Chapter 15

Halogenated Persistent Organic Pollutants and Polycyclic Aromatic Hydrocarbons in Food

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Abstract

During recent years, mass spectrometry (MS) and hyphenated chromatographic instrumentation and techniques have been a subject of dramatic developments, resulting in the introduction of various useful tools for the analysis of halogenated persistent organic pollutants (POPs) and polycyclic aromatic hydrocarbons (PAHs) in food and environmental matrices. This chapter describes state-of-the-art in the field of MS as a primary detection tool for the halogenated POPs and PAHs previously separated using either gas chromatography (GC) or liquid chromatography (LC). Since sample preparation practice plays a crucial role for obtaining optimal performance characteristics of a particular analytical method, a brief overview of sample extraction and clean-up procedures in the POPs/PAHs analysis is also briefly outlined.

Key words: Persistent organic pollutants, Polycyclic aromatic hydrocarbons, Food, Mass spectrometry, Gas chromatography, Liquid chromatography, Sample preparation

1. Introduction

Persistent organic pollutants (POPs) represent chemicals with long half-lives in all compartments of the environment including biota. Based on the Stockholm convention on POPs (last up-date in May 2009), the following groups of compounds are of main interest (Table 1) (1):

- Organochlorine pesticides (OCPs)
- Polychlorinated biphenyls (PCBs)
- Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)
- Brominated flame retardants (BFRs) including hexabromobiphenyl (HBB), tetra-, penta-, hexa-, and heptabromodiphenyl ethers (a group of compounds commonly known as polybromodiphenyl ethers, PBDEs)

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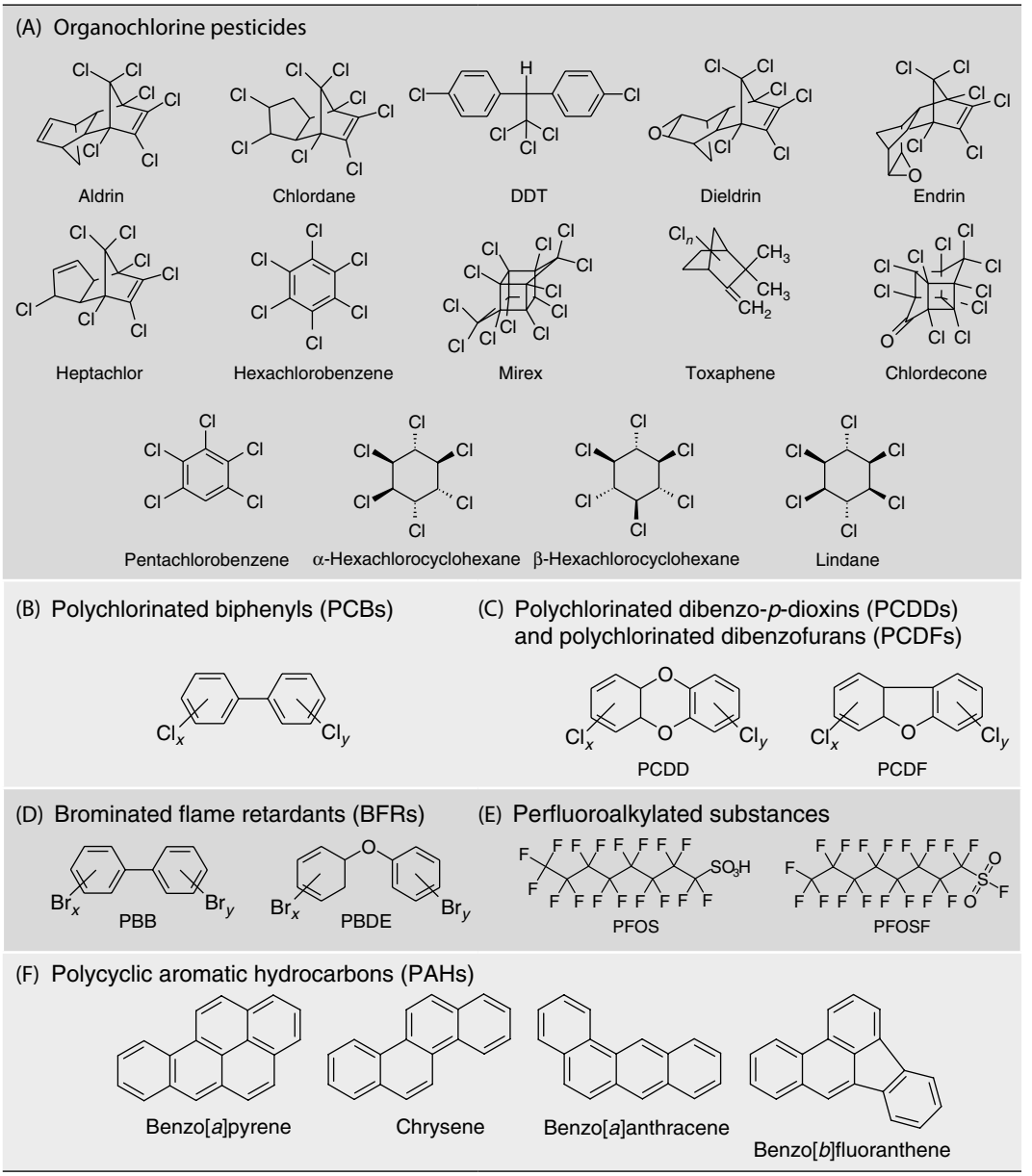
Table 1
An overview of POPs (based on the Stockholm convention, 2009) and a group of so-called "PAH4" (EFSA, 2008) (1, 2)



- Perfluoroalkylated substances represented by perfluorooctane sulfonic acid (PFOS), its salts and perfluorooctane sulfonyl fluoride (PFOSF)

While the group of OCPs, PCBs, PCDDs/PCDFs, and BFRs is accumulated mainly in lipid tissue (2), the perfluoroalkylated substances are, on the other hand, bound to proteins (3).

In addition to these halogenated POPs, polycyclic aromatic hydrocarbons (PAHs) are often included in the monitoring programs as indicators of environmental pollution. Although PAHs do not meet POPs definition (their bioaccumulative potential, due to a relatively rapid metabolism in biota, is low), they are also included in this chapter under this term. With regards to typical physicochemical properties, analytical strategies applicable for their determination are similar to those employed for POPs.

During recent years, several analytical approaches have been successfully developed not only for the "older" POPs such as OCPs, PCBs, PCDDs/PCDFs, and PAHs, but also for "emerging" contaminants such as BFRs and perfluoroalkylated substances. The determination of these analytes in complex matrices represents a challenging task since the concentrations in food samples are typically at ultra-trace levels, thus, requiring advanced analytical strategies for their accurate determination.

In practice, the methods used for the analysis of POPs/PAHs in food typically consist of the following basic steps: (1) *sampling and homogenisation*; (2) isolation of target analytes from a representative sample (*extraction step*); (3) separation of POPs/PAHs from bulk co-extracted matrix components (*clean-up step*); in this step also further *fractionation* of some groups of POPs might be required (typically to enable pre-concentration of minor analytes); (4) *separation* of the compounds of interest employing relevant chromatographic technique; (5) *identification and quantification* – nowadays mainly using mass spectrometric techniques. If the need is important enough, this is followed by (6) *confirmation* of results by an additional analysis. In the following sections, an overview of current state-of-the-art in the field of POPs/PAHs analysis in food will be presented with the attention to the applications employing mass spectrometry.

Although not discussed in this chapter, effect-based bio-analysis methods employing transcriptomics, proteomics, and biosensor-based technologies are of growing use as an efficient tool for hazard screening. For instance, CALUX (Chemically-Activated Luciferase eXpression) bioassay represents a very popular tool for rapid and easy screening of dioxins and dioxin-like PCBs (4).

2. Extraction

In general, extraction techniques rely on a favourable partition of POPs/PAHs from the sample matrix into the extraction matrix. However, the extraction procedures are typically not selective enough for the isolation of POPs/PAHs from complex food matrices, thus, additional clean-up and further fractionation steps are included in respective analytical procedures. In most cases, the samples are homogenised with sodium sulphate or

other desiccant (e.g. hydromatrix, magnesium sulphate) causing rupture of cell walls and binding water present in the sample. The flowing powder is then extracted (in some cases after overnight drying) with a suitable solvent or their mixture. The principles and the use of the most extraction techniques are summarised bellow and in Table 2 (5–8).

Liquid–liquid extraction (LLE) is applicable only to liquid matrices (e.g. milk, oils). In most cases, LLE uses about 100 mL of solvent per 5–50 g of sample. The major drawbacks of this technique are low sample throughput resulting from the need for manual concentration step, and using of large amounts of organic solvents. As far as stable emulsions are formed, centrifugation is needed to assist the separation of phases.

Solid-phase extraction (SPE) represents a feasible alternative for isolation/pre-concentration of POPs from aqueous and other liquid samples. Non-polar analytes are adsorbed by stationary phase such as octadecyl silica (C_{18}) in an extraction cartridge or disc. This approach is more advantageous compared to LLE because of reduced sample preparation time, decreased solvent usage, and improved sensitivity. SPE-based sample processing can be fully automated; and a wide range of SPE extractors are available in the market. However, problems such as clogging of the SPE cartridge can be encountered when solid particles are dispersed in the sample.

Soxhlet extraction represents the most frequently used extraction technique for isolation of lipophilic POPs from solid low moisture matrices or flowing powder obtained by desiccation of a sample (1–100 g). In common practice, extraction with polar and non-polar organic solvents such as dichloromethane, hexane–acetone, hexane–dichloromethane takes 4–18 h. To increase sample throughput, semi-automated extractor batteries are employed. Due to co-extraction of lipids and other sample components, "dirty" extracts obtained by this liquid–solid extraction, need subsequent extensive clean-up.

Accelerated solvent extraction (ASE) also known as *pressurised solvent extraction* (PLE) uses organic solvent/solvent mixtures at increased pressure during the extraction. This allows to keep the solvent(s) in liquid phase even at higher temperatures. Higher speed extraction of POPs/PAHs under these conditions is a result of their increased solubilities, better desorptions, and enhanced diffusion. The PLE system consists from a stainless-steel extraction cell, where temperature and pressure are controlled by electronic heaters and pumps. Extraction steps in the static mode involve: (1) loading the sample into the extraction cell; (2) filling the cell with an organic solvent; (3) heating and pressuring the cell to adjusted values; (4) transfer of the extract to the collection bottle and rinsing the sample with an additional solvent; and (5) purging the remaining solvent from the sample to the collection bottle using a suitable gas. Compared to Soxhlet extraction, only minutes

Table 2
Characterisation of extraction techniques employed in food analysis

	Extraction technique				
	Soxhlet	**Sonication**	**PLE**	**MAE**	**SFE**
Extraction time	3–48 h	10–60 min	5–30 min	3–30 min	10–60 min
Sample size	1–30 g	1–30 g	1–30 g	1–10 g	1–5 g
Solvent demands	100–500 mL	30–200 mL	10–100 mL	10–40 mL	2–5 mL (solid trap); 5–20 mL (liquid trap)
Investment	Low	Low	High	Moderate	High
Advantages	No filtration required	Multiple extraction	Fast extractions; low solvent volumes; elevated temperatures; no filtration required; automated systems	Fast and multiple extractions; low solvent volumes; elevated temperatures	Minimal solvent volumes; elevated temperatures; relatively selective towards matrix interferences; no clean-up or filtration needed; concentrated extracts; automated systems
Limitations	Long extraction times; large solvent volumes; clean-up step needed	Large solvent volumes; repeated extractions may be required; clean-up step needed	Clean-up step needed	Extraction solvent must be able to absorb microwaves; clean-up step needed; waiting time for the vessels to cool down	Many parameters to optimise, especially analyte collection

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are needed for the automated extraction process, but sample clean-up is still necessary, unless sorbents such as florisil, aluminium oxide are employed as fat, pigments, or other components retainers. To achieve good selectivity of the POPs/PAHs isolation, careful tuning of extraction conditions is necessary in such a case.

Ultrasonic extraction is a simple extraction technique, in which the sample is suspended in an organic solvent in a vessel and placed in an ultrasonic bath. The main parameters influencing the extraction efficiency are the polarity of the solvent, the homogeneity of the matrix, and the ultrasonication time. After extraction, the mixture of the sample and organic solvent is separated by filtration and rinsing with the solvent. Although this extraction procedure does not require expensive instruments and is not laborious, large consumption of the solvent (30–200 mL per 1–30 g of sample) is the main drawback of this technique.

Matrix solid-phase dispersion (MSPD) allows extraction of various POPs/PAHs from homogenously dispersed food samples with a sorbent phase (e.g. C_{18} silica). The homogenised sample is placed in a glass-syringe-barrel column and the POPs/PAHs are selectively eluted with suitable organic solvent (e.g. hexane), followed by the immediate instrumental analysis since the sample extraction and clean-up are conducted in one step. Compared to "conventional" extraction procedures, this technique requires a smaller sample size, has a shorter analysis time, and uses less organic solvent.

Supercritical fluid extraction (SFE) offers short sample processing times and use of a cheap environment-friendly extraction agent. In SFE, the sample is loaded in a high-pressure vessel and extracted with low viscosity supercritical fluid (in most cases carbon dioxide at pressures of 150–450 bar and temperatures of 40–150°C). The analytes are collected in a small volume of solvent or onto a solid-phase trap, from which they are rinsed with organic solvent in a subsequent step. Fat retainers (e.g. basic alumina, neutral alumina, florisil, and/or silica) can be introduced into the extraction thimble to obtain a fat-free extract. The use of SFE in POPs analysis has partially vanished during recent years because of operation problems such as a need to optimise many parameters, problems for matrices with high water content, and the high cost of automated instrumentation.

Microwave-assisted extraction (MAE) allows rapid extraction of POPs/PAHs from solid matrices by employing microwave energy as a source of heat. The partitioning of the analytes from the sample to the extractant depends upon the temperature and the nature of the extractant. Since the microwave device heats the entire sample simultaneously without heating the vessel, the solution reaches its boiling point rapidly, leading to a very short extraction time. The attraction of this technique includes also somewhat easy optimisation and it is cheaper than other modern extraction techniques (SFE, PLE).

Solid-phase microextraction (SPME) represents solvent-free isolation/pre-concentration technique employing a fused-silica fibre that is coated with an appropriate stationary phase. Analytes present in the sample are directly extracted (from the headspace or by direct immersion) and concentrated onto the fibre coating. The SPME sampling procedure is then followed by the transfer of pre-concentrated analytes into the chromatographic system using either a GC injector (thermal desorption) or an SPME–HPLC interface (desorption by the solvent). The main features of SPME include unattended operation via robotics (if a fully automated option is available) and in the case of GC-amenable analytes, elimination of maintenance of the liner and column (contamination by non-volatiles does not occur). This sample extraction technique, however, is susceptible to strong matrix effects, which can produce complications in quantification. In addition, variability of limits of detection for different analytes depends on the equilibrium between the coating material and the matrix.

Stir-bar sorptive extraction (SBDE) can be used as an effective tool for sample enrichment in aqueous solutions. A glass-lined magnetic bar is covered with a thick layer of sorbent (similar to that in SPME). By magnetically stirring the bar in the sample solution, the analytes are enriched in the sorbent phase. After this pre-concentration, the compounds are thermally desorbed from the bar with GC–MS.

In addition to these extraction techniques, sample preparation can be simplified by using a single-step organic solvent extraction and salting out effect to enhance liquid–liquid partitioning from water in the sample. This strategy is the main concept of the *QuEChERS* (Quick, Easy, Cheap, Effective, Rugged, and Safe) sample extraction method (9). During the development of this method, great emphasis was placed on streamlining this sample preparation procedure whenever possible by simplifying or omitting impractical, laborious, and time-consuming steps. The "original" QuEChERS method involves initial extraction with acetonitrile (MeCN), liquid–liquid partitioning after addition of a mixture of anhydrous $MgSO_4$ and NaCl, which reduces some polar matrix components, followed by a simple clean-up step in which the extract is mixed with primary secondary amine (PSA) sorbent and anhydrous $MgSO_4$ (dispersive-SPE). After these steps the extract is ready for GC–MS and LC–MS (directly or after dilution with water containing formic acid). The QuEChERS concept has been successfully used in the analysis of various POPs in food in its original version, or after some modifications such as change of extraction solvent (MeCN → MeCN containing acetic acid, ethyl acetate, methanol), the amount and kind of salts (NaCl → sodium acetate). While the use of MeCN or ethyl acetate is suitable for POPs such as OCPs, PCBs, and PBDEs, methanol is preferred as an extraction solvent for perfluoroalkylated substances.

3. Clean-Up

As mentioned earlier, not only are the target POPs/PAHs isolated during the extraction from the sample, but also various matrix components are co-extracted and may lead to worsened method performance. Although in some cases little or no clean-up is needed, the impact of matrix effects (caused mainly by the matrix co-extracts) such as inaccurate quantification, decreased method ruggedness, poor analyte detectability, and even reporting of false positive or negative results have to be considered. Therefore, some clean-up step is typically involved in the POPs/PAHs analysis (Table 3) (5, 8–10).

Gel permeation chromatography (GPC), sometimes referred as *size exclusion chromatography* (SEC), represents a non-destructive clean-up procedure. In most cases, spherical porous styrene–divinylbenzene copolymers (commercially available as soft Bio-Beads S-X3, or rigid types e.g. PL-gel) are used for separation of lipids (>500 Da), which are the first eluting compounds from the column, followed by the smaller molecules, including the POPs. However, size of the molecules is not the only separation mechanism in this particular case since π–π interactions of this copolymer with planar compounds may cause different elution order not reflecting the "size rule." Dichloromethane, chloroform, or mixtures of dichloromethane–hexane or ethyl acetate–cyclohexane are the most often applied eluents. The GPC can be fully automated and, contrary to adsorption chromatography, it is more suitable for the isolation of "unknown" contaminants. This method can handle a relatively large amount of lipids (up to 500 mg). However, in some cases, the use of a second GPC elution or other clean-up techniques is needed to remove all lipids. In addition, this technique does not separate individual groups of POPs, thus, follow-up fractionation, if needed, is employed to obtain different classes of POPs.

Adsorption column chromatography involves passing the extracts though adsorbent columns. Various sorbents such as alumina, silica, and florisil, available in different mesh sizes, levels of activity and column size, either separately or in combination, were successfully evaluated for this purpose to reduce sample handling and analysis time. Alumina columns have a fat capacity of ~250 mg per 10–20 g, which may not be enough in ultra-trace analysis required to remove large quantities of lipids. With regards to silica gel, it allows fractionation of the extract according to the polarity of different classes of POPs.

Dispersive solid-phase extraction (d-SPE) is a very simple clean-up procedure where suitable sorbent (primary-secondary amine – PSA, C_{18} silica, or activated charcoal) is added to an extract aliquot. After mixing and centrifugation, the extract is used for subsequent

Table 3
Characterisation of lipid removal techniques

Technique	Requirement					
	Lipid removal	Destruction of compounds	Recovery	Amount of time spent	Amount of work invested	Automation
GPC	Two steps necessary	None observed	Good	High	High	Easy
Florisil, silica, alumina	Few lipids removed or large amount of absorbent used	None observed	Good	Fair	Fair	Difficult
Sulphuric acid (and/or sulphuric acid/silica)	Good	Some OCPs	Good	Low	Fair	Difficult
Saponification	Good	Some OCPs, PCBs with fully chlorinated aryl-ring	Fair	Low	Fair	Difficult

Source: From (5) with permission

analysis employing either GC or LC. The d-SPE step reduces the amount of common matrix co-extractives typical for foods, particularly fatty acids supposing PSA is employed. As far as C_{18} silica is employed, lower recoveries of some OCPs might occur. Regarding charcoal, this non-specific sorbent was shown to be very suitable in analysis of PFOS and related perfluorinated compounds.

Destructive lipid removal includes either alkaline treatment (saponification), or oxidative dehydration by sulphuric acid treatment. In the later case, mineralisation of lipids and other bulky matrix components is realised either by direct addition of concentrated acid to the extract or by passing the crude extract through impregnated silica columns, and are the most commonly used lipid removal destructive methods. It has been shown that PBBs, PBDEs, and PCBs are stable under strong acid conditions. Basic conditions of saponification are critical as too high temperatures and too long of process time may cause degradation of highly brominated PBDEs, PBBs, and PCBs. Also, silica gel impregnated with alcoholic KOH or of a multilayer column with neutral silica, acidified silica, and basic silica can be employed.

4. Determination of POPs/PAHs by GC–MS and LC–MS

For the analysis of trace levels of lipophilic POPs (OCPs, PCBs, PCDDs/PCFDs, and BFRs), and PAHs occurring in complex matrices such as foodstuffs, high-resolution gas chromatography (GC) interfaced to mass spectrometry employing a suitable single or tandem mass analyser represents the key separation/detection technique (2, 8). For those POPs, which are either not amenable to GC due to their ionic nature (perfluoroalkylated acids) or their diastereomers are interconverted in a hot GC injector, moreover, poorly separated on conventional GC columns (e.g. HBCD), LC–MS is the method of choice for their analysis (8, 11).

Regardless of the continuously improving detection capabilities of modern GC–MS or LC–MS systems (discussed below), the sample preparation practice remains a crucial role in obtaining required performance characteristics of a particular analytical procedure (mainly limits of quantification and uncertainty of measurement might be adversely affected by sample matrix).

During the last few years, a large number of novel ambient desorption ionisation techniques, such as desorption electrospray ionisation (DESI), atmospheric-pressure solids analysis probe (ASAP), direct analysis in real time (DART), and many others have become available. Their main advantages compared to conventional techniques involve the possibility of direct sample examination in the open atmosphere, minimal or no sample preparation requirements, and, remarkably high sample throughput (chromatographic separation is not involved in this particular case) (12).

4.1. Sample Injection in GC–MS

Several of existing GC inlet systems are applicable for trace analysis of POPs/PAHs in complex food matrices; the most common being a "hot" splitless injector, a programmed temperature vaporiser (PTV), and, in the last decade, also a direct sample introduction/difficult matrix introduction injector (DSI/DMI). The choice of an optimum injection strategy depends on many factors including the concentration range of target analytes, their physicochemical properties, and, to a significant extent, on the amount and nature of matrix co-extracts present in the food sample extract (8, 13–15).

Hot *splitless injection* (250–300°C) has been in use in many laboratories concerned with routine trace analysis of POPs/PAHs. Depending on the type of injector liner and expansion volume of sample solvent, the volumes introduced onto the GC capillary are typically in the range of 1–3 μL with 0.5–2 min of splitless period. However, this inlet suffers from the potential thermal degradation (e.g. *p*,*p*′-DDT → *p*,*p*′-DDD and/or *p*,*p*′-DDE; BDE-209 → nona-BDE congeners), rearrangement (HBCD), and/or adsorption of susceptible analytes.

To overcome, or at least partly compensate for these problems, *pulsed splitless injection* can be applied. Increased column head pressure for a short time period during the sample injection splitless period (usually 1–2 min) leads to a higher carrier gas flow rate through the injector (8–9 mL/min vs. 0.5–1 mL/min during classical splitless injection), thus faster transport of sample vapours onto the GC column. In this way, the residence time of analytes, and, consequently, their interaction with active sites in the GC inlet, is fairly reduced. In addition, the detection limits can be lowered by injection of higher sample volumes (for most liners up to 5 μL) without any risk of backflash. For splitless injections >1–2 μL, a retention gap prior to the analytical column is strongly recommended to avoid excessive contamination of front part of separation column, and consequent peaks distortion.

A *programmable temperature vaporisation* (PTV) *injector* represents the most versatile GC inlet offering significant reduction of most problems typically encountered when using hot vaporising devices (splitless inlets) in trace POPs/PAHs analysis. The most important fact is that a PTV injector chamber is cool at the moment of injection. A rapid temperature increase, following withdrawal of the syringe from the inlet, allows efficient transfer of the volatile analytes onto the GC column while leaving behind non-volatiles in the injection liner. With regard to these operational features, PTV is ideally suited for thermally labile analytes and analytes representing a wide boiling range. PTV enables introduction of large sample volumes (up to hundreds of microlitres) into the GC system. This feature makes the use of PTV for POPs/PAHs analysis particularly attractive and also enables its on-line coupling with various enrichment and/or clean-up techniques such as automated solid phase extraction (SPE) approaches. From a practical point of

view, PTV is compatible with any capillary GC column diameter including microbore columns. However, to attain its optimal performance in a particular application, many parameters have to be optimised (e.g. initial and final injector temperature, inlet heating rate, venting time, flow and pressure, transfer time, injection volume, type of liner). Due to the inherent complexity of this inlet, method development might become on some occasions a rather demanding task. Despite this, the use of PTV in food analysis is becoming a gold standard.

Direct sample introduction (DSI) and its fully automated version, *difficult matrix introduction* (DMI), represent a relatively novel large volume injection (LVI) technique. The DSI approach involves adding up to 30 μL of the extract to a microvial that is placed in an adapted GC liner. After evaporating and venting sample solvent at a relatively low temperature, the injector is ballistically heated to transfer analytes at the front of a relatively cold GC column (some matrix components with similar volatility range can be pre-concentrated here). In the next phase, the column undergoes normal temperature programming to separate volatilised compounds. Then, during the cooling period, the microvial containing residues of non-volatile matrix components is removed and discarded. In the commercial DMI system, the entire liner along with the microvial is replaced after each injection. In this way, time-consuming and expensive purification step can be omitted or significantly reduced for some matrices. Since the bulk (semi)volatile matrix components introduced from the sample into the injector may influence the quantitative aspects of the injection process and/or interfere in analytes detection, instruments with MS analysers (single or tandem) providing more accurate results should be preferably used. Regardless of the sample preparation strategy, reduced demands for the GC system maintenance represents a positive feature of this technique.

4.2. Sample Separation Using GC and LC

With regard to a (typically) complex mixture of matrix components occurring in food extracts (in many cases even after purification) in fairly higher amounts as compared to concentrations of isolated toxicants, the optimisation of GC and LC separation requires careful attention to a number of important variables and their interaction. Physical (length, internal diameter, and stationary phase), parametric (temperature and flow velocity) column variables, and mobile phase composition and its additives affect the separation process. The nature of functional groups as well as the percentage of substitution of those functionalities govern the stationary phase–analytes–interferences interactions thus influencing their retentions. The choice of the separation system is closely associated with the selectivity/specificity of the detection system employed in a particular analysis. For instance, poor resolution of critical pairs (analyte–analyte or analyte–interference) by

chromatography might be compensated by the resolving power of the respective mass spectrometric detector.

Organochlorine pesticides. A conventional approach to GC analysis of OCPs employs capillary columns with low-bleed stationary phases mostly consisting of (5%-phenyl)-methylpolysiloxane (or cyanopropyl, cyanopropylphenyl or increased phenyl content up to 50%). Relatively long analytical column 30–60 m of 0.25–0.32 mm inner diameters are commonly used in routine practice, with GC analysis time approaching 1 h. However, the growing number of required analyses (not only organochlorine but also other pesticides) leads to requiring decreased analysis time using fast GC techniques (mostly in combination with MS detection), thus increasing sample throughput and reducing the laboratory operating costs (16, 17). As an example, Fig. 1 illustrates the

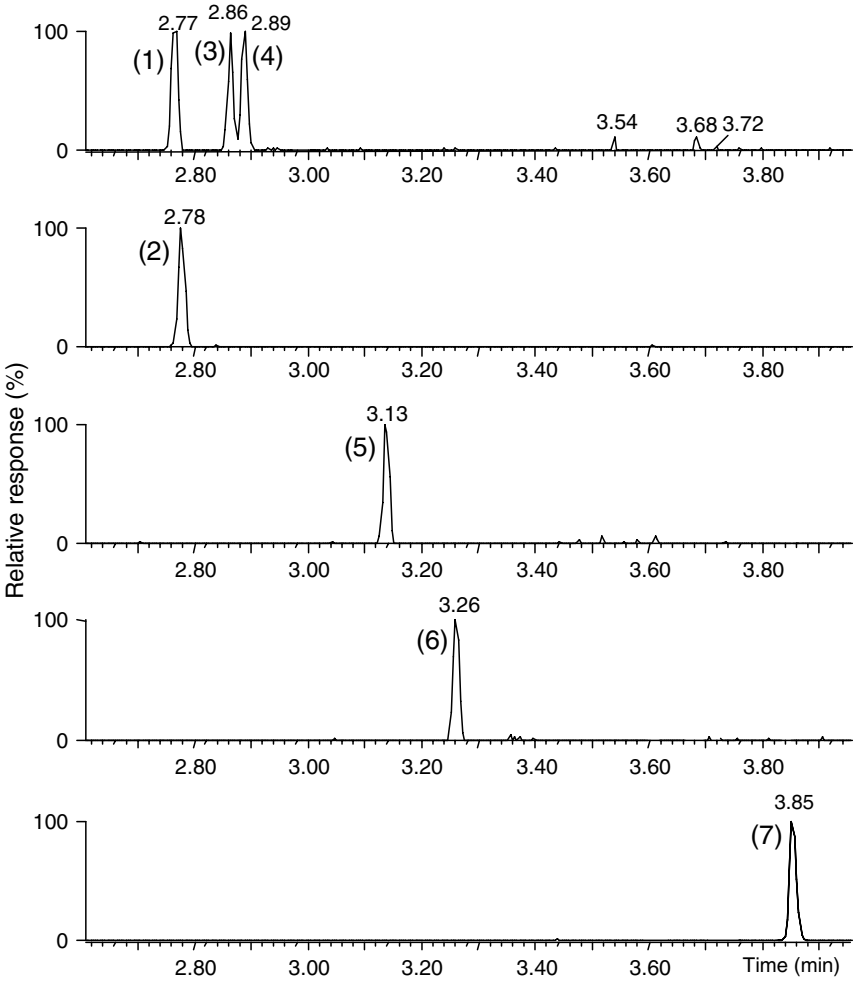

Fig. 1. PTV–LPGC–EI-HRTOFMS chromatogram of selected OCPs at a concentration of 0.01 mg/kg fish oil extract. The target ions were extracted using a 0.02 Da mass window. (1) α-HCH (*m/z* 180.938), (2) HCB (*m/z* 283.810), (3) β-HCH (*m/z* 180.938), (4) γ-HCH (*m/z* 180.938), (5) heptachlor (*m/z* 271.810), (6) aldrin (*m/z* 262.852), (7) *p,p′*-DDT (*m/z* 235.008).

rapid analysis of selected OCPs in fish oil extract at a very low level (0.01 mg/kg). In this particular case, fast GC under the conditions of a high temperature programming (60°C/min) and vacuum conditions in a megabore GC column (10 m × 0.53 mm × 0.5 μm) coupled to a restriction capillary (3 m × 0.15 mm), so called low-pressure gas chromatography (LPGC), was used. Using this chromatographic set-up, the analysis is completed within 7 min, which reduces the GC run time.

Polychlorinated biphenyls. GC combined with specific detectors, either "conventional" electron-capture detector (ECD), or currently preferred MS, are routinely used in PCBs analysis. Since even high-resolution capillaries do not allow separation of all 209 congeners, either simultaneous separation on two parallel columns differing in polarity or comprehensive two-dimensional GC (GC × GC) separation and detection with electron-capture detector (ECD) or MS is the method of choice for routine analysis. Typically, non-polar columns such as 100%-methylpolysiloxane or (5%-phenyl)-methylpolysiloxane are employed for their separation. However, because of coelution of a number of congeners (critical pairs), alternative phases such as (50%-phenyl)-methylpolysiloxane, (8%-phenyl)-polycarboranesiloxane, or (14%-cyanopropyl-phenyl)-methylpolysiloxane have to be used (5, 17).

Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans. Because the concentrations of interest are at the parts per trillion (ppt) level, the analytical methods for PCDDs/PCDFs require laborious and time-consuming sample clean-up and preconcentration processes. Additionally, detection techniques of high selectivity and high sensitivity are required since samples often contain matrix components (potential chemical interferences) at concentrations several orders of magnitude higher than those of target analytes. One of the key factor, which makes the analysis of dioxins so difficult, is the existence of many congeners (i.e. 75 PCDDs, 135 PCDFs). Differences in toxicities (expressed as toxic equivalency factors, TEFs) of several orders of magnitude exist between various isomers (with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDD, being the most toxic), thus, the separation and reliable identification/quantification of each is a crucial task for the risk assessment. Since the monitoring of all PCDDs/PCDFs is hardly attainable, the legislation reduces the monitoring only of those compounds with the highest toxicological potential (19).

For GC separation, long narrow bore capillary columns are often used (30–60 m × 0.32–0.25 mm × 0.15–0.25 μm) with different stationary phases: (5%-phenyl)-methylpolysiloxane, (50%-cyanopropylphenyl)-dimethylpolysiloxane, or 44%-methyl–28%-phenyl–20%-cyanopropylpolysiloxane–8% Carbowax 20 M (DB-Dioxin) (2).

Brominated flame retardants. Although 209 BDE congeners are theoretically possible, only a small number of these contaminants can be found in the earlier produced technical PBDE mixtures (e.g. BDE 28, 47, 99,100, 153, 154, 183, 209), which allows using a single capillary GC column that offers sufficient resolution for a congener-specific PBDE determination. A non-polar or medium-polar column, e.g. 100%-methylpolysiloxane, (5%-phenyl)-dimethyl polysiloxane, 14%-cyanopropylphenyl–86%-dimethylpolysiloxane, with a length of 25–60 m and small diameters (<0.25 mm) are most frequently employed. The use of sufficiently long columns is important for achieving enough separation between BDE congeners and possible interferences supposing selectivity of detection is not sufficient (8, 10).

Special attention is usually paid to BDE 209, not only because of its susceptibility to thermo-degradation in the GC system, but also due to relatively very long retention times, thus reduced GC throughput. For that reason, the analysis of BDE 209 is sometimes carried out separately from the analysis of the other PBDEs. To overcome this problem, relatively short column enabling reduction of residence time of this congener, can be considered. Typically, non-polar columns, 100%-methylpolysiloxane, (5%-phenyl)-methylpolysiloxane, with a length of 10–15 m and 0.1–0.2 μm film thickness are utilised (8, 10). As an example, Fig. 2 shows a rapid separation of PBDEs (including the troublesome BDE 209 congener) under the conditions of LPGC with a high temperature programming (60°C/min). In this particular case, a combination of a restriction capillary (2.5 m × 0.15 mm) and megabore column (10 m × 0.53 mm × 0.5 μm) was employed for the separation.

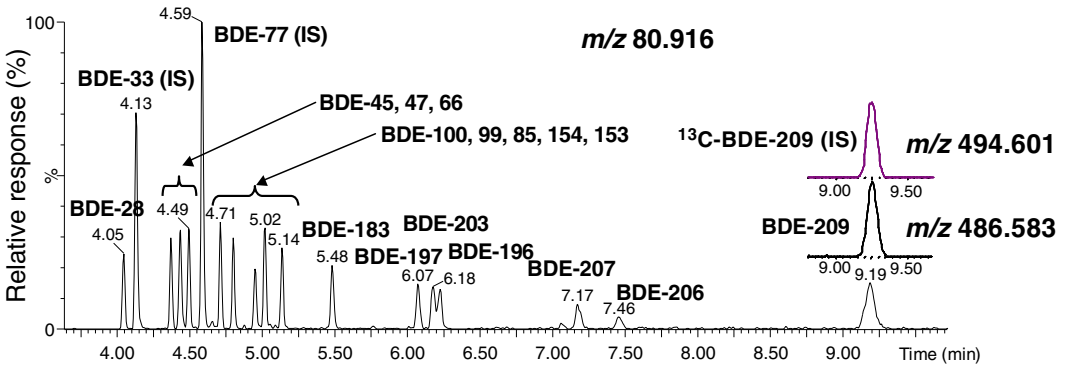

Fig. 2. LPGC–HRTOFMS chromatogram of standard solution of PBDEs detected in NCI mode (BDE-28, 47, 49, 66, 85, 99, 100, 153, 154, 183, 196, 197, 203 at a concentration of 0.5 ng/mL; BDE 206, 207, 209 at a concentration of 1 ng/mL; BDE 37 and 77 at a concentration of 5 ng/mL, and ^{13}C-BDE-209 at a concentration of 50 ng/mL; 1 μL injected). The target ions (bromine $[Br]^-$ with exception of BDE 209) were extracted using a narrow 0.05 Da mass window.

Besides PBDEs, signals of several other BFRs such as pentabromotoluene (PBT), pentabromoethylbenzene (PBEB), hexabromobenzene (HBB), bis(2,4,6-tribromophenoxy)ethane (BTBPE), octabromotrimethylphenylindane (OBIND), or decabromodiphenyl ethane (DBDPE), can be found in the chromatogram (the latter compounds elutes even after BDE 209) obtained at conditions relevant to those in Fig. 2. Determination of one of the most widely used BFR, hexabromocyclododecane (HBCD) can also be carried out using GC–MS with stationary phases similar to those typically used for PBDEs. However, such a GC set-up does not allow quantification of individual diastereomers of HBCD (α-, β-, and γ-HBCD, each having two enantiomers) since they are not separated using common GC stationary phases; moreover, they undergo interconversion at temperatures above 160°C. Contrary to GC, reversed-phase HPLC employing non-polar (C_{18}, C_{30}) or chiral columns for their separation represent a versatile tool for the isomer-specific determination of HBCD isomers (8, 10). With regards to tetrabromobisphenol A (TBBPA), acidification and derivatisation are required before the GC–MS analysis, while LC–MS allows its direct determination. For the separation, LC columns 50–250 mm × 2–4 mm × 3.5–5 μm are typically used with gradient elution employing a mixture of MeCN:H_2O, MeOH:H_2O, or MeCN:MeOH:H_2O. For optimised chromatographic separation and/or ionisation response, mobile phase additives such as formic acid, tris(hydroxymethyl) aminomethane and ammonium acetate, are often used. In the case of TBBPA, mobile phase MeOH:H_2O is preferred over MeCN:H_2O due to more stable detector baseline and thus lower LODs (8, 10).

Perfluoroalkylated substances. GC can be used for direct determination of fluorotelomer alcohols, perfluoroalkyl sulfonamidoethanols, and perfluoroalkyl sulfonamides, which have high vapour pressure. For their separation, narrow bore capillary columns (30 m × 0.25 mm × 0.20–0.25 μm) with polar stationary phases (e.g. polyethylene glycol) are employed (20). On the other hand, perfluoroalkyl sulfones, perfluorocarboxylic acids, are only amenable to LC analysis. In this case, e.g. LC columns 50–150 mm × 2–4 mm × 3.5–5 μm with C_{18} sorbent can be used with gradient elution employing MeOH:H_2O (with ammonium acetate as an additive) (11, 21).

Polycyclic aromatic hydrocarbons. For GC separation, long narrow bore capillary columns are often used (60–30 m × 0.25 mm × 0.25 μm) with stationary phases such as (5–50%-phenyl)-methylpolysiloxane (22). Separation of some isomeric PAHs (e.g. B[*a*]A/Chr; B[*b*]F/B[*j*]F/B[*k*]F; I[1,2,3-*cd*]P/DB[*ah*]A) might be a problem even on long non-polar capillaries. If comprehensive analysis of all 16 carcinogenic PAHs (2) is required, then "heavy," low volatile representatives, such as dibenzopyrenes, can be a limiting factor in GC

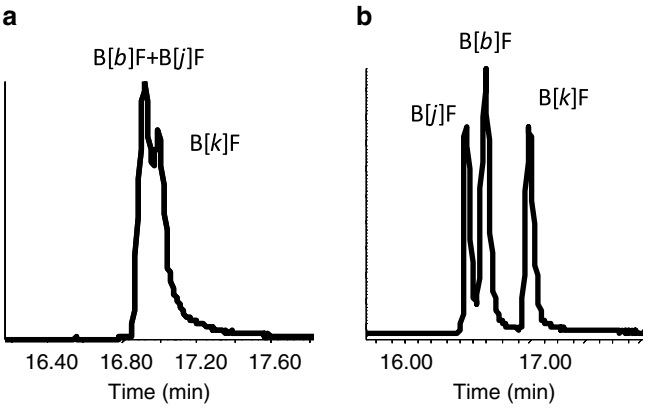

Fig. 3. GC separation of "critical" PAH isomers employing (**a**) (5%-phenyl)-methylpolysiloxane (30 m × 0.25 mm × 0.25 μm) and (**b**) 50% liquid crystalline–methylpolysiloxane (10 m × 0.18 mm × 0.1 μm). Quadrupole mass analyser used for detection (*m/z* 252 displayed).

throughput due to long retention times; moreover, band broadening might contribute to high detection limits. Under these conditions, low-bleed columns allowing programming up to high temperature limits are needed. If the analysis of these late eluting PAHs is not required, medium-polar stationary phases, 50% liquid crystalline–methylpolysiloxane stationary phase may provide a greater selectivity for some critical pairs as compared to non-polar low bleed stationary phases (Fig. 3). HPLC analysis of PAHs is very common, LC columns 100–250 mm × 2–4 mm × 3.5–5 μm with C_{18} sorbent (or some special sorbent developed for PAHs separation, e.g. LC-PAH, Chromspher 5PAH) can be used with gradient elution employing most often MeCN:H_2O (23).

4.2.1. Comprehensive Two-Dimensional GC (GC × GC)

The introduction of systems allowing continuous transfer of the entire sample from the first column to be separated on the second column with different selectivity has been made. Thanks to enormous increase of chromatographic resolution, significant improvements of both target and non-target screening of food components in a wide range of matrices is possible. This approach, called comprehensive two-dimensional gas chromatography (GC × GC), is introduced in the following sections in a greater detail (24, 25).

The heart of the GC × GC system is a modulator that connects the first dimension conventional-size column with a short microbore column in the second dimension.

There are three fundamental functions of this interface: (1) trapping of small adjacent fractions (typically 2–10 s) of the effluent from the first separation column; (2) refocusing these fractions (either in time or in space); and (3) injection of the refocused fractions as narrow pulses into the second-dimension column. The separation on the latter column is extremely fast and takes only 2–10 s vs. 20–120 min for the first dimension, and is, therefore, performed under essentially isothermal conditions.

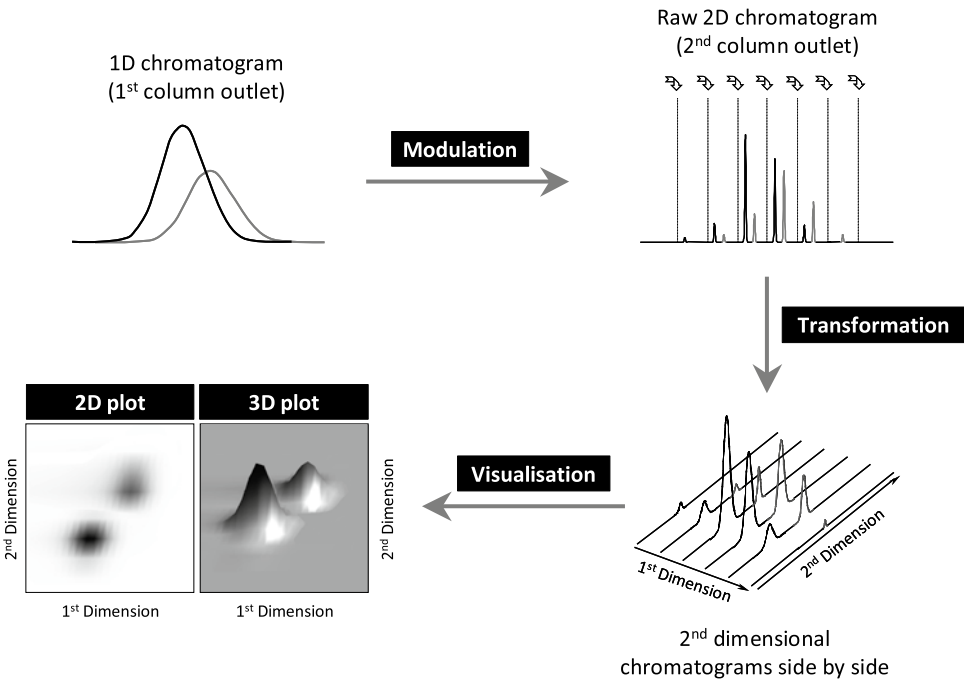

Fig. 4. Generation and visualisation of a GC × GC chromatogram.

A large series of high-speed chromatograms as the outcome of the transfer of chromatographic band from the first to the second dimension is generated during the GC × GC run. As shown in Fig. 4, these adjacent pulses are usually stacked side-by-side by special software to form a 2D chromatogram with one dimension representing the retention time on the first column (t_{R1}) and the other, the retention time on the second column (t_{R2}). The most convenient way to visualise GC × GC data is as contour plots representing the bird's eye view, where peaks are displayed as spots on a plane using colours and shading to indicate the signal intensity (Fig. 4).

Compared to conventional 1D-GC, the optimisation of GC × GC analysis requires rather more complex approach. The changes in operational parameters such as oven temperature or carrier gas flow rate have different impacts on the performance of separation columns since these differ both in their geometry and separation mechanism. Furthermore, new parameters such as modulation frequency and modulator temperature have to be optimised.

Conventional columns, typically 15–30 m length × 0.25–0.32 mm internal diameter × 0.1–1 μm film thickness, are used in the first dimension. This allows application of virtually all sample introduction techniques (split/splitless, on column, PTV, DMI/DSI). Stationary phases commonly used in first-dimension columns

are typically non-polar such as 100% dimethylpolysiloxane or (5% phenylene)-dimethylpolysiloxane on which the separation is governed mainly by analyte volatility (dispersive van der Waals forces play a key role). The size of columns for the second dimension is commonly in a range of 0.5–2 m length × 0.10–0.18 mm internal diameter × 0.10–0.18 μm film thickness. More polar stationary phases such as 35–50% phenylene–65–50% dimethylpolysiloxane, polyethylene glycol, carborane, and/or cyanopropyl–phenyl–dimethylpolysiloxane are often employed. Analytes interact with these medium-polar/polar phases via various mechanisms such as π–π interactions, hydrogen bonding etc., hence the requirement for different, independent separation principle is met. In most applications orthogonality is achieved using non-polar × polar separation mechanisms. However, for the analysis of POPs/PAHs, only highly stable stationary phases are typically used for their separation. This actually limits the use of stationary phases in most applications only to non-polar and medium-polar ones (Table 4).

Effective and robust modulation is a key process in the GC × GC analysis. In practice, a fixed modulation period, typically in a range of 1–10 s is employed during the analysis. Under ideal experimental conditions, the retention time of the most retained compound in the second dimension is shorter than the modulation time. If this is not the case, i.e. analytes do not elute in their modulation cycle, so called "wrap-around," which might cause some coelutions with less retained compounds from the following cycle. Avoiding this phenomenon can be achieved e.g. by an increase of the second dimension column temperature (if an independent oven is available) thus reducing the second dimension retention time. In any case, optimal function of modulator is essential for the quality of the separation and detection process.

The fast separation on a short and microbore second dimension column results in very narrow peaks with widths of 50–1,000 ms at the baseline. Unfortunately, conventional scanning MS detectors are typically too slow and do not provide reliable spectra and peak reproduction. At present, only time-of-flight mass spectrometers can acquire 50 or more mass spectra per second, which are required for the proper reconstruction of the chromatogram and for quantification in GC × GC.

4.2.1.1. Advantages of GC × GC in the POPs Analysis

A number of characteristics of GC × GC have been reported that documents superiority of this technique over conventional 1D-GC in POPs analysis (24, 25).

High Peak Capacity. The peak capacity, characterised as a maximal number of chromatographic peaks that can be placed side by side into the available separation space (chromatogram), is significantly enhanced. As an example, the merit in dioxin analysis resulting from the separation power is shown in Fig. 5.

Table 4
Overview of GC × GC methods used for the determination of POPs in food matrices

Matrix	Analytes	Injection	GC set-up	Column flow (mL/min)	Modulation period (s)	Ionisation/ detection	Acquisition speed (Hz)	Run time (min)	Ref.
Fish	PCBs, PBDEs, PAHs	Pulsed splitless, 280°C, 1 µl	^{1}D: BPX-5 (30 m × 0.25 mm × 0.25 µm) ^{2}D: Rt-LC35 (1 m × 0.15 mm × 0.1 µm)	1.3	2	EI-TOFMS	100	32.3	(28)
Fish oil	OCPs, PCBs, PBDEs	LV-DSI, 10 µl	^{1}D: Rtx-5Sil-MS (15 m × 0.25 mm × 0.25 µm) ^{2}D: DB-17MS (2 m × 0.18 mm × 0.18 µm)	1–1.5	3.5	EI-TOFMS	100	51.5	(29)
Fish oil	PCDDs/ PCDFs	LV-DSI, 10 µl	^{1}D: Rtx-Dioxin 2 (60 m × 0.25 mm × 0.25 µm) ^{2}D: Rtx-PCB (3 m × 0.18 mm × 0.18 µm)	2–2.5	3.5	EI-TOFMS	50	56.5	(30)
Fish	PCBs, PCDDs/ PCDFs	Splitless, 270°C, 1 µl	^{1}D: Rtx-Dioxin 2 (60 m × 0.25 mm × 0.25 µm) ^{2}D: Rtx-500 (2 m × 0.18 mm × 0.18 µm)	2.5	3	EI-TOFMS	100	35.0	(31)
Fish, pork, milk	PCBs, PCDDs/ PCDFs	Splitless, 250°C, 1.2 µl	^{1}D: Rtx-500 (40 m × 0.18 mm × 0.10 µm) ^{2}D: BPX-50 (1.5 m × 0.10 mm × 0.10 µm)	0.8	4	EI-TOFMS	60	48.0	(18)
Fish	PBDEs	Splitless, 280°C, 1 µl	^{1}D: DB-1 (30 m × 0.25 mm × 0.25 µm) ^{2}D: 007-65HT (1 m × 0.10 mm × 0.10 µm)	1.2	5	NCI-qMS	24	33.0	(32)
Tea	OCPs	Splitless, 270°C, SPME	^{1}D: BPX-5 (40 m × 0.18 mm × 0.18 µm) ^{2}D: SupelcoWax (2.5 m × 0.10 mm × 0.10 µm)	1.0	5	EI-TOFMS	125	17.2	(33)
Fruit	OCPs	Pulsed splitless, 250°C, 1 µl	^{1}D: DB-XLB (30 m × 0.25 mm × 0.1 µm) ^{2}D: DB-17 (1 m × 0.10 mm × 0.10 µm)	1.2	2	EI-TOFMS	250	22.6	(34)
Fruit	OCPs	Splitless, 250°C, 1 µl	^{1}D: Rtx-5MS (10 m × 0.18 mm × 0.20 µm) ^{2}D: TR-50MS (1 m × 0.10 mm × 0.10 µm)	1.0	4	EI-TOFMS	100	24.0	(35)
Milk	PCBs, PBDEs, OCPs	Splitless, 280°C, 1.2 µl	^{1}D: DB-1 (15 m × 0.25 mm × 0.25 µm) ^{2}D: HT-8 (1.2 m × 0.10 mm × 0.10 µm)	0.8	3	EI-TOFMS	60	50.3	(36)

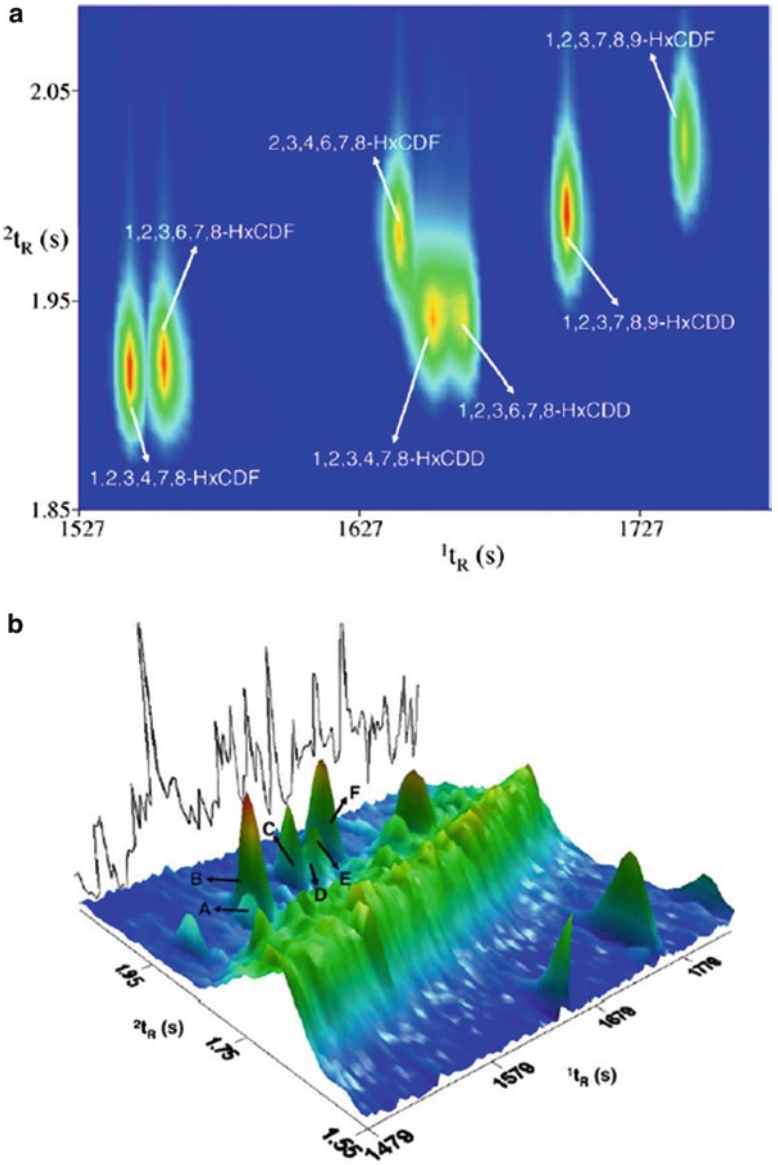

Fig. 5. (**a**) GC × GC–TOFMS contour plot of a standard solution containing 1 ng of HxCDD/Fs. The deconvoluted ion current (DIC) is based on the sum of the molecular ions corresponding to HxCDD/Fs (*m/z* 390 + 374). (**b**) Expended section of the HxCDD/F region of a GC × GC shade surface plot after injection of the clean-up fraction containing PCDD/Fs isolated from a real fish sample. DIC based on *m/z* 390 and 374. Concentrations are in the range of 2–3 pg/μL (A: 1,2,3,4,7,8-HxCDF; B: 1,2,3,7,8,9-HxCDF; C: 2,3,4,6,7,8-HxCDF; D: 1,2,3,4,7,8-Hx CDD; E: 1,2,3,6,7,8-HxCDD; F: 1,2,3,7,8,9-HxCDD). (Reproduced from (26) with permission from Elsevier).

Enhanced Detectability. Compared to 1D-GC separation, pronounced improvement of detection limits in GC × GC system is obtained thanks to compressing the peak in the modulation capillary and/or front part of the second column (following fast

chromatography avoids band broadening of focused peaks). Furthermore, thanks to improved separation of analytes and matrix interferences (chemical noise) in the GC × GC system, typically the signal to noise ratio is also improved.

Structured Chromatograms. Due to complementary separation mechanisms occurring in both columns, the chromatograms resulting from particular GC × GC set-up are ordered, i.e. molecules have their definite locations in the retention space based on their structure. In the reconstructed 2D contour plots, characteristic patterns are obtained, in which the members of homological series differing in their volatility are ordered along the first dimension axis (a non-polar capillary is typically employed in first dimension), whereas the compounds differing by polarity separated on other interaction principles are spread along the second dimension axis. The formation of clusters of the various subgroups of compounds in a GC × GC contour plot may be useful for the group type analysis (Fig. 6); in this way, information supporting identification of isomeric species is obtained.

Improved Identification of Unknowns. Non-target screening allows obtaining (even retrospectively) an overview of the sample constituents. This approach consists from: (1) peak finding and deconvolution (algorithm for recognising of partly coeluting peaks in the GC–MS chromatogram and obtaining their "pure" mass spectra); (2) library searching, and (3) further post-processing. Since a large amount of data has to be processed, automated data processing is employed.

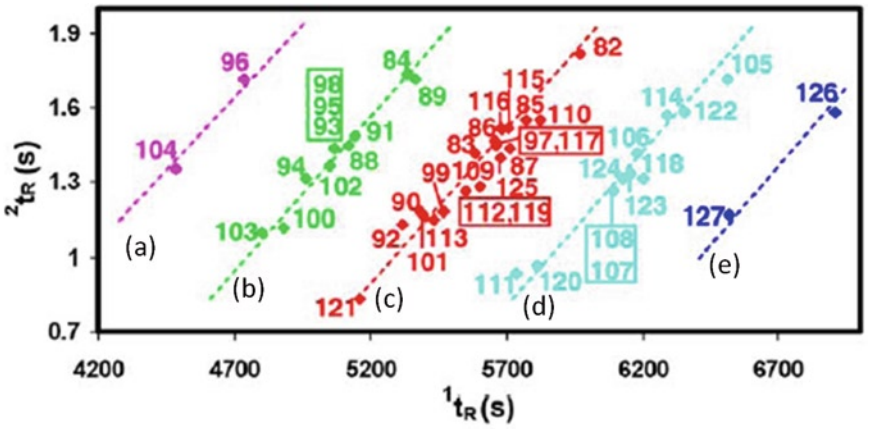

Fig. 6. GC × GC–TOFMS apex plots of the penta-CB homologue groups using the HT-8 (50 m × 0.22 mm × 0.25 μm) × BPX-50 (2.5 m × 0.1 mm × 0.10 μm) column set. A separate apex plot is shown for each homologue group. The *ortho*-substitution level is described as follow: (a) tetra-*ortho*-CBs, (b) tri-*ortho*-CBs, (c) di-*ortho*-CBs, (d) mono-*ortho*-CBs, (e) non-*ortho*-CBs. Boxes represent coeluting congeners. (Reproduced from (27) with permission from Elsevier).

4.3. Sample Ionisation and Detection Using MS

Currently, mass spectrometric detectors (MS) play a dominating role in analysis of food since they are by far the most powerful and flexible tools. The advantage over "conventional" GC detectors (e.g. ECD, NPD, FPD) is the possibility to obtain, in addition to selective detection of analyte eluted at certain retention time, also structural information, enabling either confirmation of target compounds, or identification of unknown species, supposing full spectral information was acquired during the run (17). Following the introduction of relatively inexpensive single quadrupole and ion trap mass analysers in the middle of the 90s of the last century, a lot of innovative developments have occurred in the field of mass spectrometry. Atmospheric pressure ionisation in LC–MS caused extensive spreading of its use for food analysis including determination of the parent POPs polar metabolites not amenable to GC. Also, the introduction of and, at the end of the last century, the rediscovery of time-of-flight mass analysers allowed the use of this sophisticated instrumental technique in both research as well as routine applications (37). The choice of an optimal mass analyser to be coupled to respective separation systems is determined by several key operating parameters such as (1) mass range, (2) mass resolution/mass resolving power, (3) mass accuracy, (4) spectral acquisition speed, (5) acquisition mode, (6) detectability (or often expressed as sensitivity) of the instrument, (7) linear dynamic range, (8) availability of tandem MS function, (9) versatility, and (10) cost (38). The general specifications and features of selected mass analysers hyphenated to both GC and LC are shown in Tables 5 and 6.

The most common analytical techniques used in POPs/PAHs analysis are summarised in Table 7. In the text below, more details for particular techniques are given.

Organochlorine pesticides. The types of MS instruments used for pesticide residue analysis include single mass analysers (quadrupole, ion trap operated in full scan, and TOF). When employing SIM (quadrupole) and MS/MS (ion trap, triple quadrupole) modes, settings of time segments are typically needed, which may limit the number of targeted analytes that can be detected in a particular time slot at desired low levels. The trade-off with SIM relates to the difficulty of identifying analytes due to fewer ions monitored and higher chance of matrix interferences as compared to MS/MS (16, 17, 38).

Recent progress in instrumentation design as well as the use of fast recording electronics together with improvements of signal processing techniques has led to rediscovery of TOF mass analysers for the determination of a wide range of pesticide residues. As indicated earlier, GC–TOFMS has been demonstrated as a powerful tool not only for quantification of target analytes but also for identifying non-targeted compounds in complex matrices (37).

Table 5
General specifications and features of selected mass analysers coupled to gas chromatography[a]

	Scanning				Non-scanning	
Criteria	**Quadrupole**	**Triple quadrupole**	**Ion trap**	**DF magnetic sector**	**High-speed TOF**	**High-resolution TOF**
Mass range	Up to 1,200 Da	Up to 1,500 Da	Up to 1,000 Da	Up to 4,000 Da	Up to 1,000 Da	Up to 1,500 Da
Mass resolution/ mass resolving power	Unit mass	Unit mass	Unit mass	>10,000 (10% valley definition)	Unit mass	>7,000 (FWHM)
Mass accuracy	0.1–0.2 Da	0.1–0.2 Da	0.1–0.2 Da	<5 ppm	0.1 Da	<5 ppm
Maximal spectral acquisition speed (*m/z* 50–550 Da)	12,500 amu/s (i.e. theoretically 25 Hz)		5 scans/s	7 scans/s	500 spectra/s	20 spectra/s
Acquisition mode	Full scan, SIM	Full scan, SIM, SRM, MRM	Full scan, SRM, MRM	Full scan, SIM	Full spectra	Full spectra
Sensitivity	>pg in full scan, fg in SIM	>pg in full scan, <pg in MS/MS	>pg in full scan	fg in SIM	pg	fg–pg
Linear dynamic range (orders of magnitude)	>5	>5	4–5	>5	4	4
Ionisation	EI, PCI, NCI	EI, PCI, NCI	EI, PCI, NCI	EI, PCI, NCI	EI	EI, PCI, NCI
MS/MS	None	MS^2	MS^n, $n = 2–10$	Only with special configuration	None	None
Cost	+	+++	+	++++	+++	+++

[a] *DF* double focussing, *EI* electron ionisation, *MS* mass spectrometry, *NCI* negative chemical ionisation, *PCI* positive chemical ionisation, *SIM* selected ion monitoring, *SRM* selected reaction monitoring, *MRM* multiple reaction monitoring, *pg* picogram, *fg* femtogram

Table 6
General specifications and features of selected mass analysers coupled to liquid chromatography[a]

	Scanning			Non-scanning		
Criteria	**Quadrupole**	**Triple quadrupole**	**Ion trap**	**High-speed TOF**	**High-resolution TOF**	**Orbitrap**
Mass range	Up to 1,000–3,000 Da	Up to 1,000–3,000 Da	Up to 2,000–6,000 Da	Up to 6,000 Da	Up to 30,000 Da	Up to 4,000 Da
Mass resolution/mass resolving power	Unit mass	Unit mass	Unit mass	>2,000 FWHM	>40,000 FWHM	Up to 100,000 FWHM
Mass accuracy	0.1–0.2 Da	0.1–0.2 Da	0.1–0.2 Da	<15 ppm	<3 ppm	<2 ppm
Acquisition mode	Full scan, SIM	Full scan, SIM, SRM, MRM	Full scan, SRM, MRM	Full spectra	Full spectra	Full spectra
Maximal spectral acquisition speed (*m/z* 50–1,000 Da)	≈6 scans/s		≈2 scans/s	100 spectra/s	40 spectra/s	10 spectra/s
Sensitivity	>pg in full scan, <pg in SIM	>pg in full scan, <pg in MS/MS	>pg in full scan	>pg	pg	pg
Linear dynamic range	>5	>5	4–5	>3	4–5	>4
Ionisation	ESI, APCI	ESI, APCI, APPI	ESI, APCI	ESI	ESI, APCI, APPI	ESI, APCI, APPI
MS/MS	None[b]	MS^2	MS^n, $n=2–10$	None[b]	None[b]	None[b]
Cost	+	+++	+	+++	+++	+++

[a] *APCI* atmospheric pressure chemical ionisation, *APPI* atmospheric pressure photoionisation, *ESI* electrospray ionisation, *FWHM* full width at half maximum, *MS* mass spectrometry, *SIM* selected ion monitoring, *TOF* time-of-flight, *SRM* selected reaction monitoring, *MRM* multiple reaction monitoring, *pg* picogram, *fg* femtogram

[b] MS/MS option only when hyphenated, such as QTOF

Table 7
Overview of the most common analytical techniques used in POPs and PAHs analysis[a]

Group	Separation	Ionisation	MS detection
Organochlorine pesticides	GC	EI	Q, IT, TOF, QqQ
Polychlorinated biphenyls	GC	EI, NCI	Q, IT, TOF
Polychlorinated dibenzo-*p*-dioxins and dibenzofurans	GC	EI	Sector, IT (MS^2), TOF (in combination with GC×GC)
Brominated flame retardants			
Polybrominated diphenyl ethers and related brominated aromatic compounds	GC	NCI	Q, IT, sector, TOF
Hexabromocyclododecane	GC	NCI	Q, IT, sector, TOF
	LC	ESI(−)	QqQ
Tetrabromobisphenol A	GC (deriv.)	EI	Q, IT, TOF
	LC	ESI(−)	QqQ
Perfluoroalkylated substances			
Fluorotelomer alcohols Perfluoroalkyl sulfonamidoethanols	GC	PCI (NCI)	Q, IT, TOF
Perfluoroalkyl sulfonamides Perfluoroalkyl sulfones Perfluorocarboxylic acids Perfluoroalkyl sulfonamides	LC	ESI(−)	Q, QqQ, QTOF
Polycyclic aromatic hydrocarbons	GC	EI	Q, IT, TOF, QqQ
	LC	APPI(+)	QqQ

[a]*APPI*(+) atmospheric pressure photoionisation in positive ion mode, *GC* gas chromatography, *GC*×*GC* comprehensive two-dimensional gas chromatography, *EI* electron ionisation, *ESI*(−) electrospray ionisation in negative ion mode, *IT* ion trap, *LC* liquid chromatography, *MS* mass spectrometry, *NCI* negative chemical ionisation, *PCI* positive chemical ionisation, *TOF* time-of-flight, *Q* quadrupole, *QqQ* triple quadrupole, *QTOF* quadrupole/time-of-flight

TOFMS can be performed emphasising either high speed (unit mass resolution) or high resolution, albeit at slower acquisition speed. In the case of high-speed TOFMS, GC×GC is often used for good resolution of sample components, permitting unbiased identification of sample components and achieving low LODs. The advantages of high-resolution TOF over common mass analysers (unit resolution quadrupoles and ion traps) in residue analysis, can be summarised as follows: (1) Acquisition of spectral data across a wide mass range is possible at any time during the GC run without a decrease in detection sensitivity (i.e. full spectral information searchable in spectral library is available within the elution of sample component). (2) Due to a high mass resolving power, matrix components yielding ions with the same nominal mass (isobaric interferences) as that of the target analyte can often be partially or completely resolved, and hence do not

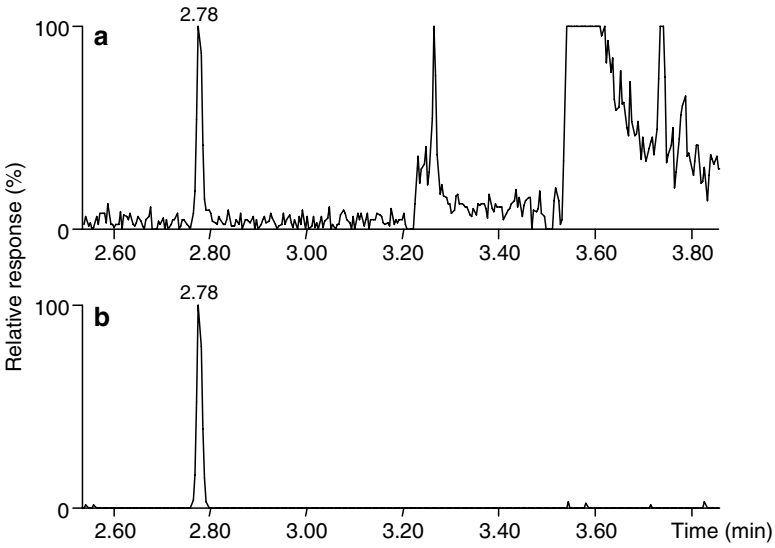

Fig. 7. PTV–LPGC–HRTOFMS chromatograms of hexachlorobenzene ($t_R = 2.78$ min) at a concentration of 0.003 mg/kg in apple baby food extract (2 µL injected). Target ion *m/z* 283.810 extracted using different mass windows. While by using (**a**) 1 Da mass window (setting corresponding to a unit mass resolution instrument) the peak-to-peak (PtP) signal-to-noise (S/N) ratio was only 9:1, setting the mass window as narrow as (**b**) 0.02 Da led to a distinctly improved PtP S/N value of 63:1. Mass measurement accuracy allowed determination of the mass of hexachlorobenzene's quantification ion [$C_6{}^{35}Cl_5{}^{37}Cl$] with the error as low as +0.7 mDa.

interfere. (3) Mass measurement accuracy permits estimation of the elemental composition of the detected ions (39). An example of improved detection of HCB at trace level is demonstrated in Fig. 7. Because of high mass resolving power and monitoring of the exact mass of a target analyte, chemical noise originating from various sources (e.g. matrix coextracts, column bleed) can be significantly reduced, resulting in an improved limit of quantification (LOQs).

Various ionisation techniques are also possible in GC–MS; nevertheless, in the case of OCPs, electron ionisation (EI) is commonly preferred. Chemical ionisation (CI) in both positive (PCI) and negative (NCI) modes, as a softer ionisation technique, tends to give lower LODs depending on the pesticide, but it is not as widely applicable in OCPs methods and does not provide as much structural information about the analyte as EI (16).

A practical example demonstrating improvement of detection of heptachlor present at trace level in fish oil extract is shown in Fig. 8; in this case the use of tandem MS led to unambiguous identification and quantification of this analyte compared to single MS (SIM mode), or by using conventional detection (ECD) (40).

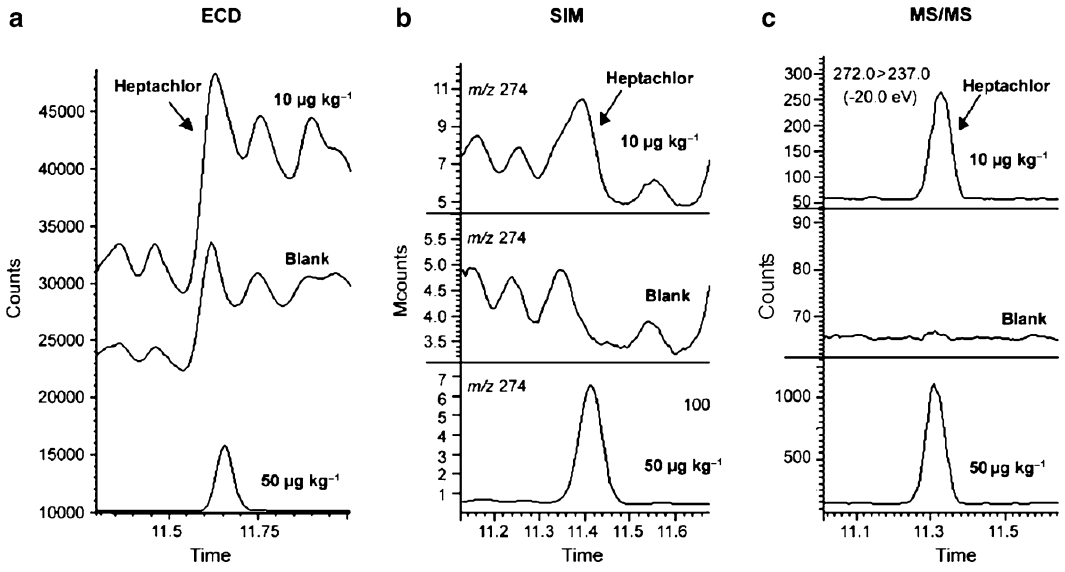

Fig. 8. Comparison of selectivity between (**a**) electron-capture detector (ECD), (**b**) selected ion monitoring (SIM) of a single quadrupole, and (**c**) tandem MS (MS/MS) of a triple quadrupole for heptachlor at 0.01 mg/kg in fish oil. Chromatograms of the corresponding blank extract and a higher matrix-matched standard are provided for contrast and identification (Reproduced from (40) with permission from Elsevier).

Polychlorinated biphenyls. GC–MS represents a reliable technique for PCBs quantification, particularly given by the availability of ^{13}C-labelled PCB standards. Although EI mode can be used, the NCI allows obtaining lower LODs of these analytes. Both single quadrupole (SIM) and ion trap (MS/MS) are frequently employed in the routine analysis of PCBs. In addition to these detection principles, triple quadrupole is becoming more and more popular in the analysis of PCBs. The use of high-resolution MS permits quantification of lower PCBs differing by two chlorines because of the high mass resolving power that allows unbiased measurement of ions. Also, the application of both high-resolution and high-speed TOFMS (the latter in combination with GC×GC replacing the "heart cut" (GC–GC) approach) represents a tool successfully applied in the analysis of PCBs (5, 38).

Polychlorinated dibenzo-p-dioxins and dibenzofurans. Quantitative determination of PCDDs/PCDFs is typically performed using GC coupled to high-resolution MS (double focusing sector analyser). Although PCI/NCI techniques can be used for their determination, a majority of laboratories employ EI. High-resolution systems (mass resolving power of >20,000 FWHM) provide higher selectivity compared to unit mass resolution instruments especially when the levels of potentially interfering compounds are too extensive (41). However, this instrumentation is very expensive, bulky, and requires operation by a highly trained specialist. Therefore, alternate analytical instruments (less expensive) have been investigated for dioxin analysis in several

laboratories. GC–MS/MS employing the ion trap analyser and GC × GC–TOFMS have been reported as a valuable technique for improved selectivity in dioxin analysis. In the case of GC–MS/MS the high selectivity (minimised chemical noise → increasing of S/N → lowered LOD) is obtained due to formation of characteristic dioxin product ions produced by the collision induced dissociation from the precursor ion(s), while in GC × GC–TOFMS the improvement of selectivity is achieved employing the secondary column with different selectivity that can better separate the target compounds from co-eluting matrix components (42, 43).

Brominated flame retardants. The determination of PBDEs and related brominated aromatic compounds is performed by GC–MS operated either in EI or NCI mode. The low-resolution MS is routinely applied compared to the high-resolution MS that requires more experienced users and is much more costly and labour intensive. The high-resolution MS (sector) has several advantages over low-resolution MS (e.g. increased sensitivity and selectivity), but is almost exclusively operated in EI mode. For low-resolution MS, NCI, in addition of EI, can be applied to obtain an increased sensitivity for higher-brominated BDE congeners (8, 10). Recently, the application potential of high-resolution TOFMS under NCI conditions in the analysis of PBDEs has been demonstrated (44). EI is preferred in the analysis of PBDEs, whenever the identification of mixed organohalogenated compounds has to be carried out. Another advantage of EI mode is the possibility to use ^{13}C-labelled internal standards. This is not applicable in NCI, since generally only the $[Br]^-$ ions (*m/z* 79 and 81) are monitored. The main benefits of NCI include efficient ionisation, lower LODs, and less fragmentation compared with EI. Recently, the application of LC techniques for the analysis of PBDEs has been described. The use of atmospheric pressure chemical ionisation (APPI) in negative mode was found to be a promising tool mainly for the BDE 209 congener, seeing the difficulties encountered for this congener during GC–MS analysis (8).

Traditionally, HBCD has been analysed using GC–MS operated in NCI (similarly to PBDEs) for which the $[Br]^-$ ions are monitored because of their high selectivity (8, 10). In the case of LC employed for the isomer-specific determination, electrospray ionisation (ESI) or APCI are utilised for ionisation. Using LC–ESI-MS/MS and single reaction monitoring, the transition $[M–H]^-$ (*m/z* 640.6) → $[Br]^-$ (*m/z* 79 and 81) is monitored. The derivatised product of TBBPA is ionised typically under GC–EI-MS conditions followed by its detection using a single mass analyser (e.g. quadrupole, ion trap, TOF). In the case of direct analysis of TBBPA employing LC, ESI in negative ion mode combined with tandem MS (e.g. QqQ, IT) is most commonly used (8).

Perfluoroalkylated substances. For GC-amenable perfluoroalkylated substances (fluorotelomer alcohols, perfluoroalkyl sulfonamidoethanols, and perfluoroalkyl sulfonamides) EI is not useful

because of the low intensity of molecular ions and the lack of specific fragments. However, this is not the case when PCI is employed for the ionisation. The fluorotelomer alcohols provide intensive protonated molecules ($[M+H]^+$) if methane is used as a reagent gas, but also some other useful selective (high *m/z*) fragments and/or adduct ions ($[M+C_2H_5]^+$). The perfluoroalkyl sulfonamides give also $[M+H]^+$ ions in PCI, but no suitable fragments, therefore, in this particular case, NCI can be used for their qualitative confirmation (formation of high *m/z* fragments). In the same way, the perfluoroalkyl sulfonamidoethanols provide intensive $[M+H]^+$ ions in PCI and also some fragments, but NCI can be also used for qualitative confirmation (20). Regarding the detection, all common GC–MS instruments (quadrupole, ion trap, and TOF) can be used for their analysis (the only requirement is the availability of chemical ionisation). However, the detection limits remain the limitation since these compounds occur in biotic matrices at ultra-trace levels.

For LC-amenable perfluoroalkylated substances (perfluoroalkyl sulfones, perfluorocarboxylic acids, and perfluoroalkyl sulfonamides) electrospray ionisation in negative mode coupled to either single MS or tandem MS has enabled to improve the analysis of these compounds. LC with a single MS (e.g. quadrupole), though a sensitive technique, requires more thorough clean-up of the sample in order to remove matrix interferences. Therefore, LC with tandem MS employing QqQ, IT, or QTOF can be considered as the current standard for the analysis of LC-amenable perfluoroalkylated substances (11, 21).

Polycyclic aromatic hydrocarbon. GC–EI-MS operated in SIM mode (quadrupole) represents probably the most common GC technique for determination of PAHs in food matrices. The problem encountered in the analysis of PAHs is separation of isomers and limited EI fragmentation, which does not allow reliable confirmation at ultra-trace levels. Although HPLC with a fluorescence detector (FLD) is also often routinely used, unfortunately, some of carcinogenic PAHs do not provide a fluorescence yield. LC–APCI-MS allows determination of PAHs without derivatisation (post column), which is typically required in LC–ESI-MS. The recently developed APPI enhances the ionisation of the PAH analytes, thus, lowering LODs. To improve selectivity, tandem MS (triple quadrupole) is preferred for their determination (22, 23).

5. Comprehensive POPs/PAHs Profiling

Most analytical methods for POPs focus on individual groups of targeted analytes. Therefore, analysis of multiple classes of POPs typically entails several sample preparations, fractionations, and

injections, whereas other chemicals of possible interest are neglected or lost. A comprehensive POPs profiling is a novel instrumental approach employing GC × GC–TOFMS. Thanks to the recent revival of TOFMS instruments several hundreds of analytes, belonging to different classes of organic pollutants such as PCBs, PAHs, BFRs, pesticides can be theoretically measured in one run. During recent years, some effort has been spent to develop such profiling approach resulting in the introduction of GC × GC–TOFMS methods, typically in combination with large volume injection to achieve low LODs of target compounds, allowing simultaneous analysis of various groups of POPs/PAHs in food and environmental matrices (18, 28, 29, 31, 36). The main benefits of such a strategy involve: (1) more efficient monitoring of POPs, POP-like compounds, and other chemicals of interest in food; (2) possibility of non-target screening (even retrospectively) since full spectral information are acquired during the GC × GC run, and (3) significantly higher sample throughput.

6. Matrix Effects

Under real-world conditions, some residues of matrix co-extractives unavoidably remain in the purified sample prepared for examination by GC or LC analysis. Inaccurate quantification, decreased method ruggedness, poor analyte detectability, and even reporting of false positive or negative results are the most serious matrix-associated problems, which can be encountered (45, 46).

Matrix-induced chromatographic response enhancement is presumably the most discussed matrix effect adversely impacting quantification accuracy of certain, particularly more polar analytes during GC analysis. In principle, during injection of particular compounds in pure solvent, adsorption and/or thermo-degradation of susceptible analytes on the active sites (mainly free silanol groups) present in the GC injection port and in GC chromatographic column may occur. On this account, the number of analyte molecules reaching GC detector is reduced. This is, however, not the case when a real-world sample is analysed. Co-injected matrix components tend to block the active sites in GC system thus reducing the analyte losses and, consequently, enhancing their signals as compared to the injection in pure solvent. If these facts are ignored and calibration standards in solvent only are used for calculation of target analytes concentration, recoveries as high as even several hundred percent might be obtained. Repeated injections of nonvolatile matrix components, which are gradually deposited in the GC inlet and/or front part of the GC column, can give rise to successive formation of new active sites, which might be responsible for the

effect, sometimes called *matrix-induced diminishment*. Gradual decrease in analyte responses associated with this phenomenon together with distorted peak shapes (broadening, tailing) and shifting the retention times towards higher values negatively impact ruggedness, i.e. long-term repeatability of analyte peak intensities, shapes, and retention times, performance characteristic of high importance in routine trace analysis.

On the other hand, LC–MS with atmospheric pressure ionisation (API) interface is considerably influenced by the composition of liquid entering the detector, i.e. the type and amount of organic mobile phase modifiers and volatile buffers, and also the type and amount of sample matrix components. These substances present in the injected sample can cause serious quantification problems when co-eluted with the analyte of interest; either by suppression or enhancement of the analyte signal. It is assumed that matrix components influence the efficiency of the ionisation processes in API interface (causing a mutual positive or negative effect in the amount of ions formed from the target analyte). Those components may also influence the ion formation in the ionisation process by altering the surface tension of electrospray droplets and by building adduct ions or ion pairs with the analytes. As a result of matrix suppression/enhancement phenomena, the response of an analyte in pure solvent standard may differ significantly from that in matrix sample.

Ways to compensate for matrix effects include: (1) method of standard addition (GC–MS, LC–MS); (2) use of isotopically labelled internal standards (GC–MS, LC–MS); (3) use of matrix-matched standards (GC–MS, LC–MS); and (4) use of analyte protectants (GC–MS). The latter approach offers the most practical and convenient solution to the problems associated with calibration in routine GC analysis of pesticide residues in diverse food samples. Essentially, analyte protectants are compounds that strongly interact with active sites in the GC system, thus decreasing degradation and/or adsorption of co-injected analytes. Therefore, the application of those compounds can minimise losses of susceptible analytes, thereby significantly improving their peak shapes and lowering detection limits. The analyte protectants are added to sample extracts and matrix-free standards alike to induce response enhancement in both instances, resulting in maximization and equalisation of the matrix-induced response enhancement effect. Various compounds have been evaluated as analyte protectants, and a mixture of 3-ethoxypropane-1,2-diol, L-gulonic acid γ-lactone, and D-glucitol (in MeCN extracts) was found to most effectively cover a wide volatility range of GC-amenable pesticides (47).

7. Quality Assurance

The analysis of POPs/PAHs occurring typically at ultra-trace levels requires an extensive quality assurance/quality control (QA/QC) regime to ensure required data quality objectives can be met. This includes, among others, following major areas:

1. *Calibrants*: Standard solutions (isotopically labelled) internal and syringe standards.
2. *Analytical procedure control*: Chromatographic parameters such as baseline, peak shape, resolution; monitoring the ion intensity ratios; recovery; procedural blanks; estimation of limit of detection (LOD) and quantification (LOQ); matrix effects.
3. *System performance/long-term stability*: Precision (repeatability, reproducibility); method stability and reliability; accuracy (use of spiked samples and certified reference materials, CRM); participation in relevant proficiency testing whenever possible.

The detailed discussion of QA/QC requirements (for regulatory purpose) can be found in various sources such as Commission Decision 2002/657/EC – Performance of Analytical Methods and the Interpretation of Results (48), SANCO/10684/2009 – Method Validation and Quality Control Procedures for Pesticide Residues Analysis in Food and Feed (49), Commission Directive 2002/69/EC – Sampling methods and the methods of analysis for the official control of dioxins and the determination of dioxin-like PCBs in foodstuffs (19), CITAC/Eurachem Guide – Guide to Quality in Analytical Chemistry (50) and in Notes section (see Notes 1–6).

8. Notes

Following recommendations should always be considered within POPs/PAHs analysis (8, 10, 21, 51):

1. *Blank analysis – BFRs*. The use of plastics should be reduced to a minimum in the analysis of BFRs, since they can contain a wide range of these compounds. In addition, higher concentrations of BDE 47 and BDE 99 can originate from the laboratory air; in the case of BDE 209 also the contamination originated from in-house dust has to be taken into account. Therefore, the laboratory glass should be placed in a closed area not allowing the deposition of PBDEs from the air/dust. The correction of the results by applying the analysis of procedural blanks can be applied only if the blank values are relatively constant. In the case of BDE 209 congener the analysis results

can be considerably affected by the degradation under influence of daylight (the use of UV filters at laboratory windows is highly recommended) and poor solubility (this should be checked before preparing stock solution or preparing highly concentrated extracts).

2. *Blank analysis – perfluoroalkylated substances.* The source of the procedural contamination involves a contact with laboratory materials made of, or containing, fluoropolymers (e.g. polytetrafluoroethylene or perfluoroalkoxy compounds). This can be, for example, polypropylene sample bottle, SPE cartridges, purified reagent water, nylon syringe filter, HPLC tubing, autosampler vial septum, the degasser and solvent-selection valves containing fluoropolymer coatings and seals. Therefore, during the validation of the method used for perfluoroalkylated substances, the possible sources of contamination have to be investigated and eliminated (e.g. replacing the type of SPE cartridges, washing the nylon syringe filter prior the filtration, replacing the HPLC tubing constructed from poly(tetrafluoroethylene) (PTFE) by stainless steel and polyetheretherketone (PEEK) tubing). The procedural blank should be run during each sample sequence. Special care should also be taken to blank analysis when replacing product(s) of the manufacturer by the other one(s).
3. *Internal and syringe standards.* The use of internal and syringe standards is highly recommended. A known amount of internal standard (surrogate) added at the beginning of the procedure is used to compensate for the losses throughout the analytical procedure, while the syringe standard is added before the injection for compensation of inter-injection fluctuations. As a general rule, both internal and syringe standards should not be present in the sample, should combine chromatographic and physical properties similar to those of target POPs/PAHs and should not co-elute with target and also non-target analytes (if MS cannot distinguish the co-eluting analytes based on the different mass spectra). During recent years, the number of available internal and syringe standards, in the case of POPs/PAHs, has rapidly increased. This includes the use of (1) unlabelled, (2) ^{13}C-labelled, and (3) D-labelled compounds. Although more expensive, the ^{13}C-labelled analogues are preferred over D-labelled standards due to risk of isotopic exchange process in non-deuterated solvent or with matrix components, but in the case of GC analysis of PBDEs/HBCD this limits the detection to EI-MS only.
4. *GC determinative step – BFRs.* Thermal degradation of BFRs should be checked and minimised by using short and narrow GC columns with thin films of stationary phase. The other aspects involve: (1) temperature during the sample injection and GC separation (should be high enough for high boiling

point POPs), (2) short residence times during injection (this can be achieved by using a pulsed splitless injection).

5. *GC determinative step – OCPs.* *p*,*p*′-DDT degrades into *p*,*p*′-DDE and/or *p*,*p*′-DDD, endrin into endrin ketone and endrin aldehyde in active or poorly deactivated injection port liners during GC injection that uses liners (splitless, PTV). Degradation products of endrin and *p*,*p*′-DDT should be checked on a regular basis (by injecting a single analyte). If the breakdown exceeds the 15% level replacement of liner (or even cutting of 10–20 cm of the front part of GC column occupied by active sites) is recommended. Also, various types of commercially available specially deactivated liners can limit the breakdown level.
6. *GC and LC determinative steps.* It is recommended to run a standard with a known amount of target analytes at the beginning and the end of each (longer) sequence. This can provide useful information on the stability of the analyte signal during the analyses and to consider instrument maintenance on the bases of signal decrease and behaviour of analyte peak shape caused by matrix coextracts (matrix effects). This includes, in the case of GC–MS replacement of the liner, cutting of 20–30 cm of the front part of GC column, cleaning of the ion source; in LC–MS, replacing the pre-column (or even the LC column), and cleaning the ion source. The analysis using LC–MS (especially if ultra-high performance LC, UHPLC, with small particles of stationary phase is employed) should always include filtration of the final extract by a syringe filter (0.22 or 0.45 μm for UHPLC or HPLC, respectively). This (simple) procedure significantly prolongs the lifetime of a particular LC column.

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