Effective Separation of Hydrophilic Compounds Using Hydrosphere C18 for High Performance Liquid Chromatography

Naohiro Kuriyama*, Noriko Shoji,
Saoko Nozawa and Taeko Nakajima

YMC CO., LTD.

Kyoto, Japan

Abstract

Silica-based packing materials for high performance liquid chromatography (HPLC) are most commonly used for the effective separations of a large number of organic compounds. Although these packing materials are well suited for analyses of neutral compounds, many hydrophilic compounds such as biomolecules or natural compounds including basic pharmaceuticals, cannot be retained enough and often tail on conventional packing materials.

To solve these problems we have developed Hydrosphere C18, a reversed-phase packing material for analytical HPLC. Hydrosphere C18 possesses a hydrophilic surface, produced from a newly developed generation of ultrapure silica with minimal trace metals. The balanced hydrophilic/hydrophobic nature of this packing material provides strong retention of hydrophilic compounds even under 100% aqueous conditions. A special endcapping procedure utilizing Lewis acid- Lewis base chemistry was applied for the endcapping process instead of conventional silylation methods to minimize the effect of residual silanols.

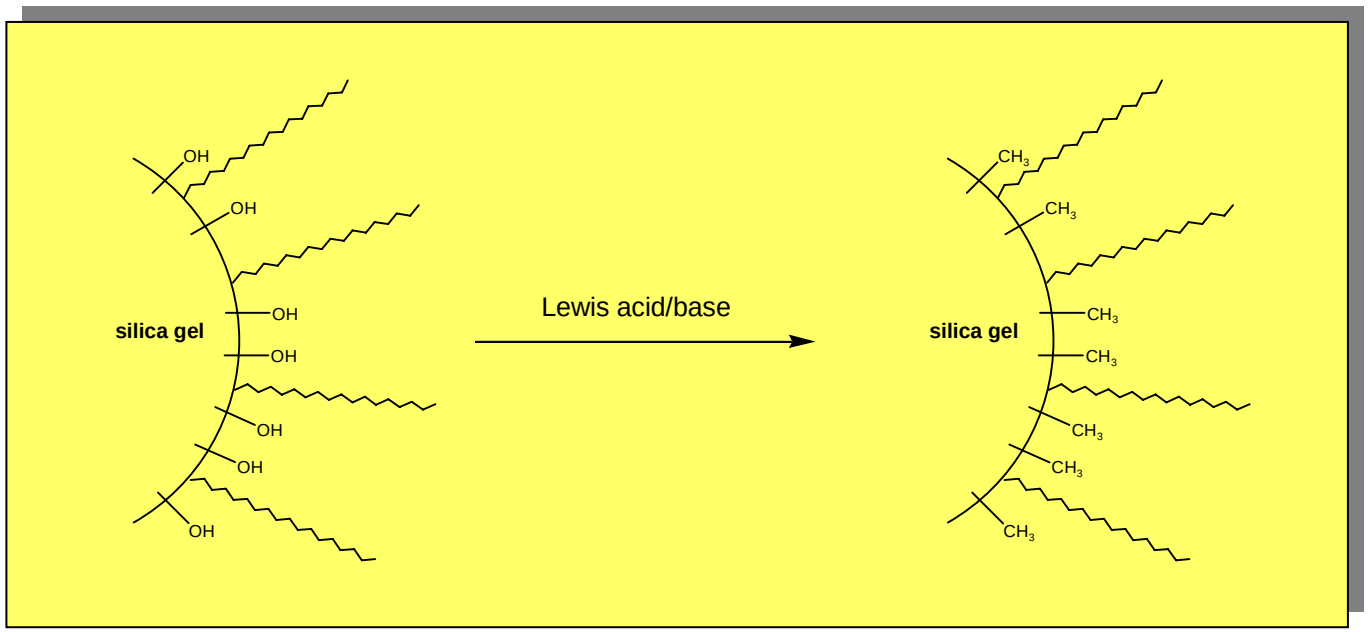
In this presentation we will show the separation characteristics of Hydrosphere C18 for reversed-phase HPLC. Use of Hydrosphere C18 allows not only the strong retention of hydrophilic compounds, but also the successful separation of complex mixtures including basic compounds.

YMC SEPARATION TECHNOLOGY

YMC *Pro* Series of ODS Columns

	Hydrosphere C18	Pro C18	Pro C18 RS
Silica	ultra pure	ultra pure	ultra pure
Particle size	3,5μm	3,5μm	5μm
Pore size	120A	120A	80A
Carbon content	12%	16%	22%
Bonded phase	monomeric C18	monomeric C18	polymeric C18
Endcapping	completely endcapped	completely endcapped	completely endcapped
Features	hydrophilic surface	excellent reproducibility	high durability

Unique Endcapping Procedure

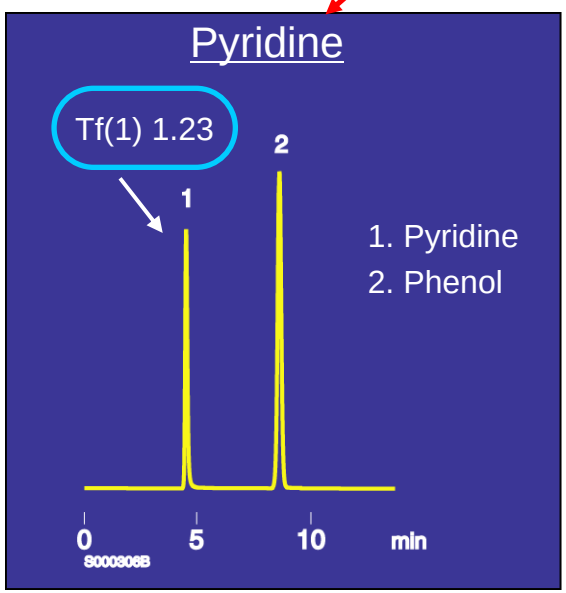


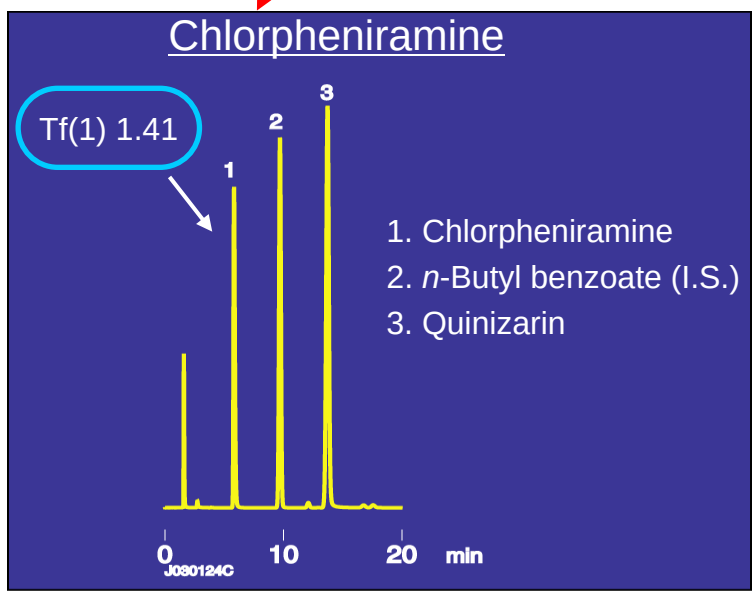
Advanced endcapping technology using Lewis acid/base reaction commonly applied for Pro series of ODS columns results in good peak shapes even for basic compounds that often elute with poor tailing shapes on competitive columns

Peak Symmetry of Basic Compounds
Pro series

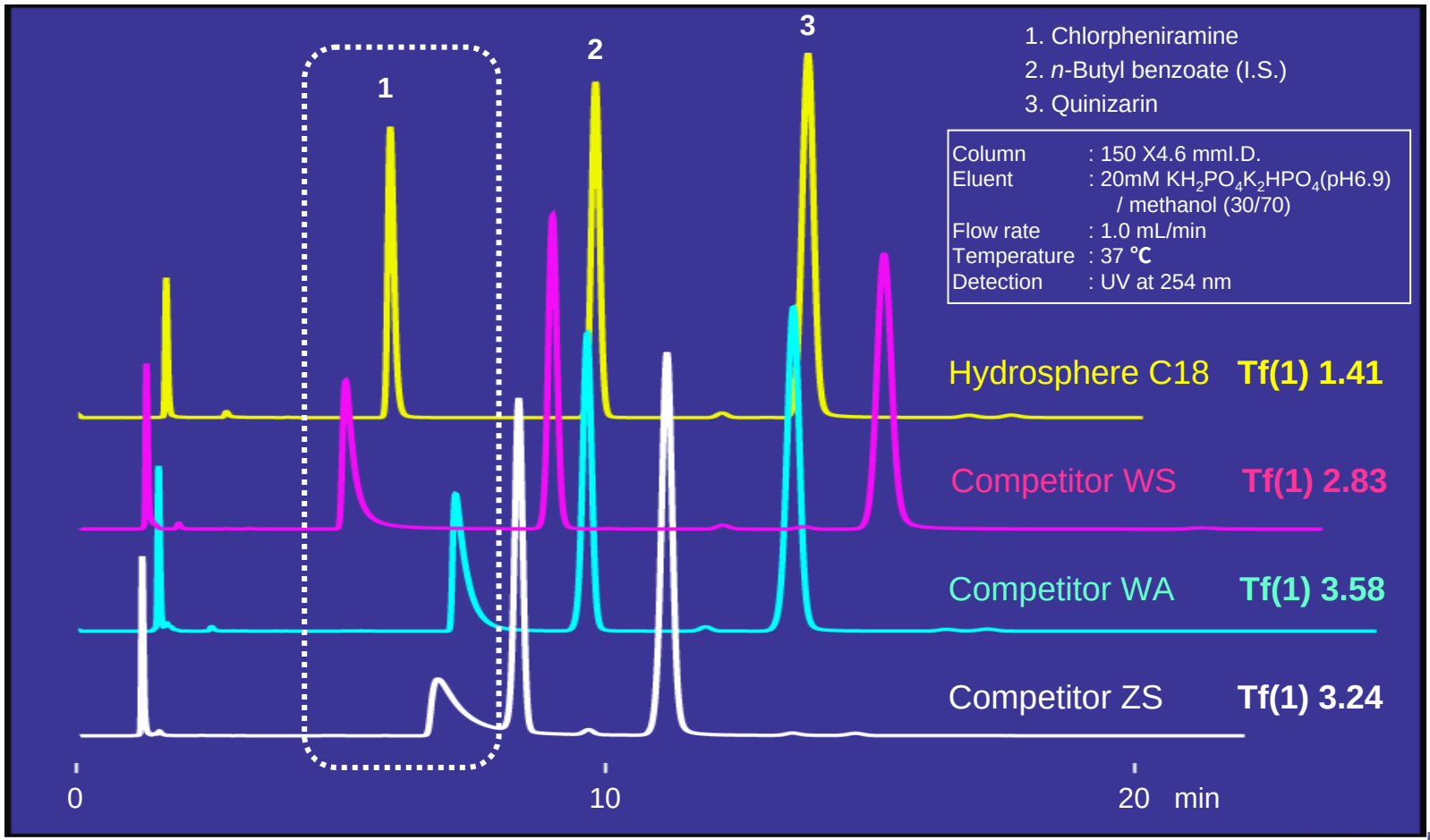
	Pyridine	Chlorpheniramine
Hydrosphere C18	○	○
Pro C18	○	○
Pro C18 RS	○	○

○：Tf≦1.5
△：1.5<Tf≦2.0
×：Tf>2.0

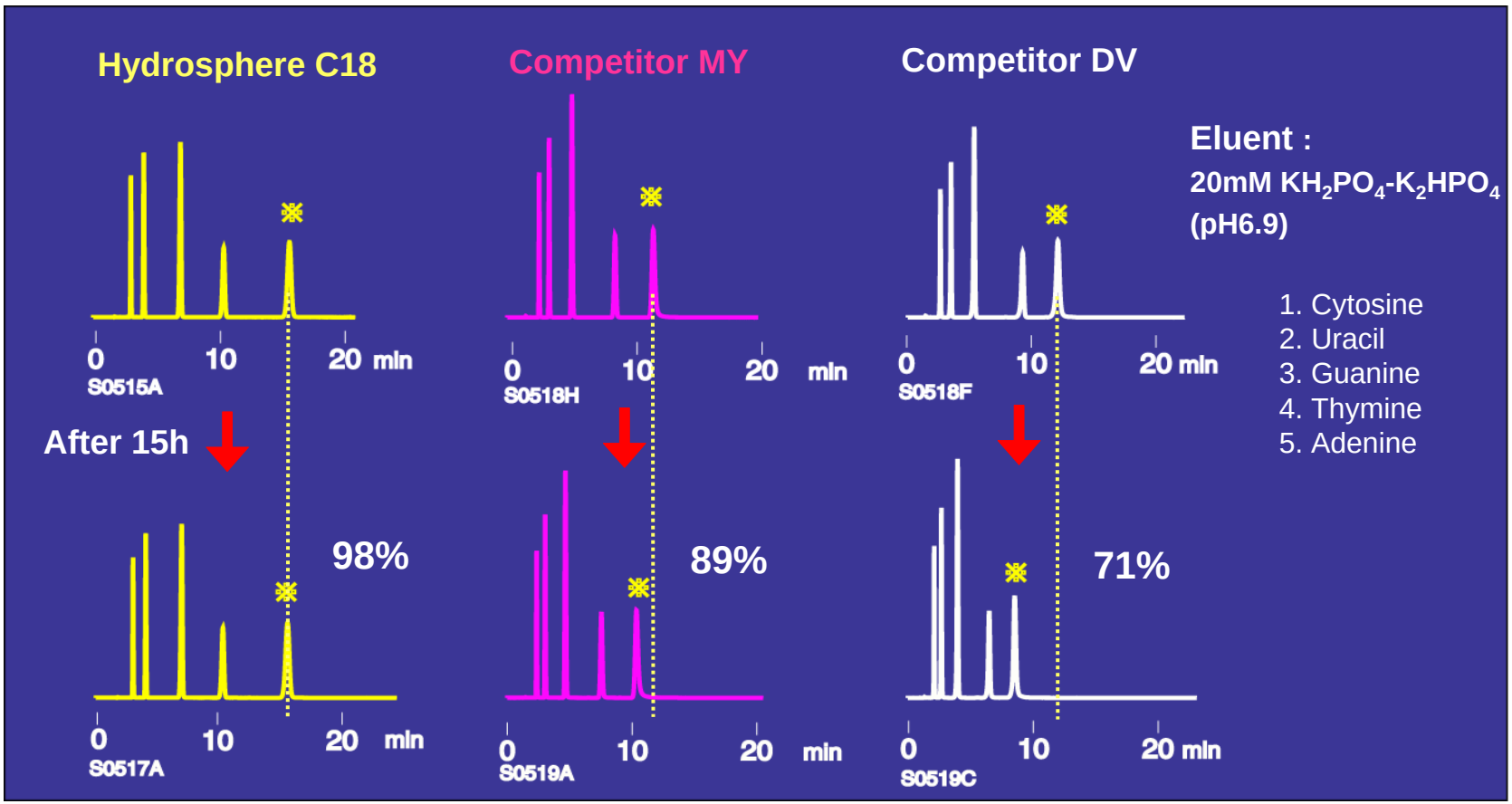




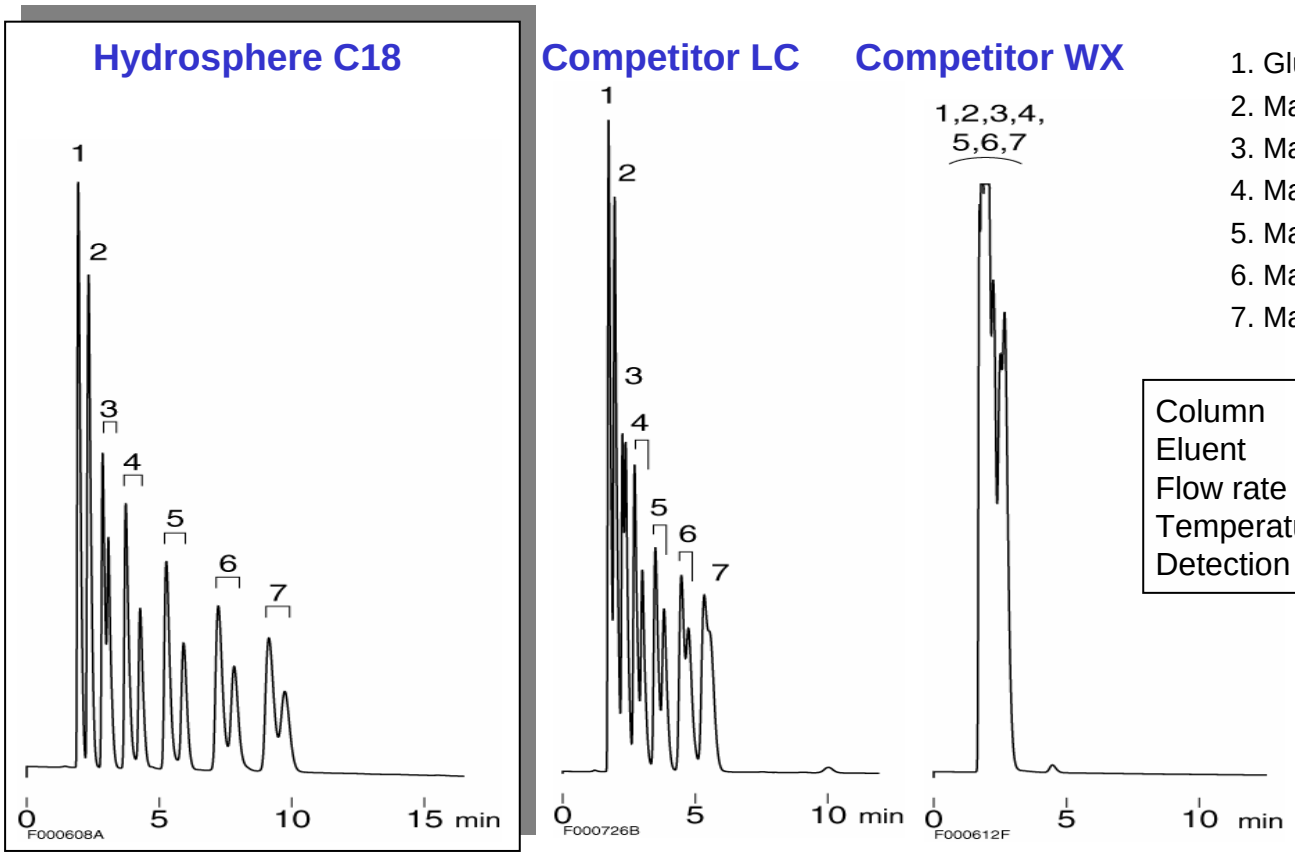
Peak Symmetry of Basic Compounds
Competitors



Reproducibility of Retention Time under 100% Aqueous Condition



Strong Retention and Unique Selectivity of Hydrosphere C18: *Maltooligosaccharides*

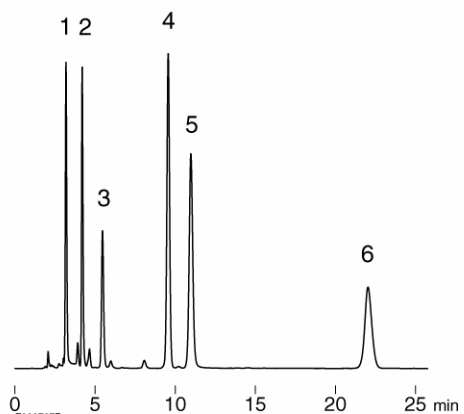


1. Glucose (G1)
2. Maltose (G2)
3. Maltotriose (G3)
4. Maltotetraose (G4)
5. Maltopentaose (G5)
6. Maltohexaose (G6)
7. Maltoheptaose (G7)

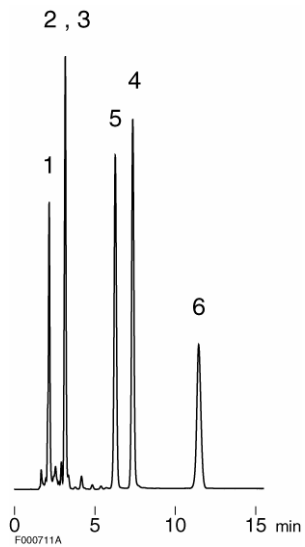
Column	: 150 X4.6 mmI.D.
Eluent	: water
Flow rate	: 1.0 mL/min
Temperature	: ambient (26℃)
Detection	: RI

Strong Retention and Unique Selectivity of Hydrosphere C18: *Coenzymes*

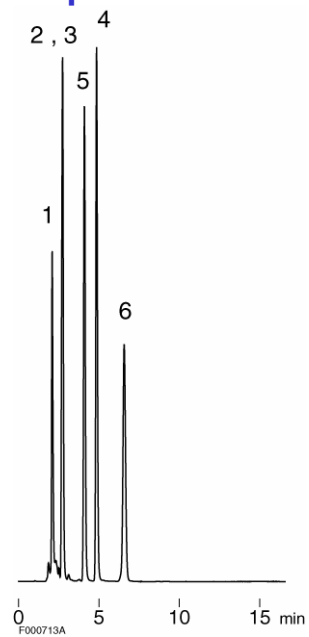
Hydrosphere C18



Competitor MY



Competitor DA



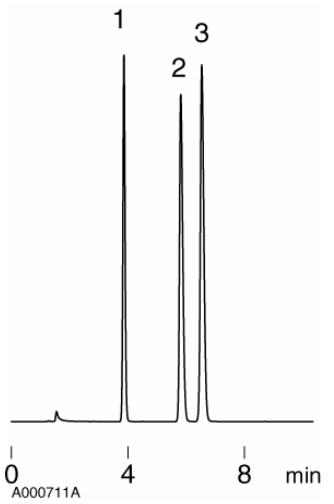
Column	: 150 X4.6 mmI.D.
Eluent	: 20mM KH_2PO_4-K_2HPO_4(pH6.9) / acetonitrile (99/1)
Flow rate	: 1.0 mL/min
Temperature	: 37 ℃
Detection	: UV at 260 nm

1. NADP
2. Nicotinic acid
3. NADPH
4. Nicotinamide
5. NAD
6. NADH

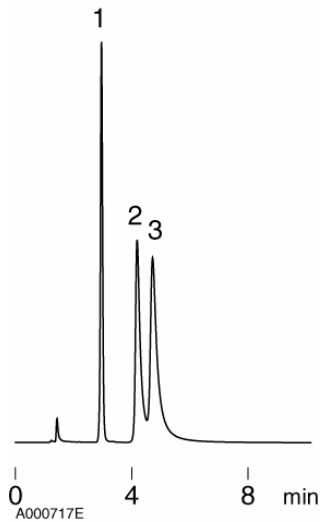
YMC SEPARATION TECHNOLOGY

Strong Retention and Unique Selectivity of Hydrosphere C18: *Orotic acids*

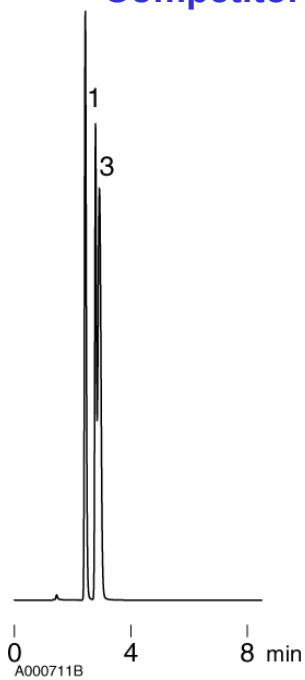
Hydrosphere C18



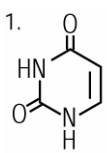
Competitor SS

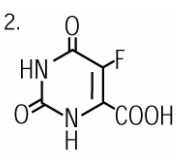


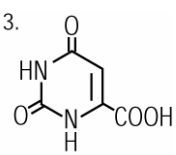
Competitor LC



Column	: 150 X4.6 mmI.D.
Eluent	: 20mM H_3PO_4
Flow rate	: 1.0 mL/min
Temperature	: 37 ℃
Detection	: UV at 254 nm

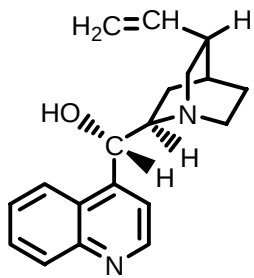
1. 
Uracil

2. 
5-Fluoroorotic acid

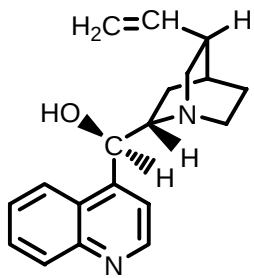
3. 
Orotic acid

Optimization of Analytical Conditions for Alkaloids(1)

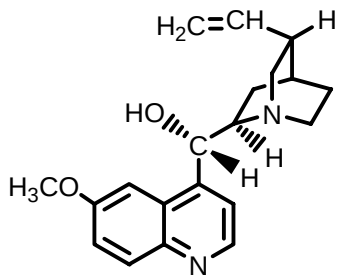
Cinchona alkaloids



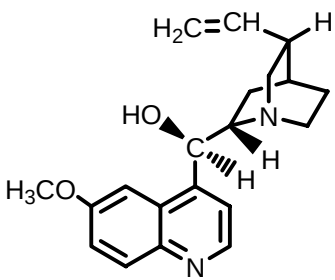
1. Cinchonine



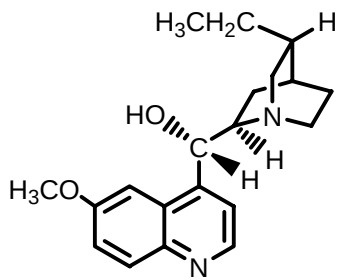
2. Cinchonidine



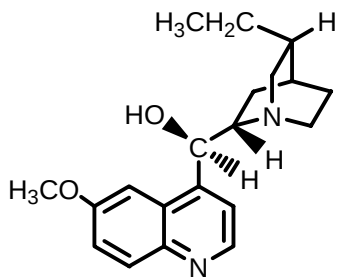
3. Quinidine



4. Quinine



5. Dihydroquinidine

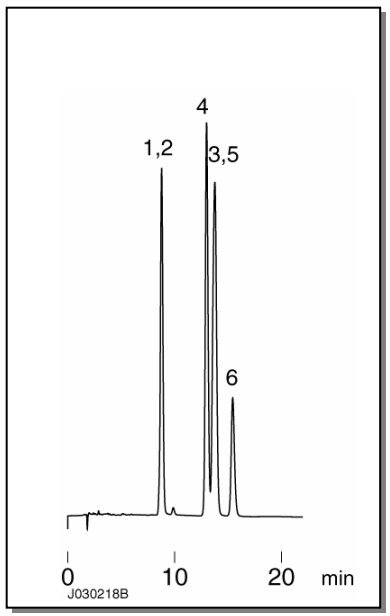


6. Dihydroquinine

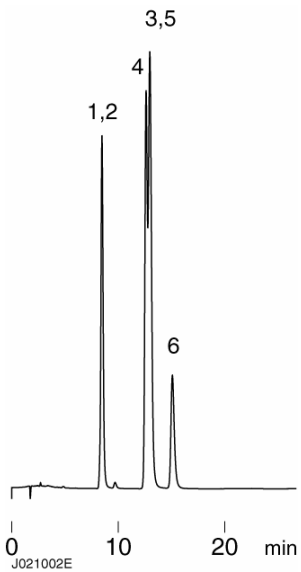
Optimization of Analytical Conditions for Alkaloids(2) : *Initial Conditions*

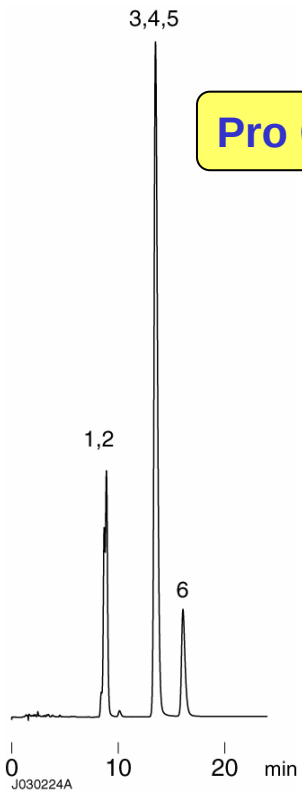
Initial Conditions: 20 mM KH_2PO_4-K_2HPO_4(pH6.9)/methanol(40/60)

Hydrosphere C18



Pro C18





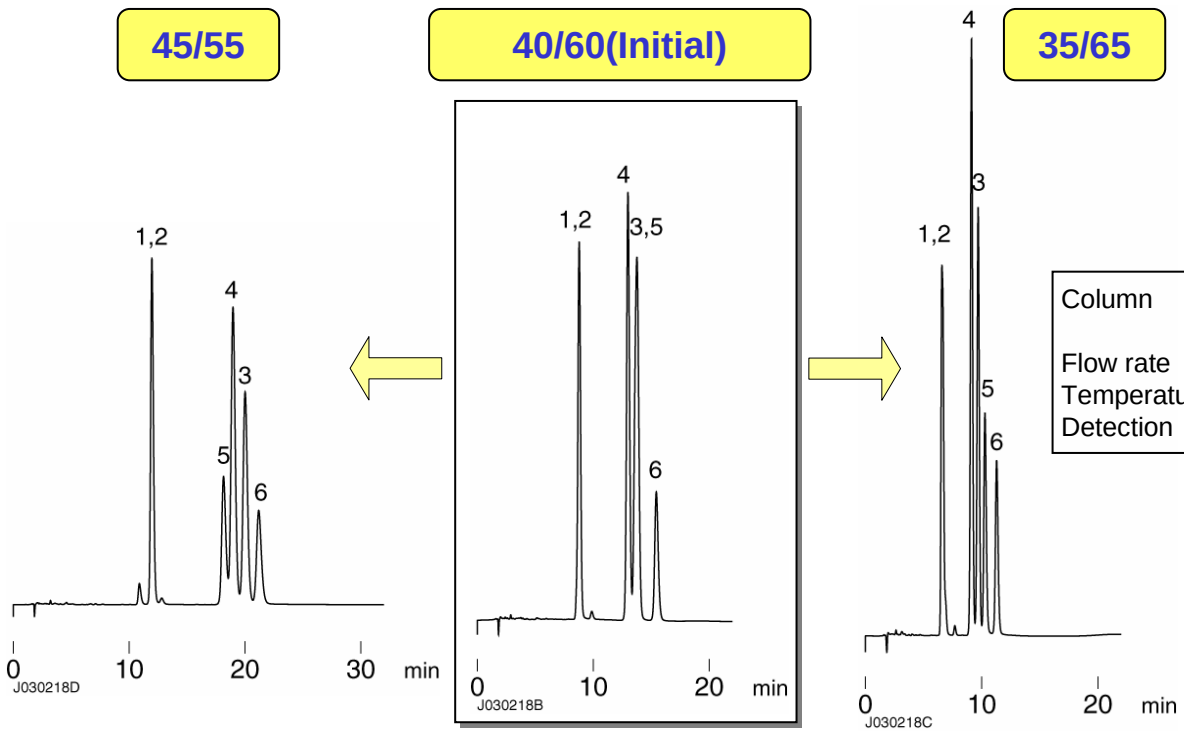
1.Cinchonine
2.Cinchonidine
3.Quinidine
4.Quinine
5.Dihydroquinidine
6.Dihydroquinine

Column	: 150 X4.6 mmI.D.
Flow rate	: 1.0 mL/min
Temperature	: 37 ℃
Detection	: UV at 235 nm

Optimization of Analytical Conditions for Alkaloids(3) : *Effect of Solvent Content*

20mM KH_2PO_4-K_2HPO_4(pH6.9)/methanol

45/55 | **40/60(Initial)** | **35/65**



1.Cinchonine
2.Cinchonidine
3.Quinidine
4.Quinine
5.Dihydroquinidine
6.Dihydroquinine

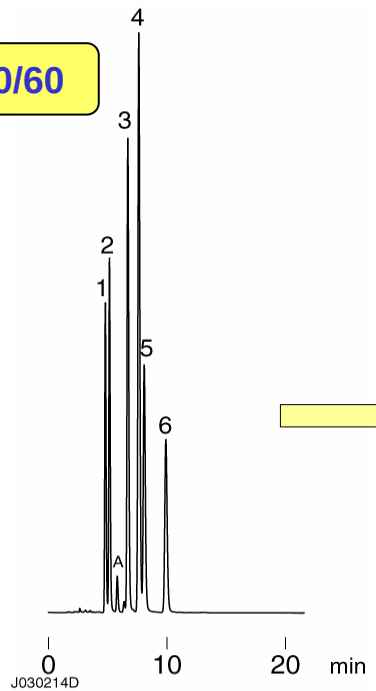
Column	: Hydrosphere C18 (5μm,120A) 150 X4.6 mmI.D.
Flow rate	: 1.0 mL/min
Temperature	: 37 ℃
Detection	: UV at 235 nm

Optimization of Analytical Conditions for Alkaloids(4) : *At Low pH*

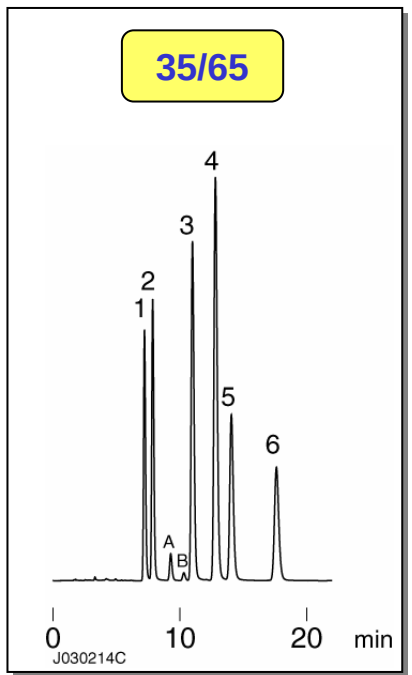
methanol /20mM KH_2PO_4 (pH4.6)

1.Cinchonine
2.Cinchonidine
3.Quinidine
4.Quinine
5.Dihydroquinidine
6.Dihydroquinine

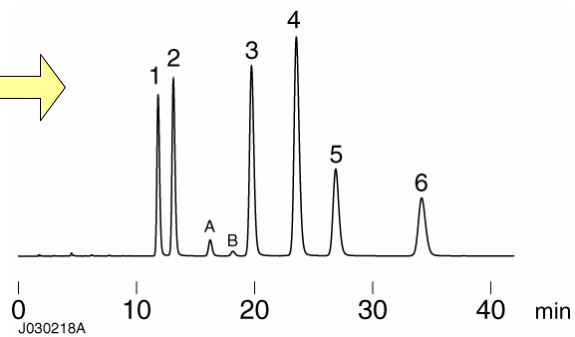
40/60



35/65



30/70

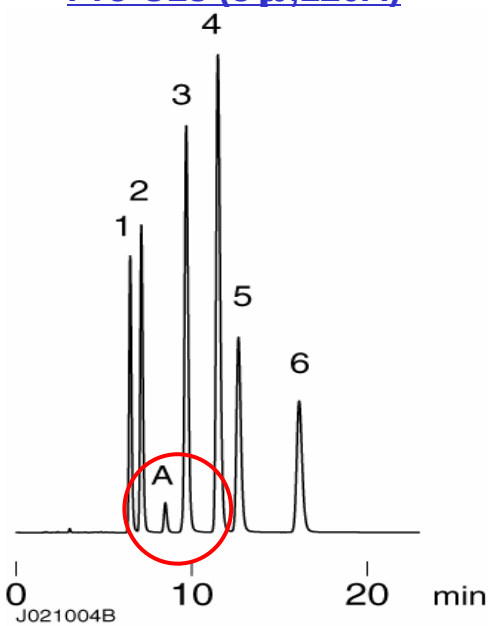


Column	: Hydrosphere C18 (5μm,12nm) 150 X4.6 mmI.D.
Flow rate	: 1.0 mL/min
Temperature	: 37 ℃
Detection	: UV at 235 nm

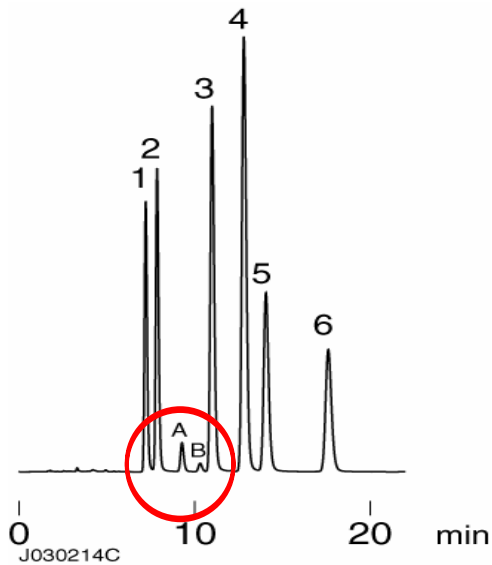
Optimization of Analytical Conditions for Alkaloids(5)

Comparison of Hydrosphere C18 with *Pro* ODS columns

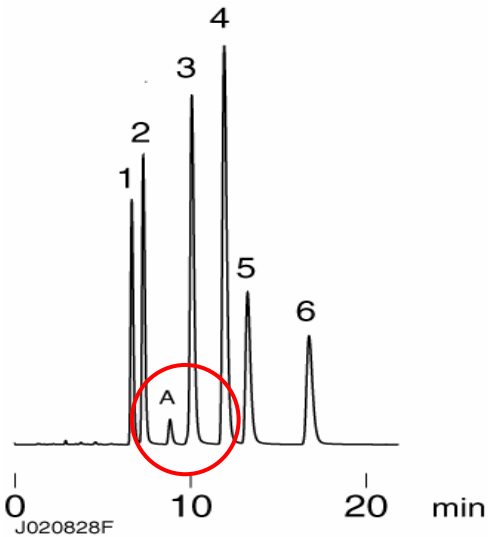
Pro C18 (5μ,120A)



Hydrosphere C18 (5μ,120A)



Pro C18 RS (5μ,80A)



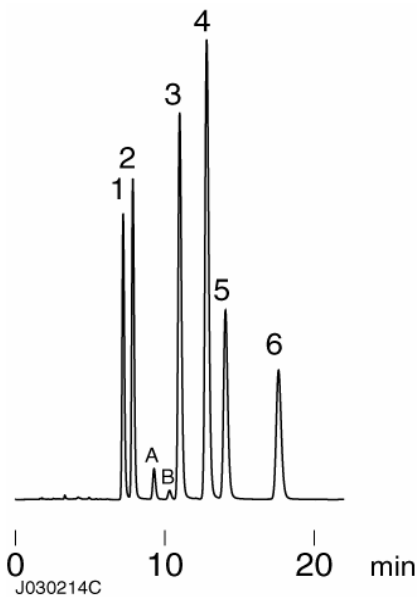
Column	: 150 X4.6 mmI.D.
Eluent	: methanol/20mM KH_2PO_4(35/65)
Flow rate	: 1.0 mL/min
Temperature	: 37 ℃
Detection	: UV at 235 nm

1.Cinchonine
2.Cinchonidine
3.Quinidine
4.Quinine
5.Dihydroquinidine
6.Dihydroquinine

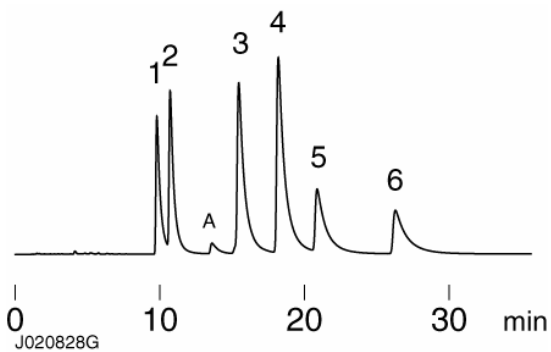
Optimization of Analytical Conditions for Alkaloids(6)

Comparison of Hydrosphere C18 with competitive columns

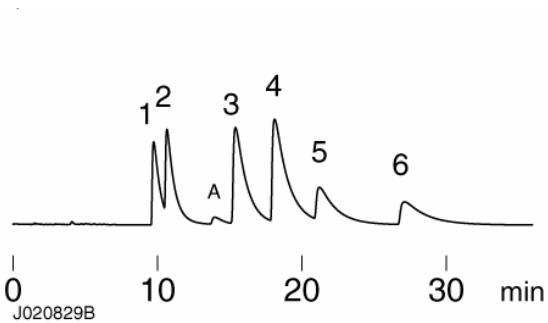
Hydrosphere C18



Competitor WA



Competitor LA



Column	: 150 X4.6 mmI.D.
Eluent	: methanol/20mM KH_2PO_4(35/65)
Flow rate	: 1.0 mL/min
Temperature	: 37 ℃
Detection	: UV at 235 nm

1.Cinchonine
2.Cinchonidine
3.Quinidine
4.Quinine
5.Dihydroquinidine
6.Dihydroquinine

Conclusions

- Unique endcapping process provides in good peak shapes even for basic compounds
- Excellent reproducibility of retention time under 100% aqueous conditions vs. conventional ODS columns
- Hydrophilic compounds can be effectively separated with strong retention under 100% aqueous conditions
- A natural complex mixture of Cinchona alkaloids including some minor impurities were successfully separated

YMC SEPARATION TECHNOLOGY