Simultaneous Analysis of Dyes by HPLC-DAD

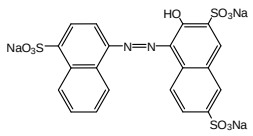
Food dyes, when added to food or drinks, play an important role of enhancing palatability. Food dyes are largely divided into natural dyes and synthetic dyes. An analysis example for six synthetic dyes is introduced here.
Six dyes analyzed here have maximum UV absorption at different wavelengths and therefore, DAD (diode array detector) was used to analyze them simultaneously. By using DAD, a chromatogram can be extracted at the optimal wavelength for each dye.
As a spectrum of a standard sample can be compared with that of a target component for confirmation, more accurate quantitative analysis is possible.

Standard samples and Structural Formulas

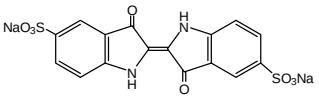
Abbrev.	English name	Extraction wavelength
R2	Amaranth	530 nm
B2	Indigo Carmine	620 nm
Y5	Sunset Yellow	480 nm
B1	Brilliant Blue FCF	620 nm
R3	Erythrosine	530 nm
R106	Acid Red 52	530 nm

[Preparation of Standard Sample]
5.0 mg of each sample was weighed and the volume was made up to 50 mL with purified water to make a 100 mg/L solution. The analytical samples for the calibration curve were prepared by diluting the standard sample to different concentrations with Eluent (A).

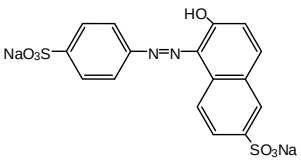
R2 : $C_{20}H_{11}N_2Na_3O_{10}S_3$ = 604.46



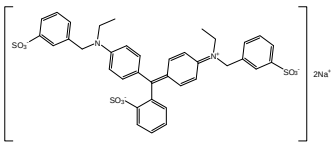
B2 : $C_{16}H_8N_2Na_2O_8S_2$ = 466.35



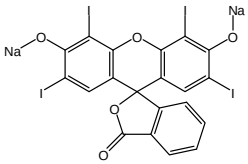
Y5 : $C_{16}H_{10}N_2Na_2O_7S_2$ = 452.36



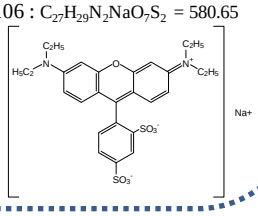
B1 : $C_{37}H_{34}N_2Na_2O_9S_3$ = 792.85



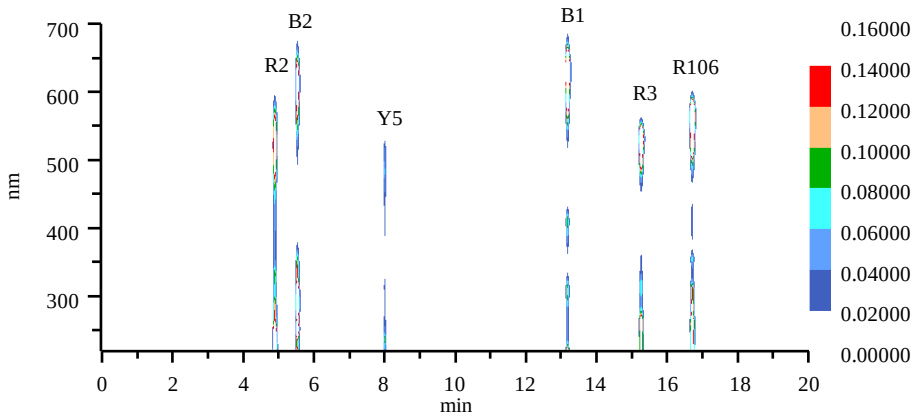
R3 : $C_{20}H_6I_4Na_2O_5$ = 879.86



R106 : $C_{27}H_{29}N_2NaO_7S_2$ = 580.65



Analysis result of standard samples



[Contour indication (50 mg/L each)]

[Analytical conditions]

Column	: HITACHI LaChrom C18 (3 µm) 4.6 mm I.D. × 150 mm	Flow rate	: 1.0 mL/min
Eluent	: (A) 10 mmol/L ammonium acetate / CH_3CN = 95/ 5 (B) 10 mmol/L ammonium acetate / CH_3CN = 50 / 50	Column temp.	: 40°C
		Detection	: DAD 254 nm, 480 nm, 530 nm, 620 nm
*Gradient	(0 min) B 2 % → (21 min) B 100 % → (27 min) B 100 %	Injection vol.	: 10 µL

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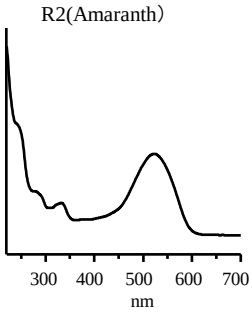
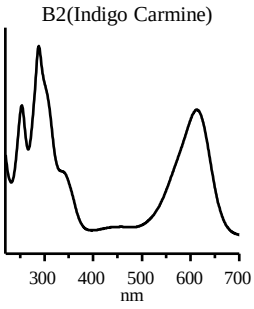
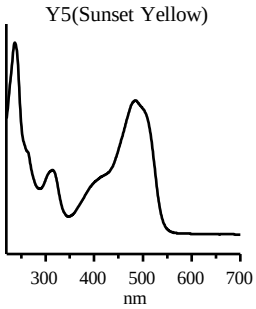
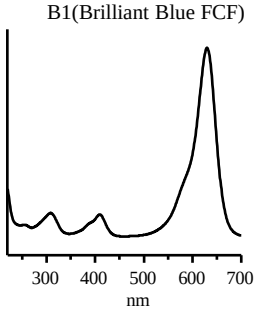
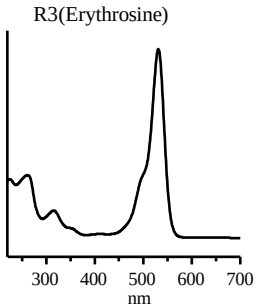
■Confirmation of Spectrum

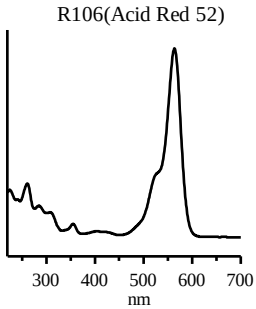




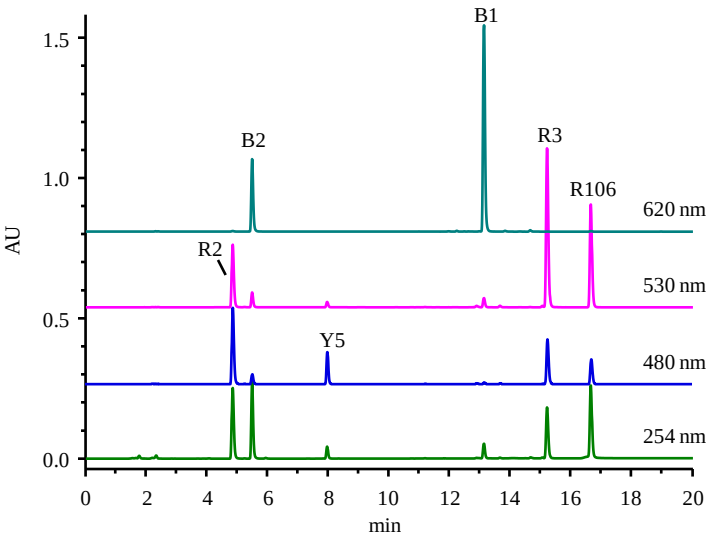








■Chromatogram Extracted at Optimal Wavelength



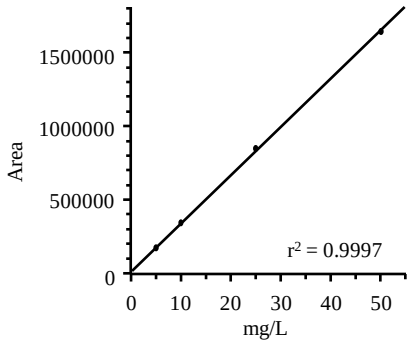
[Extraction chromatogram (50 mg/L each)]

Six dyes analyzed here have different maximum UV absorption wavelengths. Therefore, DAD was used for the analysis and a chromatogram was extracted at the optimal wavelength for each dye.
By using DAD, components having different optimal wavelengths can be analyzed simultaneously.

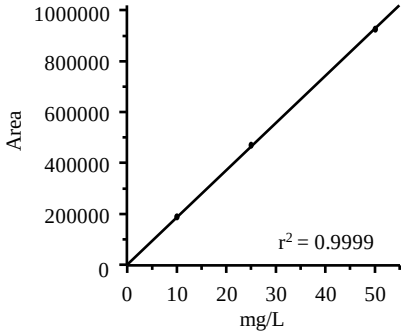
■ Linearity

e.x B1(Brilliant Blue FCF) R106(Acid Red 52)

B1(Brilliant Blue FCF) / 620 nm



R106(Acid red 52) / 530 nm



All of the calibration curves (all components in the range from 0.5 to 50 mg/L) exhibited high linearity, with $r^2 = 0.999$ or more

System configuration : Primaide 1110 Pump, 1210 Auto Sampler, 1310 Column Oven, 1430 DAD

NOTE : These data are an example of measurement; the individual values cannot be guaranteed.
The system is for research use only, and is not intended for any animal or human therapeutic or diagnostic use.