Hydrophilic Interaction Liquid Chromatography: Method Development Approaches

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Abstract

This poster summarizes the results of an investigation into hydrophilic interaction liquid chromatography (HILIC), focusing on the retention mechanisms of eleven stationary phases. Additionally to a column characterization study, the effect on retention of changing acetonitrile content, column temperature, mobile phase pH and buffer concentration was investigated.

Introduction

The ability to retain and separate polar and hydrophilic molecules can be very challenging during method development. HILIC has proved to be a viable alternative for the analysis of polar compounds. Although it has been demonstrated that the organic modifier/aqueous ratio is the predominant factor in providing the necessary separation selectivity in HILIC [1], the choice of stationary phase is also very important. Unfortunately, HILIC materials and the roles of their functional groups are not always well understood and users are often under the impression that HILIC columns are interchangeable.

In this poster we are therefore proposing a HILIC column selection scheme, derived from a characterization regime developed by Tanaka et al. [2] which will allow to classify the HILIC retention and selectivity properties for Thermo Scientific™ HILIC chemistries, according to the following :

- Selectivity for hydrophilic and hydrophobic groups, α (OH) and α (CH2);
- Configurational and positional isomer selectivity, α (V/A) and α (2dG/3dG);
- Anion and cation exchange properties, α (AX) and α (CX),
- Evaluation of the acidic-basic nature of the stationary phases, α (T_b/T_p)
- Retention factor for uridine, k uridine.

Additionally, we investigated the chromatographic parameters that have a major role in the HILIC selectivity for acidic and basic compounds:

- Organic solvent content;
- Buffer concentration;
- Mobile phase pH;
- Column temperature.

We will demonstrate how these various steps can help users during HILIC method development.

Methods

Instrumentation: Thermo Scientific™ Accela™ UHPLC
Columns as given in Table1.

Column Characterization Study

Mobile phase: Acetonitrile:ammonium acetate (20 mM in the aqueous portion, pH 4.7) (90:10, v/v).
Flow rate: 0.5 mL/min. UV: 254 nm. Inj Vol: 5 µL. Column T: 30 C.
Chromatographic test mixtures:

1. Toluene, uridine, 5-methyluridine, α (CH2).
2. Toluene, uridine, 2'-deoxyuridine, α (OH).
3. Toluene, adenosine, vidarabine, α (V/A).
4. Toluene, 2'-deoxyguanosine, 3'-deoxyguanosine, α(2dG/3dG).
5. Toluene, uracil, sodium p-toluenesulfonate, α (AX).
6. Toluene, uracil, N,N,N-trimethylphenylammonium chloride, α (CX).
7. Toluene, theobromine, theophylline, α (T_b/T_p).

Acetone was used as t_0 marker on Hypercarb.

Chromatographic Parameters Study

Mobile phases: Various mobile phases were prepared by mixing the desired volumes of acetonitrile and stock buffer solutions.
Flow rate: 1.0 mL/min. UV: 228 nm (for acid mixture) + 248 nm (for basic mixture). Inj Vol: 5 µL. Column T: 30 C.
Basic test mixture: 1. Uracil, 2. Adenosine, 3. Uridine, 4. Cytosine and 5. Cytidine
Acid test mixture: 1. Salicylamide, 2. Salicylic acid, 3. Aspirin and 4. 3,4 Dyhydroxyphenylacetic acid (dhpa)

TABLE 1. HILIC stationary phases characterized.

**** Nanopolymer silica hybrid * Porous Graphitic Carbon**

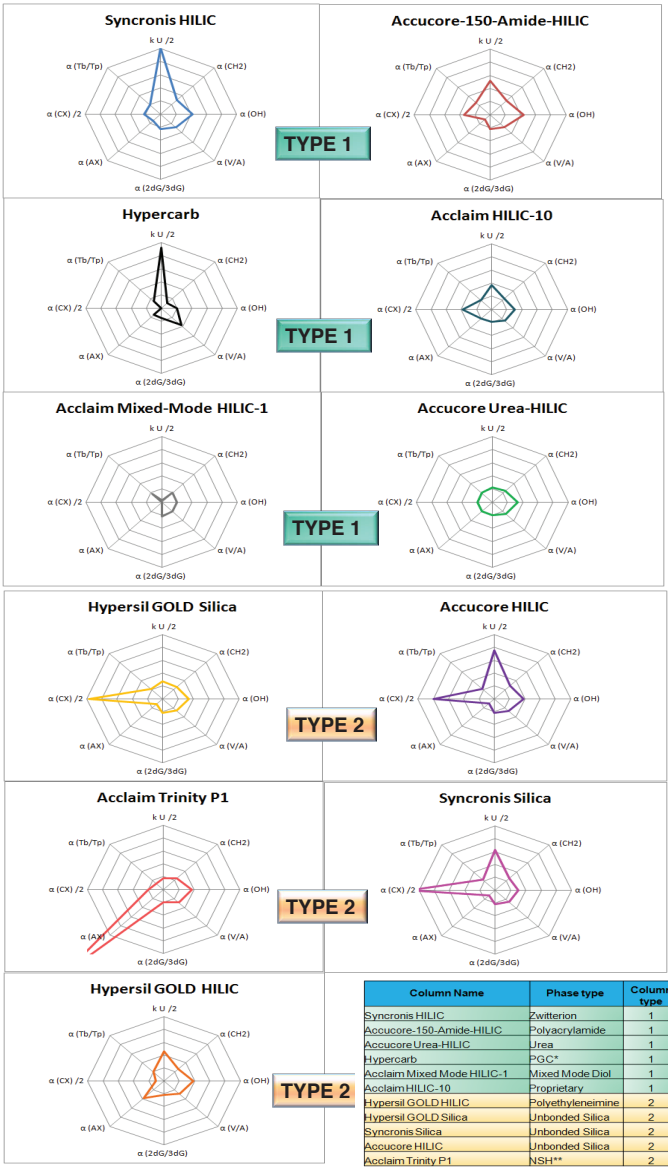
Column Name	Phase type	Column dimension (mm)	Surface area (m^2/g)	Pore size(Å)
Syncronis™ HILIC (5 µm)	Zwitterion	100 x 4.6	320	100
Hypersil GOLD™ HILIC (5 µm)	Polyethyleneimine	100 x 4.6	220	175
Hypersil GOLD™ Silica (5 µm)	Unbonded Silica	100 x 4.6	220	175
Hypersil GOLD™ Silica (1.9 µm)	Unbonded Silica	100 x 4.6	220	175
Syncronis™ Silica (5 µm)	Unbonded Silica	100 x 4.6	320	100
Accucore™ HILIC (2.6 µm)	Unbonded Silica	100 x 4.6	130	80
Acclaim™ Mixed Mode HILIC-1 (5 µm)	Mixed Mode Diol	150 x 4.6	300	120
Acclaim™ HILIC-10 (3 µm)	Proprietary	150 x 4.6	300	120
Acclaim™ Trinity P1 (3 µm)	NSH**	150 x 3.0	100	300
Accucore™ 150-Amide-HILIC (2.6 µm)	Polyacrylamide	100 x 2.1	80	150
Accucore™ Urea-HILIC (2.6 µm)	Urea	100 x 2.1	130	80
Hypercarb™ (5 µm)	PGC*	100 x 4.6	120	250

Results and Discussions

Characterization Study

The separation factors obtained from this study were displayed in radar plots, figure 1.

FIGURE 1. Radar plots for HILIC stationary phases



Column Name	Phase type	Column type
Syncronis HILIC	Zwitterion	1
Accucore-150-Amide-HILIC	Polyacrylamide	1
Accucore Urea-HILIC	Urea	1
Hypercarb	PGC*	1
Acclaim Mixed Mode HILIC-1	Mixed Mode Diol	1
Acclaim HILIC-10	Proprietary	1
Hypersil GOLD HILIC	Polyethyleneimine	2
Hypersil GOLD Silica	Unbonded Silica	2
Syncronis Silica	Unbonded Silica	2
Accucore HILIC	Unbonded Silica	2
Acclaim Trinity P1	NSH**	2

The degree of ion exchange interactions has the most significant influence on the radar plots shape, leading to the separation of the Thermo Scientific HILIC columns into two types:

TYPE 1
Columns with very little ion exchange interactions.
Syncronis HILIC, Accucore-150-Amide-HILIC and Hypercarb show greater retention and better selectivity for all of the test compounds.
Small hydrophilicity, but good selectivity are shown by Hypercarb, Accucore Urea-HILIC, Acclaim HILIC-10 and Acclaim Mixed-Mode HILIC-1.

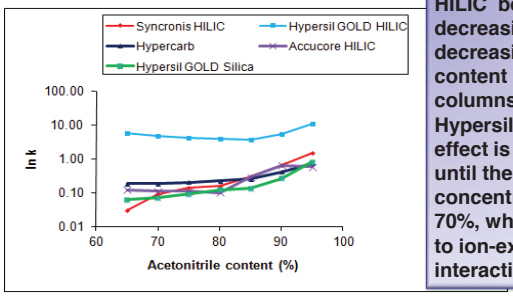
TYPE 2
Columns with ion exchange interactions.
The bare silica materials have strong cation exchange ability.
Accucore HILIC and Syncronis Silica have greater α (CH2), α (OH) and k uridine selectivity than Hypersil GOLD Silica, due to their different pore size, surface area and particle technology.
Acclaim Trinity P1 shows very strong anion exchange activity and so does (to a lesser extent) Hypersil GOLD HILIC.

Chromatographic Parameters Study

The effect of acetonitrile content on retention

The percentage of acetonitrile in the mobile phase was varied whilst keeping ammonium acetate concentration constant at 10 mM. The logarithmic capacity factors (ln k) for a model compound (salicylic acid) were plotted against the acetonitrile content (Figure 2).

FIGURE 2. Effect of acetonitrile content on the retention of salicylic acid.

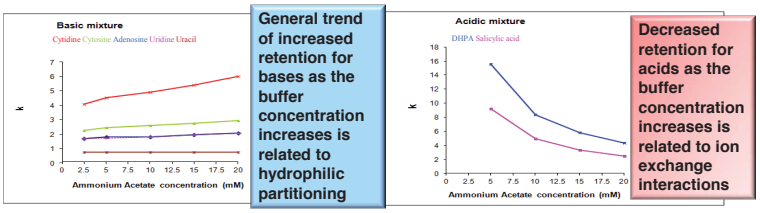


Salicylic acid shows HILIC behaviour of decreasing retention with decreasing acetonitrile content on all of the columns tested. On Hypersil GOLD HILIC this effect is not observed until the acetonitrile concentration reaches 70%, which could be due to ion-exchange interactions.

The effect of buffer concentration on retention

Separation of the two test mixtures was carried out using mobile phase: acetonitrile:water (90:10, v/v), containing ammonium acetate, whose concentration was varied from 2.5 to 20 mM. The logarithmic capacity factors (ln k) for the two model compounds were plotted against the buffer molar concentrations. Figure 3 shows the data relative to the basic and acid test mixtures obtained on Hypersil GOLD HILIC.

FIGURE 3. Effect of buffer concentration on retention of basic and acidic mixtures, for Hypersil GOLD HILIC.



General trend of increased retention for bases as the buffer concentration increases is related to hydrophilic partitioning

Decreased retention for acids as the buffer concentration increases is related to ion exchange interactions

The effect of mobile phase pH on retention

The pH of a 100 mM ammonium formate stock solution (pH~ 6.4) was adjusted with formic acid to pH 3.3, 4.0 and 4.8. Table 2 summarizes the retention data of the model compounds on four representative columns.

TABLE 2. Effect of buffer pH on retention of model compounds.

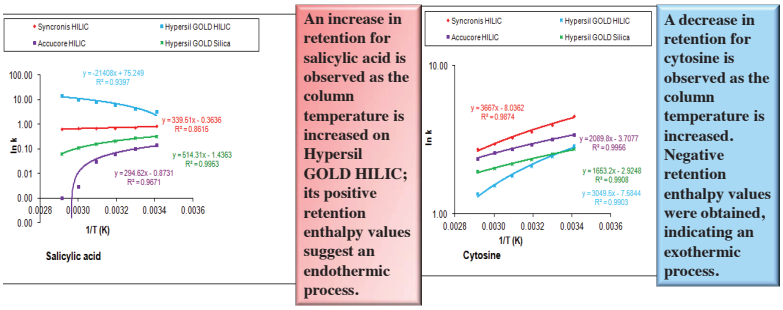
Column	pH	k salicylic acid	k aspirin	k cytosine	k cytidine
Accucore HILIC					
	3.3	0.21	0.43	3.07	3.49
	4.0	0.25	1.26	3.22	3.88
	4.8	0.24	1.61	3.12	3.80
	6.4	0.26	1.79	3.23	3.98
Hypersil GOLD HILIC	3.3	3.73	0.50	3.34	6.50
	4.0	3.89	0.54	3.42	6.56
	4.8	3.98	5.70	3.22	6.02
	6.4	4.18	5.90	3.26	6.11
Syncronis HILIC	3.3	1.11	0.97	4.88	8.40
	4.0	1.17	2.15	4.90	8.82
	4.8	1.06	2.60	4.62	8.13
	6.4	1.06	2.60	4.68	8.23
Hypersil GOLD Silica	3.3	0.40	0.61	2.41	2.55
	4.0	0.43	1.20	2.49	2.76
	4.8	0.42	1.43	2.41	2.66
	6.4	0.40	1.50	2.37	2.64

The retention is affected when the analyte ionisation state changes in the pH range considered. This is the case for aspirin, where its retention decreases with the buffer pH on the silica materials and for salicylic acid on Hypersil GOLD HILIC

The effect of column temperature on retention

The column temperature was varied from 20 to 70 ºC for the separation of salicylic acid and cytosine. Mobile phase: acetonitrile:water (90:10, v/v), containing 10 mM ammonium acetate. Figure 4 shows van't Hoff plots for salicylic acid and for cytosine.

FIGURE 4. Effect of column temperature on retention of salicylic acid and cytosine on four representative columns.

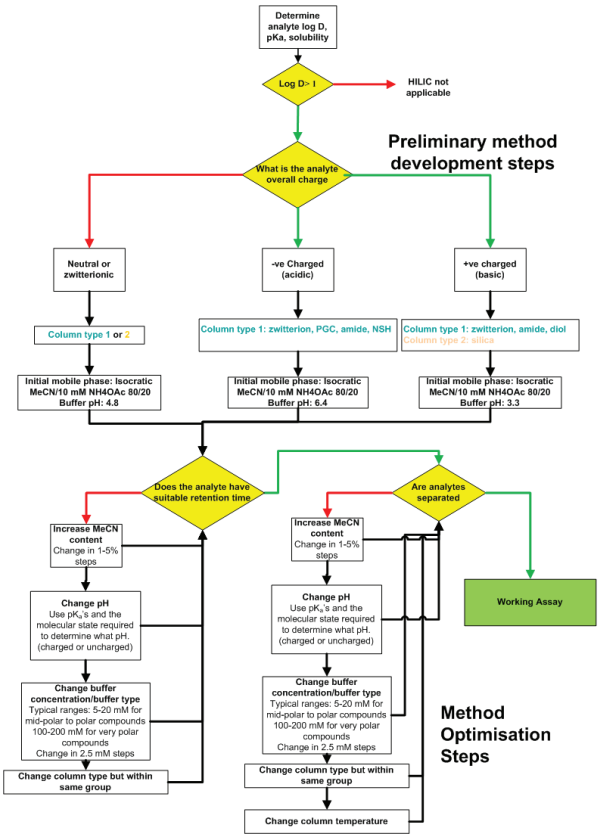


An increase in retention for salicylic acid is observed as the column temperature is increased on Hypersil GOLD HILIC; its positive retention enthalpy values suggest an endothermic process.

A decrease in retention for cytosine is observed as the column temperature is increased. Negative retention enthalpy values were obtained, indicating an exothermic process.

Conclusion

Based on our observations made here, we propose the following procedure to help during HILIC method development:



References

[1] Y. Guo, S. Gaiki, J. Chromatogr. A 1074 (2005) 71.
[2] Y. Kawachi, T. Ikegami, H. Takubo, Y. Ikegami, M. Miyamoto, N. Tanaka, J.Chromatogr. A 1218 (2011) 5903.

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