Determination of Hydrophilic Drugs and their Metabolites in Blood using a TSKgel HILIC column

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Introduction

- Reversed Phase Chromatography (RPC) and Hydrophilic Interaction Chromatography (HILIC) columns are widely utilized for separations of pharmaceutical products, which have hydrophobic and hydrophilic properties. HILIC columns possessing various hydrophilic ligands, such as hydroxyl, carbamoyl, amino and ionic groups, strongly retain hydrophilic and ionic compounds so that different selectivity can be expected in comparison with ODS columns.

- Therapeutic drug monitoring (TDM) by HPLC is required for several drug classes as the effective dosage range, varying between insufficient activity and toxic levels, is patient dependent. Although an ODS column is adopted for separating drugs in blood samples, hydrophilic compounds show poor retention times on an RPC column. We investigated the possibility of the separation of hydrophilic drugs and its metabolites in blood on a new HILIC column.

- In this poster the solvent composition and organic solvent effects on elution behavior for a new HILIC column from Tosoh Corporation, a TSK-GEL NH_2-100, 3µm column, is demonstrated. The separation of hydrophilic drugs and its metabolites in serum by on-line and off-line deproteinization procedures are also reported.

Experimental

Columns – Tosoh Corporation (Japan)

- TSKgel NH_2-100, 3µm, 4.6mm ID x 15cm
- TSKgel ODS-100V, 3µm, 4.6mm ID x 15cm
- TSKgel precolumn BSA-ODS-100V, 2mm ID x 1cm

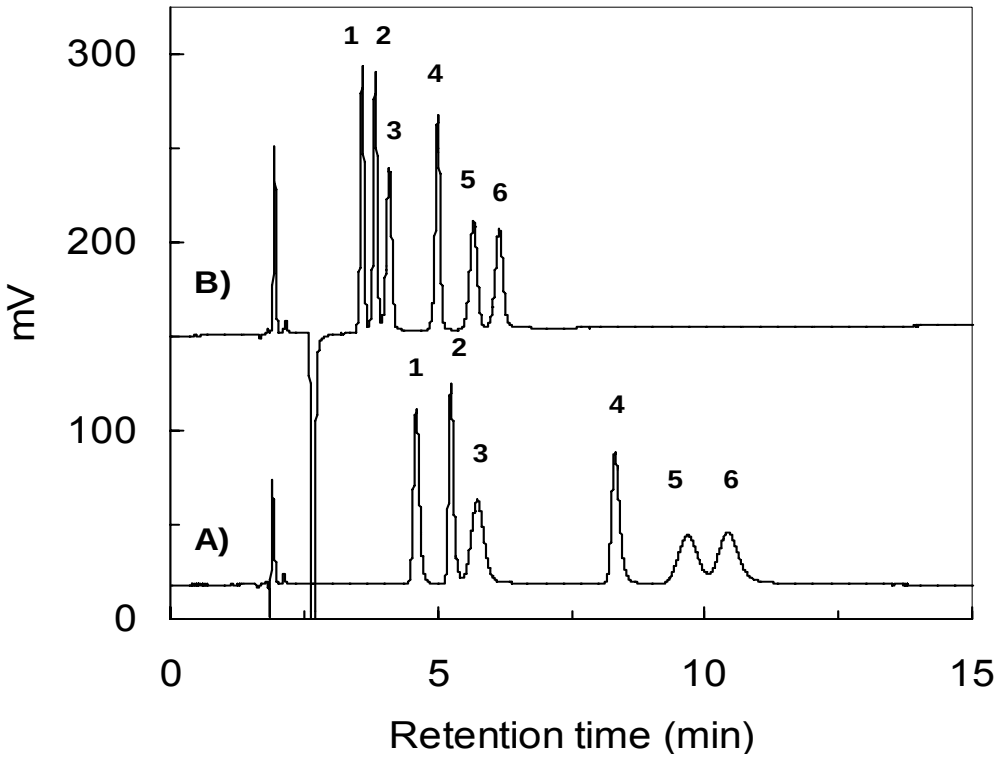
Instrumentation

Pump: DP-8020 (Tosoh)
Detector: UV-8020, RI-8020 (Tosoh)
Auto-sampler: AS-8020 (Tosoh)
Data processing: LC-8020 model 2 (Tosoh)

Chemicals and Reagents

All saccharides and reagents were purchased from Kishida Chemicals (Osaka). The pyridylaminated sugars were from Takara Bio Inc. All organic solvents were of HPLC grade from Kishida Chemicals. Human serum was purchased from Sigma.

Figure 1: Separation of Sugars on a TSKgel NH2-100, 3µm Column



TSKgel NH_2-100, 3µm, 4.6mm ID x 15cm

Eluent:	A) H_2O/Acetone (25/75)
	B) 100mmol/L TEA-FA (pH 10.0) /Acetone (25/75)
Flow rate:	1.0mL/min
Injection vol.:	10µL
Temperature:	50ºC
Detection:	RI
Samples:	1. fructose 2.sorbitol 3.glucose
	4.sucrose 5.maltose 6.lactose

Table 1: Effect of Organic Solvent on Elution Profiles of Various Sugars

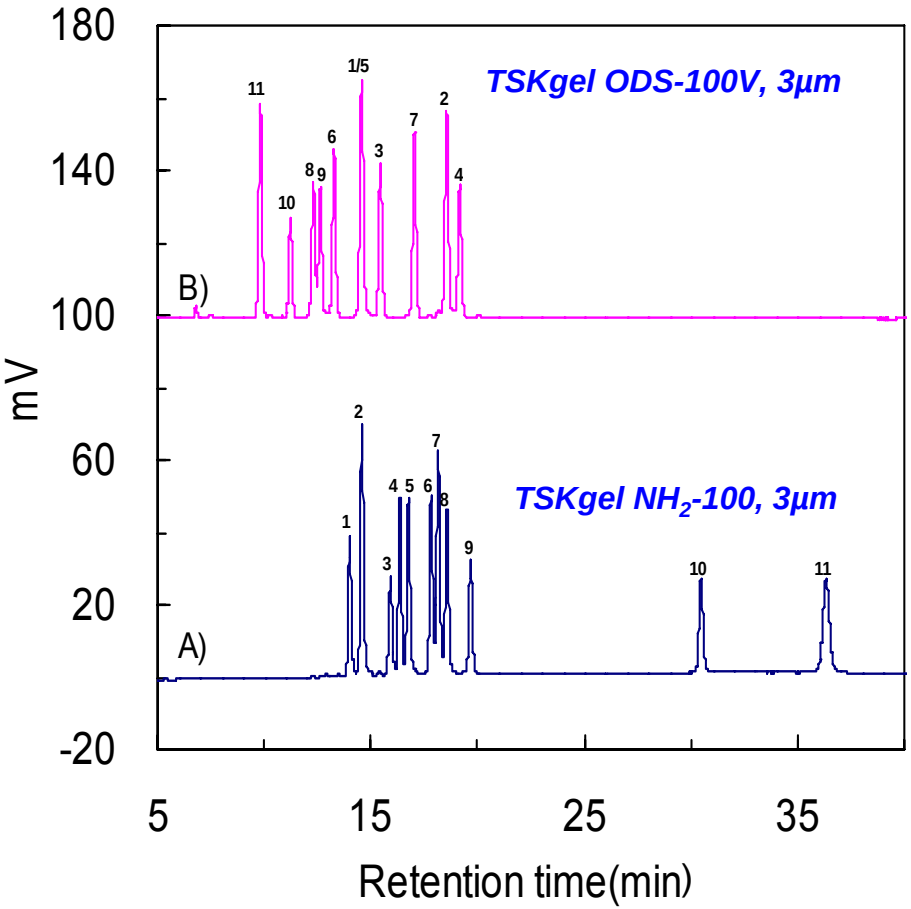
No.	Sample	TEA-FA/ACN			TEA-FA/Acetone			TEA-FA/EtOH		
		Rt (min)	Area	TP	Rt (min)	Area	TP	Rt (min)	Area	TP
1	Rhamnose	3.74	516	9,773	2.98	508	4,662	3.10	413	1,051
2	Ribose	3.84	319	13,951	3.10	367	14,511	3.38	117	7,181
3	Erythritol	4.01	784	15,498	3.13	732	15,495	3.04	756	11,041
4	Fucose	4.07	674	2,640	3.36	658	1,120	3.64	449	274
5	Xylose	4.42	493	6,463	3.43	569	7,349	3.76	274	5,189
6	Arabinose	4.59	381	6,520	3.62	424	4,026	4.09	79	1,213
7	Xylitol	4.76	831	15,677	3.54	794	15,409	3.58	808	10,820
8	Fructose	5.01	791	14,774	3.75	786	11,165	4.28	794	4,925
9	Sorbose	5.11	858	15,455	3.76	828	14,483	4.16	836	9,043
10	Sorbitol	5.69	830	15,269	3.99	827	15,185	4.27	831	10,113
11	Mannose	5.51	462	10,727	3.94	518	5,517	4.66	247	939
12	Mannitol	5.84	825	15,400	4.05	802	15,321	4.41	797	9,749
13	Galactose	6.02	470	4,125	4.35	596	1,924	5.16	176	443
14	Glucose	5.95	852	6,645	4.25	862	4,508	4.96	759	1,152
15	Sucrose	8.47	887	15,707	5.18	887	14,543	5.96	884	7,206
16	Inositol	8.79	938	12,627	6.35	972	13,576	8.30	909	3,089
17	Cellobiose	9.67	666	8,656	6.89	783	4,786	7.63	572	1,186
18	Maltose	9.78	697	7,448	5.91	788	4,505	7.50	700	1,217
19	Lactose	10.28	635	9,110	6.46	703	5,362	8.67	542	4,512
20	Trehalose	10.53	752	15,164	6.36	797	14,181	8.51	830	742
21	Gentiobiose	11.65	561	7,449	7.04	707	3,923	10.98	297	11,041
22	Glucosamine	6.32	675	8,546	4.14	703	1,950	3.83	610	213
23	N-Acetylglucosamine	5.04	898	1,759	3.70	936	792	3.55	894	145
24	Galactosamine	6.53	621	4,614	4.14	594	926	3.81	526	167

Table 1: Chromatographic Conditions

TSKgel NH_2-100, 3µm, 4.6mm ID x 15cm

Eluent:	A,B: 100mmol/L TEA-Formic acid (pH10.0) /solvent (25/75)
	C: 100mmol/L TEA-Formic acid (pH10.0) /solvent (5/95)
Flow rate:	1.0mL/min
Injection vol.:	10µL
Temperature:	40ºC
Detection:	RI
Samples:	saccharides

Figure 2: Comparison of Separation of PA Sugars on RPC & HILIC Columns



A) TSKgel NH2-100, 3µm, 4.6mm ID x 15cm
B) TSKgel ODS-100V, 3µm, 4.6mm ID x 15cm

Eluent:	A) A: 200mmol/L TEA-Acetic acid (pH6.5)/ACN (30/70)
	B: 500mmol/LTEA-Acetic acid (pH6.5) /ACN (60/40)
	B) A: 50mmol/L HCO_2NH_4/ACN (98/2),
	B: 50mmol/L HCO_2NH_4/ACN (90/10),
Gradient:	0min (0% B) 30min (100% B) 45min (100% B)
Flow rate:	1.0mL/min
Injection vol.:	10µL
Temperature:	40ºC
Detection:	Fs (Ex. 315nm, Em. 380nm)
Samples:	pyridylaminated (PA) sugars

Figure 2: Pyridylaminated Sugars

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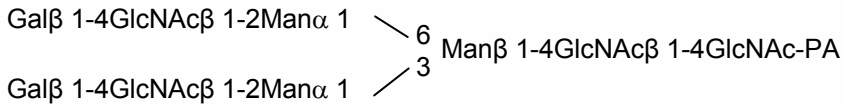

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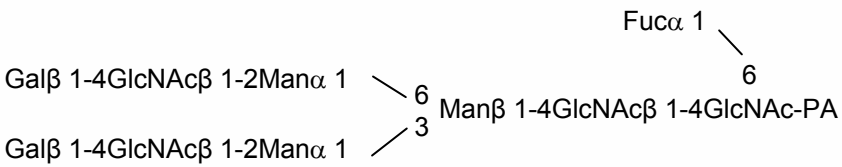

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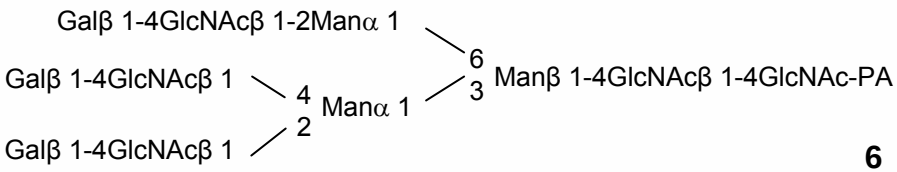

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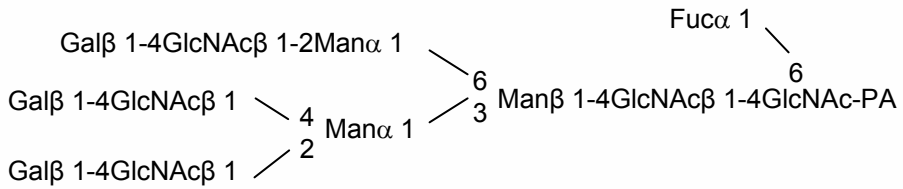

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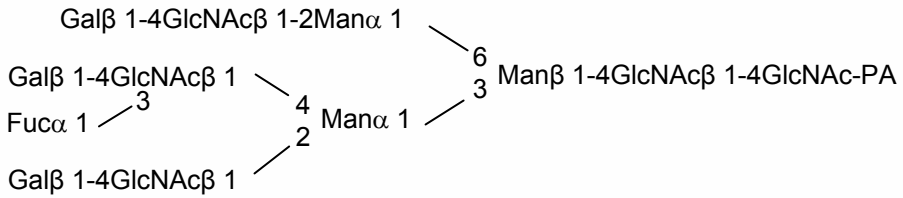

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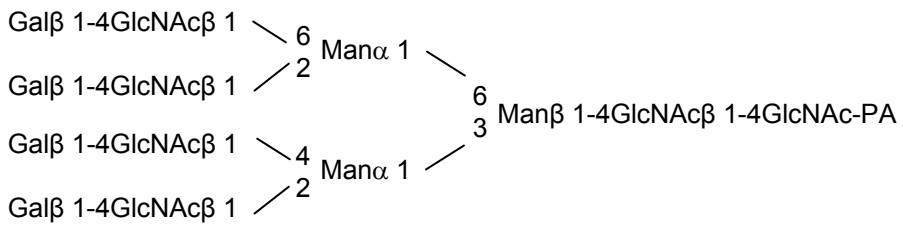

Figure 2: Pyridylaminated Sugars Continued

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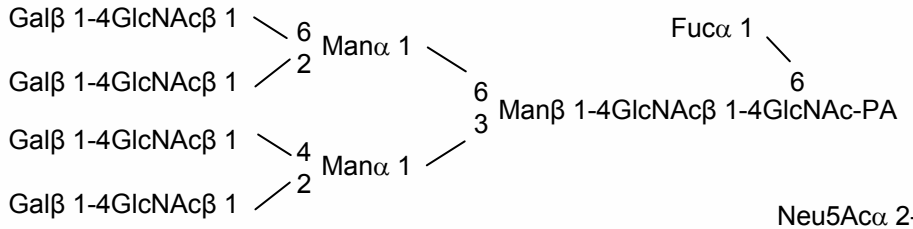

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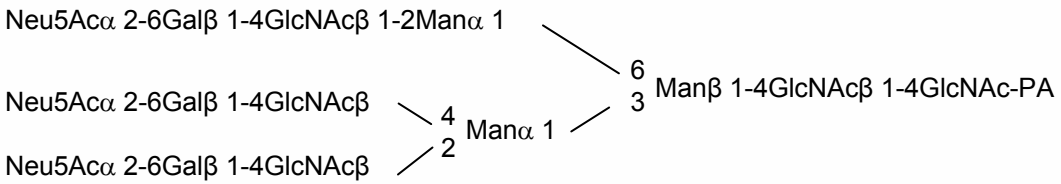

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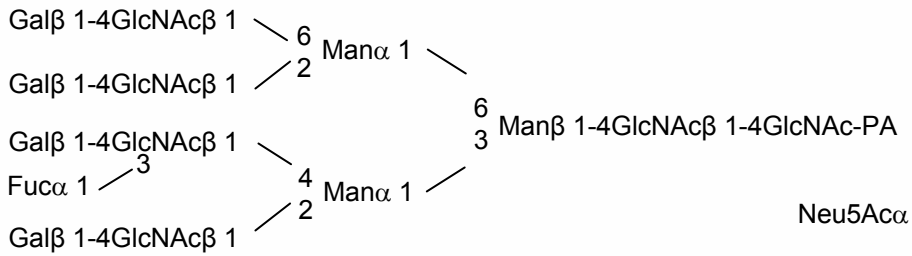

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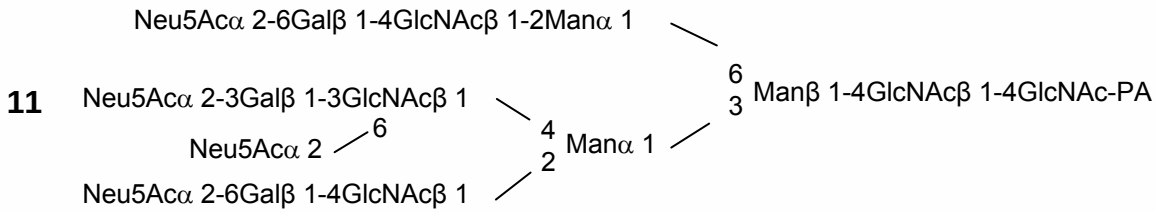

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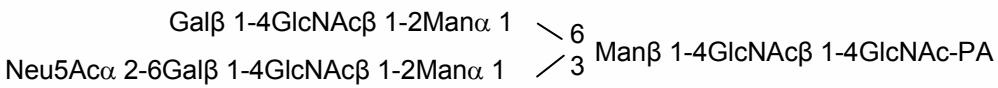
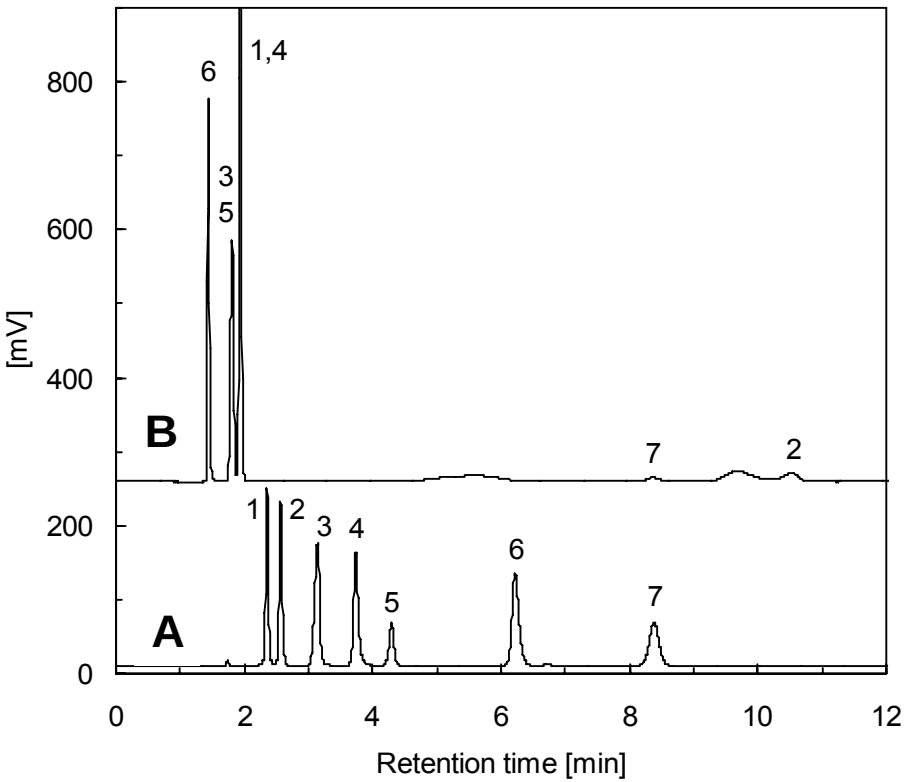


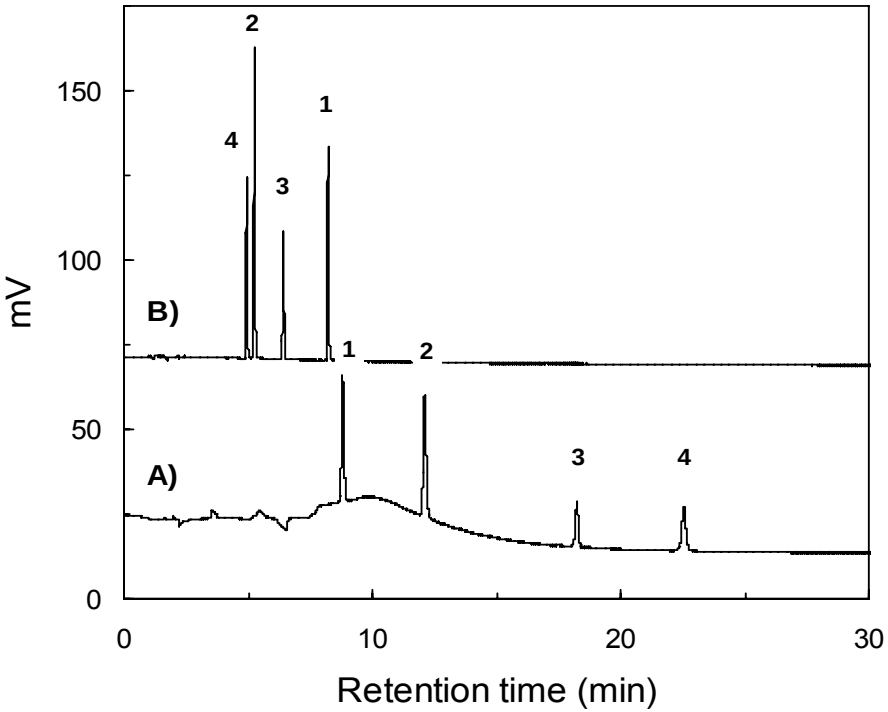
Figure 3: Separation of Vitamins on TSK-GEL HILIC & ODS Columns



A) TSKgel NH_2-100, 3µm, 4.6 mm ID x 15 cm
B) TSKgel ODS-100V, 3µm, 4.6 mm ID x 15 cm

Eluent:	A) 25 mmol/L Phosphate buffer (pH 2.5)/AcCN=30/70
	B) 25 mmol/L Phosphate buffer (pH 2.5)/AcCN=88/12
Flow rate:	1.0mL/min
Injection vol.:	5µL
Temperature:	40ºC
Detection:	UV@254nm
Samples:	1.nicotinamide 2.vitamin B2 3.pyridoxine
	4.nicotinic acid 5.vitamin C 6.vitamin B1
	7.vitamin B12

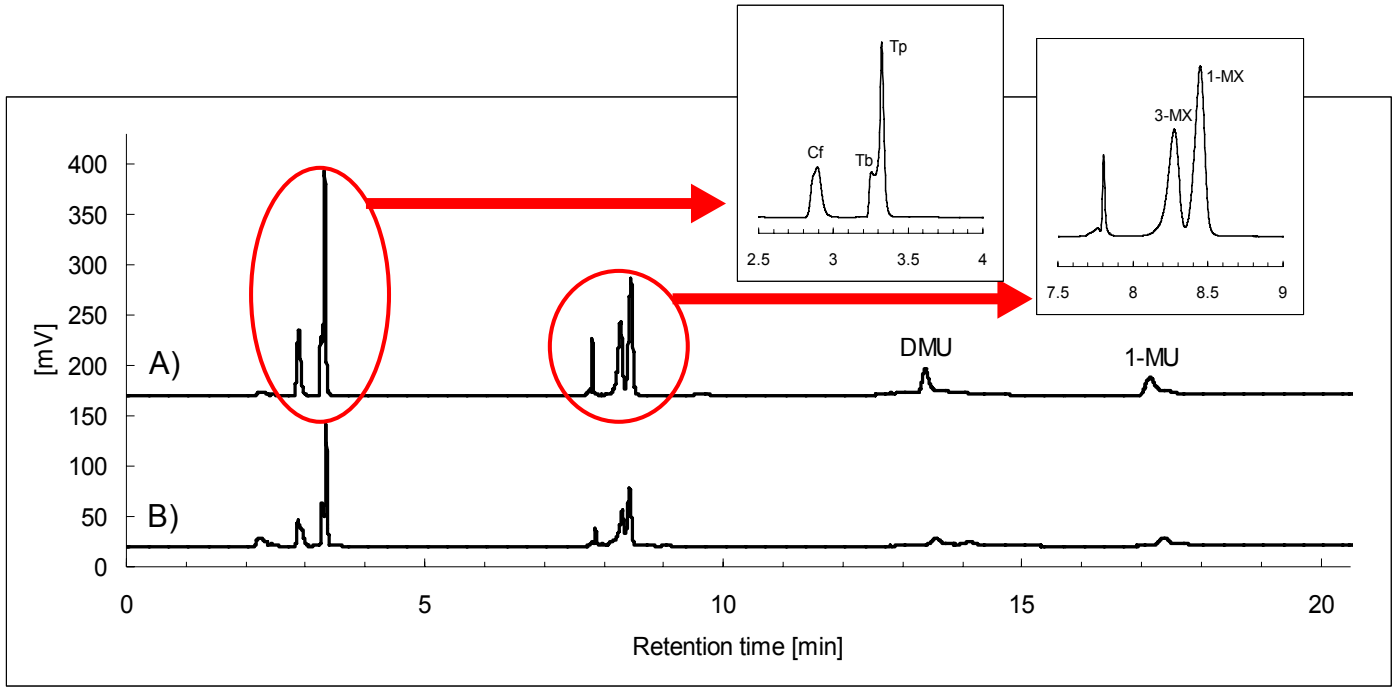
Figure 4: Comparison of Elution Pattern of Theophyline and its Metabolites on TSK-GEL HILIC & ODS Columns



A) TSKgel NH_2-100, 3µm, 2.0mm ID x 15cm
B) TSKgel ODS-100V, 3µm, 2.0mm ID x 15cm

Eluent:	A) A: 0.1mol/L Triethylamine-Formic acid (pH 10.0)/ACN (5/95)
	B: 0.1mmol/L Triethylamine-Formic acid (pH 10.0)/ACN (50/50)
	B) A: H_2O/ACN (98/2)+0.1% Formic acid
	B: H_2O/ACN (50/50)+0.1% Formic acid
Flow rate:	0.25mL/min
Injection vol.:	10µL
Temperature:	40ºC
Detection:	UV@254nm
Samples:	1. theophyline 2. 3-methylxanthine
	3. 1,3-dimethyluric acid 4. 1-methyluric acid
	(concentration: 10µg/mL for each)

Figure 5: Separation of Theophyline and its Metabolites in Serum with On-line Deproteinization



TSKgel NH_2-100, 3µm, 4.6mm ID x 15cm

TSKgel precolumn BSA-ODS-100V, 2.0mm ID x 1cm

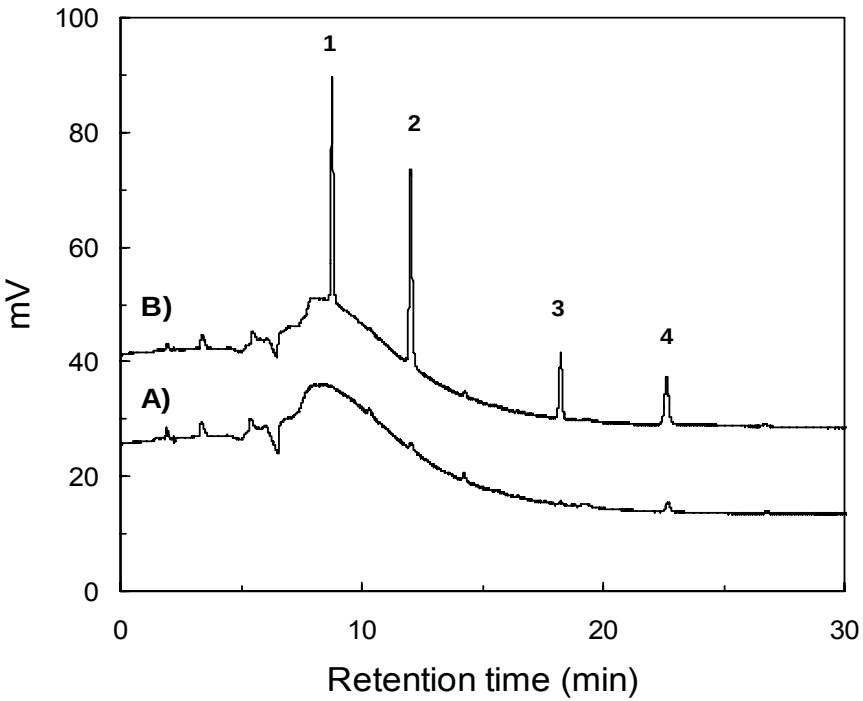
A: Standard B: Serum spiked with the standard samples

Eluent: Pretreatment: 0.2mol/L HCO_2NH_4 (pH 3.6) during 0-0.3min
A: ACN B: H_2O/ACN=15/85 C: 0.2mol/L HCO_2NH_4 (pH3.6)/ACN=30/70 (Stepwise gradient: 0.3-2.0min A, 2.0-8.0min B, 8.0-20min C)

Flow rate: 1.0mL/min Detection: UV@254nm Temperature: 40ºC Injection vol.: 5µL

Samples: 1. caffeine (Cf) 2. theobromine (Tb) 3. theophyline 4. 3-methylxanthine (3-MX) 5. 1-methylxanthine (1-MX)
6. 1,3-dimethyluric acid (DMU) 7. 1-methyluric acid (1-MU)
50µg each

Figure 6: Separation of Theophyline and its Metabolites in Serum with Off-line Deproteinization



TSKgel NH_2-100, 3µm, 2.0mm ID x 15cm

A: Supernatant of serum deproteinated with 10-fold ACN

B: Supernatant of spiked serum deproteinated with 10-fold ACN

Eluent:	A: 0.1mol/L Triethylamine-Formic acid (pH10.0)/ACN (5/95)
	B: 0.1mol/L Triethylamine-Formic acid (pH10.0)/ACN (50/50)
Gradient:	0 min-2min (0% B) 2-30 min (80% B) 30-32min (0% B)
Flow rate:	0.25mL/min
Injection vol.:	10µL
Temperature:	40ºC
Detection:	UV@254 nm
Samples:	1. theophyline 2. 3-methylxanthine 3. 1,3-dimethyluric acid
	4. 1-methyluric acid
	(concentration: 10µg/mL for each)

Conclusions

- Organic solvent effect on the retention behavior of sugars using a TSKgel NH_2-100, 3µm HILIC column was investigated. Ethanol and acetone were utilized for an organic modifier instead of acetonitrile. Lower concentration of ethanol was needed to achieve similar retention times because the polarity was higher than that of the other solvents.

- Although the elution order of pyridylaminated (PA) sugars on the TSKgel NH_2-100, 3µm HILIC column was different from that on an ODS column, two acidic PA sugars were completely separated on the TSKgel NH_2-100 column due to an additional ionic interaction. Many water soluble vitamins eluted earlier from the ODS column with poor resolution, whereas excellent separations on the TSKgel NH_2-100 HILIC column were obtained due to stronger retention for hydrophilic compounds.

- On-line drug monitoring was investigated. Theophyline and its metabolites were separated by using a column switching technique. However, hydrophobic compounds which cannot be strongly retained, eluted from the column with broader peak shapes at high concentrations of ACN. Trace amounts of samples were carried over to the next analysis. Further optimization of the operating conditions would be necessary.

- In the case of the off-line deproteinization, the spiked samples could be separated successfully. A TSKgel NH_2-100, 3µm HILIC column can be useful for analyzing hydrophilic compounds in blood and is applicable to the separation of other hydrophilic drugs.