Evaluation of Scale-up From Analytical to Preparative Supercritical Fluid Chromatography

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Abstract

An approach for reliable transfer from analytical to preparative scale supercritical fluid chromatography was evaluated. Here, we accounted for the conditions inside the columns as well to the fact that most analytical instruments are volume-controlled while most preparative scale units are mass-controlled. The latter is a particular problem when performing pilot scale experiments and optimizations prior to scaling up to production scale. This was solved by measuring the mass flow, the pressure and the temperature on the analytical unit using external sensors. Thereafter, it was revealed with a design of experiments approach that the methanol fraction and the pressure are the two most important parameters to control for preserved retention throughout the scale-up; for preserved selectivity the temperature was most important in this particular system. Using this approach, the resulting chromatograms from the preparative unit agreed well with those from the analytical unit while keeping the same column length

and particles size. A brief investigation on how the solute elution volume varies with the volumetric flow rate revealed a complex dependency on pressure, density and apparent methanol content. Since the methanol content is a parameter of great importance to control during the scale up, we must be careful when changing operational and column design conditions which generates deviations in pressure, density and methanol content between different columns.

Keywords: SFC; Chiral Separation; Transfer of method, Scale-up; Operational parameters; Design of Experiments

1 Introduction

There is today a strong trend towards the use of preparative supercritical fluid chromatography (Prep-SFC) [1,2] and today prep-SFC is utilized in 50% of all achiral separations at the mg-g scale and at 85% at the g-kg scale, at AstraZeneca R&D, Mölndal Sweden [3]. Usually, screening and optimization are first performed at analytical scale and thereafter the scale-up is done to the preparative scale. It is therefore of importance that retention and selectivity are maintained during the scale-up procedure [4–6].

Scale-up in LC is straightforward with well-known methods available based on rules of thumbs such as scaling the volumetric flow and injection volume to the square of the ratio of the column radius [4]. Therefore, the research focus in Prep-LC has in the recent years instead focused on computer-assisted optimizations of analytical system before the scale-up, i.e. how to overload the analytical-scale system in the best way for reaching maximal productivity [7,8]. In a recent computer-based study, global numerical optimizations were performed in 1000 randomly selected separation systems to obtain a more general picture of the requirements for maximal productivity [8]. For example, it was found that it is almost always beneficial to use shorter columns with high pressure drops and that the selectivity should be greater than 2 whereas the sample concentration and the column efficiency have very limited impact on the maximum productivity [8].

Scale-up in SFC is more complex, mainly due to the compressibility of the mobile phase causing possible density, pressure and temperature gradients and therefore variations of the adsorption process for the component bands which travel along the column. Thus, the simple rules from LC cannot be applied directly since they assume constant density of the mobile phase. Scale-up in SFC from analytical scale to preparative scale is complicated and depends on a large number of factors such as column geometry, particle size, modifier content, operating pressure, operating temperature, system plumbing and mobile phase flow rate [9]. Although our knowledge of scale-up in SFC is limited we know that the modifier content is the most important parameter that controls retention [10–12] and that the temperature and pressure also has an impact on retention and selectivity [12–15].

Therefore, well-understood scaling strategies in SFC are urgently needed which was also the important message of the first highlight in a recent publication by Tarafder et al [9]. Here, a strategy for scale-up and method transfer in SFC was suggested which requires that the analytical system and the scaled-up system operate at the same average density. The authors suggested that we need to "… ensure that the intrinsic and the extrinsic conditions are the same in both the systems". This should be achieved by keeping the same column length, stationary phase particles size and by precisely matching the eluent composition [9]. However, the authors did not evaluate the proposed approach in a real setting, i.e. scaling up a method from an analytical unit to a preparative unit. This adds a further challenge not studied in the above mentioned article [9], namely that modern analytical SFC units always are volume-flow controlled while most large scale preparative units are mass-flow controlled.

The methodology for measuring the actual conditions in the SFC column has been successfully applied to verify or elucidate the differences between set and measured conditions of pressure, temperature and mass flow [16–20]. All of these studies serve to illustrate the complexity of SFC and the challenge of producing reproducible research using commercial SFC instrumentation from only set values of operational conditions. In contrast, in liquid chromatography no one would question a researcher for

verifying the flow rate and the mobile phase composition in the column as compared to its bulk composition before the column inlet. Unfortunately, the measurements necessary for reliable SFC scale-up are challenging and tedious and should be adapted for the particular goal of the SFC separation. The measuring approach was recently applied in two articles where the aim was reliable determination of adsorption isotherms in SFC [21,22].

The aim of this study is to evaluate the approach suggested for scale-up in SFC by reproducing the same "intrinsic" and "extrinsic" conditions of the columns and separation system in an analytical and preparative unit, respectively [9]. For this purpose a Design of Experiments (DoE) was first initially used for a detailed evaluation of the impact of variations of the experimental parameters such as the methanol content in the mobile phase, the pressure and the temperature on the retention factors and selectivity for the particular separation system. Then, in order to ensure identical conditions, the mass flow and mobile phase composition was measured on the volume based analytical system and then transferred to the mass based preparative system. Pressure and temperature were also measured on both systems for the same reason. Finally, the chromatograms obtained on the analytical and preparative system will be compared at three different methanol levels. Additionally, the reproduction of the intrinsic and extrinsic conditions when changing flow rate was evaluated by matching average pressure, density and volumetric fraction methanol.

2 Experimental

2.1 Chemicals

HPLC grade methanol (Fischer Scientific, Loughborough, UK) and CO_2 (> 99.99%, AGA Gas AB, Sweden) were used as mobile phase and the solutes injected were (±)-trans-stilbene oxide "TSO" (98%) and 1,3,5-tri-tert-butyl-benzene "TTBB" (97%) both purchased from Sigma-Aldrich (St. Louis, MO, USA). All solutes were dissolved in methanol and filtered through a 0.45 µm PTFE syringe filter prior to injection. The

chiral stationary phase was 5 µm Lux Cellulose 4 from Phenomenex (Torrance, CA, USA) packed in a 250 × 4.6 and 250 × 50 mm column with bulk material from the same batch.

2.2 Instrumentation

The analytical scale experiments were performed using a Waters UPC^2 system (Waters Corporation, Milford, MA, USA) equipped with a 100 µL loop and 250 µL syringe. The preparative scale experiments were performed with a SuperSep 600 instrument (NovaSep, Pompey, France) equipped with a 10 mL injection loop which was filled manually for each injection using syringes of different volumes. Injections on both the UPC^2 and SuperSep 600 were carried out in mixed stream mode; after the CO_2 and modifier flows have been mixed. The UPC^2 uses a diode-array detector while the SuperSep 600 a multiple wavelength detector. The column inlet and outlet temperatures were measured with two PT-100 4-wire resistance temperature detectors with an accuracy of ± 0.2°C (Pentronic AB, Gunnebo, Sweden). The inlet and outlet pressures were measured using two absolute pressure transmitters of model EJX530A (Yokogawa Electric Corporation, Tokyo, Japan), with an accuracy of ± 1 bar. For the UPC^2, both the total and methanol mass flow were measured using two Bronkhorst mini CORI-FLOW model M12 (Bronkhorst High-Tech B.V., Ruurlo, Netherlands) Coriolis mass flow meters with an accuracy of ± 0.2% of the mass flow. The total flow was measured directly after the CO_2/methanol mixer and methanol was measured after the co-solvent pump and before the mixer. Pressure, temperature and mass flow were continuously logged during all experiments using these external sensors. For more information about the measuring of mass flow, pressure and temperature, see [22].

2.3 Procedure

Design of Experiments: A full factorial design with three center points was used in order to study the variation in retention factor and selectivity with the operational parameters of temperature, pressure and methanol fraction. Replicate injections were performed of 2 µL of 0.1 g/L TSO solutions on the 250 ×

4.6 mm column in the UPC^2 system. The backpressure regulator (BPR) was set to 105, 155 or 205 bar; the column oven was set to 24, 30 or 36°C. These pressures and temperatures are within the range of typical operations in preparative SFC. The methanol content was set at 5, 10 or 15 v%. The actual methanol content was calculated for each experiment using the approach presented in [23]. In the DoE calculations average measured temperature and pressure were used as well as average calculated fraction of methanol. The flow rate was set to 1 mL/min in all experiments; a low flow rate was used to minimize pressure and temperature gradients along the column which could otherwise result in axial heterogeneity of the retention factor [24]. The measured pressure gradient was between 8 and 12 bar and the measured temperature gradient between 0.1 and 1°C. The dead volume of the columns were determined with N_2O according to [11,25]. The chromatograms were recorded at the UV-wavelength 225 nm.

Scale-up studies: The UPC^2 instrument was set to deliver 4 mL/min at 30 °C with the dynamic component of the system backpressure regulator disabled. The UPC^2-backpressure regulator consists of a static and a dynamic part, where the dynamic part is responsible for fine-tuning the pressure to the one specified by the operator and the static part is basically a flow restrictor. This was a prerequisite to be able to equalize the column outlet pressure between the UPC^2 and SuperSep systems. Analytical elution profiles were obtained by injecting 2 µL of 0.1 g/L TSO containing 0.1 g/L TTBB and overloaded elution profiles were acquired by injecting 16.9, 33.9, 50.8 and 67.7 µL of 40 g/L TSO on the UPC^2 system. The geometric equivalent, i.e. the injection volume increased $(50/4.6)^2$ times were about 200 µL and precisely 2, 4, 6 and 8 mL for overloaded injections on the SuperSep system. The chromatograms were recorded on both systems at 225 nm and 274 nm for analytical and overloaded elution profiles, respectively. On the SuperSep, manual injections were done 2-4 times to ensure repeatability. Pressure, temperature and mass flow were continuously measured during all experiments on both systems. The studies were done at three different methanol fractions; 5, 10 and 15 v%.

Impact on retention volume by changing flow rate: Experiments to investigate the dependence of the elution volume on the set volumetric flow rate were performed on the analytical system by initially running the UPC^2 at 4 mL/min, 15 v% methanol, 22 °C and a BPR pressure of 110 bar. The inlet and outlet pressure were continuously measured. The flow was then lowered to 1 mL/min using the same BPR pressure. At this flow the back-pressure was increased to a point where the average column pressure matched the average pressure at 4 mL/min. Furthermore, the set methanol volume fraction at 1 mL/min was increased to match the calculated average methanol content at 4 mL/min. Injections of 34 µL 40 g/L TSO were made at 1 and 4 mL/min at the different back-pressures and methanol levels. To calculate the density profile along the column, temperature was assumed to be constant and equal to room temperature and the pressure drop was assumed to be linear along the column. Density could then be calculated using NIST REFPROP 9.1 [26] using the measured mass fractions of carbon dioxide and methanol according to Tarafder et al. [9]. The methanol volume fraction along the column was calculated also based on the assumption of constant temperature and linear pressure gradient and was calculated using the procedure presented in [23]. To calculate the retention volume, the volumetric flow rate first needs to be calculated. Because the density profile was found to be linear or near linear along the column, the average density together with the measured total mass flow could be used to calculate the average volumetric flow rate.

3 Results and discussion

First, the impact of variations in pressure, temperature and methanol fraction in the eluent on retention and selectivity were investigated. The validity of these results was then verified for overloaded injections using the preparative system. Thereafter, temperature, pressure and total methanol mass flow were measured using external sensors on the analytical system. With these measurements, it is possible to correlate data from the analytical volume-flow controlled system with the mass-flow controlled preparative system. The success of the scale-up was evaluated by comparing elution profiles from the

analytical system with those from the preparative scale system. Finally, scale-up was investigated from another perspective i.e. how to match elution volumes at different flow rates by carefully compensating for differences in the average pressure, density and methanol content.

3.1 The influence of pressure, temperature and methanol content

Analytical unit: A DoE investigation was made using the analytical system employing the same approach as described in [11]. The retention factors of the two enantiomers and the selectivity were chosen as responses. A polynomial function including quadratic and interaction terms was used to fit each of the responses, i.e. retention factor and selectivity, to the factors pressure, temperature and methanol fraction using multilinear regression. Prior to the regression, the factors were centered and normalized. See the Supplementary data for details.

Fig. 1a shows the calculated coefficients for the models corresponding to the retention factors of the first (k_1) and second eluting enantiomer (k_2). A large value of a coefficient means that the polynomial term for that coefficient has a large influence on the model. It can be concluded that the methanol fraction (C_M) has the largest coefficient and is therefore the most important factor; however, a significant quadratic term for the methanol fraction (C^2_M) indicate a complex degree of non-linearity for this relation. The pressure (P) is the second most important factor for the retention factors and the temperature (T) the least important one. Fig. 1b shows that the temperature and the methanol fraction and are the two most important factors for the selectivity (α) while the pressure is less important. Also here, the methanol fraction has a large quadratic term, indicating a clear non-linearity in the response of the methanol content. The selectivity increases with decreasing methanol fraction and decreasing temperature while increased pressures gave slightly higher selectivity. However, the variations in selectivity were, in absolute numbers, between 1.82 and 2.11 in the design region which indicates that for this compound the selectivity is relatively insensitive to changes in temperature, pressure and methanol fraction.

The main conclusions from the analytical DoE results on this particular system are that the methanol fraction followed by the pressure are the two most important parameters to preserve when scaling-up an analytical method.

Preparative unit: Since the DoE was done on the analytical system it was necessary to verify the conclusions in the preparative scale system. Fig. 2 shows how a reference chromatogram was compared with the results obtained when varying the following parameters: (a) methanol content (b) pressure and (c) temperature while injecting TSO. Each parameter was changed ± 20% compared to the reference settings (solid gray lines in Fig. 2a-c. A perturbation resulting in a smaller degree of overlap with the reference chromatogram indicates an influential parameter. In Fig. 2a and b we can see that a substantial increase (dotted lines) or a decrease (dashed lines) in methanol fraction and pressure, respectively, has a large impact on the overloaded profiles. This is however not the case for a change in temperature especially not for the first enantiomer (see Fig. 2c). These experiments agrees qualitatively with the observations from the DoE investigation that the most important parameters for the retention factors are the methanol content followed by pressure and that the temperature is less important. For the selectivity the temperature was important which is also evident for the overloaded elution profiles where the last eluting enantiomer is more sensitive to temperature as compared to the first eluting one (see Fig. 2c). Note, that a change in pressure or temperature will also induce a change in the volume fraction of methanol; this effect will be discussed below in section 3.3.

3.2 Scale up with identical operational conditions

Transfer of operational data in scale up: The total mass flow and mass fraction of methanol was measured in the analytical UPC^2 system and then used to set identical operational conditions on the preparative SuperSep system. Observe that the total mass flow is geometrically scaled to maintain the same linear velocity in both systems. The volumetric modifier fraction and linear flow rate on the two systems are pressure and temperature dependent, so these must also be identical. This was achieved by

measuring the column outlet pressure of both the analytical UPC^2 system and the preparative SuperSep unit and then tuning the BPR on the latter system until the column outlet pressures reached equal values. The average temperature was also matched by measuring the surface inlet as well as outlet temperature of the column and accordingly adjusting the set temperature in the SuperSep system. In Table 1, the final set and measured conditions for both systems at three different levels of methanol content in the eluent are presented. Note the significant difference between set v% and measured wt.% values which demonstrates the importance of proper conversion between these units. Especially since the methanol fraction is, in this case, the most important parameter to influence the retention time of the elution profiles (see Fig. 1).

The systems were operated at high flow rates; therefore the pressure drops generated local density gradients in both systems. However, by setting identical column outlet pressure in the 4.6 and 50 mm columns, the gradients can be assumed to be more or less identical. Hence, the volumetric flow rate gradient will also be identical. Outside the column, the local density profile is not necessarily the same due to different capillary contribution to the pressure in the systems.

Verification of scale-up: Fig. 3a-c shows the resulting profiles after injection of sample in both the analytical and preparative systems at three different methanol levels (i.e. the levels in Table 1) in the mobile phase. The black lines in Fig. 3a-c are the analytical peaks from the analytical system and the gray lines are those from the preparative system.

It is clearly seen from the insets of Fig. 3a-c that the analytical and preparative instrument shows very good agreement for analytical elution profiles. The corresponding overloaded elution profiles are presented as main figures (cf. Fig. 3a-c). Here, it can be seen that both retention and selectivity is also clearly preserved between the systems in the case of overloaded profiles, i.e. the overlap between the overloaded profiles from the analytical and the preparative unit is good for all methanol contents in Fig.

3a-c. The tails of the elution profiles are also very similar on both systems which would be expected from the comparison of the analytical injections. The difference being that the observed peak fronting is more pronounced on the 50 mm I.D column as compared to the 4.6 mm column. However, the fronting can also be observed in the UPC^2 system using the 4.6 mm column for all methanol levels, but most clearly for the first eluting enantiomer at the two highest methanol levels, i.e. Fig. 3 b and c.

A possible explanation to the more pronounced fronting of the profiles eluting on the larger scale system could be an increased radial heterogeneity in the larger column, generating a non-uniform velocity profile across the column [27]. This non-uniform velocity profile could be due to differences in frit design and column packing between the large and small column. The different system dispersions could also be a contributing factor but our experiments show that the extra-column volume actually scales close to the increase in column volume. The same wavelength is monitored in both the analytical and preparative system, but the recording frequency is different, 1 Hz on the preparative and 20 Hz on the analytical system. However, difference in the sampling frequency cannot explain the fronting behavior on the preparative system. A more likely explanation could be to the so called injection plug effects where parts of the injected sample is eluted faster because of the significantly higher elution strength in the injection solvent compared to the mixed phase [28,29]. We recently investigated the reasons for peak distortion in mixed stream injection in SFC [23] and concluded that mismatch in eluent strength was the major source for the deformation. But, since the same injection principle was used for both the UPC^2 and the SuperSep 600 (mixed-stream mode), this is likely not a reason for the much more pronounced fronting of the elution profile using the 50 mm column as compared to the 4.6 mm. A more plausible explanation might be that in the wider column the wall support does not stabilize the sample zone as good as in the narrow column. Actually the viscous fingering wavelength should be independent of column width [30]. However, if the width of the column is around or narrower than half of the wavelength, the flow instability will be stabilized. This will result in that no viscosity fingers are observed on the narrow

column even if they are present on the wider column. The viscosity fingers only originates from the speed of injection, viscosity ratio between sample solution and eluent and the dispersion [30]. A thorough investigation explaining the nature of the peak fronting would be important but is beyond the scope of this article.

The experiments presented shows that by carefully examining the actual conditions of pressure and methanol volume fractions at each set volumetric flow rate, the elution volumes can fairly well be matched. By measuring and matching the conditions of the two different systems in detail, there is no need to do any calculations of the density profile, it is per definition identical if the eluent mass composition, pressure and temperature are identical, regardless of which co-solvent or combinations thereof are used. This explains the success of the scale-up presented in this study. However, the approach presented here is not always practical since the column length and particle diameter for the analytical and prep columns cannot always be matched, then other approximate approaches have to be employed e.g. [9]. However, even the approximate approaches have difficulties as the density can only be calculated for a limited amount of carbon dioxide co-solvent mixtures.

3.3 Impact on retention volume by changing flow rate

It is well known that, increasing the flow rate will affect the density gradient over the column. To get similar retention volume at different flow rates the average pressure and/or density over the column should be matched according to Tarafder et al. [9]. In this section, it will be demonstrated what happens with the retention volume when the flow rate is increased using TSO on the 4.6 mm i.d. Lux Cellulose column as model compound. First the average density, then the average volumetric methanol fraction over the column will be matched in order to get a good agreement between the elution volumes at the two flow rates.

In Fig. 4a, the chromatograms for the later eluting enantiomer of TSO (for the full chromatogram see

Supplementary Data) of a 34 µL injection of 40 g/L TSO at a flow rate of 1 mL/min (solid black line) and 4 mL/min (dashed black line) and with set methanol fraction at 15 v% and set BPR at 110 bar are shown. Note that the response is plotted versus the elution volume in an overlaid fashion for easier comparison. Fig. 4b-d shows the interpolated values of the gradients of the column pressure, density and the methanol volume fractions. Fig. 4b shows that the column pressure profile is significantly higher at 4 mL/min (dashed black line) than at 1 mL/min (solid black line). The pressure is much more dependent on the position in the column at higher flow rates, shown by a steeper negative slope compared to an almost horizontal slope for the lower flow rate. The same trend is observed for the density gradient over the column (see Fig. 4c).

Next, the difference in elution volume between the separation conducted at 4 mL/min and 1 mL/min will be reduced by matching the average density over the column by adjusting the BPR pressure, in accordance with Tarafder et al. [9]. The average density and pressure were matched when the BPR pressure at 1 mL/min was increased from 110 to 159 bar, see solid gray lines in Fig. 4b and c. We must stress that matching average pressure will not always ensure matched average density. This is true only for ideal gases. However, in this case, the average density was matched and the retention volumes for the different flow rates show a better agreement (Fig. 4) although it is still far from good. The really interesting result is the difference in the intrinsic methanol fraction shown in Fig. 4d. Here, we calculated the methanol volume fraction according to [23]. In Fig. 4d we can see that the 4 times higher flow rate (dashed black line in Fig. 4d) results in a much higher local value of methanol fraction as compared to the lower flow rate (solid black line in Fig. 4d). Thus, the intrinsic methanol fraction varies along the column due to the pressure gradient which is likely because of the different compressibility of carbon dioxide and methanol. This is a most important finding especially since the volume fraction methanol was found to be the most important factor for controlling the retention time (see section 3.1).

Increasing the back-pressure at 1 mL/min from 110 to 159 bar, it can be assumed that the average methanol volume fraction increases; this is also what happens, see Fig. 4d solid black and gray lines. By setting the methanol fraction to 20 v% for the 1 mL/min separation, dashed gray line in Fig. 4d, the average methanol fraction was matched. In this case the increased methanol content in the system did not substantially change the measured pressure (< 1 bar) and had only some minor effect on the density gradient. Inspecting the chromatogram in Fig. 4a, we see once again a substantial improvement. However, even with the compensations for average pressure, density and methanol volume fraction, the elution volumes, in the studied case, do not agree exactly; this is most likely due to the non-linear retention behavior which the matching of average methanol fraction cannot account for. This is in line with the observed non-linear retention factor relationship to the methanol fraction presented in Section 3.1.

4. Conclusions

A successful approach for scaling up an analytical SFC method from an analytical bench-top instrument to a large scale preparative SFC system was presented, using the separation of TSO on a Phenomenex Lux Cellulose 4 column, was demonstrated. At first, a design of experiments approach was used for determining which parameters must be particularly well controlled under the scale-up process. In this case, it was found that the methanol fraction and the pressure are the most important parameters while temperature played a minor role. In other words accurate temperature regulation would be the least concern in order to achieve predictable scale up.

By matching the mass flow and mass composition of carbon dioxide and co-solvent together with column outlet pressure and column average temperature, reliable scale up is possible with from a volume-flow based instrument to a mass-flow based instrument.

By measuring and matching the conditions of two different systems in detail, there is no need to do any

calculations of the density profile, it is per definition identical if the eluent mass composition, pressure and temperature are identical, regardless of which co-solvent or combinations thereof are used. The approach of matching column conditions can be applied to any type of scale up problem, e.g. when transferring analytical or preparative methods on the same column between different manufacturers of SFC instrumentation, since the focus is shifted from the system conditions to the column conditions.

Possible non-ideal scale up phenomena such as heterogeneous column packing and/or injection solvent plug effects likely prevented a completely predictable scale up. These phenomena are not necessarily inherent to SFC but to chromatography in general and remain to be investigated and explained in the case of SFC.

A strong impact on the intrinsic methanol fraction when increasing the flow rate with otherwise identical set operation conditions was observed during experiments aimed at matching column conditions to conserve the elution volume when changing flow rate on the analytical column (see Fig. 4). It was found that not only matching the average pressure (and thus the average density) but also matching the methanol volume fraction was necessary to achieve close to overlapping elution profiles at low and high flow. More particularly, the intrinsic methanol volume fraction in the eluent changes considerably with pressure and temperature of the system. It remains to be investigated if and how this phenomenon is related to the particular SFC instrument used. Further knowledge and modeling of the intrinsic conditions should therefore be of importance for proper scale-up of various analytical conditions to preparative operational conditions.

Finally, the applicability of using matched density and volumetric methanol fraction approach relies on that the equation of state for that fluid is known. So currently, the approach is only applicable for separation conducted using methanol-carbon dioxide mixtures as eluent.

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Figure Captions

Fig. 1: The results from the DoE investigation with coefficients corresponding to centered and normalized polynomial terms from the model fit with error bars representing the 95% confidence intervals for (a) the retention factors of the first eluting enantiomer (k_1) and second eluting enantiomer (k_2), respectively and for (b) the selectivity. The analytical Waters UPC^2 system was used.

Fig. 2: Figure showing elution profiles on the SuperSep 600 system (250 x 50 mm column) at a 20 % positive or negative variation of methanol fraction in eluent, BPR pressure and column temperature after the injection of 4 mL of 40 g/L TSO at 449 g/min. The solid gray line is the reference chromatogram acquired at 8.5 wt.% methanol, a BPR of 132 bar and temperature at 32 °C. In (a) the methanol content is increased to 10.2 wt.% (dotted line) or decreased to 6.8 wt.% (dashed line). In (b) the BPR pressure is increased to 158 bar (dotted line) or decreased to 106 bar (dashed line). In (c) the temperature is changed to 38.4 °C (dotted line) or to 25.6 °C (dashed line).

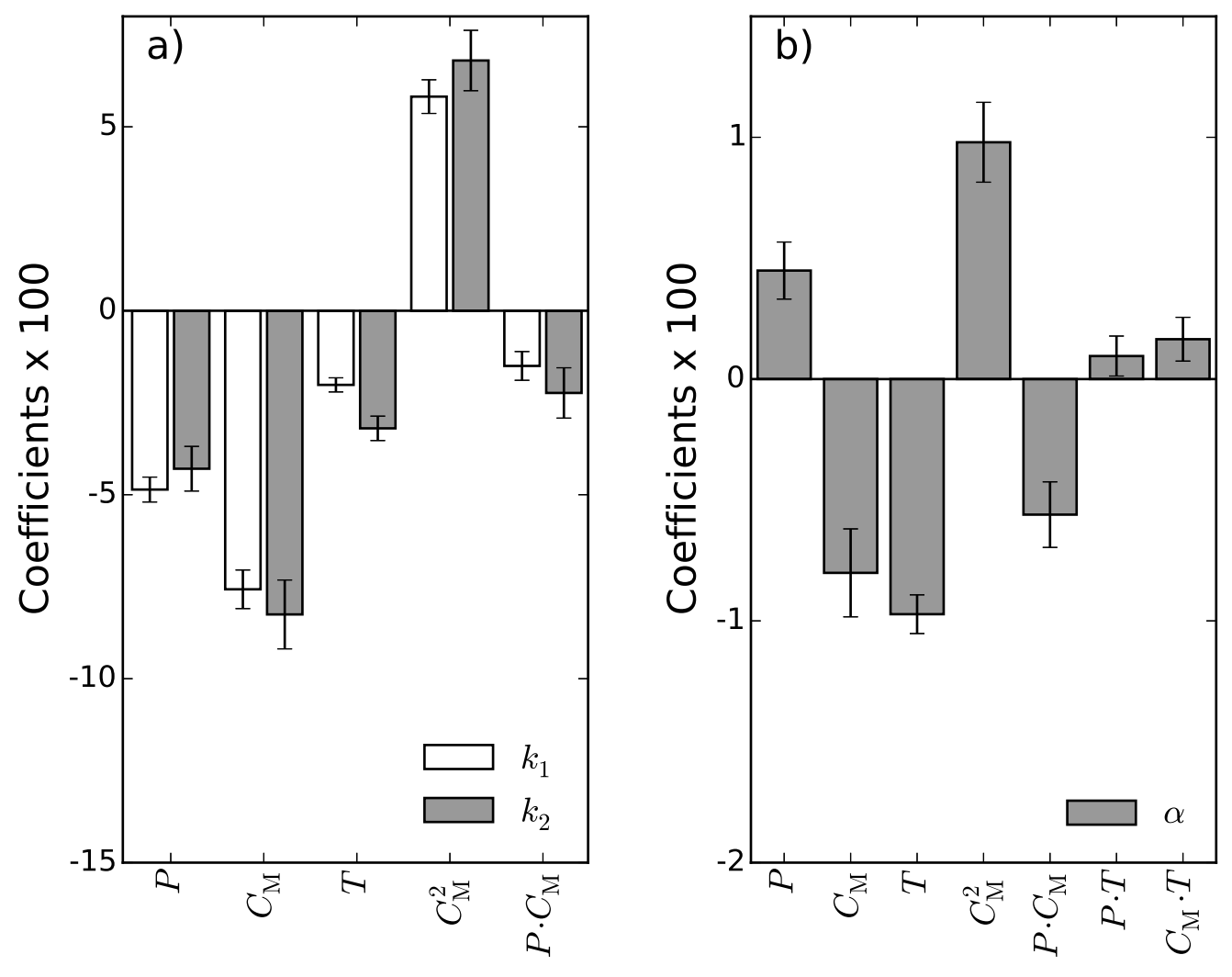
Fig. 3: The results from the matched scale-up at different eluent methanol level in wt.% units: (a) 4.2 (b) 8.5 and (c) 12.8. The black lines in all figures/insets correspond to injections on the analytical UPC^2 unit with the 250 x 4.6 mm column and the gray lines correspond to injections on the preparative SuperSep unit with the 250 x 50 mm column. The insets correspond to 2 or 200 µL 0.1 g/L analytical injections using the analytical UPC^2 and the preparative SuperSep unit, respectively. The main figures corresponds to 16.9, 33.9, 50.8 and 67.7 µL injections of 40 g/L TSO on the analytical UPC^2 unit and to 2, 4, 6 and 8 mL injections of the same sample on the preparative SuperSep unit. Corrections were made for system void volumes by matching the elution time of the TTBB marker (see section 2.2 “Scale-up studies”)

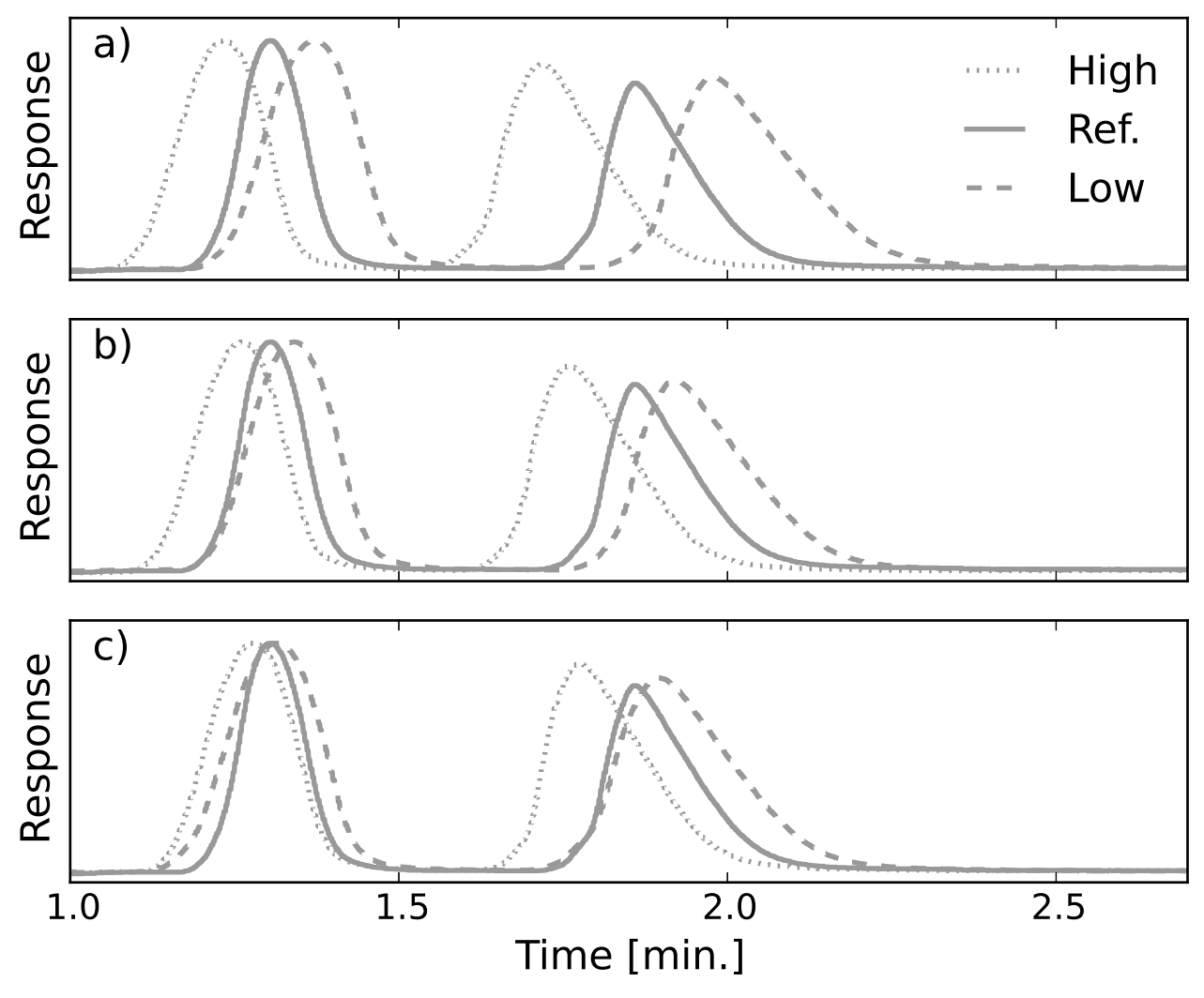
Fig. 4: Figure illustrating how the retention volume for the later eluting enantiomer (full chromatogram is presented in Supplementary data) varies with varied flow rate and how this can be compensated for. In (a) the resulting elution profiles are plotted for a 34 µL injection of 40 g/L TSO and thereafter the

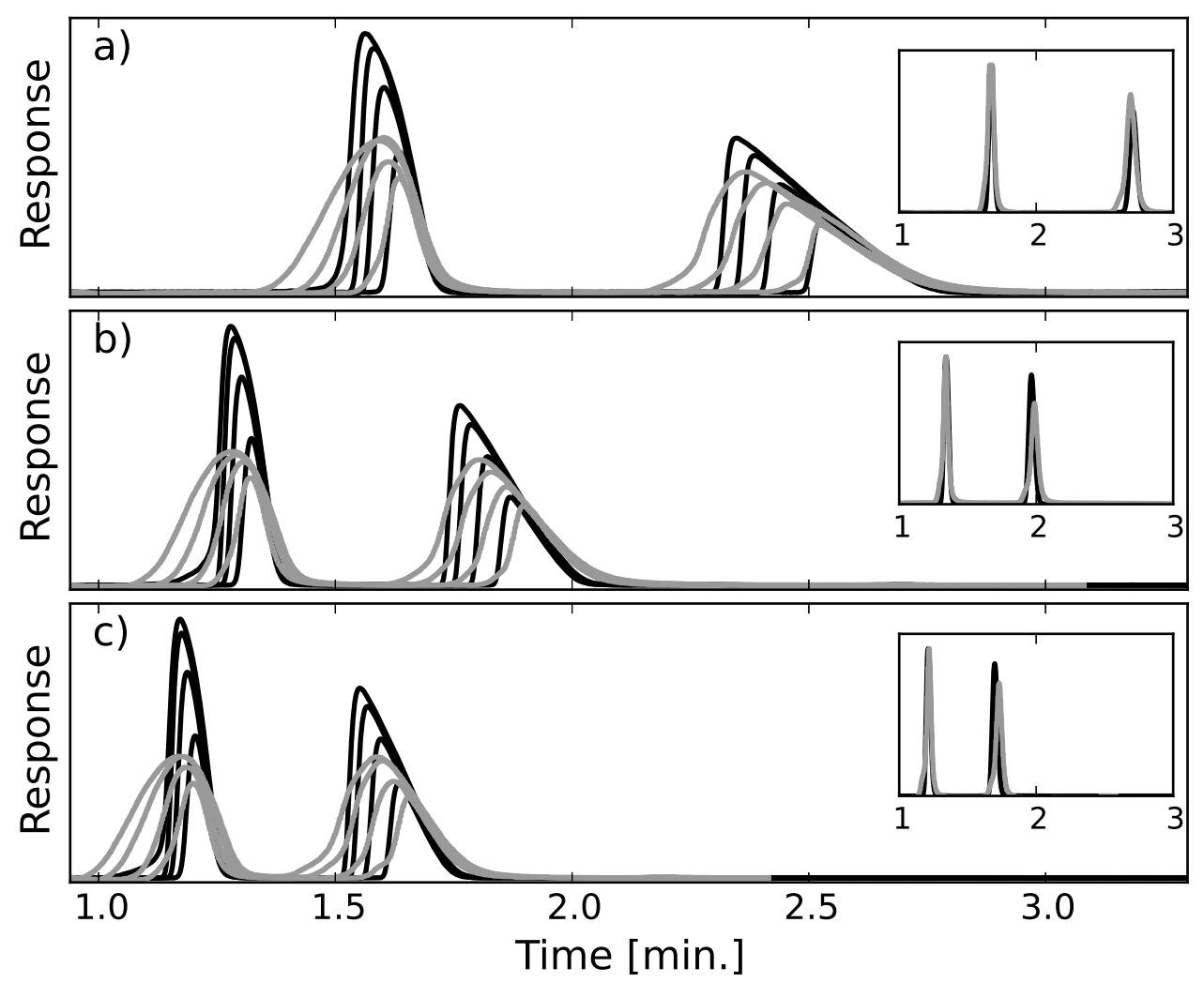
464 interpolated gradients of (b) column pressure (c) density and (d) methanol volume fraction over the
465 column. The solid black line represent data obtained at a flow rate of 1 mL/min, 15 v% methanol and 110
466 bar; the dashed black line represents data obtain at identical set conditions except that the flow rate is 4
467 mL/min. The gray lines represent data obtained at 1 mL/min, 15 v% methanol and 159 bar (solid gray
468 line) respective at 1 mL/min, 20 v% methanol and 159 bar (dashed gray line). The parameter values refer
469 to set instrumental values using the analytical Waters UPC^2 system (see Experimental section).

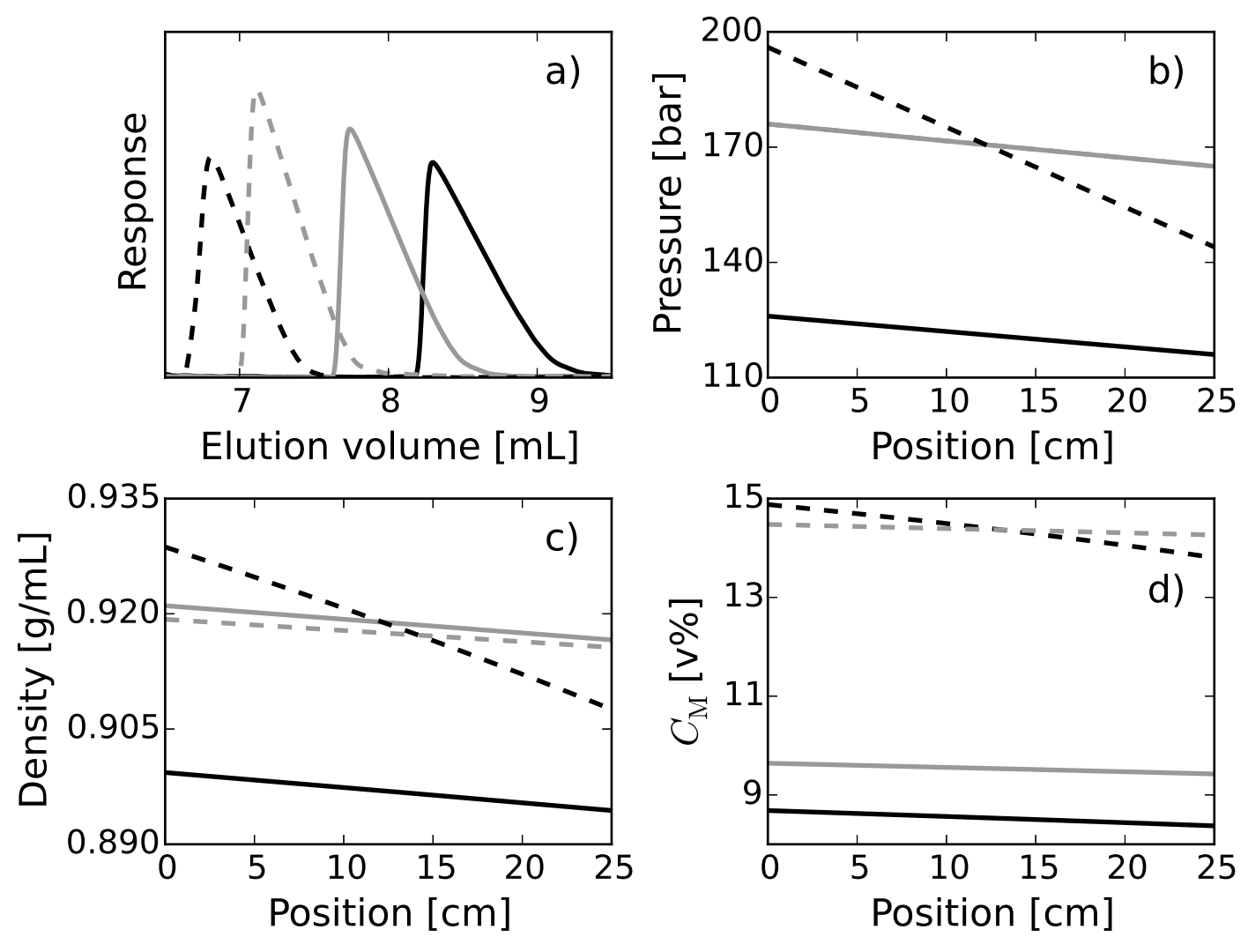
Table 1: Set and measured system conditions when scaling up with preserved temperature, pressure and methanol fraction. See experimental section and section 3.2 for details.

UPC^2 250 × 4.6 mm: Volume-controlled Analytical SFC Unit								**SuperSep 250 × 50 mm:** Mass-controlled Preparative SFC Unit					
Set				Measured				Set				Measured	
MeOH [v%]	Flow [mL/min]	BPR [bar]	Temp. [°C]	MeOH [wt.%]	Flow [g/min]	P_{out} [bar]	T_{avg} [°C]	MeOH [wt.%]	Flow [g/min]	BPR [bar]	Temp [°C]	P_{out} [bar]	T_{avg} [°C]
5	4	Off	30.8	4.2	3.84	135	30.0	4.2	453	132	32	135	30.0
10	4	Off	30.8	8.5	3.80	135	30.1	8.5	449	132	32	135	30.3
15	4	Off	30.8	12.8	3.77	135	30.2	12.8	446	132	32	135	30.4


a)
b)
Coefficients x 100
5
0
-5
-10
-15
1
0
-1
-2
k_1
k_2
α
P
C_{M}
T
C_{M}^2
$P \cdot C_{\mathrm{M}}$
$P \cdot T$
$C_{\mathrm{M}} \cdot T$


a)
b)
c)
Response
Response
Response
High
Ref.
Low
1.0
1.5
2.0
2.5
Time [min.]


a)
b)
c)
Response
Response
Response
1
2
3
1
2
3
1
2
3
1.0
1.5
2.0
2.5
3.0
Time [min.]


a)
Response
7
8
9
Elution volume [mL]
b)
Pressure [bar]
200
170
140
110
0
5
10
15
20
25
Position [cm]
c)
Density [g/mL]
0.935
0.920
0.905
0.890
0
5
10
15
20
25
Position [cm]
d)
C_{M} [v%]
15
13
11
9
0
5
10
15
20
25
Position [cm]

Supplementary Data

S1. Raw data and statistics for the DoE models

The raw data for the responses are presented in Table S1 and the statistics for the regression models are presented in Table S1. The regression model used in this study was:

$$R = p_0 + p_1 T + p_2 P + p_3 C_M + p_4 T^2 + p_5 P^2 + p_6 C_M^2 + p_7 TP + p_8 TC_M + p_9 PC_M \quad (1)$$

where R is the response, p are numerical fitting coefficients, T is the temperature, P the pressure and C_M the volumetric methanol fraction. Insignificant terms at 95% confidence level were removed.

Table S1: experimental values of retention factor (k) and separation factor (α) from the DoE.

Exp. No	P [bar]	MeOH [v/v]	T [°C]	k_1	k_2	α
1	105	5	24	1.708	3.551	2.080
2	155	5	24	1.417	2.972	2.097
3	205	5	24	1.241	2.623	2.114
4	105	10	24	1.176	2.327	1.978
5	155	10	24	1.002	1.994	1.991
6	205	10	24	0.892	1.783	2.000
7	105	15	24	0.963	1.864	1.935
8	155	15	24	0.838	1.632	1.947
9	205	15	24	0.757	1.478	1.951
10	105	5	30	1.778	3.579	2.013
11	155	5	30	1.414	2.886	2.042
12	205	5	30	1.215	2.500	2.057
13	105	10	30	1.169	2.241	1.917
14	155	10	30	0.971	1.871	1.928
15	205	10	30	0.849	1.651	1.944
16	105	15	30	0.934	1.756	1.880
17	155	15	30	0.798	1.506	1.887
18	205	15	30	0.710	1.349	1.902
19	105	5	36	1.904	3.708	1.947
20	155	5	36	1.428	2.836	1.986
21	205	5	36	1.199	2.403	2.005
22	105	10	36	1.186	2.200	1.856
23	155	10	36	0.945	1.774	1.877
24	205	10	36	0.813	1.537	1.891
25	105	15	36	0.915	1.669	1.823
26	155	15	36	0.761	1.397	1.836
27	205	15	36	0.665	1.235	1.856
28	155	10	30	0.974	1.879	1.928
29	155	10	30	0.972	1.875	1.928

30	155	10	30	0.971	1.871	1.928

Table S2: Model statistics for the regression models used for the responses.

Statistic	k_1	k_2	α
R^2	0.998	0.995	0.992
Q^2	0.997	0.992	0.981

S2. Figure showing the elution volume of both enantiomers of TSO

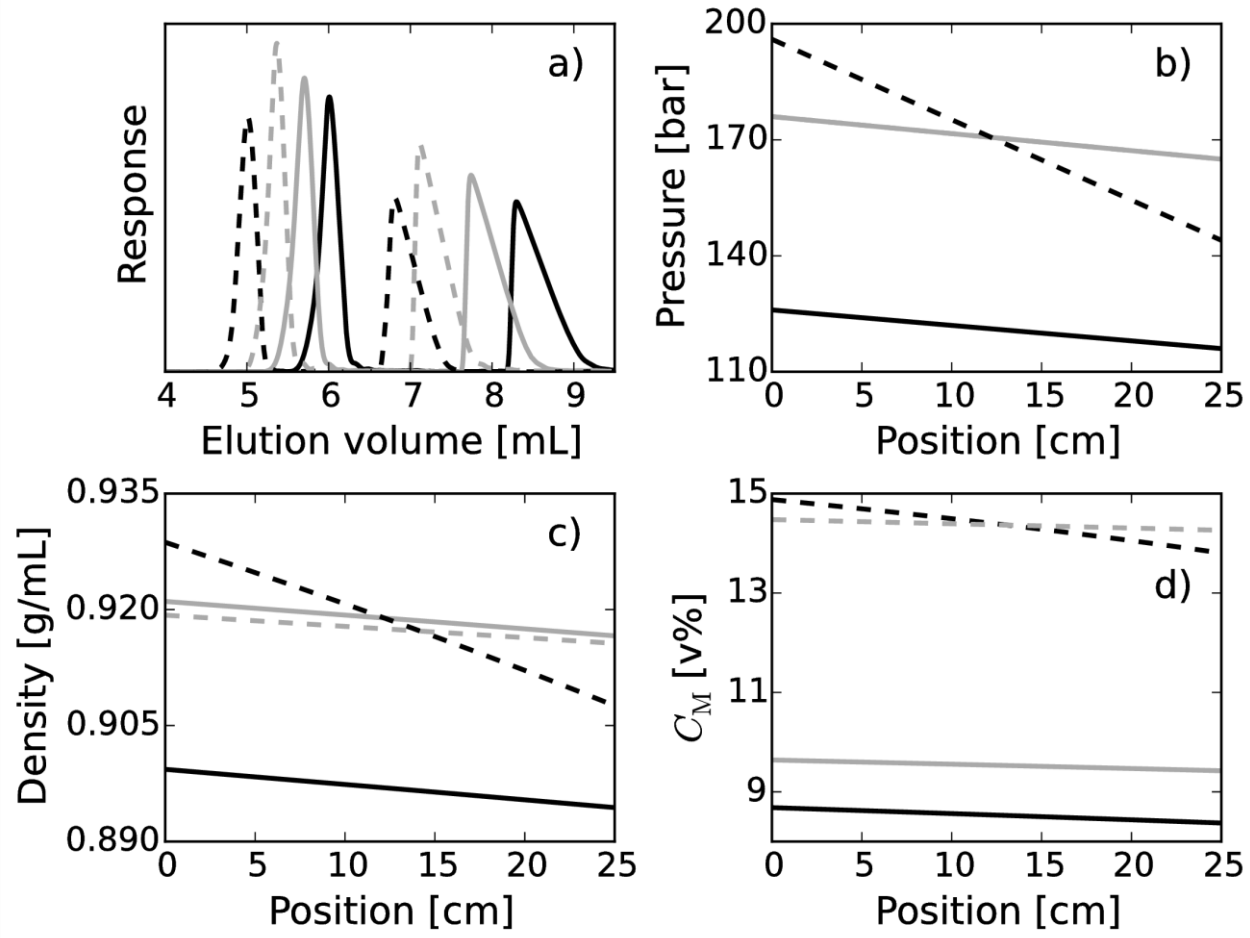


Figure S1: Figure illustrating how the retention volume varies with varied flow rate and how this can be compensated. In (a) are plotted the resulting elution profiles and thereafter the interpolated gradients of (b) column pressure (c) density and (d) methanol volume fraction. The solid black line represent data obtained at flow of 1 mL/min, 15 v% methanol and 110 bar; the dashed black line represents data obtain at identical set conditions except that the flow rate is 4 mL/min. The grey lines represent data obtained at 1 mL/min, 15 v% methanol and 159 bar (solid grey line) respective at 4 mL/min, 20 v% methanol and 159 bar (dashed grey line). *The parameter values refer to set instrumental values using the analytical Waters UPC^2 system (see Experimental section).*