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Evaluation and comparison of the kinetic performance of ultra-high performance liquid chromatography and high-performance liquid chromatography columns in hydrophilic interaction and reversed-phase liquid chromatography conditions

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ABSTRACT

An intrinsic performance comparison is made of the reduction in analysis time that can be obtained when switching from HPLC to UHPLC column formats in HILIC and reversed-phase conditions. A detailed overview of the packing characteristics of both stationary phase types is given first. It is demonstrated that HILIC columns demonstrate higher external porosity values than their reversed-phase counterparts resulting in lower flow resistance values. Column total porosity values determined from the elution time of a small marker molecule are shown to depend strongly on the composition of the mobile phase. To omit errors that might arise from an over- or underestimation of the column void time, all plate height and kinetic plot data are therefore expressed as a function of the interstitial velocity. Although only a limited number of columns are evaluated in this study, it is shown that the column efficiency of the HILIC columns is lower than that of their reversed-phase counterparts, at least for the compounds evaluated here. Despite this lower efficiency, the kinetic performance of both stationary phase types is similar, due to the much lower viscosity of the mobile phases typically used in HILIC conditions. Finally, it is demonstrated that a similar, yet slightly larger reduction in analysis time can be obtained when switching from HPLC column formats to UHPLC formats in HILIC compared to reversed-phase conditions.

1. Introduction

Hydrophilic interaction chromatography (HILIC) employs a polar stationary phase to retain polar analytes that are eluted by a mixture of an organic solvent (usually acetonitrile) and an aqueous buffer. The term HILIC was introduced by Alpert in 1990 [1] and has since then become a widespread alternative for reversed-phase liquid chromatography (RPLC) to achieve good retention and peak shapes for polar or ionizable analytes [2–5]. HILIC complements other areas of chromatography (RPLC and normal phase (NPLC)) and extends the range of separation options [6]. In addition, HILIC offers certain advantages over RPLC approaches [7,8], such as lower back pressures and improved desolvation with electrospray ionization (ESI) due to the large percentage of organic modifier in the mobile phase [9]. The lower back pressure allows using higher flow rates resulting in an increased sample throughput, longer columns and/or the use of sub-2 μm particle materials for improved resolution, while enhanced desolvation with ESI mass spectrometry results in better sensitivity and lower limits of detection [10]. Superior peak shapes can also be obtained for some compounds in HILIC compared to RPLC [7,11].

Nevertheless, HILIC also has some drawbacks [12], in particular regarding the complex and ill-understood retention mechanism. Unlike the well-known retention mechanism in RPLC [13,14], several retention mechanisms have been proposed for HILIC [6], such as analyte partitioning between a water-enriched layer on the surface of the stationary phase and a highly organic mobile phase [15], adsorption of the analyte onto the surface of the adsorbent [16,17], hydrogen bonding, electrostatic interactions, ion-exchange [18] making it quite difficult to predict the effect of a change in conditions on the separation outcome [18–20]. HILIC moreover does not offer the flexibility and applicability of RPLC and has been associated with longer re-equilibration times and problems with sample solubility.

The recent revival of HILIC has led to the introduction of a large number of stationary phases for HILIC that are available both in

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HPLC and UHPLC column formats. Columns with particle sizes of 3.0–3.5 μm are commonly used for HPLC applications. To enhance the analysis speed and efficiency of LC analyses, sub-2 μm particle size columns operated under ultra-high pressure conditions have been introduced for both RP and HILIC [21–23]. The gain in separation performance that can be obtained by switching from conventional HPLC to UHPLC conditions is well-quantified and has been extensively demonstrated for RPLC separations [24]. A number of publications also exist that focus on the comparison in separation performance between RP and HILIC columns, e.g., for the separation and quantification of ephedrines [11,25], the quantification of peptides [26] and, more fundamentally, the comparison of the intra-particle diffusivity between these two technologies [27]. Recently, McCalley et al. published a paper on the comparison of sub-2 μm column formats on the one hand and 3.5 μm particle column formats on the other hand, of bare silica HILIC and C_{18} RP columns from a fundamental point-of-view [28]. A comparison of reduced plate height curves of both column selectivities using basic and neutral solutes revealed smaller b-term coefficients for the HILIC columns, despite the larger solute diffusivity in the acetonitrile-rich mobile phases encountered in HILIC mode. This finding was attributed to the enhanced surface diffusion in the layer of acetonitrile on the surface of the RP stationary phases, which increases the b-term coefficient. Reduced c-term coefficients were found to be higher in HILIC than in RP, which was attributed to slower adsorption–desorption kinetics in HILIC. Kinetic plots moreover revealed that HILIC can present a significant improvement in performance when high efficiencies are required, resulting from the low viscosity of typical HILIC mobile phases and the low b-term coefficients.

No quantitative comparison of the gain in separation performance that can be obtained by switching from HPLC to UHPLC column formats in RP conditions on the one hand versus HILIC conditions on the other hand has, however, been made up to now. For this purpose, van Deemter and kinetic plot curves will be used in this study. A generic test mixture that can be applied to both HILIC and RP conditions under similar retention conditions will be defined.

2. Experimental

2.1. Chemicals and columns

Ammonium acetate and thymidine were obtained from Sigma-Aldrich (Steinheim, Germany), cytosine, guanine, thymine, guanosine, adenosine, adenine and uracil from Janssen chimica (Geel, Belgium). Milli-Q water was prepared using a Milli-Q gradient water purification system from Millipore (Bedford, MA, USA). HPLC grade acetonitrile (ACN) was purchased from Fisher Chemicals (Erembodegem, Belgium). HPLC grade tetrahydrofuran (THF) was from VWR (Leuven, Belgium) and dichloromethane (analytical grade) was from Fisher Chemicals. Glacial acetic acid was obtained from Merck (Darmstadt, Germany) and acenaphthene from Merck (Hohenbrunn, Germany). Details of the six evaluated columns are shown in Table 1.

Polystyrene standards with 12 different molecular weights ranging between 500 Da and 2000,000 Da were used for inverse size exclusion chromatography (ISEC) experiments and were also purchased from Sigma-Aldrich (Bornem, Belgium).

2.2. Apparatus

All experiments were performed on a UHPLC series 275 (Perkin Elmer, Massachusetts, USA) equipped with an autosampler, a binary pump, a forced-air oven and a variable wavelength detector with a detector cell of 2.6 μL. The maximum operating pressure of the system was 690 bar (10,000 psi). A stainless steel viper (125 μm I.D.) with heat exchanger (2 μL) (Dionex, Amsterdam, The Netherlands) was used between the injector and the inlet of the column. Between the outlet of the column and the detector, PEEK tubing with an internal diameter of 125 μm was used. The tubing was not altered during the experiments to avoid changing the extra-column volume. The overall system volume was determined to be 13 μL. Chromera software (Perkin Elmer) was used to control the UHPLC system and for data acquisition and analysis. The absorbance was measured at a wavelength of 254 nm. The column temperature was kept constant at 30 °C.

2.3. Sample preparation

Stock solutions of thymine, adenosine, uracil, adenosine, cytosine and thymidine were prepared in a concentration of 1000 ppm in H_2O. Guanosine and adenine were dissolved separately in a concentration of 1000 ppm in DMSO. Guanine (1000 ppm) was dissolved in 0.1 M NaOH solution. Thiourea and acenaphthene were individually dissolved in a concentration of 1000 ppm in water and acetonitrile, respectively. Fresh test samples were prepared daily by mixing and diluting stock solutions in the mobile phase for the evaluation of the column performance according to Table 1.

2.4. Methodology for the determination of column porosities

External porosity values (ε_e) were measured experimentally by inverse size exclusion chromatography (ISEC) using a set of twelve polystyrene standards (MW = 500; 2000; 3000; 10,000; 20,000; 30,000; 70,000; 150,000; 300,000; 700,000; 1000,000; 2000,000). Each standard was dissolved in a concentration of 0.1 mg/mL in pure tetrahydrofuran. The flow rate was set at 0.4 mL/min for the 2.1 mm I.D. HPLC columns, at 0.2 mL/min for the 2.1 mm I.D. UHPLC columns and at 0.8 mL/min for the 4.6 mm I.D. HPLC column to operate all columns at similar column pressures. Injection volumes were 1 μL and the detection wavelength was 254 nm. Each injection was performed in triplicate and the obtained retention volumes averaged. Retention volumes were corrected for the extra-column volume of the system. The elution volumes of the polystyrene standards were subsequently plotted against the cubic root of their molecular weight ($MW^{1/3}$). External porosities were derived by extrapolating the exclusion branches of the ISEC plots to $MW^{1/3} = 0$ [29].

Column dead volumes and total porosities were assessed from the elution time of an unretained marker (thiourea for RP columns and acenaphthene for HILIC columns) using different mixtures of acetonitrile and water. Additionally, pycnometric measurements were performed using THF and dichloromethane as pure liquids, with densities of $\rho_{THF} = 0.886\ g/cm^3$ and $\rho_{CH_2Cl_2} = 1.322\ g/cm^3$, respectively [29].

2.5. Methodology for column evaluation and theoretical comparison

For the theoretical evaluation of the column performance of RP and HILIC columns, a mobile phase consisting of ACN and ammonium acetate (NH_4Ac) buffer in varying ratios was used to keep the retention factor of the last eluting compound constant on all columns ($k''_{last} \sim 10$). The use of the zone retention factor k'' was preferred over the phase retention factor k' (based on the column void time t_0) for reasons that will be elaborated in Section 3.2. The zone retention factor k'' can be calculated as:

$$k'' = \frac{t_R \times L}{u_i} - 1 \quad (1)$$