生 薬 学 雑 誌
Shoyakugaku Zasshi
45(1), 29~34 (1991)

Application of Supercritical Fluid Chromatography to Determination of Gingerols in Zingiberis Rhizoma

KEIICHI SUTO,* KAZUHIKO SAGARA and TAKU MIZUTANI

Research Center, Taisho Pharmaceutical Co., Ltd., 1–403, Yoshino-cho, Omiya-shi, Saitama 330, Japan

(Received June 7, 1990)

Determination of (6)-gingerol, (8)-gingerol and (10)-gingerol in Zingiberis Rhizoma by coupled supercritical fluid extraction (SFE)/supercritical fluid chromatography (SFC) is described.

Zingiberis Rhizoma (ginger) was subjected to SFE with carbon dioxide. The extraction oils containing gingerols were trapped on a trimethylsilyl (TMS) silica gel column by reducing the pressure of carbon dioxide. The trapped oil was then analyzed on a silica gel column by SFC using carbon dioxide containing *n*-butanol as the mobile phase and UV adsorption monitoring at 280 nm.

Keywords——supercritical fluid chromatography; SFC; supercritical fluid extraction; SFE; ginger; Zingiberis Rhizoma; gingerol

Zingiberis Rhizoma is a useful drug in Chinese medicine, called "Syokyo" (fresh rhizome), "Kansyokyo" (dried rhizome) or "Kankyo" (dried steamed rhizome) depending upon the crude drugform, and is prescribed in many Chinese prescriptions. Zingiberis Rhizoma is used as a carminative and aromatic stimulant to the gastro-intestinal tract. As the constituents of Zingiberis Rhizoma, the essential oil, zingiberol, zingiberone and zingiberene and pungent components, gingerols and shogaols have been known. Gingerols and shogaols have effects on the central nervous, cardiovascular and on digestive systems. Analytical methods on HPLC[1] or GLC[2] have been reported for gingerol determinations. However, these methods require a manual extraction procedure. Recently supercritical fluid is used for extraction and chromatography (called SFE and SFC, respectively). A combination of the SFE and SFC technique could simplify analytical procedures. This report describes the application of the coupled SFE/SFC system to the quantitative determination of (6)-gingerol, (8)-gingerol and (10)-gingerol in Zingiberis Rhizoma, the structure of which are shown in Fig. 1.

Experimental

Plant material. Commercial Zingiberis Rhizoma used in this study was purchased from Matsuura Yakugyou Co., Ltd. (Nagoya) and was used as a dry powder.

Chemical and reagent. Solvent——carbon dioxide was of high purity of more than 99.99% (Kanto Sanso Ind., Co., Ltd., Tokyo). Methanol and acetonitrile were HPLC grade (Wako Pure Chemicals Ind., Ltd., Tokyo). Ethanol, methylenechloride, isopropanol and *n*-butanol were spectrosol grade (Wako Pure Chemicals Ind., Ltd., Tokyo).

Solutes——(6)-gingerol, (8)-gingerol and (10)-gingerol were isolated and purified in our laboratory from ginger as previously reported.[3]

Apparatus. A supercritical fluid chromatograph SUPER 200 system 3 (JASCO, Tokyo) equipped with a photodiode array detector MULTI 340 (JASCO, Tokyo) was used.

Figure 2 shows the hydraulic diagram of the SFE/SFC system used. This system consists of three sections: fluid delivery, extraction and chromatography, and fractionation, as indicated by broken-line boxes. The operation of extraction and chromatography section includes the SFE process, preconcentration process, and SFC fractionation process.

The thick-line in Fig. 2 shows the flow line of the SFE and preconcentration process, which run simultaneously. The back-pressure regulator (BR1) applied a suitable pressure to the extraction vessel (E.VSL), while the pressure

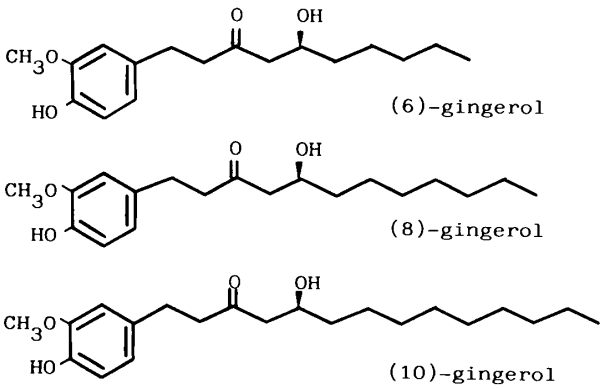

Fig. 1. Structures of Gingerols

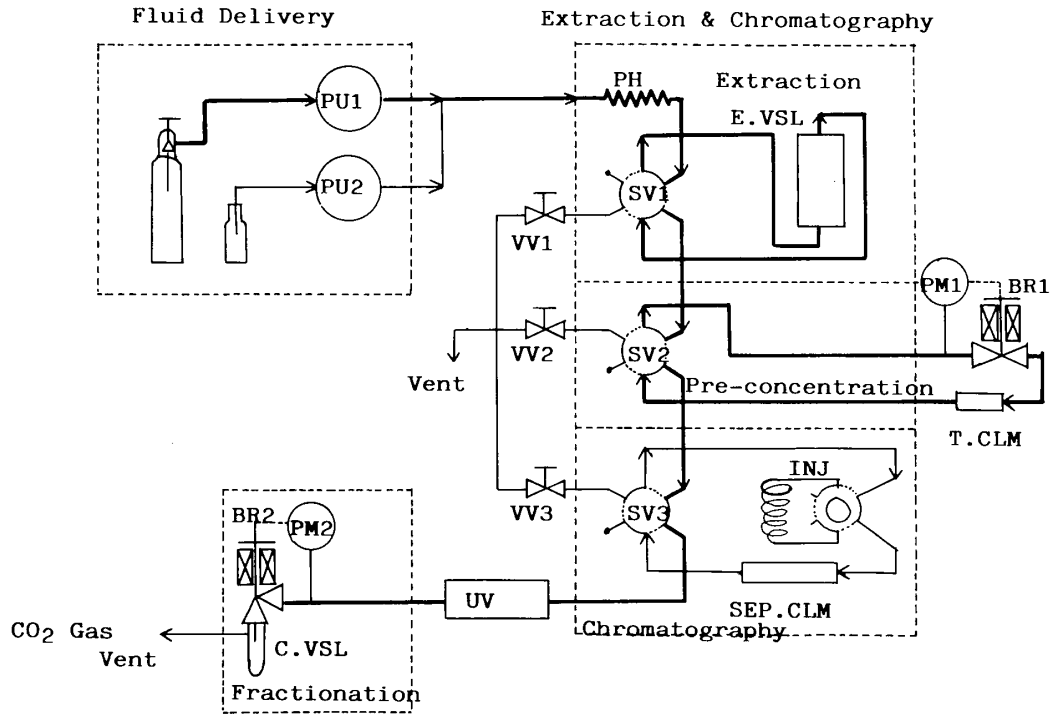

Fig. 2. Hydraulic Diagram of SFE/SFC System

Components: PU1=CO2 pump, 880-PU with cooling jacket, JASCO; PU2=modifier solvent pump, 880-PU; PH=preheat coil, 0.5 mm I.D.×5 m in length; SV1, SV2, SV3=six-way switching valves, Model 7000, Rheodyne; E.VSL=extraction vessel, 4.6 mm I.D.×50 mm length stainless-steel column; BR1=back-pressure regulator, Model 880-81, JASCO; PM1=pressure transducer, Densou Sangyou; T.CLM=trap column, 4.6 mm I.D.×150 mm length packed 5 μm TMS-silica gel; INJ=injector; SEP.CLM=separation column, 4.6 mm I.D.×50 mm length packed 5 μm silica gel; UV=photo-diode-array UV detector, MULTI-330, JASCO; BR2=back-pressure regulator; PM2=pressure mater; C.VSL=10 ml glass collection vessel; VV1, VV2, VV3=vent valves. Extraction and preconcentration flow line is indicated by the thick line.

at the trap column (T. CLM) placed downstream of the regulator was kept at a lower pressure by the second back-pressure regulator (BR2). Therefore, the density of carbon dioxide decreased rapidly in the trap column, which resulted in the deposition of extracts on the packed bed of the trap column. On completion of extraction, the flow line was switched to the SFC system, and the modifier was sent from PU2. The extraction vessel was now bypassed by means of the switching valve (SV1) and supercritical carbon dioxide containing modifier solvent flowed through the trap column on which the extract had been concentrated. The flow then moved to the separation column (SEP.CLM) *via* the first back-pressure regulator, which was now completely open. The second

back-pressure regulator applied a suitable pressure for SFC separation to the entire line, including the trap column, separation column, and UV detector. In practice, the switching valve (SV2) was first set in the position so that the trap column was bypassed. Prior to the introduction of the trapped extract, the separation column was equilibrated and then the valve (SV2) was switched over to introduce the trapped extract. The extract on the trap column was now eluted and was introduced into the separation column, where chromatographic separation took place. A photodiode array detector was used to monitor real time three-dimentional chromatograms.

SFE condition. The extraction was performed at a pressure of 300 kg/cm² and a temperature of 50°C with supercritical carbon dioxide at a flow rate of 4 ml/min as liquid carbon dioxide. The trap column was SUPERPAK SIL C1 (4.6 mm I.D.×150 mm in length, and packing material was 5 μm TMS silica gel) purchased from JASCO (Tokyo). As an extraction vessel, a 4.6 mm×50 mm stainless-steel column was used.

SFC condition. The separation column was SUPERPAK SIL (4.6 mm I.D.×50 mm in length, and packing material was 5 μm silica gel) purchased from JASCO (Tokyo). The mobile phase was supercritical carbon dioxide containing 5% (v/v% in liquid carbon dioxide) of *n*-butanol at flow rates of 4 ml/min as liquid carbon dioxide and 0.2 ml/min at a pressure of 300 kg/cm² and a temperature of 50°C.

Assay procedure. About 20 mg of dry powder of Zingiberis Rhizoma, previously weighed accurately, was placed in an extraction vessel. The sample was extracted and chromatographed under the condition of the coupled SFE/SFC system. The gingerol contents were calculated from the peak areas.

Calibration curve. Calibration curves for (6)-gingerol, (8)-gingerol and (10)-gingerol were obtained from 70.36 μg–351.80 μg, 10.10 μg–50.80 μg and 14.52 μg–72.60 μg, respectively. The regression equations were as follows; $Y=0.0402X+0.2519$ ($r=0.999$), $Y=0.0407X-0.0345$ ($r=0.999$) and $Y=0.0364X+0.0491$ ($r=0.999$), respectively, where Y is the peak area (abs.sec) and X is the concentration (μg).

Recovery test. Known amounts of gingerols were added to the dry powder of Zingiberis Rhizoma and the amount of each gingerol was determined by the present coupled SFE/SFC system. The recovery test was repeated four times. The recovery rates for (6)-gingerol, (8)-gingerol and (10)-gingerol were 97.8%, 99.3% and 98.3%, respectively. This shows that this determination method can be satisfactorily used for the quantitative determination of gingerols in Zingiberis Rhizoma.

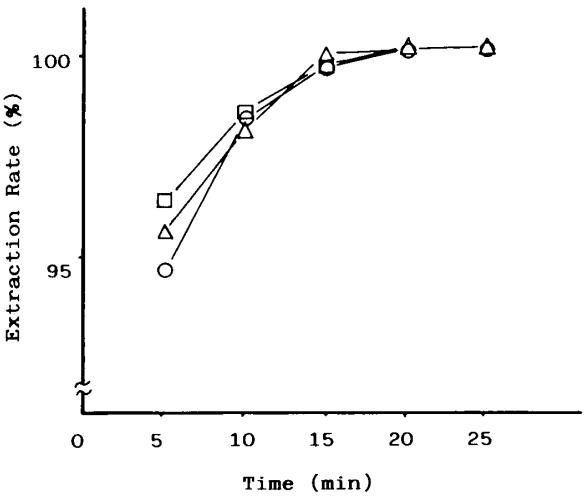

Fig. 3. Effect of Extraction Time: Fluid, carbon dioxide; flow rate, 4 ml/min as liquid carbon dioxide; pressure, 300 kg/cm²; temperature, 50°C; extraction vessel, 4.6 mm I.D.×50 mm length stainless-steel column; trap column, SUPERPAK SIL C1 (5 μm) 4.6 mm I.D.×150 mm in length (JASCO, Tokyo). Solutes: (□) (6)-gingerol; (○) (8)-gingerol; (△) (10)-gingerol.

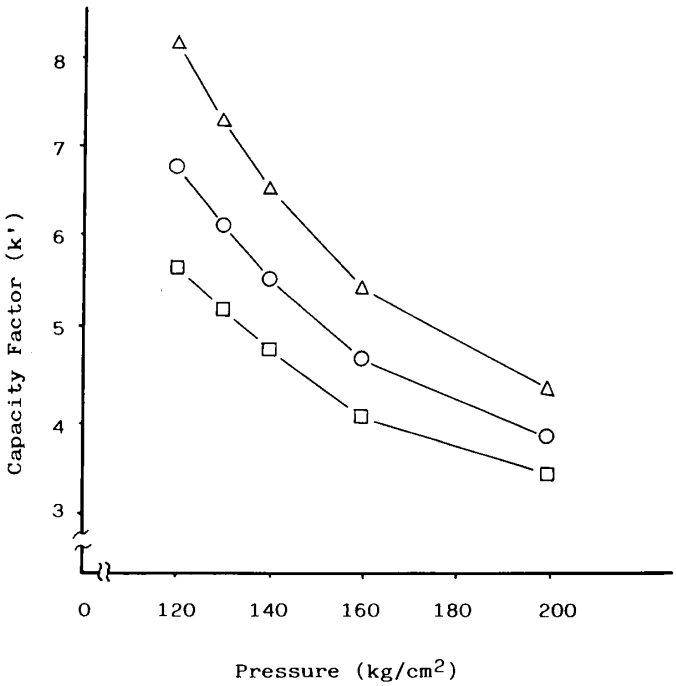

Fig. 4. Capacity Factors of Gingerols vs. Column Pressure

Solutes: (□) (6)-gingerol; (○) (8)-gingerol; (△) (10)-gingerol.

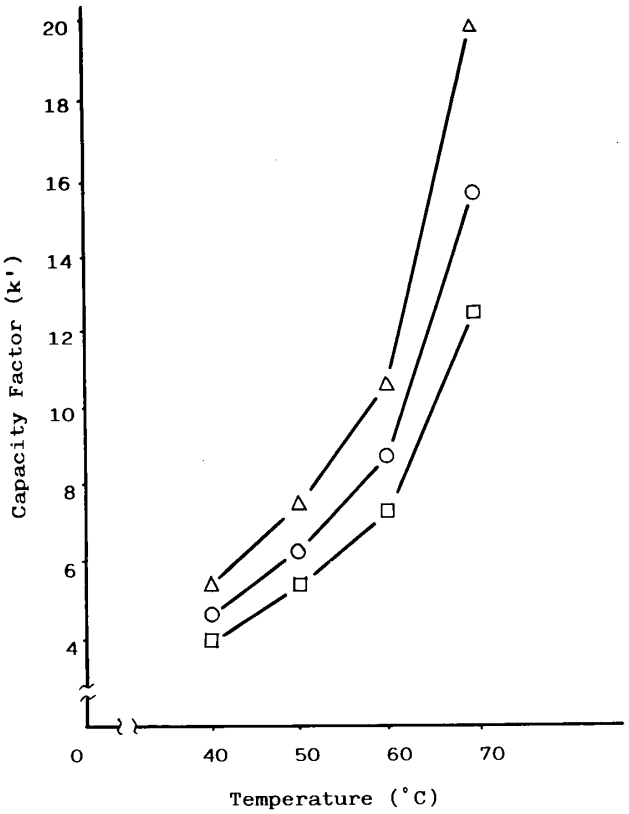

Fig. 5. Capacity Factors of Gingerols vs. Column Temperature

Solutes: (□) (6)-gingerol; (○) (8)-gingerol; (△) (10)-gingerol.

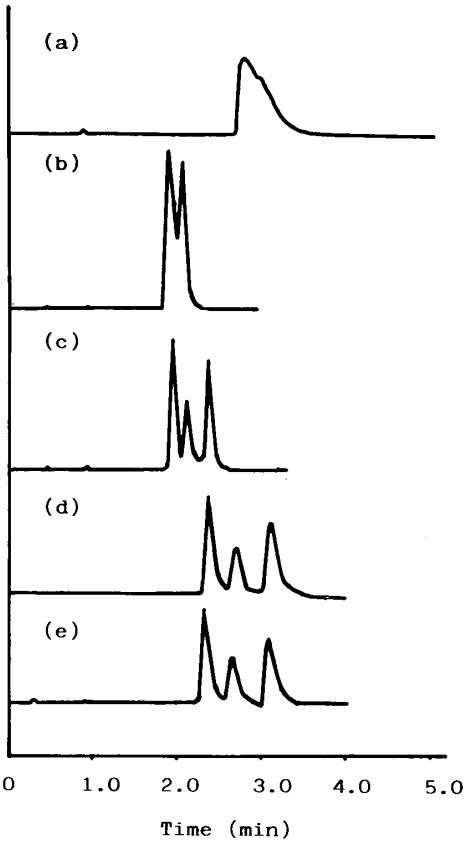

Fig. 6. Separation of Gingerols in Supercritical Carbon Dioxide Containing a Polar Modifier at Various Concentration (v/v% in Liquid Carbon Dioxide)

(a) 10% acetonitrile, (b) 5% methanol, (c) 5% ethanol, (d) 5% isopropanol, (e) *n*-butanol. Column, SUPERPAK SIL (5 μm) 4.6 mm I.D. × 50 mm in length (JASCO, Tokyo); column pressure, 130 kg/cm²; column temperature, 50°C; flow rate, 4ml/min as liquid carbon dioxide; detection, UV at 280 nm.

Result and Discussion

1. Effects of pressure, temperature and extraction time on SFE

The supercritical fluid extraction was applied to gingerols by using supercritical carbon dioxide without using an entrainer. The effects of temperature, pressure and time on extraction were examined to establish the procedure of pretreatment of SFC. The effect of temperature and pressures on the extraction efficiency of gingerols were examined at 35°C, 50°C and 70°C and at 100 kg/cm² and 300 kg/cm².

The optimum pressure for the extraction of gingerols with supercritical carbon dioxide was 300 kg/cm², but the temperatures had little influence on the extraction efficiency. At 50°C the extraction efficiency of gingerols reached an equilibrium when the time of extraction was more than 20 min (Fig. 3).

2. Effect of pressure and temperature on SFC

The physical state of carbon dioxide is characterized by two parameters: pressure and temperature or, more precisely, density and temperature. At constant temperature, the retention decreased with the density. This tendency is often explained by the solubility variations caused by density variations. In most cases, the solute solubility increases with the density. When the pressure was increased at constant temperature, the density increased and, as a consequence, the retention decreased. As, at a given

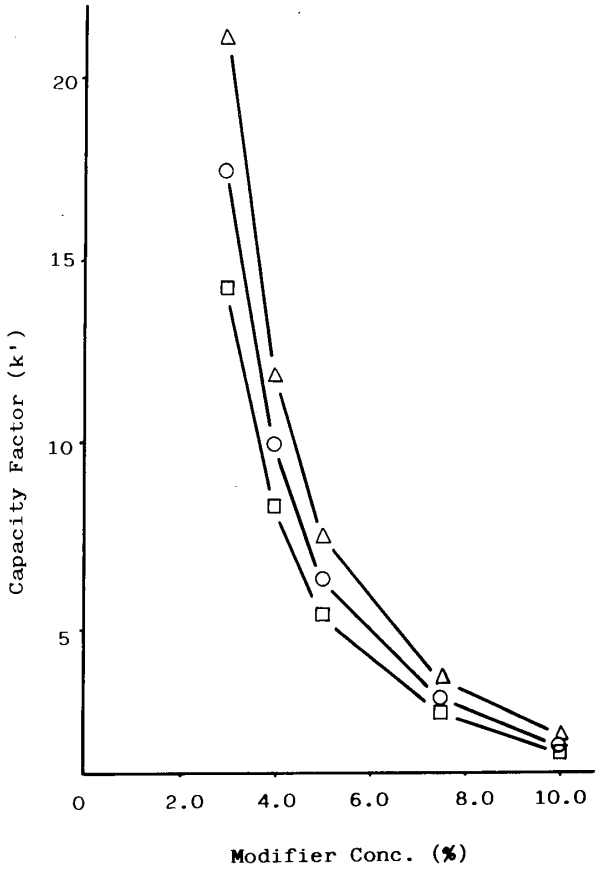

Fig. 7. Capacity Factors of Gingerols vs. *n*-Butanol Percentage in carbon dioxide: Column, SUPERPAK SIL (5 μm) 4.6 mm I.D. × 50 mm in length (JASCO, Tokyo); column pressure, 130 kg/cm^2; column temperature, 50°C; flow rate, 4 ml/min as liquid carbon dioxide; detection, UV at 280 nm. Solutes: (□) (6)-gingerol; (○) (8)-gingerol; (△) (10)-gingerol.

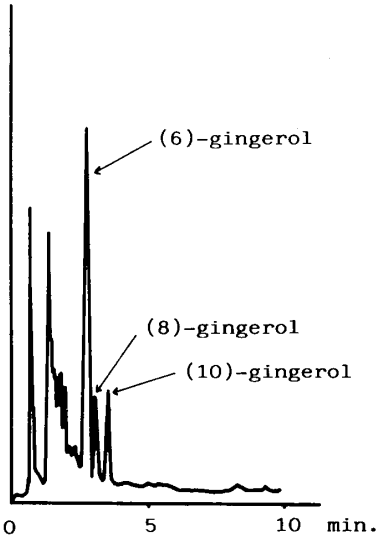

Fig. 8. Chromatogram of Gingerols in Zingiberis Rhizoma

temperature, the pressure and density are correlated, the influence of carbon dioxide density on the selectivity is weak with regard to that of temperature and modifier nature. Temperature is one of the parameters which can induce great variation in selectivity. In the temperature range examined in this study, the lower the temperature, the higher the selectivity. Because, in this temperature range, the retention increased when the temperature was increased at a constant pressure, and the shapes of peaks became broad and the separation factors decreased.

3. Effect of modifier on SFC

Another way of modifying the mobile phase polarity is to add a small amount of polar solvent.

At first, methylene chloride, acetonitrile and methanol were added to carbon dioxide in such quantities that the separation time should be roughly the same as that in the original procedure. Good separation was obtained with methanol. Ethanol, isopropanol and *n*-butanol were also examined. The less polar the alcohol, the greater the capacity factors and selectivity (Fig. 6). The influence of modifier contents on the solute retention and resolution was examined by varying the *n*-butanol concentration from 3% (v/v% in liquid carbon dioxide) to 10%. The higher the modifier concentration, the lower the retention. Good separation of gingerols was obtained at 5% (Fig. 7).

4. Effects of stationary phase and column size on SFC

A silicagel column was used in this study, because gingerols were highly retained on silica gel, although they were hardly retained on TMS-silica gel or ODS-silica gel and were eluted as one peak without separation. Taking the pressure drop[4] into consideration, we used a 4.6 mm I.D. × 50 mm column in this study.

TABLE I. Contents (%) of Gingerols in Zingiberis Rhizoma on Market

Sample	(6)-Gingerol	(8)-Gingerol	(10)-Gingerol
1	0.53	0.10	0.12
2	0.29	0.08	0.11
3	0.30	0.07	0.10
4	0.16	0.05	0.07

5. Determination of gingerols

Figure 8 illustrates the separation of three gingerols in Zingiberis Rhizoma. They were eluted within five minutes (Fig. 8). Each gingerol content in four samples of Zingiberis Rhizoma (dried rhizome) on the market was determined by the present method. The gingerol contents varied as shown in TABLE I.

Conclusion

By the directly coupled SFE/SFC system, extraction, preconcentration and chromatographic separation can be carried out in a single run. The use of supercritical carbon dioxide seems to allow an easy extraction of gingerols at low temperatures and in an oxygen-free environment, which is essential for the analysis of labile compounds. Thus, the SFE/SFC system is more simple and rapid than previous methods for the simultaneous determination of gingerols in Zingiberis Rhizoma.

References and Notes

1) a) Y. Kano, K. Saito, T. Sakurai, S. Kanemaki, M. Tanabe, M. Yasuda, *Shoyakugaku Zasshi*, **40**, 333 (1986); b) E. Steinnegger, K. Stucki, *Pharm. Acta Helv.*, **57**, 66 (1982).
2) Y. Masada, T. Inoue, K. Hashimoto, M. Fujioka, K. Shiraki, *Yakugaku Zasshi*, **93**, 318 (1973).
3) G. Kobayashi, Q.R. Huang, M. Iwamoto, J. Yamahara, H. Fujimura, *Proc. Symp. WAKAN-YAKU*, **6**, 80 (1988).
4) P.S. Schoenmaker, L.G.M. Unnk, *Chromatographia*, **24**, 51 (1987).