COMPARISON OF GC-MS/MS AND GC-HRMS RESULTS FOR THE DETERMINATION OF PCDD/FS IN FOOD AND FEED SAMPLES

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Introduction

The European Union has set maximum limits for polychlorinated dibenzo-p-dioxins and dibezofurans (PCDD/Fs), the sum of PCDD/Fs and dioxin-like polychlorinated biphenyls (dl-PCBs) and also for non-dioxin-like PCBs which are specified in EC Regulations 1259/2011[1] and 277/2012[2] for food products and feedstuff respectively. The methods of sampling and analysis for the determination of PCDD/F and dl-PCB concentrations are defined in EC Regulation 252/2012[3] for food, and 278/2012[4] for feedstuff. In both Regulations, the confirmatory method of determination is high resolution gas chromatography / high resolution mass spectrometry (GC-HRMS), this being the only acceptable method with the necessary sensitivity and specificity. However, for surveillance programs there is a need for screening methods, which include GC-MS, GC-MS/MS and bioanalytical methods, provided that specific performance-related criteria are met.

Modern gas chromatography-triple quadrupole mass spectrometry instruments have the advantage of high sensitivity and high selectivity through multiple reaction monitoring (MRM). In addition, GC-MS/MS triple quadrupole mass spectrometry instruments are less expensive and much easier to maintain than GC-HRMS instruments. However, because of its lower sensitivity than GC-HRMS, GC-MS/MS finds better application in environmental samples[5], such as emission gases and fly ash, which contain high concentrations of PCDD/Fs and PCBs, rather than food and feedstuff where regulations require the analysis of concentrations at low pg/g levels. A limiting factor for sensitivity in the case of lipid-containing samples, is the fact that most clean-up methods require a maximum of 3 g of fat per sample, therefore the levels of PCDD/F and PCB concentrations are near the limit of detection per g of fat of the GC-MS/MS method. In our lab we apply a manual clean-up method based on Carbosphere, a special kind of active carbon material that allows the processing of more than 10 g of fat[6].

In the present study we undertook the task of optimizing the parameters of the gas chromatography - triple quadrupole mass spectrometry (GC-MS/MS) method and comparing its performance in the determination of PCDD/F in real food and feed samples to that of the gas chromatography – high resolution mass spectrometry (GC-HRMS) method. Moreover, two clean-up protocols for sample preparation were tested: automated clean-up using PowerPrep apparatus by FMS Inc and our routine clean-up method including successive chromatographic steps on active carbon (Carbosphere), acid silica and basic alumina.

Materials and methods

Samples

Samples were provided by the European Union Reference Laboratory Proficiency Test on the Determination of Dioxins and PCBs in Feed and Food and by the 13th Round of the Interlaboratory Comparison Study on the Determination of POPs in Food organized by the Department of Exposure and Risk Assessment, Norwegian Institute of Public Health, Oslo, Norway.

Lipid extraction and clean-up

The lipid extraction and clean-up method applied have been described in detail elsewhere[6]. Quantification standards (^{13}C-labelled solutions of PCDD/Fs (Wellington) in toluene) were added to each sample prior to extraction. Extraction of lipids was achieved using Soxhlet apparatus.

Concerning non-automated methodology, further clean-up was performed by successive steps of active carbon chromatography (Carbosphere 80/100 mesh, Alltech) and basic alumina chromatography for PCDD/Fs. Automated clean-up was performed using the solid phase extraction system PowerPrep^{TM} (FMS Inc, Boston MA, USA).

Final eluates from both methodologies were evaporated to dryness and re-dissolved in 50 μL of nonane containing $^{13}C_6$-1,2,3,4 TCDD as injection standard.

Instrumental analysis
The eluates obtained from the clean-up process, were measured on GC-MS/MS and GC-HRMS instruments.

GC-HRMS measurement
The quantification of PCDD/Fs was performed by HRGC-HRMS (EI), on MID mode, on a Trace GC gas chromatograph (ThermoFinnigan) equipped with a CTC A 200S autosampler, coupled to a MAT-95 XP mass spectrometer (Thermofinnigan) performing at 10000 resolving power (10% valley definition). Instrumental conditions and purity control criteria are according to EPA 1613B method (U.S. EPA, 1994) and Regulation 252/2012/EC. The quantification was carried out by the isotopic dilution method. For TEQ calculations the WHO 2005 TEFs[7] were applied.

GC-MS/MS measurement
The GC-MS/MS measurements were performed on a TSQ Quantum XLS Ultra triple-quadrupole GC-MS/MS (Thermo) coupled to a Trace GC Ultra gas chromatograph equipped with a TriPlus autosampler (Thermo).
For the optimization of the multiple reaction monitoring method, different electron energy, emission current, source temperature, collision energy, scan width and scan time values were tested. Two precursor ions M+ and (M+2)+ for each PCDD/F, resulting each in one product ion (M-COCl+) were measured and the ion abundance ratio between the two monitored product ions was checked with the same ratio of a calibration standard at similar concentration, within the QC limits of ± 15 %.

The following MS/MS settings were found to give the best results:

Source temperature	250 ^{0}C
Ionization	EI
Electron energy	45 eV
Emission current	50 μA
Q2 Gas pressure (Argon)	1.5 mTorr
Collision energy	22 V
Q1 peak width	0.7 Amu
Q3 peak width	0.7 Amu
Precursor ions	M+, (M+2)+
Product ion	M-COCl+, (M+2)-COCl+
GC column	TR-DIOXIN-5MS 60m, 0.25 mm, 0.1 μm (Thermo)
Injection	Splitless
Injected volume	2 μL

The results of the GC-MS/MS measurements were compared with routine GC-HRMS.

Results and discussion
The major limitation of common clean-up methods for PCDD/F determination is the low loading capacity for fat (about 3 g), which requires higher sensitivity of quantification method. By using the Carbosphere method, this problem can be eliminated, since the Carbosphere column has a high capacity for at least 10 g of fat and can therefore be combined with instrumental methods of analysis less sensitive than GC-HRMS. For samples containing more than 1 pg TEQ / g fat, automated clean-up using the solid phase extraction system PowerPrep can be performed. As can be seen in Table 1, when the lard or the pork sausage sample is processed by Carbosphere, better results are obtained. But in both cases the MS/MS results are near the HRMS results with a deviation of less than 15%. Z-scores from the consensus values are within accepted limits ($-3<z<3$).

Table 1

Sample TEQ pg/g fat	GC-MS/MS	GC-HRMS	Deviation %	Consensus value	z-score MS/MS
Lard Carbosphere	0.994	1.007	1.29	1.18	-1.5
Lard PowerPrep	0.861	0.980	12.14	1.18	-2.7
Pork sausage Carbosphere	0.686	0.678	1.18	0.68	0.1
Pork sausage PowerPrep	0.661	0.724	8.70	0.68	-0.3
Reference solution CIL	12.543	12.385	1.27	12.51	0.03
Reindeer PowerPrep	1.544	1.598	3.38		
Halibut filet	2.786	2.802	0.57		
Cod liver oil	1.393	1.595	12.66		
Olive oil spiked	3.638	3.647	0.24	3.38	0.76

The comparison of the PCDD/F TEQ results of GC-MS/MS and GC-HRMS measurements shows that the deviation for different food samples, covering a concentration range between 0.7 and 12 pg/g fat, are below 15% in all cases.

One important criterion for the identification of the PCDD/F congeners is the ion abundance ratio between the two monitored product ions, resulting from two different precursor ions. This ratio depends on the ion abundance ratio of the selected precursor ions and the probability of the loss of $CO^{35}Cl$ or $CO^{37}Cl$ for formation of each product ion. The measured ion abundance ratios matched the respective ratios of a calibration standard at a similar concentration, within the QC limits of 15%.

Due to the very low noise in the GC-MS/MS system, the calculation of an LOQ from a signal-to-noise ratio was not possible. Therefore, the LOQ was calculated from the lowest concentration with acceptable signal-to-noise ratio, ion abundance ratio within ±15% of the theoretical value and deviation of the relative response factor from the mean value ≤ 20%. From the measurement of six GC-MS/MS sequences at the range of 0.4 pg per each individual congener per injection, LOQ was calculated at 0.1 pg/g fat when Carbosphere chromatography was used for clean-up (10 g of fat) and 0.2 pg/g fat when PowerPrep was used for clean-up (5 g of fat).

The results presented here are in agreement with previous comparisons of GC-HRMS with GC-MS/MS that have shown a deviation below 20% of WHO-PCDD/F-TEQ values calculated with the two methods[8], and a deviation of +/-10% for dl-PCBs[9]. This means that GC-MS/MS triple quadrupole mass spectrometry instruments have the capability of analyzing food and feed samples at low concentrations with results comparable with GC-HRMS when a clean-up method that can handle enough fat is used.

References:

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