Supercritical fluid extraction: a critical review of its analytical usefulness

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We examine the evolution of supercritical fluid extraction (SFE) since 1990 in order to pinpoint the reasons for its rare implementation by routine analytical laboratories despite its high analytical potential. We identify various reasons, and we propose ways to overcome the shortcomings behind them. We also discuss the great analytical potential of SFE and justify its use for routine work.

Keywords: Analytical supercritical fluid extraction

1. Introduction

The high solvent power of supercritical fluids (SFs) is becoming a major argument for laboratories engaged in innovative research to develop SFE methods for routine analyses. Thus, a number of laboratories have chosen to replace their conventional methodologies with new, SFE-based methodologies in order to minimize organic solvent consumption and boost throughput.

A wide variety of solvents is available for use as SFs, including carbon dioxide, nitrous oxide, ethane, propane, n-pentane, ammonia, fluorofom, sulphur hexafluoride and water. Carbon dioxide is currently the solvent of choice, as it can easily reach supercritical conditions and has clear advantages (e.g., low toxicity, inflammability and cost, and high purity) over other fluids. However, the use of carbon dioxide is restricted by its inadequate solvating power for highly polar analytes, which can, to some extent, be boosted by using an appropriate modifier.

SFE has consolidated in some areas, including environmental, pharmaceutical and polymer analysis; above all, however, it has found a major niche in food analysis. In the beginning, many thought the environmental industry would benefit most from SFE; nearly a decade later, and despite the introduction of several official SFE-based methods by the US Environmental Protection Agency (EPA), SFE has not had the impact that was anticipated initially. Thus, many analysts were soon frustrated by their SFE systems not living up to expectations and, as a result, a number of manufacturers of SFE equipment ceased production.

The interest in SFE methods can be charted in surveys of publications using SFE as a key term. In this work, we used papers abstracted by the Analytical Abstracts Database (Royal Society of Chemistry, UK) as the data source. Fig. 1(a) shows the number of papers published each year over the period 1990–2002 in 15 member states of the European Union (EU) (37% of all papers published in the world) and in the USA (33% of papers). As can be seen, the use of SFE rose rapidly in its early years, and the number of publications grew steadily from 1990 to 1995. However, this was immediately followed by a levelling off and, since 1997, by a decline in the number of publications.

Fig. 1(b) compares research endeavours in the EU and USA for the five-year period 1993–1997 with those for the 1998–2002 period. A considerable decline in productivity (by 39% in the USA and 6.5% in the EU) is clearly apparent. The average decrease for the world was 12.2%, which is consistent with the expected decrease in SFE-related publications.

While SFE continues to be used, it is now limited to a group of applications where it provides substantial advantages...
over alternative techniques. This article looks back on that period and analyses how SFE developed. In this respect, interesting information can be extracted from the review by Smith [1]. This reveals why many of the initial uses have vanished and why the initial promise was never fulfilled.

Based on Fig. 2, which shows the differential features of SFE in relation to other techniques that it can replace, we identify the origin of its great analytical potential and yet rare implementation and illustrate these points with examples from pertinent studies. Most reported studies comparing SFE with a conventional technique involve Soxhlet extraction (for solid samples), and extraction with a solid sorbent or distillation at a high temperature (for liquid samples).

2. Basic features of SFE favouring its analytical use

Ever since its commercial development in the early 1990s, SFE has attracted considerable attention as a sample-preparation procedure. Many analysts were quick to try the new technique, which gathered no less than 600 entries in Analytical Abstracts in the period 1990–1995. Below we discuss the most salient reasons for SFE being a major choice for sustainable chemistry.

2.1. Efficiency in sample preparation

Because SFE has several distinct physical properties, it is regarded as a promising alternative technique to conventional solvent extraction. Some of its major advantages are summarized as follows:

- Quality control of sample preparation
- Selectivity
- Accuracy/efficiency
- On-line integration of sample preparation and detection
- On-line detection
- On-line collection
- On-line screening control
- On-line separation

Main reasons for its scarce implementation in routine laboratories

- No universal method
- Extensive sample preparation
- Difficulty of extracting polar and ionic compounds
- Difficult extraction of wet or liquid samples and solutions
- Slow adoption as official method by regulatory authorities
- Erratic flow due to plugged restrictors

Figure 2. Comparison of SFE with other separation techniques.
(1) SFs manifest higher diffusion coefficients and lower viscosities than a liquid solvent. As a consequence, solubility and diffusivity in such fluids tends to be much higher than in liquids, resulting in comparatively fast reaction kinetics [2].

(2) In SFE, the solvation power of the fluid can be manipulated by changing pressure (P) and/or temperature (T); therefore, it may achieve a remarkably high selectivity. This tunable solvation power of SFs is particularly useful for the extraction of complex samples.

(3) In SFE, a fresh fluid is continuously forced to flow through the sample; therefore it can provide quantitative or complete extraction [3].

In addition to the advantages mentioned above, another distinct advantage of SFE over conventional methods is that SFE involves short extraction time and minimal usage of organic solvents. Some studies have shown that SFE for 30–60 min provides higher recoveries than several hours of Soxhlet extraction [4,5]. Thus, while Soxhlet extraction and SFE may extract similar amounts of analytes, the high collection efficiency of SFE results in much smaller losses of volatile components than the Soxhlet process. For example, Yang et al. [6] reported higher recoveries of the more volatile components of gasoline, including BTEX (benzene, toluene, ethylbenzene and xyylene) using SFE for 30 min than they obtained using Soxhlet extraction for 4 h. They concluded that the apparent higher efficiency obtained using SFE was a result of losses during the Soxhlet extraction (not poor extraction of BTEX from the soil samples by the Soxhlet process).

A number of authors have demonstrated that higher extraction efficiencies result simply from better collection efficiency by comparing optimised SFE methods and conventional liquid solvent extraction [7,8]. Examples of improved recoveries with SFE include: heavy petroleum hydrocarbons relative to ASTM Soxhlet extraction with Freon-13 [9]; SFE of aromatic amines from soil compared to Soxhlet extraction and sonication [10]; and, SFE of polyaromatic hydrocarbons (PAHs) from sediments and urban air particulates using a reactive solvent modifier [11]. The simplicity of SFE is clearly reflected in the extraction of atrazine, cyanazine, desethylatrazine and metolachlor from soil samples [12]. Supercritical CO2 extraction of an untreated soil sample for 15 min sufficed to achieve recoveries comparable to those provided by Soxhlet extraction after pre-treatment of the sample.

A good example for this type of comparison is provided by the extraction of polychlorinated biphenyls (PCBs) from sediment and mussel tissue standard reference materials (SRMs) [13]. These materials are ideal because they are well characterized, homogenous and widely available. The results obtained were comparable and SFE has several advantages over Soxhlet for PCB determinations, including reduced sample clean-up, reduced extraction time (50 min compared to 18–24 h) and reduced organic solvent usage (7.5 ml compared to 250 ml).

2.2. Wide scope of application
SFE has distinctive advantages for on-line fractionation, as it allows the extraction conditions to be fine-tuned with a view to improving specific extractions. Among other things, this allows one to separate extracted compounds into groups by adjusting operational parameters, such as the type and the proportion of liquid modifier or chelating agent, or by altering the pressure and/or temperature of the SF, for example.

Bauza et al. [14] developed a potential analytical-scale SFE method for the formation of diastereomeric salts and, depending on the solubility in the supercritical phase, the discrimination of the enantiomers of some carboxylic acids by using (R)-(+) or (S)-(−)-methylbenzylamine as the diastereomeric salt producer.

Palma et al. [15] used a two-step separation technique for the investigation of active phenol compounds in grape seeds. By using pure CO2, they obtained a fraction with strong antioxidant activity consisting mainly of fatty acids, aliphatic aldehydes and sterols; in the second step, they used CO2 modified with 20% ethanol to extract a more agrochemically active fraction containing mainly epicatechin and gallic acid.

2.3. Coupling SFE to integrate sample preparation and analytical determination
One of the greatest advantages of SFE over other sample-preparation techniques is that it can be automated; this makes it highly suitable for fast, routine analyses. The efficiency of the different SFE-collection models in on-line assemblies is very important, as it provides quantitative transfer of extracted analytes to the analytical instrument and reduces contamination levels. Four different ways of collecting SF-extracted analytes have been proposed, namely [16]:

(a) Solvent collection [17] in a vessel, such as that devised by Palma et al. [18] for adjustable flow control coupled to an H2O liquid trap for the extraction of glycosides from grapes.

(b) Solid-phase collection, which is accomplished by depressurising the SF at the inlet of a column packed with an inert material (e.g., stainless steel beads [19], a fused silica capillary [20]) or an adsorbing material, such as octadecylsilica (ODS) [21], diol and silica [22], silica gel [23], Florisil [24], Tenax [25] or alumina [26]. After the extraction has completed, the analytes are eluted from the solid-phase trap with a suitable solvent. One advantage of solid trapping is increased selectivity that can be further improved by
coupling a selective trap with a selective eluent. For example, polar compounds can be trapped on a silica gel column and subsequently eluted with appropriate solvents [27].

(c) Solid–liquid phase collection, which uses a solid-phase trap followed by a vessel containing a solvent [28]. This is well suited to highly volatile analytes; the losses of analytes from the solid-phase trap are collected in the vessel holding solvent [29]. Husers et al. [30] demonstrated that the solid–liquid trap can minimize the losses of PAHs observed with some liquid collection devices.

(d) Empty vessel trap collection [31] is done with one or several empty vessels and dispenses with the need to remove the solvent from the extracted components, which is time-consuming.

In comparison to any other collection alternative, solid-phase collection is widely used and offers high trapping efficiency for substances with high vapour pressures, since the trap temperature can be easily be reduced to −30 °C [32]. The recoveries of several PAHs were more than doubled when utilizing a cryocooled adsorbent trap compared to collection in pure dichloromethane [33]. Using solid-phase trap collection, it is also easier to obtain extracts ready for final analysis (2 ml extract volume) and to couple the trap on-line with analysis systems [34].

The on-line coupling of a SF extractor to an analytical detector provides several advantages, namely:

(a) Large amounts of extract can be passed through the instrument (virtually 100% can be transferred in a direct manner).
(b) Little sample manipulation is required, thus avoiding analyte losses.
(c) Increased throughput.
(d) Those samples requiring it can be protected from light and air.
(e) Substantially reduced amounts of solvent are used.
(f) The coupling allows the development of sample screening methods, thereby avoiding the need to chromatograph every single extract in routine analyses.

The design of the interface is one of the crucial aspects in developing a hyphenated technique involving the direct coupling of SFE to a destructive or non-destructive analytical detector. In developing such an interface, one should take into account the drastic operating conditions of the extraction process (viz. high pressures and temperatures, a SF with a high solvent strength or corrosive power) and the requirements of the detection technique to be used. Reported interfaces between a SF extractor and a molecular spectroscopic detector are either high-pressure devices (e.g., a fibre-optic-based flow cell, a windowed flow cell) or low-pressure flow injection devices (e.g., a flow-through sensor, a membrane phase separator). One of the most widely used interfaces for on-line detection with molecular spectroscopic techniques is the windowed high-pressure flow cell. Such a cell can be incorporated into a SF extractor for continuous monitoring of extracts between the extraction chamber and analyte trap sections, so that detection can be performed prior to depressurisation. The principal advantage of using these in situ detection systems is the elimination of the trapping process; this can help reduce analysis times and analyte losses through incomplete trapping and/or recovery, and avoid subsequent contamination of the experimental assembly. Also, this system provides a final extract that can be analysed using another detection technique.

Typical examples of on-line coupled systems include those used for the determination of PAHs in soil [35] and caffeine in coffee [36] by using a fluorimetric detector in the former case and a spectrophotometric one in the latter; the detector was inserted between the extraction chamber and the restrictor, so detection was done in the supercritical phase. In fact, measurements were made in the supercritical medium prior to depressurisation and analyte collection, even though the system also provided a final liquid extract for analysis.

2.4. SFE and sample-screening methods
The availability of fast, reliable screening methods is an important prerequisite for increasing the number of samples to be analysed when there is an urgent need for results. SFE has been recognized as an effective alternative to liquid-liquid extraction for the isolation of analytes from a variety of matrices. The combined liquid-liquid solvating capabilities and gas-like transport properties of SFs can provide efficient, fast extraction of analytes, thus simplifying the analytical process to a great extent.

Fat in skimmed milk, whole milk, cocoa and leather [37,38] was monitored in a screening system, samples being fully treated in a SF extractor. Fat in the milk samples was trapped on a C18 cartridge and automatically rinsed with hexane; fat in leather was trapped on stainless steel balls and eluted with a mixture of hexane and methylene chloride. The analytes were injected into a solvent stream in a simple flow injection (FI) module to measure a response in a piezoelectric detector. The limits of detection (LODs) thus achieved were 0.001% and 0.0007%. The repeatability, as relative standard deviation, was ±2.3% and ±3%, and the throughput 16 and 3 samples/h. The results were highly consistent with those obtained with using classical methods (Soxhlet-GC) for both types of sample, but the SFE method provided substantial advantages in terms of simplicity, automation, miniaturisation and speed in obtaining the yes/no binary response in the screening system.

A screening method for the determination of total polyphenols in grape marc by on-line coupled SFE and
spectrophotometry with a flow-through sensor was developed by Valcárcel and co-workers [39].

The use of these screening methods allows instruments with high purchase and maintenance costs (e.g., capillary electrophoresis or liquid chromatography equipment) to be reserved for processing only those samples for which the screening system has previously provided a reliable positive response.


3.1. Poor robustness of the early commercial equipment

Since its commercial development in the early 1990s, SFE has attracted considerable attention as a sample-preparation technique. As noted earlier, many analysts were quick to try it, but soon became frustrated with early SFE systems, which did not live up to their expectations. As a result, the number of SFE-equipment manufacturers soon declined. According to some experts, market forces have weeded out high-quality products from the undesirable, something they all agree was badly needed in the early days of SFE. Extremely poor systems were sold initially, and that hurt the field quite a bit. Improvements have been made on some of the original SFE systems so that they are suited to today's market; for the most part, however, the changes have not reflected new developments.

3.2. Lack of standard extraction procedures

SFE has had problems in catching on partly because of the lack of a universal method that works for all analytes and matrices. The SFE technique has never reached the stage where the analyst can place a sample at one end and get a result at the other; rather, it requires the operator to understand the extraction process and what goes on in between. Some routine laboratories just do not want to spend the time needed to learn how the technique works. Thus, given the wide diversity of real-world matrices, it would never be feasible for them to acquire the awareness of various extraction strategies that would be crucial for the future success of analytical SFE as a single universal strategy for all analytes.

3.3. Difficulties in extracting polar analytes

The SFE technique involves extensive sample preparation, although it does not take that much more sample preparation or method development than any of the competing methods – not even Soxhlet extraction. Although CO₂ is an excellent solvent for non-polar analytes, its most frequent limitation as an analytical extraction solvent is that its polarity is often too low to obtain efficient extractions, either because the analytes lack sufficient solubility or the extractant is poor at displacing the analytes from active matrix sites.

Most SFE applications use methanol as modifier, but, in some cases, other co-solvents, such as hexane, aniline, toluene and diethylamine, have been shown to be more efficient [40,41].

Cleland et al. [42] used 20% methanol-modified CO₂ to recover arsenic from dogfish muscle. Alcohol phenol ethoxylate, a non-ionic surfactant, was extracted from SFE disks only with the presence of modifier (10% of methanol), while the solvent strength of pure CO₂ was insufficient [43]. Despite the fact that the polarity of CO₂ can be raised by adding a modifier, this can detract from selectivity (i.e., more impurity compounds may be co-extracted with the target analyte and recovery reduced by effect of an increased amount of modifier in the collecting solvent decreasing the trapping efficiency).

3.4. Inefficiency in clean-up

One of the problems with SFE is that the resulting extracts are not always free from unwanted matrix components and thus require clean-up. For this purpose, a number of clean-up methods have been tested simultaneously with or after the extraction step. Interfering substances are often trapped with a sorbent material and, in some cases, the solvent composition is altered to increase recoveries.

One problem encountered in practice is that CO₂ is immiscible with water, but will dissolve it to a small extent; this makes the extraction of wet or liquid samples and solutions particularly difficult. Thus, extractions from most biological fluids (e.g., blood, urine and saliva) are precluded and applications to drug metabolism and toxicological studies limited. Such matrices can be more readily examined with solid-phase extraction (SPE) or solid-phase microextraction (SPME) methods [44].

One of the disappointments for SFE is its slow adoption as an official technique by regulatory authorities. This is partly a reflection of the lack of demand by users, who have frequently found that although the technique is efficient, it is also labour intensive and thus difficult to automate. Additional, less severe adverse connotations to be considered in adopting SFE include the problems caused by erratic flow caused by plugged restrictors [45], which lengthen process times, and the presence of volatile analytes, which require changes in the conditions for collection after depressurisation.

4. Trends in SFE

The saying “time is money” is increasingly endorsed in the laboratory environment. There has always been a need to control analytical costs in terms of solvent usage and disposal, consumable expenditure and administration overheads. A comparison of Soxhlet extraction, microwave-assisted extraction (MAE) and accelerated solvent extraction (ASE) with SFE reveals some interesting facts (see Table 1).
Thus, Soxhlet extraction is by far the most inexpensive technique in terms of investment costs; however, it is rather solvent- and time-consuming. Also, it requires thorough sample treatment.

MAE is an interesting alternative because of its medium investment costs and the possibility of performing multiple fast extractions with little solvent consumption; however, it invariably requires clean-up and involves long cool-down times for the extraction cells. Also, the choice of solvent may be somewhat limited as it should preferably be able to absorb microwaves.

ASE and SFE have the advantage that they constitute automated systems for unattended performance. Their main shortcoming is their very high investment cost and the need for a continuous service agreement with the instrument supplier as the instruments have several disposable parts that must be replaced on a routine basis – often by a local authorized dealer. The main advantage of ASE is that existing Soxhlet methods can, to a great extent, be converted into ASE methods with small changes. Also, the ASE technique is fairly simple to understand for people used to Soxhlet. Unfortunately, it requires external clean-up steps and uses more solvent than, e.g., SFE (which uses minimal amounts of – usually – CO₂).

Fig. 3 shows the features of ASE in various analytical fields. It can be seen that environmental analysis is the widest field of application for SFE; industrial, food and biomedical laboratory applications of this separation technique are also of high potential. The fact that these latter analytical fields have so far exploited SFE to a lesser extent is probably the result of resistance to innovation by conservatives workers who stick to solidly established methods and techniques and avoid potential complications arising from experimentation.

Carefully structured research showing the advantages of SFE over conventional extraction techniques and a critical comparison between them would probably foster usage of SFE to the extent that one would expect from its potential. Skilled personnel with a deep knowledge of the technique could aid demonstrating it in an easy, affordable way to novices, as could crash or advanced course, both of which would no doubt help to spread it. A number of official methods are bound to be replaced with SFE alternatives in the future because of the outstanding advantages of SFE.

We believe that SFE has come a long way, but much fine-tuning is still needed to develop more commercial instruments with improved extractor parts or performance in specific steps. As regards the SF, new, more polar supercritical phases can be expected to be developed to expand the scope of extracted analytes with compounds of a greater molecular weight and more ionic species. Also, mixed solvents are bound to facilitate the establishment of gradients, and ternary and quaternary mixtures of supercritical phases and/or modifiers can be expected to further expand the number of analytes amenable to SFE and to raise its selectivity. Efficiency and precision may be improved by using real-world samples, such as “in-house” matrix standards or SRMs with certified values for analytes of interest. Based

<p>| Table 1. Comparison of Soxhlet extraction, microwave-assisted extraction (MAE), accelerated solvent extraction (ASE) and supercritical fluid extraction (SFE) |</p>
<table>
<thead>
<tr>
<th>Factor</th>
<th>Soxhlet</th>
<th>MAE</th>
<th>ASE</th>
<th>SFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Investment</td>
<td>Small</td>
<td>Medium</td>
<td>Large</td>
<td>Large</td>
</tr>
<tr>
<td>Process time</td>
<td>Long (&gt; 48 h)</td>
<td>Short (&lt; 30 min)</td>
<td>Short (&lt; 30 min)</td>
<td>Large</td>
</tr>
<tr>
<td>Solvent consumption</td>
<td>High (200–500 ml)</td>
<td>Low (&lt;40 ml)</td>
<td>Medium (&lt;100 ml)</td>
<td>Minimal (&lt;0.5 ml)</td>
</tr>
<tr>
<td>Method development</td>
<td>Simple</td>
<td>Simple</td>
<td>Simple</td>
<td>Labour-intensive</td>
</tr>
<tr>
<td>Sample treatment</td>
<td>Required</td>
<td>Required</td>
<td>Required</td>
<td>Not required</td>
</tr>
</tbody>
</table>

![Figure 3. Use of SFE in different fields.](http://www.elsevier.com/locate/trac)
on results so far, “in-house” matrix standards can be effective alternatives to SRMs when these are unavailable. Such standards are made from a natural matrix containing the analyte, so they represent the true links that need to be broken to release the analyte from its natural environment.

Improvements in the extraction chamber should focus on three aspects, namely:

(a) Optimising extraction cell design in terms of cell closing and sealing in order to expedite operational changes to allow automation by robot. Cells affording sampling and sample treatment prior to extraction are also desirable.

(b) Improving the process that occurs within the cell in order to facilitate extraction (e.g., by including a derivatisation [46,47] reaction to raise or lower the polarity of the analytes). The use of alternative forms of energy (e.g., ultrasound) before or during extraction is also bound to facilitate and/or expedite the process.

(c) Developing new types of interface for connecting detection systems on-line with a view to overcoming the problems posed by existing choices and allowing new hyphenated techniques to be established in order to meet the new requirements arising from an expanded scope of extracted analytes. Another objective is the introduction of multicollection systems that would enable the automatic use of the different collection modes described earlier, depending on the properties of the particular sample and analytes.

Smart, systematic development of SFE can be expected to consolidate it into an advantageous alternative to conventional solid-liquid extraction, so that its real, great potential can be fully realized.

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