Trace analysis of polychlorinated dibenzo-p-dioxins/furans (PCDD/F) in food and feed using GC-MS/MS

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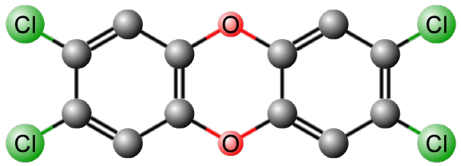
Product Specialist for GC and GC/MS

Dioxins

Organochlorines

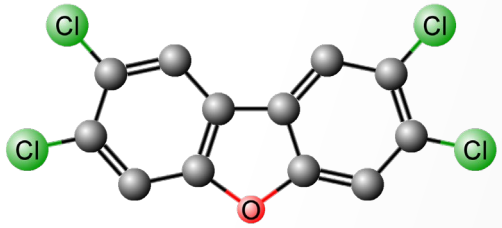
Synthesis byproduct



2,3,7,8-TCCD

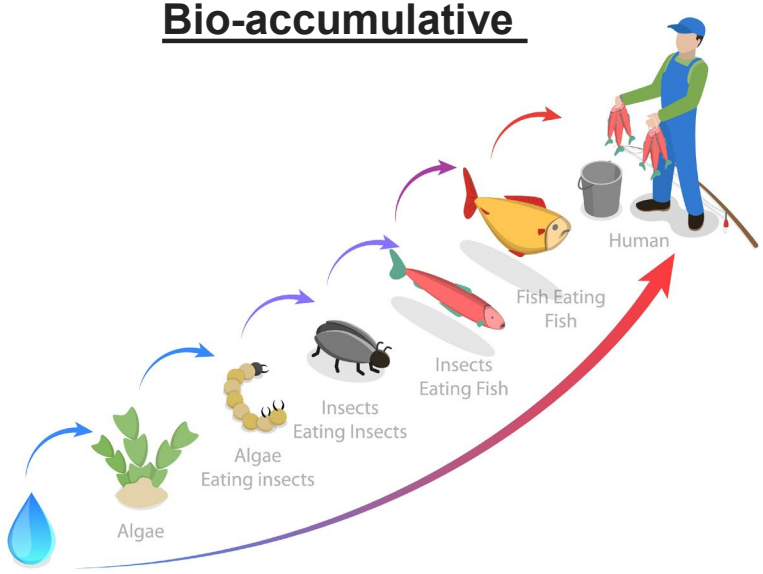
(most toxic known chemical)

Incineration byproduct



2,3,7,8-TCCDF

Bio-accumulative



Toxic

POPs regulated by Stockholm Convention

- Aldrin
- Chlordane
- DDT
- Dieldrin
- Endrin
- Heptachlor
- Hexachlorobenzene
- Mirex
- Toxaphane
- Polychlorinated biphenyls (PCB)
- Polychlorinated dibenzo-p-dioxins (PCDD)
- Polychlorinated dibenzofurans (PCDF)

The Dirty Dozen

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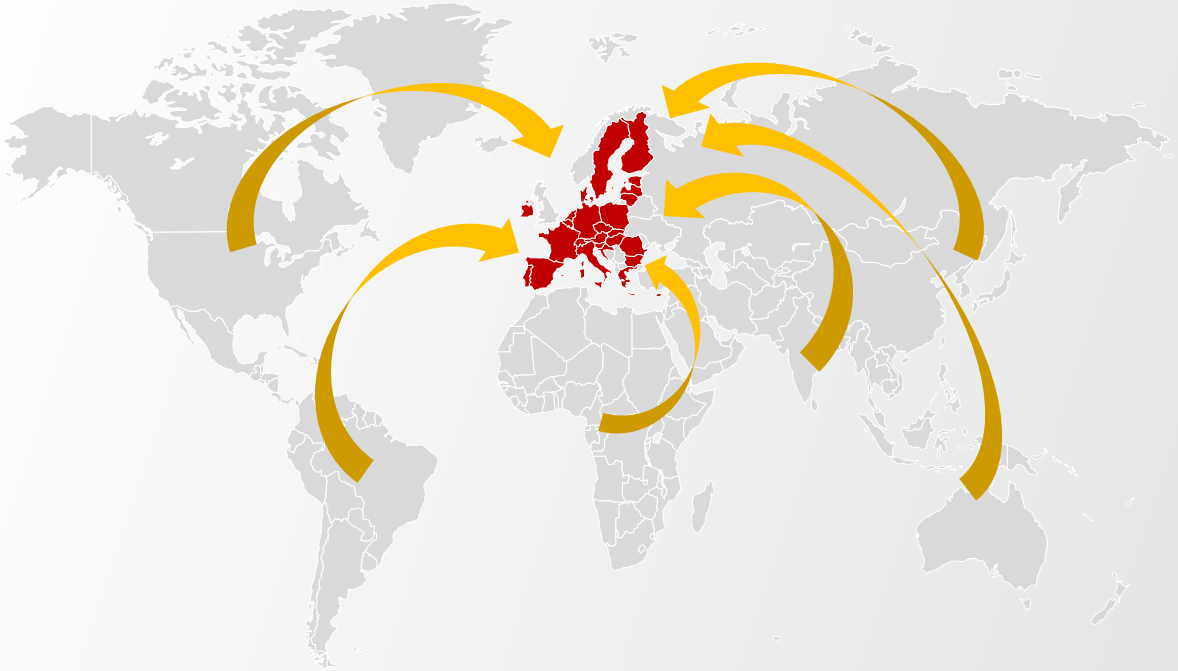
Adoption of European safety guidelines

2001 - EU Scientific Committee on Food - risk assessment of PCDD/Fs and dioxin-like PCBs in food

- Weekly intake of 14 pg WHO TEQ/kg body weight limit established
- Maximum and action limits for PCDD/F within food and feed set highlighting the need for constant monitoring

Globalization of food market

- No one jurisdiction is risk free from exposure
- Monitoring of food and feed exports and imports need to avoid future accidental exposure



Instrumentation requirements

Meeting the needs of compliance

- High resolution magnetic sector mass spectrometry (HRMS)
  - Gold standard in Dioxin analysis (Worldwide compliance)

- **2014** – EU allows GC-MS/MS for dioxin analysis in food and feed

Thermo Scientific™ DFS™ GC-HRMS

Thermo Scientific™ TSQ™ 9610 GC-MS/MS

Project Goal

Compare performance between platforms in meeting Dioxin analysis criteria in food and feed

Internal evaluation

- Thermo Fisher Scientific

External evaluation

- EURL (Freiburg. Germany)
- Wageningen Food Safety Research Institute (Netherlands)

Injection and separation methodology

Thermo Scientific iConnect PTV injector

Thermo Scientific TG-Dioxin column (60 m)

Injection		Oven temperature program	
Injector	iConnect PTV	Initial temperature (°C)	140
Liner	PTV liner with concentric baffle (P/N 453T2845-UI)	Hold time (min)	2
Injection mode	Large volume	Rate 1 (°C·min^{-1})	25
Injection volume (µL)	5	Temperature 1 (°C)	250
Split flow (mL·min^{-1})	100	Rate 2 (°C·min^{-1})	2.5
Initial injector temperature (°C)	75	Temperature 2 (°C)	260
Injection time (min)	0.4	Hold time (min)	5
Transfer rate (°C·s^{-1})	2.5	Rate 3 (°C·min^{-1})	2.5
Final temperature (°C)	300	Temperature 3 (°C)	285
Cleaning phase rate (°C·s^{-1})	14.5	Rate 4 (°C·min^{-1})	10
Cleaning phase temperature (°C)	330	Temperature 4 (°C)	320
Cleaning phase hold time (min) / flow rate (mL·min^{-1})	5 / 200	Hold time (min)	15
Carrier gas flow rate (mL·min^{-1})	1.2		

Trace analysis of polychlorinated dibenzo-p-dioxins/ dibenzofurans using GC-MS/MS in accordance with EU Regulations 2017/644 and 2017/771 for food and feed

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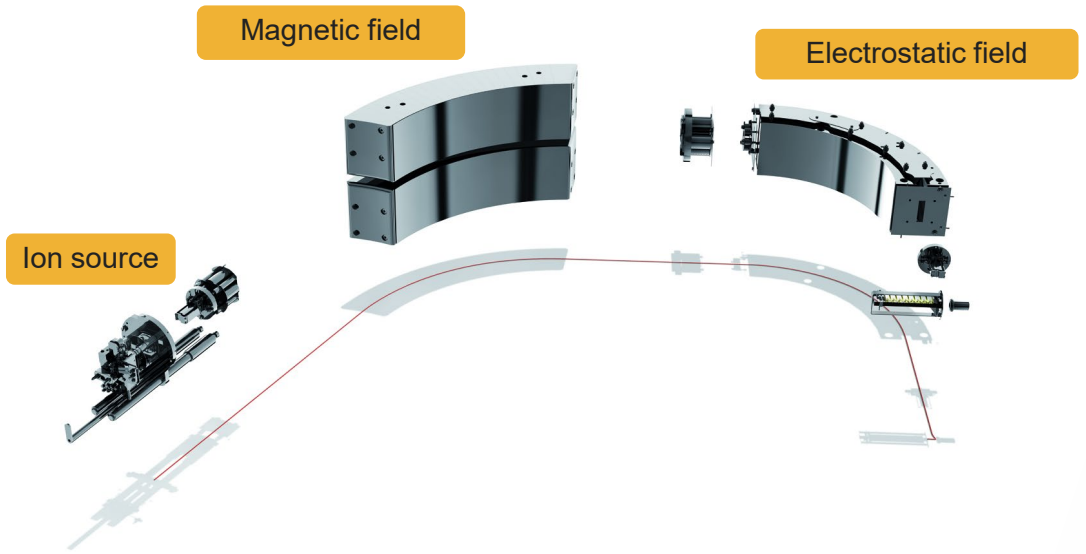
Keywords
GC-MS/MS, timed-SRM, polychlorinated dibenzo-p-dioxins, polychlorinated dibenzo-p-furans, sensitivity, Regulation (EU) 2017/644, Regulation (EU) 2017/771, feed, food

Goal
To demonstrate the suitability of the Thermo Scientific™ TSQ™ 9610 triple quadrupole GC-MS/MS system with Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software for the routine and regulatory compliance testing of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzo-p-furans (PCDD/Fs) in food and feed samples in accordance with Commission Regulations (EU) 2017/644 and 2017/771.

Introduction
Polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDD/F), or dioxins as they are commonly referred to, are classified as persistent organic pollutants (POPs) under the Stockholm Convention. Due to their chemical stability and high fat solubility, these chemicals can accumulate within food chains and pose exposure/health risks to humans through consumption of food items (i.e., diary, meat, and fish). Regulations are in place to monitor food and feed for the presence of dioxins to protect the population.[1] Current maximum levels allowable for PCDD/Fs in food and feedstuffs are at the pg·g^{-1} concentration range due to the toxicity risk they pose.[2,3] However, the European Commission has announced plans to lower the current maximum levels in certain feedstuffs in 2024 based an updated risk assessment.[4] As of 2014, a change of EU regulations permitted the use of gas chromatography-triple quadrupole mass

Differences in mass selectivity

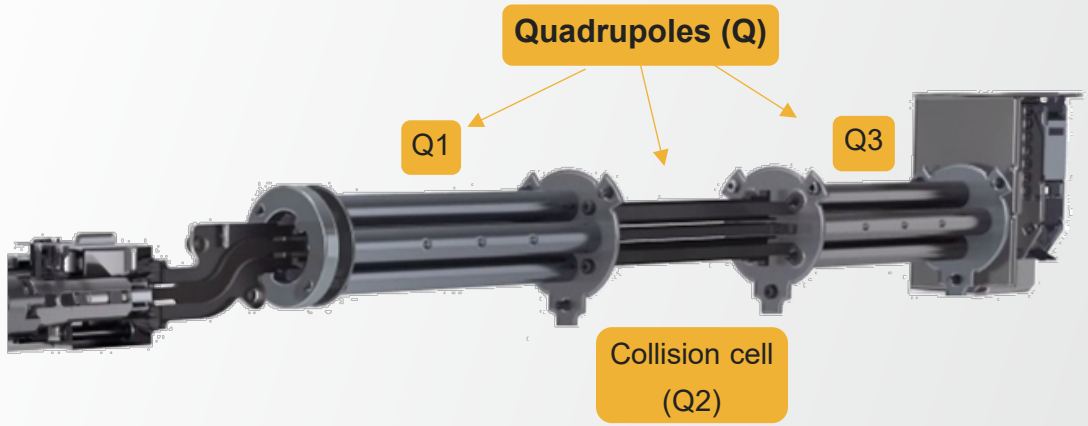
DFS



- Double focusing mass analyzer
  - Magnetic and Electrostatic focusing
  - High mass resolution data obtained
    - R = 10,000 (10% valley definition)
    - R = 20,000 FWHM
  - Robust performance from source design

VS.

GC-MS/MS



- Mass separation in alternating electric field
  - AC/DC applied across quadrupoles
  - Unit (low) mass resolution technique
- MS/MS selectivity
  - Monitor compound selective fragmentation
  - Fast scanning capabilities
    - -> 800 simultaneous transitions possible

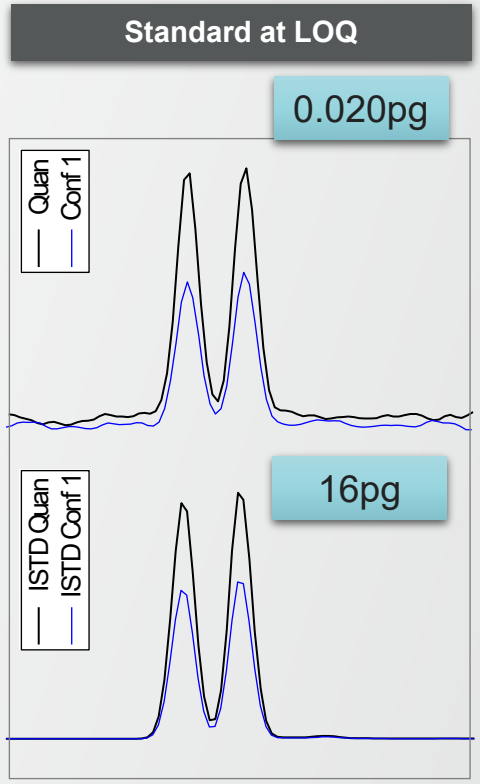
LOQ determination

Calibration based approach for GC-MS/MS analysis[1]

- Defined as the lowest level of analyte detected and meets performance criteria:
  - Within the specified retention time window (for all monitored ions).
  - ***Ion ratio intensities ≤ 15%*** of theoretical (or calculated from standards) values
  - Deviation from the average ***relative response factor ≤ 30% for all calibration points***
  - ***The LOQ calculated by taking the lowest point of the calibration curve*** and correcting for the final sample volume, sample intake weight, and associated internal standard recovery.

LOQ determined experimentally

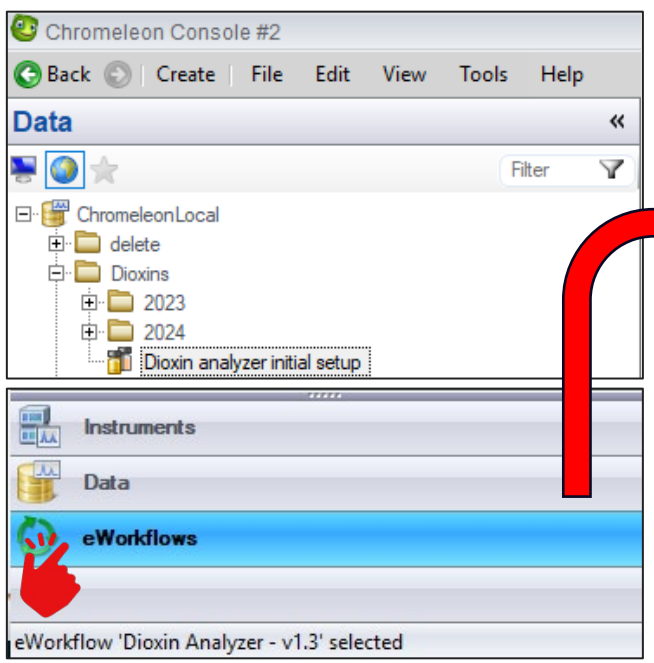
$$Sample\ LOQ\ (pg/g) = \sum_{n=PCDD/F}^{17} Min\ Conc_n(pg/\mu L) * \left(\frac{\text{Sample volume}(\mu L)}{\text{Sample weight}(g) * Recovery\ l\ (\%)}\right)$$

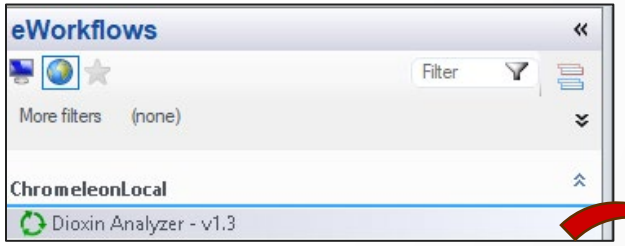


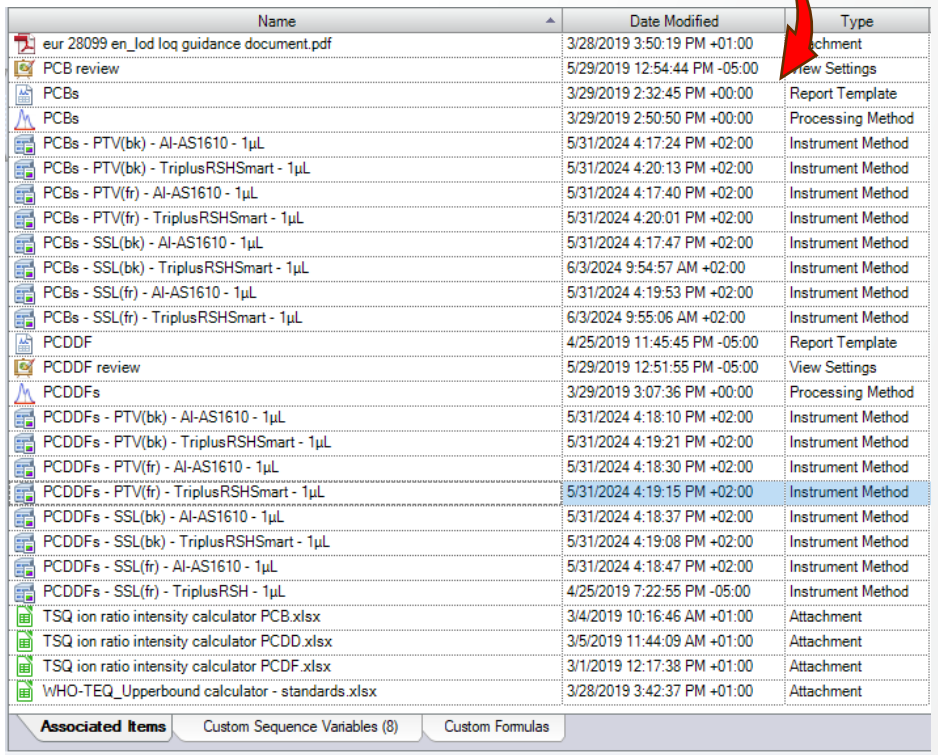
1. Wenzl, T., Haedrich, J., Schaechtele, A., Robouch, P., Stroka, J., Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food; EUR 28099, Publications Office of the European Union, Luxembourg, 2016, ISBN 978-92-79-61768-3; doi:10.2787/8931u

Thermo Scientific™ Chromeleon Chromatography Data System (CDS) 7.3.2

Dioxin eWorkflow







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PCBs	3/29/2019 2:32:45 PM +00:00	Report Template
PCBs	3/29/2019 2:50:50 PM +00:00	Processing Method
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PCBs - PTV(bk) - TriplusRSHSmart - 1µL	5/31/2024 4:20:13 PM +02:00	Instrument Method
PCBs - PTV(fr) - AI-AS1610 - 1µL	5/31/2024 4:17:40 PM +02:00	Instrument Method
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TSQ ion ratio intensity calculator PCB.xlsx	3/4/2019 10:16:46 AM +01:00	Attachment
TSQ ion ratio intensity calculator PCDD.xlsx	3/5/2019 11:44:09 AM +01:00	Attachment
TSQ ion ratio intensity calculator PCDF.xlsx	3/1/2019 12:17:38 PM +01:00	Attachment
WHO-TEQ_Upperbound calculator - standards.xlsx	3/28/2019 3:42:37 PM +01:00	Attachment

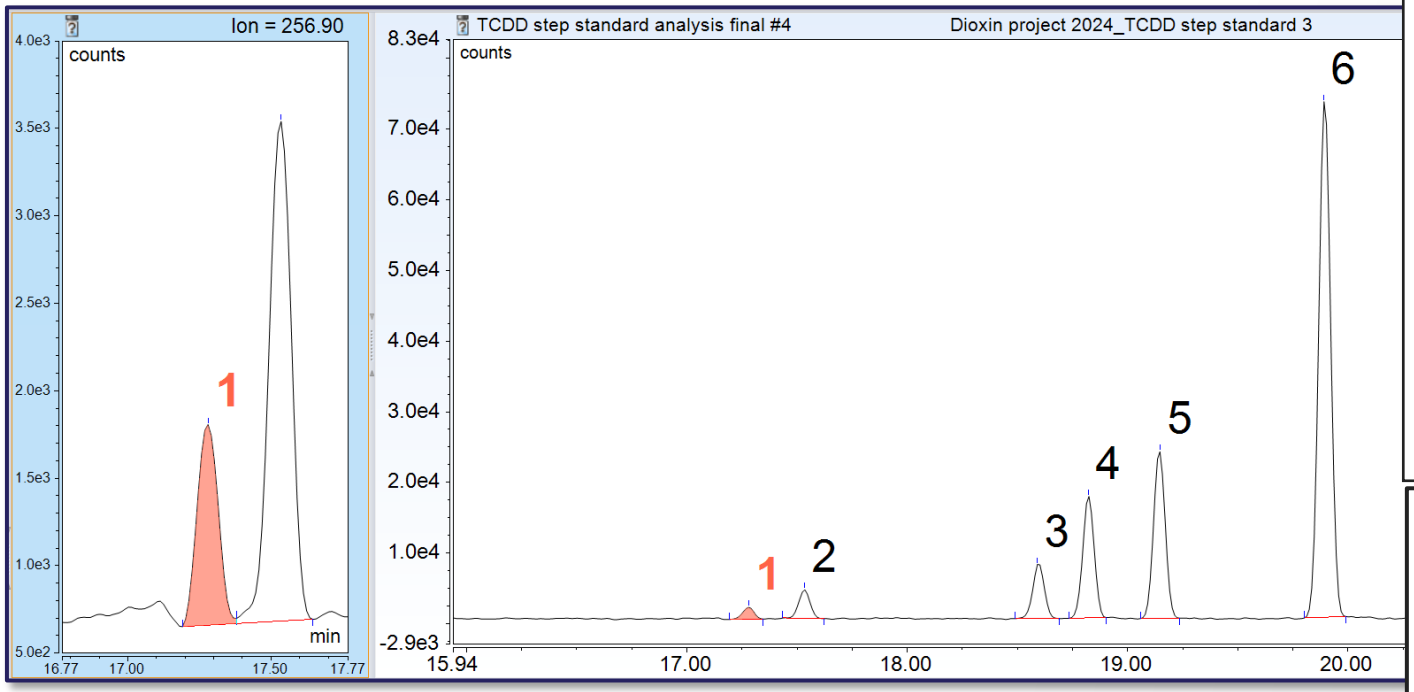
Associated Items | Custom Sequence Variables (8) | Custom Formulas

Automated full method setup for PCBs and PCDD/Fs including:

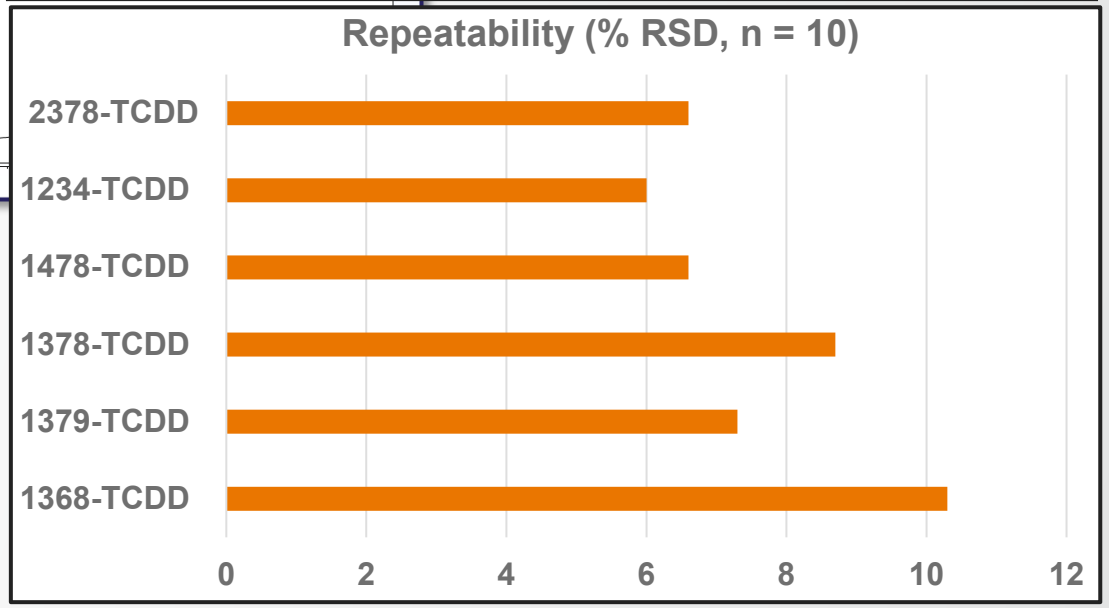
- Instrument methods for TriPlus RSH Smart and AI-AS 1610 autosamplers
- Isotopic dilution quantification methods
- Reporting layouts/templates
- Supporting compliance documentation

Meeting performance criteria

Analysis repeatability at trace levels



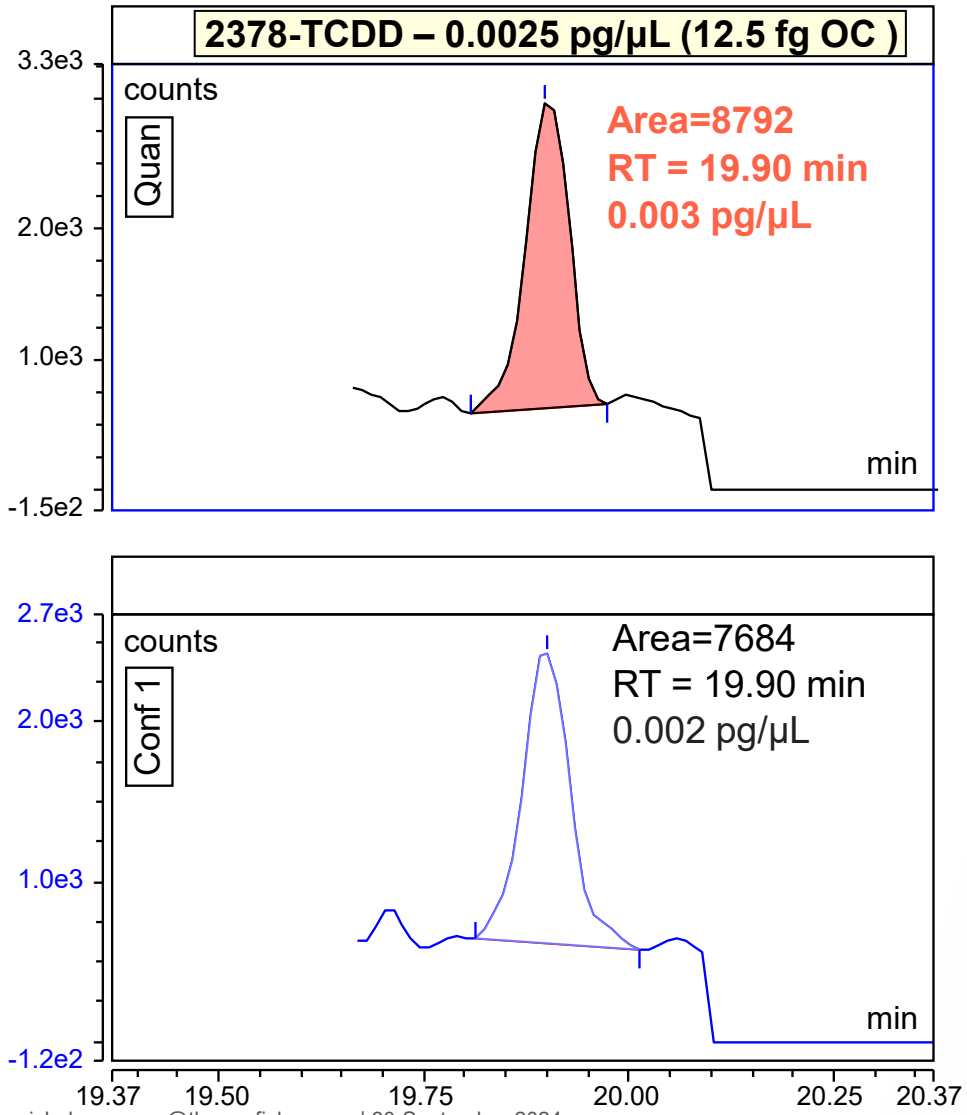
Peak number	Compound	Concentration (fg On-Column)	Repeatability (%RSD) n = 10
1	1368-TCDD	10	10.3
2	1379-TCDD	20	7.3
3	1378-TCDD	50	8.7
4	1478-TCDD	125	6.6
5	1234-TCDD	250	6.0
6	2378-TCDD	500	6.6

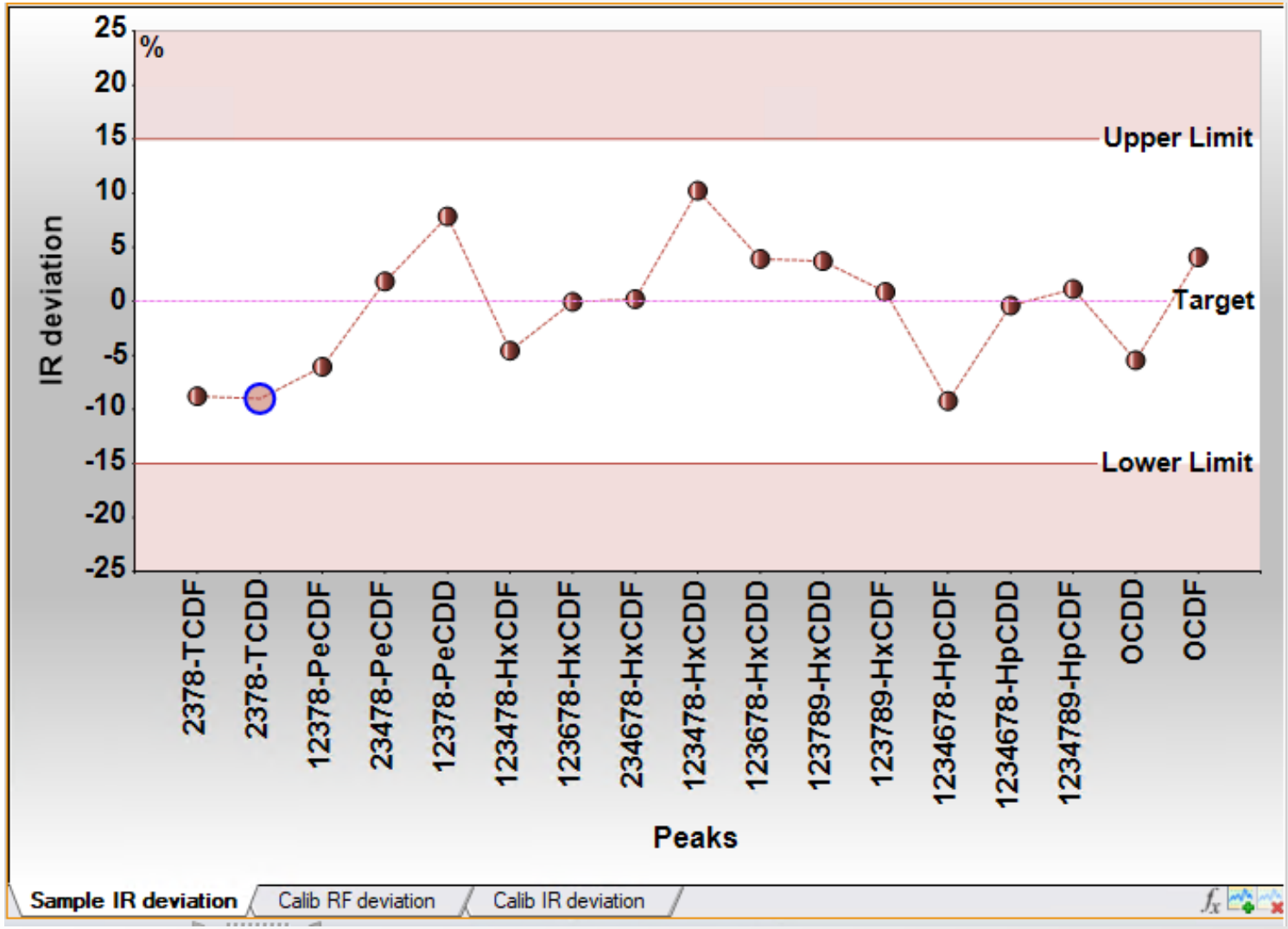


Assessment of the dioxin sensitivity using TCDD step standard ranging from 2 to 100 fg•µL^{-1} with a 5 µL injection and reproducibility over 10 injections.

Meeting performance criteria

Ion ratio performance at trace levels





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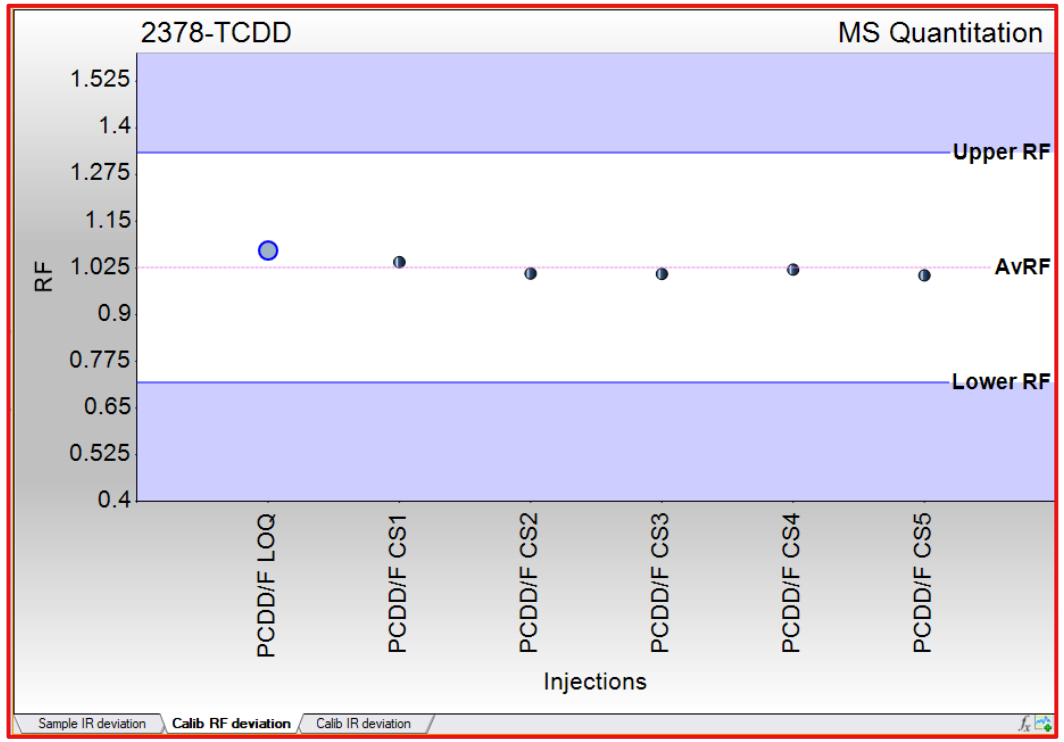
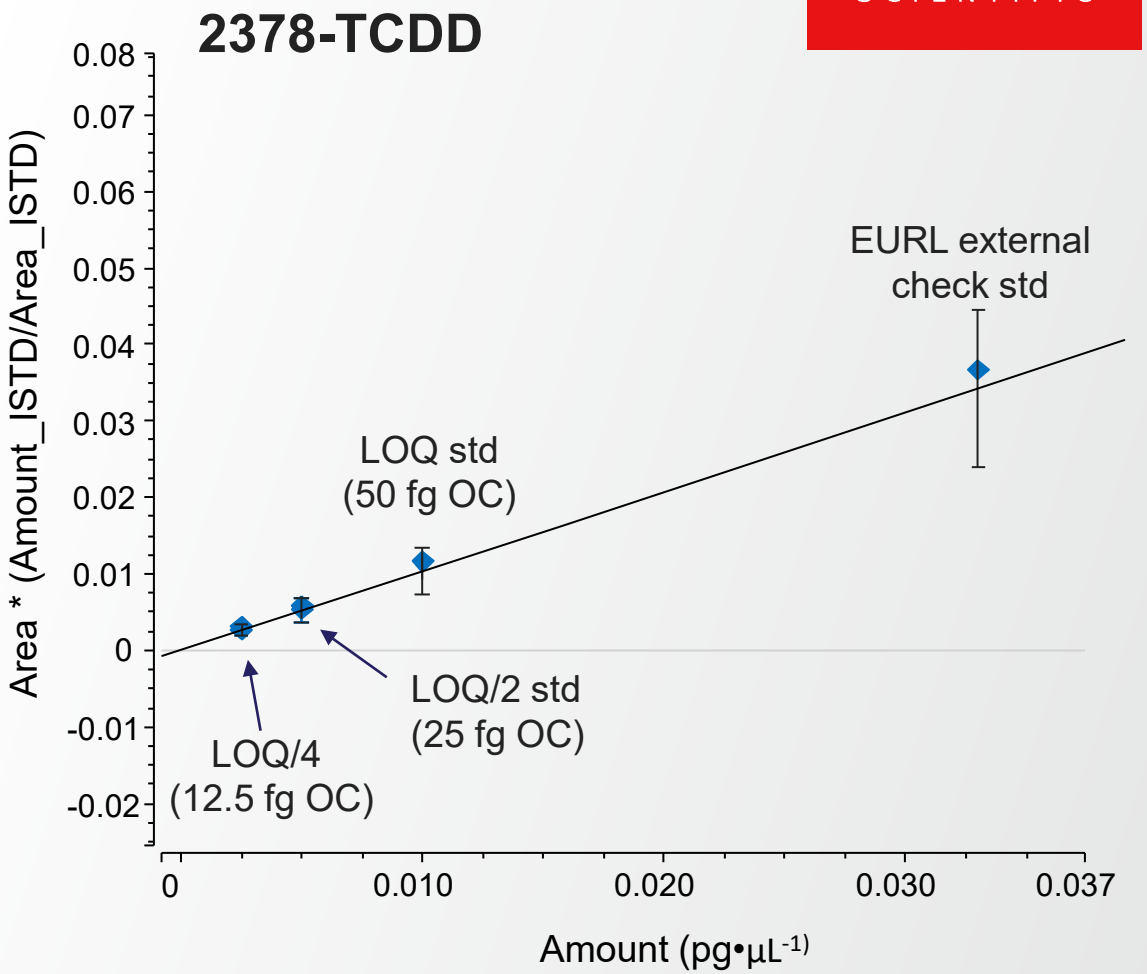
Meeting performance criteria

Response factor variance and accuracy



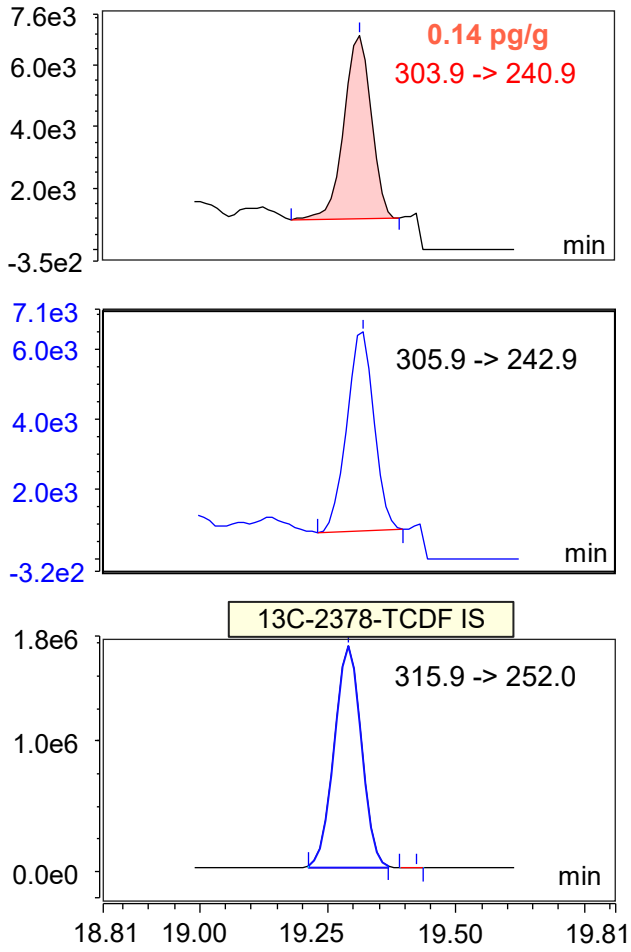


	A	B	H	I	J
1	Peak Name	Ret.Time min			
2					
3	First Injection	First Injection	EURL external check standard	EURL analysis LOQ/4 -4	EURL analysis LOQ/2 -4
5	2378-TCDF	19.292	Pass	Pass	Pass
6	*2378-TCDD*	*19.852*	*Pass*	*Pass*	*Pass*
7	12378-PeCDF	23.319	Pass	Pass	Pass
8	23478-PeCDF	24.704	Pass	Pass	Pass

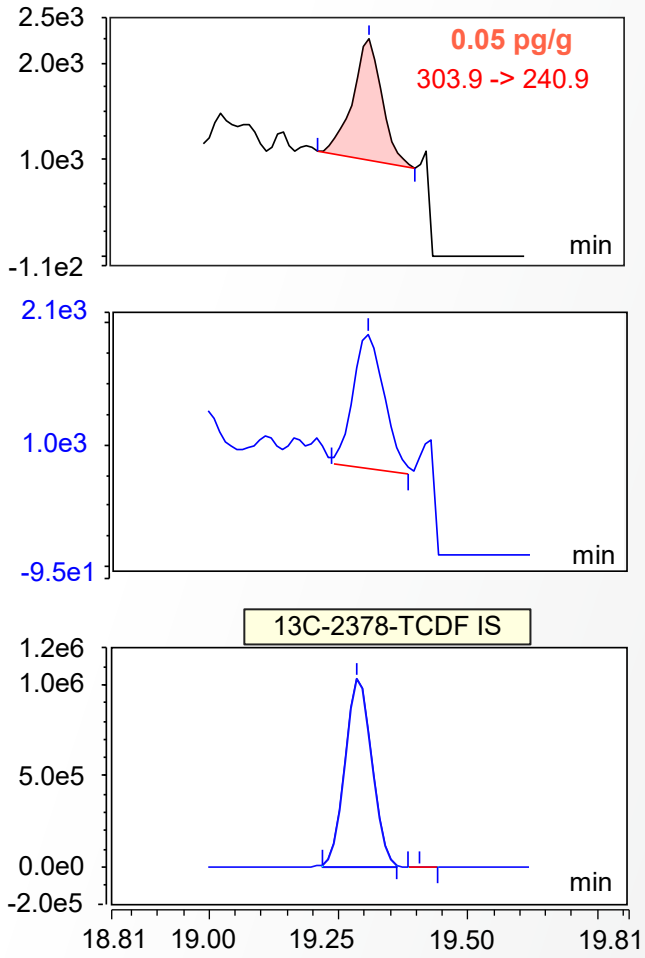
MS/MS selectivity in complex matrices

Minimal matrix impact at sub –pg/g concentrations

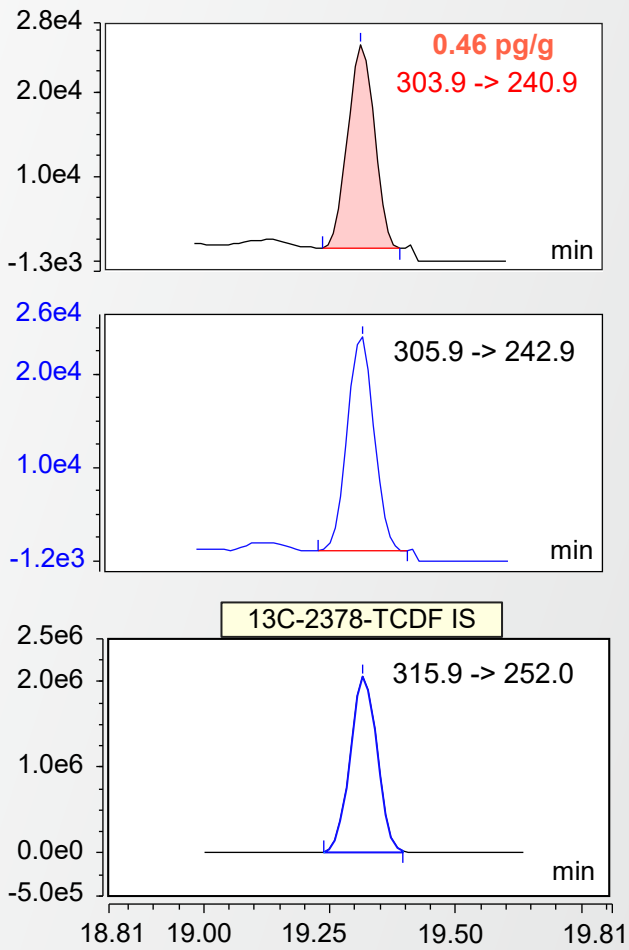
TCDF in laying hen egg using GC – MS/MS



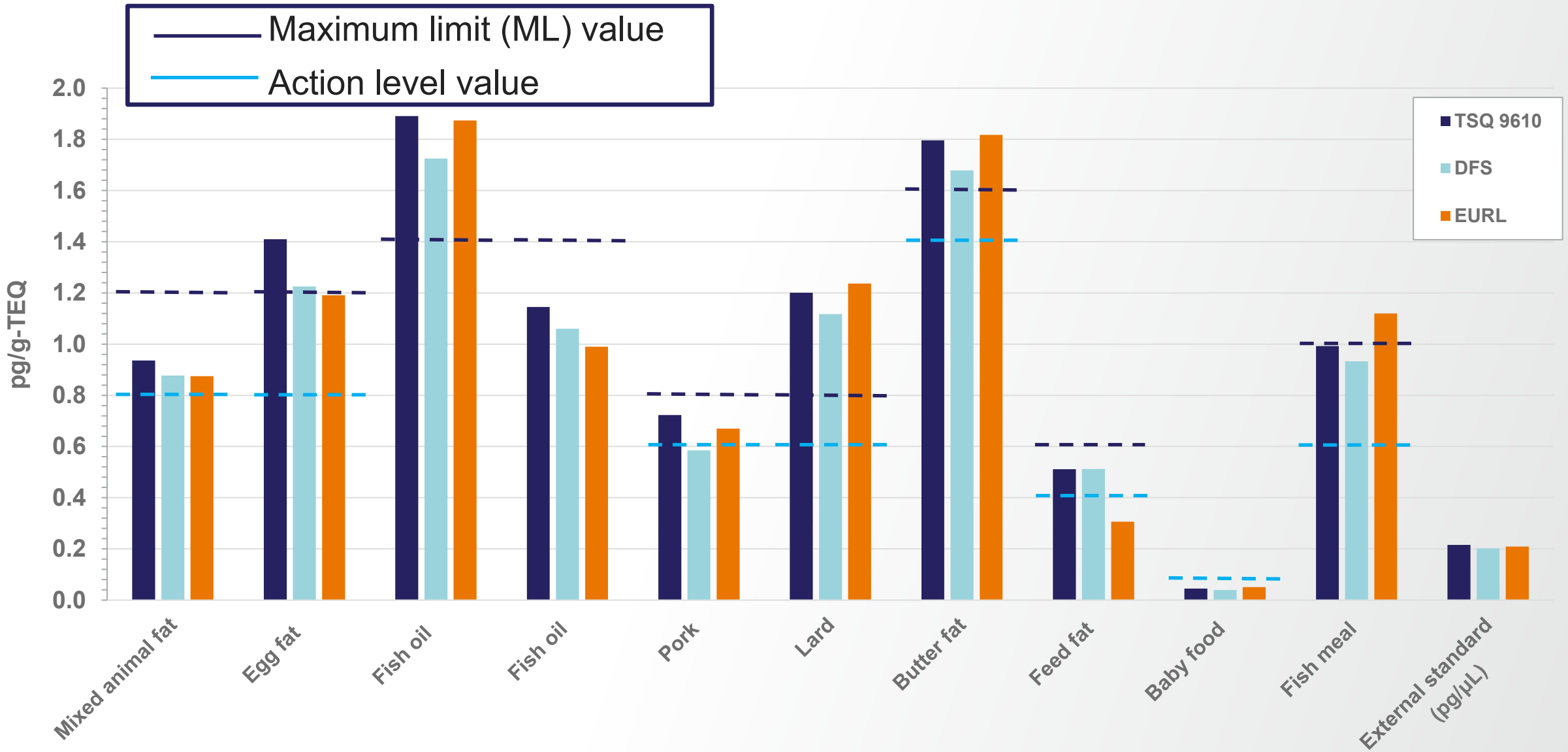
TCDF in broiler fat using GC – MS/MS



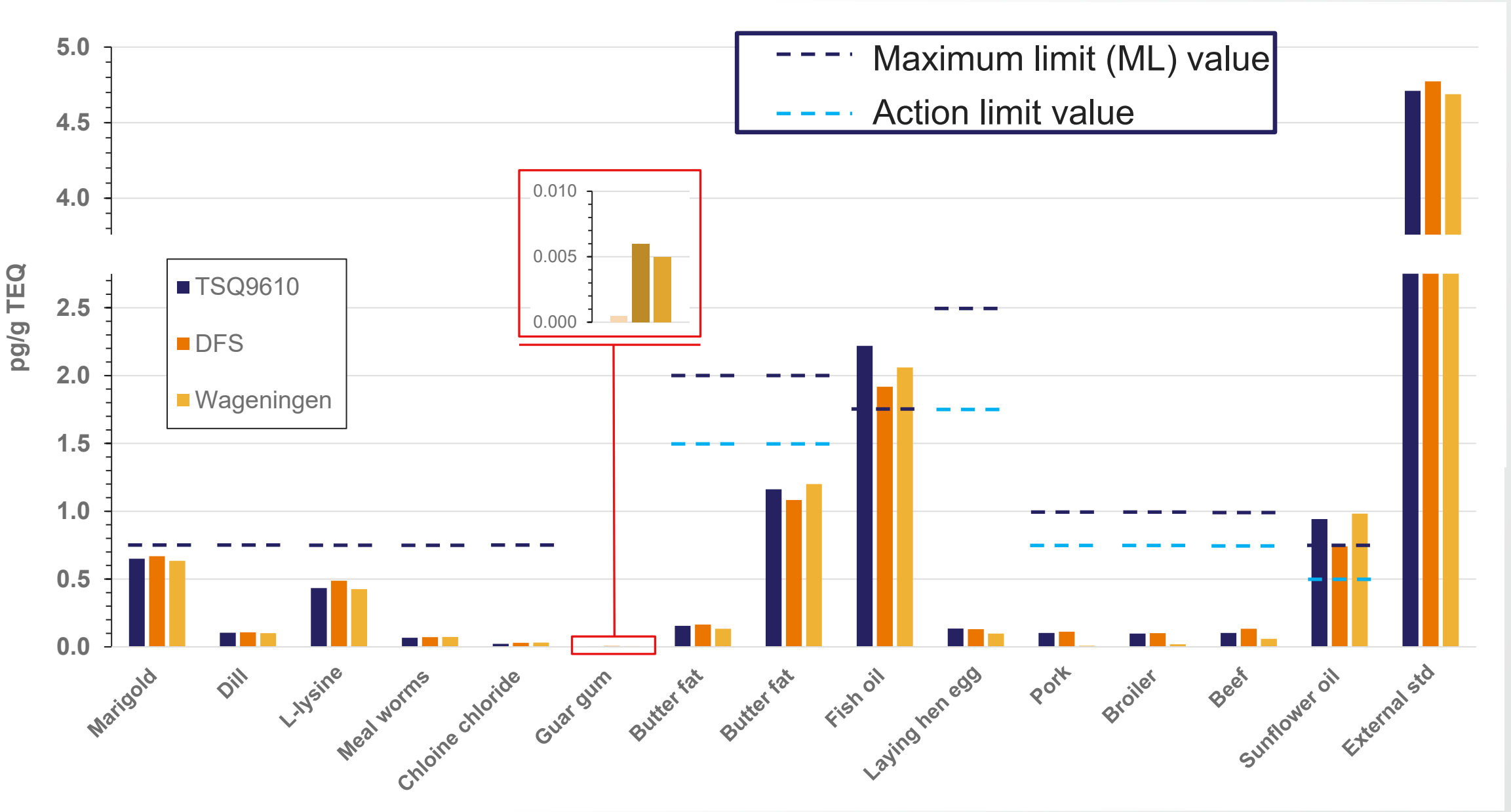
TCDF in mixed animal fat using GC – MS/MS



EURL sample analysis (Freiburg, Germany)



Wageningen Food Safety Research (Netherlands) analysis



Conclusions

- Femtogram level sensitivity achievable by TSQ 9610 GC-MS/MS in food and feed samples
- Check standard performance within ion ration thresholds for EU regulatory compliance at femtogram levels
- Good data agreement between TSQ 9610 and DFS through internal and external evaluation highlights accuracy and precision performance of the TSQ 9610
- Easy implementation with the Chromeleon 7.3.2 CDS Dioxin analyzer eWorkflow

Thank you

Any Questions