

Investigation of reverse-phase - HILIC continuous analysis using a one column

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Abstract

The simultaneous analysis of mixture sample which contains hydrophilic substances and hydrophobic substances is sometime terribly difficult. In generally we need to analyze it 2 times of intermitted analysis using reversed phase chromatography(RPC) and hydrophilic interaction chromatography(HILIC). To analyze these samples conveniently, we examined RPC/HILIC continuous analysis method. For this method, Shodex ODP2 HP column was tested. And we obtained successful result below.

1) RPC mode and HILIC mode were expressed within one column to change the ratio of water and acetonitrile mixture which is used as mobile phase.

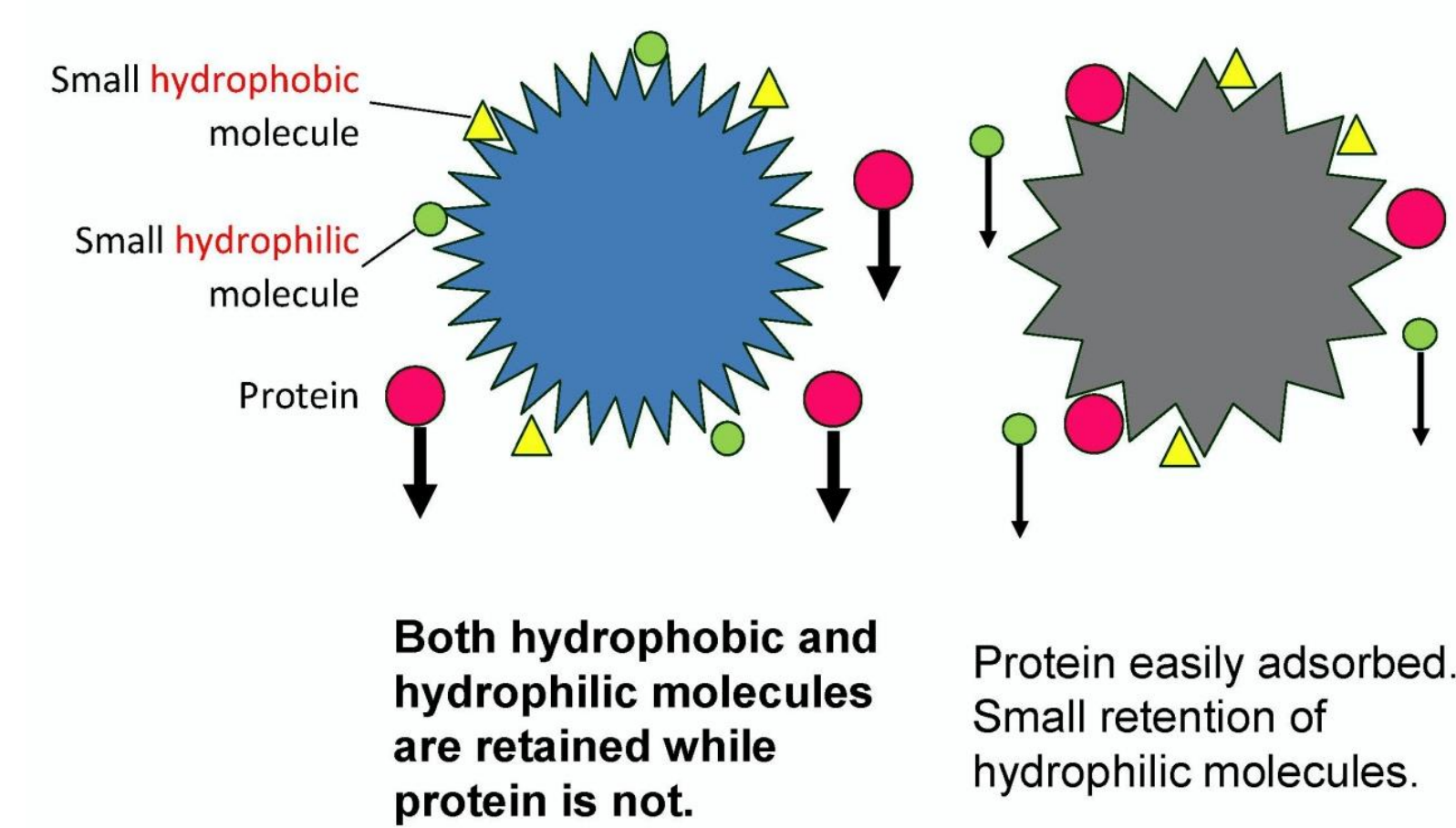
2) RPC/HILIC continuous analysis method was applied to the commercial nutritional supplement drink and the components of it were detected. Repeatability was confirmed. And the analysis time was 40 min./run including equilibration time.

Shodex ODP2 HP column has a unique feature that can be applied to both RPC and HILIC. So the sample solution contains hydrophilic substances and hydrophobic substances was analyzed within one run.

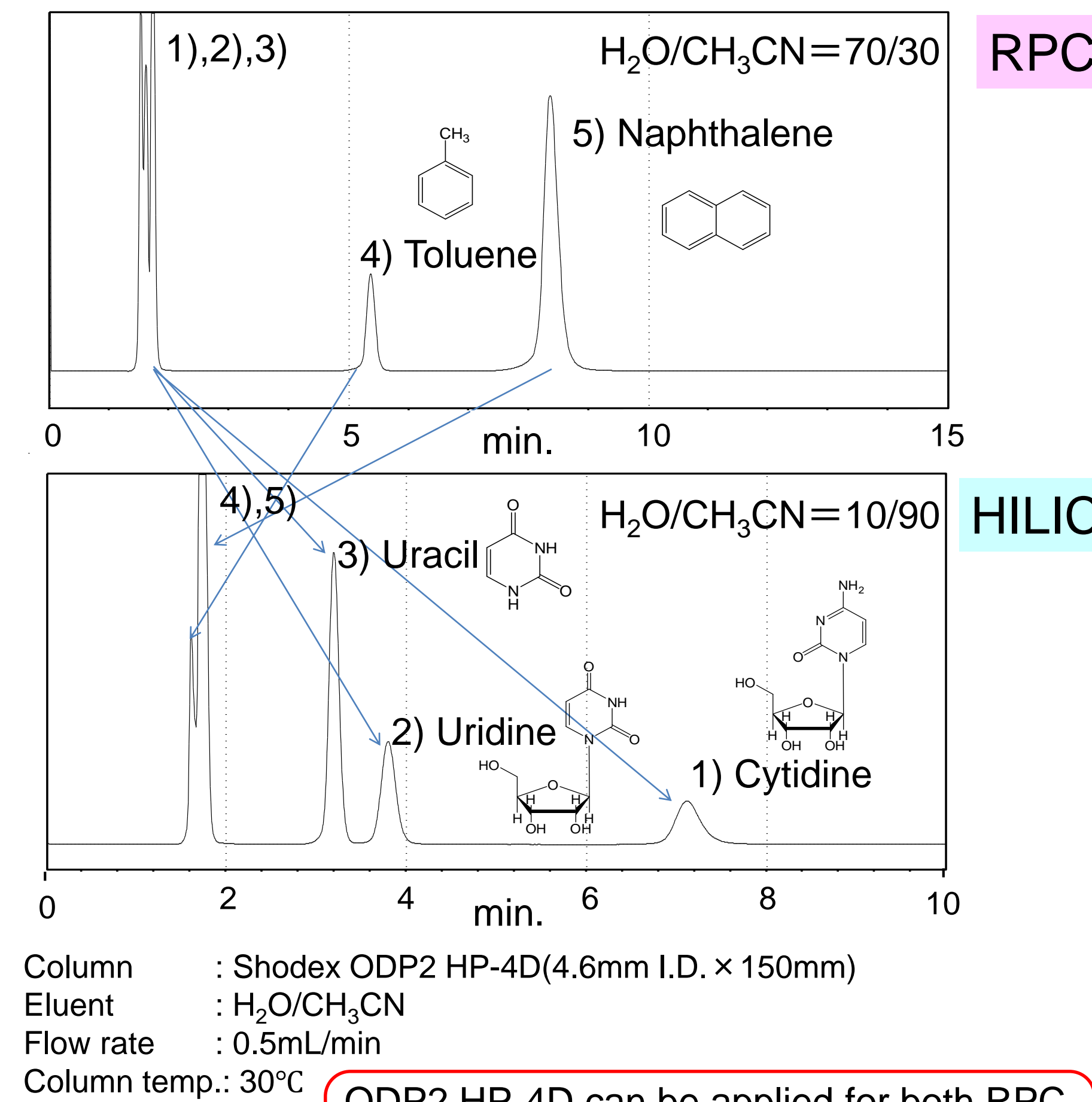
The features of Shodex® ODP2 HP

The difference of ODP2 HP and ODS

	ODP2 HP	ODS
Base material	Polyhydroxy-methacrylate	Silica
Functional group	None	Octadecyl (C18)
Average pore size (Å)	40	100 - 120
Available pH	3 - 12	2 - 8

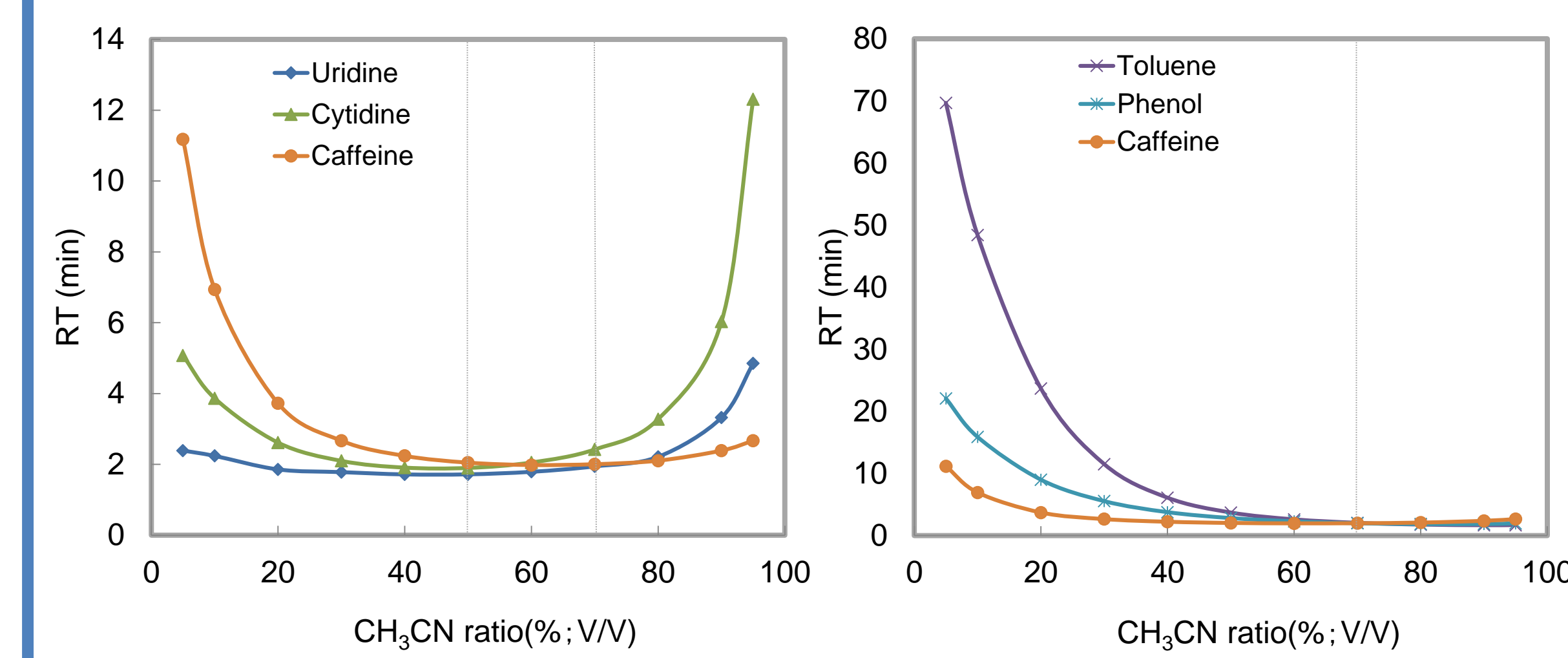


The relationship of CH₃CN concentration and elution order.

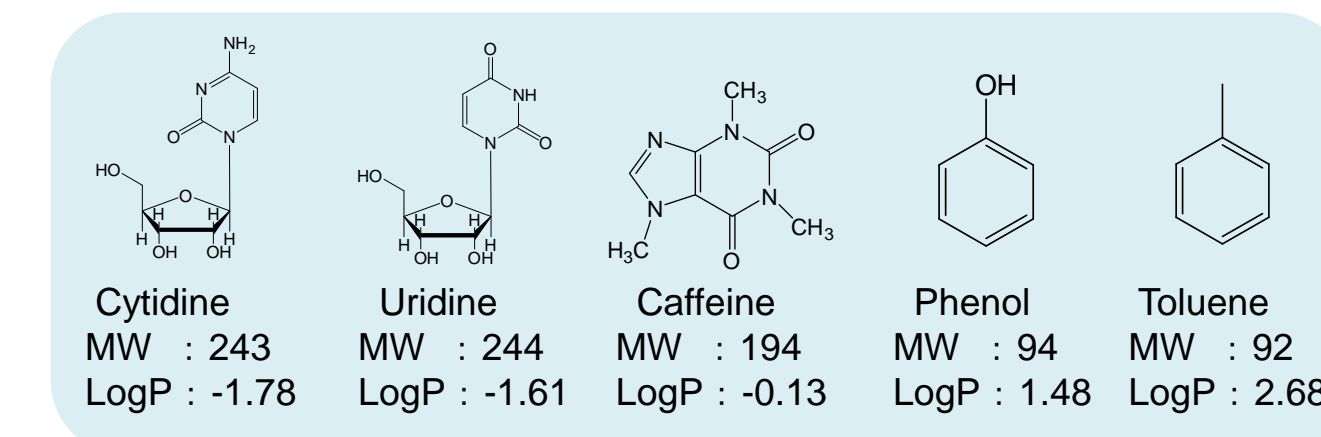


ODP2 HP-4D can be applied for both RPC and HILIC because of polymer based packing material having hydroxyl group.

The relationship of CH₃CN concentration and retention time.

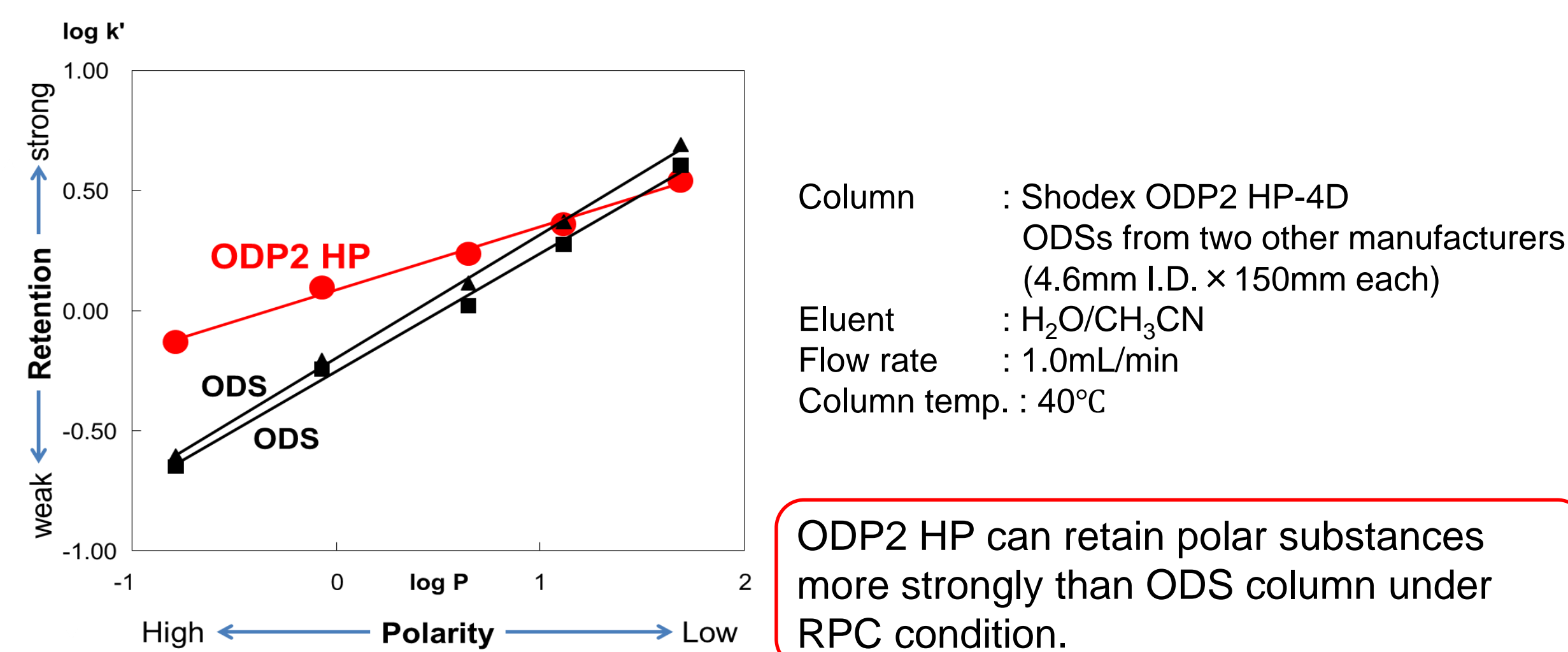


Column : Shodex ODP2 HP-4D (4.6mm I.D. x 150mm)
 Eluent : H₂O/CH₃CN
 Flow rate : 1.0mL/min
 Detector : UV(254nm)
 Column temp.: 40°C



More than 70% of CH₃CN : HILIC mode would be working.
 Less than 50% of CH₃CN : RPC mode would be working.

The relationship of polarity of analytes and retention factor.

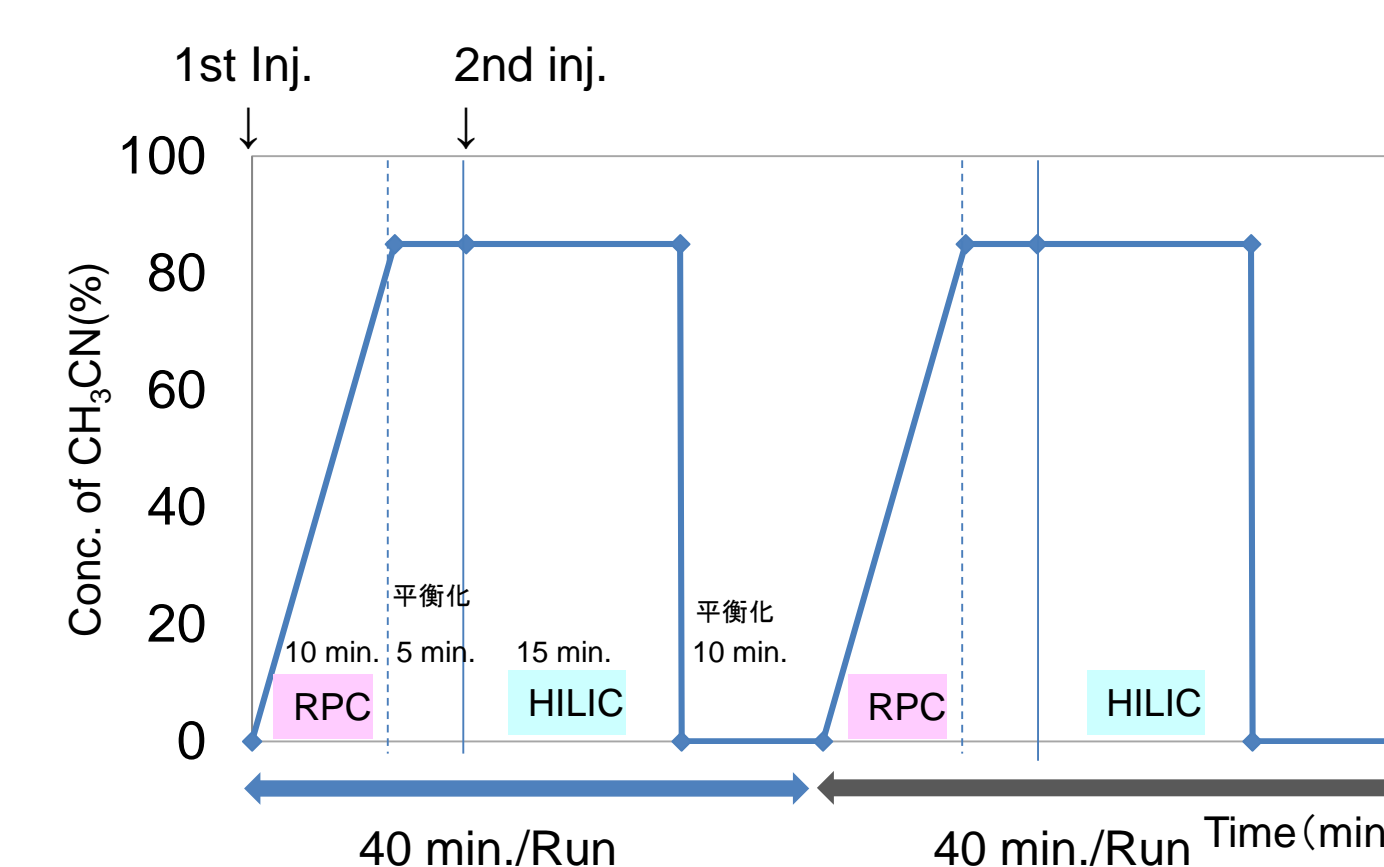


RPC/HILIC continuous LC/MS analysis (2 times injection method)

[experiment]
 RPC/HILIC continuous LC/MS analysis was tested. (Injected manually)

[sample]
 commercial nutritional supplement drink (diluted 100 times with mobile phase)

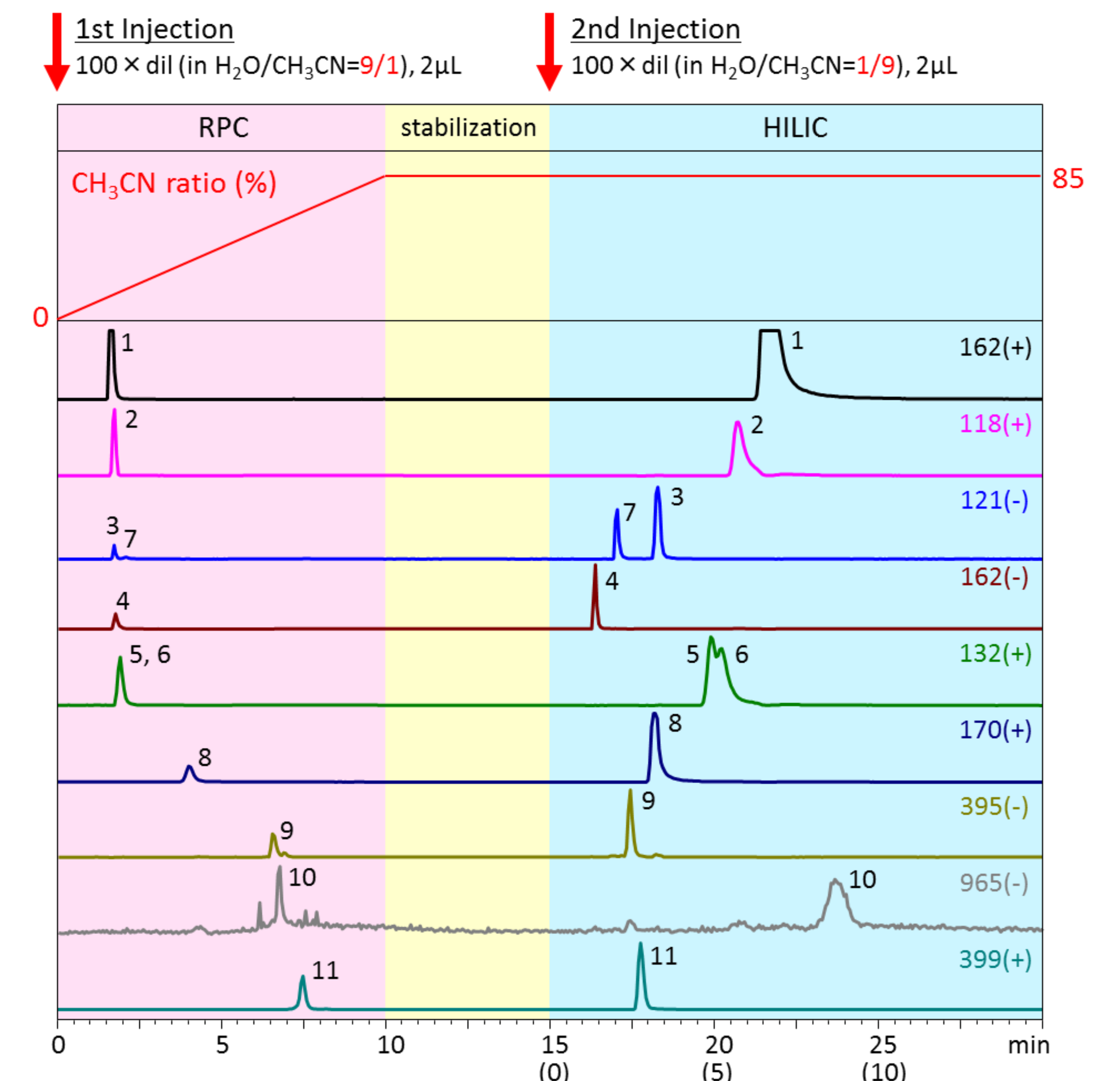
[result]
 good repeatability
 40 min./Run (including stabilization time)



#	Compound	Structure	MW	Log P
1	Carnitine		161	-4.52
2	Valine		117	0.20
3	Erythritol		122	-3.00
4	Acesulfame (K)		201	-0.31
5	Isoleucine		131	0.73
6	Leucine		131	0.73

#	Compound	Structure	MW	Log P
7	Benzoic acid		122	1.89
8	Pyridoxine		169	-1.10
9	Sucralose		397	0.68
10	Rebaudioside A		967	-1.13
11	Fursultiamine		398	2.35

LC/MS : Shimadzu Nexera / LCMS-8030 Plus
 Column : Shodex ODP2 HP-2D(2.0mm I.D. x 150mm)
 Eluent : (A) 10mM HCOONH₄ aq./ (B) CH₃CN Linear gradient ; (B%) 0%(0min)→85%(10-30min)
 Flow rate : 0.2mL/min
 Detector : ESI-MS SIM(+/-)
 Column temp.: 30°C



• Sample 9 and 11 were separated by RPC mode and sample 1, 2, 3, 5, 7 and 8 were separated by HILIC mode.
 • Sample 4 wasn't retained well both RPC mode and HILIC mode. → Other separation mode would be working.
 • Sample 10 was retained strongly both RPC mode and HILIC mode.
 • We can obtain two kinds of analysis data (RPC and HILIC) in one run.
 • We can select the appropriate data for quantitative or qualitative analysis.

HILIC characteristic evaluation

