Overview

Purpose: To show GC-MS/MS as an efficient PCDDF/Fs screening tool.

Methods: Extensive multi-step clean-up with final measurement using GC-MS/MS.

Results: Strong correlation, between the results of GC-MS/MS and GC-HRMS within acceptable limits achieved around the level of interest for a high percentage of the food and feed samples tested.

Introduction

Removing the frequency of contamination events caused by dioxins and dioxin-like substances is a high priority for governments and organizations charged with the task of protecting human health. The largest source of human dioxin exposure comes through dietary intake of food of animal origin. Consequently, there are extensive monitoring programs in place to identify potential contamination entering into the food chain, 1 when contamination is discovered at non-compliant levels (above maximum levels allowed) the consequences can be serious and widespread. Apart from the risk to human health, contamination events can have a huge economic and political impact and receive a very high level of media attention. As this is the case, there is a strong need for organizations that interact with the food chain, from food ingredient and feed manufacturers, through to consumers and regulatory bodies, to more closely monitor their own interest. The result is that the testing requirement is growing, as is the burden on confirmatory analysis capacity using high resolution (GC-HRMS) techniques. Current European Union regulations permit the use of GC-MS/MS and bioassy techniques for screening dioxins and dioxin-like PCBs at the level of interest in food and feed samples. 2 GC coupled with triple quadrupole MS is particularly suitable screening technique as isolation dilution is retained as well as the high selectivity of the MS/MS experiment. If results are determined to be at a significant level (non-compliant) then confirmatory analysis by a high resolution technique that meets the regulatory requirement must be carried out. In order for a screening technique to be suitable for regulatory dioxins analysis, it must comply with the specific regulations for screening methods and carry with it the ability to strongly correlate with the current ‘gold standard’ confirmatory technique in analytical performance and quality control.

This work describes the use of the Thermo Scientific TSQ Quantum Ultra GC-MS/MSMS as applied to high efficiency screening of PCDD/Fs/PCDFs in feed and food samples at the levels of interest and at a level of performance with ‘gold standard’ confirmatory analysis using GC-HRMS (Thermo Scientific DFS).

Methods

Sample Preparation

The extraction and clean-up process for food and feed samples was performed according Figure 1. For food samples with legal limits on fat basis, the application of a maximum of 3 g of fat for clean-up is applied for achieving low limits of quantification with this method.

GC-MS/MS Measurement

The GC-MS/MS measurements were performed using a TSQ Quantum XLS Ultra™ triple quadrupole connected to a Thermo Trace 1310 GC system.

The GC and injection parameters are found in Table 1. The mass spectrometer parameters are given in Table 2 with the SRM transitions monitored in Table 3. All measurements were performed with each quadrupole set to 0.7 Da FWHM. This ensures unit mass resolution is achieved to minimize the background and the likelihood of interfering ions during the MSMS process.

Results

Selectivity, Sensitivity and Quantitative Performance

In order for a PCDD/F screening technique to be truly efficient it needs to be able to perform at a level that closely correlates with high resolution confirmatory techniques. The first prerequisite of any such technique is sensitivity and selectivity and Fig 2 demonstrates this in real samples. Figure 2a shows an overlay of 2,3,7,8-TCDD target ions for five injections of a mixed animal fat sample at 0.13 μg/g fat. The selectivity and selectivity observed was high enough to allow comfortable, precise detection with all ion ratio integrity maintained. Figure 2b shows overlay of 1,2,3,7,8-PeCDF (0.4 ng/kg 88% dry weight) and 2,3,4,7,8-PeCDF (3.4 ng/kg 88% dry weight) for four injections of grass meal (animal feed) sample.

Ion Ratio Confirmation

During GC-MS/MS analysis, because of the nature of having two stages of MS, the ion ratios differ from that of HRMS but still form a predictable pattern in line with the isotopic composition of precursor and product masses. This allows high confidence in a strong pre-confirmation positive detection. Figure 3 shows the theoretically calculated ion ratios for SRM analysis of tetra thor octa PCDD/F congeners as well as the measured values obtained from a calibration sequence. The data obtained showed strong agreement, well within a typical ±5% GC tolerance (comparable to QC tolerances for GC-HRMS methods in EPA Method 1613 revision B).

Sample and QC Information

Another advantage of screening dioxins using GC-MS/MS is that the isotopic dilution quantification technique, common in HRMS confirmatory analysis is retained. This means that solid quantitative data can be achieved, with real TEQ calculations, as well as a good understanding of sample preparation efficiency through recovery information. Figure 4 gives recovery information for a set of food samples screened using TSQ Quantum XLS Ultra. In addition, congener provenance with profile information remains with triple quadrupole screening, which can add value to continuous monitoring. This information is lost in non-GCMS based screening techniques.

Screening Efficiency

A direct comparison of calculated WHO-PCDD/F-TEQ in pg/g fat (or wet weight for fish) was executed by analyzing the same sample extracts on both the TSQ Quantum XLS Ultra and the DFS HRMS. The data obtained are given in Figure 4. Very good correlation with HRMS data was observed in the real calculated values down to ca. 0.5 WHO-PCDD/F-TEQ pg/g level indicating that a highly efficient screening method is possible with TSQ Quantum XLS Ultra. The selectivity and selectivity obtained with the technique made this possible. This means, in addition to a very low false negative rate, very few compliant samples are likely to be directed to subsequent confirmatory analysis.

Conclusion

- The Thermo Scientific TSQ Quantum XLS Ultra is a highly applicable screening tool for PCDD/Fs in food and feed.

- Strong correlation, between the results of GC-MS/MS and GC-HRMS within acceptable limits were observed around the level of interest for a high percentage of the food and feed samples tested.

- Measured ion ratios for identity confirmation are predictable and can therefore be tested against theoretical values.

- A different approach for LOD calculation (from the signal/noise ratio, employed on HRMS systems) is required due to the inherent low noise of the GC-MS/MS system. For this, the lowest calculated concentration was used.

References
