Chromatography Today Help Desk

Issues with Scaling and Method Transfer

There are many challenges associated with moving a method whether that be on the same nominal scale or indeed from one scale to another to ensure that a successful separation is obtained. There are a variety of parameters that have to be considered, and not all of them are obvious since some of them will relate to the system that is employed. Examples are given which demonstrate the importance of considering all the aspects of the method transfer.

In the first example the helpdesk was initially asked to develop the separation for some very polar compounds for a pharmaceutical purity study. The role of the helpdesk was to improve the resolution between the active pharmaceutical compound and the impurity to allow a real sample to be analysed. The method development was successful, and a method was developed; An example chromatogram is shown in Figure 1, and although not from the original method, confidentiality issues prevent this, it does highlight the issues of method transfer.

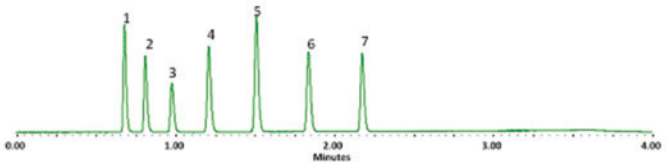
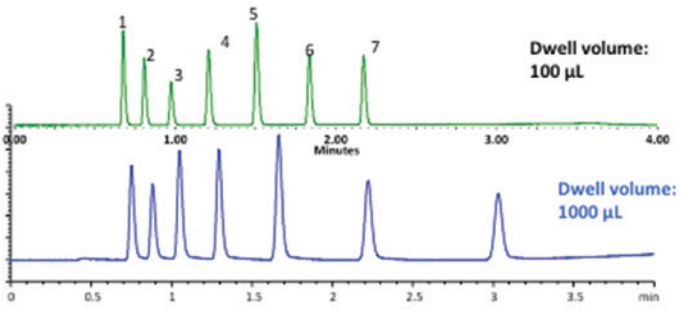


Figure 1: The method developed with the help of the Chromatography Today helpdesk team. The dwell volume on the system is 100 µL, both columns 100 x 2.1 mm, mobile phases; A – Water, B – Acetonitrile, gradient 65-95% in 2.1 min, then 95% B for 0.4 min, flow rate 400 µL/min.

The method was validated with standards and subsequently used with real samples with no issues. Eventually the customer looked to outsource the product with the analytical method, this is where issues occurred. The contractor received the method and reported back to the client that the method 'was not working', as 'there was a shift in the retention times for some of the components'. Initially the column utilised was suggested as introducing variability into the assay, so an identical replacement column was purchased which also exhibited the same retention time shifts.

The method returned to the customer to confirm that the assay was working, no issues were observed. An investigation into the root cause determined that the issue related to the types of pump that were being used, specifically the dwell volumes of the two pumps were quite different. Figure 2 demonstrates the effect that the two systems with different dwell volumes can have on the retention times for some of the peaks.



Separation Factors	Peak 1/2	Peak 2/3	Peak 3/4	Peak 4/5	Peak 5/6	Peak 6/7
100 µL	0.80	0.66	0.71	0.70	0.76	0.80
1000 µL	0.55	0.72	0.70	0.68	0.70	0.68

Figure 2: Comparison of two systems running the same analyses. The top chromatogram is obtained on a 100 µL dead volume system, whereas the bottom one is obtained using a chromatographic system with a dead volume of 1000 µL.

This example, which was based on a column packed in all cases with the same fully porous particles, was interesting since it can easily be avoided. However, the helpdesk considered if there were other situations where changes to the assay (in the first example it was the type of instrumentation) occur which might not have such an obvious effect. In the example given previously a fully porous particle was being used, however this poses an interesting question regarding changing the substrate material. One of the major differences associated with the superficially porous material packed column is that the column has less pore volume than its corresponding fully porous material packed column. Does this then mean that it may not be feasible to transfer some gradient based methods from fully porous to solid core technology because of the issues associated with the effective dwell volume of the column? For an understanding of this, it is important to understand the relative volume differences that are present in the two different packing material formats of columns.

In a fully porous column the amount of space available for mobile phase is about 70%, which means that in a 150 x 4.6 mm column the void volume of the column is:

$$void\ volume = 0.7(\pi r^2 l)$$

Which is: 1744 mm^3 (or 1.744 mL)

Comparing that to the void volume in a solid core material packed column, which has about 20-30% less void volume [1], is 1245 mm^3 (or 1.245 mL), so a potential difference of approximately 500 µL. This difference produces different absolute retention times which can be seen in Figure 3. However, it is the relative retention times that most chromatographers are interested in, and in particular when a gradient

separation is being employed, since ultimately this will determine if the separation is successful. Thus, the relationship between the column void volume and the gradient volume formation on the column needs to be considered when altering methods.

To examine this scenario, it is necessary to employ a mathematical interpretation since the retention time of a compound can be calculated by using a relatively simple but very powerful equation. This can be expressed, in a dimensionless format as follows:

$$k^* = \frac{t_G F}{S \Delta \Phi V_m}$$

Where;

k^* - gradient retention factor

t_G – gradient time

F - flow rate

S – compound specific constant

V_m – column void volume

$\Delta\Phi$ – change in volume fraction of B solvent

So, for any two columns, which have different void volumes, to produce the same retention time the flow rate must be adjusted (with all other parameters unchanged). However, as the above equation demonstrates it is also possible to alter the gradient conditions to ensure that method transfer keeps the retention times unchanged. Thus, when going from a fully porous column to a solid core column it is necessary to determine the effect that the change in the void volume will have on individual retention times and also consequently on the resolution of analytes. In some cases, the effect is hardly noticeable, Figure 3, this will not always be the case and the helpdesk will wait with anticipation for the first reader who has an issue with changing resolution on moving a method from fully porous to solid core that is not purely due to the changes in efficiency.

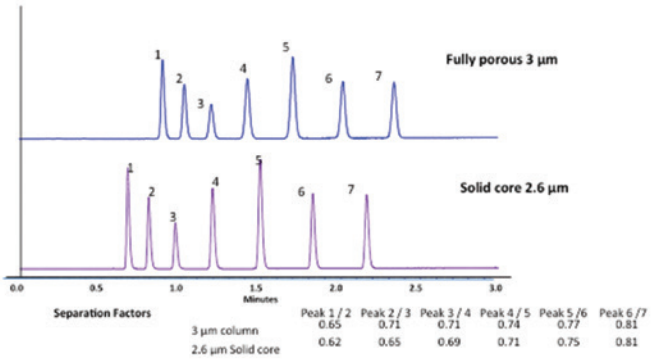


Figure 3: Effect of transferring from a 3 µm fully porous to a solid core of approximately the same particle size. Both columns 100 x 2.1 mm Mobile phases; A – Water, B – Acetonitrile, gradient 65-95% in 2.1 min, then 95% B for 0.4 min, flow rate 400 µL/min.

Transferring a method from one laboratory to another or indeed from one instrument to another, should be done carefully, as has been demonstrated. If the diameter of the column is to be increased, then the relevant calculations and considerations have to be made above and beyond the flow rate and injection volume. The equation quoted previously can be employed to ensure that this is done successfully

In terms of mass transfer, it has been shown [2] that as the diameter of a column can increase the radial mass transfer as issues associated with the wall effect are reduced, innovative column designs that cover both analytical and prep scale, look to simulate the 'infinite diameter column' to reduce the radial mass transfer effects that chromatographic columns experience [2]. Figure 4 shows an example of such a novel design which uses a patented frit technology to isolate only the middle core section of flow from the column. Increasing the column diameter will in general reduce these wall effects, however it is important to also consider how the eluant is dispersed radially at the inlet and outlets of the column.

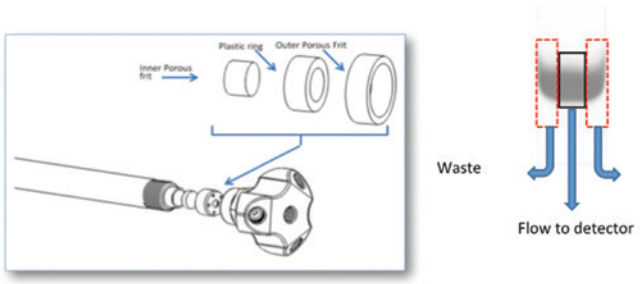


Figure 4: Schematic design of a parallel segmented column, which removes the outer flow by means of a patented frit design.

Practical issues relating to the increase in the potential energy/ pressure generated by the column when a mobile phase flows through it should also be considered. This is normally not an issue when the column is performing well since the flow is scaled according to the square of the column radius, when changing the column diameter, however if there is an issue with the chromatographic system due to a blockage then clearly the amount of potential energy associated with a large i.d. column will be larger than that associated with a smaller i.d. column, by a square factor. Manufacturers have tried to address this by the use of larger particles, which inherently have a lower back pressure, and also a lower cost. The use of larger pore frits can also be investigated, although it could be envisaged that the pressure drop associated with a frit would be substantially less than that associated with the column.

When using larger diameter columns, the thermal mass transfer should also be considered when using small particles (<2 micron). There are two modes of operation that a HPLC column can be operated under, namely forced air and still air heating (nominally isothermal and adiabatic heating modes). This results in two different temperature profiles across the column as shown in Figure 5.

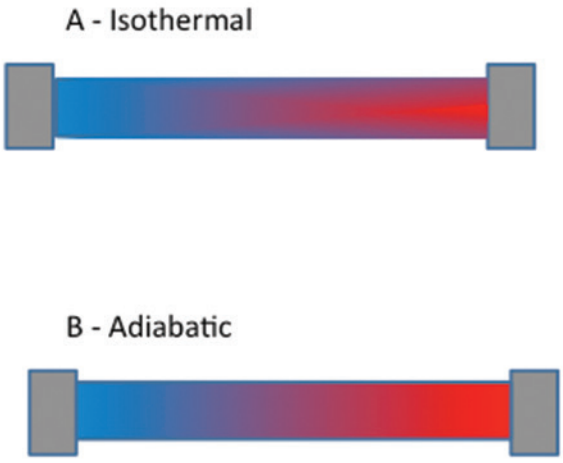


Figure 5: Schematic diagrams of temperature gradients observed with A – Forced air (Isothermal) and B – Still air (Adiabatic) operating conditions.

These two modes of operation can be considered extreme situations and although each of these extremes may be obtainable with the correct column oven configuration for analytical columns this may not be the case for larger preparative columns. In terms of a

separation to be successfully scaled the temperature profile within the column should be consistent, if this is not the case then this can result in radial and longitudinal temperature gradient profile differences between the narrow and larger diameter columns. The longitudinal and radial temperature gradients, which are generated effectively from the pressure being converted to a thermal energy, thus resulting in a higher exit temperature of the column than an inlet, will alter the speed of elution of the peaks as they travel through the column. Although there are many examples of compounds eluting quicker at higher temperatures, this is not always the case, since this is dependent on whether the interaction between the stationary phase and the analyte is endothermic or exothermic. The van't Hoff equation [3] describes the relationship between an analyte and the stationary phase, and it is evident that the combination of enthalpy and entropy to determine the overall interaction energy does allow for exothermic and endothermic interactions.

Longitudinal thermal gradients are not affected by altering the diameter of the column, however, this is not the case with radial temperature gradients. In chemical reactor design this is well understood although the lack of application of this understanding has resulted in many tragic accidents [4, 5]. In a chromatographic scenario, radial temperature gradients will result in band broadening as different parts of the column will be effectively eluting the compound at different retention times. Wider diameter columns will tend to have a greater temperature gradient and so changes to the diameter of the column may result in different performance characteristics.

In terms of issues to be considered when scaling a separation, it is the effects of flow rate, pressure and temperature that can be the most misunderstood attribute of a separation. Increasing the particle size can have a positive financial and safety affect, however it can also affect the separation. In HPLC it has been shown that the pressure can affect the separation, causing shifts in retention times [6] due to a change in the shape of the molecule for compounds such as proteins and for smaller molecules due to influencing equilibria between a charged and uncharged form of a molecule. Thus, it is important that when changing the scale of the separation that this effect is investigated as part of the method transfer procedure.

We have discussed the issues of transferring methods from one analytical system to another and also the effect of transferring a method from one scale of separation to another and the considerations that need to be taken to ensure that this process is successful. It is not enough to only consider the flow rate and injection volumes but consideration should also to be given to the effects of pressure and temperature within the chromatographic system since these both affect the resulting separation.

References

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