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Validation of a gas chromatography–triple quadrupole mass spectrometry method for confirmatory analysis of dioxins and dioxin-like polychlorobiphenyls in feed following new EU Regulation 709/2014☆

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ABSTRACT

The European Regulations laying down methods of sampling and analysis for the EU official control of levels of polychlorodibenzo-*p*-dioxins (PCDDs), polychloro-dibenzofurans (PCDFs), dioxin-like (DL) and non dioxin-like (NDL) PCBs in food and feed have been recently amended by EU Regulation Nos. 589/2014 and 709/2014. A major update is the recognition of gas chromatography (GC) triple quadrupole mass spectrometry (GC–QQQMS/MS) as a confirmatory tool for checking compliance with maximum levels (ML). These revisions have been initiated since this technology now exhibits similar performances to GC (magnetic sector) high resolution mass spectrometry (GC–HRMS). In this paper, we show a fully validated method for PCDD/Fs and DL-PCBs analysis in feed material of plant origin (vegetable oil) using GC–QQQMS/MS following the dedicated EU Regulation 709/2014. We show that individual analytical criteria (selectivity, linearity, quant/qual MRM transitions, accuracy around ML of 1.50 ng WHO_{2005}TEQ/kg, within-lab reproducibility, robustness, and background subtraction) meet the strict requirements set by the EU Regulation. We also propose a clear interpretation of instrumental limit of quantitation (iLOQ) as a 'performance-LOQ', defined in a specific way for GC–QQQMS/MS, and method limit of quantitation (mLOQ) as 'real-LOQ' that is used to report bound results. Eventually, the evaluation of measurement uncertainty, following a top-down approach and data produced with our method, demonstrates similar results than with GC–HRMS, thus offering a reliable alternative to the standard method for vegetable oil.

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1. Introduction

Humans all over the world are exposed to chemicals during their lifetime. Among the thousands of toxic compounds for humans are the polychlorodibenzo-*p*-dioxins (PCDDs), polychloro-dibenzofurans (PCDFs), and polychlorobiphenyls (PCBs). Those families of compounds are classified as persistent organic pollutants (POPs) and have been regulated [1], following the Stockholm convention for persistent organic pollutants in 2001, in order to protect human health and environment. Food consumption, and especially high fat content food, is the main route of exposure to PCDD/Fs and PCBs for humans [2,3]. Therefore the European Commission requires that any food or animal feedstuffs released on the market must be controlled and must comply with maximum levels (MLs) set by a panel of experts. More generally, a continuous food and feed control is enforced in Europe (not only for dioxins or PCBs) in a clear and defined framework of Directives and Regulations. Such legislation contributes to reducing human exposure over time and practically decreases human daily intake efficiently [4,5].

The official continuous control strategy for dioxins was implemented in early 2000s, after the dioxin crisis that happened in Belgium in 1999 had highlighted the lack of efficient control of our food web [6,7]. From post-crisis Directive 2001/102/EC [8] and Regulation 2375/2001 [9] establishing, respectively, MLs for PCDD/Fs in feeding stuffs and foodstuffs, to more recent Regulation 277/2012 [10] and Regulation 1259/2011 [11], several amendment such as the introduction of the 'action' level [12], the 'upper-bound' concept

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[13], and the inclusion of dioxin-like (DL-)PCBs [14] and six marker or indicator non dioxin-like (NDL-)PCBs (PCB 28, 52, 101, 138, 153 and 180) [11] have taken place. In order to enforce such an ambitious continuous monitoring and ensure rapid action to be taken in the framework of the Rapid Alert System for Food and Feed (RASFF) in case of non-compliance, these legislations were supported by the enactment of the screening-confirmatory strategy [13,15]. At that time, because no standardized or harmonized methods were available (in the early years two thousands), harmonized quality criteria were proposed for biological and gas chromatography coupled to mass spectrometry (GC–MS) measurements [16,17]. Appropriate analytical requirements such as sensitivity, selectivity, and accuracy were described in details. Rather than a standardized method, a performance-based measurement system (PBMS) approach was however selected to allow laboratories to use their own methods as far as they were able to demonstrate their fit for purposeness. The general concept of harmonized quality criteria was not intended to offer specific or practical solutions to the analyst's daily challenges. It actually offered the necessary flexibility to develop state-of-the-art methods, to integrate new knowledge and technologies but, also, took into account that laboratories may execute protocols in slightly different manner in daily routine or may need to modify their analytical procedure in order to control/eliminate interferences. The decisions taken at that time were clearly oriented to help analytical chemists to do and to use good science with the aim to generate effective data that fit for their intended use [15].

One of the major harmonized quality criteria for the confirmatory method was the use of ^{13}C-labeled isotope dilution (ID) process for quantitation, and GC coupled to (magnetic sector) high resolution MS (GC–HRMS) for analysis. This is based on the fact that GC–HRMS was the most sensitive and selective tool for such measurements, especially when compared to other MS analyzers such as time-of-flight MS (TOFMS), single quadrupoles MS (QMS), and quadrupole ion storage tandem-in-time MS (QISTMS/MS) [18], that did not meet required performances. QISTMS and TOFMS coupled to various types of GC have been reported to be well suited for the measurement of dioxins, but still currently suffer from a lack of sensitivity at the ML [19–22]. However, recent technical progresses and developments in the area of GC coupled to tandem-in-space MS (GC–MS/MS) using triple quadrupole analyzers (QQQ), initiated the revision of this specific criteria as this modern instrumentation exhibited similar performances to GC–HRMS [23–26]. Based on these reports, a working group formed within the network of European Reference Laboratory (EU-RL) and National Reference Laboratories (NRLs) of EU Member States has successfully investigated the capability of GC–QQQMS/MS for potential use as an alternative confirmatory method for checking compliance with MLs. Hence, some specific criteria were proposed as basic requirements for dioxin confirmatory analysis using GC–QQQMS/MS [27]. These criteria were further validated at the EU level and Regulation (EU) Nos. 252/2012 [28] and 152/2009 [29] have therefore been amended by a new Commission Regulation (EU) 589/2014 [30] and 709/2014 [31], referring to the use of GC–QQQMS/MS as an appropriate confirmatory method for checking compliance with the ML for food and feed control, respectively. Starting 23rd of June 2014, GC–QQQMS/MS has thus been recognized as a confirmatory tool that provide information enabling PCDD/Fs and DL-PCBs to be identified and quantified unequivocally at MLs and, in case of need, at the action thresholds for the official control of dioxins in food and feed. In addition to regular control at fixed levels, the investigation of low background levels is also of prime interest to allow proper exposure assessment, for establishing congener patterns in order to identify the source of a possible contamination, and to set targets for the re-evaluation of maximum and action levels. At the moment, for the measurement at such trace levels, typically below one fifth of the level of interest in food-feed, the use of GC–HRMS is still recommended to attain sufficient sensitivity.

In this paper, we report on a fully validated GC–QQQMS/MS method for the control of PCDDs, PCDFs, and DL-PCBs in a challenging feed matrix (vegetable oil). We aim to show that the entire method meets the strict requirements set by the EU Regulation in terms of performances and individual analytical criteria for the production of reliable results at MLs. In the study we do not simply compare duplicate analysis by GC–HRMS and GC–QQQMS/MS, but rather only focus on the latter as the following analytical criteria are individually studied in details: selectivity, linearity, quantitation and qualification (Quant/Qual) multiple reaction monitoring (MRM) transitions, accuracy (trueness and precision), robustness, background subtraction and blank calculation. A special attention was further dedicated to the proper establishment of limits of detection and quantitation because of the fact that GC–QQQMS/MS data are usually free of the noise typically required to estimate these values. Eventually, we assessed the measurement uncertainty of the method using data specifically produced using this instrumentation. In order to enhance the robustness of our results beyond vegetable oil, we based our investigations on fortified quality control (QC) samples, fortified vegetable oil samples, real samples (unknown), procedure blanks, and proficiency test (PT) samples.

2. Materials and methods

2.1. Chemicals and consumables

Solvents (hexane, toluene, methanol, and dichloromethane) were Picograde® reagents (LGC Promochem, Wesel, Germany). Nonane puriss analytical-reagent grade standard for GC was purchased from Fluka (Steinheim, Germany). Water was obtained from a Milli-Q Ultrapure water purification system (Millipore, Brussels, Belgium). All solvent batches were tested for studied analytes contamination before use. Disposable PTFE columns for the automated clean-up were obtained from Fluid Management Systems (FMS Inc., Waltham, MA, USA). Chromatographic pure grade helium gas, 99.9999% alphagaz 2 was purchased from Air Liquide (Paris, France). Technical N27 grade liquid CO_2 was used for PTV cooling (Air Liquide, Paris, France). All congeners of PCDD/Fs (2,3,7,8-substituted) and non-*ortho* (NO-)PCBs (PCBs #81, 77, 126, 169) were quantitated against their own ^{13}C-labeled internal standards (EDF-4144, Cambridge Isotope Laboratories (CIL, Andover, MS, USA)). Recoveries were measured with recovery standards (EDF-4145, syringe standard (CIL)). These standards were used to assess the loss of compounds during analysis (internal standard vs recovery standard). The quantitation was not affected by any loss of compounds since all analytes were quantitated by isotopic dilution against ^{13}C-labeled internal standards. Calibration curve standards were purchased from CIL for PCDD/Fs and NO-PCBs (EDF-4143). Concentrations of native and labeled compounds were summarized in a previous study [32]. The ^{13}C-labeled mono-*ortho* (MO-)PCBs (including PCBs #105, 114, 118, 123, 156, 157, 167, 189) internal standard spiking solution (MBP-MKX) was purchased from Wellington Laboratories (Guelph, Canada). The calibration curve for MO-PCBs and NDL-PCBs (PCBs #28, 52, 101, 138, 153, 180) was prepared using EC-4987, EC-5179, EC-4058 (CIL), and MBP-MKX standards. Six levels were prepared for MO-PCBs: 1-4-10-20-40-80 pg/μL and a seventh for NDL-PCBs at 1000 pg/μL. The ^{13}C-labeled PCB-80 was used as recovery standard for all PCBs. An internal standard mixture solution made of ^{13}C-labeled PCDDs, PCDFs, NO-PCBs, MO-PCB, and NDL-PCBs was prepared to facilitate spiking of samples. NO-PCBs and MO-PCBs are grouped under the term dioxin-like PCBs (DL-PCBs). Quality control (QC) samples consisted in pork fat used in routine for control charts. Procedure

blanks followed the same procedure than the samples and were recorded on the same rate than QCs.

2.2. Sample preparation

When required, the extraction of fat was performed using accelerated solvent extraction (ASE™ 350, Dionex, Thermo Fisher Scientific). The present study aimed to validate a method for vegetable oil of plant origin, which consisted in feed material. Such oil samples did not require pre-clean-up fat extraction and where directly processed though the multistep automated clean-up and fractionation procedure. The sample intake was 4 g, following our in-house accredited (ISO17025) routine lab procedure for vegetable oil. Samples were first spiked with internal standards, diluted in 10 mL hexane and then loaded on the automated PowerPrep™ system (FMS Inc, Waltham, USA) for multiple column clean-up and fractionation. The method has previously been described in details by Focant et al. [33]. Briefly, samples were loaded on a mixed bed acid/basic silica column for lipid breakdown, then passed through a partly deactivated basic alumina column for removal of interferences, and finally ended up in a carbon-based column for separation of non-planar from planar species. Two fractions were subsequently collected: the first by forward elution of MO/NDL-PCBs with a mixture of hexane/dichloromethane 50:50, the second by reverse elution of PCDD/Fs and NO-PCBs with toluene. Volumes of hexane/dichloromethane 50:50 and toluene were reduced in a dedicated tube using a sensor-equipped TurboVap II Workstation (Caliper Life Science, Teraflene, Belgium) and after transfer in GC vials using a RapidVap (Labconco, Kansas City, MO, USA). Nonane was added as keeper (90 μL for MO-PCBs and NDL-PCBs fraction, and 4 μL for the PCDD/Fs and NO-PCBs fraction) and recovery standard was added prior analysis.

2.3. Instrumentation and measurements

For analysis, we used a 7000 C GC–QQQMS/MS system from Agilent (Palo Alto, CA, USA) equipped with a 7890B GC oven, a programmable temperature vaporization (PTV) inlet, and a 7693A automated liquid sampler (ALS). We injected 5 μL of the nonane purified extract for PCDD/Fs and NO-PCBs analysis whereas we injected 2 μL of the nonane purified extract for MO and NDL-PCBs. The PTV was operated on solvent vent mode and cooled by liquid CO_2. The inlet temperature program was: start at 45 °C (3 min) and ramp at 720 °C/min until 320 °C; vent flow was 100 mL/min at pressure of 10 psi for 2.8 min. Purge flow was set to 1200 mL/min after 5 min. GC column was the classic DB-5 ms 60 m × 250 μm × 0.25 μm (Agilent). The GC oven temperature program for dioxin fraction was: start at 120 °C (5 min), ramp at 25 °C/min until 250 °C (5 min), then 3 °C/min until 285 °C (15 min) for a total runtime of 41.6 min. For the MO-PCB fraction, we started the same program but left 0 min at 285 °C. The transfer line temperature was held at 280 °C. On the MS side, we used the 7000C electron ionization (EI) ion source heated at 280 °C and operated at 70 eV, quadrupoles held at 150 °C, a nitrogen collision flow of 1.5 mL/min, and helium quench flow of 2.25 mL/min. Quads resolution was set to unit mass, which by default corresponds to peak width of 0.7 Da at half height.

Measurements were carried out in Multiple Reaction Monitoring (MRM) mode. Dwell times were selected and acquisition windows adjusted to optimize acquisition frequency to get ten data points per peak. Calibration and tune of the instrument were performed in the EI high sensitivity autotune mode every ten days and performances of the instrument were checked at the same time. We locked retention times to PCB-105 allowing to change and cut the head of the column for other purposes and bring back the system to the original configuration without adapting retention times in the acquisition and quantitation method. It was done by automatically slightly adjusting the pressure inside the column to get original retention times.

Two MRM transitions were monitored for each target for quantitation ('Quant transition') and qualification ('Qual transition') purposes. Each Quant/Qual transitions were recorded from two specific precursor ions (usually 2 Da offset) and two distinct product ions. Quantitation was performed with the Quant transition only and the Qual transition was exclusively used to verify ion ratio between Quant/Qual transitions. The expected ratio was determined experimentally using standards at constant collision energy. In case of deviation to this ratio, we checked the correct integration of peaks to fall within the acceptable tolerance interval specified by the Regulation (±15%) [31]. This procedure limited the risk of integrating wrong peaks or interferences.

QCs and procedure blanks samples were analyzed and reported on a QC chart over a discontinuous period of 9 months. The monitoring of blanks allowed to track possible fluctuations of the contamination of the laboratory environment, which was closely controlled since the average blank level was subtracted from samples and not individual blanks belonging to series. We discuss the advantage of this correction in Section 3.4.

3. Results and discussion

3.1. Selection of the validation criteria

The recent EU Regulation No. 709/2014 lists specific requirements that GC–MS methods have to comply with for confirmatory purposes. Because GC–HRMS performs in selected ion monitoring (SIM) mode and GC-QQQMS performs in tandem (MS/MS) mode, some of these criteria are dependent of the type of MS analyzer used (i.e. ion selection, LOQ determination, ...). Other requirements are however the same for both approaches (i.e. selectivity, upper-bound and lower-bound differences, ...). In the present study we considered each of these criteria and carried out a full validation of the method in accordance with the Regulation. We systematically investigated all points, parameters, and performances to reach to perform the validation. Table 1 summarizes all criteria to be met for GC–QQQMS/MS in order to use this instrumentation as a confirmatory method for official control of dioxins in feed.

Because we performed the validation using vegetable oil, a challenging feed matrix for which MLs are amongst the lowest and set at 1.5 pg WHO_{2005}-PCDD/F-PCB-TEQ/g (ppt) [10], it could easily be transposed to other feed and foodstuffs as MLs are higher and analytical criteria are the same. Levels are reported in WHO_{2005}-TEQ/g (toxic equivalent quantity per gram), which is the sum of the concentration of each individual congener corrected by a toxic equivalency factor (TEF) proposed by the World Health Organization in 2005. Criteria for NDL-PCB measurement in food-feed are generally less stringent than for PCDD/Fs and DL-PCBs [31] and MLs are at the ng/g (ppb) levels, which makes their proper measurement easier to attain. We however decided to apply the strict criteria of PCDD/Fs and DL-PCBs measurement to NDL-PCBs for the validation. As an example, despite the fact the legislation state that one precursor ion for Quant/Qual MRM transitions and two distinct product ions have to be followed for NDL-PCB measurements, we followed two specific precursor ions, as it is required for PCDD/Fs and DL-PCBs.

3.2. Method limit of quantitation and instrumental limit of quantitation

The proper establishment of the limit of quantitation (LOQ) is one of the major differences between the GC–HRMS method

Table 1
Criteria to be met for GC–QQQMS/MS for analysis of dioxin according to EU Regulation.

Criteria	PCDD/Fs and DL-PCBs GC–QQQMS/MS (EU No 589/2014)	NDL-PCBs GC–QQQMS/MS (EU No 589/2014)
Detectable quantity	- PCDD/F upper femtogram (10^{-15} g) - NO-PCB low picogram (10^{-12} g) - MO-PCB nanogram (10^{-9} g)	NDL-PCB nanogram (10^{-9} g)
Selectivity	- Chromatographic separation of 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF <25% valley peak to peak	Relative retention time ± 0.25% Internal standard signal vs analyte signal
Multiple reaction monitoring (MRM) transitions	- Monitoring 2 specific precursors with each specific product ion transition for all labeled and unlabeled analytes - Relative ion intensities max ± 15% - Resolution MS quadrupoles = unit	- Monitoring at least 1 precursor ion and 2 product ions - Tolerance ratio ± 20% if rel. intens. > 50% - Tolerance ratio ± 25% if rel. intens. 20–50% - Resolution MS quadrupoles = unit
Blank	- Used for LOQ calculation	- Used for LOQ calculation - Blank value < 30% of maximum level ML
iLOQ	- iLOQ calculated from lowest calibration point - lowest concentration point on cali. Must give acceptable and consistent deviation to the average relative response factor (RRF) - Average RRF calculated for all points - Deviation to average RRF <30%	- ditto
LOQ	- LOQ calculated from average blank level - LOQ < 20% of ML - Difference upperbound (ub) and lowerbound (lb) levels <20%ML	- ditto - Diff. ub and lb for sum NDL-PCB@ML < 20%
Accuracy	- Demonstrate performances at 0.5ML, ML, 2ML - Trueness (accuracy) ± 20% - Within-lab reproducibility (RSD) <15%	- Demonstrate performances at 0.5ML, ML, 2ML - Trueness for sum NDL-PCB@ML ± 30% - Within-lab reproducibility (RSD) <20%
Control	- QC control chart for blanks - QC control charts for sample	- QC control chart for blanks - QC control charts for sample
Recovery	- Individual internal standard in range 60–120% - Out of range OK if contribution to TEQ < 10%	- Individual internal standard in range 50–120% - Out of range OK if contribution to sum NDL-PCB < 10%
Measurement uncertainty	- Expanded measurement uncertainty - Coverage factor = 2 (Confidence level = 95%)	- Expanded measurement uncertainty - Coverage factor = 2 (Confidence level = 95%)

and the GC–QQQMS/MS method. Although a S/N (signal/noise) ratio calculation is performed on raw signals to establish LOQs in GC–HRMS, this cannot be performed for GC–QQQMS/MS because quadrupoles very efficiently filter ions with the direct consequence of reducing the noise tremendously. A noise-free signal is therefore expected next to the peaks and any S/N calculation based on such a flat baseline would lead to unrealistic S/N ratio values. In the EU Regulation, this issue is partly addressed as it is stated that '. . .the limit of quantitation may be identified as the lowest concentration point on a calibration curve that gives an acceptable (≤30%) and consistent (measured at least at the start and at the end of an analytical series of samples) deviation to the average relative response factor (RF) calculated for all points on the calibration curve in each series of samples.' It however does not explain precisely how to calculate the LOQs, and this definition of LOQs can lead to several interpretation and different ways to assess these limits. This is undesirable as improper establishment of LOQs of individual congeners might lead to overestimation of the capabilities of the method to be able to perform at levels of about one fifth of the ML (an EU requirement). Additionally, it can impact the calculation of the differences between upper-bound and lower-bound results that has to be lower than 20%.

For this validation exercise, we differentiated between the instrumental limit of quantitation (iLOQ), which is a 'performance-LOQ', and a method limit of quantitation (mLOQ), which is the 'real-LOQ' that takes possible matrix effects and blank levels into account. Based on a report from a EU core working group composed of members from EU national reference laboratories (NRLs) and expert laboratories [27], we used a statistical approach for specifically assessing iLOQ, of the GC–QQQMS/MS method. iLOQs were calculated using 8 replicate injections of the lowest acceptable calibration point, and iLOQs were further defined as 10 times the standard deviation (SD) associated to these replicates. The lowest acceptable calibration point was determined according to the two following criteria. First, the calculated RSDs of the lowest level for all congeners (Table 2) must be ≤15% (this is the 'consistent deviation to relative response factor'). The 15% criterion was not included in the standard as such but we used the typically accepted value for RSDs expectations at such low levels. Second, the relative difference between the average response factors (RF) obtained for all points (including replicates) and the average response factors obtained for only the lowest point must be ≤30%, according to the Regulation (this is the 'acceptable deviation to the relative response factor'). When these criteria were met, the linearity was acceptable in the calibration range and the resulting lowest calibration level was eventually used to determine iLOQ as explained before. In Table 2, one can see that 1,2,3,6,7,8-HxCDF had an RSD of 17.9% for triplicates of the lowest point, which is supposed to be an exclusion value. Nevertheless, we decided to keep this lowest point as the other criteria were very successfully fulfilled (−1.21% difference between the average RF of the lowest point and the average response factor of all points, and very good R^2 0.9990). Results for PCDDs, PCDFs, NO- and MO-PCBs appeared to be better than for NDL-PCBs. For NDL-PCB congeners, a lowest calibration point at 1 pg/μL was thus excluded (due to RSD > 15% and difference between RF > 30%) and iLOQs were determined based on the calibration point at 4 pg/μL for which all RSD values get back far below 15%. Three of the 6 NDL-PCBs (PCB-138, PCB-153, and PCB-180) had R^2 correlation criteria slightly below the 0.9900 ideal value but had very low differences between RF and iLOQs were kept at 4 pg/μL. Globally, iLOQ values were similar to the ones obtained for GC–HRMS (not shown) where their establishment is based on S/N ratio.

Table 2
Calibration curve data and instrumental limits of quantitation (iLOQs).

	Retention time (min)	Lowest cali point (pg/μL)	Highest cali point (pg/μL)	Lowest cali point RSD (%)	R^2 correlation coefficient	Difference RF (low)-RF (All) (%)	Average RF	iLOQ (pg/μL)
PCDDs								
2,3,7,8-TCDD	20.72	0.016	2.800	2.5	0.9960	−1.94	0.9622	0.018
1,2,3,7,8-PeCDD	24.31	0.016	0.800	12.8	0.9949	−3.72	0.8254	0.029
1,2,3,4,7,8-HxCDD	27.97	0.016	0.800	8.9	0.9949	0.11	0.9731	0.022
1,2,3,6,7,8-HxCDD	28.10	0.040	2.000	4.6	0.9996	8.23	0.8706	0.032
1,2,3,7,8,9-HxCDD	28.46	0.080	4.000	4.0	0.9962	−6.60	0.9375	0.062
1,2,3,4,6,7,8-HpCDD	32.93	0.400	10.000	3.4	0.9990	2.32	0.9088	0.053
OCDD	39.35	4.000	120.000	2.3	0.9900	−11.83	1.0206	0.465
PCDFs								
2,3,7,8-TCDF	20.30	0.016	0.800	5.5	0.9919	−11.82	0.9328	0.010
1,2,3,7,8-PeCDF	23.26	0.016	0.800	13.7	0.9969	−9.38	0.8614	0.022
2,3,4,7,8-PeCDF	24.06	0.016	0.800	7.7	0.9922	−3.25	0.8840	0.021
1,2,3,4,7,8-HxCDF	27.02	0.016	0.800	3.8	0.9990	8.99	1.0247	0.016
1,2,3,6,7,8-HxCDF	27.15	0.016	0.800	17.9	0.9990	−1.21	0.9058	0.009
2,3,4,6,7,8-HxCDF	27.79	0.016	0.800	9.3	0.9993	7.17	0.9145	0.007
1,2,3,7,8,9-HxCDF	28.94	0.016	0.800	14.2	0.9993	−7.19	0.8913	0.020
1,2,3,4,6,7,8-HpCDF	31.09	0.080	4.000	9.8	0.9946	−8.94	0.8551	0.053
1,2,3,4,7,8,9-HpCDF	33.92	0.016	0.800	14.9	0.9990	−2.23	0.8754	0.020
OCDF	39.76	0.016	0.800	12.4	0.9933	18.92	0.9089	0.027
NO-PCBs								
PCB 81	17.71	0.320	8.000	1.7	0.9933	−10.65	1.1202	0.030
PCB 77	18.02	0.320	8.000	1.4	0.9931	−10.31	1.1453	0.037
PCB 126	20.92	0.320	8.000	1.7	0.9905	−9.96	0.9978	0.077
PCB 169	24.17	0.320	8.000	2.1	0.9935	−7.19	0.9094	0.071
MO-PCBs								
PCB-105	19.66	1.000	80.000	9.8	0.9958	−0.38	1.1869	2.109
PCB-114	19.12	1.000	80.000	8.8	0.9938	−4.89	1.2161	1.504
PCB-118	18.74	1.000	80.000	8.7	0.9994	15.32	1.2420	1.930
PCB-123	18.62	1.000	80.000	11.8	0.9945	−4.86	1.1166	1.537
PCB-156	22.51	1.000	80.000	5.6	0.9892	−5.38	1.1639	1.897
PCB-157	22.71	1.000	80.000	4.2	0.9882	−7.09	1.1096	1.287
PCB-167	21.56	1.000	80.000	10.0	0.9925	−6.32	1.1848	2.067
PCB-189	25.76	1.000	80.000	2.5	0.9893	−7.48	1.0754	1.626
NDL-PCBs								
PCB-28	14.19	4.000	1000.000	2.0	0.9944	8.39	1.1619	3.928
PCB-52	14.79	4.000	1000.000	1.5	0.9983	16.98	1.4403	6.530
PCB-101	16.81	4.000	1000.000	2.4	0.9964	11.11	1.2845	2.733
PCB-138	20.46	4.000	1000.000	4.3	0.9817	−5.59	1.1996	1.587
PCB-153	19.43	4.000	1000.000	2.0	0.9780	−6.03	1.2122	1.469
PCB-180	23.14	4.000	80.000	0.4	0.9723	−1.64	0.5431	0.904

Beyond iLOQs and pure instrumental performances, the setting of proper mLOQs is critical and reflects the real analytical sensitivity of the method in real environment. Congener mLOQs are defined by analyzing blank replicates and are the one used to report upper-bound/medium/lower-bound results. As for GC–HRMS, we analyzed 12 procedural blanks for which an average value and SD are calculated for each separate congener. The mLOQs are defined as the average value of the blank plus six times SD. This definition is meaningful since, whenever levels are greater than mLOQs, signals are statistically coming from the sample and not from the background. Table 3 describes the occurrence and average blank levels found in twelve individual procedure blanks for each of the 35 congeners, as well as congener iLOQs (pg/μL) and mLOQs (ng WHO_{2005}TEQ/kg). For most congeners, in our working environment, and based on a sample intake of 4 g, blank levels were measurable and were used to calculate the mLOQs. For 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, and PCB-169, not present in blanks, we corrected iLOQs to mLOQs by correcting with the sample intake amount.

The mLOQs reported in Table 3 are the threshold values used to report upper-bound, medium-bound, and lower-bound values for checking compliance to MLs.

MLs are 0.75 ng WHO_{2005}-PCDD/F-TEQ/kg, 1.50 ng WHO_{2005}-PCDD/F-PCB-TEQ/kg (including NO-PCBs and MO-PCBs), and 10 μg/kg for the sum of the 6 NDL-PCBs. In Table 3, we have also reported the sum of the mLOQs for a direct comparison with MLs. As shown in Table 1, mLOQs must be <20% of MLs. Our method is thus compliant as we report a sum of 0.13 ng WHO_{2005}-PCDD/F-TEQ/kg, which is 18% of the ML, and a sum of 0.30 ng WHO_{2005}-PCDD/F-PCB-TEQ/kg, which is 20% of the ML. The difference between upper-bound and lower-bound results will be *de facto* always below 20% of the ML. For NDL-PCBs a sum of 4.6 μg/kg, which is above 20% of the ML. This is however due to a fairly high contamination of the laboratory environment at the time of the validation exercise, not to the method itself (also observed with GC–HRMS). Nevertheless, even under these unfavorable laboratory conditions, every single of the 29 congeners contributing to the TEQ calculation fulfill the criteria of the Regulation.

3.3. Selectivity and quant/qual transitions

No specific criteria are set for the selectivity aspect of GC–QQQMS/MS. However, GC–HRMSGC–HRMS and GC–QQQMS/MS chromatograms look different because of the ion filtering from the quadrupoles and the resulting flat baseline. We therefore always used unsmoothed chromatograms to stay as close as possible to raw data and avoid any artificial enhancement of signals. 1,2,3,4,7,8- and 1,2,3,6,7,8-hexachlorinated furans congeners (HxCDF) that are the most difficult to separate were baseline separated. We generally did not observe better results using smoothed

Table 3
Average blanks levels data and method limits of quantitation (mLOQs).

	Retention time (min)	iLOQ (pg/μL)	Average blank level (ng/kg)	Occurence in blank $n = 12$ (%)	mLOQ (ng/kg)	mLOQ (ng WHO_{2005}TEQ/kg)
PCDDs						
2,3,7,8-TCDD	20.72	0.018	–	0	0.005	0.005
1,2,3,7,8-PeCDD	24.31	0.029	–	0	0.007	0.007
1,2,3,4,7,8-HxCDD	27.97	0.022	0.001	8	0.013	0.001
1,2,3,6,7,8-HxCDD	28.10	0.032	0.011	25	0.135	0.014
1,2,3,7,8,9-HxCDD	28.46	0.062	0.003	17	0.041	0.004
1,2,3,4,6,7,8-HpCDD	32.93	0.053	0.120	100	0.440	0.004
OCDD	39.35	0.465	0.502	100	1994,000	0.001
					Sum mLOQ	0.036
PCDFs						
2,3,7,8-TCDF	20.30	0.010	0.010	33	0.101	0.010
1,2,3,7,8-PeCDF	23.26	0.022	0.149	92	0.565	0.017
2,3,4,7,8-PeCDF	24.06	0.021	0.004	8	0.082	0.025
1,2,3,4,7,8-HxCDF	27.02	0.016	0.005	17	0.072	0.007
1,2,3,6,7,8-HxCDF	27.15	0.009	0.004	25	0.060	0.006
2,3,4,6,7,8-HxCDF	27.79	0.007	0.007	33	0.077	0.008
1,2,3,7,8,9-HxCDF	28.94	0.020	0.014	50	0.180	0.018
1,2,3,4,6,7,8-HpCDF	31.09	0.053	0.136	92	0.549	0.005
1,2,3,4,7,8,9-HpCDF	33.92	0.020	0.002	25	0.024	0.000
OCDF	39.76	0.027	0.081	83	0.603	0.000
					Sum mLOQ	0.096
NO-PCBs						
PCB 81	17.71	0.030	0.593	75	3.670	0.001
PCB 77	18.02	0.037	15.455	100	49.658	0.005
PCB 126	20.92	0.077	0.364	92	1.372	0.137
PCB 169	24.17	0.071	–	0	0.018	0.001
					Sum mLOQ	0.144
MO-PCBs						
PCB-105	19.66	2.109	41.287	100	187.558	0.006
PCB-114	19.12	1.504	3.697	100	15.228	0.000
PCB-118	18.74	1.930	138.294	100	601.074	0.018
PCB-123	18.62	1.537	2.450	100	9.108	0.000
PCB-156	22.51	1.897	3.528	100	13.960	0.000
PCB-157	22.71	1.287	0.639	100	3.267	0.000
PCB-167	21.56	2.067	9.385	100	40.878	0.001
PCB-189	25.76	1.626	0.365	100	1.240	0.000
					Sum mLOQ	0.025
NDL-PCBs						
PCB-28	14.19	3.928	314.419	100	994.601	
PCB-52	14.79	6.530	600.444	100	1909.234	
PCB-101	16.81	2.733	382.301	100	1303.266	
PCB-138	20.46	1.587	42.143	100	161.674	
PCB-153	19.43	1.469	49.145	100	171.672	
PCB-180	23.14	0.904	12.171	100	34.941	
				Sum mLOQ	4575.388	

chromatogram correction neither in terms of precision (RSD) nor in terms of accuracy.

Relative retention times of individual internal standards vs their target unlabeled compound were also checked and were never different for more than 3 s (internal standard always before target). This helped to confirm that the correct peaks were integrated. We also observed a between-run variation on the retention time of the couple target-internal standard in a 4 s window without causing any adverse effect.

Correct integration as well as the absence of interference was verified manually by checking quant/qual transition ratio. The quantitation MRM transition gave the higher response for an analyte whereas the qualification transition (quantitation transition with a +2 Da offset) gave a lower response. Ratios Quant/qual transitions were determined experimentally from the calibration curve using constant MS parameters such as collision energy. The tolerance is ±15% for PCDD/Fs and DL-PCBs and more for NDL-PCBs (see Table 1). In Table 4, we reported average values calculated from calibration curve and used as a reference for all analysis. A closer look to raw data is mandatory whenever a congener is out of range to guarantee accurate result.

3.4. Background subtraction

Two common ways may be used to correct measured concentration with blank levels: subtracting an individual blank, of the same kind of the sample and prepared in the same way, that belongs to the same series of samples (for instance one blank per ten samples), or subtracting an average value for the blank and verifying that all blanks are under control over time. We chose the latter solution because it provides two advantages. First, we must carefully control background levels in a control chart, which is useful to detect trends, highlight contamination problems, and demands a proactive approach to control the contamination. Second, we lower the effect of a statistical variation of an individual blank that belongs to a unique series. Instead we consider that if the blank is under control, and therefore within a confidence interval, there is no reason to take this particular value and ignore the average value observed, which is the key point for mLOQ determination. We therefore considered that the statistical variation of the blank was implicitly included into the measurement uncertainty. The assessment of the latter in Section 3.7 shows that it does not affect the uncertainty and precision (dispersion) of the results.

Table 4
Measured average ratio between quantitative and qualitative transition.

	Mean	SD	95% confidence		RSD%	Tolerance (%)
			LCI	LCS		
PCDDs						
2,3,7,8-TCDD	96.4	9.9	76.6	116.2	10	15
1,2,3,7,8-PeCDD	81.6	17.5	46.6	116.6	21	15
1,2,3,4,7,8-HxCDD	64.7	9.7	45.4	84.0	15	15
1,2,3,6,7,8-HxCDD	64.4	11.1	42.3	86.6	17	15
1,2,3,7,8,9-HxCDD	73.3	13.8	45.6	101.0	19	15
1,2,3,4,6,7,8-HpCDD	79.7	14.0	51.7	107.6	18	15
OCDD	94.3	11.1	72.0	116.6	12	15
PCDFs						
2,3,7,8-TCDF	94.0	13.6	66.8	121.1	14	15
1,2,3,7,8-PeCDF	81.7	11.2	59.3	104.0	14	15
2,3,4,7,8-PeCDF	88.0	23.2	41.7	134.3	26	15
1,2,3,4,7,8-HxCDF	62.3	11.6	39.1	85.6	19	15
1,2,3,6,7,8-HxCDF	60.8	5.9	48.9	72.6	10	15
2,3,4,6,7,8-HxCDF	62.7	6.3	50.2	75.2	10	15
1,2,3,7,8,9-HxCDF	62.6	9.9	42.9	82.3	16	15
1,2,3,4,6,7,8-HpCDF	76.1	8.3	59.5	92.6	11	15
1,2,3,4,7,8,9-HpCDF	82.3	23.8	34.6	129.9	29	15
OCDF	93.0	18.6	55.8	130.1	20	15
NO-PCBs						
PCB 81	64.3	8.8	46.7	81.9	14	15
PCB 77	62.4	0.8	60.7	64.0	1	15
PCB 126	95.1	8.6	78.0	112.3	9	15
PCB 169	73.3	5.6	62.1	84.5	8	15
MO-PCBs						
PCB-105	30.5	1.1	28.3	32.7	4	15
PCB-114	30.0	1.0	28.1	32.0	3	15
PCB-118	30.3	0.6	29.0	31.6	2	15
PCB-123	29.9	0.8	28.2	31.5	3	15
PCB-156	46.8	1.2	44.5	49.1	2	15
PCB-157	47.6	1.2	45.2	50.0	3	15
PCB-167	47.0	1.1	44.9	49.1	2	15
PCB-189	62.7	1.0	60.8	64.7	2	15
NDL-PCBs						
PCB-28	31.8	0.6	30.6	33.0	2	25
PCB-52	63.2	0.9	61.4	65.0	1	20
PCB-101	30.5	1.0	28.5	32.4	3	25
PCB-138	47.3	1.1	45.1	49.6	2	25
PCB-153	47.4	1.1	45.2	49.6	2	25
PCB-180	62.9	1.0	60.9	64.8	2	20

3.5. Accuracy

Fortified materials (vegetable oil consisting in sunflower oil) were used to assess the bias of the method for PCDD/Fs, NO-PCBs, and MO-PCBs. Six series of samples spiked at half ML (ML/2), ML, and twice ML (2ML) were injected over 3 days (2 series per day) providing also within lab reproducibility data. Matrix blank was checked and no congener was found in the unfortified vegetable oil. All congeners were spiked in the blank matrix following a natural typical pattern to reach the appropriate levels. Table 5 shows results of the accuracy experiments.

These results are well in accordance with the EU Regulation that states that bias of the method (systematic error) must be <20% and within lab reproducibility (random error), expressed in terms of %RSD, must be <15%. Additionally, results were obtained and reported by independent operators who operated in blind without knowing target levels in samples.

We also analyzed proficiency test (PT) vegetable oil (Rikilt, Wageningen, Netherlands). Two materials were independently analyzed, one naturally contaminated by fish oil, the other artificially fortified with PCDD/Fs and DL-PCBs. Results (Table 6) illustrate the good accuracy of the method, meeting requirements of EU Regulation. Looking at the results, taking into account the measurement uncertainty (Section 3.7) and considering ML for vegetable oil, we have reported correctly non-compliant (material 1) and compliant (material 2) sample. *Z*-scores for the two materials were within ±2 interval as well. *Z*-score is used to assess the deviation of a result from a mean value in terms of unit of standard deviation. It is the difference between the result and the mean divided by the standard deviation of the population.

Table 6
Results of proficiency testing on vegetable oil (Rikilt, 2013) taking into account measurement uncertainty.

	Reported pg WHO_{2005}TEQ/g	Target value pg WHO_{2005}TEQ/g	Bias%
Material 1			
PCDD/Fs	1.10 ± 0.20	1.01	8.8
DL-PCBs	0.80 ± 0.19	0.89	−10.3
Total TEQ	1.90 ± 0.36	1.90	−0.1
Material 2			
PCDD/Fs	0.55 ± 0.11	0.48	15.6
DL-PCBs	0.82 ± 0.21	0.85	−3.0
Total TEQ	1.38 ± 0.26	1.33	3.7

3.6. Control and robustness

Blank samples and quality control (QC) samples (pork fat) prepared in the lab were injected (2 per week) during 2 months (September–October 2013) and, after a 6-months break period, during April 2014. Fig. 1 represents the QC chart for blank-corrected QC samples during this period. The average value and intervals at ±2 SD (95% confidence) were determined by 10 QC samples that are not represented on the control chart. Each of the 15 QCs is statistically distributed within the confidence interval. No difference was observed after the system was used for other purposes that required several column changes and system venting, over a 6 months period. We did not have problem to get back to the original high performances attained at the start of the validation exercise. A blank control chart has also been recorded to follow blanks and

Table 5
Bias of the method for PCDD/Fs and DL-PCBs using 6 series of samples spiked at 3 levels around maximum level (ML).

	Average ng WHO_{2005}TEQ/kg	SD	RSD%	Target ng WHO_{2005}TEQ/kg	Bias%
PCDD/Fs					
Spike level					
ML/2	0.41	0.03	7.1	0.40	2.36
ML	0.78	0.04	5.7	0.79	−1.54
2ML	1.60	0.03	2.2	1.58	1.30
DL-PCBs					
Spike level					
ML/2	0.31	0.03	9.0	0.33	−7.00
ML	0.59	0.02	3.4	0.65	−8.53
2ML	1.26	0.02	1.6	1.30	−3.42

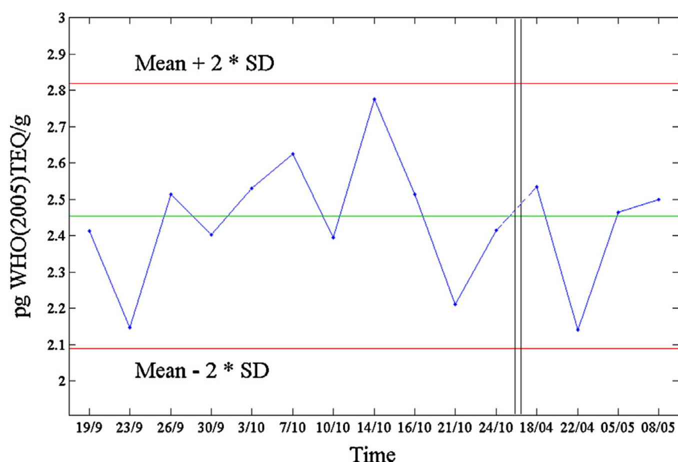

Fig. 1. QC chart over September–October period and April period.

background level over the same period (Fig. 2). It clearly appeared that blank levels were fluctuating outside the 95% confidence range but were still randomly distributed below the LOQ line during the first period of 2 months and it came back to lower and precise values for the second period after actions were taken in the laboratory. That situation did not impacted our performances as a real out-of-control blank is present when its level is higher than the mLOQ (determined as the average blank + 6 SD) since all values below mLOQs are considered as background and thereof not significant. A blank below mLOQ is therefore always accepted as far as no significant trends highlight possible out of control contamination issues, which has never been the case during this study.

3.7. Measurement uncertainty

Under the official control of food and feed within the EU, the assessment of compliance (or non-compliance), from what the release of the food or feedstuff on the market depends, requires the determination of measurement uncertainty (MU). Two major approaches are possible: top-down or bottom-up. In our view, the use of the top-down approach is preferred as it is based on the use of the validation data set produced by the analysis of real samples through the whole analytical process to assess MU. In this context, the expanded MU was calculated using a coverage factor of 2 (k = 2), according to the EU Regulation [31] with requirements of standard ISO 17025 norm.

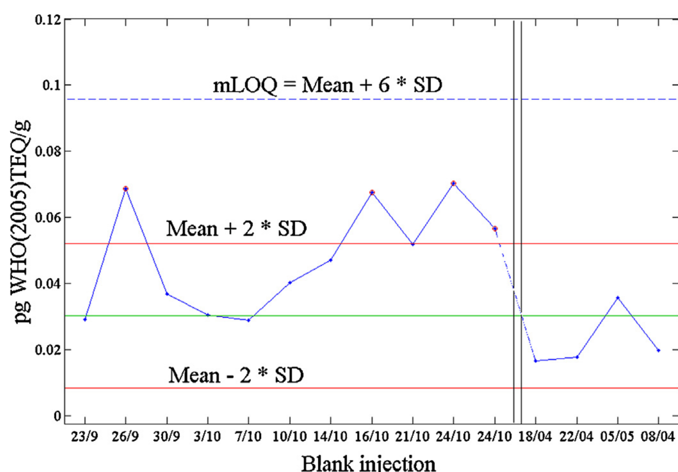

Fig. 2. Blank control chart over September–October period and April period.

We followed the trueness and precision studies performed during validation. They contribute to the relative expanded uncertainty according to the following Eq. (1):

$$\%U = 2\sqrt{\%u_{\text{bias}}^2 + \%u_{\text{Rw}}^2} \tag{1}$$

where ($\%u_{\text{bias}}$) is the relative standard uncertainty associated with the contribution of the bias; ($\%u_{\text{Rw}}$) is the relative standard uncertainty associated with the contribution of the precision study (i.e. within laboratory reproducibility).

An harmonized approach of MU for the measurement of contaminants using isotope dilution MS based technique is currently under consideration and discussed at the EU level by a working group of experts within the European network of NRLs [34].

The harmonized approach recommends the use of six proficiency testings (PT) in order to assess the systematic error. Because we participated to only one PT during the present study, we did not get data generated from six interlaboratory studies, but instead we performed 6 recovery experiments to assess the bias (Section 3.5). This approach is an alternative when few data from PTs are available and is acceptable if we can determine the bias itself as well as the uncertainty on the reference spiked value ($\%u_{\text{spike}}$) of the pure 2,3,7,8-substituted PCCD/Fs standards. This uncertainty is mainly due to the purity of internal standards (98%) and negligibly due to volumetric errors while pipetting. The contribution to u_{bias} is shown in Eq. (2). We observed a posteriori during the recovery study and PT data (respectively Tables 5 and 6) that all the measured values fell within the uncertainty interval and therefore the slight difference between the 'true' value and the observed value is the result of statistical variations taken into account in the $\%u_{\text{Rw}}$ term. Moreover, it is a good indication that the measurement uncertainty is realistic:

$$\%u_{\text{bias}}^2 = \%\text{MS}_{\text{bias}} + \%u_{\text{spike}}^2 \tag{2}$$

Results of 6 recovery experiments of artificially spiked materials in a blank sunflower oil (feed) matrix are shown in Table 5, Section 3.5. We determined for every experiment (i) the relative bias ($\%b_i$) and then the relative mean square of the bias ($\%\text{MS}_{\text{bias}}$) following Eq. (3) where n is the number of experiments (n = 6):

$$\%\text{MS}_{\text{bias}} = \sum \frac{\%b_i^2}{n} \tag{3}$$

The contribution of u_{spike} to Eq. (2) is determined using 2% uncertainty (98% purity internal standard) on the concentration set for each congener in the spike material, divided by $\sqrt{3}$ since it follows a square distribution rather than Gaussian, assumption realistic for pure standard solution according the Eurachem guide [35]. This factor is subsequently corrected by TEF values, since all the data including MU have to be expressed in TEQ units for compliance assessment, and the square root of the sum of squares of all congeners is taken to propagate errors [34] (Eq. (4)). As we show in Table 7, this contribution is completely negligible compared to the other source of uncertainty in the Eq. (2):

$$u_{\text{spike}} = \sqrt{\sum_{i}^{29} \left[\text{TEF}_i * \left(\frac{0.02 * C_i}{\sqrt{2}}\right)\right]^2} \tag{4}$$

The other contribution to measurement uncertainty is the random error ($\%u_{\text{Rw}}$) arising from the analytical process characterized by the within-lab reproducibility. This value was determined using quality control (pork fat) sample (n = 25) injected over a 9-months period and included in a QC control chart (Fig. 1). The relative standard deviation of the control chart (RSD_{CC}) simply equals $\%u_{\text{Rw}}$ and represents the dispersion of the data.

Table 7 summarizes all relative uncertainties obtained for spiked materials at ML/2, ML, and 2ML. The expanded uncertainties ($\%U$) of

Table 7
Calculation of measurement uncertainty.

Table in %	Relative uncertainties		PCDD/Fs + DL-PCBs (experimental)
	PCDD/Fs	DL-PCBs	
Validation at ML/2			
%MS (bias)@ML/2	49.9	107.0	36.4
%*u* (spike)	0.5	0.9	0.5
%*u* (bias)	7.1	10.4	6.1
%*u* (Rw)	7.3	7.9	7.2
Uncertainty %*u*	10.2	13.1	9.4
Expanded %*U*	20.3	26.1	18.8
Validation at ML			
%MS (bias)@ML	29.0	80.5	34.5
%*u* (spike)	0.5	0.9	0.5
%*u* (bias)	5.4	9.0	5.9
%*u* (Rw)	7.3	7.9	7.2
Uncertainty %*u*	9.1	12.0	9.3
Expanded %*U*	18.1	24.0	18.5
Validation at 2ML			
%MS (bias)@2ML	5.7	13.8	2.1
%*u* (spike)	0.5	0.9	0.5
%*u* (bias)	2.5	3.8	1.5
%*u* (Rw)	7.3	7.9	7.2
Uncertainty %*u*	7.7	8.8	7.3
Expanded %*U*	15.3	17.6	14.6
Expanded %*U* (HRMS)	19.5	24.2	20.1

18.1% and 24.0% were obtained experimentally at ML for the sum of PCDD/Fs and the sum of DL-PCBs, respectively. In the framework of the EU legislation, MU has to be estimated at the ML. MU (%) associated with lower levels (background levels) is indeed much higher than the value reported here in the narrow range around the ML. The estimation of MU at other levels than at ML is given for information in Table 7 but is not considered in this work. For comparison, MU determined for the GC–HRMS method by the same approach, but using PTs, gives at ML, 19.5% and 24.2% for the sum of PCDD/Fs and DL-PCBs, respectively. We show that the GC–QQQMS/MS method provides very similar results for precision and uncertainty compared with GC–HRMS, despite different technologies of mass spectrometer used. These results not only show that MS/MS can be used for dioxin analysis but that this method gives as consistent results as HRMS does. However, long-term precision and bias studies should be performed in routine conditions to evaluate if the MS/MS method could maintain this level of performance over long period.

We have determined the MU for PCDD/Fs and DL-PCBs separately, as well as the sum of the contribution PCDD/Fs + DL-PCBs experimentally. In case of separate determination of uncertainties for PCDD/Fs and DL-PCBs, the Regulation requires the calculation of the uncertainty on the total TEQ as the sum of the two uncertainties determined separately. At ML, from the recovery experiment, the spiked levels are 0.78 ± 0.14 pg WHO-TEQ/g and 0.59 ± 0.14 pg WHO-TEQ/g for the sum of PCDD/Fs and the DL-PCBs. The sum of those uncertainties is 0.28 pg WHO-TEQ/g for the whole contribution, which for a level of 1.37 pg WHO-TEQ/g represents 20.7% uncertainty. In Table 7, we show that we underestimate the uncertainty of the sum of PCDD/Fs + DL-PCBs (18.5%) when it is determined experimentally from the final result as regard as the calculated %*U* for the whole contribution (EU Recommendation).

We assessed the measurement uncertainty following current recommendations from the EU working group despite no official Regulation has been published yet. Some discussions are now also focusing on the integration of a new contribution taking variations of daily performances into account. This contribution would be added to Eq. (1) and would imply the calculation of daily limits of quantitation as well as daily check injections [34]. In our study, we however kept the classical approach to assess and give a first idea of the performances. Yet, for further use of GC–QQQMS/MS on a routine basis, we will use this daily LOQ calculation as a fine-tuning from known performances and basic parameters already determined.

3.8. Extension to other matrices

This extended validation study was carried out for vegetable oil (feed). The method can be adapted to other matrix with some optimization and modification to take possible matrix-specific interferences into account. As an example, some were observed for hen eggs under the form of coelutions with some PCBs. Therefore, in order to be able to successfully extend the findings of the present study to other matrices, we recommend the systematic verification of (1) possible coelutions with isomers or congeners (non-2,3,7,8-substituted for dioxins/furans, and non-targeted PCBs) present at particularly high levels in the matrix, and (2) interferences that are not filtered by the quadrupoles. Method optimization for specific matrices can then be carried out by simply optimizing the GC separation of interfering congeners, and by increasing the mass resolution (lowering the peak width from 0.7 to 0.5 Da for example) to allow better specific filtration by the quadrupoles. Note that the effect of the latter has to be validated as it can affect the instrument sensitivity because fewer ions can reach the detector and therefore modify iLOQ values.

4. Conclusion

A GC–QQQMS/MS method has been developed and fully validated in accordance to criteria laid down in the new EU Regulation 709/2014 that allows the use of GC–QQQMS/MS as a confirmatory method for official control of PCDDs, PCDFs, and DL-PCBs in animal feedstuffs. We demonstrated that this technology meets requirements and can achieve similar performances to GC–HRMS. We assessed a realistic measurement uncertainty in the typical range of the HRMS method and very similar analytical parameters despite the different technologies of mass analyzers. The triple quadrupole technology can therefore be considered to exhibit equal performances to the magnetic sector instrumentation in the context of ML measurement. We however noticed some lack of precision in the Regulations, which may lead to different interpretations of some key aspects like the determination of the limit of quantitation. In this study we have proposed some interpretation and kept the most stringent criteria to prove that this method provides accurate, consistent and reliable results.

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