Supercritical Fluid Extraction as a Successful Technique for Pesticides Estimation

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Abstract

Recently Supercritical fluid extraction (SFE) has been focused as successful sample preparation step for pesticide multiresidue analysis due to its numerous advantages over classical solvent extractions (mainly rapidity, selectivity, low solvent volume requirement). However, developing SFE for pesticides is linked to numerous factors like pressure, temperature inside the cell, static time, flow rate and dynamic time (or fluid volume), volume of modifier added etc. Therefore, adequate knowledge is required for a new user to optimize the extraction performance for every new class of pesticides or new matrix to be analysed. Consequently, extraction parameters need to be carefully chosen. For instance, SFE of polar pesticides is enhanced by modifiers added to the matrix or the supercritical fluid. So this article discusses detail the principal strategies for efficient extractions of pesticides from several matrices.

Key words: Supercritical fluid extraction (SFE), Pesticides, Pressure, Temperature, Matrix, Soxhlet extraction.

Introduction

The extraction process where the extractant is in its super-critical state which means that both the pressure and temperature are above their critical values is called super-critical fluid extraction (SFE). Supercritical fluids possess exceptional properties, in between those of gas and liquids, depend on the temperature, pressure and composition of the fluid (Riekkola *et al.*, 1993). They have lower viscosity and higher diffusivity than that of liquids which allows more efficient extraction. Moreover, the density (and therefore the solvent power of the fluid) may be adjusted by altering both the pressure and temperature that provides opportunity of theoretically performing exceedingly selective extraction (Camel, 2001).

Supercritical fluid extraction (SFE) although not a relatively new technique in the analytical field but is gaining its position over conventional solvent extraction methods in the past few years. This modern technique provides advantages including reduction of organic solvent consumption, faster analysis time, selective in complex matrices extractions, requirement of smaller sample size, being potentially more efficient, easier operation procedure that is, cutting costs (Ono *et al.*, 2006; Camel, 1998). Other attractive features are non toxicity of supercritical fluid (usually CO_2), relatively clear and concentrated extract and possible coupling with chromatography and automation. A typical SFE system mainly consists of

A)   a high pressure pump for delivering fluid
B)   an extraction cell that contains sample
C)   an instrumentation that maintains the extraction cell at desired pressure and temperature,
D)   pump for adding an organic solvent (modifier) to the fluid which increase the solvating properties
E)   a restrictor which maintain the pressure of the fluid along with other apparatus as shown in the above Fig. 1.

The application of SFE for trace levels of pesticides extraction (as detrimental substances) from different matrices is increased in last few years as the awareness and concern of consumers, producers and specially rules and regulations monitoring authorities of food quality control have been heightened (Ono *et al.*, 2006; Aguilera *et al.*, 2003). Increased public concern in the recent years about the health risks from pesticide residues in the diet has been led to strict regulation regarding maximum residue limits (MRL) and total dietary intake of pesticide residues in food stuffs (Rissato *et al.*, 2005). Generally, the concentration of pesticide residues in food is very low while food matrices are quite complex and consequently, the role of sample preparation by appropriate extraction, purification and quantification by sophisticated instru-ments becomes very important. On the other hand, stringent food safety law requires faster

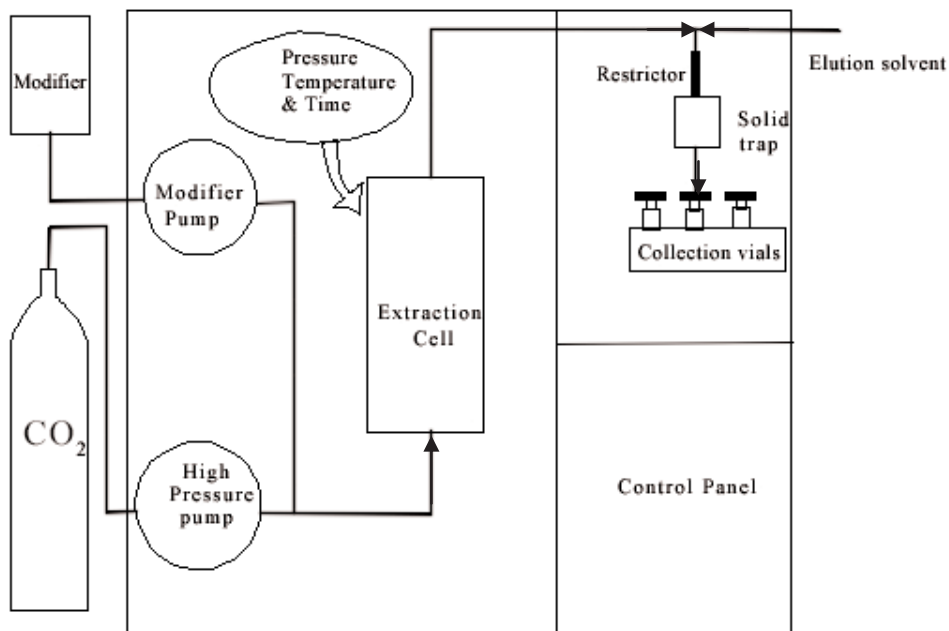* Corresponding author: E-mail: sfmahmood_du@yahoo.com

Fig. 1. Instrumentation of SFE

and more efficient analysis of pesticide residues, as well as, simultaneous analysis of many different compounds (Ono *et al.*, 2006; Rissato *et al.*, 2005a; Rissato *et al.*, 2005b; Aguilera , 2003).

As the analyses of food by GC or HPLC for trace levels of pesticides most frequently requires the removal of high molecular weight interferences such as lipids and natural resins, the extraction process is the first and a major limiting step in these kinds of analyses which often involve sample preparation followed by solvent extraction in conventional methods with all its draw backs like large volume of hazardous solvents consumptions and time utilizations. Sample preparation steps consume two thirds of the analysis time and generate one third of the errors (FAO/OMS; Codex Alimentarius, 1994). So concerns about hazards associated with solvents, their cost, solvent waste disposal, have led to the development of alternative sample extraction method as supercritical fluid extraction. In the area of agrochemicals, SFE has been used for selective extractions of organophosphorus and organochlorine pesticides, carbamates and different herbicides from different matrices. In SFE CO_2 is frequently used as a supercritical fluid due to its suitable critical temperature (31.2 ^{O}C) and pressure (72.8 atm) (Motashi *et al.*, 2000), since it can be easily removed by reducing its pressure and its suitability as an alternative to conventional methods for the extractions of different classes of pesticides from a variety of foodstuffs, including cereals (Kim *et al.,* 1998; Norman *et al.,* 2001; Ohlin *et al.,* 2001), meat

(Argauer *et al.*, 1997; Juhler, 1998) , eggs (Fiddler *et al.*, 1999), honeybees (Jones and McCoy, 1997), baby foods (Chuang *et al.*, 2001) and table ready foods (Aguilera *et al.,* 2003) has also been demonstrated. A CO_2 density of 0.8-0.9 g mL^{-1} appears to be adequate for most pesticides (Motashi *et al.*, 2000). However, one important disadvantage of SFE is the large number of variables to control, which means that the development and validation of SFE methods can be costly and very time-consuming. In addition, SFE of pesticides from the different samples mentioned above show an elevated matrix dependence, and the variables related to the preparation of the SFE sample are, in general, more critical than those affecting the extraction process. Usually, all the SFE methods proposed so far for multiresidue analysis of pesticides in vegetables use very similar extraction conditions (supercritical carbon dioxide at 300-350 atm pressure and 50-60 ^{O}C temperature). The real factors are however the type and amount of material added to the vegetable sample and the presence of water, salts, or modifiers in the SFE sample that determine the effectiveness of the method (Valverde *et al.,* 1996; Lehotay, 1997).

In order to give a concise idea about the general steps of SFE for the analysis of polychlorinated biphenyles (PCBs) and organochlorinated pesticides the following EPA method is illustrated below (Fig. 2):

Determination of pesticides is a challenge for mainly three reasons: the wide variety of physicochemical properties and

chemical structures of pesticides, many possible matrices that should be investigated and the trace concentrations at which pesticides are usually present. Therefore, an attempt is taken in this review article to highlight the optimization strategies, problems and solutions of SFE for pesticide residue analysis to guide the new users of SFE. Especially, this may provide a background knowledge to smoothly intro

polar organic solvents but for very polar and ionic compounds the modifier may be a complexing agent, ion-pairing reagents or a derivatization reagents (Rochette *et al.,* 1993; Luque de Castro and Tena, 1996; Camel, 2001). However, addition of a modifier changes values of critical pressure and temperature that results in a subcritical state not the super
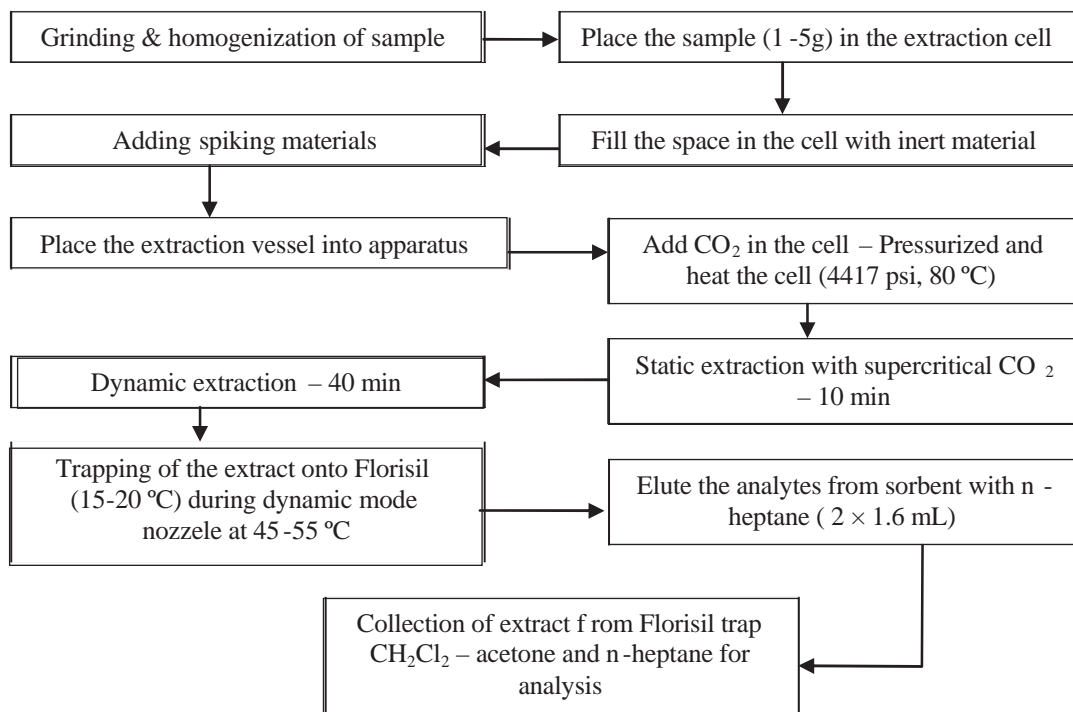
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┌─────────────────────────────────┐      ┌─────────────────────────────────┐
│ Grinding & homogenization of    │─────▶│ Place the sample (1-5g) in the  │
│ sample                          │      │ extraction cell                 │
└─────────────────────────────────┘      └─────────────────────────────────┘
                                                          │
                                                          ▼
┌─────────────────────────────────┐      ┌─────────────────────────────────┐
│ Adding spiking materials        │◀─────│ Fill the space in the cell with │
│                                 │      │ inert material                  │
└─────────────────────────────────┘      └─────────────────────────────────┘
                 │
                 ▼
┌─────────────────────────────────┐      ┌─────────────────────────────────┐
│ Place the extraction vessel     │─────▶│ Add CO₂ in the cell – Pressurized│
│ into apparatus                  │      │ and heat the cell (4417 psi, 80 ℃)│
└─────────────────────────────────┘      └─────────────────────────────────┘
                                                          │
                                                          ▼
┌─────────────────────────────────┐      ┌─────────────────────────────────┐
│ Dynamic extraction – 40 min     │◀─────│ Static extraction with          │
│                                 │      │ supercritical CO₂ – 10 min      │
└─────────────────────────────────┘      └─────────────────────────────────┘
                 │
                 ▼
┌─────────────────────────────────┐      ┌─────────────────────────────────┐
│ Trapping of the extract onto    │─────▶│ Elute the analytes from sorbent │
│ Florisil (15-20 ℃) during       │      │ with n-heptane (2 × 1.6 mL)     │
│ dynamic mode nozzele at 45-55 ℃ │      │                                 │
└─────────────────────────────────┘      └─────────────────────────────────┘
                                                          │
                                                          ▼
         ┌────────────────────────────────────────────────┐
         │ Collection of extract from Florisil trap       │
         │ CH₂Cl₂ – acetone and n-heptane for analysis    │
         └────────────────────────────────────────────────┘
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Fig. 2. Operating procedure of SFE recommended by EPA (Environmental Protection Agency) (Camel, 2001)

duce and adopt this advanced analytical technique in Agro-Research fields of developing country like Bangladesh.

Optimization strategies of super-critical fluid extraction

To optimize the extraction process a new user must have proper knowledge regarding the influencing factors as many parameters alter the extraction efficiencies, and therefore, SFE affords a high degree of selectivity although this makes the optimization quite tedious. Table I summarizes the principle factors that influence the result of extraction and these factors are discussed below:

i) Nature of solutes (Pesticides)

With regard to the nature of the compounds to be extracted, polarity is the characteristics to be taken into account. Pure CO_2 efficiently extracts non-polar to low polarity compounds (Camel, 2001). However, polar compounds require the addition of a modifier to either the supercritical fluid or the matrix to enhance the extraction. The modifiers may be

critical state for extration. In addition, modifier enhances solvating power of the fluid and reduces extraction selectivity and therefore, non target analytes are co extracted (Camel, 2001). Finally, the modifier condenses upon depressurization which results in elution of retained compounds in solid traps as collection device. So addition of modifier should be avoided or minimized whenever possible.

The applicability of SFE to the pesticide multiresidue analysis was evaluated by Ono *et al.,* (2006). In that study they examined 303 compounds and showed good recovery (70 - 120 %) for over 80% of the compounds, which indicated the usefulness of SFE method for sample preparation of pesticide multiresidue and analysis by GC/MS. The extraction conditions where CO_2 density of 0.819 g/mL (17.2 MPa, 40^OC), 15 min static time, 15 min dynamic extraction time with a dynamic flow of supercritical carbon dioxide at 2-3 mL/min for a 10 mL sample cartridge along with acetone as collection solvent was used. As the polar compounds with low K_{0w} (K: distribution coefficient between octanol and

Table I.   Parameters influencing the optimization strategy of SFE (Camel, 2001)

	Parameter	Main effect	Optimization strategy
i) Nature of the solutes	Nonpolar	Solubilization	CO_2
	Moderately polar to nonpolar	Solubilization	CO_2 + organic solvent; other fluids
	Ionic	Solubilization	CO_2 + reagent; ion pairing, complexation, derivatization
ii) Extraction parameters	Pressure	Solubilization	Increase pressure
	Temperature	Desorption; diffusion	Increase temperature
	Time	Extent of extraction	Increase extraction time
iii) Nature of the matrix	Large particles	Low extraction rate; low recoveries	Grinding the matrix
	Active sites	Solute adsorption	Addition of a modifier
	Water content	Water entrainment; low recoveries	Drying/drying agent addition

water), compounds with high K_{0w} and high molecular weight compounds like pyrethloids show lower recovery by supercritical carbon dioxide, they used phosphorous salt mixtures (A mixture of disodium hydrogen phosphate anhydrate and sodium dihydrogenphosphate anhydrate, 7:1), ion pair reagent hexanesulfonic acid sodium salt (HXS) and acetone into the sample. These improves the recovery of most pyrethloids with a range of 70 to 120% while few showed that of 60 -130%. However for polar pesticides namely acephate, methamidofos, acetamiprid and monocrotophos, most recoveries did not reach 70%. Among them methamidofos showed the lowest because of the synergistic effect of its volatility which was also the reason for the low recovery of dichlorvos, butylate, biphenyl, dichlobenil, and 2, 6-dichlorobenzamide as evaporation from acetone took place when collecting extracts during the dynamic extraction. Therecovery of chlorothalonil, captan, captafol and dichlorofluanid was also less than 70%. Low recovery seems to have been caused by decomposition within the sample. (Ono et al., 2006)

In all cases, recoveries obtained without modifier were less than those obtained using methanol as modifier, except for vinclozolin, dichlorvos, and pyrazophos, pesticides for which the mean recoveries obtained without modifier were slightly higher (Aguilera et al., 2003). The two very polar phosphoramidothioate pesticides, methamidophos and acephate, gave poor recoveries in absence of modifier (<30%). On the other hand, recoveries obtained by using ethyl acetate as modifier were, in general, slightly higher than those obtained with methanol, except again for the most polar pesticides methamidophos and acephate, for which recoveries >70% were only obtained by using methanol as modifier.

Rissato et al., (2005) in their study for the development of a supercritical fluid extraction method for simultaneous determination of organophosphorous, organohalogens, organonitrogen and pyretroids pesticides in fruit and vegetable samples like apples, lettuces, potatoes and tomatoes use the extraction conditions of $70^{O}C$ at different pressures 19971, 44935, and 69898 kPa with a flow rate of 1.5 mL/min for CO_2 or CO_2 modified with 10% of acetone and methanol. The solvating power of pure CO_2 was too low for exhaustive extraction of the pesticides investigated which reveals that pesticides such as tetradifon, etaconazole, hexaconazole, imazalil, metolachlor, prochloraz, propico-nazole, triadimenol, chlorpyrifos, diazinon, dichlorvos and dimethoate were not satisfactorily detected from some matrices or their recovery results ranged from 8 to 26%. However, the best results were obtained for organohalogens, some organonitrogens and pyrethroids with recoveries between 36 and 65%, due to the physicochemical properties of these compounds (Rissato et al., 2005). As expected, the increase in the addition of the modifier, and consequently the fluid strength, was beneficial for the extraction of most pesticides. However, the effect of the modifier in natural matrices is varied, for instance some pesticides such as bromo-propylate, chlorothalonil, endosulfan, tetradifon, buprofezin, metolachlor, dicloran chlorpyrifos, dichlorvos and dimethoate, presented maximum recoveries using acetone, whereas, under the same conditions, aldrin, dicofol, hexachlorobenzene, imazalil, trifluralin and diazinon showed the presence of co-extractives (recoveries above 140%). The best result, for the studied samples, was accomplished using CO_2 modified with 10% methanol, presented above 50% of recoveries and no result of coextractive was observed. Thus, evidently the addition of 10% methanol increases the extraction efficiency.

ii) Extraction parameters (pressure, temperature & time)

The choice of pressure and temperature in SFE directly affects the selectivity, which is the main advantage of SFE over solid liquid extraction methods. The pressure (density) of the extracting fluid is normally of great importance in the determination of the solubility of the analytes in many matrices. In 2005 Rissato *et al.,* studied the combination of three different pressures (19971, 44935 and 69898 kPa) to check the pressure behavior. They reported that pressure up to 44935 kPa increased the recovery rate of pesticides compared to the 9971 kPa initial pressure. The recoveries obtained were higher than 81% for all matrices studied by them and for 69898 kPa no significant increase in recovery was observed. Consequently, it is believed by the analytical application chemists that if an analyte is very soluble in supercritical fluid at a low density, this solubility will increase or remain the same at higher densities, or pressures. The combination of both effects of modifiers and pressures lead to the best results in the multiresidue pesticide analysis in fruit and vegetables (Rissato *et al.,* 2005 a). In general, for polar compounds the addition of modifier to directly to the matrix (before extraction) may help in altering the analyte-matrix interaction in place where static extraction should be carried out first so that sweeping the modifier out of the cell can be prevented and thus resulting enhanced extraction (Camel, 2001). In cases where the analytes do not readily derivatized, addition of a derivatization agent that can react with active sites of the matrix to enhance extraction can be useful. However, the use of modifier to CO_2 in SFE has some severe drawbacks so it should be avoided or minimized as far as possible. The presence of modifier alters the value of the critical pressure and temperature as too high a modifier content may yields temperature lower than the critical value called subcritical state with higher viscosity and lower diffusion coefficients than the supercritical state. The modifier also increases the solvating power of the fluid which reduces the selectivity of extraction as more matrix materials or non-target analytes are co-extracted. Lastly, the modifier condenses upon pressure release that results in elution of the retained compounds when a solid trap is used as collection device, since then it may behave somewhat like a chromatographic device (Camel, 2001).

Elevated temperatures usually increase recoveries of compounds in native matrices, mainly due to a better desorption from the matrix (Bowadt and Hawthorne, 1995; Hawthrone and Miller,1994). Moderate temperatures should be used whenever possible as some pesticides readily degrade at high temperatures and imposes negative effect on fluid density. Study by Aguilera *et al.,* (2003) reported that increasing the extraction temperature from 50 to 90 ^{O}C or the extraction

pressure from 300 to 500 atm while keeping other variables constant did not have a remarkable influence on the recovery of the studied pesticides (all the normalized mean recoveries obtained at 90 ^{O}C, or at 500 atm, were within the range 0.85-1.15).

Camel (1998) reported that pesticides that are difficult to extract may be improved by applying a short period of static time. The dynamic time is a measure of the total volume of fluid that percolated through the cell (determined by flow rate). Pesticides that are hardly extracted (i.e polar or compounds that strongly interact with the matrix) require large volumes (more than 4 vessel volumes) of extraction fluid.

iii) Nature of the matrix

The characteristic of the matrix (water content, percentage of organic carbon, humic materials, etc) and its physical nature (particle size or porosity) are of supreme importance for the successful achievement of extractions. As extraction is strongly dependent on the matrix which requires new optimizations each time when a new matrix is considered. High water content may prevent the extraction of non polar compounds which may also result in plugging of the restrictor so addition of drying agent to the matrix may be advisable. Lower organic carbon content in any matrix yields satisfactory extracts than with sample having higher organic carbon content for same operating conditions. In addition, the particle size of the matrix influence the extraction efficiency and grinding the sample before the extraction is highly recommended to limit the diffusion step inside the matrix and to increase the surface area, which increase the rate of extraction when it is limited by matrix effect. However, very fine particles may be swept out of the cell by the fluid and result in plugging and mechanical transfer problem (Camel, 2001).

The influence of oil content, modifier, temperature, pressure, CO_2 volume and gazpacho-Magnesium sulfate ratio on the extraction of 17 pesticides in food item (gazpacho) was studied by Aguilera et al, 2003 which showed that lower oil content results higher extraction of pesticides. They also reported that addition of small amount of drying agent (anhydrous magnesium sulfate) in the gazpacho-magnesium sulfate mixture (SFE sample) resulted higher recoveries for dichlorvos, pyrazophos, and vinclozoline (normalized recoveries 1.4-1.5), but lower recoveries were obtained for chlorothalonil, chlorpyrifos-methyl, and procymidone (normalized recoveries 0.5-0.6) (Aguilera *et al.*, 2003).

iv) Cleaning up of extracts

Clean up is necessary in order to reduce the detection limits of methods and /or to avoid interferences from the matrix.

When SFE is applied to fruit and vegetable samples, it usually provides clean extracts which can be directly analyzed by gas chromatography with out clean up steps (Anastassiades and Schwack, 1998; Eller and Lehotay, 1997). However, as SFE is an excellent method for extracting fats from various matrixes (Perretti *et al.,* 2004; Johnson and Barnett, 2003) when it is used for pesticide analysis of lipid containing samples, large quantities of lipid co-extractives can often accompany the target analytes of interest due to the substantially high solubility of lipids in supercritical carbon dioxide. Because of their high boiling points and molecular weights, many lipid species are difficult to elute under conventional gas chromatographic conditions. Hence, the lipid moieties tend to accumulate in a GC injector port, resulting in highly irregular chromatographic profiles. There are various ways that can be selected to reduce the interference of lipid co-extractives in GC assays. Cleaning extracts via gel permeation chromatography (GPC) or by SPE columns packed with different sorbent materials such as Florisil, graphited carbon black, or aminopropyl are the general approaches for purification (Schenck and Donoghue, 2000; Hengel and Shibamoto, 2000; Poustka *et al.,* 1995; Norman and Panton, 2001). Alternative cleanup techniques, such as matrix solid-phase dispersion and even the use of binary gas mixtures for SFE, have been employed by researchers in SFE extraction of products of animal origin. Also, "in-line" trapping methods have been shown to be very selective which was applied for pesticides and involves placing a sorbent between the sample and restrictor so that the lipids are retained and the pesticides are eluted in supercritical CO_2. Aguilera *et al.*, (2005), evaluated the SFE and the use of aminopropyl solid-phase material for "in-line" cleanup for residue analysis of 22 GC-amenable pesticides in wild- and white-rice samples with a fat content of 1.9 and 0.4%, respectively. Aminopropyl material is a superior fat-retention material for 'in-line' cleanup to other sorbents like Florisil, Celite, Extrelut, Hydromatrix. Their study showed that pesticide mean recoveries obtained from rice samples, at fortification levels around 0.5 mg/kg, by means of the SFE/in-line cleanup method (15-mL CO_2 volume, 50 ^{O}C temperature, 200 atm pressure, 200 µL of methanol static modifier, and a 1-cm layer of aminopropyl at the bottom of the extraction vessel), ranged between 74 and 98%, except for captafol and dimethoate for which mean recoveries lower than 21%.

In order to establish a screening method for the determination of 27 pesticides (Thiabendazole, carbaryl, imazalil, iprodione, hexaflumuron, chlorfluazuon, ethofemprox, imibenconazole metabolite, furametpyr, ben-furesate, pyrazoxyfen, methabenzthia-zuron, inabenfide, myclobutanil, ethobenzanid, pencycuron, buprofezin, dymron, tebufenozide, clofentazine, flufenoxuron, diflubenzuron, triflumizole and fenpyroximate) in fresh fruits and vegetables (potato, radish, cucumber, apple, banana) by SFE coupled with cartridge column clean up followed by estimation by HPLC, Kaihara *et al.,* (2000) performed their study and established method for qualitative and quantitative determination. As the wet samples such as fruits and vegetables were not suitable for the SFE instrument, so in their study the water in the samples was removed with an absorptive polymer (Arasorb$^®$ S-310) prior to SFE. The pesticides were extracted by SFE, the extracts trapped with Extrelut$^®$ NT + Bond Elut$^®$ C_{18} and then eluted with acetonitrile. The eluate was cleaned up with Sep-Pak$^®$ Florisil+Bond Elut$^®$ PSA cartridges. After washing with n-hexane, the pesticides were eluted from the cartridges with 15% ether/n-hexane, 15 and 50% acetone/n-hexane 20 ml for each solvent mixture. The pesticides spiked in samples at 0.5 ppm level showed satisfactory recovery except for thiabendazole, imazalil and clofentazine. However, in their latter study (Kaihara *et al.,* 2001) for multi residue analysis of 18 pesticides (Imazalil, iprodione, hexaflumuron, chlorfluazuon, furametpyr, ben-fure-sate, pyrazoxyfen, methabenzthia-zuron, inabenfide, myclobutanil, pencycuron, buprofezin, dymron, tebufenozide, flufenoxuron, difluben-zuron, triflumizole & fenpyroximate) from rice along with similar samples of previous study by liquid chromatography - electrospray ionization mass spectrometry (LC/MS (ESI), they presented higher recovery and detection limits for all the pesticides than the earlier work. In the final step of purification, the pesticides were sequentially eluted with 50 ml 50% acetone/n-hexane instead of 15% ether/n-hexane, 15 and 50% acetone/n-hexane.

Pesticides extraction using SFE from different matrices

Supercritical fluids for extraction started since mid-1980s and up to now numerous applications of this technique have been reported. A recent study by Cortés *et al.*, (2008) showed that 5 different pesticides (Dimethoate, diazinon, fenitrothion, chlorpyrifos and methidathion) were successfully extracted using SFE and analysed by RPLC-GC in lycopene and other carotenoids obtained from tomatoes. Tomatoes grown in fields are usually treated with different pesticides and these samples are used to extracts carotenoids and lycopene for application as nutritional supplements, in the cosmetic and pharmaceutical industries. Based on result Cortés *et al.,* (2008) suggested that pesticide free tomatoes must be used in this purposes.

Rodil *et al.,* (2007) optimized a simultaneous supercritical fluid extraction and clean up procedure using aluminium oxide basic and acidic silica gel in the supercritical extraction cell for determination of 15 organohalogenated pollutants (organochlorinated pesticides, polychlorinated biphenyls, brominated flame retardants) in aquaculture samples.

Decontamination of organochlorine pesticides (OCPs) in *Radix codonopsis* (a Chinese herbal medicine) by SFE followed by estimation by GC was studied by Zhao *et al.,* (2006) as OCPs possess a long half-life, accumulate extensively, exhibit potential harmful biological effects and have detrimental effects on the environment. The studied pesticides were α-, β-, γ- and δ-benzene hexachloride, PCNB (pentachloro-nitrobenzene), PCA (pentachloroaniline), HEPT (heptachlor), MPCPS (methylpentachloro-phenyl sulfide), pp©DDE [1,1-dichloro-2, 2-bis(p-chlorophenyl) ethylene], op © DDT [1,1,1-trichloro-2-(o-chlorophenyl)-2-(p-chlorophenyl)ethane], pp © DDD [1,1-dichloro-2-2-bis(p-chlorophenyl)ethane] and pp©DDT [1,1,1-trichloro-2,2-bis(p chloro-phenyl)ethane]. The optimized condition for the extraction was pure CO_2, extraction pressure 15 MPa, extraction temperature 60^OC, extraction time 20 min and

flow rate 55 mL/h and showed at least 93.5% removal of the organochlorine pesticide residues in the herb sample.

The parameters of multiresidue SFE methods for the determination of various pesticides is presented below in tabular form (Table -II)

Comparative performances of SFE and conventional Soxhlet extraction

SFE has gained increased attention as a potential replacement for conventional liquid solvent extraction (sonication or soxhlet) owing to the properties of supercritical fluids. In a comparative study by Crespo and Yusty, (2005) between SFE and conventional Soxhlet extraction for determination of polychlorinated biphenyls (PCBs) in seaweed samples revealed that despite being combined with a clean up step, SFE was much faster than Soxlet method, required amount of high purity solvent was lower with cleaner extracts that made possible the determination PCBs at lower concentrations. SFE with optimised parameters is able to yield extractions with sufficient accuracy and precision to be a significant alternative to conventional methods for the analysis of

Table II. Multiresidue SFE methods for the determination of various pesticides (organochlorine, organophosphorus, organonitrogen, pyrethroid, others) in food matrices : (Camel, 1998).

Matrices	Sample preparation	Extraction	Collection/analysis
Spiked apples	Celite and Na_2SO_4 addition	CO_2 45 ^{O}C, 189 bar	C_{18} silica Hexane /acetone 1:1 elution
Spiked apples	Frozen sample mixed with $MgSO_4.H_2O$and Hydromatrix CH_3OH addition	CO_2 50 ^{O}C, 320 bar	C_{18} silica CH_3CN elution GC/ITD (Ion trap detector)
Tomatoes	Freezing Callulose CF-1 addition	CO_2 50 ^{O}C, 350 bar	C_{18} silica Acetone elution GC/ITD
Spiked oranges, sweet potatoes, green beans	Frozen sample mixed with $MgSO_4.H_2O$ and Hydromatrix	CO_2 50 ^{O}C, 350 bar	C_{18} silica Acetone elution GC/ITD
Grapes, carrots, potatoes, broccoli	Grinding Mixing with Hydromatrix	CO_2 60 ^{O}C, 320 bar	C_{18} silica CH_3CN elution GC/MS
peaches, oranges, potatoes	Grinding Mixing with Hydromatrix and dry ice	CO_2 60 ^{O}C, 320 bar	C_{18} silica CH_3CN elution GC/MS
Spiked strawanerries	Grinding Mixing with Na_2SO_4	CO_2 +10% acetone/CH_3OH 75 ^{O}C, 440 bar	Silanized glass brads - hexane elution GC/ECD
Spiked wheat grains	Grinding	CO_2 40 ^{O}C,350 bar	Florisil Acetone elution GC/ECD
peppers, tomatoes, cucumbers	Mixing with $MgSO_4$ CH_3OH addition	CO_2 50 ^{O}C, 300bar	Ethyl acetate GC/FPD or GC/ ECD

Table III. Comparison of Soxhlet & SFE performances for the extraction of Polyaromatic hydrocarbons (PAHs) (Camel, 2001)

Technique	Solvent	Conditions	Time	Further treatment applied	Extraction results
Soxhlet	Hexane-acetone	10 g sediment; 300 mL solvent	18 h	Concentration	50.3-161%
SFE	CO_2 + 10% CH_3OH	5 g sediment; copper filings; 450 bar, 120 ^{O}C; 1-1.5 mL min^{-1} collection: CH_2Cl_2	60 min	None	31.7-171%
Soxhlet	CH_2Cl_2	10 g soil + 10 g Na_2SO_4; 150 ml solvent	24 h	Concentration	Total 1623 mg kg^{-1}
SFE	CO_2 + 20% CH_3OH	1 g soil; 250 kg cm^{-2}; 70ºC; 2 mL min^{-1} collection: CH_2Cl_2	5 min static + 30 min dynamic	Concentration	Total 1544 mg kg^{-1}
Soxhlet	CH_2Cl_2	10 g soil + 30 g Na_2SO_4; 100 ml solvent	6 h + cooling	None	Total 297.4 mg kg^{-1}
SFE	CO_2 + 20% CH_3OH	1 g soil; 250 kg cm-2; 70°C; 1 mL min^{-1} collection: CH_2Cl_2	5 min static + 60 min dynamic	None	Total 458.0 mg kg^{-1}

Table IV. Comparison of the SFE with the traditional Soxhlet extractor. (Camel, 2001)

Techniques	Time	Solvent volume	Sample size	Cells	Filtration required	Investment
SFE	10-45 min	2-5 mL (solid trap); 15-60 mL (liquid trap)	1-5 g	1-2	No	High
Soxhlet	6-24 h	100-500 mL	1-50g	1	No	Very low

PCBs in seaweed samples. Quan *et al.*, (2004) in their study on SFE and clean-up of organochlorine pesticides in ginseng also concluded with aforesaid inference. To illustrate the extraction performances of these two techniques in pesticide analysis in terms of extraction conditions, time and further treatment required comparisons are presented for several applications Table III-IV.

Conclusion

Pesticides are necessary and essential in agricultural production. The analysis of trace levels of pesticides in food frequently requires the removal of high molecular weight interferences such as lipids natural resins before the analysis by GC or HPLC. The extraction process is therefore the first and a major limiting step in the pesticide residue analysis. In recent years, SFE is incorporated as a successful analytical technique which minimizes environmental concern, time, labor and exposure of laboratory technicians to toxic chemicals. SFE simplifies the sample preparation step and acts as an alternative solvent intensive isolation procedure due to its

ability to change solvent conditions and by controlling polarity, temperature and pressure (Rissato *et al.*, 2005a). It is also a potential technique for extracting mobile or bound residues. SFE showed to be efficient and sensitive for a wide variety of matrices and can easily be modified to accommodate more compounds. For instance, scientific papers regarding successful extraction of wide variety of pesticides like organohalogen, organonitrogen, organophosphorous, pyrethroid, triazines, thiocrbamates, triazoles, phenoxy-acetic and benzoic acid, organotins etc from different matrices using SFE are reported.

As legislation will be inclined to restrict or even ban the utilization of many common hazardous solvents, SFE will in future undoubtedly supersede the traditional methods. However, due to high investment cost of this technique (Table IV), financial considerations may affect the choice of extraction technique. In this regard, Soxhlet extraction as a reference method may sustain its applicability for numerous applications. However, there is a crucial demand for the approvals of a well develop recent technique which simply

reduces hazardous and costly solvent consumption in labora-tories and time devoted to sample preparation. This proposed method could be incorporated to the routine analysis of pes-ticides for agro based countries like Bangladesh

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