Practical Effects of Sample Diluent on Peak Shape Using sub 2um Columns in Achiral SFC

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It is well known that for optimal peak shape, maximum detection sensitivity, and highest observed chromatographic efficiency, analytes should be dissolved in sample diluent matching the initial mobile phase composition [1]. This laboratory best practice serves both reverse phase achiral and chiral liquid chromatography. The impact of sample diluent is particularly important in preparative chromatography where reproducible retention times are critical to enable stacking of injections. For chiral separations performed via supercritical fluid chromatography (SFC), the sample diluent effect is less pronounced than in reversed-phase HPLC, see *Figure 1*. It is possible, even on coated chiral stationary phases, to inject mixtures of dichloromethane (DCM) and methanol with little detrimental effect to the chromatography, or observing the expected deterioration in performance of the stationary phase.

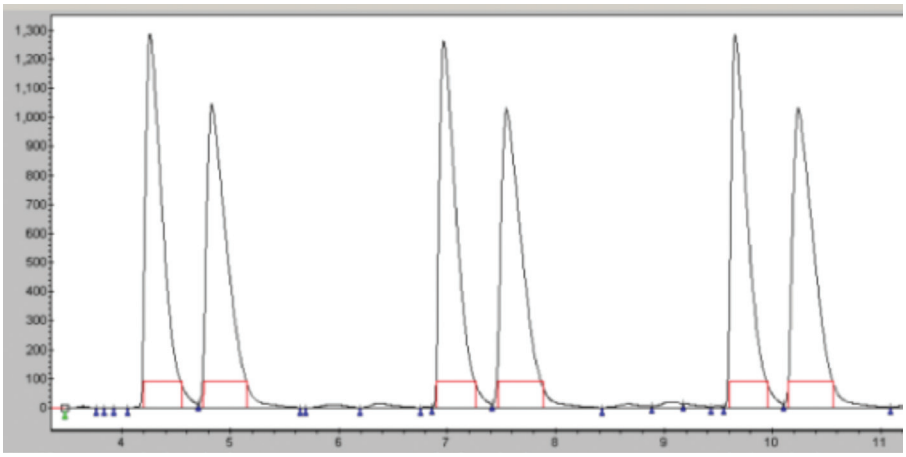

Figure 1: Racemate dissolved in 1:1 mixture of MeOH and DCM and 350 µL injected onto the SFC - into a modifier stream of methanol. An injection is made every 165 seconds, no distortion of peak shape is observed and the increase in co-solvent at the time of injection has no impact on the retention time of material already on the column [2].

When studying the effect of sample diluent on achiral ultra-performance SFC columns it is evident that it does have a major effect on chromatographic performance as previously reported [3]. This is something we have reproduced in our laboratory using the Acquity UPC^2 with the Torus 2-PIC column. Due to the physical nature of carbon dioxide at atmospheric conditions, it is impossible to use the mobile phase as an injection diluent in SFC. As with other studies, we overcame this challenge by mimicking the physical properties of super/subcritical CO_2 by using something of similar polarity, in this case, heptane.

We have carried out an experiment, incrementally increasing the injection volume of the same compound, 7-Diethylamino-4-methyl-coumarin (1 mg/mL) in three different diluents; methanol, isopropyl alcohol (IPA) and a one-to-one mixture of heptane and IPA. In each case, multiple injections were carried out on two different dimensions of the Waters Torus 2-PIC:

Column 1: 2.1 mm x 50 mm, 1.7 µm.

Column 2: 2.1 mm x 100 mm, 1.7 µm.

In each case, a gradient method of 3% to 35% methanol in CO_2 run over 1.25 minutes (50 mm length column) and 2.5 minutes (100 mm length column) was performed.

The results from both columns arrived at the same conclusion, the methanol diluent provided the worst peak shape and heptane/IPA provided the best. The detrimental effect of methanol was far more pronounced on the 50 mm length column than on the 100 mm length column, see *Figures 2a* and *2b*. These results suggest the peak deformation is either inversely proportional to the amount of time spent on the column which is a function of the bed length or the percentage of modifier required to elute the peak, both of which are less for the 50mm column.

As expected this data set confirms that peak shape and plate count can be optimised by using a non-polar diluent, see *Figure 3*. In this case the theoretical plate count, calculated as in *Figure 4*, was taken to be a measure of efficiency and therefore a judge of the quality of the chromatography. The most significant contributing factor to the theoretical number of plates in this case was the width at half peak height, peak distortion on the shorter column lead to half height peak widths of up to 8 seconds. A high number of theoretical plates and therefore efficiency is desired as the narrower the peaks the less selectivity required to give adequate resolution.

$$N = 5.545 \left(\frac{t_R}{w_h}\right)^2$$

N = number of theoretical plates
t_R = retention time
Wh = peak width at half height

Figure 4: Equation to calculate the theoretical number of plates [4].

To produce the most efficient chromatography, a diluent closely representing the mobile phase should be chosen. However, the often limited solubility of pharmaceutical compounds in heptane or even heptane/IPA mixtures

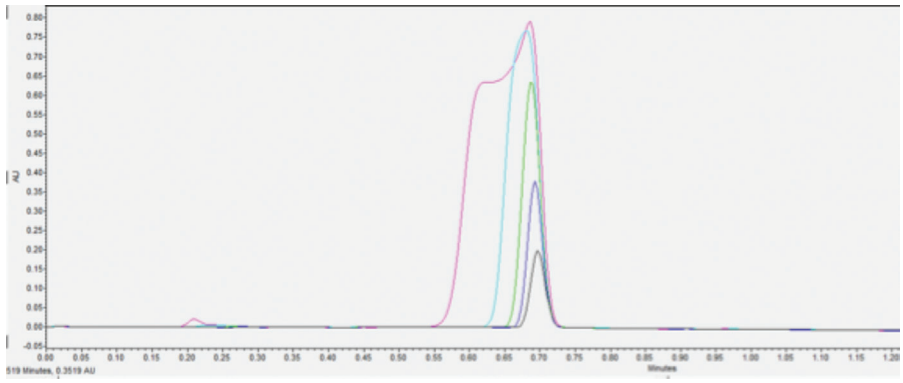

Figure 2a

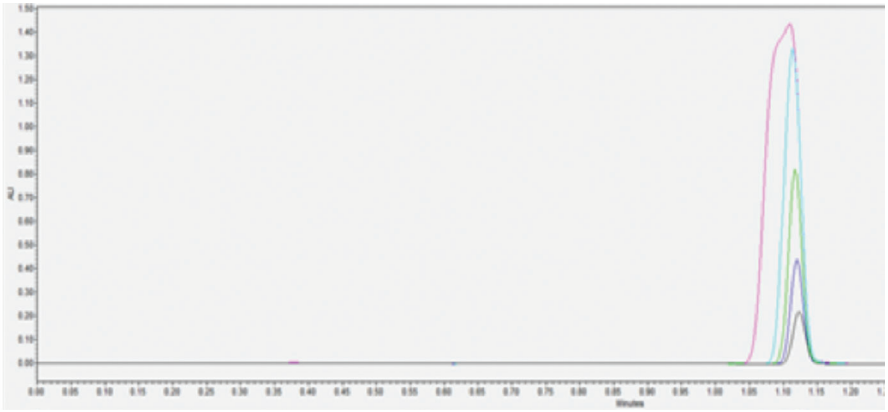

Figure 2b

Figure 2: Comparison of increasing injection volume (0.2 (Black), 0.4 (Blue), 0.8 (Green), 1.6 (Aqua), and 3.2µL (Pink)) on a 50mm (2a) and 100mm (2b) x 2.1 mm, 1.7µm column, using methanol as the diluent.

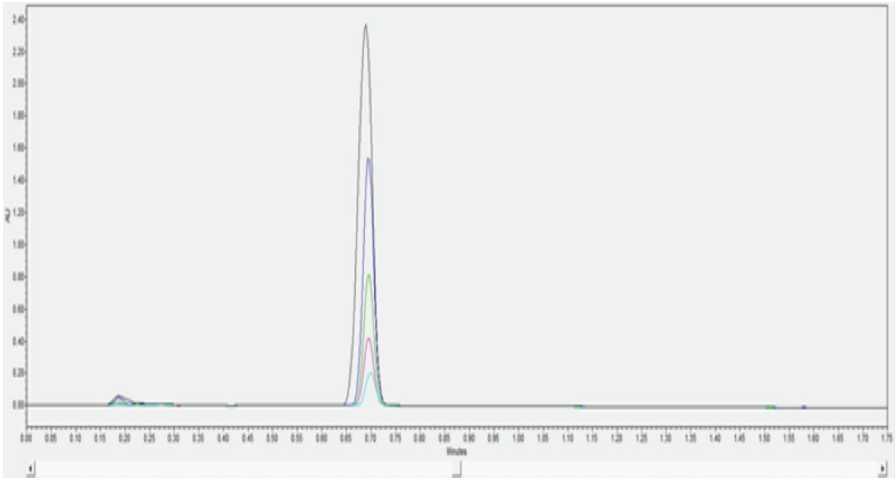

Figure 3: A series of 0.2 (Aqua), 0.4 (Pink), 0.8 (Green), 1.6 (Blue) and 3.2µL (Black) injections of Diethylamino-4-methyl-coumarin (1 mg/mL in heptane/IPA) on 2.1 mm x 50 mm, 1.7 µm.

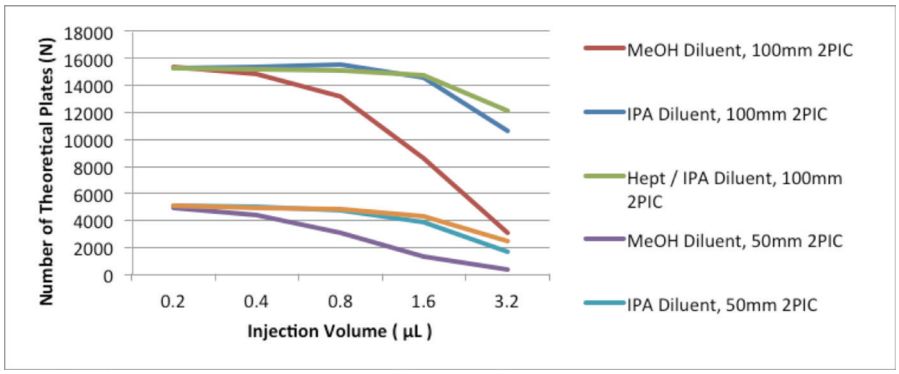

Figure 5: Number of theoretical plates (N) plotted against injection volume for the three diluents trialled.

prevents the use of these solvents as diluent and as such; a more practical approach is required.

If we ignore the difference in N between the two columns (the difference in retention factor makes this direct comparison unfair) we can compare the similarities by analysing the results in *Figure 5*:

- Deterioration in theoretical plate count is observed as the injection volume increases, this is true for all three diluents analysed on both columns.
- On both columns, the effect is most pronounced when injecting in methanol and least in heptane/IPA.
- The effect of using IPA as a diluent is only marginally worse at larger injection volumes than using a mixture of heptane/IPA.

In conclusion, injection diluent does play a major role in peak deformation when using sub 2 µm ultra-performance SFC columns. This effect can be minimised by using a solvent of similar polarity to the gradient starting conditions, although this is not always possible or practical due to poor solubility.

Compromises must be made regarding:

- Column length versus run time, ultra-performance SFC is usually chosen due to the possibility of using very short gradient methods for screening and scouting, so a 50 mm column may be essential.
- The concentration of the sample versus the volume to be injected. Often dependant on the solubility in the diluent.

One solution we have found to enable a 'one size fits most' approach is dissolve the sample at ~1 mg/mL in IPA and inject 0.5 -1.0 µL on a 100 mm column, running a 2.5 minute gradient. Although not the scientifically best approach it is the most practical for our laboratory.

For information: Reach Separations is an outsource chromatographic purification laboratory specialising in both achiral and chiral separations of small molecules. From their well-equipped labs in Nottingham (UK) they are keen advocates of the benefits of SFC particularly with its growing reputation for achiral separations.

References:

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[3] J.N.Fairchild et al, LCGC North America, Vol. 3, Iss. 4, 326-333

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