



# **Low level quantification of PCDD/Fs in animal feedstuffs using the Thermo Scientific TSQ 9000 GC-MS/MS system with AEI source**

Runcorn, UK

PP10604

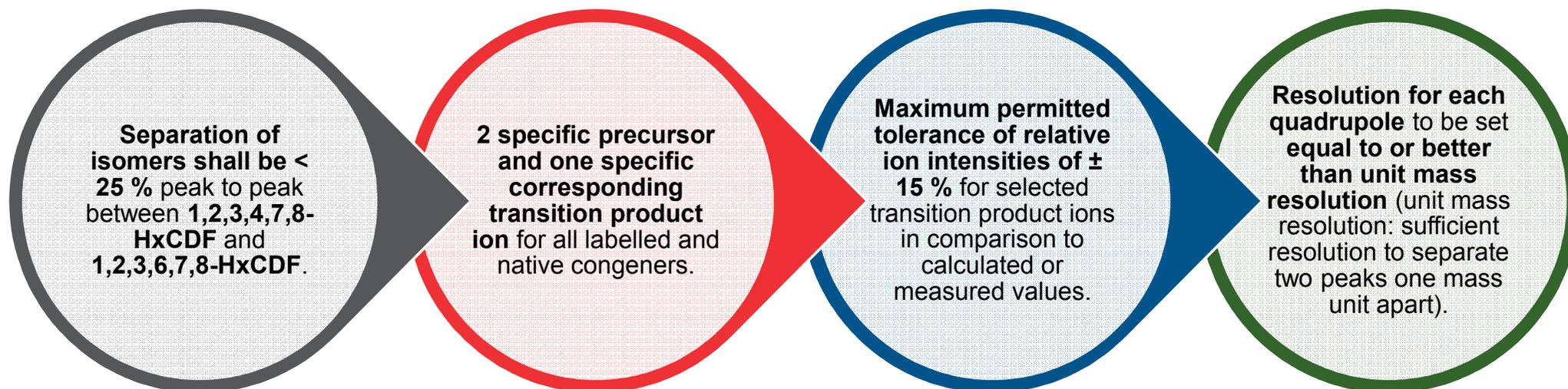
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## Introduction

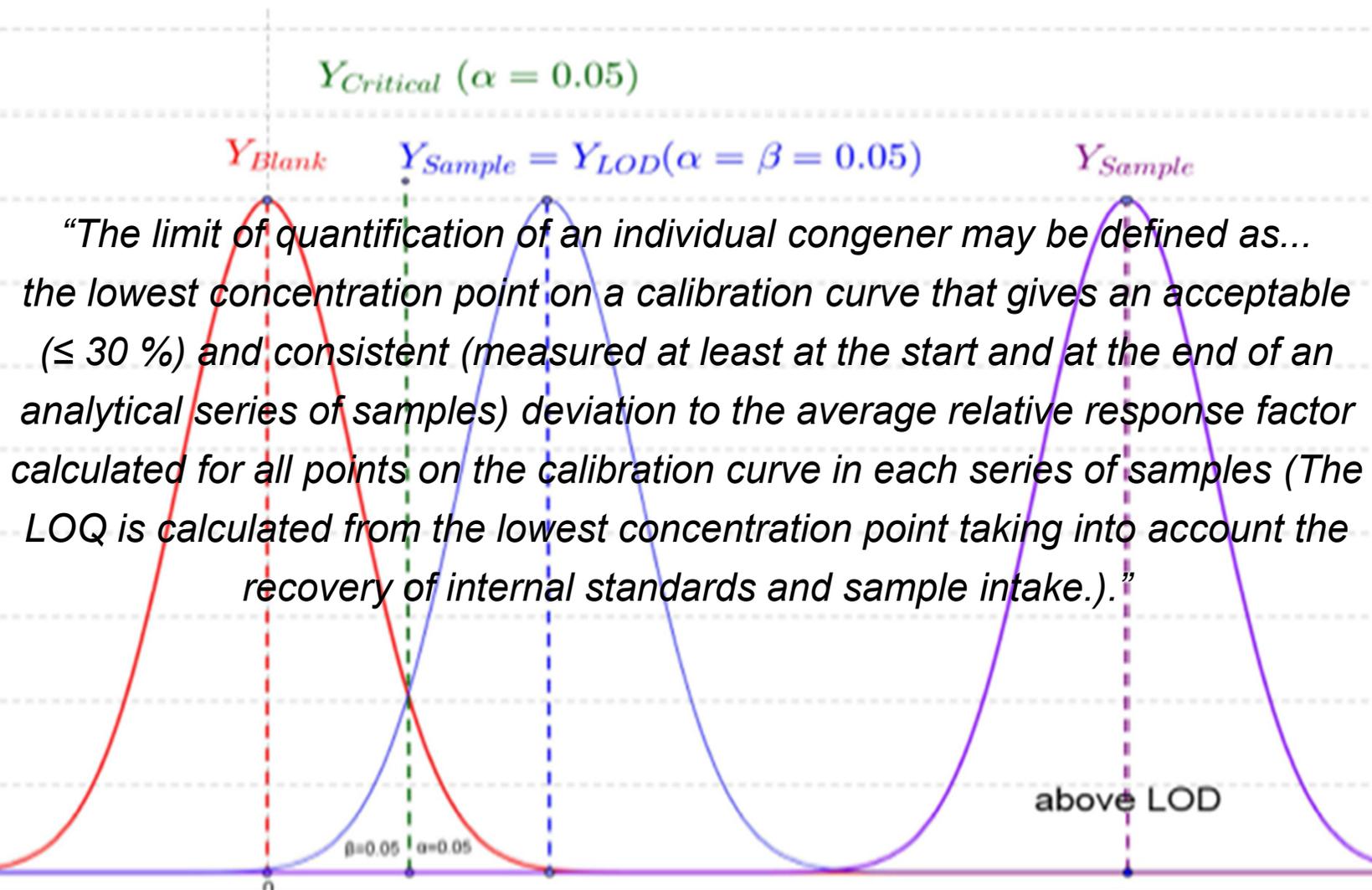
- Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzo-*p*-furans (PCDFs) commonly referred to as *dioxins*, are persistent environmental pollutants (POPs).
- Dioxins are ubiquitous in the environment and accumulate in the food chain, mainly in the fatty tissue of animals. Dioxins are highly toxic and can cause reproductive and developmental problems, damage the immune system, interfere with hormones and cause cancer.
- More than 90% of human exposure is through food ingestion, mainly meat and dairy products, fish and shellfish. Many national authorities have programmes in place to monitor the food supply.
- European Union (EU) legislation ensures strict limits are placed upon the maximum levels (MLs) which are allowable in foodstuffs and animal feed and food.

## Challenges

- Due to the high toxicity and bioaccumulative nature of dioxins, accurate confirmation and quantification of PCDD/Fs in animal food and feedstuffs is essential to minimise human exposure.
- Current EU legislation requires GC-MS/MS analytical methods to demonstrate compliance at 1/5<sup>th</sup> of the maximum levels (MLs), these include:



# LOQ challenges



# Objectives

- To assess the performance of the Thermo Scientific™ TSQ™ 9000 triple quadrupole GC-MS/MS system equipped with a new Advanced Electron Ionization (AEI) source for the analysis of PCDD/Fs in accordance with regulatory requirements.
- To show compliance at 1/5<sup>th</sup> maximum levels and demonstrate the sensitivity, selectivity and robustness required in order to operate in a routine environment.



- For all experiments described below, a Thermo Scientific TSQ 9000 triple quadrupole mass spectrometer with AEI source was used.
- Sample introduction was performed using a Thermo Scientific™ TriPlus™ RSH autosampler, and chromatographic separation was obtained with a Thermo Scientific™ TRACE™ 1310 GC system.

# GC and Injector conditions

## TRACE 1310 GC System Parameters

Injection Volume ( $\mu\text{L}$ ):	2
Liner:	Thermo Scientific™ LinerGOLD™ single taper with wool (P/N: 453A0924-UI)
Inlet ( $^{\circ}\text{C}$ ):	280
Carrier Gas, (mL/min):	He, 1.2
Inlet Mode:	Splitless (split flow 120mL/min after 2 min)
Column:	Thermo Scientific™ TraceGOLD™ TG-Dioxin GC (60m x 0.25mm, 0.25 $\mu\text{m}$ ) (P/N: 26066-1540)

## Oven Temperature Program

Temperature 1 ( $^{\circ}\text{C}$ ):	120
Hold Time (min):	0
Temperature 2 ( $^{\circ}\text{C}$ ):	250
Rate ( $^{\circ}\text{C}/\text{min}$ ):	25
Hold Time (min):	0
Temperature 3 ( $^{\circ}\text{C}$ ):	285
Rate ( $^{\circ}\text{C}/\text{min}$ ):	2.5
Hold Time (min):	0
Temperature 4 ( $^{\circ}\text{C}$ ):	320
Rate ( $^{\circ}\text{C}/\text{min}$ ):	10
Hold Time (min):	15
Total Run Time (min):	39.7



# MS conditions

## TSQ 9000 Mass Spectrometer Parameters

Transfer Line (°C):	300
Ionization Type:	EI – with AEI source
Ion Source (°C):	300
Electron Energy (eV):	50
Acquisition Mode:	Timed SRM with Dwell Time Prioritization (x10 – natives HIGH, labelled LOW) Detector gain factor x7
Tuning parameters:	AEI Full Tune (Default)
Collision gas and pressure (psi):	Argon, 70
Resolution :	0.7 (both Q1 and Q3)

**Resolution for each quadrupole to be set equal to or better than unit mass resolution** (unit mass resolution: sufficient resolution to separate two peaks one mass unit apart).



## Samples and sample preparation

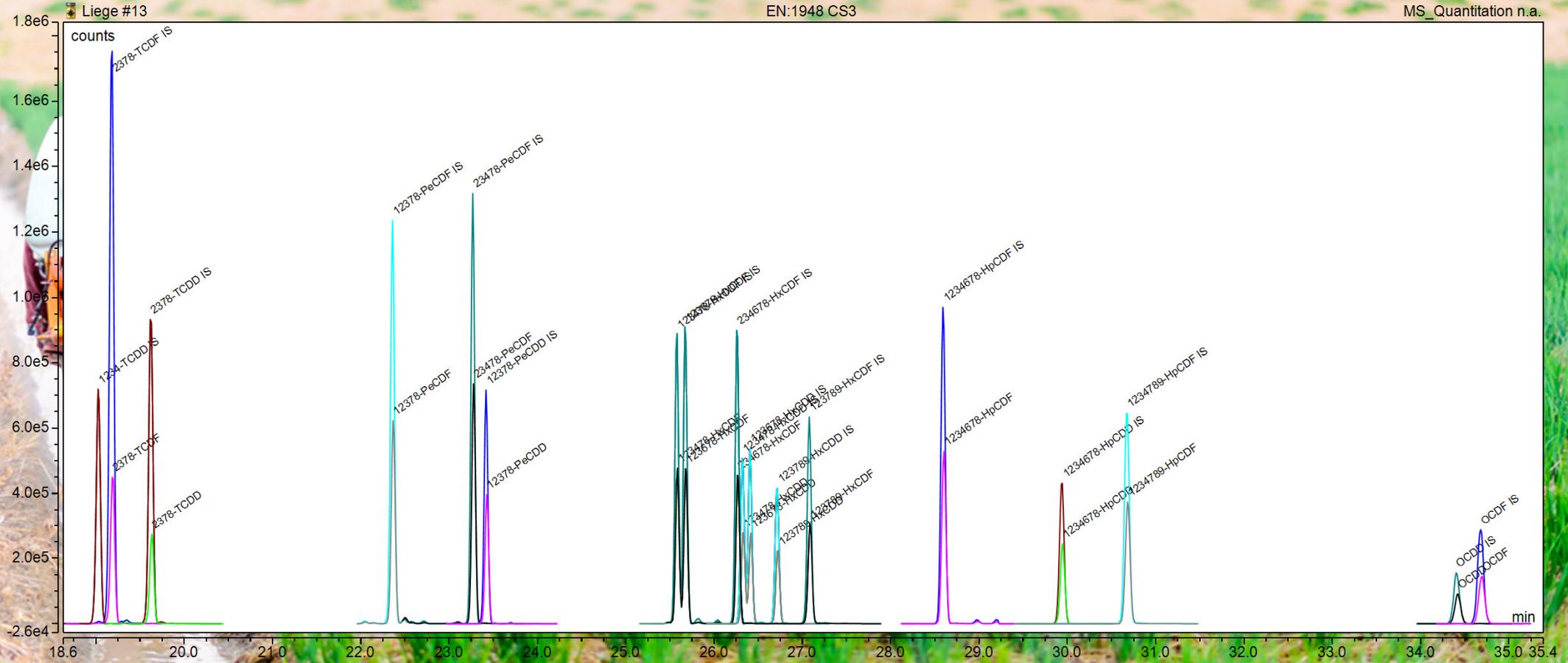
- Animal feedstuff samples (and relevant quality control samples and procedural blanks) were provided by the University of Liege. Sample types, weights and maximum levels allowed (ML) are given below.
- European method EN:1948 standard solutions (Wellington Laboratories Inc., Canada) were utilized for initial performance tests and for calibration and quantitation.

	Alfalfa	Pork Fat	Premix	Premix	Sheep	QC – Pork fat	QC - Feed (grass)
Sample intake (g)	32.13	4.57	10.17	11.1	2.55	6.8	20.71
Regulatory ML [WHO-PCDD/F-TEQ(pg/g)] *	0.75	1	1	1	2.5	1	0.75**
1/5th ML [WHO-PCDD/F-TEQ(pg/g)] *	0.15	0.2	0.2	0.2	0.5	0.2	0.15

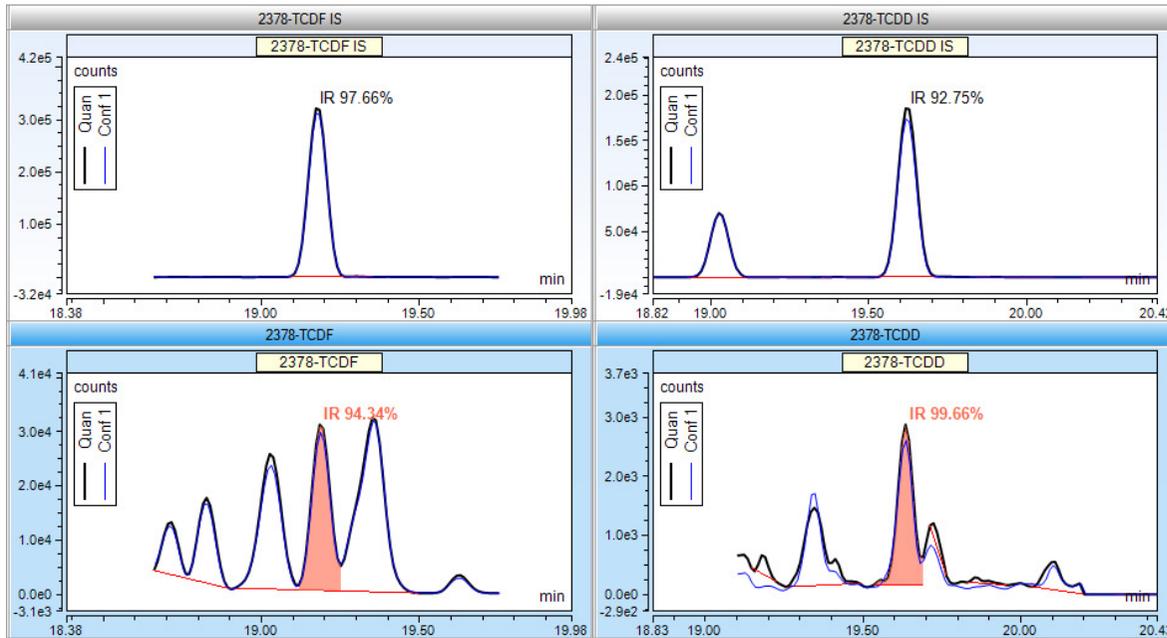
\* maximum limits taken from European directive 2002/32/EC <sup>(3)</sup>

\*\* lower limit applied

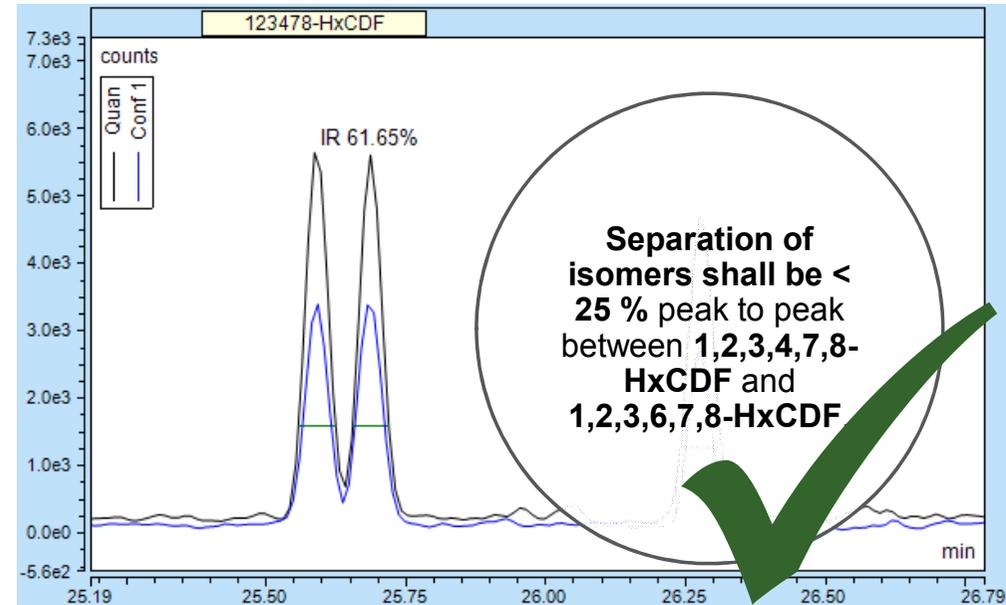
# Chromatography



# Chromatography – Separation

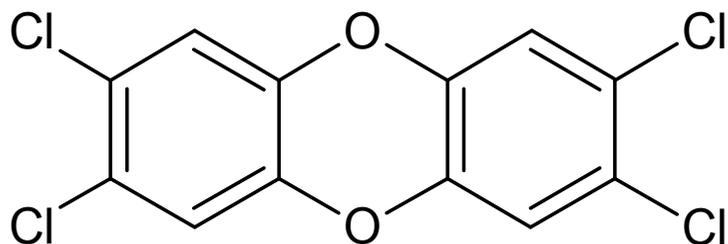


**TCDD/F congener separation on the TG-Dioxin (60m x 0.25mm, 0.25 $\mu$ m) column**

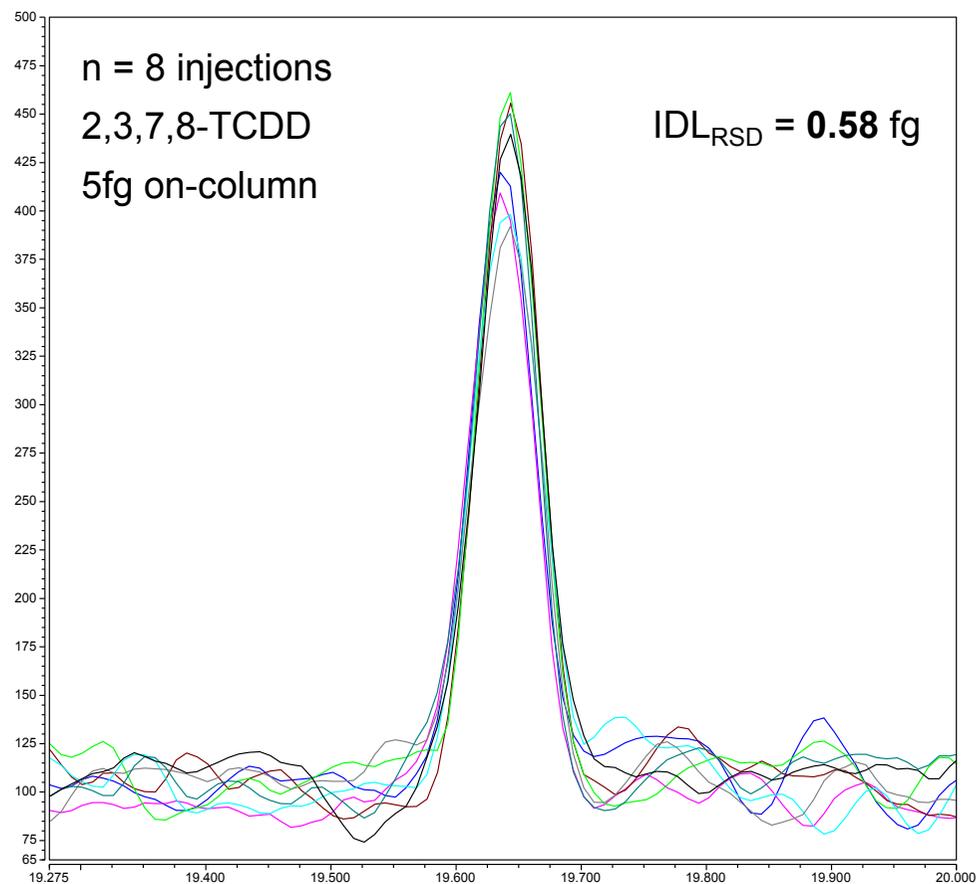


# Instrumental Detection Limit (IDL)

**IDL performance of the TSQ 9000 for  
2,3,7,8-TCDD using repeated injections  
of 5 fg on-column**



***Attogram levels of  
sensitivity!***



# Linearity

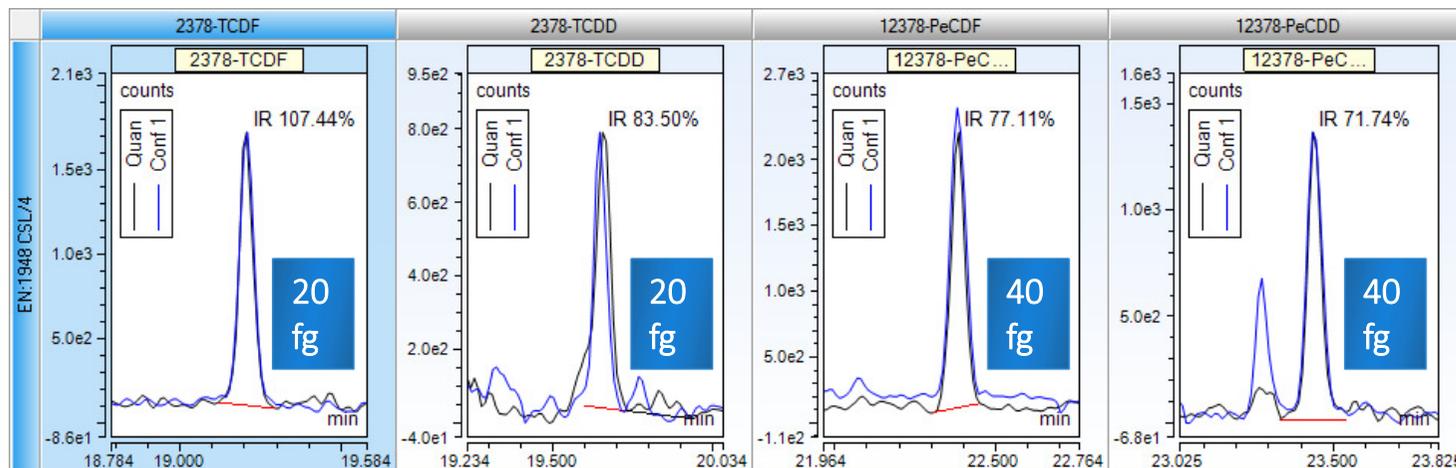
	Congener	Average RF from Calibration	RF standard deviation	RF RSD (%)	Calibration range (pg/ $\mu$ L)
Response factor	2378-TCDF	1.06	0.06	<b>5.6%</b>	0.01 - 80
	2378-TCDD	1.13	0.06	<b>5.6%</b>	0.01 - 80
	12378-PeCDF	1.02	0.02	<b>2.3%</b>	0.02 - 160
	23478-PeCDF	1.08	0.02	<b>1.9%</b>	0.02 - 160
	12378-PeCDD	1.11	0.03	<b>2.4%</b>	0.02 - 160
	123478-HxCDD	1.08	0.03	<b>3.1%</b>	0.04 - 160
	123678-HxCDD	1.04	0.04	<b>3.8%</b>	0.04 - 160
	123789-HxCDD	1.05	0.04	<b>3.7%</b>	0.04 - 160
	123478-HxCDF	1.04	0.02	<b>2.3%</b>	0.02 - 160
	123678-HxCDF	1.04	0.03	<b>2.4%</b>	0.02 - 160
	234678-HxCDF	1.04	0.04	<b>3.9%</b>	0.02 - 160
	123789-HxCDF	0.98	0.04	<b>4.4%</b>	0.02 - 160
	1234678-HpCDF	1.09	0.02	<b>1.8%</b>	0.04 - 320
	1234789-HpCDF	1.13	0.03	<b>2.5%</b>	0.04 - 320
	1234678-HpCDD	1.16	0.08	<b>7.3%</b>	0.04 - 320
	OCDD	1.15	0.05	<b>4.5%</b>	0.16 - 320
OCDF	0.99	0.06	<b>5.8%</b>	0.16 - 320	



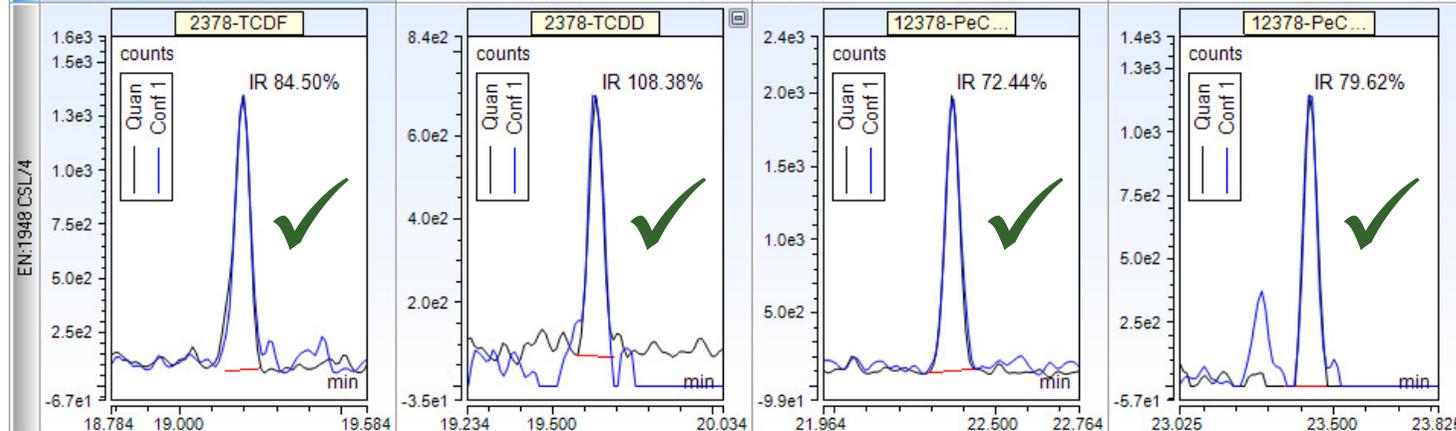
**Example of response factors over 8 calibration injections for 2,3,7,8-TCDD**

# Robustness

4<sup>th</sup> Injection



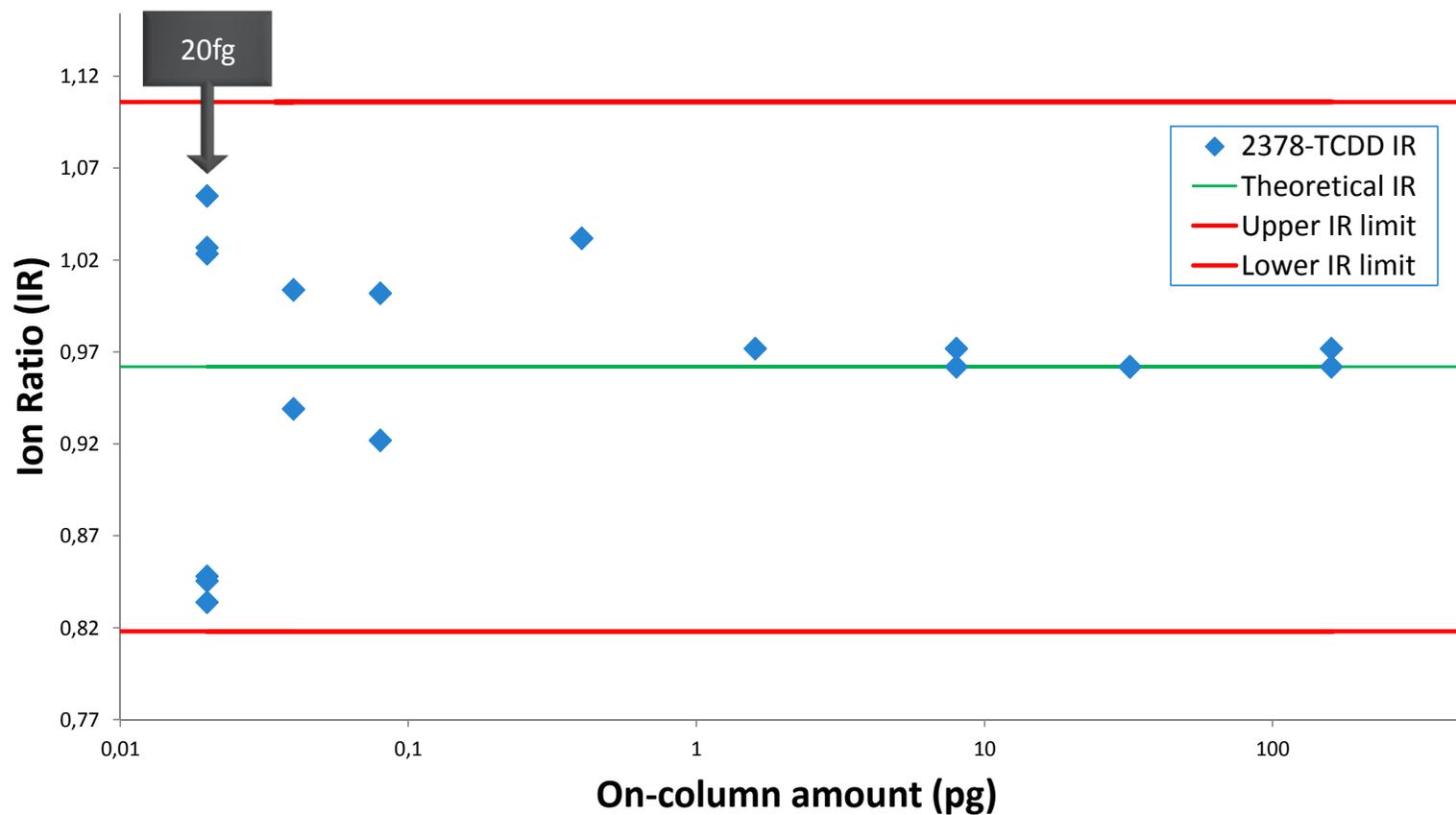
61<sup>st</sup> Injection



**Ion ratios for the lowest level calibration standard (LOQ) showing compliance from the beginning of the sequence to the end.**

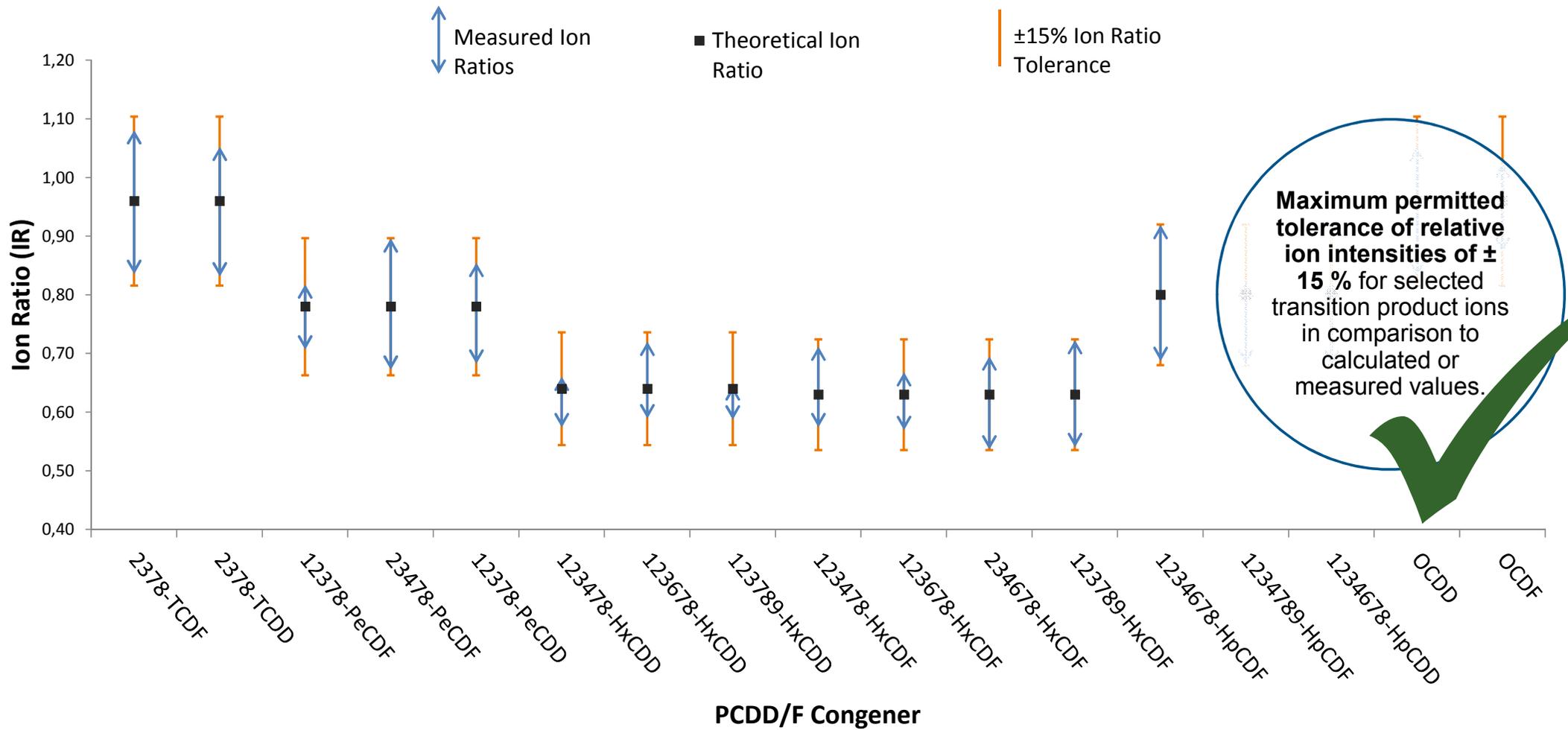
**2 specific precursor and one specific corresponding transition product ion for all labelled and native congeners.**

## Robustness – Ion ratio 2,3,7,8-TCDD



*Ion ratios shown over the full calibration range (0.01 – 80pg/ $\mu$ L) for the two native 2,3,7,8-TCDD transitions*

# Robustness – Ion ratios for all native congeners



# QC results

Sample: QC - feed				
Congener	On column LOQ (pg)	TEF (WHO 2005)	LOQ - Upperbound WHO-TEQ-PCDD/Fs (2005) pg/g	TSQ 9000 result Upperbound WHO-TEQ-PCDD/Fs (2005) pg/g
2378-TCDF	0.02	0.1	0.00048	0.02010
2378-TCDD	0.02	1	0.00483	0.01884
12378-PeCDF	0.04	0.03	0.00029	0.00304
23478-PeCDF	0.04	0.3	0.00290	0.06221
12378-PeCDD	0.04	1	0.00966	0.05556
123478-HxCDF	0.04	0.1	0.00097	0.02632
123678-HxCDF	0.04	0.1	0.00097	0.01530
234678-HxCDF	0.04	0.1	0.00097	0.01606
123478-HxCDD	0.08	0.1	0.00193	0.00389
123678-HxCDD	0.08	0.1	0.00193	0.01723
123789-HxCDD	0.08	0.1	0.00193	0.00556
123789-HxCDF	0.04	0.1	0.00097	0.00717
1234678-HpCDF	0.08	0.01	0.00019	0.01185
1234678-HpCDD	0.08	0.01	0.00019	0.00101
1234789-HpCDF	0.08	0.01	0.00019	0.01565
OCDD	0.32	0.0003	0.00002	0.00308
OCDF	0.32	0.0003	0.00002	0.00033
		<b>SUM</b>	<b>0.02844</b>	<b>0.28319</b>

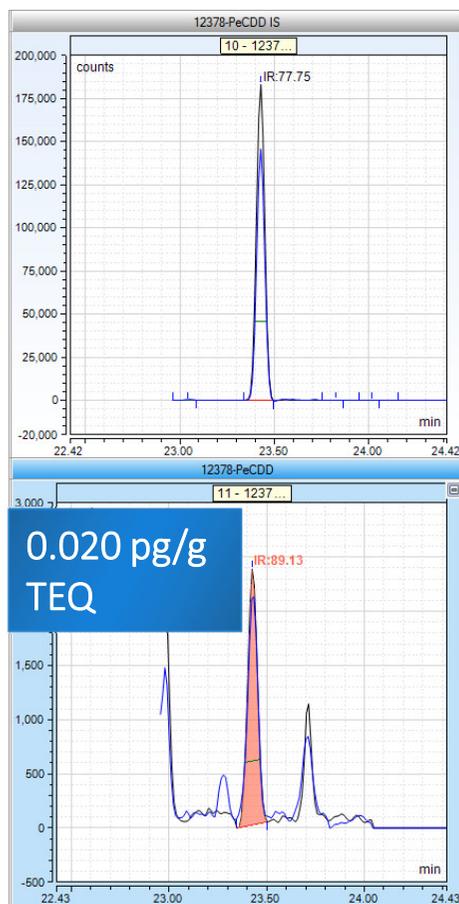
Sample: QC - feed	
Sample weight (g)	20.71
Final volume (µL)	10
Sample injection volume (µL)	2
Standard injection volume (µL)	2
EU ML (sum WHO-TEQ-PCDD/Fs [2005] pg/g)	0.750
1/5th EU ML	0.150
<b>Expected sum WHO-TEQ-PCDD/Fs [2005] pg/g derived from LOQ</b>	<b>0.028</b>

**The sum of the calculated upperbound values at the LOQ\* are significantly lower than the 1/5<sup>th</sup> EU maximum level requirements**

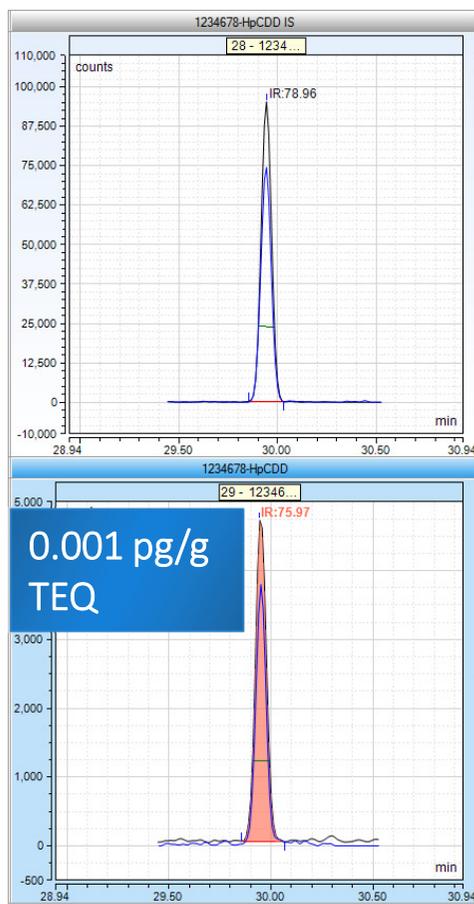
**QC result ~2/5<sup>th</sup> of ML ✓  
All congeners > LOQ**

\* - (applying sample intake weight and assuming 100% IS recovery)

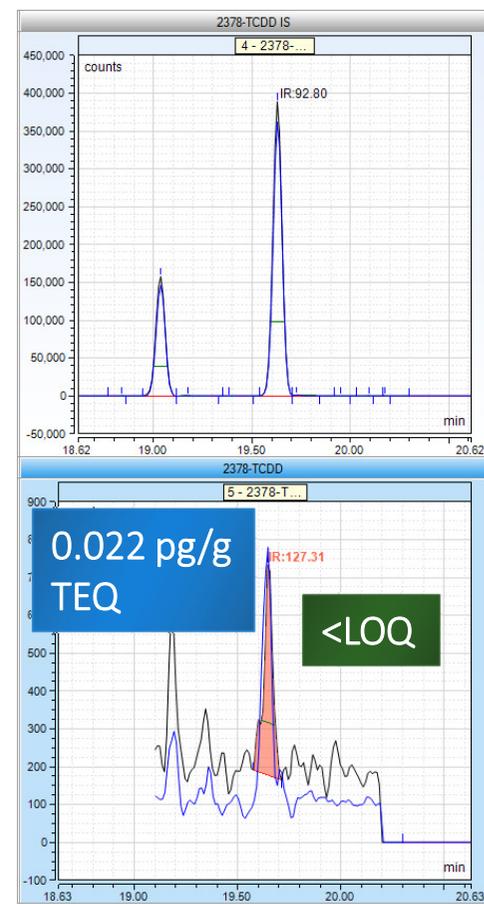
# Sample results - Chromatography



a) Alfalfa



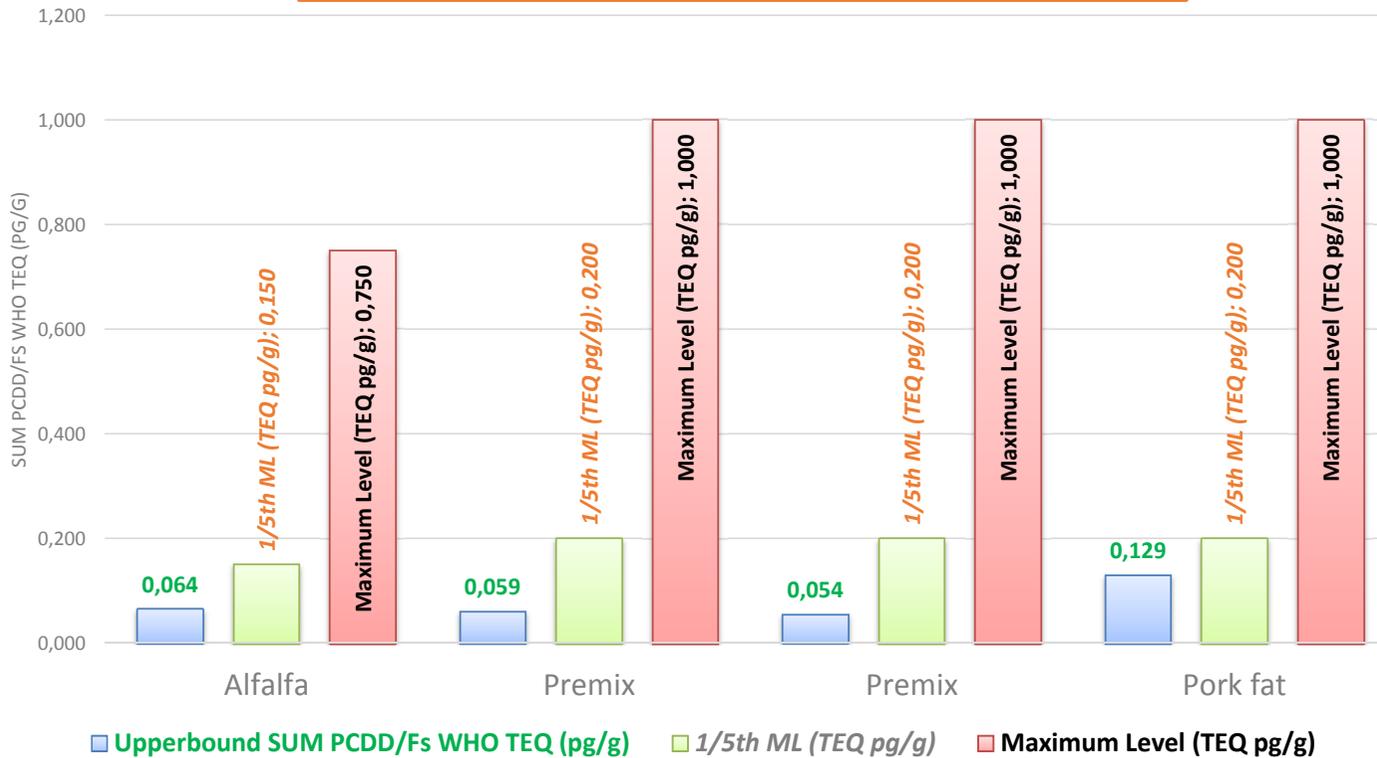
b) Premix



c) Pork Fat

# Sample results

	Alfalfa TSQ 9000 AEI	Premix TSQ 9000 AEI	Premix TSQ 9000 AEI	Pork fat TSQ 9000 AEI	Sheep TSQ 9000 AEI	Sheep GCHRMS
Upperbound SUM PCDD/Fs WHO TEQ (pg/g)	0.064	0.059	0.054	0.129	4.999	4.815
Lowerbound SUM PCDD/Fs WHO TEQ (pg/g)	N/A	N/A	N/A	N/A	4.987	4.756
Maximum Level (TEQ pg/g)	0.750	1.000	1.000	1.000	2.500	2.500
1/5th ML (TEQ pg/g)	0.150	0.200	0.200	0.200	0.500	0.500

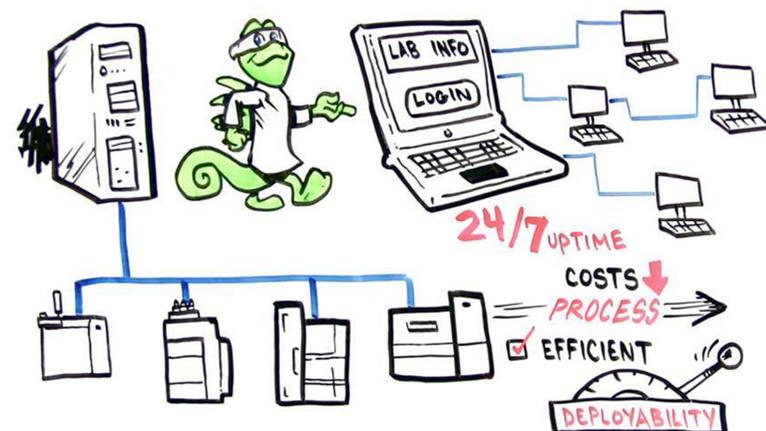


For the samples with **values less than the ML**, the calculated **Upperbound SUM PCDD/Fs WHO-TEQ (pg/g)** comfortably fall below the **1/5th ML requirements** as stated by EU regulations.

For the samples with values **greater than the ML**, the calculated Upperbound SUM PCDD/Fs WHO-TEQ (pg/g) is in excellent agreement with GCHRMS data acquired

## Software

- Chromeleon™ 7.2 Chromatography Data System (CDS) software was used for instrument control, data acquisition, processing and reporting.
- One-click Thermo Scientific™ eWorkflows™ available for simplified method, sequence creation and reporting.
- Integration with Thermo Scientific™ SampleManager LIMS™, SDMS and LES to manage the complete laboratory workflow.



## Conclusions

- The **sensitivity** achieved with the with the AEI source was proven to be repeatable and **robust** throughout a continuous two day analytical sequence.
  - Lowest detectable amount of TCDD **<0.6 fg on-column**.
  - On-column LOQs between **20 fg** (TCDD/TDCF) and **~300 fg** (OCDD/OCDF). This shows compliance with the regulations for sample intake weights as low as 5g for feed samples.
  - With this level of sensitivity Upperbound SUM PCDD/Fs WHO TEQ (pg/g) values of **<0.06pg/g** are routinely achieved in real matrix samples (ex: animal feed).
- The **stability** and **repeatability of the ion ratios** and **response factors** generated provide confidence in results at the lowest quantifiable levels.
  - Ion ratios were consistently **<±15%** throughout entire sequence for all native and labelled congeners.
  - Response factor RSDs between **1.8 – 7.3%** were achieved over the entire calibration range (**0.01 – 80 pg/μL to 0.16 – 320 pg/μL**)
- The reported upperbound WHO-TEQ results reported for low level samples below the MLs were **significantly lower than the 1/5<sup>th</sup> MLs required**, giving both reassurance and flexibility when analysing multiple sample types.
- The TSQ 9000 GC-MS/MS system configured with the AEI source **satisfies** all of the current **EU commission requirements** for the detection and confirmation of dioxins in food and feed samples.

