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.

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

[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