SIMULTANEOUS DETERMINATION OF PCDD/PCDF AND DIOXIN-LIKE PCBS IN EDIBLE VEGETABLE OILS

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1. INTRODUCTION

The U.S. Environmental Protection Agency (EPA) reassessment of the toxicity of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/PCDF) and dioxin-like polychlorinated biphenyls (PCB) identifies ingestion as a key route of human exposure to these compounds. The reassessment, however, found that several potentially key routes of ingestion had not yet been thoroughly investigated. Vegetable oils, of concern due to their high fat content, were among the food items for which only limited data (and none from North America) could be found[1]. This paper addresses experimental procedures adapted to simultaneously determine PCDD/PCDF and dioxin-like PCBs in edible vegetable oils and provides a brief discussion of results obtained for thirty oil samples.

2. EXPERIMENTAL PROCEDURES

2.1 Sample Collection

Thirty edible vegetable oil samples were collected from grocery stores throughout the United States. The survey was not intended to be a statistically representative survey of edible oil production/consumption in the United States. The survey did, however, determine a number of samples per oil type that was roughly proportional to the use of oils in the U.S. diet. Soybean oil dominates the edible oil market in the United States, both in cooking usage and in prepared foods such as baked goods, dressings and margarines. The survey's focus on this commodity reflects soybean oil's large market share. Additionally, the study examined edible oils used in a secondary capacity by the contemporary U.S. population. The 30 oil samples were distributed as follows: soybean oil - 10 samples, corn oil - 3 samples, canola oil - 2 samples, olive oil - 2 samples, peanut oil -1 sample, safflower oil -1 sample, sunflower oil - 1 sample, solid shortening - 3 samples,

margarine - 6 samples, canola oil spray -1 sample. Samples were collected in the Washington, D.C. metropolitan area, Chicago, Salt Lake City, Cincinnati, Miami, Denver, Minneapolis, San Francisco, and San Antonio.

2.2 Sample Preparation

Samples were processed using general procedures in SW846 Method 8290[2] with select improvements from EPA Method 1613[3] and several modifications for simultaneous determination of PCDD/PCDF and PCBs. Use of standardized methods had been requested for this study due to their proven success in a variety of sample matrices and their ease of adapting from one laboratory to another. Due to the ubiquitous nature of PCBs in the environment, additional steps were taken to reduce background contamination above and beyond those routinely incorporated for PCDD/PCDF analysis and recommended in Methods 8290 and 1613. These additional procedures included the following: (1) all glassware, glass wool, and anhydrous sodium sulfate used in sample preparation was heated at 450°C for a minimum of 8 hours prior to use; (2) silica and alumina cleanup column reagents were heated in a tube furnace under flowing nitrogen to minimize exposure to the atmosphere; and (3) organic solvents were pretested for PCB content before use to verify purity.

Approximately 5 g of each oil sample was weighed into a jar and dissolved in 20 mL of toluene. Each sample was spiked with Method 1613 PCDD/PCDF internal standard solution containing fifteen $^{13}C_{12}$-labeled 2,3,7,8-PCDD/PCDF. Samples were also spiked with six $^{13}C_{12}$-labeled PCBs (nos. 77, 126, 169, 105, 118). Internal standards were purchased from Cambridge Isotopes, Woburn, MA. Matrix spike and matrix spike duplicate quality control samples were also spiked with solutions containing known amounts of unlabeled PCDD/PCDF and PCBs at this time. Each sample was swirled well to mix, covered with foil, and allowed to stand for approximately 5 minutes. Samples were transferred to 125-mL separatory funnels, spiked with 2,3,7,8-TCDD-$^{37}Cl_4$ cleanup standard as described in EPA Method 1613 and mixed well. Each extract was acid/base washed (8290 Section 7.3.5.6) and processed through the following cleanup columns: acid/base silica (8290 Section 7.5.1), acid silica (8290 Section 7.3.5.6), basic alumina (1613 Section 12.4), and AX-21 carbon/celite (8290 Section 7.5.3). The AX-21 carbon/celite columns were back eluted with 30 mL of toluene rather than 20 mL as specified in Method 8290 to improve recovery of OCDD. Extracts were quantitatively transferred to concentrator tubes premarked at 20 μL with decane, spiked with 10 μL of 1,2,3,4-TCDD-$^{13}C_{12}$/1,2,3,7,8,9-HxCDD-$^{13}C_{12}$ recovery standard, and concentrated to a 20-μL final volume.

These procedures proved to be effective for all but eight of the 30 samples. Upon analysis, these eight samples had chromatographic interference in the tetra-CDD/CDF and penta-CDD/CDF windows which compromised the determination of tetra- and penta-CDD/CDF and the PCBs. The eight samples were reprocessed as described above except that extracts were put through two alumina columns instead of one and the carbon column was back eluted with 20-mL toluene in the event that the additional solvent rinse of the carbon column (originally included to enhance OCDD recovery) was removing interferences specific to these oil matrices from the carbon column. Chromatography in the reprocessed sample extracts was significantly improved.

2.3 GC/HRMS Analysis

All extracts were analyzed by GC/HRMS in the selected ion monitoring mode at a resolution of 10,000 or greater. The GC/HRMS system consisted of a VG Autospec HRMS configured with a Hewlett Packard 5890 capillary GC with cool, on-column injection. Simultaneous analysis of the seventeen 2,3,7,8-substituted PCDD/PCDF and six PCBs was performed on a DB-5 (60 m x 0.32 mm, 0.25 μm) capillary column. Injection volumes of 0.5 μL were used for sample and calibration analyses. Masses monitored for the determination of PCDD/PCDF were the same as those listed in EPA Method 1613 Table 3. Additional masses monitored for PCBs and their theoretical isotope ratios are listed in Table 1.

Table 1. PCB Masses and Isotope Ratios

Group I	M	M+2	Isotope Ratio
PCB 77	289.9224	291.9195	0.77
PCB 77-$^{13}C_{12}$	301.9626	303.9597	0.77
PCB 118, 105, 126	323.8834	325.8805	0.61
PCB 118-, 105-, 126-$^{13}C_{12}$	335.9237	337.9207	0.61
Group II	M	M+2	Isotope Ratio
PCB 156, 169	357.8444	359.8415	0.51
PCB 156-, 169-$^{13}C_{12}$	371.8817	373.8788	1.24

The GC/HRMS system was calibrated for PCDD/PCDF using response factors generated from a five-point curve at concentrations specified in EPA Method 1613. The system was calibrated for PCBs using response factors generated from a five-point curve at the levels presented in Table 2. The recovery standard for all $^{13}C_{12}$-labeled PCBs was 1,2,3,4-TCDD-$^{13}C_{12}$.

Table 2. PCB Calibration Levels (pg/μL)

Analyte	Point 1	Point 2	Point 3	Point 4	Point 5
Native PCBs (77, 126, 169, 118, 105, 156)	2	10	50	100	500
$^{13}C_{12}$-Labeled PCBs (77, 126, 169, 118, 105, 156)	100	100	100	100	100
Recovery Standard 1,2,3,4-TCDD-$^{13}C_{12}$	100	100	100	100	100

3. RESULTS

OCDD was the only analyte detected in all thirty oil samples above background levels. Concentrations of OCDD detected in the oil samples ranged from 3.55 - 33.10 pg/g compared to OCDD method blank levels of 2.79- 4.38 pg/g. With the exception of OCDD, any PCDD/PCDF analytes detected in the thirty oil samples were at, or near, detection limit levels. Detection limits, calculated according to Method 8290 Section 7.9.5.1, were generally near 1 pg/g for all analytes and ranged from 0.1 to >2 pg/g. 2,3,7,8-TCDD, the most toxic PCDD/PCDF congener, was not detected in any of the oil samples or blanks with detection limits ranging from 0.2 to 1.8 pg/g. PCBs 126 and 169 were not detected in oil samples or blanks. In spite of the efforts taken to reduce PCB background levels, PCBs 77, 118, 105, and 156 were found in all blanks. The PCB concentrations in the three blanks processed along with the oil samples showed a wide degree of variability. PCBs 118 and 105 were more prevalent as might be expected due to their higher concentration in PCB Aroclor mixtures. PCBs 77, 118, 105, and 156 were detected in all oil samples with less variability than, but concentrations comparable to, the blanks indicating that the oil samples did not contain these PCBs at levels above background. Internal standard recoveries for the 30 oils using these procedures were within Method 8290 limits of 40-135% for all analytes except OCDD-$^{13}C_{12}$, which was recovered at levels less than 40% in seven of the 30 oils. Recoveries of native analytes added to matrix spike samples ranged from 54 to 117%.

4. CONCLUSIONS

This study shows that Method 8290 can be adapted for the simultaneous analysis of trace levels of PCDD/PCDF and select PCBs in edible vegetable oils and that these compounds, with the exception of OCDD, were not present in the oils or were detected at concentrations at, or near, the detection limit levels or concentrations found in the method blanks. Detection limits achieved using these procedures, although well below the calibration ranges defined in Methods 1613 and 8290, were limited by the sample size that was easily dissolved and efficiently cleaned with Method 8290 procedures. Experimental steps which might further reduce detection limits include the following.

(1) Use of a larger sample size followed by gel-permeation chromatography (GPC) cleanup prior to Method 8290 cleanup steps. The addition of GPC may aid in removing matrix interferences thereby allowing a larger sample aliquot to be processed without overloading traditional Method 8290 cleanup columns. Alternatively, two 5-g aliquots of each sample could be spiked with half the usual level of internal standards and independently processed through cleanup procedures as presented for this study. The two aliquots for each sample could then be combined and concentrated into a single sample for GC/HRMS analysis.

(2) Method detection limit verification by spiking multiple aliquots of the sample matrix with analytes at concentrations at, or near, the detection limit and processing through all analytical procedures. Such a study would insure that detection limits currently estimated using instrumental noise heights to calculate a minimum detectable level as specified in Method 8290 Section 7.9.5.1 could be accurately achieved.

(3) Expanding the calibration range beyond the level obtained with traditional Method 1613 calibration solutions to verify the accuracy of analytes currently detected below the calibration range.

5. REFERENCES

(1) U.S. EPA, 1994; Estimating Exposure to Dioxin-Like Compounds, EPA/600/6-88/005Cb; U.S. Environmental Protection Agency, Washington, D.C., June 1994.

(2) SW846 Method 8290, "Polychlorinated Dibenzodioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by High-Resolution Gas Chromatography/High Resolution Mass Spectrometry (HRGC/HRMS)", Revision 0, November 1992.

(3) Method 1613, "Tetra-through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS", Revision A, October 1993.

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