Journal of Chromatography A, 1458 (2016) 82–89

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

The hydrophilicity vs. ion interaction selectivity plot revisited: The effect of mobile phase pH and buffer concentration on hydrophilic interaction liquid chromatography selectivity behavior

Chad D. Iverson, Xinyun Gu, Charles A. Lucy*

Department of Chemistry, University of Alberta, Gunning/Lemieux Chemistry Centre, Edmonton, Alberta T6G 2G2, Canada

ARTICLE INFO

Article history:
Received 17 March 2016
Received in revised form 16 June 2016
Accepted 18 June 2016
Available online 19 June 2016

Keywords:
Hydrophilic interaction liquid chromatography (HILIC)
Electrostatic interactions
Selectivity
Silanol
HPLC
Stationary phase

ABSTRACT

This work systematically investigates the selectivity changes on many HILIC phases from ${}_{w}{}^{w}$pH 3.7–6.8, at 5 and 25 mM buffer concentrations. Hydrophilicity ($k_{cytosine}/k_{uracil}$) vs. ion interaction (k_{BTMA}/k_{uracil}) selectivity plots developed by Ibrahim et al. (J. Chromatogr. A 1260 (2012) 126–131) are used to investigate the effect of mobile phase changes on the selectivity of 18 HILIC columns from various classes. "Selectivity change plots" focus on the change in hydrophilicity and ion interaction that the columns exhibit upon changing mobile phase conditions. In general, the selectivity behavior of most HILIC columns is dominated by silanol activity. Minimal changes in selectivity are observed upon changing pH between ${}_{w}{}^{w}$pH 5 and 6.8. However, a reduction in ionic interaction is observed when the buffer concentration is increased at ${}_{w}{}^{w}$pH $\geq$ 5.0 due to ionic shielding. Reduction of the ${}_{w}{}^{w}$pH to < 5.0 results in decreasing cation exchange activity due to silanol protonation. Under all eluent conditions, the majority of phases show little change in their hydrophilicity.

1. Introduction

Since Alpert [1] introduced the term hydrophilic interaction liquid chromatography (HILIC) in 1990, HILIC's popularity has steadily increased. This popularity is due to HILIC's ability to retain and resolve highly polar analytes that are difficult to separate by reversed phase chromatography, and to HILIC's compatibility with mass spectrometry [2–6]. HILIC has been the subject of many reviews [2,4,5,7–11] and several studies covering a wide range of HILIC stationary phases and/or mobile phase conditions [12–16]. HILIC has found utility in the analysis of small molecules such as metabolites [2,17,18], pharmaceuticals [19–24] and food chemicals [25–32], as well as larger biomolecules such as glycans [33–35] and peptides/proteins [36,37]. Today, many types of HILIC phases are commercially available, including bare silica, amine, amide, diol, and zwitterionic phases [8,38].

Retention in HILIC is due to a combination of the analyte partitioning into a surface water layer that forms on the surface of polar particles in the presence of an acetonitrile (ACN)- (or other suitable aqueous miscible polar aprotic organic solvent) rich mobile phase, and direct interactions, such as adsorption, with the stationary phase surface [7,39–41]. Secondary interactions such as dipole-dipole, hydrophilic interactions, hydrophobic interactions, and electrostatic interactions are responsible for the different selectivity classes of HILIC phases [12,15,42].

In 2011, Dinh et al. [15] characterized these interactions on 22 HILIC stationary phases using principal component analysis (PCA). Their approach successfully classified the behavior of the different phases, but was necessarily complex. Inspired by the two-dimensional RPLC selectivity plot developed by Neue and co-workers [43,44], Ibrahim et al. [45] developed several two-dimensional plots to characterize the selectivity of HILIC phases based on the relative retention of a subset of the test probes studied by Dinh et al. [15]. The objective of these two-dimensional plots was to frame HILIC selectivity in a format that was visually easier to comprehend.

One drawback of the selectivity plots of Dinh et al. [15] and Ibrahim et al. [45] are that they reflect HILIC selectivity under a single set of mobile phase conditions. Altering mobile phase conditions such as pH and buffer concentration can fine-tune HILIC selectivity by affecting water layer thickness, silanol activity and/or the ionization state of polar bonded groups [8–11,16,46–48].

Herein we investigate the effect of pH and buffer concentration on the selectivity behavior of many classes of HILIC phases.

* Corresponding author.
E-mail address: charles.lucy@ualberta.ca (C.A. Lucy).

http://dx.doi.org/10.1016/j.chroma.2016.06.061
0021-9673/

Specifically, we reconstruct the hydrophilicity vs. ion interaction selectivity plot of Ibrahim et al. [45] under three pH values and two buffer concentrations. We then focus on the changes in selectivity caused by the new mobile phase conditions.

2. Experimental

2.1. Apparatus

All experiments were performed on a Varian ProStar HPLC (Varian, Palo Alto, CA, USA) consisting of a 210 ProStar Pump and a ProStar 410 Autosampler fit with a 40 μL loop. This system was connected to a Knauer Smartline 2500 UV detector (Knauer-ASI, Franklin, MA, USA) with a 2 μL flow cell connected via fibre optic cables. The detector time constant was 0.1 s.

2.2. Chemicals and reagents

All solutions were prepared with Nanopure water (Barnstead, Dubuque, IA, USA). Cytosine, uracil, Optima-grade ACN, and HPLC-grade ammonium formate were from Sigma Aldrich (St. Louis, MO, USA). HPLC-grade ammonium acetate was from Fisher Scientific (Fair Lawn, NJ, USA). HCl was from Caledon Laboratory Chemicals (Caledon, ON, Canada) Benzyltrimethylammonium chloride (BTMA) was from Acros Organics (Fair Lawn, NJ, USA).

2.3. Tested columns and test probes

Table 1 lists the 21 columns evaluated in this study and their characteristics. Retention factors (k) for cytosine, uracil and BTMA were calculated as the average of three injections of the standards prepared in the buffered eluent. Toluene was used as the unretained dead time marker (t_0) for all HILIC phases. The standard deviations of the retention ratio measurements shown in Fig. 1 are smaller than the size of the marker symbol (RSD's typically <1%).

All analytes studied herein do not display acid/base character with the pH range studied. Thus, lower capacity buffers could be used, albeit with addition of buffer to the injected sample and extensive column equilibration (Section 2.4). With weak acid and base analytes, additional selectivity changes will result from protonation of the analyte, in which case adequate buffering capacity is essential.

2.4. Chromatographic conditions

The premixed mobile phases consisted of 80:20 v/v ACN:water containing ammonium acetate ($_w^w$pH 6.8 or 5.0) or ammonium formate ($_w^w$pH 3.7). Analytes (20 μL partial loop fill injection) were separated under ambient temperature (23 ± 3 °C) at 0.5 mL/min and detected at 254 nm. Prior to the first injection under each condition, each column was equilibrated with ≥45 column volumes of fresh eluent. The buffer strength quoted (5 or 25 mM) is that present *after* ACN addition. Buffers were prepared by titrating the appropriate amount of the above salts with HCl. The % ACN quoted in this work represents the volume of ACN relative to the total volume of the solvents including buffer and ACN. The pH for each aqueous component was measured prior to adding ACN ($_w^w$pH). Early studies suggested that the $_w^w$pH value is more representative of the surface aqueous layer in HILIC [42,48]. More recently, direct pH measurement of the buffered aqueous/organic mobile phase (after calibrating in aqueous buffers; $_w^s$pH) has been advocated [12,49,50]. As neither measure is truly an ideal descriptor of the mobile phase acidity, both the $_w^s$pH and $_w^w$pH are quoted.

Table 1
Characteristics of the stationary phases evaluated in this study.

#	Brand Name	Manufacturer	Support	Functionality	Particle size (μm)	Pore size (Å)	Surface area (m^2/g)	Column length (mm)	Column diameter (mm)
1	Zorbax HILIC Plus	Agilent	Silica	Underivatized	3.5	95	160	100	4.6
2	Chromolith Si	Merck	Silica monolith	Underivatized	N/A	130	300	100	4.6
3	Ascentis Express HILIC	Supelco	Fused core silica	Underivatized	2.7	90	150	100	4.6
4	Xbridge HILIC	Waters	Silica (BEH)	Underivatized	3.5	130	185	150	2.1
5	Cosmosil HILIC	Nacalai	Silica	Triazole	5	120	300	150	4.6
6	Ultra Amino	Restek	Silica	Aminopropyl	3	100	300	50	3.0
7	TSKgel NH_2-100	Tosoh Bioscience	Silica	Aminoalkyl	3	100	450	150	4.6
8	ZIC—HILIC	Merck	Silica	Polymeric sulfoalkylbetaine zwitterionic	5	100	180	150	4.6
9	ZIC-pHILIC	Merck	Porous polymer	Polymeric sulfoalkylbetaine zwitterionic	5	–	–	150	4.6
10	ZIC-cHILIC	Merck	Silica	Polymeric phosphorylcholine zwitterionic	3	100	180	150	4.6
11	TSKgel Amide-80	Tosoh Bioscience	Silica	Amide (polymeric carbamoyl)	5	80	450	100	4.6
12	AdvanceBio Glycan Mapping	Agilent	Poroshell silica	Proprietary amide	2.7	120	130	100	4.6
13	Fortis HILIC Diol	Fortis Techonolgies	Silica	Alkyl diol	3	100	380	100	4.6
14	Ascentis Express OH5	Supelco	Fused core silica	Penta hydroxy	2.7	90	150	100	4.6
15	FRULIC-N	AZYP LLC	Silica	High loaded propylcarbamate cyclofructan 6	5	100	440	150	4.6
16	PolyHYDROXYETHYL A	PolyLC	Silica	Poly(2-hydroxyethyl aspartamide)	5	200	188	150	4.6
17[a]	Ultra IBD	Restek	Silica	Proprietary polar alkyl embedded	5	100	300	100	4.6
18[a]	Ascentis Express F5	Supelco	Fused core silica	Pentafluorophenylpropyl	2.7	90	150	100	4.6
19[a]	Ultra PFP	Restek	Silica	Pentafluorophenylpropyl	5	100	300	100	4.6
20	PolySULFOETHYL A	PolyLC	Silica	Poly(2-sulfoethyl aspartamide)	5	200	188	150	4.6
21	Acclaim HILIC 10	Thermo Scientific	Silica	Proprietary neutral polar functionality	3	120	300	150	4.6

[a] Column was studied but excluded from plots as it exhibited reversed phase behavior under the conditions studied.