



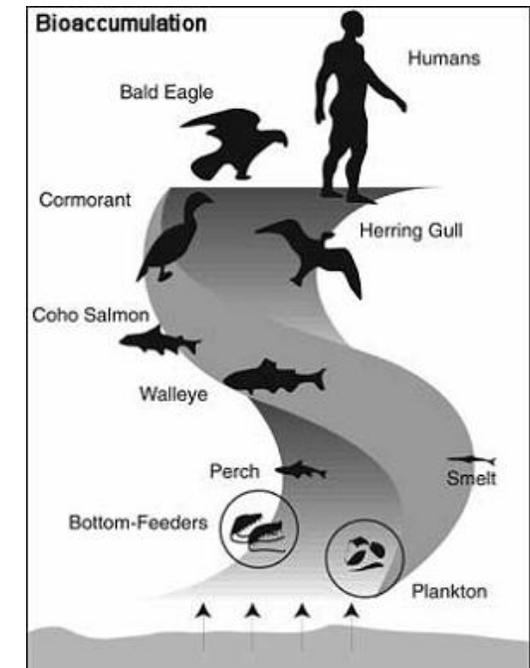
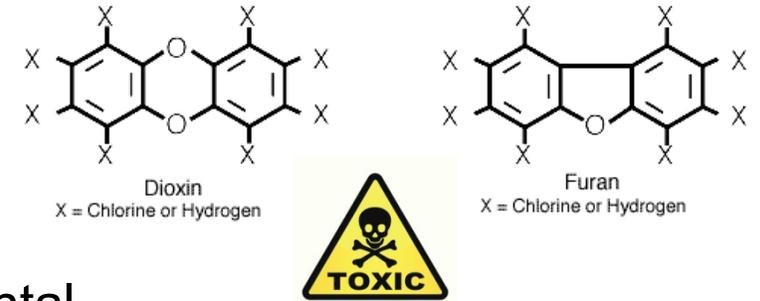
Low level quantification of PCDD/Fs in animal feedstuffs using the Thermo Scientific TSQ 9000 GC-MS/MS system with AEI source

Runcorn, UK

PP10604

Introduction

- Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), commonly referred to as *dioxins*, are persistent environmental pollutants (POPs).
- Dioxins are highly toxic and can cause reproductive and developmental problems, damage the immune system, interfere with hormones and cause cancer.
- Dioxins are ubiquitous in the environment and bioaccumulate in the food chain. Main route of exposure for humans is food. Daily intake in industrialised countries is 1-3 pg TEQ/kg body weight, close to the tolerable intake value set by WHO
- Due to the high toxicity and bioaccumulative nature of dioxins, any national authorities have programmes in place to monitor the food supply.

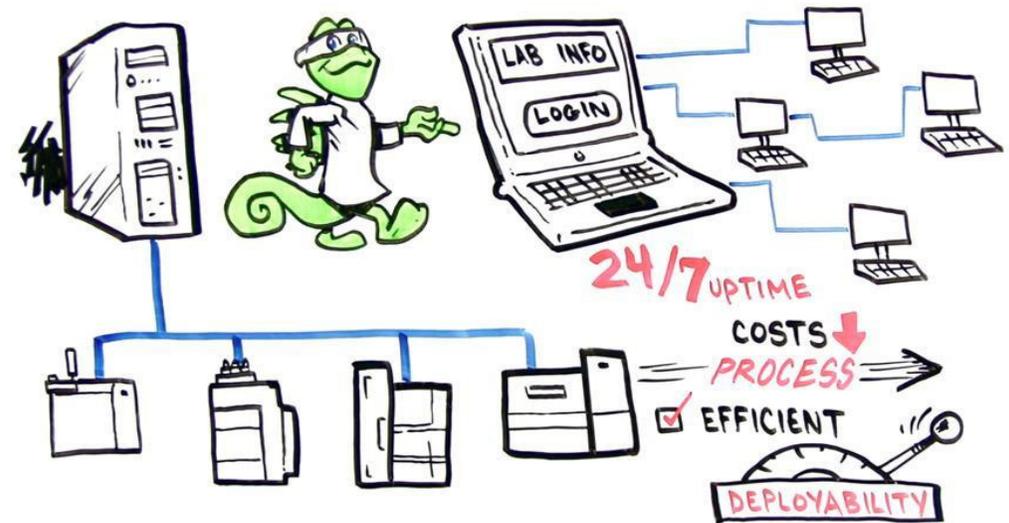


Objectives

- European Union (EU) has set maximum levels (MLs) in foodstuffs and feedstuffs to minimise human exposure.
- Current EU Regulation* requires official confirmatory analytical methods to give accurate confirmation and quantification of PCDD/Fs at **ppt level** in food and feed.



- 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
- To demonstrate its performances in terms of sensitivity, selectivity and robustness, and ultimately its capability to operate in a routine environment.



Challenges - Sensitivity

- Accurate confirmation and quantification of PCDD/Fs at **ppt level** is required in food and feed (LOQ, compliancy at 1/5th of the maximum levels (MLs))

For the calculation of LOQ, in GC-MS/MS Triple Quad instruments

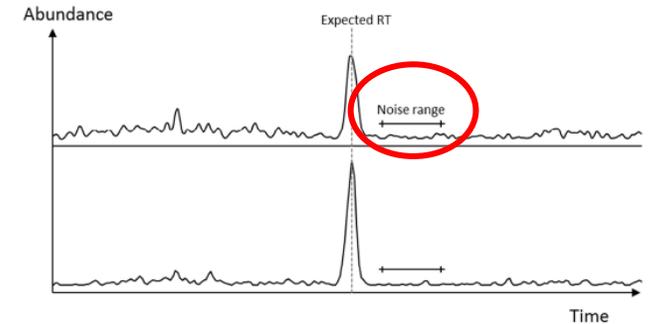
→ S/N approach is not applicable as the noise level is too small to perform a reliable signal-to-noise ratio calculation*.

→ Calibration curve approach is used instead:

*“The LOQ of an individual congener may be defined as the lowest concentration point on a calibration curve that gives an **acceptable** ($\leq 30\%$) and **consistent** (measured at least at the start and at the end of an analytical series of samples) **deviation to the ARRF** calculated for all points on the calibration curve in each series of samples .*

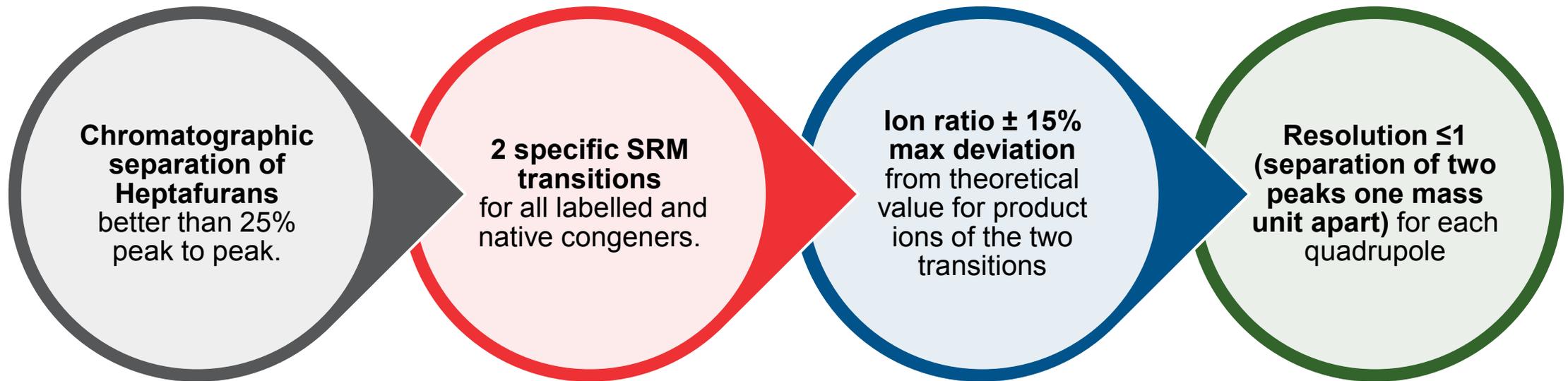
(The LOQ is calculated from the lowest concentration point taking into account the recovery of internal standards and sample intake).”

S/N in HRMS



Challenges - Robustness

- Accurate confirmation and quantification of PCDD/Fs at **ppt level** is required in food and feed.
- GC-MS/MS analytical methods are allowed for confirmatory analysis, proven to show **specific performances** in terms of selectivity, robustness and sensitivity **at the LOQ**:





- For all experiments in this study, 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 (µL):	2
Liner:	Thermo Scientific™ LinerGOLD™ single taper with wool (P/N: 453A0924-UI)
Inlet (°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, 0.25µm) (P/N: 26066-1540)

Oven Temperature Program

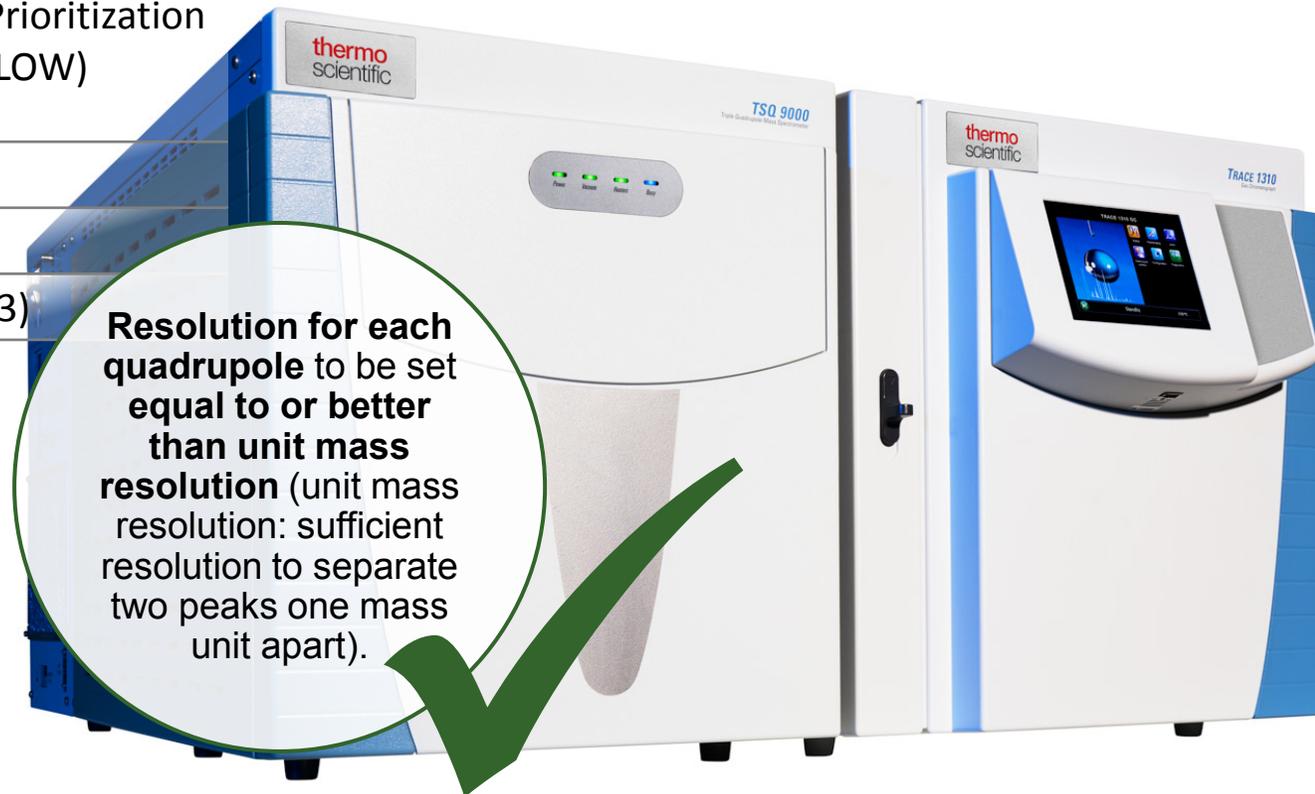
Temperature 1 (°C):	120
Hold Time (min):	0
Temperature 2 (°C):	250
Rate (°C/min):	25
Hold Time (min):	0
Temperature 3 (°C):	285
Rate (°C/min):	2.5
Hold Time (min):	0
Temperature 4 (°C):	320
Rate (°C/min):	10
Hold Time (min):	15
Total Run Time (min):	39.7



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 @ FWHM (both Q1 and Q3)



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.

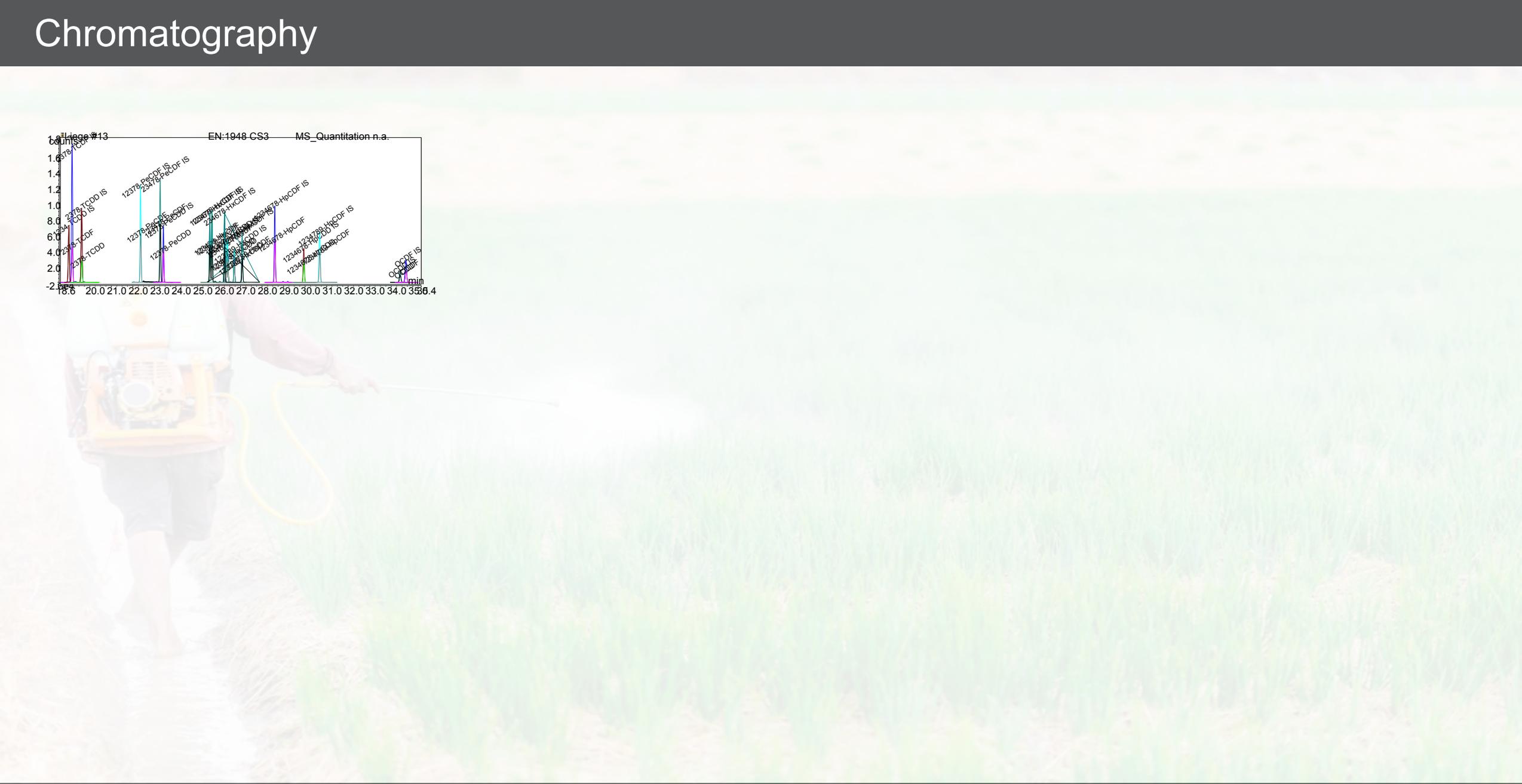
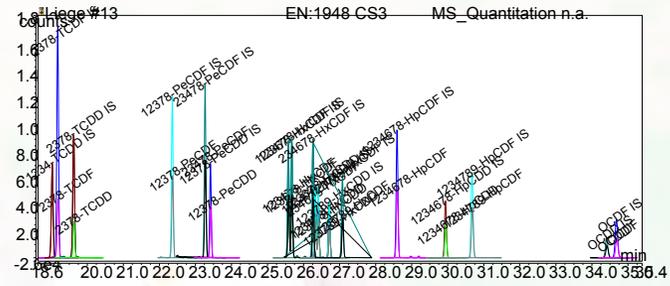
	Alfalfa	Pork Fat	Premix	Premix	Sheep	QC – Pork fat	QC - Feed (grass)
Sample intake (g)	32.13	4.57	10.17	11.1	2.55	6.8	20.71
Regulatory ML [WHO-PCDD/F-TEQ(pg/g)] *	0.75	1	1	1	2.5	1	0.75**
1/5th ML [WHO-PCDD/F-TEQ(pg/g)] *	0.15	0.2	0.2	0.2	0.5	0.2	0.15

* maximum limits taken from European directive 2002/32/EC ⁽³⁾

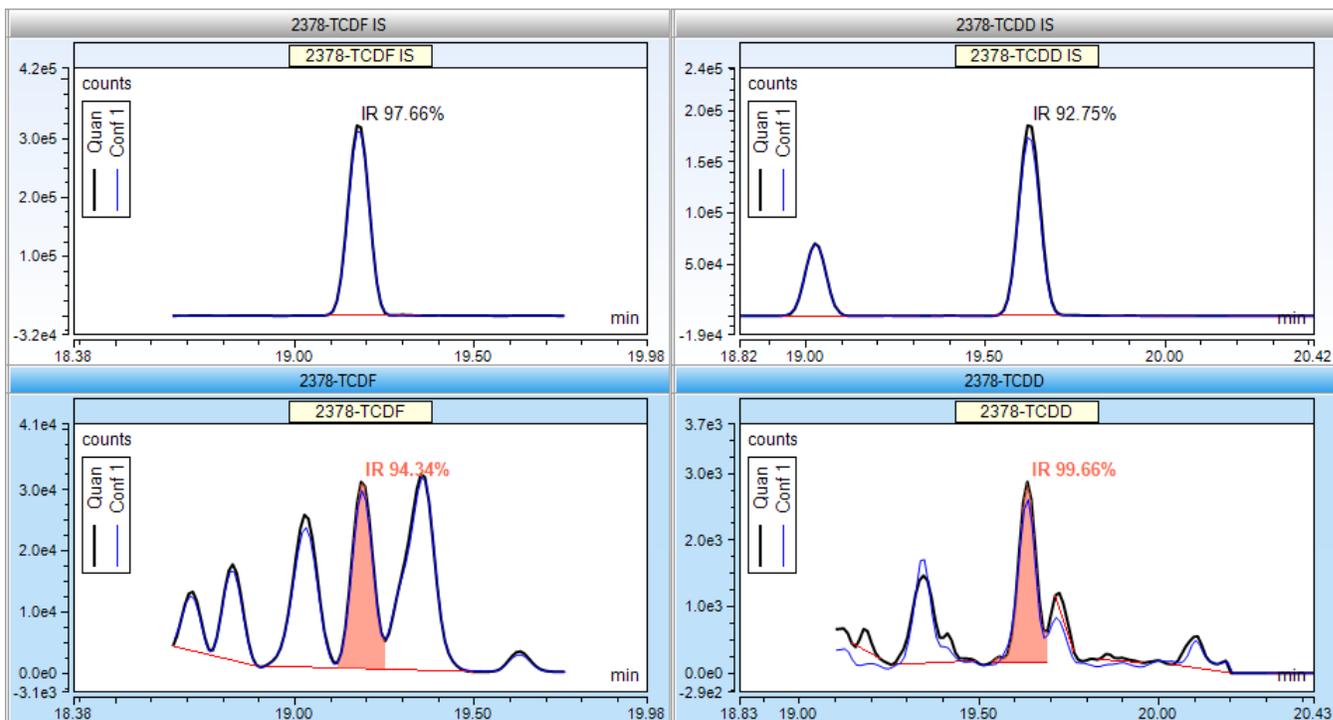
** lower limit applied

- European method EN:1948 standard solutions (Wellington Laboratories Inc., Canada) were utilized for initial performance tests and for calibration and quantitation.

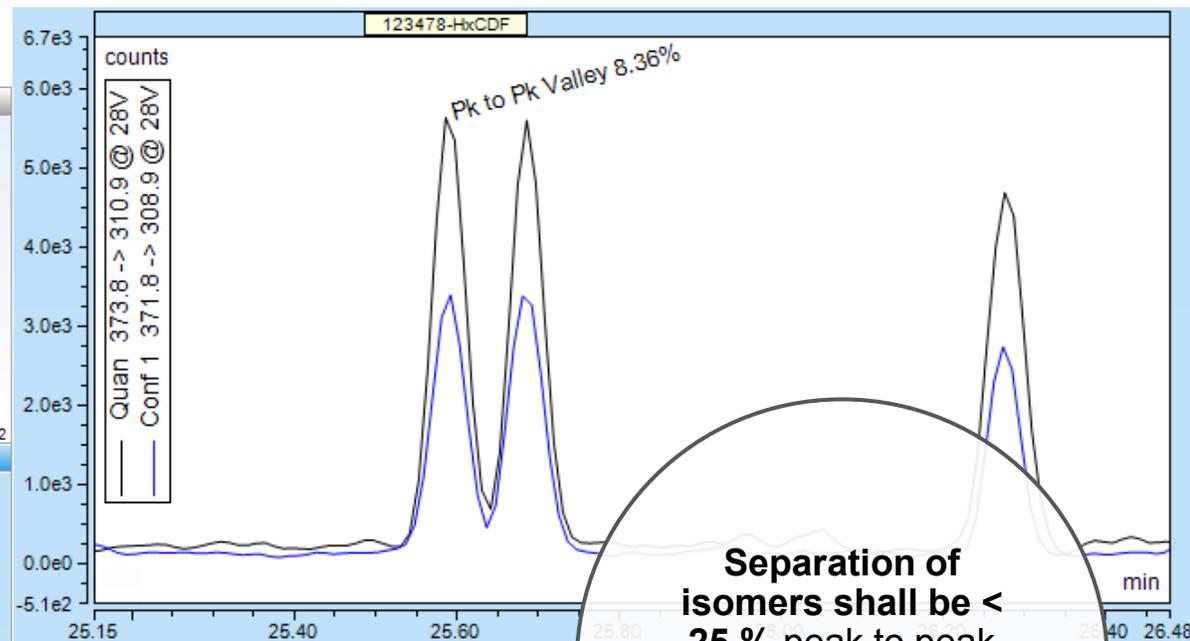
Chromatography



Chromatography – Separation



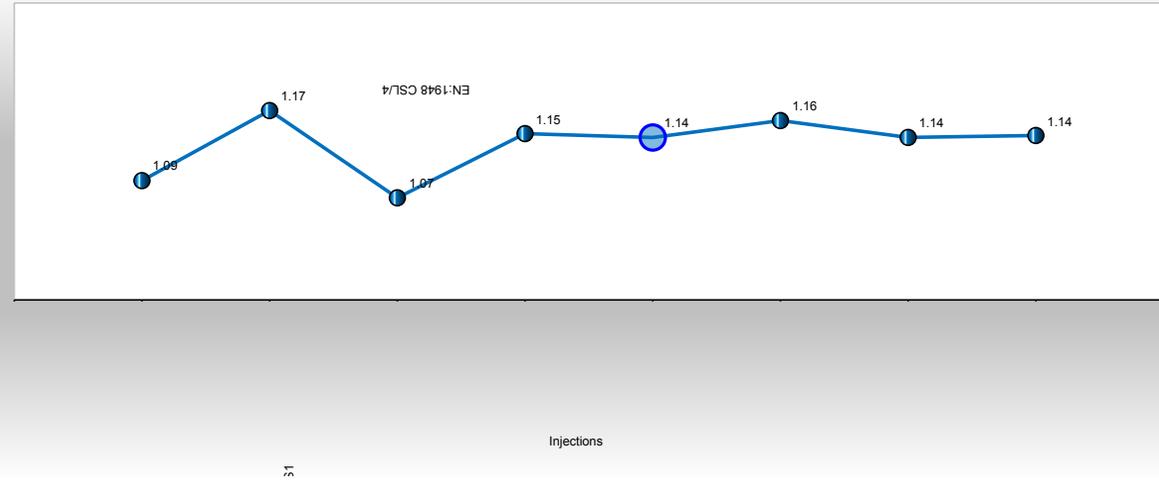
TCDD/F congener separation on the TG-Dioxin (60m x 0.25mm, 0.25 μ m) column



Separation of isomers shall be < 25 % peak to peak between 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF.

Linearity

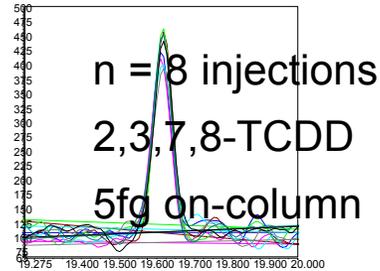
	Congener	Average RF from Calibration	RF standard deviation	RF RSD (%)	Calibration range (pg/ μ L)
Response factor	2378-TCDF	1.06	0.06	5.6%	0.01 - 80
	2378-TCDD	1.13	0.06	5.6%	0.01 - 80
	12378-PeCDF	1.02	0.02	2.3%	0.02 - 160
	23478-PeCDF	1.08	0.02	1.9%	0.02 - 160
	12378-PeCDD	1.11	0.03	2.4%	0.02 - 160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



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

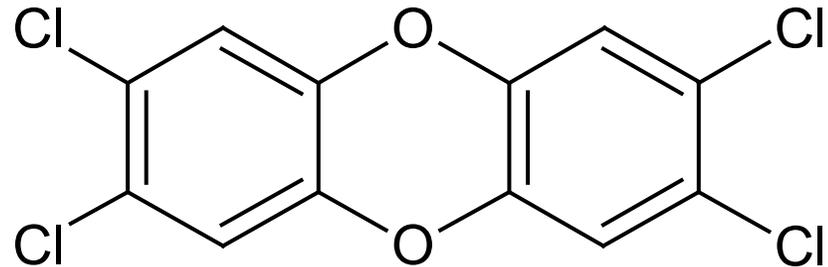
Instrumental Detection Limit (IDL)

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



$IDL_{RSD} = 0.58 \text{ fg}$

2378-TCDD



***Attogram levels of
sensitivity!***

LOQ calculation

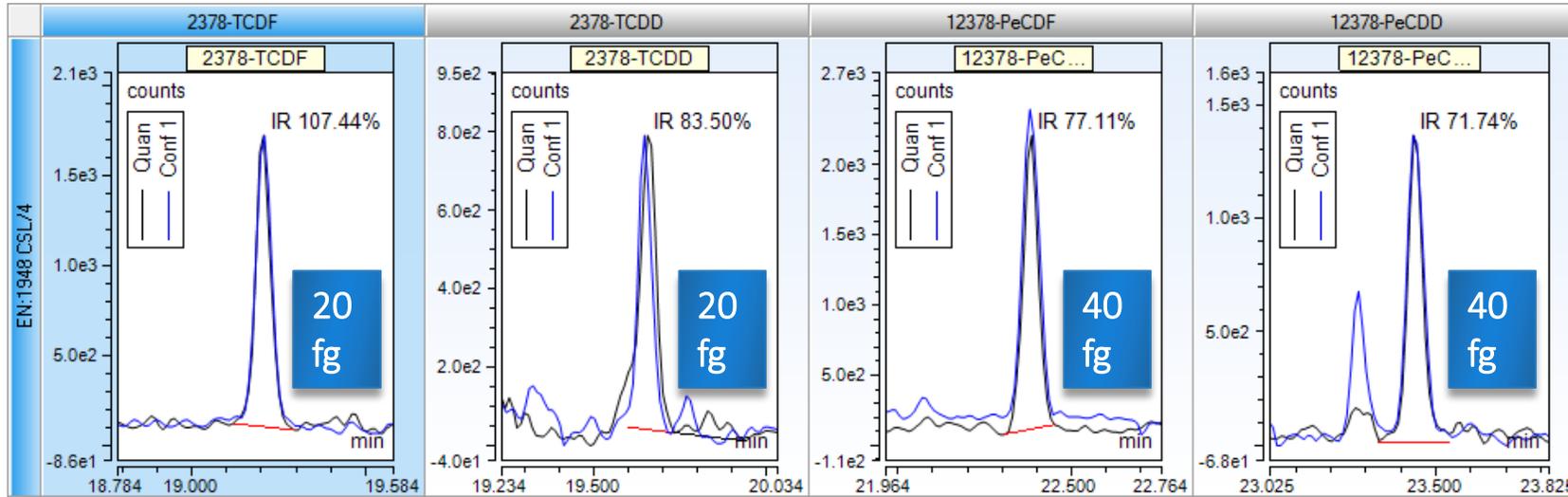
- IDL describes the suitability of a system for trace level analysis, but it does NOT represent method LOQ and cannot be used for TEQ calculation.

*“...the lowest concentration point on a calibration curve that gives an **acceptable** ($\leq 30\%$) and **consistent** (measured at least at the start and at the end of an analytical series of samples) **deviation to the ARRF...**”*

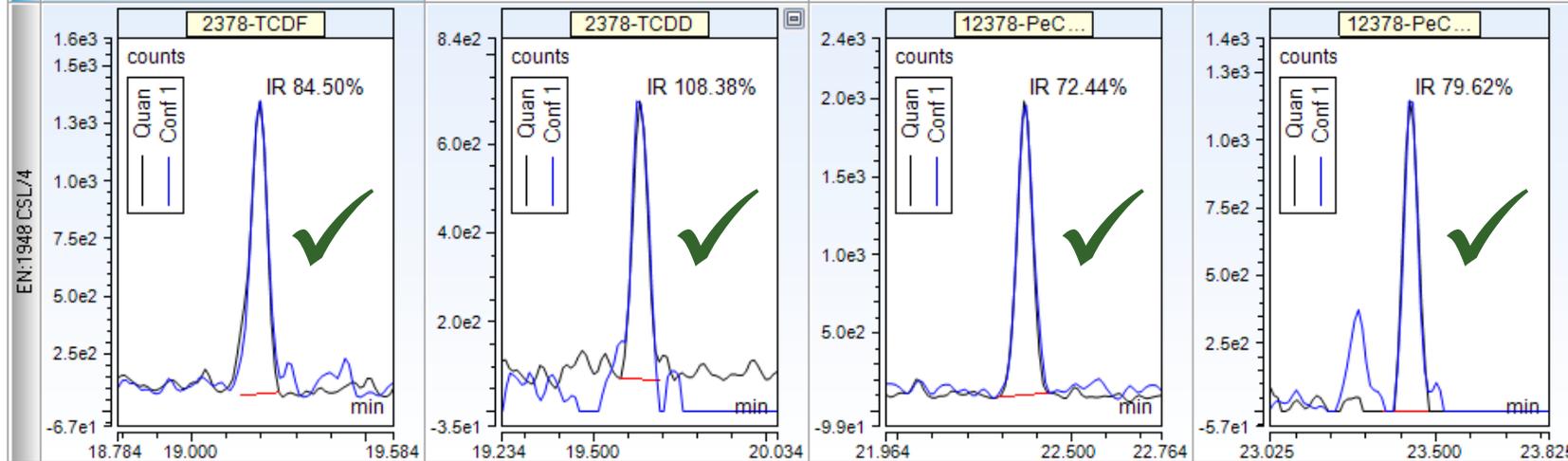
- Specific performances in terms of selectivity and robustness must be met at the LOQ.

Congener	LOQ, absolute amount injected on column (pg)
2378-TCDF	0.02
2378-TCDD	0.02
12378-PeCDF	0.04
23478-PeCDF	0.04
12378-PeCDD	0.04
123478-HxCDF	0.04
123678-HxCDF	0.04
234678-HxCDF	0.04
123478-HxCDD	0.08
123678-HxCDD	0.08
123789-HxCDD	0.08
123789-HxCDF	0.04
1234678-HpCDF	0.08
1234678-HpCDD	0.08
1234789-HpCDF	0.08
OCDD	0.32
OCDF	0.32

4th Injection



61st Injection

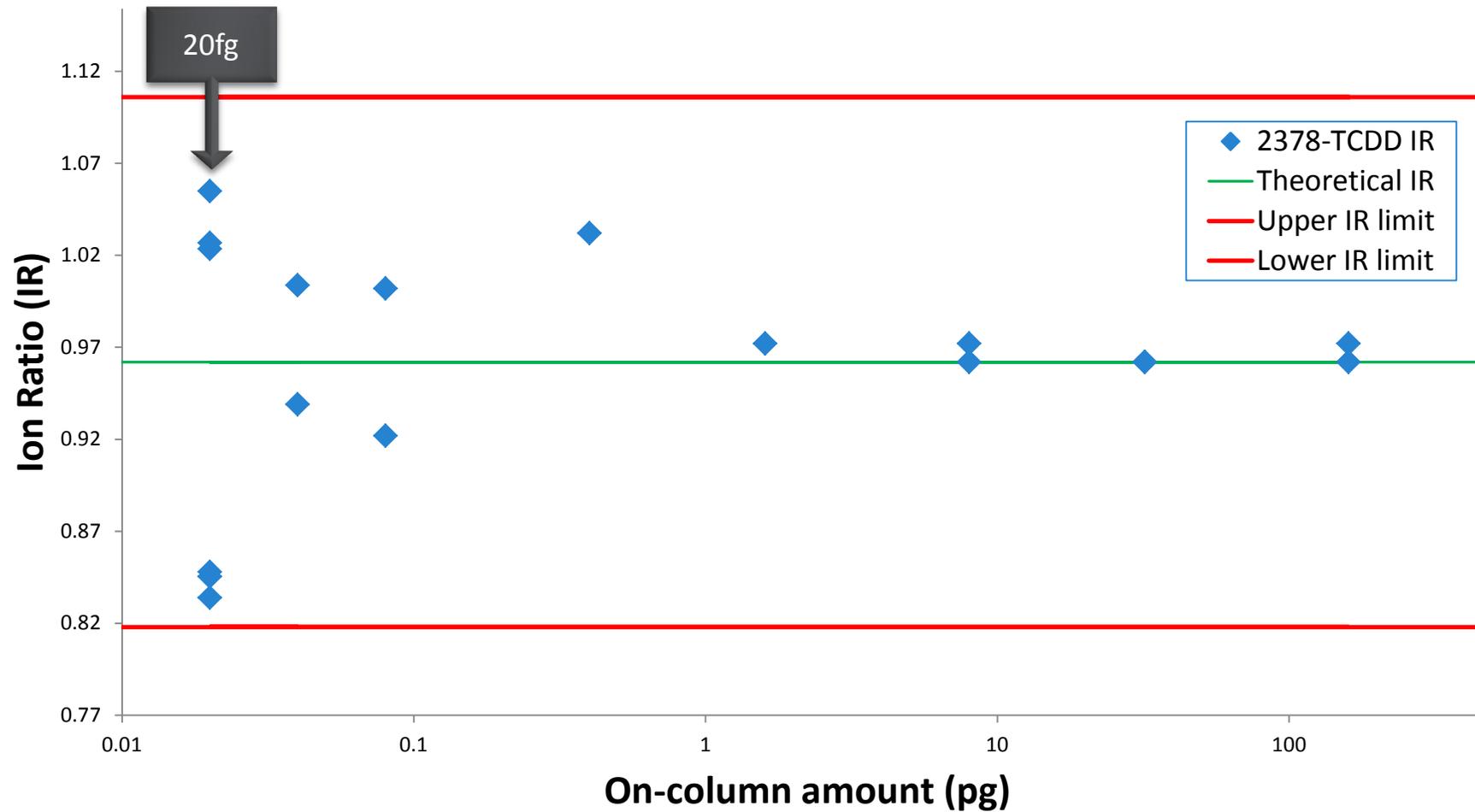


Ion ratios for the lowest level calibration standard (LOQ) showing compliance from the beginning of the sequence to the end.

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

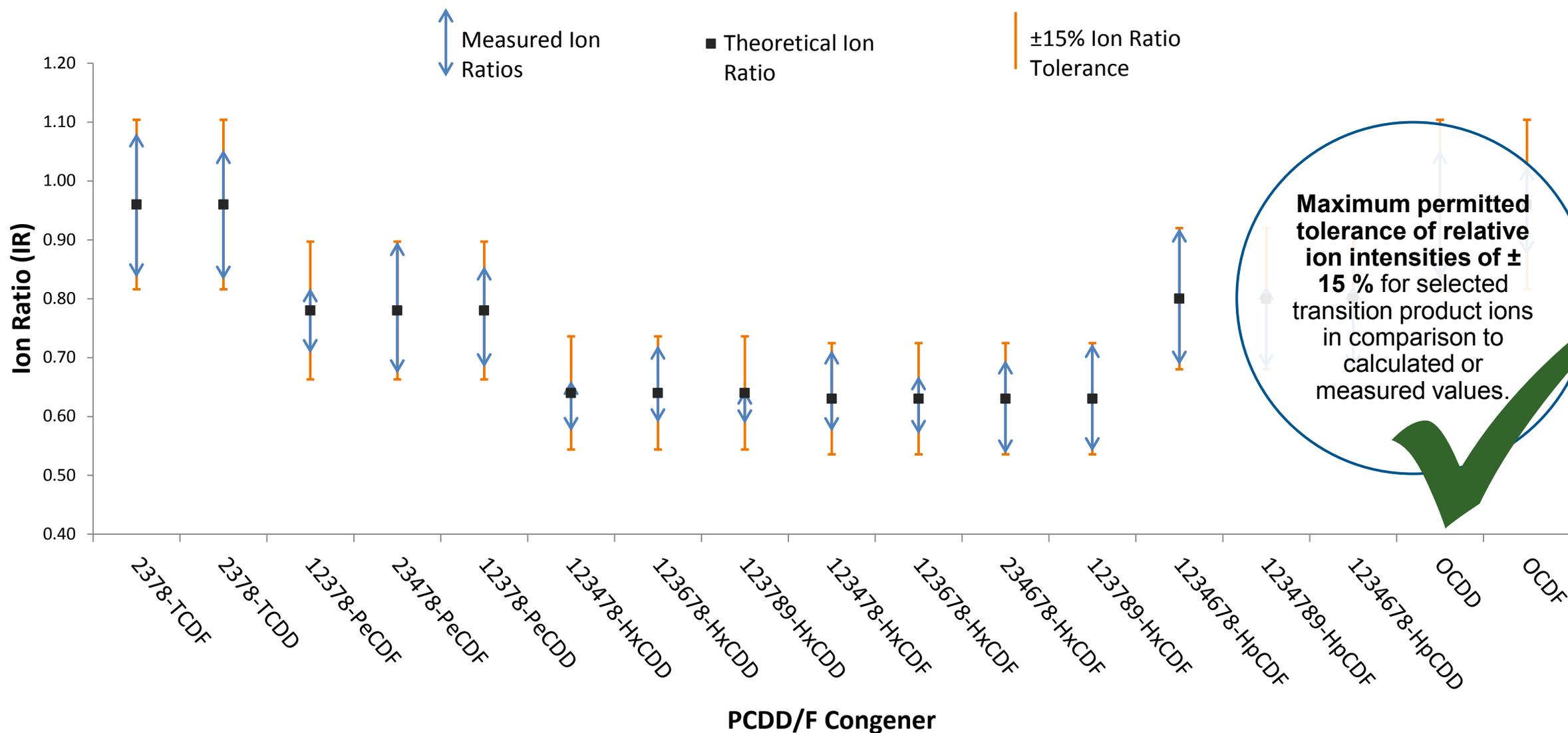


Robustness – Ion ratio 2,3,7,8-TCDD



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

Robustness – Ion ratios for all native congeners



Matrix LOQ and QC results

Sample: QC - feed				
Congener	On column LOQ (pg)	TEF (WHO 2005)	Matrix LOQ 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
		SUM	0.02844	0.28319

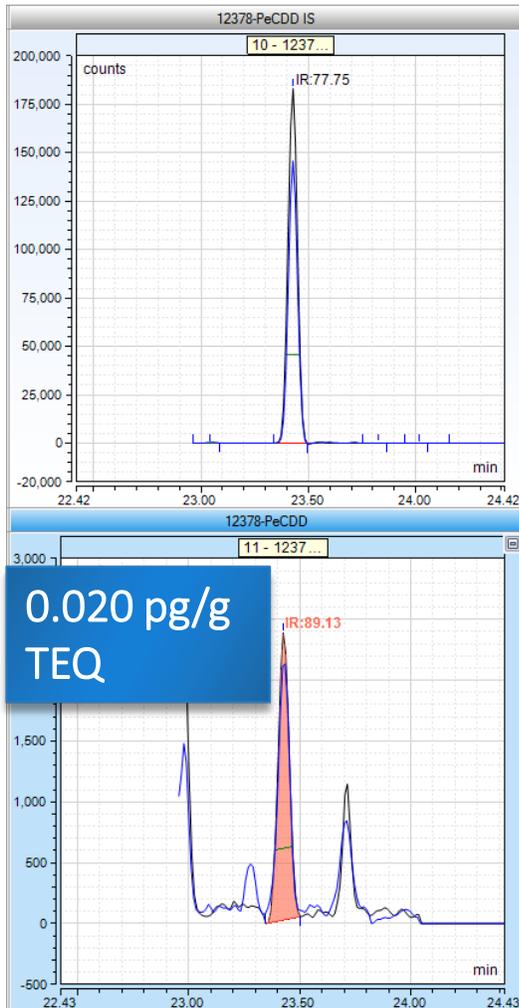
Sample: QC - feed	
Sample weight (g)	20.71
Final volume (µL)	10
Sample injection volume (µL)	2
Standard injection volume (µL)	2
EU ML (sum WHO-TEQ-PCDD/Fs [2005] pg/g)	0.750
1/5th EU ML	0.150
Expected sum WHO-TEQ-PCDD/Fs [2005] pg/g derived from LOQ	0.028

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

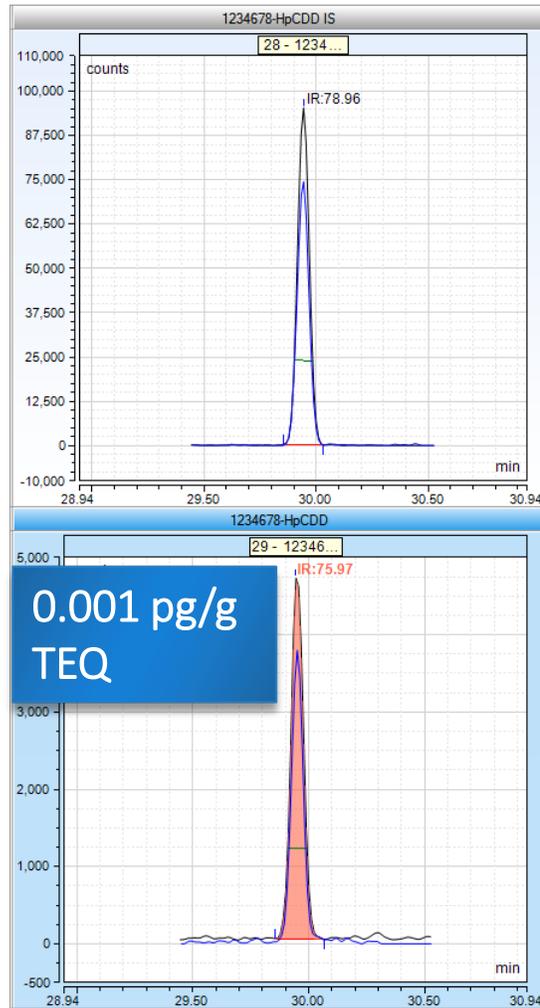
QC results, all congeners > LOQ Results in line with HRMS measurements ✓

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

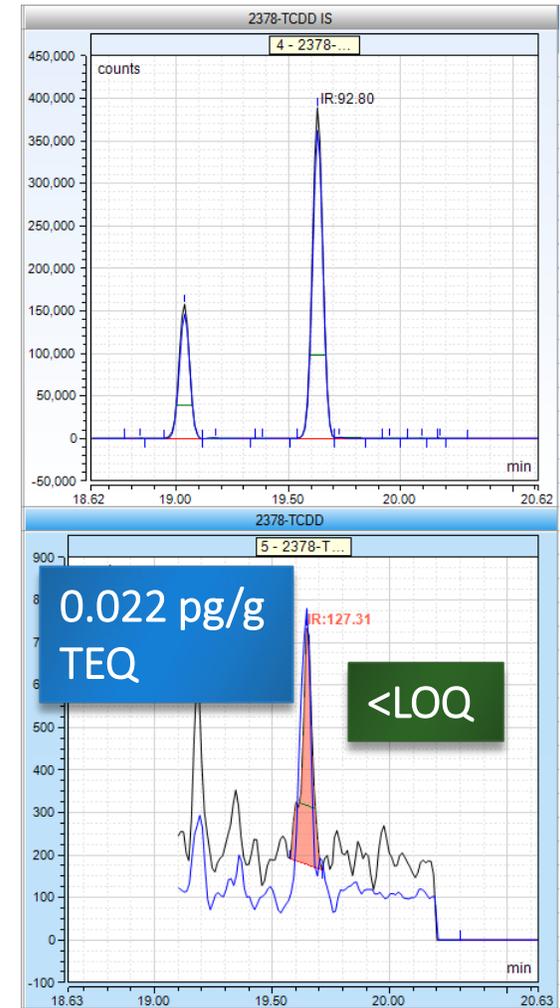
Sample results – Chromatography and quantification



a) Alfalfa

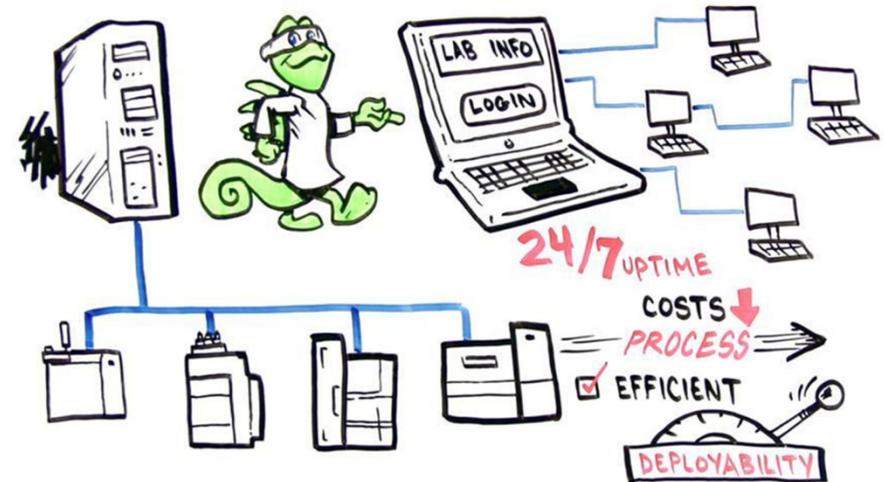


b) Premix



c) Pork Fat

- 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 new AEI source was proven to be suitable for trace level analysis.
 - 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.
- The reported **matrix LOQ** (pg TEQ/g) results reported for low level samples below the MLs were **significantly lower than the 1/5th MLs required**, giving both reassurance and flexibility when analysing multiple sample types.
- The method was repeatable and **robust** throughout a continuous two day analytical sequence, suitable for **routine work**.
- Ultimately 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.