Application Note: 30112

Confirmation of Low Level Dioxins and Furans in Dirty Matrix Samples using High Resolution GC/MS

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Key Words

- DFS
- Dioxin
- Dioxin-like PCBs
- Furans
- HRGC/HRMS

Introduction

Over the past 30 years, dioxin TEQ levels and body burden levels in the general population have been on the decline and continue to decrease[1][2]. More than 90% of human exposure to dioxins and dioxin-like substances is through food[2]. With increasingly lower dioxin levels in food, feed, and tissues, more demanding limits of detection, selectivity, sensitivity and QC checks are required to confirm their presence at these ever decreasing levels.

Figure 1: Thermo Scientific DFS High Resolution GC/MS with two TRACE GC Ultras™ and TriPlus™ Autosampler.

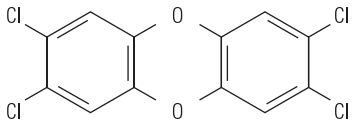

Figure 2: 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD.)

Because HRGC/HRMS possesses all of the above criteria, it has become the most efficient analytical technique for this application, and is now required for dioxin analysis in food and feed by European directives, as well as by the US EPA for Method 1613 Rev.B[3-7]. Also, because of its specificity, HRGC/HRMS is required by these directives for the positive confirmation of the existence of the analyte in the sample.

The new directives demonstrate the continuing need for even more sensitive analytical instrumentation. As an example, the new methods for confirmation require limits of quantitation (LOQ) to be 80% lower than the lowest reported level in the method. This requires the instrumentation to reach even lower levels of detection, and reduce the necessary sample volumes needed for analysis.

Modern instrumentation, like the DFS, can achieve these lower levels of detection as presented for the first time at the "Dioxin 2006" in Oslo, Norway, even at the ag level[8,9], and as a result, samples can be prepared quicker and analyzed with higher sample throughput. The high sensitivity of a DFS HRGC/HRMS system makes it the perfect solution for critical samples.

Experimental Conditions

All measurements were carried out on the Thermo Scientific DFS High Resolution GC/MS system coupled to a Thermo Scientific TRACE GC Ultra™ gas chromatograph equipped with a split/splitless injector. Samples were injected using the Thermo Scientific TriPlus™ Autosampler, see Figure 1. The injection volume was 2 µL of each sample measured. A Thermo Scientific TRACE™ TR-5MS GC column with the dimensions 60 m length, 0.25 mm ID and 0.1 µm film thickness was used for the analysis. The temperature program is shown in Table 1.

The injection was performed using the hot needle technique. The empty needle was heated up in the injector for 2-3 seconds before injecting the sample, thus eliminating any discrimination of higher boiling congeners.

The DFS mass spectrometer was set up in the multiple ion detection mode (MID) at a resolution of 10,000 (10% valley definition). FC43 was used as a reference compound to provide the inherent lock and cali masses. These reference masses are monitored scan-to-scan to insure the highest mass precision, stability and ruggedness necessary for routine target compound analysis on a high resolution mass spectrometer. For all native dioxin/furan congeners, as well as for their specific ^{13}C labeled internal standards, one quantification mass and one ratio mass were implemented in the MID set up, as shown in Table 2. The effective resolution is constantly monitored on the reference masses and documented in the data files for each MID window.

Modifications of the MID descriptor used in this application might be necessary for different applications. As an example, the EPA method 1613 standards typically do not contain the octa-furan ^{13}C labeled internal standard, so the masses in brackets in Table 2 can be deleted for a pure EPA 1613 MID set up. To set the boundaries of the MID retention time windows for each individual congener group, a window defining standard (such as a fly ash) must be used to properly set the MID time windows.

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Selecting the dioxin and furan confirmation masses requires special attention and may vary according to different analysis methods. In this set up, the ratio mass at m/z 371.82300 (52%) was used for the native hexa-furan instead of the normal furan ratio mass trace at m/z 375.81723 (81%). This was done because the the FC43 reference mass at m/z 375.980170 is too close in mass to the analyte and may interfere in the signal of the analyte ion. This alternate ion selection decreases the background noise, and increases the signal-to-noise value of the analyte mass trace. A similar situation can be seen for the hepta-dioxin ratio mass at m/z 425.77317. Here the FC43 mass m/z at 425.976977 is close and could cause interferences.

Injector temperature	260 °C
Splitless time	1.5 min, (septum purge stopped for 1.2 min)
Purge flow	50 mL/min
Column type	Thermo Scientific TRACE TR-5MS 60 m x 0.25 µm x 0.1 µm
Carrier gas flow rate	0.8 mL/min
Oven temperature program (solvent nonane)	120 °C (3 min) 19 °C/min – 210 (0 min) 3 °C/min – 275 °C (12 min) 20 °C/min – 300 °C (3 min)
Transfer line temperature	280 °C

Table 1: GC parameters.

In spite of these effects, FC43 offers practical advantages over PFK as an alternative reference compound. For dioxin analysis, FC43 provides reference masses with good intensity for all MID windows, even at reduced reference gas flows into the ion source. Together with its lower boiling point, FC43 contaminates the ion source less than PFK.

The optimization of the electron energy on the instrument is critical in obtaining the best results. On the DFS instrument used for the demonstrated measurements, an electron energy of 48 eV provided optimum sensitivity. This parameter should be determined once for a given instrument; typical optimum values are generally found between 40 and 50 eV. During the optimized procedure, the best instrument performance was achieved by autotuning the ion source on the FC43 reference mass m/z 414 with a resolution setting of 10,000 as shown in Table 3.

Ionization mode	EI positive
Electron energy	48 eV
Source temperature	270 °C
Resolution	10 000 (10% valley)

Table 3: MS tuning parameters.

MID window no. (time window)	**Reference masses (FC43) m/z L = lock mass; C = cali mass**	**Target masses m/z (n - native; is - ^{13}C int. std.)**	**MID cycle time (intensity, dwell time ms)**
1 – Tetra-PCDD/F	313.98336 (L), 363.98017 (C)	303.90088(n), 305.89813(n),	0.75 s
(9.00 – 19.93 min)		315.94133(is), 317.93838(is),	(L/C: 30, 4 ms; n: 1, 137 ms; is: 7, 19 ms)
		319.89651(n), 321.89371(n),	
		331.93680(is), 333.93381(is)	
2 – Penta-PCDD/F	313.98336 (L), 363.98017 (C)	339.85889(n), 341.85620(n),	0.80 s
(19.93 – 23.52 min)		351.89941(is), 353.85702(n !),	(L/C: 30, 4 ms; n: 1, 147 ms; is: 7, 21 ms)
		353.89646(is !), 355.85400(n),	
		365.89728(is), 367.89433(is)	
3 – Hexa-PCDD/F	375.97974 (L), 413.97698 (C)	371.82300(n), 373.82007(n),	0.80 s
(23.52 – 26.98 min)		385.86044(is), 387.85749(is),	(L/C: 30, 4 ms; n: 1, 147 ms; is: 7, 21 ms)
		389.81494(n), 391.81215(n),	
		401.85535(is), 403.85240(is)	
4 – Hepta-PCDD/F	413.97698 (L), 463.97378 (C)	407.78101(n), 409.77826(n),	0.90 s
(26.98 – 32.06 min)		419.82147(is), 421.81852(is),	(L/C: 35, 4 ms; n: 1, 169 ms; is: 7, 24 ms)
		423.77588(n), 425.77317(n),	
		435.81638(is), 437.81343(is)	
5 – Octa-PCDD/F	425.97681 (L), 463.97378 (C)	441.74219(n), 443.73929(n),	0.95 s
(32.06 – 36.00 min)		(453.78250(is)), (455.77955(is)),	(L/C: 40, 4 ms; n: 1, 183 ms; is: 7, 22 ms)
		457.73706(n), 459.73420(n),	
		469.77741(is), 471.77446(is)	

Table 2: DFS MID set up for PCDD and PCDF analysis: MID lock mode (width first lock: 0.3 u, electric delay: 10 ms).

Sample measurements

Two types of experiments were conducted to prove instrument sensitivity, stability and robustness. First, a long sequence of 72 repeated injections measuring TCDD masses of a 17 fg/µL 2,3,7,8 TCDD standard (diluted from a 100 fg/µL TCDD standard, Wellington Laborato-ries Inc., Guelph, ON, Canada) was performed. Secondly, a real life sample series was analyzed by measuring the full set of dioxin/furan masses of a blood pool sample that contained low concentrations of dioxins/furans quantified for 2,3,7,8 TCDD at 20 fg/µL. Repeat injections were made of several analytical sequences.

A method 1613 CS1 calibration standard (1:10 diluted = 50 fg/µL tetras; 250 fg/µL pentas to heptas; 500 fg/µL octas, Cambridge Isotope Laboratories Inc., Andover, MA, USA) was used to check the chromatographic separation performance of the system.

Results

A typical separation using the GC parameters from Table 1 of an EPA 1613 CS1 dioxin standard at 50 fg/µL of TCDD and TCDF is shown in Figure 3. These GC parameters were also employed for the blood sample.

Instrument sensitivity is demonstrated by the injection of a 20 fg/µL TCDD standard, as seen in Figure 5.

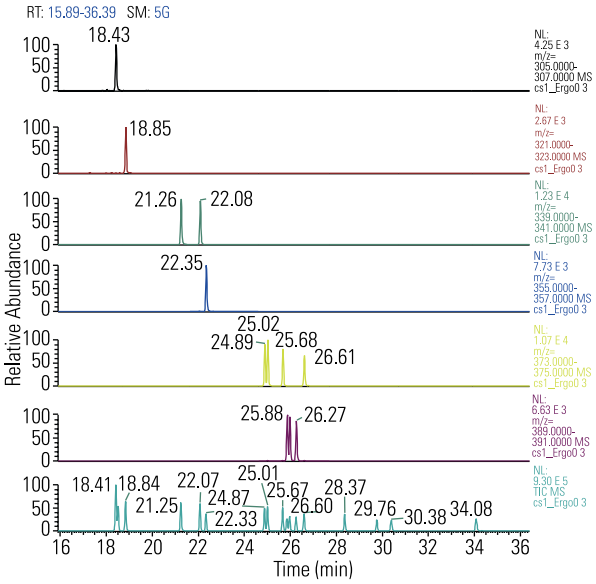

Figure 3: EPA CS1 standard (diluted 1:10, 50 fg/µL TCDD), GC parameters see Table 1.

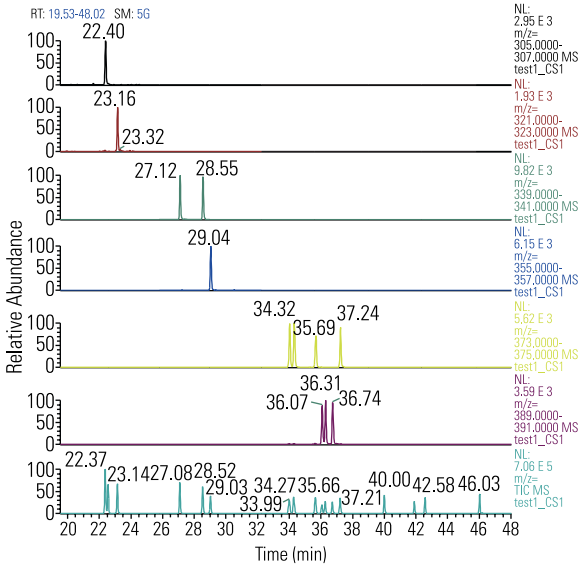

Figure 4: EPA CS1 (1:10), improved GC separation at the expense of longer analysis time, GC oven program: 120 (2)-10-220(10)-3-235(7)-4.6-310(1).

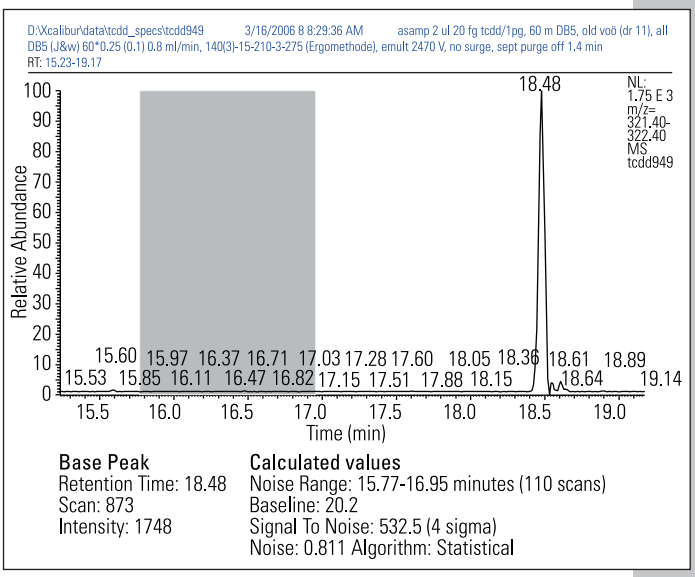

Figure 5: TCDD standard at 20 fg/µL injected providing a S/N > 500:1 on a 60 m column, GC parameters as given in Table 1.

To demonstrate how well the system performs using low dioxin concentrations in dirty matrices, repeated injections were made of a challenging real life sample, a pooled blood sample extract. A typical chromatogram showing the 2,3,7,8-TCDD traces, quantitatively determined at 20 fg/µL, is shown in Figure 6.

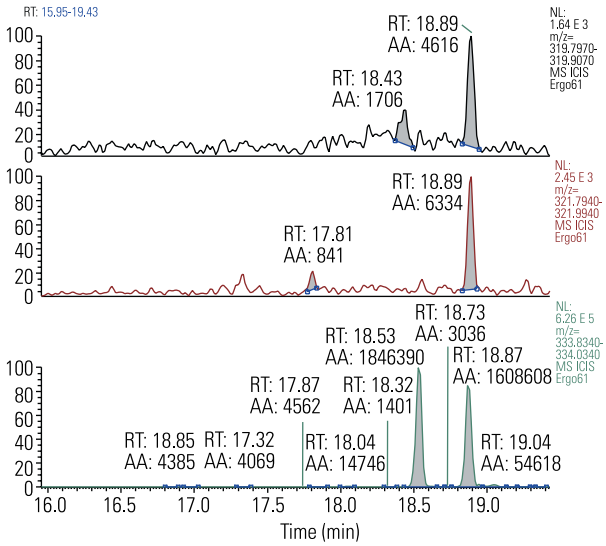

Figure 6: Blood sample extract at 20 fg/µL TCDD, analysis parameters as given in Table 1 - 3 with ratio mass native TCDD (top), quantification mass native TCDD (middle), quantification mass internal standard ^{13}C-TCDD (bottom).

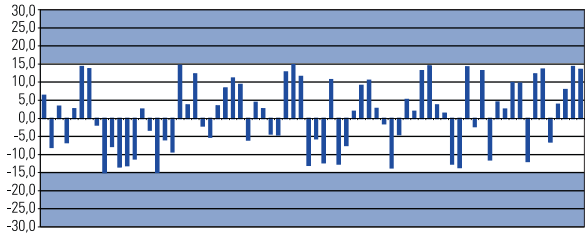

Figure 7: Confirmation of ion ratios m/z 320/322 in % for repeated injections of a 17 fg/µL TCDD standard.

The confirmation ratios (relative areas of quantification and ratio masses) for all dioxins/furans in repeated injections of a 17 fg/µL were evaluated for the standard and the blood pool sample, see Figures 7 and 8. All 2,3,7,8-TCDD results in the standard, as well as in the blood sample series, gave excellent results within the required ± 15% window at the lowest detection levels and provided the confirmation ion ratios in compliance with EPA 1613 requirements.

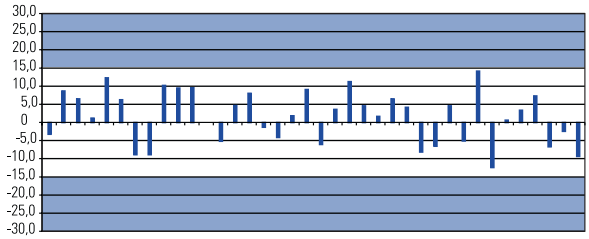

Figure 8: Confirmation of ion ratios m/z 320/322 in % for repeated injections of a blood sample extract.

Conclusions

With the DFS High Resolution GC/MS system, food, environmental, and biological samples can be analyzed for dioxins and furans in the low femtogram range. Even difficult sample types with heavy matrix effects can be successfully analyzed.

The reliability, sensitivity and long-term robustness of the DFS are demonstrated with a series of repeated injections of a "dirty" matrix blood sample. Using HRGC/HRMS, the DFS system delivers confirmatory analyses that can withstand any legal interrogation.

References

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AN30112_E 03/08C