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Possibilities of retention modeling and computer assisted method development in supercritical fluid chromatography

Eva Tyteca[a,1], Vincent Desfontaine[b,1], Gert Desmet[a], Davy Guillarme[b,*]

[a] Department of Chemical Engineering, Vrije Universiteit Brussel, Pleinlaan 2, Brussels, Belgium
[b] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, Switzerland

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ABSTRACT

The multi-modal retention mechanism in supercritical fluid chromatography (SFC) results in a non-linear dependency of log(k) on the fraction of organic solvent φ and log(φ). In the present study, the possibility of retention modeling for method development purposes in SFC was investigated, considering several non-linear isocratic relationships. Therefore, both isocratic and gradient runs were performed, involving different column chemistries and analytes possessing diverse physico-chemical properties. The isocratic retention data of these compounds could be described accurately using the non-linear retention models typically used in HILIC and reversed-phase LC. The interconversion between isocratic and gradient retention data was found to be less straightforward than in RPLC and HILIC because of pressure effects. The possibility of gradient predictions using gradient scouting runs to estimate the retention parameters was investigated as well, showing that predictions for other gradients with the same starting conditions were acceptable (always below 5%), whereas prediction errors for gradients with a different starting condition were found to be highly dependent on the compound. The second part of the study consisted of the gradient optimization of two pharmaceutical mixtures (one involving atorvastatin and four related impurities, and one involving a 16 components mixture including eight drugs and their main phase I metabolites). This could be done via individual retention modeling based on gradient scouting runs. The best linear gradient was found via a *grid search* and the best multi-segment gradient via the previously published *one-segment-per-component search*. The latter improved the resolution between the critical pairs for both mixtures, while still giving accurate prediction errors (using the same starting concentrations as the gradient scouting runs used to build the model). The optimized separations were found in less than 3 h and 8 h of analysis time (including equilibration times), respectively.

1. Introduction

Supercritical fluid chromatography (SFC) is gaining in importance as a chromatographic technique, to analyze a wide range of compounds from relatively hydrophilic to highly lipophilic [1–4]. In SFC, the retention mechanism is multi-modal, combining different interaction mechanisms, and highly dependent on the nature of the stationary phase and the type of organic modifier [5,6].

In SFC, the mobile phase generally consists of a mixture of apolar CO_2 and a limited proportion of methanol (typically up to 30%). In this case, normal phase retention behavior takes place using a polar stationary phase (such as silica, diol, amino, cyano, amide). On the other hand, using an apolar stationary phase (such as C_8, C_{18}, phenylhexyl), reversed-phase retention behavior is expected, as in the absence of H_2O, the interactions between the compounds and the stationary phase are favored, limiting the contribution of the apolar CO_2 in the mobile phase. When other stationary phases (such as alkyl bonded phases with hydrophilic end-capping or polar embedded group) are used, intermediate behavior can be expected [7]. The use of MeOH in the mobile phase introduces other interactions such as H-bonding, dipole–dipole interactions and solvent adsorption [7,8]. Solvent adsorption is also playing a major role in hydrophilic interaction chromatography (HILIC) [9].

The multi-modal retention mechanism results in a non-linear dependency of the logarithm of the retention factor log(k) on the fraction of organic solvent φ and log(φ). As such, the linear solvent strength (LSS) model, widely used to model reversed-phase retention, can no longer be applied in SFC. Also in HILIC, a multi-modal retention mechanism exists, combining partitioning, adsorption through H-bonding and electrostatic and ionic interactions [10], and non-linear retention models have been reported [11–13].

* Corresponding author. Tel.: +41 22 379 34 63.
E-mail address: davy.guillarme@unige.ch (D. Guillarme).
[1] These authors contributed equally to this work.

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Therefore, the non-linear retention models that have proven to be successful in HILIC retention modeling could also be of interest for the SFC retention modeling:

$$\ln(k) = \ln(k_w) + S_1\phi + S_2\ \ln(\phi) \quad (1)$$

$$\ln(k) = \ln(k_w) + S_1\phi + S_2\phi^2 \quad (2)$$

$$\ln(k) = \ln(k_w) + 2\cdot\ln(1 + S_2\phi) - \frac{S_1\phi}{1 + S_2\phi} \quad (3)$$

where ϕ is the fraction of water, k_w the extrapolated value of k for $\phi = 0$ (i.e., pure CO_2), S_1 the slope and S_2 the curvature coefficient [14].

The expression for the gradient retention factor can be found by solving the fundamental gradient equation:

$$t_0 = \int_0^{t_R - t_0} \frac{dt_s}{k(\phi)} \quad (4)$$

where t_0 is the column dead time and t_R is the total retention time. For multi-segment gradients, the fundamental gradient equation becomes a sum of integrals, each describing the retention during one segment of the gradient [15]. This sum of integrals can still be solved in a straightforward way, to obtain an analytical expression for the effective retention factor $k_{eff} = (t_R - t_0)/t_0$.

In reversed-phase method development (MD) schemes, isocratic and gradient data are measured and interpolated, then isocratic and gradient retention results can be interconverted (e.g. prediction of isocratic retention based on gradient scouting runs). This is possible because the retention relationships in reversed-phase behave rather "gently", with only relatively small deviations from the LSS-behavior, i.e. linear relationship between $\ln(k)$ and ϕ. In reversed-phase, the retention behavior of a component can therefore in general be examined with a set of well-chosen experiments that cover the entire intended experimental space. The retention space between experimental data points is then modeled, and computer predictions, based on these models, are used in MD processes [15–18].

Based on the accuracy of the retention models, different MD strategies can be applied. If accurate retention time predictions are possible, individual retention modeling can be used to optimize the gradient conditions. This approach was developed for reversed-phase separations [16]. In addition, HILIC separations for which an accurate retention model exists have also been optimized using this approach [13]. When the modeling is very accurate, the separation power can be increased, using multi-segments gradients. However, smart algorithms are needed to find out the optimal gradient conditions among the innumerous combinations [15]. On the other hand, if no accurate retention modeling is possible, a hybrid method called the predictive elution window shifting and stretching approach (PEWS^2) can be used for the gradient optimization in both RPLC and the HILIC mode [13,18].

Design of Experiments is another strategy that can be used to optimize a separation, without the need for retention models such as Eqs. (1)–(3). This approach was recently proposed to optimize SFC gradient conditions by Dispas et al. who reported on the optimization of t_{iso}, t_G and $\%MeOH_{start}$ for the separation of six antibiotic drugs and caffeine [19].

In the present study, the possibilities of retention modeling and gradient optimization in SFC were investigated, using the non-linear retention models of Eqs. (1)–(3). To the best of the authors' knowledge, the isocratic retention models proposed in this study have not yet been applied in SFC. Moreover, gradient retention prediction and optimization of the gradient conditions via individual retention modeling have not been reported in SFC. For this purpose, a range of mobile phase compositions %B (10 mM ammonium formate in 98% MeOH + 2% H_2O) were applied to various column chemistries (BEH, 2-EP and HSS) and several analytes possessing diverse physico-chemical properties (see Table 1) and the isocratic retention relationships were studied. Subsequently, we investigated the possibility to predict gradient retention from a limited number of isocratic or gradient runs and applied this approach to two real-life separation problems, with varying number of compounds (atorvastatin and four related impurities, a 16 component mixture including 8 drugs and their main phase I metabolites), using Eq. (3) (the Neue and Kuss-equation), as this gives the simplest expression for the gradient retention factor when solving the fundamental gradient equation (Eq. (4)), and hence requires the smallest computational effort during the optimization searches (e.g. the model in Eq. (1) requires a time-resolved numerical integration for each individual screening condition).

Atorvastatin marketed as a calcium salt is a member of the drug class known as statins, which are used for lowering blood cholesterol and for prevention of events associated with cardiovascular disease [20]. From 1996 to 2012, atorvastatin became the world's best-selling drug of all time, under the trade name Lipitor, with more than $125 billion in sales over approximately 14 years [21]. In the present study, a stability-indicating SFC method was developed for the determination of atorvastatin and four related pharmacopeia impurities.

To evaluate the interaction potential of drugs, new chemical entities, toxic substances and phytochemicals, and account for the existing risks during co-exposure, *in vitro* drug metabolism assay has to be performed in the pharmaceutical industry during the drug development process. From an analytical point of view, there is a need to develop fast methods able to discriminate a significant number of substrates and metabolites, each corresponding to a given cytochrome P450 (CYP) subfamily [22]. In the present study, a SFC method was developed for the separation of eight probe substrates and eight CYP-specific metabolites, previously investigated in LC conditions [23].

2. Material and methods

2.1. Chemicals and reagents

Methanol (MeOH) HPLC grade was purchased from Fisher Scientific (Loughborough, UK), whereas isopropanol (IpOH), ethanol (EtOH) and heptane were purchased from VWR (Radnor, PA, USA). Pressurized liquid CO_2, 3.0 grade, (99.9%) was purchased from Pan-Gas (Dagmerstellen, Switzerland). Ultrapure water was supplied by a Milli-Q Advantage A10 purification unit from Millipore (Bedford, MA, USA). Ammonium formate was purchased from Sigma-Fluka (Buchs, Switzerland).

2.2. Instrumentation and columns

All the experiments were performed on a Waters Acquity UPC^2 system (Waters, Milford, MA, USA) equipped with a binary solvent delivery pump, an autosampler that included a 10 μL loop for partial loop injection, a column oven and a two-step (passive + active) backpressure regulator (BPR). The passive component maintains pressure higher than 104 bar, while the active component allows further back pressure increase and fine backpressure adjustments. The injection volume was 1 μL and the measured dwell volume was 440 μL.

The Acquity UPC^2 system was also combined with a benchtop single quadrupole, namely Waters Acquity QDa detector fitted with a Z-spray electrospray (ESI) ionization source. Make-up solvent delivered by a Waters Isocratic Solvent Manager (ISM) pump