Supercritical Fluid Chromatography

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Summary. Chromatographic separations with a supercritical fluid as the mobile phase were suggested more than 20 years ago. Availability of commercial hardware makes this technique more widely usable today. Many separations by this method are now carried out with supercritical carbon dioxide as the mobile phase and packed liquid-chromatography columns as the stationary phase. Although carbon dioxide has many practical advantages, including its near-ambient critical temperature and minimal interference with spectrometric detection, the use of other supercritical fluids or addition of modifiers to carbon dioxide may extend the applications of this technique. Some mixtures that are difficult to analyze by other chromatographic methods may be susceptible to separation by supercritical fluid chromatography. Mixtures that have been separated with supercritical carbon dioxide include resin acids with the empirical formula $C_{20}H_{30}O_2$ and ubiquinones from bacterial cell wall extracts of *Legionella pneumophila*.

The impact chromatography has had on chemical technology over the past 40 years has been remarkable. A brief historical review will serve to indicate this and will also help to place the development of supercritical fluid technology in perspective.

Although liquid chromatography (LC) was practiced in a variety of forms after Tswett (*1*) separated pigments in 1906, it was not until 1941 that Nobel prize-winning work of Martin and Synge (*2*) formally defined liquid-liquid partition chromatography. This led to a broader understanding and use of LC. Martin and Synge also suggested the use of a gas in place of a liquid as the mobile phase and thus defined gas-liquid partition chromatography. Approximately 10 years later, James and Martin (*3*) published experimental details of the gas chromatographic separations of acids and amines, beginning modern gas chromatography (GC) and catalyzing a dramatic increase in the use of this analytical technique. The petroleum industry, in particular, had analytical needs that were easily fulfilled by the gas chromatographic technique. A wide variety of materials encountered in petroleum chemistry are volatile or can be volatilized up to 450°C, which is considered the practical limit for GC. Younger scientists may not fully appreciate the explosive growth that occurred in this field since the middle 1950's. In 1952 it was possible to count the number of gas chromatographers on one hand. Today, perhaps tens of thousands of scientists use GC routinely.

The seminal idea for supercritical fluid chromatography (SFC) was planted at an international GC meeting in 1958. Lovelock (*4*), one of the research pioneers in GC, suggested the use of a supercritical fluid mobile phase (*5*). In 1961 Klesper *et al.* (*6*) published experimental results of the use of supercritical dichlorodifluoromethane and monochlorodifluoromethane to separate the involatile nickel etioporphyrin isomers. This was followed by in-depth studies progressing from GC through "dense" gas chromatography and on to SFC by Rijnders and Sie (*7–11*) and by Giddings and co-workers (*12–16*). Work in the area of supercritical fluid technology continued. Jentoft and Gouw (*17, 18*) applied SFC to practical analyses of petroleum mixtures, including automobile exhaust. Rogers and co-workers (*19, 20*) extended the SFC technology with a variety of separations, including oligomers and silicone polymers. The advances and discoveries from 1961 to the present have been described in review articles (*21–24*) and will not be repeated here in detail.

Supercritical fluid technology has wide application beyond chromatography. Hubert and Vitzthum (*25*) discussed the supercritical extraction of hops, spices, and tobacco without the use of organic solvents. Friedrich (*26*) described the extraction of oil from soybeans with supercritical carbon dioxide, comparing the method with hexane extraction with respect to economics and purity of the product. Van Wasen and Schneider (*23*) described physicochemical applications of SFC in the determination of thermodynamic data such as thermal and caloric equation of state, phase behavior, and transport properties. These fundamental measurements have great importance in the overall understanding of all areas of supercritical technology.

In this article I review the state of the art of SFC, with specific emphasis on carbon dioxide and packed columns. I will discuss the important chromatographic operating parameters, the use of solvent mixtures consisting of a second component (a modifier) in carbon dioxide, and the spectral transparency of carbon dioxide.

Important Parameters

A key term in SFC is "supercritical." The critical temperature of a substance is the transition point where the vapor and liquid phases have the same density. Above the critical temperature, a gas cannot be liquefied, no matter how high the pressure. It is substantially higher than the boiling point (the temperature at which the vapor pressure of a substance equals 1 atmosphere) and much higher than the triple point, where the gas, liquid, and solid states coexist.

Some parameters that are important in chromatography are the diffusion coefficient, density, and viscosity of the solvent. Table 1 shows values of these properties which are representative of those present in "typical" separations in GC, SFC, and high-performance liquid chromatography (HPLC). The diffusivities are average values; within each separation mode, experimental values will range about them. For instance, it is possible in a very favorable HPLC situation, such as the separation of benzene from nitrobenzene with a liquid pentane mobile phase at 45°C and a silica stationary phase, to have a binary interdiffusion coefficient equal to or superior to that for benzoperylene in supercritical carbon dioxide at 45°C with the same silica stationary phase. However, benzoperylene, a fused six-ring polycyclic aromatic hydrocarbon, will neither dissolve in liquid pentane to an appreciable extent nor elute from an HPLC column with liquid

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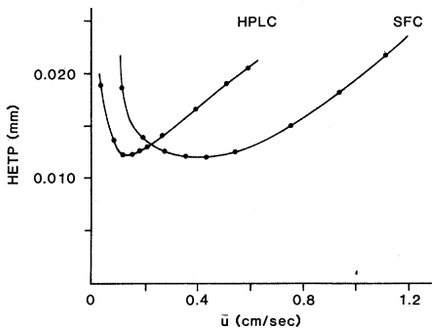


Fig. 1. Van Deemter plots for chromatographic data for HPLC and SFC elution of pyrene (*27*); *HETP* is height equivalent to a theoretical plate.

pentane in any reasonable amount of time. Yet benzoperylene is soluble in supercritical carbon dioxide at sufficient density (0.75 g/cm^3) and elutes in a few minutes.

Figure 1 shows graphs of height equivalent to a theoretical plate (H) versus average linear velocity ($\bar{u}$) of the mobile phase for two such separations (*27*). In both cases the solute is pyrene, and the stationary phase a reversed-phase octadecyl silane (ODS) at 40°C. The mobile phase for the SFC separation is carbon dioxide at an average density of 0.75 g/cm^3. The mobile phase for the HPLC separation is acetonitrile and water (70:30 by volume). These conditions provide approximately the same capacity factor (k') for pyrene in both cases.

The optimum average linear velocity for the solute pyrene occurs at 0.13 cm/sec in the HPLC separation and at 0.40 cm/sec in the SFC separation. The minimum value of H in both cases is 0.012 mm for the stationary phase, in which the particle diameter is 5 μm.

Figure 2 is a comparison of a sample separation carried out in the HPLC and then the SFC mode. With the same column and instrument (*28*) the solutes are resolved in a total separation time in SFC that is less than the retention time for the first component peak in HPLC. In general, if the solutes are soluble in carbon dioxide, as they are in the comparable HPLC solvent, the efficiency per unit time will be more favorable for the SFC mode, being directly proportional to the respective interdiffusion coefficients.

Now let us consider how to calculate the ratio of the interdiffusion coefficients for the two mobile phases by using the van Deemter curves generated experimentally. Equation 1 is a hyperbolic relation between H and $\bar{u}$, known as the plate height equation; $D_{1,2}$ is the interdiffusion coefficient of solute and solvent; d_p is the average particle diameter of the stationary phase; and A, B, and C are constants derived from the geometry, the packing, and the capacity characteristics of the separating column (*29*).

$$H = A + \frac{B}{\bar{u}} + C\,\bar{u} \qquad (1)$$

Here, $A = 2\lambda d_p$ (multipath term), $B = 2\gamma D_{1,2}$ (longitudinal diffusion), and $C = \phi(d_p^2/D_{1,2})$ (resistance to mass transfer in the mobile phase); λ is the packing factor, whose numerical value ranges between 1 and 2; γ is a constant that takes into account the restricted diffusion path in a packed column; and ϕ is a function of the capacity factor k':

$$\phi = \frac{1 + 6\,k' + 11\,k'^2}{24\,(1 + k')^2}$$

The values of A, B, and C can be used to evaluate the optimum velocity of the mobile phase (the velocity corresponding to the minimum H value). Taking the first derivative of H with respect to ($\bar{u}$) and setting it equal to zero, we find the minimum of the hyperbola or $(\bar{u})_{min}$.

$$\frac{dH}{d\bar{u}} = \frac{d}{d\bar{u}}\left(A + \frac{B}{\bar{u}} + C\,\bar{u}\right) = 0 \qquad (2)$$

and

$$(\bar{u})_{min} = \sqrt{\frac{B}{C}} \qquad (3)$$

Substituting for B and C from Eq. 1, we then obtain,

$$(\bar{u})_{min} = \left(\frac{2\,\gamma\,D_{1,2}}{\phi\,d_p^2/D_{1,2}}\right)^{1/2} =$$

$$\left(\frac{2\gamma}{\phi}\right)^{1/2}\left(\frac{D_{1,2}}{d_p}\right) = \text{constant} \times \frac{D_{1,2}}{d_p}$$

Since the same column was used, the packing parameter λ and d_p are identical. This assumption is supported by the identical $(H)_{min}$ values. The ratio of the capacity factor functions (ϕ) is approximately one, even though the experimental capacity factors were not precisely the same. Finally, the ratios of interdiffusion coefficients can be calculated from the minimum average linear velocity ratios:

$$\frac{(D_{1,2})_{SFC}}{(D_{1,2})_{HPLC}} = \frac{(\bar{u})_{min,\,SFC}}{(\bar{u})_{min,\,HPLC}} =$$

$$\frac{0.40\ \text{cm/sec}}{0.13\ \text{cm/sec}} = 3.1$$

This value can be compared with the independently measured experimental data for interdiffusion coefficients (*30–33*). Ignoring the difference in temperature and the fact that the data are for naphthalene rather than pyrene, we can estimate the ratio of interdiffusion coefficients from the data of Table 2 for carbon dioxide at 40°C and *n*-hexane at 25°C. This ratio compares very favorably with the chromatographic data.

Table 1. Typical values of parameters important in chromatographic band broadening.

Parameter	GC	SFC	HPLC
Diffusion coefficient, $D_{1,2}$ (cm^2/sec)	10^{-1}	10^{-4}	10^{-5}
Density (g/cm^3)	10^{-3}	0.8	1
Viscosity (g/cm-sec)	10^{-4}	5×10^{-4}	10^{-2}

$$\frac{(D_{1,2})_{CO_2}}{(D_{1,2})_{n\text{-hex}}} = \frac{1.15 \times 10^{-4}}{0.40 \times 10^{-4}} = 2.9$$

Table 3 gives viscosity data for carbon dioxide at various combinations of temperature and pressure. The data are from Lauer *et al.* (*32*) and confirm the discussion of viscosity effects in chromatography by Giddings *et al.* (*34*). The somewhat surprising result is that the viscosity is constant at any constant density irrespective of the combination of temperature and pressure that produces this density, in both the supercritical state and the liquid state (*32*). The viscosity increases with increasing density. The values of the viscosity of supercritical carbon dioxide are much lower than the typical values for liquid mobile phases used in LC, by at least a factor of 10. This leads to a column pressure drop (inlet minus outlet pressure) per unit linear velocity in chromatography with supercritical carbon dioxide which is one-tenth (or less) of the drop usually encountered with typical liquid mobile phases. This lower column pressure drop has at least two benefits: a higher linear velocity is achieved more easily with a given pumping system, and the lifetime of the packed column is extended.

So far I have compared SFC with HPLC in terms of efficiency per unit time with the parameters held constant—that is, under isothermal, isobaric, and constant (or no) modifier operation. But in addition to the diffusion enhancement in SFC, the pressure (and therefore density), temperature, and mobile phase modifier influence the retention process. The influence of these parameters, individually and in concert, has been discussed in detail (*28, 35, 36*).

Any one of these parameters can be programmed during a separation, and the potential gain is analogous to that with temperature programming in GC and solvent programming (gradient elution) in

HPLC (*19, 20, 37–40*). The comparative effects are quite complicated and there are no general equations on which to base a theoretical treatment; hence such a discussion is beyond the scope of this article. This is an area of intensive research in several laboratories.

In discussing SFC I have considered carbon dioxide as the mobile phase; however, the points made above are applicable to any supercritical fluid. In the following sections I will consider the ramifications of the choice of fluid on the practical aspects of chromatography, including modifier addition to carbon dioxide, transparency in infrared and ultraviolet spectra, and fraction collection and off-line mass spectrometry. The application of SFC to separate ubiquinones in bacterial cell extracts will then be discussed.

Table 2. Experimental interdiffusion coefficients for naphthalene (*32*).

Solvent	Temperature (°C)	Interdiffusion coefficient (cm^2/sec) $\times 10^{-4}$
Carbon dioxide	40	1.15
n-Hexane	25	0.40
Methyl alcohol	25	0.18
Water	25	0.10

Modifier Addition to Carbon Dioxide

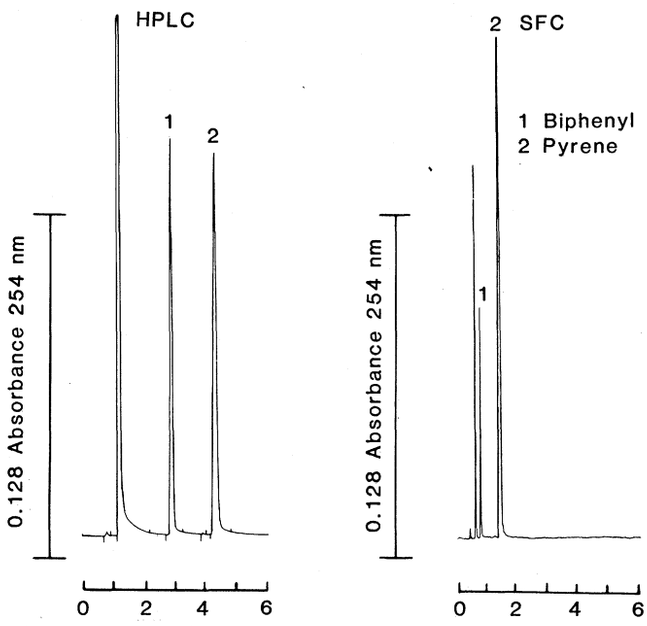
The solvent strength of carbon dioxide varies markedly with the density of the fluid. The solvent strength at a density less than 0.25 g/cm^3 is less than that of perfluorinated alkanes, while at a density of 0.98 g/cm^3 it exceeds that of hexane, perhaps approaching the solvent strength of methylene chloride. It has been found experimentally that the solvent strength can be extended somewhat by addition of miscible polar modifiers such as alcohols (*36, 41*). Most organic solvents—alcohols, ethers, tetrahydrofuran, dimethyl sulfoxide, chloroform, and others—are miscible with carbon dioxide. The use of carbon dioxide–modifier mixtures offers great flexibility since the modifier and its concentration are easily and widely variable and the solvent power can be altered to fit the analysis. For example, the xanthines are separated in about 1 minute on a silica column with a mixture of 6.5 mole percent 2-methoxyethanol in carbon dioxide. On the same column, the separation cannot be achieved with carbon dioxide alone.

Both the maximum solvent power and solvent selectivity of the mixture are determined by the identity of the modifier, just as in HPLC (*42*). For the xanthine analysis mentioned above, use of 2-propanol as the modifier gives a longer analysis time and different elution order, with caffeine and theophylline coeluting. Methylene chloride and chloroform are unsuitable for this separation in this system. The amount of the modifier affects the overall retention time as well as the elution order. With 2.5 mole percent 2-methoxyethanol in carbon dioxide, theobromine elutes before theophylline. Randall (*41*) has studied the effects in SFC of the type and amount of modifier added to carbon dioxide as the mobile phase. Chromatograms from her studies are seen in Fig. 3, which shows the separation of the xanthines with 9.5 and 6.5 percent 2-methoxyethanol and 9.5 percent 2-propanol added to the carbon dioxide.

Modifier addition can be expected to enhance SFC technology. However, a relatively small number of combinations of solute, modifier, and stationary phase have been tested, and it is too early to determine the limitations and full potential of adjusting selectivity in SFC through modifiers. It seems unlikely that selectivity change obtained with modifiers in SFC will be as great as that obtained through adjustments in solvent selectivity in HPLC.

Transparency in the Infrared and Ultraviolet

Carbon dioxide has interesting properties as a chromatographic solvent in addition to those already discussed. It is a relatively simple molecule and its infrared spectrum is also relatively simple.



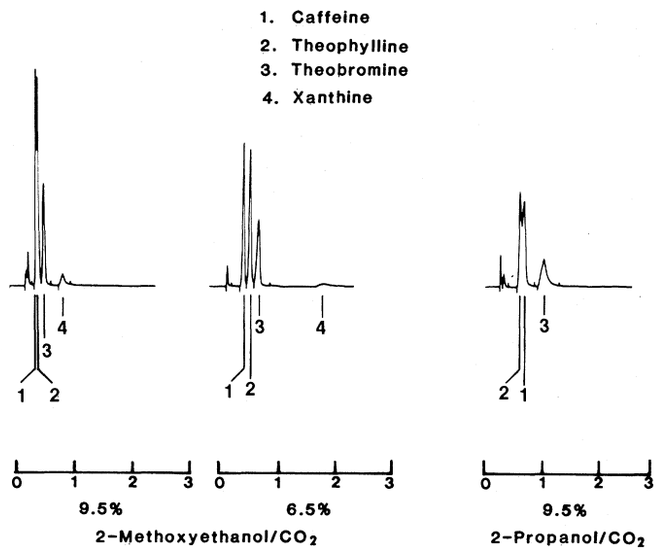


Fig. 2 (left). Chromatograms of the separation of biphenyl (peak 1) and pyrene (peak 2) at the optimum average linear velocity ($\bar{u}$) for HPLC and SFC. An ODS reversed-phase column, 10 cm by 4.6 mm, and a 5-μm particle diameter were used. For HPLC the solvent was acetonitrile and water (70:30) at 1.0 cm^3/min, linear velocity 0.13 cm/sec, and column pressure drop 62 bars. For SFC, the carbon dioxide flow rate was 2.5 cm^3/min, linear velocity 0.40 cm/sec, column pressure drop 14 bars, and average column pressure 165 bars. Fig. 3 (right). Comparison of SFC separations of xanthines, showing the effects of type and amount of modifier in carbon dioxide. A silica column, 10 cm by 4.6 mm, and a 5-μm particle diameter were used. The solvent was carbon dioxide with modifiers added. Flow rate was 4 cm^3/min, 60°C, average column pressure 346 bars, column pressure drop 14 bars, and density 0.86 g/cm^3.

Griffiths and Shafer (*43*) described SFC separations in which carbon dioxide was used as the mobile phase and a Fourier transform infrared (FTIR) spectrometer was used as the detector. They obtained easily identifiable spectra of various organic compounds with the on-line flowing cell detector. Figure 4 shows the infrared spectrum of carbon dioxide which they obtained at a pressure of 103 bars, 50°C, and a density of 0.43 g/cm^3. They noted that the spectrum becomes somewhat less usable as the density is increased.

The ultraviolet spectrum of high-purity carbon dioxide is also transparent down to and below the spectral cutoff wavelength of the variable-wavelength ultraviolet detectors used in SFC. Useful chromatograms can be obtained at a working wavelength of 190 nm if the purity of the carbon dioxide is carefully controlled and any modifier being used does not exhibit significant absorbance.

SFC appears to be applicable to some mixtures that have long been a challenge to GC and HPLC. Two features of SFC with carbon dioxide are useful here: free, underivatized acids can be easily and quickly eluted, and the transparent ultraviolet region permits detection at a wavelength where selective absorption of isomers is minimized. Resin acids are the main non-steam-volatile components in pine oleoresin and in tall oil, a by-product of kraft pulping of pinewood. These components include mixtures of isomeric diterpene acids, the most abundant being abietic acid. The resin acids abietic, neoabietic, pimaric, and levopimaric acid have the empirical formula $C_{20}H_{30}O_2$, contain one carboxylic acid functional group, and have two double bonds distributed within and without the ring structures. Dehydroabietic acid is similar to the other resin acids except that it has three double bonds. The resin acids, as well as polyunsaturated fatty acids, have been implicated in fish toxicity (*44*). Analysis of these mixtures is difficult by GC and HPLC.

Table 3. Viscosity of supercritical carbon dioxide (*32*).

Temperature (°C)	Pressure (bar)	Density (g/cm^3)	Viscosity (g/cm-sec) $\times 10^{-4}$
32	90	0.70	6.0
40	115	0.70	6.0
50	187	0.70	6.0
80	269	0.70	6.0
100	334	0.70	6.0
32	128	0.80	7.5
40	164	0.80	7.5
50	264	0.80	7.5
80	365	0.80	7.5
100	467	0.80	7.5
32	226	0.90	9.8
40	282	0.90	9.8
60	420	0.90	9.8
80	558	0.90	9.8
100	696	0.90	9.8

Figure 5 shows chromatograms of a commercial sample thought to consist of at least 85 percent abietic acid. The wavelength of maximum absorbance of this isomer is approximately 235 nm. The other isomers exhibit significantly different wavelength maxima as the distribution of double bonds changes. The lower chromatogram was obtained at 235 nm, while the upper chromatogram was obtained (for the same sample and separating conditions) at the more universal wavelength of 196 nm. The analysis at 196 nm reveals that there are considerably more constituents present, and the peak areas suggest high levels of impurity. From chromatograms at other wavelengths, the peak labeled 1 was tentatively identified as neoabietic acid. A variety of commercial samples of abietic acid and other resin acid isomers suggest that the determination of the purity and the component identification of such mixtures can be improved by SFC analysis.

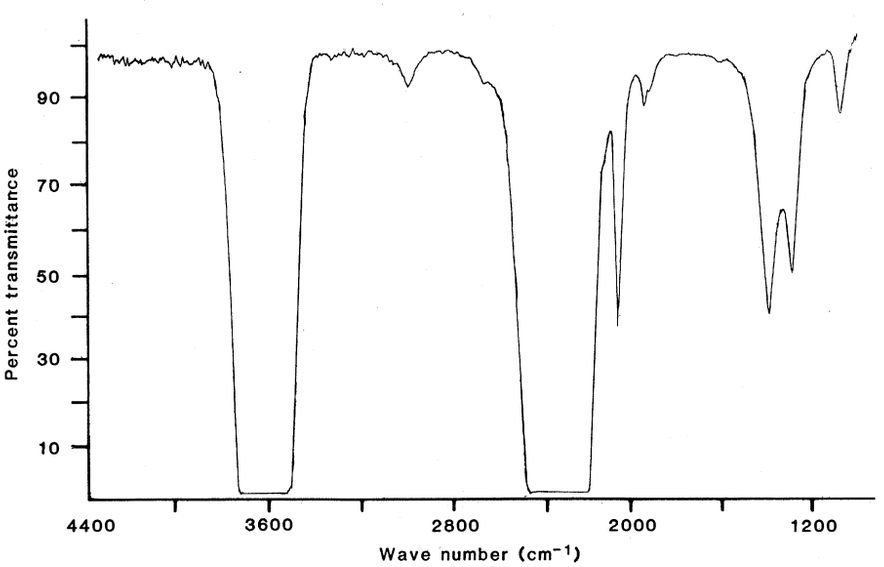


Fig. 4. Fourier transform infrared spectrum of supercritical carbon dioxide at 50°C and a density of 0.43 g/cm^3. [Spectrum courtesy of P. Griffiths (*43*)]

Fraction Collection and Off-Line Mass Spectrometry

A practical advantage of SFC with carbon dioxide is the ease of fraction collection and the simple nature of the solute in the collected state.

Figure 6 shows a chromatogram of lipids separated by Norris and Rawdon (*45*). In the normal instrument configuration, the system pressure is held at the operational column value through the ultraviolet detector and reduced to atmospheric at an exit back-pressure regulator. In order to collect a fraction, Norris and Rawdon replaced the back-pressure regulator with a stainless steel capillary tube that was crimped just enough to achieve the desired pressure. They note that the expanding gas forms a plume of small solid carbon dioxide particles and the solute precipitates out at the apex of the expanding carbon dioxide cone. By holding a mass spectrometer direct-inlet probe filament in this cone as the ultraviolet peak is displayed on the printer plotter, Norris and Rawdon collected enough sample for off-line mass spectrometric analysis.

Figure 7 shows the electron impact mass spectrum of the trilaurin (peak 5) in Fig. 6. The mass spectrum agrees precisely with catalog data (*46*) for an authentic sample of this compound. Although several laboratories have demonstrated the feasibility of on-line SFC-MS (*47–50*), no commercial interface equipment is available for routine use. However, off-line collection of fractions from SFC columns using carbon dioxide is relatively easy. The expansion of the carbon dioxide is accompanied by a rapid temperature drop; thus even volatile solutes can be trapped and desolvated on a direct-inlet probe filament. Alternatively, fractions could be collected in a small tapered glass vial for qualitative analysis. In this case, a small Teflon tube slipped over the stainless steel exit tube would facilitate collection of the expanding fluid in the tapered vial with minimal rebounding of the solid carbon dioxide out of the container. The SFC separation

of the lipid mixture on the packed column required less than 2 minutes. Fractions of the six compounds can be serially collected and introduced into the mass spectrometer inlet at a convenient time. An expensive mass spectrometer does not have to be dedicated to one particular supercritical fluid chromatograph and may be used for several chromatographs.

The trilaurin peak in Fig. 6 represents approximately 100 μg of solute collected from a single injection of the sample. The triglyceride contains three 12-carbon saturated fatty acids, and the ultraviolet absorbance results primarily from the carboxylic acid groups.

Biological Application:

Ubiquinones in Bacteria

SFC has been used to separate and detect ubiquinones (*51*), a class of compounds that exist in biological systems including bacteria. Coenzyme Q-12 is one of these isoprenoid quinones, which contain a substituted benzoquinone and a long isoprenoid tail (Fig. 8). These oligomeric compounds are constituents of bacterial plasma membranes and play a vital role in electron transport, oxidative phosphorylation, and many other subtle biochemical functions (*52–54*).

The long isoprenoid tail allows ubiquinones to be miscible with and soluble in the lipid phase of the mitochrondrial membrane. They are the only electron carriers in the respiratory chain that are not tightly bound or covalently attached to a protein. Ubiquinone Q-12 is a highly mobile carrier of electrons between the flavoproteins and the cytochromes of the electron-transport chain (*52*).

Jones and Collins (*54*) discussed the isoprenoid quinone structure in bacteria and the chemical taxonomy implications in microbiology. Their data are based on thin-layer chromatographic (TLC) measurements and are given for a wide range of bacteria. Unfortunately, isoprenoid quinones are listed only as major or minor constituents with no numerical evaluation.

Bacterial cell extracts of *Legionella* were provided by Moss (*55*). Figure 8 shows a chromatogram of the bacterial cell extract of *Legionella pneumophila*, the causative agent in Legionnaires disease. We tentatively identified the ubiquinones Q-8 through Q-13 from ultraviolet absorption spectra and relative retention information. The most significant features of the chromatogram are the absence of the Q-10 peak and the major peaks of Q-11, Q-12, and a smaller but significant Q-13 peak. Moss and coworkers (*56*) subsequently studied six *Legionella* species and observed that the ubiquinone profiles allowed further classification of *Legionella* into three distinct groups. They confirmed the structure and identity of these ubiquinones including the observation that Q-13 had not been found previously in other bacteria (*54, 57, 58*). The R_F values of the TLC work and our SFC retention data were well correlated.

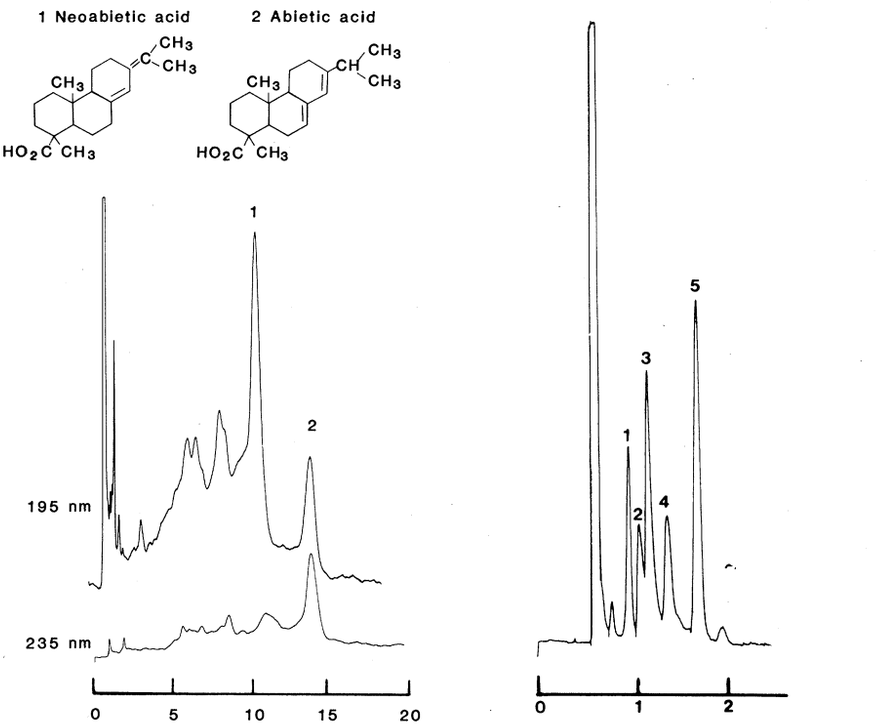


Fig. 5 (left). Comparison of SFC separations of abietic acid mixture at different ultraviolet wavelengths. Polystyrene–divinyl benzene column, 10-μm particle diameter, 15 cm by 4.1 mm. Flow rate was 2.2 cm^3/min, linear velocity 0.23 cm/sec, and temperature 100°C. Average column pressure was 320 bars, density 0.68 g/cm^3. Fig. 6 (right). Separation of lipid mixture with fraction collection (peak 5) for MS analysis. Fractions are lauric acid (peak 1), glyceryl monolaurate (peak 2), glyceryl dilaurate (peaks 3 and 4), and trilaurin (peak 5). Octadecyl silane reversed-phase column, 5-μm particle diameter, 10 cm by 4.6 mm. Carbon dioxide with 0.5 percent by weight methyl alcohol, flow rate 3.0 cm^3/min, linear velocity 0.35 cm/sec, and temperature 30°C. Average column pressure was 166 bars, column pressure drop 15 bars. Average density of the mobile phase was 0.80 g/cm^3. [Chromatogram courtesy of Norris and Rawdon (*45*)]

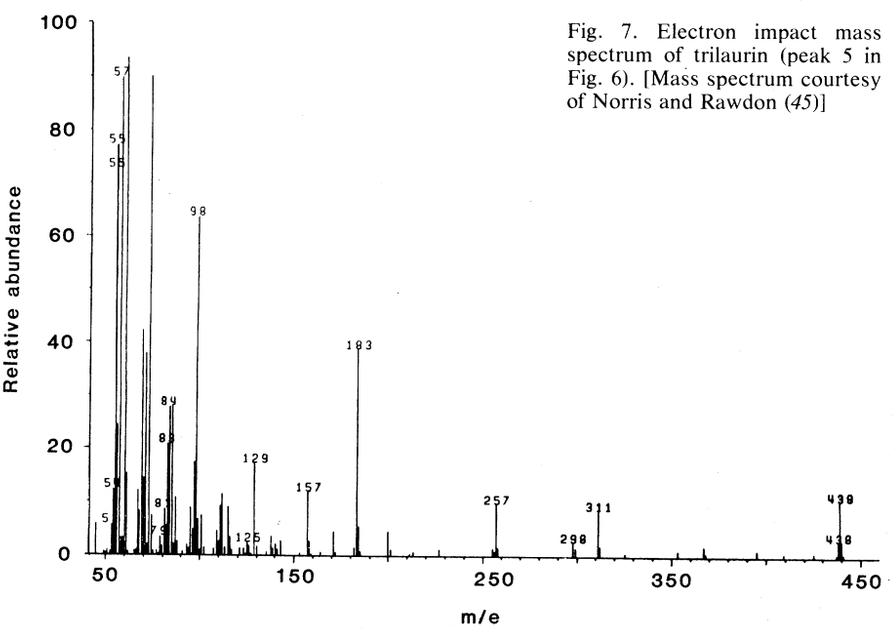


Fig. 7. Electron impact mass spectrum of trilaurin (peak 5 in Fig. 6). [Mass spectrum courtesy of Norris and Rawdon (*45*)]

Concluding Remarks

SFC with carbon dioxide as the supercritical fluid mobile phase and packed LC columns as the stationary phase represents the revival of a technique that has been around for more than 20 years. Recent availability of commercial hardware makes it possible for chromatographers (*35*) to use this technique without having to make their own high-pressure equipment. The availability of a large number of suitable supercritical fluids extends the potential applications of this technique. Wall-coated capillary columns may also widen the applicability if suitable attention is given to the somewhat different instrumentation (*59*). The separation column can also be coupled to on-line detectors other than the ultraviolet spectrometer, including the mass spectrometer, FTIR, and flame ionization and most other GC detectors.

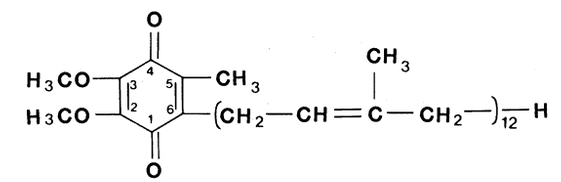
Carbon dioxide offers many practical advantages to those using this technology for the first time. It is inexpensive, available in high purity, and innocuous. Furthermore, its near-ambient critical temperature makes it attractive for use with thermally labile compounds. Finally, because of all its positive features, carbon dioxide is the solvent gas of choice in many commercial production-scale supercritical fluid extraction processes, and a chromatographic scheme using the same solvent gas is a good match with these processes—both for analysis and for pre–pilot plant surveys.

Addition of polar modifiers to carbon dioxide extends its somewhat limited solvent strength, allows investigation of exotic modifiers (ion-pairing and optically active reagents), and still allows application to thermally labile compounds.

The ultraviolet absorbance of carbon dioxide is minimal, allowing spectrometric detection down to 190 nm. Its infrared spectrum is almost as simple and allows the detection of almost any other organic compound. Because carbon dioxide is nontoxic, nonflammable, and convenient to handle, fraction collection for ancillary qualitative analysis is simple.

Application of SFC separations will undoubtedly increase in the future. Carbon dioxide is useful for separating nonpolar compounds that are currently analyzed by TLC and normal-phase HPLC. The advantages of SFC are high resolution per unit time (greater sample throughput), orthogonal column selectivity compared to GC and HPLC, and ease of fraction collection and analysis of thermally labile molecules.

As stated earlier, SFC is complementary to GC and HPLC. There are types of sample mixtures for which one of the techniques is clearly preferable. For example, if all compounds in a mixture are volatile and not thermally labile or reactive at high temperatures, GC is the best technique to use. Interdiffusion coefficients of solutes in a gas are three orders of magnitude more favorable than in supercritical fluids and perhaps four orders of magnitude more favorable than in HPLC mobile liquids. Thus, the peak width and the efficiency per unit time (isothermally) will be much more favorable in GC. In other cases, where one or more of the solutes in a mixture to be separated are thermally labile, reactive at high temperatures, or involatile, the choice is between SFC and HPLC. The decision may be based on the solubility of the solutes in a supercritical mobile phase. If the solutes of interest are soluble in supercritical carbon dioxide, for instance, it is always possible to find at least one other liquid solvent that will also dissolve the sample. However, the diffusion coefficient will be more favorable than any of the interdiffusion coefficients in the analagous HPLC mobile solvent by a factor of 3 to 5 or more. Whatever the gain factor is, the relative efficiency per unit time will be as great (in the absence of programming). Introduction of programming may further enhance the gain of SFC over HPLC in these cases. At least it will give a more sensible basis for comparison with gradient elution HPLC. It is likely that 20 to 40 percent of the solutes presently separated by HPLC are amenable to SFC separation with supercritical carbon dioxide. It is possible that most separations carried out by normal-phase HPLC may be handled with good advantage by SFC. Clearly, a great amount of experimental data will be needed to test such speculations.



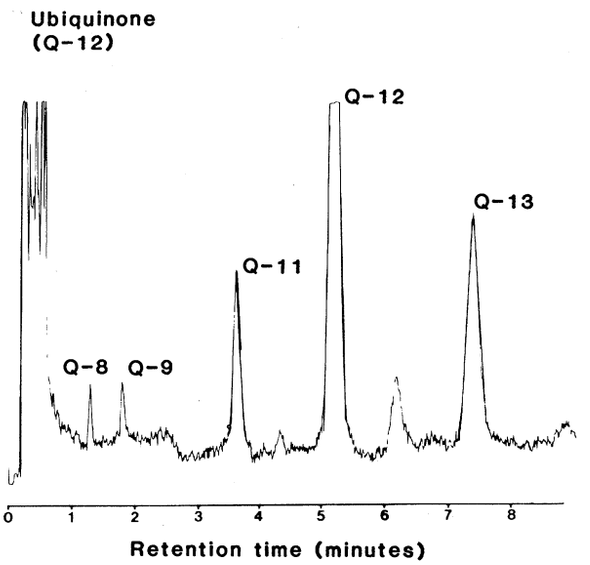


Fig. 8. SFC chromatogram of ubiquinones in bacterial cell extract of *Legionella pneumophila*. ODS reversed-phase column, 3-μm particle diameter, 10 cm by 4.6 mm. Carbon dioxide with 1.5 percent methyl alcohol, flow 3.5 cm^3/min, linear velocity 1.0 cm/sec, temperature 40°C. Average column pressure was 232 bars, column pressure drop 69 bars. Average density of the mobile phase was 0.87 g/cm^3.

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A Survey of Separative Techniques

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Summary. Separative science has recently undergone numerous advances. This article discusses many developments and trends in liquid, ion, gas, and countercurrent chromatography, field-flow fractionation, and electrophoresis.

The accompanying articles describe six areas of separation science in which major advances have recently occurred. These areas, however, are not the only ones in which progress is taking place. The entire field of separation science is evolving at a surprising rate, considering that separation is the oldest and most basic technique employed in the laboratory. The following sections discuss some of the areas where this evolution—if not revolution—is occurring most rapidly. These examples are meant to be representative rather than exhaustive.

Liquid Chromatography

High-performance liquid chromatography (HPLC), born only 20 years ago as high-pressure liquid chromatography (LC) is, with gas chromatography, one of the two most commonly used separative techniques today. Despite the maturity implied by such wide usage, it is still a rapidly evolving area. Two important current trends are the use of HPLC for separation of macromolecules, particularly those of biological origin, and the use of supercritical fluids as eluants. These are reviewed elsewhere in this issue. An equally important trend is a reduction in column size and a concomitant increase in analytical speed.

The first steps in this direction involved the use of short columns, perhaps 3 to 5 cm long. These columns were developed, in part, as a result of the use of guard columns—short, inexpensive, disposable columns fitted between the injector and the analytical column to trap extraneous materials that would foul the expensive analytical column. Investigators observed that these short columns could have as many as 5000 theoretical plates, an efficiency that is adequate for most uses. Short columns reduce analysis times and cut down on the use of expensive high-purity solvents, albeit at the cost of selectivity. Some of that selectivity can be restored, however, by using smaller particle sizes for packing the column, and 3-μm particles are becoming more common. Analyses that took 10 minutes 5 years ago can now often be done in 1 minute or less.

Solvent use can be further reduced and sensitivity increased by using columns with smaller bores. A standard HPLC column has a diameter of 4.6 mm. New columns are now available with diameters of 2, 1, or even 0.5 mm. A 2-mm column cuts solvent use by 80 percent, while microbore columns, those with diameters of 1 mm or less, cut it by at least 95 percent. The microbore columns are no more selective than conventional columns of equal length, and analysis times are equivalent. They can, however, be fabricated at reasonable costs in much longer lengths that produce very high efficiency. Their small size also allows use of exotic, expensive packing materials, such as diamond dust or finely divided noble metals, and even more expensive solvents: deuterated solvents, for example, might be used when it is necessary to analyze the collected sample by nuclear magnetic resonance. Some investigators are using columns with diameters as small as 50 μm, and it seems likely that these will eventually be commercially available. These ultramicrobore columns can, in some cases,

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