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Review

Supercritical fluids in separation science – the dreams, the reality and the future

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Abstract

The last 20 years have seen an intense interest in the use of supercritical fluids in separation science. This started with the introduction of commercial instruments first for packed and then for capillary chromatography and it looked as if this would be a technique to rival gas–liquid chromatography and HPLC. The activity developed quite rapidly into packed column supercritical fluid separations then into supercritical fluid extraction. However, in recent years there has been a decline in publications. These later techniques continue to be used but are now principally applied to a limited group of applications where they offer significant advantages over alternative techniques. This review looks back over this period and analyses how these methods were developed and the fluids, detectors and applications that were examined. It suggests why many of the initial applications have vanished and why the initial apparent promise was not fulfilled. The rise and fall of supercritical fluids represents a lesson in the way analysts approach new techniques and how we might view other new separation developments at the end of this millennium. The review looks forward to the future of supercritical fluids and their role at the end of the first century of separation science. Probably the most important idea that supercritical fluids have brought to separation science is a recognition that there is unity in the separation methods and that a continuum exists from gases to liquids. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Reviews; Supercritical fluid extraction; Supercritical fluid chromatography; Supercritical fluids

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1. Supercritical fluids in separation science

In the early and mid 1980s supercritical fluids were the exciting new topics in chromatography. At most major chromatography symposia, whole day sessions of lectures and posters were devoted to new developments in supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC). However, only a few years later, the principal chromatography meeting in Europe (ISC 98) contained only four lectures in a single session, starting with the question “Supercritical fluid chromatography. Still Alive?” [1], and only six posters were offered, three on SFC and three on SFE (at the equivalent meeting in 1999 only one lecture and four posters are planned). However, the lecturer’s question was rhetorical, SFC is still alive and there are niche applications where SFC is superior to gas–liquid chromatography (GLC) or high-performance liquid chromatography (HPLC).

What was the sudden interest in the 1980s, why did so much appear to be promised, what was the reality, why did interest then wane over a brief

20-year span, and what is the role of supercritical fluids in the future? In reviewing supercritical fluids in separation methods at the end of the millennium let us consider how this situation arose. Why did this topic provide such a spectacular rise and apparent decline? We need to distinguish the role of supercritical fluids in analytical chemistry from their wider role in synthetic chemistry, in production processes and in manufacturing. There the rise in interest has been steadier and more sustained. In these areas the properties of supercritical fluids have successfully enabled new techniques and reactions to be developed [2], which were not possible with conventional solvents. We can look at the development of supercritical fluids in analytical chemistry as a case study to show how science can sometimes be driven by dreams, excitement and the occasional exaggerated claim, almost akin to a chromatographic “gold fever”.

Initially it was a matter of curiosity lead research, the “what if” syndrome. Than it became novelty driven research, where academic researchers must always be seeking the next new topic. This serves as

the source of the next grant essential to support their work. This driving force frequently leads to whole teams jumping to a new topic, even before the old ideas have been fully examined. This rush from topic to topic, has been seen time and time again with chiral chromatography, capillary electrophoresis (CE) (just how many of the manufacturers from six years ago are still in the market place?) and now capillary electrochromatography (CEC). Sometimes when the early practitioners are more cautious and recognise the limitations from an early stage, the rise in interest is slower. Currently CEC users are trying hard to avoid the SFC syndrome. However, they may already have been overtaken by the rush to “chromatography on a chip” and “total analytical systems” (μ TAS).

Supercritical fluids are also a tale of the role and use of physical chemistry in analytical chemistry. The properties of supercritical fluids were well known even before the analyst showed an interest, but often seemed to be ignored when chromatographic claims were made. In a topic that had so much background information, why did it take so long before the hype was ignored and the practical relationship to separation science was understood? Perhaps we were so swamped with pure physical chemistry studies that it appeared that there was a strong theoretical background to what was being done. The analytical community assumed these results were important. However, often they did not lead to an understanding of the use of supercritical fluids in practical separation science, only to a greater knowledge of the physical chemistry. It seemed that more was being determined about SFC than about most LC separation methods but at the end of the day the significant pointers were still largely missed. From the physical properties, capillary SFC could never compete with GLC in speed and efficiency. Packed column SFC was always going to be limited by the low polarity of carbon dioxide to a normal-phase role.

The role of supercritical fluids in sample preparation has been more secure and is based on a robust background of chemical engineering. Importantly it has also been based on a more systematic and realistic approach, even though it has still not reached its apparent potential. The interest in these two methods can be charted in surveys of publi-

cations using SFE and SFC as key terms. In the early years there was a rapid rise of SFC, with an annual doubling of publications from 1980 to 1986 (Fig. 1a) [3]. It looked as if the technique would rapidly rise to the heights of GLC or HPLC. However, this was immediately followed by a levelling off and since 1991 there has been a steady but slow decrease in the number of publications in major journals per year (Fig. 1b). Despite all the apparent interest, the number of SFC papers per year never really exceeded 200 and is settling at about the 100 level. Over the same period SFE has shown a slower increase to a higher level but there some signs that it might now be levelling off.

This review is a personal view and other participants in the story may have different interpretations. There should be lessons that can be drawn for the future, on the interaction of theory and experiment and the role that business plays in development and publicity. Perhaps the route that was followed was the quickest method to determine the problems, successes and limitations of the SFC concept, but perhaps it has left too many open questions and an inherent distrust in users and managers of new techniques.

2. History

The critical phenomenon was discovered in 1869 by Andrews but even now most physics text books only discuss the critical point as part of a phase diagram. Few consider the properties of compounds or elements beyond the critical point, in the supercritical region. Instead for many years supercritical fluids largely remained a curiosity with an exotic name, which were ignored by the average chemist whether analytical or synthetic. The first acknowledgement of the analytical potential for supercritical fluids probably came in a note made by James Lovelock in 1958 [4]. He observed the possibility of doing critical state chromatography but this was not followed up for many years. First GLC and then HPLC were the topics of primary interest. Studies in supercritical fluids (SFs) were probably also deterred by the reportedly high pressures and temperatures that were required.

The first supercritical chromatographic separation

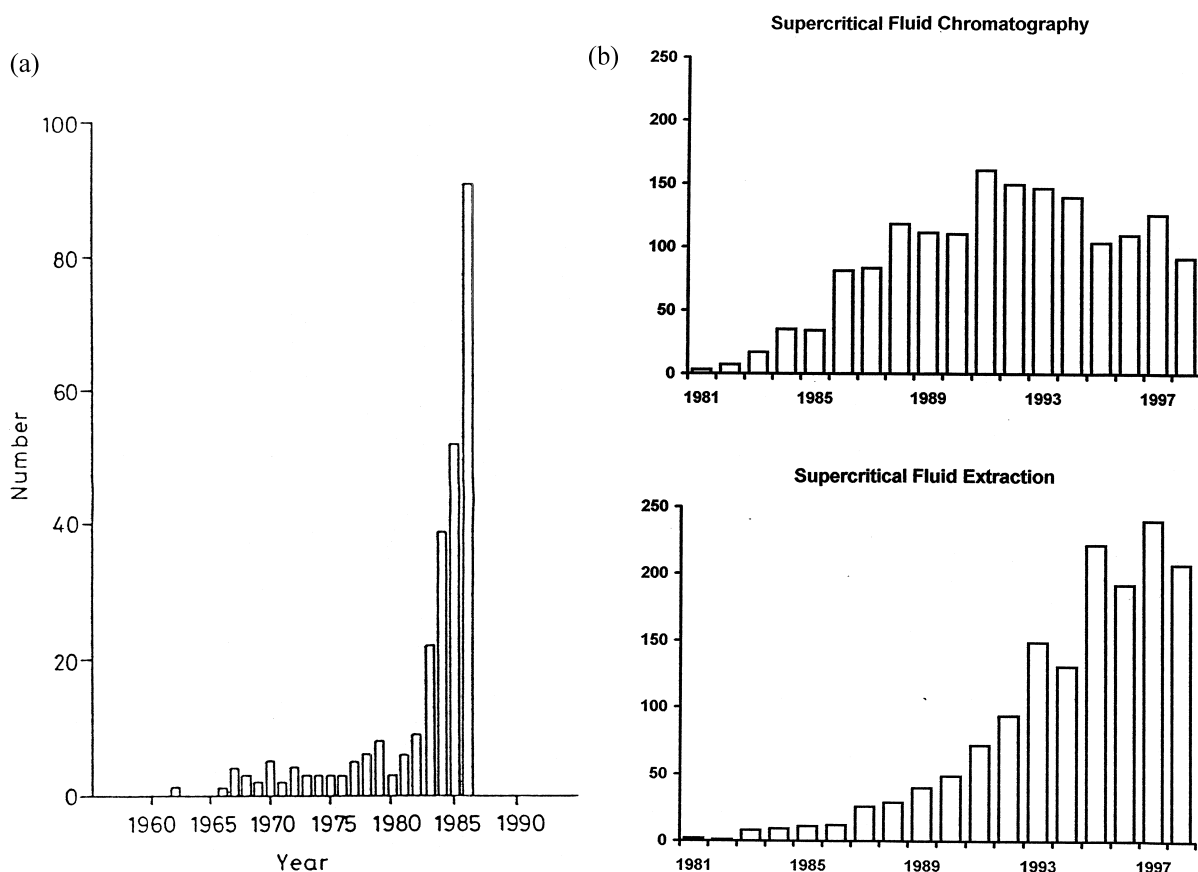


Fig. 1. Numbers of publications on the use of supercritical fluids in analytical chemistry. (a) The early rise of interest in SFC 1962–1986 [3] (reproduced with permission of the Royal Society of Chemistry). (b) Publications on SFC and SFE in major journals from 1981–1998 (based on a Science Citation Index search).

was reported in 1962 by Klesper et al. [5], who separated nickel etiporphyrin II from nickel mesoporphyrin IX dimethyl ester using dichlorodifluoromethane and monochlorodifluoromethane. Subsequent work by Sie et al. [6], Giddings et al. [7] and others employing carbon dioxide in “dense gas chromatography” was reviewed in 1972 by Gouw and Jentoft [8]. They felt at that time that the potential number of compounds that could be examined by SFC was “enormous”. Out of 10^6 known compounds only 15% were suitable for gas chromatography (GC). However, this review preceded the dramatic rise of reversed-phase HPLC following the introduction of bonded stationary phases and microparticulate silica.

3. The early analytical methods

The first “commercial” SFC instrument was offered briefly by Hewlett-Packard in 1983. It consisted of a standard packed column 1082B HPLC system fitted with a back-pressure regulator and cooled pump heads. It was offered on an unsupported experimental basis but came with a series of application sheets developed by Gere and colleagues. These included polynuclear aromatic hydrocarbons on ODS bonded and unbonded silica [9], the separation of ubiquinone from bacterial cell extracts [10], caffeine from beverages [11], paprika oleoresins and carotenoids [12], which demonstrated the application of SFC to a natural product application, and the

separation of oligomers [13] and mixtures of methyl vinyl silicones and peroxides [14]. This last paper is of interest as one of few examples of the separation of thermally labile analytes. These separations were also reviewed in a paper [15], which effectively defined the principal future direction of packed column SFC. However, few of these instruments were sold and the company's involvement was lost when a new model of HPLC instrument was developed whose pump heads could not be easily cooled. However, this work initiated interest by analytical chemists in the field. It showed how HPLC instruments could be readily adapted to provide packed column SFC systems. Other groups also examined the use of packed microbore columns [16].

About the same time Lee and co-workers were reporting their early work on SFC using capillary columns. The first ten years of their development work [17] and the basic design of the instrumentation were described in 1982 [18]. Early studies also examined application areas such as the linking of SFC to mass spectrometry (MS) [19] and comparisons of GC and SFC separations (Fig. 2). Two "A" page reviews brought this work to the attention of a wider audience [20,21].

An interest was also being generated at this time in the broader areas of supercritical fluid technology and engineering [22].

4. The rise and "fall" of supercritical fluids

SFC and in particular capillary SFC needed new equipment and a host of new companies, particularly in the USA (very few of which still survive), started to bring out chromatographic systems capable of operating at high pressures and temperatures. From about 1984, both capillary and packed SF instruments were commercially available and the trade literature had found a new topic to champion. This provoked a number of headline such as "Supercritical fluid chromatography as a routine analytical technique" [23].

Initially there was a fierce transatlantic debate whether packed or capillary columns were better. The US groups (largely converted GC users) favoured capillary columns and reporting many fantastic separations. There was also a strong manu-

facturer push behind capillary methods. However, this commercial pressure brought a number of claims, some justified but many now seen as misleading. "SFC overcomes the limitation of both GLC and HPLC" [24]. Here was a method that could apparently deliver the efficiency of GC but had a similar sample capacity and ability to handle low volatility analytes as HPLC. The result was an explosion of interest and the number of papers dramatically increased over the next five years (Fig. 1a), including a number of reviews which brought the field together and made the ideas in the original papers more accessible [25,26]. However, the publicity often omitted or used a compressed time scale on chromatograms so that a separation taking between 60 and 160 min, looked like a 20-min GC trace.

In contrast, despite the early work of Gere, much packed column development was based in Europe or Japan with chromatographers treating it as a branch of HPLC. They saw it as new liquid-like method, which offered a low viscosity solvent, a fast analysis time and a readily variable mobile phase. Packed column SFC systems tended to be laboratory-made by cooling the pump head in a HPLC system to liquefy the carbon dioxide and adding a back-pressure device [3,27,28], although a few manufacturers offered complete instruments based on their HPLC technology. Subsequently, it was seen that both packed and capillary columns had a role but they each offered different advantages and disadvantages and were suitable for different applications [29].

Specialist meetings on SFC were organised and sessions were devoted to the topic at most major chromatography symposia. Workshops and short courses in the Europe and the USA resulted in the first monographs on SFC [30,31] describing the developments for the interested analyst.

But by 1987 questioning voices could be heard even amongst the SF community. Schoenmakers asked "just how good is SFC anyway" [32]. He suggested that compared to GC, SFC offers almost exclusively disadvantages. "If GC provides satisfactory results, SFC will not do better." He identified the limitations of a non-polar eluent and noted that despite the efficiency advantages of capillary SFC, it cannot compete for speed. He subsequently also noted that many articles assign the best of everything

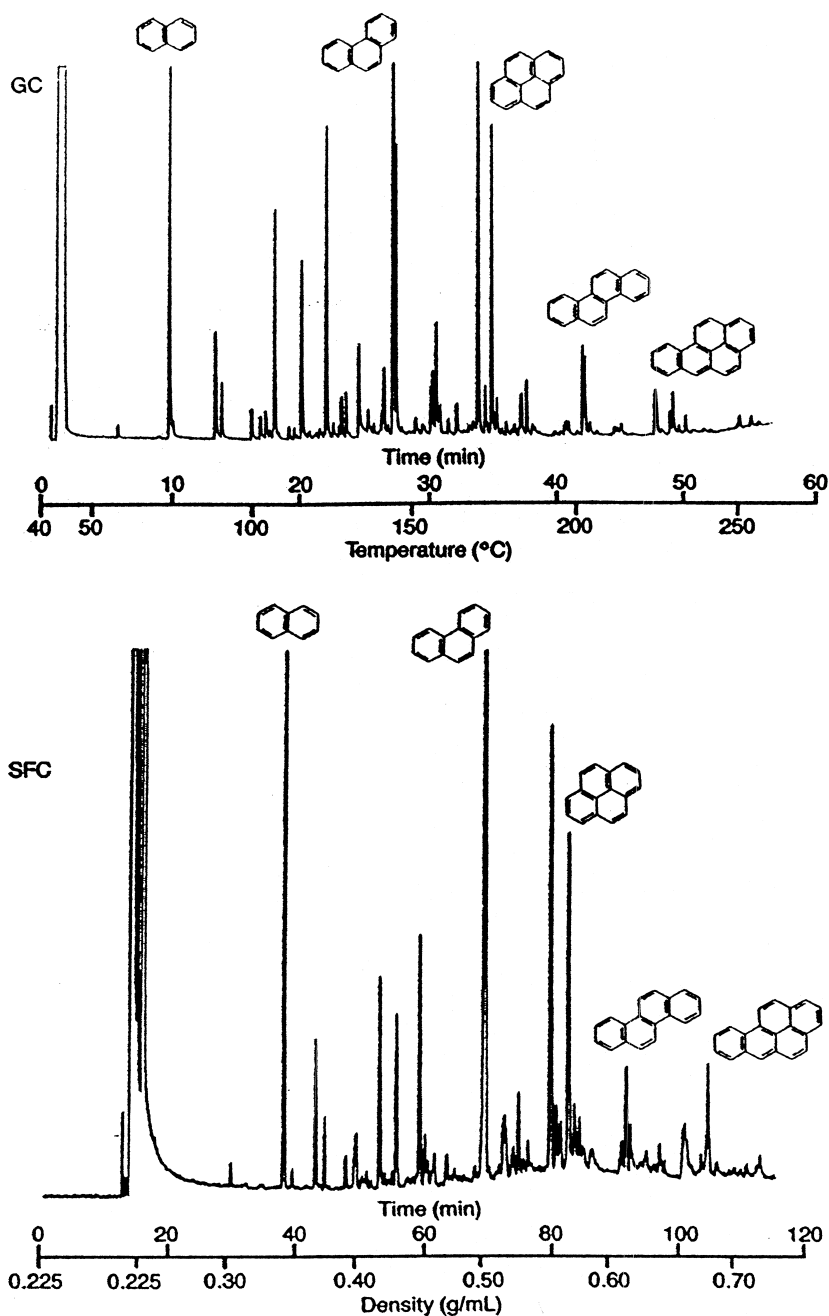


Fig. 2. Comparison of GC and SFC chromatograms of coal tar. GC conditions: mobile phase, hydrogen; column, SE-54 (20 m \times 300 μ m I.D.); temperature, 40°C for 4 min then to 265°C at 4°C min⁻¹. SFC conditions: mobile phase, carbon dioxide; column, SE-54 (34 m \times 50 μ m I.D.); temperature, 40°C; density programme, 0.225 g ml⁻¹ for 15 min then to 0.70 g ml⁻¹ at 0.005 g ml⁻¹ min⁻¹. Reprinted with permission from Ref. [21]. Copyright (1984) American Chemical Society.

to SFC but that “nature prevents the combination of the solvating powers of a liquid and the viscosity and diffusivity of a gas in a single (supercritical) fluid” [29,33]. When the diffusion rates and densities of supercritical fluid are compared with those of gases and liquid the differences are clear (Fig. 3). At low pressures and/or high temperatures, a supercritical fluid has a low density and behaves like a high viscosity gas. The solvation capacity is low and because the diffusion rates are lower than in a gas, the separation process has to be slowed to maintain the efficiency (causing long elution times). As the pressure is raised (or temperature is reduced) the fluid becomes denser and more liquid-like. The solvation strength increases but the diffusion rate decreases until the efficiency cannot be maintained with open-tubular columns. However, the fluid still has a higher diffusion rate than a liquid so better efficiencies than in HPLC can be obtained with packed columns.

Importantly, conditions do not exist where super-

critical fluid can have *both* the solvation capacity of the liquid-like phase and the high diffusion rate of the gas-like phase at the same time. However, the initial publicity talked of SFC having all the advantages of GC and LC but none of the disadvantages. This disregard of the physical properties of the fluids and the resulting problems were rapidly discovered when attempts were made to apply the method. The second problem, in a field used to the wide solvation range of reversed-phase HPLC, was that the polarity of carbon dioxide is low and many analytes of interest were simply insoluble.

However, interest continued to develop and by 1988, a review appeared titled “SFC Current status and prognosis” [34] and the method was being reviewed for the education market in the Journal of Chemical Education [35,36]. Perhaps recognising some of the limitations of SFC these reviews suggested that the future lay in SFE and in finding those applications which could uniquely be solved by SFC. SFC faced real competition from GC and LC and

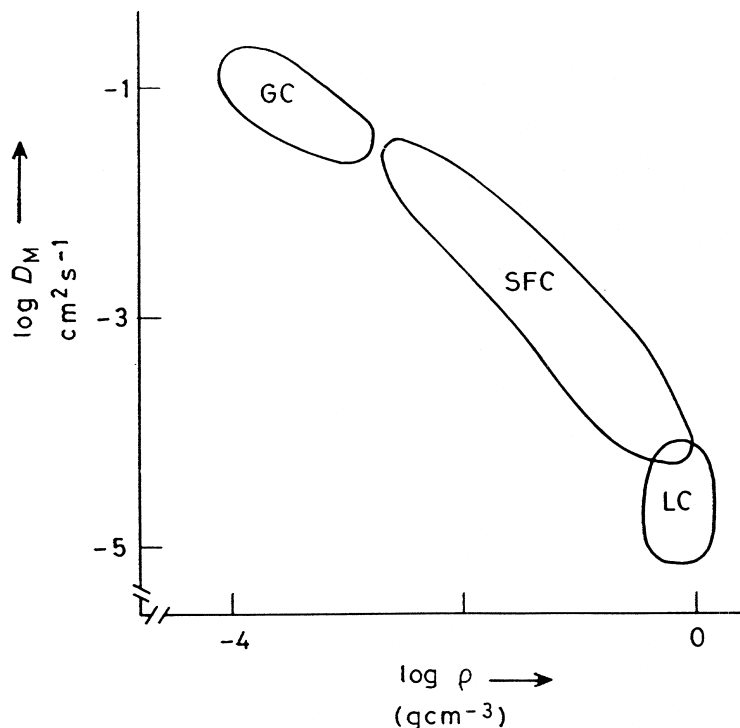


Fig. 3. SFC schematic of the relationship between the mobile phase density and diffusion coefficient of the solutes. Areas indicate typical gas (GC), liquid (LC) and supercritical fluid (SFC) operating ranges [29]. Reproduced with permission of the Royal Society of Chemistry.

needed to find problems for which it was specially suited.

Then by 1990 the heat had gone out of the SFC. It is interesting to speculate why development had suddenly stalled after such initial promise. There were perhaps two main reasons, firstly there was the new attractive research topic of CE. As a consequence, the excitement (and the grant hungry researchers) moved on to new pastures, CE offered dramatically good results, much more easily than SFC with its technological difficulties of high pressures and temperatures. However, despite the initial promise this method has also apparently slumped in many application areas with few accepted routine methods.

The second and more important reason was the recognition that the scope of SFC is essentially limited by the inherent physical chemistry of the fluid. SFC was being squeezed between developments in LC and GC, such as ultra-high-temperature open-tubular columns. Many users rapidly recognised that the older GC and LC methods could still easily carry out many of the assays with fewer instrumentation problems. Realism crept in and SFC started to be restricted to those methods where it offers a real advantage.

In SFC, the initial rush and then the slowing down seem to have been unusually dramatic but most new methods follow these stages. They all show a slow start, a surge of interest, then an attempt to apply the technique to all possible analytes, irrespective of feasibility. Finally, there is a settling down to the realistic application to those assays for which it offers real advantages of separation power or economics. Even LC developed only slowly when still a normal-phase method but exploded with the arrival of reversed-phase methods, which suddenly opened out the whole pharmaceutical industry as an application base.

In contrast, analytical SFE had a slower start and a more sustained development. It built on the industrial use of liquid carbon dioxide as a clean solvent, which has been known for many years [37]. Large scale industrial and preparative applications abounded and were discussed in journals, such as *Separation Science and Technology* (a lesson that analytical chemistry does not develop in isolation). The most widely reported examples are the use of

supercritical carbon dioxide for the decaffeination of coffee and the extraction of hops and spices [38]. Other applications included numerous extractions of natural products [39], the regeneration of activated charcoal [40], and stripping organic compounds from metal and mineral surfaces [41]. A comprehensive review by Williams [42] of the applications, methods and theory of SFE covered many of the areas later examined on an analytical scale.

However, the translation to the laboratory was slow and it took some time for analytical chemists to appreciate the potential of small scale supercritical extraction as a method of sample preparation. Early work [43] including a phase diagram of the extraction conditions and the different composition patterns that resulted from changes in conditions. In 1984 the use of dense gases for the high-pressure extraction of natural products was reviewed by Stahl et al. [44]. Subsequent reviews, such as “Extraction with supercritical fluids, Why, How and so what” [45] and the comprehensive monograph by Hugh and Kukonis [46] (and its subsequent 2nd edition [47]) provided a strong theoretical base for SFE long before most analysts showed interest. The general chemical engineering interest in supercritical fluids has continued. The proceedings of a series of ACS symposia on SFC and SFE [48] and on the wider applications of supercritical fluids [49–51] form a valuable series of reviews of the field.

5. Supercritical fluid methods

It is useful to look in detail at the various supercritical fluid strands to determine why some themes have dominated and look like providing the applications in the future.

5.1. What really is a supercritical fluid?

What is a supercritical fluid – why is it “super” – or isn’t it?

A supercritical fluid is defined as an element or compound above its critical pressure and critical temperature [52,53]. It is an unfortunate name and implies something special as it appears to confer enhanced properties that in reality are unjustified. Some researchers tried to take this definition further

[30] and required that in addition there had to be an interaction between the analyte and the eluent. This was to distinguish the method from dense gas chromatography, where it was assumed that no interactions were present. However, the extent of interaction of different gases runs from considerable to negligible.

A number of myths grew up in the early days of SFC about supercritical fluids:

- Super solvent – but most were quite weak solvents.
- Super efficient – most separations had lower efficiency than comparable GC separations and delivered fewer plates min^{-1} .
- Super selective – selectivity in extraction depends primarily on the analyte mixture and matrix and is not primarily an eluent property.
- Super fast separations – only compared to HPLC, slower than most GC separations.
- Super safe – often non-toxic but required the use of highly compressed gases so above a small scale there is a considerable safety issue.

From the numerous physical chemistry studies of supercritical fluids, we appear to know more about their solvation properties, the effects of temperature, pressure and density, and their phase diagrams [47] than about many of the solvents used for conventional LC or GC. Certainly considerable effort seems to have been put into modelling SF solubility changes and extractions compared to many liquid systems. However, while appearing to provide a useful physical chemical background for SFC and SFE, many of the models required an intimate knowledge of the properties of the analytes, which in reality would not be available. As a result, the theory often obscured the reality of the method.

The properties of supercritical fluids frequently appear to be more susceptible to the conditions than conventional solvents. This arose from the conditions used in the early studies, which deliberately examined the region around the critical point. In this region the properties of the fluids change markedly with temperature and pressure, reflecting the coming together of the properties of the gas and liquid phases. In practice, as SF methods developed, higher pressures were usually employed to increase analyte solubility. Under these conditions the eluent properties are more robust and change much less with the

applied pressures. It was also found that there was usually no significant boundary or changes in properties on going from super to subcritical temperature conditions at moderate or high pressures. The primary consideration for the chromatographer is the fluid density and generally these higher densities are preferred for their higher solvation power and higher elution strengths.

Unlike conventional liquid chromatography, as the temperature is raised (at constant pressure) in SFC, initially the retention increases because of a reduction in eluent density. With further increases in temperature, the retention then decreases as the volatility of the analyte increases (Fig. 4) [54]. The system moves from a LC-like situation controlled by the eluent strength to a gas chromatographic situation controlled by the analyte volatility. Thus under the same separation conditions, different analytes might be in different part of their individual ranges so that retention variations with temperature can be unpredictable. Only a few studies have reported negative temperature gradients to speed up assays as they are hard to control.

In the early days, particularly with capillary chromatography, the most frequently employed operating variable was a pressure gradient, which has been approached from a theoretical [55–57] and practical viewpoints [58]. These gradients gave an increasing eluent strength throughout the run [59]. Combined pressure and temperature gradients were also employed and many of the early capillary instruments had the capability for maintaining a constant density during these gradients (isoconfertic conditions). Modifier composition and pressure gradients were also combined [60].

However, there was concern that a pressure gradient along packed column could cause a deterioration of the separation [33] but this was later reinvestigated and alternative causes were suggested [61]. One advantage of supercritical solvents compared to conventional organic normal-phase solvents was that the equilibration rate after changing conditions was much more rapid even on interactive surfaces, such as silica [62].

5.2. Supercritical fluids

Although a wide range of compounds, whose

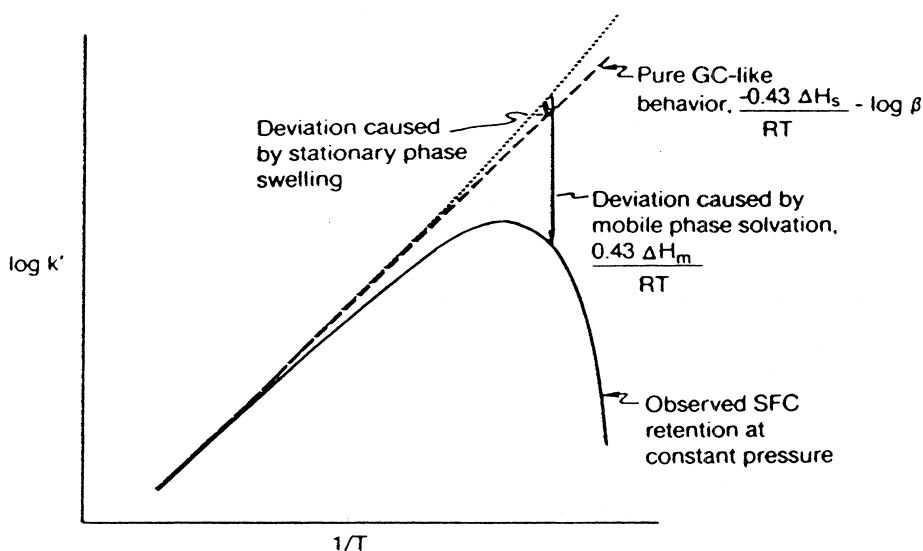


Fig. 4. Model of observed SFC retention at constant pressure as a function of temperature ($1/T$) [54]. Reproduced with permission of Wiley-VCH.

critical points are attainable under reasonable conditions, have been examined as SFE or SFC solvents, most have not been adopted to any extent. In many cases, the compounds require special handling and would not be compatible with work on an open bench. As a result, from an early stage, carbon dioxide was popular because of its low cost, readily availability and safety. It tended to be highly purified for capillary instruments but a standard good quality grade was commonly used in Europe for packed column systems. It has provided the standard gas for almost all commercial systems and is probably the only realistic solvent for use in most laboratories.

Much early work, particularly by Klesper et al. employed a number of hydrocarbons, including hexane, pentane and butane, as eluents [63]. However, these alkanes have limited solvation power and the temperatures required to reach their critical points were much above their boiling and flash points. Thus they presented such considerable health and safety risks that they were used by few other laboratories. These studies continued for some time and included the examination of gradient elution [64] with binary eluent mixtures of organic solvents and carbon dioxide and a detailed comparison of organic

and inorganic solvents for the separations of poly-aromatic hydrocarbons (PAHs) [65].

Nitrous oxide has provided the only widely examined alternative to carbon dioxide and was claimed to have a stronger eluent strength for both extraction [66,67] and chromatography [68]. There was particular interest in its application for the separation of amines [69,70], as there was a concern that basic amines would form carbamates in carbon dioxide. Although this reaction has not been confirmed, it was felt to impose a limit on the basicity on amines [71]. However, the oxidising strength of nitrous oxide has proved a hazard with oxidisable analytes or with organic modifiers [72,73] and its use for SFC or SFE is strongly discouraged.

A number of exotic and frequently toxic solvents have also been examined. Ammonia gas can readily be converted to a supercritical fluid for chromatography [74]. However, although it can be used in specially designed systems [75], it is such a strong solvent that in most cases it dissolves silica-based materials and it too aggressive and toxic for practical use. In contrast, sulphur hexafluoride [76] was a weaker eluent than carbon dioxide and of limited application. A mixture of ammonia and sulphur

hexafluoride was examined but the ammonia was corrosive and the mixture still had only a weak elution strength [77]. Sulphur dioxide has also been tried but caused serious degradation of the column and injection valves. [78].

Lately there has been an interest in haloalkanes, such as chlorodifluoromethane (Freon-22), which proved to be an excellent solvent for the chromatography of even quite polar analytes [79] and for extraction [80]. Fluoroform has also been examined [81–83]. Other organic halogens have also been studied including the more ecofriendly 134a-Freon [84,85]. None of these compounds has been widely adopted, possibly because of limited availability and high costs and environmental concerns.

A special interest case has been made for the inert gas xenon [86–88]. It is a monoatomic eluent, which makes it suitable for online SFC–Fourier transform (FT)-IR as it has no inherent background spectrum [89,90]. It has also been suggested that the related inert gas helium is supercritical in conventional GC, because of the elevated temperature and pressure in the injection port of a long open-tubular column [91].

Supercritical water has been examined although it is too aggressive for chromatography and has mainly found a role in waste remediation and the destruction of toxic waste [92]. However, in the last few years superheated (subcritical) water has attracted interest for both extraction and chromatography (see Section 7.3).

5.2.1. Eluent modifiers

Because the polarity of carbon dioxide is effectively similar to hexane, organic modifiers, such as methanol or acetonitrile, are frequently added to the eluent [93,94] but their influence is different in capillary and packed column separations. In capillary column separations, the influence of the modifier on elution strength is proportional to its concentration. It primarily alters the bulk mobile phase properties. In contrast, even very small amounts of modifier can often have a dramatic effect on packed column separations. They can enable the elution of quite polar compounds, such as acid and basic analytes [95]. This behaviour is characteristic of a normal-phase separation mechanism, where a low level of modifier acts by deactivating the stationary phase

surface. Higher levels of modifier will then act as in capillary SFC by changing the bulk eluent polarity. Some special effects of the modifiers on the matrix structure in SFE will be considered later (see Section 6.3.2)

A wide range of other modifiers has been examined [96]. In capillary chromatography, these included methanol, propan-2-ol [97], tetrahydrofuran (THF), 1-hexanol, 2-methoxyethanol, propan-1-ol [98], dimethyl sulphoxide (DMSO) [98], and many others. To avoid a response with a flame ionisation detection (FID) system, formic acid [98] and water [99] have been added as modifiers and found to give negligible background signals. There has been considerable interest in the effects and physical properties of these mixed phases compared with conventional eluents, including solvatochromic studies [100–102].

Problems of mixed methanol–carbon dioxide phases were emphasised by Page et al. [103]. They reported that frequently the chosen elution conditions will cause phase separation, which can cause deterioration in chromatographic performance. One difficulty is deciding when the level of modifier has risen to a point where it becomes the primary eluent, which has simply been diluted with the weaker carbon dioxide. This has led to studies on enhanced fluidity eluents in which moderate levels of carbon dioxide improve the properties of organic eluents (see Section 7.4).

5.2.2. Which fluids are actually useful

In practical terms, only carbon dioxide, with methanol or acetonitrile as a modifier, has reached anything like routine acceptability for extraction or chromatography. None of the other supercritical materials has shown sufficient advantages for general use, when compared with the ready availability, low cost, low toxicity (safety) and readily obtained critical conditions offered by carbon dioxide. If SFC–FT-IR was more widely used than xenon, although expensive, would probably be the eluent of choice.

5.3. Instrumentation for SFC

Many of the problems in implementing SFC came from the instrumentation. The requirement to pump

low flow-rates (under constant and gradient conditions) of a compressed but low-viscosity and high-pressure fluid at a reproducible constant flow-rate, pressure and temperature has proved difficult over a prolonged period. Just maintaining a constant back-pressure was often a problem. In addition, the sample had to be accurately introduced into this high-pressure flowing stream. These problems prevented SFC from being widely adopted as a routine method in the 1980s. It was not until second generation instruments were introduced in the early 1990s by Anachem and Hewlett-Packard, that the main problems appeared to be overcome. However, by this time the failure of the technique to deliver reproducibility meant that the market was already largely lost.

Some of the earlier attempts to improve the situation merely added new problems. To prevent problems caused by the poor mixing of modifiers with carbon dioxide, cylinders of pre-mixed eluents were proposed. However, the eluent was reported to change composition as the cylinder emptied because of differences in the volatility of the carbon dioxide and organic modifier [104]. To overcome the need to condense gaseous carbon dioxide, in order to pump it as a liquid, some suppliers provided cylinders pressurised with helium. However, the results were erratic as the helium dissolved in the carbon dioxide altering its elution and extraction strength [105,106]. Although there was a disagreement whether this also led to poor reproducibility [107]. A recent physical chemistry study suggested that the use of helium should be avoided [108].

5.3.1. Capillary instrumentation

Because the diffusion rates in supercritical fluids are lower than those in gases, the internal diameters of the open-tubular columns in capillary SFC must generally be smaller (50–100 μm) to maintain the high efficiency [109,110]. As a result the SF flow-rates are very low ($\mu\text{l min}^{-1}$). This led to the use of syringe pumps, which had to operate under precise conditions. Pressure and density gradients meant that the control of these parameters needed to be incorporated into the instrumentation. Modifier gradient elution required the use of two pumps adding considerably to the cost.

Various passive devices were adopted for maintaining the back-pressure, from capillary tubing to

integral frits so that the pressures were primarily controlled by the pumping rate. Much of the early concern in capillary instrumentation centred around the flow restrictor and these were widely compared and contrasted [111,112]. A frequent problem was that the adiabatic expansion of the carbon dioxide on depressurisation caused cooling and plugging of the restrictor. A particular difficulty in capillary chromatography was the need to be able to inject very small sample volumes (nl) [113], which was usually achieved using a timed switching valve so that only part of the contents of the sample loop could enter the column. The detection process was similarly miniaturised with UV detection being carried out across the column providing only a short path length. A frequent complaint was the poor reproducibility of injection and the difficulties of limited sensitivity often found with liquid microbore column separations. This might now be improved from developments in CE and CEC detection.

A prominent company in this area was Lee Scientific, who held a patent in the USA for capillary SFC, which they successfully defended in 1987 [114]. While preserving their position, this action appeared to have the effect of promoting packed column systems by limiting the suppliers of capillary systems. As the limitations of capillary SFC became more apparent and it suffered increased competition from high temperature GC, most SF companies either switched to packed column systems or vanished through amalgamation.

5.3.1.1. Columns for capillary GLC

It was rapidly discovered that GLC open-tubular columns could not be used for SFC as the solvent strength of the mobile phase was sufficient to strip out the liquid phases unless it was bonded to the column wall. This led to the design of dedicated phases for SFC, which were reported to have improved properties [115,116]. Recent developments have also examined the role of packed microbore columns [117] for SFC.

5.3.2. Packed column instruments

Packed column instrumentation for SFC was easier to develop and worked on a familiar scale of pressures and temperatures, with equipment common to HPLC users. In this case the advantage is that the

supercritical fluid has a higher diffusion rate and lower viscosity than most liquid solvents. The separations were therefore more efficient, suffered less from dead volume problems, and could be operated at a higher linear flow-rate than LC, reducing retention times.

In most cases it was necessary to cool the pump heads to about 0°C so that the carbon dioxide condensed and could be pumped [3,28]. However, the low viscosity of liquid carbon dioxide means that check valve leakage was more of a problem than in HPLC and the pump seals had to be selected to be resistant to the extraction of additives. Gradient elution and the use of modifiers caused some difficulties with standard LC pumps. At low levels (1–5%) the modifier pump works at the lowest limit of its specification (10–50 $\mu\text{l min}^{-1}$) and frequently fails to deliver a constant flow [118].

Injection was carried out with a standard LC rotary injection valve, although rotor wear was increased. The detectors were usually standard HPLC spectroscopic detectors fitted with a high-pressure flow cell. Usually either a mechanical back-pressure regulator or pressure relief valve was used until the introduction of the electronic regulator by Jasco [119]. Subsequently, programmable regulators have become available [120]. Although changes in pressure alter retention times, it has little effect on relative retentions or selectivity [121]. The rationale behind the design of the present generation of packed column SFC systems has been discussed by Berger [121].

5.3.2.1. Columns for packed column SFC

Almost all packed column SFC has employed conventional HPLC columns. Liquid chromatographers had already learnt that bonded phases were desirable for stability. In the earliest separations both unbonded silica and ODS bonded silica were used [15]. Other column materials, including cyano bonded silica [122,123] and polymeric materials, such as polystyrene–divinylbenzene (PS–DVB), were also examined [124]. By 1990 Taylor and Chang noted that although packed columns had been “categorically dismissed as inapplicable for SFC during the mid 1980s” they were rapidly gaining ground [125]. However, in the initial work the mechanism of retention was not always obvious,

although subsequently it has become clear that interaction with the surface silanols by a normal-phase mechanism is a major contributor to retention. Differences in the selectivity and retention effects in the presence and absence of modifiers were examined on ODS [126] and PS–DVB [127] columns. The retention and selectivity properties of different phases were also compared by Schoenmakers et al. [128] and their application and use was reviewed by Petersen [129].

The most efficient separation on a packed column SFC was reported in 1993 by Berger and Wilson [130]. They obtained 260 000 plates from ten serially linked 200 mm packed columns for the components of lemon oil (Fig. 5). The dead time was only 12 min. The separation was isocratic and useful results could be obtained in a time scale suitable for analytical applications and not dissimilar to the times needed for capillary GC. This was an important demonstration that the low viscosity of SF meant that long (or multi-linked) packed columns were practical.

It also quickly became apparent that many of the chiral columns being developed for liquid chromatography could also be used in supercritical fluid chromatography, often with greater success [131]. When the conditions were explored the enantioselectivity often increased as the temperature was reduced and consequently the preferred conditions were frequently subcritical and in some cases subambient [132]. The range of separations and chiral columns have recently been reviewed [133,134] (see Section 6.2).

5.3.3. Retention in SFC

Many early studies examined the relationship between retention and the solubility of the analyte in a supercritical fluid [43]. Schoenmakers proposed a thermodynamic view of retention in SFC, primarily based on solubility parameters using the Lee–Kesler equation of state [135]. Martire and Boehn then developed a statistical thermodynamic model [136]. Later workers tended to use the simpler Peng–Robinson equation of state to calculate solubility and this was found to provide better predictions at higher mobile phase densities [137]. This equation was also used to examine the temperature dependence of retention by Bartle et al. [138]. Later theoretical

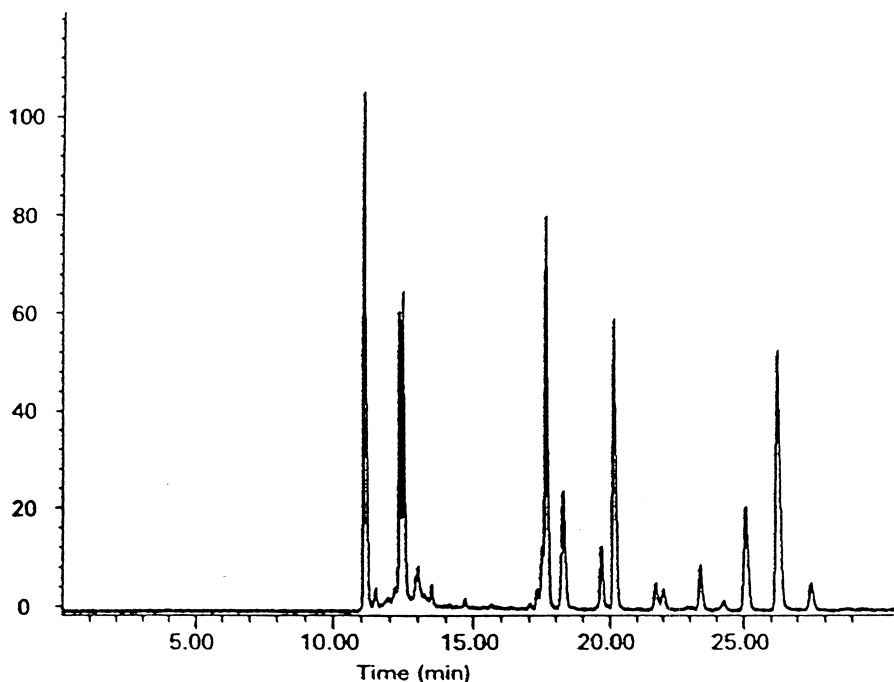


Fig. 5. Separation of Brazilian lemon oil on 10 linked packed silica columns (each 200×4.6 mm) exhibiting $>200\,000$ plates. Conditions: eluent, carbon dioxide with 2% methanol; flow-rate, 2 ml min^{-1} ; temperature/pressure, 60°C and 150 bar outlet pressure. Reprinted with permission from Ref. [130]. Copyright (1993) American Chemical Society.

studies were undertaken by Roth [139], Poe [140] and Martire [141]. However, most of these studies concentrated on model compounds, usually the polycyclic aromatic hydrocarbons, with solubility rather than polarity interactions with the stationary phase.

These papers provided a theoretical background to SFC and usually could achieve a good correlation with between theory and practice. However, they represented only a limited range of analytes and a broader extension would be limited by the lack of basic physical parameters and interaction functions. Thus although it appeared that SFC had a strong framework, it was not one that the average chromatographer could use to develop separations.

Of more application for method development were studies in 1986 by Mourier et al. [142]. They examined separations on a number of phases and reported that the retention selectivity was similar to that of a typical polar solvent, such as hexane. Wheeler and McNally [143] then compared the retention characteristics of different functional groups on capillary and packed columns. They noted

that the normal-phase characteristics of SFC offered separations not available on reversed-phase HPLC. Upmooor and Brunner [144] considered that retention was governed by the polarity of the mobile phase expressed by the Snyder parameter. They also noted that the adsorption of any modifier on residual silanols plays an important role for polar analytes.

In an attempt to systematically explain retention of analytes with different functional groups, King and Friedrich [145] suggested that Fedors solubility parameters [146] could provide a guide. Heaton et al. proposed a relationship between molecular interaction parameters and retention in packed column separations [147]. Thermodynamic theory was used to derive a linear relationship between the log retention factor and chain length of homologous *n*-alkanes in SFC [148] but appears similar to the relationship for homologues in GC. Recently, there has also been a number of studies examining the linear solvation energy relationships of supercritical fluids and retention [102,149,150].

The influence of a normal-phase mechanism can

be seen in a paper by Smith et al. [123], who examined the retention of a homologous series of ketones on a cyano-bonded silica. They suggested that it could be modelled as a combination of a normal-phase polar interaction and a volatility effect, whose relative contribution increase with the chain length. In a second study, Smith and Cocks [151] showed that there was a strong surface effect on a silica column. Fatty acid methyl esters were separated primarily according to the number of double bonds and that *cis* and *trans* isomers could be distinguished. The existence of a normal-phase type mode of retention can also be seen in chiral separations (see Section 6.2) where SFC closely resembles separations with non-polar eluents.

5.3.4. Detectors for supercritical fluid chromatography

One of the attractions of SFC was that it could use both GC- and LC-like detectors. Thus it could use the universal FID instrument for involatile and volatile analytes after separation on both capillary [152] and packed columns [153,154]. Selective responses were also obtained from a number of detectors but very few were widely studied, once a demonstration paper had been reported. Nitrogen and phosphorus responses could be obtained from a thermionic detector in capillary SFC [155,156] with both carbon dioxide and nitrous oxide eluents but there was some problems of linearity at low levels. The electron capture detector was applied to polychlorinated biphenyls (PCBs) [157] and agrochemicals [158–160]. The flame photometric detector was also examined [161,162]. The chemiluminescence detector gave a response as a selective sulphur [163,164] or nitro/nitroso detector [165] but suffered from quenching. Other GLC detectors examined included the photoionisation detector for aromatic compounds [166].

Packed column systems have also frequently used LC-like optical methods. The use of both the UV detector and diode array detector was widespread from the earliest days of SFC. However, in capillary chromatography, the cell path length is short because detection is across the column width and sensitivities were relatively poor. Only a few papers have used fluorescence detection, either for capillary [167] or packed columns [168] possibly because no commer-

cial instrument is readily available. The light scattering or mass evaporative detector has attracted a number of studies because the evaporation of the eluent is a lot easier than in LC [169–172]. The expanding supercritical fluid provides its own nebulisation, although some care is required to prevent “snow” formation [171].

One attractive detector for SFC appeared to be FT-IR [173,174] because it could offer qualitative information about the analytes [175,176]. However, the absorbance of the mobile phase and changes in the mobile phase spectrum with applied pressure meant that background correction was difficult, particularly if modifiers were used [124]. The scope of the method was much improved by the use of xenon as the eluent [89,90] as there was no background absorbance to interfere with the spectrum.

SFC coupled to mass spectrometry (MS) has been one of the most successful application of SFC [177–181]. The advantage is that it is much easier to evaporate a supercritical mobile phase into the MS source than most LC solvents. Method development was very simple because only pressure and temperature had to be controlled. Most of the practical problems were associated with interfacing [182–184] and the effect of the evaporating eluent freezing and blocking the flow. The applications have been wide ranging and included the forensic examination of controlled drugs [185], natural products [186], polymer additives [187], clinical samples [188], and drug metabolites [189,190]. Because the MS instrument is the main source of information, the reproducibility of the retention and the separation selectivity are much less important than for other SFC applications. As a result mass spectroscopists were not restrained by the limits on reproducibility, which slowed the uptake of SFC elsewhere.

Because carbon dioxide contains no protons it is also attractive as a transparent solvent for nuclear magnetic resonance (NMR) spectroscopy. A number of workers [191,192] have examined the application of SFC coupled to NMR spectroscopy for the separation and identification of complex mixtures. However, there are problems as the signals are affected by pressure and density so gradient conditions can cause drifting signals. Often in flow-NMR methods, stop-flow techniques are employed to enable greater sensitivity to be obtained. However, in

SFC–NMR the detector cell is pressurised and to ensure a constant signal the pressure must be maintained during the scanning. The major problem in SFC–NMR has been the low polarity of carbon dioxide which limits the types and number of analytes that can be examined. Typical examples have been the dialkyl phthalates and vitamin A isomers. It is difficult to employ organic modifiers as they would also generate significant NMR signals unless fully deuterated.

The use of hyphenated techniques in SFC has been reviewed [193].

5.3.5. Instrumentation for supercritical fluid extraction

With the increasing interest in supercritical fluids for SFC in the late 1980s, analytical chemists started to take an interest in SFE as a potential sample preparation method. In its simplest form, SFE was easy to implement and only needed a method of maintaining the pressure in a heated extraction cell, a device for releasing the fluid and a method for collecting the extract. The first studies examined model systems, such as the extraction of PAHs from absorbent media, and compared carbon dioxide and hydrocarbon solvents [194]. A review article by Hawthorne in 1990 [66] noted that there were only two papers on SFE in analytical journals up to 1986 and only a further 26 up to mid 1989. This review did much to alert the analytical community to the potential of SFE and started considerable interest in the field.

The development of laboratory SFE system went hand in hand with the development of restrictors for capillary SFC and back-pressure regulators for packed column SFC. Effectively the extraction cell replaced the separation column. The main practical developments (and problems) were associated with the collection process and attempts to improve the yield and recovery. The large volume of the expanding supercritical fluid generates a gas flow which can blow volatile analytes out of the system. A lot of effort went into understanding the parameters affecting analytical reproducibility, both in the extraction process and in the trapping of the extract after extraction. The trapping method could either use a small volume of solution [195], solid traps [196] or solidify the carbon dioxide, which was then allowed

to sublime [197]. A solventless expansion was also reported [198]. Care had to be taken to avoid restrictor plugging by solid carbon dioxide or extract [199]. Using a back-pressure regulator caused some problems as the extract could be deposited in the valve mechanism. If a solvent was added before the regulator, it would continually wash the extract into a collection vessel [200].

Integrated systems in which an extract was passed directly to the separation column played an important role in many early systems. These included SFE–SFC (Fig. 6) [201,202], SFE–GC [203] and combinations with MS. The advantage in each case was a reduction in sample handling and compatibility between the two solvents. The use of linked systems has been reviewed [193,204,205]. Attempts to automate SFE brought additional problems because the extraction system had to be capable of maintaining the pressure and temperatures needed. This has proved a difficult step because the instrument has to make and break a high-pressure seal for each sample.

6. Applications – real and demonstrated

As with any new analytical technique, the successful adoption of supercritical fluid methods depended on the users being able to demonstrate applications and methods that were better (more efficient, more selective, faster or cheaper) than existing methods. Many of early reports were of impressive separations of difficult analyte mixtures and suggested a bright future. Ready linkage to FID and mass spectroscopic detection made the assays even more acceptable. This led to a widespread search for the limits of SFC and SFE to determine which samples were possible and which worthwhile. However, the claims of equipment manufacturers and scientists often led users to expect capabilities that were unrealistic.

Compilations of demonstrated separations were prepared and the collection from the 1989 Symposium in Snow Bird [206] included 370 examples from almost every area of the chemical industry, pharmaceuticals, food, natural products, biomolecules, pesticides, fuels and polymer additives. Primary journals also published many examples. However, the failures were not reported but had considerable influence on the perceived value of the tech-

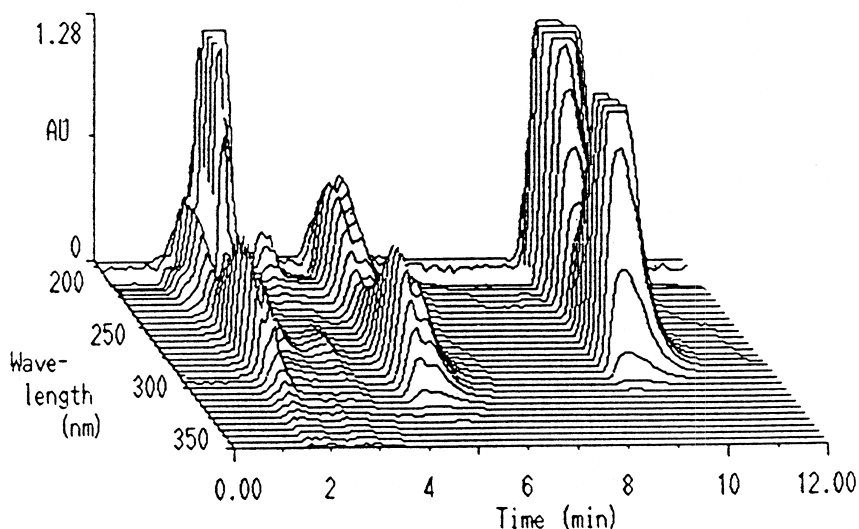


Fig. 6. SFC-diode array chromatogram from coffee powder by directly coupled SFE–SFC. SFE conditions: 200 bar at 40°C added water 20%. SFC conditions: mobile phase, carbon dioxide–2% methanol, 5 ml min⁻¹; pressure, 150 bar; temperature, 40°C; column, Fine Pak SIL C₁₈ (150×6 mm). Reprinted from Ref. [201] with permission of Elsevier Science.

nique for many industrial analysts. SFC was seen as a method for non-polar analytes but with poor reproducibility and with numerous operational problems. Even when the method worked, it frequently showed little advantage over HPLC or GLC methods.

In looking at the applications of supercritical fluids, this review will concentrate on those areas where SFC appears to be making the greatest impact. A survey of the titles/keywords of papers using supercritical fluids up to the end of 1998 (Table 1) shows that the overall numbers covering chromatography and extraction are similar. When these titles were grouped by keywords, they give a reasonable indication of the major application areas. The results reflect the non-polar nature of carbon dioxide, with significant numbers of papers on soil analysis and the extraction of PAHs, PCBs and pesticides. Other prominent areas, include food analysis, where the emphasis is on fats and vegetable oils, and petrochemicals, with interests in oil and polymers. The small number of pharmaceutical applications is noticeable and these are dominated by SFC of chiral analytes. The application of SFE to natural products and plant and essential oil analysis is probably underestimated but this area lacks common terms for searching.

A wider coverage of supercritical fluid methods

can be found in the many books on SFC [30,31,207–211] with a further three books reportedly in press at the end of 1998. One indication of the trends in SFC is that two of the more recent books have been dedicated to packed column SFC [212,213]. Although there has been special interest in bioanalytical applications [214–216] most of these assays are more easily carried out by HPLC.

6.1. Capillary column separations

Typical claims for capillary SFC, included a faster analysis time, higher sensitivity, higher resolution, minimal derivatisation for separation and the ability to separate thermally labile organic compounds [217]. However, these claims are valid only in comparison to HPLC but fall down when compared to GC. The main interest in capillary chromatography comes from the high efficiency that could be obtained for involatile samples. However, because of the limitations imposed by the smaller diffusion rates in supercritical fluids than in gases, it was necessary to use a low flow-rate and a very narrow column. A frequently reproduced separation of triglycerides [25] took over 160 min, too long to be a viable method.

The primary success of capillary SFC has been for the separation of non-polar polymers or mixtures of

Table 1
Areas of supercritical fluid separation in analytical chemistry

Categories of publication over the period 1980–1998 based on a keyword search

Topic	Papers
Supercritical fluids	3973
SFC	1532
SFE	1526

Application areas

(by title word search – linked with either chromatography or extraction if more than 50 papers)

	Total	SFC	SFE
<i>Agrochemicals</i>	4		
Herbicide	43		
Pesticide	144	43	90
<i>Environmental</i>	109	24	61
Soil	198	24	156
PCB	23		
PAH	20		
Polycyclic	89	30	53
Pollutant	28		
Sediment	69	11	50
<i>Food</i>	71	15	34
Fat	52	15	21
Fatty	55	15	19
Lipids	46		
Oil (vegetable)	42		
<i>Petroleum</i>	36		
Fuel	25		
Oil (hydrocarbon)	148	47	106
Oils (fuel)	15		
<i>Pharmaceutical</i>	25		
Drugs	61	33	23
Steroid	15		
Chiral	81	60	0
<i>Phytochemical</i>	1		
Essential oils	17		
Polymer	103	29	23

higher homologues of analytes which were too involatile for GLC. These mixtures were suitable for capillary chromatography with pressure programming, where the separation is based primarily on size/volatility rather than polarity differences. One enduring application has been in the petroleum industry, where SFC can simulate the high temperature distillation carried out in the refining process (SIMDIST) and separate mixture of alkanes from

C₂₀ to C_{>100} (Fig. 7) [218]. Natural and synthetic waxes can also be separated [219]. A closely related area is the assay of polymer additives (plasticisers, UV agent, etc.) [220] although the main attraction here has been in the use of SFE. Natural products also attracted interest but when the carotenoids were examined the authors conceded that the capillary SFC separation could only match the conventional HPLC [221].

6.2. Packed column separations

The potential advantages of packed column SFC over HPLC were the increased speed of analysis and the ability to use universal detection methods, such as FID. However, the widespread need to use organic modifiers meant that most packed column SFC separations used spectroscopic detection. Although many assays have been examined, few of these have endured. The problem was that irrespective of the nominal nature of the stationary phase, SFC is essentially a normal-phase separation mode in which a primary retention mechanism is the interaction of the analyte with the stationary phase. Thus any assay where the reversed-phase mode of separation is preferable, which includes the great majority of analytes, will probably not transfer easily to SFC. Although it may be possible to achieve a separation, the corresponding HPLC method will probably give more robust and reliable results. This problem was the cause of considerable disillusionment in the early day of SFC when pharmaceutical companies were encouraged to purchase and evaluate expensive SFC systems. This early rush to SFC was probably a mistake. Few of their samples and fewer of their matrices proved suitable. This was coupled with a lack of robust well designed SFC systems, which could satisfy the standards required for GLP. As a result most companies rapidly relegated the equipment to a back room or converted it to HPLC. A slower introduction, with demonstrated methods, would have lead to greater acceptability for those methods where SFC really does work. Robust equipment arrived on the scene too late, the bubble had burst and few companies would invest in the new generation of systems. However, some packed column SFC has succeeded and clearly there is a niche role for the technique. This is primarily as an easier

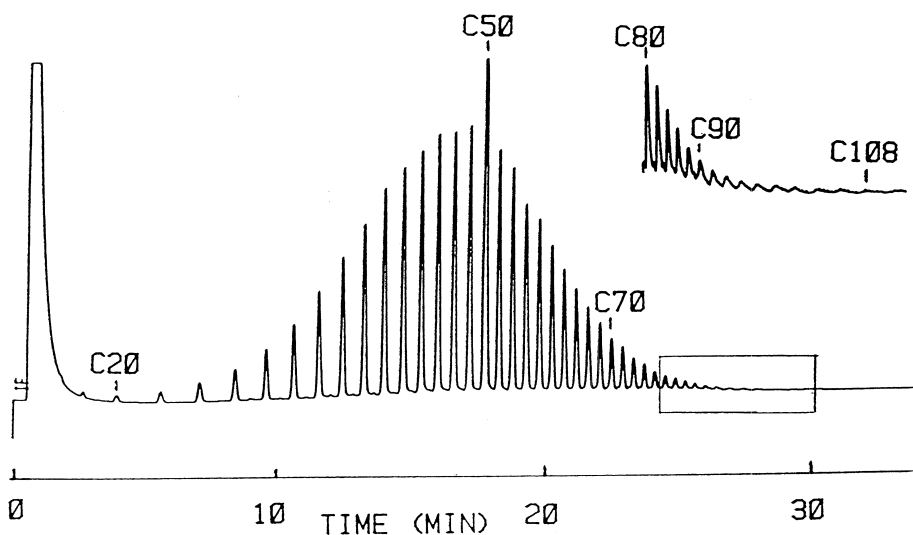


Fig. 7. SFC chromatogram of a SIMDIST calibration standard PE 740. Conditions: column, polysiloxane PM (100×1 mm); mobile phase, carbon dioxide; temperature 150°C; pressure, 2000 p.s.i. for 4 min, then 2000–5500 p.s.i. over 21 min [218]. Reproduced by permission of Preston Publications. A Division of Preston Industries, Inc.

and more robust method to carry out normal-phase separations than with non-polar organic eluents.

One theme continuing from the very first SFC paper [5] has been an interest in metal complexes [222] but even here the main interest has been in SFE. Many of the early workers separated pesticides [223] often linked with SFE from plant and soil matrices [224]. Other separations included polymer additives [225] and environmental samples [226]. SFC also showed an ability to separate groups of saturates, olefins and aromatics which is important in grading oils (Fig. 8) [227,228] and has been adopted as an official method (see Section 6.4).

Although the main market for separation techniques is the pharmaceutical industry, most drug compounds are too polar for routine SFC. Some early work examined the application of capillary SFC [229] but most studies have employed packed columns. The polarity of many drugs required the use of modifiers, for example barbiturates [230], benzodiazepines [231] and the opium alkaloids [232]. Often such a high proportion of modifier was required, as in the analysis of ranitidine [233], that questions were raised whether the eluent was supercritical or if the carbon dioxide played any useful role other than as an inert diluent. To improve the separations, even ion pair chromatography was tried

for a range of analytes, including basic drugs, although there were problems of the insolubility of the counter ion in the carbon dioxide mobile phase [234]. The potential of reverse micelles in *n*-alkane supercritical eluents were also briefly examined as a method of separating polar analytes, such as proteins [235].

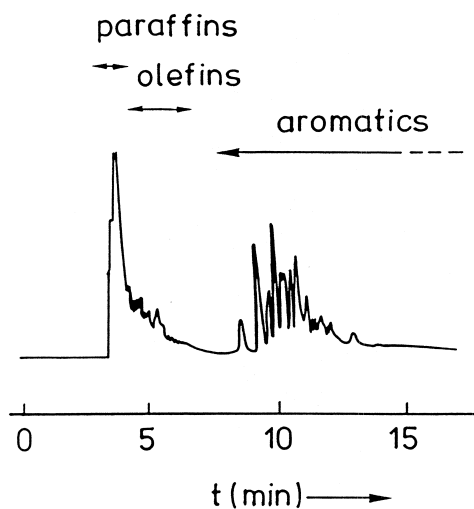


Fig. 8. Group separation of paraffinic, olefinic and aromatic fraction of a gasoline sample by packed column SFC. Reproduced from Ref. [227] with permission of Elsevier Science.

The limitations of SFC for the analysis of polar analytes were systematically addressed by Berger and Deye, who developed conditions needed to separate aromatic acids [236,237], phenols [238], anilines [239] and strong bases [240] by employing additives to control pH and surface ionisation [241]. Berger and Wilson subsequently examined a number of polar and basic nitrogen containing analytes including primary amines [242] by using a basic additive in the mobile phase. The value of these approaches was demonstrated in the separation of phenothiazine antipsychotics [243], antidepressants [244] and stimulants [245]. This work confirming that much of the concern about the limited potential of SFC for polar analytes was unfounded. In a subsequent book [212] Berger demonstrated the approach to be taken to select suitable conditions for a separation. The potential of SFC for the separation of polar analytes has been recently reviewed [246].

The most successful area for SFC separations has been in the enantioseparation of chiral analytes [131,133,247,248] including the preparative scale [249]. The normal-phase separation mode enhances the interaction between the analytes and stationary phase increasing chiral selectivity. Using a single mobile phase of carbon dioxide–methanol containing trifluoroacetic acid and triethylamine, Medvedovici

et al. [250] showed that two columns (Chiralpak AD or Chiralcel OD) would separate 95% of a set of 44 racemates of different types. The separations were carried out subcritically at 30°C and 200 bar (Fig. 9). Most of the remaining analytes tested could be resolved on Chirobiotic T, Chirobiotic V, Chirex 3022 and Chirex 3005. By changing the temperature conditions, it can be possible to reverse the elution of order of some entropically controlled analytes [251].

One problem in the pharmaceutical industry is that methods frequently have to be scaled up [252,253]. Whereas the separation can readily be achieved by SFC, it becomes a practical problem of safety engineering because of the high pressures of a compressed gas that are involved. Precautions must also be taken at the collection stage to avoid the sample being blown away by the large volumes of expanding fluid. One approach has been to trap the analyte as a solid in a liquid nitrogen cooled collection vessel and then to allow the carbon dioxide to be removed in a room temperature sublimation

A frequent claim in the early literature was that SFC had the ability to handle thermally unstable compounds but even now very few examples have been reported. The principal examples are explosive propellants [175,254,255] and thermally labile poly-

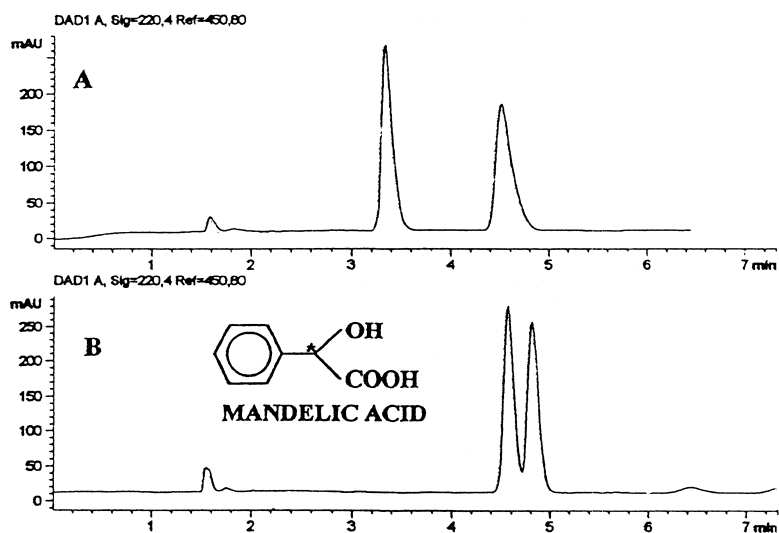


Fig. 9. Packed column separations of mandelic acid on Chiralcel OD (A) and Chiralpak AD (B) column. Conditions: mobile phase, carbon dioxide; modifier, methanol containing 0.1% triethylamine and 0.1% trifluoroacetic acid programmed from 5% to 30%; temperature, 30°C; pressure 200 bar. Reprinted from Ref. [250] with permission of Elsevier Science.

mer additives, such as fire retardants [256]. A number of unstable pesticides have also been examined [257,258].

6.3. Applications of SFE

In contrast to SFC, where the advantages compared with gas or liquid chromatography were often hard to identify, SFE frequently provides methods that are improvements in efficiency or time compared to conventional techniques. SFE can often overcome limitations in existing methods or even offer new extraction routes. This enables analysts to overcome some of the sample preparation constraints which often prove to be the stumbling block to an efficient analytical procedure. However, in a book on SFE in 1996, Taylor remarked that “SFE is not a fully mature technique” [259]. Not all samples are suitable and despite the apparent ease of the SFE method, users have to remember that the limits imposed by kinetics and thermodynamics are still present.

As with SFC, initially there were many attempts to apply the method to unsuitable matrices, there was often a hope that “super” SFE could do what other systems found to be impossible. There were many claims of selectivity and efficiency that proved to be unrealistic or at best optimistic but generally SFE has proved a greater success than SFC. However, the need for successful automation has proved a significant restriction in many routine applications. The success of SFE has led to a wide range of monographs and these are a good source of the possible applications and methods. They range from the earliest applications before its use on the analytical scale [37,39] and to descriptions of analytical methods [260–262]. More can be found in many of the books on SFC. The theory from a physical chemistry viewpoint is best described in the specialised monographs, especially McHugh and Kurkonis [47], although short descriptions are given in most texts.

6.3.1. The problems

As with SFC many of the initial papers and literature were over-optimistic, typically an extraction would be claimed to be complete because on repeating the extraction no additional extract was obtained. However, it has frequently been demon-

strated that using more powerful conditions or adding a modifier will yield an additional extract [197,263]. Extraction conditions were also reported to be 100% effective because quantitative recoveries of spiked samples had been obtained. However, extraction from reference materials or from older samples under the same conditions would then be incomplete. These problems were clearly demonstrated in the work of Burford et al., who showed that native or aged spiked samples retaining typical analytes more strongly (Fig. 10) [263].

There is a clear need for SFE to be carried out on reference materials of known composition determined by an alternative technique. Even this approach is not perfect as in a number of cases SFE gave higher yields than the “standard” Soxhlet method.

Another misleading claim in SFE was that of “super” selectivity, or an enhanced ability to discriminate between different analytes. The reality is that SFE as any extraction is a solubility and diffusion controlled process. Given enough time even a poorly soluble analyte can be extracted, or an apparently impermeable matrix can be penetrated. Every selective extraction is based on the ability to obtain a significant proportion of the desirable analyte with a minimum of undesirable components in a selected time. However, in routine applications the matrix may be variable in composition or structure. The method must then have an excess of extraction capability. This is to ensure that the extract is obtained in high yield from even the most intractable sample. As a consequence some undesired material is usually also obtained. This over-capacity for extraction is already built into established methods, usually by using a stronger solvent or a longer time than required for the ideal sample. This was necessary to ensure the robustness of the method, whereas many SFE reports examined a single clean matrix. Even the Soxhlet method can be made more selective by using a series of extraction solvents of increasing strength. The supposed enhanced selectivity of SF is thus a myth, in extraction there is always a trade off between a reproducible complete extraction and selectivity.

A practical problem is that carbon dioxide is immiscible with water but will dissolve it to a small extent so the extraction of wet or liquid samples and

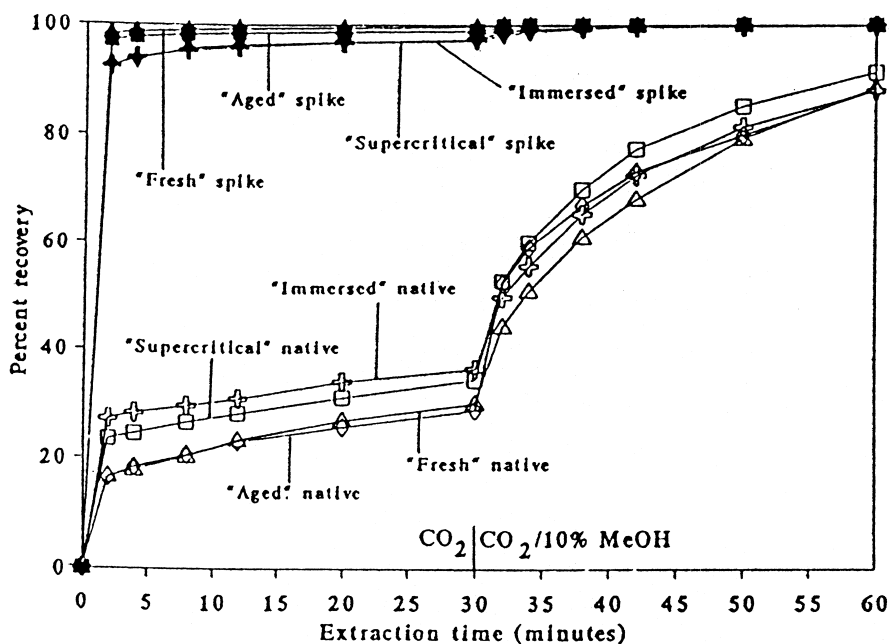


Fig. 10. Comparison of the differences in the extraction of naphthalene in spiked (as naphthalene- d_8) (solid symbols) and native form (open symbols) from urban air particulate (SRM 1649). The recoveries also demonstrate how extractions appears to go to completion and then additional naphthalene can be extraction by using modifier. Reprinted with permission from Ref. [263]. Copyright (1993) American Chemical Society.

solutions are particularly difficult. Thus extractions from most biological fluids, such as blood, urine and saliva, are precluded, limiting applications in drug metabolism studies and toxicology. Instead these matrices are more readily examined using solid-phase extraction (SPE) or solid-phase micro-extraction (SPME) methods [264].

The ideal matrix for SFE is a finely powdered solid with good permeability, allowing a large surface area for interaction. Typical examples are soils, particulates and powdered dried plant materials. Intermediate in suitability are semi-permeable solids, such as polymers, which can be partially penetrated to give qualitative but not quantitative extractions. The worst samples are wet body tissues, such as fish, as they were virtually impermeable, solid wood, rocks and liquid/solution samples. Some wet samples can be dried or blended with a drying agent [265], but in routine use this requires additional handling steps, when traditional methods of extraction, such as maceration and liquid-liquid extraction methods are much easier.

The low polarity of carbon dioxide means that many of the successful methods have been for the analysis of non-polar analytes, such as PAHs, oils and fats, and the matrix requirements have lead to environmental soil samples, dried plant materials and polymers.

6.3.2. Environmental and pesticide samples

Work in this area generated much of the interest in analytical SFE and lead to many of the studies of the extraction and trapping protocols. In early studies Hawthorne and Miller [266] used carbon dioxide to extract organic pollutants from model systems and environmental samples. They found that they could obtain the certified levels of PAHs and good results were also obtained from urban dust SRM 1649 [267] in a much shorter time than conventional methods. This work developed into a series of studies linking SFE and SFC to MS for identification [268].

Related studies have been carried out on pesticides from soil matrices. The early studies were not promising as although good recoveries of organo-

chlorine and organophosphorus pesticides could be obtained from spiked sand [269], the recoveries were poor (down to 27%) from standard reference materials (SRMs). The results were improved in later work [270] when an average of 93% was recovered from spiked soils using a 3% methanol–carbon dioxide mixture. The addition of traces of water (either in the carbon dioxide or in the soil sample) showed an improvement, indicating that consistent sample preparation was very important. A correlation was derived between ease of extraction and SFC on a packed column, for example for diuron and linuron, and suggested that the retention times could aid method development [224].

In an important study, Fahmy et al. [271] examined the effect of modifiers on the extraction of analytes from clays, soil and plant materials. They showed that the clays and plant material swelled under the influence of the modifier and that this effect could be correlated with the extraction efficiency. This work has important implications as the efficiency of extraction will be dependent on the structure and type of the soil [272]. Suitable conditions for one soil type might not be appropriate for another type. Consequently attempts to use conditions which are too selective might result in a variability of extraction.

Pesticide residues, such as chlorpyrifos, have also been extracted from plant materials [273] using a SFE–LC–MS linked system. Anthraquinone has also been extracted from paper and wood pulp [274] by SFE–HPLC/LC–EC. The extractions of a range of pesticides from environmental [275] and from food samples have recently been reviewed [276].

6.3.3. *Foods and fats*

The use of SFE to extract lipids from different food ingredients was one of the earlier applications [277]. Related work has used SFE to determine the fat content of raw and processed food [278–281] and this is one of the areas where supercritical fluids demonstrate a real advantage over alternative methods. The methods have been applied to both animal fats, such as those in beef burgers [282,283], and vegetable oils, including residual cooking oils in deep fried food [284].

SFE has also been used to extract flavour and volatile constituents from many foods and food

ingredients ([285] and see the following section), pesticide residues [286] and polymer additives which have migrated from packaging [287].

The use of SFE in food analysis has recently been reviewed [288,289].

6.3.4. *Plant materials*

Probably the broadest application of SFE has been the extraction of natural products from plant material. This area was also well established as a preparative and industrial method long before any analytical interest. Early studies by Saito and co-workers examined linked systems, such as SFE–SFC of tocopherols from wheat germ [290] or caffeine from tea and coffee (Fig. 6) [201]. Other groups have linked SFE and GC for a range of spices, such as basil, oregano [291,292], turmeric [200] and allium species [293].

The technique has also been applied to the extraction of the active components from herbal medicines, such as parthenolide from feverfew, tansy and German chamomile (Fig. 11) [294,295]. In this case, an intermediate silica trap yielded a cleaner sample in a much shorter time than conventional isolation methods. Another example has been the extraction of the yew tree to yield the anticancer taxanes [296]. Both these studies examined the conditions needed to achieve complete extraction. The application of supercritical fluids for the isolation of natural products, with particular interest in compounds with pharmaceutical activity, has been reviewed by Bevan and Marshall [297]. Other natural products, such as microbial fermentation components, have also been extracted as part of screening studies [298,299].

6.3.5. *Polymers*

The additives in polymers can often prove difficult to extract with organic solvents because of the difficulty in penetrating the solid matrix. The lower viscosities and higher diffusion rates of supercritical fluids mean that generally they can easily penetrate the polymer matrix and considerably speed up extraction [300]. However, the polymer is first extracted from the edges or surfaces of solid material and complete extraction and quantitation can be difficult unless the particle size of the sample is carefully controlled. Some of the work has examined

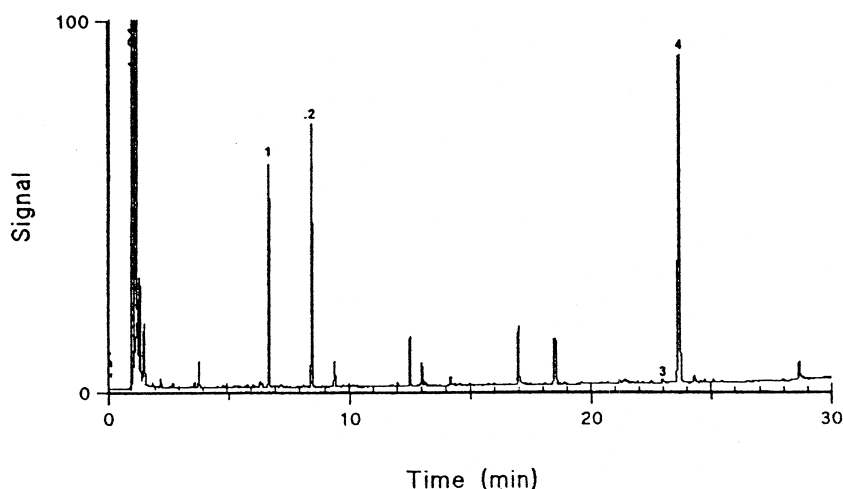


Fig. 11. Gas chromatograph of an extract of freshly dried feverfew. Extraction with carbon dioxide at 250 bar and 45°C. GC on BP1 (25 m \times 0.25 mm I.D.) temperature 60 to 300°C at 8°C min⁻¹. Peaks: 1=Camphor, 2=chrysanthenol, 3=dihydroparthenolide, 4=parthenolide. Reprinted from Ref. [294] with permission of Elsevier Science.

the polymers themselves as oligomers [301] but most has concentrated on the extraction of additives, such as plasticisers and UV-stabilisers [301–304] or thermal protection agents [256]. The ability to precisely alter the solvation power of supercritical fluids has been applied to the molecular mass fractionation of polymers [305].

6.3.6. Miscellaneous applications of extraction

The use of supercritical fluids as low polarity solvents for the extraction of metal ions as complexes has recently been reviewed [306,307] and this method has found particular interest in the nuclear industry [308].

In order to improve the extractability of some analytes, methods have been developed for their derivatisation in situ. These reactions, which have recently been reviewed by Field [309], include the methylation of 2,4-dichlorophenoxyacetic acid (2,4-D) with tetraalkylammonium salts [310], the methylation of acids with methyl iodide on an anion-exchange resin [311], and the transesterification of pyrethrins to methyl esters using methanol and acidic alumina [312].

6.3.7. Modelling the extraction process

A model to explain the kinetics of SFE was developed by Bartle and co-workers who described

the process as equivalent to the loss of heat from a hot sphere. This so-called “hot-ball model” [313,314] provided a good simulation of the kinetics of the extraction process and was subsequently refined to examine flat surfaces (infinite slab) and edges. They also examined the influence of the solubility of the analyte in the mobile phase [303]. This work provoked studies of the kinetics of extraction which confirmed the value of the model. The model can also be used to estimate the total amount of analyte even from an incomplete extraction.

6.4. Official and standard methods

One of the disappointments in SFE and SFC is their slow adoption as official methods by regulatory authorities. This is partly a reflection on a lack of demand from users who have frequently found that although the techniques are efficient, they are labour intensive and lack automation.

There was some interest in SFC as a regulatory method by AOAC [315]. The US Environmental Protection Agency (EPA) have recently adopted SFE as the official method for total petroleum hydrocarbons and PAHs from environmental matrices (EPA methods 3560 and 3561 SW-846 1995) [316] and has proposed a draft method for the extraction of

PCBs and organochlorine pesticides [317]. The ability to carry out group separations of hydrocarbons has resulted in an SFC method which has been adopted by the American Society for Testing and Materials (ASTM) for the aromatic and PNA content of diesel fuels and aviation turbine fuel [318]. The ASTM have also developed a standard guide for the purity of carbon dioxide used in supercritical fluid applications [319].

It is reported that there have been no problems in gaining acceptance by the regulatory authorities in the pharmaceutical industry for the inclusion of supercritical fluid separations as part of new drug submissions [320].

7. Related eluents

In any survey of SFE and SFC, a number of closely related techniques should be considered in which the solvent or eluent has enhanced properties but is employed between ambient conditions and the often extreme conditions of the supercritical state. One realisation from the study of the supercritical fluids is that they are not unique and there is a continuum of conditions and properties between the ambient state and the critical region. Supercritical fluids as a group should be regarded as a unifying point in chromatography. They bridge the divide between liquid and gas chromatographic mobile phases and their use emphasises that all partition separation methods are essentially the same. One set of rules can be applied and one theory can be used. The older divisions are simply a convenient classification for analytes working at moderate temperatures and near atmospheric pressures. This view was emphasised in a recent review by Chester on the mobile phase perspective of separation science [321]. He considered that the barriers between the different chromatographic techniques were “imaginary and artificial”.

7.1. Unified elution gas–fluid–liquid

One effect of the recognition of the absence of boundaries between phases was a series of studies in which a sample was eluted through a single column by a gas and then a supercritical fluid eluent [322] or

by the full range of a gas then fluid then liquid eluent [323,324]. Shen and Lee have also examined a mobile phase which changes from liquid to gas along the column [325]. A unified theory of chromatography has also been developed by Martire which covers all three techniques [141].

7.2. Pressurised solvent extraction

As an alternative to SFE with carbon dioxide or other supercritical fluids, it was proposed that heating organic solvents under pressure above their boiling point (but below their supercritical point) would enhance the speed of reaction and solvent strength. These pressurised solvent extractions (PSEs) were demonstrated [326] to be an easy method for extraction, reducing the amount of solvent required and speeding up the process.

The system was marketed as accelerated solvent extraction (ASE) and has led to a number of comparison studies with SFE and conventional extraction methods, including the extraction of environmental samples [327,328], drugs from rodent food [329] and additives from polymers [330]. Because PSE represented an extension of existing methods, it attracted attention and was rapidly adopted as an EPA method No. 3545 [331] for the pressurised fluid extraction of base/neutral compounds and acids, such as PNAs, chlorinated pesticides, herbicides, polychlorinated biphenyls and organophosphorus pesticides.

7.3. Superheated water extraction and chromatography

Although it was recognised that water could be used as a supercritical solvent [4] in practice it is too reactive and has been used for waste remediation and the destruction of toxic waste. However, on heating water under pressure, the polarity decreases markedly. First Hawthorne et al. [332] and then others demonstrated that superheated (frequently termed subcritical) water could be used as a solvent for the extraction of PCBs [333] and PAHs from soil samples [334,335]. It has since been used for Dacthral [336] and a range of analytes from different polarity matrices [337].

Superheated water can also be used as a mobile

phase for reversed-phase liquid chromatography [338,339] with both UV and fluorescence spectroscopic detection and universal FID [340,341]. The eluent can also be buffered [342] and the separation can be applied to a range of analytes including pharmaceuticals [343]. If the water is replaced by deuterium oxide then coupling to LC–NMR is enhanced as virtually no background signal is obtained [344].

7.4. Enhanced fluidity solvents

The low viscosity of carbon dioxide means that mixtures with organic solvents create eluents with properties between those of a liquid and a supercritical fluid. These enhanced fluidity solvents then take on many of the solvation properties of the organic solvent but are much less viscous and have lower diffusion rates, making them better extraction and chromatography solvents. These methods have been used for a number of studies including the extraction of phenolics from river sediment [345] and house dust [346] and have been compared with SFE and PSE for the extraction of coal [347]. The idea has also been extended to chromatography and mixtures of carbon dioxide and fluorocarbon has been used as an eluent for reversed-phase HPLC [348] and THF–carbon dioxide has been employed in size-exclusion chromatography [349].

8. Supercritical fluids in the next millennium

There is a future for supercritical fluids in analytical chemistry. Probably not the dominating role that was claimed at the start of SFC when it was suggested that it would displace GC and LC in a few years. It can still fill a viable niche in what can be seen as a continuum of separation eluents from gases to liquids. This continuum also exists across the temperature range and will include subcritical and superheated regions where the mobility and solvent power have been enhanced by temperature and pressure. Analysts should cease to focus on the critical point as defining a change in conditions but instead should take a broader global outlook. This means that they should keep an open mind on the eluents and conditions that can be used. PSE and

superheated water are both examples where the eluents have been known for years but altering the conditions caused a useful change in the solvation properties. Perhaps the time has now come to stop according supercritical fluids a unique status but to define them as solvents by their physical attributes of density and viscosity etc.

Supercritical fluids will also continue make an impact in other areas of chemistry as a solvent for unique reactions, as a clean solvent in industrial processes and as an operational medium for operations, such as spraying and micronisation. Many of these will be in the chemical engineering field and chemists need to keep a watch on developments that might be brought back to analytical chemistry in the future.

8.1. The future of supercritical fluid chromatography

After all the early claims and promises, SFC proved a partial or complete disappointment in many laboratories and it has never reached the level of acceptance that was envisaged in the early days. The reasons are clear from the earlier discussion, primarily it is a normal-phase method and cannot compete with the widespread dominance of reversed-phase HPLC in the pharmaceutical and fine chemicals fields. It also is operationally more difficult and little routine instrumentation is available.

However, carbon dioxide separations are generally superior to many existing normal-phase methods. Column/solvent equilibration is faster and eluent strengths can be easily adjusted by pressure and temperature changes. Although normal-phase separations in general are much less important than reversed-phase methods, there are also clearly a number of separations which favour this mode. The most important area is clearly that of chiral separations. This has continued to be an area where super- or often sub-critical chromatography can provide an enhanced separation to normal-phase eluent by increasing sample–stationary phase interactions.

The second major area has been in petrochemical separations. SFC is particularly suited to the separation of very high-molecular-mass analytes. These can be combined with simulated distillations (SIM-

DIST) for the characterisation of oil fractions containing alkanes with up to 100 or even 140 carbons. It also has an application in group analysis in which saturated and unsaturated hydrocarbons are resolved from different types of aromatic groups.

The ease of developing one-off SFC methods means that SFC–MS is still an attractive rapid technique for assays. The mass spectrometer providing both a universal detector and structural information. SFC–FT-IR appears not to have developed the same importance and SFC–NMR is still a fairly specialised system available in few laboratories.

8.2. Supercritical fluid extractions in the future

Supercritical fluid extraction has risen more slowly than SFC and in most cases the applications have been effective. It should have a strong role as an environmentally friendly technique taking over from the use of chlorinated solvents for the extraction of non-polar analytes. However, acceptance into routine laboratories has been limited as it faces a number of problems.

(i) Competition – relatively simple methods such as solid-phase extraction and now SPME have taken the market for many extractions from liquid samples such as metabolism studies. They can be automated and considerably reduce solvent usage.

(ii) Robustness – SFE extraction is prone to operational problems, and particular care must be taken to ensure the results are not matrix dependent and that sample recovery is complete. As noted earlier SFE has not yet matured.

(iii) Automation – the need in most laboratories is to reduce sample handling and thus save time and costs and improve reproducibility. Automated SFE is available at a price but is principally applicable to dry powered samples such as plant material or soils and cannot handle liquid samples.

It is likely that for the near future SFE methods will concentrate on the environmental area. It will also be prominent in the extraction of dried plant materials for drugs and phytochemical studies, often as a preparative or semi-preparative method, but these are largely research activities on limited numbers of samples. Its principal official capacity ap-

pears to be for the extraction of fats from food as part of nutritional studies.

9. Conclusions for the millennium

The use and role of supercritical (and subcritical) fluids were oversold in the past and some of the effort put into the method may have been misplaced. Although as part of study of the overall chromatographic concept, the investigations were worthwhile. The ready adoption of SFC was hampered for many years by problems with instrumentation, which could not deliver the sensitivity, precision or reproducibility required in industrial applications. Difficulties with back-pressure regulation, consistent flow-rates and modifier addition, sample injection, automation, and the short path in capillary flow cell were eventually overcome but by then the potential customers had been lost. It also seemed that in the early stages in the development of practical SFC, much effort was diverted into studies of the theory and modelling of retention and solubility, rather than finding out what could be actually reliably separated or extracted. Few robust applications were developed and the method went to the market place on speculations and promises which were not fulfilled. A more pragmatic view of the properties of SFC should have recognised the limitation of capillary methods at an earlier stage. Even though this technique provided the publicity to raise interest in SFs, it is now largely ignored.

As a concept supercritical fluids are now more prominent and this has led to their use in many other areas of chemistry as a unique solvent. Probably the most important idea that supercritical fluids have brought to separation science is a recognition that there is unity in the separation method and that a continuum exists from gases to liquids. The same basic principles apply from one end of the pressure/temperature spectrum to the other but that the operation of each individual separation method is governed by the physical properties (primarily the density, viscosity and diffusion rates) of the mobile phase or solvent that is used. These properties define the shape and size of the column and stationary phase and the types of analytes that can be separated. The physical chemistry of the mobile phases is

dominant and even the best claims of the marketing people cannot overcome the properties of the eluent.

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