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Short communication

Determination of magnolol and honokiol in Magnoliae Cortex using supercritical fluid chromatography on-line coupled with supercritical fluid extraction by on-column trapping

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Abstract

An assay of magnolol and honokiol in Magnoliae Cortex was established by on-line supercritical fluid chromatography (SFC) coupled with supercritical fluid extraction (SFE).

With an amino column as the trapping and separation column, magnolol and honokiol were extracted and focused at the column head as a narrow band, even if 5% methanol was added to the supercritical carbon dioxide as an entrainer. Addition of the entrainer improved extract efficiency, and recovery of the analytes was almost the same as for solvent extraction. Determination of analytes of interest in Magnoliae Cortex was achieved with only a few mg of sample. SFE was completed within ca. 1 min, and SFC was achieved within ca. 3 min. The consecutive procedures of extraction, concentration and analysis were completed within ca. 5 min. © 1997 Elsevier Science B.V.

Keywords: Magnoliae Cortex; Supercritical fluid extraction–supercritical fluid chromatography; Pharmaceutical analysis; Magnolol; Honokiol; Neo-lignans

1. Introduction

Supercritical fluid chromatography (SFC) can be used to separate thermally labile and high-molecular-mass samples unsuitable for gas chromatographic analysis, and, when compared to HPLC, it offers high separation efficiencies, shorter analysis times, and a wider range of detection possibilities [1]. The variety of stationary phases available for HPLC are available for packed column SFC. SFC coupled with supercritical fluid extraction (SFE) achieves rapid and efficient analysis by the consecutive extraction, concentration and separation. Greibrokk reviewed developments in the use of supercritical fluids in coupled systems [2]. We have published a rapid and efficient method for characterization of herbal medicine using SFE and SFC [3,4].

Magnoliae Cortex is a useful drug prescribed in many Japanese and Chinese traditional medicines as an anodyne, a sedative, a stomach medicine or a cough remedy. Neo-lignans including magnolol and honokiol are known to be components of Magnoliae Cortex. Since magnolol and honokiol have been reported to have various physiological effects, they are considered important and characteristic components of Magnoliae Cortex (Fig. 1). Analytical

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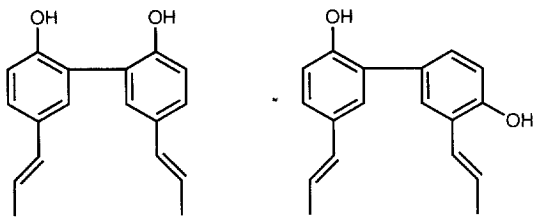

Fig. 1. Structures of magnolol and honokiol.

methods [5,6] using HPLC or GLC have been reported for their determination. However, these methods required manual extraction. Recently, SFE of neo-lignans and sesquiterpene from Magnolia species has been described [7–9].

In this report, SFE coupled with SFC by on-column trapping which directly traps SFE extracts at the head of an analytical column and the application of our coupled SFE–SFC system to the quantitative determination of magnolol and honokiol in Magnoliae Cortex are described.

In this on-line SFE–SFC method, the analytes of interest must be trapped effectively and quantitatively during SFE, and the trapped analytes must be focused at the analytical column head as a narrow band to obtain good chromatographic performance in SFC.

The goal of this study was the selection of the combination of the modifier and the packing material of the column. Combinations of the kind and the concentration of the entrainer or the modifier and the column were compared and optimized. The quantitative aspects of the method were studied.

2. Experimental

2.1. Plant material

The commercial Magnoliae Cortex used in this study was purchased from Alps Pharmaceutical (Gifu, Japan).

2.2. Chemicals and reagents

2.2.1. Solvent

Carbon dioxide was of a purity above 99.99% (Kanto Sanso, Tokyo, Japan). Methanol, ethanol and acetonitrile were of HPLC grade (Wako, Tokyo, Japan). Chloroform was of spectrosol grade (Wako).

2.2.2. Solute

Magnolol and honokiol standards for quantitative determination were purchased from Wako.

2.2.3. Apparatus

A supercritical fluid chromatograph Super 200 system 3 (Jasco, Tokyo, Japan) equipped with a photometer 875-UV (Jasco) was used.

2.2.4. Column

NH_2 column (Capcell Pak NH_2 (5 μm), 35 mm×4.6 mm I.D., Shiseido, Tokyo, Japan), CN column (Finepak CN-10P (10 μm), 50 mm×4.6 mm I.D., Jasco), SiO_2 column (Finepak SIL-5P (5 μm), 50 mm×4.6 mm I.D., Jasco), Diol column (Finepak OH-10P (10 μm), 50 mm×4.6 mm I.D., Jasco) or ODS column (TSK gel 80Ts (5 μm), 150 mm×0.46 mm I.D., Tosoh, Tokyo, Japan)

2.3. On-line SFE–SFC conditions

2.3.1. SFE conditions

The extraction was performed for 1 min at a pressure of 20 MPa and a temperature of 45°C with supercritical carbon dioxide containing 5% methanol at a flow-rate of 4 ml/min. Extracts were passed directly to the SFC column, and preconcentrated on the head of the column as a narrow band by the on-column trapping method.

2.3.2. SFC conditions

After SFE, the extraction vessel was bypassed by manual switching of a valve, the conditions were changed to those for SFC, and the trapped analytes were consecutively analyzed. Now the methanol concentration of mobile phase was raised to 15%. Other conditions were the same as for SFE. Magnolol and honokiol were UV monitored at 300 nm.

2.4. Assay procedure

About 2 mg of dry powder of Magnoliae Cortex, previously weighed accurately, was placed in the extraction cell (Cartridge Guard Column E, 35 mm×

4 mm I.D., an empty column, GL Science, Tokyo, Japan). The sample was extracted and chromatographed under the conditions described above. The contents of magnolol and honokiol were calculated from the peak areas.

3. Result and discussion

First, the SFE–SFC system (Fig. 2) was tested with an ODS trapping column. In this method, the extract was trapped on the column bed by reduction of fluid solubility following sudden pressure drop to the atmospheric pressure in the trap column.

A trapping column similar to an ODS column, which shows almost no adsorption of the analytes was used in order not to disturb the SFC resolution. An entrainer could not be used with this method, since it resulted in elution of the analytes. It was difficult to extract the analytes completely in a short time solely with supercritical carbon dioxide. To extract a sufficient amount of the analytes in a short period of time, an entrainer was needed.

Secondly, an NH_2 column was tested as the trap column. Since it exhibited strong retention of the analytes, now an entrainer could be used and the analytes were selectively trapped on the column just by passage through the column without decreasing the pressure. Extraction efficiency was greatly improved by the addition of the entrainer and the extraction of the analytes from dry powder of Magnoliae Cortex was completed within only 1 min.

3.1. Comparison of retention behavior among combinations of modifier and stationary phase

First, combinations of chloroform, diethyl ether or methanol as the modifier, and NH_2 column, CN column, SiO_2 column or Diol column as the stationary phase were compared. The retention behaviors of magnolol and honokiol were compared by adding 10% of each modifier. The combination of methanol and the NH_2 column, diethyl ether and diol column and chloroform and the diol column yielded satisfactory separation of analytes with a short retention time (Table 1).

A more detailed study of the retention behavior of the analytes with these three combinations was carried out by changing the concentration of the modifier. With the combination of methanol and an NH_2 column, the k of magnolol was more than about 20 with the addition of less than 5% methanol. Magnolol was retained to a high degree on the head of the NH_2 column, if the extraction time was limited to a few minutes. Magnolol and honokiol were eluted and completely separated within a few minutes with the addition of methanol at concentrations above 15% (Fig. 3). The same retention behavior in SFC was found for the analytes with the

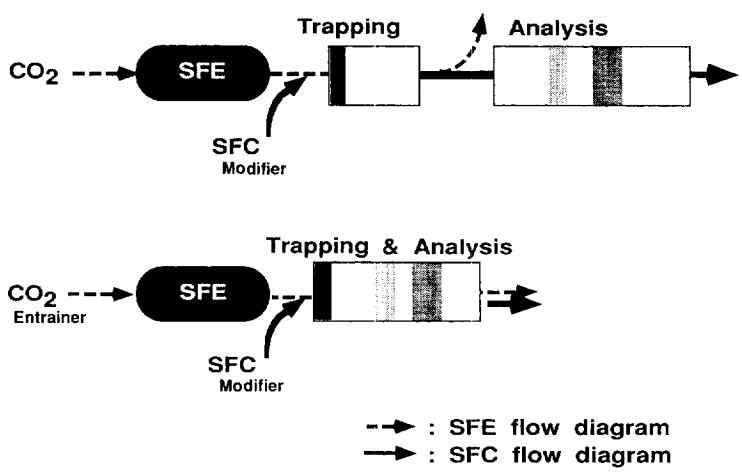

Fig. 2. On-line SFE–SFC system.

Table 1
Retention data for magnolol for various combinations of modifiers and stationary phases

	NH_2		Diol		Silica		CN	
	k	α	k	α	k	α	k	α
Methanol	5.9	2.9	0.4	2.6	0.3	2.1	0.2	1.0
Chloroform	>60	–	9.5	1.5	14.0	1.4	1.0	1.0
Diethyl ether	>60	–	7.2	1.8	4.5	1.9	0.6	1.3

Concentration of each modifier was 10%.
The hold-up time was determined with 1,3,4-tri-*tert*.-butylbenzene.

combinations of diethyl ether and diol column or chloroform and diol column, but the combination of methanol and NH_2 column yielded the best separation of analytes, which was presumed to be due to the high selectivity of the NH_2 column for phenols (Fig. 4).

3.2. Extraction rate

The extraction time was fixed at 1 min. The yield found did not change when the extraction time was increased. The extraction rates were compared with 1 min, 3 min and 10 min as the extraction times. The results were almost the same for each, and 1 min was sufficient for extraction of analytes in SFE using methanol as the entrainer. The results were also almost the same as those obtained by the HPLC method with the solvent extraction described in the literature [5]. The ratios of magnolol and honokiol to the values obtained by HPLC were respectively 103.4% and 97.1%.

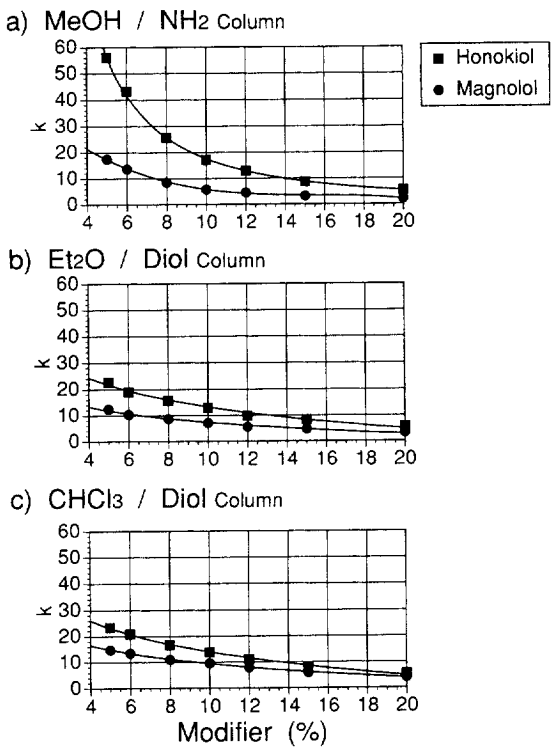

Fig. 3. Effect of modifier concentration on the retention of magnolol and honokiol.

3.3. Determination of magnolol and honokiol in Magnoliae Cortex

Fig. 5 illustrates the results of separation and determination of magnolol and honokiol for six samples of Magnoliae Cortex on the market. The three samples in the upper row were produced in

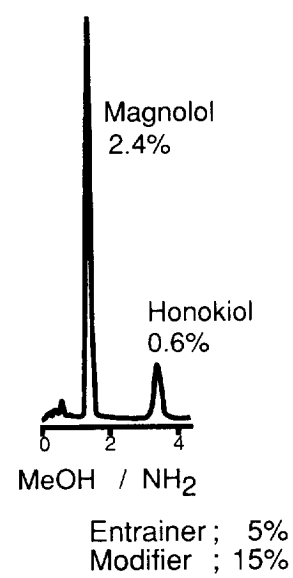

Fig. 4. Analytical result for MeOH/NH_2 combination.

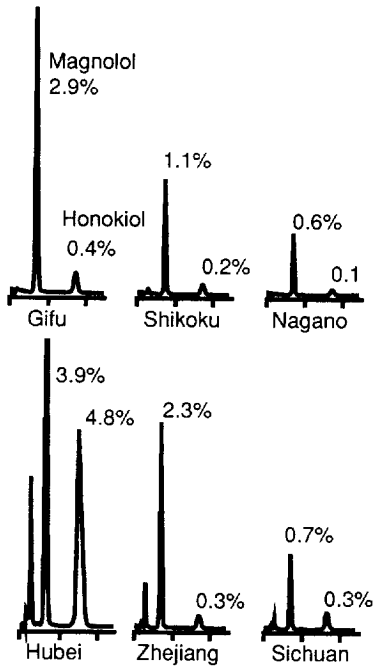

Fig. 5. Contents of magnolol and honokiol in commercial Magnoliae Cortex.

Japan, and the three samples in the lower row were produced in China. The content of magnolol was higher than that of honokiol in five samples. Only one sample, produced in Hubei, contained more honokiol than magnolol. Chinese samples contained characteristic components which were eluted fast.

3.4. Calibration curve

Calibration curves for both magnolol and honokiol were obtained from 16.95–84.75 μg. The regression equations were the following: $y=4.3289x+40.997$ (0.999) and $y=5.6592x+13.797$ (0.998), respectively, where y is the peak area and x is the concentration (μg).

4. Conclusion

It was possible to determine magnolol and honokiol concentrations in Magnoliae Cortex with the use of an NH_2 column for the trapping and SFC analysis. The NH_2 column showed a strong retention of magnolol and honokiol. These analytes were focused at the column head during SFE, even if 5% methanol was added as the entrainer. After extraction, the analytes were desorped quickly and separation of the analytes was successfully performed in SFC by the increase of the methanol concentration to 15% as the modifier.

In this study, the analytes were focused at the column head, if the amount of modifier added gave k values of the analytes higher than 10.

Magnolol and honokiol in Magnoliae Cortex were determined from only a few mg of crude drug powder. Only 1 min was needed for the SFE extraction, and only 3 min for the SFC analysis. Within only 5 min, the rapid analysis was completed, from extraction as a pretreatment to analysis including the system change from SFE to SFC. The entrainer could be used in the present on-column trapping method, and the extraction efficiency was greatly improved [10,11]. It is thus possible to apply the on-column trapping method to the SFE with entrainers. The limit of detection of analyte was greatly spreaded by this on-line SFE–SFC system. It appears that the application of our system to crude drugs and herbal medicines is extensive. In particular, it is useful for the application to an analysis including a comparatively complex extraction procedure of analytes as a pretreatment.

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