Application of a Combined POPs Analysis Method for Quantification of PCDD/DFs, DL-PCBs, and PCNs in Environmental Extracts using APGC-MS/MS

J. Stubleski[1*], J. Robinson[1], C. Frid[1], N. Riddell[1], V. Raja[1], F. Dorman[2], B. Chittim[1]

[1] Wellington Laboratories Inc., 345 Southgate Drive, Guelph N1G 3M5, Canada
[2] Waters Corporation, 34 Maple Street, Milford, MA 01757, USA

1 Introduction

Although legacy persistent organic pollutants (POPs) including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (DL-PCBs) have been listed in the Stockholm Convention since 2001, historic contamination incidents, their persistence in the environment, as well as their ability to bioaccumulate and biomagnify has resulted in continued monitoring and precedence within the environmental field(1,2). Extensive research has shown that exposure to these compounds can have detrimental impacts on human health, such as carcinogenic effects, endocrine disruption, and the promotion of diabetes mellitus(2). Polychlorinated naphthalenes (PCNs), which were added to the Stockholm Convention in 2015, have also been shown to exhibit dioxin-like effects in animal studies with tentative toxic equivalence factor (TEF) values reported in multiple publications(3,4). Ideally, samples containing PCDDs, PCDFs, DL-PCBs, and PCNs would be analyzed together to calculate a total TEQ. However, most existing analytical methods are based on magnetic sector technology, which can only maintain sensitivity and mass resolution with a limited number of target compounds. This results in sample preparation and analysis being focused on single compound classes. Fortunately, advances in mass spectrometer technology have produced instruments capable of accurately quantifying a wide range of analytes with one injection; therefore, sample preparation and analysis can be tailored towards multiple compound classes at the same time. Within the last ten years, high resolution gas chromatography-tandem mass spectrometry (HRGC-MS/MS) has been validated as a reliable alternative for the analysis of POPs in environmental samples and has been shown to target over one hundred analytes in one acquisition(5,6,7). This is due to a faster scan rate, increased sensitivity, and larger dynamic range compared to magnetic sector instruments. The HRGC-MS/MS technology should therefore be capable of analyzing and accurately quantifying PCDDs, PCDFs, DL-PCBs, and PCNs present in environmental samples in a single injection. To test this hypothesis, PCDDs/PCDFs, DL-PCBs, and PCNs were extracted from sediment reference materials with known concentrations using an in-house method and analyzed with a combined POPs (SRM acquisition) method. The concentrations determined using the combined POPs method were then compared to the reported certified reference values (CRVs) and informational values (IVs) to evaluate accuracy and recovery.

2 Materials and Methods

Sample extraction and cleanup was carried out on lab blanks (clean baked sand), spiked blanks (fortified blank baked sand), and sediment certified reference materials WMS-01 (Wellington Laboratories Inc., ON, Canada) and NIST 1944 (NIST, Gaithersburg, MD, USA) using an in-house method modified from EPA Method 1613B. Samples were Soxhlet extracted with 90:10 toluene:acetone (Caledon, ON, Canada) for 16-20 hours at a rate of 3-4 cycles per hour and then evaporated to 0.5-1.0 mL. Hexane (Caledon, ON, Canada) was added to each extract to a final volume of 7 mL before being treated with activated copper (Restek, Bellefonte, PA, USA) to remove sulfur. Further sample clean-up included a multi-layer column consisting of precleaned silica (Si) gel, 30% H_2SO_4 Si, 23% NaOH Si, and $NaSO_4$ (eluted with hexane (Caledon, ON, Canada)). The extracts were then concentrated using a rotary evaporator to 0.5-1 mL and air dried until near dryness prior to loading on 10% deactivated PX-21 carbon columns with 2 mL hexane (Caledon, ON, Canada). The extracts were eluted with 18 mL toluene and concentrated using a rotary evaporator to near dryness. All samples were spiked with 100 µL of an extraction standard (DFPCN-ES (Wellington Laboratories Inc., ON, Canada); 20-40 ng/mL) prior to Soxhlet extraction. Spiked blanks were also spiked with native precision and recovery standards: PCN-STK-A (Wellington Laboratories Inc., ON, Canada; 10 ng/mL), WP-N-1.0 (Wellington Laboratories Inc., ON, Canada; 1.0 ng/mL), and EPA-1613PAR DLN-A (Wellington Laboratories Inc., ON, Canada; 0.8-8.0 ng/mL). Finally, DFPCN-IS (Wellington Laboratories Inc., ON, Canada; 200 ng/mL) was added to each extract as an injection standard (10 µL) prior to acquisition on an Agilent 8890 GC (Agilent, Santa Clara, CA, USA) coupled to a Waters Xevo TQ-XS MS/MS (Waters Corporation, Milford, MA, USA). All standards and samples were injected using a 10:1 split and target compounds were chromatographically separated using a HP-5MS UI (Agilent, Santa Clara, CA, USA; 60 m x 0.25 mm x 0.25 µm) column. Confirmation data for PCDD and PCDF analytes were acquired using a Rtx-Dioxin2 column (Restek, Bellefonte, PA, USA; 40 m x 0.18 mm x 0.18 µm). Analysis was performed using a 17 function DFPCN SRM method while operating in API+ mode. Target PCDD/PCDF, DL-PCB, and PCN concentrations, their TEQ values, as well as total PCN concentrations were determined using TargetLynx software (Waters Corporation, Milford, MA, USA).

3 Results

In accordance with our ISO 17025 procedures, an initial evaluation of instrument linearity, precision, and sensitivity (signal to noise; S/N) was completed on the APGC TQ-XS MS/MS for the analyte groups of interest prior to analysis of environmental samples. The DFPCN combined POPs analysis surpassed the requirements detailed in Table 1: the 7-point calibration RRF %RSD was less than 7% for all analytes indicating that the system was linear for the expected concentration range, the %RSD of 10 injections of the lowest calibration standard concentration (CS1) was less than 8% for all analytes demonstrating excellent instrument precision, and the S/N for selected compounds with the lowest response were all greater than 10:1 with considerably higher values than that observed for the same concentration on the magnetic sector HRMS. This preliminary data indicated that the TQ-XS was more sensitive than the magnetic sector for this type of analysis, but performance ultimately needed to be tested using real matrix samples.

Table 1: Initial instrument validation results (linearity, precision, and S/N) for DFPCN analyses on the APGC TQ-XS MS/MS

Validation Parameter	**Instrument Linearity**		**Instrument Precision**		**Signal to Noise (S/N)**
Requirement	5-point Cal RRF %RSD < 20%		%RSD n=10 reps of CS1 < 15%		S/N > 10:1 in CS1
PCDDs/ PCDFs	7-point: 0.1/0.5/1.0-200/1000/2000 ng/mL		0.1/0.5/1.0 ng/mL		0.01 pg 2378-TCDD on column
	RRF %RSD	1.28% - 4.30%	CS1 %RSD	4.58% - 6.87%	S/N (PtP) = 53 (over 30x > Magnetic Sector)
DL-PCBs	7-point: 0.4-800 ng/mL		0.40 ng/mL		0.04 pg PCB-77 on column
	RRF %RSD	1.88% - 6.56%	CS1 %RSD	1.94% - 6.39%	S/N (PtP) = 669 (over 30x > Magnetic Sector)
PCNs	7-point: 0.25-500 ng/mL		0.25 ng/mL		0.025 pg PN-1 on column
	RRF %RSD	1.32% - 4.82%	CS1 %RSD	1.56% - 7.33%	S/N (PtP) = 12 (over 15x > Magnetic Sector)

Certified reference materials, NIST 1944 and WMS-01, as well as spiked blank sand samples were used to evaluate the accuracy of the DFPCN sample preparation and instrumental analysis method. As seen in Figure 1, all target PCDD, PCDF and DL-PCB concentrations that were reported for the NIST 1944 and WMS-01 reference materials were within ±20% of the CRVs and the spiked blank sand concentrations were all within ±20% of the spiked concentration.

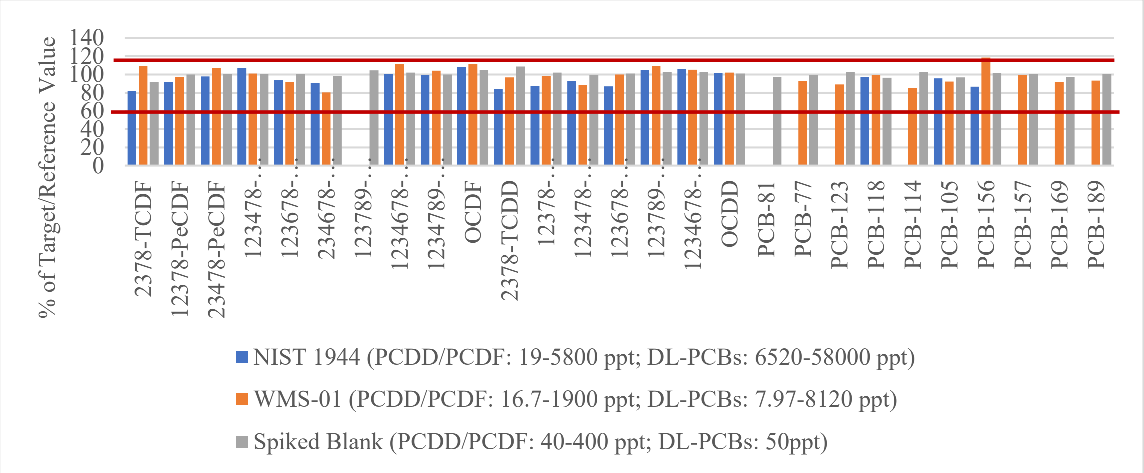

Figure 1: Percentage of Target/Reference Values for PCDD/PCDFs and DL-PCBs in NIST 1944 (blue), WMS-01 (orange) and associated spiked blank (grey).

Unfortunately, certified reference values for PCNs were not available to verify the accuracy of the developed method for this analyte group using the NIST 1944 reference material. Instead, agreement with informational values (median values obtained from 6 independent labs with no measurement uncertainty) reported on the CofA were utilized to evaluate method accuracy for PCNs. As seen in Figure 2, all reported PCN analytes were within ±20% of the stated informational values except for PN-23 and PN-75. Since the recovery of all PCN analytes met the ±20% criteria in the associated spiked blank (except for PN-18 which was within 30%), a matrix interference not sufficiently removed during sample cleanup, as a result of excluding a direct sulfuric acid extraction, was suspected. Significant losses of lower chlorinated PCNs were observed during method development upon exposure of extracts to sulfuric acid, likely resulting in the formation of naphthalene sulfonic acids. However, recoveries were found to be acceptable if only a multi-layer column was utilized instead.

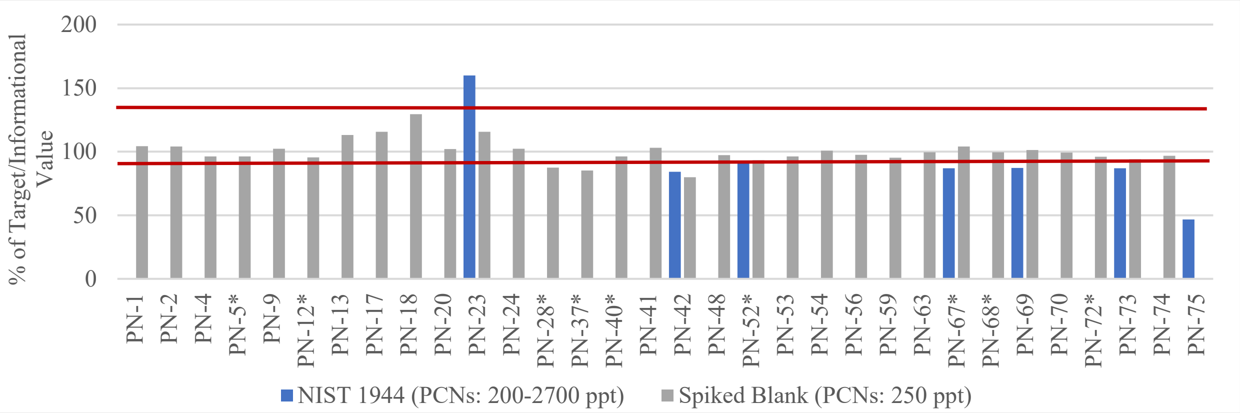

Figure 2: Percentage of Target/Informational Values for PCNs (PN-#) in NIST 1944 (blue) and associated spiked blank (grey).

In addition to analyzing spiked blanks with every sample batch, the effectiveness of the DFPCN sample extraction and cleanup method for recovering target analytes was further evaluated using the percentage of spiked ^{13}C-surrogates still present in the final extract. An acceptable range of ^{13}C-surrogate recoveries was determined to be between 50%-130% and the average surrogate recovery (n=4) of the WMS-01 and NIST 1944 SRMs ranged between 58% - 130% for all ^{13}C-surrogates, which indicates that the sample preparation method adequately retained the target compounds.

4 Discussion

This research project was possible because of the increased sensitivity of the APGC TQ-XS MS/MS and its ability to target an increased number of compounds compared to the magnetic sector due to its noise filtering capability and faster scanning rate. These qualities were important for troubleshooting crosstalk between the overlapping PCDD, PCDF, DL-PCB, and PCN channels. Specifically, crosstalk was observed between the ^{13}C-TCDD and hexa-PCN channels, ^{13}C-penta-PCB and hexa-PCN channels, as well as the ^{13}C-tetra-PCB and penta-PCN channels. Due to the increased sensitivity of the TQ-XS MS/MS we were able to choose alternate SRM transitions which used precursor masses of lower relative abundance to eliminate crosstalk between the ^{13}C-penta-PCB and hexa-PCN channels as well as the ^{13}C-tetra-PCB and penta-PCN channels. Unfortunately, the crosstalk between ^{13}C-TCDD and hexa-PCN channels could not be resolved with alternative transitions since there was complete overlap in nominal precursor masses. Therefore, GC columns with varying selectivity including a 60m HP-5MS UI, 60m Rtx-Dioxin2, 60m DB-17ms, and 60m DB-35ms were tested to determine if the peaks could be resolved chromatographically. The HP-5MS UI 60m column provided the best separation for these compounds, which allowed us to accurately quantify the hexa-PCNs.

In addition to optimizing acquisition methods, modifications to our in-house PCDD/PCDF/DL-PCB sample preparation procedure needed to be made in order to ensure adequate recovery and accuracy of target compounds. When using our validated method developed for the extraction and cleanup of PCDDs, PCDFs and DL-PCBs in sediment samples, we observed significant losses in ^{13}C-mono, di, and tri-PCNs in all samples. Sources of these losses were investigated by isolating each step in the sample preparation method and calculating the percentage of ^{13}C-surrogates recovered from each step. The low recoveries were mainly attributed to acid treating the samples as well as issues with eluting the PCNs from the PX-21 column effectively. As previously stated, it was also found that direct sulfuric acid extractions could not be performed therefore we were limited to multi-layer silica columns for sample cleanup. The highest recoveries were obtained from the samples that did not undergo acid treatment ranging from 82-92% for all ^{13}C-PCNs, while lower recoveries were obtained from the samples that were acid treated while being cooled in an ice bath (51-87% for all ^{13}C-PCNs), and the worst recoveries were obtained for samples that were acid treated at ambient temperature (29-58% for all ^{13}C-PCNs). Decreased recoveries from the PX-21 column step were investigated by testing the sorption capacity of the PCNs to the PX-21. A deactivated PX-21 column was tested resulting in increased recoveries of ^{13}C-PCNs (61-79%) compared to the activated PX-21 (25-67%).

NIST 1944 and WMS-01 reference materials, as well as spiked blanks (spiked clean baked sand) were used to evaluate the developed DFPCN sample preparation and analysis methods for accuracy and recovery. Reported concentrations within ±20% of the reference, informational, or spiked value were considered accurate. All PCDDs, PCDFs, and DL-PCBs were accurately reported in the spiked blanks as well as both reference materials using the combined methodology. Unfortunately, more variation was observed in the PCN data. In order to determine if this was the result of the acquisition, the NIST 1944 was also processed and acquired for the PCN analytes only. Figure 3 illustrates that the results of the PCN specific method agreed quite well to those obtained using the DFPCN method. In order to increase confidence associated with the values of PN-23 and PN-75, an environmental reference material with certified reference values would need to be examined; unfortunately, at the time this work was completed, no such material existed to the best of our knowledge. This is significant because the contribution of the estimated PCN TEQ against the total DFPCN TEQ in WMS-01 was calculated as 12.3%.

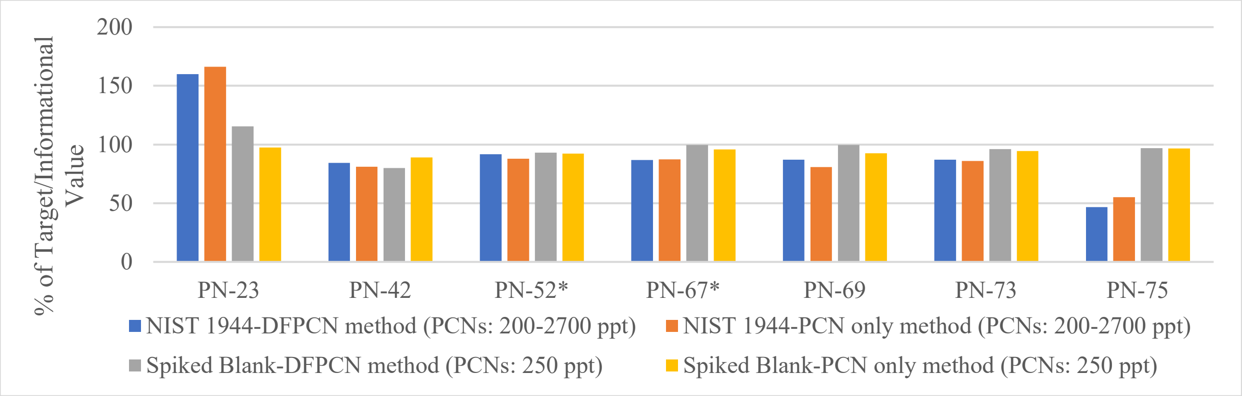

Figure 3: Comparison of Target and Informational Values for PCNs (PN-#) using the DFPCN sample preparation (NIST 1944 in blue and associated spiked blank in grey) and PCN-only sample preparation and analysis method (NIST 1944 in orange and associated spiked blank in yellow).

5 Conclusions

Unlike the magnetic sector HRMS, the APGC TQ-XS MS/MS can analyze significantly more analytes and compound classes at one time without sacrificing linear range, precision, sensitivity, or accuracy. This opens the door to more comprehensive sample preparation and analysis methods that can provide more information from a single injection. Although increasing the number of target compounds can come with additional challenges such as troubleshooting channel crosstalk, the increased sensitivity and reliability of the APGC-MS/MS makes overcoming these obstacles and applying modifications to existing methods possible. Unfortunately, it was difficult to verify the method accuracy of PCNs in complex environmental samples using our combined DFPCN method due to the lack of suitable reference materials. These materials are greatly needed as research has indicated PCNs exhibit dioxin-like effects. If consensus TEF values are to be determined for PCNs, it is essential that accurate reporting of these compounds in complex matrices can be achieved first. It would be very useful to be able to report a total TEQ for PCDDs, PCDFs, DL-PCBs, and PCNs with confidence.

6 Acknowledgments

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