Food safety

Fully automated method implementation for compliant analysis of polychlorinated dibenzodioxins/furans by GC-MS/MS

Polychlorinated dibenzo-*p*-dioxins/furans (PCDDs/PCDFs), commonly known as dioxins, are a group of persistent organic pollutants (POPs) that have been the focus of environmental scrutiny and regulation for decades. Due to their extreme toxicity, the European Union (EU) has set strict thresholds on allowable limits of dioxins within food and feed ($pg \cdot g^{-1}$ concentration) to protect public health. This requires constant monitoring of food and feed items imported into the EU from jurisdictions with different threshold limits or less stringent regulatory requirements.[1,2]

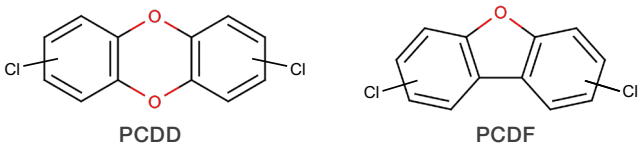

Figure 1. Chemical structure of polychlorinated dibenzodioxins/furans (PCDD/F)

Performance-based methodology for dioxin analysis is applied within the EU to ensure accurate reporting of dioxin levels within food and feed. Results are evaluated according to strict quality control and reporting criteria. For analytical testing laboratories, this often means a significant time investment for adoption and implementation of the methodology for dioxin analysis, as well as a requirement for highly skilled operators. Even for laboratories experienced with GC-MS-based test procedures, the addition of dioxin testing to the overall portfolio is demanding. To simplify this process, a dedicated eWorkflow™ template is available within the Thermo Scientific™ Chromeleon™ Chromatography Data System. This eWorkflow template is available for the Thermo Scientific™ TSQ™ 9610 triple quadrupole GC-MS/MS system and is free to download from the Thermo Scientific™ AppsLab™ Library of Analytical Applications (Dioxin eWorkflow).

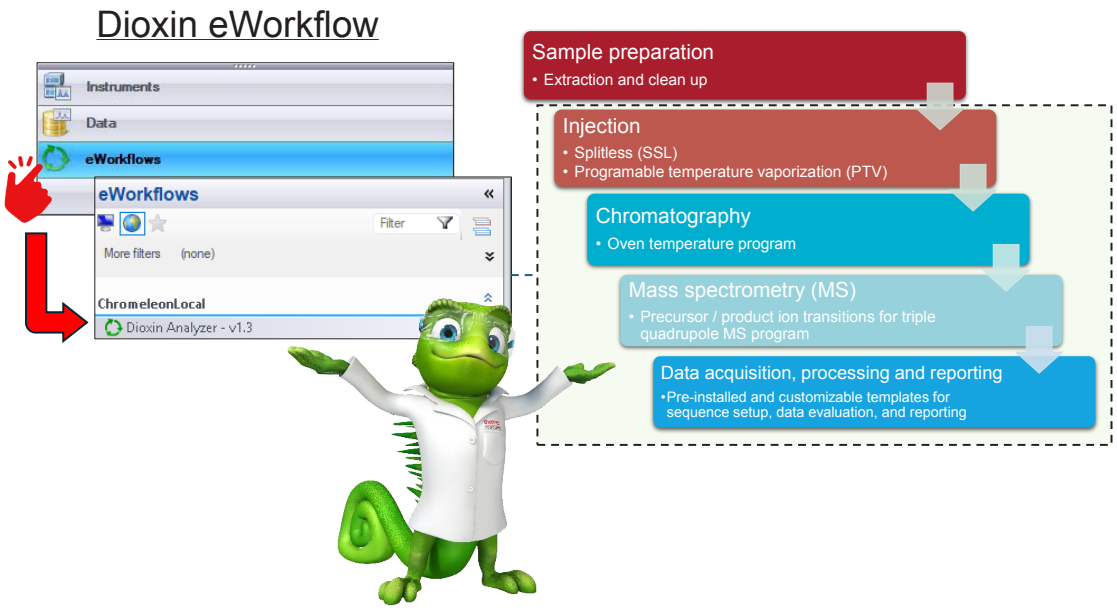

Figure 2. Overview of Dioxin eWorkflow template and its contents in Chromeleon CDS

The eWorkflow template provides all tools required for implementation of a fully compliant analytical method for dioxin analysis. It includes pre-optimized settings for injection, separation, and mass spectrometer-based data acquisition as well as pre-installed and customizable templates for data evaluation and reporting (Figure 2).

Processing methods within the Dioxin eWorkflow template use isotopic dilution for quantitation, allowing users to perform data acquisition and real-time quantitation within the same software. Spiking levels of both syringe and internal standard can be defined per injection, permitting samples spiked with different levels of internal or syringe standard concentration to be analyzed in a single sequence. This can be seen in Figure 3 where internal standard concentration level within the sequence is defined at different levels for calibration standards (i.e., PCDD/F CS1), check standards (LOQ, LOQ/2, LOQ/4), and blank/sample injections (Sample).

A summary of the sample results is presented in an interactive table (Figure 4). The information provided by default includes the following parameters:

- Internal standard recovery – recovery assessment from analyte extraction/cleanup process.
- Targeted and observed ion ratio confirmation – validates that the detected compound meets the theoretical ratio between quantification and qualifier ions.
- Limit of quantification (LOQ) – choice of lowest calibration standard or user defined limit.
- Lower, middle, and upper bound concentration – concentrations reported when all results below LOQ are replaced with a zero value (lower bound), half the value of the LOQ (middle bound), or the value of the LOQ (upper bound).
- Deviation upper/lower bound – difference between upper and lower bound limits.

A dedicated sample LOQ is calculated for each component for every sample injection, accounting for the analyte extraction recovery (or internal standard recovery), injection volume, and sample weight. In addition, lower, middle, and upper bound values of the World Health Organization PCDD/F Toxic Equivalence (WHO-PCDD/F-TEQ)[1] are automatically calculated together with percent deviation between upper and lower bound limits, which is required for reporting purposes.

Name	Type	*CM7:IntStd_Level
Dioxin analysis CS1	Calibration Standard	PCDD/F CS1
Dioxin analysis CS2	Calibration Standard	PCDD/F CS1
Dioxin analysis CS3	Calibration Standard	PCDD/F CS1
Dioxin analysis CS4	Calibration Standard	PCDD/F CS1
Dioxin analysis CS5	Calibration Standard	PCDD/F CS1
Dioxin analysis LOQ/4	Check Standard	PCDD/F LOQ/4
Dioxin analysis LOQ/2	Check Standard	PCDD/F LOQ/2
Dioxin analysis LOQ	Check Standard	PCDD/F LOQ
Dioxin analysis RBW - Reagent blank	Blank	Sample
Dioxin analysis QK1 - Mixed animal fat	Unknown	Sample
Dioxin analysis QK3 - Egg fat	Unknown	Sample
Dioxin analysis QK6 - Fish oil	Unknown	Sample

Internal standard concentration is defined per injection, allowing analysis of samples/standards containing varying levels of internal standard

Figure 3. Interactive injection sequence table

Peakname	Ret.Time	ISTD recovery	Target IR	Obs IR	IR deviation	TEF	Sample LOQ	<LOQ?	Lowerbound	Middlebound	Upperbound	SUM Upperbound	Deviation Upper/Lowerbound
	min	%	%	%	%		pg/g		WHO-PCDD/F-TEQ (pg/g)	WHO-PCDD/F-TEQ pg/g)	WHO-PCDD/F-TEQ pg/g	WHO-PCDD/F-TEQ pg/g	
2378-TCDF	19.32	85.26	93.1	94.7	1.7	0.1	0.044		0.046	0.046	0.046		
2378-TCDD	*19.90*	*74.29*	*96.0*	*90.4*	*- 5.9*	*1*	*0.050*		*0.070*	*0.070*	*0.070*		
12378-PeCDF	23.36	80.30	79.7	88.8	11.3	0.03	0.232	<LOQ	0.000	0.003	0.007		
23478-PeCDF	24.75	91.77	77.5	79.2	2.2	0.3	0.203		0.072	0.072	0.072		
12378-PeCDD	25.00	90.59	78.5	76.6	- 2.4	1	0.206		0.347	0.347	0.347		
123478-HxCDF	28.14	88.71	64.2	61.6	- 4.0	0.1	0.210	<LOQ	0.000	0.011	0.021		
123678-HxCDF	28.25	91.33	62.8	61.5	- 2.0	0.1	0.204	<LOQ	0.000	0.010	0.020		
234678-HxCDF	28.93	87.78	64.5	52.5	- 18.7	0.1	0.213	<LOQ	0.000	0.011	0.021		
123478-HxCDD	29.04	80.16	63.9	68.3	6.8	0.1	0.233		0.059	0.059	0.059		
123678-HxCDD	29.12	86.13	64.9	61.9	- 4.6	0.1	0.217		0.123	0.123	0.123		
123789-HxCDD	29.43	86.13	64.2	66.0	2.8	0.1	0.217		0.066	0.066	0.066		
123789-HxCDF	29.79	99.20	63.5	70.4	10.8	0.1	0.188	<LOQ	0.000	0.009	0.019		
1234678-HpCDF	31.43	90.29	79.8	84.6	6.1	0.01	0.207		0.003	0.003	0.003		
1234678-HpCDD	32.86	90.88	79.6	79.2	- 0.6	0.01	0.205		0.091	0.091	0.091		
1234789-HpCDF	33.59	111.19	79.6	92.5	16.3	0.01	0.168	<LOQ	0.000	0.001	0.002		
OCDD	37.45	106.88	96.0	93.9	- 2.2	0.0003	0.349		0.002	0.002	0.002		
OCDF	37.68	106.88	93.3	86.9	- 6.9	0.0003	0.349	<LOQ	0.000	0.000	0.000	0.968	9.3%

Sample SUM Upperbound LOQ | Summary | Sample summary | Check standard | Calibration | Audit Trail

Upper/lower bound deviation should not exceed 20%

Full overview of Internal standard recovery and ion ratio performance

Sample LOQ automatically corrected for injection volume, sample weight, and internal standard recovery

Figure 4. Sample summary table provided with the Dioxin eWorkflow template within Chromeleon CDS

Within the data processing tab of Chromeleon CDS, templates have been designed to facilitate data evaluation. An example of this can be seen in Figure 5, in which the interactive charts function as part of the processing method templates shows acceptable ion ratio range according to regulated criteria (±15% from theoretical ion ratio). In cases where poor integration occurs, the results are displayed visually, allowing for quick data evaluation without the need to cycle through each injection. Correction to the integration automatically updates the results, which now fall within the regulated method criteria range, drastically speeding up processing time.

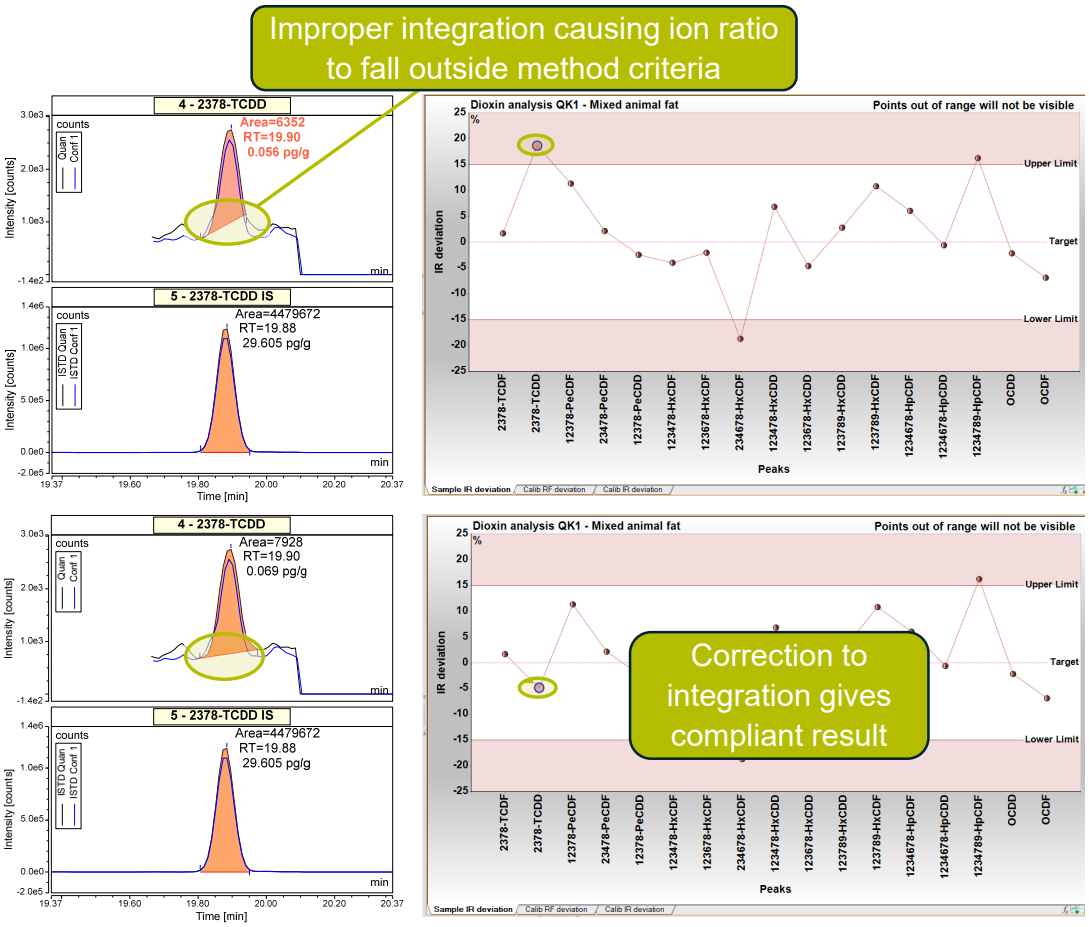

Figure 5. MS component and ion ratio interactive chart views of the Dioxin eWorkflow template in Chromeleon CDS. (A) Incorrect integration of quantification or qualifier peak causes ion ratio to fall outside specified regulatory limits and (B) correction of integration with automatic updating of interactive chart results.

The Dioxin eWorkflow template allows analytical testing laboratories to easily implement compliant analytical methodology for dioxin analysis. It delivers validated data acquisition/processing methods and reporting templates for dioxin analysis, helping remove time-consuming steps around method development and data processing.

Included within the Dioxin eWorkflow template:

- Injection sequence templates with pre-optimized analytical parameters for method injection and data acquisition.
- Incorporation of isotopic dilution quantitation, removing the need for additional software or script writing to perform data calculations.
- Interactive tables and charts to facilitate quick data evaluation for compliance purposes.
- Fully customizable data evaluation and report layout templates based on the analyst's needs.

References

1. Commission Regulation (EU) 2017/644 (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 and repealing Regulation (EU) No 589/2014.
2. Commission Regulation (EU) 2017/771 (2017). Amending Regulation (EC) No 152/2009 as regards the methods for the determination of the levels of dioxins and polychlorinated biphenyls.

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