Preparative Separation of Active Components from Natural Products using Low-pressure Gradient Preparative HPLC

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Introduction

Natural products contain a variety of active components. Many medicines have their origin in natural products and it is said that they account for 50% of total medicines. Preparative HPLC is used to obtain active components from complex mixture such as natural products. In this poster, we will show a preparative separation of rosemary. Rosemaries have several effects such as odor elimination, antibacterial activity, and antioxidant activity, so it has been used for meat dishes in Europe for a long time. Rosemaries contain rosmarinic acid, carnosic acid, and carnosol among their active components. Rosmarinic acid has the ability to control allergic symptoms. Carnosic acid and carnosol exert a revitalizing influence on biological defense mechanisms

and enhance detoxification. It is also reported that carnosic acid has the ability to improve memory performance and to facilitate creation of nerve growth promoting substances which has a significant role in maintaining nerve cells. We used a newly released low-pressure gradient preparative HPLC system in this study. This system enables combination of four mobile phases, so it is useful for method development. Shim-pack PREP-ODS was used as the column for preparative separation and water, methanol and 2% formic acid water solution were used as mobile phases. As a result, rosmarinic acid, carnosic acid, and carnosol were successfully separated from other components.

Materials

Reagents and standards

Reagents: Rosmarinic acid, Carnosol, Carnosic acid, acetophenone, propiophenone, butyrophenone, valerophenone, and formic acid were purchased from Sigma-Aldrich. Water was made in house using a Millipore Milli-Q Advantage A10 Ultrapure Water Purification System. Methanol was purchased from Honeywell.
Samples: 10mg of acetophenone, propiophenone,

butyrophenone, and valerophenone were dissolved in 10mL of methanol. It was diluted 10 times with water and transferred to a 1.5 mL vial for analysis.
Rosemary was purchased from a local supermarket. 1g of the rosemary was extracted with 10mL of methanol and then the supernatant was filtered using a 0.45 µm filter and transferred to a 1.5 mL vial for analysis.

System

The alkylphenone mixture and active components were separated using a Shimadzu Prominence low-pressure gradient preparative HPLC system consisting of LC-20AP preparative pump, FCV-200AL low-pressure gradient unit,

DGU-10B helium degassing unit, SIL-10AP autosampler, SPD-20AV UV-VIS detector, and a CBM-20A system controller.

Fig.1   Prominence low-pressure gradient preparative HPLC system

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Results

Reproducibility test

Reproducibility of the Prominence low-pressure gradient preparative HPLC system was tested using a 100 mg/L alkylphenone mixture. Table 1 shows the analytical conditions and Fig. 2 shows the chromatograms. Table 2 shows the reproducibility of retention time of alkylphenone mixture (n=6). As a result, less than 1% relative standard deviation of retention time was obtained.

Table 1 Analytical Conditions

System	: Prominence low-pressure gradient preparative HPLC System
Column	: Shim-pack PREP-ODS (150 mm L. x 20 mm I.D., 15 μm)
Mobile Phase	: A: Water
	B: Methanol
Time Program	: B Conc. 60 % (0 min) - 100 % (15 min) - 60 % (15.01-20 min)
Flow Rate	: 20 mL/min
Column Temperature	: Ambient
Injection Volume	: 200 μL
Detection	: 245 nm
Cell	: Cell for SPD-10Avp
Sample	: Alkylphenone mixture (100 mg/L each)

■ Peaks
1. Acetophenone, 2. Propiophenone, 3. Butyrophenone, 4. Valerophenone
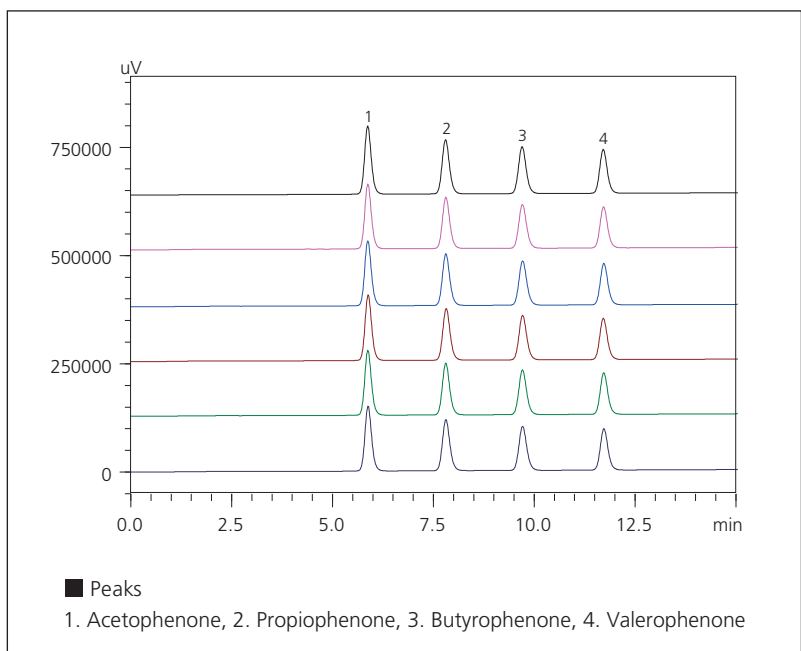
Fig.2   Chromatograms of repeated analysis of an alkylphenone mixture

Table 2   Reproducibility of retention time of an alkylphenone mixture

	Peak 1	Peak 2	Peak 3	Peak 4
1st	5.872	7.801	9.699	11.711
2nd	5.876	7.810	9.708	11.719
3rd	5.875	7.809	9.712	11.726
4th	5.883	7.818	9.712	11.713
5th	5.876	7.807	9.709	11.724
6th	5.881	7.810	9.711	11.727
Average	5.877	7.809	9.709	11.720
Std Dev	0.004	0.005	0.005	0.007
%RSD	0.069	0.070	0.051	0.058

Separation of Active Components in Rosemary Extract

Table 3 shows the analytical conditions. We set water, methanol, and 2% formic acid in water as mobile phase A, B, and C respectively. The FCV-200AL low-pressure gradient unit enables gradient elution using up to four solvents. By setting the mobile phases as mentioned above, one can set any concentration of formic acid one would like without changing mobile phases. Fig. 3 shows the chromatogram of rosemary extract. The active components were successfully separated from other components.

Table 3 Analytical Conditions

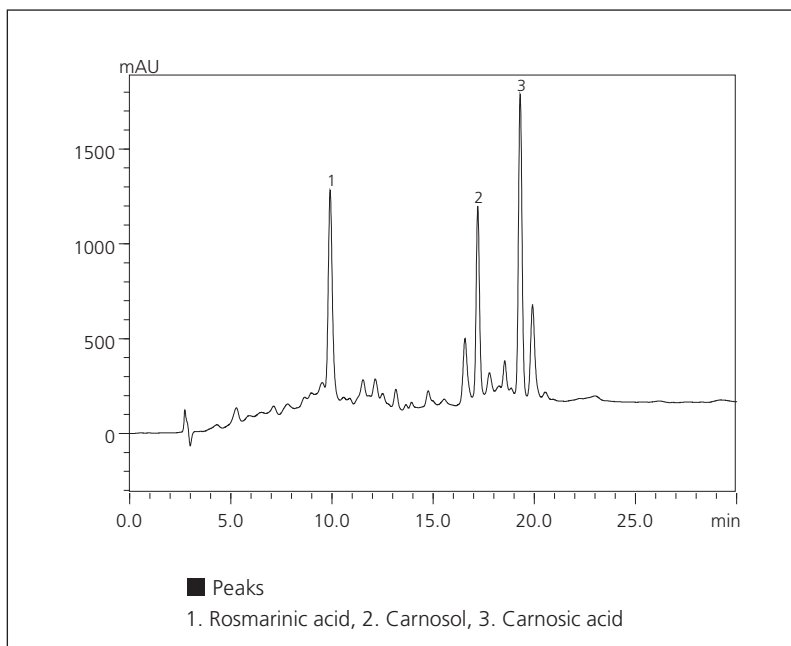|	
System	: Prominence low-pressure gradient preparative HPLC System
Column	: Shim-pack PREP-ODS (150 mm L. x 20 mm I.D., 15 μm)
Mobile Phase	: A: Water
	B: Methanol
	C: 2% Formic acid in water
Time Program	: B Conc. 30 % (0 min) - 95 % (15-30 min) - 30 % (30.01-45 min)
	C Conc. 5%
Flow Rate	: 20 mL/min
Column Temperature	: Ambient
Injection Volume	: 200 μL
Detection	: 230 nm
Cell	: Cell for SPD-10Avp
Sample	: Rosemary extract

Fig.3   Chromatograms of rosemary extract

Conclusions

1. Prominence low-pressure gradient preparative HPLC system enables reliable preparative isolation of active components from natural products.
2. Prominence low-pressure gradient preparative HPLC system facilitates method development by the use of up to four solvents.

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