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Note

Capillary supercritical-fluid chromatography with flame-ionization detection: reduction of detection artifacts and extension of detectable molecular weight range

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When a supercritical fluid is used as a chromatographic mobile phase, the resulting process, supercritical-fluid chromatography (SFC), has characteristics of both high-performance liquid chromatography (HPLC) and gas chromatography (GC)[1–4]. For example, mobile phase solvation in SFC allows relatively low-temperature elution of materials, but the gas-like viscosity of the mobile phase results in higher optimum velocities and shorter analysis times than when using liquids in the same columns.

Increased analysis speed is important, but is overshadowed by genuine, new capability. With CO_2, N_2O, NH_3 and several other SFC mobile phases, it is possible to use a flame-ionization detector[4–7]. This provides the long-sought combination of a solvating mobile phase and sensitive, universal detection.

Many analysis problems fall between the capabilities of GC and HPLC. With GC, both volatility and thermal stability are required for a successful analysis. Although these are not required for HPLC, sensitive, universal detection is not possible today with liquid mobile phases. Unitl the combination of SFC–flame-ionization detection (FID), there was no convenient, sensitive capability for determining low volatility or thermally labile analytes that do not strongly absorb light.

Since the mobile-phase pressure must be reduced at the SFC-column–flame-ionization detector interface, solutes can condense, often resulting in noisy, spiked speaks[4–6]. This has limited the application of FID and other GC detection methods to SFC. Fjeldsted and co-workers[4,5], have been successful at reducing the consequences of spiking in their straight-walled, capillary restrictors by the use of electronic filtering. Rawdon has adapted a flame-ionization detector for packed-column SFC which is reported free of spiking to solute molecular weights of 2000[7]. Our attempts at producing similar, pinched restrictors for capillary SFC have not been successful. However, we have been successful in significantly reducing spiking with capillary restrictors. This has allowed an increase in the molecular-weight range of capillary SFC–FID.

EXPERIMENTAL

Our supercritical fluid chromatograph is similar to the one described by Pea-

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don *et al.*[8]. A high-pressure syringe pump (Model 8500, Varian, Palo Alto, CA, U.S.A.) was modified for pressure control[9]. Provision was made to supply the pressure reference signal either from the pump front panel or an external source. Pressure programming was accomplished using an Apple IIe computer with an Adalab interface (Interactive Microware, State College, PA, U.S.A.). Our arrangement provided 11-bit pressure resolution (*ca.* 0.05%) from 0 to 400 atm. The injector is an electrically actuated, internal loop valve with 0.1-μl injection volume (Model EC14W.1, Valco, Houston, TX, U.S.A.). Room-temperature split sampling was used[8].

The injector tee, venting restrictor, and column were placed in the oven of a gas chromatograph equipped with a flame-ionization detector (Model 5700, Hewlett-Packard). The column outlet was connected to a fused-silica capillary restrictor using a 1/32 in. zero dead volume union with capillary ferrules (Valco). This detector restrictor tubing had a 14-μm I.D. and was about 20 cm in length. The last 5 cm of the restrictor were drawn out in a flame to reduce the inside diameter and to remove the polyimide coating. The outlet I.D. was sized using a light microscope, and the tube cut in a position to give an outlet orifice of about 2 μm. This restrictor was inserted through the flame-ionization detector so that the outlet was even with the jet tip.

Commercial columns with 100-μm I.D. and film thicknesses of 0.1 and 0.25 μm were used. The stationary phases used were BP-10 (SGE, Austin, TX, U.S.A.), Methyl Silicone, and CPS-2 (Quadrex, New Haven, CT, U.S.A.). Even though these phases had been cross-linked and were essentially non-extractable, conditioning with supercritical fluid at the temperature and upper pressure limit to be used was always necessary.

RESULTS AND DISCUSSION

We have taken two specific actions to help prevent condensation in the flame-ionization detector restrictor and extend the molecular weight range. First, tapering the restrictor outlet tends to keep the pressure higher through the length of the restrictor, making the pressure gradient more abrupt at the outlet. Second, when a potential condensation problem exists, we get as much heat into the restrictor as possible. The detector block is kept at 400°C, for example. However, the most effective means of providing additional heat is to raise the mobile-phase temperature just prior to decompression. The easiest way to accomplish this is simply to raise the column oven temperature if the solutes are thermally stable.

We typically run our columns at 90 to 120°C and have experienced very little condensation trouble at these temperatures for compounds up to about C_{60}. The effect of mobile phase temperature on spiking is clearly seen in Fig. 1 where the conditions were adjusted to deliver the analyte peak (glycerol tristearate) to the detector at approximately the same rate (that is, similar peak widths and areas) in both chromatograms. The differences in detection are due only to the effect of the mobile phase temperature. Secondary advantages of operating the column at higher temperatures, when possible, are the increases expected in the solute diffusion coefficients, and the improved linearity of the mobile phase density-pressure isotherm. This results in faster optimum velocities, and in more even peak spacing in pressure-programmed chromatograms of homologues and oligomers.

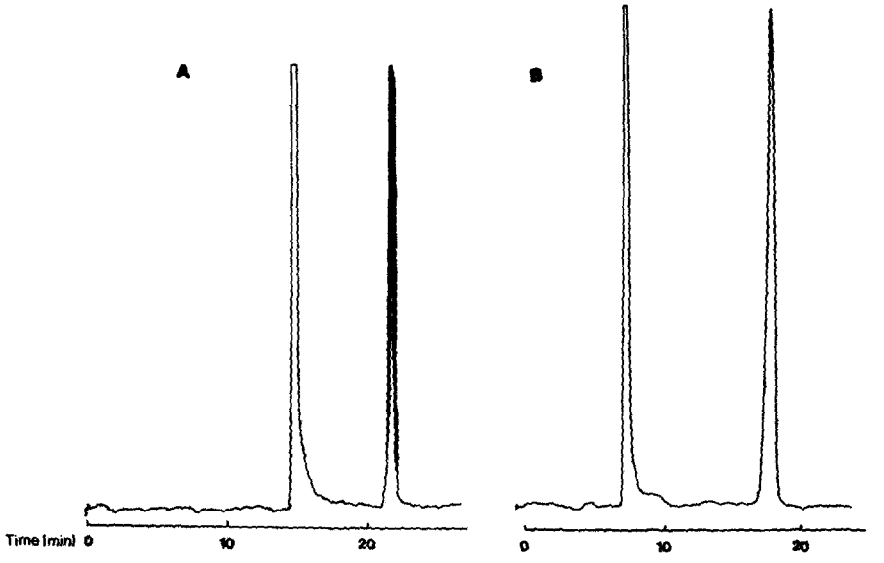

Fig. 1. Isobaric chromatograms of glycerol tristearate at (A) 60°C, 200 atm and (B) 90°C, 250 atm. Conditions: CO_2 mobile phase; methyl silicone column, 15 m × 100 μm I.D., 0.25-μm film thickness.

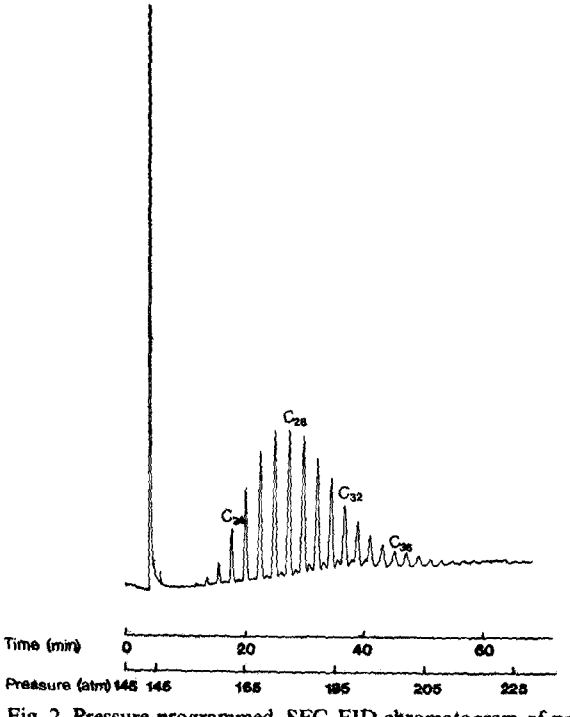

Fig. 2. Pressure-programmed, SFC–FID chromatogram of paraffin wax. Conditions: CO_2 mobile phase; column temperature, 90°C; BP-10 column, 9 m × 100 μm I.D., 0.1-μm thickness.

Fig. 2 is a pressure-programmed chromatogram of paraffin wax, obtained from the raw (non-filtered) flame-ionization detector output signal. (The combined time constant of the electrometer output and the strip-chart recorder used was approximately 300 ms.) No condensation or spiking is evident in this example. Compounds as small as C_{24} were reported to cause spiking in earlier work[6].

SFC–FID, with a CO_2 mobile phase, is very well suited for fat analysis. Fig. 3 shows a separation of underivatized fatty acids. Special commercial columns, such as the CP™ Wax 57 CB (acid treated) (Chrompak, Bridgewater, NJ, U.S.A.), are available for GC determinations of free acids. However, upper temperature limits of this column restrict the acids that can be determined to those lighter than C_{12}. SFC–FID gives good peak shape and precision for the acids shown, and potential to determine even heavier acids. (The relative standard deviation for C_{18}-acid at a concentration of 0.2% in the test solution was 2.4% using external standards, and 0.6% with an internal standard.)

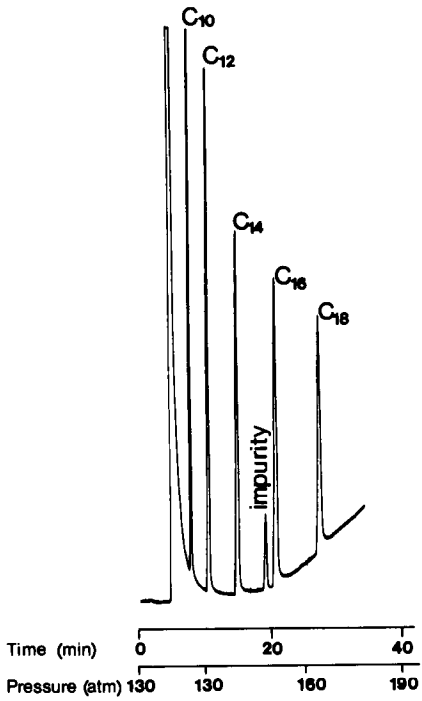

Fig. 3. Pressure-programmed, SFC–FID chromatogram of even-numbered straight chain free fatty acids from C_{10} through C_{18}. Conditions: CO_2 mobile phase; column temperature, 90°C; column as in Fig. 2.

Mixed glycerides can be analyzed straightforwardly by SFC–FID. Fig. 4 shows the components in an industrial-grade glycerol monostearate. The last peak, glycerol tristearate, has a molecular weight of 890. The sample preparation in this (and all other examples) only involved dissolving the test sample in a solvent.

Non-ionic surfactants are also easily analyzed by SFC–FID. Separations of aromatic, ethoxylated surfactants such as Triton X-100 (Rohm and Haas, Philadel-

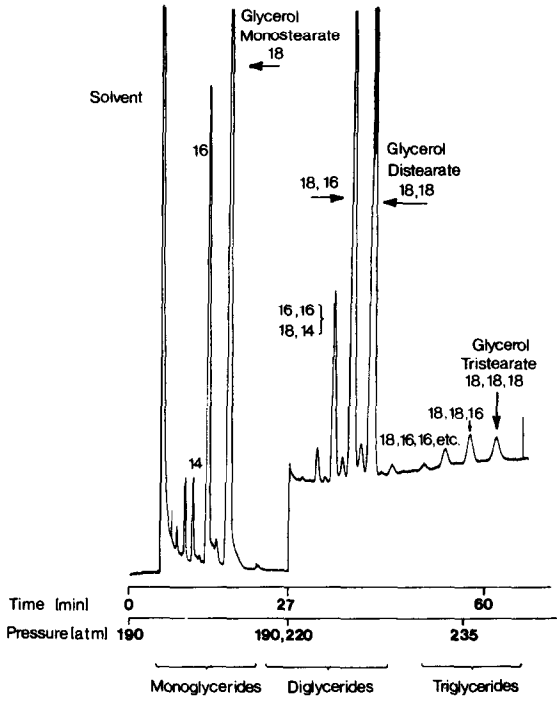

Fig. 4. Pressure-programmed SFC–FID chromatogram of industrial glycerol monostearate in trichloromethane. Conditions. CO_2 mobile phase; oven temperature, 90°C; column as in Fig. 2 but 12 m in length.

phia, PA, U.S.A.) are possible by HPLC[10]. Triton X-100 has also been fractionated by packed-column SFC using solvent programming and UV detection[11]. Fig. 5 shows the capability using capillary SFC–FID. In addition to the better resolution provided by capillary SFC over both packed-column HPLC and SFC, FID clearly shows impurity peaks present that presumably are not good UV absorbers since they have not been visualized with UV-detection. Also, determination of averge degree of polymerization cannot be done directly from UV-detected chromatograms because it is not known if response factors of all the peaks are identical[10]. However, with FID, relative response factors (normalized to carbon content) are easily predictable once peak assignments are made. Thus, determination of degree of polymerization should be straightforward by SFC–FID.

The real potential is illustrated in Fig. 6, however, where the components of an alkyl, ethoxylated surfactant, Neodol 23-6.5 (Shell Chemicals, Houston, TX, U.S.A.), are separated. This material is composed of C_{12}- and C_{13}-alkyl chains with varying degrees of ethoxylation. All of the components are clearly visible ranging from the C_{12}- and C_{13}-alcohols through the ethoxylate numbers up to about 22, as well as some impurities. Molecular weights of the last peaks approach 1200.

Detector spiking is a function of the restrictor geometry, temperature, pressure, flow-rate, solute volatility and solute delivery rate. The combined effects of volatility and delivery rate are seen in Fig. 4 where spiking occurs on a large diglyceride peak but not on the smaller triglyceride peaks that follow. With the physical conditions

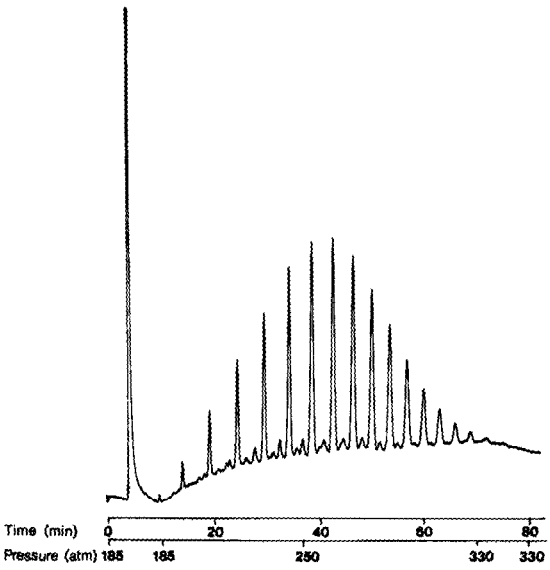

Fig. 5. Pressure-programmed SFC–FID chromatogram of Triton X-100. Conditions: CO_2 mobile phase; column temperature, 110°C; column as in Fig. 2.

constant, we can imagine a "spiking delivery rate threshold" that varies directly with solute volatility.

A unique advantage of SFC over both GC and HPLC is the selectivity control that is possible with temperature selection. For constant-pressure conditions, parti-

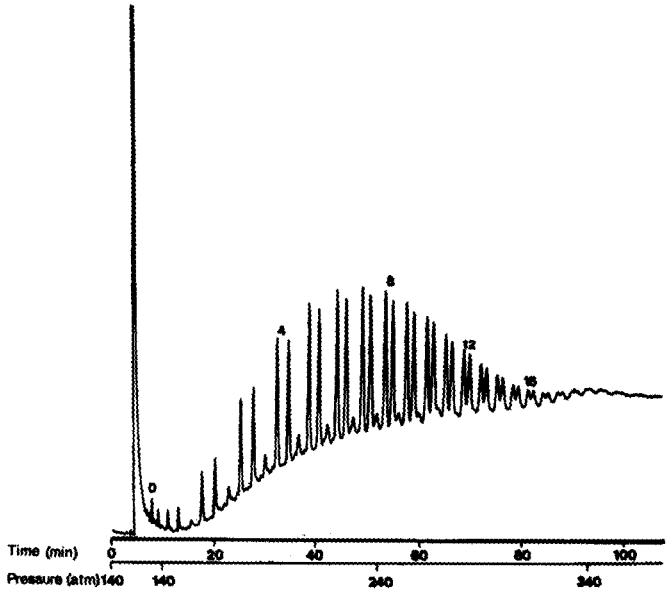

Fig. 6. Pressure-programmed SFC–FID chromatogram of Neodol 23-6.5, an alkyl ethoxylate mixture of the formula $H(CH_2)_n(OCH_2)_eOH$, where n is 12 or 13 and e varies from 0 to at least 22. Some e values are indicated on the chromatogram. Conditions: CO_2 mobile phase; column temperature, 110°C; column as in Fig. 2.

tioning at low temperature is controlled by the solute–mobile phase interactions, and decreases with increasing temperature (as the mobile phase loses density). However, at even higher temperatures, partitioning into the mobile phase can increase again due to the increase in solute volatility. A pair of solutes might have similar partitioning behavior at low temperature, but different boiling points, or *vice versa*. Thus, temperature-dependent selectivity changes can be exploited in SFC in those cases where higher temperatures can be tolerated. An example is shown in Fig. 7 where an 85°C temperature change reversed the elution order of the two analytes. This behavior must be considered, in addition to solute thermal stability and propensity to spiking, when selecting an operating temperature.

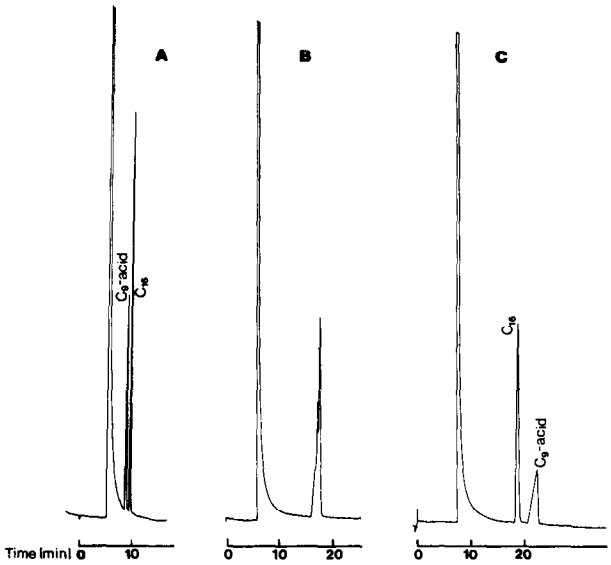

Fig. 7. Isobaric chromatograms of nonanoic acid and hexadecane at 111 atm and (A) 140°C, (B) 95°C and (C) 55°C. Conditions: CO_2 mobile phase; column as in Fig. 2.

Finally, we have not used electronic filtering in any of the chromatograms shown. Its use would further increase the molecular weight range of materials detectable by SFC–FID beyond the range shown here.

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