Dioxins Analysis with New GC-MS/MS System and Software "TQ-DioK"

Imane Senoussaoui[1], Masahiro Hashimoto[1],
Thomas Lehardy[2], Anais Venisseau[2], Philippe Marchand[2]

[1] JEOL (EUROPE) SAS, France

[2] LABoratoire d'Etude des Résidus et Contaminants dans les Aliments (LABERCA), Nantes, France

The Sector Mass spectrometer (MS) combined with gas-chromatograph (GC-HRMS) was the official method used for polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) analysis due to its high selectivity and high sensitivity for those compounds. It is still mainly used in expert laboratories. The European commission regulation has been amended in 2014 (EU589/2014) and now allows the use of the GC-triple quadrupole MS system for PCDDs and PCDFs (PCDD/Fs) analysis to check the compliance of samples [1]. In this study, we have quantified PCDD/Fs using the new JEOL GC-triple quadrupole MS called JMS-TQ4000GC. The software TQ-DioK which is dedicated to dioxins analysis was used to process the data.

Introduction

Dioxins are a general term for PCDD/Fs. Their structures consist of two chlorinated rings. Many congeners differ in term of number of chlorine atoms and binding sites (**Fig. 1**).

These substances are considered as persistent organic pollutants (POPS) due to their presence in the environment and the health risks associated. A World Health Organization (WHO) study has demonstrated the health risks (carcinogenic and immunotoxic) when population are exposed to them [2]. In addition, PCDD/Fs have been regulated by the Stockholm convention on POPs in May 2001 [3].

In particular, 17 substances have to be monitored because they are regulated (7 PCDDs and 10 PCDFs). The highest toxic compound is the 2378-TeCDD (**Fig. 2**).

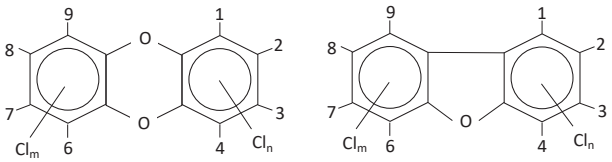
Currently, dioxins analysis can be done not only using GC-HRMS but also with GC-MS/MS according to European commission regulation (2014). GC-triple quadrupole MS is interesting in terms of handling, instrument size and operating costs.

Recently, JEOL has developed a new GC-triple quadrupole MS (JMS-TQ4000GC) and a new dedicated dioxins analysis software called TQ-DioK. In this study, we evaluated JMS-TQ4000GC with TQ-DioK using dioxins standard samples and several real samples.

Instrument JMS-TQ4000GC

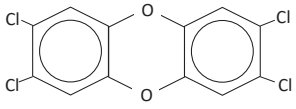
The collision cell of JMS-TQ4000GC is a unique technology called short collision cell (Patent No.: US8692191, EP2469578, US8604420) [4]. It allows to carry out high speed and high sensitivity quantitative measurements. The switch between "high speed" and "high sensitivity" modes is easy to do, by changing accumulation time for ions in the short collision cell. Besides, new compounds can be added on the SRM data file using the SRM optimization tool. Therefore, dioxins data obtained can be analyzed smoothly.

Fig. 1 The structures of PCDDs(A) and PCDFs(B)



(A) PCDDs (B) PCDFs

Fig. 2 The structure of 2378-TeCDD



〉〉 Espace Claude Monet, 1 Allee de Giverny 78290, Croissy-sur-Seine, France | E-mail: hashimoto@jeol.fr.

TQ-DioK

JEOL has introduced a dedicated GC-triple quadrupole MS software specialized in MS/MS data analysis: TQ-DioK. It is a quantitative analysis software dedicated to dioxins analysis. The data processing is very special, because dioxins compounds consist of many isomers with the same basic composition and an isotopic dilution technique is required for their determination. JEOL already provides a quantitative analysis software for dioxins analysis (DioK) dedicated to the GC-Sector MS. Its features were ported to TQ-DioK. Therefore, dioxins data obtained can be analyzed smoothly.

The TQ-DioK analysis window is a "three-layer chromatogram window". On the upper part, the chromatogram of native compounds is represented, and on the lower part of the window we can find the chromatogram of internal standard (IS) compounds and between the 2 chromatograms the calculated retention time (RT).

As a result, the peak can be identified and assigned to a specified isomer by comparing the peak position between the chromatogram of native compounds, the chromatogram of IS compounds and calculated RT of compounds. Since the RT axis always synchronizes with each isomer, it is easy to check the chromatogram of each isomer, and identify the peak status by an associated coloring system **(Fig. 3)**.

Experimental
Standard sample measurement Samples

The standard PCDD/Fs solutions (DF-IS-A, DF-ST-A and DF-LCS-C from *WELLINGTON Laboratories* (CANADA)) were used for the measurement. Then, the range of concentrations for calibration curve was prepared from 0.025 to 1 pg/µL (OCDD and OCDF: 0.05 - 2 pg/µL) (**Table 1**).

GC-MS/MS measurement conditions

Table 2 shows the GC-MS/MS measurement conditions. A split/splitless inlet was used, and nitrogen gas was applied as collision gas. **Table 3** shows the precursor ion, product ion and collision energy (CE). Two specific precursor ions from each non-labeled compound and labeled compound were set.

Results for standard samples

The GC-MS/MS method was validated in term of chromatographic separation, sensitivity and RRF. Some criteria, especially separation and RRF have to comply with EU commission regulation.

Fig. 3 Analysis view of TQ-DioK

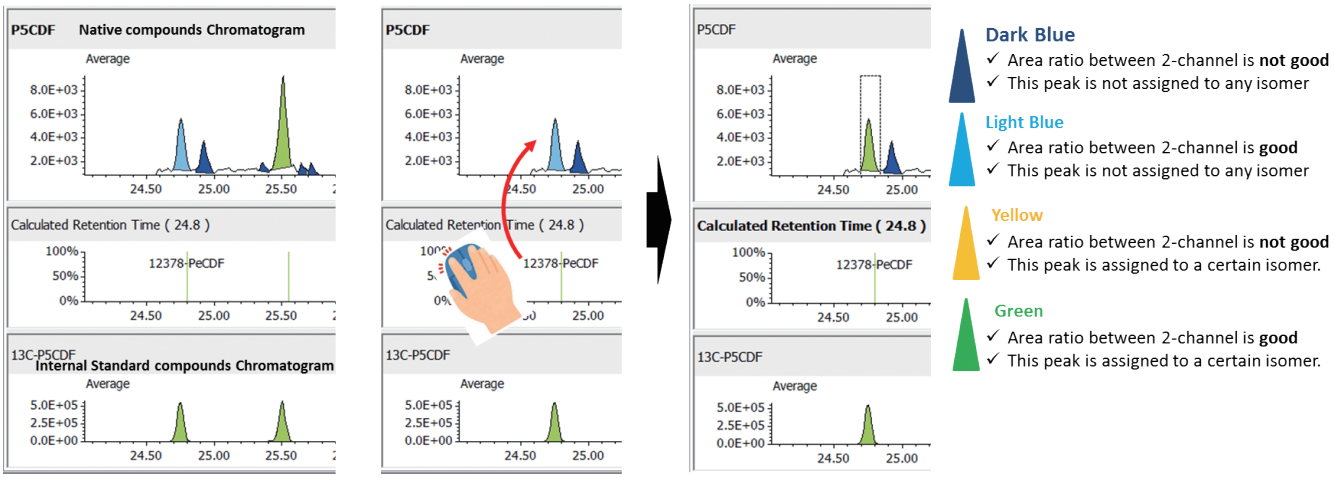


Table 1 Concentrations of each calibration point

PCDD/Fs	Concentration ^{12}C (pg/µL)	Concentration ^{13}C (pg/µL)
Cal. 1	0.025(OCDD and OCDF 0.05)	1.25(OCDD and OCDF 2.5)
Cal. 2	0.05(OCDD and OCDF 0.1)	1.25(OCDD and OCDF 2.5)
Cal. 3	0.1(OCDD and OCDF 0.2)	1.25(OCDD and OCDF 2.5)
Cal. 4	0.25(OCDD and OCDF 0.5)	1.25(OCDD and OCDF 2.5)
Cal. 5	0.5(OCDD and OCDF 1.0)	1.25(OCDD and OCDF 2.5)
Cal. 6	1.0(OCDD and OCDF 2.0)	1.25(OCDD and OCDF 2.5)

Table 2 Measurement conditions

[GC]	
Inj. volume:	2 µL
Inlet type:	Split/Splitless
Inj. mode:	Splitless
	(Purge time 1 min, Purge flow 20 mL/min)
Inlet temp.:	280 °C
Column flow:	1 mL/min (Constant flow)
GC column:	DB- 5 MS (60 m × 0.25 mm, 0.25 µm)
Oven temp.:	120 °C (3 min) → 50 °C/min → 200 °C (0 min) → 4 °C/min → 300 °C (5 min) → 40 °C/min → 325 °C (5 min)

[MS]	
MS:	JMS-TQ4000GC
Ionization:	EI+
Acquisition mode:	High sensitivity mode
IS temp.:	280 °C
ITF temp:	280 °C

Table 3 Precursor ion, product ion and CE

No.	Compound name	Group name	Precursor ion	Product ion	Precursor ion	Product ion	CE(V)
1	13C-2378-TeCDF	13C-T4CDF	315.9	252	317.9	254	25
2	2378-TeCDF	T4CDF	303.9	240.9	305.9	242.9	
3	13C-1234-TeCDD	13C-T4CDD	333.9	270	331.9	268	25
4	13C-2378-TeCDD	13C-T4CDD	331.9	268	333.9	270	
5	2378-TeCDD	T4CDD	321.9	258.9	319.9	256.9	
6	13C-12378-PeCDF	13C-P5CDF	351.9	287.9	353.9	289.9	30
7	12378-PeCDF	P5CDF	339.9	276.9	341.9	278.9	
8	13C-23478-PeCDF	13C-P5CDF	351.9	287.9	353.9	289.9	
9	23478-PeCDF	P5CDF	339.9	276.9	341.9	278.9	
10	13C-12378-PeCDD	13C-P5CDD	367.9	303.9	369.9	305.9	25
11	12378-PeCDD	P5CDD	355.9	292.9	357.9	294.9	
12	13C-123478-HxCDF	13C-H6CDF	385.9	321.9	387.9	323.9	30
13	123478-HxCDF	H6CDF	373.8	310.9	375.8	312.9	
14	13C-123678-HxCDF	13C-H6CDF	385.9	321.9	387.9	323.9	
15	123678-HxCDF	H6CDF	373.8	310.9	375.8	312.9	
16	13C-234678-HxCDF	13C-H6CDF	385.9	321.9	387.9	323.9	
17	234678-HxCDF	H6CDF	373.8	310.9	375.8	312.9	
18	13C-123478-HxCDD	13C-H6CDD	401.9	337.9	403.9	339.9	25
19	123478-HxCDD	H6CDD	389.8	326.9	391.8	328.9	
20	13C-123678-HxCDD	13C-H6CDD	401.9	337.9	403.9	339.9	
21	123678-HxCDD	H6CDD	389.8	326.9	391.8	328.9	
22	13C-123789-HxCDD	13C-H6CDD	401.9	337.9	403.9	339.9	
23	123789-HxCDD	H6CDD	389.8	326.9	391.8	328.9	
24	13C-123789-HxCDF	13C-H6CDF	385.9	321.9	387.9	323.9	30
25	123789-HxCDF	H6CDF	373.8	310.9	375.8	312.9	
26	13C-1234678-HpCDF	13C-H7CDF	419.8	355.9	421.8	357.9	30
27	1234678-HeCDF	H7CDF	407.8	344.8	409.8	346.8	
28	13C-1234678-HpCDD	13C-H7CDD	435.8	371.9	437.8	373.9	30
29	1234678-HpCDD	H7CDD	423.8	360.8	425.8	362.8	
30	13C-1234789-HpCDF	13C-H7CDF	419.8	355.9	421.8	357.9	30
31	1234789-HpCDF	H7CDF	407.8	344.8	409.8	346.8	
32	13C-12346789-OCDD	13C-O8CDD	471.8	407.8	469.8	405.8	30
33	12346789-OCDD	O8CDD	459.7	396.8	457.7	394.8	
34	13C-12346789-OCDF	13C-OCDF	455.8	391.8	453.8	389.8	30
35	12346789-OCDF	OCDF	443.8	380.8	441.8	378.8	

Separation

123478-HxCDF and 123678-HxCDF peaks were separated perfectly by using GC-MS/MS method (**Fig. 4**). Indeed EU commission regulation in force, allows a 25% valley between these 2 peaks.

Sensitivity

All target compounds in the lowest calibration point were detected (**Fig. 5**). In addition, the lowest calibration point was measured 8 times for the instrument detection limit (IDL) determination. Then, IDL was calculated using 2378-TeCDD. As a result, IDL value was equal to 4 fg (**Fig. 6**).

RRF

Fig. 7 shows calibration curves of 2378-TeCDD and 2378-TeCDF. Indeed **Table 4** shows the result of relative standard deviation (RSD) of relative response factor (RRF) for the lowest calibration point, average of RRF, RSD of RRF and limit of quantification (LOQ). RSD of RRF obtained with the lowest calibration point was between 2.2 and 12.9%. Average of RRF value was between 0.94 and 1.14.

According to EU regulation, RSD for RRF has to be under 15%. Here, RSD of RRF from the average of all calibration points was within 9.1%. Regarding LOQ, its value was calculated by signal-to-noise (S/N = 3) of the lowest calibration point. As a result, obtained LOQ value was between 0.08 and 1.69 fg/µL.

Food samples Measurement Extraction and purification

The collected samples were freeze-dried or only dried and then grinded to make a homogenous powder. Subsequently, dioxins were extracted from the powder sample using the Büchi "SpeedExtractor E-914" automated instrument. The extracted samples were purified using the "MIURA GO-4 HT" system. Finally, the purified samples were measured by both GC-HRMS and GC-MS/MS instruments and the results were compared.

Result
Ratio of selected two transition product ions

The tolerance of ratio of the selected two transitions product ions for average value or calculated value should be < ±15% according EU regulation.

Average value of each compound was calculated using all calibration points. Those ratios for each compound were within ±15% of average value (**Fig. 8**).

Comparison of GC-HRMS and GC-MS/MS systems

Grass, Egg and Pork fat were measured by both GC-HRMS and GC-MS/MS systems. Toxic Equivalent Quantity (TEQ) was calculated using Toxic Equivalency Factors (TEF) based on WHO 2005 [5]. **Fig. 9** shows the comparison data of Grass, Egg and Pork fat. The TEQ calculated for each compound by GC-MS/MS was similar to GC-HRMS result. By consequence the difference between the Dioxin OMS-TEQ in ng/kg of matrix calculated by GC-HRMS and the GC-MS/MS TEQ(dioxins) was within 20%.

Fig. 4 Separation of 123478-HxCDF and 123678 HxCDF

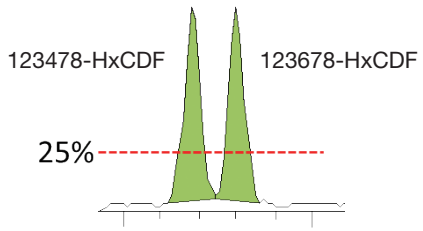


Fig. 5 Average SRM chromatograms of PCDDs(A) and PCDFs(B) in calibration point 1 (0.05 pg injected)

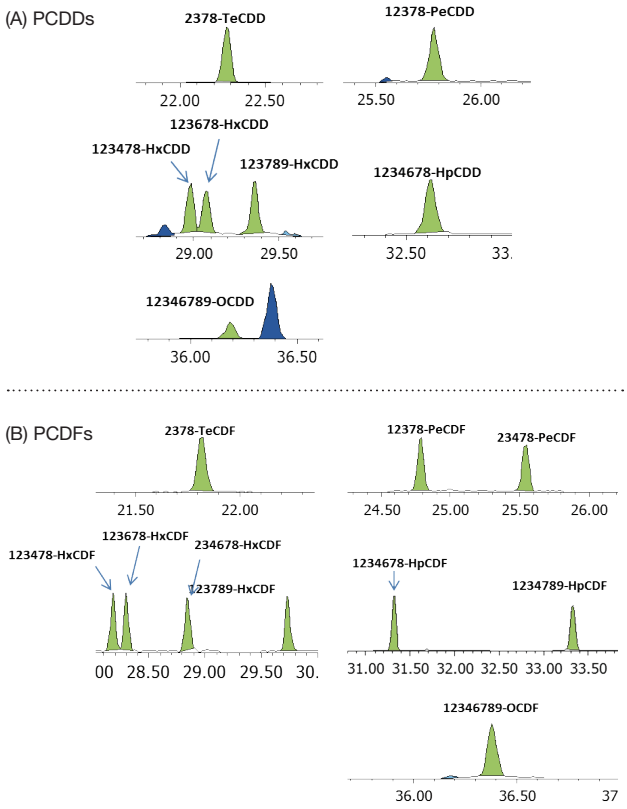
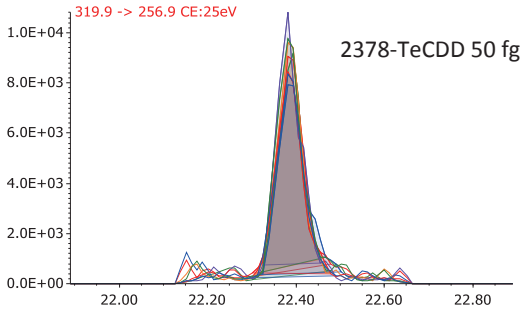


Fig. 6 IDL data by 2378-TeCDD



Average	Standard deviation	CV%	IDL [fg]
69077.5	1847.6	2.7	4.0

Fig. 7 Calibration curve of 2378-TeCDD(Left) and 2378-TeCDF(Right)

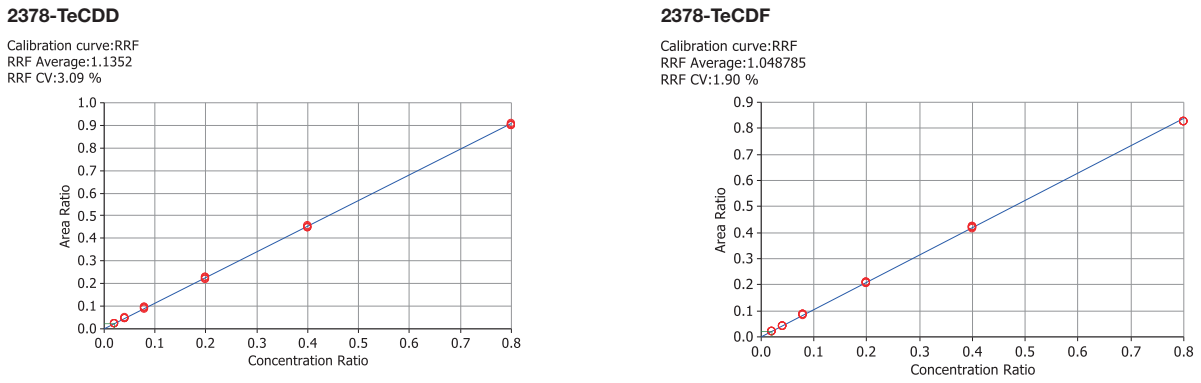


Table 4 Results of RSD of RRF for the lowest calibration point, average of RRF, RSD of RRF and LOQ

Compound	Lowest calibration point (pg/μL)	RSD of RRF by the lowest calibration point (%)	Average of RRF	RSD of RRF (%)	LOQ (fg/μL)
PCDFs					
2378-TeCDF	0.025	2.2	1.04	1.8	0.13
12378-PeCDF	0.025	2.3	1.04	2.4	0.45
23478-PeCDF	0.025	4.0	1.05	3.9	0.68
123478-HxCDF	0.025	3.7	1.00	2.9	0.40
123678-HxCDF	0.025	12.9	1.00	6.1	0.36
234678-HxCDF	0.025	8.7	1.03	5.6	0.42
123789-HxCDF	0.025	5.3	1.01	3.5	0.35
1234678-HeCDF	0.025	4.2	1.07	4	0.08
1234789-HpCDF	0.025	5.5	1.04	4	0.08
12346789-OCDF	0.050	4.1	0.95	9.1	1.19
PCDDs					
2378-TeCDD	0.025	3.6	1.14	3.1	0.41
12378-PeCDD	0.025	5.4	1.00	4.1	0.21
123478-HxCDD	0.025	12.5	1.00	6.4	1.46
123678-HxCDD	0.025	12.5	0.94	8.6	1.69
123789-HxCDD	0.025	5.9	1.03	4.5	1.11
1234678-HpCDD	0.025	8.9	1.10	5.8	0.22
12346789-OCDD	0.050	8.3	1.03	9	0.90

Fig. 8 Ratios of selected two transition product ions

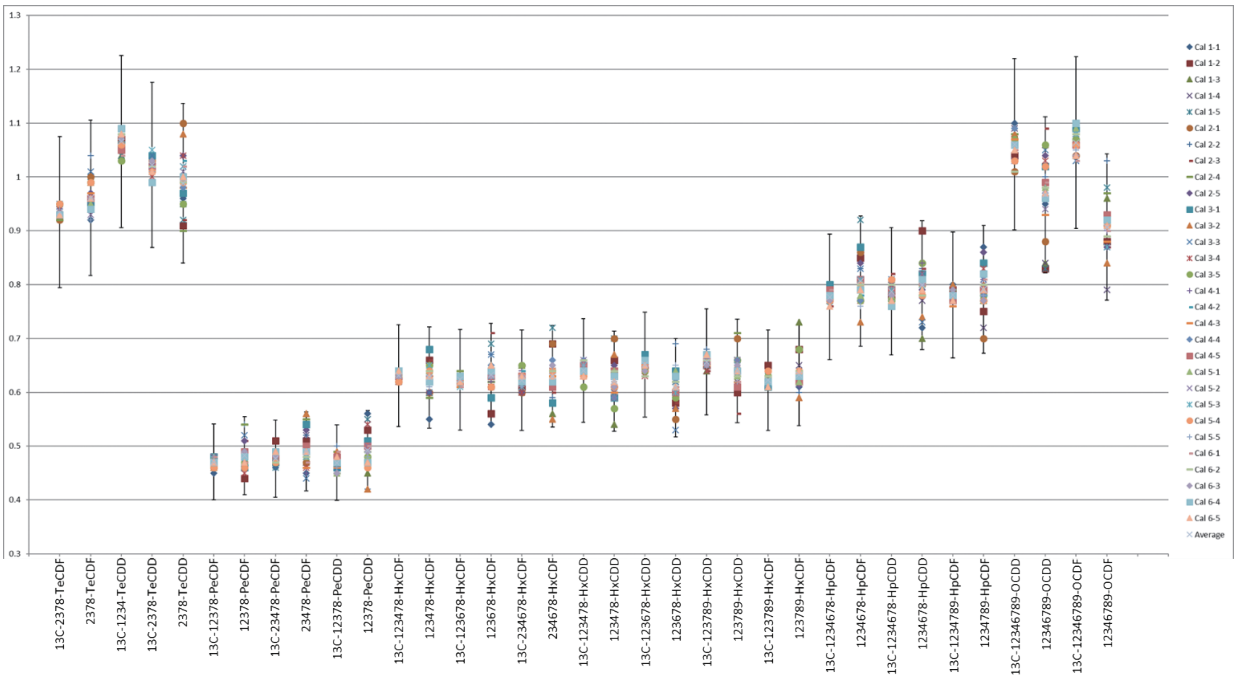
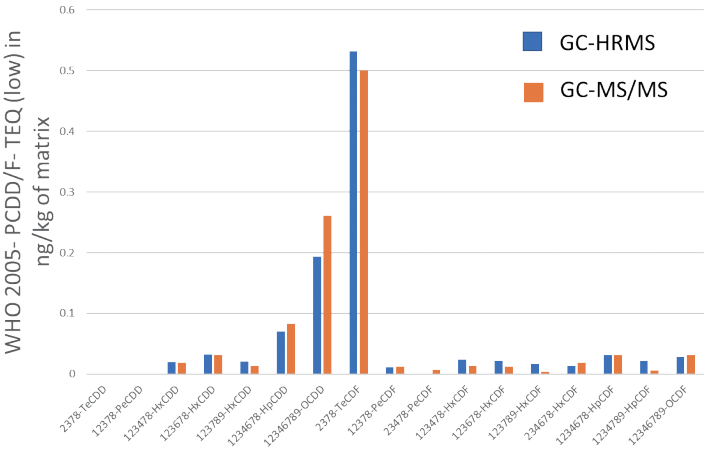


Fig. 9 Comparison data of GC-HRMS and GC-MS/MS

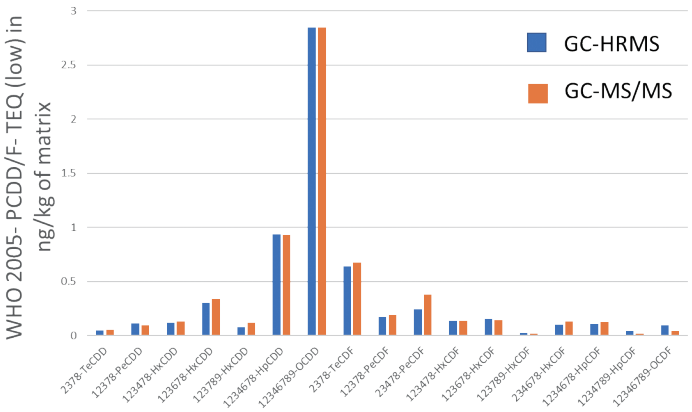
(A) Grass

	GC-HRMS	GC-MS/MS
2378-TeCDD	-	-
12378-PeCDD	-	-
123478-HxCDD	0.020	0.019
123678-HxCDD	0.033	0.032
123789-HxCDD	0.021	0.013
1234678-HpCDD	0.070	0.083
12346789-OCDD	0.193	0.262
2378-TeCDF	0.532	0.500
12378-PeCDF	0.012	0.012
23478-PeCDF	-	0.008
123478-HxCDF	0.025	0.013
123678-HxCDF	0.022	0.013
123789-HxCDF	0.017	0.004
234678-HxCDF	0.014	0.019
1234678-HpCDF	0.031	0.032
1234789-HpCDF	0.022	0.007
12346789-OCDF	0.028	0.032
OMS2005- Dioxin- TEQ (low) in ng/kg of matrix	0.070	0.065



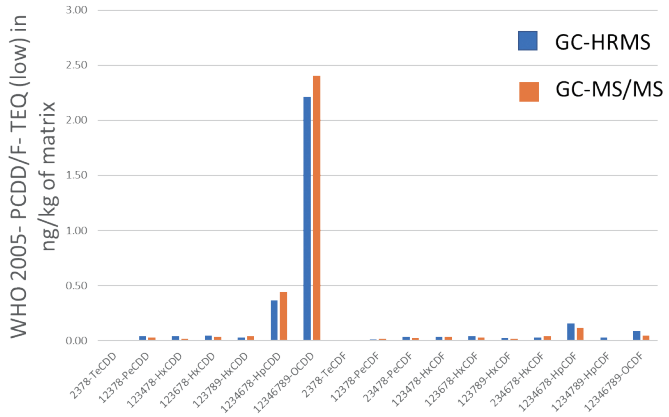
(B) Egg

	GC-HRMS	GC-MS/MS
2378-TeCDD	0.045	0.051
12378-PeCDD	0.112	0.097
123478-HxCDD	0.121	0.133
123678-HxCDD	0.300	0.340
123789-HxCDD	0.079	0.117
1234678-HpCDD	0.936	0.931
12346789-OCDD	2.845	2.848
2378-TeCDF	0.641	0.673
12378-PeCDF	0.173	0.191
23478-PeCDF	0.245	0.381
123478-HxCDF	0.135	0.135
123678-HxCDF	0.156	0.141
123789-HxCDF	0.026	0.021
234678-HxCDF	0.103	0.131
1234678-HpCDF	0.108	0.125
1234789-HpCDF	0.045	0.019
12346789-OCDF	0.093	0.040
OMS2005- Dioxin- TEQ (low) in ng/kg of matrix	0.404	0.449



(C) Pork fat

	GC-HRMS	GC-MS/MS
2378-TeCDD	-	0.001
12378-PeCDD	0.042	0.032
123478-HxCDD	0.041	0.020
123678-HxCDD	0.048	0.038
123789-HxCDD	0.030	0.040
1234678-HpCDD	0.368	0.442
12346789-OCDD	2.216	2.406
2378-TeCDF	0.000	0.009
12378-PeCDF	0.014	0.016
23478-PeCDF	0.036	0.022
123478-HxCDF	0.037	0.036
123678-HxCDF	0.042	0.028
123789-HxCDF	0.026	0.019
234678-HxCDF	0.031	0.042
1234678-HpCDF	0.157	0.119
1234789-HpCDF	0.033	0.004
12346789-OCDF	0.091	0.050
OMS2005- Dioxin- TEQ (low) in ng/kg of matrix	0.084	0.069



Conclusion

The JMS-TQ4000GC was evaluated for dioxins quantification. The results have shown that the JMS-TQ4000GC instrument complies with the EU commission regulation. In addition, it is easy to identify the chromatogram of each isomer and to confirm the peak status using TQ-DioK software. These results show that the JMS-TQ4000GC system associated with TQ-DioK software are a powerful tool for dioxins analysis.

Acknowledgement

All measurements and evaluation about the basic performances of JMS-TQ4000GC were organized and tested by the LABoratoire d'Etude des Résidus et Contaminants dans les Aliments (LABERCA), Nantes, France.

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