A CONFIRMATORY METHOD FOR THE QUANTIFICATION OF PCDDs AND PCDFs IN FOOD AND FEED BY GC-MS/MS IN COMPLIANCE WITH EU REGULATION 589/2014

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Introduction

The term dioxins is commonly used in reference to polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). These are a group of chemically related compounds, known to be toxic and persist as ubiquitous pollutants. Therefore, dioxins are restricted internationally under the Stockholm Convention[1], along with other nationally enforced regulations. Given the persistence and toxicity of PCDDs and PCDFs, reliable and robust analytical methodology is required to ensure food safety and consumer protection.

Due to legislative requirements in Europe and America, the gold standard for dioxin analysis uses gas chromatography coupled with high resolution mass spectrometry (GC-HRMS), traditionally operated by electron impact (EI). Following recent technological advances, however, and after a detailed evaluation, the European Commission has legislated that tandem quadrupole (GC-MS/MS) may be used as a confirmatory method[2].

Regulation EU 589/2014 sets out performance criteria with respect to specificity, sensitivity, confirmation ions, resolution and calibration for the confirmation of dioxins in food and feed. This regulation is enforced in conjunction with existing requirements[3-5]. The use of atmospheric pressure gas chromatography (APGC) coupled with a high sensitivity tandem quadrupole mass spectrometer will be demonstrated as a sensitive and robust option for confirmatory analysis of PCDDs and PCDFs in compliance with 589/2014/EU.

Materials and methods

Standards EPA-1613 CSL to CS5 were purchased for calibration curves, containing both native and ^{13}C labelled PCDD, PCDF and TCDD compounds. For sample preparation the following standards were purchased: EPA-1613 PAR, EPA-1613 LCS and TF-TCDD-MXB along with ^{13}C labelled EPA-1613 ISS PCDD and PCDF congeners. All standards were purchased from Wellington Laboratories (Ontario, Canada). A further dilution of the CSL standard was made in nonane to give a 10 fg.μl^{-1} standard.

Sample preparation was completed in accordance with standard methods in accordance with Commission Regulations 252/2012/EC and 152/2009/EC[3-5].

Results and discussion

Coupling atmospheric pressure gas chromatography (APGC) with MS/MS achieves excellent sensitivity and selectivity required for the confirmation of PCDDs and PCDFs in food and feeds in accordance with recent changes to European Commission Regulation 589/2014/EU and associated analytical requirements. Operating in MRM mode at least two specific precursors to fragment transitions were monitored per analyte, as required by Regulation 589/2014/EU, where sufficient resolving power and selectivity is required in order to differentiate between the analytes of interest and interfering compounds.

All data, for labelled and unlabelled standards was acquired in MRM mode, providing excellent selectivity for two transitions per compound. These transitions are shown in Table 1, where two specific precursor ions are monitored, each with a specific corresponding product ion. This complies with paragraph 6.5 in Annex III of Regulation 589/2014/EU.

The excellent selectivity and specificity afforded by APGC- MS/MS is shown in Figure 1 for all unlabelled standards, where an overlay of all PCDD and PCDF congeners is given. This figure supports the requirements of Paragraph 5.2 (Annex III) requiring high selectivity in order to differentiate between the 17 congeners of 2,3,7,8-substituted PCDD/Fs

	Dioxin	pg.µl⁻¹
1	TCDF	0.01
2	TCDD	0.01
3	1,2,3,7,8-PCDF	0.05
4	2,3,4,7,8-PCDF	0.05
5	PCDD	0.05
6	1,2,3,4,7,8-HxCDF	0.05
7	1,2,3,6,7,8-HxCDD	0.05
8	2,3,4,6,7,8-HxCDF	0.05
9	1,2,3,4,7,8,-HxCDD	0.05
10	1,2,3,6,7,8-HxCDD	0.05
11	1,2,3,7,8,9-HxCDD	0.05
12	1,2,37,8,9-HxCDF	0.05
13	1,2,3,4,6,7,8-HpCDF	0.05
14	1,2,3,4,6,7,8-HpCDD	0.05
15	1,2,3,4,7,8,9-HpCDF	0.05
16	OCDD	0.1
17	OCDF	0.1

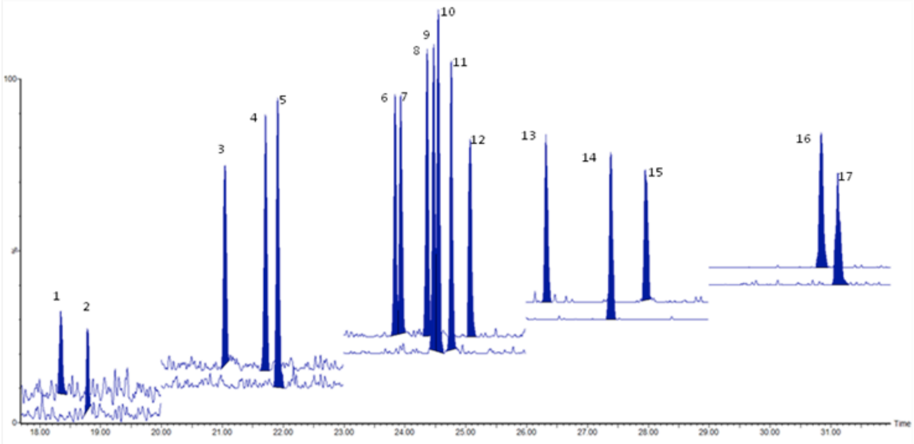

Figure 1: Overlay of all 17 PCDD and PCDF analytes at one in ten dilution of CSL standard

Furthermore, the MRM transitions facilitates the simple calculation of abundance ratios where the two isotopic precursors ($[M^{+\bullet}\,{}^{35}Cl]$ and $[M^{+\bullet}\,{}^{37}Cl]$) are made relative to their product ion (predominant loss of $[CO^{35}Cl]$ and $[CO^{37}Cl]$ respectively). An example of this is shown in Figure 2. In this study, good agreement was achieved for abundance ratios of these fragments for complex samples, relative to the calibration standards. All results were found to be compliant with Regulation 589/2014/EU.

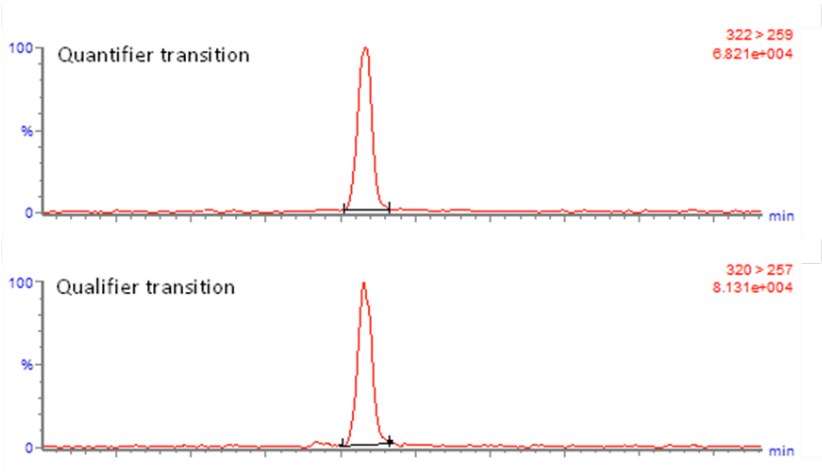

Figure 2: Quantification and qualification ions for TCDD in egg sample, yielding an intensity ratio of 0.876. This is compliant with Regulation 589/2014/EU tolerance of ±15 % (calculated ratio of 0.868).

Excellent linearity ($R^2 > 0.995$) was determined for all PCDD and PCDF analytes over a working range of 0.01 to 40 pg.μl^{-1}. All relative response factors were compliant with the maximum tolerance set out in Regulation 589/2014/EU. These results are summarized in Table 1 showing MS/MS conditions along with linearity and response factors determined for all unlablled PCDD and PCDF standards.

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Table 1: GC-MS/MS conditions and results for PCDDs and PCDFs, including internal standards used

	Dioxin	Precursor	Product	Collision Energies (eV)	Retention time (min)	TEF	Relative response factor (RRF)	Co efficient of determination (R^2)
1	TCDF	304 306	241 243	40 40	18.36	0.1	7.9	> 0.999
2	TCDD	320 322	257 259	30 30	18.79	1	3.3	> 0.999
3	1,2,3,7,8-PCDF	338 340	275 277	40 40	21.01	0.1	2.8	> 0.999
4	2,3,4,7,8-PCDF	338 340	275 277	40 40	21.72	0.1	2.1	> 0.999
5	PCDD	354 356	291 293	30 30	21.91	1	2.8	> 0.999
6	1,2,3,4,7,8-HxCDF	374 376	311 312	40 40	23.85	0.1	6.3	> 0.999
7	1,2,3,6,7,8-HxCDD	374 376	311 312	40 40	23.94	0.1	5	0.999
8	2,3,4,6,7,8-HxCDF	374 376	311 312	40 40	24.37	0.1	3	> 0.999
9	1,2,3,4,7,8,-HxCDD	390 392	327 329	30 30	24.48	0.1	4	> 0.999
10	1,2,3,6,7,8-HxCDD	390 392	327 329	30 30	24.56	0.1	4.1	> 0.999
11	1,2,3,7,8,9-HxCDD	390 392	327 329	30 30	24.77	0.1	3.5	> 0.999
12	1,2,37,8,9-HxCDF	374 376	311 312	40 40	25.08	0.1	8	0.999
13	1,2,3,4,6,7,8-HpCDF	408 410	345 347	40 40	26.33	0.01	4	> 0.999
14	1,2,3,4,6,7,8-HpCDD	424 426	361 363	30 30	27.39	0.01	5.8	> 0.999
15	1,2,3,4,7,8,9-HpCDF	408 410	345 347	40 40	27.97	0.01	3.3	> 0.999
16	OCDD	458 460	395 397	30 30	30.58	0.0003	1.8	> 0.999
17	OCDF	442 444	379 381	40 40	31.12	0.0003	8.3	0.998
18	^{13}C TCDF	316 318	252 254	40 40		-	-	-
19	^{13}C TCDD	332 334	268 270	30 30		-	-	-
20	^{13}C PCDF	350 352	286 288	40 40		-	-	-
21	^{13}C PCDD	366 368	302 304	30 30		-	-	-
22	^{13}C HxCDF	386 388	322 324	40 40		-	-	-
23	^{13}C HxCDD	402 404	338 340	30 30		-	-	-
24	^{13}C HpCDF	420 422	356 358	40 40		-	-	-
25	^{13}C HpCDD	436 438	372 374	30 30		-	-	-
26	^{13}C OCDD	470 472	406 408	30 30		-	-	-

Having demonstrated APGC- MS/MS compliance with Regulation 589/2014 results from the quantitative and confirmatory analysis of complex samples will be discussed, showing excellent agreement with previously acquired GC- HRMS data.

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