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Review

Collection in analytical-scale supercritical fluid extraction

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Abstract

This review is a comprehensive summary of available collection techniques in supercritical fluid extraction (SFE), with emphasis on which parameters are especially important for a successful analyte collection. Environmental, biological and agricultural applications, including several types of sample matrices and analyte groups, are discussed with respect to choice of collection mode and optimization of collection conditions. This review also includes discussions about collection when a modifier is used or when the sample contains large amounts of fat or water, as well as possibilities to achieve enhanced selectivity.

Keywords: Reviews; Collection techniques, SFE; Trapping; Supercritical fluid extraction

Contents

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1. Introduction

Analytical-scale supercritical fluid extraction (SFE) is a well-recognized alternative to conventional solvent-based extraction techniques. SFE has the main advantages of being environmentally benign and available as fully automated instruments. Therefore, with the objective to minimize organic solvent consumption and increase sample throughput, many analytical routine laboratories chose to replace their conventional methodologies with new ones based on SFE.

SFE comprises two integrated parts: extraction of the analytes from the sample matrix and subsequent collection (trapping) of the analytes. The collection can be achieved either on-line into a chromatographic instrument such as GC or SFC, or off-line by depressurizing the supercritical fluid (SF) into a collection device. The collection device can be an empty vessel, a vessel containing a small volume of organic solvent, a solid-phase trap, or a cryogenically cooled capillary. Hence, there are many possibilities for achieving collection in SFE, and they all have their advantages and disadvantages, as well as different parameters to optimize.

The importance of a proper collection of analytes in SFE has been emphasized lately [1–5]. It was discovered that faulty collection rather than non-quantitative extraction could explain many of the reported low extraction recoveries. There are several recent research papers describing effects of varying different collection parameters on the extraction recovery of analytes from both spiked inert materials and real samples. All this information, regarding different modes of collection, types of analytes and variety of applications, has not yet been collected in a review article. However, there are publications in the form of books [6–10], book chapters [11–13] and review papers [14–16] treating several aspects on SFE, also including some aspects on collection in SFE.

This review article is entirely focused on collection in analytical-scale SFE employing carbon dioxide as extraction fluid. Collection and fractionation in process-scale SFE will not be covered here, since that topic has been well described by others [17,18]. Instead, this article will give a brief background on theory of different collection-modes in analytical-scale SFE, discuss important parameters and demonstrate how they should be optimized, review representative applications and point out recent trends and developments within this topic.

2. Collection modes

In this study, the collection modes have been grouped into four major classes: (i) solvent collection, in which collection is achieved in a vessel containing solvent; (ii) solid-phase collection, which includes collection on a column packed with a solid material that is adsorbing or inert; (iii) on-line collection, in which the collection device is connected to a chromatograph; and (iv) alternative collection, which comprises collection in empty vessels, inside fused-silica capillaries and on combined solid-phase–solvent traps. Brief theory on each collection mode will be given below, followed by descriptions of the most important parameters that should be optimized for a successful collection of different types of analytes.

2.1. Solvent collection

Collection in a solvent is most commonly achieved by keeping the restrictor outlet immersed into a vessel containing a small volume of an organic solvent, such as methanol, hexane or acetone. There

are several parameters to consider, including solvent type, solvent volume, solvent temperature, restrictor flow-rate, restrictor temperature and pressurization of the collection vessel. Fig. 1 demonstrates the different steps of analyte collection employing a linear restrictor dipped into a vessel containing solvent.

As shown in Fig. 1, the analyte undergoes four major steps during the collection process: (1) exit from the restrictor; (2) diffusion through the gas bubble to the gas–liquid interface; (3) solvation into the liquid solvent phase; and (4) maintained stability in the solvent.

The first step implies that the analyte should not adsorb to the inside of the restrictor, or by any other means reside inside the restrictor. Ideally, the entire pressure drop is at the outer tip of the restrictor, which means that solutes should have full solubility in the SF all the way out to the tip. This is nearly true for most modern instruments, which are equipped with automatic variable restrictors. Moreover, a uniform heating of the entire restrictor minimizes problems with restrictor plugging by extracted components [19] or ice [1]. For example, Langenfeld et al. used a heat gun to hinder ice formation in the restrictor [1]. Unfortunately, this crude heating technique caused analyte losses for lower-molecular-mass PAHs. This could be overcome by using a temperature-controlled heating block set at 5 °C, which resulted in analyte collection efficiencies of over 95% for all PAHs in methylene chloride.

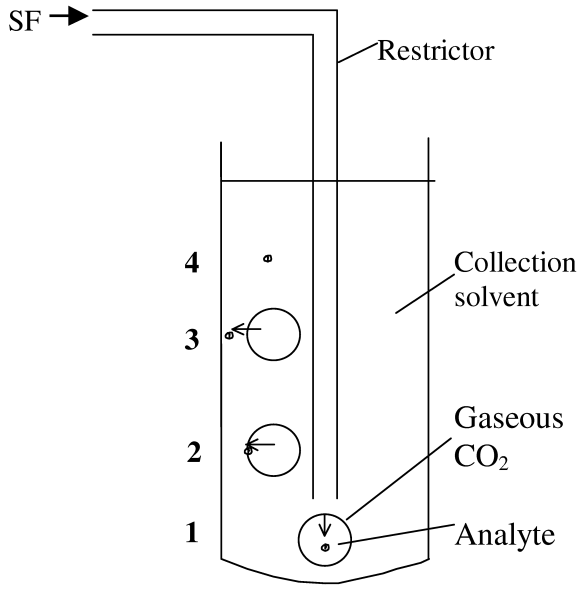

Fig. 1. Schematic of solvent collection, showing the four main steps of the collection procedure: (1) exit; (2) diffusion; (3) solvation; and (4) maintained stability.

The second step is controlled by the diffusion constant of the analyte in the gas phase. Smaller bubble sizes results in shorter average diffusion paths, which means that the analytes will reach the gas–liquid interface faster. Smaller bubble sizes can be achieved by applying a lower restrictor flow-rate [20] or by using a solvent of higher viscosity [19]. In addition, a higher solvent viscosity results in longer rising time of the bubbles, which gives the analytes a longer time to reach the gas–liquid interface [19]. The same effect can be obtained by using a higher column of solvent in the vessel [1,19]. For example, Bøwadt et al. demonstrated that the average recoveries for six PAHs were increased from 48 to 75% when 10 ml of solvent was used instead of 4 ml [21]. However, Thompson et al. [22] and Stone and Taylor [23] did not find any correlation between solvent viscosity and collection efficiency, which implies that this parameter is not always critical.

The third step, solvation of the analytes into the solvent, is naturally mostly determined by the solvent strength of the collection solvent. A good match of the solubility parameters of solvent and target analytes is an important parameter in analyte collection, which also has been demonstrated using simple thermodynamic models [24]. The solvent should well dissolve both analytes and coextracted materials, to avoid losses in the form of aerosol [25]. A slightly higher solvent temperature may improve the solubility of some compounds [20]. However, a lower temperature is usually preferred since this results in lower vapor pressure of the analytes [19], which improves the collection of especially the more volatile analytes. A slight pressurization of the collection vessel also improves the collection efficiency of volatiles significantly [23], which in addition minimizes evaporation of the solvent and formation of aerosols. A condenser placed on top of the collection vessel has also demonstrated reduced purge losses of volatiles and aerosols during extraction [26]. With such as set-up 16 PAHs were quantitatively collected in ethyl acetate including to

naphthalene (96% recovery) together with five nitro-PAHs including nitrobenzene (99% recovery).

In the fourth part of analyte collection the collected analytes should be maintained in the collection vial during the whole extraction procedure until the samples are taken for further analysis. A lower collection temperature is normally preferable. Bøwadt et al. showed that decreasing the restrictor temperature from 100 to 80 °C for the collection of PAHs in 10 ml of acetone improved the average recovery from 75 to 100% [21]. Moreover, the restrictor temperature should not be too hot, as this may cause degradation of thermally labile compounds [20,21].

Compared to solid-phase collection (see Section 2.2), solvent collection is less prone to analyte breakthrough losses when the sample contains large amounts of fat or water, or when a modifier is used. However, volatile analytes are more difficult to trap in a solvent than on an adsorbing solid-phase trap. Compared to on-line collection (see Section 2.3), larger samples can be processed employing solvent collection. However, the sensitivity is lower due to the obvious dilution effect. Hence, samples containing large amounts of fat or water, such as many types of food products, oilseeds, fresh plants, fruits and vegetables, water samples and animal tissues, are beneficially collected in SFE employing solvent collection.

2.2. Solid-phase collection

In solid-phase collection, the decompressed extraction fluid passes a device filled with a sorbent material [e.g., chromatographic material such as octadecylsilica (ODS), diol, silica, Florisil] or an inert material (e.g., stainless steel beads) [27–31]. After completed extraction, the analytes are eluted from the solid-phase trap with a suitable solvent. If the SFE unit is not equipped with a solid-phase trap device, a solid-phase extraction (SPE) cartridge can be utilized [32,33]. A schematic picture of a solid-phase trap is shown in Fig. 2.

The most common way to investigate the solid-phase trapping efficiency during the method development is to extract a known amount of analytes from an inert material placed in the extraction cell [34–36]. Another approach is to apply the analytes directly at the inlet of the solid-phase trap and

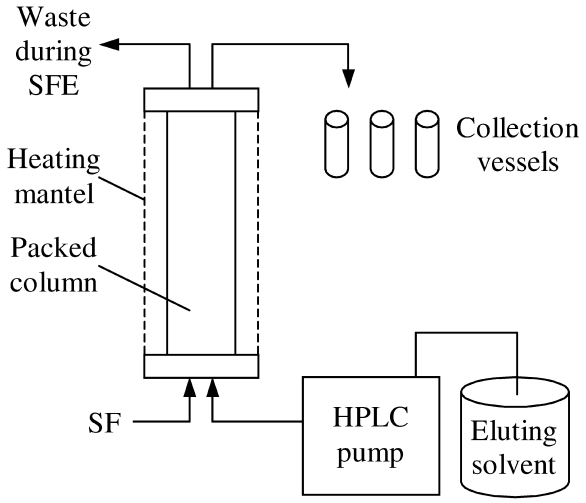

Fig. 2. Schematic of a solid-phase trap.

perform an extraction with subsequent fractionated elution of the trap [37]. Additionally, the trap can be connected to a LC system in order to investigate the elution profiles [38,39]. Standard solutions are conveniently injected with the LC injector and the elution solvent is pumped with the LC pump. Fractions can then be collected for further analysis or directly detected with a detector.

The choice of trapping material combined with the elution solvent or solvent mixture is of great concern in solid-phase collection. The effect of different trapping materials have been investigated for a number of applications such as extractions of pesticides from vegetables [40] and soil [41] as well as PCBs from sewage sludge [42]. In some cases, a combination of different packing materials can be successful [38]. The capacity of the trapping material may become a problem, resulting in breakthrough losses of analytes [34]. Even if the target analytes do not exceed the limit of the trap, losses may occur caused by overloading the trapping material with co-extracted matrix components, such as fat. Another problem with co-extracted material is deactivation of the trap packing material and the need of further clean-up steps of the extracts.

Eckard and Taylor studied the capacity of different types of trap materials as mixtures of ODS–glass beads and Porapak Q–glass beads for the extraction of a polar test mixture [29]. With a total sample

amount of 100 mg, ca. 65% was trapped using a trap filled with 0.5 g ODS and 100% using a trap filled with 0.4 g Porapak Q. These results indicate the higher sample capacity for this type of material compared to ODS. However, one way of avoiding capacity problems is to perform a fractionated extraction/elution procedure, i.e., to rinse the trap at certain time intervals during the extraction [34].

Concerning the choice of rinse solvent, the elution characteristic of the trap such as elution volume and recovery has to be investigated [37,38]. It is usually favorable to minimize the volume needed for total recovery. In addition, the choice of solvent in relation to the final analysis must be considered.

There is a major drawback when using solid-phase trapping together with water-containing samples or when high concentrations of modifier are added to the extraction fluid. At these conditions condensation of water or modifier in the trap may cause breakthrough losses of analytes and consequently lower recoveries. A common solution is to keep the trapping temperature above the boiling point of the modifier. For example, extractions of a model fat sample using 15% of methanol as modifier and ODS as trapping material have shown that by increasing the trap temperature from 80 to 90 °C the trapping efficiency increased from ca. 33 to 95% [34]. However, a high trap temperature may cause degradation of thermolabile analytes. Moreover, the analytes of interest may not trap effectively at the high temperature required to vaporize the modifier, leading to analyte breakthrough losses [4,27]. In general, a modifier with a high vapor pressure is preferable, since it makes it possible to keep the trap at a relatively low temperature even at a relatively high modifier concentration. In contrast, when trapping onto inert materials as stainless steel beads, the modifier may coat the beads and act as a trapping phase with increased recoveries as a result [43].

Selectivity in SFE is an expanding research area. Most work has been focused on the extraction step, i.e., the outlet-side of the extraction cell has been filled with an adsorbent, which retains matrix components (e.g., fat components) extracted from the sample. The most commonly used fat retainer is basic alumina, but several others such as silica and Florisil have been utilized. For example, selective extractions of PCBs from a model fat sample, containing PCBs, triglycerides and phospholipids were obtained by placing the sample upstream a layer of basic alumina in the extraction cell [44,45]. The same principle is applicable in the solid-phase trap, i.e., by a proper combination of packing material and rinsing solvent, selectivity can be introduced in the collection step. Studies have shown improved selectivity and reduced elution volume by using a mixture of activated carbon with ODS as trapping material and hexane–methylene chloride or toluene as rinsing solvent [38]. In this case toxic planar fractions of environmental pollutants (e.g., PCDDs, PCDFs and PCBs) could be fractionated from non-planar fractions (e.g., ''bulk'' PCBs and pesticides). In another work, a long trap (ca. 4 ml) was compared with a standard trap (ca. 1 ml) filled with ODS (Hewlett-Packard 7680T system), demonstrating that fat-soluble vitamins could be selectively eluted from co-extracted fat components [39]. Fig. 3A and B shows the elution profiles for the long and the short trap, respectively. In the same work, it was also shown that by employing different packing materials (ODS, cyanopropyl silica and aminopropyl silica were investigated) in the long trap, different elution profiles with varying selectivity could be obtained for aromatic amines.

Solid-phase collection can also be performed ''in-line'', which has been described by several research groups [46–48]. The trapping material is then placed after the sample and prior to the restrictor, and selectivity can be introduced as in trapping under depressurized conditions.

In comparison to solvent collection, the solid-phase trapping technique offers high trapping efficiency for substances with high vapor pressures since the trap temperature can easily be reduced to −30 °C [42]. The recoveries of several PAHs were more than doubled when utilizing a cryocooled adsorbent trap compared to collection in pure dichloromethane [49]. Using solid-phase collection it is also easier to obtain extracts ready for final analysis (ca. 2 ml extract volume) and to couple the trap on-line with chromatographic analysis systems [50,51].

2.3. On-line collection

SFE has been coupled on-line to chromatographic techniques such as SFC, GC and LC, of which SFC naturally provides the highest compatibility and

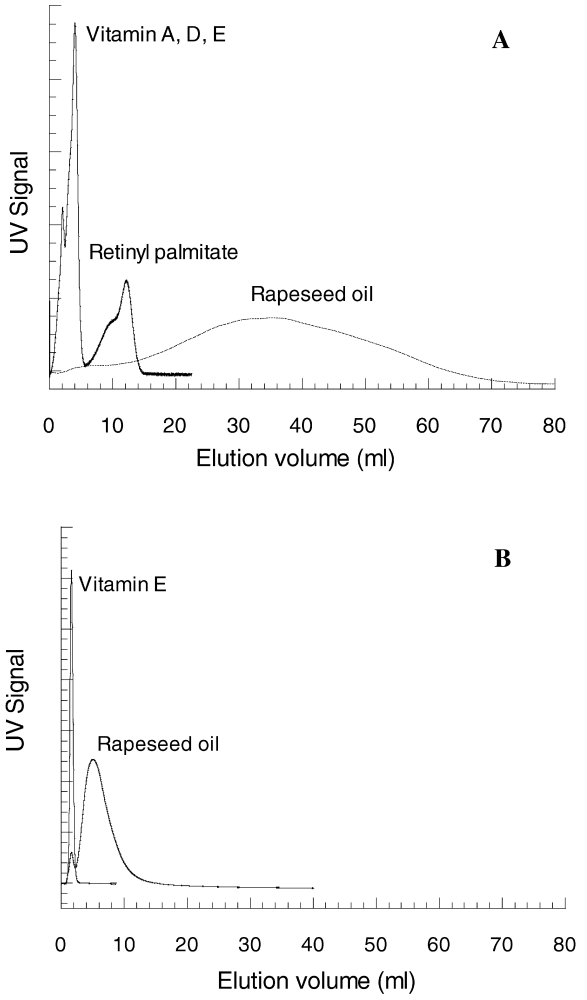

Fig. 3. Elution of fat-soluble vitamins and rapeseed oil from a long trap (A), and a standard Hewlett-Packard trap (B) (from Ref. [39]).

thereby the simplest solutions for interfaces. A collection device interfaced to a chromatographic system has two major requirements: quantitative trapping of the analytes and quantitative transfer of the analytes as a narrow band to the chromatographic system. This can be achieved by trapping the analytes on a cryogenically cooled surface, which may be adsorbing or inert, followed by thermal or chemical desorption of the analytes from the trap and transfer to the chromatographic column. The collection device can be a small piece of fused-silica capillary (uncoated or coated with a stationary phase) or a small column filled with an inert or adsorbing material. Alternatively, the collection can be achieved directly at the head of the analytical column, if packed SFC or HPLC is employed, or directly into the injector of a capillary GC system.

In SFE–capillary SFC, the most commonly employed interface for trapping the analytes during the extraction is inside a cryogenically cooled capillary [50,52,53]. This is typically an uncoated fused-silica capillary of inner dimensions around 10 cm×0.2 mm, which is directly coupled to the outlet of the restrictor. A schematic diagram of a cryogenic on-line trap is shown in Fig. 4.

It is important to cool the capillary to temperatures of −40 to −50 °C in order to obtain quantitative collection of even the most volatile analytes [52]. However, since the SFE restrictor is connected to the trapping capillary, a too low temperature may cause plugging of the restrictor. Daimon and Hirata found that collecting temperatures of below −20 °C may cause the SC-CO_2 to liquefy, which makes the capillary less efficient for trapping the solutes [50]. In addition, small amounts of solvent may also be trapped in the collection capillary, which produces a large solvent front peak in the separation system [52]. However, this problem can be avoided by purging the extraction/collection system with nitrogen before and after each extraction [52], or by using a solvent of high vapor pressure, such as methylene chloride [54].

An alternative to the uncoated capillary described above is a capillary coated with a chemically bonded stationary phase. Such a capillary enables more efficient trapping of some analytes, and the self-cooling effect of the expanding SC-CO_2 has shown to be sufficient for quantitative collection of non-volatile analytes [50].

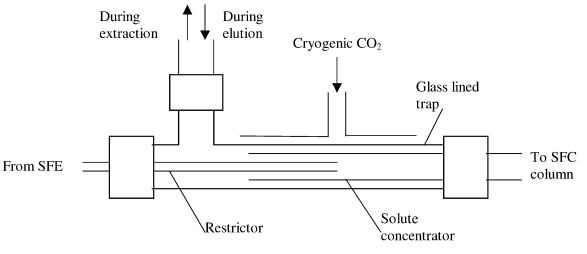

Fig. 4. Schematic of a SFE–capillary SFC interface (from Ref. [52]).

In SFE–packed column SFC, the collection is commonly achieved on a small separate trapping column [54–56], or directly at the head of the analytical column [57,58]. Analyte collection at the beginning of a solid-phase trap packed with an adsorbing material, as described above (see Section 2.2), offers efficient collection of the analytes. However, desorption to the chromatographic column is more difficult, depending on how strongly the analytes are retained to the trap. Analyte desorption is usually achieved by increasing the trap temperature to around 200 °C [54,56], or by adding large amounts of modifier to the SF mobile phase [57,59].

Parameters that need to be carefully optimized, with regards to both collection and desorption, include column packing material, temperature, type of modifier and concentration, extraction time and flow-rate. The column packing material must strongly retain the analytes, even if a modifier needs to be used during the extraction. If the trapping column also is the separation column, the same packing material must release the analytes and provide sufficient separation efficiency in order to enable quantification of the target analytes. Suto et al. demonstrated that magnolol and honokiol could be efficiently retained on an NH_2 column in an SFE–packed column SFC system with SC-CO_2–methanol (95:5, v/v) as extraction fluid, and then desorbed and separated using SC-CO_2–methanol (85:15, v/v) as carrier fluid [57].

In SFE–GC, inert materials are generally employed for collection of analytes, e.g., a trap filled with glass beads [60], using the same principles for collection and desorption as described above for fused-silica capillaries. It is of utmost importance to properly cool the trapping material, since the collection solely relies on cryogenic cooling without any significant adsorbing interactions. In general, there is a pertinent risk of analyte breakthrough losses during the extraction if a too high flow-rate is applied [60] or if a modifier is used [51].

An alternative mode of collection in SFE–GC is to collect the analytes directly in the GC injector [61]. Lou et al. showed that PAHs could be quantitatively trapped without discrimination in a heated split/splitless injector connected to a column of 50 °C when modified SC-CO_2 was used for extraction [61]. In the same study, it was also demonstrated that higher sensitivity could be obtained by instead using an injector liner packed with an adsorbing bonded-phase material. However, this latter approach required a more careful optimization of the collection temperature (i.e., the temperature of the liner) when a modifier was used.

Compared to solvent collection and solid-phase trapping in ''off-line'' mode, the on-line technique offers higher sensitivity since the entire portion of extracted components can be transferred to the chromatographic column. In addition, the technique requires less sample handling, which consequently may give smaller standard deviations as well as shorter analysis times. A major drawback is that many on-line approaches are sensitive to coextracted fat, water and sometimes modifier, and depending on the type of interface there is an inherent risk of overloading and ruining the analytical column. Therefore, only small samples are usually extracted when the SFE is on-line coupled to a chromatograph. For a more detailed discussion regarding on-line coupling of SFE to chromatographic instruments, the reader should confer the excellent review articles [62,63].

2.4. Alternative collection modes

In process-scale SFE, collection is commonly achieved in one or several empty vessels, to eliminate the tedious step of removing the solvent from the extracted components. Collection in an empty vessel has also been employed in analytical-scale SFE, even though it may result in lower recoveries compared to collection in a vessel containing solvent [64]. Glass beads [65] or glass wool [66] can be utilized to increase the total collection-surface inside the vessel. The extraction temperature affects the collection temperature, unless the vessel is cooled by other means than solely expanding CO_2. A modifier may change the surface of the collection vessel, and thereby change its properties of retaining analytes. An excessively long collection time can lead to losses of volatile analytes [67], which naturally is a problem if a long dynamic extraction time must be applied to achieve quantitative extraction recovery. In addition, if the analytes are sensitive to oxidative degradation, it is advantageous to instead collect in a solvent containing a protective antioxidant.

Miller et al. employed a wide stainless steel tube (178 μm I.D.) inserted into an empty 10-ml vessel to obtain fast depressurization after a static extraction step, and thereby minimized the risk of restrictor plugging [67]. They demonstrated that recoveries between 85 and 95% could be obtained for analytes as volatile as *n*-heptane by carefully optimize parameters such as extraction temperature, modifier and collection time.

Vejrosta and co-workers have demonstrated that analyte collection can be achieved in a minimal volume of organic solvent by using a fused-silica capillary (30 cm×500 μm I.D.) as collection device and condensed modifier as trapping solvent [68–70]. The end of a linear restrictor was placed inside the collection capillary. The amount of condensed modifier formed during CO_2 expansion was linearly dependent on the temperature of the collection capillary, e.g., −30 °C gave rise to approximately 300 μl of liquid methanol when SC-CO_2–methanol (90:10, v/v) was used as extraction solvent [68]. The layer of condensed modifier (and trapped analytes) was continuously moving out from the capillary and into a capped collection vial. Vejrosta et al. also showed that quantitative collection recoveries could be obtained for *n*-alkanes, PAHs, and a mixture of acetophenone, *N*,*N*-dimethylaniline, and naphthalene in a total solvent volume of less than 1 ml [70]. However, the collection technique is not recommended for analytes as volatile as the collection solvent.

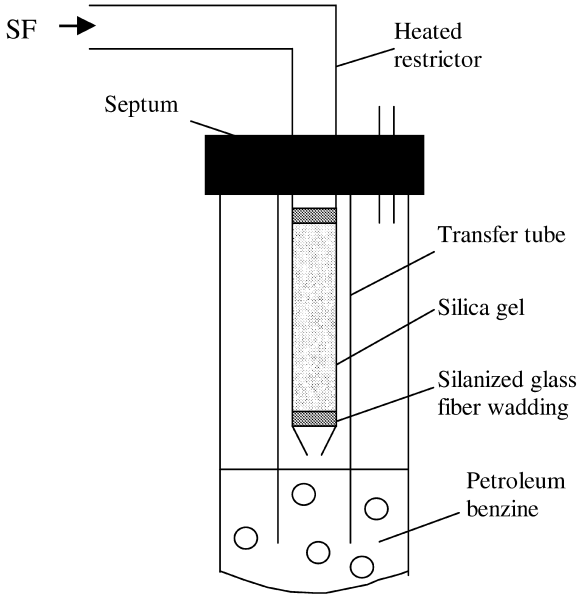

Fig. 5. A combined solid-phase–solvent collection trap (redrawn from Ref. [28]).

In a combined solid-phase–solvent collection device, breakthrough-losses of analytes from the solid-phase trap can be collected in the subsequent vessel containing solvent [28,29,71]. One example of such collection device is shown in Fig. 5.

The solid–liquid trap turned out to be especially valuable in the evaluation of trapping efficiencies of different solid-phase materials [28,29,71]. For example, Hüsers and Kleiböhmer showed that the collection efficiency of PAHs using a silica gel–*n*-hexane trap was not affected by flow-rate or modifier addition [28].

3. Applications

3.1. Environmental samples

In the mid 1980s extraction of environmental pollutants such as PAHs and PCBs from solid matrices (e.g., soils and sediments) was a main starting point for applying supercritical technology in analytical scale on real world samples [72]. Several investigations have been devoted to elucidate solvent collection and solid-phase trapping of these types of organic contaminants and both trapping techniques are common in environmental applications. In general, SFE is very well suited for extraction of hydrophobic organic contaminants from environmental solids and both solvent collection and solid-phase trapping have been demonstrated robust once suitable collection parameters are established. Also, it was early demonstrated that on-line connection to GC was possible [73] and several studies have been devoted to optimizing various on-line collection strategies [63]. During the last few years rather few publications have been presented related to collection efficiencies of organic contaminants extracted from environmental samples since much of this information already is available in the literature. Additionally, most instrument producers also provide their customers with application notes with relevant

extraction and collection parameters for the determination of the desired analytes. Another important aspect is that many research groups have turned their interest to a more recent extraction technique called accelerated solvent extraction which is a strong competitor to SFE for environmental samples [74].

3.1.1. PAHs

Several organic solvents have been tested for the collection of PAHs. Langenfeld et al. found that methylene chloride, chloroform and acetone worked reasonably well while methanol and hexane gave unsatisfactory recoveries [1]. Collection in methylene chloride of PAHs extracted from a marine sediment (SRM 1941) has also been shown to give quantitative data for PAHs with a molecular mass down to phenanthrene [19]. However, in this case a designed solvent collector was used in which the solvent could be cooled independently of the temperature of the restrictor. This device was successful despite the fact that the marine sediment contained 30% (w/w) sulfur, which normally causes severe clogging, but did not occur in this case as the restrictor was heated to 200 °C. Methylene chloride has also been tested in a thorough investigation of three different types of restrictors in off-line collection of PAHs in order to find a good solution to clogging problems encountered for real world samples [75]. The best approach according to the authors was an 11-cm aluminum-block heated restrictor (100–150 °C) dipped into the collection solvent giving recoveries of 90–95%.

Acetone is another possible solvent used for efficient collection of PAHs [21], however, both the collection solvent volume and the restrictor temperature was of great importance. The preferable restrictor temperature was 80 °C using 10 ml of solvent, which gave quantitative efficiencies as compared to lower solvent volumes (4 ml) and higher restrictor temperatures (100 °C). The effects seen from larger solvent volumes were explained by longer contact time between bubbles and solvent due to deeper solvent. Reindl and Höfler made similar observations when extracting PAHs from soil samples [76]. At a restrictor temperature of 180 °C the recoveries in acetone increased from ca. 80 to 95% when increasing the solvent volume from 5 to 15 ml. They also observed that if 15 ml of solvent was used, restrictor temperatures up to 200 °C gave quantitative recoveries, but at 250 °C the recoveries dropped dramatically to 60%.

From the above discussion it is clear that the restrictor must be heated to avoid clogging. However, if the trapping solvent reaches too high temperatures analytes might be lost. Therefore the restrictor should preferably be heated independently of the solvent, or at least to a somewhat moderate temperature. Both methylene chloride and acetone have demonstrated suitable as collection solvents. From the point of reducing the amount of chlorinated organic solvent in the environment, acetone is the better choice and is also less toxic.

An interesting approach for minimizing solvent usage in off-line collection by means of restrictors was presented by Vejrosta et al. using a moving liquid layer [69]. By continuously introducing small volumes of an organic solvent flowing down through the fused-silica capillary restrictor the PAHs could be quantitatively collected in a final deposited solvent volume of 0.3–0.75 ml of methanol, where no solvent was present in the collection vial prior to the extraction step. Later Vejrosta et al. presented a similar setup for off-line collection where the expanding SF effluent was mixed with overheated organic solvent vapor inside a fused-silica capillary [70]. Impressive collection data were achieved for acenaphthene, fluorene, phenathrene, pyrene and chrysene in 10 different collection solvents demonstrating that the developed collection methodology was nearly independent of the solvent as seen in Table 1.

Only two solvents (cyclohexane and diethyl ether) gave incomplete trapping. Additionally the total solvent collection volume for a 15 min extraction was between 0.1 and 0.2 ml meaning that the extracts were at least 10 times more concentrated than with other collection techniques.

Solid-phase trapping on silica gel with a subsequent liquid trapping step in light petroleum has been used by Meyer and co-workers for the analysis of PAHs in soil and sediment [77,78]. This collection device was a modification of a previously developed trap [19]. With the modified setup it was possible to determine PAHs with a molecular mass down to fluorene. A nice feature with the collection system was that the solid-phase trap was used as clean-up

Table 1
Average recoveries of five different PAHs trapped inside a fused-silica capillary where expanding SF effluent was mixed with overheated organic solvent vapor using various organic solvents

Organic solvent	Recovery (%)
Ethanol	99
Chloroform	99
Acetonitrile	99
CCl_4	98
2-Propanol	97
Methanol	96
Acetone	96
Tetrahydrofuran	96
Ethyl acetate	96
Dichloromethane	94
Cyclohexane	89
Diethyl ether	87

Data from Vejrosta et al. [70].

column after the finalized extraction cycle, with no extra clean-up step needed. The collection solvent was simply passed through the collection column (silica gel) and PAH elution was then completed by rinsing the column with a few milliliters of light petroleum–toluene (3:1, v/v).

One of the most crucial parts in coupling SFE to GC is trapping of the desired analytes [63]. In general this step can be divided in two groups, either external trapping or internally directly on the column. Other important parameters to consider are extraction flow-rate, trapping temperature and the thickness of the column stationary phase. An early approach for coupling SFE directly to GC was demonstrated by Hawthorne and Miller [79]. They extracted automobile exhaust organics trapped on Tenax-GC, where the organics were recovered from the trap by SC-CO_2 and cryogenically trapped in the GC column. A crucial part of coupling SFE on-line to GC is the inner diameter of the restrictor. A good solution was the usage of a 25 μm restrictor coupled to a wide-bore thick-phase column [73]. With this set-up it was possible to obtain quantitative data for PAHs spiked on river sediment as well as for certified urban dust. However, when coupling SFE on-line to GC–MS a 150 μm I.D. fused-silica capillary had to be used as interface to avoid too high pressures in the ion source. A similar approach for qualitative analysis of PAHs in urban dust particles utilized an on-column retention gap (53 μm I.D.) where a 5 μm fused-silica restrictor was inserted and analytes where trapped prior to GC–FID [80]. Later Hawthorne et al. used a conventional split/splitless port and performed on-line analysis of SFE extracts deposited in a heated injector port [81]. The split injector made it possible to directly analyze samples containing high amounts of sulfur and water, which normally could not be handled with conventional off-line and on-line SFE–GC systems. Good quantitative data was obtained for a number of PAHs in certified sediment materials (NIST 1941).

3.1.2. PCBs

PCBs have successfully been collected by solvent collection. One of the best solvents is acetone and to determine collection efficiencies, Arochlor 1260 was spiked on sand and extracted with neat CO_2 at 150 °C and 400 bar [21]. The results were strongly depending on restrictor temperature but to a less extent on solvent volume. Recently Nilsson et al. performed a thorough investigation of collection efficiencies of a large number of PCBs in acetone at a restrictor temperature of 80 °C but with varying extraction parameters (40–150 °C, 150–400 bar) [82]. In the automated system, a pressurized collection feature was utilized, and nearly all extraction conditions gave 100% recovery. Only at long extraction times (60 min) some small analyte losses were observed due to solvent evaporation (decreased collection solvent height), but this was easily adjusted for by automated collection solvent replenishment.

Other collection solvents that have been used for the extraction of PCBs from soils are hexane and isooctane and their collection efficiencies have been compared at 50 °C and 200 bar [83]. Isooctane was the better choice allowing for collection also of the lighter, less chlorinated PCBs. For example, the recovery of PCB 28 in isooctane was 105% while in hexane only 82%.

A common problem when utilizing solvent collection and restrictors for the extraction of PCBs from natural samples is losses due to clogging of the restrictor. This is often caused by co-extracted sulfur, which is present in the sediment due to anerobic bacterial action. This can be overcome by heating the restrictor (150–200 °C) or alternatively by putting

some copper granules at the outlet side of the extraction cell [19]. The latter solution is today common procedure in many PCB sediment applications [84–86].

When using sorbent traps there are several factors affecting the collection efficiency. Bøwadt et al. investigated the collection efficiency of PCBs on three sorbent traps (ODS, silica and Florisil) and one solid surface (stainless steel) using neat and modified CO_2 [42]. The solid surface trap had the poorest trapping performance while ODS and Florisil were the best packing materials. Extracting trace levels of aged PCBs from a certified sewage sludge using neat CO_2, with Florisil as trap packing material and *n*-heptane as trap eluent, generated very clean extracts ready for analysis with GC–ECD. The choice of eluent was not random. It had previously been shown that heptane gave the narrowest elution profile for the analytes as well as quantitative recoveries from the trap [2].

In a publication by Hartonen et al., a thorough investigation of trapping efficiencies for several organic pollutants like polybrominated benzenes, polybrominated biphenyls, and polybrominated diphenyl ethers on solid-phase traps (ODS, Florisil), extracted from sediments at different extraction conditions, were presented [31]. Florisil was said to be the best choice for samples like this due to its stronger interactions with the analytes. In the same investigation it was also demonstrated that extraction parameters in general had little effect on the trapping efficiency. However, big effects in terms of trapping efficiency were seen when the solid-phase trap was used in combination with different modifiers. Even though the trap was heated to the boiling point of the modifier used, trapping efficiencies rarely exceeded 90% with modifier in the extraction process.

Nilsson et al. compared two automated SFE systems, one with solid-phase trapping using Florisil (HP 7680T) and the other with solvent collection in acetone (Isco SFX 3560) for the determination of PCBs in sediments [86]. Quantitative recoveries were obtained in both cases, but direct injection of the final extracts without further clean-up revealed large differences in cleanness of the chromatograms. While the extract from the solid-phase system showed basically no interferences, the liquid collection system had a much more noisy baseline. An example of chromatograms obtained for these two systems can bee seen in Fig. 6.

On-line SFE–GC–ECD was tested for Arochlor 1254 spiked onto sediment, however, in this early investigation only semi-quantitative data were presented [73]. On-line SFE–GC data for PCBs spiked on Tenax have also been reported by Nielen et al., but recoveries were relatively poor, around 60% [87]. In fact the number of on-line SFE–GC applications for PCBs in solid matrices are very limited and instead off-line collection is by far the most common way of determining PCBs using SFE.

3.1.3. Dioxins

Solvent collection of 2,3,7,8-TCDD has been performed in *n*-hexane [88]. No loss of this analyte was reported at a pressure of 314 bar and 40 °C during the course of a 30-min extraction. Collection of a large number of PCDDs and PCDFs in hexane has also been performed with success in other large-scale studies involving extraction with both CO_2 and nitrous oxide under various extraction conditions [46,89].

Other applications have involved solid-phase collection using a trap packed with ODS followed by elution with 2-propanol [90]. With the developed SFE methodology, obtained dioxin concentrations were close to those obtained with Soxhlet. However, as pointed out by Larsen and Facchetti, ODS combined with 2-propanol will generate extracts containing a large number of unwanted components including PCBs and PAHs which are eluted together with the dioxins and furanes [91]. In order to fully utilize the solid-phase trapping device, it should be filled with activated carbon allowing for the PCDDs and PCDFs to be eluted in one fraction. Such a system has been developed by van Bavel et al. as described above combining SFE on-line with LC [38].

A similar approach involved solid-phase trapping of 13 different PCDDs and PCDFs on a trap packed with activated carbon–Celite (1:5, w/w) with a total mass of 370 mg as done by Mannila et al. [92]. In the first solvent elution step, 4 ml of hexane was used to elute unwanted interfering compounds such as PCBs. It was demonstrated that these types of compounds were eluted already after the addition of 2 ml of solvent. The analytes of interest were then

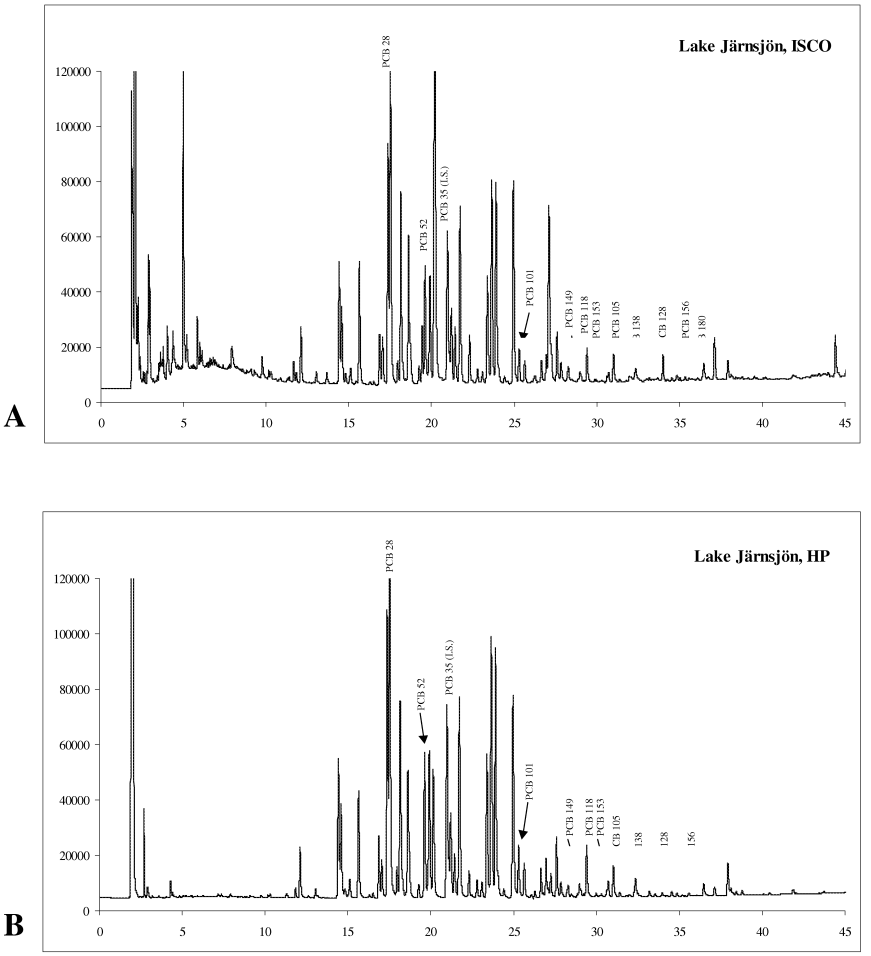

Fig. 6. Direct injection into the GC–ECD system of untreated extracts obtained from two automated SFE systems: (A) Isco 3560 and (B) HP 7680T, equipped with solvent collection (acetone) and solid-phase trap (ODS), respectively.

eluted with 15 ml of xylene. Individual recoveries ranged from 81 to 118% with RSDs between 3 and 18% ($n=10$).

A combined solid–liquid collection device has been used by Friedrich and Kleiböhmer for trapping of PCDDs and PCDFs [71]. The collection device consisted of silica impregnated with 10% sulfuric acid followed by hexane as organic solvent. This was demonstrated efficient for trapping analytes extracted from soil samples using CO_2 modified with toluene–trifluoroacetic acid.

3.1.4. Pesticides

Solvent collection of pesticides was investigated by van der Velde et al. in 1992 [83] where hexane was compared to isooctane for several organochlorine pesticides such as HCHs, HCBs, DDE, DDT and dieldrin. By using isooctane good collection recoveries could be achieved also for the more volatile pesticides while hexane for some analytes had recoveries of 40%. Similar studies performed by Wenclawiak et al. demonstrated that CBs were efficiently trapped in pure toluene [93]. However in order to quantitatively collect various HCHs, obstacles such as glass beads had to be introduced in the gas flow path. The optimized trap was tested on contaminated soil and generated recoveries in the range of 80–97% for the individual CBs and HCHs.

In a later investigation, quantitative recoveries of pesticides such as α-HCH could be achieved by placing a Dewar condenser on the top of the off-line collection solvent vial when collecting analytes in ethyl acetate [26].

A comprehensive study of suitable solid-phase traps and elution solvents, for the determination of a number of pesticides in soil, was performed by Koinecke et al. [41]. Out of five solid-phase traps and four solvents, diol combined with ethyl acetate gave quantitative recoveries for fenpropimorph, pirimicarb, parathion-ethyl, triallate and fenvalerate. With the optimized trap, extraction efficiencies of three pesticides aged for 3 and 31 days in soil using SFE with 5% methanol in CO_2 were compared to slurry extraction and Soxhlet. SFE gave comparable data to the other two techniques, but with the main advantage of generating extracts ready for analysis with no further sample clean-up needed.

3.1.5. Aliphatic hydrocarbons

Several experiments have been performed by Porter et al. on solvent collection of *n*-alkanes [19]. Initially they studied possible losses of light alkanes C_9 to C_{17} from 15 ml of methylene chloride when purging this solvent with gaseous CO_2. However, none of the spiked *n*-alkanes were lost at 5 °C after 40 min of purging. They also demonstrated that nonane was more efficiently trapped (96%) when a pre-cooled collection solvent was used as compared to uncooled solvent (80%), while the higher alkanes were quantitatively collected without pre-cooling. A severe problem occurring for higher-molecular-mass alkanes (C_{28} and C_{36}) was clogging of the restrictor unless the whole restrictor was heated to 75 °C with an additional heating of the collection solvent (iso-octane) to 40 °C. However, when properly heated, C_{15}, C_{28} and C_{36} could be quantitatively recovered (101–107%). It was also demonstrated that heating the restrictor worked nicely also when collecting alkanes in the range of C_{9-17} as long as the solvent was cooled, but in order to manage independent heating of the restrictor while cooling the collection solvent, a special dual-chamber-trapping vial had to be developed.

The problems seen when collecting more volatile analytes such as *n*-alkanes by a normal capillary restrictor dipped in the collection solvent has also been well demonstrated by Burford et al. [75]. The best collection efficiencies for volatiles such as heptane, octane and nonane collected in methylene chloride were obtained with no restrictor heating. But, as the authors concluded, this represents a rather unrealistic scenario for real-world samples due to the risk of clogging. Collection of volatile alkanes is therefore not recommended with this technique. An alternative approach for overcoming restrictor-clogging problems when extracting alkanes from real-world matrices such as petroleum sludge is to use a variable-flow control device so that samples containing large amounts of water and co-extractable material could efficiently be extracted with quantitative collection of alkanes as volatile as octane [94]. The system allowed the restrictor to be heated to over 200 °C while still cooling the collection solvent to low temperatures assuring good collection efficiencies.

Collection of *n*-alkanes (C_{10}–C_{32}) on solid reversed-phase traps was investigated by Mulcahey et al. [95]. Phenyl, C_8, and C_{18} traps were tested and most of them worked nicely apart from phenyl, which gave somewhat low recoveries for the lower *n*-alkanes. When using normal-phase traps (OH, SiO_2, CN and NH_2) less favorable recoveries were obtained in all cases for the lower-molecular-mass *n*-alkanes. A thorough comparison of solid-phase and solvent collection of volatile petroleum hydrocarbons in soils was performed by Yang et al. [96]. Two commercially available systems were investigated (Hewlett-Packard solid-phase trap and Isco solvent collection), and it was found that both collection systems (solid-phase Porapak Q at 5 °C and methylene chloride with restrictor at 80 °C) gave comparable data for analytes as volatile as toluene, C_8 and ethyl benzene. However, for very volatile analytes such as hexane and benzene, solid-phase trapping was more efficient generating quantitative data. The situation was improved for solvent collection by utilizing special options on the Isco system, such as cooling the solvent down to −20 °C and pressurizing the collection vial to 2 bar overpressure, but hexane was still never collected to more than 60% as compared to 90% for the solid-phase trap.

On-line SFE–GC of *n*-alkanes from urban dust has been performed with nitrous oxide as extraction solvent [97]. The total time for one analysis was 1 h

and the authors claimed the advantages to be maximum sensitivity and minimal analyte loss. Burford et al. developed a quantitative SFE–GC method for the determination of gasoline and diesel range organics from a number of environmental solid samples including soils and sediments [98]. A cryogenic trapping temperature of −25 °C was used to trap analytes as low as *n*-pentane on a DB-1 column (100% dimethylpolysiloxane, bonded phase) with a film thickness of 5 μm. Applying a split ratio of 1:100 allowed for 1-g samples to be analyzed avoiding column overloading. It was also nicely demonstrated that by adding drying agents, samples could be analyzed as received (without air drying) and still not suffer from freezing water with accompanying restrictor plugging.

3.2. Biological samples

For biological applications, drugs are normally trapped by means of solvent collection, whereas for environmental contaminants solid-phase trapping is preferred. Only a few papers deal with on-line modes and alternative collection such as in-line trapping.

Most published work on biological matrices has been performed on solid and semi-solid samples such as tissues with only a few reports dealing with direct SFE of liquid matrices such as blood and urine. Collection from liquid matrices is complicated due to the relatively high solubility of water in the SF. The co-extracted water may clog the restrictor, create a two-phase mixture with the collecting organic solvent, or deactivate the solid-phase trapping material, with lowered trapping efficiency and breakthrough losses of analytes as a consequence. In contrast, collection from tissue samples presents problems as co-extraction of fat and other endogenous materials, resulting in complex extracts, which may need further clean-up steps before chromatographic analysis.

3.2.1. POPs

The first publications on SFE from tissue and blood samples concerned extractions of various xenobiotics, collected in ice-cold hexane [99,100]. But overall, SFE applications on biological samples were during the 1990s mainly dealing with POPs in tissue samples, using solid-phase trapping as collection mode.

Bøwadt et al. employed an optimized solid-phase trapping procedure for extractions of PCBs from lyophilized fish tissue (tuna muscle with native PCB concentrations in the range of 3–84 ng/g) [101]. The trap was filled with Florisil and kept at 20 °C when neat CO_2 was used and at 65 °C when methanol was added as modifier. PCBs were eluted with either heptane or dichloromethane and all extracts were subjected to acid silica clean-up after completed extraction to avoid deterioration of the GC column. Hale et al. extracted chlorinated pesticides and PCBs from 644 fish fillets and eight bird mesentery samples [102] using a trapping performance optimized in a previous paper [103]. Two types of trapping materials were examined, namely (i) 100–120 mesh silanized glass beads and (ii) 20–30 μm C_{18} mixed (1:1) with 80–100 mesh Unibeads 2S, as well as different trapping temperatures and elution procedures. The inclusion of a C_{18} material in the trap provided improved recoveries, and optimal retention of PCBs during extraction was obtained on the C_{18}–Unibeads trap at −30 °C. The analytes were eluted from the trap with 2 ml of isooctane at 80 °C. Fish extracts could be directly injected into the GC, but the bird mesentery extracts required an additional clean-up step using Florisil. However, the authors experienced no restrictor plugging during the course of over 700 extractions.

Comparing CO_2 and CHF_3 as extracting fluids, Yoo and Taylor extracted PCBs and chlorinated pesticides from a standard reference material of mussel tissue [104]. The analytes were trapped onto ODS at −10 °C during the extraction, followed by elution with 3×1.5 ml of isooctane at 70 °C. Compared to Soxhlet extraction, the recoveries of the SFE method were equal or higher. The group of van Bavel et al. trapped ^{13}C-labeled PCBs, extracted from human adipose tissue samples, on a mixture of 5.4 mg PX-21/g ODS [105]. The non-planar fraction was eluted with 6 ml of hexane–methylene chloride and the planar fraction with 9 ml of xylene. Recently, Gonzalez Amigo et al. extracted PAHs from bird liver samples, collecting the analytes on an ODS trap at 75 °C with subsequent elution with four 1.5 ml portions of acetonitrile [106]. Recoveries of 90–115% were achieved for spiked samples and levels in

the range of 0.2–2.3 μg/kg were found in real samples of a bird of prey (*Tyto alba*).

Johansen et al. have reported both on-line SFE–GC and on-line SFE–HPLC for extractions of PCBs from crab and cod tissues [32,36]. In the case of GC [32], the collection of the analytes was achieved in the retention gap during the extraction by cryofocusing with nitrogen. The trapping efficiency was investigated at various temperatures from −1 to −50 °C with the highest recoveries obtained at −20 to −30 °C. After completed extraction the analytes were transferred to the analytical column by thermal desorption and by helium carrier gas. In the case of on-line coupling to HPLC [36], the compounds were collected on a HPLC column made of PYE [2-(1-pyrenyl)-ethyl) dimethylsilylated silica gel], with hexane used as mobile phase. Recently, on-line SFE–CE has also been reported for extractions of cresol and chlorophenols from human urine [107]. Extracted analytes were collected on a solid-phase trap filled with diol, and eluted from the trap at 25 °C using 1.5 ml of methanol. This volume was then directly transferred to the CE instrument across an interface, which consisted of a programmable arm and the autosampler of the CE equipment.

3.2.2. Drug residues

Solvent collection with methanol (10 ml) maintained at ca. 1 °C was used in an early paper by Cross et al. for the extraction of incurred sulfamethazine from swine muscle tissue [108]. The obtained results were in good agreement with reference values provided. Houpalahti and Henion performed a similar approach by collecting extracted growth-promoting anabolic steroids from spiked bovine tissue (muscle and liver) in 1 ml of precooled (−5 °C) methanol [109].

Applications of SFE in forensic science have increased over recent years focusing on extractions of opiates and steroids in samples such as blood, hair and tissues. The drug temazepam was extracted from whole blood samples from authentic forensic blood specimens [110] and the extracted analyte was collected in methanol at the flow outlet. Compared to a conventional SPE method, the SFE method was more efficient towards other benzodiazepines and gave cleaner extracts with recoveries above 80%. In two papers by Allen and co-workers, opiates were extracted from whole blood and urine for forensic studies [111,112]. The analytes were collected by expansion in methanol before the derivatization step prior to GC–MS. Recently Brewer et al. reported determinations of cocaine, benzoylecgonine (a cocaine metabolite), codeine and morphine from a hair standard reference material [113]. The SC-CO_2 was depressurized through a stainless steel restrictor into 2 ml of methanol. Compared to a conventional method based on acid hydrolysis, the SFE method gave higher recoveries, and the found amounts of opiates were in good agreement with reported levels.

Cirimele et al. has also extracted opiates (codeine, morphine, 6-monoacetylmorphine), in this case from hair samples of drug addicts, with collection on a solid-phase trap filled with Tenax [114]. 1 ml of a modifier solution (methanol–triethylamine–water, 2:2:1) was added directly to the sample, and neat CO_2 was used as extraction fluid. The trap was kept at 25 °C and eluted after completed extraction with 1.8 ml of chloroform at 30 °C. The recoveries obtained for spiked samples were 61, 53 and 96% for the three analytes, respectively. The low recoveries in some cases might have been caused by breakthrough losses due to modifier condensation in the trap. For the samples from drug addicts, the concentrations of the opiates were found to be in the range of 0.5 to 15 ng/mg.

Another application in which solid-phase trapping was employed is the extraction of androstenone from boar fat trapped on ODS with elution of the extracted material with cyclohexane before final analysis by GC–MS [115]. The SFE–GC–MS method was shown to be in good agreement with a routine method based on radioimmunoassay (RIA). In a similar work, β-agonists such as clenbuterol and salbutamol were extracted from spiked and incurred bovine liver tissue samples using a commercial SPE cartridge containing 2 g of neutral alumina attached as a trap directly after the restrictor [116]. After completed extraction the cartridge was removed and eluted with 4 ml of methanol–water (70:30) prior to quantification utilizing enzyme immunoassay.

When extracting the corticosteorid ^{3}H-budesonide from human blood plasma, Karlsson et al. found that a high trapping temperature (110 °C) of the solid-phase trap (ODS) was needed for full recovery of the steroid, when high concentration of methanol was

used as modifier [117]. However, excessive temperatures were also shown to cause thermal degradation of the analyte. Interestingly, some analyte loss occurred if depressurization of the CO_2 was performed prior to the elution of the trap. When the order of depressurization and elution was reversed, the recovery was significantly improved, from 82 to 95%.

In order to minimize difficulties with analyte losses associated with off-line solid-phase collection, Parks and Maxwell developed an in-line trapping technique for veterinary residues (sulfonamides) from spiked chicken tissue samples [47]. The extraction vessel was packed with 4 g of neutral alumina placed after the sample, separated with frits. During the extraction, the extracted target analytes were trapped in the alumina bed with the vast majority of fat components remaining in the SF. The lipophilic material was then collected off-line after CO_2 decompression on an additional SPE column. When the extraction was completed, the alumina was poured into an empty SPE column and the analytes were eluted with the HPLC mobile phase. Recoveries of sulfametazine, sulfadimethoxine and sulfaquinoxaline in chicken liver, breast and thigh are listed in Table 2.

Compared to conventional off-line trapping mode, recoveries were in all cases higher with the in-line mode. Additionally, the chromatographic determination was much easier with less interferences. However, when the same trapping technique was used in the study of zoalene from spiked chicken liver [33], it was found that the addition of water to the sample might cause breakthrough losses of analytes from the in-line alumina trap.

Maxwell et al. applied an in-line SPE column PTFE sleeve trap assembly for the extraction of steroids from spiked chicken liver samples [118]. This collection mode was compared to an in-line alumina bed as well as an off-line alumina trap. After completed extraction, the SPE column was removed and the analytes were eluted in 1–2 ml of solvent. In this case it is not necessary to transfer the sorbent bed to an empty SPE column after the extraction. Additionally, the in-line column is directly compatible for integration with SPE processing systems using vacuum manifolds. Stolker et al. employed the same trap assembly for the extraction of trace levels of nortestosterone, testosterone, metyltestosterone from spiked, hydrolyzed bovine urine [119]. The analytes were retained in-line while co-extracted material was trapped off-line after CO_2 depressurization. After completed extraction, the SPE column was removed and eluted with 3 ml of methanol–water (70:30) mixture, which was directly injected into the HPLC or GC–MS systems.

3.2.3. Miscellaneous

Using supercritical ammonia as extracting solvent, Jacobson et al. extracted ^{11}C-labeled tracer compounds (acetyl-carnitine and methylpiperidyl benzilate) from kidney and brain in rats [120]. These compounds are of interest for in vivo and in vitro studies of physiological and biochemical processes. The extracts were trapped in only 1 ml of water and the collected fractions were heated to 40 °C under reduced pressure for 2 min to remove most of the ammonia before final analysis. However, it was found that only 66% of the radioactive acetyl-carnitine was trapped in the collected fractions and 12%

Table 2
SFE recoveries of sulfonamides from fortified chicken tissues comparing off-line and in-line trapping modes [47]

Tissue		Sulfamethazine		Sulfadimethoxine		Sulfaquinoxaline	
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Liver	Off-line	60	3	69	7	54	2
	In-line	90	2	97	1	76	3
Breast	Off-line	67	4	79	6	64	6
	In-line	86	2	92	2	75	4
Thigh	Off-line	75	5	84	10	73	8
	In-line	91	4	95	3	80	3

remained in the extraction vessel. For the more lipophilic methylpiperidyl benzilate, 93% was collected and less than 1% remained in the extraction vessel.

Another application is the extraction of bile acids from human feces [121]. The bile acids were collected in 5 ml of methanol mixed with ricinoleic acid, which was used as the internal standard. The results were comparable to those obtained with solvent extraction. Pyo and Shin reported extractions of microcystins from cyanobacterium [122]. The extracted material was collected in an empty vessel but since they used aqueous methanol to modify the CO_2, the extracts were collected in a liquid state in the collection vessel. The extracts could be directly analyzed using HPLC. Arancibia et al. extracted $Pb(DDC)_2$ from human blood, urine and milk from patients diagnosed with plumbunemy, and the collection of the extracts was achieved in the modifier solution [123].

3.3. Agricultural and food samples

Agricultural samples are here defined as any type of crop material, including grains, seeds, plants, flowers, fruits and vegetables, growing or harvested from a field or manufactured as a product. Food samples are regarded as any type of products that has been manufactured for eating, which in addition to edible agricultural products also include products of animal origin.

Fats and oils extracted from agricultural and food samples are commonly collected in a solvent or in an empty vessel, while flavors and fragrances are preferably collected on a cryogenically cooled adsorbing trap. For pesticides and fat-soluble vitamins, both solvent collection and solid-phase trapping are common modes of collection in analytical-scale SFE.

3.3.1. Fats and oils

In general if a larger amount of fat is extracted from a sample, solvent collection may be preferred to solid-phase collection, due to the inherent risk of overloading the solid-phase trap with fat. For example has the loading capacity of a standard-1-ml ODS trap (HP 7680T) been estimated to be around 80–100 mg of fat, which is a relatively low amount of material for many applications [44].

In a work by Gonzáles-Vila et al., methanol was used as collection solvent for the isolation of various lipid components from *Eucalyptus globulus* wood [124]. If the collection vessel also is pressurized, even relatively volatile compounds can be quantitatively trapped in a solvent. Eller and King employed pressurized vessels cryogenically cooled to 0 °C, for the extraction and collection of an essential oil from cedarwood chips (CWO) [125]. A mixture of anhydrous sodium sulfate, 1 ml of water saturated with sodium sulfate and 2 ml of diethyl ether was used for collection of the CWO and simultaneous removal of excess water. SFE gave higher extraction yields of CWO compared to the conventional steam distillation procedure, as well as a CWO more resembling the original cedarwood chips in terms of odors (as evaluated of a sensory panel).

Berg et al. discovered the disadvantages of employing a solid-phase trap when the samples contain large amount of fat or when a modifier is used [126]. The trap was therefore bypassed, and collection was achieved in an empty vessel. This approach enabled quantitative determination of lipid classes and total fat in meat samples using SC-CO_2 containing 8% of ethanol as extraction fluid. Hence, a collection solvent is not always necessary when the amount of extracted fat is high (which will then act as a collection solvent). The same was experienced by Taylor and King, who employed an empty collection vessel, pressurized and cooled to 0 °C, for the enrichment of ferulate-phytosterol esters (FPEs) from corn bran oil [127]. Moreover, the AOAC official method for determination of oil in oilseeds recommends collection in empty vessels with $\geq$1.0 g of glass wool at the bottom [128].

3.3.2. Pesticides

For the determination of pesticides in agricultural and food samples there is no obvious trend in the selection of collection mode. Both solvent collection and solid-phase trapping are common approaches, depending on the nature of the sample and the available instrument. A 10-ml volume of methanol was used for the collection of fenpyroximate, which was determined in spiked apple samples [129]. Chuang et al. used SFE with solvent collection for the determination of atrazine, carbofuran, chlorpyrifos and metolachlor in canned baby food [130]. Not

surprisingly, the use of dichloromethane as collection solvent gave higher recoveries when compared to pure water, especially for the unstable chlorpyrifos.

A comparison of solid-phase traps (ODS, diol, Tenax and Porapak-Q) and rinse solvents (acetone, ethyl acetate, acetonitrile and methanol) was done by Lehotay and Valverde-García [40], for the collection of 56 different pesticides extracted from spiked fruits and vegetables. Acetone eluted the pesticides in the smallest volume from almost all of the investigated traps, and the ODS trap gave the most consistently high recoveries.

King et al. utilized a Florisil trap for the collection of organochlorine, organophosphorus and organonitrogen pesticides from wheat grains (spiked samples) [131]. Acetone was used for rinsing the trap, and recoveries above 80% were obtained. In a similar work, thionazin, methyl parathion, fenthion, methidation, pyrazophos, phosalone, vinclozolin and procymidone were determined in orange juice (spiked samples) [132]. Recoveries were between 85 and 100% using an ODS trap at 20 °C, and rinsing with chloroform. Pensabene et al. employed a Florisil trap for the determination of chloramphenicol in whole eggs [48]. Methanol–water (55:45) was used as rinsing solvent, and the results agreed very well with those obtained by conventional solvent extraction.

3.3.3. Flavors and fragrances

Collection on a cryogenically cooled trap filled with an adsorbing material generally provides the highest collection efficiency for volatile compounds. For example, a Tenax TA (Chrompack) solid-phase trap was used for the collection of olive oil aromas [133]. After completion of the extraction, the trap was installed in a GC–MS injector port, and the analytes were thermally desorbed at 220 °C. In another work, Larrayoz et al. investigated various collection parameters for the determination of volatiles in cheese [134]. It was found that higher collection efficiency was obtained by employing an ODS trap instead of a trap filled with stainless steel beads, by applying −5 °C of trap temperature compared to 0 °C, and by using *n*-hexane–acetone (2:1, v/v) as rinse solvent as compared to pure *n*-hexane. The optimized parameters were applied in a subsequent study for the analysis of real cheese samples [134,135].

Blanch et al. compared extraction recoveries of wine aromas employing three different collection set-ups [136]: (i) an ODS-trap cooled to −5 °C; (ii) a programmed temperature vaporizer (PTV) set to 10 °C (a quartz liner 100 mm×1 mm I.D., filled with Thermotrap TA material from Chrompack); and (iii) the PTV described above, on-line coupled to GC and cooled to −5 °C. The results using standard solutions of wine aromas showed that the highest collection recoveries could be obtained using either set-up (i) or (iii). However, set-up (iii) demonstrated significantly higher sensitivity compared to both set-ups (i) and (ii), and was therefore used for analysis of real wine samples. Unfortunately, no recovery data were given.

Palma and Taylor demonstrated that 5-hydroxymethyl-2-furaldehyde could be quantitatively determined in raisins employing SFE with collection on an ODS trap held at 75 °C, using 20% of methanol as modifier [137]. It was shown that lower modifier concentration gave rise to poorer extraction and higher modifier concentration resulted in breakthrough losses of the analyte.

3.3.4. Fat-soluble vitamins

Fat-soluble vitamins are prone to oxidation, a degrading process that is accelerated by light and heat. Therefore, an antioxidant is usually added to the collection solvent/rinse solvent to protect the analytes. Fat-soluble vitamins commonly occur at low concentrations (~ppm levels) in food and agricultural materials. Moreover, the fat content of many of these materials is high, which also makes the extraction and the following collection of fat-soluble vitamins difficult. Basically all different types of collection approaches in analytical SFE have been used for fat-soluble vitamins, including solvent collection [138–141], solid-phase collection [142–147], and collection in an empty vessel [66,148]. However, the latter approach is only suitable if the fat content of the sample is high and the vitamin concentration is not too low.

Solvent collection has not been used as much as solid-phase collection for the analytical determination of fat-soluble vitamins in food and agricultural samples. However, Turner and Mathiasson success-

fully utilized SFE with solvent collection for the determination of vitamins A and E in milk powder [139]. It was found that an equal mixture of ethanol and diisopropyl ether (total volume of 16 ml) gave higher vitamin E recoveries than pure isopropanol, and that a collection temperature of 5 °C resulted in higher vitamin E recoveries compared to 10 and 15 °C. The optimized method gave recoveries of 99 and 96% for vitamins A and E, respectively, when compared to conventional saponification/solvent extraction. In two subsequent publications, considering the determination of vitamins A and E in milk powder and infant formula [140] and in dairy and meat samples [141], 10 ml of ethanol–2-propanol (8:2) and 10 ml of pure ethanol were used for collection, respectively. A smaller volume of a relatively polar solvent or solvent mixture could be used, since lipase-catalyzed hydrolysis was coupled on-line to the extraction, thereby providing sample clean-up as well as transformation of vitamin A esters into vitamin A.

Carotenoids were extracted from freeze–dried carrot tissues using SFE with solvent collection in 10 ml of hexane–acetone (9:1, v/v) containing 0.005% (w/v) BHT [138]. The extraction recoveries were similar to those obtained by conventional solvent extraction.

Marsili and Callahan determined α- and β-carotene in fresh vegetables utilizing SFE with solid-phase collection [142]. It was discovered that when a modifier was used, an ODS trap gave higher recoveries than a trap filled with stainless steel beads. Hexane was used as rinse solvent to quantitatively remove the carotenoids from the ODS trap. Methylene chloride and chloroform efficiently removed the carotenoids from the trap, but resulted in severe degradation. The optimized SFE procedure gave equal or higher recoveries compared to solvent extraction.

β-Carotene, β-cryptoxanthin and zeaxanthin were determined in *Spirulina pacifica* algae employing SFE with collection on an ODS-filled trap [147]. CO_2 modified with 15% of ethanol was used as extraction fluid, and the temperature of the trap was set to 80 °C during the 70 to 100 min dynamic extraction. Different rinse solvents were tested, showing that tetrahydrofuran was more efficient than acetonitrile and hexane. The obtained carotenoid recoveries were very similar to those obtained by conventional solvent extraction.

Berg et al. also employed an ODS trap (HP 7680T system) for the determination of vitamins A and E in minced meat, liver paste, milk and milk powder [146]. In order to avoid breakthrough losses of analytes because of the high fat content of the samples, the trap was rinsed after the 5 min static extraction time, two times during the dynamic extraction (after 1.25 and 20 min, respectively), as well as after completion of the extraction. The trap was held at 90 °C during the extraction. Hexane–dichloromethane (1:1, v/v) was used as rinsing solvent. In the same work, a similar method was applied for the food samples using SFE with solvent collection at 5 °C in 16 ml of ethanol–diisopropyl ether (1:1, v/v) containing 10 mg of palmitoyl ascorbic acid as antioxidant (Isco 3560 system). The obtained results were similar to those obtained by conventional saponification/solvent extraction.

4. Conclusions

The best choice of collection mode in analytical-scale SFE depends on sample and analyte properties, extraction parameters as well as final analysis technique. In general, if the sample contains large amount of fat or water, or if a modifier is used, collection in a solvent or in an empty vessel are usually preferred. If the analytes are volatile, solid-phase trapping offers the most efficient collection. For most other types of samples, any collection mode can be selected. Several different advantages and disadvantages can be recognized in terms of simplicity of optimization and use, selectivity, sensitivity and compatibility with extraction parameters as well as with the final analysis.

The three major types of collection, in a solvent, on a solid-phase trap and on-line coupling to a chromatograph, all require quite extensive optimization procedures. However, solvent collection is probably the simplest system to use, and in many cases also the easiest to optimize. In contrast, solid-phase trapping may offer selectivity in the extraction method by its two-step performance including trapping and chromatographic elution. Highest sensitivity is often obtained using on-line collection, since

the entire extract is introduced into the chromatographic column. However, this approach is mainly applicable on small sample sizes and/or low analyte concentrations. Solid phase collection is normally more sensitive than solvent collection, as it gives more concentrated extracts. Some extraction parameters can if they are extreme require a certain type of collection, e.g., high SF flow-rates work best with solid-phase collection using an adsorbing trap material.

5. Nomenclature

BHT	Butylated hydroxytoluene
CB	Chlorobenzene
CE	Capillary electrophoresis
CWO	Cedar wood oil
DDC	Diethyl dithiocarbamate
DDE	Dichloro diphenyl dichloroethylene
DDT	Dichloro diphenyl trichloroethane
GC	Gas chromatography
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HPLC	High-performance liquid chromatography
LC	Liquid chromatography
MS	Mass spectrometry
ODS	Octadecyl silica
PAH	Polynuclear aromatic hydrocarbon
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzodioxine
PCDF	Polychlorinated dibenzofurane
POP	Persistent organic pollutant
PTV	Programmed temperature vaporizer
SC-CO_2	Supercritical carbon dioxide
SF	Supercritical fluid
SFC	Supercritical fluid chromatography
SFE	Supercritical fluid extraction
SPE	Solid-phase extraction
2,3,7,8-TCDD	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin

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