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Application News

No.L471

High Performance Liquid Chromatography

Comprehensive Two-Dimensional Analysis of Polyphenols in Red Wine Using Nexera-e Coupled with SPD-M30A

Phenolic compounds are created as secondary metabolites in plants. In many cases, their structures contain multiple aromatic rings and a hydroxyl group. These polyphenols are known to display antioxidant effects, and are said to be effective in preventing arteriosclerosis and cerebral infarction. Red wine is known to contain large amounts of polyphenols, including flavonoids and phenolic acids, and in recent years has attracted considerable attention due to the health benefits associated with these substances. Regarding the analysis of polyphenols in red wine, separation and quantitation of these polyphenols by simultaneous analysis using HPLC is difficult due to the presence of many coexisting substances. The Nexera-e comprehensive two-dimensional liquid chromatograph can be very effective for such an analysis.

■ **Comprehensive Two-Dimensional Separation with SPD-M30A at Appropriate Wavelengths for Respective Analytes**

By using the Nexera-e with a photodiode array detector (PDA), a target component can be separated from a complicated mixture of coexisting substances, and then quantitated, all in a single analysis. In the analysis, assuming ethyl gallate, tyrosol and rutin as the target compounds, the ChromSquare dedicated software was applied to generate a contour plot using the respective optimal wavelengths, thereby permitting both separation and quantitation. Fig. 1 shows the contour plots at the respective optimal wavelengths, along with the 1D and 2D gradient profiles generated using the auto-gradient feature.
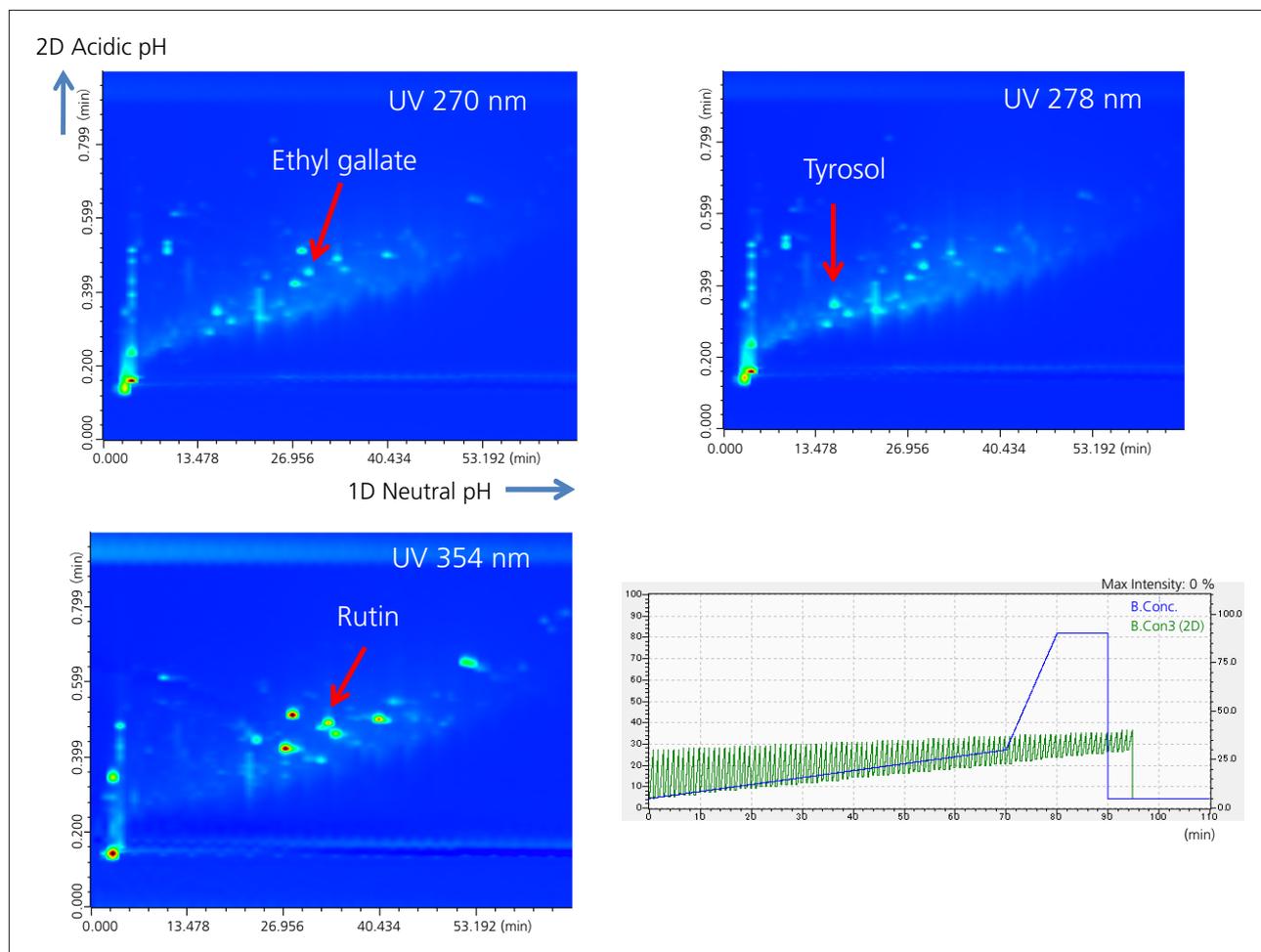

Fig. 1 Comparison of Comprehensive-2D Separation of Commercial Red Wine with Appropriate Wavelength Obtained Using "Auto-Gradient" Profile Feature

■ Quantitation of Ethyl Gallate, Rutin, and Tyrosol in Red Wine

The analytical conditions are shown in Table 1. We attempted to conduct separation by reversed-phase × reversed phase using a neutral phosphate buffer solution in the 1st dimension, and an acidic phosphate buffer solution in the 2nd dimension.
The calibration curve for each substance was generated using 6 concentrations in the range of 5 - 250 mg/L. The red wine sample was filtered through a 0.22 μm membrane filter, and then injected. Fig. 2 shows the calibration curve for ethyl gallate. Also, Table 2 shows the repeatability of the total and 2D retention times, respectively, for the three analytes, the blob area repeatability values corresponding to the peak areas, the calibration curve coefficient of determination (R^2) values, and the respective quantitation values.

Table 1 Analytical Conditions

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1D Column	: Shim-pack XR-ODS Ⅱ (100 mm L. × 1.5 mm I.D., 2.2 μm)
Mobile Phase	: A : 10 mM (sodium) phosphate buffer pH = 6.8
	B : acetonitrile
Flowrate	: 0.05 mL/min
Time Program	: B Conc. 5 % (0 min) → 30 % (70 min) → 90 % (80 min) → 90 % (90 min)
	→ 5 % (90.1 min) → STOP (110 min)
Column Temp.	: 40 °C
Injection Vol.	: 3 μL
Loop Vol.	: 50 μL (Modulation time: 60 sec)
2D Column	: Kinetex XB-C18 (50 mm L. × 3 mm I.D., 2.6 μm)
Mobile Phase	: A : 10 mM (sodium) phosphate buffer pH = 2.6
	B : acetnitrile
Flowrate	: 2 mL/min
Time Program	: Auto-gradient: Initial. B Conc. 5 % (0 min) → 30 % (0.75 min) → 5 % (0.76 min)
	→ STOP (1 min)
	Final. B Conc. 30 % (0 min) → 40 % (0.75 min) → 30 % (0.76 min) → STOP (1 min)
	The initial and final B Conc. were changed in a stepwise manner.
Detector	: SPD-M30A photodiode array detector (high sensitivity cell 1 μL, wavelength = 270 nm, 278 nm, 354 nm)
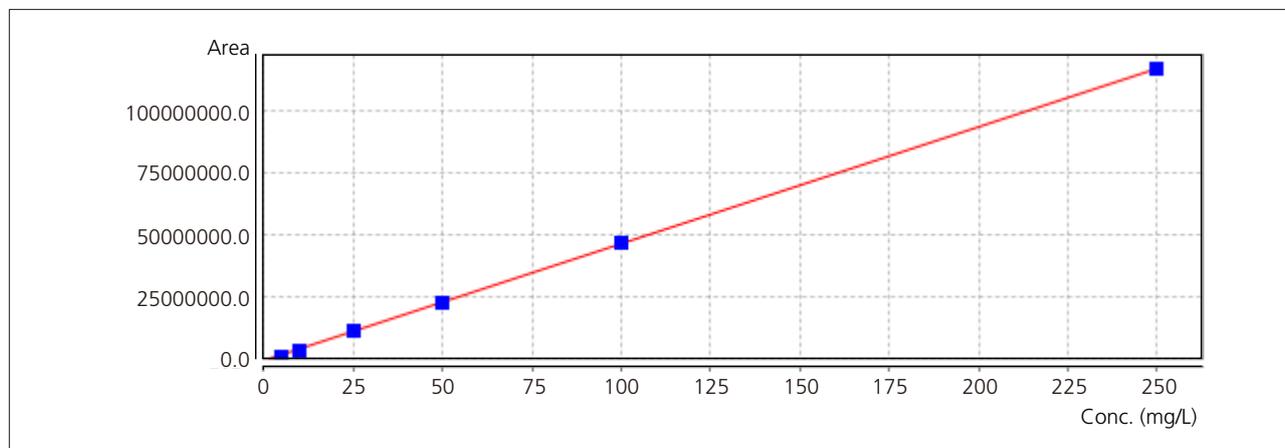
Fig. 2 Calibration Curve for Ethyl Gallate

Table 2 Repeatability of Retention Time and Blob Area (%RSD, n = 5), R^2 Value and Concentration (mg/L)

Compound	Total retention time	Retention time (2D)	Area		R squared	Concentration
Tyrosol	0.007	0.159	2.7		0.999804	101.5
Ethyl gallate	0.007	0.49	3.8		0.999864	15.1
Rutin	0.007	0.52	4.1		0.998805	14.2

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