APPLICATION OF APGC-MS/MS FOR THE DETERMINATION OF PCDD/Fs AND PCBs IN FEED AND FOOD MATRICES

Kotz A[1*], Traag W[2], Winterhalter H[1], Malisch R[1], Dunstan J[3]

[1] European Union Reference Laboratory (EU-RL) for Dioxins and PCBs in Feed and Food, State Institute for Chemical and Veterinary Analysis (CVUA), Freiburg, Germany;
[2] RIKILT – Institute of Food Safety, Wageningen, Netherlands;
[3] Waters, Manchester, UK

Introduction

Methods of analysis for the official control of levels of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs) in certain food stuffs and feeding stuffs are laid down in Commission Regulations (EU) No 252/2012[1] and (EC) No 152/2009[2], amended by Commission Regulation (EU) No 278/20121, respectively. As confirmatory method for the unequivocal identification and quantification of PCDD/Fs and DL-PCBs at the level of interest, these regulations require the application of gas chromatography coupled with high resolution mass spectrometry (GC-HRMS). For screening purposes GC-MS methods and bioanalytical screening methods can be applied. On the basis of the results of several studies on the applicability of GC-MS/MS methods for the determination of PCDD/Fs and DL-PCBs, the network of European Union Reference Laboratory (EU-RL) and National Reference Laboratories (NRLs) of EU Member States for Dioxins and PCBs in Feed and Food developed further analytical criteria for the application of GC-MS/MS as confirmatory method for determination of PCDD/Fs and DL-PCBs[3]. These criteria were based on experiences of institutes with GC-MS/MS systems in electron ionization (EI) mode[4,5,6,7].

Recently also MS/MS systems equipped with an atmospheric pressure GC source (APGC) showed sufficient sensitivity for determination of PCDD/Fs and DL-PCBs at low concentrations in food and feed samples. The soft ionisation at atmospheric pressure in N_2 plasma under charge transfer conditions can increase the intensity of the monitored molecular ion of PCDD/Fs and DL-PCBs and reduce the fragmentation in the ion source. Especially for the low maximum and action levels applicable for PCDD/Fs in feed and food, low LOQs for the individual congeners are of importance.

The object of this evaluation is to check the applicability of the Waters APGC-MS/MS system for the determination of PCDD/Fs and DL-PCBs in feed and food, and ability to meet the criteria developed for the use of GC-MS/MS as confirmatory method.

Materials and methods

GC-MS/MS measurement

The APGC-MS/MS measurements were performed using an APGC Xevo TQ-S provided by Waters, Manchester, UK, at the Waters application laboratory in Manchester using standards and samples provided by the EU-RL and RIKILT, and at the EU-RL.

APGC-MS/MS (Waters APGC Xevo-TQ-S) parameters applied at the EU-RL for PCDD/Fs:

Ionisation	API+
Source	Dry N_2
Source temperature	150 °C
Corona current	2.0 µA
Sampling cone voltage	35 V
Cone gas flow	200 L/h
Auxiliary gas flow	300 L/h
GC make-up flow	150 ml/min

Multiple reaction monitoring	
Precursor Ion	$M^{+\cdot}$
Product Ion	$M\text{-}COCl^{+\cdot}$
Collision energy	31 V
Collision gas flow	0.3 ml/min (Argon)
Cycle time	ca. 0.4 s
Dwell time	48 ms

For each 2,3,7,8-substituted PCDD/F congener and the respective ^{13}C-labeled internal standard two precursor ions each with one product ion were monitored.

Injector and GC settings (Agilent GC 7890A) applied at the EU-RL:

Injector settings (PCDD/Fs)	
Injector	Multimode injector, solvent vent mode
Injector liner	Deactivated dimpled cold splitless liner, 2mm ID
Injected volume	5 µl
Vent pressure / flow / end time	50 kPa, 10 ml/min, 0.5 min
PTV programme	100 °C, 0.5 min, 700 °C/min, 340 °C, 20 min

GC settings (PCDD/Fs)	
Analytical column	DB-5MS 60 m x 0.25 mm, 0.25 µm (Agilent)
Uncoated column (heated transfer line)	Rxi guard column ca. 0.5 m x 0.25 mm (Restek)
Carrier gas / flow	Helium, 1.4 ml/min (constant flow)
GC oven programme	90 °C, 1.5 min, 20 °C/min to 250 °C, 2.5 °C/min to 285 °C, 25°C/min to 340 °C, 6.5 min
Heated transfer line	360 °C

Extraction and Clean-up

The extraction and clean-up processes for food and feed samples for APGC-MS/MS are the same as for GC-HRMS and are described elsewhere. For comparison the identical extracts were measured on APGC-MS/MS and GC-HRMS. Additionally a modified clean-up with a reduced number of clean-up steps, skipping the carbon column and florisil for further cleaning of the PCDD/F fraction, was applied.

Calibration

For calibration of the APGC-MS/MS system the following calibration solutions (table 1) were applied. For checking of the performance of the GC-MS/MS system in the low concentration range additionally 1:2, 1:5, 1:10 and 1:20 dilutions of the lowest calibration standard Cal 1 were measured.

Taking into account the injection volume of 5 µl, the dilutions of the calibration standard Cal 1 reflect an absolute amount of 2,3,7,8-TCDD on column of 31.3 fg, 12.5 fg, 6.3 fg and 3.1 fg, respectively.

Concentration [pg/µl]	Unlabeled congeners					^{13}C-labeled congeners
	Cal 1	Cal 2	Cal 3	Cal 4	Cal 5	Cal 1 – Cal 5
2,3,7,8-TCDD	0.0125	0.025	0.05	0.2	0.5	0.5
1,2,3,7,8-PeCDD	0.025	0.05	0.1	0.4	1	1
1,2,3,4,7,8-HxCDD	0.025	0.05	0.1	0.4	1	1
1,2,3,6,7,8-HxCDD	0.0625	0.125	0.25	1	2.5	2
1,2,3,7,8,9-HxCDD	0.025	0.05	0.1	0.4	1	0.5
1,2,3,4,6,7,8-HpCDD	0.125	0.25	0.5	2	5	2
OCDD	0.25	0.5	1	4	10	6
2,3,7,8-TCDF	0.0125	0.025	0.05	0.2	0.5	0.5
1,2,3,7,8-PeCDF	0.0125	0.025	0.05	0.2	0.5	0.5
2,3,4,7,8-PeCDF	0.0625	0.125	0.25	1	2.5	2
1,2,3,4,7,8-HxCDF	0.025	0.05	0.1	0.4	1	1
1,2,3,6,7,8-HxCDF	0.025	0.05	0.1	0.4	1	1
1,2,3,7,8,9-HxCDF	0.0125	0.025	0.05	0.2	0.5	0.5
2,3,4,6,7,8-HxCDF	0.0125	0.025	0.05	0.2	0.5	0.5
1,2,3,4,6,7,8-HpCDF	0.025	0.05	0.1	0.4	1	1
1,2,3,4,7,8,9-HpCDF	0.0125	0.025	0.05	0.2	0.5	0.5
OCDF	0.05	0.1	0.2	0.8	2	1

Table 1: Concentrations of individual congeners in calibration standards for APGC-MS/MS

Results and discussion

Sensitivity

For checking of the sensitivity of the APGC-MS/MS system the absolute peak areas of the calibration standards and the dilutions were compared for the transition 320 > 257. For 2,3,7,8-TCDD the absolute concentrations on

column ranged between 2.5 pg and 3.1 fg. The peak areas showed a good correlation to the absolute injected amount on column with an $R^2 > 0.99$ also for the concentration range below 100 fg (figure 1).

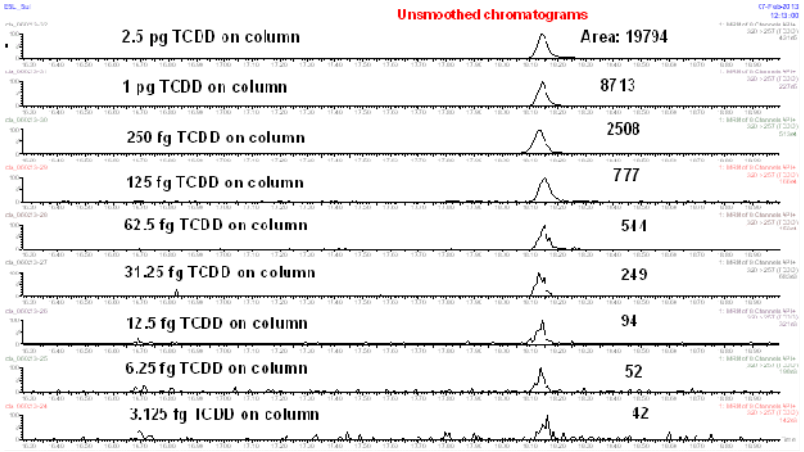

Figure 1: Correlation of peak areas and concentrations for 2,3,7,8-TCDD

Ion abundance ratio

One important criterion for the unequivocal identification of the PCDD/F congeners is the ion abundance ratio between the 2 monitored product ions, resulting from 2 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 calculated ion abundance ratio is comparable with the measured ratios, if an identical collision energy and collision gas pressure is applied for both transitions. The measured ion abundance ratios in calibration matched the calculated theoretical values within the QC limits of ± 15 % (figure 2).

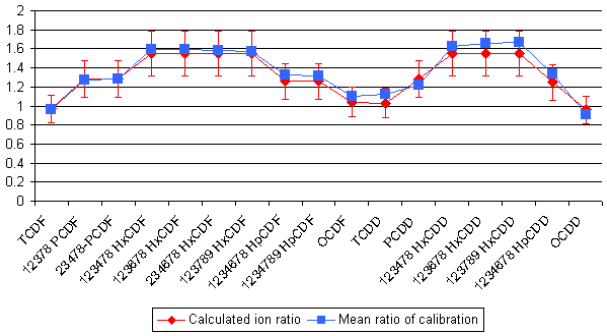

Figure 2: Comparison of calculated and measured ion abundance ratios in calibration (Cal 1 1:5 – Cal 5)

Calculation of the limit of quantification (LOQ)

As for the APGC-MS/MS system a considerable noise level is visible, one option for calculating the LOQ would be the application of the signal-to-noise ratio. However, in order to check the reliability of the results in this low concentration range, the ion abundance ratio and the relative response factors are important additional criteria. The deviations of the theoretical ion abundance ratio should be within ± 15 % of theoretical value and the deviation of the relative response factor of the mean value ≤ 30 % (with a CV ≤ 20% for the complete calibration). Applying these criteria, LOQs were calculated based on calibration standards and dilutions. For 2,3,7,8-TCDD and 2,3,7,8-TCDF LOQs were obtained in the range of 10 – 30 fg on column.

Heated transfer line

Due to the fact that the ion source is operated at atmospheric pressure conditions, the transfer of the higher chlorinated PCDD/Fs from the heated GC oven into the ion source is a critical point. In order to reduce column bleeding in the transfer line, the analytical column is connected with an uncoated column at the end. Transfer line temperatures below 360 °C cause a considerable peak tailing for higher chlorinated congeners, especially

OCDD and OCDF. For the routine application with a maximum oven temperature of 340 °C the transfer line temperature is set to 360 °C.

Results for sample extracts

Results of APGC-MS/MS measurements were compared with assigned values from PTs and mean values of quality control materials obtained from GC-HRMS analysis. The APGC-MS/MS system showed sufficient sensitivity to analyse samples in the range of action and maximum levels. Deviations of the results of APGC-MS/MS from reference values were below 20 % for the WHO-PCDD/F-TEQ (figure 3). Also deviations for individual congeners were in an acceptable range. The fish oil sample was analysed after applying normal clean-up (A), clean-up without carbon column (B) and clean without carbon and florisil column (C). For fish oil sample C considerable differences between the assigned value and the APGC-MS/MS result could be observed due to unresolved interferences on PCDFs.

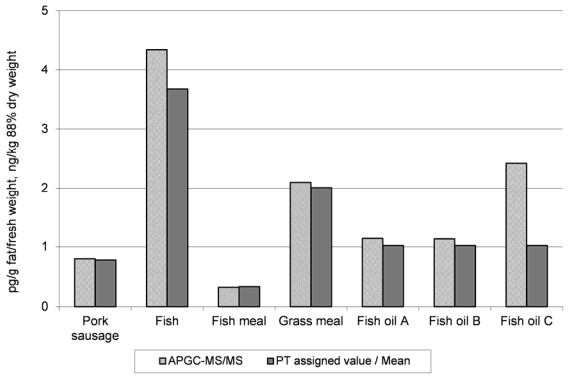

Figure 3: Comparison of APGC-MS/MS results with PT assigned values and GC-HRMS mean values

Summary

First experiences showed sufficient sensitivity of the APGC-MS/MS for monitoring of maximum and action levels for PCDD/Fs in feed and food matrices. As previously shown for other GC-MS/MS systems, the monitoring of two transition product ions provides selectivity for the analysis of PCDD/Fs, which is comparable to GC-HRMS at resolution 10'000. The proposed amendments of EU regulations for application of GC-MS/MS as confirmatory method can also be met by the APGC-MS/MS system. The application of different clean-up methods for fish oil shows, that the extraction and clean-up steps have considerable influence on the quality of the analytical results.

Acknowledgements

We would like to thank the European Commission for the financial support of the work of the European Union Reference Laboratory for Dioxins and PCBs in Feed and Food (EU-RL), Freiburg, Germany, and Waters for providing an APGC-MS/MS testing system.

References

1. Commission Regulation (EU) No 252/2012 of 21 March 2012 repealing Regulation (EC) No 1883/2006 (OJ L 84, 23.3.2012, p. 1–22)
2. Commission Regulation (EC) No 278/2012 of 28 March 2012 amending Regulation (EC) No 152/2009 of (OJ L 91, 29.3.2012, p. 8–22)
3. Kotz A et al. (2012) Organohalogen Compd 74:156-159
4. Ingelido AM et al. (2011) Rapid Commun. Mass Spectrom, 26: 236-242
5. Bernsmann T et al. (2011) Proceedings of Dioxin 2011
6. Sandy C et al. (2011) Organohalogen Compd 73: 1370-1371
7. Kotz A et al. (2011) Organohalogen Compd 73 : 688-691