Evaluation of Core Shell Type HILIC Amide Column as First Choice

Tomoyasu Tsukamoto and Norikazu Nagae

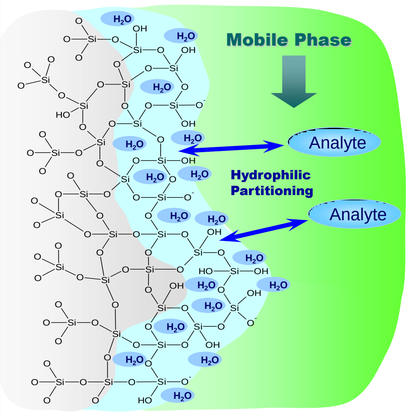
Innovations United, 300 East 57th Street, Suite 11J, New York, NY 10022
Tel: 212-204-0075 Fax: 414-313-6368 Email: info@innovationsunited.com

Abstract

Hydrophilic Interaction liquid Chromatography (HILIC) proposed by Alpert in 1990 has been applied for analysis of many hydrophilic compounds. Amide, diol, polyol, bare silica, ion exchange and zwitter ion phases have been used as a hydrophilic stationary phase along with an organic solvent rich mobile phase for HILIC. It is said that HILIC separation is achieved by partition between a mobile phase and a water rich layer on the stationary phase. Therefore, it is important for HILIC that a stable water rich layer can be formed on the stationary phase. We believe that a HILIC column as a first choice must have the following four characteristics; (1) the base material must be highly hydrophilic, (2) the functional group must have high water retention capacity and be densely bonded on the base material, resin or silica, (3) the functional group and base material must have a little electrostatic interaction, and (4) the spacer chain between the functional group and the base material must have a reduced hydrophobicity. In this study, we selected amide groups for a HILIC stationary phase due to non-electrostatics and high hydrophilicity. The amide type core shell particle was evaluated whether it satisfied the characteristics of the above requirements. The characteristics of SunShell HILIC-Amide used as a core shell type amide column was investigated using the evaluation method for HILIC reported by Kawachi and coworkers [1]. The bonding density of amide groups of SunShell HILIC-Amide was about 3.3 μmol/m^2, and the efficiency was achieved up to 220,000/m. This HILIC column showed not only hydrophilicity as high as PolyHYDROXYETHYL and Amide-80 column using the uridine test method but also very low anion and cation exchange interactions. In case of comparison of core shell type HILIC columns on separation of nucleobases, SunShell HILIC-Amide indicated the longest retention time. Finally analyses of glycosides, melamine and synthetic sweeteners were achieved.

[1]. Y. Kawachi, et al., J. Chromatogr., A 1218 (2011) 5903-5919.

HILIC Mechanism



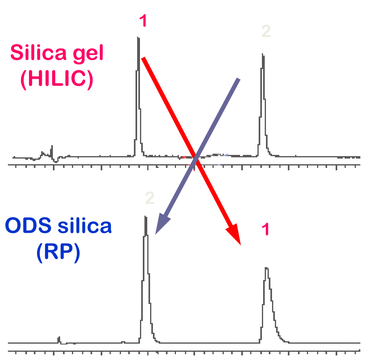


Figure 1. Image of HILIC separation mechanism

Character of HILIC

- Polar organic solvent such as methanol can be used.
- HILIC separation bases on partition of sample between mobile phase and water layer formed on stationary phase.
- The higher polarity of a compound, the longer their retention time.

Stationary Phase for HILIC

Hydrophilic interaction

Electrostatic interaction | **Ion exchange interaction** | **Hydrogen Bonding**

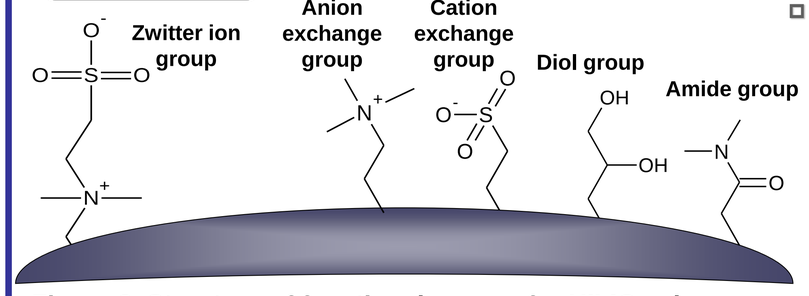
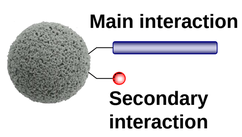


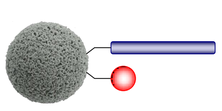
Figure 2. Structure of functional groups for HILIC columns

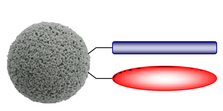
Comparing with RP column

- Main interaction is hydrophilic partition but secondary interaction also works relatively strong.
- All columns aren't end-capped.
- Electrostatic functional groups are often used for HILIC

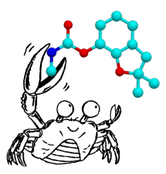
Work of the Secondary Interaction





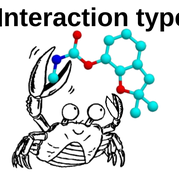


Existing adsorbent



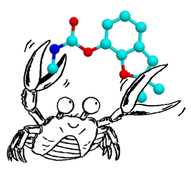
Residual silanol group

Controlled secondary Interaction type



A polar group embedded C18

Mix mode type



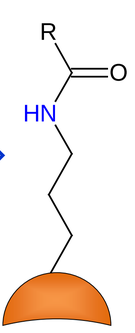
Mix mode column

- The secondary interaction was used to change the selectivity of the columns.
- The secondary interaction also was reduced by an end capping for C18.
- It is very difficult to lose the secondary interaction on adsorbents completely.

What is Needed as First Choice for HILIC

1. The base material must be highly hydrophilic.
2. The functional group must have high water retention capacity and be densely bonded on the base material, resin or silica.
3. The functional group and base material must have a little electrostatic interaction.
4. The spacer chain between the functional group and the base material should be as short as possible.

selected



Amide group

Evaluation of Hydrophilicity

Table 1. Comparison of hydrophilicity

	U	A	V
Column	*k* (U)	*k* (A)	*k* (V)
ZIC-HILIC (5 μm)	2.11	1.55	2.32
ZIC-HILIC (3.5 μm)	2.10	1.51	2.28
Nucleodur HILIC (3 μm)	2.20	2.33	3.40
Amide-80 (5 μm)	3.30	3.80	4.90
XBridge Amide (3.5 μm)	2.55	2.81	3.64
PolySULFOETHYL (3 μm)	1.58	1.15	1.39
PolyHYDROXYETHYL (3 μm)	3.92	3.75	4.93
CYCLOBOND I (5 μm)	0.70	1.36	1.68
LiChrospher Diol (5 μm)	1.50	2.50	3.30
Chromolith Si	0.31	0.73	0.85
HALO HILIC (2.7 μm)	0.64	1.59	1.87
COSMOSIL HILIC (5 μm)	1.60	2.20	3.00
Sugar-D (5 μm)	1.58	1.88	2.72
NH_2-MS (5 μm)	2.44	2.13	2.90
SunShell HILIC-Amide (2.6 μm)	2.93	3.55	4.84

Uridine (U) Vidaravine (V) Adenosine (A)

Conditions: Mobile phase: Acetonitrile/ammonium acetate buffer (20 mM, pH = 4.76) = 90:10 [v/v]Linear velocity; 1.0 mm/s, UV detection wave length; 254 nm, Column oven temperature; 30 ℃

Columns were evaluated their hydrophilicity using uridine

↓

The longer retention time of uridine, the higher hydrophilicity

HILIC columns have different hydrophilicity

↓

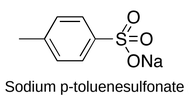
It is necessary to choose a HILIC column properly as well as a RP column.

SunSell HILIC-Amide showed the third highest hydrophilicity in these columns.

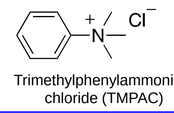
Evaluation of Ion Exchange

Table 2. Comparison of ion exchange ability

	U	SPTS		U	TMPAC	
Column	k (U) 20 mM	k (SPTS)	α(SPTS/U)	k (U) 20 mM	k (TMPAC)	α(TMPAC/U)
ZIC-HILIC (5 μm)	2.11	0.69	0.33	2.11	3.32	1.57
ZIC-HILIC (3.5 μm)	2.10	0.56	0.27	2.10	3.45	1.64
Nucleodur HILIC (3 μm)	2.20	1.13	0.51	2.20	3.14	1.43
Amide-80 (5 μm)	3.30	0.89	0.27	3.30	4.57	1.38
XBridge Amide (3.5 μm)	2.55	0.74	0.29	2.55	1.89	0.74
PolySULFOETHYL (3 μm)	1.58	0.25	0.16	1.58	1.38	0.87
PolyHYDROXYETHYL (3 μm)	3.92	0.87	0.22	3.92	3.34	0.85
CYCLOBOND I (5 μm)	0.70	3.32	4.73	0.70	0.45	0.63
LiChrospher Diol (5 μm)	1.50	0.95	0.63	1.50	1.73	1.16
Chromolith Si	0.31	0.06	0.19	0.31	5.25	16.94
HALO HILIC (2.7 μm)	0.64	0.20	0.31	0.64	9.03	14.11
COSMOSIL HILIC (5 μm)	1.60	1.28	0.80	1.60	0.78	0.49
Sugar-D (5 μm)	1.58	3.00	1.90	1.58	0.39	0.25
NH_2-MS (5 μm)	2.44	2.01	0.82	2.44	0.69	0.28
SunShell HILIC-Amide (2.6 μm)	2.93	1.29	0.48	2.93	1.18	0.44



Sodium p-toluenesulfonate (SPTS)



Trimethylphenylammonium chloride (TMPAC)

Ion exchange interaction was evaluated using the above ion compounds and uridine.

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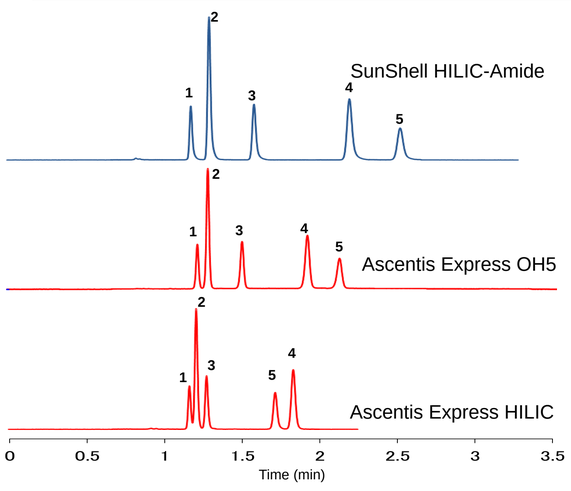
The smaller the separation factor of uridine and ion compounds, the lower ion exchange interaction.

The separation factors of both on SunShell HILIC-Amide were small. Consequently, SunSehll HILIC-Amide had low ion exchange interaction.

Amide vs other HILIC phase



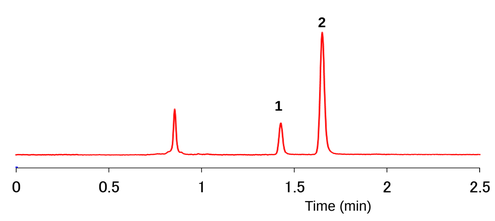
Column:
SunShell HILIC-Amide 2.6 μm, 100 x 4.6 mm
Ascentis Express OH5 2.7 μm, 100 x 4.6 mm
Ascentis Express HILIC 2.7 μm, 100 x 4.6 mm
Mobile 1.0 mL/min
Temperature: 40 ºC
Detection: UV@250 nm,
Sample : 1.Tymine, 2. Uracil, 3. Uridine, 4. Cytosine, 5. Cytidine

Figure 3. Comparison of three kinds of HILIC column

Comparing between SunShell HILIC-Amide and other HILIC columns • • •

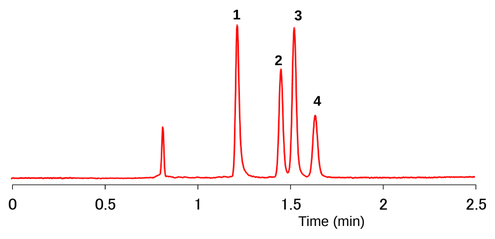
- **SunShell HILIC-Amide showed stronger retention than others.**
- **Amide showed different selectivity comparing with bare silica HILIC column.**

Applications of HILIC



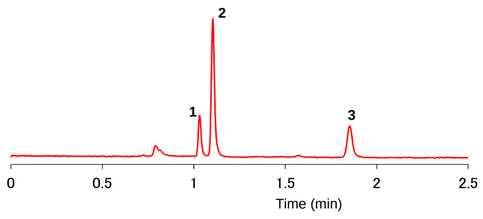
Column: SunShell HILIC-Amide 2.6 μm, 100 x 4.6 mm
Mobile phase: acetonitrile:5 mM phosphate Buffer (pH6.9) = 75:25
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Detection: UV@220 nm
Sample: 1. Cyanuric acid, 2. Melamine

Figure 4. Separation of melamine and cyanuric acid



Column: SunShell HILIC-Amide 2.6 μm, 100 x 4.6 mm,
Mobile phase: acetonitrile: 25 mM phosphate Ammonium (pH4.9) = 8:2
Flow rate: 1.0 mL/min
Temperature: Ambient
Detection: UV@215 nm
Sample: 1. Helicin, 2. Salicin, 3. Arbutin, 4. Rutin

Figure 5. Separation of glycosides



Column: SunShell HILIC-Amide 2.6 μm, 100 x 4.6 mm,
Mobile phase: acetonitrile:25 mM phosphate buffer (pH2.5) =8:2
Flow rate: 1.0 mL/min
Temperature: Ambient
Detection: UV@215 nm
Sample: 1. Aspartame, 2. Saccharin, 3. Acesulfame K

Figure 6. Separation of synthetic sweeteners

Conclusions

- **Sunsehll HILIC-Amide had high hydrophilicity and low ion exchange comparing with other HILIC columns.**
- **SunShell HILIC-Amide showed the longest retention time in all core shell type HILIC columns.**
- **Characteristics of a HILIC column depends on a functional group which shows different selectivity individually as well as a RP columns.**