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Review

Pressurized liquid extraction in the analysis of food and biological samples

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Abstract

Originally, the use of the pressurized liquid extraction technique (PLE) was mainly focused on the extraction of environmental pollutants present in soil matrices, sediments, and sewage sludge. However, more recently the distinct advantages of this technique are being exploited in diverse areas, including biology, and the pharmaceutical and food industries. The aim of the present review is to explore recent analytical applications of this extraction technique (PLE) in the extraction of contaminant compounds and matrix components in food and biological samples, placing special emphasis on the strategies followed to obtain a rapid, selective, efficient and reliable extraction process.

Keywords: Review; Pressurized liquid extraction; Food samples; Biological samples; Contaminants; Matrix components

Contents

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1. Introduction

Owing to the complexity of the sample matrix, the analysis of food involves several important difficulties. Analyses of solid and semi-solid food and biological samples are at a disadvantage with respect to those associated with liquid samples, in which they usually require fewer pretreatment steps, owing to their liquid form. The traditional extraction method for the determination of a wide variety of compounds in this kind of sample is Soxhlet extraction. However, Soxhlet methods may be sometimes inefficient and slow, and they may consume large quantities of organic solvents. In an attempt to overcome these limitations, in recent years several other extraction techniques have been developed. Among them, microwave-assisted extraction (MAE), supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) have recently been under intensive study, good efficiency and reliability being reported.

Pressurized liquid extraction is a sample preparation technique that combines elevated temperature and pressure with liquid solvents to achieve fast and efficient extraction of the analytes from the solid matrix. The use of higher temperatures implies a reduction in solvent viscosity, thereby increasing the solvent's ability to wet the matrix and to solubilize the target analytes. Temperature also assists in breaking down analyte–matrix bonds and encourages analyte diffusion to the matrix surface. PLE has been shown to have significant advantages over competing techniques as regards time saving, solvent use, automation and efficiency. For example, PLE has an advantage over MAE in that no additional filtration step is required, since the matrix components that are not dissolved in the extraction solvent may be retained inside the sample extraction cell. This is very convenient for the purposes of automation and on-line coupling of the extraction and separation techniques.

Since the introduction of the first commercial PLE instrument a few years ago, application of this technique has focused on the extraction of environmental pollutants present in soil matrices, sediments, sewage sludge and fly ash, and some reviews have been published summarizing the main applications of PLE in this field [1–3]. More recently, however, the distinct advantage of PLE, such as a significantly reduced extraction time and the low solvent volumes required, are being exploited in different areas, including biology, ant the pharmaceutical and food industries. Thus, the number of papers addressing PLE in this field has increased considerably, as can be seen in Fig. 1.

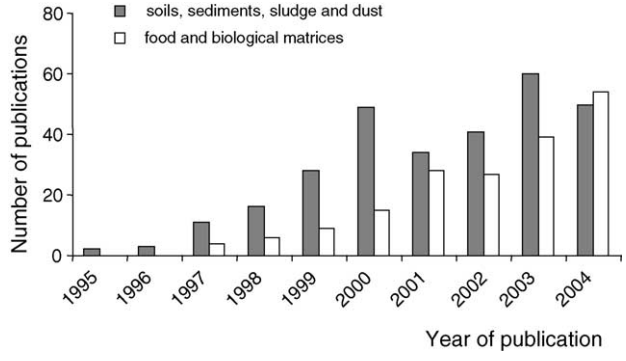

Fig. 1. Growth in the number of publications on pressurized liquid extraction (PLE) over the period 1995–2004.

The aim of the present review is to explore the most recent analytical applications of this technique (PLE) in the extraction of contaminant compounds and matrix components prior to their determination.

2. Pressurized liquid extraction

This technique, which involves extraction with solvents at a high pressure and temperature without their critical point being reached, has received different names, such as accelerated solvent extraction (ASE), pressurized fluid extraction (PFE), pressurized liquid extraction (PLE), pressurized hot solvent extraction (PHSE), high-pressure solvent extraction (HPSE), high-pressure, high temperature solvent extraction (HPHTSE) and subcritical solvent extraction (SSE). The use of these different terms may lead to confusion and here we use the term PLE, which is the most widely accepted designation, even though since 1996 the EPA has adopted the term PFE to refer to this technique in Method 3545. When water is employed as the extraction solvent, the authors tend to use a different name to highlight the use of this environmentally-friendly solvent. Thus, terms such as subcritical water extraction (SWE), hot water extraction (HWE), pressurized hot water extraction (PHWE), high-temperature water extraction (HTWE), superheated water extraction or hot liquid water extraction can be found in literature. Nevertheless, it is important to note that although referring to the same technique, in this case water is employed instead of another organic solvent. The dramatic changes in the physical–chemical properties of water, especially in its dielectric constant (ε), at elevated temperatures and pressures enhance its usefulness as an extraction solvent. The dielectric constant (as a measure of the polarity of the solvent) is a key parameter in determining solute-solvent interactions, and – in the case of water – increasing the temperature under moderate pressure can significantly decrease this constant. At ambient pressure and temperature, water is a polar solvent with a high dielectric constant ($\varepsilon = 78$) but at 300 °C and $P = 23$ MPa this value decreases to 21, which is similar to the value for ethanol ($\varepsilon = 24$ at 25 °C) or acetone ($\varepsilon = 20.7$ at 25 °C). This means that at elevated temperatures and moderate pressures the polarity of water can be reduced considerably and the solvent (i.e. water) can act as if ethanol or acetone were being used. The main effect of this drop in the dielectric constant when working at elevated temperatures and pressures is that water can be used instead of another organic solvent to extract medium- or low-polarity compounds.

Pressurized liquid extraction – with either an aqueous or an organic solvent – can be accomplished in the static mode,

the dynamic mode, or a combination of both. In the static mode, sample and solvent are maintained for a user-specified time at constant pressure and temperature, whereas in the dynamic mode the solvent flows through the sample in a continuous manner. However, the different names used for this technique fail to indicate which mode is used. Because in most cases the dynamic mode uses water as extractant, several authors have preferred to use the term PHWE to refer to dynamic extraction with water as solvent, and the term pressurized liquid extraction with water to refer to static extractions with water. Several studies have shown that a combination of both extraction modes can result in improved extraction [34,44,108,114].

A more detailed description of the basic principles of PLE and the influence in the extraction process of different parameters that affect performance – such as sample size and composition; the nature, volume and flow of the solvent; temperature, extraction time, the number of cycles, and pressure – is available in number of excellent review articles in the literature [2,4] and these are therefore not discussed here. Thus, the aim of this review is to discuss the most relevant aspects of PLE processes developed in the fields of biology and food science, placing special emphasis on the strategies followed to obtain a rapid, selective, efficient and reliable extraction process.

3. Pressurized liquid extraction process

3.1. Pre-treatment of the sample

Prior to loading in the extraction cell, the sample is often pretreated in some way. Pre-treatment usually involves sieving or grinding of the sample because the diffusion of analytes from the sample to the solvent extract can be increased considerably by decreasing particle size. Drying the sample is also important, since any moisture in it may diminish extraction efficiency. This step is especially important when non-polar solvents are to be used in extraction, and it is usually accomplished by incorporating a desiccant into the PLE cell. Drying agents such as sodium sulphate, diatomaceous earth or cellulose have frequently been employed for this purpose. Other alternatives that have been used to dry samples include vacuum ovens, freeze-drying or lyophilization, although these are not recommended in the case of extraction of volatile compounds. The use of more polar solvents (acetonitrile, methanol, ethyl acetate, etc.) or solvent mixtures (hexane/acetone, hexane/acetonitrile, etc.) can assist in the extraction of wet samples, making this drying step less crucial. Despite good selection of the drying agent, some water may sometimes be co-extracted, thus interfering in later steps (clean-up, extract concentration, or direct analysis). In this case, some authors have proposed direct addition of anhydrous sodium sulphate to the collection vial [35,37]. The extraction of wet samples using water as the extraction solvent is very useful because, in some cases, it permits the elimination of this drying step, thereby minimizing sample pre-treatment [42–44,76,78,107,108,111,113,115,116].

In order to avoid the aggregation of sample particles that might alter extraction efficiency, PLE often requires dispersion of the sample with an inert material. Diatomaceous earth or sand have been the dispersing agents most frequently used in PLE, although other inert dispersion media such as high-density glass beads (Filter Aid), soda lime glass beads (Q-Beads) or Teflon particles have been tested in the extraction of arsenicals from standard reference materials for fish [56].

3.2. Optimization of the extraction process

Optimization of the extraction process generally begins with an appropriate choice of the extraction solvent. Often, the same solvent used for conventional extractions, such as Soxhlet extractions, is initially tested in pressurized liquid extraction.

The extraction solvent must be able to solubilize the analytes of interest, minimizing the co-extraction of other matrix components. When choosing the extraction solvent, it is also important to take into account the compatibility with the later treatment steps (extract clean-up, target analyte preconcentration, or the analytical technique), as well as the volatility of the solvent if extract concentration is necessary.

The polarity of the solvent should be close to that of the target compound. Thus, non-polar and water-immiscible solvents such as hexane, pentane, etc. or a combination of non-polar with medium-polarity solvents, such as pentane/dichloromethane or cyclohexane/ethyl acetate, have frequently been used in the extraction of apolar and lipophilic compounds. On the other hand, more polar solvents, such as acetonitrile, methanol, ethyl acetate or water, have been employed in the case of polar and hydrophilic compounds. Mixtures of low- and high-polar solvents generally provide more efficient extractions than single solvents when analytes with a wide range of polarities are extracted. Some authors have developed another strategy to obtain the selective extraction of analytes with a broad range of polarities; this consists of carrying out two PLE extractions: one with a non-polar solvent, to extract the less polar compounds, and the second one with a more polar solvent to extract the more polar analytes [89,103].

In static mode, pure organic solvents or solvent mixtures have been used. Less frequently, water has been used as a single extraction solvent in the static mode [53,54,66], although it is the extractant most frequently used in the dynamic mode [49,67,76,78,83,96,97,106,107,110,111,113,115,116].

Some authors have managed to improve the extraction process by adding modifiers to the extraction solvent. As an example, water modified with a surfactant – sodium dodecyl sulphate (SDS) – has been used as a solvent to extract PAHs from fish tissues [19] or to extract ginsenosides at low temperatures [85]. Surfactants have the ability to form micelles that can solubilise different compounds, including

very hidrophobic analytes, without the need for any strong increase in temperature. Water can also be modified with certain organic solvents such as methanol, acetone or acetonitrile in low proportions in order to decrease its dielectric constant and hence its polarity [40,42,43,52,55]. The addition of an acid or a base has been employed by some authors to alter pH and improve extraction yields [65]. Modifiers are used less frequently with solvents other than water [57,59,60].

Optimization of the extraction conditions (sample size, sample particle size, volume, the nature and flow (in the case of the dynamic mode) of the solvent, temperature, extraction time, the number of cycles, and pressure) is normally accomplished using the classical one-variable-at-a-time method, in which the optimization is assessed by systematic alteration of one variable while the others are kept constant. However, regarding the determination of interactions between parameters and finding the most suitable PLE conditions, minimizing the number of experiments, some authors have recently reported the use of "experimental design" for this purpose. In the experimental design strategies the values of all the factors under study are varied in each experiment in a programmed and rational way. It is thus possible to detect the influencing factors while the number of trials can be kept to a minimum. For example, optimization of the extraction of seven PCBs from a naturally contaminated fish meal and two feed samples by means of a factorial design has recently been reported by Holst et al. [18]. The variables studied were extraction solvent, extraction temperature and flush volume. Pallaroni and von Holst [69] also applied a statistical design approach to evaluate the influence of several extraction parameters – such as temperature, time, and the solvent extraction mixture – on the extraction of a mycotoxin (zearalenone) from cereals.

Among the variables affecting PLE, the nature of the extraction solvent and temperature generally have a profound effect on the PLE process. However, several studies have shown that pressure is usually a minor variable for the resulting efficiency and that it is only required to maintain the extractant in the liquid phase.

3.3. Clean-up and enrichment of the extract

In spite of a good optimization of all the extraction parameters to obtain a selective PLE method, high molecular weight matrix components such as lipids, pigments, or resins are frequently present in the extract and must be eliminated to minimize adverse effects affecting the detection of compounds of interest. Thus, the removal of co-extracted matrix components is necessary and for this, different clean-up procedures have been developed.

Co-extractives are frequently removed during the post-extraction clean-up steps, although in order to offer a fast and efficient connection between extraction and analysis attention is currently focused on the automation of the clean-up step and on the developed of in situ clean-up steps during extraction. Commonly used post-clean-up procedures include adsorption columns or gel-permeation chromatography (GPC). Traditionally, adsorption columns using florisil, neutral alumina, or silica gel have been applied, especially in the case of fatty samples in which it is necessary to remove co-extracted lipids. However, gel-permeation chromatography (GPC) – sometimes referred to as size-exclusion chromatography (SEC) – is becoming increasingly popular, mainly because it can be readily automated. In GPC, separation is accomplished on the basis of molecular size, and has the advantage that the column can be used over several months with no effects on clean-up capacity; accordingly, the procedure can be fully automated. Divinylbenzene-linked polystyrene gel (Bio Beads SX-3) is the most widely used material for GPC.

Sulphuric acid-impregnated silica gel columns have been also used for the destructive removal of lipids and other oxidizable components from biological and food samples. The cleaning-up of fatty samples is very tedious and time-consuming, and sometimes more than one step is required to remove lipids (e.g. GPC and silica columns [27], sulphuric acid-impregnated silica gel and alumina columns [10]; acid, basic and neutral silica columns [15], etc.).

In order to avoid the exhaustive clean-up of extracts prior to analysis and to increase the possibilities of automation, recent reports have focused on the development of in situ clean-up methods. In the case of fatty samples, in situ elimination of lipids can be achieved by adding fat-retaining sorbents such as florisil, alumina, silica gel, 2,3-dihydroxypropoxypropyl, cyanopropyl-bonded silica or sulphuric acid-impregnated silica gel to the PLE cells, preventing lipids and other co-extractable materials from coming out in the extract.

The matrix solid-phase dispersion technique (MSPD) has also been employed by some authors as in situ clean-up procedure in the extraction of trace compounds from kidney [40,63], bovine milk [49,67], fruit and vegetables [36], meat and infant food [66], and medical food [131]. During the MSPD procedure, the sample matrix is mixed with an appropriate material and the mixture is ground until total disruption of the sample matrix. The matrix solid-phase dispersion technique can be adjusted to retain particular compounds by choosing an appropriate dispersion material in addition to using a specific eluent. Most applications have utilized C_{18}, although recent applications have demonstrated that clean extracts can be achieved with a cross-linked acrylic polymer (XAD-7 HP), which is able to retain lipid components (fatty acids, sterols and triglycerides) in addition to proteinaceous matter.

Other way to minimize matrix interferences in sample extracts has been reported by other authors [51,61,64,94]; it consists of application of a preliminary PLE extraction with a non-polar solvent in order to eliminate the hydrophobic compounds present in the sample (fat, resins, oils, chlorophylls, etc.) before the extraction of the compounds of interest.

The use of the PLE in the extraction of trace compounds generally involves the preconcentration of the trace compounds present in the extract and hence the clean-

up procedures also frequently serve as analyte-enrichment techniques. The main techniques used are liquid–liquid extraction (LLE) [34], solid-phase extraction (SPE) [19,21,31,39,47,52,54,55,64,99] including immunoaffinity-based solid-phase material [71] and ion-exchange materials [68,73], solid-phase microextraction (SPME) [40,42,43] or spin-bar sorptive extraction (SBSE) [42,43]. This clean-up and preconcentration step is especially necessary when PLE in the dynamic mode is carried out, because higher extract volumes are obtained and the analytes are diluted in the liquid extract. After dynamic extraction, the analytes are generally pre-concentrated by liquid–liquid extraction [76,78,106–108,110,111,114] or using solid-phase extraction [86,96,113]. Solid-phase extraction is the most widely used preconcentration technique in this mode because it can be coupled on-line to the extractor outlet, affording excellent results.

4. Analytical applications

In the analysis of food and biological samples, the PLE technique has been used for two main purposes: the extraction of contaminants and the isolation of matrix components.

In the next section, the experimental conditions for PLE procedures implemented in food and biological samples will be reviewed, and the results and drawbacks will be discussed. In an attempt to simplify this review, and in view of the different PLE treatments used, contaminants and matrix components will be dealt separately.

4.1. Contaminants compounds

Different applications of PLE for the determination of contaminant compounds in a variety of food and biological samples are presented in Table 1.

4.1.1. Polycyclic aromatic hydrocarbons, polychlorinated compounds and alkylphenols

Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dibenzofurans (PCDFs) and dibenzo-*p*-dioxins (PCDDs) form a group of persistent organic compounds that have considerable impact on the environment. Thus, many authors have focused their efforts on studies of their extraction from different matrices. These compounds have low water solubility and low vapour pressure. Since they are apolar and highly lipophilic, the highest concentrations are found in fatty foods, rather than in vegetables, cereals or fruit.

Pressurized liquid extraction has been successfully applied to these compounds as a step prior to their determination. Frequently, non-polar, water-inmiscible solvents (e.g. pentane or hexane), or a combination of non-polar solvents with medium polarity such as dichloromethane (DCM), have been used. One of the major problems with these fatty matrices is the presence of large amounts of co-extracted lipids, which means that a post-clean-up of the extract is required to carry out lipid elimination. Acid digestion or saponification is a destructive method for the removal of lipids that is commonly applied for this purpose. Alternatively, studies aimed at removing these high lipid contents without destruction have been carried out using gel permeation chromatography (GPC). In order to reach a higher sample throughput, Focant et al. [15] replaced slow GPC purification by a high-capacity disposable silica column containing 28 g of acidic, 16 g of basic, and 6 g of neutral silica; this allowed up to 4 g of lipids for each sample to be retained.

Owing to the strong retaining properties of carbon as regards certain planar aromatic systems, especially those with adjacent aromatic rings and electronegative substituents, carbon-based sorbents have been used to fractionate the planar dioxins, furans and PCBs from other classes of aromatic compounds, thereby improving sample clean-up. Kitamura et al. [25] proposed a method for the determination of dioxins in lipid-rich biological matrices based on a combination of PLE, using DMSO/ACN at 180 °C, and DMSO/ACN/hexane partitioning to reduce the large amount of lipids generated during extraction. Following this, a multilayer silica gel-activated carbon (MLS-AC) column was used to separate the mono-ortho-PCBs and non-ortho-PCBs/PCDDs/Fs fractions.

The possibilities of in situ removal of the lipids from biotic samples have been investigated by including several fat-retaining sorbents in the PLE cells to prevent lipids and other co-extractable materials from coming out in the extract.

Several retainer sorbents – florisil, alumina, silica gel, 2,3-dihydroxypropoxypropyl and cyanopropyl bonded silica – were tested by Gómez-Ariza et al. [17] in high-lipid content samples. The authors concluded that although quantitative recoveries are observed with all sorbents, the cleanest extracts are obtained using florisil in the extraction cell, using a dichloromethane-pentane mixture at a temperature of 40 °C. The florisil sorbent is ground with the sample (2:1, w/w) to form a homogeneous mixture and is loaded into the extraction cell on top of a layer of florisil. Sulphuric acid-impregnated silica gel has also been used successfully as a retainer with complex fatty materials such as cod-liver oil and milk powder sorbent by Muller et al. [14]. The in-cell fat removal procedure consists of packing the sample dispersed in a sand-Na_2SO_4 mixture (1:1, w/w) on top of a multilayer column containing acid silica and neutral silica in the bottom part to prevent the sulphuric acid from leaving the extraction cell.

These selected extractions contribute to eliminating the exhaustive clean-up of extracts prior to analysis, reducing sample manipulation and total time, and hence increasing the possibilities of automation.

Water modified with a surfactant, sodium dodecyl sulphate (SDS), was also used as solvent to extract PAHs from fish tissues by Morales-Muñoz et al. [19]. These authors found that the surfactant concentration did not appear to be significant with regard to recoveries when working above this critical micellar concentration. A solid-phase extraction step was then required to remove the surfactant and preconcen-

Table 1
PLE procedures for the analysis of contaminant compounds in food and biological samples

Matrix	Compounds	Pre-treatment	PLE				Post-treatment[a]	Technique[b]	Analyte level (ng/g)	Reference
			Solvent[c]	T (°C)	Cycles	Extraction time (min)				
PAHs, PCCs and alkylphenols										
Oyster (CRM)[d]	PCBs	Sample/DE[e] (1:1, w/w)	Isooctane	100	1	5	Not required	GC/ECD	50–150	[6]
Mussel(CRM) and fish(CRM)	PAHs, PCBs	Sample/Na_2SO_4	DCM	100	1	5	GPC + Cc.	GC/MS	2–2000	[7]
Smoked food	PAHs	Sample/Na_2SO_4/C_{18}	DCM/ACN (90:10, v/v)	100	2	5	H_2SO_4 + florisil column	GC/MS		[8]
Fish tissue	9 PCBs	Sample/hydromatrix + Al_2O_3 layer.	DCM	125	3	3	Na_2SO_4 + Cc.	GC/ECD	80–400	[9]
Oyster tissue	6 PCBs	Sample/hydromatrix	Hexane/acet.	100	1	5	$AgNO_3$/H_2SO_4 silica gel + alumina columns + Cc.	GC/ECD	50–150	[10]
Fish tissue	PCB Aroclor™ standard	Sample/hydromatrix dry in a microwave	Hexane	125	2	5	Cc. + sulphuric acid	GC/ECD	210–1800	[11]
Fish tissue	14 PCBs and 9 dioxins	Sample/Na_2SO_4 + Al_2O_3 layer	Hexane	100	2	5	Not required	GC/ECD		[12]
Cod liver and fish	PCBs, DDT, toxaphene, chlordane, hexachlorobenzene, hexachlorocyclohexanes, dieldrin	Sample/Na_2SO_4	EtAc/cyclohexane (1:1, v/v)	125	2	10	Filtration through Na_2SO_4 +GPC	GC/ECD		[13]
Cereal-based foodstuff, milk powder (CRM), cod-liver oil (CRM)	7 PCBs	Sand/Na_2SO_4 (1:1,w/w) + sulphuric acid/silica gel layer	Hexane	100	2	5	Not required	GC/MS	20	[14]
High-fat-content samples	PCDDs/Fs, PCBs	Sample/Na_2SO_4	Hexane	–	2	5	Acid + basic + neutral silica columns	GC/HRMS	$(0.05–2) \times 10^{-3}$	[15]
Fish tissue	Nonylphenol and its ethoxylates	Sample/Na_2SO_4 (1:4, w/w)	DCM	–	3	10	SPE aminopropyl silica	HPLC/Fl	4–40	[16]
Eggs and mussels (CRM)	PCBs	Sample/florisil (1:2, w/w) + florisil layer	DCM/pentane (15:85, v/v)	40	2	10	Not required	GC/ECD	4–600, 4–150	[17]
Fish meal and feed samples	7 PCBs		*n*-Heptane	100						[18]
Trout and sardine	PAHs	Not required	Water + SDS[f]	200	4	15	SPE C_{18}	HPLC/Fl		[19]
Fish liver	4-*t*-octylphenol, nonylphenols, bisphenol A	Sample/florisil						LC/(ESI)-MS	5–20	[20]
Egg and fish tissue	alkylphenols and alkylphenols ethoxylates	Sample/Na_2SO_4 dessicator over night	ACN (egg sample) DCM (fish tissue)	amb[g]	3	10	Amimopropyl SPE + SPE C_{18}	LC/MS–MS; LC/Fl	16–38 (Fl) 4–22 (MS)	[21]
Animal feed	PCDDs/Fs, PCBs	Not required	DCM/hexane (3:1, v/v)	180	3	10	H_2SO_4 + LLE + silica and alumina columns	HRGC/HRMS		[22]
Plant matrices	14 PAHs	Not required	*n*-Hexane	40, 120	3	10	GPC	GC/MS		[23]
Blood samples	PCBs, PCDD/Fs	Lyophilization	Acet./hexane (1:1, v/v)	150	2	10	LLE + TS-ML-AC	HRGC/HRMS		[24]
Meat and fish	PCBs, PCDD/Fs	Not required	DMSO/ACN (1:9, v/v)	180	1	15	LLE + MLS-AC	HRGC/HRMS		[25]
Fish meal and animal feed	PCBs	Sample/Na_2SO_4 + sulphuric acid/silica layer	*n*-Heptane	100	2	5	Not required	GC/ECD		[26]
Organ tissue	59 organohalogenated compounds	Sample/hydromatrix	DCM/acet. (1:1, v/v)	100	2	–	GPC + silica gel columns	GC/MS		[27]
Smoked meat	10 PAHs	Sample/poly(acrylic acid)/sodium salt	*n*-Hexane	100	2	10	GPC	GC/MS		[28]
Fish tissue	PAHs	Sample/Na_2SO_4	Hexane/acet. (1:1, v/v)	100	1	5	GPC	HPLC/Fl		[29]
Fish tissue	PCBs, organochlorinated pesticides	Sample/Na_2SO_4	Hexane/DCM hexane/acet.	90–120	3	5	GPC	HRGC/ECD		[30]
Marine organisms	Alkylbencene sulphonates	Lyophilized sample + Na_2SO_4	Two extractions:				SPE C_{18}	HPLC/Fl	5–15	[31]
			(1) Hexane	100	3	5				
			(2) MeOH	100	3	5				
Pesticides										
Fruit	Organophosphorus pesticides	Sample/extrelut	EtAc	100	1	5	GPC + Cc.	GC/FPD	30–220	[32]
Tomatoes	58 pesticides	Sample/fibrous cellulose powder(CF-1) 1:1 or 2:1 (w/w)	ACN	60	1	2	NaCl + Na_2SO_4 + Cc.	GC/ITD	0.4–220	[33]
Fruit and vegetables	Fungicides (thiabendazole, carbendazim)	Sample/glass beads (1:1, w/w)	Water	75	Static (5–20 min) + dynamic mode (2–20 mL min^{-1})		LLE (EtAC) + Cc.	HPLC/UV; HPLC/Fl	10–800	[34]

Eight foods	17 *N*-methylcarbamates	Sample/extrelut	ACN	100	1	5	NaCl + Na_2SO_4 in collection vial + Cc. + carboxylic acid mini-column	HPLC/Fl	200	[35]
Fruit and vegetables	7 carbamates	MSPD[h]	Water	50	dynamic mode (1 mL min^{-1}, 3 min)		Not required	LC/MS	2–10	[36]
Potato, banana; wheat grains	12 organochlorine pesticides; 29 pesticides and herbicides	Sample/hydromatrix	Hexane/10% acet. or ACN	100	1–2	5	Na_2SO_4 in collection vial + Cc.	GC/ECD	8–100	[37]
Apple and carrot puree	26 organophosphorus pesticides	Sample/hydromatrix (1:1, w/w)	EtAC/cyclohexane or DCM/acet.	100	1–2	5	GPC + Cc.	GC/FPD	50	[38]
Baby food and adult-diet samples	4,4′-DDE; 4,4′-DDD; 4,4′-DDT chlorpyrifos and malathion	Sample/extrelut (1:1,w/w) + Na_2SO_4 and sand layer	ACN	80	3	5	SPE Envi-carb	ELISA and GC/MS	0.3–110	[49]
Beef kidney	atrazine	MSPD	Water/ethanol	100	1	10	SPME	GC/ITD-MS	20	[40]
Fruit and vegetables	28 pesticides (8 classes)	Sample/hydromatrix (8:5, w/w)	acet./DCM (3:1, v/v)	110	2		LLE (DCM)[i] + SPE (Florisil + Na_2SO_4 + ENVI-Carb)	GC-ECD; GC-FPD	2–140	[41]
Strawberries	Organochlorine and chlorobenzenes	Not required	Water/acet. (90:10, v/v)	120	2	10	SPME or SBSE	GC/MS	2–5; 1–40	[42]
Fruit and vegetables	Organochlorine and chlorobenzenes	Not required	Water/acet. (90:10, v/v)	120	2	10	SPME or SBSE	GC/MS	50	[43]
Pear, apple and cucumber	5 *N*-methylcarbamates	Not required	Water	75	static + dynamic mode (30 min)		Sorbent column C_{18}	HPLC/Fl	1000	[44]
Honey	6 acaricides		Hexane/propanol (1:3, v/v)	95	1	8		HPLC	10–200	[45]
Oil seeds	25 pesticides and metabolites	Not required	Hexane/ACN	60	1	10	GPC	GC/ITD-MS		[46]
Tobacco	Organochlorines, anilines, acylalanines, organophosphorus, halogen compounds and *N*-methylcarbamates	Sample/hydromatrix (7:3, w/w)	Acet.	100	3	3	SPE	GC/MS–MS; HPLC/Fl	50	[47]
Calabash chalk	Lindane, endrin,endosulphan, p-p′-DDD	Not required	Acet.	100	1	5	Not required	GC/MS		[48]
Bovine milk	6 carbamates	MSPD	Water	90	Dynamic mode (1 mL min^{-1}, 5 min)		Not required	LC/MS	1–5	[49]
Animal feed	11 chlorinated pesticides	Not required	*n*-Hexane/acet. (3:2, v/v)	100	2	9	Adsortion + GPC	GC/MS		[50]
Metals										
Fish samples and CRM	Arsenicals	Freeze-dried sample/glass beads; pre-PLE: acet. (amb. 3 cycles, 5 min)	MeOH/water (50:50, v/v)	amb.	3	5	Dry and re-dissolved	IC/ICP-MS		[51]
Ribbon kelp	3 arsenosugars	Freeze-dried sample/glass beads	MeOH/water (30:70, v/v)	amb.	1	1	Cc. + SPE C_{18}	IC/ICP-MS; IC/MS–MS		[52]
Fresh plants and CRM	8 arsenicals	Fine-grounded	Water	120	1	5	Not required	IC/ICP-MS		[53]
Carrots	As(III), As(V), monomethylarsonic acid, dimethylarsinic and arsenobetaine	Freeze-dried sample/Ottawa sand	Water	100	3	1	SPE C_{18}	LC/ICP-MS		[54]
Ribbon kelp	9 arsenicals	Sample/glass beads	MeOH/water (30:70, w/w)	amb	3	1	Cc. and SPE C_{18}	IC/(ESI)-MS–MS		[55]
Fish (CRM)	As(III), As(V), disodium methylarsenate, dimethylarsinic acid and arsenobetaine	Sample/teflon	Two extractions: (1) Acet. (2) Water/MeOH	amb.	3	1	Dry and re-dissolve	IC/ICP-MS		[56]
Seafood (CRM)	Organometallic species of As, Sn, Hg	Sample/hydromatrix	Acetic acid/MeOH (50:50, v/v)	100	5	3		GC/ICP-MS LC/ICP-MS		[57]
Fish (CRM)	Speciated arsenic							HPLC/ICP-MS		[58]
Marine (CRM)	Arsenicals	Not required	Acetic acid/MeOH (50:50, v/v)	100	5	3	Cc.	HPLC/ICP-MS		[59]

Table 1 (*Continued*)

Matrix	Compounds	Pre-treatment	PLE				Post-treatment[a]	Technique[b]	Analyte level (ng/g)	Reference
			Solvent[c]	T (°C)	Cycles	Extraction time (min)				
Drug residues										
Feed	Antibacterial (Lasalocid)	Sample/sand	MeOH + 0.3% acetic acid	80	1	5	–	HPLC/UV		[60]
Bovine liver	Corticosteroids (dexamethasone, beta-epimer betamethasone, flumethaxone)	Sample/hydromatrix; pre-PLE: hexane (60 °C, three cycles, 5 min)	Hexane/EtAc (1:1, v/v)	50	1	5	Not required	LC/MS–MS		[61]
Feed	5 antibiotics (avoparcin, bacitracin, spiramycin, tylosin, virginiamycin)	Sample/sand	Acet./water (65:35, v/v) pH 2.0	80	2	5	Not required	IST (screening test)		[62]
Kidney	Antibiotic (avoparcin)	MSPD	21.5 mM TEAP[j] water/ethanol (70:30, v/v)	75	3	5	HILIC-SPE	HILIC/UV	500	[63]
Kidney fat	7 anabolic steroids (6 gestagens, 1 androgen)	Alumina layer + sodium sulphate layer + sample pre-PLE: hexane (60 °C, 1cycles, 5 min)	ACN	50	1	5	SPE (C_{18})	LC/MS–MS	2	[64]
Feed	Antimicrobials (13 quinolones)	–	0.2%MPA[k] water/ACN (70:30, v/v) pH 2.6	–	–	–	Automated SPE (OASIS HLB)	LC/DAD; LC/Fl	400–1500	[65]
Meat and infant food	13 sulphonamides	MSPD	Water	160	1	15	Not required	LC/MS–MS	0.4–3	[66]
Bovine milk	Aminoglycoside antibiotics	MSPD	Water	70	dynamic mode (1 mL min^{-1}, 4 min)		Not required	LC/MS–MS	2–13	[67]
Natural toxins										
Corn and rice	Fumonisins B1 and B2	Sample/hydromatrix	Ethanol/water (30:70, v/v)	80	2	5	Anion-exchange SPE	LC/Fl		[68]
Wheat and corn	Zearalenone	Sample/hydromatrix	MeOH/ACN	80	2	5	Not required	LC/(ESI)-MS	4	[69]
corn	Zearalenone	Sample/hydromatrix	MeOH/ACN	80	2	5	Not required	LC/(ESI)-MS		[70]
225 Samples	Zearalenone	Sample/NaCl/hydromatrix	ACN/water (85:15, v/v)	40	3	20	IAC	HPLC/Fl	1	[71]
Corn	Zearalenone	Sample/hydromatrix	TEA[l] 1% water/isopropanol	80	2	5	Not required	LC/(ESI)-MS		[72]
Maize and wheat	Deoxynivalenol, fumonisin B1, zearalenone	Sample/DE	ACN/water (75:25, v/v)	40	–	–	Two SPE (anion exchange, Mycosep column)	LC/(APCI)-MS–MS	3–20	[73]
Wheat, corn, rye, barley, rice and swine feed	Zearalenone, α-zearalenol	Not required	MeOH/ACN (50:50, v/v)	50	1	5	Cc.	LC/Fl	2–6	[74]

[a] Cc., concentration step; GPC, gel permeation chromatography; HILIC, hydrophilic interaction chromatography; IAC, immunoaffinity columns; LLE, liquid-liquid extraction; MLS-AC, multilayer silica gel-activated carbon; SBSE, stir bar sortive extraction; SPE, solid phase extraction; SPME, solid phase microextraction, TS-ML-AC, tandem simplified multilayer-activated carbon.

[b] APCI, atmospheric pressure chemical ionization; DAD, diode array detection, ECD, electron-capture detector; ELISA, enzyme-linked immunosorbent assay; ESI, electrospray ionization; Fl, fluorescence detection, FPD, flame photometric detection; GC, gas chromatography; HILIC, hydrophilic interaction chromatography; HPLC, high-performance liquid chromatography; HRGC, high-resolution gas chromatography; HRMS, high-resolution mass spectrometry; IC, ion-chromatography; ICP, inductively coupled plasma; IST, inhibitory substance test; ITD, ion-trap detection; LC, liquid chromatography; MS, mass spectrometry; SRM, selected reaction monitoring, UV, ultraviolet detection.

[c] Acet., acetone; ACN, acetonitrile; DCM, dichloromethane; DMSO, dimethyl sulfoxide; EtAC, ethyl acetate; MeOH, methanol.

[d] CRM, certified reference material.

[e] DE, Diatomaceous earth.

[f] SDS, sodium dodecyl sulphate.

[g] amb. ambient temperature.

[h] MSPD, matrix solid-phase dispersion.

[i] LLE of the aqueous layer extracted with DCM.

[j] TEAP, triethylammonium phosphate.

[k] MPA, methaphosphoric acid.

[l] TEA, triethylamine.

trate the analytes. In that study, pressurized liquid extraction was monitored on-line by coupling the extractor to a flow-injection manifold and quantitative recoveries were obtained after optimization of the PLE variables.

Alkylphenol ethoxylates (APEOs) are non-ionic surfactants widely used in a variety of industrial processes and also in cleaning products. Their biodegradation products, especially the fully deethoxylated nonylphenol (NP), may alter the endocrine system, and hence their use in industrial applications has been restricted. The relatively low polarity of some of these compounds explains their bioacumulation in aquatic organisms and therefore PLE has also been used for the extraction of these compounds from fish tissues. Smith et al. [21] proposed a method to determine alkylphenol and alkylphenol ethoxylates by PLE using an SPE clean-up step with amino propyl-based cartridges. This cartridge eliminated approximately 80% of the lipids and other interferences from the extract, although the remaining co-extractives interfered in the LC/MS–MS determination and hence an additional SPE clean-up step had to be performed. A florisil SPE sorbent was used, but this failed to produce a cleaner extract, because lipids have physical–chemical properties similar to those of the higher ethoxylates and coelute with them. However, the use of a C_{18} cartridge provided adequate results in extract purification.

4.1.2. Pesticides

As pesticides different chemicals, mainly organophosphorus, *N*-methylcarbamates, organonitrogen, organosulphur and chlorinated compounds, are used, all exhibiting a broad range of physical–chemical properties. As it is known, an important consideration when developing an extraction method for pesticide multi-residue analysis is the need to cover a wide range of different compounds with different properties in a single procedure.

Pesticides residues are often present in non-fatty foods, such as fruit, vegetables or cereal-based foods, which have a high- or medium-water content. Sample drying prior to extraction is an efficient way to handle this type of sample. Drying is normally accomplished by direct addition of a drying agent such as sodium sulphate, diatomaceous earth (Hydromatrix™ or Extrelut®) or cellulose. While sodium sulphate works well for soils and sediment samples, Hydromatrix or cellulose is a good choice for wet-tissue samples. Sodium sulphate is not recommended for use with methanol or other polar solvents because it may become solubilized in the extraction process and deposited in the exit lines. However, anhydrous sodium sulphate has been added to the collection vial to absorb co-extracted water [35,37].

The water present in this kind of wet-samples prevents non-polar organic solvent from reaching the analytes and hence more polar solvents (acetonitrile, methanol, ethyl acetate, dichloromethane) or solvent mixtures (hexane/acetone, cyclohexane/ethyl acetate, hexane/acetonitrile, hexane/propanol) have frequently been used to help the extraction process. Water-based extraction of pesticides from these food samples is also appropriate, since the wet-sample matrix does not need to be dried prior to the extraction step [42–44]. The low cost and the environmental friendliness of water make this solvent a good alternative to organic solvents for the extraction of pesticides in food samples. As a fully automated alternative, Herrera et al. [44] proposed a combination of static-dynamic pressurized hot-water extraction coupled with on-line filtration–preconcentration–chromatographic separation by HPLC and fluorescence derivatization–detection for the determination of *N*-methylcarbamates from fruit and vegetables. Using low temperature (75 °C) to avoid compound degradation, recoveries ranging from 80 to 104% were obtained for all five *N*-methylcarbamates. As an extraction solvent, water can be modified with organic solvents such as methanol, ethanol, acetonitrile or acetone in low proportions in order to decrease its dielectric constant, and hence its polarity, with no need to implement strong increases in temperature. Water/acetone (90:10, v/v) mixture was employed by Wennrich et al. [42,43] to extract organochlorine and chlorobenzenes from fruit and vegetables, and Curren et al. demonstrated the effectiveness of ethanol as a co-solvent during PHWE of triazine pesticides [5,40].

The problem of co-extractive compounds, which may lead PLE extracts to appear as turbid or highly coloured, has been overcome by incorporating post-clean-up procedures that also often serve as a preconcentration step for the target analytes, such as liquid–liquid extraction (LLE) [34], gel permeation chromatography (GPC) [32,38,46,50], solid-phase extraction (SPE) [39,41,44,47], solid-phase microextraction (SPME) [40,42,43] or stir-bar sortive extraction (SBSE) [42,43]. However, in situ clean-up procedures have also been employed. Curren and King [40] developed a water-based PLE method followed by a solid-phase microextraction (SPME) for the removal of atrazine from beef kidney using in situ matrix solid-phase dispersion (MSPD). During the MSPD procedure, the sample matrix was combined with an acrylic polymer resin (XAD-7HP). Finally, ethanol-modified water was used to elute the atrazine from the dispersed matrix, although higher amounts of co-extracted compounds were also removed due to the presence of ethanol.

4.1.3. Metals

Among metals, almost only inorganic and organic arsenic has been determined in food samples using PLE. Generally, inorganic arsenic is considered to be the most toxic form, followed by dimethylarsinic acid (DMA), monomethylarsonic acid (MMA), arsenosugars (associated with seafood), and finally non-toxic arsenobetaine (AsB), also associated with seafood. Extraction procedures have been developed and optimized to release as much of each arsenic species present in a food as possible without causing degradation products. The separation of arsenic species is generally accomplished

via ion chromatography (IC) in both the cation and anion modes, using inductively coupled plasma mass spectrometer detectors (ICP-MS) due to their sub ng g^{-1} detection capacities.

Fish and seafood can accumulate considerable amounts of organic arsenic from their environment. Thus, the speciation of arsenic in seafood and fish is an active area of research and most studies in the literature consulted, based on PLE extraction, determine arsenic in these matrices. One of the first publications describing the use of this technique for arsenic speciation is that of McKiernan et al. [51]. These authors reported a comparison of the PLE extraction of arsenicals from fish samples using a mild extraction solvent mixture (methanol/water) and a previously reported sonication method. The results of this study revealed that PLE is a useful method for extracting arsenicals from fish using two extractions: a de-fatting extraction using acetone, and a speciation extraction using MeOH/water 50:50 (v/v). A small percentage of arsenic ($\leq$5.1%) was removed with acetone in all fish studied, while most ($\geq$71.8%) of the arsenicals were collected in the second extraction. Arsenosugars (relatively non-toxic arsenicals) produce erroneous results owing to chromatographic co-elution with other toxic arsenic species. Gallager et al. [52] showed that PLE was capable of extracting three arsenosugars from ribbon kelp using methanol/water mixtures. These arsenosugars were structurally identified and characterized by ion chromatography coupled with electrospray ionization tandem mass spectrometer (IC-ESI-MS/MS) in order to investigate possible false-positive signals observed using IC/ICP-MS.

A PLE method for the extraction of arsenite, arsenate, MMA, DMA and AsB from carrots was developed by Vela et al. [54], using water as the extraction solvent and Ottawa sand as the dispersing agent. The authors found that the use of three static cycles of 1 min duration each was sufficient to extract 95–97% of total arsenic when a temperature of 100 °C was used. Gallagher et al. [56] evaluated another three dispersion media – Filter Aid (high density glass beads), Q-beads (soda lime glass beads) and Teflon particles – in terms of their arsenic extraction recoveries. The results of these authors indicated that Filter Aid is not a suitable dispersion medium because it retains inorganic arsenic, MMA and DMA. No analyte losses were observed with Q-Beads or Teflon but the former needed and acid pre-cleaning to reduce the arsenic blank.

The extraction of these compounds using acetic acid/methanol mixtures as extraction solvents is currently being investigated using certified reference materials (CRM) for seafood [57,59].

One of the most recent publications of a PLE method for organometallics in food is that of Wahlen et al. [57]. In this paper, the authors describe a method for the simultaneous extraction of species of Sn and As and, for the first time, the extraction of methylmercury (MeHg) in certified reference materials for seafood using an acetic acid/methanol mixture as extraction solvent.

4.1.4. Drug residues

The sample preparation technique required in drug residue analysis is a very critical aspect because these analytes must often be isolated from complex biological matrices such as animal tissues (liver or kidney). Among these drug residues, steroids and antibiotics have been determined using PLE.

Corticosteroid drugs are synthetic hormone analogues that are widely employed to combat inflammatory diseases in food-producing animals, although they are also commonly used as growth promoters. They tend to accumulate and persist in animal liver or kidney. Very few papers have addressed the analysis of steroids by PLE procedures. One of the first publications reporting the use of this technique for three corticosteroids in bovine liver was that of Draisci et al. [61]. This team developed a highly automated procedure, including de-fatting and extraction step. Efficient fat removal was achieved using hexane along three static cycles of 5 min at 60 °C. Extraction of the target drugs was then achieved with a mixture of hexane/ethyl acetate (1:1, v/v) in a single 5 min cycle at 50 °C. The entire procedure required about 35 min. Hooijerink et al. [64] proposed an in-cell clean-up for the extraction of seven anabolic steroids from kidney fat. In this procedure, the extraction cell was filled, from bottom to top, with 5 g of alumina, followed by sodium sulphate, and then the melted sample. The authors applied two pressurized liquid extractions, using hexane and acetonitrile as solvents. In the first extraction with hexane, the analytes were retained on the alumina layer, extracting the fat matter of the sample, whereas in the second one acetonitrile was used to elute the steroids. Acetonitrile was used as the elution solvent instead of methanol/water mixtures because, under elevated pressure and temperature, these mixtures dissolved some of the sodium sulphate, clogging the tubing lines of the PLE system.

Antibiotics are synthetic, active antimicrobial compounds widely used in human and veterinary medicines. Only a few procedures based on PLE are available for the determination of antibiotics. A water-soluble antibiotic, avoparcin, was determined in kidney by Curren and King [63] using the hot-water procedure previously developed for atrazine in the same matrix [40]. As well as the clean-up step based on the MSPD technique, they also included an organic buffer – triethylammonium phosphate (TEAP) – with the aim of reducing the co-extraction of lipids and proteins. The aqueous extract was then concentrated by SPE in the hydrophilic interaction chromatography (HILIC) material polyhydroxyethyl aspartamide.

Sulphonamides residues were also extracted from meat and infant foods by Gentili et al. [66] using MSPD, with C_{18} as in situ clean-up. The use of water as extraction solvent permitted direct injection of the extract into the LC–MS/MS system after precipitation of the fat and other co-extracted compounds. Precipitation was accomplished by cooling the extract at −18 °C over 1 h.

The use of medicated feedstuff has also led to the development of procedures for the determination of antibiotics in feed samples [60,62,65]. For instance, thirteen quinolones

were successfully extracted by Pecorelli et al. [65] from fish and swine feed using a 0.2% metaphosphoric acid in water/acetonitrile mixture (70:30, v/v) at pH 2.6 as extraction solvent. Automated SPE with a polymeric sorbent (Oasis HLB) was employed as the post-clean-up/preconcentration step.

4.1.5. Natural toxins

The contamination of cereal crops by naturally occurring mycotoxins caused by moulds is a common phenomenon. The determination of mycotoxins in food and feeds is of general interest because of their different toxic effects on humans and animals. Among such compounds, zearalenone (ZON) is a non-steroidal estrogenic mycotoxin that is stable even at high temperatures and that has been detected in certain cereal-based foods and feedstuffs. Thus, the extraction and determination of this compound has been addressed by several authors. ACN/water, MeOH/water and ACN/MeOH are the three mixtures most commonly used in the extraction of ZON from cereals by other extraction techniques, such as conventional liquid shaking or MAE. Thus, they are also the mixtures most used in PLE as extraction solvents.

Pallaroni and von Holst [69,70] have described the application of PLE to the analysis of ZON in corn and wheat by LC–MS without any additional clean-up step. A statistical design approach was applied to optimize the PLE parameters. In the optimized procedure, a mixture of acetonitrile/methanol was selected as the extraction solvent, applying a temperature of 80 °C. Extraction recoveries of ZON of over 100% were obtained under these conditions. Recently, those authors have developed an alternative extraction method using a less toxic extraction solvent mixture 1% TEA in water/isopropanol (1:1, v/v) instead of the ACN/MeOH (1:1, v/v) mixture [72]. ZON is almost insoluble in water but its solubility increases in alkaline aqueous solution. However, since ZON is not stable in alkaline conditions, those authors had to find a compromise and therefore tested various solvent mixtures. The mixture of 1% TEA in water/isopropanol (1:1, v/v) fulfilled these criteria.

Recently, Urraca et al. [74] have developed a method for the determination of ZON and one of it metabolite, α-zearalenol, using LC with fluorescence detection. Recoveries between 94 and 104% were obtained for these two compounds in all the matrices tested using a methanol/acetonitrile mixture as extraction solvent.

4.2. Matrix components

Different applications of PLE for the isolation of matrix components in a variety of food and biological samples are presented in Table 2.

4.2.1. Pharmacologically active compounds

An interesting and increasingly important new application area of PLE is in the extraction of chemical constituents from plants or herbal materials of pharmacological interest. Water, methanol, or water/methanol mixtures have been almost exclusively used as the extraction solvent for these compounds. When aqueous solutions are chosen as extraction solvents, PLE in dynamic mode has frequently been employed. Suomi et al. [78] reported a comparison between PHWE in its static and dynamic mode and HWE at atmospheric pressure for the extraction of two iridoid glycosides in plants, determination being carried out with micellar electrokinetic capillary chromatography (MECC). The authors concluded that HWE was the most effective extraction technique, with very good repeatability.

The use of aqueous surfactant solutions instead of plain aqueous or organic solvents has been studied by some authors. Choi et al. [85] reported that the presence of surfactant micelles increases the solubility of ginsenosides at lower extraction temperatures. In that study, the authors employed an aqueous non-ionic surfactant solution (water + TritonX-100), obtaining excellent recoveries for all compounds even at 50 °C. Ong and Len [86] also developed a method for the analysis of glycosides in medicinal plants using what they called surfactant-assisted PHWE. The results obtained with this technique were at least equivalent to or even better than those obtained with Soxhlet extraction using an ethanol/water (95:5, v/v) mixture. The extraction of eight coumarins of biological interest was carried out by Waksmundzka et al. [89] using two PLE extractions owing to the wide range of polarities of these compounds. In the first, petroleum ether was employed, extracting the furanocoumarins, whereas the more polar coumarins were extracted with methanol.

4.2.2. Polyphenols

The vast majority of phenolic compounds found in medicinal herbs and foods can accurately be called polyphenols: another term for “polyhydroxy phenols”. Their molecular structure, a ring of six carbon atoms with more than one –OH group attached, means that polyphenols are readily oxidized. Thus, many polyphenols have antioxidant properties and much attention is now focused on their extraction from plants because of recent increased demand for natural antioxidants. These natural antioxidants are important in the food industry not only because of their usefulness as a preservation method but also because of their beneficial effects on health. Polyphenols with several hydroxyl groups are hydrophilic, so methanol – although mainly water – have been used as solvents in their extraction. The aim of several authors has been to determine the polyphenolic profiles of different species using a PLE method. For instance, Alonso et al. developed a methanol-based PLE technique to identify and quantify the main extractable polyphenols of varieties of maturing cider apple [90] and also in *Golden* apples [91]. In this study, no clean-up steps were necessary prior to HPLC/DAD determination. Papagiannopoulos et al. [99] also determined the 41 characteristic polyphenols of carob (*Mediterranean leguminosae*) pods using an acetone/water mixture for their extraction. In this case, a polyamide SPE cartridge was used after PLE to clean-up the extract.

Table 2
PLE procedures for the analysis of matrix components in food and biological samples[a]

Matrix	Compounds	Pre-treatment	PLE				Post-treatment	Technique[b]	Reference
			Solvent	T (°C)	Cycles	Extraction time (min)			
Pharmacologically active compounds									
Japanese yew bark	Taxanes	Oven-dried sample	MeOH/water (90:10, v/v)	150					[75]
Kava root	Kavalactones	Not required	Water	175	dynamic mode (1 mL min^{-1}, 20–40 min)		LLE (DCM)	GC/FID-MS	[76]
Medicinal plants	Aristolochic acids	Sample/sand	MeOH	120	dynamic mode (1.5 mL min^{-1}, 20 min)		Not required	LC/DAD	[77]
Plant leaves	Glycosides (catalpol, aucubin)	Not required	Water	100	dynamic mode (30 min)		LLE (diethyl ether)	MECC/DAD	[78]
Medicinal plants	Aristolochic acids	Sample/sand	MeOH	120	dynamic mode (1 mL min^{-1}, 20 min)		Not required	CZE/UV	[79]
Medicinal plants	Alkaloids (berberine, strychnine)	Sample/sand	MeOH	120	dynamic mode (1 mL min^{-1}, 20 min)		Not required	CZE/UV	[80]
Coca leaves	Cocaine, benzoylecgonine	Sample/sand (1:3, w/w)	MeOH	80	dynamic mode (1 mL min^{-1}, 10 min)		Cc.	GC/FID; CE/UV	[81]
Medicinal plants and health supplements	Ginsenosides	Sample between sand layers	MeOH	140	dynamic mode (1 mL min^{-1}, 20 min)		Not required	HPLC/DAD	[82]
Medicinal plants	Alkaloids (berberine, baicalein, glycyrrhizin)	Sample/sand	Water or water/ethanol	95, 140	dynamic mode (1 mL min^{-1}, 40 min)		Cc.	HPLC/UV	[83]
Natural health products	Ephedrine, pseudoephedrine and four metabolites	Sample/Ottawa sand	Water/3% MeOH	90	3	5	Not required	FI-ESI-FAIMS-MS	[84]
Ginseng	ginsenosides	Dried sample	Water + TritonX-100	50–120	1	10	Not required	HPLC	[85]
Medicinal plants	Three glycosides	Sample/sand	Water + TritonX-100	95	dynamic mode (1 mL min^{-1}, 40 min)		SPE C_{18}	LC/(ESI)-MS	[86]
Fruit	Furanocoumarins	Sample/neutral glass	MeOH	100		10	Cc. + re-dissolve	HPLC/UV-VIS	[87]
Kava root	6 kavalactones	Sample between Ottawa sand layers	MeOH	60	–	–	Cc. + re-dissolve	LC/UV; LC/MS	[88]
Pastinaca sativa fruit	8 coumarins	Sample/neutral glass	Two extractions:				Dry and re-dissolve	HPLC/UV	[89]
			(1) Petroleum ether	100	–	–			
			(2) MeOH						
Phenols and polyphenols									
Cider apple	16 polyphenols	Freeze-dried sample/DE (1:1, w/w)	MeOH	40	2	5	Not required	HPLC/DAD	[90]
Golden apple	12 polyphenols	Sample/DE (1:1, w/w)	MeOH	40	2	5	Cc.	HPLC/DAD	[91]
grape seeds and skins	9 phenolic compounds	Sample/sea sand	MeOH	100, 150	3	10	Not required	HPLC/DAD	[92]
Malt	5 proanthocyanidins	Sample/DE	Acet./water (4:1, v/v)	60	2	10	Automated SPE	LC/UV-MS	[93]
Hops	4 polyphenols	Pre-PLE: pentane (60 °C, 10 min, two cycles)	Acet./water (4:1, v/v)	60	2	10	Automated SPE	LC/UV-MS	[94]
Grapes	6 phenolic compounds	sample + LiChrolut EN sorbent layer	Two extractions				Not required	HPLC/DAD	[95]
			(1) Water 40 °C, 150 atm		3	10			
			(2) MeOH 100 °C, 40 atm						
Aromatic plant (sage)	Phenolic diterpenes (carnosic acids, carnosol, methyl carnosate) phenolic acids (rosmarinic)	Sample/sea sand	Water	100	Dinamic mode (1 mL min^{-1}, 60 min)		SPE on-line (C_{18} or cyclohexyl sorbent)	HPLC/(ESI)-MS	[96]
rosemary leaves	Phenolic diterpenes (carnosol, rosmanol, carnosic acid, methyl carnosate), flavonoids (cirsimaritin and genkwanin)	Not required	Water	25–200	Dinamic mode (1 mL min^{-1}, 30 min)		Freeze-dry and re-dissolved	LC/MS LC/DAD	[97]
Sambucus nigra L	Flavonols (rutin, isoquercitrin)							HPLC	[98]
Carob pods (Mediterranean leguminosae)	41 polyphenols	Sample/DE (1:2, w/w)	Acet./water (1:1, v/v)	60	2	5	Polyamide SPE	HPLC/UV-ESI-MS	[99]
Tea leaves and grape seeds	Flavanols (catechin, epicatechin)	Sample/sea sand	MeOH	130	2	5	–	HPLC/DAD-Fl	[100]
Microalga	Polyphenols	Not required	Hexane, light petroleum, ethanol, water	115, 170	1	9, 15	Cc. or freeze-dry	MECC/DAD	[101]
Aromatic plants	7 phenolic diterpenes and 1 phenolic acid	Not required	Water	60, 100	1	25	Freeze-dry	CE/(ESI)-MS	[102]
Soybean food	Isoflavones	Sample/SPE-ed^{TM}	Two extractions:				Cc. + re-dissolved	HPLC/ED	[103]
		matrix/florisil/Ottawa sand	(1) Hexane	100	2	5			
			(2) 60% MeOH/0.3% FA[c]	100					
Soybeans	Isoflavones	Freeze-dried sample/sand	Ethanol/water (70:30, v/v)	100	3	7	Not required	HPLC/DAD HPLC/MS	[104]

Essential oils									
Rosemary	Terpenes oxygenates	Not required	Water	150	Dynamic mode (2 mL min^{-1}, 30 min)		LLE (hexane)	GC/FID	[105]
Peppermint	Oxygenates, caryophyllene	Air dried sample	Water	125-150	Dynamic mode (1 mLmin^{-1},)		LLE (chloroform)	GC/MS	[106]
Majoram leaves	Terpenes, pinenes alcohols	Not required	Water	150	Dynamic mode (2 mL min^{-1}, 15 min)		LLE (hexane)	GC/FID-MS	[107]
Medicinal plant (fennel)	Monoterpenes, oxygenates	Not required	Water	150	Static-dynamic mode (30 min + 20 min, 2 mL min^{-1})		LLE (hexane)	GC/FID-MS	[108]
Laurel	Essential oils	Not required	Water	150	Static-dynamic mode (15 min + 25 min, 2 mL min^{-1})		LLE (hexane)	GC/FID-MS	[109]
Savory and peppermint	Terpenes, oxygenates	Air dried sample	Water	100–175	Dynamic mode (12–40 min)		LLE (DCM)	GC/FID-MS	[110]
Oregano	11 Oregano oil compounds	Not required	Water	125	Dynamic mode (1 mL min^{-1}, 24 min)		LLE (hexane)	GC/FID-MS	[111]
Lemon grass	Essential oils (neral, geranial, geraniol, limonene, citronellal, P-myrcene)	Not required	Hexane, DCM, acet. MeOH	40	3	10	Not required	GC/FID	[112]
Thymbra spicata L.	Essential oils	Not required	Water	150	Dynamic mode (2 mL min^{-1}, 30 min)		SPE C_{18}	GC/TOF-MS	[113]
Lime peel	Essential oils (neral, geranial, geraniol, linalool, terpineol)		Water/MeOH or ethanol	130	Static-dynamic mode (5 min +15 min, 1 mL min^{-1})		LLE (hexane)	GC/FID; GC/MS	[114]
Chinese medicine	Essential oils	Not required	Water	150	Dynamic mode (1 mL min^{-1}, 5 min)		SPME	GC/MS	[115]
Chinese medicine	Essential oils	Not required	Water	160	Dynamic mode (1 mL min^{-1}, 5 min)		(HS)-LPME[d]	GC/MS	[116]
Fat matter									
Cereal lipids and animal tissues	Total fatty acids	Sample/celite	Chloroform/MeOH isopropanol/hexane	100–120	2–3	5	Not required	GC	[117]
Powdered infant formula	Total fat	Sample/hydromatrix	Hexane/acet. (4:1, v/v)	125	3	5	Cc. and dry at 100 °C	Gravimetric and GC/FID	[118]
Meat	Total fat	Sample/hydromatrix + dry step	Petroleum ether or hexane	125	2	1 or 2	Cc. and dry at 100 °C	Gravimetric	[119]
Dried milk products	Total fat	Not required	Hexane/DCM/MeOH	80	3	1	Cc. and dry	Gravimetric, GC HPLC/ELSD	[120]
Chocolate	Total fat	Sample/hydromatrix	Petroleum ether	125	3	3	Cc. and dry at 102 °C	Gravimetric	[121]
Dairy products	Total fat	Sample/hydromatrix	Hexane/isopropanol petroleum ether/acet petroleum ether/acet./isopropanol	100–120	1 or 3	1 or 2	Cc. and dry at 102 °C + re-dissolve (petroleum ether) + Cc.	Gravimetric	[122]
Oilseeds	Oil	Wet sample/Na_2SO_4 dried sample/sand	Petroleum ether	105	3	10	Cc.	Gravimetric	[123]
Snack foods and dog biscuits	Unbound fat	Wet sample/Na_2SO_4 dried sample/sand	Petroleum ether, hexane, chloroform, hexane/isopropanol, chloroform/ethanol	125	1–3	5–25	Cc.	Gravimetric	[124]
Egg-containing food	Oxysterol	Sample/celite	Hexane/isopropanol (3:2, v/v)	60	2	10	Cc.	GC	[125]
Dairy products	Fat		Hexane, DCM, MeOH, petroleum ether, acet., ethanol, isopropanol	80–120		8–10	Cc. and dry	Gravimetric	[126]
Poultry meat	Total lipids	Sample/hydromatrix	Chloroform/MeOH (2:1, v/v)	120	2	10	LLE (saline solution) + Na_2SO_4 + Cc.	TLC; CGC	[127]
Wheat germ	Oil	Not required	Hexane	105	3	10	Cc.	GC/FID	[128]
Corn and oats	Polar and non-polar lipids		Hexane, DCM, isopropanol, ethanol	100					[129]
Others									
Freshwater fish	Polycyclic musk compounds	Sample/hydromatrix + alumina layer	EtAC/hexane (1:5, v/v)	80	2	5	Cc. + re-dissolve	GC/MS	[130]
Medical foods	Vitamin K1	MSPD	EtAC	50	1	5	Cc	LC/Fl	[131]
Processed food	12 carotenoid food additives	Sample/hydromatrix + Na_2CO_3 or acetic acid (if neccessary)	MeOH/EtAC/light petroleum (1:1:1, v/v/v)	40	3	2	NaCl in vial + Cc. + re-dissolve	LC/(APCI)-MS	[132]
Seeds and nuts	Tocopherols	Sample/hydromatrix	ACN	50	2	5	Not required	HPLC/ED	[133]
Green algae	Carotenoids	Lyophilized sample between Ottawa sand layers	Acet. or DCM/MeOH (1:3, v/v)	40	3	5	Cc. + re-dissolve	LC/UV; LC/MS	[88]
Palm pressed fiber	Carotene, tocopherols and tocotrienols	Dried sample	*n*-Hexane	80	2	10	Cc. + re-dissolve	NPLC/UV	[134]

[a] For acronyms, see Table 1.

[b] CE, capillary electrophoresis; CGC, capillary gas chromatography; CZE, capillary zone electrophoresis; ED: electrochemical detection; ELSD: evaporative light scattering detector; FI-ESI-FAIMS-MS, flow injection-electrospray ionization-high field asymmetric waveform ion mobility spectrometry-mass spectrometry; HT-CG: high temperature capillary gas chromatography; MECC, micellar electrokinetic capillary chromatography; NPLC, normal phase liquid chromatography; TLC: thin-layer chromatography; TOF, time-of-flight.

[c] FA, formic acid.

[d] (HS)-LPME, headspace liquid-phase microextraction.

In a recent study, Ibañez et al. [97] have demonstrated the possibility of tuning selectivity for antioxidant extractions by means of a small change in water temperature. They carried out dynamic extractions of rosemary at different temperatures from 25 to 200 °C. At 25 °C, the more polar compound (rosmanol) was the mayor component of the extract, although an increase in the extraction ability of water towards the less polar compounds was observed when the temperature was increased from 25 to 200 °C. Therefore, using the process described by these authors it is possible to obtain extracts enriched with different types of polyphenols.

Papagiannopoulos et al. [93] reported a new instrumental setup for the automated analysis of solid sample materials by on-line coupling of PLE, automated SPE, and HPLC, and described its successful application in the determination of proanthocyanidins in malt samples. The determination of polyphenols in hops was also carried out by these authors using the instrumental setup mentioned above [94]. In this case, a preliminary PLE extraction with pentane (60 °C, 10 min, two cycles) had to be carried out in order to eliminate the hydrophobic compounds present in the hops (resins, oils and chlorophylls) that interfered in the subsequent automated SPE step.

4.2.3. Essential oils

Essential oils are mixtures of compounds that can be divided into two fractions: a volatile fraction that constitutes approximately a 90–95% of the whole oil, and a non-volatile fraction that contains hydrocarbons, fatty acids, sterols, carotenoids, flavonoids, etc. The volatile fraction contains monoterpenes and sesquiterpene hydrocarbons and their oxygenated derivatives, aliphatic aldehydes, esters and alcohols. Owing to the widespread use of these aromatic compounds in perfumery and in the pharmaceuticals and food industries, new extraction processes for the isolation of essential oils are currently being developed. As can be seen in Table 2, pressurized liquid extraction of essential oils from plant materials has been almost exclusively carried out in dynamic mode, using water as the extraction solvent [106,107,110,111,113,115,116]. Nevertheless, Gámiz and Luque de Castro [108] performed a hybrid static-dynamic water extraction of the main components of the essential oil from fennel by making a simple change in the dynamic extractor. They concluded that joint use of both extraction modes affords better quality oil and higher selectivity because the composition of the extract can be manipulated. In most works, the compounds were removed from the aqueous extract by a LLE step using chloroform [106], hexane [107–109,111,114] or dichloromethane [110], and were detected by gas chromatography–flame ionization detection (GC/FID).

4.2.4. Fat matter

The determination of fat in certain food products is difficult due to the binding of the fat by the matrix. Thus, traditional methods used to extract fat include a pretreatment step, generally with ammonium hydroxide, to denature or destroy the matrix structure and hence gain access to the fat (Mojonnier method). Despite this, several works have demonstrated the ability of PLE to extract fat matter without the use of such aggressive pre-treatments, obtaining results similar to those obtained with the traditional pre-treatment/extraction methods [118]. Additionally, PLE has probed to be a good alternative to replace other extraction methods, such as Soxhlet extraction [127,128] or Folch extraction (a solid–liquid extraction with chloroform/methanol 2:1, v/v) [117,125,127].

Non-polar solvents such as petroleum ether [119,121] or hexane [119,128] are good solvents for extracting non-polar compounds but their ability to extract more polar lipids, such as phospholipids, is often poor. In this case, the use of binary solvent mixtures such as chloroform/methanol [117,127] and hexane/isopropanol [122,124]; or even ternary solvent mixtures such as hexane/dichloromethane/methanol [120] or petroleum ether/acetone/isopropanol [122] has proved to be a successful choice for total lipid extraction. The effect of different binary or ternary extraction mixtures on the extraction of total fat has been described by several authors [117,122,124,126,127]. Such studies are necessary because some mixtures may lead to the extraction of a non-lipid fraction that includes nitrogen-containing compounds, as has been described by Boselli et al. [125].

4.2.5. Others

Recent applications have demonstrated the advantage of PLE for the extraction of other economically valuable nutraceuticals such as vitamins or carotenoids. For instance, Delgado-Zamarreño et al. [133] has proposed a method for the extraction of vitamin E isomers from seeds and nuts with acetonitrile using a low temperature, 50 °C, in order to avoid degradation of the vitamins. No additional clean-up step is necessary and the extract is injected directly into the chromatographic system. Recently, Breithaupt [132] has developed a method for the routine extraction of 12 carotenoid food additives from several kinds of food matrices using a ternary solvent system (methanol/ethyl acetate/light petroleum). In the reported applications, low temperatures were used during the PLE procedure because these nutraceutical compounds are thermally unstable.

The PLE technique has also proved to be an advantageous choice for the extraction of other natural compounds such as musk aroma. Musk compounds are natural compounds widely used as fragrances in cosmetics products and also as flavours in the food industry and fish farming. Their lipophilic characteristics involve the bio-accumulation of polycyclic musk compounds, especially in freshwater fish, human adipose tissue and human milk. A selective single-step extraction and clean-up was performed by Draisci et al. [130] for the extraction of polycyclic musk compounds in freshwater fish, using ethyl acetate/hexane as the extraction solvent. The use of alumina as an adsorbent inside the extraction cell permitted the collection of extracts clean enough for direct injection into the GC/MS system.

5. Conclusions

As can be appreciated from the present review, the pressurized liquid extraction technique can be successfully applied for the extraction of almost all types of compounds from very different and complex food and biological samples. The suitability of this technique in this field has been confirmed and the number of papers addressing this issue has grown considerably: from the four papers reported in 1997 to the 54 published in 2004. The strategies followed to obtain a rapid, selective, efficient and reliable extraction process are very different and vary as a function of the sample matrix and the compounds studied. Currently, these strategies mainly focus on two basic aims: partial or total automation of the whole analytical process, and the development of highly selective extractions of compounds of different polarities.

The possibility of coupling PLE with other steps in the analytical process is one of the most interesting aspects of this methodology. Although static PLE is the most widely used mode, it is less flexible in terms of modifications or coupling with other techniques, usually, commercial devices are employed. However, dynamic PLE is especially interesting with respect to automation of the analytical process because of its dynamic nature and because it can be accomplished using flexible laboratory-built configurations. Water is the solvent most frequently used for dynamic extractions and has been used in many recent studies owing to its low cost and its environmental friendliness. In addition to the flexibility of the extraction system, automation of the whole process also implies the need to minimize the co-extraction of other matrix components. The easy removal of these co-extractives is also an important aim of recent applications and attention is currently focused on the direct coupling of the clean-up step and on the developed of in situ clean-up steps during extraction. Another strategy consists of the application of a preliminary PLE extraction with a suitable solvent to eliminate interfering compounds before extraction of the analytes of interest.

Selective extraction methods, especially in case of the extraction of analytes with a broad range of polarities, are also of great interest. The main strategies followed in these cases are the use of mixtures of low- and high-polarity solvents or the use of two sequential PLEs for the same sample: one with a non-polar solvent to extract the less polar compounds, and the second one with a more polar solvent to extract the more polar analytes. When water in the dynamic mode is employed for this purpose, selective extractions have been achieved by changing the water temperature during the extraction.

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