Application Note

Food & Agriculture

Dioxins Analysis in Food and Feed by Intuvo 9000/7010 GC-QQQ System

Authors

Rebecca Veeneman and
Anna Cali
Agilent Technologies, Inc.

Abstract

Dioxins belong to the so-called “dirty dozen”—a group of chemicals known as persistent organic pollutants (POPs). Dioxins and furans are the more commonly used names that have been associated with polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), with the most toxic being 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Dioxins and furans are characterized by the chemical backbone and the number of chlorines substituted on the backbone.

Introduction

Dioxins and furans are persistent environmental pollutants that have been extensively studied and shown to bioaccumulate in the environment.

Historically, high resolution mass spectrometry (HRMS) was needed to confirm and quantify trace levels of dioxins, as in EPA Method 1613B.[1] However, as of June 2014, the European Union (EU) has instituted regulation (709/2014) governing the levels of PCDDs, PCDFs, dioxin-like PCBs, and non-dioxin-like (NDL) PCBs in food and feed that enables the use of gas chromatography/tandem quad mass spectrometry (GC/TQ MS) systems in confirmatory testing for compliance with EUMLs.[2] This change was due to the realization that triple quadrupole mass spectrometers could provide performance similar to that seen with HRMS systems, and was previously demonstrated on an Agilent 7890B/7000C GC/TQ MS system.[3]

With the introduction of the Intuvo 9000 GC and its direct heating technology, new possibilities for a faster separation can be explored for this critical analysis.

Experimental

The evaluation of performance was demonstrated using an Agilent Intuvo 9000 GC configured with a split/splitless inlet, coupled to an Agilent 7010 Series triple quadrupole using the high efficiency ion source (HES). Two methods were developed to meet various needs. The first method has a run time of 52.5 minutes, and uses a temperature program that may also be applied to traditional GC ovens. A faster method was developed, taking advantage of the direct column heating available on the Intuvo 9000 GC. The instrument conditions for both methods are listed in Table 1.

Sample preparation

The most frequently used methods for the determination of PCDD/PCDF and DL-PCB in foodstuffs and animal feed combine fat extraction (Soxhlet) with cleanup steps using different column chromatographies (silica gel coated with sulphuric acid, florisil, alumina, and active carbon).

Manual dioxin sample preparation is tedious and comprehensive; multicolumn automated systems have been made to automate dioxin sample extraction to reduce analysis times and attempt to reduce costs according to the 1613 method.

Table 1. Intuvo 9000 GC System parameters.

Parameter	52.5 Minute Method	31.12 Minute Method
GC Conditions		
Injection Port Liner	p/n 5181-3315 and 5190-2293	
Injection Volume	1 µL	1 and 0.5 µL
Pulsed Splitless	60 psi for 0.6 minutes; 50 mL/min at 0.8 minutes	
Column	DB-5MS UI (60 m × 250 µm, 0.25 µm)	
Column Flow Rate	1.6 mL/min	1.6 mL/min
Oven	130 °C (2 minutes) 20 °C/min to 220 °C (15 minutes) 5 °C/min to 235 °C (7 minutes) 5 °C/min to 330 °C (2 minutes)	130 °C (1 minute) 100 °C/min to 200 °C (6 minutes) 7.5 °C/min to 235 °C (4 minutes) 20 °C/min to 310 °C (11 minutes)
Guard Chip	310 °C	310 °C
Bus	325 °C	325 °C
MS Conditions		
Operation Mode	Electron ionization (EI), Multiple Reaction Monitoring (MRM)	
Transfer Line Temperature	330 °C	330 °C
Source Temperature	280 °C	280 °C
Quadrupole Temperature	150 °C	150 °C

Results and discussion

A standard containing Native PCDDs, Native PCDFs, syringe standards, and ^{13}C-labeled internal standards (Table 2) was evaluated with both the 52.5-minute and 31.12-minute method. Chromatograms displaying the separations under both sets of conditions are shown in Figures 1 and 2.

Calibration curves were analyzed for regression analysis against both separation methods. For the original, longer method, a six-point calibration curve was analyzed, with concentration details provided in Table 3. Correlation of determination was used as an evaluation of the linearity, and the resulting values are shown in Table 4.

Table 2. Compounds tested.

Compound	Type
2378-TCDD	Native
2378-TCDF	Native
12378-PCDD	Native
12378-PCDF	Native
23478-PCDF	Native
123478-HxCDD	Native
123478-HxCDF	Native
123678-HxCDD	Native
123678-HxCDF	Native
123789-HxCDD	Native
123789-HxCDF	Native
234678-HxCDF	Native
1234678-HpCDD	Native
1234678-HpCDF	Native
1234789-HpCDF	Native
OCDD	Native
OCDF	Native
1234-TCDD-ISS	Syringe Standard

Compound	Type
2378-TCDD-LCS*	ISTD
2378-TCDF-LCS*	ISTD
12378-PCDD-LCS*	ISTD
12378-PCDF-LCS*	ISTD
23478-PCDF-LCS*	ISTD
123478-HxCDD-LCS*	ISTD
123478-HxCDF-LCS*	ISTD
123678-HxCDD-LCS*	ISTD
123678-HxCDF-LCS*	ISTD
123789-HxCDD-ISS	Syringe Standard
123789-HxCDF-LCS*	ISTD
234678-HxCDF-LCS*	ISTD
1234678-HpCDD-LCS*	ISTD
1234678-HpCDF-LCS*	ISTD
1234789-HpCDF-LCS*	ISTD
OCDD-LCS*	ISTD
OCDF-LCS*	ISTD

LCS = Labeled Compound Standard
ISS = Internal Syringe Standard

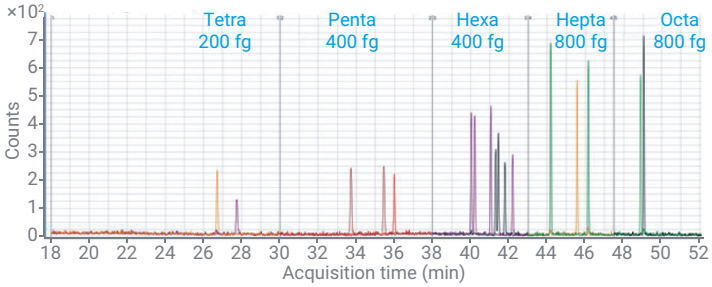

Figure 1. 52.5-minute method, 1 µL injection volume, level 2 standard.

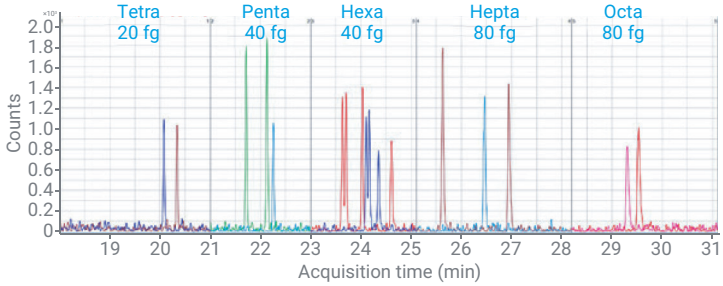

Figure 2. 31.12-minute method, 1 µL injection volume.

Table 3. Calibration curve for the 52.5-minute method; 1 µL injection volume.

fg/uL	Tetra	Penta	Hexa	Hepta	Octa
L1	50	100	100	200	200
L2	200	400	400	800	800
L3	1000	2000	2000	4000	4000
L4	4000	8000	8000	16000	16000
L5	20000	40000	40000	80000	80000
L6	80000	160000	160000	320000	32000

Table 4. Compound specific coefficient of determination results for the 52.5-minute method.

Analyte	R^2
2378-TCDD	0.99997
12378-PCDD	0.99983
123478-HxCDD	0.99978
123678-HxCDD	0.99998
123789-HxCDD	0.99928
1234678-HpCDD	0.99998
OCDD	0.99999
2378-TCDF	0.99991
12378-PCDF	0.99987

Analyte	R^2
23478-PCDF	0.99991
123478-HxCDF	0.99999
123678-HxCDF	0.99994
234678-HxCDF	0.99999
123789-HxCDF	0.99999
1234678-HpCDF	0.99996
1234789-HpCDF	0.99999
OCDF	0.99993

A more detailed statistical evaluation of the data is provided in Table 5. In this table, the transitions used for each target compound is provided, along with instrument-specific limits and signal-to-noise values. The instrumental limit of quantitation (iLOQ) was calculated using 10 replicate injections at the lowest calibration point.

Method 2: Faster temperature program

Using the accelerated temperature program shown in Table 1, a second calibration curve was generated using a 1 µL injection of prepared standards shown in Table 6.

In similar fashion to the longer method evaluation, the calibration standards were run in sequence, then processed using a data system where correlations of determination were generated for each compound. The transitions applied to each compound were kept consistent with those provided in Table 5. Table 7 displays the correlation values.

Table 5. RT, MRM transitions, LOQ, and LOD.

Name	RT	Transition	Conc. RSD	MDL (fg/µL)	LOQ	LOD	Noise	S/N
2378-TCDF	26.64	303.9 → 240.9	2.6	3.8184	13.5334	4.06	1.92	18.54
2378-TCDD	27.835	319.9 → 256.9	3.9	5.7684	20.445	6.1335	1.61	14.62
12378-PCDF	33.638	339.8 → 277.0	4.9	14.8674	52.6945	15.8083	2.02	17.6
23478-PCDF	35.359	339.8 → 277.0	6.4	20.1087	71.271	21.3813	1.99	21.43
12378-PCDD	35.921	355.9 → 292.9	6.5	20.0992	71.2375	21.3713	1.48	19.69
123478-HxCDF	39.96	373.8 → 310.9	6.4	19.4782	69.0366	20.711	1.55	24.45
123678-HxCDF	40.141	373.8 → 310.9	4.6	14.2778	50.6047	15.1814	1.55	25.08
234678-HxCDF	41.148	373.8 → 310.9	3.4	10.0481	35.6132	10.684	1.96	22.2
123478-HxCDD	41.237	389.8 → 326.9	4.2	12.5373	44.4357	13.3307	1.51	23.82
123678-HxCDD	41.533	389.8 → 326.9	8.1	24.7315	87.6558	26.2967	1.45	25.78
123789-HxCDD	41.737	389.8 → 326.9	7.3	21.5291	76.3055	22.8916	1.55	26.1
123789-HxCDF	42.135	373.8 → 310.9	4.9	14.8626	52.6774	15.8032	1.39	30.68
1234678-HpCDF	44.133	407.8 → 344.8	4	23.4349	83.06	24.918	1.76	46.85
1234678-HpCDD	45.674	423.8 → 360.8	3.5	20.7641	73.594	22.0782	1.9	34.19
1234789-HpCDF	46.105	407.8 → 344.8	8.7	52.986	187.7979	56.3394	1.52	52.61
OCDD	48.83	457.7 → 394.8	2.8	16.7276	59.2874	17.7862	1.33	44.22
OCDF	48.995	441.7 → 378.8	2.5	14.9213	52.8855	15.8656	1.35	49.63

Table 6. Calibration curve for the 32.12-minute method; 1 µL injection volume.

fg/uL	Tetra	Penta	Hexa	Hepta	Octa
SC1	20	40	40	80	80
SC2	80	40	160	320	320
SC3	400	200	800	1600	1600
SC4	1600	800	3200	6400	6400
SC5	8000	4000	16000	32000	32000
SC6	32000	16000	64000	128000	128000

Table 7. Correlations of determination for the accelerated method.

Analyte	R^2
2378-TCDD	0.99995
12378-PCDD	0.99984
123478-HxCDD	0.99975
123678-HxCDD	0.9999
123789-HxCDD	0.99931
1234678-HpCDD	0.99998
OCDD	0.99999
2378-TCDF	0.99992
12378-PCDF	0.99985
23478-PCDF	0.99991
123478-HxCDF	0.99999
123678-HxCDF	0.99993
234678-HxCDF	0.99979
123789-HxCDF	0.99998
1234678-HpCDF	0.99997
1234789-HpCDF	0.99999
OCDF	0.99993

Advances in mass spectrometry, most notably the HES available on the 7010 TQ, shows promise for lower detection, and subsequently smaller sample volumes. To evaluate the impact of the HES on the accelerated separation method, a smaller injection volume was added, providing data for both 1 and 0.5 µL sample injections at the lowest calibration point. Table 8 shows the comparison of injection volume, using RSD% for seven replicates of the SC1 standard listed in Table 6.

Conclusion

Regulatory agencies are recognizing the ability of tandem quadrupole systems to effectively identify and quantify concerning dioxin and furan compounds, as shown by European Union Commission Regulations No. 589/2014 and No. 709/2014, which adds GC/TQ as an option for confirmatory analysis of certain foodstuffs. Compared to high resolution MS systems (HRAM), GC/TQ is a more affordable system to analyze potentially contaminated samples. Advances in MS sources, most notably the HES option evaluated in this work, allow for improved detection with smaller injection volumes, without a loss in data confidence or precision. Likewise, advances in gas chromatography, such as the Intuvo 9000 system, accelerate separations with direct heating capabilities, which opens a pathway for rapid screening and faster throughput.

In this work, two separation methods were developed to demonstrate performance using faster separations and smaller sample volumes. These outcomes are possible due to the partnership between the innovative technology embedded in the Intuvo 9000 GC and the robust performance of the 7010 TQ Mass spectrometer.

Table 8. Comparison statistics using two different injection volumes (1 and 0.5 µL) on the accelerated separation of targeted dioxins and furans.

Name	Transition	1 µL RSD	0.5 µL RSD
2378-TCDF-LCS-REC	315.8 → 252.0	2.3	3.2
2378-TCDD	319.9 → 256.9	4.0	3.9
2378-TCDD-LCS-REC	331.8 → 268.0	3.2	3.7
2378-TCDF	303.9 → 240.9	5.3	6.1
23478-PCDF	339.8 → 277.0	5.9	6.8
12378-PCDF-LCS-REC	351.9 → 287.9	2.0	2.1
23478-PCDF-LCS-REC	351.9 → 287.9	1.3	2.1
12378-PCDD	355.9 → 292.9	8.0	7.2
12378-PCDD-LCS-REC	367.8 → 304.0	3.6	3.4
12378-PCDF	339.8 → 277.0	5.2	5.4
123478-HxCDF-LCS-REC	385.8 → 322.0	2.0	3.1
123478-HxCDD	389.8 → 326.9	4.1	7.1
123678-HxCDF-LCS-REC	385.8 → 322.0	1.6	4.5
123478-HxCDF	373.8 → 310.9	6.8	7.0
123678-HxCDF	373.8 → 310.9	7.6	8.5
1234678-HpCDD	423.8 → 360.8	6.8	7.0
234678-HxCDF-LCS-REC	385.8 → 322.0	0.6	3.7
234678-HxCDF	373.8 → 310.9	7.5	7.5
1234678-HpCDF	407.8 → 344.8	8.5	8.7
123789-HxCDF-LCS-REC	385.8 → 322.0	3.6	2.4
OCDD	457.7 → 394.8	7.7	7.7
123789-HxCDF	373.8 → 310.9	5.4	5.4
123478-HxCDD-LCS-REC	401.8 → 338.0	3.8	2.3
OCDF	441.7 → 378.8	8.7	8.5
123678-HxCDD-LCS-REC	401.8 → 338.0	3.6	4.6
123678-HxCDD	389.8 → 326.9	4.5	5.2
123789-HxCDD	389.8 → 326.9	7.8	7.7
1234678-HpCDF-LCS-REC	419.8 → 356.0	1.9	1.0
1234678-HpCDD-LCS-REC	435.8 → 372.0	2.5	4.4
1234789-HpCDF-LCS-REC	419.8 → 356.0	2.6	3.0
1234789-HpCDF	407.8 → 344.8	6.0	5.6
OCDD-LCS-REC	469.7 → 405.8	1.8	3.5
OCDF-LCS-REC	453.7 → 389.8	2.7	4.0

References

1. US EPA Method 1613 "Tetra- through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS", U.S. EPA Office of Water Engineering and Analysis Division (4303) 401 M Street S.W. Washington, D.C. 20460. **1994**.
2. Commission Regulation (EU) No 709/2014 of 20 June 2014 amending Regulation (EC) No 152/2009 as regards the determination of the levels of dioxins and polychlo-rinated biphenyls, Offic. *J. Eur. Commun.* **2014**, *L188*, 1–18.
3. Riener, J. Validation of a Confirmatory GC/MS/MS Method for Dioxins and Dioxin-like PCBs to Meet the Requirements of EU Regulation 709/2014. *Agilent Technologies Application Note*, publication number 5991-6590EN, **2016**.

www.agilent.com/chem

DE.6419560185

Printed in the USA, July 1, 2020
5991-9399EN

Agilent
Trusted Answers