Supercritical fluid extraction: a critical report of its analytical usefulness

Miguel Valcárcel[*], Mohammed Zougagh[a] and Angel Ríos[a]

Department of Analytical Chemistry, University of Córdoba, Campus de Rabanales, Edificio Marie Curie, E-14071 Córdoba, Spain. E-mail: qa1meobj@uco.es, Tel. +34 957 218616

[a]Present address: Department of Analytical Chemistry and Food Technology, University of Castilla–La Mancha, Campus de Ciudad Real, E-13004 Ciudad Real, Spain

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.

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. 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 shows the number of papers published each year over the period 1990–2005 in the world. 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. 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.

I. 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.

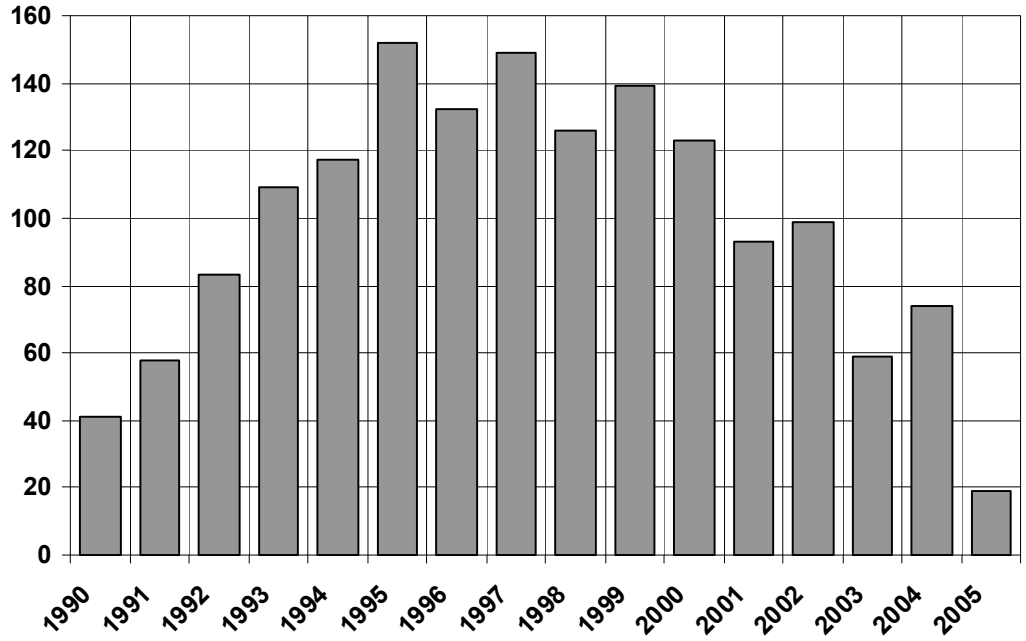

Figure 1. Number of SFE publications from the world, 1990-2005

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:

(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 [1].
(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 [2].

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 [3,4]. 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.

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. [5] developed a potential analytical-scale SFE method for the formation of diasteromeric 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 diasteromeric salt producer.

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 [6]:

(a) Solvent collection [7] in a vessel, such as that devised by Palma et al. [8] 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, a fused silica capillary) or an adsorbing material, such as octadecylsilica (ODS), diol and silica, silica gel, Florisil, Tenax or alumina. 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 [9].
(c) Solid–liquid phase collection, which uses a solid phase trap followed by a vessel containing a solvent [10]. This is well suited to highly volatile analytes; the losses of analytes from the solid-phase trap are collected in the vessel holding solvent [11]. Husers et al. [12] demonstrated that the solid–liquid trap can minimize the losses of PAHs observed with some liquid collection devices.
(d) Empty vessel trap collection [13] 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.

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.

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. A screening systems for the determination of total polyphenols in grape marc and PAHs in sediments and olive oil samples by on-line coupled SFE and spectrophotometry or fluorescence were developed by our research group [14-16]. The use of these screening systems 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.

II. MAIN REASONS FOR THE DECLINE IN THE USE OF SFE FOR ROUTINE ANALYSES (1992–2005)

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 to 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.

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.

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 [17,18]. Cleland et al. [19] used 20% methanol-modified CO2 to recover arsenic from dogfish muscle. 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 [20]. 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).

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 [21]. 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 [22], which lengthen process times, and the presence of volatile analytes, which require changes in the conditions for collection after depressurisation.

III. TRENDS IN SFE

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 realworld samples, such as ''in-house'' matrix standards or SRMs with certified values for analytes of interest. Based 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 [23,24] 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.

REFRENCE

[1] Mira, B., Blasco, M., Subirats, S., J. Supercrit. Fluids. Vol.14, **1999**, p.95.
[2] Stashenko, E.E., Puertas, M.A., Combariza, M.Y., J. Chromatogr. A Vol.752, **1996**, p.631.
[3] Reindl, S., Hofler, F., Anal. Chem. Vol.66, **1994**, p.1808.
[4] Lee, H.B., Peart, T.E., Hong-you, R.L., J. Chromatogr. A. Vol.636, **1993**, p.263.
[5] Bauza, R., Ríos, A., Valcárcel, M., Anal. Chim. Acta. Vol.386, **1999**, p.234.
[6] Yang, Y., Hawthorne, S.B., Miller, D.J., J. Chromatogr. A. Vol.699, **1995**, p.26.
[7] Thompson, P.G., Taylor, L.T., Richter, B.E., Porter, N.L., Ezzell, J.L., J. High Res. Chromatogr. Vol.16, **1993**, p.713.
[8] Palma, M., Taylor, L.T., Zoecklein, B.W., Douglas, L.S., J. Agric. Food. Chem. Vol.48, **2000**, p.775.
[9] Smith, R.M., Burford, M.D., J. Chromatogr. A. Vol.600, **1992**, p.175.
[10] Husers, N., Kleibohmer, W., J. Chromatogr. A. Vol.697, **1995**, p.107.

[11] Eckard, P.R., Taylor, L.T., J. High Res. Chromatogr. Vol.19, **1996**, p.117.
[12] Husers, N., Kleibohmer, W., J. Chromatogr. A, Vol.697, **1995**, p.107.
[13] Miller, D.J., Hawthorne, S.B., McNally, M.E.P., Anal. Chem. Vol.65, **1993**, p.1038.
[14] L. Arce, A.G. Lista, A. Rıos, M. Valcárcel, Anal. Lett. Vol.34, **2001**, p.1461.
[15] M. Zougagh, A. Ríos, M. Valcárcel, Anal. Chim. Acta. Vol. 524, **2004**, p.279.
[16] M. Zougagh, A. Ríos, M. Valcárcel, Anal. Chim. Acta Vol. 525, **2004**, p.265.
[17] J.J. Langenfeld, S.B. Hawthorne, D.J. Miller, J. Pawliszyn, Anal. Chem. Vol.66, **1994,** p.909.
[18] Y. Yang, A. Gharaibeh, S.B. Hawthorne, D.J. Miller, Anal. Chem. Vol.67, **1995**, p.641.
[19] Cleland, S.L., Olson, L.K., Caruso, J.A., Carey, J.M., J. Anal. Atom. Spectrom. Vol.9, **1994**, p.975.
[20] Kane, M., Dean, J.R., Hitchen, S.M., Dowle, C.J., Tranter, R.L., The Analyst 120, **1995**, p.355.
[21] Pawliszyn, J., Solid Phase Microextraction. Theory and Practice, Wiley–VCH, New York, **1997**.
[22] Luque de Castro, D., Valcarcel, M., Tena, M.T., Analytical Supercritical Fluid Extraction, Springer, Berlin, **1994**.
[23] Field, J.A., J. Chromatogr. A. Vol.785, **1997**, p.239.
[24] Bauza, R., Ríos, A., Valcarcel, M., Anal. Chim. Acta Vol.450, **2001**, p.1.