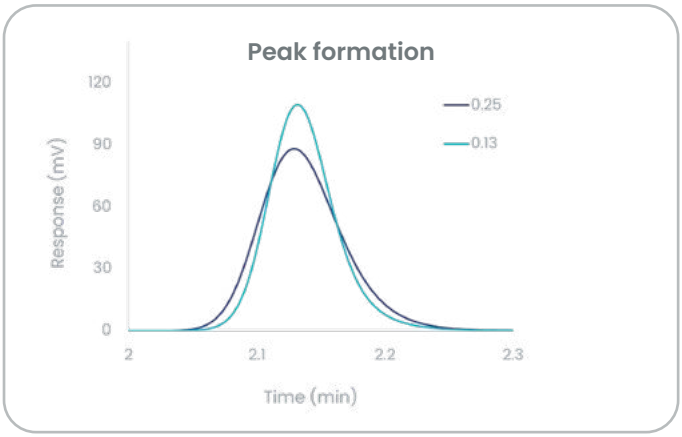

Figure 2b. Zoomed in view of butyrophenone.

For example, acetophenone, an early eluter, showed substantial improvement. This occurs because early eluters have a lower affinity for the stationary phase, making them more susceptible to dispersion from the autosampler. In contrast, analytes that stay on the column longer experience less peak broadening, as their bands narrow during their extended interaction with the stationary phase in the column.

Post-Column Tubing Internal Diameter and Length

Similar to the pre-column tubing, post-column tubing can have an effect on peak dispersion. To verify this, three different IDs were tested: 0.25, 0.13, 0.10 mm, again each having a different total volume. Because this tubing is post-column, a gradient elution was used with two additional phenones, hexanophenone and heptanophenone. Results are shown in Figure 3a and zooming in on butyrophenone, there are differences in peak height and form between the different IDs tested. The smaller the ID, the sharper the peak. As shown in Table 2, decreasing the ID of post-column tubing may increase peak height up to 50%, compared to 0.25 mm ID tubing.

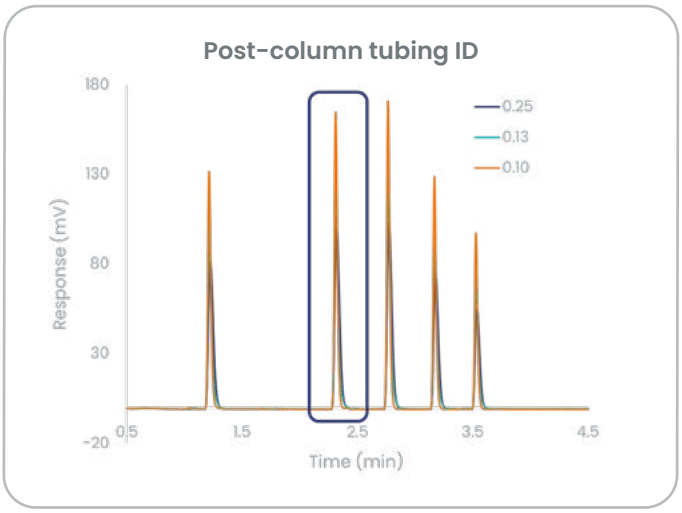

Figure 3. Peak formation after testing different post-column tubing ID's. Figure 3a. Overlay showing all 5 compounds tested.

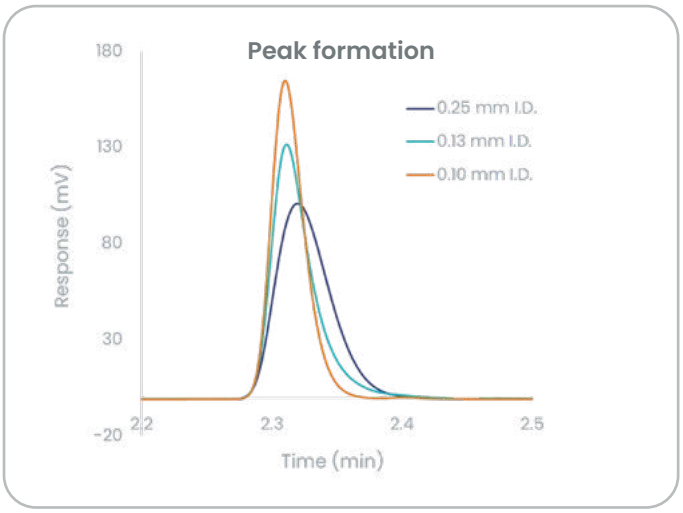

Figure 3b. Zoomed in view of butyrophenone.

Influence on peak height			
Compound	**0.25 mm ID**	**0.13 mm ID**	**0.10 mm ID**
Acetophenone	100%	121%	146%
Butyrophenone	100%	121%	146%
Valerophenone	100%	121%	147%
Hexanophenone	100%	121%	150%
Heptanophenone	100%	122%	153%

Table 2. Effect of post-column tubing ID on peak height, assessed by the efficiency calculation:

$$\frac{\textit{Peak height 0.13 mm ID or 0.1 mm ID}}{\textit{Peak height 0.25 mm ID}} \textit{ x 100 = \% Efficiency}$$

Another important factor to consider is the post-column tubing length. To test this, a further experiment using the 0.13 mm ID tubing and both the default 60 cm of tubing along with a piece of tubing of 215 cm were tested. Both were then compared to the standard 0.25 mm ID with a length of 60 cm. The results are shown in Figure 4 and Table 3 with no big difference between the two 0.13 mm ID's. The only difference shown was with the larger 0.25 mm ID capillary. These results demonstrate that with the total volume being higher, ID is a more important factor to consider compared to the length of the tubing.

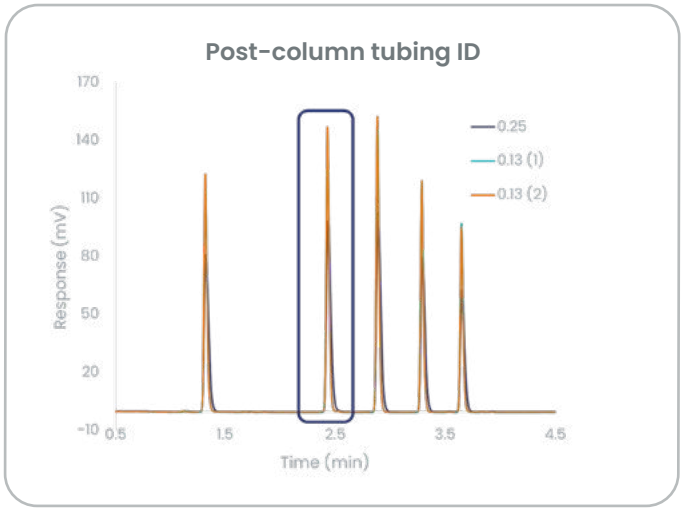

Figure 4. Peak formation after testing different post-column tubing lengths at 0.13 mm ID. 0.13 (1) = 60 cm (standard length tested, 0.25 mm ID was also 60 cm) 0.13 (2) = 215 cm. Figure 4a. Overlay showing all 5 compounds tested.

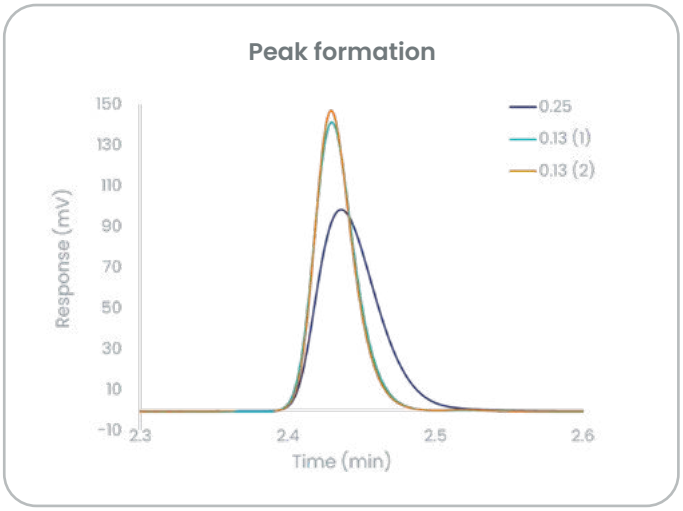

Figure 4b. Zoomed in view of butyrophenone.

Influence on peak height			
	0.25 mm ID	0.13 mm ID (1)	0.13 mm ID (2)
Acetophenone	100%	132%	140%
Butyrophenone	100%	131%	135%
Valerophenone	100%	132%	135%
Hexanophenone	100%	134%	137%
Heptanophenone	100%	135%	139%

Table 3. Effect of post-column tubing length on peak height, assessed by the efficiency calculation:

$$\frac{\textit{Peak height 0.13 mm ID (1) or (2)}}{\textit{Peak height 0.25 mm ID}} \textit{ x 100 = \% Efficiency}$$

Sample Loop Internal Diameter

The sample loop is an important part of the autosampler as it is located on the injection valve. It determines how much sample can be injected, and after it is filled with sample it is part of the pre-column flow path. Two different sample loops with IDs of 0.25 and 0.50 mm were tested, to see what effect this has on peak dispersion. The 0.25 mm ID sample loop was used as the standard level and compared to the 0.5 mm ID loop. Moreover, the loops have the same volume, but different IDs. The results are shown in Figure 5 and observing a zoomed in view of butyrophenone in Figure 5b and the efficiency calculations from Table 4, there are no differences, indicating that the sample loop ID has little influence on peak dispersion.

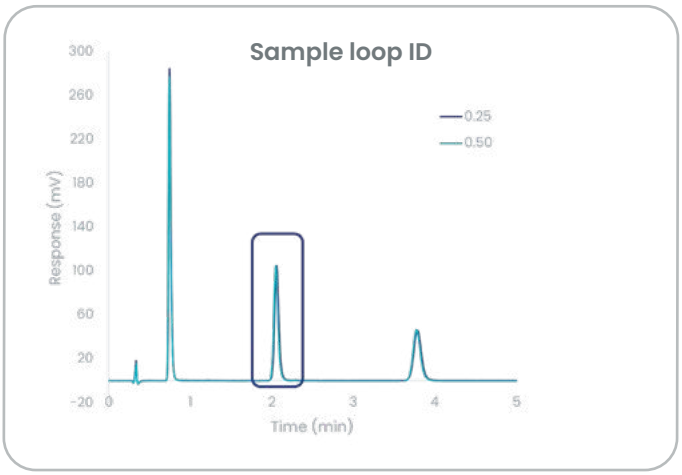

Figure 5. Peak formation after testing different Sample loop IDs, blue= 0.25 mm and red 0.5 mm ID. Figure 5a. Overlay showing all three compounds tested.

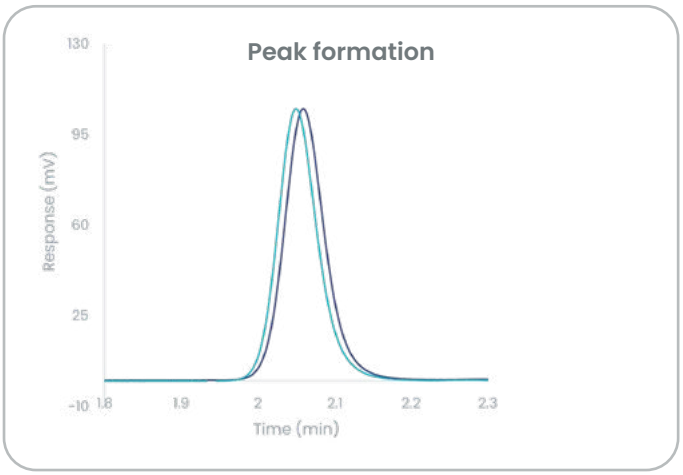

Figure 5b. Showing a zoomed in view of butyrophenone.

Influence on peak height

Compound	0.25 mm ID	0.5 mm ID
Acetophenone	100%	99%
Butyrophenone	100%	101%
Valerophenone	100%	103%

Table 4. Effect of sample loop ID on Peak Height, assessed by the efficiency calculation:

$$\frac{\textit{Peak height 0.5 mm ID}}{\textit{Peak height 0.25 mm ID}} \textit{ x 100 = \% Efficiency}$$

Injection Valve

The next experiments test how different valves have an influence on peak dispersion. The first test was with the injection valve, as it is a critical part of the autosampler used for the injection. Two different valves were tested, HPLC and UHPLC, and the main difference is the volume of the HPLC valve being 4.4 times larger than the UHPLC valve. The results are shown in Figure 6 and Table 5. As seen in Figure 6b, there is a difference in peak formation when comparing the two valves visually as the UHPLC peak is higher and in Table 5, it indicates that having a smaller volume valve can improve peak height up to 20%. Moreover, Table 6 shows that not only peak height, but also peak areas are different. This can be explained because of the difference in port-to-port volume between the two valves, as the HPLC is larger than the UHPLC valve.

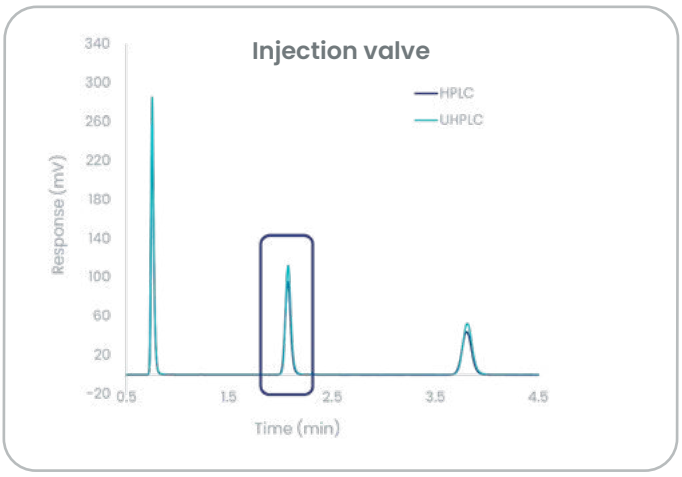

Figure 6. Chromatogram overlays of the different injection valves tested. Figure 6a. Shows the three compounds tested.

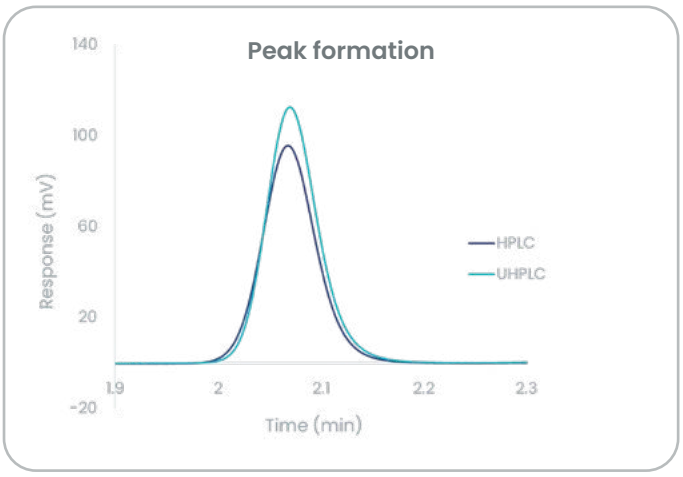

Figure 6b. A zoomed in view of butyrophenone.

Influence on peak height		
Compound	**HPLC**	**UHPLC**
Acetophenone	100%	117%
Butyrophenone	100%	120%
Valerophenone	100%	120%

Table 5. Effect of the injection valve on Peak Height, assessed by the efficiency calculation:

$$\frac{\textit{Peak height UHPLC valve}}{\textit{Peak height HPLC valve}} \textit{ x 100 = \% Efficiency}$$

Influence on peak height		
	HPLC	**UHPLC**
Peak area	3.55×10^5	4.12×10^5
Peak height	8.97×10^4	1.07×10^5

Table 6. Influence of injection valve on peak area and peak height for butyrophenone

Diverter valves

Diverter valves are useful components of an LC flow path as they can direct the LC flow either to the detector or the waste, keeping important parts of the detector clean. In this set of experiments, similar to the injection valve tests, two different post-column diverter valves—HPLC and UHPLC—were evaluated. They were then compared to the standard setup where no diverter valve was present. Overlays of the different chromatograms can be seen in Figure 7. Table 7 shows that by placing a UHPLC diverter valve in the flow path, minimal change can be observed in terms of peak shape. Using an HPLC valve, a decrease of 7% peak height can be expected. The likely cause of this difference is the port-to-port volume of both valves, as the HPLC valve is much larger compared to the UHPLC valve and is showing to have an influence on peak dispersion.

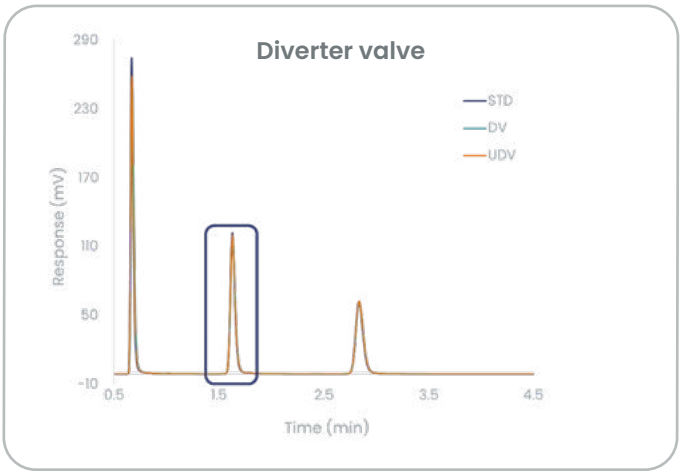

Figure 7. Peak formation after testing diverter valves, STD= standard setup (no diverter valve), DV=HPLC diverter valve, UDV= UHPLC diverter valve. Figure 7a. Overlay showing all three compounds tested.

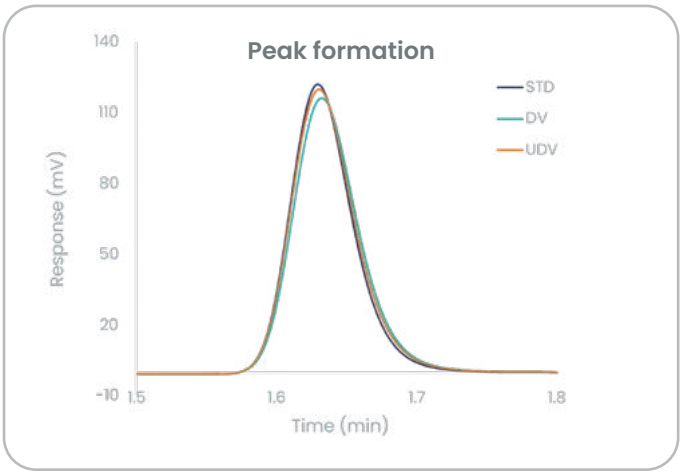

Figure 7b. Showing a zoomed in view of butyrophenone.

Influence on peak height			
Compound	**Standard**	**HPLC**	**UHPLC**
Acetophenone	100%	93%	96%
Butyrophenone	100%	95%	98%
Valerophenone	100%	97%	100%

Table 7. Effect of a diverter valve on peak height, calculated with the following equation:

$$\frac{\textit{Peak height diverter valve}}{\textit{Peak height standard}} \textit{ x 100 = \% Efficiency}$$

Column Selection Valve

A column selection valve is another useful option to add to a UHPLC flow path. This techniqe allows for multiple colums to be permanently installed in a column oven, giving users the flexibility to automatically switch between different methods without physically changing between columns. To test the effect a column selection valve (CSV) has on peak dispersion, experiments with the standard flow path and with the setup of a pre-column CSV and post-column manifold were performed. As seen in Figure 8, where overlays of the different experiments are depicted, the peak heights were only slightly different with only a 7% difference between the two experiments as observed in Table 8. This shows that having a CSV can affect peak dispersion and needs to be considered when designing a flow path.

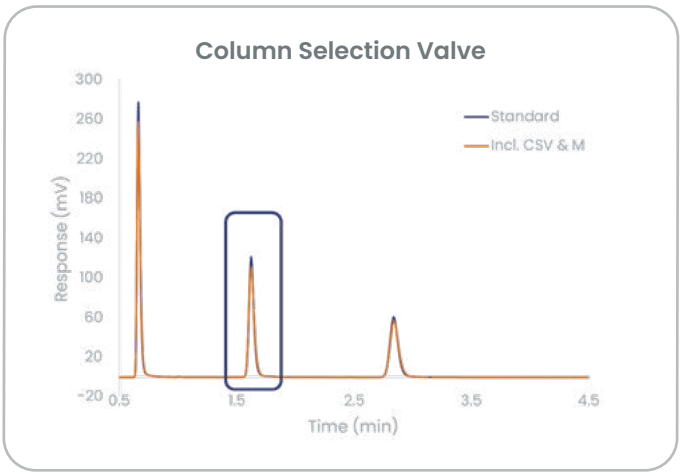

Figure 8. Peak formation after testing for the CSV in the flow path compared to the standard setup. Figure 8a. Shows chromatogram overlays of the three compounds tested.

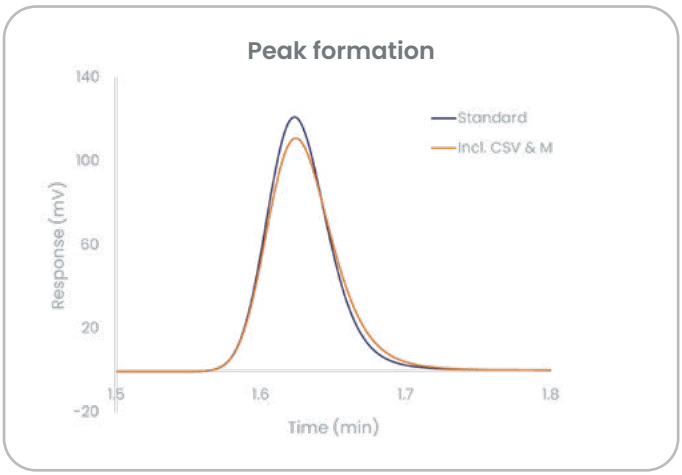

Figure 8b. Shows a zoomed in view for butyrophenone.

Influence on peak height

Compound	Standard	CSV
Acetophenone	100%	95%
Butyrophenone	100%	93%
Valerophenone	100%	94%

Table 8. Effect of a CSV on peak height, calculated with the following equation:

$$\frac{\textit{Peak height CSV}}{\textit{Peak height standard}} \textit{ x 100 = \% Efficiency}$$

Flow path component	Effect on peak dispersion
Injection Valve	**
Diverter Valve	**
Column Selection Valve	**
Pre-Column Tubing ID	***
Post-Column Tubing ID	***
Tubing Length	*
Sample Loop ID	*

Peak dispersion and the choices to be made

Setting up a liquid chromatography system involves weighing several pros and cons, as each component of the flow path can greatly influence the analysis. One critical factor to consider, as demonstrated in this article, is peak dispersion. If minimizing peak dispersion is important for your lab, you'll need to carefully evaluate the trade-offs between adding components or selecting tubing with specific internal diameters, as these choices can have a significant impact on system performance. The table below gives an idea of which parameters have the most effect with a *** and then the least effect with a single *.

For more information please also refer to Spark Holland B.V. white paper comparing different LC injection designs where dispersion is also addressed: The comparison of LC autosampler injection designs - Spark Holland

References

Carr, P. W., & Majors, R. E. (2008). Glossary of HPLC/LC separation terms. LCGC North America, 118-168.

O'Haver, T. (2018, July). Integration and peak area measurement. Opgehaald van A pragmatic introduction to signal processing: https://terpconnect.umd.edu/~toh/spectrum/Integration.html