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Sample introduction and elution method for preparative supercritical fluid chromatography

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ABSTRACT

The loading capacity in preparative supercritical fluid chromatography with different injection and elution methods was investigated. With the ordinary valve injection method using a syringe, the maximum loading capacity onto a column (250 mm × 10 mm I.D.) packed with 5-μm silica gel was only a few milligrams. However, this value was increased by a factor of ten (a few hundred milligrams) for a column (125 mm × 10 mm I.D.) packed with 10–20 μm silica gel using a programmed extraction–elution method employing a small extraction vessel (1 ml) connected to the column and stepwise pressure gradient elution.

INTRODUCTION

The advantage of preparative supercritical fluid chromatography (SFC) is that the supercritical carbon dioxide mobile phase becomes gaseous under normal ambient conditions, permitting the easy separation of solutes simply by pressure reduction. Therefore, even though the solute concentration in the mobile phase is lower than that in LC, a low-concentration or broad peak can be fractionated efficiently without removing a solvent by evaporation as required in preparative liquid chromatography (LC).

There are two approaches to the establishment of preparative SFC. The first is to enlarge the dimensions of the column and peripheral devices in proportion to the required sample loading capacity[1–3]. The other approach is to increase the column lodading capacity by improving the sample introduction and elution method.

In SFC and LC, the maximum loading capacity for a compound is restricted by the solubility of the compound in the mobile phase. Solubilities in supercritical carbon dioxide are generally very low (*e.g.*, a few percent even for a favourable compound) compared with those in liquid solvents. Therefore, the capacity is lower in SFC than in LC as long as the conventional elution method is employed. Hence, the most crucial point is to increase the sample loading capacity by improving the sample introduction and elution method.

We have previously reported a programmed extraction–elution method which significantly increased the loading capacity by nearly 20% of the stationary phase

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mass[4,5]. This paper describes a comparison of the maximum sample loading capacity by the programmed extraction–elution method with that obtained by the ordinary valve injection method.

EXPERIMENTAL

Materials

Coffee (UCC Original Blend, Ueshima Coffee, Kobe, Japan) was purchased from a grocery store.

Carbon dioxide (standard grade) was purchased from Toyoko Kagaku (Kanagawa, Japan) and was used as the mobile phase. A test mixture was prepared by dissolving naphthalene and anthracene (Wako, Osaka, Japan) in dichloromethane.

The columns used were a Jasco SuperPak SIL (250 mm × 4.6 mm I.D.) packed with 5-μm silica gel, referred to as column A, a SuperMegaPak SIL column (250 mm × 10 mm I.D.) packed with 5-μm silica gel, referred to as column B, and a SuperMegaPak SIL column (125 mm × 10 mm I.D.) dry-packed with 10–20 μm silica gel, referred to as column C.

Apparatus

A Jasco Super-200 system 3[6–8] was used. A Model 7125 injection valve (Rheodyne, Cotati, CA, U.S.A.) was used in the ordinary valve injection method. An extraction vessel was used for the programmed (stepwise pressure gradient elution) extraction-elution method as described previously[4].

RESULTS AND DISCUSSION

Ordinary valve injection method

Before investigating the sample loading capacity on columns A and B, the optimum flow-rates were established with reference to the H–u curves (H = plate height, u = linear velocity) shown in Fig. 1. They were found to be 3.0 and 5.0 ml/min for columns A (4.6 mm I.D.) and B (10 mm I.D.), respectively. Preparative SFC was carried out using these optimum flow-rates.

Fig. 2 shows the chromatograms of the test mixture containing various amounts (0.01, 0.60, 1.5, 3.0 and 6.0 mg each) of naphthalene and anthracene separated on column B. When column B was loaded with 0.30 mg of the compounds the peaks started to tail, and with more than 0.60 mg their shapes were distorted and split into two parts, exhibiting an overloaded state.

This may be due to a partial increase in the solvating power of the dichloromethane used as a sample solvent and overloading of the stationary phase. The sample solubility in dichloromethane is higher than that in supercritical carbon dioxide. Therefore, when an excessive amount of the sample solutes dissolved in dichloromethane was injected, the solutes were introduced into the column as an oversaturated solute band in the mobile phase. Dichloromethane accompanied part of the solute as it moved in the column, but soon it moved faster than this solute band. This caused the band to be left as oversaturated in the mobile phase. The remainder was adsorbed on the stationary phase up to saturation. In this way, the solutes saturated in the stationary phase travelled immediately after the over-saturated solute band,

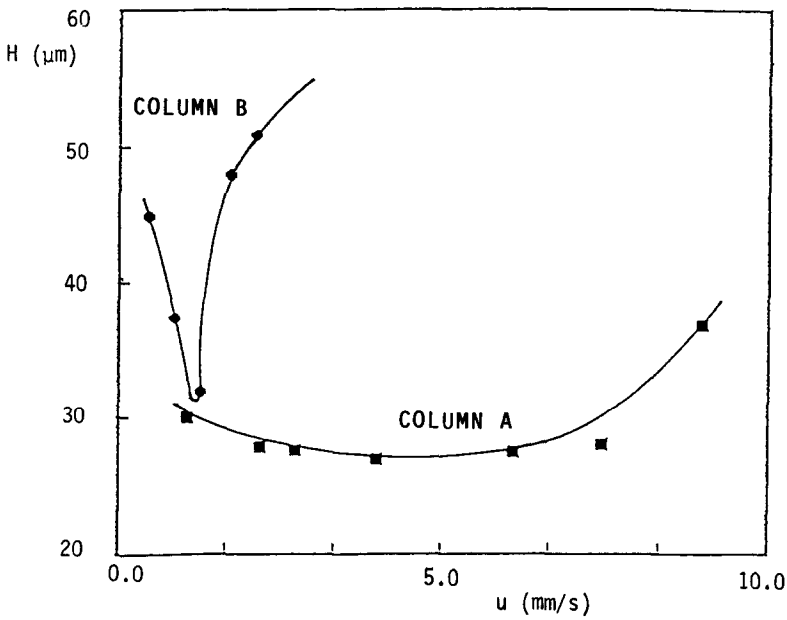


Fig. 1. *H–u* curves for columns A and B. Conditions: mobile phase, carbon dioxide; column temperature, 40°C; back-pressure, 20 MPa; detection, UV at 300 nm; Sample, anthracene.

resulting in a Gaussian peak shape adjoining a trapezoid with a logarithmic increase and an exponential decay.

The results of the programmed extraction–elution method, in which no solvent was used for dissolving the sample solutes, support the above interpretation because such peaks were not observed.

Programmed extraction–elution method (column C)

Fig. 3 shows a chromatogram of the test mixture obtained by the programmed extraction–elution method with a stepwise pressure gradient. The test mixture containing 100 mg of each of the compounds was loaded as a solid material using a 1-ml extraction vessel. Naphthalene was eluted in 30 min at 60°C and 10 MPa, whereas anthracene was retained in the column. By changing the back-pressure to 25 MPa, anthracene then started to elute. With this method, the mixture of 100 mg each of naphthalene and anthracene was separated completely.

At 10 MPa 60°C, the compounds were extracted in the vessel and flowed into the column without the aid of an organic solvent as in the ordinary valve injection method discussed above. With this method, the elution profile of naphthalene was a simple logarithmic increase and exponential decay.

However, the elution profile of anthracene was not as simple as that of naphthalene, and was wavy. This may be due to the low solubility of the compound in supercritical carbon dioxide, which caused variations in concentration resulting from the change in surface area during the extraction process.

It is remarkable that even though the column used (column C) was packed with 10–20 μm material and had only 1000 plates and the amounts of sample injected were

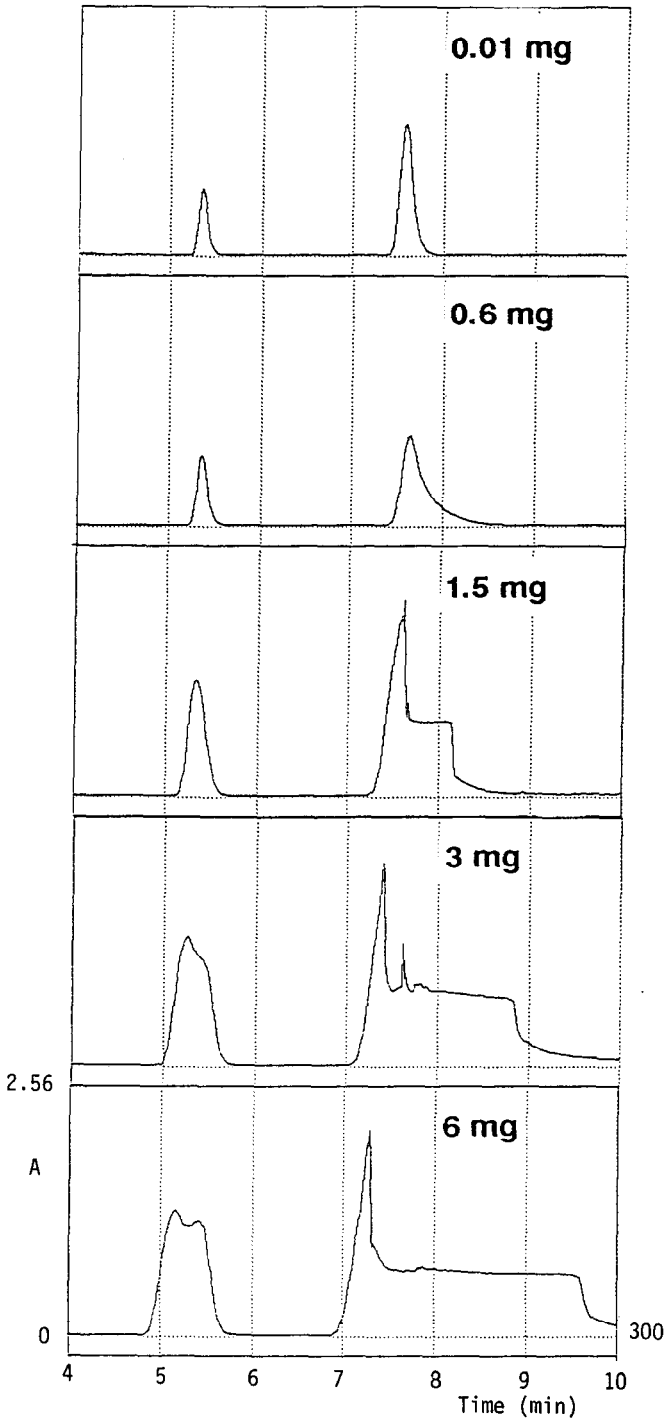


Fig. 2. Chromatograms for various sample loadings on column B. Conditions: carbon dioxide flow-rate, 5.0 ml/min (at −5°C); column temperature, 40°C; back-pressure, 20 MPa.

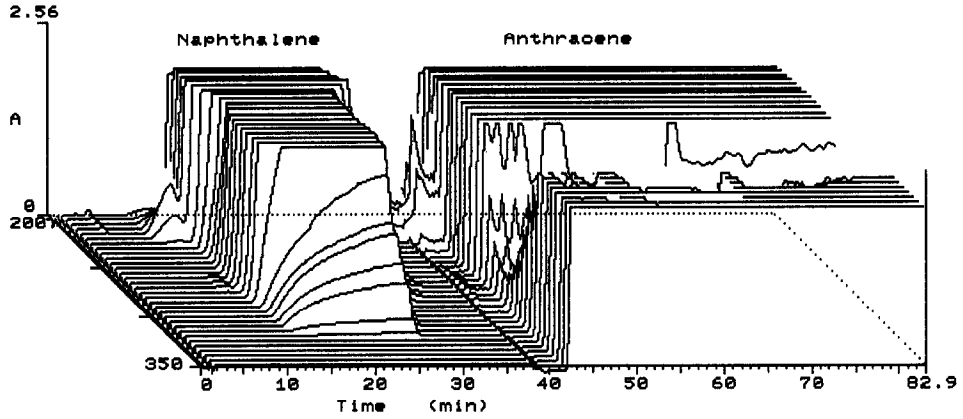


Fig. 3. Preparative chromatogram obtained with the programmed extraction–elution method using column C. Conditions: carbon dioxide flow-rate, 5.0 ml/min (at −5°C); column temperature, 60°C; back-pressure, 10 MPa for 0–30 min and 25 MPa for 30–120 min; sample, naphthalene and anthracene (100 mg of each).

about ten times higher than those in the valve injection method using a highly efficient column (column B, having 10 000 plates), the two compounds were completely separated.

Practical applications to fractionation of coffee extract

SFC fractionation of coffee powder was performed with the proposed method. A 3.50-g amount of coffee powder was loaded in the 10-ml extraction vessel and column C was used for separation and fractionation of the extract. The column outlet pressure was kept at 20 MPa for the first 40 min, then ethanol was added at a

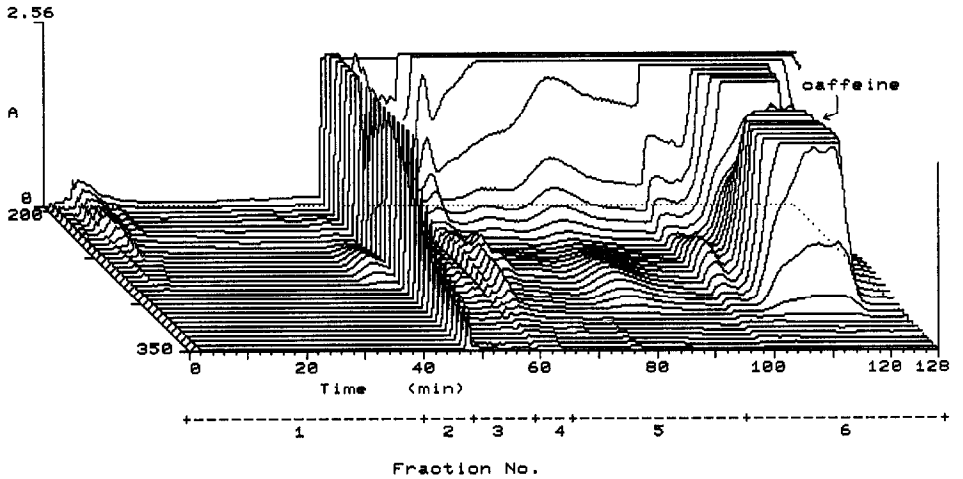
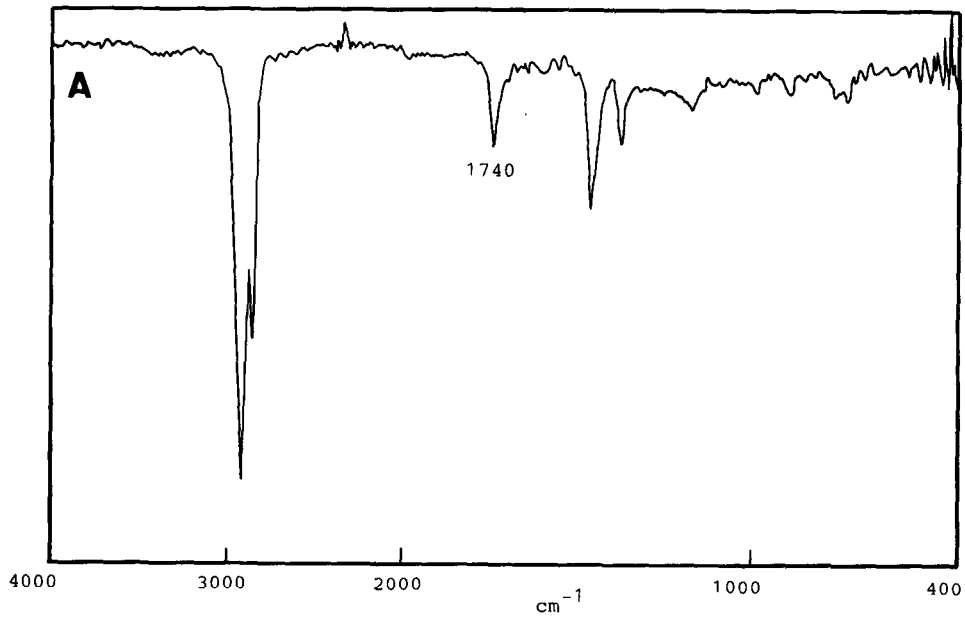


Fig. 4. Preparative chromatogram of coffee extract obtained with the programmed extraction/elution method. See text for details.


A
1740
4000
3000
2000
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400
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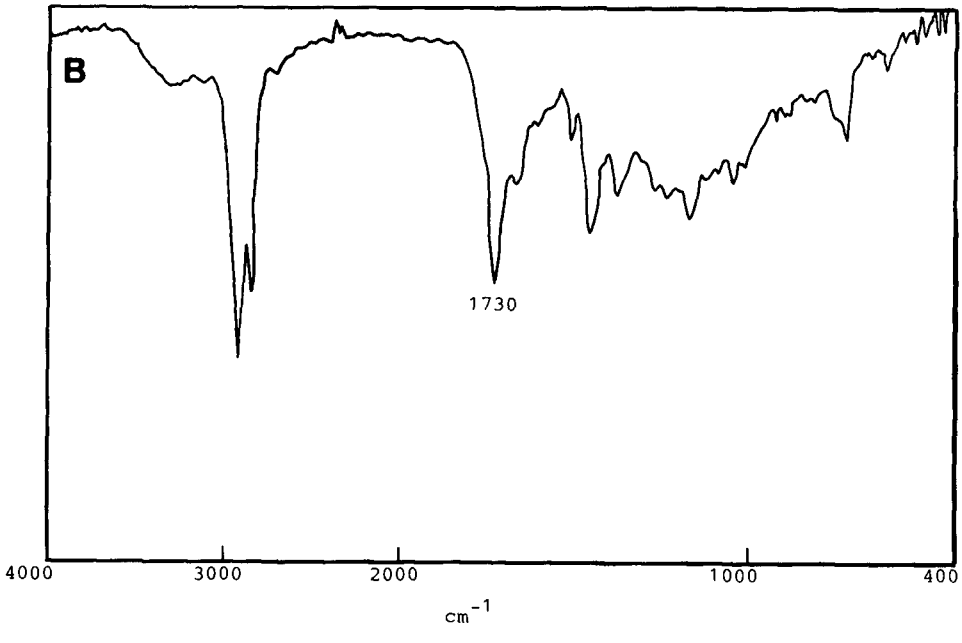
B
1730
4000
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2000
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400
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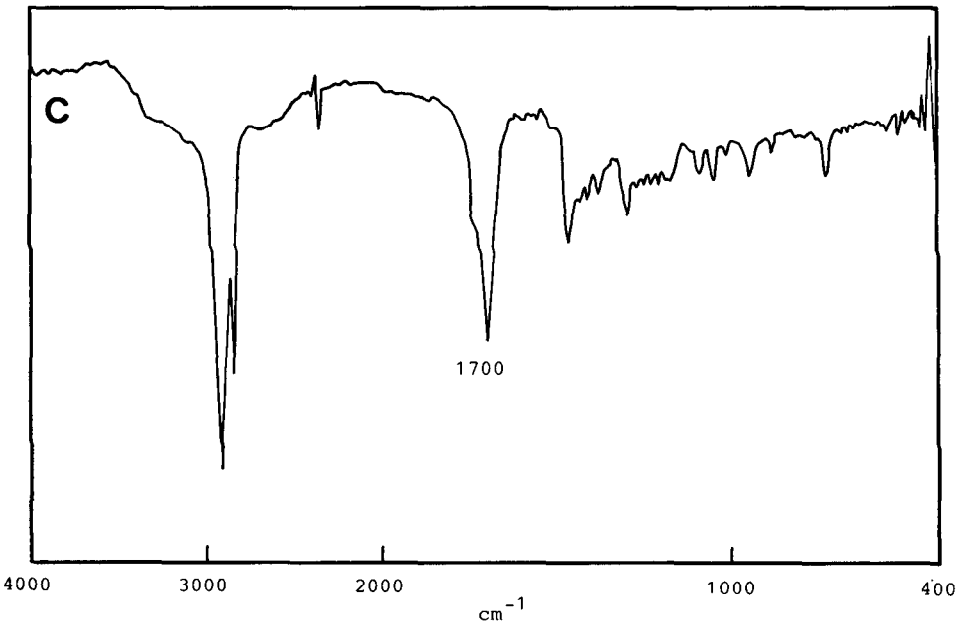
Fig. 5.



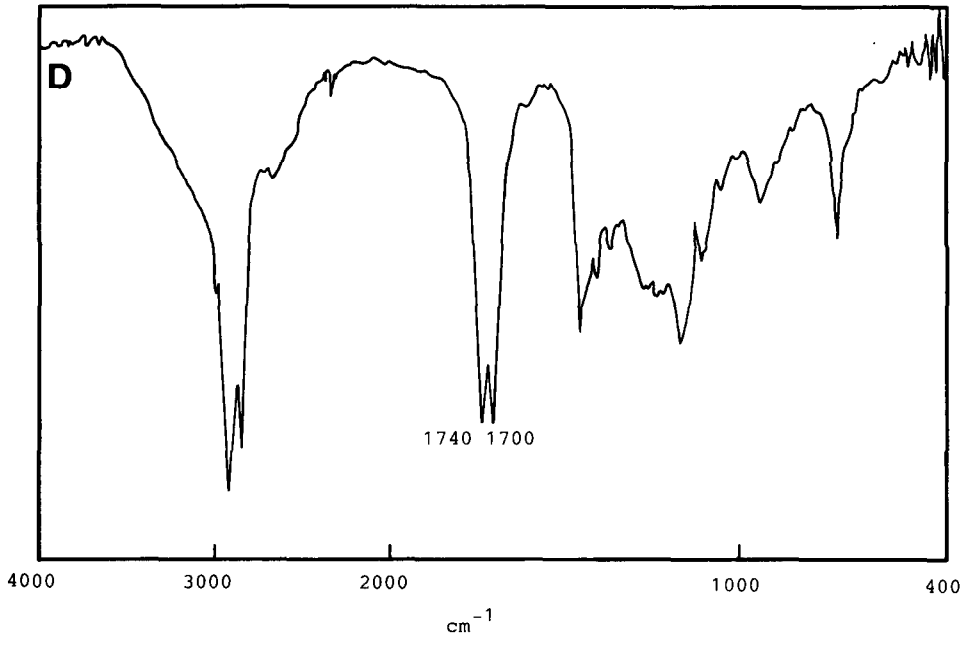


Fig. 5.

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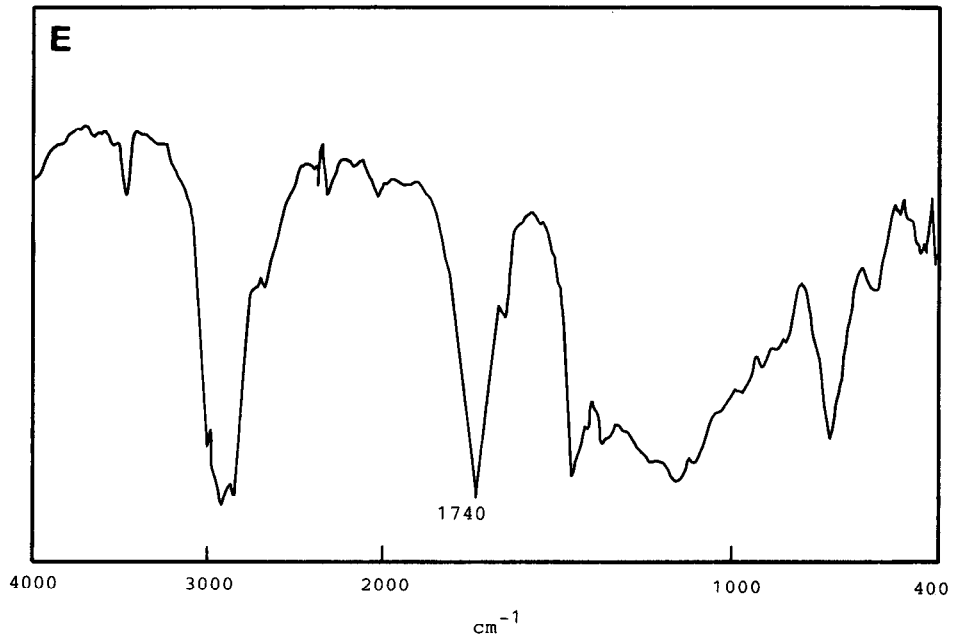

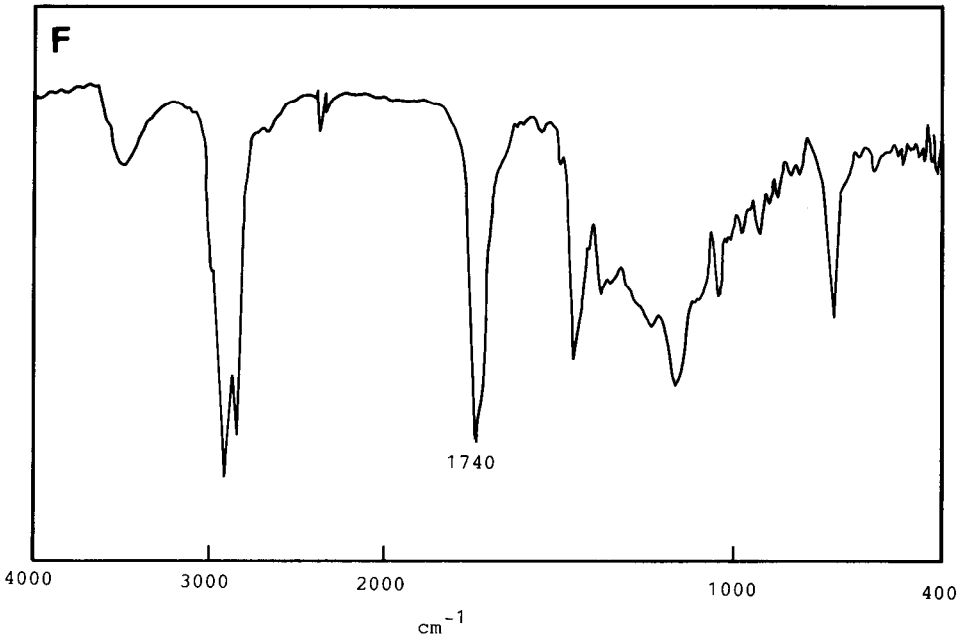

Fig. 5. IR spectra of each fraction in Fig. 4. (A) Fraction 1; (B) fraction 2; (C) fraction 3; (D) fraction 4; (E) fraction 5; (F) fraction 6.

flow-rate of 0.05 ml/min for 10 min, then increased stepwise to 0.1 ml/min, maintained at this level for 45 min, and finally increased to 0.2 ml/min for 30 min. The carbon dioxide flow-rate was kept constant at 6.0 ml/min (−5°C) and the column temperature was also constant at 60°C.

Fig. 4 shows the three-dimensional elution profile of the coffee extracts obtained by the above preparative SFC run. The numbers shown under the time axis are the time frames and the corresponding fractions collected with reference to real-time UV spectrum monitoring. The amount extracted was measured to be 190 mg by weighing the sample before and after the run.

Fraction 1, weighing 1.3 mg, was oil. It smelled completely off-flavour from coffee flavor. The IR spectrum shown in Fig. 5A, suggested that this fraction contained substances having long alkyl chains and keto groups.

Fraction 2 was a flavour component and was collected as an ethanolic solution. It smelled like deeply roasted coffee with a bitter taste. The IR spectrum shown in Fig. 5B suggested that this fraction contained fatty acid esters. Fraction 3 was also a flavour component smelled like coffee flavour with an acidic taste. The IR spectrum shown in Fig. 5C suggested that this fraction contained fatty acids. Fraction 4 was also a flavour component and smelled similar to fraction 3. The IR spectrum shown in Fig. 5D suggested that this fraction contained two types of substances, fatty acids and triglycerides.

Fraction 5, weighing 100 mg, was oil without a smell. The IR spectrum shown in Fig. 5E suggested that the main constituent was triglyceride. Fraction 6, weighing 72.4 mg, was also oil without a smell. The IR spectrum shown in Fig. 5F suggested that the main constituent was triglyceride containing caffeine. Caffeine was identified by SFC analysis monitored with a multi-wavelength UV detector.

CONCLUSION

The programmed extraction–elution method offers a high loading capacity in preparative SFC. In addition, even a solid sample can be applied without dissolution in a solvent. Insoluble components of the sample remain in the extraction vessel, and such components can readily be extracted and separated by LC. A delicate change in the mobile phase strength can be achieved rapidly and precisely by simply varying the density of the fluid. It is not easy to change the mobile phase strength in LC as quickly as in SFC, and LC cannot be applied to the programmed extraction–elution method.

Theoretical aspects of this method are currently under investigation.

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