The Application of Supercritical Fluid Extraction and On-line Alumina Column Cleanup for the Determination of Polychlorinated Biphenyls in a Fish Tissue Candidate Reference Material - *CARP-2*.

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Abstract

Supercritical fluid extraction (SFE) coupled with on-line alumina column cleanup was successfully used to quantify selected polychlorinated biphenyl congeners in a fish tissue candidate reference material, CARP-2. The analyte recoveries were 65 % or better for the five replicate analyses. The incorporation of on-line alumina column cleanup eliminated the post-extraction cleanup steps. Samples were ready for GC determination about two hours after first starting the extraction. The results were in good agreement with the certified values for a similar certified reference material, ***CARP-1***.

1. Introduction

A major project of the Chemical Metrology Group of the Institute for National Measurement Standard, National Research Council of Canada is the production of Certified Reference Materials (CRMs). Our current catalogue lists several types of CRMs - three seawater CRMs, one river water CRM, three sediment CRMs and five biological tissue CRMs. One of our biological tissue samples, ***CARP-1*** is certified for selected priority PCDDs, PCDFs and PCBs[1]. An imprtant criterion for establishing a certified value for an analyte in a certified reference material is that values determined by at least two independent analytical methods (often from more than one expert laboratory) be available. The methods for determining organochlorine compounds in a biological tissue sample typically consist of several steps -- extraction, bulk lipid removal, cleanup and fractionation, and finally analysis by GC-ECD or GC-MS. The objectives of our study were threefold : 1) demonstrate the suitability of a CRM as a method-development aid, 2) investigate the applicability of SFE as an extraction technique[2] and 3) investigate the usefulness of on-line alumina column cleanup[3,4,6]. If this approach was successful, then it could be considered as an alternative method for in-house use.

2. Experimental Methods

Instrumentation

All extractions were carried out using a Suprex PrepMaster Supercritical Fluid Extractor. A Suprex 50-ml sample cell was used to contain the sample plus 50g of anhydrous sodium sulphate.A second extraction cell, 10 ml in volume was used to contain the alumina for on-line cleanup. This second cell, serving as a cleanup column was installed between the sample select

valve and the fused silica restrictor, and was mounted in a vertical orientation on a retort stand external to the SFE instrument. Although the PrepMaster has a large sample ove and valve chamber, the use of the large 50 ml sample cell precluded the installation of the alumina cleanup cell in the same oven. Two heat guns were used to maintain the external cleanup cell, associated tubing and the fused silica restrictor / collection vial region above 40°C. Two thermocouples, one mounted just below the cleanup column and the other mounted on the stainless steel union that connected the stainless steel tubing to the fused silica restrictor, were used to monitor the temperature of these areas. The effluent from the restrictor was collected in a 22-ml vented Pyrex vial containing 14 ml of iso-octane as the trapping solvent. Figure 1 is a schematic diagram of the setup and the CO_2 flow path.

The SFE conditions were as follows :

Solvent :	CO_2, no modifiers
Pressure :	400 atm
Temperature :	40°C
Flow rate :	3-4 ml/min
Restrictor :	20 mm x 52 µm fused silica tube
Total CO_2 flow :	250 ml (5 sample cell volumes)
Initial 'static' mode :	1 min
'Dynamic' mode :	Remainder of run ~ 80 min

Sample handling

The CARP-2 material is in the form of an aqueous slurry stored under nitrogen in a sealed Pyrex ampoule. Each ampoule contains approximately 10 g of material with a moisture content of 85% and a lipid content of 3.92 %[5] . Each ampoule was emptied into a mortar containing 50 g of anhydrous sodium sulphate (previously conditioned at 450°C overnight). The sample was ground and mixed with the sodium sulphate for approximately 15 minutes until it was dry and free flowing. The sample was then spiked with the following PCB recovery standards :

PCB 30 (110 ul at 26,400 pg/ul)
PCB 188 (83 ul at 29,100 pg/ul)
PCB 204 (42 ul at 22,787 pg/ul)

The sample was allowed to air dry for 5 min and then mixed again. It was then poured into the 50 ml sample extraction cell. The remaining volume above the sample was filled with 2 -3 grams of Fluka Alumina (Product 06290, Type 5016 A basic, previously conditioned at 450°C overnight) placed at the outlet end of the extraction cell. The external 10-ml SFE cell was also filled with 8 - 9 g of the same alumina.

After the desired 5 cell volumes of CO_2 had passed through the sample cell, the extraction was stopped and the collection vial was removed. The iso-octane was gently evaporated under a stream of nitrogen to a volume of 500 µl. The extract was transferred to a 2-ml volumetric flask containing 486 pg of PCB 194 (instrument standard). Several washings of the collection vial were combined to bring the solution close to the 2-ml mark. Pure iso-octane was added to bring the sample exactly to the 2-ml volume. This sample was then diluted 1/10 prior to injection on a Varian 3300 GC equipped with an electron-capture detector.

Gas Chromatography:
Column : J & W DB-5 (60 m x 0.25mm x 0.25µm film thickness)
Carrier gas : Helium at 30 psig
Injection : Splitless with a vent time of 1 min after injection
Temperature program :
120°C for 2 min
120°C to 180°C at 20°C/min
180°C to 300°C at 4°C/min
9 min hold for a total run time of 44 min

Each sample extract solution was injected 5 times. A calibration standard was injected after every two sample injections. Method blank solutions containing the recovery standards were also injected. Peak heights were measured using the Varian Star Chromatography system. Calculations and data reduction were performed in custom software written in a spreadsheet program (Quattro Pro).

3. Results and Discussion

To establish optimal conditions, several method blanks were carried out to determine the extraction parameters, check for system blanks and determine if cross-contamination and/or sample carryover would be a problem. No problems were observed. The measured recoveries for PCBs 78, 188 and 204 spiked onto conditioned sodium sulphate were 90 % or better. When the procedure was applied to the CARP-2 material, very high recoveries were observed for PCB 78 (100 - 200 %). As it turned out, a sample with no PCB 78 added, yielded a recovery of 136 %. It was decided that a coeluting compound was the source of the problem and PCB 30 was substituted for PCB 78. PCB 30 has a retention time similar to that of PCB 78 but elutes in a region free from any interferences. The observed recoveries for the five sample runs reported here average 85 % with a RSD of 17%. Table 1 contains the recoveries for all five sample extractions. A total CO_2 flow of 250 ml (5 cell volumes at 3-4 ml/min) was optimal for maximizing analyte recovery while minimizing lipid breakthrough.

Table 1 Observed PCB recoveries

PCB Spike	Run 1	Run 2	Run 3	Run 4	Run 5
30	81	110	84	73	109
188	71	97	75	67	97
204	76	100	81	66	89
Average‡	76	102	80	69	98

‡ This value used to calculate analyte recoveries for each respective sample run

Table 2 lists the PCB concentrations of selected congeners in CARP-2 determined in this study along with the certified values for the same compounds in CARP-1. The PCB contents of CARP-1 and CARP-2 are very similar.

Table 2 CARP-2 PCB concentrations compared to CARP-1 certified values

IUPAC #	CARP-1 certified values ng/kg (wet weight)	CARP-2‡ ng/kg (wet weight)
52	124 ± 32*	175 ± 36†
101/90	124 ± 37	127 ± 28
105	54 ± 24	79 ± 15
118	132 ± 60	165 ± 15
138/163/164	102 ± 23	116 ± 16
153	83 ± 39	103 ± 17
170/190	22 ± 8	6.6 ± 0.8
180	46 ± 14	37 ± 7
187	36 ± 16	15 ± 2

‡ results of five replicate analyses
* 95% confidence interval
† standard deviation

Three of the determined PCB concentrations (PCBs 52, 170/190 and 187) fall outside of the 95% confidence intervals for the certified reference material. The differences were significant for PCBs 170/190 and 187 after their analytical uncertainties were taken into account.

The on-line alumina cleanup eliminates the post-extraction Florisil/alumina column cleanup typically performed on the extracts. This results in a time saving of 7.5 hours overall and a reduction of 350 ml of solvents per sample relative to our standard method of Sohxlet extraction followed by post-extraction Florisil and silica column cleanup . Judging from the quality of the chromatograms, the extracts obtained in this study are just as clean as those obtained with our standard procedure. Future work includes re-analysing the SFE extracts using GC-MS and extracting new samples spiked with ^{13}C-PCB surrogate standards and then analysing by GC-MS.

4. Conclusion

Supercritical fluid extraction coupled with on-line alumina cleanup is a rapid and efficient extraction and cleanup method for the determination of PCBs in a fish tissue candidate reference material.

6. Literature Cited

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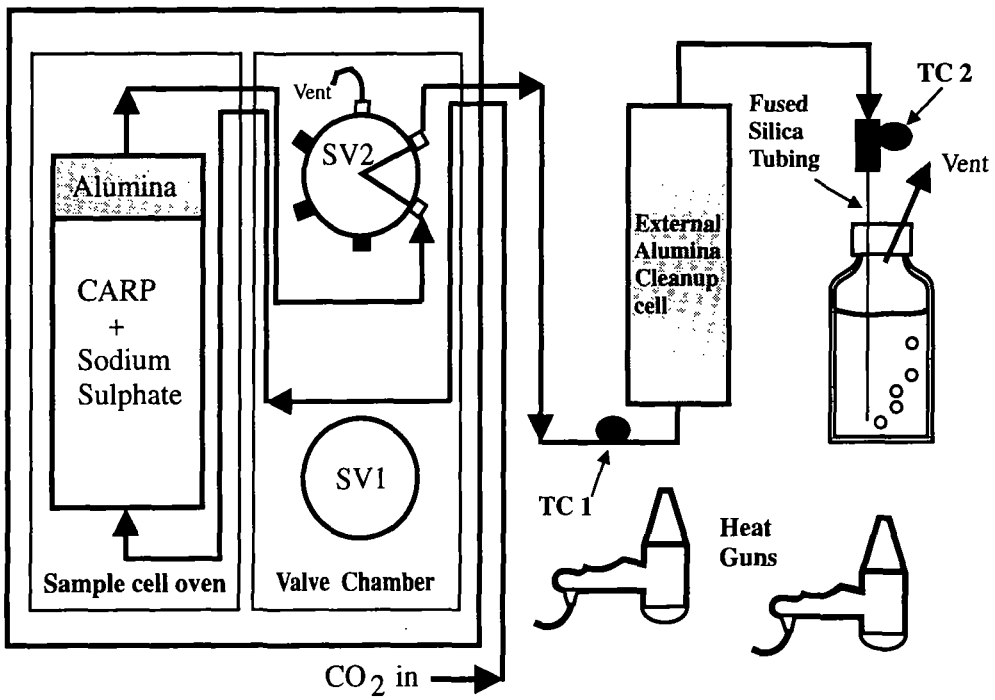

Suprex PrepMaster

Fig. 1