APPLICATION NOTE

Analysis of dioxins by GC-TQMS

KEY WORDS: TQMS, dioxins, regulatory standards, MRM

INTRODUCTION

Dioxins refers to polychlorinated dibenzo-p-dioxins (PCDDs) and related compounds polychlorinated dibenzofurans (PCDFs) also called as "furans" (Figure 1). These two groups of compounds are among the most toxic chemicals and are classified as Persistent Organic Pollutants (POPs).

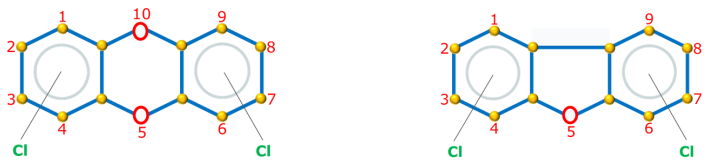

Figure 1. Dioxins and Furans

Dioxins are anthropogenic compounds produced unintentionally as by-products in some industrial activities and waste combustion processes, mainly. In addition to be present in the environment, dioxins are fat-soluble and bio-accumulative compounds in the tissues of animals and humans. Consumption of food is the more important exposure for humans, in particular, fish, shellfish, dairy products and meat.

Dioxins can invoke serious health effects in humans such as loss of body weight, hormone disruption, reproductive disorders, skin toxicity, immune system disorders and cancer, among other diseases. Since 2014, GC-MS/MS is an EU-accepted technique as confirmation method for dioxin analysis in accordance with EU 589/2014 and subsequent regulations implemented in this regard.

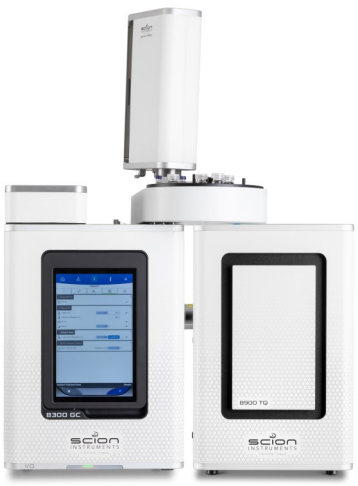

Figure 2. SCION Instruments 8300 GC coupled to a 8900 TQMS with a 8400 Autosampler

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REGULATIONS

GC-HRMS of dioxins in food and environmental samples has been the approved technique for most regulatory bodies due to high selectivity and sensitivity.

More recently, triple quadrupole MS instruments have demonstrated the improved sensitivity and selectivity needed for ultra-trace analysis of dioxins in complex matrices. In addition, GC-TQ instruments require minimal tuning and have lower maintenance requirements, are easier to operate and offer fewer chromatography interferences compared to GC-HRMS.

Therefore, since 2014, GC-MS/MS is an EU-accepted technique as a confirmation method for dioxin analysis in accordance with EU 589/2014 and subsequent regulations.

GC-TQMS SPECIFICATIONS

Table 1. GC-TQMS Configuration for Dioxin Analysis

SCION 8300 GC	
Carrier gas	Helium, 1.5 mL/min, constant flow
Injector	1177 split/splitless, 280 ºC
Injection volume	1 µL, pulsed splitless
Insert	4 mm ID fritted liner
Column	BR-Dioxins, 60m x 0.25mm, 0.25 micron film thickness
Column temp.	140 ºC, hold 1 min→200 ºC at 20 ºC min. hold 1min→ 320 ºC at 5 ºC/min hold 14 min,
Autosampler	SCION 8400
8900 GC-TQMS	
Source	EI, 280ºC
X-line temp.	280 ºC
Collision gas	Ar, 1.5 mTorr
MS mode	Dynamic MRM
Scan time	Automatic, EDR
Quad resolution	Q1=0.7 mass unit, Q3= 0.7 mass unit
Software	Compass TQ

EXPERIMENTAL

Dioxins are the only group of compounds losing COCl fragment (63 Da). This is a very selective transition producing clean MRM chromatograms with very few interferences.

Two precursor ions are selected for each compound, each with one specific MRM product ion

Table 2: MRM transitions for dioxin analysis by GC-TQMS

Nº	Compounds	RT (min)	Quan. transition	CE (eV)	Confirm. transitions	CE (eV)	Nº	Compounds	RT (min)	Quan. transition	CE (eV)	Confirm. transitions	CE (eV)
1	^{13}C-1,2,3,4-TCDD	22.77	332 > 268	-22	334 > 270	-22	19	1,2,3,4,7,8-HxCDD	29.49	390 > 264	-40	388 > 262	-40
2	^{13}C-2,3,7,8-TCDF	22.88	316 > 252	-30	318 > 254	-30						390 > 327	-25
3	2,3,7,8-TCDF	22.89	304 > 171	-55	306 > 243	-30	20	^{13}C-1,2,3,6,7,8-HxCDD	29.56	402 > 338	-22	404 > 340	-22
					304 > 206	-40	21	1,2,3,6,7,8-HxCDD	29.58	390 > 264	-40	388 > 262	-40
4	^{13}C-2,3,7,8-TCDD	23.29	332 > 268	-22	334 > 270	-22						390 > 327	-25
5	2,3,7,8-TCDD	23.30	322 > 259	-22	320 > 194	-40	22	^{13}C-1,2,3,7,8,9-HxCDD	29.87	402 > 338	-22	404 > 340	-22
					322 > 196	-40	23	1,2,3,7,8,9-HxCDD	29.87	390 > 327	-25	388 > 262	-40
6	^{13}C 1,2,3,7,8-PCDF	25.57	352 > 288	-32	350 > 286	-32						390 > 264	-40
7	1,2,3,7,8-PCDF	25.58	340 > 277	-30	338 > 205	-55	24	^{13}C-1,2,3,7,8,9-HxCDF	30.21	386 > 322	-32	388 > 324	-32
					338 > 240	-40	25	1,2,3,7,8,9-HxCDF	30.22	372 > 309	-30	374 > 311	-30
8	^{13}C-2,3,4,7,8-PCDF	26.41	352 > 288	-32	350 > 286	-32						372 > 239	-55
9	2,3,4,7,8-PCDF	26.42	340 > 277	-30	338 > 205	-55	26	^{13}C-1,2,3,4,6,7,8-HpCDF	31.76	420 > 356	-35	422 > 358	-35
					338 > 240	-40	27	1,2,3,4,6,7,8-HpCDF	31.78	408 > 345	-35	410 > 347	-35
10	^{13}C-1,2,3,7,8-PCDD	26.57	368 > 304	-22	370 > 306	-22						408 > 275	-55
11	1,2,3,7,8-PCDD	26.57	356 > 293	-25	354 > 291	-25	28	^{13}C-1,2,3,4,6,7,8-HpCDD	33.15	436 > 372	-22	438 > 374	-22
					356 > 230	-40	29	1,2,3,4,6,7,8-HpCDD	33.15	424 > 361	-25	422 > 226	-70
12	^{13}C-1,2,3,4,7,8-HxCDF	28.73	386 > 322	-32	388 > 324	-32						424 > 298	-42
13	1,2,3,4,7,8-HxCDF	28.74	374 > 311	-30	372 > 239	-55	30	^{13}C-1,2,3,4,7,8,9-HpCDF	33.87	420 > 356	-35	422 > 358	-35
					372 > 309	-30	31	1,2,3,4,7,8,9-HpCDF	33.90	408 > 345	-35	410 > 347	-35
14	^{13}C 1,2,3,6,7,8-HxCDF	28.83	386 > 322	-32	388 > 324	-32						408 > 275	-55
15	1,2,3,6,7,8-HxCDF	28.83	374 > 311	-30	372 > 239	-55	32	^{13}C-OCDD	37.77	470 > 406	-25	472 > 408	-25
					372 > 309	-30	33	OCDD	37.78	458 > 395	-25	460 > 397	-25
16	^{13}C-2,3,4,6,7,8-HxCDF	29.41	386 > 322	-32	388 > 324	-32						458 > 332	-42
17	2,3,4,6,7,8-HxCDF	29.42	374 > 311	-30	372 > 239	-55	34	OCDF	38.04	444 > 381	-30	442 > 379	-30
					372 > 309	-30						444 > 309	-60
18	^{13}C-1,2,3,4,7,8-HxCDD	29.48	402 > 338	-22	404 > 340	-22							

Analysis of dioxins by GC-TQMS

RESULTS AND DISCUSSION

The SCION instruments 8900 TQMS is capable of resolving the different classes of dioxins using the power of MRM (Figure 3). Further, the power of the 8300 GC Is evidenced by the chromatographic resolution of the HxCDF isomers, well below the 25% peak to peak separation stipulated in the regulations for quantification (Figure 4).

The inclusion of stable isotope labelled internal standards is a key part of the regulatory framework. The Compass TQ software package for automatic quantitation allows a fast and easy overview of the results. Users can set an extensive number of evaluation rules to meet any stringent analytical regulation. These rules are applicable to any compound in the method and values can be customized by compound.

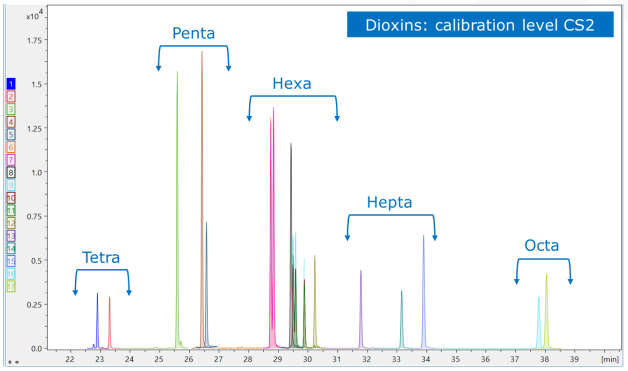

Figure 3. MRM resolution of dioxin classes

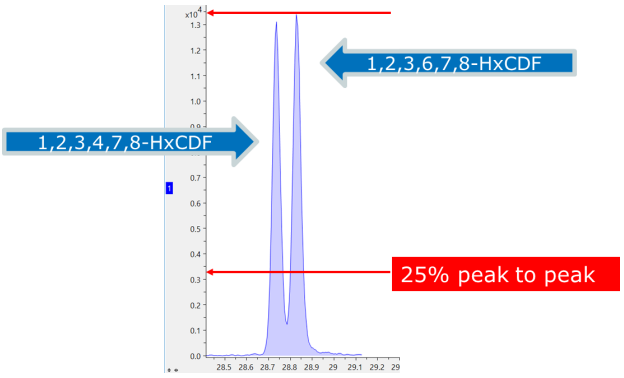

Figure 4. Chromatographic resolution of dioxin isomers

RQ	Score	RT Score	Ions Score
	+++	++	++
	+++	++	++
	+++	++	++
	+	++	+
	+	++	+
	+	++	+
	---	++	---
	---	++	---

Figure 5. Easy visualisation of scoring

All analytical performance criteria described in EU 644/2017 have been included in the method. If the reported values are higher than the setpoint, software will trigger a red flag to warn user that the compound needs to be reviewed (Figure 5).

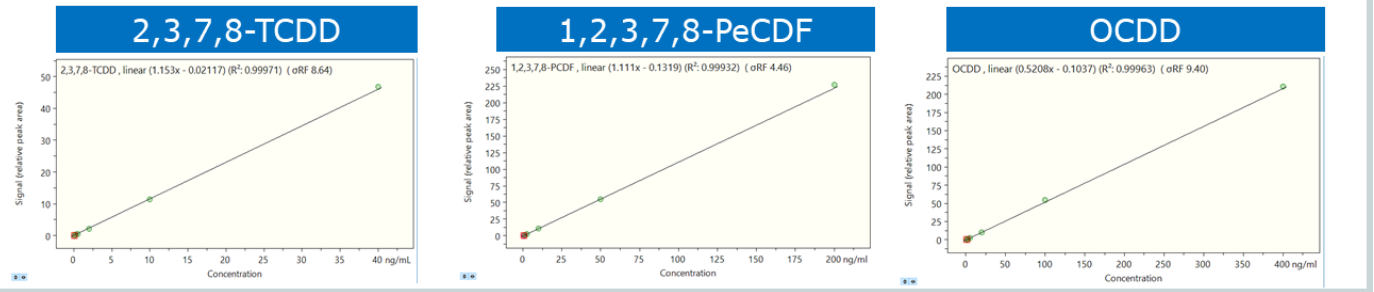

Figure 6. Linearity of response

The calibration curves (Figure 6) yielded a R^2 value > 0.99 with a RSD of < 13%. Retention time drift was low (<±0.1m) with recovery ranging from 94-107%.

To experimentally determine signal-to-noise ratio (s/n), the lowest calibration level CSL was diluted 10-fold and 20-fold with n-nonane. 1µL of each was injected. Both ions (quan+qual) were detected at LOQ (10fg) and LOD (5fg) levels.

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ANALYSIS OF FLY ASH

To validate the method, several commercially available certified reference materials were analyzed. Reference materials were split in two aliquot parts: one analyzed by 8900 GC-TQMS and the other sent to an external laboratory for analysis by GC-HRMS.

One such comparative analysis was of fly ash (Certified material BCR-615) . The data (Table 3, Figure 7) showed an accuracy of ± 20% with good corelation between the HRMS and TQMS results.

DETERMINATION OF DIOXINS IN ANIMAL FEED

To check performance of the method in real samples, an animal feed sample was doped with dioxins at very low levels. Recovery rates of stable isotope labelled internal standards was 60-120%. For every analysis, Compass TQ software checks ion ratios tolerance (RQ score) for each target compound allowing the user to have confidence in compliance with regulatory standards. Ion ratio QC was found to have a tolerance of ± 15%. Figure 8 shows the stability of the ion ratios for 2,3,7,8-TCDD once the complete batch performed for calibration standards and samples is completed.

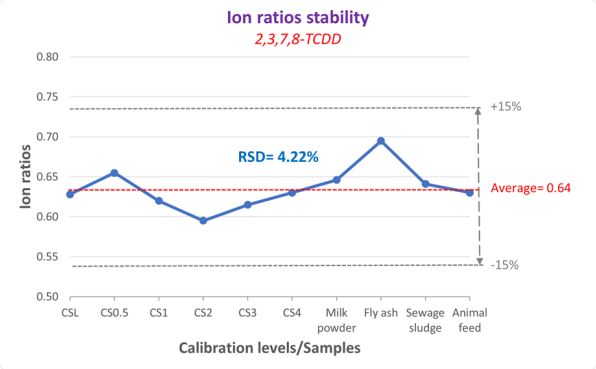

Figure 8. Stability of ion ratios across sample set

Table 3. Results of key compound quantification by HRMS and TQMS compared to certified values

Compound	Certified value *(ng/Kg)*	GC-HRMS *(ng/Kg)*	GC-MS/MS *(ng/Kg)*
2,3,7,8-TCDD	27	31.5	25.8
1,2,3,7,8-PeCDD	92	97.5	108.6
1,2,3,4,7,8-HxCDD	74	69.9	70.7
1,2,3,6,7,8-HxCDD	103	100.4	100.9
1,2,3,7,8,9-HxCDD	108	104.7	89.0
1,2,3,4,6,7,8-HpCDD	870	845.6	885.4
OCDD	1750	1907.5	2037.0
2,3,7,8-TCDF	86	93.3	85.2
1,2,3,7,8-PeCDF	176	162.5	156.7
2,3,4,7,8-PeCDF	125	129.7	111.3
1,2,3,4,7,8-HxCDF	203	185.4	189.6
1,2,3,6,7,8-HxCDF	204	204.1	182.3
1,2,3,7,8,9-HxCDF	13.3	9.6	11.2
2,3,4,6,7,8-HxCDF	130	170.6	151.4
1,2,3,4,6,7,8-HpCDF	750	703.8	723.0
1,2,3,4,7,8,9-HpCDF	61	66.4	61.1
OCDF	290	274.7	318.3

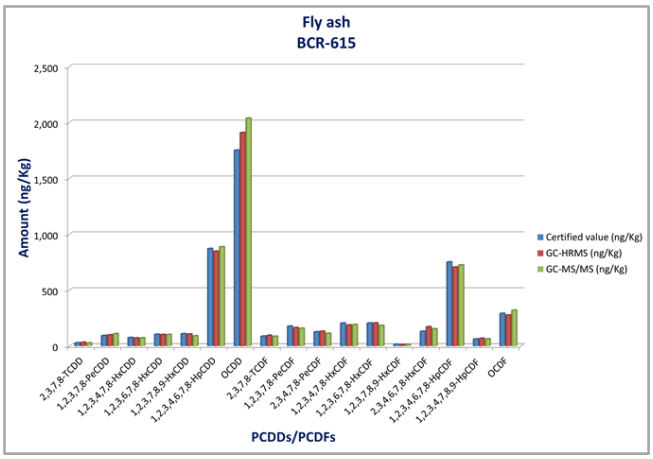

Figure 7. Corelation of HRMS, TQMS and certified values

CONCLUSION

Analytical performance obtained with 8900 GC-TQMS demonstrate that this is a powerful instrument to analyze dioxins in food, feed and environmental samples with confidence. High selectivity with specific MRM transitions in addition to extensive QC parameters allows unambiguous identification and confirmation of dioxins avoiding false positives/negatives. Superb sensitivity at lower femtogram on-column level equivalent to low pg/g -TEQ levels in original samples, injecting a small sample volume (1 µL).

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