ANALYTICAL CRITERIA FOR USE OF MS/MS FOR DETERMINATION OF DIOXINS AND DIOXIN-LIKE PCBS IN FEED AND FOOD

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Introduction

In the European Union, methods of sampling and analysis for the official control of levels of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (DL-PCBs) in feeding stuff are laid down in Commission Regulation (EC) No 152/2009, amended by Commission Regulation (EU) No 278/2012[1], and for levels in certain foodstuffs in Commission Regulation (EC) No 252/2012[2], respectively. These methods are based on harmonized quality criteria of 2001 for chemical and bioanalytical analysis[3,4] and the revised criteria of 2010 for application of bioanalytical methods for screening of feed and food [5].

The above mentioned regulations require the application of gas chromatography coupled with high resolution mass spectrometry (GC-HRMS) as confirmatory method for the unequivocal identification and quantification of PCDD/Fs and DL-PCBs at the level of interest. In order to identify foods or feedingstuffs exceeding the maximum or action levels, samples can be screened using cost-effective, high sample-throughput screening methods with the aim of avoiding false-compliant results. These methods can be GC-MS methods or bioanalytical screening methods.

Since the late 1980s GC-MS/MS methods (tandem mass spectrometry and ion trap systems) have been applied for the determination of PCDD/Fs and DL-PCBs, mainly in environmental matrices[6]. When the analytical criteria for determination of PCDD/Fs in feed and food were developed[3], GC-MS/MS based methods were considered to be able to provide the required specificity, however, had a considerably higher working range than GC-HRMS instruments. The further development of the tandem mass spectrometry systems in previous years resulted in considerably lower working ranges and the applicability of these systems also for determination of the low PCDD/F and DL-PCB concentrations in food and feed samples. In 2011 different studies on the applicability of GC-MS/MS for determination of PCDD/Fs and DL-PCBs in feed and food samples were published[7,8,9,10]. These results of the comparison of GC-MS/MS and GC-HRMS results indicated that GC-MS/MS has the potential as an alternative confirmatory method.

For further discussion of the aforementioned results, a MS/MS core working group was formed within 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.

MS/MS core working group and network of EU-RL and NRLs

In 2010 the core working group on "Possible use of new MS techniques/instruments for determination of dioxins and PCBs in feed and food" was formed. Core working group members from NRLs and expert laboratories held five meetings with the scope of "Evaluation of new technical developments for sensitive GC-MS/MS determination of dioxins and PCBs in food and feed and development of analytical criteria for possible use of these new techniques". The discussions and conclusions within the working group were based on the experience from different laboratories applying GC-MS/MS systems from different vendors. The results were partly published or presented at meetings of the working group. All laboratories evaluating the performance of the GC-MS/MS systems were expert laboratories with a long experience in PCDD/F and PCB analysis using GC-HRMS.

Results were presented for discussion at three workshops of the EU-RL/NRL network held in 2011 and 2012. The derived criteria as presented here were unanimously agreed at the workshop in May 2012.

Evaluation of GC-MS/MS systems

The evaluation of the available GC-MS/MS systems that were reported to have sufficient sensitivity in different laboratories included the application of

- different extraction and clean-up steps, resulting in different possible interferences in GC-MS/MS,
- different GC-MS/MS systems and parameters,
- different approach of evaluating the performance of the systems.

Important parameters for the evaluation included

- definition of the working range, especially focusing on the lower end of the working range,
- linearity, reproducibility and robustness of the systems,
- applicability in routine analysis,
- comparison of GC-MS/MS and GC-HRMS results (or in comparison with PT consensus values).

From these parameters, the direct comparison of GC-MS/MS and GC-HRMS results from the same sample extracts provides the most important indication on the reliability of the results. This direct comparison covers the possible influence of different food and feed matrices, the influence of possible interfering co-extracted analytes, taking into account the different selectivity of high resolution MS (R = 10000) or MS/MS, and a wide concentration range, including the range of action and maximum levels and also the low background levels.

The conclusions of the evaluation of different available sensitive GC-MS/MS systems showed that latest GC-MS/MS systems from different vendors are applicable for routine analysis of PCDD/Fs and DL-PCBs in feed and food at the levels of regulatory interest and thus have a potential as an alternative confirmatory method.

Proposed amendments of current criteria

<u>Definition of screening and confirmatory methods</u>

Current EU regulations on methods of sampling and analysis (Commission Regulation (EC) No 152/2009 and Commission Regulation (EC) No 252/2012) include the definitions of screening and confirmatory methods in context with the two different goals of monitoring for the presence of PCDD/Fs and DL-PCBs: selection of those samples exceeding maximum or action levels and determination of levels of PCDD/Fs and DL-PCBs.

For better clarification, the here proposed amendments include separate definitions for screening and confirmatory methods. For confirmatory methods the definition now also includes the possibility of using gas chromatography coupled with tandem mass spectrometry (GC-MS/MS) systems with sufficient sensitivity as confirmatory methods. However, for the determination of low background levels, i.e. below $1/5^{th}$ of the level of regulatory interest, the application of GC-HRMS is required.

Limit of quantification of individual congeners

An important criterion is the definition of the limit of quantification of individual congeners. Current legislation defines the "accepted specific limit of quantification of an individual congener' [as] the concentration of an analyte in the extract of a sample which produces an instrumental response at two different ions to be monitored with an S/N (signal/noise) ratio of 3:1 for the less intensive signal and fulfilment of identification criteria as described, for example, in standard prEN 16215 (Animal feed — Determination of dioxins and dioxin-like PCBs by GC/HRMS and of indicator PCBs by GC/HRMS) and/or in EPA method 1613 revision B."

The evaluation of the GC-MS/MS systems showed, that the calculation of a realistic limit of quantification (LOQ) from the signal-to-noise ratio is not always possible due to the very low noise level or even the absence of noise. Differences regarding the recording of the electronic or chemical noise for different GC-MS/MS systems would then result in different LOQ calculations from the signal-to-noise ratio.

Additionally for GC-HRMS systems, the calculation of the LOQ from a signal-to-noise ratios seems to be possible for sample extracts, but with further electronic optimization of these systems and decreasing of the noise level the possibility of very high signal-to-noise ratios and therefore unrealistically low LOQs increases.

For comparability of the different GC-MS/MS and GC-HRMS systems, an approach independent of the noise level and the signal-to-noise ratio is therefore necessary.

The proposed amendments include two different approaches for calculating the limit of quantification for individual congeners. Generally, 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 for example in standards EN 16215[11] and EPA methods 1613[12] and 1668[13] as revised.

Approach 1: The LOQ can be calculated from the signal-to-noise ratio as already defined in the current regulations.

Approach 2: As an alternative approach, if the signal-to-noise ratio does not provide reliable results due to a very low, or no discernable noise level, the limit of quantification is based on the calibration curve. The limit of quantification is then defined as the lowest concentration point on a calibration curve that gives an acceptable ($\leq$ 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 conversion of the limit of quantification from the calibration curve to the sample, the recovery of the internal standards of the respective congener and the sample intake has to be taken into account.

Specific requirements for confirmatory methods

Current EU regulations include specific requirements for GC-MS methods for screening and confirmatory purposes including difference between upper and lower bound calculation, addition of internal standards and limits for recoveries, removal of interfering substances, gas chromatographic separation and range of calibration. Further criteria for GC-HRMS are referred to in EN 16215 and EPA method 1613.

As the inclusion of GC-MS/MS as a confirmatory method required new criteria, the specific criteria for GC-HRMS as a confirmatory method are also further specified. Therefore, besides the reference to EN 16215 and EPA methods 1613 (PCDD/Fs) and 1668 (PCBs), the HRMS resolution is defined as being typically greater than or equal to 10000 for the entire mass range at 10 % valley.

The new criteria for GC-MS/MS as a confirmatory method are based on established criteria in Commission Decision 2002/657/EC[14] and EPA methods 1613 and 1668.

Comparable to HRMS, the application of GC-MS/MS requires the monitoring of at least 2 specific precursor ions, each with one specific corresponding transition product ion for all labelled and unlabeled analytes in the scope of analysis.

The maximum permitted tolerance of relative ion intensities are set to ± 15% for selected transition product ions in comparison to calculated or measured values (average from calibration standards), requiring the application of identical MS/MS conditions, in particular collision energy and collision gas pressure, for each transition of an analyte.

The resolution for each quadrupole must be set equal to or better than unit mass resolution (unit mass resolution: sufficient resolution to separate two peaks one mass unit apart) in order to minimize possible interferences on the analytes of interest.

Additionally, further criteria as described, for example, in standard EN 16215 and/or in EPA methods 1613 and 1668 as revised, except the obligation to use GC-HRMS have to be followed.

Conclusion

The proposed amendments of the current criteria introduce the possibility of using GC-MS/MS systems as an alternative to GC-HRMS systems as confirmatory methods. As for HRMS, these amendments set comparable strict criteria also for MS/MS systems in order to maintain the requested high analytical quality and reliability for confirmation of the levels of PCDD/F and PCB. Finally, it is of paramount important to recognize that apart from the detection system (in this case HRMS and MS/MS) the extraction and clean-up process has a profound influence on the quality of the analytical results.

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