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Application News

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High Performance Liquid Chromatography

Analysis of Kakkonto by Nexera-e and SPD-M30A Photodiode Array Detector (Part 1)

Kakkonto (traditional Chinese medicine) is believed to be effective against cold, headache, stiff shoulders, etc. because it has the properties to promote sweating and provide anti-inflammatory and analgesic action. It consists of puerariae radix, ephedra, licorice, paeoniae radix, etc. It contains many compounds such as ephedrine, glycyrrhizic acid, and cinnamic acid. The Nexera-e comprehensive two-dimensional liquid chromatograph is useful to separate such a complex sample.

In general, different separation modes are selected for 1st and 2nd dimension for comprehensive two-dimensional chromatography. That can enable a high resolution which is not achieved by each individual separation mode. Here, we tried a comprehensive separation by using a semi-micro reversed-phase column and neutral pH mobile phase for 1st dimension and a ultra-high speed reversed-phase column and acidic pH mobile phase for 2nd dimension. Herbal medicines contains many relatively-polar compounds, so pH is said to be important parameter. Glycyrrhizic acid (See the red arrow in Fig. 1) was quantitated.

Improved Separation by Auto-Gradient Program Function

The composition of organic solvent in fractions that are eluted from 1st dimension and introduced to 2nd dimension change according to gradient of 1st dimension. The composition might cause distortion of peak shape in the 2nd dimension because of the solvent's elution power. To prevent such a problem, the auto-gradient program function, which gradually changes the initial and final concentration of 2nd dimension gradients is used. The left figure of Fig. 1 shows the contour plot obtained by the gradient whose initial and final concentration are kept constant and the right figure shows the contour plot obtained by the gradient whose initial and final concentration are gradually changed. Improved separation for glycyrrhizic acid was observed.

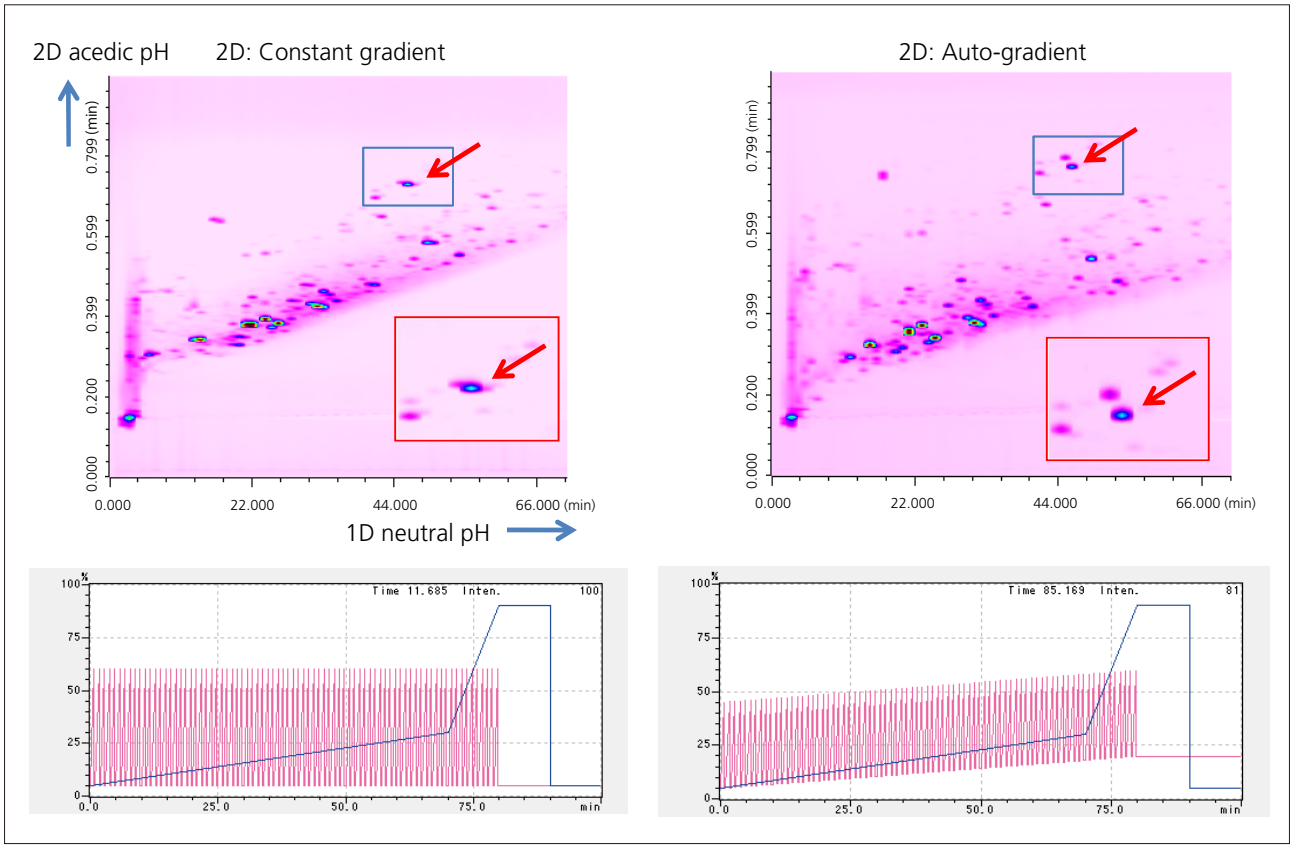

Fig. 1 Comprehensive-2D Separation of Commercial Kakkonto (Traditional Chinese Medicine) Product Obtained with/without "Auto-Gradient Program Function"

Repeatability Test of Peak Retention Time and Peak Area, and Quantitation of Glycyrrhizic Acid in Kakkonto

Table 1 shows the analytical conditions. Neutral and acidic phosphate buffer were used for 1st and 2nd dimensions, respectively. Fig. 2 shows sample preparation.

Glycyrrhizic acid, an active ingredient in Kakkonto extract, was detected at UV 254 nm (See the red arrow in Fig. 1). Fig. 3 shows the calibration curve from 50 to 1000 mg/L and Table 2 shows repeatability of five replicate analyses for the blob area which corresponds to the peak volume and linearity of the calibration curve. Glycyrrhizic acid was quantitated as 608.4 mg/L.

Table 1 Analytical Conditions

1D Column	: Shim-pack XR-ODS Ⅱ (100 mm L. × 1.5 mm I.D., 2.2 µm)
Mobile Phase	: A: 10 mmol/L (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.	: 2 µL
Loop Vol.	: 50 µL (Modulation time : 60 sec)
2D Column	: Phenomenex Kinetex XB-C18 (50 mm L. × 3 mm I.D., 2.6 µm)
Mobile Phase	: A: 10 mmol/L (sodium) phosphate buffer pH= 2.6 B: acetonitrile
Flowrate	: 2 mL/min
Time Program	: Without Auto-gradient: B Conc. 5 % (0 min) → 60 % (0.75 min) → 5 % (0.76 min) → STOP (1 min) With Auto-gradient: Initial. B Conc. 5 % (0 min) → 45 % (0.75 min) → 5 % (0.76 min) → STOP (1 min) Final. B Conc. 20 % (0 min) → 60 % (0.75 min) → 20 % (0.76 min) → STOP (1 min) The initial and final B conc. has been changed by a stepwise method
Detector	: SPD-M30A Photo diode array detector (standard cell 1 µL, wavelength = 254 nm)

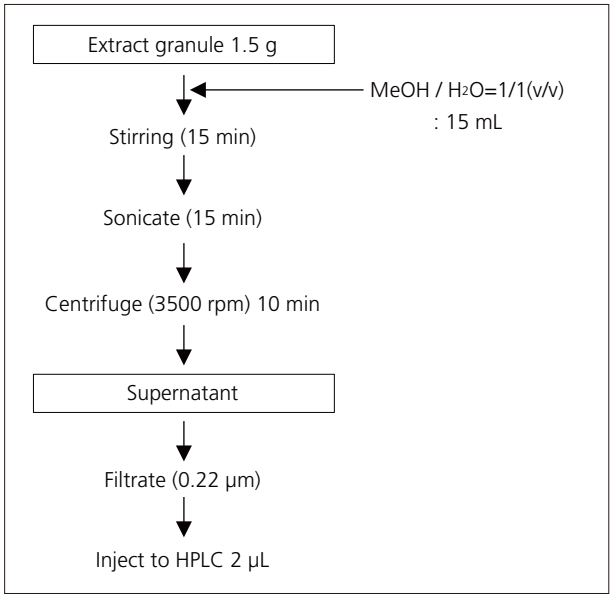

Fig. 2 Sample Preparation

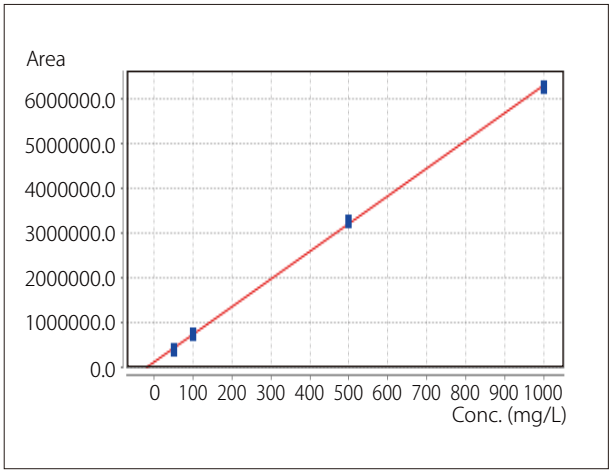

Fig. 3 Calibration Curve of Glycyrrhizic Acid

Table 2 Repeatability of 5 Replicate Analyses in %RSD and Linearity of 50-1000 µg/L for Glycyrrhizic Acid

Compound	Total retention time	Retention time (2D)	Area	R squared
Glycyrrhizic acid	0.007	0.37	5.4	0.999778

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