Technical Note: No. SE1004

ChromaNik Technologies Inc.

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Characteristics of SunShell HILIC-Amide

HILIC (Hydrophilic Interaction Chromatography) named by Alpert at 1990 is a normal phase mode separation and also a technique to separate highly hydrophilic, ionic, and polar compounds which can't be separated in a reversed phase mode. Regarding separation of sugars, amino acids and nucleic acid bases, an aqueous mobile phase is needed on reversed phase chromatography, while a mixture of organic solvent and buffer solution with high concentration of an organic solvent in HILIC. The latter mobile phase gives better sensitivity for mass spectrometry detection because of the high concentration of an organic solvent. It is said that HILIC is more suitable than reversed phase chromatography for LC/MS. Many HILIC columns are available at the present time. These HILIC columns sometimes have not only a hydrophilic interaction but also an ion exchange interaction with the intention or no intention of doing. Furthermore someone is highly hydrophilic and the other one is lowly hydrophilic. So it is reported that separation is different among many HILIC columns

This report describes comparison of hydrophilicity, selectivity and position isomers 15 kinds of HILIC column using the parameter proposed by Dr Tohru Ikegami (1)

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

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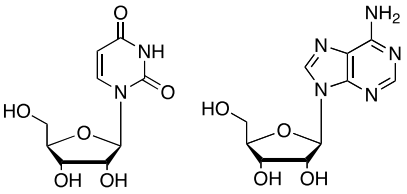
[[Comparison of hydrophilicity]]

Retention factor of Uridine (U) was used as a parameter of hydrophilicity . And retention factor of adenosine (A) and vidaravine (V).

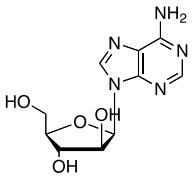
Condition: Mobile phase: Acetonitrile/ammonium acetate buffer (20 mM, pH = 4.76) = 90:10 [v/v],

Linear velocity: 1.0 mm/s, UV detection wave length: 254 nm, Column oven temperature: 30 ℃.

	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
TSKgel 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)　　Adenosine (A)



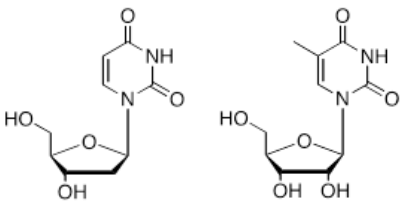
Vidaravine (V)

When uridine was used as a parameter of hydrophilicity, The most hydrophilic column was PolyHYDROXYETHYL, the second one was TSKgel Amide -80 and the third one was SunShell HILIC-Amide. In the case of comparison of retention factor of adenosine and vidaravine, those three columns showed almost same retention factor. The higher a hydrophilicity, the longer a retention time. So high hydrophilicity is better for a HILIC column.

[[Comparison of selectivity regarding exist of OH and CH_2]]

Selectivity regarding exist of OH and CH_2 was evaluated by separation factor of uridine and 2'-deoxyuridine and 5-methyluridine. Separation condition was as same as that in comparison of hydrophilicity.

	U	2dU	α(OH)	5mU	α(CH_2)
Column	*k* (U)	*k* (2dU)		*k* (5mU)	
ZIC-HILIC (5 μm)	2.11	1.04	2.03	1.26	1.67
ZIC-HILIC (3.5 μm)	2.10	1.02	2.07	1.23	1.71
Nucleodur HILIC (3 μm)	2.20	1.42	1.55	1.72	1.28
TSKgel Amide-80 (5 μm)	3.30	1.98	1.67	2.60	1.27
XBridge Amide (3.5 μm)	2.55	1.50	1.70	1.98	1.29
PolySULFOETHYL (3 μm)	1.58	0.74	2.13	1.07	1.48
PolyHYDROXYETHYL (3 μm)	3.92	2.04	1.92	2.88	1.36
CYCLOBOND I (5 μm)	0.70	0.58	1.21	0.62	1.13
LiChrospher Diol (5 μm)	1.50	1.10	1.36	1.30	1.15
Chromolith Si	0.31	0.31	1.00	0.28	1.12
HALO HILIC (2.7 μm)	0.64	0.60	1.08	0.56	1.16
COSMOSIL HILIC (5 μm)	1.60	1.00	1.60	1.40	1.14
Sugar-D (5 μm)	1.58	0.91	1.74	1.10	1.44
NH_2-MS (5 μm)	2.44	1.30	1.88	1.88	1.30
SunShell HILIC-Amide (2.6 μm)	2.93	1.65	1.78	2.29	1.28



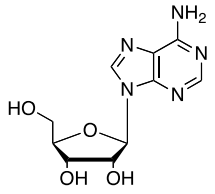
2'-deoxyuridine (2dU)　　5-methyl uridine (5mU)

Selectivity is much dependent upon a stationary phase. The zwitterion phase like ZIC-HILIC and Nucleodur showed high selectivity of exist of both OH and CH2, while the bare silica phase like Chromolith Si and Halo HILIC showed poor selectivity.

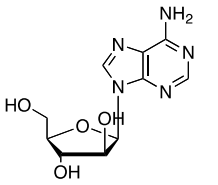
[[Comparison of regioisomer selectivity]]

Selectivity of regioisomer was evaluated by separation factor of adenosine and vidaravine. Separation condition was as same as that in comparison of hydrophilicity.

Column	A *k* (A)	V *k* (V)	α(V/A)
ZIC-HILIC (5 μm)	1.55	2.32	1.50
ZIC-HILIC (3.5 μm)	1.51	2.28	1.51
Nucleodur HILIC (3 μm)	2.33	3.40	1.46
TSKgel Amide-80 (5 μm)	3.80	4.90	1.29
XBridge Amide (3.5 μm)	2.81	3.64	1.30
PolySULFOETHYL (3 μm)	1.15	1.39	1.21
PolyHYDROXYETHYL (3 μm)	3.75	4.93	1.31
CYCLOBOND I (5 μm)	1.36	1.68	1.24
LiChrospher Diol (5 μm)	2.50	3.30	1.32
Chromolith Si	0.73	0.85	1.16
HALO HILIC (2.7 μm)	1.59	1.87	1.18
COSMOSIL HILIC (5 μm)	2.20	3.00	1.36
Sugar-D (5 μm)	1.88	2.72	1.45
NH_2-MS (5 μm)	2.13	2.90	1.36
SunShell HILIC-Amide (2.6 μm)	3.55	4.84	1.36



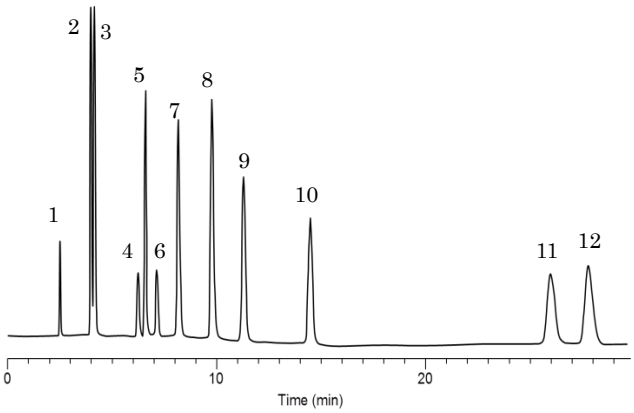
Adenosine（A）



Vidaravine（V）

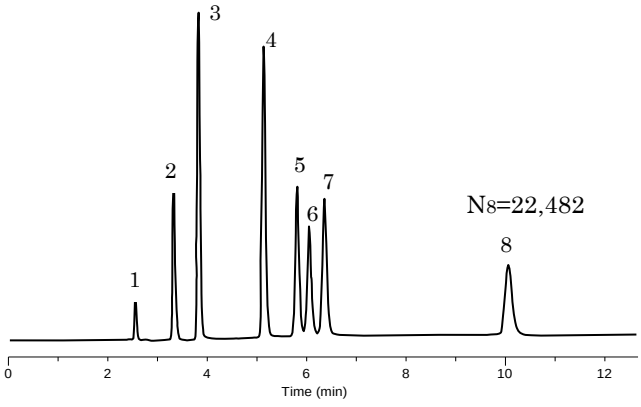
The zwitterion phase showed high selectivity of regioisomer although its retention was not long. SunShell HILIC-Amide showed the largest value for selectivity of regioisomer among the three phases which showed high retention.

[[Separation for each parameter]]



Column: SunShell HILIC-Amide (2.6 μm, 4.6 mm×100 mm),
Mobile phase: Acetonitrile/20 mM Ammonium acetate buffer (pH 4.8) = 90:10 (v/v),
Flow rate: 0.549 ml/min,
Back pressure: 3.9 MPa,
Temperature: 30 ℃,
Detection: UV@254 nm.
Samples: 1, Toluene; 2, Theobromine; 3, Theophylline;
4, 4-Nitrophenyl-β-D-glucopyranoside; 5, 2'-Deoxyuridine;
6, 4-Nitrophenyl α-D-glucopyranoside; 7, 5-Methyluridine;
8, Uridine; 9, Adenosine; 10, Vidarabine;
11, 3'-Deoxyguanosine; 12, 2'-Deoxyguanosine.

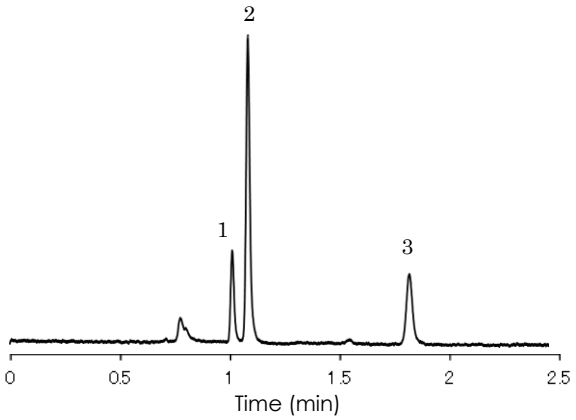
[[Separation of uridine and analog ofuridine]]



Column: SunShell HILIC-Amide (2.6 μm, 4.6 mm×100 mm),
Mobile phase: Acetonitrile/20 mM Ammonium acetate buffer (pH 4.8) = 90:10 (v/v),
Flow rate: 0.549 ml/min,
Back pressure: 3.9 MPa,
Temperature: 30 ℃,
Detection: UV@254 nm.
Samples: 1, Toluene; 2, Tegafur; 3, Trifluorothymidine;
4, 2'-Deoxy-2'-fluorouridine; 5, Thymidine;
6, 5'-Deoxy-5-fluorouridine; 7, 2'-Deoxy-5-fluorouridine;
8, 5-Fluorouridine.

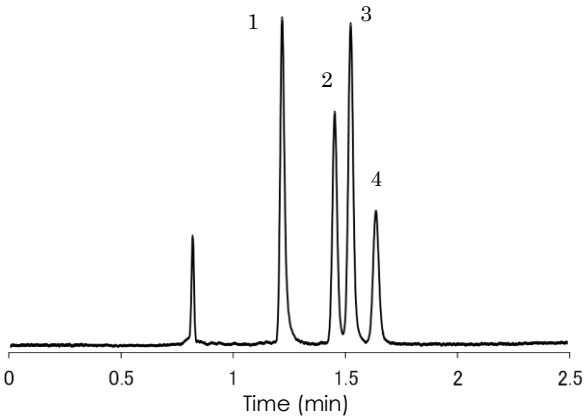
* SunShell HILIC-Amide showed the highest hydrophilicity of the phases bonded with a silane reagent. Furthermore a core shell particle made efficiency high. I deeply appreciate that Dr Tohru Ikegami offered all data showed in the above.

[[Separation for artificial sweeteners]]



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.

[[Separation for glycoside]]

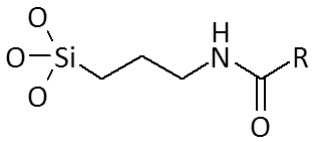


Column: SunShell HILIC-Amide, 2.6 µm 100 x 4.6 mm,
Mobile phase:
Acetonitrile/25 mM phosphate ammonium (pH4.9) =8:2
Flow rate: 1.0 mL/min ,
Temperature: Ambient,
Detection: UV@215 nm,
Sample:
1, Helicin; 2, Salicin; 3, Arbutin; 4, Rutin.

Characteristics of SunShell HILIC-Amide (USP L68)

	Core shell silica							
	Particle size	Pore diameter	Specific surface area	Carbon content	Bonded phase	End-capping	Maximum operating pressure	Available pH range
SunShell HILIC-Amide	2.6 µm	9 nm	150 m^2/g	3%	Amide	no	60 MPa or 8,570 psi	2 - 8

Stationary phase of HILIC-Amide



R: Hydrophilic group

Ordering information

	Inner diameter (mm)	2.1	3.0	4.6	
	Length (mm)	Catalog number	Catalog number	Catalog number	
SunShell HILIC-Amide	30	CH6931	CH6331	CH6431	
	50	CH6941	CH6341	CH6441	
	75	CH6951	CH6351	CH6451	
	100	CH6961	CH6361	CH6461	
	150	CH6971	CH6371	CH6471	

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