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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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 Italian Mozzarella, Irish pork, etc.) causing public concern and heavy economic impacts demonstrate that constant efforts have to be taken in this monitoring activity.
Regulations as in the European directives define maximum permitted levels in food and feed, which require analytical instruments being able to detect and quantify absolute amounts of Dioxins / Furans in the mid to the low femtogram range in routine. Efficient tools to develop and optimize instrument methods which meet these requirements and fast tests which routinely allow checking and confirming the validity of the current instrument performance are desirable.
The concept of Instrument Performance standards – a set of two special designed standards - can help to reach the above described benchmarks.

Material and methods

All measurements were carried out on a Thermo Scientific DFS (high resolution sectorfield 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 a SSL injector.
The concept for the Instrument Performance standards was developed and defined at the Thermo Scientific POPs Application Laboratory in Bremen (Germany). The standards were kindly prepared and provided by Wellington Laboratories (Ontario, Canada) and were recently commercialized by Campro Scientific (Germany).

Results and Discussion

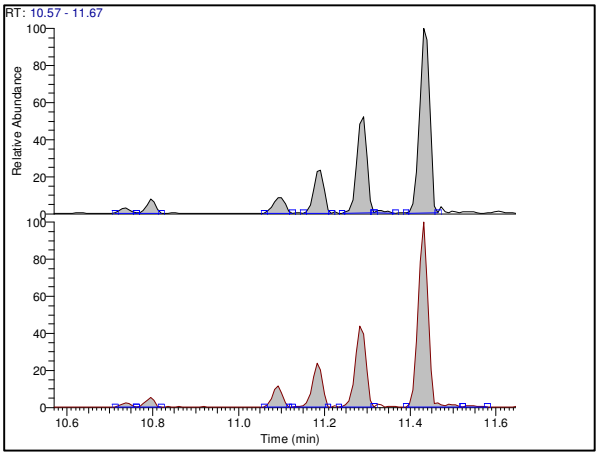

Figure 1: Sensitivity Standard: quantification and ratio mass trace of tetra Dioxins; TCDD congeners from left to right: 1368 - 2 fg/ul, 1379 - 5 fg /ul, 1378 - 10 fg/ul, 1478 - 25 fg/ul, 1234 - 50 fg/ul and 2378 -100 fg/ul (in nonane); 1 ul injected

For reasons given above the current performance capabilities of an analytical instrument used for Dioxin / Furan analysis need to be checked on a constant 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 real instrument performance for very low levels (e.g. as to the linearity in this range).
The special Instrument Performance standard 1 ("sensitivity/linearity standard") as illustrated in **Figure 1** 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 standard is included at 5 pg/ul (2378 ^{13}C TCDD).

This standard is used for method development and optimization or for routine performance checks of a GC/MS. From a single injection it can be checked for the following parameters at 6 different concentration levels:

- lowest level detected
- isotope ratios at different levels
- Signal-to-Noise at different levels
- Correct relative peak areas on quantitation and ratio mass trace
- Single injection calibration curve (**Figure 2**)

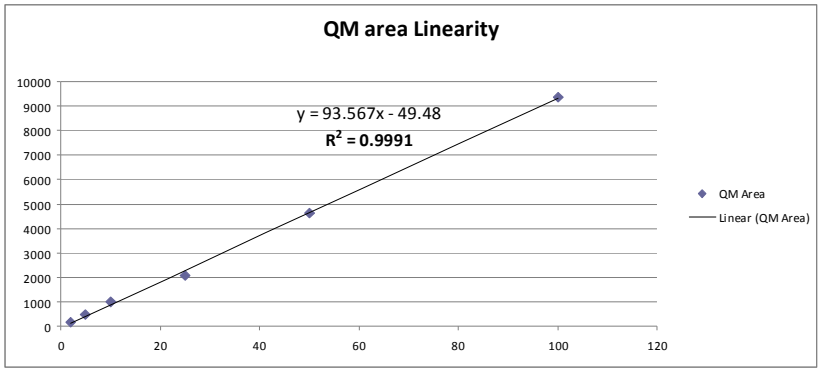

Figure 2: 6 point single injection calibration curve generated with the sensitivity standard (see Figure 1), Peak areas / concentration (fg/ul)

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.

A second standard containing the same congeners but all at the same concentration level completes the instrument performance standard set. All congeners of this "repeatability/stability" standard are equally concentrated (5 pg/ul, **Figure 3**). This standard can be used for proving how similar response factors for the different congeners are, but also allows defining correction factors in this aspect.
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 or RRFs). Thus for example RSDs at different concentrations can be calculated and

compared. Experiments have been carried out for levels down to 2 fg/ul extending the statistical basis by performing 4 repeated injections for each level (4 x 6 = 24 values) .
For very low level analysis the injection volume (1, 2,..5 ul) could be optimized very efficiently by such experiments finding the minimum injection volume were acceptable RSDs are achieved.

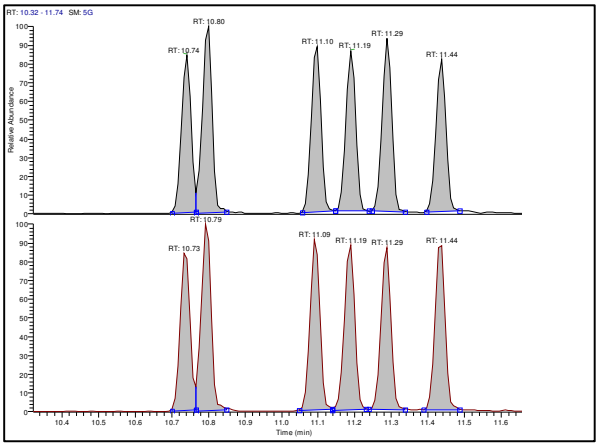

Figure 3: Repeatability Standard: quantification and ratio mass trace of tetra Dioxins;
TCDD congeners from left to right: 1368, 1379, 1378, 1478, 1234, 2378;
here: 1 / 50 diluted standard giving 100 fg/ul for all congenes (in nonane); 1 ul injected

Using the set of above described instrument performance standards the method or instrument performance can be evaluated fast and efficiently with a high precision allowing comparison of different analytical methods, instruments or technologies. It should also be noted in this context that the standards mainly check for the MS performance as deviations due to GC conditions can be supposed to be equal for all 6 components from a single injection process (e.g. deviations due to injection volume or transfer injector to column).

In addition, many further aspects of optimization are possible, e.g. optimizing the number of sampling points over the peak or the relative distribution of dwell times between natives and internal standards both for minimized RSDs and thus best performance.

Acknowledgements
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