Journal of Chromatography, 505 (1990) 237–246
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 22 257

Fractionation of lemon-peel oil by semi-preparative supercritical fluid chromatography[a]

YOSHIO YAMAUCHI and MUNEO SAITO*
JASCO, Japan Spectroscopic Co., Ltd., 2967-5 Ishikawa-cho, Hachioji City, Tokyo 192 (Japan)

SUMMARY

Fractionation of cold-pressed lemon-peel oil into several compound types, namely hydrocarbons, alcohols and aldehydes, esters and others, was demonstrated using a semi-preparative supercritical fluid chromatographic system. By utilizing stepwise pressure and modifier programming, it was possible to load 0.5 ml (about 0.5 g) of lemon-peel oil onto 50 mm × 7.2 mm I.D. column packed with silica gel (10–20 μm). The technique can be used to produce new flavours by remixing fractions in different proportions, and for simple removal of terpenes from the oil.

INTRODUCTION

Problems with supercritical fluid chromatography as a preparative separation method

Although there have been useful reports on preparative supercritial fluid chromatography (SFC) by Jentoft and Gouw[1], Hartmann and Klesper[2] and Perrut and Jusforgues[3], the technique has not yet been widely accepted, because fractions are collected in high-pressure vessels when a fluid such as carbon dioxide is used. Therefore, in order to obtain the fractions, the operator needs to wait until the last fraction has been collected, and then the pressure in the vessel must be reduced to atmospheric pressure. Hence the fractions cannot be dealt with as easily as those obtained in preparative liquid chromatography.

For easy operation of a preparative SFC system, it was necessary to develop a back-pressure regulating and fractionating system, which allowed operation under normal atmospheric pressure. For this purpose we developed a back-pressure regulator having an internal volume of less than 10 μl[4]. This back-pressure regulator is suitable for collecting solutes, in addition to applying a back-pressure, even for semi-preparative SFC, owing to the extremely low internal volume which prevents the remixing of solutes, as reported previously[5].

Lemon-peel oil

Citrus essential oil is a fairly expensive material used in the perfume and flavour

[a] Presented in part at the *International Conference on Supercritical Fluids, Nice, October, 1988.*

industries. In general, the oil is isolated from citrus-fruit peel with a cold-press machine. However, fresh cold-pressed oil deteriorates easily and develops an off-flavour (deteriorated odour), owing to the instability of terpene hydrocarbons, including limonene. Although these hydrocarbons are major components of the oil, they contribute little to the characteristic citrus aroma and flavour of the oil and are readily oxidized to off-flavour-producing compounds. Therefore, in order to increase the stability of the oil as a commercial product, the terpenes are removed by distillation. On the other hand, oxygenated compounds, such as aldehydes, alcohols and esters, are enriched because these are responsible for the flavour of the oil[6,7].

Recently, the extraction and enrichment of lemon peel oil with supercritical carbon dioxide have been examined by Calame and Steiner[8], Stahl and Gerard[9], Coppella and Barton[10] and Sugiyama and Saito[11]. Fractionation of components by supercritical-fluid extraction (SFE) was also investigated Stahl and Gerard[9] but was not very successful because a pressure near the critical pressure gave a higher selectivity for the terpenes relative to the oxygenated compounds, but a low extraction yield, whereas a higher pressure gave a higher yield but a lower selectivity.

This paper describes the fractionation of lemon-peel oil into several compound types, namely hydrocarbons, alcohols and aldehydes, esters and others by a semi-preparative SFC method.

EXPERIMENTAL

Materials and column

Cold-pressed lemon oil was donated by Mr. Sugiyama of Morinaga (Yokohama, Japan).

Standard-grade carbon dioxide was purchased from Toyoko Kagaku (Kawasaki, Japan) and was used as the mobile phase. High-performance liquid chromatographic (HPLC)-grade ethanol was purchased from Wako (Osaka, Japan) and used as a modifier for chromatographic elution. A JASCO (Tokyo, Japan) SuperPak SIL column (50 mm × 7.2 mm I.D.) packed with silica gel (10–20 μm) was used for the separation of the oil.

Apparatus

Fig. 1 shows a schematic diagram of the system. A JASCO Model 880-PU HPLC pump (1) with a cooling jacket kept at −5°C was used for the delivery of liquified carbon dioxide. Two 880-PU pumps (2 and 3) were used for solvent delivery. As the amounts of the lemon-peel oil fractions obtainable were estimated to be of the order of 10^{-3}–10^{-2} g based on the previous investigations[11], and as the fractions consist of volatile compounds, a third pump (3) was added in addition to the modifier pump. This pump delivered ethanol at a low flow-rate (0.05 ml/min), which was mixed with the column effluent at a tee-joint[12] placed downstream of the detector[11] to collect small amounts of volatile fractions efficiently. By this arrangement, the fractions could be obtained as ethanol solutions.

A six-way switching valve (7) (Model 7000; Rheodyne, Cotati, CA, U.S.A.) and a needle value (8) (Model 02-0120; SSI, State College, PA, U.S.A.) were used for by-passing the column and for releasing the fluid compressed and stored in the column when the column was removed from the line. This configuration is important for

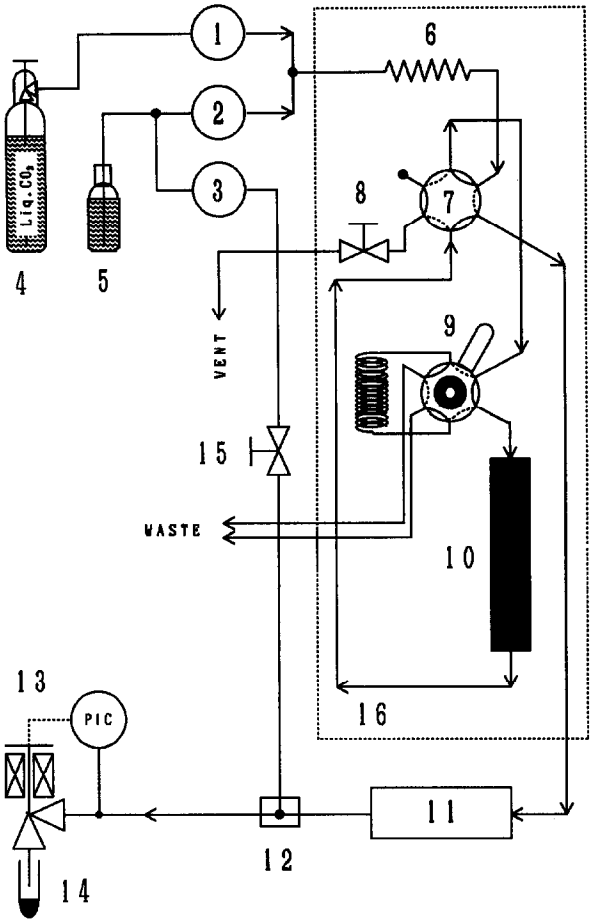

Fig. 1. Schematic diagram of coupled SFE–semi-prepararative SFC system. Components: 1 = carbondioxide pump; 2 = modifier pump; 3 = solvent pump; 4 = carbondioxide cylinder; 5 = modifier solvent; 6 = pre-heating coil; 7 = six-way switching valve; 8 = needle valve; 9 = injection valve; 10 = column; 11 = photodiode-array UV detector; 12 = tee-joint; 13 = back-pressure regulator; 14 = collection tube; 15 = shut-off valve; 16 = oven.

safety when a column larger than 4.6 mm I.D. is used, because such a column accumulates a large amount of energy in the form of compressed gas. For example, several litres of carbon dioxide are compressed to a few millilitres, and this energy can be explosively released if a column end-fitting is accidentally loosened. In the above arrangement, the column can be by-passed from the main line by means of the six-way valve (7), and the pressure in the column can be released slowly to atmospheric pressure by opening the needle valve (8). The column can then be disconnected from the line.

A Rheodyne Model 7125 injector (9) with a 0.5-ml loop was used for injecting cold-pressed lemon-peel oil.

A JASCO Model Multi-330 photodiode-array multi-wavelength UV detector (11) was used for monitoring the column effluent. A JASCO Model 880-81 back-pressure regulator (13) with a spring-loaded collection tube (14) was used for application of a back-pressure and for collecting components. The pre-heating coil (6), six-way valve (7), injector (9) and column (10) were placed in an oven (16) (JASCO Model 865-CO).

An HP 5890A capillary gas chromatograph (Hewlett-Packard, Avondale, PA, U.S.A.) was used for gas–liquid chromatographic (GLC) analysis of SFC fractions of the oil.

RESULTS AND DISCUSSION

SFC fractionation of cold-pressed oil

SFC fractionation of the cold-pressed oil was performed by injecting 0.5 ml of the oil directly onto the separation column using the ordinary valve injector. The column outlet pressure was kept at 10 MPa for the first 9.0 min, then increased to 20 MPa and held for 4.0 min, and finally ethanol was added at a flow-rate of 0.05 ml/min. The carbon dioxide flow-rate was kept constant at 2.2 g/min throughout the elution. The column temperature was constant at 40°C. The above conditions were determined bearing in mind the real-time monitoring of the spectrum using the multi-wavelength UV detector. The collection tube was changed manually every time the pressure was changed or modifier was added.

Fig. 2 shows a three-dimensional chromatogram of the oil obtained by the above procedure. The numbers shown under the time axis are time frames, which correspond to fractions collected.

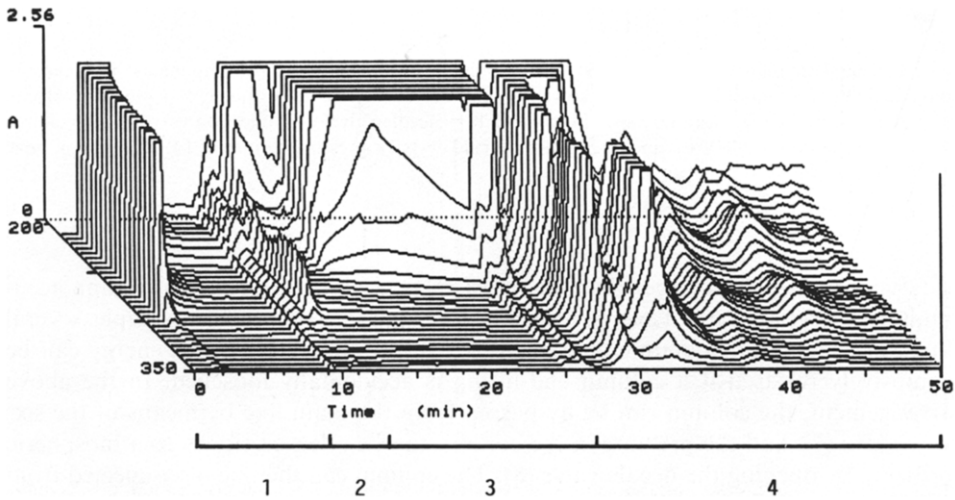

Fig. 2. Three-dimensional cromatogram of cold-pressed lemon peel oil obtained by semi-preparative SFC. The numbers under the time axis indicate fraction numbers, which are refered to as fractions 1–4 in the text and Table I. (From ref. 12).

GLC analyses of cold-pressed oil and its SFC fractions

Cold-pressed oil. Fig. 3 shows the GLC of the original cold-pressed oil without SFC fractionation, which we used as the reference. Peak assignment was performed by comparing the retention times of the components with the chromatogram of the same oil obtained in previous experiments[11] and by reference to the chromatograms of cold-pressed oil in the literature[6,10]. In these experiments, component peaks were identified by gas chromatography-mass spectrometry. Table I lists the compound names and the percentage peak area for each peak. As shown, the most abundant component is limonene, the peak area of which occupies more than 65% of the total area for all assigned peaks.

Fraction 1. This fraction was a colourless solution, which first smelt like a fresh lemon when it was collected. However, this aroma deteriorated to an off-flavour in a

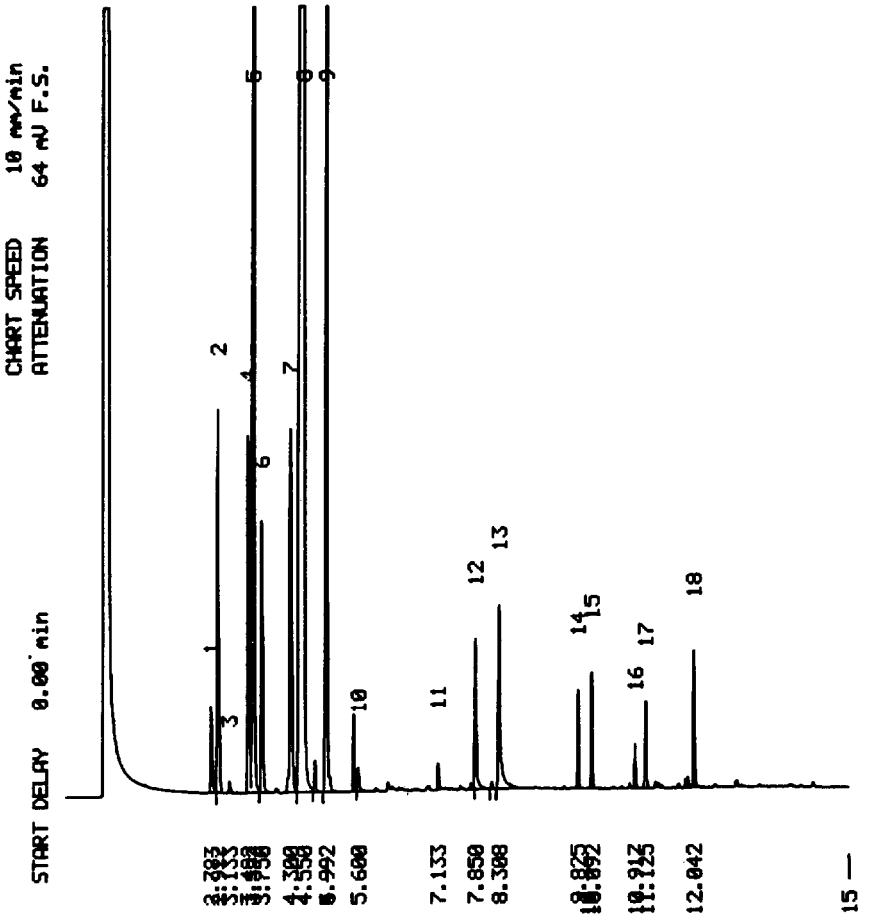

Fig. 3. GLC of cold-pressed oil. Peak numbers correspond to those in Table I. GLC conditions: column, Ultra-1 (20 m × 0.2 mm I.D.; Hewlett-Packard); detector, flame ionization; column temperature, 80°C, held for 3 min, then increased at 10°C/min to 180°C; injection volume, 2 μl (splitting ratio = 1:100); carrier gas, helium at 180; kPa. Numbers at the horizontal axis are retention times in min. (From ref. 12).

few days. Fig. 4 shows the GLC of fraction 1 obtained from the semi-preparative SFC of the oil, the chromatogram of which is shown in Fig. 3. Components are also listed in Table I. It is clear that this fraction contains only terpenes and no oxygenated compound except for peak 17 (α-bergamotene).

Fraction 2. This fraction was also a colourless solution. Its smell was slightly acidic like the aroma arising from a whole lemon being juiced in a kitchenmixer. Fig. 5 shows the GLC of this fraction. As shown in Table I, the major components are neryl acetate and geranyl acetate.

Fraction 3. This fraction was also colourless. Its aroma was similar to that of fraction 2, but less acidic. Fig. 6 shows the GLC of this fraction. It contained only aldehydes and alcohols as listed in Table I.

Fraction 4. This fraction was slightly yellow. its smell was completely off flavour, *i.e.*, like old shellaced wooden furniture. As shown in Fig. 2, this fraction included strongly UV- absorbing substances, which were suggested to be compounds containing benzene rings. There was no significant peak in the chromatogram, which is not shown here. This fraction contained slight precipitation, indicating that the substances in the fraction have higher molecular weights than those in fractions 1–3, and that the substances are non-volatile.

TABLE I

AMOUNTS (%) OF COMPONENTS OF SFC FRACTIONS OF COLD-PRESSED OIL

Peak No.	*Component*	*SFC fraction*			*Cold-pressed*[a]
		1	*2*	*3*	
1	α-Thujene	0.44			0.44
2	α-Pinene	2.01			1.92
3	Camphene	0.19			0.06
4	Sabinene	0.69	0.31		1.98
5	β-Pinene	11.96	0.22		12.37
6	Myrcene	1.47	1.09		1.44
7	α-Terpinene	3.00	0.95	0.18	2.53
8	Limonene	69.89	4.85	0.53	66.80
9	γ-Terpinene	9.12	0.34		8.02
10	Citronellal		2.07	2.52	0.17
11	Terpineol		0.44	11.67	0.12
12	Neral			24.52	0.80
13	Geranial			59.52	1.26
14	Neryl acetate		39.12	0.18	0.41
15	Geranyl acetate		50.06	0.41	0.49
16	β-Caryophyllene	0.21			0.20
17	α-Bergamotene	0.42	0.56		0.40
18	β-Bisabolene	0.61			0.59

[a] Cold-pressed oil without fractionation, given as a reference.

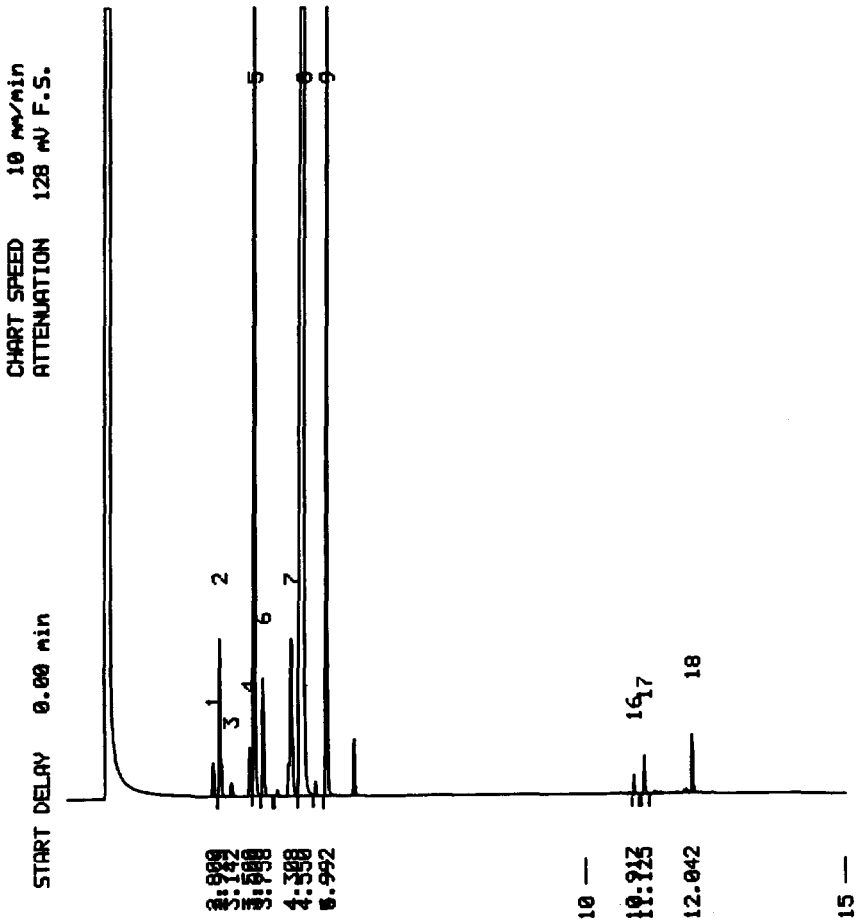

Fig. 4. GLC of fraction 1. Conditions as in Fig. 3. (From ref. 12).

CONCLUSION

Fractionation of lemon-peel oil was successfully performed by semi-preparative SFC. It is remarkable that 0.5 ml (about 0.5 g) of the oil could be fractionated into several compound types by using a relatively small column of 50 mm × 7.2 mm I.D. This means that the amount injected was about 20% of the weight of stationary phase in the column. This is higher than would be expected from the solubility of a hydrocarbon in supercritical carbon dioxide (only a few percent by weight). This high loading capacity is due to the stepwise pressure and modifier programming, which result in step-by-step injection by the stepwise change in the mobile phase strength.

The results suggest that the solute migration mechanism in our experiment was not based on an equilibrium distribution of the solute between the stationary phase

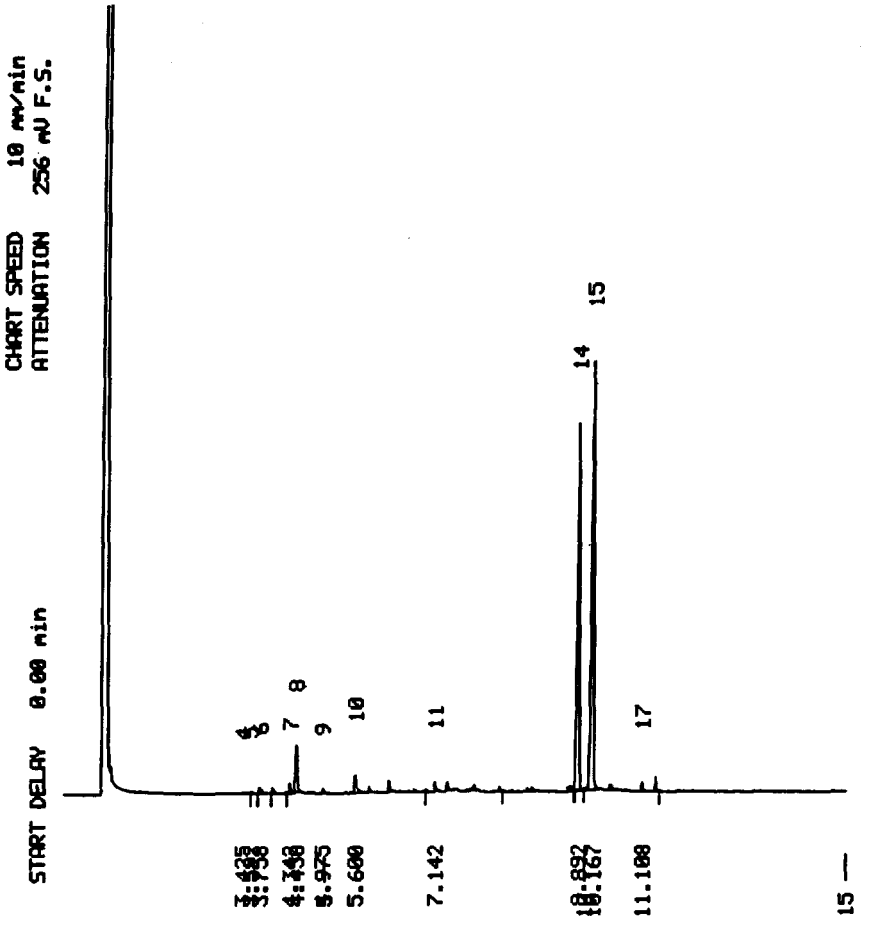

Fig. 5. GLC of fraction 2. Conditions as in Fig. 3. (From ref. 12).

and the mobile phase. Rather, it is based on absorption–desorption by the stepwise change in the mobile phase strength. Therefore, the injection is also made step-by-step, which can be regarded as programmed extraction on the column head. This may be the reason why such a high loading was possible.

This technique is more suitable for compound-type fractionation than separation into single compounds. The high loading capacity makes the preparative separation of SFE extracts of natural products feasible. The present technique can be used to produce new flavours by remixing fractions in different proportions and for the removal of terpenes from the oil.

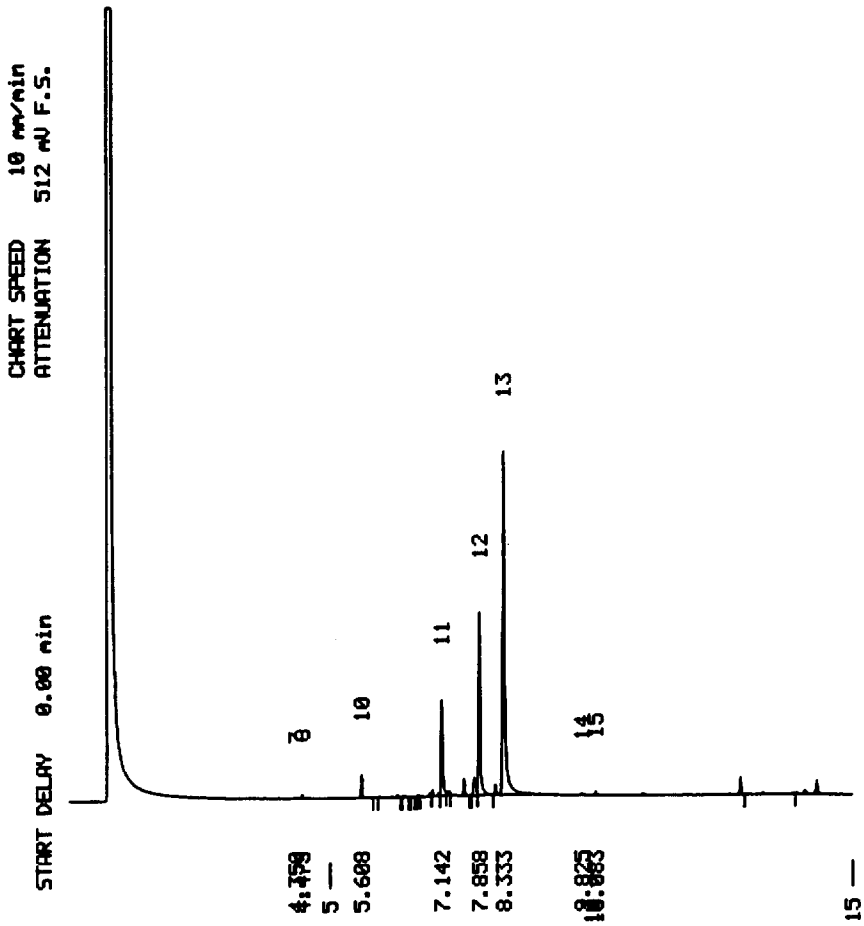

Fig. 6. GLC of fraction 3. Conditions as in Fig. 3. (From ref. 12).

ACKNOWLEDGEMENT

The authors thank the Institut National Polytechnique de Lorraine, France for the permission to reproduce Figs. 2–6.

REFERENCES

1 R. E. Jentoft and T. H. Gouw, *Anal. Chem.*, 44 (1972) 681–686.
2 W. Hartmann and E. Klesper, *J. Polym. Sci., Polym. Lett. Ed.*, 15 (1977) 713–719.
3 M. Perrut and P. Jusforgues, *Entropie*, 132 (1986) 3–9.
4 M. Saito, Y. Yamauchi, H. Kashiwazaki and M. Sugawara, *Chromatographia*, 25 (1988) 801–805.
5 M. Saito, Y. Yamauchi, K. Inomata and W. Kottkamp, J. Chromatogr. Sci., 27 (1989) 79–85.
6 Analytical Methods Committee, *Analyst (London)*, 109 (1984) 1343–1360.
7 F. Temelli, R. J. Braddock, C. S. Chen and S. Nagy, *ACS Symp. Ser.*, 336 (1988) 109–126.
8 J. P. Calame and R. Steiner, *Chem. Ind. (London)*, 19, June (1982) 399–402.
9 E. Stahl and D. Gerard, *Perfum. Flavor.*, 10 (1985) 29–37.

10 S. J. Coppella and P. Barton, *ACS Symp. Ser.*, 329 (1987) 202–212.
11 K. Sugiyama and M. Saito, *J. Chromatogr.*, 442 (1988) 121–131.
12 Y. Yamauchi, M. Saito, M. Senda, J. L. Millet and E. Castiglioni, in M. Perrut (Editor), *Proceedings of the 1st International Symposium on Supercritical Fluids, Nice, October 1988*, Vol. 1, Institut National Polytechnique de Lorraine, Nancy, 1988, p. 503.