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Hydrophilic-subtraction model for the characterization and comparison of hydrophilic interaction liquid chromatography columns

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ABSTRACT

Nowadays more and more hydrophilic interaction liquid chromatography (HILIC) columns with diverse functional groups have become commercially available, which pose a challenge to select an appropriate one. However, there is no universal model to provide guidance for selecting HILIC columns. To handle this problem, a retention model named "hydrophilic-subtraction model" was developed to characterize and compare HILIC columns. The hydrophilic-subtraction model, which was designed based on the widely recognized HILIC retention mechanisms including hydrophilic partitioning, hydrogen-bonding and electrostatic interactions, was established by the retention of 41 solutes with various properties on 8 representative HILIC columns. High correlation coefficients ($R^2 \geq 0.990$) and small standard deviations ($SD \leq 0.041$) indicated that this model correlated effectively the retention with solute descriptors and column parameters. To evaluate reliability of the model, the model was further applied to characterize 15 additional HILIC columns using 41 solutes. The results of multiple linear regression confirmed the significance of the model. The regression coefficients of the model were used to investigate retention mechanisms occurring in different chromatographic systems. Based on these regression coefficients, selectivities of HILIC stationary phases were exhibited intuitively by an angle graph and a spider diagram, which could be used as guidance for researchers to select appropriate columns for HILIC separation. Additionally, a rapid and convenient procedure was proposed for characterizing HILIC columns.

1. Introduction

Hydrophilic interaction liquid chromatography (HILIC) is an effective technique for the separation of polar compounds. The term "HILIC" was coined in 1990 to describe a chromatographic system that separates analytes between a polar stationary phase and an aqueous-organic mobile phase [1]. Initially, the main retention mechanism for HILIC, suggested by Alpert [1], was partitioning between the bulk mobile phase and a stagnant water layer enriched on the stationary phase surface. The formation of the stagnant water layer has been demonstrated using a ^{2}H nuclear magnetic resonance approach [2] and a chromatographic method via the injection of small hydrophobic solutes [3]. Recently, various studies have indicated that electrostatic interactions [4,5] and hydrogen-bonding interactions [6,7] were also the principal interactions contributing to the HILIC retention. Additionally, other interactions, such as dipole–dipole interactions and even hydrophobic interactions, could be involved. At the current state, a mixed-mode retention mechanism is widely accepted in the HILIC mode [8–10].

HILIC has been applied to the field of glycomics [11–13], peptidomics [14,15], metabolomics [16,17] and pharmaceuticals [18,19]. HILIC closes the application gap for analytes that are poorly retained on reversed-phase liquid chromatography (RPLC), insoluble in the mobile phase of normal-phase liquid chromatography (NPLC), or lack of charges for ion-exchange chromatography (IEC). In recent years, more and more commercial HILIC columns have become available, including bare silica, diol, amino, amide, saccharide and zwitterionic stationary phases [8,20,21]. Such diverse stationary phases provide researchers with more choices to meet separation, but pose a challenge to select a "right" one for separating compounds interested. Therefore, it is important to develop a method for the characterization, comparison and classification of these stationary phases.

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Many methods have been successfully developed to characterize reversed-phase columns [22], while few publications on characterization of HILIC columns have been reported. One method is based on the retention behaviors of different solutes to characterize HILIC columns. For instance, nucleoside derivatives, phenyl glucoside derivatives, xanthine derivatives, sodium *p*-toluenesulfonate and trimethylphenylammonium chloride were employed to characterize 14 HILIC columns in terms of hydrophilicity, charge effects, structural selectivity and separation efficiency [23]. Dinh et al. [24] selected 21 test solutes to probe the interaction mode of 22 HILIC columns and confirmed that the HILIC retention mechanisms were contributed by partitioning, electrostatic interactions and multipoint hydrogen bonding. However, the universally recognized HILIC test, just like RPLC test, has not been established. Another way is the application of quantitative structure-retention relationships (QSRRs). This method can relate the retention of a given solute to the physicochemical and structural parameters, allowing one to get some insights into the retention mechanisms. Based on this method, Quiming et al. developed a HILIC retention prediction model for ginsenosides on a polyamine-bonded stationary phase [25], and adrenoreceptor agonists and antagonists on unmodified silica [26], diol-bonded [27] and polyvinyl alcohol-bonded [28] stationary phases. Although Michel et al. [29,30], Buszewski et al. [31] and Jandera et al. [32] used linear solvation energy relationships (LSERs) to characterize and compare different HILIC stationary phases, this kind of characterization and comparison only focused on a specific class of analytes, such as peptides [29,31], pesticides [30], phenolic acids and flavone compounds [32]. Recently, two additional molecular descriptors on electrostatic interactions were introduced by Chirita et al. [33] to the LSER model for the investigation of the chromatographic behaviors of zwitterionic stationary phases. Using this modified LSER model, Schuster et al. [34,35] comparatively characterized 23 HILIC columns at pH 3.0 and 5.0. The resulting correlation coefficients (R^2) of LSER equations ranged from 0.487 to 0.854, indicating that LSER models had certain limitation to characterize the broad set of columns. The main reason was that the unadjusted solute descriptors of ionic species were computed based on their neutral forms. To the best of our knowledge, there was no universal model to characterize diverse HILIC columns when neutral, acidic and basic analytes were simultaneously concerned.

The aim of this work was to develop a retention model for the characterization and comparison of HILIC stationary phases. Furthermore, the retention mechanisms occurring in different types of HILIC stationary phases were probed. Comparison of column selectivity was also performed to present a simple graph for chromatographers to select an optimal one for separating compounds interested. In addition, a fast method for the characterization of HILIC columns was proposed.

2. Experimental

2.1. Reagents and chemicals

Acetonitrile (ACN) of HPLC grade was purchased from Merck (Darmstadt, Germany). Ammonium formate (NH_4FA) and formic acid (FA) were from Acros (Geel, Belgium). Water (H_2O) used in this study was purified by a Milli-Q water purification system (Billerica, MA, USA).

The probe solutes were of analytical grade and purchased from J&K Chemical (USA), Sigma–Aldrich (USA) and Aladdin Reagent Co. Ltd. (China) in Table 1 and their structures are showed in Fig. 1. All standard solutions of different solutes were prepared in ACN/H_2O (50/50, v/v) with the concentration of 1 mg/mL and stored at 4 °C before use. All HILIC stationary phases used in this study are presented in Table 2 and their structures are showed in Fig. 2.

2.2. Instrumentation

The HPLC system (Waters, MA, USA) consisted of an Alliance 2695 quaternary pump, an autosampler, a column oven and an ultraviolet (UV) detector. The Empower 3.0 workstation software was used to control the HPLC system and acquire the chromatographic data.

2.3. Chromatographic conditions

In order to compare column selectivity, the same mobile phase was used throughout the whole experiment. The mobile phase consisted of a mixture of ACN and buffer solution (85/15, v/v). The buffer solution contained 100 mM NH_4FA and was adjusted to pH 3.3 with FA. The corresponding ACN-buffer pH was 5.3. The buffer pH was measured before addition of organic solvent, while the ACN-buffer pH was measured after addition of organic solvent. The flow rate for all columns was set at 1 mL/min, apart from BEH Amide, BEH HILIC and PolySulfoethyl A at 0.1, 0.1 and 0.2 mL/min, respectively. The column temperature was maintained at 30 °C. The detection wavelength was 240 nm for melamine, codeine, dopamine, huperzine A and scopolamine, and 260 nm for the rest of the probe solutes. The injection volume was 1 μL to avoid the interference of the sample solvent. The retention time of each solute on each column was measured for three times. Toluene was used as the marker to determine the void time (t_o) for all columns.

2.4. Data analysis

The p*K* (including acidic and basic p*K*) and the octanol-water distribution coefficient ($\log D_{o/w}$) at pH 3.3 of the probe solutes were calculated using the ACD/I-Lab Web service. $\log D_{o/w}$ is the logarithm of the ratio of the equilibrium concentrations of the neutral species of a molecule in octanol to the un-ionized and ionized species in the water phase. It differs from $\log P_{o/w}$ in which the un-ionized species are considered. In this work, $\log D_{o/w}$ was used because parts of the probe solutes were ionizable at the working pH. The data calculation was performed on Microsoft excel 2010 and Origin 8.0.

3. Results and discussion

3.1. Theory

The retention mechanisms occurring in HILIC were investigated. In HILIC, the analytes are postulated to partition between the water-enriched layer on the stationary phase and the organic-rich mobile phase. The hydrophilic properties of analytes partly determine their retention behaviors in the HILIC mode. $\log D_{o/w}$ can be used to measure the hydrophilic properties of analytes. The relationship between $\log k$ and $\log D_{o/w}$ (pH 3.3) on 8 HILIC columns is showed in Fig. 3. $\log k$ is inversely correlated to $\log D_{o/w}$ (pH 3.3), indicating that the stronger retained analytes are those with lower $\log D_{o/w}$ values, namely the more hydrophilic analytes. It also means that hydrophilic partitioning plays a significant role in HILIC. Additionally, the correlation coefficients for all analytes on 8 columns range from 0.196 to 0.621, denoting that other interactions exist in the HILIC mode.

As reported in literatures, both partition and adsorption mechanisms are involved in the HILIC mode. Hydrophilic partitioning of the solute between the organic-rich mobile phase and a water-enriched layer immobilized on the stationary phase surface is the main retention mechanism [1,8]. Adsorption mechanisms,