MS Systems for the Analysis of Dioxins and PCBs

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Goal

1 To create an overview of the performance specifications of available MS systems for the analysis of Dioxins and PCBs

2 To offer a general insight in the possible techniques and the differences between them

Figure 1 Thermo Scientific™ DFS™ Magnetic Sector GC-HRMS System

Introduction

The use for high resolution mass spectrometry (HRMS) was for years a prerequisite for official analysis of dioxins and PCBs in the US[1,2] and Europe[3,4]. For these analysis, magnetic sector HRMS became the standard technique due to its superior sensitivity. However, sector HRMS required extensive training of its operators and is costly in operation, maintenance and purchase.

Yet, up to the second decade in this millennium sector HRMS was used in most laboratories. By the second decade of this millennium a demand for cheaper and easier systems to operate grew as the commercial exploitation of dioxin analysis started to raise. This was accelerated by incidents with dioxin contamination in the years before[5] and the resulting maximum limits (MLs) set by the authorities for susceptible commodities[3,6]. In 2014 the EU allowed after careful

consideration[7] tandem low resolution mass spectrometry (MSMS) for the confirmatory analysis of food[8] and feed[9].

Currently several manufacturers are offering instruments for dioxin analysis and although information is available, it still needs to be requested or withdrawn from the online available sources. With this study a starting point for comparing the performance of these instruments and there suitability is aimed to be provided.

Instruments

Historical three manufacturers of sector HRMS instruments dominated the market for Dioxin analysis, but now with the acceptance of MSMS other manufacturers could enter the playground. For sector HRMS systems the following instruments were considered:

- Thermo Scientific™ DFS™
- JEOL JMS-800D
- Waters Autospec Premier (discontinued)

And for MSMS:

- Thermo Scientific™ TSQ™ 9000
- Shimadzu GCMS-TQ8050
- Waters Xevo TQ-S with APGC
- Agilent 7010B

Figure 2 Shimadzu GCMS-TQ8050 Triple Quadrupole Gas Chromatograph Mass Spectrometer
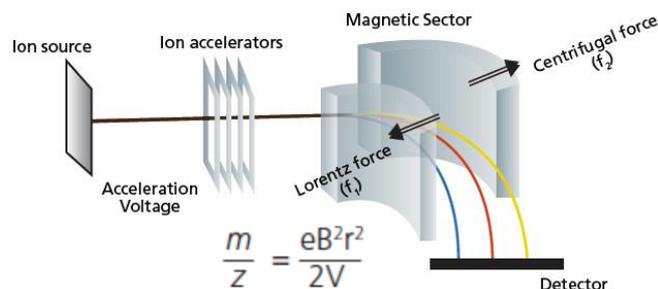
Figure 3 Schematic of magnetic sector HRMS and principle of ion separation. B magnetic flux density, z charge of the ion, e elementary charge, v velocity of the ion, m mass of the ion, r path radius, V acceleration voltage applied to ions

$$\frac{m}{z} = \frac{eB^2r^2}{2V}$$

Fundamentals

Magnetic sector HRMS and MSMS are both highly selective method to identify and quantify molecules. Their selectivity however, is obtained differently. In magnetic sector HRMS ions are separated by accelerating ions to a equal velocity and applying a magnetic field which deflects ions according Fleming's left-hand rule (fig, 3). One of the great benefits of this technique is the high transmission of ions generated in the source to the detector at higher resolutions. The sensitivity obtained with such systems depends very much on the number of ions produced in the source. As HRMS requires deep vacuum, electron ionization (EI) is typically applied to create ions.

In MSMS selectivity is not obtained by resolution, but by specific fragmentation of an ion generated in the source. The number of ions reaching the detector depend not only on the number of ions generated in the source, but also by the fragmentation efficiency of the selected fragment. For dioxins and furans loss of COCl and for PCBs CL_2 results in the positively charged fragments used for multiple reaction monitoring (MRM) measurements.

As ionization is not selective, the most abundant isotope ion created in the source is generally selected in HRMS. In MSMS however, the number of ions reaching the detector also depends on the fragmentation and other ions might result in a higher signal on the detector. For dioxins and PCBs fragmentation follows probability statistics in MSMS due to the loss of Cl and its distinct isotope clusters (fig. 4). For dioxins and furans the same ions are favourable to select in the source, but for PCBs, due to the loss of 2 Cl in MSMS, other isotopes will result in a higher signal on the detector.

So disregarding efficiency of the ion beam transmission through the analysers, in MSMS, the number of ions reaching the detector is intrinsically lower than in HRMS for dioxins and PCBs. Yet, for MSMS systems other types of ionization with better yields, like atmospheric pressure chemical ionization (fig. 5) are easier to implement as MSMS systems do not require such low operating pressures as for HRMS.
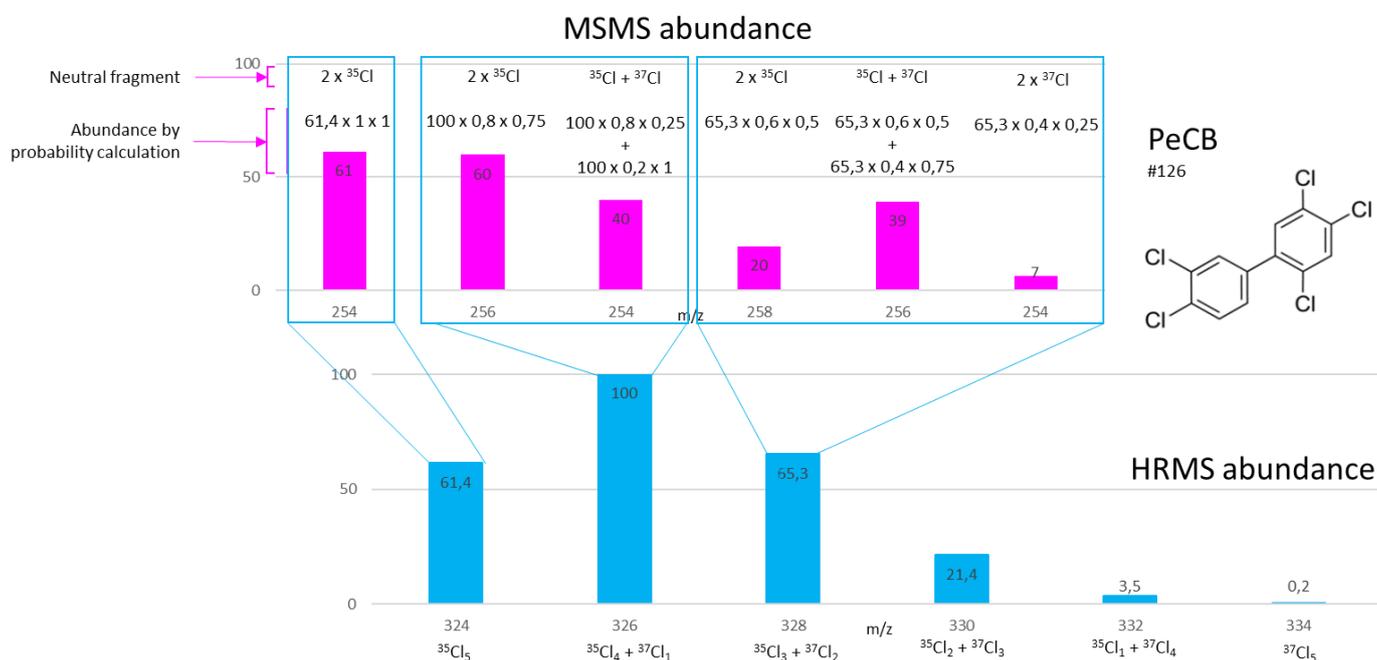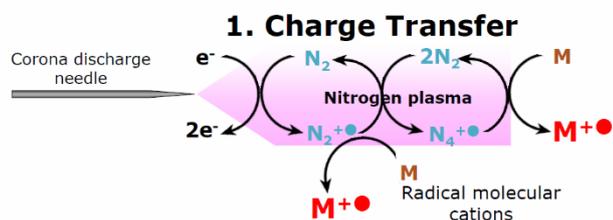

Figure 4 Theoretical HRMS (lower) and MSMS (upper) spectrum based on isotope abundance and probability calculation for fragmentation assuming equal experimental conditions for each transition.

1. Charge Transfer

"Dry" source conditions
Favoured by relatively **non-polar compounds**

Figure 5 Ionization mechanism for dioxins and PCBs in APGC.

Performance requirements

For the analysis of dioxins and PCBs several regulations describe the performance requirements[3, 10, 11]. Taking these criteria to instrument level, two parameters are of upmost importance for MS systems, being sensitivity and stability of the response.

As dioxins are of interest at already very low levels in samples systems should be able to detect an amount of 25 fg on-column accurately at the lower end of the spectrum (considering a sample feed oil, an intake of 2.5 gram and injection of 1/5 of the sample). Considering the EU guideline[12], for a standard of 25 fg on-column the ion ratio should be within ± 15 % while the deviation from the average relative response factor should be within ± 30 % for the lowest standard. The accuracy and stability of the response of a system need to be sufficient to reach these criteria within routine use and accuracy and repeatability of the relative response factors should be well within the ± 20 % and 15 %[3]. The stability of the absolute response is important to obtain consistent LOQ's as they should be corrected for recovery.

In addition to the regulatory requirements, the linear range is from practical perspective of interest. Is certain types of samples, like fish oil and palm fatty acid distillate, levels of dioxins and PCBs can vary extensively from below LOQ (below 0.05 pg/g) to over 100 pg/g within a sample. For these kind of samples a linear range of over 4 to 5 orders of magnitude would be desirable to measure all congeners in one measurement.

Specifications from application notes

Information was retrieved from application notes (table 1) to create an overview of the specified

sensitivity and linear range (table 2). In addition, an overview of reported chromatograms is given in figures 6 till 14. From the given information in the application notes and inspection of the chromatograms LODs for the systems were estimated and also given in table 2.

All systems meet the supposed required LOD of 25 fg on-column. Yet, the lowest amount measured is generally higher and for a thorough evaluation the sensitivity should be demonstrated as well as the stability and accuracy of the responses at this level. It should also be noted that the experimental conditions, instrument settings and the way signal to noise ratios (S/N) are calculated are not always comparable. Though, the standard deviations over injections of a fixed concentration, relative standard deviations over a calibration curve and stability of ion ratios should be comparable unlike S/N which are more subjective to instrumental settings and data processing.

Table 1 Application notes accessed for this study

Instrument	Application note(s)
Thermo Scientific™ DFS™	[PDF] Thermo HRMS 1.pdf [PDF] Thermo HRMS 2.pdf
JEOL JMS-800D	[PDF] JEOL HRMS 1.pdf
Waters Autospec Premier	[PDF] Waters HRMS 1.pdf [PDF] Waters HRMS 2.pdf
Thermo ScientificTM TSQ™ 9000	[PDF] Thermo MSMS 1.pdf
Shimadzu GCMS-TQ8050	[PDF] Shimadzu MSMS 1.pdf
Waters XevoTQ-S with APGC	[PDF] Waters MSMS 1.pdf
Agilent 7010B	[PDF] Agilent MSMS 1.pdf [PDF] Agilent MSMS 2.pdf

Table 2 Analytical conditions, sensitivity and linear range

Type	Magnetic Sector HRMS			Quadrupole MS-MS			
Manufacturer	Thermo Scientific	Waters	Jeol	Thermo Scientific	Waters	Shimadzu	Agilent
Model	DFS	Autospec	800D	TSQ Quantum XLS Ultra	Xevo-TQS	TQ8050	7010B
GC-column	TR-5MS	DB5-MS	DB5-MS	DB5-MS	DB5-MS	SH-Rxi-5Sil MS	DB5-MS
Dimensions	60 m x 0.25 mm x 0.1 µm	40 m x 0.18 mm x 0.18 µm	60 m x 0.25 mm x 0.25 µm	60 m x 0.25 mm x 0.25 µm	60 m x 0.25 mm x 0.25 µm	60 m x 0.25 mm x 0.25 µm	60 m x 0.25 mm x 0.25 µm
Oven temperature program	120 °C (3 min) 19 °C/min – 210 (0 min) 3 °C/min – 275 °C (12 min) 20 °C/min – 300 °C (3 min)	140 °C,(4 min) 9 °C/min to 220 °C, 1.4 °C/min to 260 °C 4 °C/min to 310 °C (6 min)	120°C (3 min) 20 °C /min to 170 °C 3 °C/min to 260°C 25 °C/min to 300 °C (3 min)	120°C 17 °C /min to 250 °C 2.5 °C/min to 285°C (13 min)	-	150 °C (1min) 20 °C/min to 220 °C 2 °C /min to 260 °C (3 min) 5 °C /min to 320 °C (3.5 min)	100 °C (2 min) 30 °C/min to 220 °C (16 min) 2 °C/min to 240 °C (5 min) 5 °C/min to 270 °C (4 min) 15 °C/min to 330 °C (6 min)
Injection volume	2 µl	1 µl	2 µl	5 µl	5 µl	2 µl	1 µl
Flow rate	0.8 ml/min	0.7 ml/min	1 ml/min	-	1.4 ml/min	45.6 cm/sec	1.056 ml/min
RT 2378-TCDD	18.48 min	27.20 min	-	-	15.41 min	22.83 min	31.77 min
Resolution	10,000 (10% valley)	10,000 (10% valley)	10,000	0.7 amu	-	-	-
Electron energy	48 eV	30 eV	40 eV	40 eV	-	-	-

Type	Magnetic Sector HRMS			Quadrupole MS-MS			
Manufacturer	Thermo Scientific	Waters	Jeol	Thermo Scientific	Waters	Shimadzu	Agilent
Model	DFS	Autospec	800D	TSQ Quantum XLS Ultra	Xevo-TQS	TQ8050	7010B
2378-TCDD							
ion 1	319.89651	319.8965	-	319.90>256.90	320>257	319.9>256.9	319.9>256.9
ion 2	321.89371	321.8940	321.8936	321.89>258.89	322>259	321.9>258.9	321.9>258.9
Channel time	136 ms	-	-	-	-	200 ms	-
[c] measured	34 fg on-column	50 fg on-column	100 fg on-column	62.5 fg on-column	2 fg on-column	50 fg on-column	200 fg on column
Linear range reported	-	50 fg - 400 pg on-column	-	-	100 fg - 40 pg on column	50 fg - 2 pg on-column	200 fg - 2.5 ng on-column
RSD or R^2 calibration curve	-	2 %	-	-	>0.998	8 %	0.996, 6 %
LOD (*estimated, 'standard solution, "sample)	10 fg*" on column, 0.2 fg' on-column	4.8 fg" on-column	<1.5 fg*' on-column	-	10 fg*' - 30 fg*' on-column	1 fg*', 20 fg" on-column	1 fg*' on-column

The linear range was reported for only one HRMS system and was a factor 8,000, while for MSMS systems the reported linear range varied from a factor 40 to 12,500. As the desired linear range is at least over factor 1,000 the required performance in sense of linear range was not demonstrated for all systems.

The stability (repeatability) of the RRF in all systems over the calibration curves were good with reported correlation coefficients of over 0.99 or RSDs <10%. Although the variability was determined in different concentration ranges and by different approaches, the information does provide a decent insight on the performance of the systems.
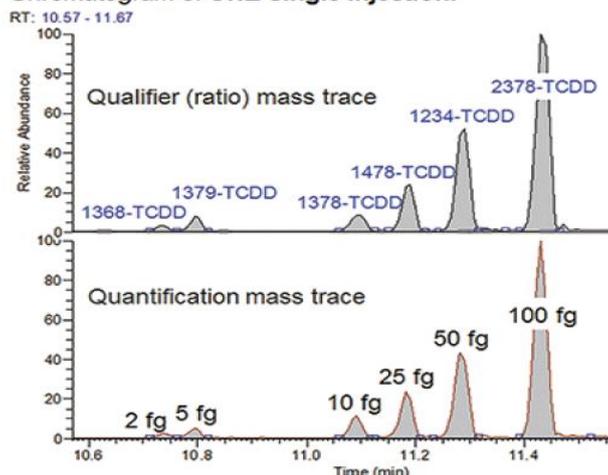

Figure 6 Thermo Scientific™ DFS™, response for tf-tcdd-mxb

Information on ion ratios are reported by different approaches, e.g. in the lowest standard or spiked by a spiked sample. As expected, the ion ratios were within the set criterium of ± 15 % for nearly, if not all observations. Numeric results on ion ration however, are scarce and are mostly reported in the discussion as meeting the criterium.

Potential competitors

For the analysis of dioxins and PCBs GC-sector HRMS has been the golden standard for many years and recently GC-MSMS is acquiring a substantial part of the market. These are the systems nowadays readily considered when new instrumentation is considered. However, also other HRMS systems, GC-Orbitrap
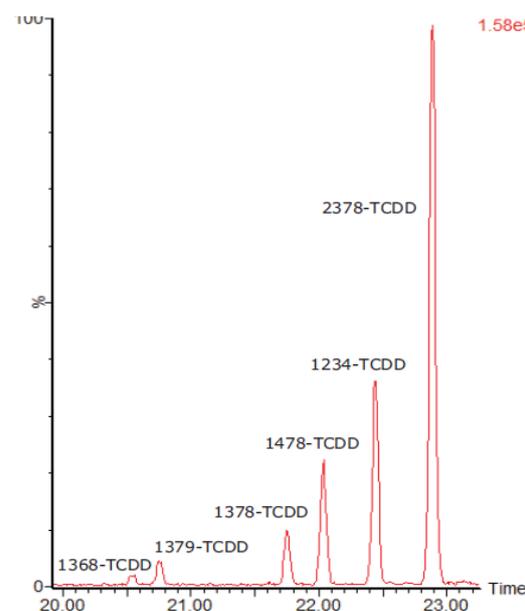

Figure 7 JEOL JMS-800D, response 100 fg TCDD on column

HRMS and GCxGC-TOF HRMS, meet the selectivity requirements as they are able to operate at 10,000 (10% valley) resolution. Till now they are not generally considered as a competitor as they have not proven

to be able to compete in sensitivity and linear range with sector HRMS or financially with MSMS. Recent studies show that such systems might at least be of scientific advantage for their screening capabilities. Furthermore, the sensitivity of these systems are approaching those of sector HRMS and MSMS systems[13, 14].

Figure 8 Waters Xevo TQ-S with APGC, response for tf-tcdd-mxb
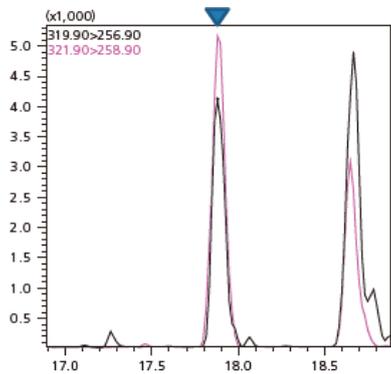
Figure 9 Shimadzu GCMS-TQ8050, standard solution 100 fg 2378-TCDD on column (S/N 285)
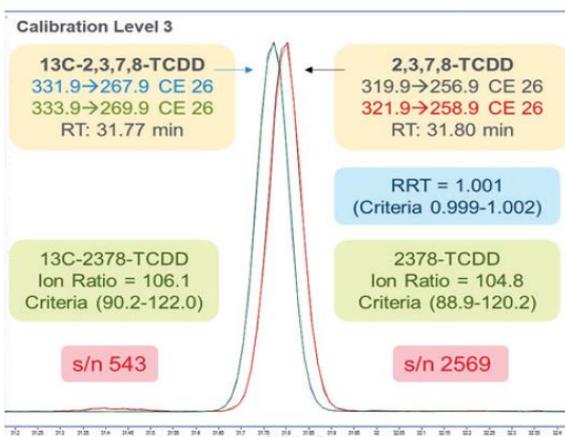

Figure 10 Agilent 7010B, standard solution 1000 fg 2378-TCDD on column

Figure 11 Thermo Scientific™ DFS™, response for 40 fg 2378-TCDD on column in a blood sample extract

Summary

Dioxin and PCB analysis often require dedicated instrumentation and purchase of such systems should be well considered. Different types of techniques are nowadays available and manufacturers are keen in promoting the advantages. As buyer one should determine well to which requirements a system should meet and request a demonstration. Although, requirements are generally thought of performance specifications such as sensitivity, linear range and repeatability, also applicability in their own laboratory should be considered.

Based on the current stingiest maximum limits for feed in the EU and actual concentrations in samples systems should meet the following criteria:

- LOD 25 fg on-column
  - ± 30 % accuracy
- Ion ratio ± 15%
- Linear range 25 fg – 25 pg on-column
  - RSD ≤ 20 %
  - ± 20 % accuracy individual standards

References

1) US Environmental Protection Agency, Method 1613, Tetra- through Octa-chlorinated Dioxin and Furans by Isotope Dilution HRGC/HRMS, revision A, (1994)

2) US Environmental Protection Agency, Method 1668, chlorinated biphenyl congeners in water, soil, sediment, biosolids and tissue by HRGC/HRMS, revision A, (2003).

3) Commission Regulation (EC) No 278/2012 of 28 March 2012 amending Regulation (EC) No 152/2009 of (OJ L 91, 29.3.2012, p. 8–22), laying down the methods of sampling and analysis for the official control of feed

4) Commission Regulation (EU) No 252/2012 of 21 March 2012 repealing Regulation (EC) No 1883/2006 (OJ L 84, 23.3.2012, p. 1–22), laying down methods of sampling and analysis for the official control of levels of dioxins and dioxin-like PCBs in certain foodstuffs

5) Hoogenboom 2019, ECVPH Food safety assurance: 7 - Pages: 503 - 528, Incidents with dioxins and dioxin-like PCBs in the food chain
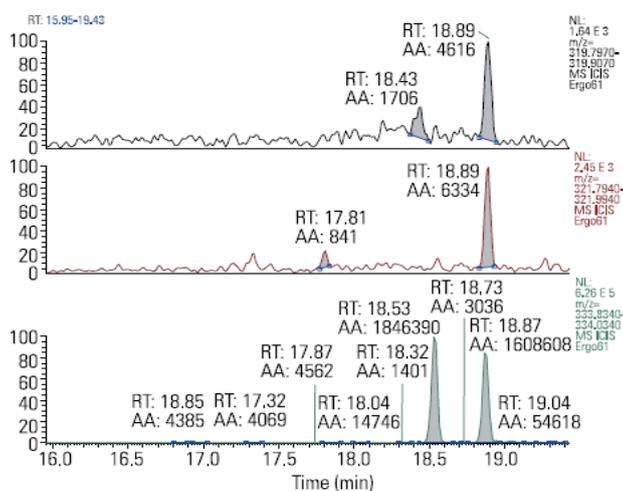
6) Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs

7) Kotz et al. 2012, Organohalogen Compounds Vol. 74, 156-159, Analytical criteria for use of MS/MS for determination of dioxins and dioxin-like PCBs in feed and food

8) Commission Regulation (EU) No 589/2014 of 2 June 2014 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EU) No 252/2012, Official Journal of the European Union, (2014) L364/365-324
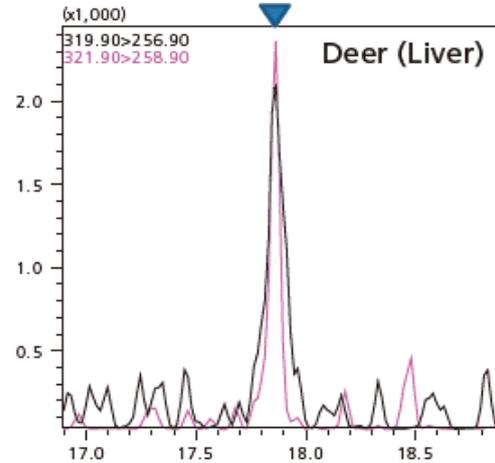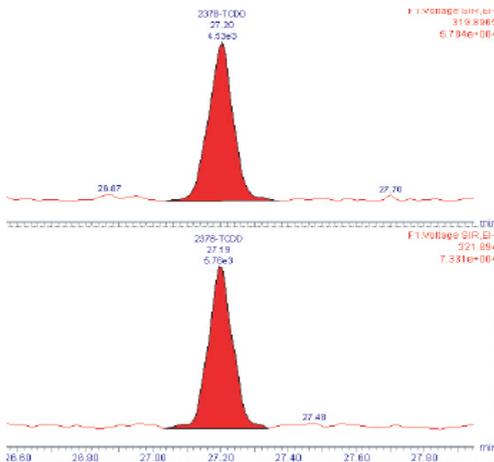
Figure 12 Waters Autospec Premier, response for 100 fg 2378-TCDD spiked to citruspulp
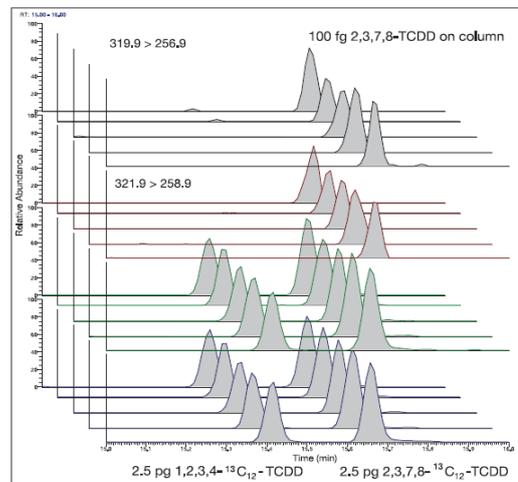
Figure 13 Thermo Scientific™ TSQ™ 9000, overlay of 2378-TCDD chromatograms for 0.13pg/g animal fat

Figure 14 Shimadzu GCMS-TQ8050, 60 fg 2378-TCDD on column in a deer liver sample extract

9) Commission Regulation (EU) No 709/2014 of 20 June 2014 amending Regulation (EC) No 152/2009 as regards the determination of the levels of dioxins and polychlorinated biphenyls Text with EEA relevance. Official Journal of the European Union, Official Journal of the European Union, (2014) L188/181-118.

10) Commission Regulation (EU) 2017/644 of 5 April 2017 laying down methods of sampling and analysis for the control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs

11) EPA; U.S. Environmental Protection Agency (1994) Method 1613: Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS.

12) Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food

13) Jean-François (Jef) Focant, LECO Corporation , Oct. 26, 2019, GC×GC–TOF-MS in Food and Feed Control: Going Beyond Dioxin Measurements

14) Paul D. Jones and John P. Giesy, Quantification of Dioxins by GC-Orbitrap MS