Retention modelling in hydrophilic interaction chromatography (HILIC)

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HILIC

Hydrophilic interaction chromatography (HILIC) has been used successfully in diverse application areas such as pharmaceutical discovery and development, drug metabolism and pharmacokinetics, biochemistry, forensics, agriculture/food chemistry, natural products and proteomics/metabolomics/glycomics. [1-3]

HILIC typically uses a polar stationary phase with MeCN-enriched aqueous mobile phases. This makes it a complementary separation technique to the ubiquitously employed reversed-phase chromatography (RPC). It is ideally suited to the analysis of polar and hydrophilic analytes (i.e., both neutral and ionized polar species with $\log D$ values, typically, <0) which are difficult to retain by RPC (Figure 1). It is a mass spectrometry-friendly technique and transferring a separation from RPC to HILIC can deliver a 10–1000 fold increase in sensitivity for hydrophilic analytes.
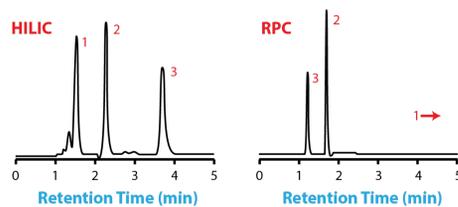

Figure 1: Illustrated separation of peptides under HILIC and RPC conditions.

The separation mode of HILIC is reported to be more complicated than RPC due to the multi-retention mechanisms that may contribute to the overall retention of analytes. Major HILIC interactions retention mechanisms are believed to be:

Partitioning (between the water-enriched adsorbed layer at the surface of the stationary phase and the water-deficient mobile phase)

Adsorption (i.e., hydrogen bonding interaction of neutral polar species and electrostatic interactions of ionized species—both attractive and repulsive)

The dominancy/proportion of each will depend on factors such as:
- Physicochemical properties of the stationary phase
- Analyte
- Type and composition of the mobile phase (i.e., type of solvents, buffer concentration, and pH)

HILIC encompasses a wide range of phases of diverse natures, which exhibit different retention and selectivity characteristics. Exploring differing stationary phases is, therefore, a major selectivity tool in proposed HILIC method development strategies. The downside, however, is that the chromatographer needs to have a good understanding of the nature of the phases in order to maximize the probability of obtaining the desired separation.

The literature contains numerous examples of the influence of pH, proportion of MeCN:water, buffer concentration, temperature, and stationary phase chemistry on the retention of various analytes; these findings have assisted in a recommendation of method development strategies for HILIC separations. [2] However, there have been surprisingly few reports on the accuracy of retention modelling in HILIC [4] and none to our knowledge have used commercially available retention modelling software.

Description of Investigation

In this work we set out to evaluate the accuracy of retention modelling using equation 1 (Figure 2) and peak shape in HILIC as a function of:

Mobile phase pH	Buffer concentration
Proportion of MeCN:water	Temperature

1) $k = (t_R - t_M)/t_M$

The following standard chromatography equations have been proposed to describe HILIC retention behavior in terms of mechanistic considerations:

2) $\log (k) = a_1 + b_1 x$ 3) $\log (k) = a_2 + b_2 x + c_2 x^2$

4) $\log (k) = a_3 + b_3 x + c_3 x^{1/3}$ 5) $\log (k) = a_4 + b_4 \log(x)$

6) $\log (k) = a_5 + b_5 x + c_5 \log(x)$ 7) $\log (k) = a_6 + b_6 \log(x) + c_6 (\log(x))^2$

8) $\log (k) = a_7 + b_7 x + c_7 \log(1 + d_7 x)$

The following equations were used to consider the effect of temperature

9) $\log (k) = a_8 + \dfrac{b_8}{T}$ 10) $\log (k) = a_9 + \dfrac{b_9}{T} + \dfrac{c_9}{T^2}$ 11) $\log (k) = a_{10} + \dfrac{b_{10}}{T} + c_{10}\log(T)$

The aim and novelty of this work was to evaluate the accuracy of retention modelling using a range of equations in HILIC as a function of mobile phase pH, buffer concentration, proportion of MeCN:water, and operating temperature on three common classes of HILIC stationary phase chemistries in a workflow similar to that used in RPC method development strategies (i.e., using a commercially available retention modelling software package).

Method

Instrumentation—Agilent 1290 Infinity UHPLC system controlled by ChemStation. Column oven model G1316C; photodiode array detector model G4212A equipped with a 1 μL/10 mm pathlength flow cell. A 12-position/13-port solvent selection valve, G1160A, was used to allow the automated selection of up to 12 different eluents from mobile phase line C. The diode array detector was set to monitor a wavelength of 254 nm with a reference at 360 nm. The data sampling rate was set at >0.063 min (0.13 s, 40 Hz).

Software

ACD/LC Simulator for modelling of HILIC separations

ACD/Percepta for determination of $\log D$ and pK_a

MODDE for factorial designs

SAS JMP for statistical evaluation to assess the relative importance of operating parameters (v10.0.2)

Compounds—54 pharmaceutically relevant compounds covering a diverse range of pK_a and $\log D$ values—a set of four were used for further analyses. [5]

Chromatography

The proprietary ACE prototype HILIC-A, HILIC-B, and HILIC-N columns (5 μm, 100 Å, 150 × 4.6 mm I.D. format) were supplied by Advanced Chromatography Technologies Ltd. (Aberdeen, Scotland, UK). The phases represent acidic, basic, and characters, respectively.

Peak width and symmetry were determined at half height as reported by ChemStation software. The first disturbance of the baseline on the injection of toluene was used as the dead time (tM) marker. A flow rate of 1.5 mL min^{-1}, 2 μL injection was used in all experiments, and a column temperature was maintained at 25˚C unless otherwise stated; analytes typically eluted within 20 min. ≥100 column volumes of the appropriate mobile phase were flushed through the column prior to commencing the testing or on changing the mobile phase conditions due to concern over the slow equilibration of HILIC phases. Chromatographic values reported are the average of duplicate injections.
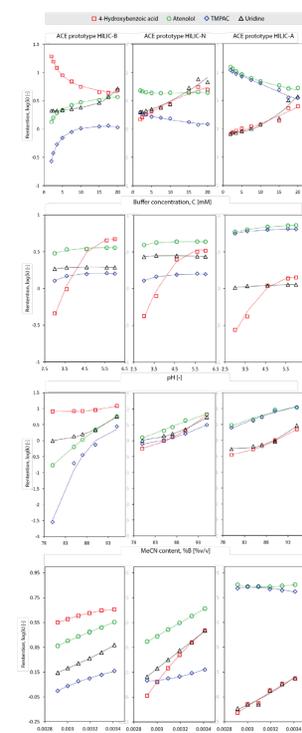
Results

Figure 2: Van't Hoff plots illustrate the retention behavior of 4 diverse compounds on different columns under different conditions.

Figure 3: Normalized chromatography showing selectivity differences using a mobile phase containing 10 mM ammonium formate w/w pH 3 (top) and pH 6 (bottom) in MeCN/water (90:10 v/v) for the ACE prototype stationary phases: HILIC-B, HILIC-N, HILIC-A. Peak assignment: 1 4-hydroxybenzoic acid; 2 theophylline; 3 uridine; 4 adenine; 5 nicotinic acid; 6 salbutamol; 7 tryptophan.

Fifty four compounds were chromatographed in 10 mM ammonium formate (w/w pH 3, 4.7, and 6) in MeCN/water (90:10 v/v), 230 and 254 nm. Four diverse compounds were selected. For these, the best prediction of retention behavior across the different conditions under consideration (pH, temperature, and proportion of MeCN in the mobile phase) was found to be provided by equations 3, 7, and 11 [5].
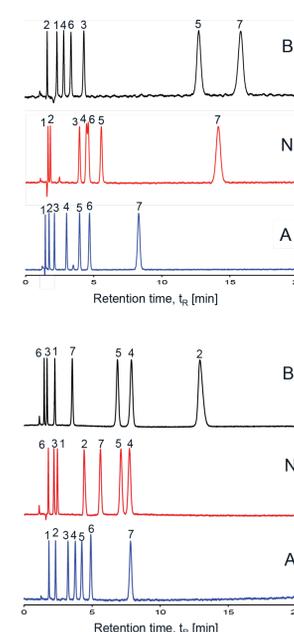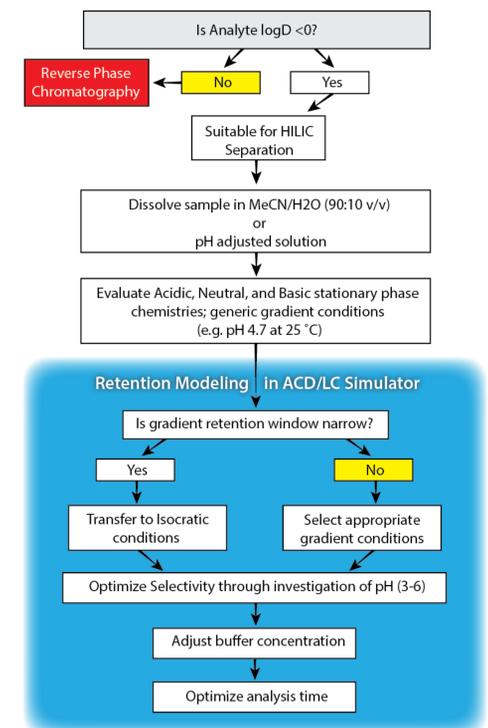

Figure 4: Proposed HILIC method development strategy using ACD/LC Simulator and using equations 3, 7, and 11 for retention modelling.
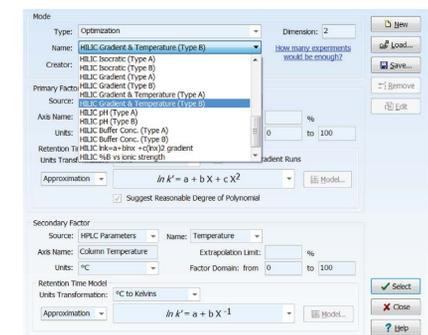

Figure 5: ACD/LC Simulator offers the capability of modeling chromatographic behavior for various types of chromatography

Conclusion

A method development strategy is proposed which consists of screening acidic, basic, and neutral columns (Figures 3 and 4). For the best column, retention is adjusted by optimization of the proportion of MeCN and pH. Finally, fine-tuning of the method is performed by adjusting the buffer concentration and temperature. As a result of these investigations, robust HILIC methodologies can be readily developed using reliable and robust *in silico* retention modelling (Figure 5).

A relative ranking of the importance of the retention and selectivity of HILIC operating parameters has been determined using statistical approaches.

For retention, the order of importance was observed to be:
Organic content > stationary phase > temperature ≈ mobile phase pH (i.e., pH 3–6 which mainly effects the ionization of the analyte) ≈ buffer concentration.

For selectivity, the order of importance was observed to be:
Nature of the stationary phase > mobile phase pH > buffer concentration > temperature > organic content.

References

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