

Strategies for supercritical fluid extraction of polar and ionic compounds

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The methods used to facilitate the supercritical fluid extraction (SFE) of polar and ionic compounds are based on two general principles, either increasing the polarity of the supercritical fluid (SF) (usually CO₂) used as extractant or reducing the polarity of the analytes to be leached from the solid matrices. Changes in the pressure and temperature of the SF, the use of SFs more polar than CO₂ or of modified CO₂, ion pair formation, esterification, the use of and organometallics, or complex formation are the most common ways of increasing the extraction efficiency for polar and ionic analytes.

1. Introduction

The combined gas-like mass transfer and liquid-like solvating characteristics of supercritical fluids (SFs) first led analytical chemists to use them as chromatographic mobile phases [1], and later as fluids capable of removing species from solid samples. The latter use established the improperly named supercritical fluid extraction, which in fact consists of a leaching process because the samples are almost always solid, and solubilization of only one or several species is achieved in most cases.

Despite the fact that SFs have been considered to be 'super solvents', this is far from true, as seen by comparing their solvating powers with those of liquids. Nevertheless, several other features of SFs, i.e., higher solute diffusivities, lower viscosities, and solvent strengths that are easier to control than those of liquid solvents, as well as additional aspects such as their inertness, purity, non-toxic character and low cost (in most cases) have increased their use for sample pretreatment [2].

The majority of analytical supercritical fluid extractions (SFE) have focused on the use of supercritical CO₂ because of its reasonably critical

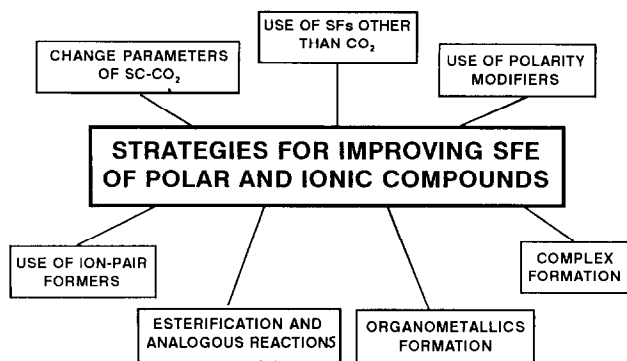


Fig. 1. Strategies for supercritical fluid extraction of polar and ionic compounds.

properties, low toxicity and chemical inertness. Although it is an excellent solvent for non-polar organics, the most frequent limitation of CO₂ 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 has a poor ability to displace the analytes from active matrix sites. Nevertheless, the properties of supercritical CO₂ in analytical SF have been exaggerated, mainly because of its excellent extraction of analytes spiked on inert supports. The far-from-real solid samples behaviour of these systems has led people to consider supercritical CO₂ as the ideal leacher in SFE. However, the matrix-analyte interactions dramatically reduce the extraction efficiency, which reduces more as the polarity of the analytes increases.

The implementation of special SFE strategies has been mandatory from the early days of the technique in order to overcome the drawbacks which hinder quantitative leaching of polar and ionic species. These strategies range from simple handling of the physical parameters of the extractant, such as the pressure and temperature, to complex derivatization steps, and include the manipulations summarized in Fig. 1 which are mainly aimed at raising the polarity of the extractant and reducing that of the extracted species.

2. Change of physical parameters of supercritical CO₂

At constant temperature, the extraction of non-polar analytes is favoured at low pressures, while

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the extraction of polar analytes requires increased pressure. This behaviour is used for class-selective extractions, an example of which is the sequential extraction of air particulates with supercritical CO₂. Alkanes are extracted at 75 atm (45°C), whereas PAHs remain unextracted until the pressure is raised to 300 atm [3].

Most of the CO₂-SFEs have been performed using mild temperatures (e.g. 50°C) while the pressure has been increased as the polarity of the target analytes was greater, in order to obviate the risks to thermolabile samples. However, Langenfeld et al. [4] have demonstrated that the influence of temperature is paramount on the extraction efficiency of polar and/or strongly retained compounds in environmental matrices such as sediments. The effect of temperature can be related to a reduction of kinetic limitations associated with overcoming the energy barrier of the desorption step, or to thermal alterations of the matrix.

3. Use of supercritical fluids other than CO₂

An alternative approach to improving SFE efficiencies is to choose pure fluids that are more polar and selective than CO₂ [5]. Unfortunately, the selection of other fluids is limited by the desire to have reasonably critical parameters, chemical inertness, low toxicity and cost, and low environmental impact. The use of supercritical N₂O has been proved to increase the extraction efficiency of high molecular weight PAHs and chlorinated dibenzo-*p*-dioxins from fly ash and sediment [5], but this extractant does not always improve extraction efficiencies [6] and can also be explosive in the presence of reactive organics. Other polar fluids such as CHClF₂ (Freon-22) have been shown to increase extraction efficiencies of nitrated and non-nitrated PAHs from diesel-exhaust particulate matter [7]. The CHClF₂-SFE of steroids has proved to be significantly better than that with supercritical CO₂ and the extraction time required was much shorter [8]. A comparison of the use of Freon-22, N₂O and CO₂ for the SFE of native pollutants including PCBs from standard reference material, PAHs from petroleum waste sludge, and PAHs from railroad-bed soil showed that CHClF₂ yielded the highest extraction efficiencies, most likely because of its high dipole moment. Trifluoromethane has also been assayed as an extraction solvent for sulphonylurea herbicides [9]. Nevertheless,

the environmental impact of these extractants dramatically detracts from their use. Supercritical methanol is also an excellent solvent but is less attractive because it is a liquid under ambient conditions, which complicates sample concentration after extraction [10]. Supercritical ammonia would also be an attractive solvent from a strength standpoint [11], but is difficult to pump because it tends to dissolve pump seals, is chemically reactive, and is likely to be too dangerous for routine use.

One of the most promising alternatives to the use of supercritical CO₂ is supercritical H₂O. Like CO₂, water is an environmentally acceptable solvent, but has not yet received attention as an analytical extraction solvent for environmental solids because, in contrast to the low polarity of supercritical CO₂, water is too polar to efficiently dissolve most non-ionic organics that are associated with environmental solids. At ambient temperature and pressure, water has a dielectric constant of ca. 80, but fortunately this parameter can be dramatically lowered to allow much milder and potentially useful conditions by simply raising the temperature at moderate pressures. Thus, by changing temperature a tremendous range in the solvent polarity of water can be achieved (e.g. changes in the dielectric constant from about 90 to 20 by raising the temperature from ambient to 300°C). In contrast the dielectric constant of supercritical CO₂ ranges only from about 1 to 1.6. Thus, the selective extraction from ionic to non-polar analytes could, in principle, be achieved by raising the temperature of water from sub- to supercritical values. This behaviour has been reported by Hawthorne et al. [12] in the class-selective extraction of polar organics, low-polarity organics, and non-polar organics, by sequentially increasing the extraction temperature from 50°C to 250°C (subcritical water) and finally to 400°C (supercritical H₂O at a pressure of 221 bar).

4. Polarity modifiers

Because of the practical difficulties involved in using polar fluids such as ammonia for SFE, the extraction of highly polar analytes has usually been carried out using CO₂ containing a few percent of added organic modifier [13], which when added to CO₂ increases its polarity. The modifier exerts its effect mainly in two basic ways: by interacting with the analyte/matrix complex to promote rapid

desorption into the supercritical fluid, and by enhancing the solubility properties of supercritical CO₂. Yang et al. demonstrated that the combination of modifier and high temperature is highly effective [14].

The modifier can either be added to the sample in the extraction cell prior to SFE or be mixed with the CO₂. The latter is more effective since the modifier is continuously passed through the sample, whereas in the former the modifier is swept from the extraction cell when the SF starts to circulate through the sample. A popular alternative to using two pumps has been the use of premixed fluid mixtures prepared by suppliers of high purity gases, which eliminates the complexity and expense of operating a second high-pressure pump in the SFE system. However, this practice is inadvisable because of the concentration shifts taking place during cylinder depletion [15].

Most SFE applications use methanol as modifier, but in some cases other cosolvents such as hexane, aniline, toluene or diethylamine have been shown to be more efficient [13,14]. The modifier is normally added in the range 1–10%, although Cleland et al. [16] used 20% methanol-modified CO₂ to recover arsenic from dogfish muscle. Paschke et al. [7] found increased recoveries of nitrated polycyclic aromatic hydrocarbons with CO₂ modified with CHClF₂ or toluene, while Johansson et al. [17] recovered organometallic compounds such as trimethyllead (96%), triethyllead (106%) and diethyllead (80%) from sediment samples and urban dust samples with CO₂ and methanol as a modifier added to the extraction cell. Alcohol phenol ethoxylate, a non-ionic surfactant, was extracted from SPE disks only with the presence of modifier (10% of methanol), while the solvent strength of pure CO₂ was insufficient [18].

5. Ion-pair formers

Neutralization of charged species by ion-pair formation represents another way of reducing the polarity of ionic compounds and thus increasing their solubility in supercritical CO₂ when polar-cosolvent addition is ineffective. Secondary alkylsulphonate and linear alkylbenzenesulphonate surfactants were quantitatively determined in sewage sludges by SFE–ion-pair formation, followed by injection-port derivatization GC, using tetrabutylammonium hydrogensulfate and supercritical CO₂ [19]. Another quaternary ammonium cation,

trimethylphenylammonium, has been used as counterion for the quantitative recovery of sulphonamides with CO₂ as extractant at 40°C and 281 bar [20]. Camphorsulfonate has also been used by Jiménez-Carmona et al. [21] as an ion-pair reagent in order to improve the extraction of clenbuterol hydrochloride from spiked food samples; the ammonium salt provided better results than the acidic form.

6. Esterification and analogous reactions

In situ chemical derivatization under supercritical conditions is another method used to increase the extraction efficiency of polar organics, as it promotes the conversion of the analyte polar groups (hydroxyl and carboxyl) into other less polar functions (ether, ester and silyl derivatives) which makes the derivative more readily soluble in supercritical CO₂. Moreover, it conditions the analytes for their subsequent chromatographic determination.

The on-line derivatization by using catalysts such as solid alumina [22] or immobilized enzymes [23] to carry out the transesterification of triglycerides to methyl esters cannot be included among the strategies aimed at improving the recovery, as prior analyte extraction is mandatory.

Hawthorne et al. [24] have proposed the derivatization and extraction under supercritical conditions of the acid herbicides (2,4-dichlorophenoxy)acetic acid and Dicamba from soil and sediment, microbial phospholipid fatty acids (as their methyl esters) from whole cells, and wastewater phenolics (as their methyl ethers) from water and from C₁₈ sorbent disks using reagents including trimethylphenylammonium hydroxide and boron trifluoride in methanol. Phenoxy-carboxylic acid has also been extracted in CO₂ after ester formation with pentafluorobenzyl bromide in acetone and K₂CO₃ [25]. Croft et al. [26] have used methyl iodide to methylate the same herbicides. They compared two different procedures for introducing the methyl iodide, and found that the addition by pipetting methyl iodide onto prepared samples was less demanding of reagents and equipment and gave more repeatable results than the method in which the derivatizing reagent was continuously supplied to the extraction cell. The latter reagent has been also used by Field and Monohan [27] for the in situ esterification–SFE of a non-polar hydrophobic pesticide (Dacthal) and its

mono- and dicarboxylic acid metabolites from soil and ground water, after SPE on strong anion exchange Empore disk samples.

In addition to increasing the SF solubility of polar compounds, the derivatization reagents may compete with analytes and displace them from the active sites of the matrix. In this way, the extraction of non-reactive substances can also be improved when the active sites of the matrix are targets of the derivatizing reagent. Hills et al. [28] demonstrated that the addition of a commercially available silylation reagent, hexamethyldisilane-trimethylchlorosilane, directly to the sample matrix prior to SFE of roasted coffee beans, roasted Japanese tea, and marine sediment, improved the extraction yield of both derivatized (as trimethylsilyl derivatives) and underivatized species. For PAHs, which are analytes which lack reactive groups, the SFE efficiency was also enhanced by addition of the same reagent [29].

7. Organometallics formation

Supercritical fluid extraction has proved to be a suitable tool for the speciation of organometallic compounds. Thus, Wai et al. [30] have demonstrated that methylmercuric chloride could be quantitatively extracted from a cellulose-based matrix by neat CO₂ when a small amount of water was used as a matrix modifier. Liu et al. [31] have reported the determination of organotin compounds in environmental samples by SFE and GC with atomic emission detection, and Oudsema and Poole [32,33] have also proposed the determination of organotins by on-line SFE-SFC with formic acid-modified CO₂ with flame ionization detection. The SFE behaviour of metals depends on the number of organic molecules forming the organometallics and has led to new ways of reducing the polarity of analytes by organometallic formation, as shown by Cai et al. [34] who developed a procedure for the simultaneous determination of tributyltin, diphenyltin and triphenyltin in sediment by in situ derivatization with ethylmagnesium bromide in the extraction cell. A similar procedure has also been used by these authors for the SFE of tributyltin and its degradation products from sea water via liquid-solid phase extraction [35]. Speciation of organic and inorganic arsenic after extraction from sand supports has been performed by GC after SFE-in situ derivatization with thioglycolic acid methyl ester [36].

8. Complex formation

Since the first reported extraction by Lainz et al. [37] of metals from aqueous solutions and silica by in situ chelation-SFE, the contributions in this area have made use of several types of ligands to implement strategies based on complex formation for the extraction of metals which has been followed in most cases by ion-pair formation.

8.1. Dithiocarbamate derivatives

These have been used by Wang and Marshall [38] in order to study the influence of the type of tetraalkylammonium dialkyldithiocarbamate on the recovery of metals from aqueous media. In this way they corroborated the hypothesis that the non-polar character of the ion pair formed depends primarily on the chain length of the alkyl substituent on the carbamate nitrogen, and to a lesser extent on the chain of the alkyl substituent on the ammonium counterion. These authors have recently used tetrabutylammonium dibutyldithiocarbamate for the characterization of cadmium, copper and zinc bound to metallothioneins previously isolated from rabbit liver by SFE with supercritical CO₂ and on-line detection by atomic absorption spectrometry [39]. The approach shows great promise as a speciation technique for protein-bound heavy metals. On the other hand, Liu et al. [40] have evaluated the off-line complexation-SFE of 13 organotin compounds from soil and sediments using diethylammonium diethyldithiocarbamate. The extracted material was then treated with pentylmagnesium bromide to convert the ionic organotin compounds into their neutral derivatives, which are amenable to gas chromatography with atomic emission detection. The outstanding results of this study are the better behaviour of both modified-CO₂ vs. neat CO₂, and high pressure (450 atm) vs. low pressure (250 atm). As was foreseeable, the recovery was higher for tetrasubstituted compounds and decreased when the number of organic substituents was reduced. This behaviour has been confirmed recently by Chau et al. [41].

8.2. Fluorinated dithiocarbamate

These derivatives yield metal complexes with solubilities two to three orders of magnitude greater than those of the ligands without fluorine, as claimed by Wai et al. in the extraction of inorganic

mercury from cellulose matrices [30]. This behaviour is also seen with other metals such as Zn, Cd, Co and Cu [42].

8.3. Fluorinated β -diketones

These have been used mainly for the extraction of lanthanides and actinides, as more effective ligands than fluorinated dithiocarbamates for complexation with *f*-block elements. The studies of Wai et al. [43] in this area demonstrated that the extraction of trivalent lanthanide- β -diketone complexes with neat supercritical CO₂ is incomplete. However, quantitative extraction is achieved when a small amount of water with 5% of methanol is used as matrix and solvent modifier, respectively [43]. In addition, a strong synergistic effect on the SFE of actinides [44,45] and lanthanides from both cellulose [46] and acidic solutions [47] was observed when a mixture of tributylphosphate (TBT) and fluorinated β -diketone was used. A logical explanation of this behaviour is the competition of TBT with the matrix for the unoccupied coordination sites of lanthanides and actinides. Thus, the formation of adducts with the complexes of these analytes with fluorinated β -diketones in supercritical CO₂ makes their removal from the solid matrix easier.

8.4. Ionizable crown ethers

The use of ionizable crown ethers as complexing agents for mercury is one of the most recent examples of the complex-formation strategy. The solubility of *tert*-butyl-substituted dibenzobistriazolo crown ether in methanol-modified CO₂ has permitted its use for the quantitative extraction of Hg²⁺ from sand, cellulose filter paper, and liquid samples using mild SFE conditions (60°C and 200 atm). Other divalent metal ions, including Cd²⁺, Co²⁺, Mn²⁺, Ni²⁺, Pb²⁺ and Zn²⁺ are virtually unextractable under these conditions [48].

9. Final remarks

The establishment of methods which generate minimal solvent waste and reduce the exposure of laboratory personnel to toxic gases, i.e., the development of clean methods, is a major goal of analytical chemistry at present.

Supercritical fluid extraction is one of the most promising ways for the implementation of such methods. At present, one of the shortcomings of SFE is its low capability for leaching polar or ionic analytes, although this problem can be partially overcome by using the strategies reported here.

Some of the strategies commented on above, such as in situ derivatization, chelation or ion-pairing SFE have only been evaluated for spiked inert supports or samples in which binding phenomena (e.g. covalently bonded drugs to proteins or carbohydrates) or preferential reactivity of the reagent towards other matrix components do not occur. In some cases, recoveries are not reported [28], or they were reduced by more than 30% [34] when a real sample or certified material was processed. Only a few of the papers cited here [16,44] have used real samples or certified materials in order to demonstrate the success of SFE for the removal of polar or ionic compounds. Simple systems, such as spiked supports, may induce an overestimation of the SFE performance. In this context, the incipient use of supercritical H₂O could be a challenge as it constitutes the ideal solvent for the development of clean methods. In principle, the main drawbacks of supercritical H₂O are its high temperature and pressure requirements, which make its routine use for sample preparation unlikely from a practical standpoint. Its use for analytical applications is further reduced by its reactive nature, both in terms of analyte degradation and corrosivity, and the liquid state of water under ambient conditions complicates concentration after extraction. However, these drawbacks could be partially or totally overcome by the following facts.

- Only non-polar compounds require the use of the supercritical state of this extractant, because of its lower dielectric constant at increased temperatures, while conditions which are not particularly drastic are mandatory for polar and ionic analytes.
- The use of sorption columns located after the restrictor could efficiently retain and concentrate the extracted target analytes, with subsequent elution using the most appropriate type and volume of eluent.

In the above ways, the complementary use of supercritical CO₂ and sub- and supercritical H₂O, or even their mixtures together with the in situ application of focused microwaves, would enable the development of solid sample preparation methods which will cover the whole range of polarities of the target compounds to be extracted.

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