Instrument Performance Standards: A new Concept for fast Routine Performance Checks and Method Development in GC/MS Analysis of Dioxins and Furans

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Overview

Purpose: Develop a concept and tool for fast and profound evaluation of instrument performance in Dioxin/Furan GC/MS analysis.

Methods: A set of two special standards (Std. 1: sensitivity std.; Std. 2: reproducibility std.) was defined and elaborated. Analytical measurements were carried out on a Thermo Scientific DFS high resolution mass spectrometer for feasibility, evaluation and validation of the concept.

Summary: The presented concept of instrument performance standards allows to:

- check the current instrument performance in a fast and efficient way
- optimize analysis methods on the basis of sensitivity and reproducibility
- validate the instrument regarding EU criteria for screening or confirmation methods

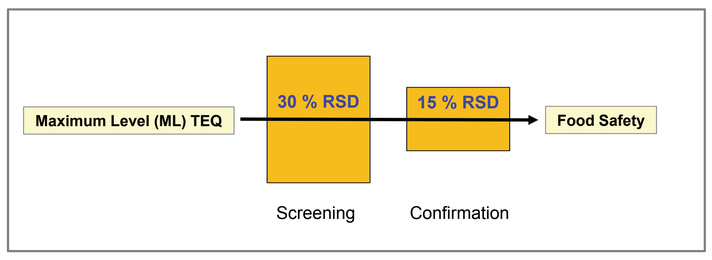
Introduction

Due to their high toxicity, persistence and bioaccumulation properties Dioxins and Furans need to be monitored at lowest levels in food and feed. Repeatedly reoccurring regional incidents of Dioxin/Furan contamination in the food chain (e.g. in 2008 Italian Mozzarella, Irish pork) causing public concern and heavy economic impacts demonstrate that constant efforts have to be taken in this monitoring activity. In this context high efficiency is required for screening methods and for confirmatory methods.

European regulations [1] define analytical performance criteria for high throughput screening and for highly precise confirmation methods. In this context screening methods are permitted to show an RSD of < 30 % at the level of interest whereas confirmatory methods are required to show an RSD < 15% (**Figure 1**). While bioassays or GC-MS/MS may be used for screening purpose the use of GC-HRMS is mandatory for confirmatory analysis.

The very low maximum levels (ML) in food and feed as defined by the European legislation [2] require highly sensitive analytical instrumentation as much for screening as for confirmation purpose. The ability to detect and quantify absolute amounts of Dioxins/Furans in the mid to the very low femtogram range in routine analysis is mandatory.

FIGURE 1: EU Performance Criteria for Screening and Confirmation Methods



Further requirements for screening methods:

- False negatives < 1%
- High sample throughput

In this context the concept of “instrument performance standards” and their application in the laboratory is here presented as a helpful and efficient tool for a required quality control reaching the above described benchmarks in terms of instrument sensitivity and reproducibility.

Materials and Methods

All measurements were carried out on a Thermo Scientific DFS (high resolution magnetic sector mass spectrometer) coupled to two Thermo Scientific Trace GC Ultra supported by an extra-wide Thermo Scientific Triplus autosampler.

GC columns used included Thermo Scientific TR-Dioxin 5ms 30 m x 0.25 mm (0.1 um) and TR-Dioxin 5ms 60 m x 0.25 (0.25 um) coupled to an SSL injecto

TCDD Spec:
20 fg TCDD - S/N 200:1 (4 σ)

The concept for the instrument performance standards was developed and defined at the Thermo Scientific POPs Application Laboratory in Bremen, Germany. The set of two standards was kindly prepared and provided by Wellington Laboratories in Ontario, Canada and was recently commercialized by Campro Scientific, Germany [3].

Results & Discussion

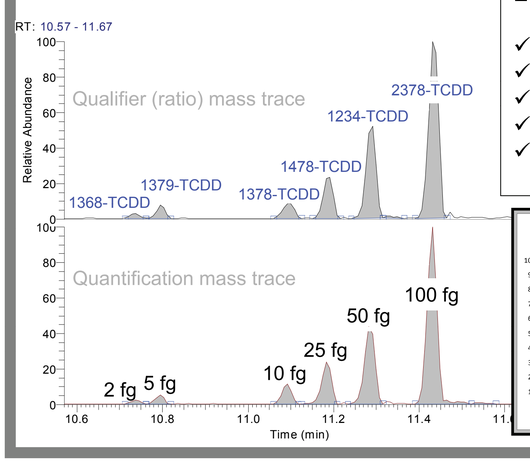
For reasons given above the current performance capabilities of an analytical instrument used for Dioxin/Furan analysis need to be checked on a regular basis in a routine lab. Typically this could be done by analyzing a low concentrated standard. Often the limits of detection and quantitation are then extrapolated from the higher concentration levels in these standards down to far lower values. Real measurements of these very low levels seldom take place and would require repeated injections of decreasing concentrations until the point of minimum Signal-to-Noise values are met.

In most laboratories the time for preparing series of diluted standards and measuring them is not given. This leaves significant uncertainty concerning the current real instrument performance for very low levels (e.g. as to the linearity in this range).

FIGURE 2. Instrument Performance Std. 1: Sensitivity Standard

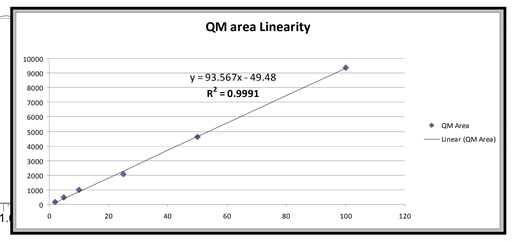
One standard with 6 different native tetra Dioxin congeners at concentrations of 2, 5, 10, 25, 50, 100 fg/µL

Chromatogram of **ONE single injection:**



Evaluation of:

- ✓ Lowest level detected
- ✓ Isotope ratios at different levels
- ✓ Signal-to-Noise at different levels
- ✓ Correct relative peak areas
- ✓ Single injection calibration curve (graph below)



The special instrument performance standard 1 (“sensitivity/linearity std.”) as illustrated in **Figure 2** contains 6 different native tetra Dioxin congeners which can easily be separated via GC on 30 or 60 m 5-Phenyl type apolar columns. The concentrations of the different congeners rise from the first to the last eluting analyte (2, 5, 10, 25, 50 and 100 fg/uL). One internal labeled standard is included at 5 pg/µL (2378-^{13}C-TCDD). This performance standard is used for method development and optimization or for routine performance checks of a GC/MS in terms of sensitivity. From a single injection the following parameters can be checked at 6 different concentration levels:

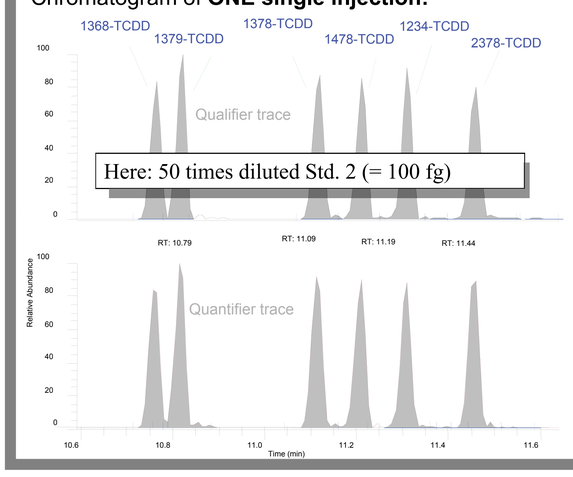
- lowest level detected
- isotope ratios at different levels
- Signal-to-Noise at different levels
- single injection calibration curve

Based on the results of such measurements it can be decided if further method optimization is needed to meet the sensitivity requirements in a given situation (e.g. injection volume), how much sample should be processed to reach overall low level analysis goals of the complete method, if maintenance is needed to re-establish the former instrument performance, etc.

FIGURE 3. Instrument Performance Std. 2: Reproducibility Std. (“RSD” Std.)

One standard with 6 different native tetra Dioxin congeners at a concentration of 5 pg/µL all

Chromatogram of **ONE single injection:**



The „RSD“ standard allows for:

- fast & efficient statistics
- estimation of RSDs from single injection
- method optimization, etc.
- check conformity with requirements for Screening/Conformation methods

A second standard containing the same congeners but all at the same concentration level completes the instrument performance standard set (**Figure 3**). All congeners of this “repeatability/stability” standard are equally concentrated at 5 pg/µL.

Using this standard statistical data can be acquired in a very fast and efficient way, giving 6 values from a single injection (e.g. peak areas, ratios or RRFs). Thus for example RSDs at different concentrations can be calculated and compared from single injections at each level. Experiments have been carried out to evaluate this approach diluting the original standard of 5 pg/µl down to levels of 100, 20 and 10 fg/µL (**Figure 4).** The statistical basis was extended by performing 4 repeated injections at each concentration level (4 x 6 = 24 values).

RSDs for the specific individual congeners calculated from the 4 sequential injections at each level were in the range of 7-10% for 10 fg, 4-7% for 20 fg and 3-5% for 100 fg TCDDs injected. Even more interesting the RSDs across the different congeners in one single injection at the different levels was well in the range of the congener specific RSDs as given above. Thus from a single injection a good estimation of RSDs is feasible.

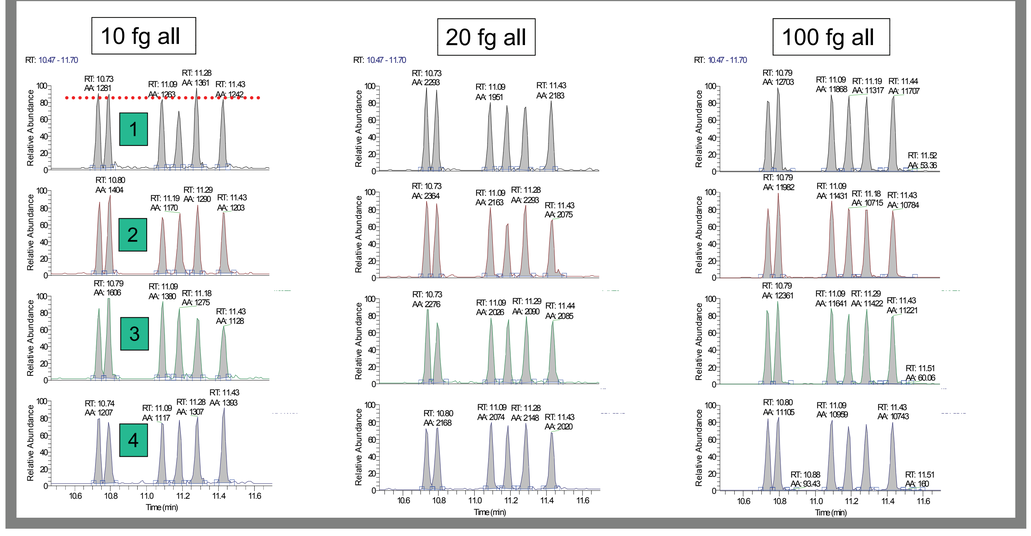
Instrument analysis methods can be optimized on the basis of single injections RSDs, e.g. as for:

- injection volume: (1, 2,..5 µL) determining the minimum injection volume with acceptable RSD
- optimizing the number of sampling points over the peak as for minimum RSD
- optimizing the relative distribution of dwell times for optimum RSD

Likewise from the instrument RSDs sample preparation parameters like sample amount and concentration factors could be determined to meet analysis method performance criteria as defined by EU regulations for screening (RSD < 30%) and confirmation (RSD < 15%) methods (**Figure 1**).

FIGURE 4. Standard 2 („RSD“ Std.): Tetra Dioxins at 10, 20 and 100 fg/µL

4 injections for each concentration level, QM mass traces shown here



Conclusions

Using a set of two specific instrument performance standards – a sensitivity and a reproducibility standard - the instrument performance for GC/MS Dioxin/Furan analysis can be evaluated in a fast and efficient way.

The sensitivity standard is ideally suited to check the current instrument performance in routine analysis and to decide whether the analysis process can go on or if maintenance is mandatory. The reproducibility standard assists in the optimization process during analytical method development and can be used to check whether analysis performance criteria meet requirements given by European regulations for screening and confirmation methods.

References

1. Commission Regulation (EC) No. 1883/2006
2. Commission Regulation (EC) No. 199/2006, amending Regulation (EC) No. 466/2001
3. www.campro.eu

Acknowledgements

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