Journal of Chromatography, 515 (1990) 515–520
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 1859

Supercritical fluid extraction and chromatography of steroids with Freon-22

S. F. Y. LI*, C. P. ONG, M. L. LEE and H. K. LEE
Department of Chemistry, National University of Singapore, 10 Kent Ridge Crescent, Singapore 0511 (Singapore)

ABSTRACT

The use of Freon-22 in supercritical fluid extraction and chromatography was investigated. For the extraction of steroids, the extraction efficiency with supercritical Freon-22 was found to be significantly better than that with supercritical carbon dioxide and the extraction time required was much shorter. Preliminary results for the chromatographic analysis of steroids also indicated that polar compounds could be eluted much more easily with Freon-22 than carbon dioxide as the mobile phase.

INTRODUCTION

Until now carbon dioxide has been the most widely used supercritical fluid for supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC), mainly owing to its inert properties, its compatibility with universal detectors (*e.g.*, flame ionization detector) and its availability in high purity. In addition, carbon dioxide is supercritical at moderate temperature and pressure, thus making it a suitable choice from an instrumentation point of view. However, a severe limitation is that it is relatively non-polar and consequently the extraction and chromatography of highly polar substances are relatively difficult. Attempts have been made to use alternative supercritical fluids[1,2] and to add polar modifiers to carbon dioxide[3–5] to extend the polarity range. However, the use of these alternatives has not been entirely satisfactory. For instance, when ammonia is used, the effects of corrosion and possible chemical reactions with the analytes have to be considered. The most commonly used modifier, methanol, not only has a very high critical temperature but also is flammable and therefore a flame ionization detector cannot be employed.

Freons have been used successfully in a few SFC applications[6]. In this investigation, chlorodifluoromethane (Freon-22) was selected based on considerations of the physical and chemical properties of a wide range of supercritical fluids. Table I compares the properties of carbon dioxide, methanol and selected Freons. It is noteworthy that the critical temperature of Freon-22 is much lower than that of methanol. Further, the dipole moment of Freon-22 is not only much higher than that of carbon dioxide and the other Freons but is also similar to that of methanol.

0021-9673/90/$03.50

TABLE I

COMPARISON PROPERTIES OF CARBON DIOXIDE, METHANOL AND FREON-22

Compound	*Critical temperature, T_c (K)*	*Critical pressure, P_c (atm)*	*Dipole moment (Debye)*
Carbon dioxide	31.3	72.9	0.0
Methanol	239.5	81.0	1.7
Freon-11	198.1	43.5	0.5
Freon-12	111.7	39.4	0.2
Freon-22	96.1	49.1	1.4

Therefore, the polarity range that can be covered by Freon-22 would be much greater than that by carbon dioxide. From an environmental point of view, Freon-22 has a much lower ozone-depleting and global warming potential than Freon-11 and -12 and has been suggested as their substitute in certain applications[7].

In this investigation, we evaluated the use of Freon-22 as an SFE solvent and the feasibility of using it as an alternative to or modifier of carbon dioxide in the SFC of polar compounds. As test substances seven steroids were studied.

EXPERIMENTAL

SFC experiments using carbon dioxide were performed with a Model SFC 3000 system (Carlo Erba), equipped with a flame ionization detector. Fused-silica capillary columns, SE-52 (column I, 10 m × 100 μm I.D., coating thickness 0.45 μm) and RSL-300 (column II, 12.5 m × 100 μm I.D., coating thickness 0.20 μm) were used. Tapered restrictors rated nominally at 8 ml/min (J&W Scientific) were connected after the column for pressure reduction. The actual flow-rate measured using a bubble flow meter was 8 ml/min at a detector temperature of 320°C. Injections were made with an air-actuated Valco VICI injection valve with a 1-μl loop. The injection time was 1 s. The chromatographic data were collected and analysed with a Hewlett-Packard Model 3390A integrator. The temperatures of the injection port, splitting outlet and detector were set at 45, 250 and 320°C, respectively.

The preliminary results for the SFC analysis using Freon-22 were obtained on a laboratory-built instrument. A Shimadzu LC-6A pump was fitted with a laboratory-made cooling jacket around the pump head. The temperature of the cooling jacket was maintained at 5°C by a refrigerating circulator consisting of a Thermomix 1442D temperature controller and a Frigomix-S cooling unit (Braun). A Hewlett-Packard 5790 GC oven was used to control the column temperature. A Rheodyne 7520 micro-injection valve fitted with a 0.5-μl injection loop was used for sample introduction. A Carlo Erbo Micro-UVis 20 detector was used for detection. The wavelength was set at 254 nm. An RSL-300 capillary column (10 m × 100 μm I.D., coating thickness 0.2 μm) was used for the analysis. A tapered restrictor fabricated in our laboratory with a measured flow-rate of 34 ml/min at 40°C was used for flow restriction and the tip of the restrictor during the chromatographic runs was immersed

in methanol to prevent blocking of the restrictor. A chart recorder (Houston Instruments) was used to record the chromatograms.

The seven steroids (Fig. 1) were obtained from Fluka and were of purum or puriss grade. Standard solutions were prepared in HPLC-grade methanol (J. T. Baker). The concentrations of estrone, testosterone, 17α-methyltestosterone and 17α-hydroxyprogesterone were 500 ppm and those of estriol, cortisone and hydrocortisone were 1000 ppm.

CP-grade carbon dioxide (British Oxygen) of 100% purity was used for the chromatographic work and purified-grade carbon dioxide for the extraction. Freon-22 was supplied by Atochem and had a purity exceeding 99.8%.

The design of the supercritical fluid extraction system has been described elsewhere[8–10]. The extraction pressures were 13.8, 15.2 and 18.0. MPa. For carbon

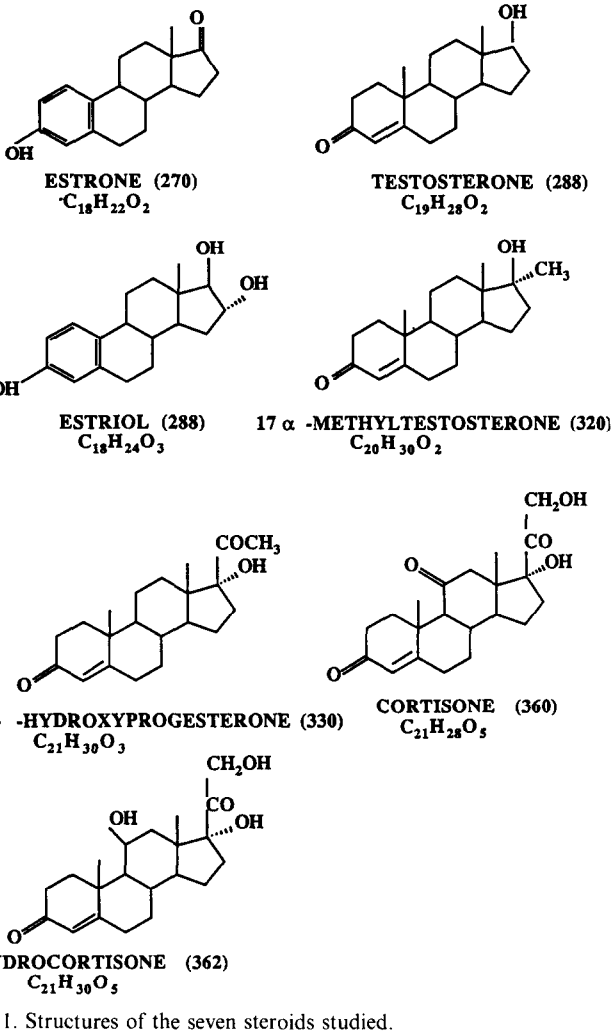

Fig. 1. Structures of the seven steroids studied.

dioxide an extraction time of 30 min was used and the temperature was maintained at 50°C, whereas for Freon-22 the extraction time was 15 min and the temperature was maintained at 100°C. For the Soxhlet extraction procedure, methanol was used as the solvent and the extraction time was extended to 7 h. In the latter instance, the extracts were periodically analysed until no further improvement in the extraction efficiency could be obtained with further increases in the extraction times. For the calculation of the extraction efficiency of each type of extraction procedure, the recoveries of estrone from spiked glass-wool were determined.

RESULTS AND DISCUSSION

The result of the supercritical fluid extraction studies are illustrated in Fig. 2. Although the extraction time for carbon dioxide was twice that of Freon-22, the recoveries obtained were much lower. Further, very little improvement could be made by increasing the extraction pressure to 18 MPa and a maximum recovery of only 16% was obtained. However, with Freon-22, 100% recovery could be achieved within 15 min. The results indicated that Freon-22 was not only capable of achieving higher extraction efficiencies but also required a shorter time. In Fig. 3, the results of the Soxhlet extraction procedure are shown. It can be seen that in order to obtain 100% recovery, an extraction time of more than 7 h would be needed. Therefore, the SFE procedure using supercritical Freon-22 would provide a significant reduction in analysis time.

Figs. 4 and 5 illustrate the chromatograms of the seven steroids obtained with pure carbon dioxide as the mobile phase using columns I and II respectively. For column I, which is the non-polar column, the selectivity was insufficient to separate testosterone and methyltestosterone. Estriol was not eluted even after an analysis time of more than 1 h at a column temperature of 115°C. However, with column II, which is of medium polarity, complete separation of all seven peaks was achieved using pure carbon dioxide as the mobile phase and the retention times obtained were not excessively long. Therefore, the use of a highly polar mobile phase did not seem necessary. However, to explore the potential of employing Freon-22 for SFC analysis,

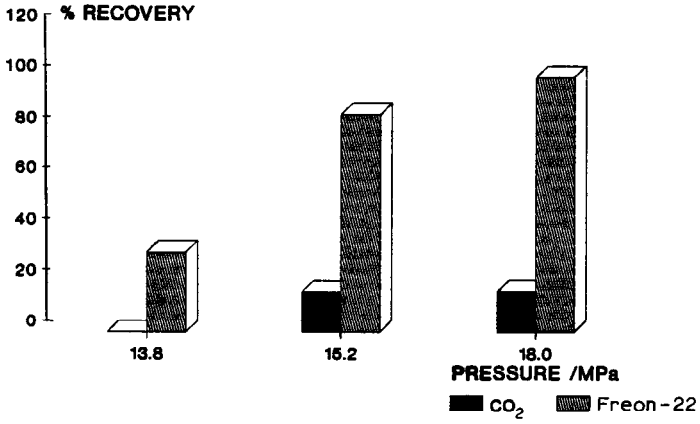

Fig. 2. Results of supercritical fluid extraction. Extraction times: carbon dioxide, 30 min; Freon-22, 15 min.

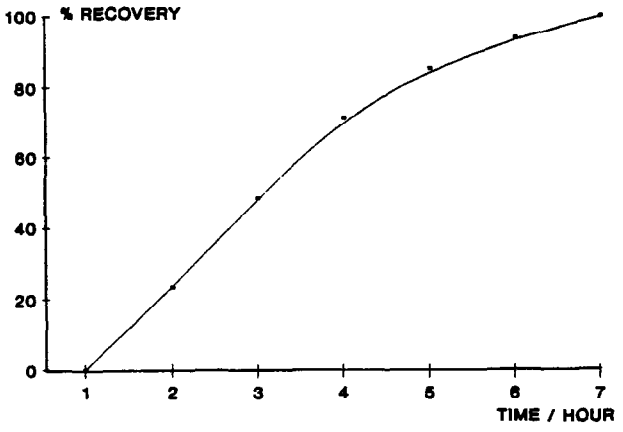

Fig. 3. Results of Soxhlet extraction using methanol.

some preliminary results were obtained to illustrate the faster elution times obtainable compared with carbon dioxide. The compound selected for this investigation was testosterone. The chromatograms obtained are shown in Fig. 6. The results show that the elution of testosterone with Freon-22 was much faster than that with carbon

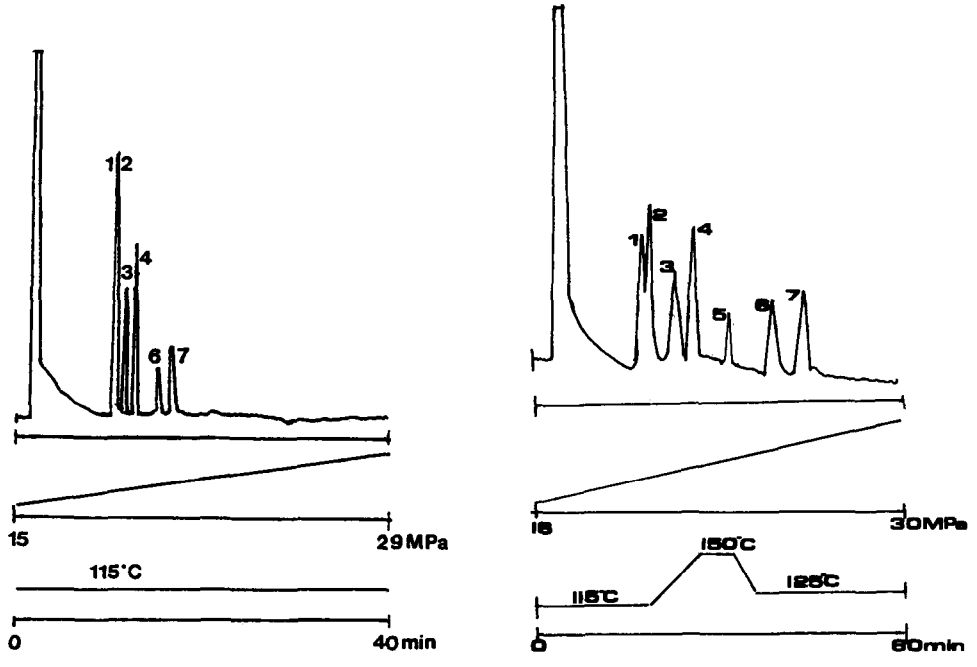

Fig. 4. Chromatogram of steroids obtained using column I and carbon dioxide as mobile phase. Peaks: 1 = testosterone; 2 = 17α-methyltestosterone; 3 = estrone; 4 = estriol; 5 = 17α-hydroxyprogesterone (not detected); 6 = cortisone; 7 = hydrocortisone.

Fig. 5. Chromatogram of steroids obtained using column II and carbon dioxide as mobile phase. Peaks as in Fig. 4.

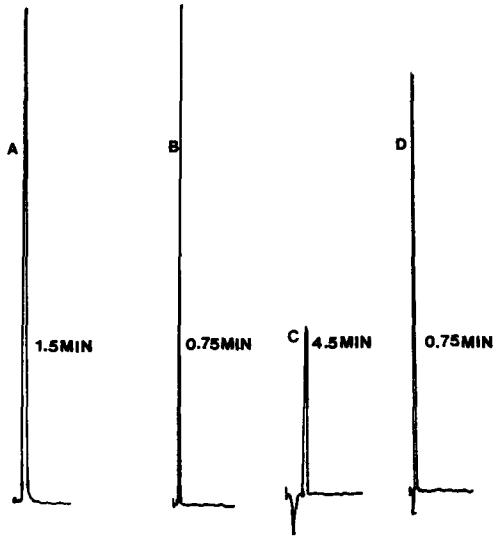

Fig. 6. Chromatograms for testosterone obtained using (A) Freon-22 at 5 MPa, (B) Freon-22 at 10 MPa, (C) carbon dioxide at 15 MPa and (D) carbon dioxide at 20 MPa.

dioxide. A lower operating pressure can be used to obtain similar or shorter retention times. These observations are consistent with the results of the extraction studies. Further, it was noted that with Freon-22 as mobile phase, peaks with higher signal-to-noise ratios were obtained. Therefore, a higher detection sensitivity could be achieved using Freon-22 with UV detection at 254 nm. In Fig. 6, chromatograms A and B were obtained using Freon-22 and the concentration of testosterone employed was 833 ppm, whereas chromatograms C and D were obtained using carbon dioxide with 2500 ppm of testosterone. Also, the UV cut-off for Freon-22 was found to be similar to that of methanol and therefore no solvent peak was observed. With carbon dioxide, a small negative peak was noted, indicating that the absorbance of supercritical carbon dioxide may be slightly higher than that of methanol. As 254 nm is the most widely used setting for UV detection, the enhanced sensitivity would certainly help to maximize the potential of SFC as a chromatographic technique.

Further work is being carried out to explore applications of Freon-22 in other supercritical fluid separation processes. However, from the promising results obtained so far, it can be concluded that there are significant advantages in using supercritical Freon-22 as a substitute for carbon dioxide in SFE and SFC.

REFERENCES

1 C. Fujimoto,T. Watannabe and K. Jinno, *J. Chromatogr. Sci.*, 27 (1989) 325.
2 Y. Hirata, *J. Chromatogr.*, 315 (1984) 39.
3 D. E. Raynie, S. M. Fields, N. M. Djordevic, K. E. Markides and M. L. Lee, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 11 (1989) 51.
4 T. A. Berger, J. F. Dye, M. Ashrof-Khorassani and L. T. Taylor, *J. Chromatogr. Sci.*, 27 (1989) 105.
5 S. Hara, A. Dobashi, K. Konoshita, T. Hondo, M. Saito and M. Senda, *J. Chromatogr.*, 371 (1986) 153.
6 D. Leyendecker, D. Leyendecker, F. P. Schmitz and E. Klesper, *J. Liq. Chromatogr.*, 10 (1987) 1917.
7 P. S. Zurer, *Chem. Eng. News*, July 24 (1989) 7.
8 C. P. Ong, H. K. Lee and S. F. Y. Li, paper presented at the *10th Australia Analytical Conference, August 28–September 2, 1989, Brisbane.*
9 C. P. Ong, H. M. Ong, S. F. Y. Li and H. K. Lee, *J. Microcolumn Sep.*, (1990) in press.
10 C. P. Ong, H. K. Lee and S. F. Y. Li, *J. Chromatogr.*, 515 (1990) 509.