Congener Specific Analysis of Toxaphene in Serum

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Abstract

A method for the determination of environmentally relevant Toxaphene congeners from serum is described.

Introduction

Toxaphene is one of the major organochlorine pesticide contaminants in fish, polar bears and other mammals in North America and Europe[1-3]. The presence of Toxaphene in human milk samples has also been reported[(2)]. At least 20 norbornane congeners (PCCs) have potential for toxicity due to some combination of their abundance in fish/animal tissue and human milk[1-3]. To assess potential human health effects, an accurate congener specific methodology is needed, to focus on the most toxic congeners. Progress in developing such a methodology has been slow. However, with the isolation and purification of 22 environmentally relevant congeners, Toxaphene analytical chemistry has passed its infancy stage[4].

The congener specific methodology developed under the present project, to determine the baseline levels of Toxaphene in Canadian Native Communities is presented here.

Experimental

Chemicals and reagents

Decachlorobiphenyl (PCB 209) was purchased from Aldrich chemicals. Carbopack - C (80-100 mesh) was obtained from Supelco Canada Inc. Sodium sulphate, florisil and silica gel were heated to 500°C in a muffle furnace for 48 h and then stored in an air tight glass jars. Hexane and dichloromethane(DCM) were glass distilled and free of interfering residues as tested by GC/MSD after concentration from 15 mL to 50 µL. All glassware was washed twice with acetone and hexane then heated at 500°C for 20h.

Instrumental analysis

Analyses of Toxaphene was performed on a Hewlett - Packard 5890 series II GC equipped with MS Engine(HP 5989 - A). The gas chromatographic (GC) operating conditions were as follows: GC column, DB - 5 (30 m x 0.25 mm id x 0.25 µm film thickness, J & W Scientific USA); Carrier gas Helium with a flow rate of 1.09 mL/min; on column injection, 3µL using HP 7673A autosampler; injector temperature 70°C (0.5 min),then raised to 240°C at the rate of 160°C/min and was kept at this temperature for 23 min. Initial oven temperature 70°C (1 min),then 22.5°C/min to 225°C, 1°C/min to 230°C, 5 min (hold) and 20°C/min to 290°C, 5 min (hold). The MS was operated in electron capture negative ion mode (ECNI) with methane reagent gas at source pressure of 1.9 torr, electron energy 180ev, ion source temperature at 120°C and interface temperature at 280°C. Hewlett - Packard Apollo series 400 work station data system was used in the quantification of Toxaphene.

Extraction

The serum samples were thawed and vortexed. An aliquot (4 mL) was placed into a centrifuge tube . The sample was spiked with a standard solution for recovery experiments.After equilibration at room temperature for 10 min, the analytes were extracted with hexane / DCM (9:1 (V/V), 4.0 mL), vortexed (1 min) and centrifed at 1800 rpm for 2 min. The top organic layer was drawn into a clean centrifuge tube . The extraction was repeated twice with

hexane/ DCM(2 x 4.0 mL). The combined organic extract was concentrated to 0.5 mL for fractionation on SPE column.

Fractionation

The fractionation of sera extracts were performed on SPE column (disposable pipette, contains from bottom to top: sodium sulfate (0.1g), silica gel (2g), florisil (2g), carbopack (0.2g) and sodium sulfate (0.3g) respectively) using Visiprep System (Supelco Canada Inc). Prior to fractionation the assembled column on a Visiprep System was prewashed with DCM/hexane (1:4, 4 x 2.0 mL) followed by hexane (4 x 2.0 mL). The concentrated extract was added on, to the top of the column. The extract tube was rinsed with hexane (3 x 0.3 mL) and each portion was transferred to the column. The elution profile used was as follows: The first fraction (F1) was eluted with hexane (10.0 mL), contained Toxaphene (0-5%), PCB congeners and pesticides from group I. This fraction was evaporated to 0.5 mL, transferred to a microvial containing 40 µL of PCB 209 (1 ppb) internal recovery standard, adjusted to final volume of 40 µL and retained for the analysis for Toxaphene and PCB congeners on GC/NCIMS. The second fraction (F2), contained Toxaphene, was eluted with hexane/DCM (1:4, 20.0 mL). After evaporation to 0.5 mL, contents of tube were transferred to a microvial containing 40 µL of PCB 209 (1 ppb) internal recovery standard, the adjusted final volume of 40 µL was analyzed for Toxaphene on GC/NCIMS.

Results and discussions

Figure 1 shows chromatograms of a standard solution of 22 Toxaphene congeners (A) and human serum extract (B), obtained by plotting the total ion current of the following $[M]^-$ and $[M-Cl]^-$ ions: 6-Cl=339.7,341.7; 7-Cl=340.7,342.7; 8-Cl=376.7,378.7; 9-Cl=410.7,412.7; 10-Cl=446.7 and 448.7, respectively.

The following four major modifications were incorporated into our original method[5]: 1) during the extraction use of acetic acid and sulfuric acid was eliminated; 2) introduction of a new SPE column as above; 3) analytes were analyzed using on column injection technique;

4) a new standard consisting of 22 Toxaphene congeners was used for quantification purpose.

From these sera samples, we have observed four prominent peaks (congener #26, 40+41, 44 and 50)[4] and 4-5 other minor Toxaphene peaks. The Toxaphene congener numbering system is that of Parlar (see reference 4). These

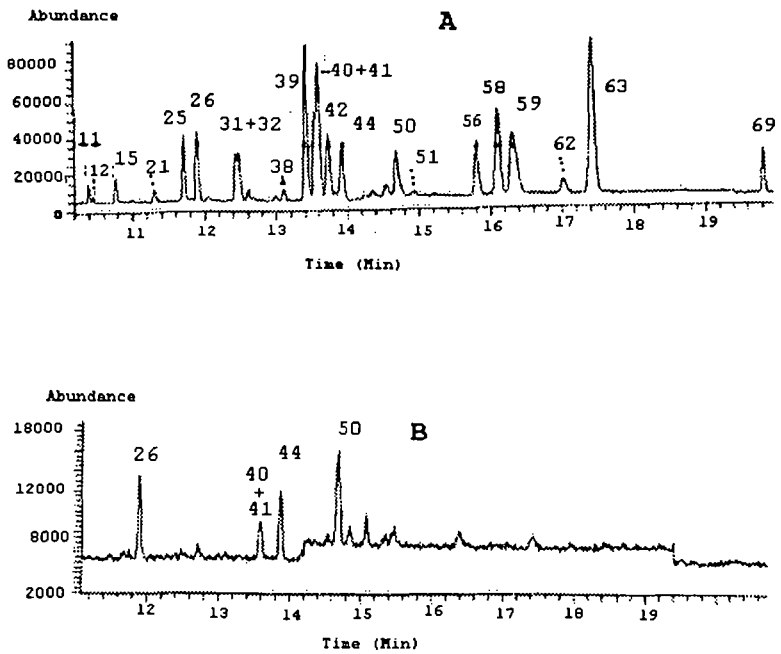

Figure 1 TIC chromatograms of Toxaphene congener mixture(A) and serum extract (B)

four peaks acount for 95% of total toxaphene values (2 - 200ppt) in these samples. These findings are in agreement with our earlier results on whole blood[5]. Similarly, these four peaks constitute about 90% of the total Toxaphene in adipose tissue[6]. The use of a new congener standard has facilitated positive identification and quantification of toxaphene .

Furthermore, the confirmation of toxaphene peaks in serum extracts was carried out by removing PCB and other interferences using nitration. Experiments were conducted to check the efficency of nitration procedure[7]. A standard solution containing 35 PCB congeners (100 ppb each) was subjected to nitration. After work - up and reconcentration no peaks were observed in the retention windows of these analytes either by GC/MSD or GC / NCIMS method.

Conclusions

We have demonstrated that the use of Toxaphene congener standard and GC / NCIMS system permits rapid, routine and confirmatory screening for Toxaphene congeners in human blood/serum. Method provides sufficient sensitivity and selectivity for the analysis of biological samples such as serum and blood. These developments in Toxaphene analysis will enhance the baseline studies in human exposure and toxicological interpretations of results.

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