307

Egypt. J. Chem. **Vol. 63,** No. 10 pp. 3961 - 3974 (2020)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Trends and Strategies for Quantitative Analysis of PCBs in Foods

Ahmed A. El-Kady[a,*] and Tatsuya Kunisue[b]

[a]Food Toxicology & Contaminants Department, National Research Centre, Cairo, Egypt
[b]Center for Marine Environmental Studies (CMES), Ehime University, 2-5 Bunkyo-cho, Matsuyama-shi, Ehime, 790-8577, Japan

Abstract

Food matrices are naturally complex and many ingredients such as lipids, proteins, pigments, and others are co-extracted with PCBs. So, reliable and effective analytical techniques are needed to determine PCBs in foodstuffs at low concentrations. This review is exploring and summarizes the current available techniques for extraction, clean-up, and separation techniques to quantify the PCBs in different foods. Several extraction approaches have been improved to overcome the issues associated with regular techniques involves a large amount of solvent and time consuming. Accelerated solvent extraction is a promising and effective extraction technique using a reasonable amount of solvents. Acidified silica gel alone or in combination with alumina is succeeded to purify most extract matrices. Besides, gel permission chromatography is widely used to eliminate large size molecules and other co-extracted impurities. As a reference technique, GC-HRMS is the most effective technique for quantification of dioxins and dioxin-like PCBs. As alternative techniques, GC coupling with tandem MS is optimized to analyze dioxin and PCBs in foodstuffs and represent a cost effectiveness technique compared to GC-HRMS. GCxGC-HRTOF-MS is evaluated and validated to examine dl-PCBs and indicator PCBs and the obtained results were satisfactory well equivalent to those results obtained by GC-HRMS.

Keywors: PCBs; foodstuffs; extraction; clean-up; instrumentation

Introduction

It is believed that the PCB-like chemical was discovered as a by-product of coal tar in 1865 and two German scientists first synthesized the PCB in 1881. In 1929, PCBs were first manufactured commercially by the Swan Corporation, which later became part of Monsanto Chemical Company of St. Louis, Missouri [1]. In 1966, a Swedish scientist recognized PCBs for the first time as an environmental concern in various fish species over different places in Sweden [2]. Production of PCBs has probably been limited to ten countries in the world and it is estimated that total amounts are about 1.5 million metric tons since 1929 [1, 3]. After forbidding the production of PCBs in the US in 1976, the global production of PCBs was continued at a rate of 36 million pounds annually between 1980 and 1984 and then became decreased to reach 22 million pounds annually till 1989 [4]. Although the PCBs production was completely banned in North America in 1977 and most European countries in the 1980s, PCBs are present in closed system applications even now. It has been estimated that about one third of the PCB amounts produced ever are still "environmentally available" [5].

Thus far, it is estimated that about 30% of the total PCB amount produced has been released into the environment and remaining are either still present in electrical equipment or have been deposited in landfills and storages. Even though PCBs are no longer produced, additional release into the environment continues. Commercially, PCBs have been sold not only as technical grade liquids, e.g., Aroclor, Phenoclor, Clophen, Kanechlor, Sovol and so on but also as liquid mixtures ready for a specific application. A well-known example of the latter is the mixture of trichlorobenzene and PCBs (35:65%), called Askarel, which has used on a very large scale in electrical equipment [1]. Theoretically, there are 209 PCB congeners with chlorine numbers ranging

_____

_____

from 1 to 10. PCBs have unique physico-chemical properties such as thermal stability, resistance to acids, oxidation, hydrolysis, and low vapor pressure. Due to their chemical stability, high lipophilicity, and less biodegradation, PCBs are considered as persistent organic pollutants (POPs) concentrating in food webs and therefore found predominantly in animal fat [6-9]. With the exception of occupational exposure, food consumption accounts 90-98% of human exposure to PCBs [10]. Especially, it is known that fatty foods from animal origin such as eggs, as well as fish and seafood, are the major sources for PCBs [11, 12]. To identify and determine PCB congeners in different foodstuffs, extraction and clean-up processes and chromatographic separation technique are required to isolate the analytes from the food matrices and interfering compounds. Finally, before instrumental analysis, the solutes should be concentrated to be above the limit of detection (LOD) and quantification (LOQ).

Determination of PCBs in foodstuffs

To determine the PCBs in foodstuffs, some effectual sample preparation processes are required because their concentrations are generally low and the matrices are rather complex [13]. For these reasons, reliable analytical methods should be applied and analytical chemists have to contend with the measurement of very low concentrations of PCBs including not only the parent compounds but also their metabolites. Most regulatory bodies established very low levels as permissible limits for POPs including PCBs in foodstuffs, and due to the complex nature of food matrices, dilution of the samples and an efficient chain of sample treatment are important aspects for food analysis [14-17]. For analysis of PCBs in foodstuffs, sample preparation, extraction, clean-up, and instrumentation are the main steps.

Quality control and quality assurance

Prior to extraction, 13C-labeled surrogates are spiked into the samples to verify the recoveries of target analytes. Alternative surrogates, which are not detected in the environment, e.g., PCB 30, PCB 201 [18], PCB 103, 198, 4,4-dibromooctafluoro biphenyl (DBOFB) [19], PCB 112 [20] are infrequently used. Using isotope dilution technique based on commercially available $^{13}C_{12}$-labeled internal standard resulting in accurate identification of the peak by means of retention time comparisons between native (^{12}C) and labeled (^{13}C) compounds, as well as by comparison of peak areas and heights [21]. No quantitative recovery of the analyte is needed once the sample and spike have been equilibrated during preparation, separation and purification steps.

One of the most suitable types of isotope dilution methods to examine inactive compounds is direct isotope dilution technique through dilution with an active compound [22]. This technique minimizes the differences in chemical behavior in case isotope dilution includes the measurement of isotopes of the same element [22]. In this case, isolation of the same compound in a pure form is needed, so appropriate treatment of the sample mixture should be applied.

Extraction

Sample preparation may affect the recoveries of PCBs, as reported in freeze-dried biota and sediment samples [23-25]. This may be due to tighter binding and occlusion of the residues in the dried matrices [26]. Generally, it is recommended to keep the sample fresh to minimize the potential contamination from laboratory air and to avoid loss of volatile low chlorinated PCB congeners [27]. Mixing of homogenized samples ground with drying materials such as anhydrous sodium sulphate and hydromatrix celite is the most common treatment to be dehydrated from solid samples [18, 25]. The extraction technique is considered the first step to isolate PCBs from the matrices. Many different extraction techniques have been established over the years to extract the organochlorine compounds (OCCs) including PCBs from several types of food matrices. Typical extraction techniques for OCCs are as follows:

Liquid-liquid extraction (LLE)

To extract PCBs from aqueous media, liquid-liquid extraction (LLE) or what is called solvent extraction is considered a suitable procedure. Because the different distribution of the target components between two phases is the key to the separation technique, one of the most sensitive issues in LLE is selection of appropriate solvent. The extraction solvent must have a miscibility gap with the other phase and at the same time should have higher solubility for the solutes (PCBs) than the aqueous media [28]. Although this technique requires large-volumes of pure solvents and is time-consuming, it considered as a standard method using frequently due to efficiency, simplicity, and robustness. Automation has overcome the time-consuming problem, but multistage operation and emulsion formation counteract this capability [29].

Soxhlet extraction (SE)

Soxhlet extraction (SE) is one of the most frequently used liquid-solid extraction method invented and developed by Franz von Soxhlet in 1879, which has been routinely using for extraction

_____

_____

of PCBs from various foodstuffs and environmental samples such as sediment, soil, fly ash [21, 30, 31]. It required 10-200 mL of organic solvent for 1-30 g of tissue or more (350 mL) for 5 g fly ash [32]. SE is a solvent- and time-consuming (~ 18 -24 h) technique as a result of slow analyte diffusion, that is, desorption from the matrix to the extraction solvent, and it cannot be automated [21, 25].

Ultrasonic extraction (UE)

As an alternative SE technique, ultrasonic extraction (UE) was introduced by Beard et al. [32] which was effective in terms of extraction time (shortening from 24 h to 4.5 h for fly ash samples) and used solvent volume (reduction from 350 mL to 60 mL). Assigning the extraction efficiency of SE as 100%, that of UE was 70% within 1.5 h for a fly ash sample. Triplicate extraction (1.5 h x 3 = 4.5 h) of the same sample reached the identical recovery as SE technique [32]. The ultrasonication time, homogeneity of the sample, polarity degrees of the used solvent play important roles in the extraction efficiency [25]. Due to the limited contact time between the sample and the solvent, UE for environmental samples such as soil, sediments, sludge may be an unsuitable method compared with SE.

A new extraction stage began with the use of solid-phase extraction (SPE) techniques; the main idea for SPE is to trap the analytes on activated sorbent [33]. Many sorbents including alumina, silica, magnesium silicate (florisil), and activated carbon are available and applicable for the food sector [34, 35].

Solid-phase extraction (SPE)

The SPE technique was introduced in the mid-1970s and became a commercially available technique in the early 1980s. Since then SPE is widely accepted as an alternative extraction/cleanup method to LLE for liquid samples [13, 34, 36]. It is well known that SPE cannot be considered as a universal method that can be applied for different analytes. For many years, n-alkyl silica has been established as a sorbent for SPE before introducing other types of solid phase [36]. There are three main groups of sorbents are used in SPE cartridges, which classified into inorganic oxides, low-specificity sorbents, and compound and class-specific sorbents [34]. The highly important aspect of SPE is the selection of the sorbent. C-18 bonded silica and styrene/divinyl benzene co-polymers are most frequently used. This technique is widely applied to water samples [37]. For foodstuffs in liquid forms, such as juices, wine, and milk, acceptable recoveries can be achieved. Although the main advantage of

using SPE is the clean-up potential, one or more clean-up steps, e.g., using a florisil column, are generally needed for the determination of PCBs in liquid form samples [38]. Another technical problem is clogging the cartridge, which is associated with the use of unfiltered liquid samples [39]. SPE is a plastic material, which may adsorb analytes and/or increase interferences with the target [40]. Different SPE sorbents i.e., florisil, alumina, aminopropyl (NH2), C-18, Envi-carb, florisil-alumina, and NH2-alumina were investigated for their clean-up efficiency for determination of OCCs (OCPs and PCBs) in low fat fish. The most efficient sorbents in the removal of co-extracted interference are florisil, alumina, and NH2 with average recoveries of 102.4%, 93.5%, and 86.5%, respectively for 6 indicator PCBs and CB-118, CB-209 [41].

Solid-phase microextraction (SPME)

Solid-phase microextraction (SPME) was developed by Arthur and Pawlyszin [40] and has been widely manufactured by Supelco since 1993. Direct extraction is mainly suitable for clean samples while headspace extraction is the better choice for dirty liquid samples. In this technique, a small fiber coated with the stationary phase is placed into the aqueous sample (3-5 mL). Then, lipophilic analytes are adsorbed and partitioned into the stationary phase. After that, the analytes are thermally desorbed in the column GC injection port, normally taking 1-10 min [39, 42].

The main advantage, compared to SPE, is that SPME does not suffer from plugging or channeling. Additionally, organic solvents are eliminated and the extraction time is reduced to a few minutes. On the other side, the fiber is coated by a maximal thickness of 100 μm polydimethylsiloxane (about 0.6 μL) [43] and so the efficiency of SPME in terms of low recoveries for specific applications is mainly due to limitation of sorptive agents and consequently decreasing the mass of extracted analytes [44,45]. Unfortunately losses of volatile analytes may occur when transferring the SPME unit from the sample to the GC injector. Additionally there is a constant risk that some analytes can be lost during the cryo-focusing step. Another disadvantage is that the relatively long equilibration times (up to 1h) are needed when performing SPME [39].

Stir-bar sorptive extraction (SBSE)
In 1999, a new extraction technique was developed by Baltussen and co-workers [46]. In this technique known as stir-bar sorptive extraction (SBSE), a stir

_____

_____

bar coated typically with 500-1000 μm of polydimethylsiloxane (about 125 μL) is used to stir aqueous samples and extract the solutes (PCBs) into the polydimethylsiloxane layer [43, 47]. After the extraction, thermal or solvent desorption can be employed [46]. The extraction mechanisms and advantages are similar to those of SPME, but the enrichment factor, which is determined by the amount of extractive phase, is up to 100 times higher [47, 48]. The major advantages of this technique are ease of use, improved sensitivity, high accuracy of analysis, and reduced risk of contamination [25].

Supercritical fluid extraction (SFE)

Supercritical Fluid Extraction (SFE) is suitable for tissue samples and solid foods. One of the most critical points for the usage of SFE is selectivity, which enables extraction of a wide range of micro-pollutants including PCBs, PAHs, and OCPs [49]. Carbon dioxide is the common supercritical fluid used in this technique. Supercritical fluids above the critical points of temperature and pressure can diffuse through solids like a gas and dissolves analytes like a liquid [35].

Other advantages are acquisition of clean extract, short time for extraction, and less solvent consumption [50]. In some cases, lipids in sea food matrices are co-extracted with the PCBs [39] and so a further clean-up step may be required. A comparison study was conducted by Crespo and Yusty [51] to examine the efficiency of SFE in the determination of PCB congeners in seaweed samples in reference to SE (Table 1).

The comparison was conducted at two concentration levels (25 and 400 μg/kg) of some PCB congeners. At the higher level of 400 μg/ kg, there were no significant differences in PCB congener concentrations between the two techniques, except for CB-28, -52, -77, and -101. However, the recoveries for PCB congeners at the level of 25 μL/kg ranged between 42-67% for SFE and between 82-92% for SE, respectively.

Accelerated solvent extraction (ASE)

Accelerated solvent extraction (ASE) was introduced and described in detail by Richter and Co-workers [52] as a new technique for sample extraction, which is performed by application of elevated temperature and pressure. This technique is also called pressurized fluid extraction (PFE) or pressurized liquid extraction (PLE) and mainly used for the extraction of solid and semisolid sample matrices that can be retained in the extraction cell [52]. Recoveries of PCB congeners (28, 52, 101, 138, 153, and 180) from oyster tissue at concentration levels of 50-150 ng/g were examined using ASE and the values were from 86.9 to 90%. [52] When dried biota samples were extracted using ASE, matrix-pike recoveries ranged from 90 to 106% [19]. In another study for fish conducted by El-Kady et al. [53], the recoveries for ^{13}C labeled internal standards of PCBs and dioxins ranged from 70 to 103%. One important key in this technique is the high temperature (50-200 ^{o}C) as it accelerates the extraction process by increasing the diffusion rate of solvent and the solubility of analytes in the solvent and decreasing the surface tension and viscosity of the solvent, which increase the penetration of the solvent within the matrix particles and improve mass transfer. Elevated pressure (1500 – 2000 psi) keeps the used solvent below its boiling point, thus enabling safe and rapid extractions [54].

Matrix solid-phase dispersion (MSPD)

In 1989, matrix solid-phase dispersion (MSPD), an extraction process for solid and semi-solid samples was first reported by Barker and co-workers [55]. A bonded-phase, such as silica (C18), which acts as an abrasive solid support material, is blended with the sample, producing shearing and grinding forces that disrupt the sample. Then the sample is dispersed over the surface of the bonded-phase support material [56].

Table 1. Comparison between SFE and SE techniques for etermination of PCB congeners in seaweed

Items	SFE	SE
Wt. of sample	0.5 g	1 g
Sample preparation	Mixed with 3 g of deactivated alumina & 1 g alumina and 200 μL of methanol are added to the sample	Mixed with 15 g sea sand
Solvent	CO_2, 7.5 mL	250 mL (n-hexane: Dichloromethane; 1:1)
Extraction time	50 min	7 h
Clean-up technique	Silica SPE	Sep-Pak Silica cartridge

SFE, Supercritical fluid extraction; SE, Soxhlet extraction

_____

_____

Briefly, a solid or semi-solid sample is mixed and blended with a solid support material at a rate from 1:1 to 1:4 (sample: solid support material) to destroy and disperse the sample onto the sorbent. Then the mixture is placed onto an empty column or onto a SPE column and the analytes are directly eluted from the column. After interfering compounds retained on the column are washed down, the analytes need to be eluted by different solvent. In some cases, a clean-up step is needed or the MSPD column is conducted with a SPE column to remove the interfering components [57].

Microwave assisted extraction (MAE)

A domestic microwave oven was used to extract analytes for the first time in 1975 by Abu-Samra and Co-workers [58]. The microwave-assisted extraction (MAE) technique is similar to ASE as it works at elevated temperature and pressure. Additional clean-up procedures such as alumina/silica column are generally required for PCBs determination in environmental matrices [19, 53, 59]. Good adequate recoveries have been achieved for PCBs in certified harbour sediments and for PCBs and dioxins in marine and sediment samples by applying MAE (acetone: cyclohexane, 1:1 at 100 ^{o}C), compared to SE technique [60].

Performance of three extraction methods (UE, MAE, and ASE) for determination of PCB congeners (CB-118, -138, -153, and -180) in eggs was evaluated under each specific condition (given in Table 2). The result indicated that there were no significant differences in recoveries of PCBs among these extraction techniques [17].

Table 2. Conditions of UE, MAE, and ASE for determination of PCB congeners (CB CB-118, -138, -153, and -180) in an egg samples at three levels

	UE	MAE	ASE
Sample Wt.	3 g	1 g	1 g
Extraction	30 ^{o}C	95 ^{o}C	100 ^{o}C 1500 Psi
Solvent used	Petroleum ether	n-hexane	Petroleum ether
Solvent	10 mL	20 mL	40 mL
Total	30 min	20 min	18 min
Filtration	+	+	-

UE, Ultrasonic extraction; MAE, Microwave assisted extraction; ASE, Accelerator Solvent Extractor

Clean-up procedures

During the extraction of foodstuffs, many types of interfering compounds, eg., lipids, carbohydrates, chlorophyll and so on, get co-extracted. So, additional clean-up processes are needed. Single or multi-sequential liquid chromatographic technique is applied and reported by many analytical scientists to separate PCBs from the extract. Alumina, silica gel,

florisil, and carbon are the typical adsorbent agents used frequently [19, 53, 61]. The main clean-up and purification procedures are shown in Fig. 1.
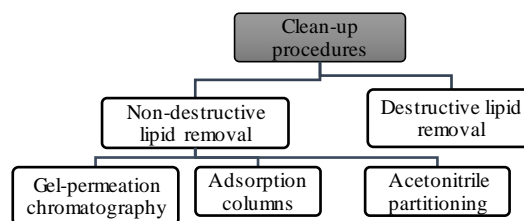

Fig. 1. Main clean-up procedures for PCB analysis

As a destructive method, acid digestion or saponification is commonly applied for the removal of lipids in the extract [62]. Silica gel treated with sulfuric acid has been also used for the removal of lipids from food matrices [53, 61, 63]. Adsorption chromatography applying the SPE technique is the most commonly used method of clean-up [64]. However, column chromatography using silica gel, alumina, and florisil, or a combination of these adsorbents, cannot necessarily guarantee lipid-free extracts. This technique has been successfully applied to the analysis of PCBs in different food matrices [65]. Since the molecular sizes of potentially co-extracted lipids, proteins, carbohydrates, pigments and so on are much bigger than the analytes, gel permeation chromatography (GPC) can be very useful tool to separate them. Because its separation principle is based on size exclusion, GPC is strongly recommended to use for separating large size molecules from lipid-rich extracts [15, 66].

An activated carbon adsorbent with surface area of 1400 m^{2}/g and grain size of 600 μm is used to separate di- and mono-ortho PCBs from non-ortho PCBs and PCDD/Fs in enriched fatty foods. In the technique, the lipid fraction (10 g of fat) which dissolved in dichloromethane (DCM) is passed through 1.8 g of activated carbon, and then the carbon column is refluxed for 120 min using 30 mL of DCM, then the column is rinsed with 20 mL of toluene and refluxed with 30 mL of toluene to elute non-ortho PCBs for up to 120 min. This fraction of non-ortho PCBs is dissolved in 5 mL n-hexane and passes through silica gel treated with 44% H$_2$SO$_4$/alumina column (1:10, w/w) as a second cleanup step for non-ortho PCBs which is eluted with a mixture of n-hexane: DCM (1:1). To isolate mono-ortho and 6 non-dl-PCBs, a half gram of lipid fraction is dissolved in 5 mL n-hexane and passed through silica get treated with H$_2$SO$_4$ and eluted with n-hexane. As described above, another silica/alumina column is used and the mono-ortho and 6 non-dl-PCBs are eluted with a mixture of n-hexane: DCM (1:1) [53,

_____

_____

61, 67]. A similar technique was applied by Bernsmann et al. [68] to determine dioxins, PCBs and PBDEs in food matrices. In this method, the extracts are passing through silica gel column coated with H_2SO_4 to eliminate lipid content, and then active carbon column is used to separate dioxin-like PCBs (dl-PCBs), non dioxin-like PCBs (ndl-PCBs) and PBDEs by a mixture of n-hexane, cyclohexane and DCM in the first fraction and PCDD/Fs by toluene in the second fraction. Further florisil column is used to clean-up PCDD/Fs fraction eluted by toluene. An alumina column is used to separate mono-ortho and ndl-PCBs eluted by a mixture of n-hexane: DCM, 98:2 from non-ortho PCBs and PBDEs eluted by a mixture of n-hexane: DCM, 1:1.

A combination of extraction and clean-up techniques has been conducted by many scientists over the past years to reduce time and cost and to minimize the potential loss of the analytes during the sample preparation [69-72]. In this combination method, ASE is generally applied as an extraction and clean-up tool: a tissue homogenate sample is placed on the top of the multi-layer sorbent, which consists of 10 g alumina, mixture of 5 g celite and 0.5 g carbopack, 10 g florisil, and 5 g silica from top to bottom, in the extraction cell. After adding surrogates and standing for equilibrium (20 min), ndl-PCBs and mono-ortho PCBs are eluted from the extraction cell using DCM: Hex (1:1), as non-ortho PCBs and PCDD/Fs are retained in the mixture of celite and carbopack. Toluene is used to elute non-ortho PCBs and PCDD/Fs from the celite/carbopack layer [71]. For the amount of adsorbents used, the result revealed no significant differences in the recoveries of PCBs and PCDD/Fs by increasing the ratio between fish composite and alumina or silica. So, the optimal mass of the adsorbent mixture was adopted as 30.5 g (10 g alumina, mixture of 5 g celite and 0.5 g carbopack, 10 g florisil, and 5 g silica) for 10 g fish composite. To separate dl-PCBs from PCDD/Fs, the mixture of DCM: n-hexane was used as a first fraction: the recoveries for dl-PCBs and PCDD/Fs were 96.5% and 6.7%. Then toluene was used as a second fraction and the recoveries were 5% and 90.7% for dl-PCBs and PCDD/Fs, respectively. In case we used toluene as a first fraction, the recoveries for both analytes were 94.2% and 96.2%, respectively, and the dl-PCBs and PCDD/Fs could not be separated if we employed toluene as a first fraction. Regarding the co-extracted materials that can be interfering with the analytes, florisil and celite can be eliminated and remove the high molecular materials. So there is no need to run the extract for GPC as a clean-up tool [71].

Table 3 lists some extraction and clean-up techniques for different food matrices reported previously. Among the extraction techniques available for foodstuffs, ASE is widely used for different food matrices and n-hexane and DCM are commonly selected as the extraction solvent. For a common clean-up method, silica gel treated with H_2SO_4 alone or in combination with alumina is adopted by many scientists, particularly for animal tissue samples. GPC is also used on a large scale to eliminate large size molecules co-extracted with analytes.

In 2003, a very simple preparation technique, called QuEChERS method, intended for pesticide analysis in vegetables and fruit was introduced by Anastassiades et al. [73]. Since the blending of the test portion with organic solvent is replaced by shaking in this method, the homogenization of the sample is an important aspect to increase surface area. Acetonitrile (ACN) that co-extracted interfering compounds can be sharply reduced compared with acetone or ethyl acetate is used as a polar extraction solvent [74, 75]. Many scientists applied this technique for the determination of PCBs in different food matrices such as fish and shrimp [74-76].

Chromatographic analysis of PCBs

GC equipped with electron capture detector (GC/ECD)

The determination method of PCBs utilized extensively until around 2000 is gas chromatography (GC) with electron capture detector (ECD) due to its sensitivity for halogenated hydrocarbons and cost-effectiveness. However, it is not suitable or recommended for non-ortho PCBs (PCB congeners 77, 126 and 169) analysis due to the lower detection limit of ECD (0.1 ng/g) and the levels of planar PCBs within range of pg/g [18]. Temperature is also an important factor that affects the response of ECD, which is normally conducted at above 300 ^{o}C, however lower temperature (180-200 ^{o}C) permit a good ECD response for some PCBs. In addition, $^{13}C_{12}$-labeled standard cannot use for PCBs quantifications [77, 78]. Other problems associated with the usage of ECD: (1) non-linear response across a relatively narrow range and (2) a wide variation in response even within a particular PCB-homologue group. In addition, a sophisticated clean-up method is required to reduce and remove interferences [25].

_____

_____

Table 3. Extraction and clean-up methods used for determination of PCBs and dioxins in different food matrices

Matrices	Target Analytes	Extraction technique	Solvent and composition	Extraction time	Clean-up technique	Ref.
Powder milk	PCDD/Fs & non-ortho PCBs	SE	Pentane : DCM (1:1)	Overnight	GPC & silica/alumina/carbon columns	[93]
Liquid milk		SPE	n-hexane	Few minutes		
Meat		ASE	n-hexane	20 min		
Mussels	PCBs & OCPs	SE	n-hexane : DCM (1:1)	8 h	Florisil column	[94]
Fish	PCBs & PCDD/Fs	ASE	n-hexane : acetone (75:25)	20 min	Silica treated with sulfuric acid & carbon cartridge	[53]
Seal fat	PCBs & PCDD/Fs	SE	DCM	8 h	GPC & Silica/alumina/carbon columns	[95]
Meat, fish, dairy product, egg, oil	PCBs	US	n-hexane : acetone	-	Alumina/florisil column	[96]
Shrimp	PCDD/Fs & DL-PCBs	SE	Toluene: cyclohexane (1:1)	24 h	Multilayer silica, alumina and carbon	[67]
Fresh & canned fish	Indicator PCBs	SE	Acetone : n-hexane (1:1)	-	H_2SO_4 to remove lipid contents & florisil 60/100 mesh	[97]
Fish	PCBs & OCPs	ASE	DCM	20 min	GPC & alumina/silica column	[19]
13 kinds of foodstuffs	PCDD/Fs & DL-PCBs	ASE	n-hexane : DCM (1:1)	20 min	Silica/alumina column	[98]
Fish, beef and feed	PCDD/Fs & DL-PCBS	ASE	n-hexane : DCM (1:1)	20 min	Silica gel treated with sulfuric acid & activated carbon	[99]
Milk	PCBs	UE & LLE	n-hexane: ACN: Ethanol (25:5:1)	-	Combined system of Extrelut-3/ Extrelut-1 cartridge with addition of 0.36 g C18 & Florisil column	[100]
Poultry Eggs	18 PCBs & PCDD/Fs	ASE	MeOH : Toluene (70:30)	20 min	Acidified silica gel for fat oxidizing & florisil. Active carbon columns	[101]

DCM= Dichloromethane; MeOH= Methanol

On the other hand, GC equipped with high resolution MS (GC-HRMS) is the most reliable tool for quantitation of dioxins and dl-PCBs due to its high sensitivity and selectivity for these analytes [79]. The European Committee (EC) legislation recognizes HRGC/HRMS as a reference and confirmatory method for suspected and positive dioxin and dl-PCBs findings in foodstuffs coupled with isotope dilution method. Due to the operation and maintenance cost, of HRGC/HRMS, and the requirement of highly qualified and competent persons, alternative techniques have been evaluated to replace the reference method or at least to minimize analysis costs by their use in preliminary screening [80].

Time-of flight mass spectrometry (TOF-MS)

Another alternative method to GC-HRMS exists, and one of the most promising is TOF-MS. As compared to SIM with sector instruments, TOF-MS offers more MS information because it monitors all masses at once within a range. The coupling of GC/GC separation and TOF-MS detection presents no difficulties and there is no peak broadening. Consequently, the GC/GC-TOF-MS coupling is a powerful instrument combining improved

chromatographic resolution of GC/GC and the analytical resolving power of the TOF-MS [81, 82]. The GCxGC coupled with high speed low resolution (LR) time of flight (TOF)-MS has been developed to analyze dioxins at an acquisition rate of 50 Hz [83]. This technique separate PCDD/Fs from PCBs and give detection limit for tetrachlorodibenzo-p-dioxin (TCDD) of 0.25 pg, but is not successful with low concentration levels (pg/L) in complex matrices with low resolution. On the other hand, Xia et al. [84] develop a GC x GC-HR TOF-MS method to determine 12 dl-PCBs and 6 indicator PCBs in one injection. In this optimization, GCxGC was coupling to micro ECD to maximize chromatographic separation which is confirmed by the two-dimensional chromatogram for target PCBs. A narrow mass (0.02 Da) was selected to obtain good separation and to increase the accuracy and selectivity. A comparative study was conducted to evaluate GCxGC-HRTOF-MS with the reference method (GC-HRMS) for dl-PCBs and 6 indicator PCBs in fish samples. The results of GCxGC-HR TOF-MS were comparable to those obtained by GC-HRMS. For indicator PCBs (28, 52, 101, 138, 153, and 180) the difference between two methods for three types of fish ranged from 4.7 to 23.9% suggesting that the GCxGC-HRTOF-MS results were consistent with the results of GC-HRMS (Fig. 2). Regarding 12 dl-PCBs, the congeners with concentration above 0.9 pg/g have similar results
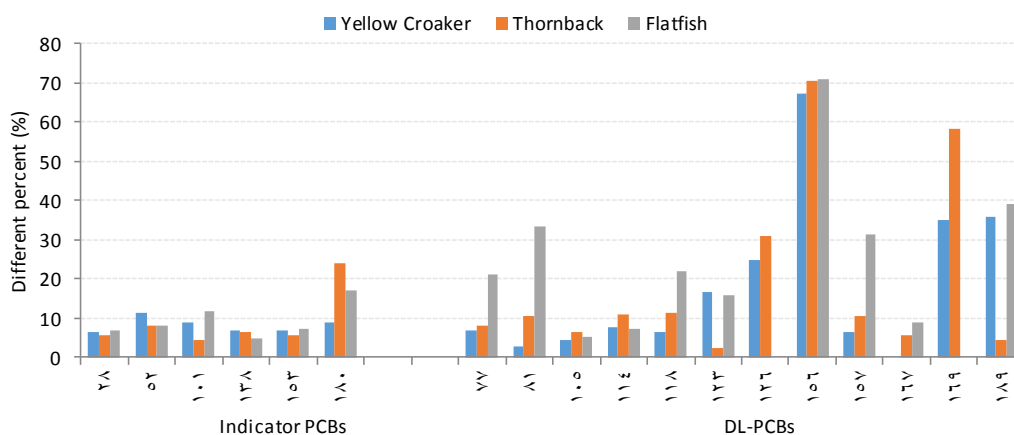
_____

_____

Fig. 2. Different percent of GC×GC-HRTOF-MS and GC-HRMS results for 6 indicator PCBs and 12 dl-PCBs in three species of fish
Different percent= (values of GC-HRMS subtract the values of GC×GC-HRTOF-MS)/ the value of GC-HRMS.

with those obtained by GC-HRMS with different percent below than 39.3% for most dl-PCBs (Fig. 2).

Tandem MS/MS

Although GC/Ion trap- mass spectrometry (MS) and GC/low resolution-MS showed good and promising results for the determination of PCBs, the sensitivity became limited by every working day of analysis as well as the intensive purification steps required to overcome the problems associated with the occurrence of co-extracted compounds [85]. Due to enhancement of ion source optics, an alternative low costly and sensitive technique like GC- triple quadrupole mass spectrometry (MS/MS) for quantitation of dioxins and PCBs is recently applied at low levels in food and feed [86-91]. So, with the usage of GC/ion trap equipment having tandem mass spectrometry capability (GC/MS/MS), the high sensitivity and specificity are being applied to determine the organic pollutants such as PCBs, dl-PCBs, and dioxins [80]. The capabilities of the GC-MS/MS technique were evaluated by the European reference Lab with different food and feedstuffs containing different concentration levels of dioxins and PCBs. The optimization of the GC-MS/MS for 12 DL-PCB and 6 NDL-PCBs (indicator PCBs) is conducted by Fürst at al. [85]. For dl-PCBs, the agreement between GC-HRMS and GC-MS/MS for 80 feed and foodstuffs is in almost within 10% and 15% differences at level above 1 pg/g or 0.1 pg/ g, respectively. For NDL-PCBs, the agreement between GC-HRMS and GC-MS/MS at concentrations ranged from 0.5 to 10 ng/ g is within ± 10%. Also, full validation including instrumental and method limit of

quantitation (iLOQ and mLOQ), accuracy, recovery and uncertainty measurement for dioxin and dl-PCBs analysis in foodstuffs was conducted by applying GC-MS/MS [92]. While the iLOQ for non-ortho PCBs and mono-ortho PCBs ranged between 0.030 and 2.109 pg/μL, the iLOQ for ndl-PCBs showed a higher range (0.904 and 6.530 pg/μL) compared with dl-PCB congeners. The mLOQ ranged between 0.018 and 601 pg/g for dl-PCBs and between 34.9 and 910 pg/g for ndl-PCBs. The variation coefficients for matrix spike recoveries at three concentration levels ranged from 3.4 to 8.5%. Based on these results, usage of GC- MS/MS is becoming approved for dioxin and dl-PCBs in animal tissues since the performance is comparable to GC-HRMS.

Conclusions

It is believed that about one third of the total amount of polychlorinated biphenyls (PCBs) has been released into the environment. Due to their physico-chemical properties, PCBs concentrated in food webs. Food consumption constitutes about 90% of human exposure to PCBs. Food matrices are naturally complex and many ingredients are co-extracted with PCBs. To determine PCBs in different foodstuffs, extraction, purification, and chromatographic separation techniques are mandatory steps to isolate PCBs from food matrices. This article reviews the approached techniques for the determination of PCBs in foodstuffs. Several extraction techniques have been assessed and established i.e. LLE, SE, UE, SPE, SPME, SBSE, SFE, ASE, MSPD, and MAE. Among those extraction methods, ASE is widely used for different

_____

food matrices using n-hexane and DCM as extraction solvents. Because a variety of impurities e.g., lipids, proteins, and pigments are co-extracted with PCBs, an adequate clean-up process is necessary to isolate PCB congeners at detectable levels. Silica gel treated with sulfuric acid or in combination with alumina is an effective technique for clean-up. GPC technique is also used widely to eliminate large size molecules from the extracts. An isotope dilution method using GC-HRMS is the most and approved effective technique to detect and quantify low concentrations of dioxins and dl-PCBs in foodstuffs. An alternative low costly and sensitive technique using GC coupled with tandem MS (GC-MS/MS) is fully validated for analysis of dioxin and dl-PCBs in food and feed stuffs. This technique is becoming approved and considered as an equivalent method as GC-HRMS by the commission Regulation (EU) No.589/2014 and No.709/2014 [85]. In the same regard, GCxGC-HRTOF-MS is approved to be an alternative of GC-HRMS since the results agreed well with those obtained by GC-HRMS for 18 PCBs (12 dl-PCBs and 6 indicator PCBs) as reported by Xia et al. [84].

Conflicts of interest

The authors declare that they have no conflict of interest.

References

1. Voot, P.D.E., Brinkman, U.A.Th., Production, properties and usage of polychlorinated biphenyls. In: TOPICS IN ENVIRONMENTAL HEALTH 4, Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products. R.D. Kimbrough & A.A. Jensen (eds.), 2nd fully revised edition. p. 3-45, Elsevier, Amsterdam, (1989).

2. Jensen, S., Report of a New Chemical Hazard. NEW SCIENTIST. 32, 612 (1966).

3. Bletchly, J.D., Study of measures to avoid dispersion into the environment of PCBs and PCTs from existing installations; report to the CEC, Aylesbury (1985).

4. Thomas, K.B., Colborn, T., Organochlorine Endocrine Disruptors in Human Tissue," in Theo Colborn and Coralie Clement, editors, CHEMICALLY-INDUCED ALTERATIONS IN SEXUAL AND FUNCTIONAL DEVELOPMENT: THE WILDLIFE/HUMAN CONNECTION [Advances in Modern Environmental Toxicology Vol. XXI] (Princeton, N.J.: Princeton Scientific Publishing Co., pgs. 342-343 (1992).

5. Espandiari, P., Glauert, H.P., Lehmler, H-J., Lee, E.Y., Srinivasan, C., Robertson, L. W., Polychlorinated biphenyls as initiators in liver carcinogenesis: resistant hepatocyte model. *Toxicology and Applied Pharmacology*, **186**, 55-62 (2003).

6. Bloom, M.S., Vena, J.E., Swanson, M.K., Moysich, K.B., Olso, J.R., Profiles of ortho-polychlorinated biphenyl congeners, dichlorodiphenyldichloroethylene, hexachlorobenzene, and mirex among male Lake Ontario sport fish consumers: the New York State Angler cohort study. *Environmental Research*, **97**, 178-194 (2005).

7. Fitzgerald, E.F., Belanger, E.E., Gomez, M.I., Hwang, S., Jansing, R.L., Hicks, H.E., Environmental exposures to polychlorinated biphenyls (PCBs) among older residents of upper Hudson River communities. *Environmental Research*, **104**, 352-360 (2007).

8. Arnich, N., Tard, A., Leblanc, J-C., Bizec, B.L., Narbonne, J-F., Maximilien, R., Dietary intake of non-dioxin-like PCBs (NDL-PCBs) in France, impact of maximum levels in some foodstuffs. *Regulatory Toxicology and Pharmacology*, **54**, 287-293 (2009).

9. Nassar, H.F., Monitoring and Risk Assessment of Selected Pesticide Congeners (PCBs) in Nile River Waters, Egypt. *Egyptian Journal of Chemistry*, **60**, 977- 984 (2017).

10. Malisch, R., Kotz, A., Dioxins and PCBs in feed and food-Review from European perspective. *Science of the Total Environment*, **491-492**, 2-10 (2014).

11. Bocio, A., Domingo, J.L., Falcó, G., Llobet, J.M., Concentrations of PCDD/PCDFs and PCBs in fish and seafood from the Catalan (Spain) market: estimated human intake. *Environment International*, **33**, 170-175 (2007).

12. Bergkvist, C., Oberg, M., Appelgren, M., Becker, W., Aune, M., Ankarberg, E.H., Berglund, M., Håkansson, H., Exposure to dioxin-like pollutants via different food commodities in Swedish children and young adults. *Food and Chemical Toxicology,* **46**, 3360-3367 (2008).

13. Picó, Y., Fernández, M., Ruiz, M.J., Font, G., Current trends in solid-phase-based extraction techniques for the determination of pesticides in food and environment. *Journal of Biochemical* and *Biophysical Methods,* **70**, 117-131 (2007).

14. Albero, B., Brunete, C.S., Tadeo, J.L., Multiresidue determination of pesticides in juice by solid-phase extraction and gas chromatography–mass spectrometry. *Talanta,* **66**, 917-924 (2005).

15. Liu, L.-B., Hashi, Y., Qin, Y.-P., Zhou, H.-X., Lin, J.-M., Development of automated online gel permeation chromatography gas chromatograph mass spectrometry for measuring multiresidual

_____

pesticides in agricultural products. *Journal of Chromatography B,* **845**, 61-68 (2007).

16. Soler, C., Pico, Y., Recent trends in liquid chromatography tandem mass spectrometry to determine pesticides and their metabolites in food. *Trends in Analytical Chemistry,* **26**, 103-115 (2007).

17. Sandra, P., David, F., Vanhoenacker, G., Advanced sample preparation techniques for the analysis of food contaminants and residues. *Comprehensive Analytical Chemistry*, **51**, 131-174 (2008).

18. Muir, D., Sverko, E., Analytical methods for PCBs and organochlorine pesticides in environmental monitoring and surveillance: a critical appraisal. *Analytical and Bioanalytical Chemistry,* **386**, 769-789 (2006).

19. El-Kady, A.A., Wade, T.L., Sweet, S.T., Sericano, J.L., Distribution and residue profile of organochlorine pesticides and poly chlorinated biphenyls in sediment and fish of Lake Manzala, Egypt. *Environmental Science and Pollution Research,* **24**, 10301-10312 (2017).

20. Asamoah, A., Essumang, D.K., Muff, J., Kucheryavskiy, S.V., Søgaard, E.G., Assessment of PCBs and exposure risk to infants in breast milk of primiparae and multiparae mothers in an electronic waste hot spot and non-hot spot areas in Ghana. *Science of the Total Environment,* **612**, 1473-1479 (2018).

21. Liem, A.K.D., Important developments in methods and techniques for the determination of dioxins and PCBs in foodstuffs and human tissues. *Trends in Analytical Chemistry,* **18**, 499-507 (1999).

22. Becker J.S., Inorganic Mass Spectrometry of Radionuclides, in: Handbook of Radioactivity Analysis 3rd Ed., 2012 (Ed. Michael F. L'Annuziata), Chapter 13, P. 833-870 (2012).

23. Wells, D.E., Chiffoleau, J-F., Klungsoyr, J., QUASIMEME: a preliminary study on the effects of sample handling on the measurement of trace metals and organochlorine residues in mussels. *Marine Pollution Bulletin,* **35**, 109-124 (1997).

24. Wells, D.E., Hess, P., Separation, clean-up and recoveries of persistent trace organic contaminants from soils, sediment and biological matrices. In: Barceló E (ed) Sample handling and trace analysis of pollutants, techniques, applications and quality assurance. Elsevier, Amsterdam, pp 73-116 (2000).

25. Ahmed F.E., Analysis of polychlorinated biphenyls in food products, 2003. *Trends in Analytical Chemistry*, **22**, 170-185 (2003).

26. Söderström, M., Nylund, K., Jarnberg, U., Alsberg, T., Asplund, L., Airborne PCB as a contamination risk on freeze-drying of sediment. *Chemosphere,* **58**, 355-366 (2005).

27. Kohler, M., Tremp, J., Zennegg, M., Seiler, C., Minder-Kohler, S., Beck, M., Lienemann, P., Wegmann, L., Schmid, P., Joint sealants: an overlooked diffuse source of polychlorinated biphenyls in buildings. *Environmental Science and Technology,* **39**, 1967-1973 (2005).

28. Müller, E., Berger, R.; Blass, E., Sluyts, D., Pfennig, A., Liquid-Liquid Extraction in Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VCH, Weinheim, 250-300 (2008).

29. Lambropoulou, D.A., Albanis, T.A., Liquid-phase micro-extraction techniques in pesticide residue analysis. *Journal of Biochemical* and *Biophysical Methods,* **70**, 195-228 (2007).

30. Fernandes, D., Zanuy, S., Bebianno, M.J., Porte, C., Chemical and biochemical tools to assess pollution exposure in cultured fish. *Environmental Pollution*, **152**, 138-146 (2008).

31. Yu, H.-Y., Guo, Y., Bao, L.-J., Qiu, Y.-W., Zeng, E.Y., Persistent halogenated compounds in two typical marine aquaculture zones of South China. *Marine Pollution Bulletin,* **63**, 572-577 (2011).

32. Beard, A., Naikwadi, K., Karasek, F.W., Comparison of extraction methods for polychlorinated dibenzo-p-dioxins and dibenzofurans in fly ash using gas chromatography-mass spectrometry. *Journal of Chromatography A,* **589**, 265-270 (1992).

33. Żwir-Ferenc, A., Biziuk, M., Solid phase extraction technique- Trends, opportunities and applications. *Polish Journal of Environmental Studies,* **15**, 677-690 (2006).

34. Poole, C.F., New trends in solid-phase extraction. *Trends in Analytical Chemistry*, 22, 362-373 (2003).

35. Beyer, A., Biziuk, M., Applications of sample preparation techniques in the analysis of pesticides and PCBs in food. *Food Chemistry*, **108**, 669-680 (2008).

36. Hennion, M-C., Solid-phase extraction: method development, sorbents, and coupling with liquid chromatography. *Journal of Chromatography A,* **856**, 3-54 (1999).

37. Carabias-Martinez R., Rodriguez-Gonzalo, E., Herrero-Hernandez, E., Behaviour of triazine herbicides and their hydroxylated and dealkylated metabolites on a propazine-imprinted polymer - comparative study in organic and aqueous media. *Analytical Chimica Acta*, **559**, 186-94 (2006).

38. Westbom, R., Thörneby, L., Zorita, S., Mathiasson, L., Björklund, E., Development of a solid-phase extraction method for the determination of polychlorinated biphenyls in

_____

_____

water. *Journal of Chromatography A,* **1033**, 1-8 (2004).

39. Björklund, E., Holst, C.V., Anklam, E., Fast extraction, clean-up and detection methods for the rapid analysis and screening of seven indicator PCBs in food matrices. *Trends in Analytical Chemistry*, **21**, 39- 52 (2002).

40. Arthur, C.L., Pawliszyn, J., Solid Phase Microextraction with Thermal Desorption Using Fused Silica Optical Fibers. *Analytical Chemistry*, **62**, 2145-2148 (1990).

41. Beyer, A., Biziuk, M., Comparison of efficiency of different sorbents used during clean-up of extracts for determination of polychlorinated biphenyls and pesticide residues in low-fat food. *Food Research International*, **43**, 831-837 (2010).

42. Sauret-Szczepanski, N., Mirabel, P., Wortham, H., Development of a SPME-GC-MS/MS method for the determination of pesticides in rain water. *Environmental Pollution,* **139**, 133-142 (2006).

43. Bicchi, C., Iori, C., Rubiolo, P., Sandra, P., Headspace sorptive extraction (HSSE), stir bar sorptive extraction (SBSE), and solid phase microextraction (SPME) applied to the analysis of roasted arabica coffee and coffee brew. *Journal of Agricultural and Food Chemistry,* **50**, 449-459 (2002).

44. Risticevic, S., Vuckovic, D., Lord, H.L., Pawliszyn, J., Solid-Phase Micro- extraction, Comprehensive Sampling and Sample Preparation, Academic Press, Oxford., **221**, 419-460 (2012).

45. Barba, C., Thomas-Danguin, T., Guichard, E., Comparison of stir bar sorptive extraction in the liquid and vapour phases, solvent-assisted flavour evaporation and headspace solid-phase microextraction for the (non)-targeted analysis of volatiles in fruit juice. *LWT - Food Science Technology,* **85**, 334-344 (2017).

46. Baltussen, E., Cramers, C.A., Sandra, P.J.F., Sorptive sample preparation- a review. *Analytical and Bioanalytical Chemistry,* **373**, 3-22 (2002).

47. David, F., Sandra, P., Stir bar sorptive extraction for trace analysis, Review. *Journal of Chromatography A,* **1152**, 54-69 (2007).

48. Nakamura, S., Nakamura, N., Ito, S., Determination of 2-methylisoborneol and geosmin in water by gas chromatography-mass spectrometry using stir bar sorptive extraction. *Journal of Separation Science,* **24**, 674-677 (2001).

49. Burford, M.D., Hawthorne, S.B., Miller, D.J., Extraction rates of spiked vs. native PAHs from heterogeneous environmental samples using supercritical fluid extraction and sonication in

methylene chloride. *Analytical Chemistry*, **65**, 1497-1505 (1993).

50. Ahmed F.E., Analyses of pesticides and their metabolites in foods and drinks. *Trends in Analytical Chemistry*, **20**, 649-661 (2001).

51. Crespo, M.O.P., Yusty, M.A.L., Comparison of supercritical fluid extraction and Soxhlet extraction for the determination of PCBs in seaweed samples. *Chemosphere,* **59**, 1407-1413 (2005).

52. Richter, B.E., Jones, B.A., Ezzell, J.L., Porter, N.L., Accelerated solvent extraction: a technique for sample preparation. *Analytical Chemistry*, **68**, 1033-1039 (1996).

53. El-Kady A.A., Abdel-Wahhab, M.A., Henkelmann, B., Belal, M.H., Morsi, M.K., Galal S.M., Schramm, K-W., Polychlorinated biphenyl, polychlorinated dibenzo-p-dioxin and polychlorinated dibenzofuran residues in sediments and fish of the River Nile in the Cairo region. *Chemosphere*, **68**, 1660-1668 (2007).

54. Giergielewicz-Możajska, H., Dąbrowski, L., Namieśnik, J., Accelerated Solvent Extraction (ASE) in the Analysis of Environmental Solid Samples- Some Aspects of Theory and Practice. *Critical Reviews in Analytical Chemistry,* **31**, 149-165 (2001).

55. Barker, S.A., Long, A.R., Short, C.R., Isolation of drug residues from tissues by solid phase dispersion. *Journal of Chromatography A,* **475**, 353-61 (1989).

56. Barker, S.A., Matrix solid phase dispersion (MSPD). *Journal of Biochemical* and *Biophysical Methods,* **70**, 151-162 (2007).

57. Kristenson, E.M., Ramos, L., Brinkman, U.A.T., Recent advances in matrix solid-phase dispersion. *Trends in Analytical Chemistry*, **25**, 96-111 (2006).

58. Abu-Samra, A., Morris, J.S., Koirtyohann, S.R., Wet ashing of some biological samples in a microwave oven. *Analytical Chemistry,* **47**, 1475-1477 (1975).

59. Kodba, Z.C., Marsel, J., Microwave-Assisted Extraction and sonication polychlorinated biphenyls from river sediments and risk assessment by toxic equivalency factors. *Chromatographia*, **49**, 21-27 (1999).

60. Eskilsson, C.S., Björklund, E., Analytical-scale microwave-assisted extraction. *Journal of Chromatography A,* **902**, 227-50 (2000).

61. Kedikoglou, K., Costopoulou, D., Vassiliadou, I., Bakeas, E., Leondiadis, L., An effective and low cost carbon based clean-up method for PCDD/F and PCBs analysis in food. *Chemosphere,* **206**, 531-538 (2018).

62. Lambropoulou, D.A., Konstantinou, I.K., Albani, T.A., Coupling of headspace solid phase

_____

_____

microextraction with ultrasonic extraction for the determination of chlorinated pesticides in bird livers using gas chromatography. *Analytical Chemica Acta*. 673-574, 223-230 (2006).

63. Justino, C.I.L., Duarte, K.R., Freitas, A.C., Panteleitchouk, T.S.L., Duarte, A.C., Rocha-Santos, T.A.P., Contaminants in aquaculture: overview of analytical techniques for their determination. *TrAC Trends in Analytical Chemistry*, **80**, 293-310 (2016).

64. Żwir-Ferenc, A., Biziuk, M., An analysis of pesticides and polychlorinated biphenyls in biological samples and foods. *Critical Reviews in Analytical Chemistry,* **34**, 95-103 (2004).

65. Agustin, M.R.R., Park, H.M., Hong, S.M., Ryu, J.C., Lee, K.B., Determination of mono- to octachlorobiphenyls in fish oil using Florisil adsorption followed by headspace solid-phase microextraction and gas chromatography with time-of-flight mass spectrometric detection. *Journal of Chromatography A,* **1085**, 278-284 (2005).

66. Zheng, G., Han, C., Liu, Y., Wang, J., Zhu, M., Wang, C., Shen, Y., Multiresidue analysis of 30 organochlorine pesticides in milk and milk powder by gel permeation chromatography-solid phase extraction-gas chromatography-tandem mass spectrometry. *Journal of Dairy Science,* **97**, 6016-6026 (2014).

67. Pemberthy, D., Quintero, A., Martrat, M.G., Parera, J., Ábalos, M., Abad, E., Villa, A.L., Polychlorinated dibenzo-p-dioxins, dibenzofurans and dioxin-like PCBs in commercialized food products from Colombia. *Science of Total Environment,* **568**, 1185-1191 (2016).

68. Bernsmann, T., Albrecht, M., Fürst, P., Fast sample preparation for routine determination of PCDD/F, PCB and PBDE in food and feed. *Organohalogen Compound,* **76**, 1281-1284 (2014).

69. Wiberg, K., Sporring, S., Haglund, P., Björklund, E., Selective pressurized liquid extraction of polychlorinated dibenzo-p-dioxins, dibenzofurans and dioxin-like polychlorinated biphenyls from food and feed samples. *Journal of Chromatography A,* **1138**, 55-64 (2006).

70. Zhang, Z., Ohiozebau, E., Rhind, S.M., Simultaneous extraction and clean-up of polybrominated diphenyl ethers and polychlorinated biphenyls from sheep liver tissue by selective pressurized liquid extraction and analysis by gas chromatography-mass spectrometry. *Journal of Chromatography A,* **1218**, 1203-1209 (2011).

71. Subedi, B., Usenko, S., Enhanced pressurized liquid extraction technique capable of analyzing

polychlorodibenzo-p-dioxins, polychlorodibenzo furans, and polychlorobiphenyls in fish tissue. *Journal of Chromatography A,* **1238**, 30-37 (2012).

72. Oziolor, E.M., Apell, J.N., Winfield, Z.C., Back, J.A., Usenko, S., Matson, C.W., Polychlorinated biphenyl (PCB) contamination in Galveston Bay, Texas: Comparing concentrations and profiles in sediments, passive samplers, and fish. *Environmental Pollution*, **236**, 609-618 (2018).

73. Anastassiades, M., Lehotay, S.J., Stajnbaher, D., Schenck, F.J., Fast and easy multiresidue method employing MeCN extraction/partitioning and ''dispersive solid-phase extraction'' for the determination of pesticide residues in produce. *Journal of AOAC International*, **86**, 412-431 (2003).

74. González-Curbelo, M.Á., Socas-Rodríguez, B., Herrera-Herrera, A.V., González-Sálamo, J., Hernández-Borges, J., Rodríguez-Delgado, M.Á., Evolution and applications of the QuEChERS method. *Trends in Analytical Chemistry*, **71**, 169-185 (2015).

75. Chamkasem, N., Lee, S., Harmon, T., Analysis of 19 PCB congeners in catfish tissue using a modified QuEChERS method with GC-MS/MS. *Food Chemistry*, **192**, 900-906 (2016).

76. Norli, H.R., Christiansen, A., Deribe, E., Application of QuEChERS method for extraction of selected persistent organic pollutants in fish tissue and analysis by gas chromatography mass spectrometry. *Journal of Chromatography A,* **1218**, 7234-7241 (2011).

77. Gómara, B., Herrero, L., Bordajandi, L.R.,́González, M.J., Quantitative analysis of polybrominated diphenylethers in adipose tissue, human serum and foodstuff samples by gas chromatography with ion traptandem mass spectrometry and isotope dilution. *Rapid Communications in Mass Spectrometry,* **20**, 69-74 (2006).

78. Van Leeuwen, S.P.J., de Boer, J., Advances in the gas chromatographic determination of persistent organic pollutants in the aquatic environment. *Journal of Chromatography A*, **1186**, 161-182 (2008).

79. Focant, J-F., Pirard, C., Eppe, G., De Pauw, E., Recent advances in mass spectrometric measurement of dioxins. *Journal of Chromatography A,* **1067**, 265-275 (2005).

80. Focant, J.F., Eppe, G., De Pauw, E. Optimisation and use of tandem-in-time mass spectrometry in comparison with immunoassay and HRGC/HRMS for PCDD/F screening. *Chemosphere,* **43**(4-7), 417-24 (2001).

81. Focant, J.F., Reiner, E.J., Macpherson, K., Kolic, T., Sjödin, A., Patterson, D.G.Jr., Reese,

_____

_____

S.L., Dorman, F.L., Cochran, J., Measurement of PCDDs, PCDFs, and non-ortho-PCBs by comprehensive two-dimensional gas chromatography-isotope dilution time-of-flight mass spectrometry (GC x GC-IDTOFMS). *Talanta,* 63, 1231-40 (2004).

82. Korytár, P., Haglund, P., Boer, J.de., Brinkman, U.A.Th., Comprehensive two-dimensional gas chromatography for the analysis of organohalogenated micro-contaminants. *TrAC Trends in Analytical Chemistry*, 25, 373-396 (2006).

83. Hoh, E., Mastovska, K., Lehotay, S.J., Optimization of separation and detection conditions for comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry analysis of polychlorinated dibenzo-p-dioxins and dibenzofurans. *Journal of Chromatography A*, 1145, 210-221 (2007).

84. Xia, D., Gao, L., Zheng, M., Wang, S., Simultaneous analysis of polychlorinated biphenyls and polychlorinated naphthalenes by isotope dilution comprehensive two dimensional gas chromatography high-resolution time-of-flight mass spectrometry. *Analytica Chimica Acta,* **937**, 160-167 (2016).

85. Fürst, P., Bernsmann, T., Baumeister, D., Optimization of GC-MS/MS for the Determination of Dioxins and PCBs in Feed and Food and Comparison of Results with GC-HRMS in: The Handbook of Environmental Chemistry, Dioxin and Related Compounds. (Ed) Mehran Alaee. ISSN 1867-979X, Springer, Switzerland, Volume 49, PP: 95-120 (2016).

86. Fürst, P., Bernsmann, T., Baumeister, D., Sandy, C., Determination of polychlorinated dibenzo-p-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) in foodstuffs and animal feed using the Agilent 7000 Triple Quadrupole GC/MS System. Application Note, 5990-6594 EN (2010).

87. Fürst, P., Bernsmann, T., Baumeister, D., Sandy, C., Determination of dioxin-like and nondioxin-like polychlorinated biphenyl congeners in foodstuffs and animal feed using the Agilent 7000 Triple Quadrupole GC/MS System. Application Note, 5990-6950 EN (2011).

88. García-Bermejo, A., Ábalos, M., Sauló, J., Abad, E., González, M.J., Gómara, B., Feasibility of triple-quadrupole GC-MS/MS as an alternative technique to GC-HRMS for the analysis of PCDD/Fs and DL-PCBs in food and feedstuff samples. *Organohalogen Compound,* **73**, 653-656 (2011).

89. Sandy, C., Fürst, P., Bernsmann, T., Baumeister, D., Determination of polychlorinated biphenyl congeners in foodstuffs and animal feed using a Triple Quadrupole GC-MS/MS Instrument. *Organohalogen Compound,* **73**, 1370-1371 (2011).

90. Ingelido, A.M., Brambilla, G., Abballe, A., di Domenico, A., Fulgenzi, A.R., Iacovella, N., Iamiceli, A.L., Valentini, S., De Felip, E., PCDD, PCDF, and DL-PCB analysis in food: performance evaluation of the high-resolution gas chromatography/low-resolution tandem mass spectrometry technique using consensus-based samples. *Rapid Communications in Mass Spectrometry,* **15**, 236-242 (2012).

91. Leondiadis, L., Zafeiraki, E., Vassiliadou, I., Costopoulou, D., Comparison of GC-MS/MS and GC-HRMS results for the determination of PCDD/Fs in food and feed samples. *Organohalogen Compound,* **74**, 153-155 (2012).

92. L'Homme, B., Scholl, G., Eppe, G., Focant, J.F., Validation of a gas chromatography-triple quadrupole mass spectrometry method for confirmatory analysis of dioxins and dioxin-like polychlorobiphenyls in feed following new EU Regulation 709/2014. *Journal of Chromatography A,* **1376**, 149-58 (2015).

93. Focant, J.-F., Eppe, G., Pirard, C., Massart, A.-C., André, J.-E., De Pauw, E., Levels and congener distributions of PCDDs, PCDFs and non-ortho PCBs in Belgian foodstuffs Assessment of dietary intake. *Chemosphere,* **48**, 167-179 (2002).

94. Khaled, A., El Nemr, A., Said, T.O., El-Sikaily, A., Abd-Alla, A.M.A., Polychlorinated biphenyls and chlorinated pesticides in mussels from the Egyptian Red Sea coast. *Chemosphere,* **54**, 1407-1412 (2004).

95. Imaeda, D., Kunisue, T., Ochi, Y., Iwata, H., Tsydenova, O., Takahashi, S., Amano, M., Petrov, E.A., Batoev, V.B., Tanabe, S., Accumulation features and temporal trends of PCDDs, PCDFs and PCBs in Baikal seals (Pusa sibirica). *Environmental Pollution,* **157**, 737-747 (2009).

96. Schecter, A., Colacino, J., Haffner, D., Patel, K., Opel, M., Päpke, O., Birnbaum, L., Perfluorinated Compounds, Polychlorinated Biphenyls, and Organochlorine Pesticide Contamination in Composite Food Samples from Dallas, Texas, USA. *Environmental Health Perspectives*, **118**, 796-802 (2010).

97. Miklavčič, A., Stibilj, V., Heath, E., Polak, T., Tratnik, J.S., Klavž, J., Mazej, D., Horvat, M., Mercury, selenium, PCBs and fatty acids in fresh and canned fish available on the Slovenian market. *Journal of Food Chemistry,* **124**, 711-720 (2011).

98. Shen, H., Guan, R., Ding, G., Chen, Q., Lou, X., Chen, Z., Zhang, L., Xing, M., Han, J., Wu, Y., Polychlorinated dibenzo-p-dioxins/furans

_____

_____

(PCDD/Fs) and polychlorinated biphenyls (PCBs) in Zhejiang foods (2006−2015): Market basket and polluted areas. *Science of Total Environment,* **574**, 120-127 (2017).

99. Sun, H., Wang, P., Li, H., Li, Y., Zheng, S., Matsiko, J., Hao, Y., Zhang, W., Wang, D., Zhang, Q., Determination of PCDD/Fs and dioxin-like PCBs in food and feed using gas chromatography-triple quadrupole mass spectrometry. *Science China Chemistry*, **60**, 670-677 (2017).

100. Ferrante, M.C., Fusco, G., Monnolo, A., Saggiomo, F., Guccione, J., Mercogliano, R., Clausi, M.T., Food contamination by PCBs and waste disposal crisis: Evidence from goat milk in Campania (Italy). *Chemosphere*, **186**, 396-404 (2017).

101. Pajurek, M., Pietron, W., Maszewski, S., Mikolajczyk, S., Piskorska-Pliszczynska, J. Poultry eggs as a source of PCDD/Fs, PCBs, PBDEs and PBDD/Fs. *Chemosphere*, **223**, 651-658 (2019).

_____