AXYS Analytical Services Ltd.

ANALYSIS OF POLYCHLORINATED DIOXINS AND FURANS, AND PCB CONGENERS IN BLOOD SERUM BY HRGC/MS

Summary

All samples were spiked with ^{13}C-labelled surrogate standards prior to analysis. Samples were extracted by shaking with solvent. Extracts were subjected to a series of chromatographic column cleanup procedures. During the Florisil chromatographic column cleanup procedure the PCB and Dioxin/Furan were separated into different fractions. Each fraction was separately analyzed by high-resolution gas chromatography with high-resolution mass spectrometric detection (HRGC/HRMS).

1. EXTRACTION PROCEDURES

An aliquot of surrogate standard solution was added to each sample prior to extraction, and an aliquot of cleanup efficiency standard added after extraction. The surrogates added were as follows:

Dioxin/furan Surrogates: ^{13}C-labelled analogues of:

2,3,7,8-tetrachlorodibenzodioxin,
1,2,3,7,8-pentachlorodibenzodioxin,
2,3,4,7,8-pentachlorodibenzofuran,
2,3,4,6,7,8-hexachloro-dibenzodioxin,
1,2,3,6,7,8-hexachlorodibenzofuran,
2,3,4,6,7,8-hexachlorodibenzofuran,
1,2,3,4,6,7,8-heptachlorodibenzofuran,
octachlorodibenzodioxin

2,3,7,8-tetrachlorodibenzofuran,
1,2,3,7,8-pentachlorodibenzofuran,
1,2,3,6,7,8-hexachlorodibenzodioxin,
1,2,3,4,7,8-hexachlorodibenzofuran,
1,2,3,7,8,9-hexachlorodibenzofuran,
1,2,3,4,6,7,8-hepta-chlorodibenzodioxin,
1,2,3,4,7,8,9-heptachlorodibenzo-furan,

PCB Congener Surrogates: ^{13}C-labelled analogues of:

PCB 1	PCB 3	PCB 4	PCB 15	PCB 19	PCB 37	PCB 54
PCB 77	PCB 81	PCB 104	PCB 105	PCB 114	PCB 118	PCB 123
PCB 126	PCB 155	PCB 156	PCB 157	PCB 167	PCB 169	PCB 188
PCB 189	PCB 202	PCB 205	PCB 206	PCB 208	PCB 209	

Total lipid analysis was performed externally using a 300 µL subsample from each sample.

An accurately weighed sample of serum was placed into a round bottom flask with aliquots of surrogate standards, and allowed to equilibrate. The sample was solvent extracted by shaking with a solution of ethanol, hexane, and saturated ammonium sulphate for 30 minutes. The extract was backwashed with water, and aliquots of clean-up standards ($^{37}CL_4$-2,3,7,8-TCDD and $^{13}C_{12}$-PCB 28, $^{13}C_{12}$-PCB 111, and $^{13}C_{12}$-PCB 178) were added. The extract was concentrated by rotary evaporation prior to chromatographic cleanup procedures

2. CHROMATOGRAPHIC CLEANUP PROCEDURES

The extract was cleaned up sequentially on the following columns:

Gel Permeation: Biobead SX-3 column

Layered Silica: layered (neutral, basic, neutral, acidic, neutral) silica gel column

Florisil: The extract was fractionated on Florisil. Fraction 1 (F1) contained the PCBs, and fraction 2 (F2) contained the PCDD/Fs.

Alumina: The extract was cleaned-up on an alumina column calibrated for PCB's. The eluate was reduced in volume, transferred to an autosampler vial and an aliquot of ^{13}C-labelled recovery standard ($^{13}C_{12}$-PCBs 9, 52, 101, 138 and 194) was added. The vial was capped in preparation for GC/MS analysis for PCB congeners.

Carbon/Celite: PCDD/F were isolated on a carbon column by forward and reverse elution. The eluate (Fraction E2) was collected, blown down to almost dryness and redissolved in hexane.

Layered $AgNO_3$ Silica: The PCDD/F fraction was cleaned-up on a layered (neutral, $AgNO_3$, neutral, basic, neutral, acidic, neutral) silver nitrate/silica gel column. The eluate was concentrated.

Alumina: The PCDD/F fraction was eluted through an alumina column calibrated for dioxins. The eluate was evaporated to near dryness, transferred to an autosampler vial and an aliquot of ^{13}C-labelled recovery standards (1,2,3,4-tetrachlorodibenzodioxin and 1,2,3,7,8,9-hexachlorodibenzodioxin) was added. The vial was capped in preparation for GC/MS analysis for PCDD/PCDF.

3. HIGH RESOLUTION GC/MS ANALYSIS

PCDD/PCDF

Calibration

Calibration procedures were performed in accordance with Section 10.0 of EPA Method 1613B. Initial calibration was performed using a six-point linearity, utilizing an additional CS-0.2 standard solution (a 5 fold dilution of the CS-1 standard) for higher sensitivity.

Analysis

Polychlorinated dibenzodioxins (PCDD) and dibenzofurans (PCDF) were analyzed on an Autospec Ultima mass spectrometer equipped with a Hewlett Packard 6890 gas chromatograph, a CTC autosampler and an Alpha workstation running VG OPUS software. The mass spectrometer was tuned daily to have a static mass resolution of 10,000 or greater and operated in the electron impact ionization mode. Data were acquired in the voltage selected ion recording mode (SIR) to enhance sensitivity. At least two ions were used to monitor each of the target analytes and ^{13}C-labelled surrogate standards. Five additional ions were monitored to check for interference from chlorinated diphenylethers. Chromatographic separation was achieved with a DB-5 capillary chromatography column (60 m, 0.25 mm i.d. x 0.1 μm film thickness). PCDD/F analyses were carried out in accordance with the specifications found in EPA method 1613B.

PCB Congeners

Calibration

Calibration procedures were performed in accordance with Section 10.0 of EPA Method 1668A. Initial calibration was performed using a six-point linearity, including the CS-0.2 standard solution for higher sensitivity.

Analysis

HRGC/HRMS analysis of PCB congeners was carried out using a VG high resolution mass spectrometer (MS) equipped with a Hewlett Packard 6890 gas chromatograph, a CTC autosampler and a data system running VG OPUS software. The MS was operated at a 10,000 static mass resolution in the electron ionization (EI) mode. Data were acquired in the voltage selected ion recording mode (SIR) to enhance sensitivity, acquiring two ions for each target analyte and surrogate standard. Chromatographic separation was achieved with an SPB-octyl capillary chromatography column (30 m, 0.25 mm i.d. x 0.25 μm film). Chromatographic separation of the PCB 156/157 pair was achieved using a secondary DB-1 chromatography column (30 m, 0.25 mm id, 0.25 μm film thickness). PCB analyses were performed in accordance with the specifications documented in USEPA Method 1668A Revision A.

4. QUANTIFICATION PROCEDURES

Concentrations for native analytes with exact labelled surrogate standards were calculated using the isotope dilution method of quantification. Analytes were quantified by comparing the total area of the quantification ions to that of the corresponding ^{13}C-labelled surrogate standard and corrected for response factors. Response factors from the initial calibration were used in the quantification.

For PCBs, those target analytes not contained in the linearity standards were quantified by the internal standard quantification method, by comparing the total area response of the native analyte ions to that of the average response from the labelled surrogate standards of the associated homologue group and corrected for response factors. Response factors from a single point calibration, analyzed at the beginning of the twelve-hour instrumental analysis run, were used in the quantification.

Concentrations of the native analytes were automatically recovery corrected by the analysis procedure. Sample detection limits were reported for each target analyte, based on an area determined from three times the average instrumental noise signal from the acquisition window of the target analyte chromatogram. The final results were not corrected for any laboratory background detected in the batch blanks.

Concentrations were reported in pg/g wet weight and calculated lipid weight basis. Percent lipid values were determined by weight basis from the independent lipid determination results.