Chromatography Today Help Desk

SFC Method Transfer and Scaling Considerations

The help desk has covered the issues of transferring and scaling HPLC methods in previous issues of Chromatography Today, however the issues associated with method transfer in other forms of chromatography has not been discussed. In this issue of Chromatography Today we will look at some of the issues associated with transferring assays when using Supercritical Fluid Chromatography (SFC).

To better understand the issues associated with method transfer in SFC it is first important to go through some of the basic properties of a supercritical fluid. The definition of a supercritical fluid is "any substance at a temperature or pressure above its critical point, where distinct gas and liquid phases do not exist, resulting in there being no transition points, i.e. boiling points". Supercritical fluids have often been described as having higher diffusion than liquids and also greater solvation than gases, which would make them an ideal solvent for chromatographic separations, see Table 1. This statement, whilst being correct, perhaps oversimplifies the reality, as actually there is a continuum between the liquid and gas states and an analyst varying the temperature or pressure would move between the physical characteristics of a gas or a liquid without incurring the challenge of going through a phase transition.

Within SFC there are a variety of parameters that the separation scientist controls either directly through the instrumentation or indirectly as a consequence of varying another parameter. These variables are;

- Co-solvent fraction, typically % methanol
- Temperature
- Pressure
- Viscosity
- Diffusion
- Density

Gas and liquid chromatographers will look at this list and relate to this, however in LC and GC, these parameters are more controlled, and their impact can be readily defined, and indeed there are a variety of popular software packages which aid the separation scientist to develop robust methods [2, 3].

Each of these parameters will be discussed in relation to the stability of the assay, as this will be indicative of how easy the assay will be to transfer or indeed to scale-up.

Co-Solvent

The most significant parameter within most chromatographic systems is the stationary phase, and this is also the case with SFC. It is, however, assumed that the stationary phase would not be a parameter readily altered during method transfer. Of the parameters that are affected during method transfer the most significant is the co-solvent fraction, for simplicity methanol will be considered to be the co-solvent throughout this article. A common relationship that is used [4-6], which relates the retention time to the volumetric methanol fraction, is given by;

$$Ln(k) = Ln(k_0) - SC_M + dC_M^2$$

Where;

$$k = \frac{t_r - t_0}{t_0}$$

t_r – retention time of solute peak

t_0 – retention time of unretained peak

C_M – volumetric methanol fraction

S, d – constants

k_0 – retention factor with no modifier

In LC this relationship is often linear and given by;

$$Ln(k) = Ln(k_0) - SC_M$$

Where;

S – constant

It is evident from the effect that the co-solvent has on the elution time in SFC, that extra column volumes which cause a delay to the gradient reaching the column will potentially have a greater impact than in LC, where extra column dwell volumes have already been shown to cause issues with method transfer [7]. Thus, when transferring a method or scaling a method from analytical to preparative scale, it is important to be aware of the impact that the co-solvent can have on the elution time on individual components. In isocratic systems this will not be affected by the delay volume, however with gradient

Table 1. Properties of carbon dioxide in different physical states, liquid, gas and supercritical fluid [1].

	Gas	Liquid	Supercritical Fluid
Density (g/cm³)	(0.6~2.0)×10^{-3}	0.6~1.6	0.2~0.9
Viscosity (Pa-s)	(1~3)×10^{-5}	(0.2~3.0)×10^{-3}	(1~9)×10^{-5}
Diffusion coefficient (cm² /s)	0.1~0.4	(0.2~2.0)×10^{-5}	(0.2~2.0)×10^{-3}

elution the gradient delay volume will effectively change the co-solvent composition on the column from that programmed into to the SFC system and for different SFC systems this could result in different elution times for analytes. The nature of the relationship between retention time and co-solvent composition means that this will be more impactful in SFC compared to HPLC.

Temperature

The next most significant parameter is temperature, where the van't Hoff relationship still applies, assuming that all other parameters are equal, however it should be stated that within SFC it has been noted by a range of authors that this relationship is only linear over a small temperature range [8, 9].

$$Ln(k) = \left(\frac{-\Delta H^0}{RT}\right) + \left(\frac{\Delta S^0}{RT}\right) + Ln(\emptyset)$$

Where;

K - retention factor, ΔH = enthalpy (note negative term),

ΔS - entropy R = Gas constant

T = absolute temperature, ø= phase ratio

Assuming that the phase ratio does not change with temperature, the result is a linear relationship between Ln(k) and 1/T. The gradient is indicative of either an exothermic or endothermic adsorption isotherm, meaning that the retention time can increase or decrease with increasing temperature. Since different compounds can have different enthalpies and entropies, it is feasible that at a certain temperature co-elution of the compounds can exist, and that increasing or decreasing the temperature will result in a separation occurring. This concept is particular useful to know when separating chiral compounds, where temperature is often used to enhance a separation [10, 11]. It should also be noted a large pressure drop across the column can result in a high temperature drop across the column, although this is limited for SFC to highly compressible areas in the temperature pressure phase space [1], which from a practical perspective when using co-solvents is not applicable.

Pressure

Pressure has been shown to affect the retention time of certain compounds in LC, however the examples within the academic literature are not common [12,13]. In SFC it is well know that the pressure can have a substantial impact on the retention time [14, 15], and can be used by the separation scientist to reduce analysis times. This effect is more pronounced at lower co-solvent compositions, and as an example Berger [16] showed that at 5% MeOH, retention is nearly halved when the BPR pressure is increased from 100 to 300 bar. However, at higher MeOH concentrations, pressure becomes progressively less important. The use of smaller particles and/or higher flow rates can results in higher pressure drops across the column suggesting that the assay may become more sensitive to smaller modifications that can occur in method transfer.

Viscosity

The viscosity of a fluid is affected by temperature, which in turn will affect the diffusion rates of analytes within the mobile phase. In liquid chromatography, this does affect the optimal flow rate but it is not impactful, however in SFC this has a greater implication in the retention time of individual analytes. Table 1 gives a summary of the typical properties of the three states off nature that are commonly employed within a chromatographic system. It can be seen that the difference in the viscosity between a liquid and a supercritical fluid is quoted as a factor of ten. It should be noted that this is very dependent on where in the phase space the parameters are chosen, and that the properties of a supercritical fluid should be considered as a continuum rather than being discrete values. This highlights one of the issues associated with the use of SFC in that the range of viscosities and diffusions that are present within a system can be very large and so consequently the system impact can be very pronounced, if care is not taken in considering the effects that the pressure drop associated with tubing etc. It should also be stated that in many cases though this is not an issue the analytical scientist needs to be aware of the potential consequences so that it can become an effective part of the separation scientist's toolbox for troubleshooting.

Diffusion

One of the advantages of SFC is the relatively high diffusion rates than are observed in the physical state when compared to liquids. For small changes that may be observed when scaling or transferring a method the diffusion term is not impactful, however it should be noted that by varying the temperature or pressure and moving around the phase diagram that very different diffusion rates can be observed which will affect the fluid dynamics, with the dispersion being very dependent on the molecular diffusion rates.

Density

One of the most controversial parameters often highlighted as an important variable, particularly in earlier texts, is density, where early authors believed that altering the density could change the properties of the mobile phase from very non-polar to a polarity akin to isopropyl alcohol. One approach suggested that could be used to modify the density was the addition of small amounts of alcohol to the mobile phase. This, it was speculated, would modify the density but not the elutropic strength of the mobile phase, thus the retention time would alter due to the pressure differences. It was concluded that, actually, the elutropic strength of the solvent did indeed alter significantly with the addition of small amounts of additives, although it should also be stated that the retention time is affected by the density of the mobile phase, however not to the extent that early researchers believed.

Density, as a control parameter has been discussed at length in a previous edition of Chromatography Today. The conclusion is copied here as a summary of a very nice article by Berger on the topic [16].

"The relationship between density and viscosity of MeOH/CO_2 mixtures used in SFC is complex. In fact, at higher modifier concentrations, or higher pressures, the relationship is confused or essentially opposite to most users' perceptions. This makes density less than useless, and, in fact, incorrect in determining retention or

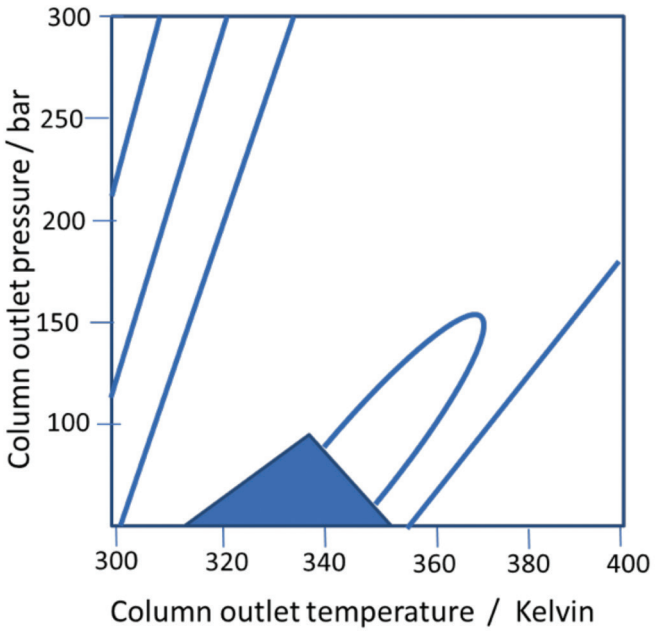

Figure 1: A schematic plot showing a 'Pressure drop across the column' contour plot showing effect of column pressure and temperature. The shaded area represents the conditions for a different physical state.

pressure drops at higher MeOH concentrations or pressures. This is counter to most of the recent SFC literature recommendations which stress relationships between density and retention. Changes in viscosity, not density, explains both pressure drops and changes in diffusion coefficients with pressure and modifier concentration. Unfortunately, viscosity data are nearly non-existent."

This presents an interesting perspective though as it suggests that if the density is not considered that there is a non-linear relationship with other parameters, and ultimately the retention time. The final consequence of this non-linearity is that the analytical system may be susceptible to small perturbations. Thus it is probably this term that needs to be considered the most when transferring methods as it can be impactful on the retention times. Unfortunately, it is not feasible to measure the density within a chromatographic system. Most commercial systems at best will have one or two pressure sensors placed at one end or both ends of the column, and from this a nominal density could be calculated. To determine a pressure or a calculated density reading along the column assumes that the pressure drops evenly per unit length, which may not be the case. Figure 1 highlights the issue. In this figure the outlet pressure of the column is plotted against the temperature, with the pressure drop across the column represented by the contour lines. It can be seen that small differences in the experimental arrangement can result in either increase or decreases in the pressure which will result in retention time variability.

Scaling strategies

There are a range of numerical models that can be used to aid scale-up and can also be used to determine the stability of the assay performance. These models include the Equilibrium Dispersive model, the Lump-Kinetic and general rate models. These models do need experimental input data to allow a degree of characterisation of the analytical system, however they are typically only employed in an academic environment. A common approach to optimising a method in industry is to use an empirical approach, although even here there are some general rules that will help establish a more robust methodology. Guillarme suggested the following;

The rules are;

- the stationary phase chemistry in the target system should be identical to the original system
- the ratio between the column length (L) and the particle diameter (dp) of the target system should be the same as the original system (the L/dp rule)
- the ratio between the injection volume and the column void volume in both the systems should be the same
- the reduced linear velocity in both the systems should be the same.

Conclusion

As with all method transfer and method scale up it is important to be aware of the impact that each parameter can have on the analytical performance. The challenge with SFC is that the flexibility of the technology results in a greater potential for instabilities to be built into the analysis, and this will ultimately result in challenges for the separation scientist. As with all chromatography the solution is to ensure that the operators have a thorough understanding of the practical and theoretical aspects of using a supercritical fluid. In particular a thorough understanding of the subtle interplay between co-solvent, pressure, temperature, diffusion and viscosity is key to ensure the development of robust methodologies.

References

1. A. Tarafder, C. Hudalla, P. Iraneta, K. Fountain, J. Chromatogr. A, 1362 (2014) 278-293
2. http://molnar-institute.com/drylab
3. http://www.chromsword.com/
4. D. Asberg, M. Enmark, J. Samuelsson, T. Fornstedt, J. Chromatogr. A. 1374 (2014) 254-260
5. P. Macaudière, A. Tambuté, M. Caude, R. Rosset, M.A. Alembik, I.W. Wainer, J. Chromatogr. 371 (1986) 177.
6. R.J. Smith, D.R. Taylor, S.M. Wilkins, J. Chromatogr. A 697 (1995) 587.
7. Chromatography Today, Aug/Sept. 2018, 44-46
8. G. Guiochon, A. Tarafder, J. Chromatogr. A, 1218 (2011), pp. 1037-1114
9. K. De Klerck, D. Mangelings, Y. Vander Heyden, J. Pharm. Biomed. Anal., 69 (2012), pp. 77-92
10. L.C. Harps, J.F. Joseph, M.K. Parr, J Pharm. Biomed. Anal., 162 (2019) 47-59;
11. C. West, A. Bouet, S. Routier, E. Lesellier, J. Chromatogr. A. 1269 (2012) 325-335
12. A. Kristl, P. Lokošek, M. Pompe, A. Podgornik, J. Chromatogr. A, 1597 (2019) p89-99
13. D. McCalley, TRAC, 63 (2014) p31-p43
14. C. Wang, Y. Zhang, J. Chromatogr. A. 1281 (2013) 127-134
15. D. Åsberg, M. Enmark, J. Samuelsson, T. Fornstedt, J. Chromatogr. A. 1374 (2014) 254-260
16. T. Berger, Chromatography Today, Feb/Mar 2019, 28-31