YNU

Dual-Mode Gradient Low-Capacity Cation-Exchange Chromatography of 14 Nucleo-Bases and Nucleosides

Shuji Torii [1], Ryuichi Sugimoto[2], Akihiro Shimura [2], Yukio Yokoyama [1]

[1] *Yokohama National University*, [2] *Mitsubishi Chemical Corporation*

Objective

The simultaneous determination of nucleo-bases and nucleosides is very important and substantially useful for the chemical diagnosis, pharmaceutical and food sciences. In general, the reversed-phase HPLC is often used for the purpose, but their chromatographic duration time is relatively long [1]. This time, we developed a new low-capacity cation-exchange resin selective to organic cations by sulfo-functionalizing poly(ethylstyrenedivinylbenzene) copolymers. This paper presents simultaneous separation and determination of nucleo-bases and nucleosides by dual-mode gradient HPLC technique with diode array detection. The application to the analyses of some beer and beer-like beverages is also presented.

[1] S. Hou, M. Ding, Anal. Sci. 26 (2010) 111–114.

Instrumentation

Pump: SHIMADZU LC-20AD ×2
Degasser: SHIMADZU DGU-$20A_3$
Data processing: SHIMADZU LCsolution data acquisition program
Diode array detector: SHIMADZU SPD-M20A
Column oven: SHIMADZU CTU-20AC

Analytes and samples

Analytes: Uracil (Ura), xanthine (Xan), thymine (Thy), hypoxanthine (Hyp), guanine (Gua), cytosine (Cyt), adenine (Ade), uridine (Urd), xanthosine (Xao), thymidine (dT), inosine (Ino), guanosine (Guo), cytidine (Cyd) and adenosine (Ado) were used.

Samples: Five beer and beer-like beverages was subjected to the analysis. All samples were degassed for 30 min using an ultrasonic bath, and then filtered through a 0.20-μm membrane filter prior to injection.

Results and discussion

It was found through a careful optimization study that the KT4 column showed excellent selectivity to the organic cations and could separate the 14 analytes in 9min as shown in **Fig.1**, using a binary gradient program was listed in **Table 1**. However, the separation between Xao and Thy was unsatisfactory.

In order to improve the separation, a dual-mode gradient elution program was considered and optimized by controlling column temperature in addition to the solvent delivery ratio, as given in **Table 2**, which enabled to separate of the 14 analytes in 9 min with a resolution of 1.0 for the critical peak pair as shown in **Fig.2**.

The method was very repeatable and quantitative as given in **Table 3**. The method was applied to the analysis of some beer and beer-like beverages. **Fig.3** and **Table 4** give analytical results for the samples.

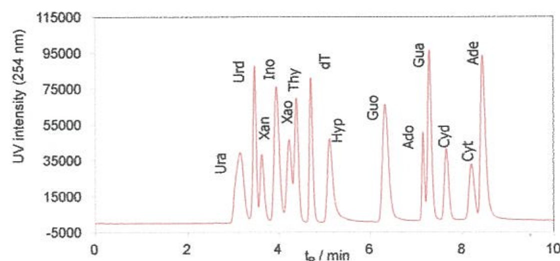

Fig. 1 Linear gradient chromatogram of 14 analytes at 45 °C. The cycle time was less than 15 min.

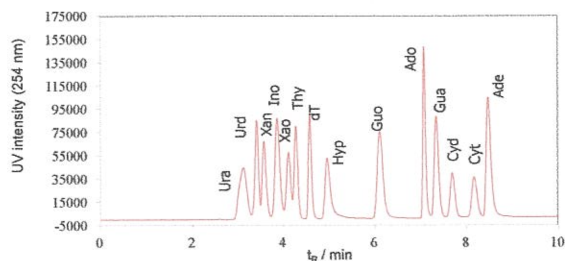

Fig. 2 Dual-mode gradient chromatogram of 14 analytes. The cycle time was 15 min.

Table 3 Quantification data

Analyte	Retention time [a] % RSD (n = 5)		Area intensity [b] % RSD (n = 5)		Test range (μmol/L)	Linearity R^2	Detection limit [c] (μmol/L)
	Intra - day	Inter - day	Intra - day	Inter - day			
Ura	0.18	0.086	0.27	0.28	0.5 – 50	0.9999	0.33
Urd	0.15	0.40	0.40	0.92	0.5 – 50	0.9996	0.14
Xan	0.20	0.29	0.74	0.76	0.5 – 50	0.9999	0.21
Ino	0.35	0.30	0.48	0.83	0.5 – 50	0.9994	0.16
Xao	0.17	0.40	0.41	1.5	0.5 – 50	0.9995	0.23
Thy	0.21	0.26	0.65	0.97	0.5 – 50	0.9996	0.17
dT	0.20	0.26	0.58	0.87	0.5 – 50	0.9992	0.15
Hyp	0.068	0.091	0.49	1.1	0.5 – 50	0.9994	0.27
Guo	0.59	0.065	0.68	0.50	0.5 – 50	0.9990	0.19
Ado	0.041	0.072	0.29	0.41	0.5 – 50	0.9991	0.089
Gua	0.11	0.17	0.42	0.39	0.5 – 50	0.9995	0.16
Cyd	0.11	0.052	0.85	0.65	0.5 – 50	0.9999	0.35
Cyt	0.10	0.12	0.62	0.79	0.5 – 50	0.9990	0.36
Ade	0.097	0.056	0.77	0.46	0.5 - 50	0.9993	0.13

[a] of the data on the sample size of 50 μmol/L × 20 μL. [b] of the data on the sample size of 50 μmol/L × 20 μL. [c] calculated on S/N = 3.

Chromatographic condition

The binary gradient elution was carried out by mixing two solvents A: 15 mM H_3PO_4 /7.0(v/v)% CH_3CH and B: 20 mM NaH_2PO_4/30(v/v)% CH_3CN with solvent delivery program as given in **Table 1,** and with a dual-mode gradient program as given in **Table 2**. Other chromatographic conditions were: column temperature, 45 °C fixed or 44 and 50 °C for the dual-mode gradient elution; monitoring wavelength, 190–300 nm; quantification wavelength, 254 nm; sample size, 20 μL; and the flow rate, 0.8 mL/min.

The analytical column used was a prototype low-capacity cation-exchange column (150 mm × 4.6 mm i.d. particle size 3.67 μm), packed with a sulfonated macroreticular poly(ethylstyrenedivinylbenzene), which was manufactured by Mitsubishi Chemical Corporation (Tokyo, Japan), referred to as KT4 (development code).

Table 1 Linear gradient program on constant column temperature at 45 °C

Time (min)	Solvent A [a] (%)	Solvent B [b] (%)
0.00 → 2.30	100 →40	0 → 60
2.31 → 3.30	40	60
3.31 → 4.00	40 → 20	60 → 80
4.01 → 5.00	20 → 5	80 → 95
5.01 → 8.00	5	95
8.01 → 9.00	100	0

Table 2 Dual-mode gradient program

Time (min)	Solvent A [a] (%)	Solvent B [b] (%)	Column temperature (°C)
0.00 → 0.30	100 → 40	0 → 60	44
0.31 → 2.20			50
2.21 → 3.30	40	60	
3.31 → 4.00	40 → 30	60 → 70	
4.01 → 5.00	30 → 5	70 → 95	44
5.01 → 8.00	5	95	
8.01 → 9.00	100	0	

[a]: 15 mM H_3PO_4/7(v/v)% CH_3CN. [b] 20 mM NaH_2PO_4/30(v/v)% CH_3CN

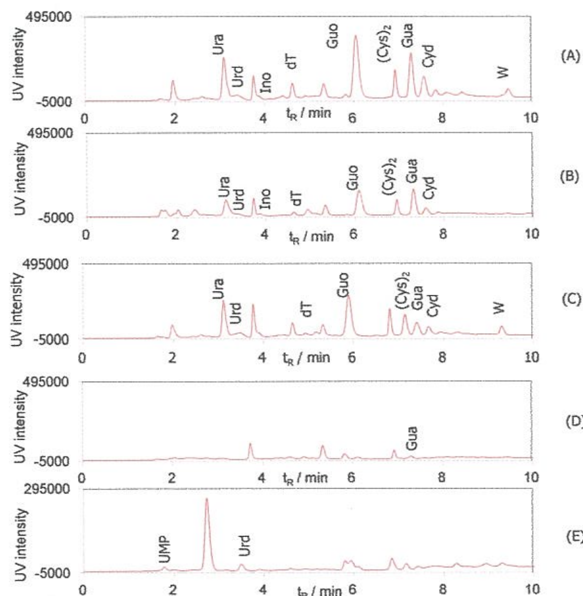

Fig. 3 Typical chromatograms for (A) beer, (B) low-malt beer, (C) beer-like liqueur, (D) low purine liqueur, and (E) non-alcohol beer. All assigned peaks were identified by their UV absorption spectra individually.

Table 4 Analytical concentration of compounds found in beverages

Analyte	A (mg/L)	B(mg/L)	C(mg/L)	D(mg/L)	E(mg/L)
Ura	16.7	7.2	15.3		
Urd	8.6	5.4	10.1		2.8
Ino	2.8	1.9	10.2		
dT	16.0	3.9			
Guo	64.6	27.7	40.4		
Gua	22.0	12.7	10.6	1.2	
Cyd	4.1	12.5	26.6		

Summary

The low-capacity cation-exchange column was selective to nucleo-bases and nucleosides. The simultaneous separation and determination of 14 nucleo-bases and nucleosides was relatively simple and fast, which was applicable to the analyses of beer and beer-like beverages. The 14 analytes was successfully separated in 9 min, which is three times faster than those seen in the latest report [1].

12th ASIANALYSIS 2013 Japan

Correspondence: S. Torii, torii-shuji-rj@ynu.ac.jp