LAAN-A-LC-E169

SHIMADZU

Application News

High Performance Liquid Chromatography No.L390

High Speed with High Resolution Analysis (Part 29) High Efficiency Analysis of Ferulic Acid in Berry Juice Using Automated Column Switching System

Typically, when conducting high-speed micro-level analysis of samples containing many impurities, sample preparation and setting of the analytical conditions often require a great deal of time and effort. Here, we introduce an example of analysis of ferulic acid in berry juice using a system constructed specifically for efficient handling of this application. Shimadzu's Prominence UFLC ultra fast LC system forms the base of the system, which also includes a column switching system comprising the first dimension separation column, a trap column, and the second dimension separation column.

■ Principle of the System

A flow diagram of the column switching system used for this application is shown in Fig. 1. The sample injected from the autosampler is first directed through the flow path (solid line) which includes Column-1 (first dimension separation column) for separation of the sample components. The target compounds eluted from Column-1 are directed to Column-2 by switching of the valve to position 2. The eluent from Column-1 is diluted by the mobile phase coming from Pump-2 to allow effective sample concentration on Column-2 (trap column). After concentration, the valve turns back to position 1 and Pump-3 activates to direct flow through Column-3 (second dimension separation column, dotted line), where additional separation is achieved.

Fig. 2 shows the results of analysis of 3 phenolic acids including the compound of interest, ferulic acid, and Table 1 shows the analytical conditions used. In this case, after ferulic acid is eluted from Column-1 over about 0.25 minutes, it is concentrated in Column-2 and then introduced into Column-3. The mobile phase used for the first dimension (Column-1) has a different pH than used for the second dimension (Column-3).

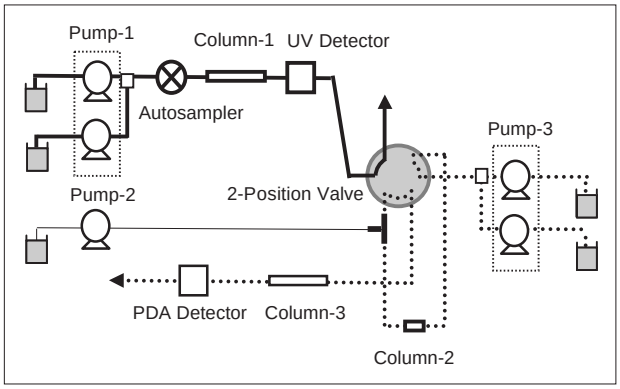

Fig. 1 Flow Diagram

Table 1 Analytical Conditions

[Column-1]	: Shim-pack XR-ODS (75 mm L. × 4.6 mm I.D., 2.2 µm)
Mobile Phase	: A: 20 mmol/L (Sodium) phosphate buffer (pH 2.5)
	B: Acetonitrile, A / B = 75 / 25 (v/v)
Flow Rate	: 1.5 mL/min (0-1.6 min)
Column Temp.	: 40 ˚C
Injection Volume	: 4 µL
Detection	: SPD-20A (314 nm) with Semi-micro Cell
[Column-2]	: Shim-pack GVP-ODS (10 mm L. × 4.6 mm I.D., 4.6 µm)
Mobile Phase	: 20 mmol/L (Sodium) phosphate buffer (pH 2.5)
Flow Rate	: 0.2 mL/min (0-1.36 min), 4.5 mL/min (1.37-1.8 min)
[Column-3]	: Shim-pack XR-ODS (75 mm L. × 4.6 mm I.D., 2.2 µm)
Mobile Phase	: A: 50 mmol/L (Ammonium) acetate buffer (pH 4.7)
	B: Acetonitrile, A / B = 80 / 20 (v/v)
Flow Rate	: 1.0 mL/min
Detection	: SPD-M20A (314 nm) with Semi-micro Cell

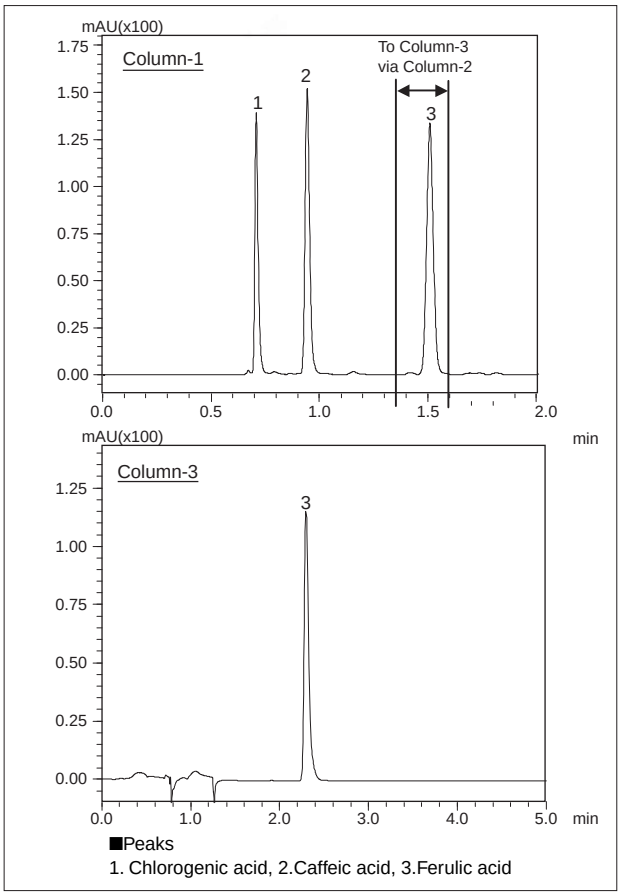

Fig. 2 Chromatograms of a Standard Mixture of 3 Phenolic Acids (50 mg/L each)

■ Linearity and Repeatability

Separation is conducted sequentially by Column-1 and then Column-3 in this analysis, and the time required to complete the analysis was about 7 minutes for one cycle through both columns. Valve switching and other operations are set within the software time program, thereby enabling automated, continuous analysis.
Fig. 3 shows the calibration curve of ferulic acid over concentration range of 0.04-20 mg/L. The correlation coefficient (R^2) was an excellent 0.99999, and the peak area repeatability for the concentration of 20 mg/L was 0.21 % RSD (n = 6).

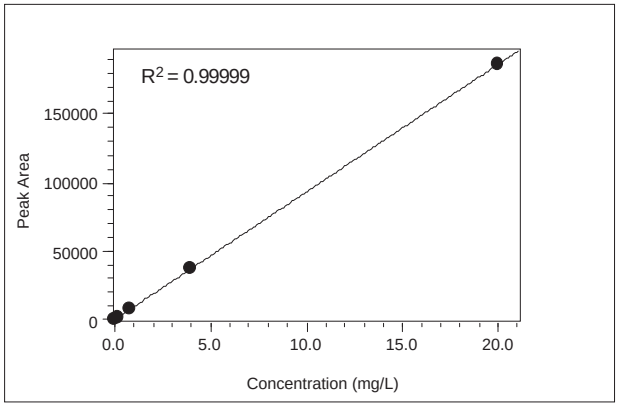

Fig. 3 Calibration Curve of Ferulic Acid

■ Analysis of Berry Juice

Fig. 4 shows the results of analysis of ferulic acid in commercially available berry juice using this system. After filtering the sample through a 0.22 μm membrane filter, 4 μL was injected.
The ferulic acid elution segment of the Column-1 chromatogram was separated into several peaks as shown in the chromatogram of Column-3, confirming the efficiency of the analysis. The peak area repeatability (n = 6) after separation by Column-3 was 0.6 % RSD.

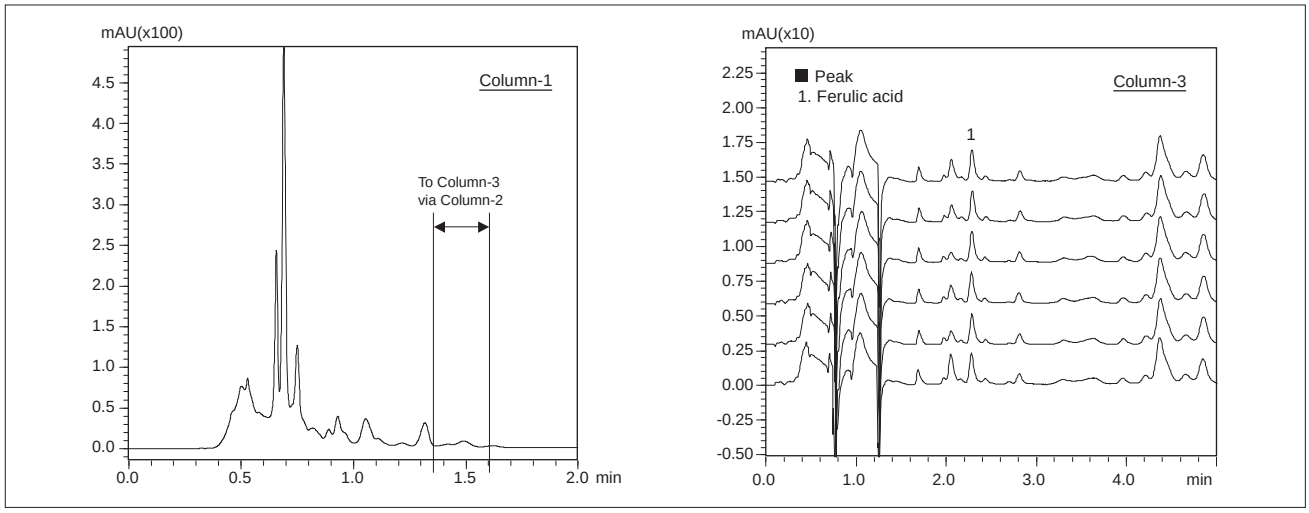

Fig. 4 Chromatograms of Berry Juice

In addition, Fig. 5 shows overlaid absorption spectra of the ferulic acid peak (Column-3) and that of a ferulic acid standard substance obtained using the SPD-M20A photodiode array detector. The close correlation of these absorption spectra is unmistakably clear, demonstrating the effectiveness of the trap column for isolating ferulic acid.

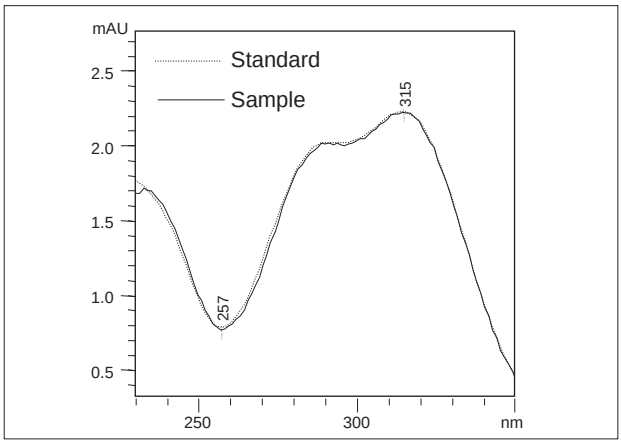

Fig. 5 Peak Spectra

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