The Effect of Temperature, Pressure and Co-Solvent on a Chiral Supercritical Fluid Chromatography Separation

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The importance of temperature, pressure and co-solvent fraction on the separation of racemic 1-phenyl-1-propanol on a cellulose-based chiral stationary phase was investigated using a chemometric approach where the true instead of the set operational variables were used as input data. More particular, the real temperatures, pressures and co-solvent fractions inside the column and the real mass flows over the column were measured with external sensors and mass flow meters and used in the calculations to ensure physically correct and instrument-independent results. The co-solvent fraction was found to be the most important factor for both retention and selectivity; therefore, the methanol dependence was investigated further by a more detailed determination of the adsorption behaviour at different methanol fractions.

1 Introduction

Both instrument manufacturers and the pharmaceutical industry have increased their interest in supercritical fluid chromatography (SFC) especially for preparative applications [1]. The reasons for this growing interest are the lower environmental impact combined with considerable higher productivities, through the much shorter cycle times, as compared to preparative-LC [2,3]. One drawback with SFC is that it is more complex than LC, mainly due to the compressibility of the mobile phase; SFC is a 'rubber variant of LC' where everything considered as constant in LC, changes in SFC. This ultimately results in radial and axial density and temperature gradients in the column [4] which affects the thermodynamics of adsorption as well as generating a volumetric flow rate gradient through the column. The phenomena result in a series of important practical consequences such as (i) 'non-intuitive' system behaviour when changing operational conditions such as pressure, temperature and solvent composition (ii) system-dependent chromatography and poor reproducibility between different vendors of bench-top SFC instruments and for the preparative chromatographer and (iii) unpredictable scale-up from analytical SFC instruments to preparative SFC instruments. The latter has the consequence that optimisations in process and preparative SFC must be done in large scale which is expensive, consumes significant amounts of
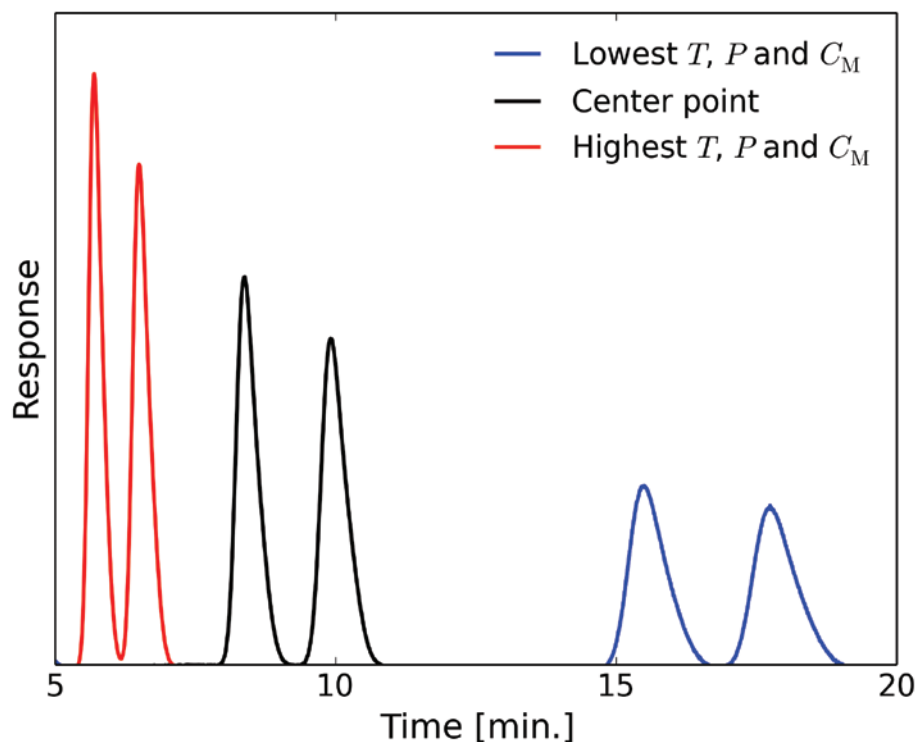

Figure 1. Chromatograms showing the two extreme points with highest and lowest pressure (P), temperature (T) and methanol fraction (C_M) and the centre point in the experimental design for racemic 1-phenyl-1-propanol.

energy and may even be impossible in some cases. Therefore a proper understanding of how operator parameters such as pressure, temperature and solvent composition affect the separation is of paramount importance.

The aim of this study was to use a chemometric methodology to elucidate how pressure, temperature and solvent composition affects the separation of racemic 1-phenyl-1-propanol as a model compound, using the real conditions over the column as inputs. Adsorption isotherms will also be determined to gain a deeper understanding of how the solvent composition in the eluent affects adsorption and retention. The compound 1-phenyl-
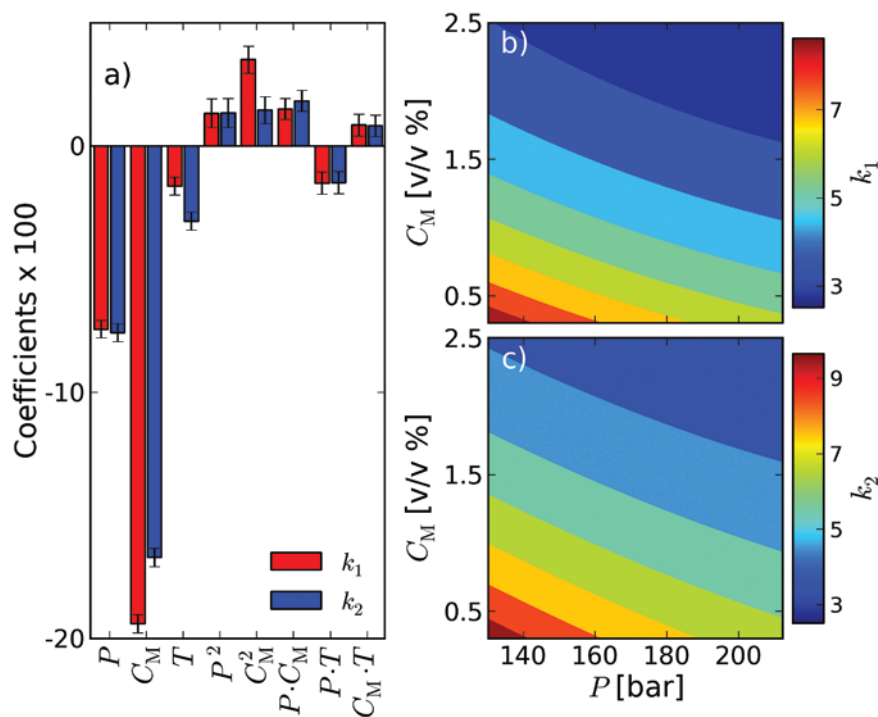
Figure 2. a) Relative importance for the first and second retention factor, respectively, by centred and normalised coefficients from the model fit; the error bars represents the 95% confidence interval of the coefficients. Figure b and c shows contour plots for the retention factor as a function of pressure (P) and amount of methanol in the eluent (C_M) for (b) the first and (c) second retention factor, respectively. The column temperature was 30°C.

1-propanol was chosen because it can be studied at very low fractions of methanol in the mobile phase and even with pure CO_2 as mobile phase. This is an interesting experimental region where small variations in pressure and temperature can give large changes in the chromatography. 1-phenyl-1-propanol is also a common neutral model compound previously used in both SFC and LC.

2 Experimental

2.1 Chemicals and instrumentation

CO_2 (>99.99%, AGA Gas AB, Sweden) modified with HPLC grade methanol (Fischer Scientific, Loughborough, UK) was used as mobile phase. (R,S)-1-phenyl-1-propanol "RS-PP", (≥97%), (R)-(+)-1-phenyl-1-propanol "R-PP", (99%) and nitrous oxide (99.998 %) were used as solutes and purchased from Sigma-Aldrich (St. Louis, MO, USA). The column was a 5 µm Kromasil CelluCoat 100 × 4.6 mm column (AkzoNobel Eka, Bohus, Sweden) with a void volume equal to 1.21 mL measured with N_2O. The chromatographic system was a Waters UPC^2 system (Waters Corporation, Milford, MA, USA) with a system void volume of 70 µL from the auto sampler to the detector (measured with pure methanol as mobile phase and the column replaced with a zero-volume union). Additional temperature and pressure transmitters were attached and connected to the column inlet and

outlet to continuously monitor and compare instrument set and real values of temperature and pressure. Furthermore, two Coriolis based mass flow meters were simultaneously monitoring the mass flow of carbon dioxide and methanol during all experiments. For more detailed information about the sensors the reader is referred to [5].

2.2 Procedure

A design space spanning back pressures of 120 to 200 bar, temperatures of 24 to 36°C, and methanol fractions between 0.5 and 2.5%, was used to study the variation of retention factors and selectivities of the enantiomers of the racemic model compound, RS-PP. In Figure 1 the chromatograms for the lowest, highest temperature, pressure and methanol fraction are shown together with the centre point. A full factorial design with two centre points (29 experiments) was used. The flow rate was set to 0.70 mL/min to minimise any gradients of temperature and pressure along the column during the experiments. Column temperature, pressure and mass flow were recorded online during the experiments with the external sensors and used in all calculations, for a detailed description of the external sensors the reader is referred to [5,10]. For example, at the centre point the back pressure was set to 160 bar giving the actual pressure equal to 173 and 169 bar

at the column inlet and outlet respectively, while the methanol fraction was set to 1.50% v/v and the real fraction was 1.33% v/v. The accuracy of the temperature sensors were ±0.2°C, the accuracy of the pressure sensors were ±1 bar and the accuracy of the mass flow meters were ±0.2% of the measured mass flow. Chemometric regression analysis was done using MODDE 7 (Umetrics AB, Sweden). The responses were log transformed and the factors were normalised and centred before the regression.

The adsorption isotherms of R-PP (the first eluting enantiomer) were determined on 0, 0.5, 1, 1.5, 2, and 2.5% v/v methanol for a 20 µL injections of 150 g/L of R-PP using the elution by characteristic points method [6]. The flow rate was 1 mL/min and the column temperature was 30°C. To keep the density constant on each methanol plateau the pressure was adjusted from 128 to 140 bar. The adsorption data was analysed using adsorption energy distribution calculations [7]. All energy distributions were unimodal as is the case for the Langmuir adsorption isotherm model.

3 Results and Discussion

3.1 Impact of temperature, pressure and co-solvent on retention factor and selectivity

In chiral SFC, the co-solvent fraction, pressure and temperature in the column affects the retention and selectivity [1,3]. Using design of experiment, the relative importance of these three factors on retention and selectivity was evaluated and quantified for racemic 1-phenyl-1-propanol, 'RS-PP'. A quadratic polynomial with interaction terms was applied to relate the factors to a response. All regression models had an excellent fit to the experimental data with an adjusted R^2-value above 0.998 in all cases.

The coefficients for the retention factor (k) models are shown in Figure 2a; here it is easy to view the relative importance of the operational parameters for the retention factor of enantiomer 1 (R-PP) and 2 S-PP), respectively. A large absolute value of the coefficient means that the corresponding parameter has a large effect on the response and a positive value means that an increase in this coefficient gives an increase in the response and vice versa. In this case, the methanol fraction (C_M) is the most important factor governing the retention for both enantiomers whereas the back pressure (P) is the second most important factor; an
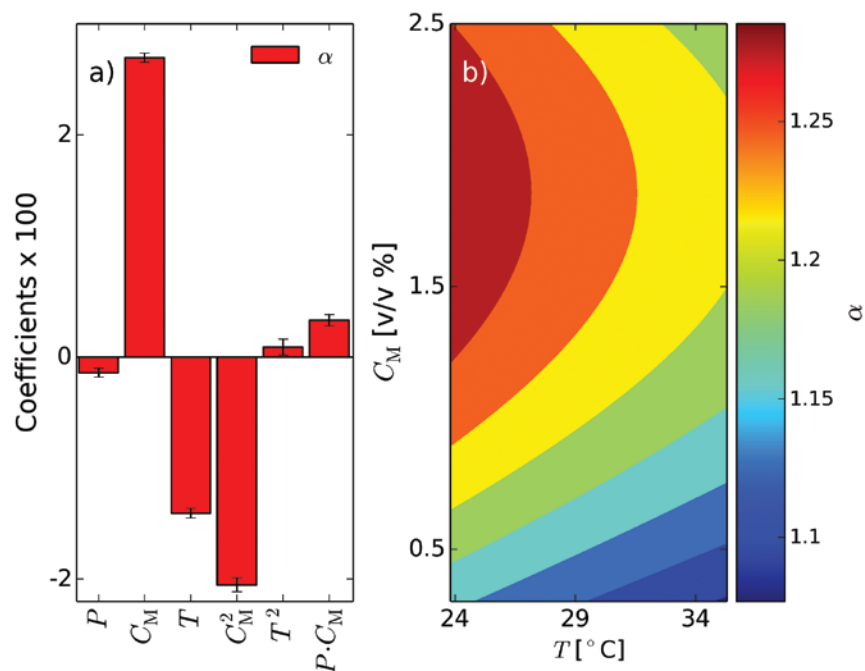
Figure 3. Same as Fig. 1, but the selectivity was studied instead of the retention factors.
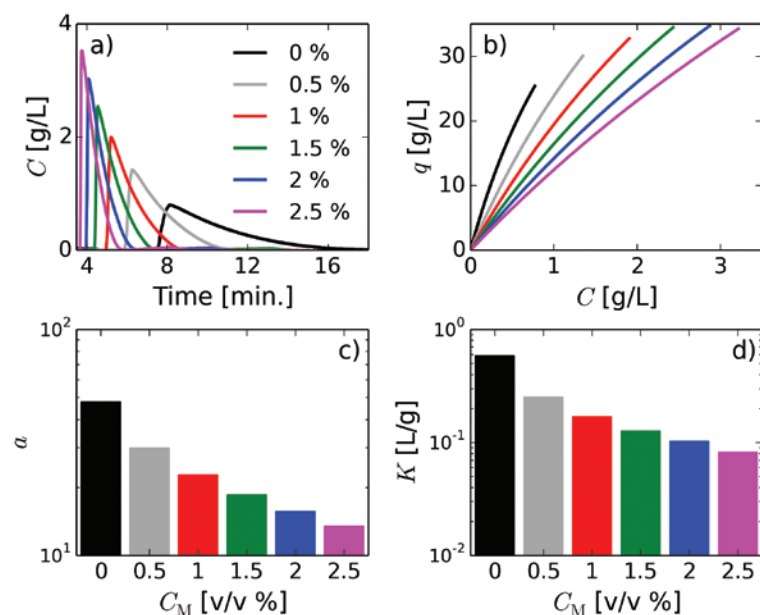

Figure 4. a) Overloaded elution profiles for R-1-phenyl-1-propanol at different amounts of methanol in the eluent and b) estimated adsorption isotherms for each of the overloaded profiles. The c Figures shows the estimated adsorption isotherm parameters for the distribution coefficient (constant a) at different fractions of methanol in the eluent; in Figure d the same is shown but for the association equilibrium constant b.

increase of the latter results in a decrease of the retention factor, because of increased mobile phase density. The temperature has two, counteracting, effects in SFC. On the one hand, it modifies retention in the same way as in LC, i.e. the retention decreased if the temperature is increased for an exothermic adsorption. On the other hand, an increased temperature will decrease the density of the mobile phase and lower its elution strength. In this case increasing the temperature leads to a decrease in the retention which indicates that the classical 'LC-like' temperature effect dominates.

Figure 2b and c shows the combined effect of the pressure and the methanol fraction for R-PP and S-PP, respectively. These contour plots confirm the trends in Figure 2a and it can also be seen that the interaction and squared terms give rise to some curvature.

The relative importance of the operational parameters for the selectivity (α) is shown in Figure 3a. It can be seen that the parameters which affect selectivity, shown in the coefficient plot, are rather different from those that affect the retention factors in that the temperature cannot be neglected. This

is because the enantiomers have different temperature dependence, see Figure 2. The methanol fraction is still the most important parameter, with a strong quadratic relationship but here the temperature is also playing a certain role. The combined effect of methanol fraction and temperature is presented in Figure 3b where the quadratic coefficient from the methanol fraction (cf. Figure 3a) leads to a rather unusual form of the contour plot. High methanol fraction and low temperature gave good selectivity, but there seems to be a maximum around 2% v/v methanol at all three pressures.

3.2 A thermodynamic study of the methanol dependency

Since the methanol fraction was the most important factor for both the retention factor and the selectivity, it was studied in more depth by determining the adsorption isotherms on five methanol fractions between 0 - 2.5% v/v. The adsorption isotherms of R-PP is determined from the diffuse part of overloaded elution profiles, at different methanol fractions in the eluent (Figure 4a), using the elution by characteristic points method [6]. The Langmuir adsorption isotherm [8] was fitted to the adsorption data with nonlinear regression and the fit was excellent. The Langmuir model is written as

$$q(C) = \frac{aC}{1 + KC} \quad (1)$$

where q is the concentration of the solute absorbed on the stationary phase, C is the concentration of the solute in the mobile phase, K is the association equilibrium constant and a is the distribution coefficient (initial slope of the adsorption isotherm). The retention factor is correlated to these parameters as k = F·a, where F is the phase ratio. The Langmuir adsorption isotherms for R-PP are shown for all methanol fractions in Figure 4b. For a certain mobile phase concentration the amount adsorbed on the stationary phase increases with decreasing methanol fraction giving steeper slopes and longer retention times.

The distribution coefficient decreases with methanol fraction, Figure 4c, and the association equilibrium constant decreases, Figure 4d; the figures are presented with logarithmic y-axis. According to previous results in SFC, a quadratic relationship between the log transformed retention factor and methanol fraction is often necessary for a good description [9–11],

this was also the case in this study and such a relationship was also very good at describing how the association equilibrium constant and distribution coefficient changed with the methanol fraction, as can be observed in Figure 4c-d.

4 Conclusions

In this study design of experiments was used to study the influence of temperature, pressure and co-solvent fraction on the retention and selectivity of racemic 1-phenyl-1-propanol on tris-(3,5 dimethylphenyl) carbamoyl cellulose stationary phase. The pressure, temperature and fraction of co-solvent inside the column was measured and used to get physically correct inputs and instrumental-independent results.

The methanol fraction was found to be the most important factor for governing the retention factors followed by the pressure whilst the temperature did not have a significant effect on the retention factor over the parameter range investigated. Increasing any of these factors resulted in a reduction in the retention factor. For the selectivity the methanol fraction in the eluent are also the most important factor followed by the temperature whereas the pressure played only a minor role. The selectivity

increased with increasing methanol fraction and decreased with increasing pressure and temperature. The log transformed responses had a clear quadratic dependency on the methanol fraction. From the more detailed adsorption study it could be concluded that the distribution coefficient and the equilibrium constant decreased with increasing methanol fraction.

It was necessary to combine the chemometric calculations with experiments utilising external measurements of the system parameters for physically correct results; this was also verified through adsorption isotherm measurements. The application of chemometric methodology to SFC methods development can be used as a successful approach to dealing with the complexity of SFC. It can be expected that a chemometric approach will lead to a more robust and easily scaled-up SFC methods.

Acknowledgement

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