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Review

Application of ultrasound-assisted extraction to the determination of contaminants in food and soil samples

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ABSTRACT

The application of ultrasound-assisted extraction (UAE) to the sample preparation of environmental and food samples has increased in the last years. This technique has been used in the development of methods for the analysis of numerous contaminants, including organic compounds (pesticides, pharmaceuticals, polycyclic aromatic hydrocarbons, polyhalogenated flame retardants, etc.) and heavy metals. The aim of this work is to review the application of this extraction procedure to the analysis of contaminants in food and soil and the comparison of its use with other well-established extraction procedures. The advantages and disadvantages of this technique together with the possibility of coupling UAE with other analytical techniques will be also discussed.

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1. Introduction

A wide range of organic and inorganic contaminants are found in the environment as a consequence of diverse anthropogenic activities or natural processes. Their presence in the different environmental compartments may cause adverse effects on human health and animals, including cancer and disruption of the immune system. In addition, these compounds can persist in the environment and bioaccumulate along the food chain. Soil is a primary terrestrial reservoir of persistent contaminants and water and the atmosphere are their main transport vectors. On the other hand, food can be contaminated after treatment with pesticides and biocides or by indirect exposure to these chemicals or other substances through the environment and during storage or food processing.

Traditional sample preparation procedures for the analysis of contaminants consist in a solvent extraction or digestion step, generally followed by a purification of the extract in the case of organic contaminants. Proper pre-treatment of contaminated samples, including leaching of strongly bound compounds, is crucial and considered to be the most laborious step of the analytical process. Traditional methods for sample preparation are laborious, time consuming and usually involve large amounts of solvents for organic compounds or acids for inorganic contaminants. In addition, more than one clean-up stage is usually required for organic compounds in food analysis prior to detection. As a consequence, a variety of sample preparation methods have been developed over the past decades with the objectives to improve the extraction performance as well as to reduce overall analysis time and cost. During the last years, several fast extraction techniques were developed to overcome the limitations of conventional methods. Pressurised liquid extraction (PLE), also named accelerated solvent extraction (ASE), and microwave-assisted extraction (MAE) are techniques that can be used instead of Soxhlet for the extraction of organic compounds, because they are rapid compared to the several hours needed for Soxhlet extraction and, in turn, much less solvent is required.

Ultrasonic energy causes an effect known as cavitation, which generates numerous tiny bubbles in liquid media and mechanical erosion of solids, including particle rupture. Sonication provides an efficient contact between the solid and the extractant, usually resulting in a good recovery of the analyte [1]. Sonication is used in sample preparation to assist the treatment of solid samples, in the extraction, digestion and slurry formation, as well as in liquid sample preparation to assist liquid–liquid extraction (LLE), homogenisation or emulsification.

Ultrasound-assisted extraction (UAE) is used for the extraction of analytes from solid samples, applying ultrasound radiation in a waterbath or with other devices, such as probes, sonoreactors or microplate horns [2]. The most available and cheapest source of ultrasound irradiation is the ultrasonic bath [3–5], but at present a more efficient system using a cylindrical powerful probe for the sonication of samples has been developed [6–8]. The choice between baths and probes depends on the requirements for the particular analysis of contaminants. If the aim is the total solid-liquid extraction, the use of a powerful probe could be better because the necessary time for extraction is lower, however, when a great number of samples need to be analysed the bath is the better option.

The extraction efficiency of a contaminant from a sample by UAE depends on each specific situation, because not all contaminants behave identically having different matrix-contaminant interactions. Then, to maximise extraction it is necessary to optimise different factors such as type of solvent and irradiation conditions (temperature and amplitude of sonication). Other parameters that influence extraction efficacy are: sonication time, sample particle size, sample amount and the ultrasound device employed.

Currently, special attention is paid to the analytical sample preparation procedures in order to ensure reduction of the extractant amount used. An interesting application of ultrasound radiation is the dynamic ultrasound-assisted extraction (DUAE), where the sample is introduced in an extraction cell placed in an ultrasonic waterbath [9,10] or in a waterbath equipped with an ultrasonic probe. DUAE can be used as an open system where fresh extraction solvent is continuously pumped through the sample increasing the analyte transfer or as a closed system, where the extraction solvent is recirculated avoiding the dilution of the extract (Fig. 1). Moreover, it is possible the online coupling of the sample preparation and the instrumental analysis with DUAE.

The aim of this review article is to give an overview of the application of UAE to the analysis of contaminants in soil and food samples.

2. Organic contaminants

2.1. Soil

The contamination of soil is caused mainly by man-made chemicals due to the application of these compounds, the leaching from landfills or direct discharge of industrial wastes along other possible sources of pollution. Among the organic compounds frequently analysed in soil we have selected five groups: pesticides, polycyclic aromatic hydrocarbons (PAHs), flame retardants, pharmaceuticals and other industrial organic compounds, all considered as substances showing high exposure and potential health hazards.

2.1.1. Pesticides

The analytical methods, based on UAE, developed for the determination of pesticides in soil are summarised in Table 1. Initially, the determination of pesticides in soil using UAE was performed employing large volumes of solvent [25], but nowadays the aim is to achieve good extraction efficiencies with a low solvent consumption. The application of UAE to the extraction of pesticide residues from soil is generally carried out using an ultrasonic bath [4,5,10,13,15,21–23]; however, an analytical method applying sonoreactors for the determination in soil of Cl-containing herbicides has been recently reported by Ueno et al. [26]. In the case of multiresidue analysis, where pesticides and their degradation products include non-polar and mid-polar compounds, a polarity of the extraction solvent compatible with all the analytes should be selected.

In the optimisation of ultrasonic extraction, different solvents with a wide range of polarities, like n-hexane, ethyl acetate, acetone and a mixture of petroleum ether and acetone have been assayed. A miniaturised extraction technique based on ultrasound radiation, named sonication assisted extraction in small columns (SAESC), was developed for the determination of pesticides in soil by Sánchez-Brunete et al. [21]. Fig. 2 shows a schematic diagram of the extraction of soil samples using SAESC. The analysis of fungicides, insecticides and herbicides in soil samples was reported by this research group using SAESC with three different solvents, ethyl acetate, methanol and acetone. In general, recoveries obtained with methanol and acetone were lower than those obtained with ethyl acetate, due to the low extraction efficiency of methanol for non-polar compounds and the high concentration of co-extractive impurities obtained with acetone., This technique was also applied to the extraction of carbamate pesticides, whose polarity is higher, and in this case, methanol was selected as extraction solvent since the recovery results obtained ranged from 82 to 99% for all the carbamates studied [15]. The same solvent was used in the determination of the herbicide metribuzin and its metabolites and N-methyl carbamates in soil, as reported by Huertas-Perez et al. [4]

Table 1
Analytical methods developed for the determination of pesticides in soil.

Compounds	Sonication extraction conditions				Clean-up	Determination					Ref.
	Solvent [ml]	Time (min)	Temp. (°C)	Type		Technique (derivatisation)	Recovery (%)	RSD (%)	LOD (μg/kg)	Levels found (μg/kg)	
Alachlor-metabolites	MeOH–water (75:25, v/v) [40]	10 × 2	RT	UB	SPE-Oasis: AcEt, MeOH	GC–MS			n.a.	100	[11]
Chlorothalonil	Acetone [50]	30 × 2	RT	UB	SPE-Oasis: DCM SPE–10% Florisil: DCM–n-hexane (6:4, v/v)	GC–MS (BSTFA)	80–91	4–6	1–5	n.d.	[12]
Chlorpyrifos, endosulfan	AcEt [10]	15 × 2	RT	UB		GC–ECD	93–99	3–5	10	1–150	[13]
Diuron, linuron and metabolites	Acetone, MeOH, DCM [21]	20 × 3	35	UB		LC–UV–DAD	60–85	0.5–5	0.6–4.6	7.6–36.0	[14]
Metribuzin and metabolites	MeOH [45]	20	RT	UB	SPE-Lichrolut: MeOH:AcEt (4:1, v/v)	MEKC	87–104	5.5–9.6	19–23.4		[4]
Carbamates	MeOH [10]	15 × 2	RT	UB		LC–FD (OPA)	82–99	0.4–10	1.6–3.7		[15]
Methyl carbamates	ACN [5]	15 × 2	40	UP	C_{18}: MeOH	LC–FD (OPA)	96–105	4.6–5.4	12		[16]
OC	Petroleum ether–acetone (1:1, v/v) [25]	20	RT	UB	SPE–2% alumina: n-hexane–AcEt (7:3, v/v)	GC–ECD	88–102	2–6	1.6–3.4	13.5–21.4	[17]
OC	DCM [20]	15 × 2	RT	UB		GC–ECD	80–109	0.8–13.5	0.1–1		[18]
OC	n-Hexane–acetone (5:2, v/v) [60]	20 × 2	RT	UB	SPE-GCB: 10% acetone–ACN	GC–ECD, GC–MS	79–106	0.4–5.7	0.002–0.005, 0.03–0.5		[19]
Acidic herbicides	40% ethanol–20% MeOH in buffered water pH 12 [20]	10 × 2	RT	UB		FPIA	80–132		0.08–5		[20]
Acidic herbicides, neutral-basic herbicides	MeOH pH 2 [10], AcEt [10]	15 × 2	RT	UB		GC–MS (F_3B–MeOH), GC–MS	85–99	1–2.8	10, 10		[21]
Insecticides	AcEt [10]	15 × 2	RT	UB		GC–MS	90–108	1–11	10	20–160	[22]
Fungicides	AcEt [10]	15 × 2	RT	UB		GC–ECD, GC–NPD, GC–MS	80–104	1–8	2–10		[23]
Insecticides and herbicides	DCM–acetone–AcEt–cyclohexane (2.1:1:1, v/v/v) [20]	1 × 2	RT	UB		GC–ECD, GC–NPD	67–120	2–15	3.6–53.1	5–230	[3]
Multiclass	AcEt [10]	15 × 2	RT	UB		GC–MS	87–106	2.4–10.6	0.02–1.6	5–228	[5]
Multiclass	Acetone–DCM (1:1, v/v) [100]	5 × 2	RT	UP	SPE–6% Florisil: diethyl ether–n-hexane (1:1, v/v)	GC–ECD	72–129	1.1–10.7	0.2–0.9	1.5–365	[24]

AcEt: ethyl acetate; ACN: acetonitrile; BSTFA: N-O-bis(trimethylsilyl)trifluoroacetamide; DCM: dichloromethane; ECD: electron-capture detector; F_3B: boron trifluorure; FD: fluorescence detector; FPIA: fluorescence polarization immunoassay; GC: gas chromatography; GCB: graphitized carbon black; LC: liquid chromatography; LOD: limit of detection; MEKC: micellar electrokinetic chromatography; MeOH: methanol; MS: mass spectrometry; n.a.: not available; n.d.: not detected; NPD: nitrogen-phosphorus detector; OC: organochlorine; OPA: o-phthaldehyde; RT: room temperature; SPE: solid-phase extraction; UB: ultrasonic bath; UP: ultrasonic probe.

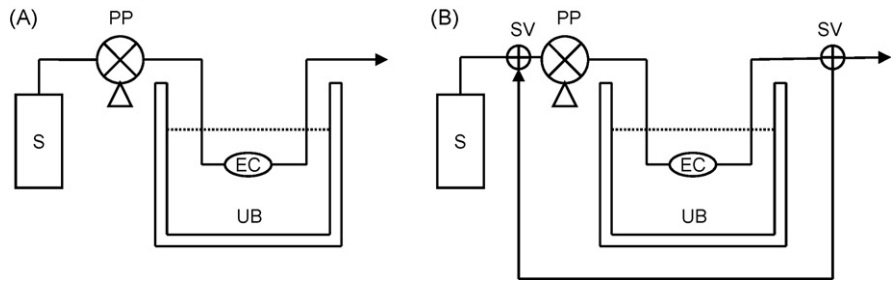

Fig. 1. Schematic diagram of the dynamic ultrasound-assisted extraction (DUAE): (A) as an open system and (B) as a closed system. S: solvent; PP: peristaltic pump; EC: extraction chamber; UB: ultrasonic bath; SV: switching valve.

and Caballo-López and Luque de Castro [27], respectively. Recently, a SAESC method was applied for the simultaneous analysis of 50 pesticides of different chemical classes and with a wide range of polarity with ethyl acetate, obtaining a good reproducibility (relative standard deviation (RSD) < 11%) and low limits of detection (LODs) [5]. In the case of acidic herbicides, such as chlorophenoxy acids, UAE was carried out, after the acidification of the sample, with methanol or aqueous mixtures of ethanol and methanol [20,21]. For industrial soils, a solvent of medium polarity like acetone is preferred in order to break out soil aggregates and allow intensive contact between particles and solvent [17].

Other extraction parameters of UAE, such as solvent volume, number of sonication cycles and extraction time have to be taken into account in order to obtain satisfactory results (Table 1). Vagi et al. [18] reported that with the different solvent volumes assayed in the extraction of organochlorine pesticides similar recoveries were obtained, and the increase of the volume of solvent had no effect on the extraction efficiency. Nevertheless, the optimal volume of solvent depends on the type and quantity of matrix used and the target compound analysed. In some of the methods presented in Table 1, the sonication cycles are usually performed with a 10 ml volume of solvent for a 5 g sample of soil. The effect of sonication time on the extraction of pesticides was studied and it was observed that the recoveries did not clearly improve when the extraction time increased from 30 to 40 min [17]. The performance of two 15 min sonication cycles allowed obtaining satisfactory pesticide recoveries [13,15,21–23]. The complete UAE procedure for pesticides in soil normally takes 15–60 min before their determination (Table 1).

In general, UAE is performed at room temperature, which allows the extraction of pesticides without noticing degradation, and an enhancement in the recovery yields due to a temperature increase is usually not observed.

After the extraction of pesticides from soil and sediment samples, a further clean-up step is frequently necessary due to the interferences co-extracted along with the target compounds [17,19,24,29]. Solid-phase extraction (SPE), carried out with different sorbents, has been often chosen as the purification procedure. Hladik and Kuivila [12] reported a SPE with Florisil for the clean-up of extracts in the determination of chlorothalonil and three degradates. The same sorbent was used in the determination of pyrethroid, organophosphate and organochlorine pesticides [24]. Oasis and graphitized carbon black cartridges have also been used, particularly to remove polar materials [12,19].

Various studies have compared different extraction techniques for extracting pesticides from soil samples. Lesueur et al. [6] applied a new ultrasonic system, based on an cylindrical probe, for extraction of 24 pesticides from soil samples and the results were compared with those obtained by different extraction methods, such as PLE, QuECheRS and the European Norm DIN method. The results revealed that this new UAE method was successful to recover the selected pesticides with a good repeatability (mean standard deviation of 8.5%), whereas some of the pesticides studied were not recovered with the other methods.

Soxhlet, PLE and UAE were compared by Villaverde et al. [29] for the extraction of pesticides from sediment samples. Although all extraction techniques produced acceptable recoveries for the pesticides studied, the UAE method was selected due to the simplicity of the extraction procedure.

In the last years, hyphenation of sonication with other sample extraction techniques, such as MAE [27] and solid-phase microextraction (SPME) [28], has been developed for the analysis of pesticides in soil. Caballo-López and Luque de Castro [27] performed the analysis of dacthal and its di-acid metabolite in soil and sediments after UAE and in situ focused microwave-assisted derivatization prior to gas chromatography (GC) with electron-capture detection (ECD), with a substantial shortening of the extraction time. The same authors have published a simultaneous SPE and continuous ultrasound irradiation system for the analysis of N-methylcarbamates pesticides in soil with recoveries higher than 96% for all the pesticides studied [16]. Lambropoulou and Albanis [28] have developed a method for the extraction of fungicides in soil samples where SPME was coupled to UAE. The extraction involved the homogenisation of the sample with water-acetone assisted by sonication and pesticides were isolated with a fused silica fibre. This method was shown to be an inexpensive and fast sample preparation method for determination of target analytes at low nanogram per gram levels in soils.

The determination of pesticides has been predominantly carried out by GC coupled to sensitive and specific detection systems, such as the ECD for halogenated pesticides [10,13,19,23] and the nitrogen-phosphorus detector (NPD) [10,23] for pesticides with

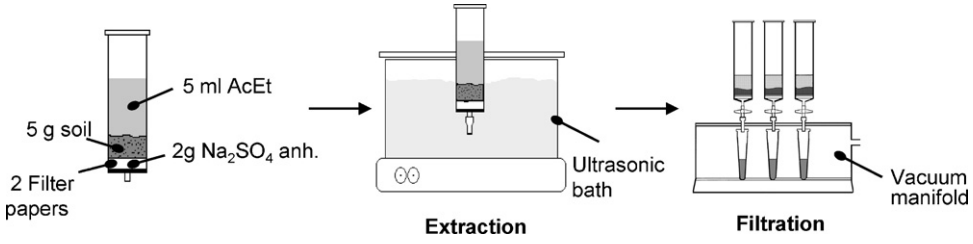

Fig. 2. Schematic diagram of SAESC (sonication assisted extraction in small columns) procedure.

Table 2
Analytical methods developed for the determination of PAHs in soil.

No. of PAHs analysed	Sonication extraction conditions				Clean-up	Determination					Ref.
	Solvent [ml]	Time (min)	Temp. (°C)	Type		Technique	Recovery (%)	RSD (%)	LOD (μg/kg)	Levels found (μg/kg)	
2	Isopropanol–water (8:2, v/v) [30]	10 × 2	55	UB	SPE-Oasis-Florisil: DCM–diethyl ether (8:2, v/v)	GC–MS	46–110	4–40	80	n.d.	[30]
13	Cyclohexane [6]	30 × 2	RT	UB		LC–FD	70–98	2–15	>0.2 μg/l	15–282	[31]
16	Acetone [20]	15 × 2	RT	UB	SPE-C_{18}: acetone	LC–FD LC–UV	80–97	1–4	10–15	500–1700	[32]
16	Acetone–water (1:1, v/v) [90]	5 × 3	RT	UB	SPE-silica: n-hexane–DCM (1.5:1, v/v)	GC–FID GC–MS			2–40	1470–6610	[33]
16	Acetone–petroleum ether (1:1, v/v) [30]	15	RT	UB	SPE-silica: petroleum ether–DCM (3:2, v/v)	GC–MS	75–120	<5	2–42	16–3884	[34]
16	Acetone–toluene (1:1, v/v) [100]	20 × 2	RT	UB		LC–FD LC–DAD	88–110	3–12			[35]
16	AcEt [10]	15 × 2	RT	UB		GC–MS	91–100	0.4–9.3	0.03–0.3	0.08–1.1	[36]
16	DCM [80]	75	RT	UB	SPE-C_{18}: acetone	LC–UV	81–90	<21	5–500	10–13	[37]
16	n-Hexane–acetone (1:1, v/v) [30]	15 × 2	RT	UB	SPE-silica: n-hexane–DCM (10:1, v/v)	GC–MS	93–110	3.5–6.9	1.0	50–2500	[38]
16	n-Hexane–DCM (1:1, v/v) [30]	10 × 3	RT	UB	SPE-alumina: n-hexane–DCM (10:1, v/v)	GC–MS	44–114	<20	0.1–2		[39]
27	AcEt [10]	15 × 2	RT	UB		GC–MS	90–102	<15	0.03–0.3	42.8–61.3	[40]
29	DCM [60]	15 × 2	RT	UB	SPE-silica: n-hexane–DCM (9.5:0.5, v/v)	GC–MS	80–120	<15	1–3	2–5000	[41]

AcEt: ethyl acetate; DAD: diode array detector; DCM: dichloromethane; FID: flame ionization detector; FD: fluorescence detector; GC: gas chromatography; LC: high performance liquid chromatography; LOD: limit of detection; MS: mass spectrometry; n.d.: not detected; PAHs: polycyclic aromatic hydrocarbons; RT: room temperature; SPE: solid-phase extraction; UV: ultraviolet detector; UB: ultrasonic bath.

nitrogen or phosphorus in the molecule. In the case of acidic [21] and phenolic pesticides a derivatization of these compounds is usually performed to reduce their polarity, increase their volatility and improve the LOD for its determination by GC. On the other hand, a fluorescent derivative is obtained for their analysis by liquid chromatography (LC) [6,12]. Gas chromatography–mass spectrometry detection (GC–MS) has been also used in multiresidue methods for pesticide analysis in soil [5,21,22,24]. Carbamate pesticides are polar compounds and can be determined directly by LC with fluorescence detection (FD) after post-column derivatization [15,16] or with ultraviolet (UV) or diode array detection (DAD) [14]. The chromatographic techniques described above provide LODs in the order of μg/kg.

2.1.2. PAHs

Table 2 summarises the analytical methods developed for the determination of PAHs in soil that have applied UAE. In general, the extraction of PAHs from soil is carried out in an ultrasonic bath [30–32,36,37,40,41].

Some authors have reported that the extraction of PAHs from soil with polar solvents, such as methanol and acetonitrile, is not effective, however, when solvents with lower polarity such as dichloromethane, ethyl acetate and acetone are employed good extraction yields are achieved. Acetone and ethyl acetate were the solvents most used since they are relatively less toxic than other solvents like dichloromethane. Ethyl acetate was used in the extraction of PAHs from uncontaminated soil [36,40] whereas for highly contaminated soils acetone was the solvent chosen [32]. The SAESC procedure described above was also applied for the analysis of PAHs with good results (recoveries > 90%) [36,40].

The appropriate extraction time for the UAE procedure and its influence in the recovery assays were also studied. It was observed that prolonged sonication cycles, longer than 60 min, did not improve the extraction efficiency due to the evaporation of the most volatile PAHs during the extraction process. Although different extraction periods were applied (15–75 min), in general two cycles of 15 min were carried out in the extraction of these compounds. The temperature of ultrasonic bath was also evaluated and similar recoveries were obtained for these compounds when UAE was carried out at different temperatures. Thus, the extraction from soil samples is generally performed at room temperature. Moreover, Banjoo and Nelson [38] reported that when the extraction of these analytes was carried out at high temperatures, losses up to 16% of thermally labile PAHs were observed due to volatilisation and oxidation.

In general, no clean-up of sample extracts is required for agricultural soils. On the other hand, industrial soil samples frequently contain pollutants that either may interfere in the determination or are capable of damaging the analytical column. Therefore, a purification of extracts is often necessary to minimise interferences. SPE, in columns or cartridges, is the technique usually applied for the purification of extracts. Different kinds of sorbents with a wide range of polarities, such as octadecyl-bonded silica (C_{18}), Florisil, silica, Oasis, etc., can be used depending on the nature of the analyte. In the methods summarised in Table 2 where a clean-up was carried out, the purification procedure usually consists on a column chromatography with silica, C_{18}, alumina or Florisil, and elution with a mixture of dichloromethane and n-hexane [33,38,39,41] followed by the concentration of the eluates.

The extraction of PAHs from soil comparing different methodologies has been carried out by several authors. As an example,

Hollender et al. [35] reported the extraction of PAHs in two contaminated soils using four different extraction procedures: UAE, Soxhlet extraction, supercritical fluid extraction (SFE), and PLE, with solvents of different polarities. In the case of UAE, recoveries higher than 88% were achieved when the extraction was performed with acetone–toluene for the uncontaminated soil and with acetone–ethanolamine for the highly contaminated one. Barco-Bonilla et al. [42] also compared PLE and UAE, and similar results were achieved in the recovery assays carried out with both techniques. PLE requires lower solvent volumes and shorter extraction times, reducing the costs and toxic wastes generated, nevertheless, the high cost of PLE equipment is an important drawback to be taken into account and thus UAE can be considered a good alternative.

Bossio et al. [30] developed a method based on UAE for the determination of PAHs together with other persistent pollutants, such as personal care products and fire retardants, in soil and sediment samples. These authors demonstrated that UAE is an attractive and effective procedure for the extraction of contaminants from solid matrices. The major advantages of UAE in comparison to other extraction methods such as PLE or Soxhlet include ease of use and low cost, and moreover it is less time consuming than Soxhlet.

The extraction of PAHs in contaminated soils where four different extraction procedures, Soxhlet, UAE, Curie point pyrolysis and MAE, were compared using the same solvent was reported by Buco et al. [43]. Although similar results were achieved in the recovery assays carried out with the different extraction techniques, Curie point pyrolysis seems to be a good alternative for low-molecular-mass PAHs with small subsample size and short operating time.

An interesting approach to enhance extraction yields is the application of UAE to assist other extraction procedures, such as SFE and Soxhlet extraction. In the case of SFE, an ultrasound transducer was installed inside the extractor [44] whereas for the Soxhlet extraction, UAE was applied, by means of a probe, in the sample cartridge zone before the siphoning of the Soxhlet chamber [45]. Ultrasound-assisted pressurised solvent extraction (UAE-PSE) for the extraction of aliphatic and polycyclic aromatic hydrocarbons from soil is another example of the hyphenated methods that provide good extraction efficiencies [46].

The determination of PAH residues has been carried out by GC with flame ionisation detection (FID) or GC–MS but it can be also performed by LC with FD and UV detection (Table 2). When the analysis was carried out with gas chromatography coupled to tandem mass spectrometry (GC–MS/MS), due to the high selectivity of the triple quadrupole, no further clean-up steps were necessary [42]. The LODs achieved with the different detection systems are of the order of μg/kg. The PAH levels encountered when real samples were analysed were generally low and phenanthrene, pyrene and benzo[a]pyrene were the most frequently detected PAHs in the soil samples.

2.1.3. Pharmaceuticals

Several extraction methods have been developed to determine the concentration of pharmaceutical compounds, including antibiotics and hormones, in soil samples. Table 3 shows the extraction conditions and determination characteristics of these methods.

The extraction of different classes of antibiotics, based on ultrasonication with different extraction solvents, such as aqueous solutions or weakly acidic buffers combined with organic solvents, has been recently reported [47,48]. Many antibiotics contain polar functional groups, therefore, depending on the pH of the medium, they can be protonated or dissociated and buffer solutions are needed. Furthermore, tetracyclines (TCs) form chelate complexes with metal ions and bind to proteins and silanol groups, therefore, Na_2EDTA (disodium ethylendiamine tetracetate), a chelating agent, is used to improve the extraction of these compounds [47].

Table 3
Analytical methods developed for the determination of pharmaceutical compounds in soils.

Compounds	Sonication extraction conditions				Clean-up	Determination				Ref.
	Solvent [ml]	Time (min)	Temp. (°C)	Type		Technique (derivatisation)	Recovery (%)	RSD (%)	LOD (μg/kg)	Levels found (μg/kg)	
Antibiotics	MeOH–EDTA-buffer (50:25:25, v/v/v) [15]	10 × 3	RT	UB	SPE-SAX SPE-Oasis: MeOH	LC–UV LC–FD	61–89	7–16	18–40		[47]
Antibiotics	Na_2EDTA–MeOH [90]	10 × 3	RT	UB	SPE-C_{18}: buffer LLE: n-hexane	LC–MS/MS	74–105	2–12	0.49–25	0.04	[48]
Qs, FQs	$MgNO_3$ (50%, w/v) + 4% ammonia [8]	30	RT	UB		LC–UV	83–104	5.8–11.7	40–80		[49]
FQs	MeOH–ammonia (75:25, v/v) [8]	30	45	UB	SPE-MIP: MeOH	LC–UV	88–104	4.8–6.0	40–70		[50]
Estrogens progestogens	Acetone–MeOH (1:1, v/v) [55]	5 × 3	RT	UB	SPE-C_{18}: ACN	LC–DAD	64–100	6–19	10–20	0.07–22.8	[51]
						LC–MS			0.4–10		
Estrogens	Acetone–hexane (1:1, v/v) [50]	30	RT	UB	SPE-Oasis: ACN	GC–MS	60–127	5–12	1.5–5		[52]
Estrogens	Acetone–MeOH, (1:1, v/v) [13]	10	RT	UB	SPE-silica: n-hexane–acetone	GC–MS/MS (MSTFA)	94–99	11–23	0.2–0.4	0.9–2	[53]
Pharmaceuticals, EDCs, hormones	Acetone–AcEt, 10% acetic acid [20]	5 × 4	RT	UB	SPE-C_{18}: pH 3 AcEt	GC–MS (MTBSTFA)	81–118	2.1–6.6	0.2–12	0.55–9.08	[54]

AcEt: ethyl acetate; ACN: acetonitrile; DAD: diode array detector; EDCs: endocrine disrupting compounds; EDTA: ethylenediaminetetracetic acid; FD: fluorescence detector; FQs: fluoroquinolones; GC: gas chromatography; LC: liquid chromatography; LLE: liquid–liquid extraction; LOD: limit of detection; MeOH: methanol; MIP: molecularly imprinted polymers; MS: mass spectrometry; MS/MS: tandem mass spectrometry; MSTFA: N-methyl-N-trimethyl silyltrifluoro-acetamide; MTBSTFA: N-methyl-N-(tertbuthyldimethylsilyl)trifluoroacetamide; Qs: quinolones; RT: room temperature; SAX: strong anion exchange; SPE: solid-phase extraction; UV: ultraviolet detector; UB: ultrasonic bath.

Blackwell et al. [48] reported a simple and rapid UAE method for the simultaneous extraction of three antibiotics, oxytetracycline, sulfachloropyridazine and tylosin. The extraction was performed with a mixture of methanol, ethylenediaminetetracetic acid (EDTA) and buffer at pH 7, followed by the clean-up of the extracts by a tandem SPE method using anion exchange and Oasis polymeric cartridges.

In the case of quinolones (Qs) and fluoroquinolones (FQs), due to the strong sorption to soil together with their different acid–base properties, an exhaustive optimisation of the extraction step is required. Turiel et al. [49] developed a method for the simultaneous analysis of several Qs and FQs in soil samples. The method was based on the extraction of these analytes by SAESC and the formation of antibiotic-Mg(II) complexes. These same authors developed a method for the determination of FQs in soil using molecularly imprinted solid-phase extraction (MISPE) in cartridges [50]. Various molecular imprinted polymers (MIP) were prepared to obtain a MIP-based material with the proper characteristics to be used both as a selective sorbent for MISPE and as a selective stationary phase. The main advantage of this method is the fact that the analytes can be separated from the matrix-interfering compounds in the MIP column directly connected to the UV detector.

The analysis of antibiotics has been usually carried out by LC with UV or FD (Table 3). However, the current trend in the analysis of antibiotics in environmental samples is the use of liquid chromatography–mass spectrometry (LC–MS). Martinez-Carballo et al. [47] applied liquid chromatography–tandem mass spectrometry (LC–MS/MS) for the determination of TCs, sulfonamides, trimethoptim and FQs in soil. This method achieved high sensitivity and selective detection, therefore, a less rigorous clean-up was required.

Estrogens, natural and synthetic, are another group of pharmaceutical products determined in soil. In general, the extraction is carried out using polar or semipolar organic solvents or mixtures (acetone–methanol or acetone–n-hexane), followed by a clean-up stage with SPE cartridges. The purification procedure usually consists on a column chromatography with C_{18} or Oasis, and elution with acetonitrile [51] or alkaline acetonitrile [52] followed by the concentration of the eluates before GC or LC analysis.

Estrogens are polar compounds and, therefore, are usually determined by LC with UV or MS detectors. Nevertheless, the analysis can be also carried out by GC–MS with or without derivatisation (Table 3). In general, due to the high polarity of these compounds, a derivatisation is conducted prior to GC–MS to reduce the polarity, enhance their mobility on the GC column and improve the LODs.

Termes et al. [53] proposed a method for the determination of estrogens with GC–MS/MS after silylation with MSTFA (N-methyl-N-trimethyl silyltrifluoro-acetamide). This method required derivatisation of the estrogens to improve peak shape and sensitivity in their GC–MS determination. In previous works, different silylation agents, such as MSTFA, BSTFA (N-O-bis(trimethylsilyl)trifluoroacetamide) and MSTFA/TMCS (trimethylchlorosilane), were used for determination of estrogens.

The presence of multiple chemicals in the environment, though each at very low concentrations, may exert additive effects that result in significant detrimental impacts on wildlife and humans. Thus, it is necessary to develop analytical methods that allow the simultaneous determination of different contaminants at trace levels. UAE was employed to determine six different pharmaceuticals, three endocrine disrupting compounds and estrone in soil. Soil was extracted with acetone–ethyl acetate followed by a SPE C_{18} clean-up procedure and analysis by GC–MS after derivatisation with MTBSTFA [54].

2.1.4. Polyhalogenated flame retardants

Polyhalogenated aromatic hydrocarbons (PAHs) represent a large family of highly lipophilic and environmentally persistent substances, of which polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polybrominated biphenyls (PBBs), tetrabromobisphenol-A (TBBPA), tetrachlorobisphenol-A (TCBPA) and hexabromocyclododecane (HBCD) have been used as flame retardants. It has been reported that TBBPA may degrade to bisphenol-A (BPA) during anaerobic soil processes and, therefore, BPA may be used as a marker of contamination due to the use of flame retardants. Table 4 shows the analytical methods, based on UAE, that have been developed for these compounds in soil.

PCBs are compounds with a low polarity, thus, extraction solvents such as n-hexane, petroleum ether, acetone, dichloromethane and mixtures are the most commonly used. In general, mixtures of acetone and n-hexane in different proportions have been found to provide the best recoveries [55,56].

Aydin et al. [55] applied a miniaturised UAE method for PCBs in soil samples that used less solvent than traditional methods, reducing the cost associated with solvent purchase and waste disposal, with recoveries that ranged from 90 to 98% and lower LODs (3–6 ng/kg) than other techniques.

The purification of extracts usually consists on a column chromatography with polar adsorbents, alumina or Florisil among others, and elution with a non-polar organic solvent such as n-hexane followed by the concentration of the eluates. Another column chromatography applied in the clean-up stage of PCB extracts is gel permeation chromatography (GPC) performed with Bio-beads equilibrated with a mixture of cyclohexane–ethyl acetate (Table 4).

There are several papers in the available literature comparing different methods to extract PCBs from soils. Bianco et al. [56] concluded that using the same extraction solvent, acetone–n-hexane, UAE provided extraction efficiencies comparable to those achieved with MAE. Sporring et al. [61] reported the extraction of PCBs from soil where six different extraction techniques, Soxhlet, Soxtec, UAE, SFE, MAE and PLE, were compared using the same solvent, n-hexane:acetone (1:1, v/v). Similar results were achieved in the recovery assays carried out with the different extraction techniques.

The determination of PCBs in soil, after the extraction and purification of the sample, is generally performed with GC given that these compounds have a high thermal stability and low polarity. The detection systems used with GC were ECD, since PCBs are organochlorine compounds, or MS, obtaining LODs in the subnanogram per gram level.

PBDEs are another group of flame retardants studied in soil. Sánchez-Brunete et al. [57] developed an UAE method for the determination of these compounds where soil was extracted twice with ethyl acetate in an ultrasonic bath for 15 min. PBDEs are generally determined by capillary GC, using preferably selected ion monitoring (SIM)-MS, both in electron impact (EI) [57] or negative chemical ionisation (NCI) mode and GC–MS/MS [62]. Fig. 3 shows the chromatogram and a partial view of the SIM chromatogram, with the corresponding mass spectra, of a soil collected in an industrial area that contained residues of two PBDEs. The analysis of eight hydroxylated brominated diphenyl ethers (OH-PBDEs) by UAE with hexane:dichloromethane (1:1, v/v) was reported by Mas et al. [58]. The thermal instability and low volatility of these PBDE metabolites make GC analysis difficult and entail the prior derivatisation of the analytes to increase their sensitivity, volatility and selectivity, therefore, this research group applied LC coupled to negative ion-spray ionisation (ISP) MS/MS for the simultaneous analysis of OH-PBDEs in soil with LODs at picogram per gram levels.

An isotope dilution method for the determination of TBBPA, TCBPA and BPA by GC–MS in agricultural and industrial soil samples was developed in our laboratory [59]. The compounds were

Table 4
Analytical methods developed for the determination of polyhalogenated flame retardants compounds in soil.

Compounds	Sonication extraction conditions				Clean-up	Determination				Ref.
	Solvent [ml]	Time (min)	Temp. (°C)	Type		Technique (derivatisation)	Recovery (%)	RSD (%)	LOD (μg/kg)	Levels found (μg/kg)	
PCBs	Acetone–petroleum ether (1:1, v/v) [2]	5 × 3	RT	UB	SPE-5% alumina: n-hexane	GC–MS	90–98	3–7	0.003–0.006		[55]
PCBs	Acetone–n-hexane (0.75:1, v/v) [100]	15 × 2	RT	UP	GPC: cyclohexane:AcEt	GC–ECD GC–MS	46–97	3.5–7.2	0.01–0.04 0.001–0.01	0.6–62	[56]
PBDEs	AcEt [10]	15 × 2	RT	UB	SPE-Florisil: n-hexane–DCM (1:2, v/v)	GC–MS	81–104	1–9	0.002–0.03	1.3–5.6	[57]
OH-PBDEs	n-Hexane–DCM (1:1, v/v) [90]	10 × 3	RT	UB	H_2SO_4	LC–ISP-MS/MS	99–108	4–9	0.2–1.4		[58]
TBBPA, TCBPA, BPA	AcEt [10]	15 × 2	RT	UB	SPE-Florisil (37% HCl)	GC–MS (BSTFA)	88–108	1.0–4.2	0.03–0.09	0.3	[59]
HBCB, TBBPA	Acetone [5]	10	RT	UB		LC–MS/MS	101–108	2.8–5.1	0.002–0.02	0.2–1.6	[60]
AWIs	Isopropanol:water (80:20, v/v) [10]	10 × 2	RT	UB	SPE-Oasis-Florisil: DCM:diethyl ether (80:20, v/v)	GC–MS	46–110	7.6–25.0	0.06–0.11	1.7–3.7	[30]

AcEt: ethyl acetate; AWIs: organic anthropogenic waste indicators; BPA: bisphenol-A; BSTFA: N-O-bis(trimethylsilyl)trifluoroacetamide; DCM: dichloromethane; ECD: electron-capture detector; GC: gas chromatography; GPC: gel permeation chromatography; HBCD: hexabromocyclododecane; ISP-MS/MS: negative ion-spray ionization tandem mass spectrometry; LC: liquid chromatography; LOD: limit of detection; MS: mass spectrometry; MS/MS: tandem mass spectrometry; OH-PBDEs: hydroxylated polybrominated diphenyl ethers; PBDEs: polybrominated diphenyl ethers; PCBs: polychlorinated biphenyls; RT: room temperature; SAX: strong anion exchange; SPE: solid-phase extraction; TBBPA: tetrabromobisphenol-A; TCBPA: tetrachlorobisphenol-A. UB: ultrasonic bath; UP: ultrasonic probe.

extracted from soil by SAESC with a low volume of ethyl acetate. For dirty soil samples, such as industrial soil, a simultaneous clean-up on an acidified Florisil–anhydrous sodium sulphate mixture was carried out to remove interferences. Due to the high polarity of TBBPA and TCBPA, poor chromatographic peaks were obtained and derivatisation was necessary when they were determined by GC. After the extraction, solvent was evaporated and analytes were derivatised with BSTFA and determined by isotope dilution gas chromatography with electron impact mass spectrometric detection in the selected ion monitoring mode (GC–MS-SIM), using $^{13}C_{12}$-labelled compounds as internal standards. Suzuki et al. [60] developed a rapid UAE method to determine TBBPA together with HBCD diastereoisomers in soil using acetone that did not require a clean-up step, obtaining good extraction yields. In this case the analytes were determined by LC–MS and LC–MS/MS.

Organic anthropogenic waste indicators (AWIs) are a broad range of industrial, agricultural and household chemicals that include products such as flame retardants, hormones, steroids, pesticides and personal care products. Bossio et al. [30] applied UAE for the determination of AWIs from soils collected in Washington. The extraction was performed twice with isopropanol:water in an ultrasonic bath for 10 min followed by a SPE clean-up and the extracts were analysed by GC–MS. The developed method makes evident that UAE can be employed to extract simultaneously organic contaminants that vary considerably in their physicochemical properties.

The coupling of UAE with other sample preparation techniques has also been applied for the analysis of flame retardants in soil samples. In this way, Salgado-Petinal et al. [62] developed a method for the determination of PBDEs where UAE, including a simultaneous "on batch" clean-up step, was carried out followed by headspace solid-phase microextraction (HS-SPME) and, thus, being a suitable alternative for PBDEs extraction from environmental solid matrices. Yu and Hu [63] combined stir-bar sorptive extraction (SBSE) with UAE for the determination of non-polar PBDEs and polar TBBPA. This novel method was found to be rapid and sensitive. The extraction involved the homogenisation with acetone assisted by sonication and subsequently PBDEs and TBBPA were isolated with fused polydimethylsiloxane (PDMS) bars. This method was shown to be a fast and simple procedure for the determination of target analytes at low nanogram per gram levels in soil.

2.1.5. Other industrial contaminants

UAE has been applied to the determination of other industrial contaminants in soil as it is shown in Table 5. Campillo et al. [64] reported the extraction of chlorophenols (CPs) from soil using during 30 s an ultrasounds probe directly immersed in the suspension prepared. Due to the high polarity of CPs many authors have recommended the derivatisation of these compounds to convert them into less polar forms, thus improving peak shape and sensitivity during GC. The derivatisation procedure most frequently used is acetylation using acetic anhydride. A purge-and-trap concentration system coupled to a GC equipped with a microwave-induced atomic emission detector was used to determine CPs.

Llompart et al. [65] reported a comparative assessment of the extraction of o-cresol, m-cresol and p-cresol in soil by three different extraction procedures, SFE, MAE and UAE with or without a one-step in situ derivatisation. Although similar results were achieved in the recovery assays carried out with the different extraction techniques, SFE and MAE appeared to be good alternatives, although the high cost of the equipments is a drawback to be considered when compared with UAE.

Nonylphenol (NP) and nonylphenol ethoxylates (NPEOx) can be extracted from soil samples by UAE followed by an enrichment step onto C_8 SPE cartridges prior to separation using LC–FD [66].

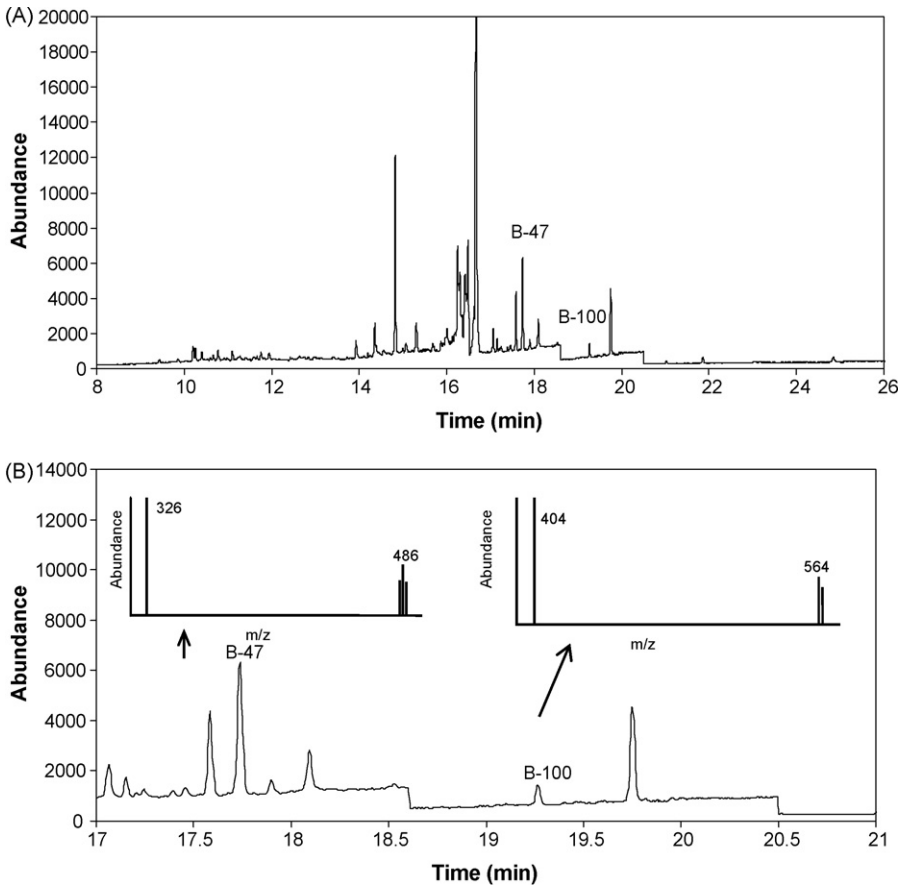

Fig. 3. Chromatograms of a soil sample collected in an industrial area containing B-47 (4.6 ng/g) and B-100 (1.3 ng/g): (A) a GC–MS chromatogram showing the presence of PBDEs B-47 and B-100 and (B) a partial view of the SIM chromatogram with the corresponding mass spectra (from Ref. [55]).

The commercially known parabens are esters of the p-hydroxybenzoic acid widely used as preservatives in pharmaceutical preparations, personal care products and food and beverages. Nuñez et al. [67] have studied the concentration of parabens in soil with a method based on the sample preparation by SAESC, followed by LC–MS/MS with low limits of quantitation (LOQs).

2.2. Food

The presence of residues of organic contaminants in food can be the result of the use of pesticides and veterinary drugs in crops and food-producing animals or the exposure of food via environmental routes. The application of UAE to the analysis of organic contaminants in food is reviewed in this section. The compounds studied have been divided in four main groups: pesticides, PAHs, pharmaceuticals, and other industrial contaminants.

2.2.1. Pesticides

In the last years, an increasing number of works have been published where pesticide residues in food of plant and animal origin have been determined using UAE as extraction technique (Table 6). The selection of solvent used in the extraction depends on the polarity of the target analyte and the food product analysed. For pesticides with a low polarity the extraction may be carried out with non-polar solvents such as n-hexane [68], however for more polar compounds solvents such as acetone, ethyl acetate, dichloromethane, or mixtures of these provide better extraction efficiencies, When pesticide residues belonging to different chemical classes with a wide polarity range are determined simultaneously, such as the analysis of multiclass pesticides (Table 6), the solvent selection is critical. In the particular case of multiresidue methods for the determination of pesticides in fruits or vegetables, which have low lipid content, the extraction is performed with a semipolar solvent as acetonitrile or ethyl acetate [78–80]. On the other hand, food with a high lipid content such as eggs or honey require a low polarity solvent (i.e. n-hexane or petroleum ether) to enhance the analyte extraction from the matrix, although other solvents (acetone or ethyl acetate) are added to increase the polarity due to the nature of the target analyte [69]. In the case of carbamates and other polar pesticides, such as quaternary ammonium compounds, the extraction was carried out using buffer solutions and methanol [16,73,74,81].

Although UAE is primarily used in the extraction of pesticides from solid samples using the appropriate solvent, it has been also applied in the LLE of fungicides and organophosphorus insecticides from beverages, such as wine and must with the addition of NaCl to enhance the partitioning from the aqueous phase to the organic phase due to the "salting out" effect [70,71].

The ultrasonic extraction is carried out using several devices such as waterbaths, probes and sonoreactors but, in general, UAE of pesticides in food has been mainly performed in an ultrasonic bath at room temperature without temperature control (Table 6). However, the new ultrasonic waterbaths provide temperature control allowing the optimisation of this parameter. A different approach in the application of ultrasonic radiation is the DUAE, where it is possible to couple online the sample preparation with the instrumental analysis. This new technique was used by Caballo-López and Luque de Castro [16] for the determination of N-methylcarbamates in fruits and vegetables using a flow injection system coupled to an UAE chamber and a SPE minicolumn for analyte concentra-

Table 5
Analytical methods developed for the determination of industrial compounds in soils.

Compounds	Sonication extraction conditions				Clean-up (derivatization)	Determination				Ref.
	Solvent [ml]	Time, (min)	Temp (°C)	Type		Technique	Recovery (%)	RSD (%)	LOD (μg/kg)	Levels found (μg/kg)	
Chlorophenols	Potassium carbonate (5%, w/v) [20]	0.5	RT	UP	(Acetic anhydride)	GC–AED	75–129	8–18	0.08–0.5		[64]
Phenols	Dichloromethane [30]	3 × 3	RT	UP		GC–MS					[65]
Nonylphenol and nonylethoxylates	Water–MeOH (30:70, v/v) [5]	15 × 2	45	UB	SPE-C_8: MeOH and ACN	LC–FD	78–97	3.5–15.6	60–520		[66]
Parabens	ACN [9]	15 × 2	RT	UB		LC–MS/MS	83–110	2.7–10.3	0.04–0.14	0.63–6.35	[67]

ACN: acetonitrile; AED: atomic emission detector; GC: gas chromatography; LC: liquid chromatography; FD: fluorescence detector; MeOH: methanol; MS: mass spectrometry; MS/MS: tandem mass spectrometry; SPE: solid-phase extraction. UB: ultrasonic bath; UP: ultrasonic probe.

tion before the chromatographic system. The extraction chamber was introduced in a waterbath at 40 °C and a probe provided the ultrasound radiation. The authors compared their method with the reference method of the Environmental Protection Agency (Method 8313) based on manual shaking with acetonitrile, solvent exchange and SPE clean-up. Although the recoveries obtained with both methods were similar, the proposed method allows the extraction of the analytes in 2 min whereas the official method required 4 h for this step.

Following the extraction, a centrifugation or filtration of the extracts is normally required before concentration and/or clean-up stages. In general, the clean-up of the extracts after the UAE is performed with SPE columns or cartridges with different adsorbents, such as Oasis MCX (mixed mode cation exchange), C_{18}, NH_2-sorbent, and Florisil (Table 6). On the other hand, for food rich in lipids, as animal tissues or eggs, it is usually necessary a more thorough purification of the extracts to remove lipidic interferences that may hinder the chromatographic analysis. Hence, Zhao et al. [69] performed a sulphuric acid–silica gel clean-up before the SPE purification with Florisil when determining α-HCH in meat and eggs.

The extraction of pesticides in food based on UAE has been compared with other procedures by several authors. Kolbe and Andersson [72] carried out the extraction of o-phenylphenol from citrus fruits with UAE and steam distillation using a modified Clevenger apparatus combined with LLE. The authors concluded that the latter was time consuming (2 h reflux) and labour extensive (manual LLE). UAE was compared with the traditional shake flask extraction method in the determination of pesticides in honey [75] and it was observed that with UAE the recoveries were higher (71–75% with the shake flask method and 92–94% with UAE) and the method was faster than the conventional procedure.

An interesting approach in sample preparation is to couple UAE with other extraction techniques to take the most of both procedures in order to achieve good extraction yields, with lower solvent consumption, and be cost effective. Table 7 shows the analytical methods developed for the determination of pesticide residues where UAE has been coupled to other extraction techniques. Matrix solid-phase dispersion (MSPD) assisted with ultrasonic radiation was used by Albero et al. [82,85–87] and Sánchez-Brunete et al. [83,84] for the determination of pesticides in honey, pollen and fruit juices. After the MSPD procedure, where the sample was blended with an adequate adsorbent and transferred to a column for its subsequent elution with an appropriate solvent, the columns were placed in an ultrasonic bath during 10–15 min. The assistance of ultrasound radiation improved the extraction of pesticide residues and the MSPD procedure allowed the simultaneous extraction and clean-up shortening the sample preparation process and avoiding possible analyte loss. Recently, an ultrasonic-assisted MSPD method was developed for the determination of triazines and organophosphorus pesticides in fruits and two different sonication devices, an ultrasonic bath and a sonoreactor, were tested [88]. In this case, a 1 min sonication with the sonoreactor yielded higher extraction efficiencies than those obtained when the waterbath was employed. The determination of acaricides in honey by direct immersion solid-phase microextraction (DI-SPME) performed in an ultrasonic bath instead of by magnetic stirring is another example of the coupling ultrasound with other extraction procedures [89]. Shrivas and Wu [91] developed an analytical method for the determination of organochlorine pesticides in fish combining UAE with single-drop microextraction (SDME). The UAE was performed with methanol for 10 min followed by the SDME procedure that was carried out with 0.6 μl of toluene. This procedure was compared with a method based on Soxhlet extraction followed by SPME, and the extraction time was 96 times lower and the recovery higher with this new method.

Table 6
Analytical methods for the determination of pesticide residues in food.

Analytes	Matrix	Solvent [ml]	Time (min)	Temp. (°C)	Type	Clean-up	Determination				Ref.
							Technique	Recovery (%)	RSD (%)	LOD (μg/kg)	
OC insecticides	Medicinal plants	n-Hexane [25]	5	Ice and salt bath	UP	Florisil cartridge: n-hexane + n-hexane–DCM (85:15)	GC–ECD	>66		30–3000 (LOQ)	[68]
α-HCH	Meat and eggs	Acetone:petroleum ether (1:1) [120]	5 × 3	RT	UP	Sulfuric acid silica gel column: petroleum ether	GC chiral–ECD	88–95	1.0–6.9		[69]
OP insecticides	Must and wine	Acetone:DCM (1:1) [20]	10	RT	UB		GC:ECD, NPD, MS	82–107	2.1–16.5	0.02–0.1 ng	[70]
Fungicides	Must and wine	Acetone:DCM (1:1) [20]	10	RT	UB		GC:ECD, NPD, MS	77–107	1.9–13.7	0.02–0.1 ng	[71]
O-phenylphenol	Citrus fruit	DCM [150]	15 × 2	RT	UB		GC:AED, GC–MS	101–106	3–8	2	[72]
N-methylcarbamates	Pear, apple, cucumber	Water (pH 10) [5]	2	40	UP DUAE	C_{18} Hydra: MeOH	LC–FD post-column derivatization	85–101	1–3	3–12	[16]
Chlormequat and mepiquat	Fruits, vegetables, juices, baby food, bread, mushrooms, beer, coffee powder	1:4 MeOH:ammonium formate buffer solution (100 mM; pH 3.5) [25]	10	RT	UB	ENVI-18 SPE cartridge	LC–MS/MS	>78	<11	0.03 pg (CQ), 0.1 pg (MQ)	[73]
Chlormequat	Fruits	MeOH–ammonium formate (100 mM; pH 3.5; 75 + 25) [50]	10	RT	UB	ENVI-18 SPE cartridge	LC–MS/MS	86–92	7.9–13.4	30	[74]
Atrazine and simazine	Honey	Benzene–water (1:1) [60]	20 × 3	<35	UB		TLC-video densitometry	92–94	2.4–2.8		[75]
Amitraz and metabolites	Pears	AcEt [25]	15	RT	UB		LC–MS/MS	70–106	9–19	<10	[76]
Triazines	Potato	UHQ water [15], DCM/acetone/n-hexane (1:1:1) [10]	10 × 2	RT	UB	Oasis MCX: NH_4OH/MeOH (15:85)	Non-aqueous CE	93–116	6–8	1.7–4.0	[77]
Multiclass	Potato and carrots	ACN [3]	45	RT	UB		LC–MS/MS	70–90	<5	0.5–2	[78]
Multiclass	Apples	ACN [50]	2	RT	UP	L–L partitioning, drying and NH_2-SPE with acetone	GC–MS	70–110	1–20	0.07–18.84 (LOQ)	[79]
Multiclass	Leafy vegetables	AcEt [45]	35	25–45	UB		LC–MS/MS	83–98	0.5–2.4	0.3–1.4 (LOQ)	[80]
Multiclass	Fruit, vegetables, cereals	Ammonium acetate–acetic acid solution 20 mM in MeOH–water (95:5) [40]	30	RT	UB		LC–MS/MS	70–120	<20	10–60	[81]

AcEt: ethyl acetate; ACN: acetonitrile; AED: atomic emission detector; CE: capillary electrophoresis; CQ: chlormequat; DCM: dichloromethane; DUAE: dynamic ultrasound-assisted extraction; ECD: electron-capture detector; FD: fluorescence detector; GC: gas chromatography; LC: liquid chromatography; LOD: limit of detection; MCX: mixed mode cation exchange; MeOH: methanol; MQ: mepiquat; MS: mass spectrometry; MS/MS: tandem mass spectrometry; NPD: nitrogen-phosphorus detector; OC: organochlorine; OP: organophosphorus; RT: room temperature; SPE: solid-phase extraction; TLC: thin layer chromatography; UB: ultrasonic bath; UHQ: ultra high quality; UP: ultrasonic probe.

Table 7
Hyphenated analytical methods for the determination of pesticide residues in food.

Coupled extraction technique	Analytes	Matrix	Solvent	Time (min)	Type	Determination				Ref.
						Technique	Recovery (%)	RSD (%)	LOD (μg/kg)	
MSPD	Multiclass	Honey	n-Hexane:AcEt (9:1)	15 × 2	UB	GC–ECD	80–113	0.6–10	0.5–15	[82]
	Multiclass	Honey	n-Hexane:AcEt (9:1)	15 × 2	UB	GC-NPD	>80	1–10	6–15	[83]
	Fipronil	Pollen	ACN	10 × 2	UB	GC-ECD	98–103	1.9–4.1	1	[84]
						GC–MS	90–101	2.8–4.9	0.2	
	Endosulfan and endosulfan sulphate	Tomato juice	AcEt	15 × 2	UB	GC-ECD GC–MS	81–100	2–10	1	[85]
	Herbicides	Fruit and vegetable juices	AcEt	15 × 2	UB	GC–MS	82–115	2–10	0.1–1.6 μg/l	[86]
	OP	Fruit juices	AcEt	15 × 2	UB	GC–NPD GC–MS	70–105	1.1–10.8	0.1–0.6 μg/l	[87]
	OP and triazines	Fruits	AcEt	1	Sonoreactor	GC–MS	73–118	8–24	1–42	[88]
DI-SPME	Acaricides	Honey	Ultrapure water	30	UB	GC–MS	100	7–11	2–18	[89]
SBSE	Multiclass	Fruits and vegetables	MeOH	10	UB	GC–MS				[90]
SDME	OC	Fish	MeOH	10	UB	GC–MS	82–95	9.4–10	0.5	[91]

AcEt: ethyl acetate; ACN: acetonitrile; DI-SPME: direct immersion solid-phase microextraction; ECD: electron-capture detector; GC: gas chromatography; LOD: limit of detection; MeOH: methanol; MS: mass spectrometry; MSPD: matrix solid-phase dispersion; NPD: nitrogen-phosphorus detector; OC: organochlorine; OP: organophosphorus; SBSE: stir-bar sorptive extraction; SDME: single-drop microextraction; UB: ultrasonic bath.

The determination of pesticide residues in food has been predominantly performed with LC or GC (Table 6). For volatile or thermally stable compounds, GC systems with selective detectors, such as NPD or ECD have been employed, but nowadays GC coupled to mass spectrometry is a well-established instrumental technique for the analysis of trace contaminants since it allows the determination and the confirmation of the identity of residues at trace levels. Although GC has been widely employed in the determination of pesticide residues, LC–MS/MS is becoming at present an essential analytical tool taking into account the number of works where this technique has been used.

2.2.2. PAHs

The determination of PAH residues in food has been mainly focused on the 16 PAHs included in the US-EPA (United States Environmental Protection Agency) priority list or on those considered as mutagenic or carcinogenic by the Scientific Committee on Food of the European Commission. The analytical methods developed for the determination of PAHs in food based on UAE are shown in Table 8. The extraction of PAH residues from food is usually carried out employing solvents with a low polarity such as n-hexane or dichloromethane. Rodríguez-Sanmartín et al. [92] studied the possibility of using ultrasound radiation in the extraction of total PAHs from mussels. They selected an ultrasound waterbath device with temperature control, since it had been previously reported a decrease in PAH recovery attributed to the aging of ultrasound probes and to the increase of temperature observed during extended sonication periods. Finally, the extraction was carried out in two steps, using two different organic solvents; dichloromethane and n-hexane in an ultrasonic bath for 10 min. The extracts were combined, evaporated and reconstituted in n-hexane for its purification by adsorption chromatography with a column packed with Florisil and eluted with a mixture of n-hexane and dichloromethane.

In the analysis of hydroxylated PAH metabolites and their corresponding conjugates in infant food including milk formulae and infant cereals, the extraction was carried out with a mixture of acetonitrile and ethyl acetate, due to the polarity of these compounds, in the presence of tert-butyl hydroquinone, an antioxidant, to avoid the losses of these compounds [93]. To obtain the free hydroxylated metabolites it was necessary an enzymatic hydrolysis in buffer solution and in absence of oxygen in order to reduce their degradation. The clean-up of the extracts was performed with C_{18} cartridges where different elution mixtures were assayed and the best results were obtained with a mixture of methanol and ethyl acetate.

Although the determination of PAHs is usually carried out in fatty food due to the lipophilic nature of these compounds, Nieva-Cano et al. [94] developed an analytical method for the determination of 16 PAHs in non-fatty food, such as potato (raw and mashed) and toasted bread. After a sonication period of 8 min with a mixture of ethyl ether–methylene chloride (1:1, v/v) and water (except for raw potato due to its high water content), the extract was centrifuged and the supernatant evaporated to dryness and redissolved in acetonitrile for LC analysis. In this case, no clean-up was necessary and the recoveries were in the range of 70–86%.

The application of ultrasound radiation to improve the efficacy of other extraction techniques has also been used in the determination of PAHs. Our research group developed a MSPD method assisted with sonication to determine 16 PAHs in honey [95]. The method, based on a previous work where pesticide residues in honey were determined [83], allowed recoveries higher than 80% and the simultaneous extraction of the analytes and clean-up of the extracts. The coupling of UAE and MSPD has advantages such as the increase in the extraction yield, due to ultrasound radiation, and the avoidance of a further purification step as the eluates

Table 8
Analytical methods for the determination of PAH residues in food.

Analytes	Matrix	Solvent [ml]	Time (min)	Temp. (°C)	Type	Coupled	Clean-up	Determination				Ref.
								Technique	Recovery (%)	RSD (%)	LOD (μg/kg)	
Total PAHs	Mussels	DCM [65]	10 × 2	RT	UB		Adsorption chromatography column: Florisil: n-hexane–DCM (60:40)	Spectrofluorimetry	94–100	2–3	21	[92]
		n-Hexane [25]										
PAHs and hydroxylated PAHs metabolites	Infant food	Native PAH: n-hexane [30]	10 × 3	RT	UB		Native PAH: silica cartridges	LC-FD	92–103	2–7	0.01–0.7	[93]
		Hydroxylated PAH: ACN:AcEt (7:3) with 0.8g/l tert-butyl hydroquinone [20]					Hydroxylated PAH: centrifuged; dried and redissolved NH_4Ac/HAc pH 5.5 incubation + ACN and C_{18} cartridge MeOH:AcEt (1:1)	LC–MS/MS				
16 PAHs	Potato toasted bread	Ethyl ether–DCM (1:1) [3]	8	RT	UB			LC–FD	70–86	4–11	0.042–33	[94]
16 PAHs	Honey	n-Hexane–AcEt (9:1) [10]	15 × 2	RT	UB	MSPD		GC–MS	80–101	6–15	0.04–2.9	[95]

AcEt: ethyl acetate; ACN: acetonitrile; DCM: dichloromethane; FD: fluorescence detector; GC: gas chromatography; HAc: acetic acid; LC: liquid chromatography; MeOH: methanol; MS: mass spectrometry; MS/MS: tandem mass spectrometry; MSPD: matrix solid-phase dispersion; PAHs: polycyclic aromatic hydrocarbons; RT: room temperature; UB: ultrasonic bath.

obtained by MSPD are usually clean enough and ready for direct injection.

PAHs are fluorescent because of their aromatic structure, therefore, spectrofluorimetry and LC–FD have been frequently used in the determination of PAH residues in food (Table 8).

2.2.3. Pharmaceuticals

Pharmaceuticals, including antibiotics, are mainly determined in food of animal origin due to the veterinary treatment of food-producing animals with these compounds. Table 9 summarises the analytical methods based on UAE developed for the determination of antibiotics and other pharmaceuticals in food. TCs, a group of broad-spectrum antibiotics, have been studied in salmon [96], propolis [97] and swine muscle and kidney [98]. In these three works, UAE was carried out using pH 4 Na_2EDTA–McIlvaine buffer, a mixture of citric acid and disodium hydrogen phosphate, due to the polar nature of tetracyclines. Na_2EDTA is a chelating agent used to avoid complex formation of the antibiotics with inorganic ions present in the matrix. The use of Na_2EDTA–McIlvaine buffer solution combined with SPE has been the standard method for the extraction of TCs from tissue matrices. The purification of the extracts was carried out with Oasis HLB cartridges where polar and non-polar interactions are established with these analytes. For propolis samples, a two-step purification was performed, first with an Oasis HLB cartridge and afterwards with a carboxylic acid cartridge, whereas in the case of salmon and swine tissues trichloroacetic acid was added after the UAE for the denaturalization of proteins, avoiding the second clean-up of the extracts.

FQs and Qs present acid–base properties and are compounds with a relatively high polarity, thus the extraction is usually carried out with high or moderately polar solvents such as methanol, acetonitrile or acetone. In the case of baby food, Díaz-Alvarez et al. [99] developed a method where good recoveries were accomplished when the extraction was carried out with methanol followed by two different SPE clean-up procedures, using a strong anion-exchange (SAX) cartridge or a MIP. The clean-up performed with SAX cartridges allowed the simultaneous determination of Qs and FQs, whereas the MIP approach enabled the analysis of FQs exclusively but with lower LODs. FQ residues have also been determined in other products of animal origin such as bovine milk [100] and royal jelly [101] using EDTA to avoid complex formation. In the case of royal jelly, acidic KH_2PO_4 was used to precipitate proteins and extract FQs, because the extraction with organic solvents did not provide good recoveries due to amount of lipophilic interferences co-extracted. After extraction, the clean-up was carried out with C_{18} cartridges and analytes were eluted with methanol containing 5% of ammonia, in particular for FQs with NH groups.

Although acidic aqueous solutions containing chelating agents have been often used in the determination of TCs residues in food, acetonitrile has been frequently used in the analysis of other antibiotics [102–106] and drugs as barbiturates and benzodiazepines [107,108]. In this way, Hammel et al. [102] developed a method to analyse in honey a total of 42 antibiotics, belonging to different chemical classes, where four subsequent LLE steps assisted by sonication were carried out. All the extractions were performed using acetonitrile as solvent but in each stage a different reagent was added to improve either the extraction or the chromatographic determination. As an example, in the third extraction step non-afluoropentanoic acid (NFPA) was added as ion pairing agent since aminoglycosides do not present an adequate retention on the chromatographic column selected.

A Chinese research group developed two analytical methods for the determination of barbiturates and benzodiazepines in pork applying UAE with acetonitrile in an ultrasonic waterbath at 30 °C and a SPE procedure to clean-up the extracts [107,108]. The novelty of these works is the use of a new SPE absorbent made of mul-

Table 9
Analytical methods for the determination of antibiotic and pharmaceutical residues in food.

Analytes	Matrix	Solvent [ml]	Time (min)	Temp. (°C)	Type	Coupled	Clean-up	Determination				Ref.
								Technique	Recovery (%)	RSD (%)	LOD (μg/kg)	
Tetracyclines	Salmon muscle	pH 4 Na_2EDTA–McIlvaine buffer and n-hexane [20]	15	Ice/salt	UB		Oasis HLB: 1% TFA in MeOH	LC–FD	84–93	4.1–5.8	50 (LOQ)	[96]
Tetracyclines	Propolis	Na_2EDTA–McIlvaine buffer; 0.1 M [40]	30 × 2	50	UB		Oasis HLB: AcEt Carboxylic acid cartridge: 0.01 M oxalic acid (pH 4)–ACN (6:4; v/v)	LC–UV	86–99	2.8–6.3	100–150 (LOQ)	[97]
Tetracyclines	Swine muscle and kidney	pH 4 Na_2EDTA–McIlvaine buffer [50]	15	Ice/salt	UB		Oasis HLB: 1% TFA in MeOH	LC–FD, LC–MS	65–90		50 (muscle) 100 (kidney)	[98]
Quinolones and fluoroquinolones	Baby food	MeOH [8]	15	RT	UB		SAX cartridges 85% formic acid and 6% formic acid in MeOH) MIP cartridge: 1:1. MeOH–acetic acid	LC–UV	87–100	2.1–13.1	SAX: 30–110, MIPS: (FQ) 9–45	[99]
Fluoroquinolones	Bovine milk	EDTA–McIlvaine buffer; pH 4 [10]	15	RT	UB		SPE-Bond Elut Plexa cartridge: MeOH	LC–MS/MS	63–94	0.4–7.9	0.002–0.409	[100]
Fluoroquinolones	Royal jelly	K_2HPO_4 (0.1 M) + Na_2EDTA (3%; w/v) pH 2.5 [30]	30 × 2	25	UB		C_{18} cartridge: MeOH–ammonia (95:5; v/v)	LC–FD	62–89	4.8–13.2	2–40 (LOQ)	[101]
Antibiotics	Honey	ACN [4]	2 × 4	RT	UB			LC–MS/MS	68–98		27–80	[102]
Sulfonamides	Chicken meat	ACN [5]	1		UP	PMME		CE–UV	96–104	2.3–8.5	3.5–16.7	[103]
Sulfonamides	Pork and chicken	ACN [5]	10	RT	UB			LC–UV	67–83	1.4–5.6	4.6–7.3 ng/l	[104]
Ivermectin	Milk and mozzarella cheese	ACN [10]	10 × 2	RT	UB		C_{18} cartridge: terbuthylmethyl ether	LC–FD	78–83	10–13	0.2 ppb	[105]
Ivermectin and moxidectin	Milk; curd and cheese	ACN + deionized water [2] ACN [2]	8 × 2	RT	UB		C_{18} cartridge: MeOH	LC–FD	82–92	2.2–12.2	Milk: 0.1 μg/l, curd and cheese: 0.25	[106]
Benzodiazepines	Pork	ACN [40]	10	30	UB		SPE MWCNT: hexone–acetone (92.5:7.5; v/v)	GC–MS	75–104	1.3–10	2–5	[107]
Barbiturates	Pork	ACN [50]	30 × 2	30	UB		SPE MWCNT: acetone–AcEt (3:7; v/v)	GC–MS/MS	75–96	2.1–7.8	0.1–0.2	[108]

AcEt: ethyl acetate; ACN: acetonitrile; AED: atomic emission detector; CE: capillary electrophoresis; EDTA: ethylenediaminetetracetic acid; FD: fluorescence detector; FQ: fluoroquinolones; GC: gas chromatography; LC: liquid chromatography; LOD: limit of detection; LOQ: limit of quantitation; MeOH: methanol; MIP: molecularly imprinted polymer; MS: mass spectrometry; MS/MS: tandem mass spectrometry; MWCNT (multi walled carbon nanotubes) PMME: polymer monolith microextraction; RT: room temperature; SAX: strong anion exchange; SPE: solid-phase extraction; TFA: trifluoroacetic acid; UB: ultrasonic bath; UP: ultrasonic probe; UV: ultraviolet detector.

tiwalled carbon nanotubes (MWCNT). This new material has very interesting structural and mechanical properties such as a large specific surface area, hence its applicability as sorbent. The authors assayed the absorption capacity of MWCNT in comparison with C_{18} and concluded that this new material was particularly more effective in the extraction of diazepam and phenobarbital.

UAE has also been applied coupled to other extraction procedures exploiting the benefits of combining techniques when analysing antibiotics. As an example, Li et al. [103] carried out the extraction of sulfonamides from chicken meat using an ultrasonic probe and the extracts were subject to a polymer monolith microextraction (PMME), an alternative to SPME.

As described above, antibiotics are polar compounds therefore LC is the appropriate analytical tool for the determination of residues in food using UV or FD (Table 9). In the case of ivermectin, the extracts were derivatised by acetylation in the presence of methylimidazole as catalyst before FD [105,106]. LC–MS/MS was applied in the determination and confirmation of a high number of antibiotic residues, 22 FQs in bovine milk [100] and 42 antibiotics in honey samples [102].

2.2.4. Industrial contaminants

A summary of the analytical methods, based on UAE, where industrial contaminants in food have been studied is shown in Table 10. Nania et al. [109] monitored perfluorinated compounds in fish from the Mediterranean Sea. The samples were extracted three times with methyl tert-butyl ether at room temperature for 15 min each time. After the extraction, the combined extracts were evaporated to dryness and reconstituted in methanol for its direct analysis by LC–MS/MS without any purification stage. Other industrial contaminants analysed were CPs in clams [110]. Several extraction solvents were assayed and the best results were obtained with the mixture methanol–water with 5% of triethylenamine (TEA) with the ultrasonic bath set at 30 °C for 10 min. In this case, the clean-up step could not be avoided due to the co-extraction of matrix components, such as proteins and lipids, which interfered with the determination of CPs by ion chromatography, and Oasis HLB cartridges eluted with methanol and dichloromethane were used as the SPE procedure.

In September 2008, the contamination of infant formulae with melamine in China was reported. Unfortunately, this has not been the first time this compound has been encountered in food or feedingstuffs. Two research groups developed analytical methods, based on UAE, for the determination of melamine residues in dairy products, fish and fish feed [111,112]. In both works, the extraction was carried out with aqueous solutions containing trichloroacetic acid, to precipitate proteins, since melamine is a strong polar compound. Xu et al. [112] added lead acetate solution after the extraction to deposit proteins and carried out a purification step with a cation exchange cartridge, whereas Yan et al. [111] did not performed clean-up of the extracts before the analysis with capillary zone electrophoresis. As mentioned above, melamine is a strong polar compound; therefore the GC determination with a weak polar column is not suitable due to the peak tailing. However, strong polar columns provide symmetrical and sharp peaks but produce high column bleeding and are not appropriate for GC–MS. To overcome this drawback, Xu et al. [112] proposed the coupling of two chromatographic columns, a short polyethylene glycol column with strong polarity on top of a long DB-5 column with a quartz capillary connector obtaining good results with this approach.

3. Inorganic contaminants

Among the inorganic contaminants of special concern are the heavy metals. The term heavy metal includes many definitions,

Table 10
Analytical methods for the determination of industrial compound residues in food.

Analytes	Matrix	Solvent [ml]	Time (min)	Temp. (°C)	Type	Clean-up	Determination				Ref.
							Technique	Recovery (%)	RSD (%)	LOD (μg/kg)	
PFOA and PFOS	Fish	MTBE [12]	15 × 3	RT	UB		LC–MS/MS	90–113	3–20	1.5–2	[109]
Chlorophenols	Clams	MeOH:water; (4:1) containing 5% TEA [40]	10 × 2	30	UB	Oasis HLB: 5 ml MeOH and 5 ml DCM	IC–APCI-MS	80–98	4.6–12.6	0.05–0.5	[110]
Melamine	Dairy products and fish	Liquid samples: 1 ml 10% TCA + 7 ml deionized water + 1 ml chloroform Solid samples: 1 ml 10% TCA + 9 ml deionized water + 1 ml chloroform	10	RT	UB		CZE-DAD	93–104	0.6–4.6	0.01 μg/ml	[111]
Melamine	Dairy products	1% TCA [17]	15	RT	UB	Cation exchange cartridge: 3 ml of 5% ammonium hydroxide in MeOH	GC–MS	93–102	3.1–8.7		[112]

APCI: atmospheric pressure chemical ionization; CZE: capillary zone electrophoresis; DAD: diode array detector; DCM: dichloromethane; GC: gas chromatography; HLB: hydrophilic-lipophilic balance; IC: ion chromatography; LC: liquid chromatography; LOD: limit of detection; MeOH: methanol; MS: mass spectrometry; MTBE: methyl tert-butyl ether; PFOA: perfluorooctanoic acid; PFOS: perfluorooctane sulphonate; RT: room temperature; TCA: trichloroacetic acid; TEA: triethylamine; UB: ultrasonic bath.

some based on density, some in atomic number or atomic weight and some in chemical properties or toxicity. The application of UAE to the determination of heavy metals as toxic, persistent and accumulative metals in soil and food is the scope in this work, and it includes elements such as As, Cd, Pb, Cr, Cu, Hg, Ni, Se, Zn, Sn, Mo and Ge though not every one is dense, or entirely metallic.

The analysis of metals can be accomplished by the commonly available atomic absorption or emission spectroscopic techniques and, among them the following techniques have been considered in this work: flame atomic absorption spectrometry (FAAS); flame atomic emission spectrometry (FAES), beam injection flame furnace atomic absorption spectrometry (BIFF-AAS), graphite furnace atomic absorption spectrometry (GF-AAS), hydride generation atomic absorption spectrometry (HG-AAS), cold vapour atomic absorption spectrometry (CV-AAS), electrothermal atomic absorption spectrometry (ETAAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). ICP-AES has the capability of simultaneous multi-element analysis with low enough LODs. Nevertheless, when a lower LOD is required the use of ICP-MS is a more appropriate technique. When no multi-element analysis is required ETAAS is a useful technique with low LODs. Another suitable technique for the determination of hydride forming elements, such as As, Sn, Se and Sb, is HG-AAS. Analysis of metals in solid samples by these instrumental techniques commonly requires the total dissolution of the sample to avoid the adverse effects of the matrix on the analysis. To obtain a suitable solution for analysis, it is necessary to transfer the analyte to a liquid phase by slurry or dissolution of sample with the assistance of chemical reagents, heat or pressure. This process is carried out to determine the metal species or the total metal content in a sample.

The total metal content in soil or food is a poor indicator of the bioavailability, mobility or toxicity. The interaction of metals with biota is dependent on their chemical forms, being the impact of some metals highly related with their oxidation state and/or inorganic/organic structure, rather than to their total concentration. The analysis to identify and quantify one or more chemical forms of an element present in a sample is known as speciation.

In this section a general view of the application of UAE techniques to the determination of total metals and their species in soil and food is given.

3.1. Soil

Soil is a complex system with physico-chemical characteristics that vary over time and space. Major components include Fe, Al, Mn, Mg and Ca, among others. Other elements are described as trace elements when they are beneficial and as heavy metals when they are present in amounts considered excessive or are hostile to living organism at any level like Cd, Cu, Zn, Hg, Ni, Pb, As, Cr, Mo, Se, and Ge. In addition, the presence of heavy metals in soil can result in the accumulation of heavy metals in food supplies.

3.1.1. Total metal content

The total metal content in soil includes fractions that are not immediately available to plants, micro-organisms and fauna, but correlation between total metal content and uptake by plants has been demonstrated [113].

The conventional techniques employed for sample preparation in the determination of total heavy metals have been dry ashing, wet acid digestion and microwave digestion. These techniques employ high amounts of acid and long sample preparation times. In addition, the use of concentrated acids or oxidizing agents to extract metals produces nitrous vapours formation after organic matter destruction and cause matrix interferences in the determination of metals, as well as contamination of sample extracts. To avoid these drawbacks, the assistance of ultrasounds for sample preparation has gained importance as an alternative method.

The UAE of metals from solid samples is based on the leaching of metal ions from powdered materials in slurries containing a solvent, generally a diluted acid solution. For inorganic matrices as soil, the most important benefit of ultrasound is the particle fragmentation and the micro-cracks that produce a better penetration of solvent to extract the metals. The mechanical effect of ultrasound helps to reduce particle size and disperse the particles to dissolve a solid sample in a liquid, whereas the chemical effect of ultrasound increases the reactions in sample digestion using the temperature and pressure produced by ultrasound irradiation.

To optimise the extraction several factors such as type of solvent, irradiation conditions, sonication times, sample characteristics and type of sonication device, as indicated above, have to be taken into account. Table 11 shows some of the analytical methods used to determine total heavy metals in soil based on UAE sample preparation. To validate the method, some authors use contaminated soil as standard reference material or comparison with the traditional procedures. The amount of soil employed varies between 5 g and 2 mg and the particle size from 200 μm to 75 μm. The best particle size for UAE depends on several parameters as sample matrix composition, ultrasonic processor, operating frequency, type of acid and its concentration and sonication time. Usually, small particles facilitate the extraction as a result of the increased contact of the surface with the liquid medium, moreover, when the particle size diminishes, the concentration of acid and sonication time also decreases and less amount of sample is required.

Table 11 shows that the reagents commonly employed are HNO_3, HCl or a mixture of both. Though this mixture was efficient to extract Hg from a soil sample, Collaisol et al. [114] preferred the use of HNO_3 + KCl to decrease the medium corrosivity and increase the formation of a stable mercury halide compound, which is in accordance with the analytical technique used for the final determination of this metal. An exception to the reagents commonly employed was the use of concentrated hydrofluoric acid (HF) in the determination of Ge by ETAAS, where the treatment by ultrasound (the tip was close to the tube that contained the soil with the acid and was immersed in a waterbath) and subsequent extraction of Ge from the acid medium in organic solvent and aqueous phase before determination by ETAAS gave complete extraction of Ge from soil avoiding sample mineralisation and losses by volatilisation [115].

Extraction procedures in aqua regia may cause problems in the ETAAS measurements due to the presence of chloride. These interferences could be avoided by the addition of an adequate matrix modifier [119] or in the case of ICP-OES, with measurements optimising the plasma conditions [120].

On the other hand, the time required to total recovery of heavy metals from contaminated soils is also a function of temperature and time of sonication. In this sense, Jamali et al. [121] reported that extraction of Cd, Cr, Ni, Pb and Zn from soil required 10–20 min and temperatures from 70 to 90 °C, whereas Cu required a longer sonication time (30 min) at 90 °C.

3.1.2. Speciation in soil

The toxicity, bioavailability, bioaccumulation and transport properties of a metal depend on its chemical form, therefore, quantification of these forms give more information about the metal behaviour than the quantification of the total metal content. Then, the manner in which an element is bound to soil influences the mobility and ultimately the bioavailability and toxicity of the element to organisms. Speciation can be divided into three classes according to Ure [122]: (a) classical, regarding the specific chemical compounds or oxidation states of elements [As (III)–As (V) or Cr (III)–Cr (VI)]; (b) functional, in relation to the observed behaviour

Table 11
Determination of total metal content in soil by UAE.

Metal	Sonication extraction conditions				Determination				Ref.
	Device	Amount/particle size	Digestion liquid	Time (temperature)	Technique	Recovery (%)[a]	RSD (%)	LOD (μg/g)	
Hg	UP	0.1–1 g/<121 μm	HNO_3 (30% v/v) + KCl (0.15%, m/v)	180 s (<30 °C)	CV-AAS	94–101	1.4–12.7	0.2 μg/l	[114]
Ge	UP	0.01–0.3 g/powder	1 ml HF	10 min (RT)	ETAAS	81–111	1.0–6.1	0.015	[115]
Mo	UP	2–20 mg/powder	1 ml HNO_3 (10%, v/v) + HCl (14%, v/v)	10 s (RT)	ETAAS	78–95	5.1–18.2	0.98–0.1 for 2–20 mg/ml	[116]
As	UP	10–100 g/<100 μm	20 ml HCl (30%)–ozone during 40 min	3 min + 3 min for degasification (n.a.)	HG-AFS	69–113	7.5–11.5	0.03–0.15	[117]
Mn; Pb	UB	5 g/n.a.	50 ml HNO_3 1 M	25 min (n.a.)	FAAS	108–121	3.0–13.4	n.a.	[118]
As; Cd; Cu	UB	0.5 g/200 μm	5 ml HNO_3:HCl (1:3) + 5 ml H_2O	3 min × 3 (40 °C)	ETAAS	89–108	0.6–13.1	5 (As); 0.04 (Cd); 0.26 (Cu)	[119]
As; Cu; Pb; Sb; Zn	UB	0.5 g/200 μm	5 ml HNO_3:HCl (1:3) + 5 ml H_2O	3 min × 3 (50 °C)	ICP-OES	60–98	1.1–13.0	LOQ:2.9 (As); 1.8 (Cu); 5.4 (Pb); 2.7 (Sb); 3(Zn)	[120]
Cu; Zn; Cd; Pb; Cr; Ni	UB	0.2 g/<75 μm	3 ml HNO_3:HCl (1:3) + 2 ml H_2O	10–30 min (70–90 °C)	FAAS ETAAS	97–101	2.6–8.2	n.a.	[121]

[a] Versus certified value CV-AAS: cold vapour atomic absorption spectrometry; ETAAS: electrothermal absorption spectrometry; FAAS: flame atomic absorption spectrometry; HG-FAS: hydride generation-atomic fluorescence spectrometry; ICP-OES inductively couple plasma-optical emission spectrometry; LOD: limit of detection; LOQ: limit of quantitation.; n.a. not available; RT room temperature; UB: ultrasonic bath; UP: ultrasonic probe.

of the element and it is characterised by terms such as plant available or mobile species; and (c) operational, taking into account the situation where the reagent used to extract the sample defines the species, e.g. acetic acid soluble or moderately reducible species.

Operational speciation is referred to sequential extraction as a method for fractionation of potentially toxic elements. The evaluation of metal bioavailability or speciation in a solid matrix as sediment or soil is usually carried out by single extraction or sequential extraction procedures.

The two protocols most widely used for sequential extraction were initially developed for the assessment of the potential impact of sediment bound to the potentially toxic elements and were developed by Tessier et al. [123] in 1979 and by the Community Bureau of Reference of the Commission of the European Communities (BCR) [124]. The Tessier procedure used five extraction steps: (1) fraction exchangeable, the soil sample is extracted with a volume of 0.5 M $MgCl_2$ at pH 7 adjusted with NaOH or HCl, after centrifugation the residue is washed with water; (2) fraction bound to carbonates, the residue from step 1 is extracted with 0.5 M CH_3COONa at pH 5 adjusted with CH_3COOH; (3) fraction bound to Fe–Mn oxides, the residue from step 2 is extracted with 0.04 M $NH_2OH{\cdot}HCl$ at pH 2 adjusted with CH_3COOH; (4) fraction bound to organic matter and sulphides, the last residue is extracted with 0.02 M HNO_3 and H_2O_2 at pH 2; after centrifugation the residue is washed with 3.2 M CH_3COONH_4; (5) residual, the residue is extracted with aqua regia. The method developed by BCR used three steps, where the first two steps of the Tessier method were replaced by an extraction in CH_3COOH.

The major limitation to the use of sequential or single extractions has been the time consuming for routine analysis, because a long mechanical shaking time is needed. In addition, large amounts of sample and extractant volumes were used. Then, ultrasonic extraction emerged as an alternative to develop more rapid sequential or single extraction methods. However, the application of ultrasound energy can change the extraction mechanism as compared to the common procedures [125].

The procedures that use ultrasound energy to determine the bioavailability of heavy metals in soil usually validate the extraction method by comparison with the results obtained when one of the traditional procedures is carried out in the same soil, using the reagents recommended in the original protocols. Sun et al. [126] used an ultrasonic bath to accelerate the sequential method of Tessier obtaining similar or higher extraction efficiencies than the conventional Tessier method for Cu, Fe, Mn, Ni, Pb and Zn in soil, and a reduction of the total operational time from 18 to 8 h. In a similar manner, Väisänen and Kiljunen [127] optimised an UAE procedure for the analysis of As, Cd, Cu, Pb and Zn in soil by comparing the obtained results for each fraction with those obtained when the Tessier method was employed. They noted that re-adsorption of As with Fe after fractionation 2 occurred when UAE is performed, but the results produced by the UAE and Tessier methods were comparable. The Tessier method was shortened from 20 h to 54 min, more samples can be pre-treated for analysis and low volume of reagents were needed when UAE was employed. Marín et al. [128] used a probe sonicator for the sequential extraction of Zn from soil, shortening the BCR procedure from 50 to 2.5 h.

The metal that can be dislodged by washing procedures in a simple extraction has been considered as the sum of exchangeable, carbonate and reducible fractions extracted by the traditional sequential procedure. Chelating agents such as EDTA, diethylenetriamine pentaacetic acid (DPTA) and nitriloacetic acid (NTA) are commonly used to determine this mobile metal fraction from soil that is a function of metal–soil interactions. Different authors have used ultrasound energy to carry out this single extraction procedure, evaluating the best solvent and sonication time for each metal [128–130].

On the other hand, the classical classification of metal speciation according to Ure [122] has been also performed by single extraction, but using separation techniques such as LC to determine one or more individual chemical species [128,131,132]. Table 12 gives a summary of the methods used for metal speciation with UAE. In all cases, the sonication conditions were selected after a previous optimisation. The sonication time was of great importance in the determination of As(III), where a time higher than 30 s produced the transformation of As(III), being also very important the selection of the extraction medium [132]. An online procedure using flow injection (FI) reported the extraction of Cr(VI), which was carried out by continuous UAE and where the leaching reagent was circulated through the soil sample, contained in a extraction chamber and immersed in a waterbath at 50 °C, with the probe placed at 1 mm from the top surface of the extraction chamber. The direction of the extractant was changed every 45 s. The leaching extract was conducted subsequently to a pre-concentration minicolumn packed with SAX resin, where the analyte retained was eluted and, after reaction with DPC (1,5-diphenylcarbazide), the Cr–DPC coloured complex was determined by photometry at 540 nm [133].

3.2. Food

Sample preparation of food to determine metals has been carried out by traditional methods of sample digestion in oxidizing acids using hot plates or microwave heating. The applicability of these techniques are dependent on the food type, as carbohydrates are completely mineralised with nitric acid at 180 °C while protein, fats and amino acids are incompletely digested and need a strong acid to be completely digested. Alternative methods are dry ashing, solvent extraction, pressure-assisted chelating extractions and UAE with acids or enzymatic hydrolysis. The main instrumental techniques used for the analysis of trace elements in food samples are those described above for the determination of metal in soils.

3.2.1. Total metal content

The extraction of total metal content by UAE is normally called acid leaching extraction, pseudo-digestion or slurry extraction, because it involves the dissolution of metals without the sample destruction occurring with the digestion techniques. These terms are employed indistinctly in many occasions, but whereas acid leaching extraction or pseudo-digestion employs strong reagents, soft reagents are generally used to prepare a slurry sample.

Therefore, the slurry sample, which combines the advantages of both liquid and solid sampling, is generally applied when samples are easy to prepare, do not require aggressive chemical pre-treatment and involve minimal sample handling, although it requires particle sizes lower than 100 μm that need much time for grinding, mainly for food with high content in fibre and fat tissues. Typical slurry sample preparation is used in the control of metal content in food. Sola-Larrañaga and Navarro-Blasco [134] determined mineral content in infant formulae using an ultrasonic probe to accelerate alkali solubilisation of powder infant formula in a solution of tetramethylamonium hydroxide for Ca or in ammonium hydroxide for P, Mg, Zn, Fe, Mn and Cu, followed by quantification by ICP-OES or FAAS, and obtained acceptable results when certified standard materials were analysed. In this way, Figueiras et al. [135] used a probe ultrasonic processor to achieve the solubilisation of metals (Ca, Cd, Mg, Mn, Pb and Zn) from plant tissue (0.1 g, particle size <50 μm) in an alkali solution (0.1 M EDTA at pH 10) and subsequent determination by FAAS and ETAAS.

The collapse of bubbles, created by sonication of sample solutions in a liquid (generally an acid), generates high local temperature and pressure that along with the oxidative power of the liquid result in quantitative metal extraction.

Table 12
Speciation of metals in soil using UAE.

Metal species	Sonication extraction conditions				Determination				Ref.
	Device	Amount/particle size	Digestion liquid	Time (temperature)	Technique	Recovery (%)[a]	RSD (%)	LOD (μg/ml)	
Zn	UP	0.2 g/n.a.	25 ml DPTA 0.005 M	10 min (RT)	FAAS	99–100	1.1–2.8	0.012	[128]
Cr(VI)	UP	1 g/1 mm	1 ml amonium buffer (0.04 M) 1.25 ml/min Direction changed each 45 s	5 min (50 °C) Tip of probe position: 1 mm from extraction cell	FI with SAX resin-PD	85–101[b]	0.1–0.5	1.23 ng	[133]
Sb(III); Sb(V)	UB	0.5 g/0.01 mm	10 ml citric acid (pH 2.08)	45 min (RT)	LC–ID–ICP-MS	n.a.	n.a.	n.a.	[131]
As(III); As(V); MMA; DMA	UP	100 mg/n.a.	10 ml H_3PO_4 (1 M)	30 s × 2 (RT)	LC–ICP-MS	63–85[c]	0.1–1.8	13–20	[132]
As(III); As(V); MMA; DMA	UP	0.2 g/n.a.	25 ml DPTA 0.005 M	10 min (RT)	LC–HG-AAS	94–100	1.9–2.1	0.012–0.026	[138]

[a] Versus certified value; [b] versus the EPA3060A method; [c] calcutated as [As total (sum of species)/As certified value] × 100. AAS: atomic absorption spectrometry; DMA: dimethylarsinic acid; DTPA: diethylenetriamine pentaacetic acid; FI: flow injection; LC: liquid chromatography; HG-AAS: hydride generation-atomic absorption spectrometry; ID-ICP-MS: isotope dilution -inductively coupled plasma–mass spectrometry; LOD: limit of detection; MMA: monomethylarsonic acid; PD photometric detector; n.a.: not available; RT: room temperature; SAX: strong anion exchange; UB: ultrasonic bath; UP: ultrasonic probe.

When UAE is employed as sample preparation procedure in food samples, the same factors indicated in the sample preparation for metals determination in soil have to be taken into account. For this purpose, procedures involving optimisation of several variables simultaneously have been developed [136–138].

Table 13 summarises the methods used for the determination of toxic metals in food by UAE as sample preparation procedure. Generally, the slurry or leaching extracts are diluted after sonication for subsequent quantification of metals. The methods summarised in Table 13 are the ones selected after their optimisation by the corresponding authors. For this reason, toxic metals can be included together with the non-toxic metals indicating the optimum compromise conditions for the determination of several metals by the reported instrumental technique.

The use of sonication probes versus ultrasonic baths minimises the inhomogeneity of energy distribution, but ultrasonic tips are more expensive, have shorter lifetimes and allow to process a lower number of samples. In an ultrasonic bath, the maximum intensity is obtained in the region over the transducer and the intensity decays with the distance. To improve the sonochemical effects in an ultrasonic bath, the iodine method has been used. The I_2 formed from a potassium iodide (KI) solution by radicalar and redox reaction with H_2O_2 formed in the water sonication indicated the cavitation process, when highest amount of I_2 was formed a higher cavitation was produced. With this procedure, Nascentes et al. [140] studied the effect of several parameters such as the water volume in the ultrasonic bath, the temperature, the addition of detergent to the waterbath and the horizontal and vertical position of the sample tube in the bath. Arain et al. [137] reported that the temperature of the ultrasonic waterbath is important for the recovery of Pb from fish samples.

The selection of size particle depends on the sample matrix composition, the analyte–particle interactions and the slurry concentration, besides other variables of the UAE. To obtain particle size reduction in a low time, some authors ground the material in a cryogenic mill, obtaining an adequate size particle with homogeneous distribution between fibre and fat content in food [139,145]. They used a particle size <62 μm to extract Pb from vegetal samples using an ultrasonic probe or bath, whereas a higher size particle can be used to extract Pb from animal samples using an ultrasonic probe.

The amount of sample is related with the ultrasound device used, generally, when ultrasonic probes are employed a sample weight of up to 0.2 g is used whereas for ultrasonic baths this weight can be higher, 0.5 g. Figueiras et al. [135] and Krishna and Arunachalam [146] noticed a significant decrease in the recovery of some metals when the amount of sample was larger than 0.1–0.15 g, using an ultrasonic probe.

The reagents more widely used for metal analysis in food samples by UAE are acids, though an alkali solution can be also employed. In general, more sonication time requires lower concentration of acid and the use of the ultrasonic probes need also lower acid concentration than ultrasonic baths. Nevertheless, the best conditions for ultrasonic extraction are dependent on analyte–matrix interactions, the type of matrix and the technique of quantification used. Higher acid concentrations were needed for Pb than for Cd extraction from vegetal tissues, whereas both analytes were extracted with the same acid concentration in animal tissues [139]. Furthermore, a higher acid concentration was needed to extract Zn from fish than from mussel and to extract Zn than Cd and Cu from fish [142]. For some metals, the re-adsorption phenomenon occurs when high concentration of acid is employed [138]. On the other hand, mixtures of acid with H_2O_2 are often used for extraction of metals from different food matrices, because oxidation of food samples with high organic matter content is usually incomplete when only acids are employed [121,137,144]. Copper extraction in mussel was low when H_2O_2 was not used, indicating that organic matter had an important role in controlling the release of Cu. Cypriano et al. [141] used a mixture of HCl and H_2O_2 in the sample preparation of palm oil, because the use of HNO_3 produced organic-nitro compounds that interfere with electroanalytical techniques and the use of HCl or H_2O_2 alone do not cause clarification of the oil. Cava-Montesinos et al. [143] reported that the residual HNO_3, after sonication of milk with aqua regia, causes a decrease in fluorescence signal of the hydride generation atomic fluorescence spectrometry (HG-AFS) that was minimised adding hydroxylamine to liberate the metal ions (As, Sb, Se and Te).

The temperature is other important factor, a temperature around 50 °C normally increases the extraction efficacy but when the temperature of the extraction medium is similar to that of the ebullition of the liquid phase, sonication efficiency decreases due to the diminished surface tension of the medium and increased vapour pressure inside the bubbles, which cause a reduction in the shock waves [147].

3.2.2. Dynamic ultrasound-assisted extraction

When the concentration of metals in a matrix is below the LODs of the detector used, it is necessary to increase it using procedures such as pre-concentration with a chelating agent, an ion changeable resin or a solid sorbent. In order to determine trace metals using conventional AAS, the use of FI sample pre-concentration has been reported. In this case, the coupling of continuous ultrasonic extraction with FI is successful to achieve quantitative recoveries of trace metals from several matrices. This technique is usually called DUAE and is widely used when FAAS (with low sensitivity) is the detector employed. In this procedure, the sample is introduced in an extraction cell and placed in an ultrasonic waterbath or in a waterbath using an ultrasonic probe near the cell and the extractant flows through the sample in a continuous manner [148]. In this dynamic extraction the drawback is the dilution of the extract. Nevertheless, to avoid this disadvantage the same volume of solvent can circulate continuously through the solid in the same direction or the flow direction can be changed at time intervals using programmed peristaltic pumps. These DUAE techniques are usually online coupled with the instrumental analysis for metals determination.

Some examples of analyses of metals using DUAE are summarised in Table 14. Whereas Ruiz-Jiménez et al. [148] did not coupled DUAE with FI, other authors coupled DUAE with FI and the analytical techniques used for the determination of toxic metals in food samples [149–152]. The sonicated acid extract was homogenised in a mixing coil, after the addition of a buffer solution in order to obtain the optimum pH value, and conducted to a pre-concentration minicolumn where the metals were retained by formation of metal chelates. Then metals were eluted and conducted to the detector where they were continuously monitored. This method allowed a total sampling frequency of 13–28 samples per hour [151]. DUAE conditions were optimised for each toxic metal in each food sample. In general, the acid concentration, the sonication time and the amount of sample were the variables with the highest effects.

3.2.3. Enzymatic hydrolysis

An alternative to the acid digestion procedures is the sample treatment by enzymatic hydrolysis, which can be employed to avoid the use of concentrated acids and the treatment of wastes. This technique uses moderate temperature and pH, preventing losses by volatilisation, permits high selectivity because the enzymes act only on certain chemical bounds, can distinguish between fractions of elements bonded to the different components of the matrix and can minimise organometallic species alteration. The enzymes used for chemical speciation are hydrolases (proteases, amylases and lipases among others) that act by breaking

Table 13
Food sample preparation by UAE for metal analysis.

Metals	Matrix	Sonication extraction conditions				Determination				Ref.
		Device	Amount/particle size	Digestion liquid	Time (temperature)	Technique	Recovery (%)[a]	RSD (%)	LOD (μg/kg)	
Cd; Pb	Vegetable and animal	UB and UP	50–200 mg/ < 212 or <63 μm	5 ml HNO_3 0.7–2.8 M	5–10 min (40–70 °C)	BIFF-AAS	96–111	7.1–10.7	33(Cd) 1600(Pb)	[139]
Cu; Zn; Cd; Pb; Cr; Ni	Vegetables	UB	0.2 g/ < 75 μm	2 ml HNO_3:H_2O_2 (2:1) + 2 ml H_2O	30 min (70–90 °C)	FAAS ETAAS	94–111	2.8–6.8	n.a.	[121]
Ca; Mg; Mn; Zn	Vegetables	UB with 0.2% (w/v) of detergent	0.3 g/<75 μm	15 ml HNO_3 0.14 M	10 min (25 °C)	FAAS	72–106	0.3–5.2	n.a.	[140]
Ca; Cd; Mg; Mn; Pb; Zn	Vegetables	UP	0.1 g/ < 50 μm	5 ml EDTA 0.1 M at pH 10	3 min	FAAS–GF-AAS	92–108	0.7–10.2	2000(Ca); 100 (Mg); 1300 (Mn); 700(Zn) 8(Cd); 93(Pb)	[135]
Cu; Pb	Palm oil	UB	100 μl (0.09 g)	2 ml HClconc:H_2O_2 (1:1)	60 min (25 °C)	SCP	98–105	0.7–10.2	13 (Cu); 50(Pb)	[141]
Ca; P; Mg; Zn; Fe; Cu; Mn	Infant formula	UB	0.4 g/powder	10 ml of aqueous solution with 250 μl of TMAH (10%) or 250 μl NH_4OH (25%)	2–5 min	FAAS ICP-OES	97–103	0.6–3.9	30-400 in ICP-OES 190–8600 in FAAS	[134]
As; Cd; Pb	Fish	UB	0.2 g/ < 65 μm	3 ml HNO_3:H_2O_2 (1:1)	5 min (80 °C)	ETAAS	98–99	4.0–7.5	n.a.	[137]
Cd; Cu; Zn;	Fish and mussel	UB	0.5 g/≤300 μm	6 ml HNO_3(4M):HCl (4 M):H_2O_2 (0.5 M) (1:1:1)	30 min (56 °C)	FAAS (Zn) GF-AAS (Cd; Cu)	81–153	2.9–15.1	20 (Cd); 130 (Cu); 630 (Zn)	[142]
As; Se; Ni; V	Fish and shellfish	UP	0.01 g/<100 μm	1.5 ml HNO_3 (3% or 0.5% for Se)	3 min	ETAAS	93–106	2.6–9.3	600 (As); 300 (Se); 200 (Ni); 400(V)	[138]
As; Sb; Se; Te; Bi	Milk	UB	1 g slurry	2 ml HNO_3:ClH (1:3) + 1 ml antifoam A + 0.25 g hydroxylamine hydrochloride	10 min (25 °C)	HG-AFS	95–102[b]	1.2–3.8	2.5(As); 1.6(Sb); 3(Se); 6(Te); 7(Bi) ng/l	[143]
As; Ni; Mg; Zn; Pb; Cd; Al; Fe	Tea	UB	100 mg/powder	2 ml HNO_3:H_2O_2(2:1)	15–20 min (80 °C)	ETAAS	87–100	2.2–13.3	n.a.	[144]
Cu; Fe; Mn; Zn; Ca; K; Na; Mg	Seaweed	UB	0.2 g/<50 μm	7 ml HNO_3 (3.7 M)	35 min (65 °C)	FAAS FAES	97–103	0.2–4.4	40(Cu); 240(Fe); 20(Mn); 500(Zn); 4750(Ca); 500(K); 2370(Na); 240(Mg)	[136]
Ca; Mg; Mn; Zn	Fish feed	UP	0.1 g//<60 μm	10 ml HCl 0.1M	10 s 3×	FAAS	98–100	1.3–1.7	MQL:5800(Ca); 4200(Mg); 1400(Mn); 2100(Zn)	[145]
Major; minor and trace elements	Lichen and mussel	UP	0.1 g/200–300 mesh	5 ml HNO_3 (1%)	4 min	ICP-MS and ICP-AES	>90 and <10 (Fe, Al)	3.3–15.4	n.a.	[146]

[a]Versus certified value; [b]versus spiked samples AES: atomic emission spectrometry; BIFF-AAS: beam injection flame furnace atomic absorption spectrometry; EDTA: ethylenediaminetetracetic acid; ETAAS: electrothermal atomic absorption spectrometry; FAAS: flame atomic absorption spectrometry; FAES: flame atomic emission spectrometry; GF-AAS: grafite furnace atomic absorption spectroscopy; HG-AFS: Hydride generator-atomic fluorescence spectrometry; ICP-OES: inductively coupled plasma-optical emission spectrometry; LOD: limit of detection; MQL: Method quantification limit; MS: mass spectrometry; n.a.: not available; SCP: Stripping chronopotentiometry; TMAH: tetramethylammonium hydroxide; UB: ultrasonic bath; UP: ultrasonic probe.

Table 14
Food sample preparation by dynamic ultrasound extraction (DUAE) for metal analysis.

Metal	Matrix	Sonication extraction conditions				Determination				Ref.
		Device	Amount/particle size	Reagent	Time (temperature)	Pre-concentration/detector	Recovery (%)[a]	RSD (%)	LOD (μg/g)	
Pb; Cd	Plants	UP	250 mg/n.a.	2 ml HNO_3 (8%; w/v) 0.25 ml/min; direction changed each 80 s	5 min (n.a.), tip of probe position: 1 mm from extraction cell	Without/ETAAS off line	98–100	0.8–10	0.04(Cd) 2.6(Pb) μg/l	[148]
Pb; Cd	Mussel	UB	50–80 mg/<30 μm	2 ml HNO_3 3 M 3.5 ml/min Direction changed each 30 s	2 min for Cd; 3 min for Pb (RT)	Chelating resin/FAAS online	100.5–99.5	3.0–4.5	0.011 (Cd) 0.25 (Pb)	[149]
Cr; Co	Seafood	UB	60 mg/ < 30 μm	2 ml HNO_3 3 M 3.5 ml/min Direction changed each 20 s	2.5 min for Cr; 3 min for Co (RT)	Chelating resin/FAAS online	98–99[b]	1.9–3.8	0.09 (Cr) 0.11 (Co)	[150]
Ni	Seafood; cereal; meat; legume; dried fruit and cheese	UB	60 mg/<30 μm	2 ml HNO_3 3 M (for legume) and 1.5 M (for the other) 3.5 ml/min Direction changed each 20 s	0.5 min for cheese; 1.5 min for legume and dried fruit; 2.5 min for seafood; 3 min for meat and cereal (RT)	Chelating resin/FAAS online	105–100	2.2–2.6	0.12	[151]
Cr	Mussel	UB	35 mg/<100 μm	1 ml HCl 3 M + HNO_3 (3 M)	5 min (20 °C)	FI-FAAS	97–102	n.a.	0.12	[152]

[a]Versus certified value; [b]versus spiked samples; ETAAS: electrothermal atomic absorption spectrometry; FAAS: flame atomic absorption spectrometry; FI-FAAS: flow injection- flame atomic absorption spectrometry; LOD: limit of detection; n.a.: not available; RT: room temperature; UB: ultrasonic bath; UP: ultrasonic probe.

Table 15
Ultrasound-assisted enzymatic digestion (UAED) of metals in food.

Metal	Matrix	Sonication extraction conditions				Determination				Ref.
		Device	Amount/particle size	Reagent	Time (temperature)	Detector	Recovery (%)[a]	RSD (%)	LOD	
Se	Yeast; oyster and mussel	UP	10 mg for yeast 50 mg for oyster and mussel/n.a.	1 mg protease XIV (for yeast) or 10 mg (for oyster and mussel) + 1 ml H_2O	5 s	ICP-MS	106–96	5.5–6.2	n.a.	[154]
SeMet	Yeast	UP	10 mg/n.a.	1 mg protease XIV + 1 ml H_2O	30 s	LC–ICP-MS	n.a.	n.a.	n.a.	[154]
As (total); As(III); As(V); DMA; MMA	Rice	UP	300 mg/<125 μm	(1) 10 mg α-amilase + 3 ml H_2O and sonication (2) +30 mg protease XIV and sonication	60 s (with amilase) + 120 s with protease	ICP-MS (for total As) LC–ICP-MS (for speciation)	99.7[b]	0.8	0.05 (As(III)) and 0.2 (for all the other species) μg/kg	[155]
As; As(III); As(V); DMA; MMA; AsB; AsC	Chicken and fish	UP	150 mg for chicken; 100 mg for fish/n.a.	30 mg protease XIV + 3 ml H_2O (for chicken); 5 ml water	240 s (for chicken) 60 s (for fish)	ICP–MS (for total As) LC–ICP-MS (for speciation)	80–82[b]	0.5–4.2	13.6(AsB); 19.6(As(III)); 12.7(DMA); 14.3(MMA); 19.4(As(V)) ng/l	[132]
As; Al; Cd; Cr; Cu; Fe; Mn; Ni; Pb; Zn	Mussel	UB	200 mg/powder	7 ml PDHP/PHP (0.5 M for pancreatin or 0.2 M for tripsin) at pH 8 or 7 ml of NaCl 1% at pH 1 for pepsin	30 min (37 °C)	ICP-AES	67–113	0.9–9.5	n.a.	[156]
As; Cd; Cr; Cu; Fe; Mn; Ni; Pb; Zn	Seaweed	UB	200 mg/powder	7 ml PDHP/PHP (0.3 M) at pH 6 for α-amylase or pH 8 for tripsin) or 7 ml of NaCl 1% at pH 1 for pepsin	30 min (37 °C)	ICP-AES	3.3–103	0.8–7.0	n.a.	[157]

[a]Versus certified value; [b]calcutated as [As total (sum of species)/As certified value] × 100; AES: atomic emission spectrometry; AsB: arsenobetaine; AsC: arsenocholine; DMA: dimethylarsenic acid; ICP-MS: inductively coupled plasma-mass spectrometry; LC: liquid chromatography; LOD: limit of detection; MMA: monomethylarsonic acid; PDHP:potassium dihydrogen phosphate; PHP: potassium hydrogen phosphate; SeMet: seleniomethonine; UB: ultrasonic bath; UP: ultrasonic probe.

Table 16
Speciation of metals in food.

Metal	Matrix	Sonication extraction conditions				Determination				Ref.
		Device	Amount/particle size	Reagent	Time (temperature)	Separation of species/detector	Recovery (%)[a]	RSD (%)	LOD (ng/g)	
Hg^{2+}; $MeHg^{+}$	Fish	UB	300 mg/n.a.	3 ml KOH 25% (w/v) in MeOH (3 times after heating in waterbath at 70 °C for 30 min)	30 min × 3 (70 °C)	LC–ICP-MS	99	3–4	n.a.	[158]
				3 ml TMAH 25% (w/v) in MeOH (3 times after heating in waterbath at 70 °C for 30 min)	30 min × 3 (70 °C)	LC–ICP-MS			n.a.	
				5 ml HCl 5 M	5 min (RT)	LC–ICP-MS			n.a.	
Se(IV); Se(VI); Te(IV); Te(VI)	Milk	UB	2000 mg	4 ml HNO_3:HCl (1:3) + 2 ml antifoam A + 5 ml H_2O	10 min (RT)	Without pre-reduction/HG-AFS	93–108[b]	3.9–12.5	0.012 ng/ml (Se IV), 0.023 (Te IV)	[159]
MBT; DBT; TBT	Mussel; oyster	UP	100 mg (mussel); 500 mg (oyster)	5 ml MeOH/HAc (1:1)-centrifugation 2×	30 s ×2	Derivatization with $NaBH_4$/MISPE clean-up/GC–FPD	86–124	1.1–13.5	3 for all matrices and species	[160]
As(III); As(V); DMA; MMA	Fish; mussel	UB	1000 mg/powder	10 ml HNO_3 (3 M) + Triton XT 114 (0.1%)	20 min (RT)	Not required/HG-AFS	93–106[b]	3–6	0.62 (As(III)); 2.1 (As(V)); 1.8 (MMA); 5.4 (DMA)	[161]
As(III); As(V); DMA; MMA	Vegetables	UB	1000 mg/powder	10 ml H_3PO_4 (1 M) + Triton XT-114 (0.1%)	10 min (RT)	Not required/HG-AFS	91–100[b]	0.1–0.8	3.1 (As(III)); 3.0 (As(V)); 1.9 (MMA); 1.5 (DMA)	[162]
As(III); As(V)	Mushroom	UB	1000 mg/powder	10 ml H_3PO_4 (1 M) + Triton X-100 (0.1%) + 0.5 ml antifoam A	10 min (RT)	Not required/HG-AFS	91–108[b]	4–10	Around 6.5 for As(III) and As(V)	[163]

[a]Versus certified value; [b]versus spiked samples AFS atomic fluorescence spectrometry; DBT: dilbuthyltin; DMA: dimethylarsinic acid; FPD: flame photometric detector; GC: gas chromatography; HAc: acetic acid; HG: hydride generation; LC: liquid chromatography; LOD: limit of detection, ICP: inductively coupled plasma; $MeHg^{+}$: methyl mercury; MeOH:methanol; MBT:monobutyltin; MISPE: molecular imprinted solid-phase extraction; MMA: monomethylarsonic acid; MS: mass spectrometry; RT: room temperature; TBT: tributyltin; TMAH: tetramethylamonium hydroxide; UB: ultrasonic bath; UP: ultrasonic probe.

molecules by catalysing the introduction of water under certain conditions, such as pH, temperature and ionic strength. Nevertheless, the main drawback of the enzymatic hydrolysis is the long time required to complete the process, usually from 12 to 25 h. Therefore, the application of ultrasound energy to accelerate the enzymatic hydrolysis procedures has been recently used with different types of samples and it is called ultrasound-assisted enzymatic digestion (UAED). A tutorial about of UAED was presented by Vale et al. [153] indicating the factors that affect its efficiency, such as pH hydrolysis conditions, temperature, type of enzyme and sonication device, time and amplitude. Table 15 shows some examples of the works using UAED for metal extraction in food samples.

Capelo et al. [154] employed a protease for extraction of Se in several matrix samples. They reported that the use of acidic conditions or longer sonication times did not improve the extraction efficacy of Se, and optimised the relation enzyme/matrix, selecting 2 mg/10 mg and reporting that the use of a buffer solution was not necessary to achieve a good enzymatic activity. This procedure was carried out in only 5 s to extract the total content of Se and in 30 s for the extraction of selenomethionine (SeMet) using an ultrasonic probe, versus 5–24 h in the conventional enzymatic hydrolysis. Evaluation of different treatments for extraction (solvent and enzymes), sample/enzyme ratios, sonication time and amplitude, temperature and immersion depth of the probe into the solution was carried out for Sanz et al. [132,155] in rice, fish and chicken to select the best extraction conditions. They concluded that sonication produces a disruption of the cell membranes which alleviates enzyme attack and then an extraction enhancement is obtained. They also reported a strong inter-relationship between the nature of the sample and the optimum extractant for maximum species recovery [132].

Peña-Farfal et al. [156] reported that the main variables affecting the extraction of several metals from mussels, using enzymatic hydrolysis and ultrasonic energy, were the ultrasound frequency, pH, sonication temperature and ionic strength. All metals studied can be extracted using the same conditions for each of the protease enzyme assayed (trypsin, pepsin and pancreatin), where variables inherent to the enzymatic activity, such as pH, ion strength and temperature were significant. The authors have also reported that the lowest LODs were obtained using trypsin and pancreatin. Nevertheless, the extraction of metals, except Cr and Fe, in seaweeds was only quantitative when pepsin was used in the enzymatic hydrolysis [157].

3.2.4. Metal speciation in food

The measurement of the total metal concentration in food does not give enough information of the potential risk of this element to biota. Nowadays, it is clear that the assessment of the environmental impact and the risk to human health is based on the identification and quantification of the different chemical forms of the elements. In the sample treatment procedure for analysis of element species, these can be quantitatively extracted avoiding species interconversion and, after isolation from the matrix, they have to be separated, identified and quantified. At present, the use of hyphenated techniques, which implies the combination of the separation technique with a sensitive detector, is usually selected as the best procedure.

The speciation of As in food samples has been most studied using ultrasound energy as the procedure for sample preparation, since the inorganic forms As(III) and As(V) have been related to an increase of cancer risk. In the case of Hg, the two major species found in environmental and biological samples are Hg^{2+} and methylmercury ($MeHg^{+}$), being the last one of the most toxic Hg species. The toxicity effect of organotin compounds are strongly dependent on the compound and matrix considered, being butyltins the most toxic compounds. Table 16 summarises the analysis of some metal species in food when using an extraction procedure with ultrasonic energy.

A comparison of different reagents used in the UAE of Hg species in fish was carried out by Reyes et al. [158]. They reported that alkali digestion versus acid digestion provided the best extraction efficiency for total Hg content and caused the lowest levels of transformation of the Hg species (from Hg^{2+} to $MeHg^{+}$ and vice versa). Cava-Montesinos et al. [159] also reported the absence of losses or transformation of the Se and Te species during sonication of milk samples in aqua regia, but no optimisation of the ultrasonic conditions was carried out. In a study of Sn speciation in food samples, Gallego-Gallegos et al. [160] used a sonication probe for the extraction of Sn species and reported that adsorption into the probe tip increased the standard deviation between consecutive sample extractions. To solve this problem, they added a second extraction cycle, after centrifugation of extracts, with the aim to avoid possible re-adsorption of the extracted species. The amplitude and the time of sonication were also evaluated, reporting that methylbutylin was re-adsorbed when ultrasonic time was longer than 30 s. In this work low LODs, using GC–FPD (flame photometric detection) analysis, were obtained after applying a clean-up step based on the use of an imprinted polymer especially designed for tributyltin.

The speciation of As has been the procedure most studied in food. Some authors have used acid reagents with a surfactant to extract As species by UAE in an ultrasound bath and these species have been determined without subsequent separation by chromatographic techniques [161–163].

4. Conclusions

The main advantage of UAE versus traditional extraction techniques, in the preparation of samples to determine contaminants in soil and food, is the reduction of the preparation time. In addition, other advantages such as the low reagent consumption under milder conditions of temperature and pressure, which diminishes laboratory wastes and possible errors in trace analysis caused by volatilisation or sample contamination, together with the relative low cost of ultrasonic equipment and its simple use have also to be taken into account. The possibility of coupling UAE with other extraction techniques like SPME and MSPD may offer additional advantages in the analysis of contaminants. Moreover, DUAE can also be coupled online with the instrumental techniques often employed in the determination of analytes. Further development of these lines will surely increase the application of UAE to the analysis of contaminants in soil and food.

References

[1] J.L. Capelo, A.M. Mota, Curr. Anal. Chem. 1 (2005) 193.
[2] H.M. Santos, J.L. Capelo, Talanta 73 (2007) 795.
[3] M. Lyytikainen, J.V.K. Kukkonen, M.J. Lydy, Arch. Environ. Contam. Toxicol. 44 (2003) 437.
[4] J.F. Huertas-Perez, M. del Olmo Iruela, A.M. Garcia-Campaña, A. Gonzalez-Casado, A. Sánchez-Navarro, J. Chromatogr. A 1102 (2006) 280.
[5] C. Sanchez-Brunete, B. Albero, J.L. Tadeo, J. Agric. Food Chem. 52 (2004) 1445.
[6] C. Lesueur, M. Gartner, A. Mentler, M. Fuerhacker, Talanta 75 (2008) 264.
[7] J.L. Capelo-Martinez, Ultrasound in Chemistry: Analytical Applications, 1st ed., Wiley–VCH, Weinheim, 2008.
[8] M.D. Luque de Castro, F. Priego-Capote, Analytical Applications of Ultrasound, 1st ed., Elsevier, Amsterdam, 2007.
[9] C. Domeño, M. Blasco, C. Sánchez, C. Nerín, Anal. Chim. Acta 569 (2006) 103.
[10] T. Hyötyläinen, M.L. Riekkola, Trends Anal. Chem. 26 (2007) 788.
[11] S. Rodriguez-Cruz, S. Lacorte, J. Agric. Food Chem. 53 (2005) 9571.
[12] M.L. Hladik, K.M. Kuivila, J. Agric. Food Chem. 56 (2008) 2310.
[13] J. Castro, C. Sánchez-Brunete, J.A. Rodríguez, J.L. Tadeo, Fresenius Environ. Bull. 11 (2002) 578.
[14] V.I. Boti, V.A. Sakkas, T.A. Albanis, J. Chromatogr. A 1146 (2007) 139.
[15] C. Sánchez-Brunete, J.A. Rodríguez, J.L. Tadeo, J. Chromatogr. A 1007 (2003) 85.
[16] A. Caballo-López, M.D. Luque de Castro, J. Chromatogr. A 998 (2003) 51.
[17] A. Tor, M. Emin, S. Ozcan, Anal. Chim. Acta 559 (2006) 173.

[18] A.C. Vagi, A.S. Petsas, M.N. Kostopoulou, M.K. Karamanoli, T.D. Lekkas, Desalination 210 (2007) 146.
[19] M.S. Kim, T.W. Kang, H. Pyo, J. Yoon, K. Choi, J. Hong, J. Chromatogr. A 1208 (2008) 25.
[20] V.S. Morozova, S.A. Eremin, P.N. Nesterenko, N.A. Klyuev, A.A. Shelepchikov, I.V. Kubrakova, J. Anal. Chem. 63 (2008) 127.
[21] C. Sánchez-Brunete, R.A. Perez, E. Miguel, J.L. Tadeo, J. Chromatogr. A 823 (1998) 17.
[22] J. Castro, C. Sánchez-Brunete, J.L. Tadeo, J. Chromatogr. A 918 (2001) 371.
[23] C. Sánchez-Brunete, E. Miguel, J.L. Tadeo, J. Chromatogr. A 976 (2002) 319.
[24] J. You, D.P. Weston, M.J. Lydy, Arch. Environ. Contam. Toxicol. 47 (2004) 141.
[25] D.B. Hill, E.H. Stobbe, J. Agric. Food Chem. 22 (1974) 1143.
[26] S.I. Ueno, T. Fujita, D. Kuchar, M. Kubota, H. Matsuda, Ultrason. Sonochem. 16 (2009) 169.
[27] A. Caballo-López, M.D. Luque de Castro, Anal. Bioanal. Chem. 386 (2006) 341.
[28] D.A. Lambropoulou, T.A. Albanis, Anal. Chem. Acta 514 (2004) 125.
[29] J. Villaverde, A. Hildebrandt, E. Martinez, S. Lacorte, E. Morillo, C. Maqueda, P. Viana, D. Barceló, Sci. Total Environ. 390 (2008) 507.
[30] J.P. Bossio, J. Harry, C.A. Kinney, Chemosphere 70 (2008) 858.
[31] N. Kayali-Sadayi, S. Rubio-Barroso, C.A. Diaz-Diaz, L.M. Polo-Díez, Fresenius J. Anal. Chem. 368 (2000) 697.
[32] L. Sarrazin, C. Diana, E. Wafo, V. Pichars-Lagadec, T. Schembri, J.L. Monod, J. Liq. Chromatogr. Rel. Technol. 29 (2006) 69.
[33] S.G. Chu, H.H. Liu, L.-L. Ma, B. Xiu, Bull. Environ. Contam. Toxicol. 70 (2003) 972.
[34] L.L. Ma, S.G. Chu, X.T. Wang, H.X. Cheng, X.F. Liu, X.B. Xu, Chemosphere 58 (2005) 1355.
[35] J. Hollender, B. Koch, C. Lutermann, W. Dott, Int. J. Environ. Anal. Chem. 83 (2002) 21.
[36] C. Sánchez-Brunete, E. Miguel, J.L. Tadeo, J. Sep. Sci. 29 (2006) 2166.
[37] P. Oleszczuk, S. Baran, Commun. Soil Sci. Plant Anal. 36 (2005) 1083.
[38] D. Banjoo, P. Nelson, J. Chromatogr. A 1066 (2005) 9.
[39] E. Martinez, M. Gross, S. Lacorte, D. Barceló, J. Chromatogr. A 1047 (2004) 181.
[40] C. Sánchez-Brunete, E. Miguel, M.D. Delgado, J.L. Tadeo, Fresenius Environ. Bull. 17 (2008) 1601.
[41] C. Planas, A. Puig, J. Rivera, J. Caixach, J. Chromatogr. A 1113 (2006) 220.
[42] N. Barco-Bonilla, J.L. Martinez Vidal, A. Garrido French, R. Romero-Gonzalez, Talanta 78 (2009) 156.
[43] S. Buco, M. Moragues, P. Doumenq, A. Noor, G.J. Mille, J. Chromatogr. A 1026 (2004) 223.
[44] E. Riera, Y. Golas, A. Blanco, J.A. Gallego, M. Blasco, A. Mulet, Ultrason. Sonochem. 11 (2004) 241.
[45] J.L. Luque-Garcia, M.D. Luque de Castro, J. Chromatogr. A 1034 (2004) 237.
[46] P. Richter, M. Jimenez, R. Salazar, A. Maricán, J. Chromatogr. A 1132 (2006) 15.
[47] E. Martinez-Carballo, C. Gonzalez-Barreiro, S. Scharf, O. Gans, Environ. Pollut. 148 (2007) 570.
[48] P.A. Blackwell, H.C. Holten Lutzhoft, H.P. Ma, B. Halling-Sorensen, A.B.A. Boxall, P. Kay, Talanta 64 (2004) 1058.
[49] E. Turiel, A. Martin-Esteban, J.L. Tadeo, Anal. Chim. Acta 562 (2006) 30.
[50] E. Turiel, A. Martin-Esteban, J.L. Tadeo, J. Chromatogr. A 1172 (2007) 97.
[51] M.J. Lopez de Alda, A. Gil, E. Paz, D. Barceló, Analyst 127 (2002) 299.
[52] K. Hajková, J. Pulkraboá, J. Schurek, J. Hajslová, J. Poustka, M. Napravniková, V. Kocourek, Anal. Bioanal. Chem. 387 (2007) 1351.
[53] T.A. Termes, H. Andersen, D. Gilberg, M. Bonerz, Anal. Chem. 74 (2002) 3498.
[54] J. Xu, L. Wu, W. Chen, A.C. Chang, J. Chromatogr. A 1202 (2008) 189.
[55] M.E. Aydin, A. Tor, S. Ozcan, Anal. Chim. Acta 577 (2006) 232.
[56] G. Bianco, G. Novario, D. Bochcchio, G. Anzilotta, A. Palma, T.R.I. Cataldi, Chemosphere 73 (2008) 104.
[57] C. Sánchez-Brunete, E. Miguel, J.L. Tadeo, Talanta 70 (2006) 1051.
[58] S. Mas, O. Jáuregui, F. Rubio, A. de Juan, R. Tauler, S. Lacorte, J. Mass Spectrom. 42 (2007) 890.
[59] C. Sánchez-Brunete, E. Miguel, J.L. Tadeo, J. Chromatogr. A 1216 (2009) 5497.
[60] S. Suzuki, A. Hasegawa, Anal. Sci. 22 (2006) 469.
[61] S. Sporring, S. Bowadt, B. Svensmark, E. Bjorklund, J. Chromatogr. A 1090 (2005) 1.
[62] C. Salgado-Petinal, M. Llompart, C. Garcia-Jares, M. Garcia-Chao, R. Cela, J. Chromatogr. A 1124 (2006) 139.
[63] C. Yu, B. Hu, J. Chromatogr. A 1160 (2007) 71.
[64] N. Campillo, N. Aguinaga, P. Viñas, I. Lopez-Garcia, M. Hernández-Cordoba, Anal. Chim. Acta 552 (2005) 182.
[65] M.P. Llompart, R.A. Lorenzo, R. Cela, K.L. Jacqueline, M.R. Belanger, J.R. Jocelyn Paré, J. Chromatogr. A 774 (1997) 243.
[66] L. Nuñez, E. Turiel, J.L. Tadeo, J. Chromatogr. A 1146 (2007) 157.
[67] L. Nuñez, J.L. Tadeo, A.I. Garcia-Valcarcel, E. Turiel, J. Chromatogr. A 1214 (2008) 178.
[68] C.M. Lino, L.C.M. Guarda, M.I.N. Silveira, J. AOAC Int. 82 (1999) 1206.
[69] R. Zhao, S. Chu, R. Zhao, X. Xu, X. Liu, Anal. Bioanal. Chem. 381 (2005) 1248.
[70] J. Oliva, A. Barba, N. Vela, F. Melendreras, S. Navarro, J. Chromatogr. A 882 (2000) 213.
[71] S. Navarro, A. Barba, G. Navarro, N. Vela, J. Oliva, J. Chromatogr. A 882 (2000) 221.
[72] N. Kolbe, J.T. Andersson, J. Agric. Food Chem. 54 (2006) 5736.
[73] X. Esparza, E. Moyano, M.T. Galceran, J. Chromatogr. A 1216 (2009) 4402.
[74] R. Castro, E. Moyano, M.T. Galceran, J. AOAC Int. 84 (2001) 1903.
[75] I. Rezic, A.J.M. Horvat, S. Babic, M. Kastelan-Macan, Ultrason. Sonochem. 12 (2005) 477.
[76] N. Tokman, C. Soler, M. Farré, Y. Picó, D. Barceló, J. Chromatogr. A 1216 (2009) 3138.
[77] E. Rodríguez-Gonzalo, R. Carabias-Martínez, E. Miranda-Cruz, J. Domínguez-Álvarez, J. Hernández-Méndez, J. Sep. Sci. 32 (2009) 575.
[78] A.C. Hogenboom, M.P. Hofman, S.J. Kok, W.M.A. Niessen, U.A.Th. Brinkman, J. Chromatogr. A 892 (2000) 379.
[79] A. Hercegová, M. Dömötörová, E. Matisová, M. Kirchner, R. Otrekal, V. Stefuca, J. Chromatogr. A 1084 (2005) 46.
[80] J. Pan, X. Xia, J. Liang, Ultrason. Sonochem. 15 (2008) 25.
[81] K. Granby, J.H. Andersen, H.B. Christensen, Anal. Chim. Acta 520 (2004) 165.
[82] B. Albero, C. Sánchez-Brunete, J.L. Tadeo, J. AOAC Int. 84 (2001) 1165.
[83] C. Sánchez-Brunete, E. Miguel, B. Albero, J.L. Tadeo, J. AOAC Int. 85 (2002) 128.
[84] C. Sánchez-Brunete, E. Miguel, B. Albero, J.L. Tadeo, Span. J. Agric. Res. 6 (2008) 7.
[85] B. Albero, C. Sánchez-Brunete, J.L. Tadeo, J. Chromatogr. A 1007 (2003) 137.
[86] B. Albero, C. Sánchez-Brunete, A. Donoso, J.L. Tadeo, J. Chromatogr. A 1043 (2004) 127.
[87] B. Albero, C. Sánchez-Brunete, J.L. Tadeo, J. Agric. Food Chem. 51 (2003) 6915.
[88] J.J. Ramos, R. Rial-Otero, L. Ramos, J.L. Capelo, J. Chromatogr. A 1212 (2008) 145.
[89] R. Rial-Otero, E.M. Gaspar, I. Moura, J.L. Capelo, Talanta 71 (2007) 1906.
[90] A. Kende, Z. Csizmazia, T. Rikker, V. Angyal, K. Torkos, Microchem. J. 84 (2006) 63.
[91] K. Shrivas, H.F. Wu, J. Sep. Sci. 31 (2008) 380.
[92] P. Rodríguez-Sanmartín, A. Moreda-Piñeiro, A. Bermejo-Barreda, P. Bermejo-Barreda, Talanta 66 (2005) 683.
[93] L. Rey-Salgueiro, E. Martínez-Caballo, M.S. García-Falcón, C. González-Barreiro, J. Simal-Gándara, Food Chem. 115 (2009) 814.
[94] M.J. Nieva-Cano, S. Rubio-Barroso, M.J. Santos-Delgado, Analyst 126 (2001) 1326.
[95] B. Albero, C. Sánchez-Brunete, J.L. Tadeo, J. AOAC Int. 86 (2003) 576.
[96] A. Pena, C.M. Lino, M.I.N. Silveira, J. AOAC Int. 86 (2003) 925.
[97] J. Zhou, X. Xue, Y. Li, J. Zhang, F. Chen, L. Wu, L. Chen, J. Zhao, Food Chem. 115 (2009) 1074.
[98] A. Pena, C.M. Lino, R. Alonso, D. Barceló, J. Agric. Food Chem. 55 (2007) 4973.
[99] M. Díaz-Alvarez, E. Turiel, A. Martín-Esteban, Anal. Bioanal. Chem. 393 (2009) 899.
[100] H. Zhang, Y. Ren, X. Bao, J. Pharm. Biomed. 49 (2009) 367.
[101] J. Zhou, X. Xue, F. Chen, J. Zhang, Y. Li, L. Wu, L. Chen, J. Zhao, J. Sep. Sci. 32 (2009) 955.
[102] Y.A. Hammel, R. Mohamed, E. Gremaud, M.H. LeBreton, P.A. Guy, J. Chromatogr. A 1177 (2008) 58.
[103] T. Li, Z.G. Shi, M.M. Zheng, Y.Q. Feng, J. Chromatogr. A 1205 (2008) 163.
[104] J. He, S. Wang, G. Fang, H. Zhu, Y. Zhang, J. Agric. Food Chem. 56 (2008) 2919.
[105] A. Anastasio, M. Esposito, M. Amorena, P. Catellani, L. Serpe, M.L. Cortesi, J. Agric. Food Chem. 50 (2002) 5241.
[106] F.A. Imperiale, M.R. Busetti, V.H. Súarez, C.E. Lanusse, J. Agric. Food Chem. 52 (2004) 6205.
[107] L. Wang, H. Zhao, Y. Qiu, Z. Zhou, J. Chromatogr. A 1136 (2006) 99.
[108] H. Zhao, L. Wang, Y. Qiu, Z. Zhou, W. Zhong, X. Li, Anal. Chim. Acta 586 (2007) 399.
[109] V. Nania, G.E. Pellegrini, L. Fabrizi, G. Sesta, P. De Sanctis, D. Lucchetti, M. Di Pasquale, E. Coni, Food Chem. 115 (2009) 951.
[110] M. Jin, Y. Zhu, J. Chromatogr. A 1118 (2006) 111.
[111] N. Yan, L. Zhou, Z. Zhu, X. Chen, J. Agric. Food Chem. 57 (2009) 807.
[112] X.M. Xu, Y.P. Ren, Z.X. Cai, J.L. Han, B.F. Huang, Y. Zhu, Anal. Chim. Acta 650 (2009) 39.
[113] P.S. Hooda, D. Mcnulty, B.J. Alloway, M.N. Aitken, J. Sci. Food Agric. 73 (1997) 446.
[114] A. Collasiol, D. Pozebon, S.M. Maia, Anal. Chim. Acta 518 (2004) 157.
[115] I. López-García, N. Campillo, I. Arnau-Jerez, M. Hernández-Córdoba, Anal. Chim. Acta 531 (2005) 125.
[116] M. Felipe-Sotelo, M.J. Cal-Prieto, A. Carlosena, J.M. Andrade, E. Fernández, D. Prada, Anal. Chim. Acta 553 (2005) 208.
[117] C. Fernández-Costas, I. Lavilla, C. Bendicho, Spectrosc. Lett. 39 (2006) 713.
[118] M.H. Özkan, R. Gürkan, A. Özkan, M. Akçay, J. Anal. Chem. 60 (2005) 529.
[119] A. Väisänen, R. Suontano, J. Anal. Atom. Spectrom. 17 (2002) 739.
[120] A. Väisänen, A. Ilander, Anal. Chim. Acta 570 (2006) 93.
[121] M.K. Jamali, T.G. Kazi, M.B. Arain, H.I. Afridi, N. Jalbani, A. Memon, J. Agron. Crop Sci. 193 (2007) 218.
[122] A.M. Ure, Microchim. Acta 2 (1991) 49.
[123] A. Tessier, P.G.C. Campbell, M. Bisson, Anal. Chem. 51 (1979) 844.
[124] A.G. Rauret, J.F. López-Sánchez, A. Sahuquillo, R. Rubio, C. Davidson, A. Ure, P. Quevauviller, J. Environ. Monit. 1 (1999) 57.
[125] J.R. Bacon, C.M. Davidson, Analyst 133 (2008) 25.
[126] F.S. Sun, Z.Y. Zhan, K.S. Zhang, Y. Wang, J. Environ. Sci. 16 (2004) 957.
[127] A. Väisänen, A. Kiljunen, Int. J. Environ. Anal. Chem. 85 (2005) 1037.
[128] A. Marín, A. López-Gonzálvez, C. Barbas, Anal. Chim. Acta 442 (2001) 305.
[129] S. Ehsan, S.O. Prasher, W.D. Marshall, J. Environ. Qual. 35 (2006) 2084.
[130] Z. Zou, R. Qju, W. Zhang, H. Dong, Z. Zhao, T. Zhang, X. Wei, X. Cai, Environ. Pollut. 157 (2009) 229.
[131] S. Amereih, T. Meisel, R. Scholger, W. Wegscheider, J. Environ. Monit. 7 (2005) 1200.
[132] E. Sanz, R. Muñoz-Olivas, C. Cámara, J. Chromatogr. A 1097 (2005) 1.

[133] J.L. Luque-García, M.D. Luque de Castro, Analyst 127 (2002) 1115.
[134] C. Sola-Larrañaga, I. Navarro-Blasco, Food Chem. 115 (2009) 1048.
[135] A.V. Figueiras, I. Lavilla, C. Bendicho, Fresenius J. Anal. Chem. 369 (2001) 451.
[136] N. Landra-Ramos, R. Domínguez-González, A. Moreda-Piñeiro, Atom. Spectrosc. 26 (2005) 59.
[137] M.B. Arain, T.G. Kazi, M.K. Jamali, N. Jalbani, H.I. Afridi, R.A. Sarfraz, A.Q. Shah, Spectrosc. Lett. 40 (2007) 861.
[138] I. Lavilla, P. Vilas, C. Bendicho, Food Chem. 106 (2008) 403.
[139] P.C. Aleixo, D. Santos-Junior, A.C. Tomazelli, I.A. Ruffini, H. Berndt, F.J. Drug, Anal. Chim. Acta 512 (2004) 329.
[140] C.C. Nascentes, M. Korn, M.A.Z. Arruda, Microchem. J. 69 (2001) 37.
[141] J.C. Cypriano, M.A. Costa Matos, R. Camargo Matos, Microchem. J. 90 (2008) 26.
[142] N. Manutsewee, W. Aeungmaitrepirom, P. Varanusupakul, A. Imyim, Food Chem. 101 (2007) 817.
[143] P. Cava-Montesinos, M.L. Cervera, A. Pastor, M. de la Guardia, Talanta 62 (2004) 175.
[144] N. Jalbani, T.G. Kazi, B.M. Arain, M.K. Jamali, H.I. Afridi, Chem. Spec. Bioavailab. 19 (2007) 163.
[145] R.C.F. Neves, P.M. Moraes, M.A.D. Saleh, V.R. Loureiro, F.A. Silva, M.M. Barros, C.C.F. Padilha, S.M.A. Jorge, P.M. Padilha, Food Chem. 113 (2009) 679.
[146] M.V.B. Krishna, J. Arunachalam, Anal. Chim. Acta 522 (2004) 179.
[147] A. Elik, Talanta 66 (2005) 882.
[148] J. Ruiz-Jiménez, J.L. Luque-García, M.D. Luque de Castro, Anal. Chim. Acta 480 (2003) 231.
[149] M.C. Yebra-Biurrun, S. Cancela-Pérez, A. Moreno-Cid- Barinaga, Anal. Chim. Acta 533 (2005) 51.
[150] M.C. Yebra-Biurrun, S. Cancela-Pérez, Anal. Sci. 23 (2007) 993.
[151] M.C. Yebra, S. Cancela, R.M. Cespón, Food Chem. 108 (2008) 774.
[152] M.C. Yebra, A. Moreno-Cid, R.M. Cespón, S. Cancela, Atom. Spectrosc. 24 (2003) 31.
[153] G. Vale, R. Rial-Otero, A. Mota, L. Fonseca, J.L. Capelo, Talanta 75 (2008) 872.
[154] J.L. Capelo, P. Ximénez-Embún, Y. Madrid-Albarrán, C. Cámara, Anal. Chem. 76 (2004) 233.
[155] E. Sanz, R. Muñoz-Olivas, C. Cámara, Anal. Chim. Acta 535 (2005) 227.
[156] C. Peña-Farfal, A. Moreda-Piñeiro, A. Bermejo-Barrera, P. Bermejo-Barrera, H. Pinochet-Cancino, I. Gregori-Henríquez, Anal. Chem. 76 (2004) 3541.
[157] C. Peña-Farfal, A. Moreda-Piñeiro, A. Bermejo-Barrera, P. Bermejo-Barrera, H. Pinochet-Cancino, I. Gregori-Henríquez, Anal. Chim. Acta 548 (2005) 183.
[158] L.H. Reyes, G.M. Mizanur Rahman, T. Fahrenholz, H.M. Skip Kingston, Anal. Bioanal. Chem. 390 (2008) 2123.
[159] P. Cava-Montesinos, A. de la Guardia, C. Teutsch, M.L. Cervera, M. de la Guardia, J. Anal. Atom. Spectrom. 19 (2004) 696.
[160] M. Gallego-Gallegos, M. Liva, R. Muñoz-Olivas, C. Cámara, J. Chromatogr. A 1114 (2006) 82.
[161] P. Cava-Montesinos, K. Nilles, M.L. Cervera, M. de la Guardia, Talanta 66 (2005) 895.
[162] M.N. Matos Reyes, M.L. Cervera, R.C. Campos, M. de la Guardia, Talanta 75 (2008) 811.
[163] A. Gonzálvez, A. Llorens, M.L. Cervera, S. Armenta, M. de la Guardia, Food Chem. 115 (2009) 360.