Evaluation of amide and C28 phases for separation of hydrophilic compounds

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Abstract

Hydrophilic Interaction liquid Chromatography (HILIC) proposed by Alpert in 1990 has been applied for analysis of 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. A polar group embedded C18 or a long alkyl chain phase such as C30 or C28 also have been used to separate hydrophilic compounds without change in retention using an aqueous mobile phase on a reversed-phase mode. The reason why these reversed-phases can showed no change in retention under an aqueous condition is that a low contact angle of water on the surface of the pore of these reversed-phase packing materials makes an aqueous mobile phase keep in the pore because pressure yielded by capillarity is less atmospheric pressure, so that retention doesn't change. Both HILIC stationary phases and reversed-phases have completely opposite characteristics each other. Therefore both HILIC and reversed-phase modes are useful for separation of hydrophilic compounds. It is important to understand separation behavior of each mode. In this study, an amide column and a C28 column were compared and evaluated to separate hydrophilic compounds. SunShell HILIC-Amide and Sunniest RP-AQUA (C28) and SunShell RP-AQUA (C28) were used to separate nucleobases, amino acids and hydrophilic vitamins. When nucleobases were separated on HILIC and reversed-phase modes using an amide column and a C28 column, each elution order of samples is said to be opposite. Only uracil, however, showed a specific elution. It was considered that the polarity of uracil under an organic solvent rich condition was different from that on water rich condition to be due to keto-enol tautomerization. LC/MS analysis of amino acids was achieved using C28 column and a mobile phase added 5 mM heptafluorobutyric acid under gradient elution conditions.

Collapse or Depermeating

C18 phases exhibit decreased and poorly reproducible retention under more than 98% aqueous conditions. This problem traditionally has been explained as being the result of ligand collapse. Nagae[1-3] ascertained, however, that the mobile phase was being expelled from the pores of the packing material.

When the surface of packing materials isn't wet by water, water used as a mobile phase expels from the pore of the packing material by capillarity. This is a reason why reproducibility in retention is low under 100% aqueous conditions. Reversely pressure around the packing materials makes water permeate into the pore of the packing material to overcome a force worked by capillarity.

1) N. Nagae, T. Enami and S. Doshi, LC/GC North America October 2002.
2) T. Enami and N. Nagae, American Laboratory October 2004.
3) T. Enami and N. Nagae, BUNSEKI KAGAKU, 53 (2004) 1309.

What does "Dewetting" mean?
A surface state changes from wetting to un-wetting? The surface of C18 is always un-wetting even if water exists in the pore, so that expression of "dewetting" is not scientific. Depermeating is a scientific expression!

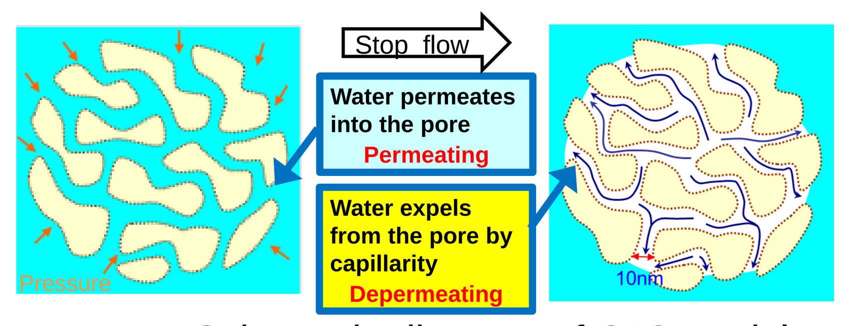


Figure. 6 Schematic diagram of C18 particle

Formula of Capillarity: $h = 2\gamma\cos\theta/(r\rho g)$
γ : Surface tension
ρ: Density of liquid

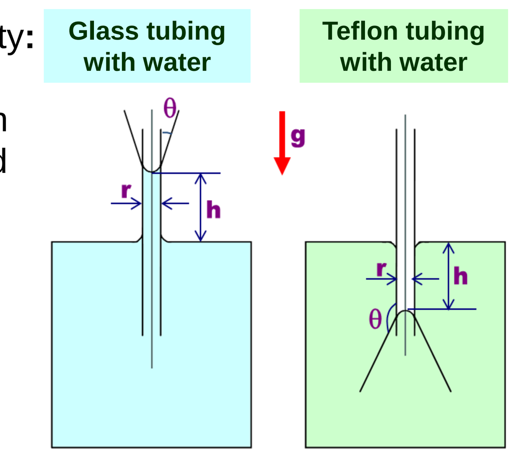
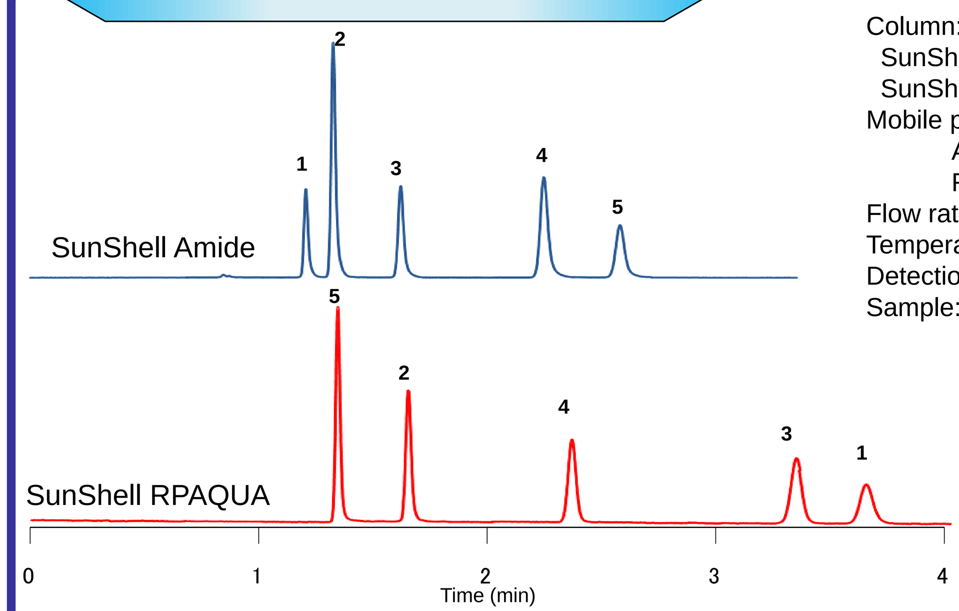


Figure. 7 Schematic of capillarity

Amide vs C28



Column:
SunShell Amide 2.6 μm, 100 x 4.6 mm
SunShell RPAqua 2.6 μm, 100 x 4.6 mm
Mobile phase:
Amide: Acetonitrile: 20 mM ammonium acetate(pH4.7) =8:2
RPAqua: 20 mM phosphate buffer(pH7.0)
Flow rate: 1.0 mL/min
Temperature: 40 °C
Detection: UV@250 nm
Sample: 1. Tymine, 2. Uracil, 3. Guanine, 4. Cytosine, 5. Cytidine

Figure. 1 Chromatograms of nucleobases

Comparing between amide and C28 column • • •

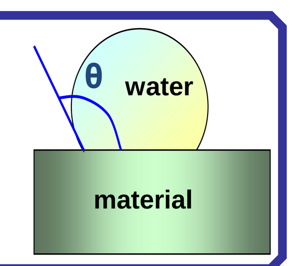
- **Each elution order of samples was almost opposite.**
- **Only elution order of uracil did not change**

Repellency and Hydrophobicity

Repellency

Water-shedding property

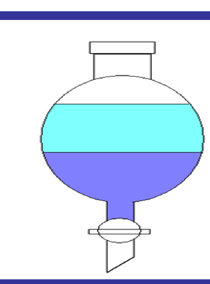
- Repellency is expressed as a contact angle of water on a material.
- The larger a contact angle, the stronger repellency, if the contact angle is more than 90 degree.



Hydrophobicity

Difficult to mixing with water

- Hydrophobicity is expressed as the ratio of concentrations of a compound between water and n-octanol using a mixture of both solvents.
- This value is well known as LogPow.



Repellency ∝̸ Hydrophobicity

- Repellency and hydrophobicity are independent each other.
- Those two parameters are out of proportional.
- When hydrophobicity is high, it doesn't mean that repellency is always high.
- Capillarity depends on a contact degree.

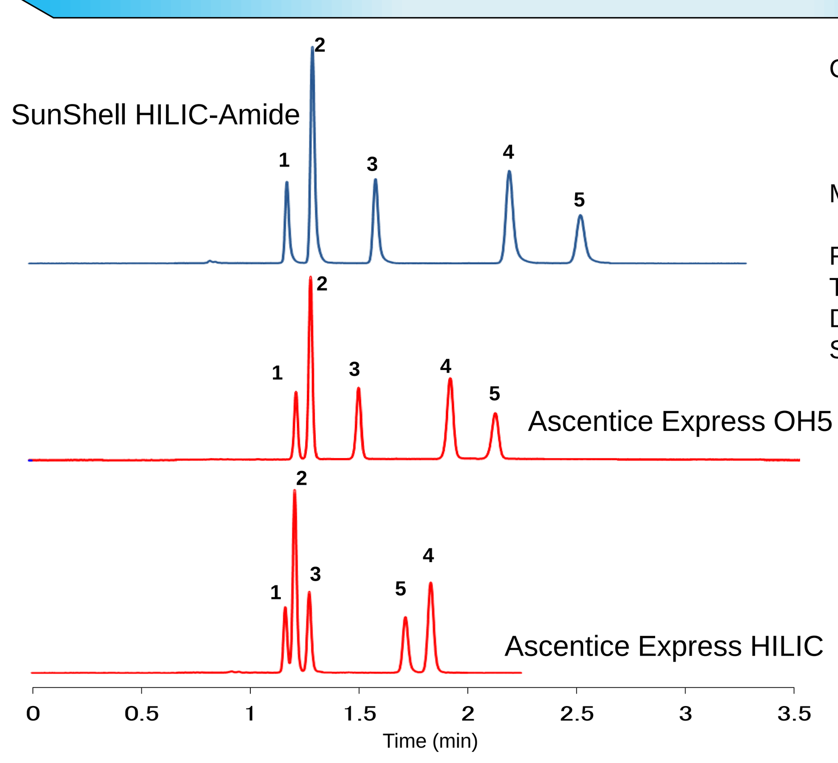
Table. 1 Physical property of each compounds

	Trifluoromethane	Octadecane (C18)	Octane (C8)	Octacosane (C28)
Contact angle(θ)	120°	126°	140°	108°
Partition coefficient (LogP)	0.64	9.18	5.18	14.09
Solibility(mg/L)	4090	0.006	0.66	8.84×10⁻¹⁰

T. Enami and N. Nagae, BUNSEKI KAGAKU, 53 (2004) 1309.

- **C28 has the smallest contact degree comparing with C18 and C8.**
- **In C28, the pressure for a mobile phase to go out from pore is smaller than atmospheric pressure.**
- **Aqueous mobile phase isn't expelled from the pore of C28 phase.**

Amide vs other HILIC phase



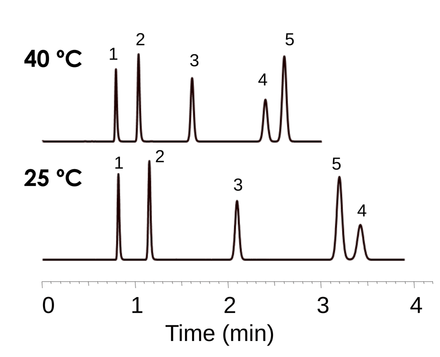
Column:
SunShell HILIC-Amide 2.6 μm, 100 x 4.6 mm
Ascentice Express OH5 2.6 μm, 100 x 4.6 mm
Ascentice Express HILIC 2.6 μm, 100 x 4.6 mm
Mobile phase: acetonitrile:
20 mM ammonium acetate(pH4.7) =8:2
Flow rate: 1.0 mL/min ,
Temperature: 40 °C
Detection: UV@250 nm,
Sample : 1.Tymine, 2. Uracil, 3. Guanine, 4. Cytosine, 5. Cytidine

Figure. 2 Different separation of nucleobases with HILIC columns

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

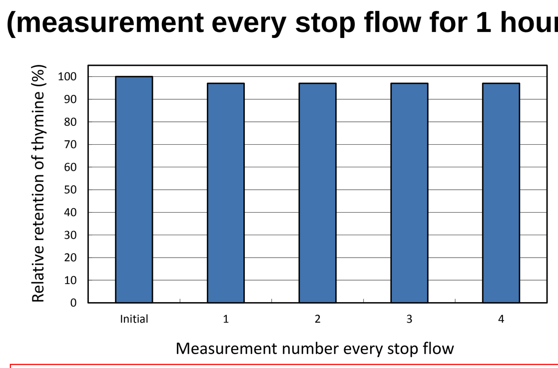
- **SunShell HILIC-Amide showed stronger retention than others.**
- **Amide showed different selectivity comparing with bare silica HILIC column.**

Stability of C28



Column: SunShell RP-Aqua 2.6 μm, 75 x 4.6 mm
Mobile phase: 10mM Phosphate buffer pH7.0
Flow rate: 1.0 mL/min
Temperature: 40 °C and 25 °C
Sample: 1. Cytosine, 2. Uracil, 3. Thymidine,
4. Uridine, 5. Thymine

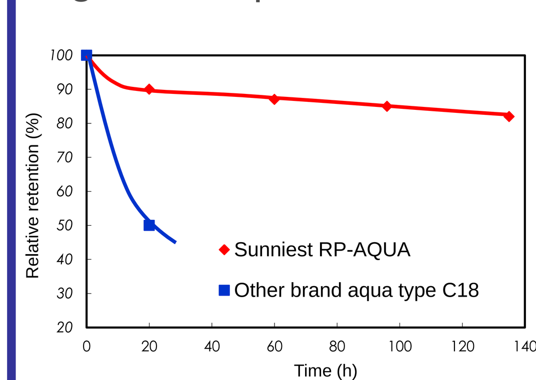
Change of retention of thymine at 40 °C (measurement every stop flow for 1 hour)



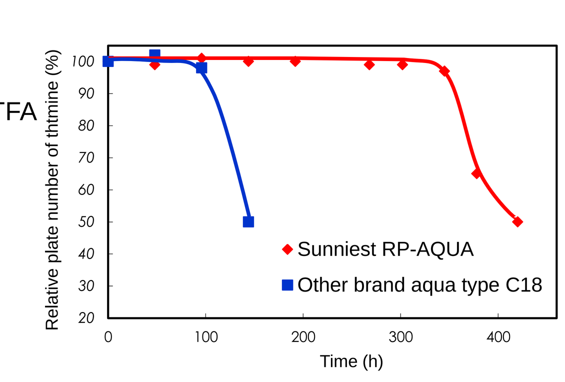
SunShell RP-Aqua showed more than 97% of reproducibility for retention using 100% aqueous buffer as a mobile phase.

Figure. 8 Separation of nucleobases and retention time stability

- **C28 column can be used under the 100% aqueous mobile phase condition.**
- **C28 column is useful column for separation of hydrophilic compounds.**
- **C28 column has better stability to acidic and basic conditions than a polar group embedded C18 column.**



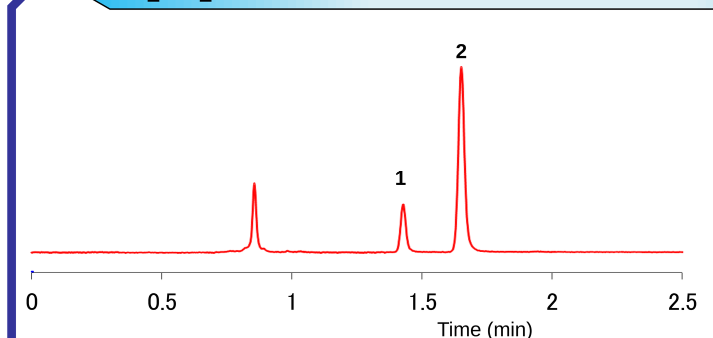
Test condition
Mobile phase: 0.5% TFA
Temperature: 60 °C



Test condition
Mobile phase:
20 mM phosphate buffer (pH 8.0)
Flow rate: 1.0 mL/min
Temperature: 40 °C

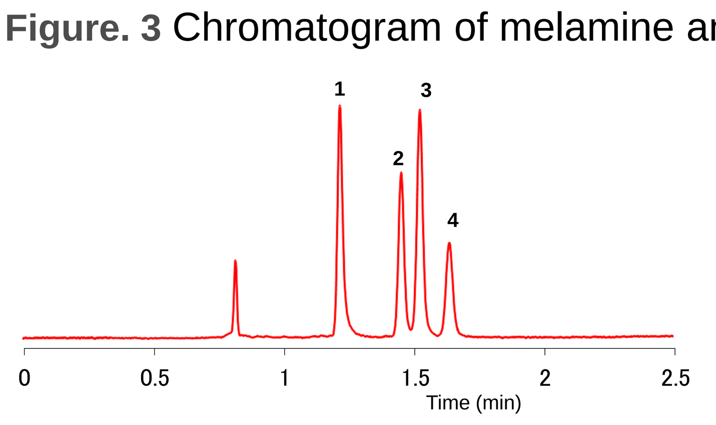
Figure. 9 Stability of C28 under acidic and basic conditions

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 °C
Detection: UV@220 nm
Sample: 1. Cyanuric acid, 2. Melamine

Figure. 3 Chromatogram 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

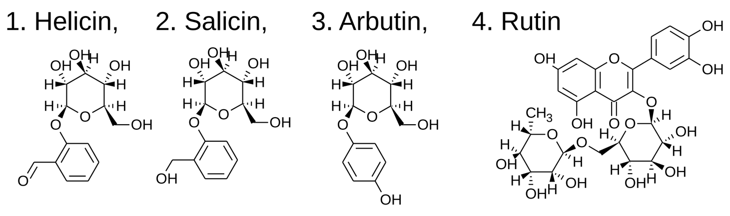
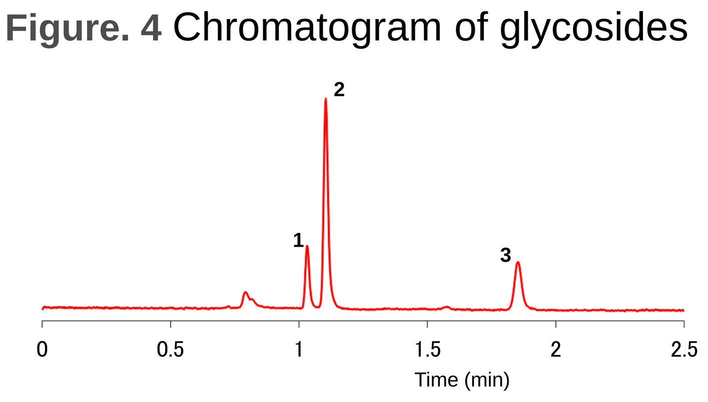


Figure. 4 Chromatogram of glycosides



Column: SunShell 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

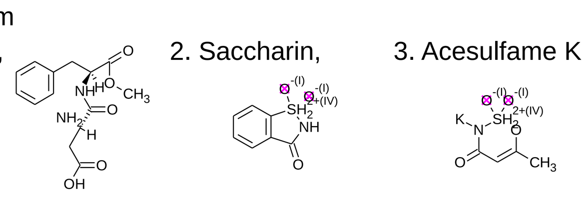
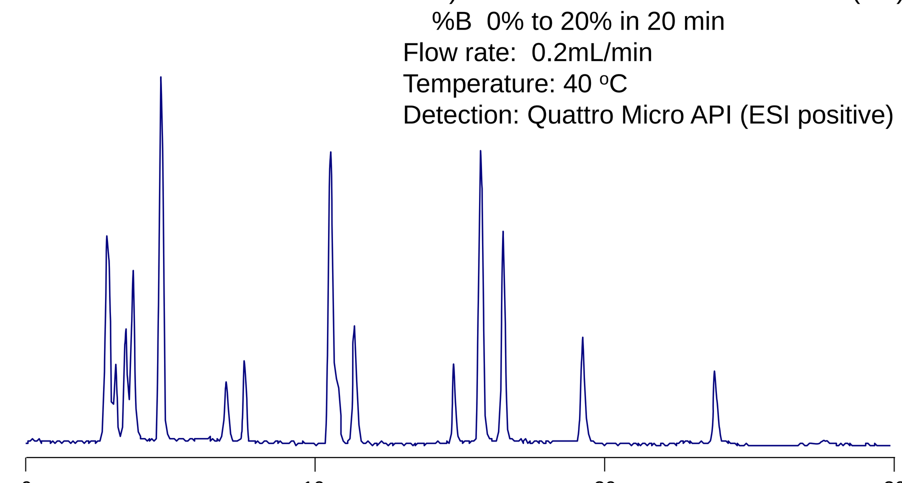


Figure. 5 Chromatogram of synthetic sweeteners

Separation of amino acids with C28

Column: Sunniest RP-AQUA 5 μm, 150 x 2.0 mm
Mobile phase:
A) 5mM HFBA (Heptafluorobutyric acid)
B) 5mM HFBA in Acetonitorile/water(9/1)
%B 0% to 20% in 20 min
Flow rate: 0.2mL/min
Temperature: 40 °C
Detection: Quattro Micro API (ESI positive) SIM



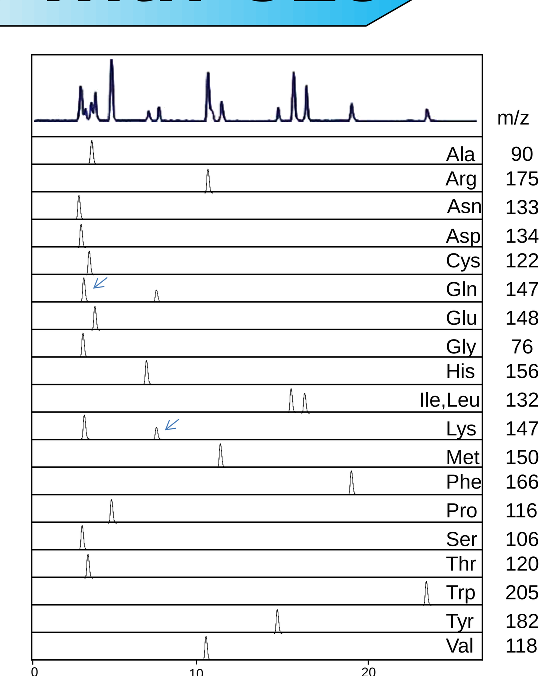


Figure. 10 Chromatogram of amino acids and MS detection

Conclusions

- Nucleobases were separated using an amide column and a C28 column, each elution order of samples is said to be opposite.
- Only uracil showed a specific elution. It was considered that the polarity of uracil under an organic solvent rich condition was different from that on water rich condition to be due to keto-enol tautomerization.
- LC/MS analysis of amino acids was achieved using C28 column and a mobile phase added 5 mM heptafluorobutyric acid under gradient elution conditions.
- Both amide and C28 column were useful for analysis of hydrophilic compounds