APPLICATION OF AMENDED CRITERIA FOR CONFIRMATORY METHODS FOR DETERMINATION OF PCDD/FS AND DL-PCBS IN FEED AND FOOD

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Introduction

Within the European Union, methods of sampling and analysis for the official control of levels of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), dioxin- like polychlorinated biphenyls (DL-PCBs) and non-dioxin-like PCBs (NDL-PCBs) in food and feed, and legal limits for these analytes are defined in directives and regulations[1,2,3,4]. The introduction of new analytical methodologies and/or detection methods or further improvement of those already existing may lead to the amendment of legal requirements. In 2012 revised criteria for application of bioanalytical methods for screening of food and feed for PCDD/Fs and DL-PCBs entered into force. In the same year, revised analytical criteria for the use of GC-MS/MS as confirmatory method for determination of PCDD/Fs and DL-PCBs in feed and food were proposed to be adopted into legislation. Different studies had shown the applicability of GC-MS/MS for determination of PCDD/Fs and DL-PCBs in food and feed in the range of the level of interest[5].

One important criterion is the applicability of new criteria in routine analysis. Therefore, the amended criteria for confirmatory methods for determination of PCDD/Fs and DL-PCBs in feed and food are checked for feasibility with special focus on the use of GC-MS/MS methods for confirmation.

Materials and methods

Bioanalytical screening methods and GC-HRMS confirmatory methods are established at the European Union Reference Laboratory (EU-RL) for Dioxins and PCBs in Feed and Food, for determination of PCDD/Fs and PCBs in various feed and food matrices.

Extraction and Clean-up

Basis for the high quality of analytical results is the complete extraction of the analytes of interest from the food or feed matrix and the appropriate clean-up of the extract for the respective detection methods.

For confirmatory methods, the extraction is based on various extraction techniques (Twisselmann/Soxhlet, pressurized liquid extraction or liquid/solid extraction) most suitable for the respective food or feed matrices of interest. For cleaning and fractionation of the obtained sample extracts clean-up methods based on automated and manual steps can be applied. Figure 1 gives an overview on the extraction and clean-up method for food and feed.

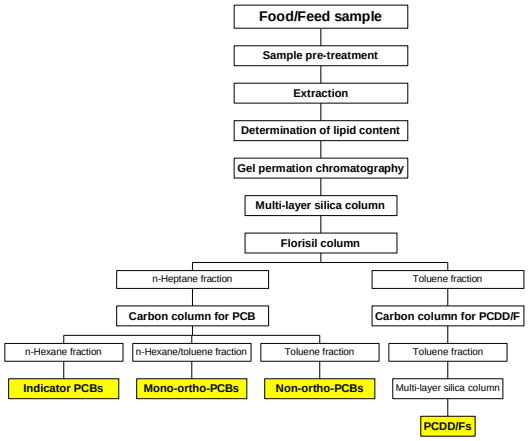


Figure 1: Extraction and Clean-up for PCDD/Fs and PCBs in feed and food

GC-MS measurement

GC-MS measurement is performed using gas chromatography coupled with high resolution mass spectrometry at resolution of 10 000 at 10 % valley. For the development and testing of the proposed analytical criteria for use of GC-MS/MS as confirmatory methods additionally measurements were performed using two different GC-MS/MS systems with different ionization. The results of the evaluation of these GC-MS/MS systems are described elsewhere[6,7].

Criteria for confirmatory methods
The following newly proposed and already established criteria were checked for their feasibility in routine analysis, with special focus on the applicability of GC-MS/MS as confirmatory method for confirmation of compliance or non-compliance:

Analytical criteria for TEQ values:
For confirmatory methods criteria for trueness (-20 % to + 20 %) and within-laboratory reproducibility (< 15 %) are defined and refer to the total TEQ (as sum of PCDD/Fs and dioxin-like PCBs) or separately for PCDD/Fs and dioxin-like PCBs. An illustration of these criteria is shown in figure 2.
Additionally the performance of the method shall be demonstrated in the range of the level of interest with an acceptable CV and a limit of quantification of about or less than 1/5th of the level of interest. The difference between upperbound level and lowerbound level shall not exceed 20% for confirmation of the exceedance of maximum levels or in case of need of action levels.

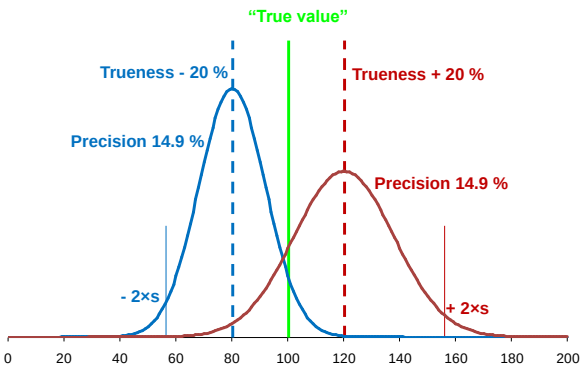


Figure 2: Trueness and within-laboratory reproducibility for TEQ values for confirmatory methods

Criteria for individual congeners:
The calculation of the limit of quantification (LOQ) of individual congeners can be based on signal-to-noise (S/N) ratio or lowest calibration concentration, in case the S/N ratio doesn't provide reliable results.

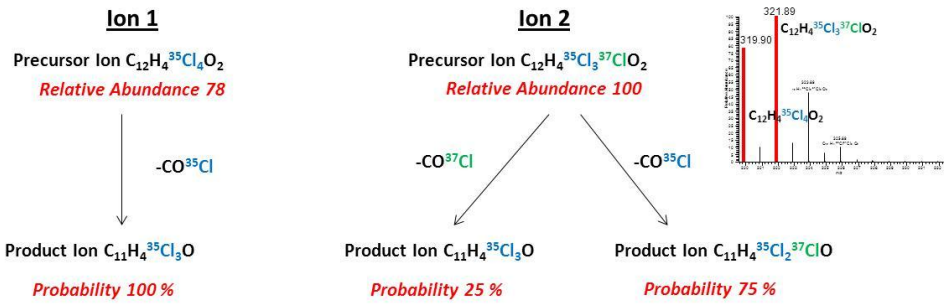
Specific criteria for GC-MS/MS as confirmatory method
The following specific criteria are proposed:

- Monitoring of at least 2 specific precursor ions, each with one specific corresponding transition product ion
- Resolution for each quadrupole equal to or better than unit mass resolution
- Maximum permitted tolerance of relative ion intensities of ± 15% in comparison to calculated or measured values

Results and discussion
Analytical criteria for TEQ values:
Different studies showed the applicability of GC-MS/MS systems for determination of PCDD/Fs and DL-PCBs for food and feed matrices in the range of established maximum levels. For the concentration range below maximum and especially action levels, additional factors such as sensitivity and working range of the specific GC-MS/MS system and the applied clean-up, especially the amount of sample used for extraction and clean-up, has to be considered. In routine analysis it is in the responsibility of the laboratory to show, that their applied extraction and clean-up methods in combination with their GC-MS/MS system can meet the established criteria. Future studies within the network of EU-RL and National Reference Laboratories (NRLs) will additionally focus on the reliability of the analysis of PCDD/Fs and DL-PCBs in feed at levels lower than the respective maximum levels.

Ion abundance ratios
One very important criterion for the correct identification of the analytes of interest, besides the retention time in comparison with the respective ^{13}C-labeled internal standards, is the maximum permitted tolerance of relative ion intensities (ion abundance ratio). For GC-MS/MS at least two specific precursor ions, each with one specific corresponding transition product ion for all labelled and unlabelled analytes have to be monitored. The

maximum permitted tolerance of relative ion intensities is ± 15% for the selected transition product ions in comparison to theoretically calculated or measured values (calculated as average from calibration standards). These strict criteria are comparable to those established for GC-HRMS.
The theoretical calculation of the ion abundance ratios and comparison with the measured values in calibration standards and sample extracts not only gives information about the correct identification of the analytes of interest, but also on the general performance of the GC-MS/MS system. However, it has to be taken into account that theoretical ion abundance ratios and measured values can only be compared, if identical MS/MS conditions, in particular collision energy and collision gas pressure, are applied for each transition of an analyte. The calculation of the ion abundance ratios can be performed as described for 2,3,7,8.TCDD in figure 3.



$$\frac{\text{Rel. Abundance (Ion 1)} \cdot \text{Probability (Ion 1)}}{\text{Rel. Abundance (Ion 2)} \cdot \text{Probability(Ion 2)}} = 1.04$$

Figure 3: Calculation of theoretical ion abundance ratio for 2,3,7,8-TCDD (GC-MS/MS methods)

The comparison of the theoretically calculated and measured values in calibration standards and sample extracts showed that the tolerable QC limits of ± 15% can be met in the relevant concentration range. Higher deviations may indicate either interferences or too low concentrations of the analytes.

Limit of quantification of individual congeners

In general the accepted specific limit of quantification of an individual congener in a sample is the lowest content of the analyte that can be measured with reasonable statistical certainty, fulfilling the identification criteria as described in internationally recognised standards. For the calculation of the LOQ of an individual analyte two options are defined:

- the concentration of an analyte in the extract of a sample which produces an instrumental response at two different ions to be monitored with a S/N (signal/noise) ratio of 3:1 for the less intensive raw data signal.
- 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 calculated for all points on the calibration curve in each series of samples. For application of this LOQ to samples, the recovery of internal standards for the sample and the sample intake has to be taken into account.

The second option is included in order to have an approach for the calculation of the LOQ, which is independent of the detection method and the noise level. Especially for GC-MS/MS very low noise levels, which can't be used for the calculation of a S/N ratio, have been observed.

For the calculation of the LOQ on basis of the calibration curve, it is necessary to measure the normal calibration standards (or at least one representative standard) and additional dilutions of lowest regular calibration standard, especially in the range of the estimated LOQ. Figure 4 shows a fourfold injection of the normal calibration standards and four dilutions (1:2, 1:5, 1:10 and 1:20). For the calculation of the LOQ the respective ion abundance ratios and the relative response factors are compared with the defined limits.

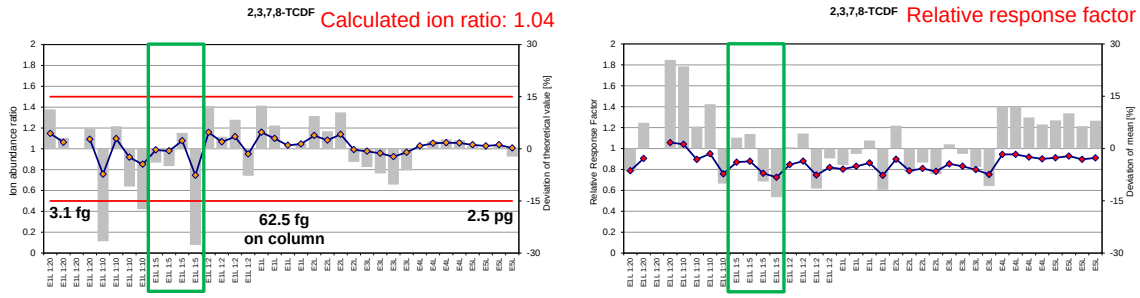


Figure 4: Comparison of ion abundance ratios and relative response factors for 2,3,7,8-TCDF for calibration standards and dilutions

In general it is necessary to measure several different dilutions of the lowest calibration standard as the sensitivity of the system can change from sequence to sequence and the LOQ for all congeners can't always be calculated on basis of the same calibration standard. For checking of the stability of the system the measurement is necessary at least at the beginning and the end of sequence.
On the basis of these calculations and the additional consideration of recovery and sample intake for every congener and sample rather complex calculations have to be performed. Additionally in contrast to the use of the signal-to-noise ratio for an analyte, the LOQ derived from the calibration can't cover all specific matrix effects.

Conclusions

Proposed criteria for GC-MS/MS as confirmatory method for determination of PCDD/Fs and DL-PCBs in feed and food can be met also in routine applications. The reliability of results in concentration ranges below the maximum levels has to be re-checked, especially focusing on application of different extraction and clean-up methods and various GC-MS/MS systems. The comparison of the measured ion abundance ratios with theoretically calculated values gives similar confidence as those obtained for GC-HRMS. The calculation of the LOQ according to the calibration method is more complex compared to the determination of the S/N-ratio and needs further calculations and also additional measurements of suitable standard solutions. Additionally, for a calculation of the LOQ including the full extraction and clean-up method a combination of the instrument LOQ with the reagent blank contribution has to be considered.

Acknowledgements
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