The performance of atmospheric pressure gas chromatography–tandem mass spectrometry compared to gas chromatography–high resolution mass spectrometry for the analysis of polychlorinated dioxins and polychlorinated biphenyls in food and feed samples

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ABSTRACT

Recently, gas chromatography tandem mass spectrometry (GC–MS/MS) has been added in European Union (EU) legislation as an alternative to magnetic sector high resolution mass spectrometry (HRMS) for the analysis of dioxins and dioxin like polychlorinated biphenyls (dl-PCB) in food and feed. In this study the performance of APGC–MS/MS compared to GC–HRMS is investigated and compared with EU legislation. The study includes the legislative parameters, relative intermediate precision standard deviation (S_{Rw},rel), trueness, sensitivity, linear range and ion ratio tolerance. In addition, over 200 real samples of large variety and spanning several orders of magnitude in concentration were analyzed by both techniques and the selectivity was evaluated by comparing chromatograms. The S_{Rw},rel and trueness were evaluated using (in-house) reference samples and fulfill to EU legislation, though the S_{Rw},rel was better with GC–HRMS. The sensitivity was considerably better than of GC–HRMS while the linear range was similar. Ion ratios were mostly within the tolerable range of ±15%. A (temporary unresolved) systematic deviation in ion ratio was observed for several congeners, yet this did not lead to exceeding of the maximum ion ratio limits. The APGC–MS/MS results for the non-dioxin-like-PCBs (ndl-PCBs) were negatively biased, particularly for PCB138 and 153 in contaminated samples. The selectivity of APGC–MS/MS was lower for several matrices. Particularly for contaminated samples, interfering peaks were observed in the APGC chromatograms of the native compounds (dioxins) and labeled internal standards (PCBs). These can lead to biased results and ultimately to false positive samples. It was concluded that the determination of dioxins and PCBs using APGC–MS/MS meets the requirements set by the European Commission. However, due to generally better selectivity and S_{Rw},rel, GC–HRMS is the preferred method for monitoring purposes.

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1. Introduction

Polychlorinated dibenzo-*p*-dioxins and -furans (PCDD/Fs, further called 'dioxins') and polychlorinated biphenyls (PCBs) are persistent organic pollutants (POPs) that are globally distributed. Dioxins and PCBs are frequently detected in the environment and in food and feed [1]. These contaminants are listed under the Stockholm Convention because of their persistency, bioaccumulative and toxic properties. In the last century, these properties were regrettably demonstrated in several studies covering dioxin contaminations in Vietnam. The defoliant Agent Orange was contaminated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-

TCDD) which was sprayed and spilled over land. To date, high levels of dioxins are found in the environment [2], food [3,4] and in blood from people in the effected regions [5]. This high exposure resulted in significant health effects [6].

In recent history, several incidents occurred with dioxins and PCBs entering the food chain. A well-known case was in 1999 where oil containing approx. 50 kg sum of marker PCBs and 1 g TEQ of dioxins were accidently added to recycled fat for the production of 500 t of feed in Belgium [7]. This feed was fed to animals, including laying-hens, where this accident was discovered. Before the accident was discovered, food of the contaminated animals entered the food chain. Although this contamination does not seem to have caused an adverse public health effect, the typical pentachlorodibenzofuran (PeCDF) congeners remained traceable in plasma from the background pollution in 2000 [8,9]. There are many other examples of dioxin contamination incidents [10–18] and to secure food safety, authorities worldwide require monitoring of dioxins and PCBs and removal of potential sources that contaminate the food and feed chain. In order to reduce the dietary exposure of the population to these compounds. In Europe several maximum limits have been established in a wide array of foods (e.g. fish, pork, dairy products, vegetable oils) [19] and feed (compound feed, ingredients) [20]. This calls for a large capacity on dioxin and PCB testing and intensive monitoring of levels in food and feed samples is nowadays performed in the EU.

The monitoring efforts and regulations are focused on a group of seventeen 2,3,7,8-substituted dioxins and 12 planar PCBs (so called 'dioxin-like' PCBs (dl-PCBs)), to which a Toxic Equivalency Factor (TEF) was assigned by the World Health Organisation (WHO) [21]. In addition to that, often six non-dioxin-like-PCBs (ndl-PCBs) are monitored (also known as the 'indicator PCBs'). For decades, the instrumental analysis of dioxins and PCBs was performed by gas chromatography (GC) coupled to magnetic sector high resolution mass spectrometers (HRMS), typically operated at 10,000 resolution. The use of HRMS is a prerequisite for food and feed compliance testing in the US [22,23] and in Europe. However, since 2014 the European Commission allowed the use of low resolution (LRMS) tandem mass spectrometric (GC–MS/MS) approaches for confirmatory analysis in food [24] and feed [25].

Previously, GC–MS/MS methods were mostly applied for environmental samples while the higher working range was not suitable for analysis of food and feed where dioxins are typically present at fg levels and PCBs are present at ng to pg-level [26,27]. Over the years the sensitivity of GC–MS/MS systems improved and became useful for dioxin and PCB analysis of food and feed through methodological adaptations. Yet the instrumental sensitivity was still not in the range of GC–HRMS [28–31] hampering the analysis of particularly low level food and feed samples such as vegetable oils. To improve sensitivity, chemical ionization (CI) has been used for the analysis of PCBs in the 1970s [32,33]. So far, CI was not combined with MS/MS for the analysis of dioxins and PCBs. Several research teams in the 70's and 80's gave the first impulse to generate hardware for atmospheric pressure ionization [34–37]. In 2005, McEven and McKay patented an atmospheric pressure ion source able to be coupled with both LC/MS and GC/MS [38–40]. This CI source should be capable of reaching the required sensitivity and stability to meet the stringent analytical requirements for the official European control of dioxins and PCBs. This improved CI source, atmospheric pressure gas chromatography (APGC), is like CI a soft ionization source. In APGC a corona discharge forms a plasma with an incoming stream of nitrogen under atmospheric conditions. After GC separation the compounds exit the chromatographic column into the plasma and ionization of the compounds takes place through primary or secondary charge transfer [41,42]. For other, more polar compounds, proton transfer results in higher ion yields. Besides the type of compound also the amount of mois-

ture in the source, which can be controlled, will affect the type of ionization. Before, during and after the ionization process the molecules and ions are transferred to the analyzer through the gas flow and the vacuum in the analyzer. The soft ionization leads to much higher molecular ions yields compared to electron ionization (EI) where substantial in-source fragmentation takes place. Therefore, APGC shows improved sensitivity over EI in GC–MS and GC–MS/MS applications.

Unambiguous identification and quantification is essential for analysis of dioxins and PCBs in the working range acceptable for food and feed. By combining APGC with MS/MS, another technique than GC–HRMS provides the opportunity to perform analyses with the required sensitivity and selectivity for this field of work. Recently, four studies showed the potential of the technique for the selective and sensitive detection of dioxins and PCBs [41–44]. However, in-depth information on this type of analysis is still limited since APGC–MS/MS is only recently introduced and only little is known about its performance, particularly on (complex) routine food and feed samples. The aim of this study was to investigate the applicability of APGC–MS/MS for the detection and quantification of dioxins and PCBs in food and feed and compare that to the traditional 'reference' method using GC–HRMS. The APGC–MS/MS performance was examined in terms of sensitivity, selectivity and dynamic range. Additionally, we have investigated the technique's performance on more than 200 food and feed samples in a wide dioxin and PCB concentration range. We compared the results with our HRMS equipment and also performed interlaboratory tests on certified and in house reference materials shipped to CART (Liege, Belgium), another NRL laboratory equipped with APGC–MS/MS instruments. Finally, we have evaluated to what extent APGC–MS/MS complies with EU regulations on the analysis of dioxins in food and feed [24]. In this study, we refer to the technique as APGC–MS/MS, as the results specifically refer to the instrument tested. It should be noted that this includes also the mode of ionization (APCI), which by definition is independent of the instrument or manufacturer.

2. Material and methods

All chemicals used were obtained from Actu-All Chemicals (Randmeer, The Netherlands) and were of persistent environmental contaminants (PEC) grade.

2.1. Dioxin and PCB standards and reference samples

Native and labelled standard mixtures ED-906-B-5, ED-980, EF-909-B-5, EF-982, ED-998, EF-999, ED-911, ED- 907, EC-4187, EC-4188, EC-4058, EC-4986, EC-4987, and EC-5179 originated from Cambridge Isotope Laboratories (Tewksbury, MA, USA). Certified reference material SRM1947, fish tissue, was obtained from NIST (Gaithersburg, USA) and an in-house reference material, sunflower oil (29950), was used for routine quality control. The sunflower oil is spiked in our laboratory at 0.92 pg/g dioxin-TEQ, 0.79 pg/g PCB-TEQ and 16 ng/g for the sum of 6 ndl-PCBs. Proficiency test samples for the 2015 feed proficiency test were received from the EU Reference Laboratory (EU-RL) for Dioxins and PCBs in Feed and Food (Chemisches und Veterinäruntersuchungsamt, Freiburg, Germany).

2.2. Samples and sample preparation

A large number of samples, blanks and control samples (n > 200), including food, feed and environmental samples were analyzed to investigate the performance of APGC–MS/MS and for comparison with GC–HRMS. Most samples were from Dutch national monitoring programs for primary agricultural products (animal products such as milk, eggs, pork, bovine meat), fishery products and feed