Chromatography with Supercritical Fluids

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In supercritical fluid chromatography (SFC) the mobile phase is neither a gas nor a liquid, but is a supercritical fluid. As a result of the unique properties of supercritical fluids, SFC is rapidly becoming a prominent separation technique for the analysis of reactive, thermally labile, and nonvolatile compounds. This article reviews the history, instrumentation, and practice of the technique. Particular emphasis is placed on the different programming methods that allow elution to be selectively controlled in ways that are unique to SFC.

AT PRESSURES AND TEMPERATURES ABOVE BUT CLOSE TO its critical point, a substance is called a supercritical fluid. It has densities, viscosities, and other properties that are intermediate between those of gases and those of liquids. Table 1 compares the most important properties of gaseous, liquid, and supercritical fluids used as mobile phases in chromatography. Since diffusion coefficients are highest and viscosities are lowest in gaseous mobile phases, gas chromatography (GC) offers the highest separation efficiency per unit time of all of the chromatographic methods. This, plus its compatibility with a wide variety of sensitive, selective, and universal detectors, makes GC the preferred chromatographic method if it is applicable to the sample of interest. Unfortunately, only 15 to 20% of known organic compounds (for which chromatographic analysis is desired) can be analyzed by GC. Either the thermal instability or the low volatility of the remaining compounds, or both, limit the use of GC for their analysis.

If GC cannot be used, either supercritical fluid chromatography (SFC) or liquid chromatography (LC) should be considered next. In LC, high resolution does not rely on separation efficiency, as is the case in GC. Both mobile-phase and stationary-phase compositions can be varied to achieve selective interactions with solutes. Since supercritical fluids have viscosities and densities intermediate between those of gases and liquids and possess solvating powers similar to those of liquids, SFC exhibits efficiencies and analysis times intermediate between those of GC and LC. It can be used for high-resolution separations of thermally labile and nonvolatile compounds at relatively low temperatures. Figure 1 compares the application ranges of the common column-chromatographic techniques as a function of molecular weight and size. Thus, LC and SFC can be applied over molecular weight ranges that are several orders of magnitude greater than GC, while size exclusion chromatography (SEC) can be applied to compounds with molecular weights as high as 10^7 daltons.

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History of SFC

The unique nature of supercritical fluids was noted as early as 1822, when Cagniard de la Tour (*1*) observed the disappearance of the meniscus between the gaseous and liquid states in a closed system at a "critical temperature." In 1879, Hannay and Hogarth (*2*) showed some of the similarities in solvating properties of liquid and supercritical fluids by demonstrating that the absorption spectra of several inorganic salts that were dissolved in supercritical fluids were identical to the spectra of the same salts dissolved in a liquid.

The first report of the use of supercritical fluids as mobile phases in chromatography was published in 1962 by Klesper and co-workers (*3*), who used supercritical Freons as the carriers for migration of metal porphyrins through a chromatographic column. This study came only 10 years after the pioneering work of James and Martin in GC (*4*) and actually predated modern LC. This initial report of SFC generated considerable interest in the analytical community. Unfortunately, the new technique was plagued by problems with instrumentation and with inadequate fundamental theory, especially in the understanding of the supercritical state. However, in the 20 years after the initial publication of Klesper *et al.*, a number of research groups pursued some excellent fundamental studies. In 1964, Giddings and co-workers (*5–7*) reported results of what they described as "dense gas chromatography." This group described various theoretical aspects of the use of chromatography with dense gas mobile phases and demonstrated the ability to work at extremely high pressures (up to 2000 atm). In 1966–67, Sie *et al.* (*8*) published a series of thorough studies on the fundamental theory and the applications of SFC. These investigators were also the first to use the term "supercritical fluid chromatography" to describe the technique. Other research groups (Schneider, Corwin, Gouw, Jentoft, Rogers, and others) played key roles in developing the foundation upon which the recent increased activity in SFC has been built. Several excellent comprehensive reviews (*9*) of the results of these pioneering studies provide more detail than is possible within the scope of this article. Despite the efforts of these pioneering researchers, the level of interest in SFC remained relatively low, dampened by the difficulties encountered with home-built instrumentation and by the fact that many groups turned their attention to the newly emerging analytical technique of high-performance liquid chromatography.

The resurgence of interest in SFC in recent years can be traced to a number of developments. One notable contribution was the growing interest in and application of supercritical fluids as extraction and process solvents. In 1982, a symposium devoted exclusively to the properties of materials above their critical points yielded a wealth of information on supercritical fluids (*10*). Another important factor was the development of reliable pumping systems (primarily for LC) that were capable of operating at high pressures. Furthermore, injection systems and sensitive, small-volume detectors had been developed for LC that were suitable for SFC. Advances in LC

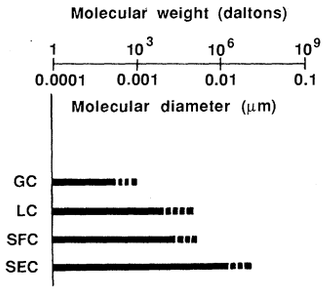

Fig. 1 (left). Range of molecular weights and sizes over which various column chromatographic techniques can be applied.

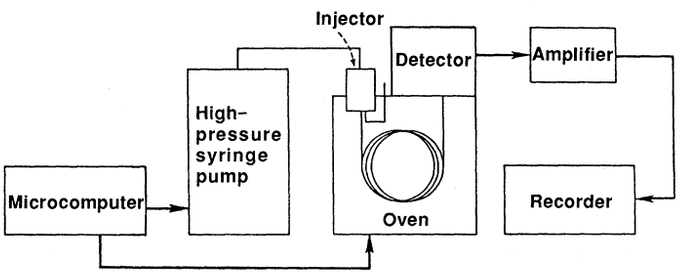

Fig. 2 (right). Schematic diagram of instrumentation for capillary SFC.

packed column and in GC capillary column technology also provided researchers with essential new technology.

In the early 1980s, two significant developments gave considerable impetus to the growth of SFC. In 1981, a collaborative effort by the research groups of Novotny and Lee led to the publication of the first study (*11*) on the use of open tubular-column SFC. Their efforts were nearly coincidental with the first reports on SFC from a major analytical instrument manufacturer. In 1982, researchers at Hewlett-Packard reported results of studies on the use of packed column SFC (*12*). At the same time, they made a commitment to produce the first commercial SFC system, which was offered as a modification package for one of their LC systems. The new and promising reports of capillary SFC and commercially available packed column SFC instrumentation marked a new era in the technique. In 1986, two major scientific journals published special issues devoted exclusively to SFC (*13*). The growth of SFC is witnessed by the commercial availability of instrumentation from at least four sources. Chromatographic supplies intended specifically for SFC are available from several major vendors.

Packed and Open Tubular Columns

The historical developments mentioned above identify the two approaches to modern SFC: the use of columns of either the packed LC type or the open tubular (capillary) GC type. Earlier studies involved exclusively the use of packed columns, while recent efforts have largely centered on the use of capillary columns. For packed column SFC, typical LC columns that contain small particle packings (3, 5, and 10 μm in diameter) are used. However, in capillary SFC, the columns used are smaller in diameter than those typically used in capillary GC. Theoretical calculations (*14*) and practical experience show that capillary columns of less than 100 μm inside diameter (i.d.) are necessary to achieve acceptable efficiencies (>3000 plates per meter) in reasonable analysis times (<1 hour). A typical 20-m-long column of 50 μm i.d. can produce greater than 10^5 theoretical plates in approximately 1 hour with supercritical CO_2 used as the mobile phase. For comparison, a typical packed column (10 cm by 4.6 mm i.d., packed with particles 5 μm in diameter) yields approximately 80,000 plates per meter or 8,000 total theoretical plates. If a complex mixture is to be analyzed and a large number of theoretical plates are required, a capillary column will produce a much better separation than a packed column. However, for simple mixtures that require rapid analysis, packed columns can produce a higher number of plates per unit time. Even so, capillary SFC can be competitive in terms of analysis speed through the use of rapid density programming, even though there is a considerable sacrifice of resolution introduced by programming in this manner. Wright and Smith (*15*) have demonstrated the near-baseline separation of six carbamate and acid pesticides in less than 2 minutes. This was achieved with a 0.9 m by 25 μm i.d. capillary column by pressure programming at 100 atm/min.

Total efficiency and speed of analysis are not the only factors that should be considered in selecting the column type. With packed columns mobile-phase flow rates typically range between 2 and 20 ml/min, which prohibits the use of many detectors, such as the flame-based detectors commonly used in GC. Capillary column flow rates range from 1 to 10 μl/min, which can be easily handled by both LC and GC detection systems including mass spectrometry. If large sample capacity is needed for preparative scale separations, a capillary column cannot be used.

A compromise between the two extremes of open tubular and packed columns is the use of microbore or packed capillary columns. Hirata (*16*) has achieved excellent separations of polystyrene oligomers up to a molecular weight of over 7000 with a 58 cm by 0.2 mm i.d. column packed with 10-μm particles that produced only 8000 theoretical plates.

Instrumentation

The instrumentation for SFC includes components that are used in both GC and LC. High-pressure pumps, developed originally for LC, can be easily adapted for SFC if pressure is controlled. In addition, accurately controlled ovens typically used in GC are necessary to control the column temperature. Although SFC injectors are common to LC, detectors are common to both GC and LC. A schematic diagram of the instrumentation needed for capillary SFC is shown in Fig. 2. Since low mobile-phase flow rates are characteristic of capillary SFC, syringe pumps are preferred. For packed columns, reciprocating pumps with cooled pump heads are more appropriate. The only other major difference between instrumentation for capillary and packed column SFC is the variety of detectors that can be used.

The majority of packed column work has been done with ultraviolet (UV) absorbance and fluorescence detectors. Limited use of the flame ionization detector (FID) has been reported by splitting the column effluent, but splitting to the detector greatly reduces the sensitivity. By comparison, the use of the flame ionization, thermionic ionization, and flame photometric detectors with capillary SFC has greatly increased the attractiveness of the technique. The coupling of capillary SFC to more sophisticated detection systems, such as mass spectrometry (*17*), Fourier transform infrared spectrometry (*18*), ion mobility spectrometry (*19*), and supersonic jet spectroscopy (*20*), also holds much promise.

Table 1. Properties of mobile phases used in chromatography.

Mobile phase	Density (g/ml)	Viscosity (poise $\times 10^{-4}$)	Diffusion coefficient (cm^2/sec)
Gas	$(0.6–2.0) \times 10^{-3}$	0.5–3.5	0.01–1.0
Supercritical fluid	0.2–0.9	2.0–9.9	$(0.5–3.3) \times 10^{-4}$
Liquid	0.8–1.0	30–240	$(0.5–2.0) \times 10^{-5}$

Mobile Phases

Carbon dioxide is the most popular mobile phase for SFC because of its low critical temperature (32°C), chemical inertness, and low background response in most detection systems. Alternative fluids are listed in Table 2 along with various critical parameters, dipole moments, and liquid densities. Fluids with low critical temperatures have been the preferred mobile phases because of their usefulness in the analysis of thermally labile compounds. Fluids with low critical pressures are also preferred because of the pressure limitations of available pumping systems. Nitrous oxide is slightly more polar than CO_2, and ammonia is the most polar fluid usually considered. The solvating properties of various fluids for the analytes of interest are important for the selection of the appropriate mobile phase. Other fluid properties that must be considered are density range, viscosity, selectivity, detector compatibility, stability, and toxicity.

The polarity of a supercritical mobile phase can be increased by the addition of a polar modifier. This method of tailoring the selectivity of the mobile phase is considered in more detail in the following section.

Programming Methods

One of the greatest advantages of SFC is the availability of methods to control solute retention. GC has long used temperature programming to control solute retention and to extend the analytical range of the technique. An analogous method used in LC is programmed control of mobile-phase composition. SFC offers the possibilities of both of these programming methods plus the additional powerful option of programming the density of the mobile phase through pressure programming.

Pressure (density) programming. The most widely applied programming method in SFC is pressure (density) programming. Giddings (*6*) predicted the utility of pressure programming when he noted in 1966 that pressure-induced equilibrium shifts could be used to separate solutes in a chromatographic system. The practical realization of pressure programming in SFC was first demonstrated by Jentoft and Gouw (*21*).

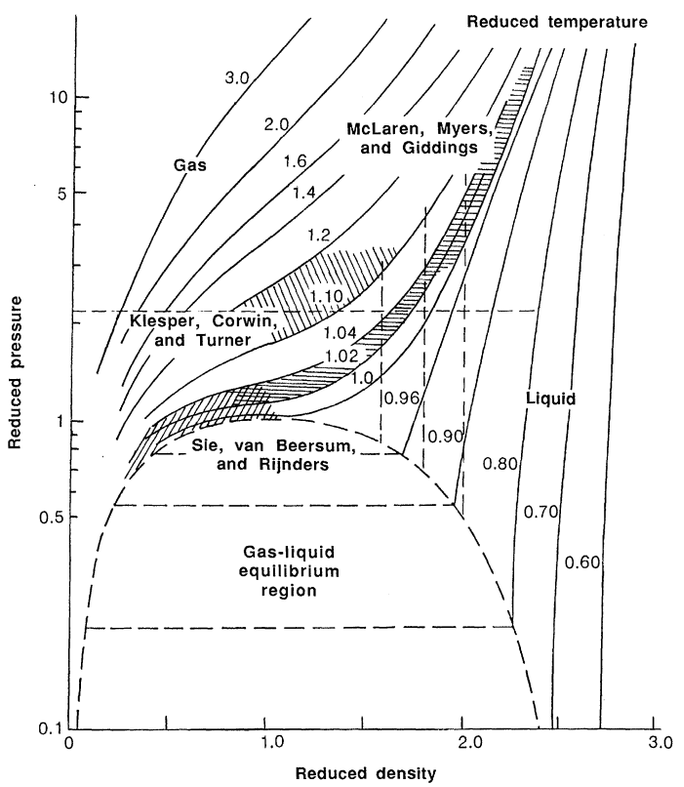

Fig. 3. Phase diagram showing pressure and density regions explored in early research activity in SFC. [Reproduced from (*7*)]

Pressure itself has only an indirect effect on the solvating ability of the supercritical fluid. In 1880, Hannay (*22*, p. 486) concluded from studies of inorganic salts dissolved in supercritical fluids that

> The liquid condition of fluids has very little to do with their solvent power, but only indicates molecular closeness. Should this closeness be attained by external pressure instead of internal attraction, the result is that the same or even greater solvent power is obtained. . . .
>
> The gas must have a certain density before it will act as a solvent, and when its volume is increased more than twice its liquid volume, its solvent action is almost destroyed.

The density of the supercritical mobile phase is the parameter of interest and not the pressure per se. Nieman and Rogers (*23*) were among the first SFC researchers to address directly the issue of whether pressure or density should be the programming parameter.

Since density (or pressure) programming plays such a significant role in SFC, it is appropriate to consider in greater detail the relation between density and pressure. Supercritical fluids are often compared on the basis of their reduced parameters, which include reduced temperature ($T_r = T/T_c$), reduced pressure ($P_r = P/P_c$), reduced volume ($V_r = V/V_c$), and reduced density ($\rho_r = \rho/\rho_c$). In these expressions T_c, P_c, V_c, and ρ_c are the critical temperature, pressure, volume, and density, respectively. A generalized phase diagram for a supercritical fluid is shown in Fig. 3. Different areas of the phase diagram are highlighted to indicate the different conditions various SFC researchers have used. This illustration helps to clarify the different terminologies, such as "supercritical fluid chromatography," "dense gas chromatography," and "near critical chromatography," which were used to describe some of the early studies.

For example, Giddings and his co-workers (*5–7*) (dense gas chromatography) used such high pressures ($P_r > 3.0$) that their mobile phases approached, or in some cases actually surpassed, the corresponding liquid densities. This approach avoided some of the sensitive fluctuations of density with pressure that occur very near the critical point. Klesper, Corwin, and Turner (*3*) worked at more moderate reduced pressures, but at somewhat higher reduced temperatures ($T_r \geq 1.1$). In their approach they avoided the sensitive critical-point region by working in a region where the pressure-density isotherm was more linear. On the other hand Sie *et al.* (*8*) worked above, but very near, the critical point. In this regard their work was more strictly "supercritical fluid chromatography" than that of the other two groups previously mentioned. In addition to the studies performed above the critical temperature, some researchers have investigated chromatography with fluids below, but very near, their critical temperatures. Lauer *et al.* (*24*) designated this the near-critical region. Several studies have reported on work done in this near-critical region (*25*).

By referring to Fig. 3, we can emphasize several points with respect to density programming in SFC. Because the pressure-density relation in the dense gas region of Giddings is almost linear, density programming and pressure programming are practically equivalent; a linear pressure program yields a linear density program. To a lesser extent, the same can be said for the work of Klesper *et al.*, for which the isotherms are approximately linear. In contrast, the isotherms in the region very near the critical point deviate greatly from linearity. Therefore, linear pressure programming gives a decidedly nonlinear density program in this region. Near the critical point, very slight variations in pressure or temperature can have significant effects on the fluid density and thus on its

Table 2. Physical parameters of selected supercritical fluids.

Fluid	Dipole moment (debyes)*	T_c (°C)*	P_c (atm)*	ρ_c (g/ml)*	ρ_{400} atm† (g/ml)	ρ_L(g/ml)*‡
CO_2	0.00	31.3	72.9	0.47	0.96	0.71 (63.4 atm)
N_2O	0.51	36.5	72.5	0.45	0.94	0.91 (0°C) 0.64 (59 atm)
NH_3	1.65	132.5	112.5	0.24	0.40	0.68 (−33.7°C) 0.60 (10.5 atm)
n-C_5	0.00	196.6	33.3	0.23	0.51	0.75 (1 atm)
n-C_4	0.00	152.0	37.5	0.23	0.50	0.58 (20°C) 0.57 (2.6 atm)
SF_6	0.00	45.5	37.1	0.74	1.61	1.91 (−50°C)
Xe	0.00	16.6	58.4	1.10	2.30	3.08 (111.75°C)
CCl_2F_2	0.17	111.8	40.7	0.56	1.12	1.53 (−45.6°C) 1.30 (6.7 atm)
CHF_3	1.47	25.9	46.9	0.52		1.51 (−100°C)

*Data taken from *Matheson Gas Data Book* and *CRC Handbook of Chemistry and Physics* (*42*). †The density at 400 atm (ρ_{400} atm) and T_r = 1.03 was calculated from compressibility data in (*29*), pp. 605–629, according to J. C. Fjeldsted, thesis, Brigham Young University (1984). ‡Measurements were made under saturated conditions if no pressure is specified, or was performed at 25°C if no temperature is specified.

solvating ability. This sensitivity near the critical point can be both detrimental and helpful. Without precise control of the temperature and pressure, supercritical fluid chromatography near the critical point can be expected to be highly irreproducible. However, with proper control of these parameters, the sensitivity of the density-pressure relation can be used to advantage in the chromatographic separation. A wide range of solvent densities, and therefore mobile-phase selectivities, is obtained by delicate control within a moderate pressure range. Fjeldsted *et al.* (*26*) took advantage of this characteristic of the mobile phase in one of the first studies in SFC which went beyond pressure control and dealt specifically with density. Wilsch and Schneider (*27*) have recently reported some significant aspects of density programming in SFC. It should be noted that although the density of a supercritical fluid most accurately describes its solvating power, the reproducibility of results is usually the main issue of practical concern. This reproducibility is achieved by precise control of all of the chromatographic parameters; simple pressure programming is often sufficient. The choice of programming mode depends mostly on the nature of the mixture to be separated. For example, the members of a homologous series elute at regular intervals under asymptotic density programming (*26*). Such density programming might be preferred over simple linear density or pressure programming in this application. Smith *et al.* (*28*) discussed some of these considerations in more detail for programmed elution in SFC.

Although density was the parameter of choice to control the solvent strength of the mobile phase in SFC, instrumental limitations forced early researchers to control pressure and not density. Supercritical fluids show significant deviations from Boyle's law; that is, molar volume is not inversely proportional to pressure. Consequently, density is not a simple function of pressure but depends on the nature, molecular size, and polarity of the supercritical fluid. For this reason, and because in a dynamic system pressure was (and still is) much more readily measured and controlled than density, most programming in SFC has been pressure programming, and only indirectly has it been density programming. For the most part, the more recent studies that focused on density have sought to electronically translate pressure programming into density programming and to maintain the SFC system under pressure control. While direct monitoring of density (and thus its control) could be achieved with available technology, all currently available instrumentation is controlled by pressure. The approach of Fjeldsted *et al.* (*26*) is representative of the current approaches to density programming. They approximated the pressure-density relation of a supercritical fluid with a simple generalized equation of state that was modified by a fluid-specific parameter which depended on vapor pressure (the acentric factor) (*29*). This approach showed good general applicability with reasonable results. This approximation has some flaws, particularly at higher pressures. Other more elaborate equations of state could be used that more closely approximate the actual pressure-density isotherms. The sophistication of the present equations of state allows for good modeling of the pressure-density relation if the necessary parameters and coefficients are available. However, ultimate accuracy is sometimes sacrificed in favor of general utility and applicability. The combination of readily available computer control with accurate mathematical mod-

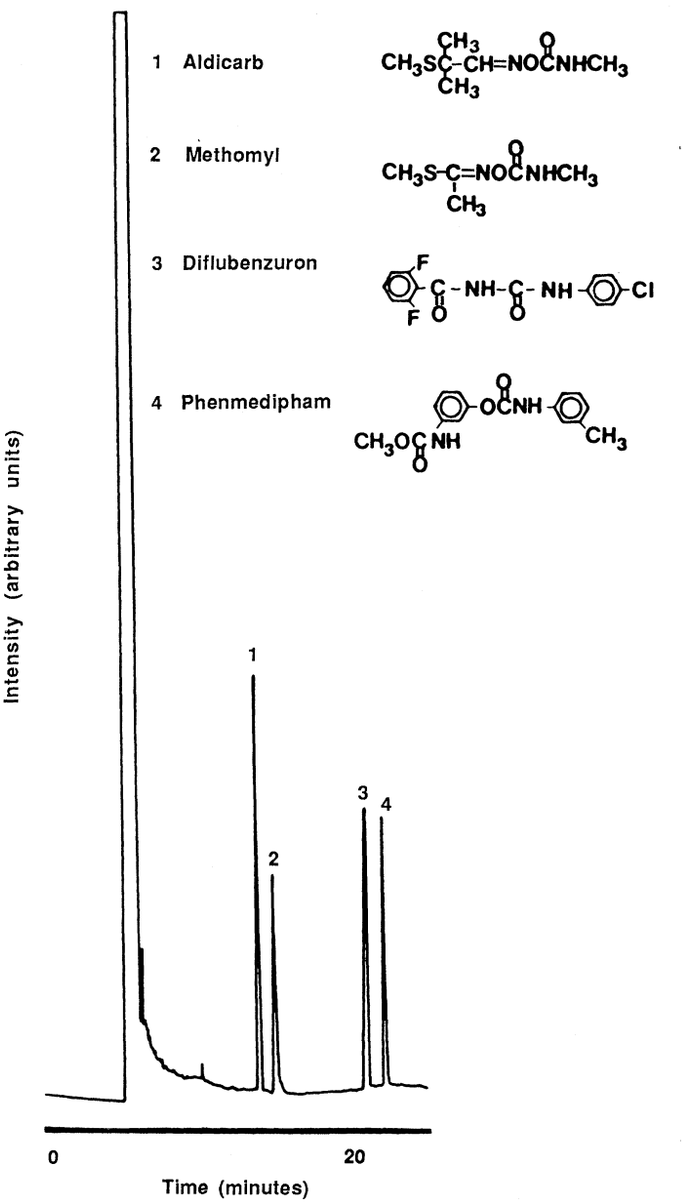

Fig. 4. Capillary supercritical fluid chromatogram of thermally labile carbamate pesticides. Conditions: 15 m by 50 μm i.d. fused silica column coated with a methylpolysiloxane stationary phase; CO_2 mobile phase at 100°C, density-programmed from 0.25 to 0.55 g/ml with a multilinear density program; FID detection. [Adapted from (*40*)]

els of supercritical fluids should make more precise density programming possible in the future.

Temperature programming. Another parameter that is programmable in SFC is the system temperature. Temperature has several effects in SFC, the first of which relates to the thermodynamics of the system. As temperature increases, physical processes such as diffusion and kinetics favor high efficiency in the chromatographic separation. At the same time, density decreases with increasing temperature at constant pressure and also contributes to an increase in chromatographic efficiency. In fact, some early SFC research on programmed elution used reversed temperature programming to elute solutes of increasing molecular weight. As the temperature decreased, the density of the mobile phase increased, which increased its solvating power. However, this reversed temperature programming worked in a counterproductive manner with regard to the thermodynamics of the system. Fields and Lee (*30*) used a combination of temperature and density programming to achieve high-resolution separations of samples of crude oil. In this study they also pointed out that the efficiency of the SFC separation was highest for the highest temperature at which the solutes and the chromatographic system itself were still stable. Other recent studies showed similar results for temperature programming in SFC (*31*).

Fluid composition (gradient) programming. One asset that SFC shares with LC is the ability to control the mobile-phase selectivity.

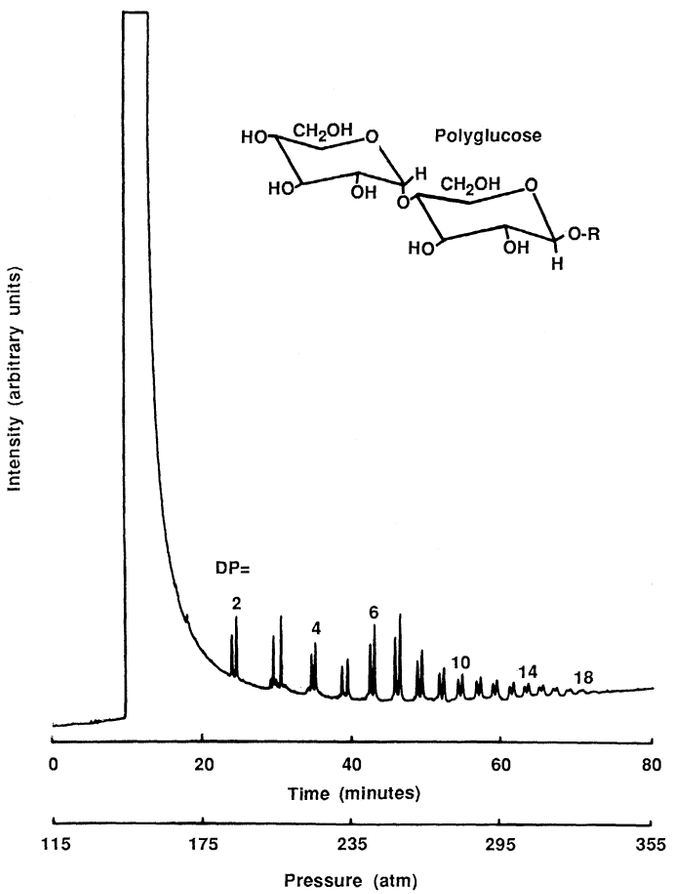

Fig. 5. Capillary supercritical fluid chromatogram of glucose oligosaccharides and polysaccharides of high molecular weight from silylated corn syrup. Degree of polymerization (DP) is indicated above the chromatographic peaks. Conditions: 10 m by 50 μm i.d. fused silica column coated with a methylpolysiloxane stationary phase; CO_2 mobile phase at 89°C, pressure programmed from 115 to 355 atm with a linear pressure program; FID detection. [Reproduced from (*41*) with permission. Copyright, Dr. Alfred Huethig Publishers]

The density programming already discussed is one example of this. In addition, the choice of supercritical fluid can be tailored to fit the chemical nature of the solutes of interest in much the same way as can be done in LC. Giddings *et al.* (*7*) developed a guideline of solvent strengths for mobile phases in SFC based on the Hildebrand solubility parameter, δ. This parameter depends on two fluid characteristics: the state effect (for example, density or intermolecular distances) and the chemical effect (for example, polarizability, acid-base properties, or hydrogen-bonding tendencies). After assuming that the van der Waals equation of state was valid, Giddings defined δ of a fluid in terms of its reduced density and the critical pressure:

$$\delta = 1.25\, P_c \frac{\rho_r}{\rho_{r(liq)}} \quad (1)$$

where $\rho_{r(liq)}$, the reduced density of the liquid phase, equaled 2.66. The critical-pressure term represents the chemical effect whereas the ratio of the reduced densities represents the state effect. Randall (*32*) applied this solubility parameter to predict the optimum composition for a number of mobile phases and mobile-phase combinations in SFC.

The simplest way to alter solvent strength in the SFC mobile phase is by adding modifiers. Some of the first studies of SFC that used modifiers doped into the mobile phase of a supercritical fluid showed profound effects on solute retention upon addition of only a very small percentage of the modifier. For example, in 1971 Novotny *et al.* (*33*) showed that less than 0.5 percent of isopropanol doped into *n*-pentane decreased the retention times of solutes markedly. However, the results of studies in which well-deactivated capillary columns were used were at variance with those mentioned above. Wright and Smith (*34*) found little effect on retention with the addition of polar modifiers in CO_2 at levels less than 1 percent for capillary SFC. This apparent discrepancy, however, is easily reconciled by examination of the systems used in the studies. The studies which showed a significant change in retention upon addition of a small amount of modifier can be explained by the effect of the modifier on the stationary phase and support, rather than by the increase in solvating power of the mobile phase itself. By covering active sites on the silica packing material, the polar modifier had less effect on the retention of solutes. This conclusion was substantiated by Conaway *et al.* (*35*) and by Board *et al.* (*36*). In the latter studies, the effect of the modifier was carefully isolated to investigate the effect of the modifier only on the solvent strength of the mobile phase. Thus the choice of modifier in SFC must take into account both its effect on the stationary phase or the support (or both) and its effect on the solvent strength of the mobile phase.

The solvent gradient programming used so widely in LC is, from one perspective, a dynamic addition of a modifier to the mobile phase. This same programming option is available in SFC. Schmitz *et al.* (*37*) demonstrated gradient programming with *n*-pentane and *p*-dioxane as the mobile phase by eluting polystyrene oligomers in packed column SFC. Blilie and Greibrokk (*38*) published preliminary results of simultaneous gradient-density programming. The requirement of precise pressure control in the solvent delivery system and the very small mass flows that are used combine to make gradient programming in capillary SFC a delicate task. To our knowledge, no capillary SFC gradient elution studies have been published to date.

Both temperature and gradient programming pose questions with regard to density. As mentioned earlier, a change in temperature changes the supercritical fluid density, which necessitates compensation by changing the pressure. Therefore, a series of isotherms must be calculated, as opposed to the single isotherm necessary in simple density programming. However, a change in mobile-phase compo-

Table 3. Published application areas of SFC.

Polysiloxanes	Tricothecenes
Polyenes	Carbamate pesticides
Polyols	Organophosphorus pesticides
Polysaccharide esters	Herbicides
Polyglycerol esters	Azo, aniline, and anthraquinone dyes
Free fatty acids	Pentaerythritol tetrastearate
Mono-, di-, and triglycerides	Glycerol tetraether lipids
Steroids	Prostaglandins
Erythromycin	Polycyclic aromatic compounds
Tetracyclines	Aliphatic hydrocarbons
Catecholamines	Long-chain alcohols
Penicillins	Nonionic surfactants
Cannabinoids	Isocyanates

sition will be accompanied by changes in density, critical temperature, and critical pressure. In most cases, the critical parameters and behavior of solvent mixtures have not been well characterized; the experimental data on mixtures of supercritical fluids have been sparse at best. A number of methods have been used extensively by chemical engineers in estimating the critical parameters of mixtures. However, most of the methods have been applied only to hydrocarbon mixtures or to mixtures of hydrocarbons with CO_2, H_2S, CO, and the inert gases. Reid *et al.* have compiled an excellent review of these methods (*39*). For fluid mixtures that include nonhydrocarbons, these methods are less accurate, and they become increasingly unreliable as the solvent polarity increases. Thus, as the polarities of the fluids in the mixture increase, the need increases for component-interaction parameters to compensate for deviations from the behavior observed with nonpolar fluids. Fortunately, the recent interest in supercritical fluids has prompted considerable research into the behavior of supercritical fluid mixtures. Consequently, much more experimental information is being produced, and more sophisticated and universally applicable estimation procedures are being proposed (*10*).

Although some aspects of programmed elution in SFC involve complex considerations, for the most part the practice of SFC programming is fairly straightforward. Instrumentation for SFC is available that incorporates appropriate hardware and software to make control of programmed elution, particularly pressure or density programming, relatively simple.

Applications

The test of the usefulness of an analytical technique is its applicability to a wide variety of sample types. Table 3 lists many of the compound classes that have been successfully analyzed with SFC. In many cases, SFC is competitive with LC and, to a lesser extent, GC. However, numerous examples continue to be identified for which SFC is clearly the most desirable technique. These examples can generally be grouped into one (or more) of three classes of compounds: reactive, thermally labile, and nonvolatile. In many cases, SFC is preferred compared to LC because the FID is available in SFC for quantitation and is the deciding factor over the use of LC. Two examples have been selected to illustrate several of these points. Figure 4 illustrates the separation of several thermally labile carbamate pesticides (*40*), which are difficult to analyze by other techniques. The use of CO_2 at 100°C preserves the integrity of these compounds. The results are easily quantified with the FID. The analysis of glucose oligosaccharides and polysaccharides of high molecular weight (*41*) in a silylated corn syrup is illustrated in Fig. 5. Two forms exist for each oligomer, the α and β anomers, which elute as paired peaks. The compound indicated toward the end of the chromatogram is composed of 18 repeating glucose units and contains 56 hydroxyl groups. After silylation, this molecule has a molecular weight of 6966, which is well beyond the capabilities of GC. Furthermore, these oligomers do not possess a chromophore in their structure, and they cannot be detected with the UV-absorbance and fluorescence detectors typically used in LC.

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