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Recent advances in mass spectrometric measurement of dioxins

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Abstract

Past years, many efforts have been dedicated to the development of alternative analytical methods for the measurement of dioxins in various types of matrices. Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) are compounds that are present in samples at part-per-billion (ppb) or part-per-trillion (ppt) level. Their measurement requires the use of very sensitive analytical methods. Gas chromatography (GC) coupled to quadrupole ion storage mass spectrometry (QISTMS), fast GC (FGC) coupled to time-of-flight mass spectrometry (TOFMS) and comprehensive two-dimensional gas chromatography (GC × GC) coupled to TOFMS are the more promising tools challenging the reference GC high resolution mass spectrometry (HRMS) based on sector instruments. We report herein some of the advances we achieved in the past years in our laboratory on the development of alternative measurement methods for those compounds.

Keywords: Quadrupole ion storage mass spectrometry; Tandem in time mass spectrometry; Time-of-flight mass spectrometry; Polychlorinated dibenzo-*p*-dioxins; Polychlorinated dibenzofurans; Polychlorinated biphenyls

1. Introduction

Humans all over the world are exposed to chemicals during their lifetime. Among the thousands of existing anthropogenic compounds, some are persistent and remain in the environment for years once generated. The variation in measured levels mainly depends on the fact that some are (were) synthesised as industrial products although others are released accidentally or as by-products. Broad ranges of toxicities can be observed. The duality level-toxicity usually indicates if measurements of particular chemical or family of chemicals should be implemented. Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs) are the persistent organic chemicals that are the most often measured in various types of matrices during food safety programs, environmental monitoring, and epidemiological studies. All together, they represent more than 400 individual molecules (congeners), which have to be separated from each other to ensure distinctive quantification of the target ones. Information on their toxicities and levels at which they can be measured can be found elsewhere [1,2].

Accurate measurement of dioxins and related compounds requires high standard analytical strategies. Those complex multistep strategies include sample extraction, sample cleanup and analyte measurements under strict quality assurance/quality control (QA/QC) criteria. Several non-instrumental and instrumental automated approaches are available for both extraction and cleanup. Soxhlet extraction and liquid–liquid extraction have long been the most used tools for non-instrumental extraction of solids and liquids, respectively. They have proven to be very efficient but some limitations encouraged the development of other approaches based on instrumental techniques. Depending on the physico-chemical properties of the sample matrix, instrumental techniques are based on solid phase extraction (SPE) [3], matrix solid phase dispersion (MSPD) [4], solid phase micro-extraction (SPME) [5], stir-bar sorptive extraction (SBSE) [6], pressurised liquid extraction (PLE) [7], microwave assisted extraction (MAE) [8], and supercritical fluid extraction (SFE) [9]. Preparative liquid chromatography (LC) using silica-based sorbents and size exclusion chromatography (SEC) are the most common techniques used for sample

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clean-up and additional sorbents like Florisil, basic alumina, porous graphitic carbon (PGC) and 2-(1pyrenyl)ethyl (PYE) are commonly used to fractionate the extracts in subgroups of compounds (PCDDs, PCDFs, PCBs), which can be analyzed separately [10–13]. Furthermore, the coupling of the extraction and clean-up steps can result in high-throughput sample preparation methodologies, which allow the processing of a large number samples for many types of matrices [14,15]. Details on sample preparation for dioxin analyses are available in review articles [16,17].

Because of the semi-volatility of the analytes of interest, gas chromatography (GC) is the preferred approach for the final separation stage prior measurement of the individual species. The chromatographic separation relies on capillary GC columns made of appropriate lengths of specialty phases (polar and apolar) and allows to differentiate between the different congeners inside the fractionated sub-groups of compounds.

Although micro electron-capture detectors (μECD) offer the required sensitivity for the measurement of selected PCBs, accurate peak identification can sometimes be difficult and mass spectrometric (MS) detectors are usually preferred. High resolution (HR) MS based on sector instruments has long been, and still is, the reference measurement method for PCDD/Fs. It offers the required sensitivity and specificity in addition to valuable mass spectral information [18]. The high sensitivity (down to the low femtogram level) is achieved using electron impact (EI) ionisation, which produces abundant molecular ions, but also by operating the MS in selected ion monitoring (SIM) mode. In SIM, a restricted number of relevant masses corresponding to the analytes of interest are selected, this increases the time spent on particular masses (dwell time) and consequently improves the sensitivity. The high selectivity results from the elevated mass resolution (ca. >10,000) of sector instruments. The use of isotope dilution (ID) based on commercially available $^{13}C_{12}$-labelled internal standard offers accurate peak identification by means of retention time comparisons between native (^{12}C) and labelled (^{13}C) compounds, as well as accurate peak quantification by comparison of peak areas/heights [19]. The measurement of the two most intense ions in the molecular cluster of native and labelled compounds allows to measure the theoretical isotope ratio and serves as a confirmatory procedure for peak identification.

Because HRMS instruments requires high investment cost and highly skilled scientists, their use results in high analysis prices. Moreover, as one observed during the 1999 Belgian dioxin crisis [20], rapid high-throughput and cost-effective analytical methods are requested for emergency response. In addition, the completion of large scale monitoring programs requires affordable analytical methods to fit the limited budgets. This can barely be attained using HRMS instruments and alternative measurement methods are desirable. From the "MS islands" presented by Brunée in 1987 [21], quadrupole ion storage mass spectrometry (QISTMS) as well as time-of-flight mass spectrometry (TOFMS) appear to be the most promising ones when coupled to suitable GC methods such as large volume programmable temperature vapourizer injection (PTV-LV) GC, fast GC (FGC) or comprehensive two-dimensional gas chromatography (GC × GC). Some of the modern developments based on those techniques for environmental analysis are available in the literature [22,23]. The potential decrease in selectivity due to the low mass resolution, relatively to HRMS sector instruments, can be counterbalanced by operating the instrument in tandem mode or by improving the chromatographic separation. The present paper reports some of the results we obtained last years in the development of alternative mass spectrometric methods for the measurement of dioxin and selected PCBs at the ultra-trace level.

2. Experimental

2.1. Chemicals

All details on sample preparation procedures and consumables are available elsewhere [12–15]. Chromatographic pure grade helium gas, 99,9999% was purchased from Air Products (Vilvoorde, Belgium). The internal standard solution of the seventeen 2,3,7,8-chloro-subsituted $^{13}C_{12}$ congeners labelled PCDD/Fs (EDF-4144), the calibration standard solution (EDF-4143) and the syringe standard (EDF-4145) were purchased from Cambridge Isotope Laboratory (CIL, Andover, MS, USA). The EDF-4143, EDF-4144 and EDF-4145 concentrations of the natives and labelled congeners are summarised in a previous report [13]. The $^{13}C_{12}$-labeled PCB internal standard spiking solution (EC-5023), as well as the 10-points calibration solutions (EC-5022) were obtained from CIL.

2.2. Sample preparation and quality control

Details on sample preparation have been reported earlier [13–15]. Extractions were carried out either using SPE cartridges or using PLE, depending on samples. Further sample clean-up was achieved using an automated system (PowerPrep™, Fluid Management Systems Inc., Waltham, MA, USA) [17]. Various quality control (QC) samples as well as ring-test samples were routinely used in the laboratory to ensure high quality standard performance of the methods. QC matrices were typically made of beef fat, yolk, bovine serum, animal feeding stuffs and milk. Additionally, unknown samples were set in series in which QC samples and instrumental as well as procedural blanks (BCs) were always included to ensure full control of the method.

2.3. Instrumentation

2.3.1. Gas chromatography–high resolution mass spectrometry (GC–HRMS)

The HRMS experiments were either performed on an Autospec Ultima (Micromass, Manchester, United King-

dom) or a MAT95XL (ThermofinniganMAT, Bremen, Germany). The HRMS was connected by a heated transfer line (275 °C) to a Agilent 6890 Series (Palo Alto, CA, USA) gas chromatograph equipped with a A200SE autosampler (CTC Analytics AG, Zwingen, Zwitzerland). The column was a 40 m RTX-5 MS (0.18 mm i.d. × 0.18 μm df) (Restek, Interscience, Louvain-La-Neuve, Belgium). Helium was used as the carrier gas at constant flow rate of 1.2 ml/min. Two microlitres of the final extract in nonane were injected into a split/splitless injector held at 275 °C in splitless mode. The HRMS instrument was operated in SIM mode. Additional GC and HRMS parameters, including performing conditions, quantification and insurance quality control for measurements were described previously [13].

2.3.2. Gas chromatography–quadrupole ion storage mass spectrometry in tandem mode (GC–QISTMS/MS)

Part of the MS/MS analyses were carried out with a Saturn 2000 GC/MS/MS coupled with a Star 3400CX gas chromatograph and a 8200CX autosampler (Varian, Walmut Creek, USA). The Saturn 5.1 software version of the workstation was used. PCDD/Fs were separated on a DB-5 MS (30 m × 0.25 mm i.d. × 0.25 μm df) capillary column (J&W Scientific, Folsom, USA). The GC conditions were: on-column injection of 1 μl at 240 °C, initial oven temperature of 150 °C for 1 min, increased at 30 °C/min to 210 °C, then increased at 1 °C/min to 250 °C and held for 7 min. Helium (N60, Air Liquide, France) has been used as carrier gas. The ion trap temperature was set at 200 °C with the transfer line at 250 °C and a maximum number of 5000 ions in the trap. All details are reported elsewhere [24]. The PCB (mono-*ortho* and indicator PCBs) fraction was injected splitless (1 μl) at 140 °C, initial oven temperature of 140 °C for 1 min, increased at 25 °C/min to 180 °C held for 1 min, then increased at 2 °C/min to 210 °C held for 8 min, finally increased at 3 °C/min to 280 °C and held for 2 min. Details are available elsewhere [12].

Large volume programmable temperature vapouriser injection GC–QISTMS/MS experiments were performed on a Finnigan PolarisQ ion trap (Austin, TX, USA) held at 250 °C. The GC transfer line (300 °C) connected the MS to a Thermoquest Trace GC 2000 (Milan, Italy) gas chromatograph equipped with a Combi Pal autosampler (CTC Analytics AG). The analyses were carried out using a 40 m (0.18 mm i.d. × 0.18 μm df) RTX-5 MS column with Helium at constant flow rate of 1.2 ml/min. The column was directly connected to a BEST PTV injector. The liner used was a Silco Sleeve liner with glass wool from Restek Corp. The oven temperature was maintained at 100 °C for 6 min, ramped at 52 °C/min to 200 °C; ramped at 2.9 °C/min to 250 °C for 6 min; ramped at 2.9 °C/min to 260 °C, and finally ramped at 10 °C/min to 300 °C for 5 min. Further details are available elsewhere [25].

2.3.3. Fast gas chromatography- and comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (FGC and GC × GC–TOFMS)

The FGC–TOFMS instrument was the Pegasus III (Leco Corp., St Joseph, MI, USA) equipped with an Agilent 6890 GC. The GC oven was programmed as follows: 140 °C for 0.5 min, then to 330 °C at 25 °C/min. Helium was used at a constant flow of 5 ml/min. The inlet temperature was 260 °C for splitless injections of 2 μl using a Restek Uniliner. A DB-XLB (25 m × 0.20 mm i.d. × 0.33 μm df) capillary column (J&W) was used. The transfer line temperature was 280 °C. The ion source temperature was 220 °C with an electron-impact filament bias voltage of −70 V. The data acquisition rate was 10 scans/s for a mass range of 120–520 amu. The detector voltage was 1800 V. Data processing was performed using the Leco ChromaTOF^{TM} software. Peak apex finding was performed automatically and further manually corrected when required. Additional details are available in a previous report [26].

The GC × GC–TOFMS instrument was the Pegasus 4D (Leco Corp.). This system is based on a non-moving quad-jet modulator made of two permanent cold nitrogen jets and two pulsed hot-air jets, which are responsible for trapping and refocusing of compounds eluting from the first dimension ^{1}D column. This modulator was mounted in an Agilent 6890 GC oven and liquid nitrogen was used to create the cold jets. Many types of column phase combination and temperature programs have been used and experimental condition will be mentioned when required. Details regarding the system have been reported elsewhere [27,28]. Data processing and display of the GC × GC chromatograms were achieved using the integrated Leco ChromaTOF^{TM} software. Peak apexes were found automatically and further manually corrected when required.

3. Results and discussion

3.1. GC–QISTMS/MS

QIST mass spectrometers have the capability to store selected ions [29]. The lack of selectivity due to the unit mass resolution is compensated by operating the instrument in the tandem mode (MS/MS or MS^2). This is referred as tandem-in-time mass spectrometry because the process takes place in three successive steps: (1) selected precursor (parent) ions are isolated in the ion trap after ionisation, (2) their dissociation by collision-induced dissociation (CID) occurs, and (3) the product ions (daughters) are sequentially ejected from the trap according to their mass and further detected by an electron multiplier [21].

The use of ion trap MS/MS for PCDD and PCDF analysis is based on the specific loss of a COCl$^{\bullet}$ fragment through a unique fragmentation reaction that produces the daughter ions [30]. For each analyte, it is necessary to monitor the production of at least two different daughter ions to check the

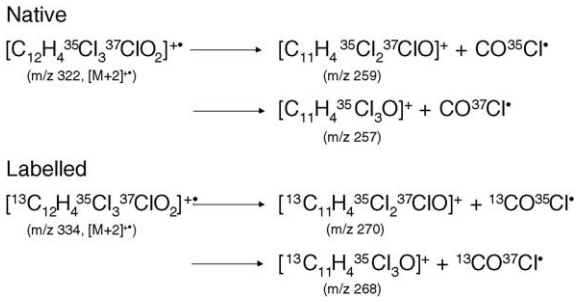

Fig. 1. Production of daughter ions from both native and ^{13}C-labelled TCDD species using QISTMS/MS.

isotope ratio. Precursor species containing at least one ^{37}Cl atom ($[M+2]^{\bullet+}$) must be isolated to ensure the production of both $[M-CO^{35}Cl^{\bullet}]$ and $[M-CO^{37}Cl^{\bullet}]$ ions for both native and labelled compounds (Fig. 1). The use of multiple reaction monitoring (MRM) allows to monitor the production of selected daughter ions for chromatographically co-eluting native and labelled compounds and perform ID [31]. The instrument alternatively scans in the native and label MS region and ion current can be reconstructed from those channels.

The ionisation (EI) can either occur in an internal source inside the ion trap, or in an external source with further acceleration of the produced ions through lenses and their introduction into the ion trap analyzer. In the case of PCDD/F and PCBs, no significant ionisation differences are observed when using one or the other approach. The isolation of molecular ions from the produced ions has to be optimised for each congener. Depending on the elution order (the chlorination level), segments are defined and specific isolation parameters are applied for each of them. The isolation of both native (^{12}C) and ^{13}C-labelled precursors is optimised to satisfy to ID requirements.

Although at least $[M+2]^{\bullet+}$ species have to be considered as precursor (Fig. 1), the choice of the parent ions is not only related to the relative isotope abundances (the more parents you isolate, the more daughters you potentially produce), but also by the isotopic ratio of the produced daughter ions. In fact, because the isotopic ratio check is carried out on the daughter ions, it is desirable to get similar abundances for both daughters to ensure accurate measurement of both isotope species at low concentrations. Therefore, as illustrated in Table 1, $[M+2]^{\bullet+}$ or $[M+4]^{\bullet+}$ parent ions are often selected. The loss in abundance of the parent ion gets limited when moving up in the chlorination level and is counterbalanced by better daughter ratios.

Optimum trapping efficiency of the precursor ions is ensured by the presence of helium, which acts as a buffer gas in the trap and slows down ion motion. As the instrument sensitivity is limited by the trapping efficiency of the precursor ion, recent instruments permit the tuning of

Table 1
Principal parameters for the MS/MS measurement of PCDD/Fs using QISTMS/MS

Segment #	Congeners	Isolation[a]	Dissociation[b]	QA/QC		
		Molecular ions (*m*/*z*)	Excitation amplitude (V)	Isotope ratios	Daughter ions (*m*/*z*)	Validity (±20%)
1	TCDD ^{12}C	322 (M + 2)	1.3 (5)[c]	0.33	257/259	0.26 < 0.33 < 0.4
	TCDD^{13}C	334 (M + 2)	1.3 (5)	0.33	268/270	0.26 < 0.33 < 0.4
	TCDF^{12}C	306 (M + 2)	1.6 (5.5)	0.33	241/243	0.26 < 0.33 < 0.4
	TCDF^{13}C	318 (M + 2)	1.6 (5.5)	0.33	252/254	0.26 < 0.33 < 0.4
2	PeCDD^{12}C	358 (M + 4)	1.3 (6)	0.66	293/295	0.53 < 0.66 < 0.8
	PeCDD^{13}C	370 (M + 4)	1.3 (6)	0.66	304/306	0.53 < 0.66 < 0.8
	PeCDF^{12}C	342 (M + 4)	1.6 (6)	0.66	277/279	0.53 < 0.66 < 0.8
	PeCDF^{13}C	354 (M + 4)	1.6 (6)	0.66	288/290	0.53 < 0.66 < 0.8
3	HxCDD^{12}C	392 (M + 4)	1.3 (6)	0.5	327/329	0.4 < 0.5 < 0.6
	HxCDD^{13}C	404 (M + 4)	1.3 (6)	0.5	338/340	0.4 < 0.5 < 0.6
	HxCDF^{12}C	376 (M + 4)	2 (6)	0.5	311/313	0.4 < 0.5 < 0.6
	HxCDF^{13}C	388 (M + 4)	2 (6)	0.5	322/324	0.4 < 0.5 < 0.6
4	HpCDD^{12}C	426 (M + 4)	1.5 (6)	0.4	361/363	0.32 < 0.4 < 0.48
	HpCDD^{13}C	438 (M + 4)	1.5 (6)	0.4	372/374	0.32 < 0.4 < 0.48
	HpCDF^{12}C	410 (M + 4)	2 (6)	0.4	345/347	0.32 < 0.4 < 0.48
	HpCDF^{13}C	422 (M + 4)	2 (6)	0.4	356/358	0.32 < 0.4 < 0.48
5	OCDD^{12}C	462 (M + 6)	1.5 (6)	0.6	397/399	0.48 < 0.6 < 0.72
	OCDD^{13}C	474 (M + 6)	1.5 (6)	0.6	408/410	0.48 < 0.6 < 0.72
	OCDF^{12}C	446 (M + 6)	2 (6)	0.6	381/383	0.48 < 0.6 < 0.72
	OCDF^{13}C	458 (M + 6)	2 (6)	0.6	392/394	0.48 < 0.6 < 0.72

[a] The q_z values were 0.3 and 0.45 when the damping gas flow was 0.3 and 1.7 ml/min, respectively.
[b] The excitation time was 10 ms.
[c] Values in parenthesis are voltages at damping gas flow of 1.7 ml/min and using an external source QISTMS.

the helium damping gas pressure inside the ion trap for each separate segment. In practice, although default damping gas flow values are 0.3 ml/min, higher flows (1.7 ml/min) have been optimized for PCDD/Fs [25,32]. The enhancement of the trapping efficiency, hence the later production of daughter ions, resulted in a three to five-fold sensitivity enhancement. Similar data were recently reported by Kemmochi et al. [33]. By summing the two 2,3,7,8-TCDD daughter ion masses (*m*/*z* 257 and 259), the reconstructed ion current (RIC) permitted to reach instrumental limit of detections (iLODs) of 200 fg, compared to the 1–5 pg range classically attained when low damping flows are used [24,34].

The CID process, responsible for the fragmentation of precursor ions in product ions, mainly depends on the excitation mode, the CID time, the excitation voltage and the stability parameter q_z, which is issued of the Mathieu second-order differential equation that accounts for the ion motion in the trap [35]. Two excitation modes (resonant and non-resonant) are available to increase the vibrational energy of the parent ions and conduct to their fragmentation with sufficient yields. Most of the MS/MS experiments described in the literature use resonant excitation mode. Application of high frequency dipole field to the end-cap electrodes of the ion trap allows to match the secular frequency of the trapped ion and results in an increase of the kinetic energy of the ion. The kinetic energy is transformed into internal energy upon collisions with the helium present in the trap. This internal energy is adequate to allow fragmentation reactions involving rearrangements via the breakage of multiple chemical bonds (loss of COCl•). The excitation voltage and the stability parameter q_z are closely linked and an optimum of the couple (CID voltage, q_z) has to be found for each congener. An optimum q_z value of 0.3 has been reported earlier for PCDD/Fs in conjunction with CID voltages ranging between 1.3 and 2 V [24]. Similar CID values were reported by other groups using resonant excitation [36]. The higher voltages correspond to PCDFs, which require higher activation energy for the loss of COCl• [37].

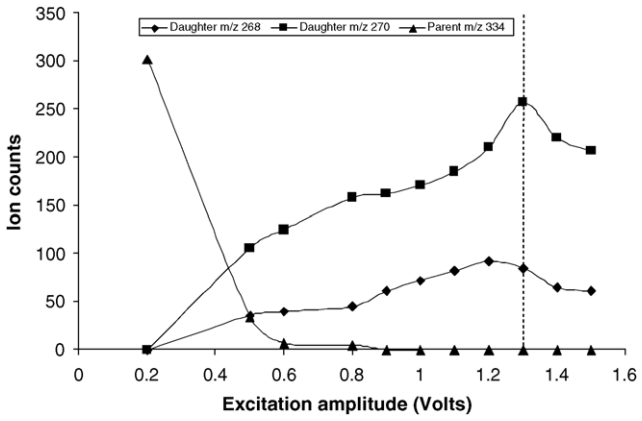

Fig. 2. QISTMS/MS fragmentation curves for the resonant excitation of 1,2,3,4-TCDD with a q_z value of 0.3. The maximum intensity for the daughter ions is obtained for a CID value of 1.3 V.

An example of dissociation curve is given for 1,2,3,4-TCDD in Fig. 2. Because helium also operates as a collision gas during the CID process, the use of higher values of damping gas flow during the isolation step influences the production of the daughter ions. In practice, because precursor ion motion is damped, more energy is required to excite the ions for CID fragmentation and, thus, higher CID amplitudes are required. CID voltages of 5–6 V have to be applied to the endcap electrodes to fragment tetra through octa-CDD/Fs. In that case, an optimum q_z value of 0.45 has been reported [25]. Table 1 summarises the optimized MS/MS parameters for the PCDD/Fs segments. Finally, relatively short excitation time values tend to offer better signal to noise ratio than longer ones, and a value of 5–10 ms is used with a typical scanning rate of 3 scans/s [23,34].

The MS/MS approach has been used to measure PCDD and PCDF levels in various types of matrices. Our early efforts, when the iLODs were not low enough to consider biota samples, were dedicated to the screening of contaminated fly ashes in conjunction with enzyme immunoassay (EIA) [24]. MS/MS data were highly correlated to HRMS data ($R^2 = 0.9987$) in a four orders of magnitude dynamic range. MS/MS relative standard deviations (RSDs) ranged between 10 and 15% (5 and 10% for HRMS). The combination of EIA for the first sorting out of samples and the use of physico-chemical MS/MS method for positive sample confirmation showed to be a viable one. More recently, we evaluated the use of a large volume programmable temperature vapourizer injection GC–QISTMS/MS method for measurement of dioxins in food and feed [25]. It appeared that a 10 μl injection volume of toluene extracts was the maximum to avoid facing excessive presence of toluene in the trap for hours and subsequent sensitivity drop. Such a limitation point out a drawback of QISTMS/MS compared to triple-stage quadrupoles (TSQ) MS/MS where species are separated in space rather than in time. If similar sensitivity is attained using QISTMS/MS and TSQMS/MS [38], the later suffers less from matrix effects due to the intrinsic difference in the CID process. In terms of sample preparation requirements, the 15-year-old report from Reiner et al. [40] stating that MS/MS and HRMS can filter out different types of interferences but that neither technique can remove all interferences is still true and efficient cleanup has to be implemented independently of the MS used.

The use of a back-flush valve in the injector permitted larger volume injections but significant losses of analytes were also observed because of the high boiling point of the solvent. The validation of the PTV-LV–GC–MS/MS alternative was carried out on various types of biological matrices to cover commonly encountered interferences. Fig. 3 illustrates the very good compound-specific correlation between HRMS and MS/MS data, even if higher standard deviations for the MS/MS method were obtained. In TEQ, the results indicate that no bias between the methods was observed in the range of 0.2–25 ng WHO–TEQ/kg using different matrices. Other reports also demonstrated the efficiency of QISTMS/MS for

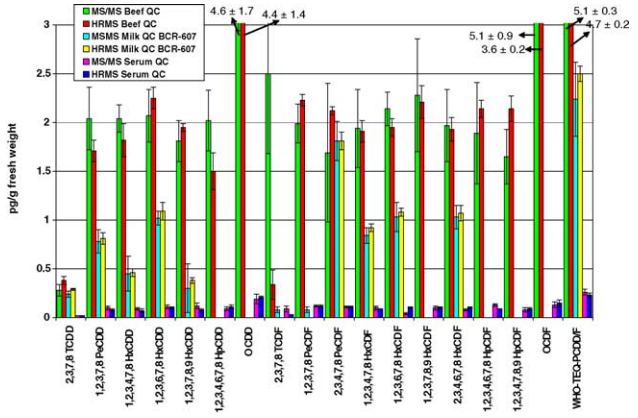

Fig. 3. Comparison of PTV-LV–GC–QISTMS/MS and GC–HRMS for the measurement of PCDD/Fs at the low pictogram level in biological matrices.

the measurements of PCDD/Fs in foodstuffs at low pictogram level [36,39].

QISTMS in tandem mode can also be used to measure PCB levels. Selectivity is ensured by monitoring the loss of a Cl_2 fragment through a unique fragmentation reaction that produces the daughter ions. Optimisation of the main parameters (Table 2) conducted to a rugged method, which has been applied to the measurement of the mono-*ortho* PCBs as well as the seven indicator PCBs (Aroclor 1260 mixture) in various types of biological matrices [12]. Malavia et al. [41] also reported the use of QISTMS/MS for the measurement of four non-*ortho* PCBs in biota samples using a similar approach.

A QISTMS-based method has also been developed for the measurement of PBDEs in biota samples [42]. EI was also used, instead of the more commonly used negative chemical ionisation (NCI), to ensure the monitoring of ^{13}C-labeled species for ID. Mass spectra are dominated by $M^{\bullet+}$ and $[M - Br_2]^{\bullet+}$ species for low and high degrees of bromination, respectively. The dissociation of the parent in daughter ions by CID was also congener-dependent, with loss of Br_2 (such as PCBs lose Cl_2) or loss of $COBr^{\bullet}$ (such as dioxins lose $COCl^{\bullet}$).

3.2. FGC–TOFMS

Reports on general principles and developments of TOFMS are available in the literature [43,44]. In TOFMS instruments, ions are accelerated to high velocity by an electric field in a flight tube. Since all ions have the same kinetic energy, the time ions take to traverse the flight tube is proportional to their masses. Light mass ions travelling faster than high mass ions. As a result of small kinetic energy distribution, a corrective electrostatic field (reflectron) is also applied at the end of the flight tube to ensure refocusing of similar masses before detection. The time to acquire a complete mass spectrum is limited by the flight time of the highest mass under analysis. A full mass spectrum can therefore be collected in less than 100 μs [45]. A unit *m*/*z* resolution TOFMS instrument is capable to acquire 5000 transients/s. Because several spectra have to be averaged to improve *S*/*N*, it results in 500 summed complete mass spectra/s for the mass range from 10 to 1000 *m*/*z*. Conversely to sector and quadrupole instruments, which offer limited scanning rates (ca. <20 scans/s) due to either the time required for electromagnets to change field strength or the limited ring electrode voltage ramp to be applied to maintain QISTMS unit *m*/*z* resolution, TOFMS analysers are a non-mass-scanning device because all ions are virtually collected at the same time.

Fast GC (FGC) type separations are appealing in terms of sample turnover but also because sharper and taller peaks are produced with potential subsequent improvement of the method sensitivity. The use of TOFMS as the detection device permits the accurate characterisation of those narrow peaks without the drastic loss in peak resolution usually observed when using low scan rate instruments and SIM or MS/MS mode [46]. TOFMS matches the speed of fast gas chromatographic separations and allows reliable reconstruction of re-

Table 2
Principal parameters for the MS/MS measurement of PCBs using QISTMS/MS

Segment #	Congeners	Isolation[a] Molecular ions (*m*/*z*)	Dissociation[b] Excitation amplitude (V)	Daughter ions (*m*/*z*)
1	TriCB^{12}C	258 (M + 2)	1.8	186/188
	TriCB^{13}C	270 (M + 2)	1.8	198/200
2	TeCB^{12}C	292 (M + 2)	1.2	220/222
	TeCB^{13}C	304 (M + 2)	1.2	232/234
3	PeCB^{12}C	326 (M + 4)	1.6	254/256
	PeCB^{13}C	338 (M + 4)	1.6	266/268
4	HxCB^{12}C	360 (M + 2)	1.8	288/290
	HxCB^{13}C	372 (M + 2)	1.8	300/302
5	HpCB^{12}C	396 (M + 4)	1.9	324/326
	HpCB^{13}C	408 (M + 4)	1.9	336/338
6	DeCB^{12}C	500 (M + 6)	2.1	428/430
	DeCB^{13}C	512 (M + 6)	2.1	440/442

[a] The q_z values were 0.3 and 0.45 when the damping gas flow was 0.3 ml/min and 1.7 ml/min, respectively.
[b] The excitation time was 10 ms.

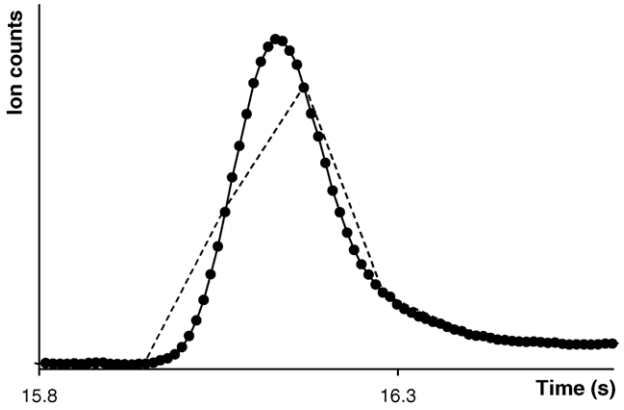

Fig. 4. Chromatographic peak profiles at scan rates of 10 (dashed lines) and 100 (continuous line) scans per second for a 300 ms peak.

sulting chromatograms (Fig. 4). Oppositely to the use of SIM mode with sector or quadrupole instruments, which consists in pre-selection of masses that will be collected during the analysis, a reconstructed ion chromatogram (RIC) can be extracted based on any ion included in the collected mass range once data collection is completed. By comparison to scanning MS, it is as if full scan data had been collected and that only few masses (native and labelled for example) were used to reconstruct the current, no SIM descriptors are required to improve sensitivity. Additionally, because all ion fragments represent the same time point on the chromatographic peak profile, there is no concentration bias and the ion ratio remains the same, ensuring spectral continuity. This important feature allows MS deconvolution of overlapping peaks if the fragmentation pattern is different. This backs up potentially poor chromatographic resolution situations [26].

Deconvoluted ion current (DIC) can thus be used to solve chromatographic co-elution problems that might arise while time-compressing the chromatograms. A method for high-throughput analysis of human serum for the 38 most prevalent PCBs in 8 min has been developed, based on the use FGC–IDTOFMS (Fig. 5) [26,47]. The separation of the congeners was carried out either chromatographically or using MS deconvolution. The instrument and the method (5 ml of serum) limit of detections (LODs) were 0.5 pg/μl and 20 pg/μl, respectively (*S/N* greater than 3), which is not as good as the one achieved using HRMS but allows the detection and quantification of the prevalent PCBs present in real human serum samples. Isotope ratio verification (^{35}Cl, ^{37}Cl) was carried out during the data processing using the two most intense masses for all native and $^{13}C_{12}$-labelled PCBs and several characteristic masses were summed for quantification. The dynamic range covered three orders of magnitude (0.5 pg/μl up to 1000 pg/μl). In terms of analyte concentration, the comparison with the HRMS reference method was good and some separation improvements were observed. Identical sample preparation steps were performed for the methods comparison. However, it appeared that the TOFMS instrument required less maintenance than the sector instrument in terms of ion source cleaning although full scan data obtained with the TOFMS instrument pointed out the poor quality of the extracts. The FGC–TOFMS method allows the analysis of 100 samples per day per instrument. Furthermore, because other POPs were present in the PCB cleanup fraction, we extended the measurement procedure to selected organochlorine pesticides (OCPs) [47]. The many new co-elutions between PCBs and OCPs were easily solved by MS deconvolution because the characteristic ion clusters were different.

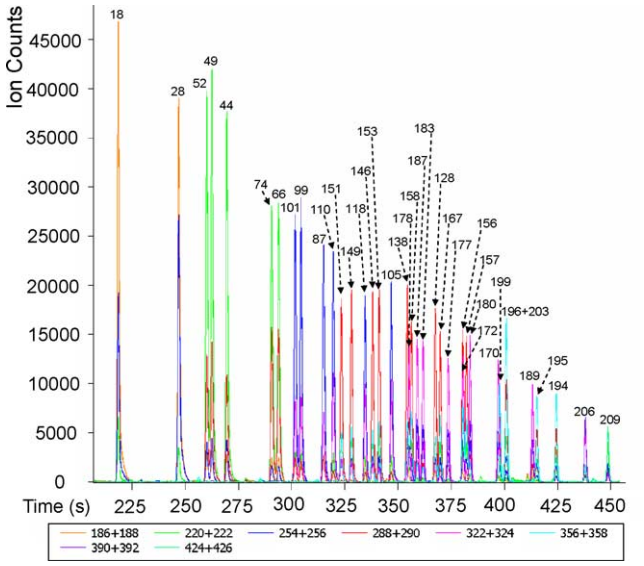

Fig. 5. FGC–IDTOFMS RIC chromatogram of 38 prominent PCBs found in human samples using a DB-XLB (25 m × 0.20 mm i.d. × 0.33 μm df) (J&W) column [26].

One could also mention the use of FGC–IDTOFMS as a screening tool capable to sort-out large biological sample batches prior further investigation. In fact, it appears that most of the total toxic equivalency (TEQ) of those samples is due to very few congeners (Table 3). The use of 2,3,7,8-TCDD, 2,3,7,8-TCDF, 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF as 'screening congeners' can allow the use of simplified ^{13}C-

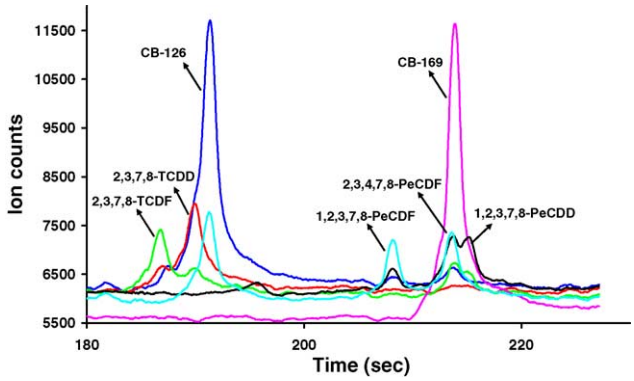

Fig. 6. Monitoring of selected 'screening congeners' by FGC–IDTOFMS using a DB-5 MS (15 m × 0.10 mm i.d. × 0.10 μm df) (J&W) column [49]. The cycle time was less than 10 min.

Table 3
Relative contributions of selected congeners to the PCDD/F TEQ in selected matrices

Matrices		Contributions to the TEQ (%)[a]		
		1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF	2,3,7,8-TCDD and 2,3,7,8-TCDF	Sum
Terrestrial	Horse	68	11	79
	Lamb	74	15	89
	Beef	79	6	85
	Pork	68	6	74
	Chicken	69	17	85
	Eggs	60	12	71
	Cream	86	0	86
	Butter	85	0	85
	Milk	75	8	83
	Powder milk	81	0	81
	Cheese	72	13	85
Aquatic	Prawns	55	36	91
	Mackerel	41	58	99
	Trout	58	40	98

[a] See [48] for the source of the data.

label standard mixtures, the time compression of the GC run and slight simplification of the samples preparation step [48]. Fig. 6 shows the type of separation that can be achieved in few minutes using classical GC injector and oven.

3.3. GC × GC–TOFMS

As we saw, TOFMS is well suited for the analysis of toxicants like PCBs. However, this type of instrument has a rather limited sensitivity, which does not allow low picogram detection with sufficient reliability. Comprehensive GC × GC is a relatively new technique that rests on the use of two different GC column phases to improve the chromatographic separation efficiency. Its comprehensive aspect is due to the fact that all eluents from the first dimension column (^{1}D) are re-injected into the second dimension column (^{2}D) with conservation of the resolution already achieved in ^{1}D. Extensive review of the principles of the technique is available in the literature [49,50]. GC × GC offers several advantages over classical GC [51]. Among them, on the side of the significant peak capacity enhancement, an increase in peak intensity is obtained after zone compression due to the modulation of the eluents of ^{1}D. Because narrow peaks are produced after modulation, mass conservation ensures higher peak intensities [52]. This is of prime interest when LODs of a detector need to be improved, as it is the case for TOFMS.

The coupling of GC × GC with TOFMS has been presented as a comprehensive three-dimensional system in which gas sample components go through three dissimilar separation mechanisms based, for example, on analyte volatility, polarity, and mass [53]. Early work on GC × GC–TOFMS were limited by the data handling and processing but more recent reports presented GC × GC–TOFMS as a promising tool for the analysis of complex mixture of analytes at the pictogram level [54–56]. Classically, ^{2}D peak widths are 100–200 ms. The coupling between GC × GC and TOFMS is thus symbiotic because the GC × GC component allows signal enhancement and improvement of the TOFMS LODs, although TOFMS is the fast mass analyser of choice for the description of narrow ^{2}D peaks. Very recently, a robust GC × GC–TOFMS instrument has been launched on the market. This contributed to move the technique from its childhood stage to a more mature status, making it a tool to be evaluated in various areas of separation science.

The use of GC × GC–TOFMS for the isotope dilution measurement of dioxins and related compounds in environmental matrices such as soils and ashes showed to be correlated to GC–IDHRMS data [57]. The use of a ^{1}D RTX-Dioxin2 specialty phase combined with a more polar RTX-500 phase permitted the resolution of all PCDD/F congeners of interest in ^{1}D although remaining matrix interferences were kept away from analyte peaks in ^{2}D. The iLODs were as low as 0.2–0.5 pg for 2,3,7,8-TCDD. The calibration curves showed good correlation coefficients for the 17 PCDD/Fs in the concentration range of 0.5–200 pg (Table 4). Such sensitivity should allow to fulfil requirements such as the EU maximum levels in foodstuffs [58], depending on congener profile. Current developments are carried out to ensure efficiency at the EU target level. The comprehensive mass analysis of the TOFMS further permits the identification of other contaminants of concern such as polychlorinated naphthalenes (PCNs), polycyclic aromatic hydrocarbons (PAHs), PCBs and PBDEs present in the samples. Measurements of PCDD/Fs and PCBs in human and foodstuff matrices using GC × GC–IDTOFMS have also been reported recently [59]. The sample preparation-fractionation procedure designed for GC–IDHRMS analyses can be modified because several fractions can be combined and a single injection can be carried out for the measurement of non-*ortho* PCBs, mono-*ortho* PCBs, and indicator PCBs, as well as PCDDs and PCDFs. For simultaneous measurement of PCBs, OCPs and PBDEs in human serum samples [60], a 10-times sensitivity enhancement resulted from the compromise between GC × GC zone compression and the TOFMS scanning rate. This is illustrated in Fig. 7 for 2,3,7,8-TCDF. Because the modulation process

Table 4
Trueness of the GC × GC–IDTOFMS response for 2,3,7,8-TCDF in the working dynamic range based on ^{12}C native (*m*/*z* 304 and 306) and ^{13}C labelled (*m*/*z* 316 and 318) ions

Expected concentration	Average measured concentration	R.S.D. (n = 3) (%)
200	198.0	3.0
40	41.5	1.4
10	10.3	3.8
2	1.9	3.9
0.5	0.5	6.4

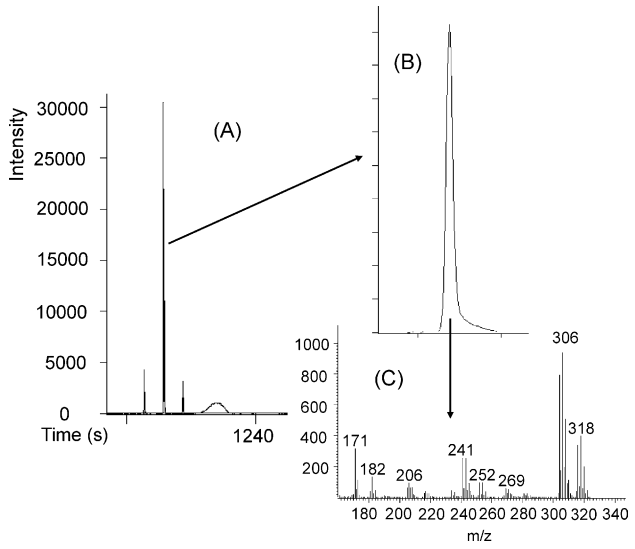

Fig. 7. Signal enhancement for 200 pg of ^{12}C 2,3,7,8-TCDF. (A) The 3 pulses correspond to the modulated GC × GC peaks and the small Gaussian peak is the classical ^{1}D signal, (B) zoom on the base peak of the cluster showing both traces for ^{12}C native (*m*/*z* 306, black) and ^{13}C label (*m*/*z* 318, grey), (C) mass spectra of the base peak.

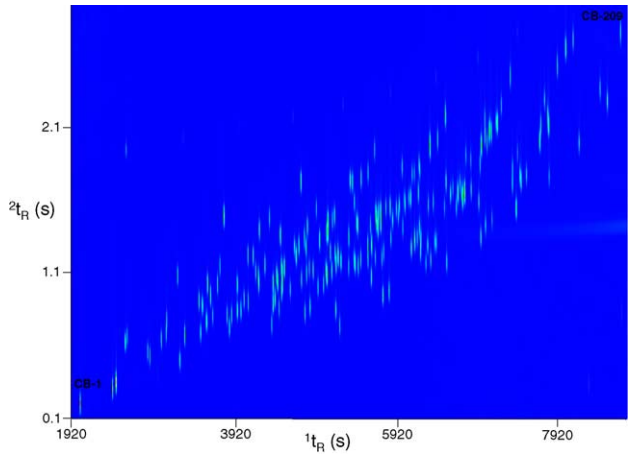

Fig. 8. GC × GC–TOFMS contour plot of the 209 PCBs. The distribution of the peaks was highly structured in the chromatographic space and based on the degree of *ortho*-substitution within each separated homologue series. A total of 192 congeners were resolved (chromatographically or by mean of deconvolution) in 146 min (1.3 analyte per min) using this column set. The 12 toxic dioxin-like congeners and the 7 European Union marker PCBs were separated from any interfering congeners. The column set was made of a HT-8 (50 m × 0.22 mm i.d. × 0.25 μm df) (SGE) as ^{1}D and a BPX-50 (2.5 m × 0.10 mm i.d. × 0.10 μm df) (SGE) as ^{2}D. See previous report for complete peak assignment [28].

generates up to five peaks for each analyte, the peak integration procedure is more complex than in the case of classical GC and peak combination has to be carried out to perform the quantification. This resulted in a significant increase of the processing time and enlargement of file size, which can be up to several hours and several Gb for a calibration curve, respectively.

Other column sets have been investigated in the area of dioxin and PCB separation using GC × GC [61]. Very fine-tuning of the phase combination is possible to get the best chromatographic separation for very complex mixtures such as the 209 PCB congeners (Fig. 8) [28,62]. In such a case, if one cannot strictly talk about fast GC conditions (total run time of 140 min), the number of analytes separated per unit of time (analytical speed) is at least as good as in classical GC (1.3 analyte per min). The use of μECD instead of TOFMS can improve the system LODs [63,64]. Korytár et al. reported LOD values of 90 fg for 2,3,7,8-TCDD. The choice between electron capture or MS type detectors depends on the specific application area of the method. The relatively cheap μECD better matches the needs for screening of well defined analytes although TOFMS offers mass spectral data that can be used to confirm the presence of expected analytes and to identify unexpected analytes when seeking for new toxicants. In addition, TOFMS produces less post-column band broadening than μECD and narrower peak widths might permit easier peak identification when retention times are tightened. In cases where chromatographic resolution has to be compromised in favour of speed, the mass spectral deconvolution capability of the TOFMS can be a valuable tool as well. The next generation of GC × GC instruments will probably simultaneously offer both type of detection to combine the sensitivity of the μECD and the selectivity of the TOFMS.

Finally, because of the resulting zone compression after modulation, another field of application for GC × GC is its use as a signal enhancer, rather than to increase the peak capacity of the chromatographic separation. A current area of efforts is the coupling between GC × GC and sensitive sector HRMS instruments. In the case of PCDD/Fs, where a good separation of the seventeen 2,3,7,8-substituted congeners can be achieved in less than 40 min with classical GC, the use of the GC × GC modulator with a short piece of open tube as ^{2}D can improve instrument LODs. Early promising results were in the low femtograms-high attograms range for 2,3,7,8-TCDD [65]. Improvements of some aspects like sector MS scanning rate and data handling still need to be carried out to offer the robustness required for routine use of this extremely sensitive tool for ultra-trace analysis.

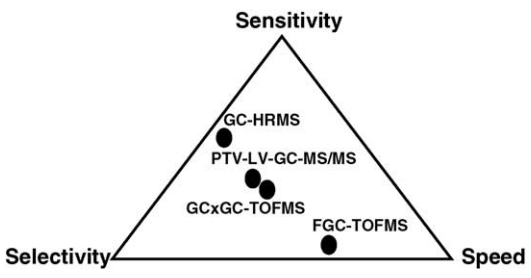

Fig. 9. Analytical triangle of the investigated measurement methods.

Table 5
Comparison between the main characteristics of the MS-based analytical methods

	GC–IDHRMS	PTV-LV–GC–IDMS/MS	FGC–IDTOFMS	GC × GC–IDTOFMS
Investment cost (€)	350,000	140,000	170,000	240,000
Operating cost	+++	++	+	+
Sample turnover	+	+	+++	+
Number of analytes per unit of time	+	+	+++	+++
iLODs	+++	++	+	++
PCDD/F measurement	+++	++	–	++
PCB measurement	+++	+++	+++	+++
Unknown measurement	–	–	++	+++

4. Conclusions

The implementation and feasibility of efficient measurement campaigns depend on several factors among which the versatility of the analytical methods to be used is of prime importance. The cost, the rapidity, and the robustness of a method have to be optimised for it to be a commercially viable tool. However, efforts in that direction are confined in a working area where the quality of the results cannot be compromised. Alternative MS tools exist in addition to the reference sector HRMS instruments for the measurement of dioxins and related compounds. PTV-LV–GC–IDMS/MS based on QISTMS, FGC–IDTOFMS and GC × GC–IDTOFMS are among the most investigated ones. Although none of them offers the sensitivity usually attained by GC–IDHRMS, they consist in viable approaches in terms of versatility, sample turnover, and cost. Fig. 9 shows a comparison of those techniques regarding sensitivity, selectivity and speed. The PTV-LV–GC–IDMS/MS and GC × GC–IDTOFMS can be found in an area where a relatively good compromise is taken between those parameters. PTV-LV–GC–IDMS/MS and GC × GC–IDTOFMS have similar iLODs (0.2 pg) but the later is the most suited to fulfil both selectivity and speed requirements simultaneously. Additionally, TOFMS instruments, especially when coupled to GC × GC, seem to be able to handle more matrix interferences than QISTMS instruments, potentially reducing the cleanup requirements of the method. The various techniques have advantages and limitations (Table 5) and their applicability depends on the specific field of application and on the set of analytes to be reported. The coming years will show us how those alternative techniques will evolve and which place they can reach in the field of dioxin and related compound measurement.

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