



Retention modeling and method development in hydrophilic interaction chromatography



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ABSTRACT

In the present study, the possibility of retention modeling in the HILIC mode was investigated, testing several different literature relationships over a wide range of different analytical conditions (column chemistries and mobile phase pH) and using analytes possessing diverse physico-chemical properties. Furthermore, it was investigated how the retention prediction depends on the number of isocratic or gradient trial or initial scouting runs. The most promising set of scouting runs seems to be a combination of three isocratic runs (95, 90 and 70%ACN) and one gradient run (95 to 65%ACN in 10 min), as the average prediction errors were lower than using six equally spaced isocratic runs and because it is common in Method development (MD) to perform at least one scouting gradient run in the screening step to find out the best column, temperature and pH conditions. Overall, the retention predictions were much less accurate in HILIC than what is usually experienced in RPLC. This has severe implications for MD, as it restricts the use of commercial software packages that require the simulation of the retention of every peak in the chromatogram. To overcome this problem, the recently proposed predictive elution window shifting and stretching (PEWS²) approach can be used. In this computer-assisted MD strategy, only an (approximate) prediction of the retention of the first and the last peak in the chromatogram is required to conduct a well-targeted trial-and-error search, with suggested search conditions uniformly covering the entire possible search and elution space. This strategy was used to optimize the separation of three representative pharmaceutical mixtures possessing diverse physico-chemical properties (pteridins, saccharides and cocktail of drugs/metabolites). All problems could be successfully handled in less than 2.5 h of instrument time (including equilibration).

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1. Introduction

Reversed phase liquid chromatography (RPLC) is the preferred elution mode in high performance liquid chromatography. In RPLC, the linear solvent strength (LSS) is the most widely used theory to model the retention of analytes as a function of the organic solvent fraction [1]:

$$k = k_w \times \exp(-S\varphi) \quad (1)$$

$$\ln(k) = \ln(k_w) - S\varphi \quad (2)$$

where φ is the fraction of organic solvent, k_w is the extrapolated value of k for $\varphi=0$ (i.e. pure water) and S is the solvent strength parameter which is a constant for a given compound and organic solvent [2,3]. The LSS model is the simplest theory but it is only valid for narrow φ -ranges. It relies on the assumption that there

is a linear relationship between the logarithm of the retention factor and the mobile phase composition. It should be noted that, in methanol–water systems, a linear model can provide a good prediction of the retention, but this is rarely the case in acetonitrile–water systems [4]. In reality, nearly all $\ln(k)$ vs. φ relationships show a curvature [4].

The values of k_w and S can be obtained either from two isocratic experiments with different φ or from two gradient runs with two different slopes and all other conditions kept constant. When k_w and S are known, the retention time t_R can be calculated for virtually any gradient times and conditions [1], which constitutes the basis of several HPLC simulation packages [5].

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

$$t_0 = \int_0^{t_R-t_0} \frac{dt_s}{k(\varphi)} \quad (3)$$

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where t_R and t_0 are the total retention time and the column dead time, respectively [5]. Using the LSS-model and including the system dwell time (t_D), the effective retention factor k becomes [8]:

$$k = \frac{t_D}{t_0} + \left(\frac{\phi_{0,1} + 1 + S_2 \phi_{0,1} / S_1 \ln(\beta_1 k'_w S_1 (t_0 - t_D / k_0) \exp(-S_1 \phi_{0,1} / 1 + S_2 \phi_{0,1}))}{1 - S_2 (1 + S_2 \phi_{0,1}) / S_1 \ln(1 + \beta_1 k'_w S_1 (t_0 - t_D / k_0) \exp(-S_1 \phi_{0,1} / 1 + S_2 \phi_{0,1}))} - \phi_{0,1} \right) \frac{1}{\beta_1 t_0} \quad (8)$$

$$k = \frac{t_R - t_0}{t_0} = \frac{t_D}{t_0} + \frac{1}{S \times \beta \times t_0} \ln \left(\left(t_0 - \frac{t_D}{k_0} \right) \times S \times \beta \times k_0 + 1 \right) \quad (4)$$

where β is the gradient slope, defined as $(\varphi_e - \varphi_0)/t_G$ and k_0 is the isocratic retention factor for $\varphi = \varphi_0$.

In method development (MD) schemes, isocratic and gradient data are measured and interpolated, and isocratic and gradient retention results can be interconverted (e.g. prediction of isocratic retention based on gradient scouting runs). It is therefore important to correctly describe the retention relationship. In general, the retention behavior of a component is 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 [6–8].

All reversed-phase retention theories propose more complex models for an accurate evaluation of retention [4]. For example, the Bosch–Rosés team investigated the usage of the polarity index as a measure of the mobile phase elution strength. This polarity index exhibits a non-linear relationship with the volumetric solvent composition [9]. Vivó-Truyols et al. [10] demonstrated that the curved relationship proposed by Bosch and Rosés led to a better prediction of retention than the linear relationship and resulted in better modeling of isocratic and gradient retentions. Polynomial equations can also be used to describe retention behavior more accurately than linear models. Schoenmakers et al. [11] therefore proposed the following quadratic relationship between k and φ :

$$\ln(k) = \ln(k_w) + S_1 \varphi + S_2 \varphi^2 \quad (5)$$

The most important drawback of the polynomial models is that the solution to the fundamental gradient equation becomes very elaborate [4]. Schoenmakers et al. solved Eq. (3) to obtain an analytical expression for the gradient retention factor k_{eff} using the second-order polynomial (Eq. (5)) and a linear gradient profile, i.e. $\phi = \phi_0 + \beta \times t_s$ where β is the gradient slope, but the analytical solution consists of a complex combination of error and exponential functions [11], which limits its practicality. In theory however, the quadratic model can be used for gradient predictions, as the error function can be approximated by a polynomial. Eq. (3) can also be solved numerically, but this may lead to long calculation times when used for method optimization purpose [12]. Moreover, the extrapolation of a polynomial equation outside the measurement range often results in physically impossible values [4]. To avoid these problems, Neue and Kuss proposed the following empirical model [4]:

$$k = k'_w (1 + S_2 \varphi)^2 \exp \left(\frac{-S_1 \varphi}{1 + S_2 \varphi} \right) \quad (6)$$

$$\ln(k) = \ln(k'_w) + 2 \ln(1 + S_2 \varphi) - \frac{S_1 \varphi}{1 + S_2 \varphi} \quad (7)$$

where k'_w is the extrapolated intercept, S_1 is the slope and S_2 is the curvature coefficient. Note that if this curvature coefficient approaches zero, Eq. (6) turns into the classical linear model (Eq. (1)). Furthermore, no physical meaning is associated to the curvature coefficient. To experimentally determine the values of k'_w , S_1 and S_2 , three isocratic experiments with different ϕ_0 or three gradient runs with varying slopes need to be performed [4]. The

Neue-model also shows a strong similarity to the equation of Bosch and Rosés [4]. Solving Eq. (3) with Eq. (7) again gives the expression for the gradient retention factor:

This retention model can also easily be extended to multi-segmented gradients, as shown elsewhere. Even for very complex gradient profiles, the retention could be predicted very accurately (<2%).

In normal phase liquid chromatography, the retention is based on surface adsorption and this behavior can be described with the following equation (Greco et al. [13]):

$$\ln(k) = \ln(k_w) - S \ln(\varphi) \quad (9)$$

where φ is the fraction of strongest organic solvent. Solving Eq. (9) with Eq. (7) again gives the expression for the gradient retention factor [14]:

$$k = \frac{t_D}{t_0} + \frac{1}{t_0 \times \beta} \left[(S + 1) \times \beta \times (k_0 \times t_0 - t_D \times \varphi_0^S) + \varphi_0^{S+1} \right]^{1/(S+1)} - \frac{\varphi_0}{\beta} \quad (10)$$

Hydrophilic interaction chromatography (HILIC) is becoming more and more popular, for the determination of polar compounds, not retained in RPLC mode. In addition, it has been demonstrated that HILIC was also a viable alternative to RPLC for the analysis of ionizable compounds [15]. HILIC retention can be considered as a mixed-mode mechanism, combining hydrophilic partitioning of the analyte between the organic-rich mobile phase and the water enriched layer partially immobilized on the stationary phase, compounds adsorption through hydrogen bonds, and numerous types of possible electrostatic and ionic interactions [13]. The dependency of $\ln(k)$ on both φ and $\log(\varphi)$ in the HILIC separation mode generally does not follow a perfect linear relationship [16,17]. Based on these observations, Liang and coworkers proposed a mixed model to describe the retention behavior of polar compounds in HILIC [16,19]:

$$\ln(k) = \ln(k_w) + S_1 \varphi + S_2 \ln(\varphi) \quad (11)$$

where φ is the fraction of water. The quadratic model (Eq. (5)) has also been proposed in literature to describe the HILIC retention [13]. Greco et al. reported determination coefficients R^2 above 0.99 for 14 benzoic acids (on a zwitterionic stationary phase) using both Eqs. (5) and (11). In all cases, the determination coefficients improved with respect to Eqs. (1) and (9). The necessity to have one additional term in the regression was confirmed by calculating the P -values for the hypothesis that the coefficients S_2 in Eqs. (4) and (8) were different from zero [13]. The good fitting of Eq. (8) suggests the contribution of adsorption-like processes, besides hydrophilic partitioning. The predominant mechanism may depend on solute characteristics, nature of polar phases and mobile phase composition [19].

In the present study, the possibility of both isocratic and gradient retention modeling in the HILIC separation mode were investigated over a broad range of conditions. The isocratic retention relationships were not only evaluated using the determination coefficients (R^2 values) such as reported in the studies of Greco et al. and Jin et al. [13,16], but also using the predictive sum of squares (Q^2 values). For this purpose, a significant number of analytical conditions (column chemistries and mobile phase pH) were applied to several analytes possessing diverse physico-chemical properties. Furthermore, the possibilities of isocratic and gradient retention prediction based on a limited number of isocratic or gradient runs for MD

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