

Separations of Long-chain Hydrocarbons and Mink Oils Using Pressure Programming Supercritical Fluid Chromatography

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The advantages of supercritical fluid chromatography (SFC) have recently been realized in particular its rapidity, flexibility, and ability to allow the analysis of substances which cannot be analysed by gas chromatography (GC). The separating power of open-tubular column GC is unparalleled, but its applicability is restricted by the limited volatility and thermal stability of many organic compounds; less volatile compounds can be analysed by high performance liquid chromatography (HPLC), but long analysis times and very small column diameters are required for efficient separations because of the limitations of solute diffusion in the mobile phase. SFC overcomes these difficulties and permits high resolution at low temperatures with short analysis times.

Above its critical point a substance such as carbon dioxide has properties which make its use as a chromatographic mobile phase very favourable. The ability of supercritical fluids to dissolve substances was first recorded in 1879, when Hannay and Hogarth¹ studied the solubility of cobalt and iron chlorides in supercritical ethanol. Numerous applications of extraction with supercritical solvents have been described, and the underlying theory fully understood.

Following a suggestion by Lovelock² in 1958 that a supercritical fluid might be used as a mobile phase in chromatography, Klesper *et al.* first demonstrated³ SFC by the separation of nickel porphyrins using supercritical chlorofluoromethanes as mobile phases. Sie and Rijnders⁴ and Giddings⁵ developed the technique further, both practically and theoretically, and many applications have been reported.

Supercritical fluids may be defined as fluids that are at temperatures and pressures above their critical temperatures and critical pressures. Supercritical fluids are now widely used in extraction, fraction, and chromatography.⁶⁻⁸ Supercritical fluid separations of the triglycerides in mink oils have not been reported yet, although other triglycerides in fish oil, rapeseed oil, soybean oil, and butterfat were separated⁹ and those of butterfat were identified using mass-spectrometry.¹⁰

In addition to their critical temperatures and pressures, the chromatographically important properties of supercritical fluid are the density, viscosity, and the diffusion coefficients of solutes.⁸ Above its critical point, a substance has density and solvating power approaching that of a liquid, but viscosity similar to that of a gas, and diffusivity intermediate

between those of a gas and liquid. Hence, the supercritical fluids have properties which make their use as chromatographic phase very favourable. As long as intermolecular interactions are sufficiently strong, supercritical fluids are able to dissolve a variety of solutes, even those with high molecular mass and low volatility. The density of supercritical fluids, and hence the solubility and chromatographic retention of solutes, can easily be changed by changing the applied pressure. This paper deals with the supercritical fluid separations of long-chain hydrocarbons and mink oils that are very difficult to be separated by either GC or HPLC, using pressure programming technique.

Experiments

A HP (Hewlett Packard, Palo Alto, CA) Model 5890 gas chromatograph was reconstructed in the laboratory as a supercritical fluid chromatograph. This system was equipped with a C14W loop injector (Valco) and a flame ionization detector (Figure 1). SFC-grade carbon dioxide (Scott Specialty Gases) was used as a basic mobile phase. Supercritical experimental conditions are summarized as the followings;

(1) Separations of long-chain hydrocarbons

Column	Nucleosil CN 1 × 50 mm	
Mobile phase	CO ₂	
Pressure programming	Initial pressure	1, 500 psi
	Initial time	3 min
	Pressure rate	40 psi/min
	Final pressure	5000 psi
Oven temperature	70 °C isothermal	
Restrictor	stainless integral restrictor 10 mL/min at 1, 500 psi	
Detector	FID	
	Temperature	350 °C
	Hydrogen flow	30 mL/min
Sample solvent	Air flow	
	300 mL/min	
Sample solvent	CHCl ₃	

(2) Separations of mink oils

Column	deltabond C ₈ 1 × 100 mm
Mobile phase	CO ₂

Pressure programming	Initial density	0.40 g/mL
	Initial time	7 min
	Density rate	0.002 g/mL per min
Oven temperature	150°C isothermal	
Restrictor	stainless integral restrictor 10 ml/min at 1,500 psi	
Detector	FID	
	Temperature	350 °C
	Hydrogen flow	30 mL/min
	Air flow	300 mL/min
Sample solvent	hexane	

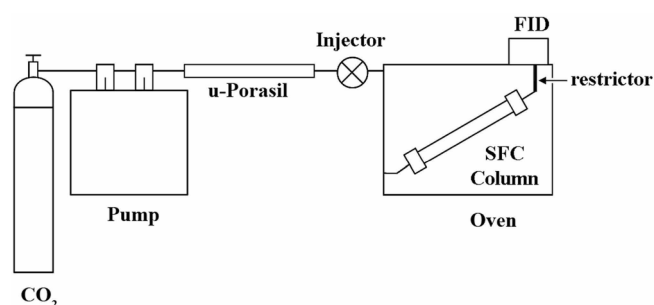


Figure 1. Schematic diagram of supercritical fluid chromatography system used for the separations of long-chain hydrocarbons and mink oils.

Results and Discussion

Supercritical fluid chromatography is of importance because it permits the separation and determination of a group of compounds that are not conveniently handled by either gas or liquid chromatography. These compounds are either nonvolatile or thermally labile so that gas chromatographic procedures are inapplicable and contain no functional groups that make possible detection by the spectroscopic or electrochemical techniques employed in liquid chromatography.

Long-chain hydrocarbons are a good example of such intractable species. It is nonvolatile since the length of carbon chain is so long. Thus long-chain hydrocarbons are the compounds beyond the volatility range of GC, and contain no UV-absorbing functional groups which make UV detection possible in HPLC.

Figure 2 illustrates the supercritical fluid chromatographic long-chain hydrocarbons. Detection was made by the flame ionization detector (FID). A major advantage of SFC over HPLC is that FID of gas chromatography can be employed. FID detector exhibits a general response to organic compounds, is highly sensitive, and is largely trouble free.

Figure 2(a) shows the chromatograms of $C_{12}H_{26}$, $C_{13}H_{28}$, $C_{14}H_{30}$ and $C_{16}H_{34}$ hydrocarbons, and Figure 2(b) shows the chromatograms of five long-chain hydrocarbons ($C_{22}H_{46}$, $C_{24}H_{50}$, $C_{26}H_{54}$, $C_{28}H_{58}$ and $C_{30}H_{62}$). An interesting experimental result is worth noticing and additional mentioning.

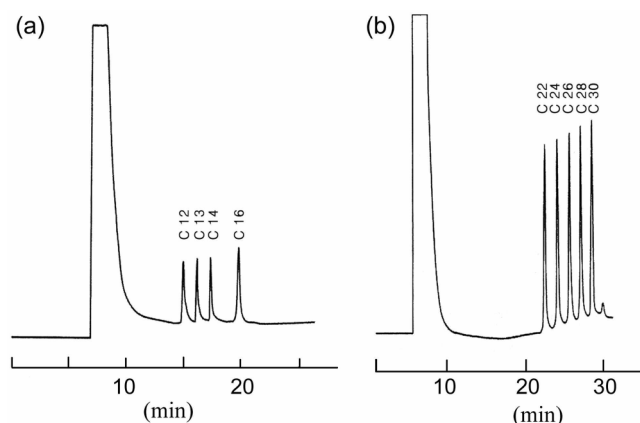


Figure 2. SFC chromatograms of long-chain hydrocarbons.

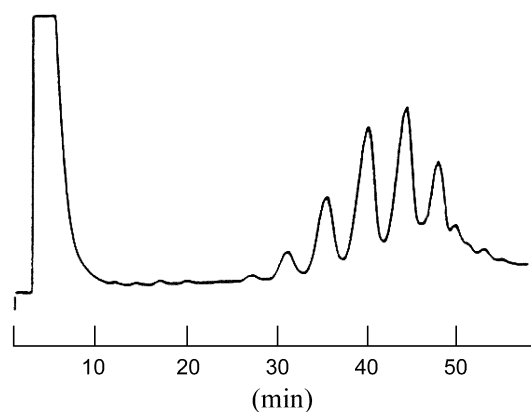


Figure 3. SFC Chromatogram of Mink Oil.

That is, the peak height of the last eluted compound ($C_{30}H_{62}$) in Figure 3(b) is not reduced compared with early eluted compounds ($C_{22}H_{46}$, $C_{24}H_{50}$, $C_{26}H_{54}$, $C_{28}H_{58}$). This result supports that the mobile phase of supercritical fluid chromatography can dissolve the hydrocarbon solutes well and the volatilization process which normally is used in gas chromatography does not occur.

Figure 3 illustrates the supercritical fluid chromatographic separation of mink oil. The oligomers of mink oil were well separated. Detection was also made by FID. The chemical species of mink oil are long-chain triglycerides which are nonvolatile and contain very weak chromophores.

In this work, pressure programming and density programming were adopted for separations of long-chain hydrocarbons and mink oils respectively. Pressure or density changes in supercritical fluid chromatography have a pronounced effect on the retention or capacity factor. This effect is a consequence of the increase in density of the mobile phase with increase in pressure. Such density increases cause a rise in solvent power of the mobile phase, which in turns the elution time for eluents.

The influence of pressure program on separations in SFC is similar to that of density program. Because of the non-linear relationship between pressure and density, linear density program at constant temperature imply non-linear

pressure ramps and the other way round. The density of the mobile phase is the most important parameter to influence and optimise separations in supercritical fluid chromatography. Density programming during an analytical run is as common in SFC as temperature programming in GC or programming of eluent composition in HPLC. The influence of density on the solvent properties can be demonstrated using the concept of solubility parameters, first introduced by Hildebrandt and Scott,¹¹ and extended to supercritical phases by Giddings.^{12,13} Following this concept, the solubility parameter, δ , of a solvent depends on its density, ρ :

$$\delta = 1.25 P_C^{1/2} (\rho/\rho_{\text{liq}})$$

P_C : critical pressure

ρ_{liq} : reference density in the liquid

The solubility parameter δ varies from 0 up to liquid-like values of 10 at high densities. To solubilise a substrate, the solubility parameters of the substrate and of the solvent should be nearly equal. At low eluent densities, solubility parameters of the mobile phases in SFC are low compared to those of the substrates, but they increase with increasing densities. Therefore, capacity ratios, k' , decrease at higher densities¹⁴ indicating higher solvent strength. The slope of the decrease is not the same for different classes of compounds.

Looking at separation efficiencies, similar observations were made. Due to lower mass transport rates at higher densities, diffusion coefficients of the substrates and the self diffusion coefficient of the mobile phase also decrease¹⁴ leading to peak broadening. Assuming constant retention behaviour at the same time, *i.e.*, $k' = \text{constant}$, this results in van Deemter Plots of h versus u , the increase of which after passing the typical minimum is less steep at lower densities than at high densities.

In conclusion, supercritical fluid chromatography was successfully applied to separation of long-chain hydrocarbons

and mink oils that are very intractable to be separated by either gas chromatography or liquid chromatography. Excellent chromatograms were obtained by pressure and density programming. The pressure and density profiles used in this work are constant for a given length of time followed by a linear increase to a final state. These kinds of separations based on the use of supercritical fluids promise to play an important role in the analysis of nonvolatile, no chromophore containing compounds in environmental, biomedical and food samples.

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