Evaluation of a GC-MS/MS for PCDD/F Analysis Incorporating Calibration Gas

Kjell Hope1*, Jose Del Pozo1, Patrick Pond1, Amit Gujar 2, Adam Ladak3

¹ Pacific Rim Laboratories Inc, 19575 55a Ave, Surrey V3S8P8, Canada, kjell@pacificrimlabs.com

² Thermo Fisher Scientific, 2215 Grand Ave. Pkwy., Austin, TX 78717 USA

³ Thermo Fisher Scientific, Macclesfield, SK11 8BJ, UK

1 Introduction

Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans have been analyzed by combinations of gaschromatography-high resolution mass spectrometry (GC/HRMS) for decades. New regulations that came into effect in 2017 in Europe¹ allowed for GC-MS/MS to be used as a confirmatory method for certain food and feed samples. This shift in regulations can have effects when it comes to routine dioxin analysis. With the continuous advancement of analytical equipment and techniques, it is becoming more feasible to achieve greater sensitivity with low resolution instruments. One of the challenges standing in the way of the MS/MS is the requirement to monitor for calibration gas (PFK or FC 43). The calibrate is monitored as a lock mass to correct for drift in HRMS systems that can be caused by matrix interferences. Typically, low resolution instruments do not need to monitor for drifts in the precis masses as they monitor a much wider mass window of 1 amu. In these systems, calibration gas is typically only used for tuning the instrument; thus, modifications need to be made in order to obtain a constant flow on calibration gas into the system to monitor stability. Although monitoring the calibrant gas does not enhance the analysis of dioxins on GC-MS/MS it can be monitored to satisfy regulatory needs.

Previous work done at Pacific Rim Laboratories which involved creating a robust method using a GC-MS/MS showed that, at that time, instruments were able to achieve the sensitivity and selectivity required for EPA 1613b as well as linearity from 0.02 pg/ μ L – 200 pg/ μ L for TCDD/F with %RSDs within the acceptable levels. This work is looking to expand on this by monitoring the stability of FC 43 thought a dioxin sequence while still retaining sensitivity on a throughout the run.

2 Materials and Methods

Standards and Chemicals. To evaluate the linearity of the system, a 7-point calibration curve was used (Wellington Laboratories Inc., Canada) ranging from 0.02 pg/ μ L to 200 pg/ μ L. Rinsing solvents such as methanol and dichloromethane were purchased from Fisher Scientific, Canada.

Instrumentation. A triple quadrupole GC-MS/MS (Thermo Fisher Scientific, Austin, USA) equipped with an advance electron ionization (AEI) source and coupled to a gas chromatograph (Thermo Fisher Scientific, Austin, USA) was used for analysis. Injections of 1 µL were run using the split/splitless inlet at a temperature of 260 °C. Carrier gas used was helium at a constant flow of 1.3 mL/min. Separation was performed using a 60 m x 0.25 mm x 0.20 µm column (Phenomenex, Torrance, USA). The GC temperature program starts at 90 °C (1 min) ramp 1 at 40 °C/min to 235 °C, ramp 2 at 2 °C/min until 240 °C, ramp 3 at 0.5 °C/min to 246 °C, ramp 4 at 6 °C/min to 260 and ramp 5 at 25 °C/min to 330 °C for a total runtime of 33 min. Transfer line temperature was set to 250 °C and the source at 350 °C. The MS was operated in MRM mode with the option to monitor calibration gas turned to EI mode. All data was quantified using TargetQuan 4 and Xcalibur.

3 Results

An initial comparison was done between two calibrations, one was run using calibration gas and the other without. This was to determine if the gas would affect sensitivity of the system. Table 1 shows that both methods are able to produce linear calibration results that would be acceptable for a 6-point curve.

The transition used to monitor FC 43 was 414 > 264. As the only difference in the two calibrations was the use of calibration gas, it was determined that if the amount of gas bled into the system was lowered, the instrument would become more sensitive. Two different solutions were tested to solve this problem.

Initially, an external line coming from a vial of FC 43 was connected to the vacant CI junction on the transfer line. The flow of this would be controlled using a needle valve and pressurized N_2 . This external system did slightly lower the amount of gas bled into the source, although it did not work consistently. Getting the needle valve and the N_2 to the "correct" position required too much fine tuning and occasionally, the vial of FC 43 would disconnect from the contraption due to the high internal pressure cause from the nitrogen gas.

No Calibration Gas									
	CS 5	CS 4	CS 3	CS 2	CS 1	CS 0.2	CS 0.04		
Native Standards	200-1000	40-200	10-50	2.10 m m/m I	0.5-2.5	0.1-0.5	0.02-0.1	Average	RSD [%]
	pg/uL	pg/uL	pg/uL	2-10 pg/uL	pg/uL	pg/uL	pg/uL	RF	
2378-TCDD	1.01	1.11	1	1.08	1.14	1.39	1	1.1	12.52
12378-PeCDD	1.07	1.05	1.04	1.05	1.09	1.1	1.04	1.06	2.39
123478-HxCDD	1.03	1.03	1.04	1.05	1.06	1.1	1.02	1.04	2.53
123678-HxCDD	1.05	1.07	1.07	1.08	1.05	0.92	1.03	1.04	5.21
123789-HxCDD	1	1.03	1.07	1.07	1.09	1.08	0.99	1.05	3.95
1234678-HpCDD	1.04	1.01	1.01	1.02	1.07	0.96	1.02	1.02	3.36
OCDD	1.01	0.97	0.99	0.98	0.99	0.96	1.02	0.99	2.23
2378-TCDF	1.02	1.02	1.02	1.01	1.12	1.1	1.02	1.05	4.26
12378-PeCDF	0.79	0.83	0.78	0.82	0.88	0.91	0.77	0.83	6.32
23478-PeCDF	0.93	0.92	0.92	0.94	0.95	0.96	0.91	0.93	2.04
123478-HxCDF	0.98	0.99	0.98	0.98	1.04	0.96	0.99	0.99	2.49
123678-HxCDF	0.93	0.98	0.94	0.96	1	0.97	0.94	0.96	2.9
234678-HxCDF	0.99	1	1	1.03	1.03	0.99	1.02	1.01	1.56
123789-HxCDF	0.95	0.97	0.96	1.01	1.02	1.22	0.94	1.01	9.36
1234678-HpCDF	1.03	1.04	1.02	1.04	1.06	1.02	1.01	1.03	1.52
1234789-HpCDF	1	1	0.99	0.99	0.99	1.01	0.97	0.99	1.02
OCDF	1.41	1.25	1.28	1.28	1.29	1.43	1.47	1.34	6.55
Calibration gas Used									
2378-TCDD	0.95	0.97	1.08	1.09	1.14	1.19	No Peaks	1.07	8.74
12378-PeCDD	0.94	0.97	1.01	1.03	1.01	1.12		1.01	5.82
123478-HxCDD	1.02	1.03	1.1	1.11	1.07	1.21		1.09	6.43
123678-HxCDD	1	1.04	1.05	1.1	1.02	1.14		1.06	5.01
123789-HxCDD	0.97	1	1.04	1.1	1.16	0.99		1.04	6.97
1234678-HpCDD	0.95	1.01	1.05	1.08	1.21	1.2		1.08	9.54
OCDD	0.95	0.99	1.05	1	1.14	1.16		1.05	8.31
2378-TCDF	0.96	1.01	1.03	1.01	1.06	1.17		1.04	6.88
12378-PeCDF	0.72	0.73	0.83	0.84	0.85	0.87		0.81	8.02
23478-PeCDF	0.88	0.85	0.92	0.95	1	1.2		0.97	13.31
123478-HxCDF	0.98	0.95	0.99	1	1.07	0.93		0.99	4.71
123678-HxCDF	0.94	0.94	0.97	1.05	1.05	1.1		1.01	6.51
234678-HxCDF	1.02	1.01	1.04	1.06	1.07	1.31		1.09	10.47
123789-HxCDF	0.94	0.96	0.99	1.03	1.01	1.12		1.01	6.43
1234678-HpCDF	0.97	0.97	1.03	1.06	1.04	1.21		1.04	8.4
1234789-HpCDF	0.93	0.96	1	0.99	1.02	1.08		1	5.23
OCDF	1.43	1.47	1.53	1.48	1.53	1.64		1.51	4.69

Table 1: RF values for two calibration curves for PCDDs and PCDFs.



Figure 1: Rough sketch of external calibration gas line

The second solution involved using a modified calibration gas manifold directly from the manufacturer. This manifold, rather than feeding into the transfer line and then into the source, was directly feeding in calibration gas into the ion source. Since the gas would not be fed into the source directly, the dispersion effect would mean that less of the gas would enter the ion source directly. Since this module is the same as the one used to tune the instrument, the same power source could be used.



Figure 2: New calibration gas module connected above the ion source from instrument manufacturer.



Figure 3: 20 fg TCDD injection while using new manufacturer module to introduce a steady flow of calibration gas (FC 43) into the system. Top left image shows the calibration gas transition monitored at 414 > 264 during the run.

As shown in Figure 3, the new system allowed for a finely-controlled introduction of calibration gas into the system which achieved the detection of 20 fg of 2378-TCDD. All RSD values were within the acceptable range when looking at a 6 or 7-point calibration.

With these promising results, further calibrations were run to determine the robustness of the new calibration gas module. When too much calibrant was added into the system the tuning and sensitivity were affected. These errors were fixed by cleaning the source thoroughly.

4 Discussion

Upon reviewing the calibration data, there is still uncertainty at the lowest end of the curve where, as suspected, sensitivity becomes an issue. EPA 1613b indicates that a sample detection limit is represented by a peak giving a signal/noise of 2.5x background noise. This is not the case for MS/MS. The instruments on the market today have very little electronic noise, therefore using calculations based on signal-to-noise is irrelevant. The detection limit is only as good as the lowest calibration standard – known as estimated quantitation limit (EQL). For MS/MS work, the EQL is used to determine the detection limit which is why it is important to operate the instrument as sensitive as possible in order to achieve detection limits comparable to those attained when using a HRMS.

5 Conclusions

GC-HRMS remains the gold standard for analysis of dioxin-like compounds. In order to add GC-MS/MS as an additional confirmatory tool, it must follow all requirements set out in EPA 1613b, such as monitoring calibration gas. More reproducible data is needed to show the effectiveness of the system at lower concentrations. Optimization is being performed to show that GC-MS/MS can perform dioxin analysis in accordance with 1613b.

6 Acknowledgements

Thank you to Amit Gujar and Adam Ladak from Thermo Fisher Scientific for the opportunity to test out this system as well as everyone from PRL that helped with the project.

7 References

 Commission Regulation (EU) No 2017/644 of 5 April 2017 laying down method of sampling and analysis for the control of levels of dioxin, dioxin-like PBCs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EU) No 589/2014 (OJ L 92, 06.04.2017, p. 9-34)