Application Note: 52266

High Efficiency, Quantitative Dioxins Screening at the Level of Interest in Feed and Food using Advanced GC-MS/MS

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Key Words

- Compliance
- Confirmation
- Dioxins
- GC-MS/MS
- PCBs
- Screening

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 though 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 consumer suppliers 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 bioassay techniques for screening dioxins and dioxin-like PCBs at the level of interest in feed and food samples.[2] GC coupled with triple quadrupole MS is particularly suitable screening technique as isotope 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. These minimum requirements for Total-TEQ (toxic equivalent quotient) from the aforementioned regulations are given in Table 1.

	Screening Methods	Confirmatory Methods
False Negative Rate	<1%	–
Trueness	–	-20% to +20%
Precision (RSDR)	<30%	<15%

Table 1: Commission Regulation (EC) No 152/2009 (Feed), No 1883/2006 (Food)

This application note describes the use of the Thermo Scientific TSQ Quantum XLS Ultra GC-MS/MS as applied to high efficiency screening of PCDDs/PCDFs in feed and food samples at the levels of interest and the level of agreement with "gold standard" confirmatory analysis using GC-HRMS (Thermo Scientific DFS).

Materials and Methods

Extraction and Clean-up

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.

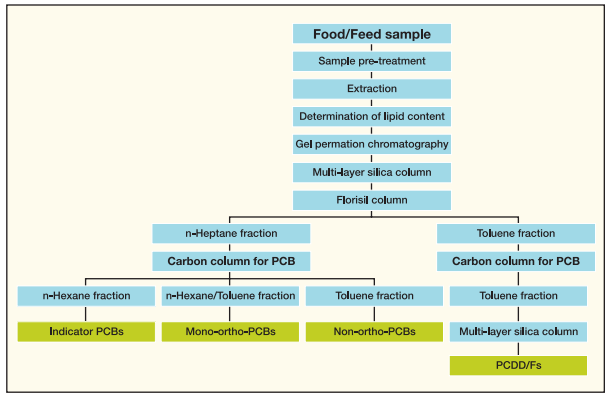

Figure 1: Extraction and clean-up for determination of PCDD/Fs and PCBs in food and feed samples

GC-MS measurement

The GC-MS/MS measurements were performed using a TSQ Quantum XLS Ultra™ GC-MS/MS system.

The following MS/MS settings were applied:

Source Temperature	250 °C
Ionization	EI
Electron Energy	40 eV
Emission Current	50 µA
Q2 Gas Pressure (Argon)	1.5 mTorr
Collision Energy	22 V
Q1 Peak Width	0.7 amu
Q3 Peak Width	0.7 amu

Table 2: Mass spectrometer parameters

The collision cell (Q2) gas pressure and collision energy were optimized for PCDD/F measurement. The monitored SRM transitions as well as the GC conditions are given below in Table 3.

PCDD/F	Precursor	Product
TCDF	303.90	240.94
TCDF	305.90	242.94
^{13}C TCDF ISTD	315.94	251.97
^{13}C TCDF ISTD	317.94	253.97
TCDD	319.90	256.90
TCDD	321.89	258.89
^{13}C TCDD ISTD	331.94	267.97
^{13}C TCDD ISTD	333.93	269.97
PeCDF	339.86	276.90
PeCDF	341.86	278.89
^{13}C PeCDF ISTD	351.90	287.93
^{13}C PeCDF ISTD	353.90	289.93
PeCDD	355.85	292.85
PeCDD	357.85	294.85
^{13}C PeCDD ISTD	367.90	303.90
^{13}C PeCDD ISTD	369.89	305.89
HxCDF	371.82	308.86
HxCDF	373.82	310.86
^{13}C HxCDF ISTD	383.86	319.90
^{13}C HxCDF ISTD	385.86	321.89
HxCDD	387.82	324.82
HxCDD	389.82	326.82
^{13}C HxCDD ISTD	399.86	335.86
^{13}C HxCDD ISTD	401.86	337.86
HpCDF	407.78	344.82
HpCDF	409.78	346.82
^{13}C HpCDF ISTD	419.82	355.86
^{13}C HpCDF ISTD	421.82	357.85
HpCDD	423.78	360.78
HpCDD	425.77	362.77
^{13}C HpCDD ISTD	435.82	371.82
^{13}C HpCDD ISTD	437.81	373.81
OCDF	441.76	378.80
OCDF	443.76	380.79
^{13}C OCDF ISTD	453.78	389.82
^{13}C OCDF ISTD	455.78	391.81
OCDD	457.74	394.74
OCDD	459.74	396.74
^{13}C OCDD ISTD	469.78	405.78
^{13}C OCDD ISTD	471.78	407.78

Table 3: Target congener groups SRM transitions

The results of the GC-MS/MS measurements were compared with routine GC-HRMS measurements using the DFS High Resolution MS (Thermo Scientific, Bremen, Germany).

PTV Injection (PCDD/Fs)	
Injected Volume	5 µL (toluene)
Injection Speed	5 µL/s
Liner	Open Silcosteel® liner (Restek®)
Injection Temperature	100 °C
Vent Flow	20 mL/min
Transfer Rate	13.3 °C/s
Final Transfer Temperature	340 °C
GC Programme (PCDD/Fs)	
GC Column	DB-5MS (60 m, 0.25 µm, 0.25 mm)
Initial Temperature	120 °C
Rate 1	17 °C/min to 250 °C
Rate 2	2.5 °C/min to 285 °C
Final Temperature	285 °C for 13 min

Table 4: GC and injector conditions

Results and Discussion

Selectivity, Sensitivity and Quantitative Performance

In order for a 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. Figure 2 shows an overlay of 2,3,7,8-TCDD target ions for five injections of a mixed animal fat sample at 0.13 pg/g fat. The sensitivity and selectivity obtained was high enough to allow comfortable, precise detection with all ion ratio integrity maintained. Figure 3 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. Figure 4 shown native PCDD/Fs SRM chromatograms for the bottom calibration level for this methodology.

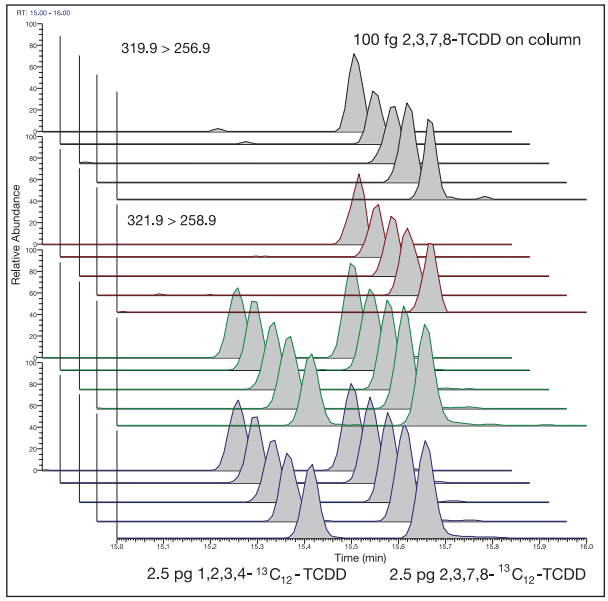

Figure 2: Overlay of 2,3,7,8-TCDD target ions for five injections of a mixed animal fat sample at 0.13 pg 2,3,7,8-TCDD/g fat. 12% CV was achieved on the real calculated amount.

Ion Ratio Confirmation

Most frequently, during routine dioxins analysis using HRMS, an ion ratio comparison of a detected congener is performed against theoretically calculated values. If the value obtained is within acceptable tolerance then the peak has passed that part of the confirmation check. In 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 4 shows the theoretically calculated ion ratios for SRM analysis of tetra thru octa PCDD/F congeners as well as the measured values obtained from a calibration sequence using the TSQ Quantum XLS Ultra. The data obtained showed strong agreement, well within a typical ±15% QC tolerance (comparable to QC tolerances for GC/HRMS methods in EPA Method 1613 revision B).

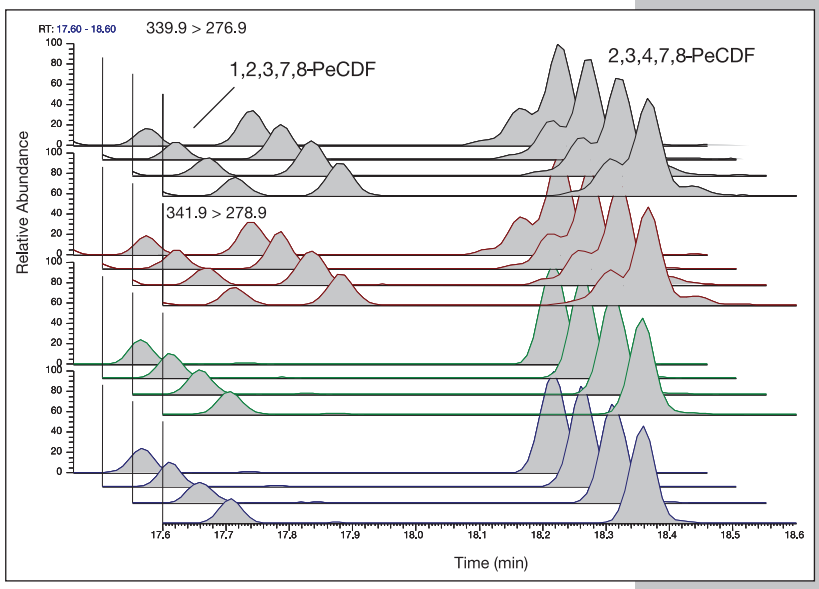

Figure 3: Overlay of 1,2,3,7,8-PeCDF (0.4 ng/kg 88% dry weight) and 2,3,4,7,8-PeCDF (3.4 ng/kg 88% dry weight) for four injections of grass meal sample

Sample and QC Information

Another advantage of screening dioxins using GC-MS/MS is that the isotope 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. Table 5 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 data. This information is lost in non-GC/MS based screening techniques.

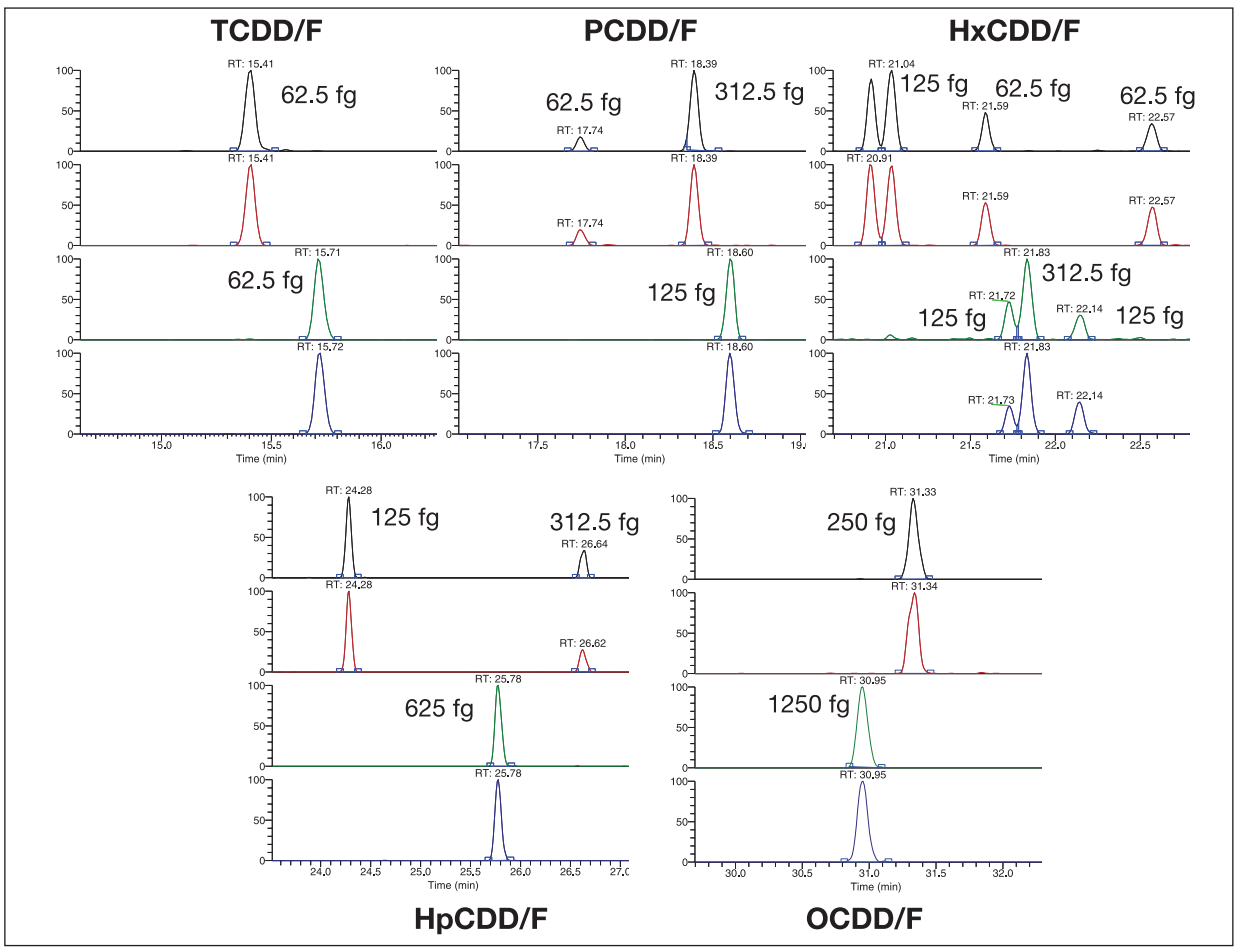

Figure 4: SRM chromatograms of native PCDD/F congeners from the lowest calibration level for the analysis. On column injected amounts are given for each congener. Dibenzofurans can been observed in the top two traces for each congener group and dibenzodioxins in the bottom two.

	Mean Recovery (%)	Relative Standard Deviation (%)
2,3,7,8-TCDF	83	11
1,2,3,7,8-PeCDF	105	12
2,3,4,7,8-PeCDF	101	13
1,2,3,4,7,8-HxCDF	106	14
1,2,3,6,7,8-HxCDF	107	15
2,3,4,6,7,8-HxCDF	104	17
1,2,3,7,8,9-HxCDF	97	17
1,2,3,4,6,7,8-HpCDF	105	18
1,2,3,4,7,8,9-HpCDF	99	18
OCDF	90	26
2,3,7,8-TCDD	87	12
1,2,3,7,8-PeCDD	105	13
1,2,3,4,7,8-HxCDD	110	13
1,2,3,6,7,8-HxCDD	108	14
1,2,3,7,8,9-HxCDD	104	16
1,2,3,4,6,7,8-HpCDD	104	17
OCDD	94	24

Table 5: Recoveries of ^{13}C-labeled internal PCDD/F standards for food samples (n = 42)

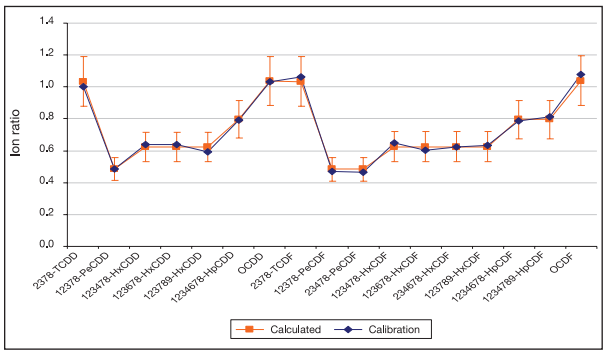

Figure 5: Theoretically calculated ion ratios for SRM analysis of tetra thru octa PCDD/F congeners in addition to the real values obtained from a calibration sequence using the TSQ Quantum XLS Ultra. Error bars show typical ±15% QC tolerance.

Screening Efficiency

A direct comparison of calculated WHO-PCDD/F-TEQ in pg/g fat (or wet weight for fish) was made by analyzing the same sample extracts on both the TSQ Quantum XLS Ultra and the DFS HRMS. The data obtained are given in Figure 6. 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

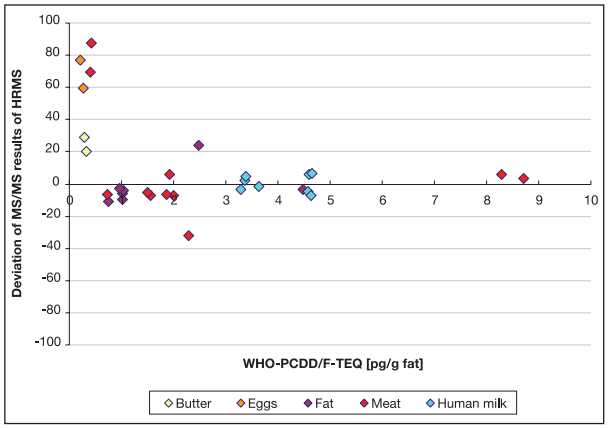

Figure 6: Deviations of WHO-PCDD/F-TEQ of GC-MS/MS results of GC-HRMS (%) for food and human milk samples

efficient screening method is possible with TSQ Quantum XLS Ultra. The sensitivity 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.

Conclusions

- 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 LOQ 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 calibrated concentration was used.

References

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