Quantitative determination of Toxaphene congeners in human serum by high resolution GC / high resolution MS analysis

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Introduction

Technical toxaphene is a complex mixture of several hundred polychlorinated C_{10} terpenes, with the chlorinated bornanes predominating [1]. More than a million tons of this insecticide has been produced worldwide. US and European production was halted in the 1980's due to concern about its toxicity, but certain congeners are very persistent in the biosphere, and have for example been identified in human breast milk [2] and in fish, birds and seals [3] in Sweden although it is reported not to have been used in large amounts in this country [2].

The commercial availability of some of the more environmentally relevant toxaphene congeners in pure form in recent years has led to a considerable improvement in the ability to quantitate these congeners in biological matrices [4]. In this paper we report a high sensitivity method for high resolution GC / high resolution MS quantitation of toxaphene congeners in human serum.

Experimental

Standards

Mixtures of toxaphene congeners were obtained from Ehrenstorfer (Augsburg, Germany) via Axact Standards (Commack, NY) as listed in Table 1 using the nomenclature of Parlar [5]. $^{13}C_{12}$ labelled PCB 77 internal recovery standard was obtained from Cambridge Isotopes.

Sample Cleanup Procedures

Toxaphene congeners were extracted from 4 ml aliquots of pooled human serum and cleaned up according to an alumina microcolumn method [6] without major modifications. Recovery was estimated at more than 50% using this method.

GC/MS Analysis

All analyses were performed using a Micromass 70SE-4F magnetic sector mass spectrometer operated in the SIM mode at a resolving power of 10,000 (10% valley), interfaced to an HP 6890 GC. Split/splitless injection with the injector at 200° C was followed by separation on a 20m long, 100μm ID, 0.1μm film thickness DB-5 column, using He at a constant velocity of 23 cm/s. The

oven temperature program was as follows: initial temperature of 70° C, hold for 1 minute; ramp to 165° C at 20° /min, hold 1 minute; ramp to 280° C at 15° /min, hold 3.5 minutes. The transfer line was held at 280° C and the ion source was at 170° C. (Although this resulted in some cold-trapping, a low source temperature gave the highest sensitivity for toxaphenes).

The dichlorotropylium fragment ions from toxaphenes at m/z 158.9768 and 160.9739 ($C_7H_7Cl_2$) were monitored by EI at 35 eV, along with the 162.0872 biphenyl fragment ion from the isotopically labelled PCB. Mass calibration was performed using hexadecane, since a significant background interference was found to be present on the toxaphene 158.9768 channel when the more usual perfluorinated calibrants PFK and FC-43 were employed.

Quantitation was carried out with the mixture of 5 congeners, Parlar no.s 26, 32, 50, 62 and 69 (Table 1) using the labelled PCB 77 as a recovery standard. No correction for serum lipid content was made.

Parlar Number	IUPAC Name
11	2,2,3-exo,8,9,10 hexachlorocamphene
12	2-exo,3-endo,8,8,9,10 hexachlorocamphene
15	2-exo,3-endo,7,8,9,10 hexachlorocamphene
21	2,2,5,5,9,10,10 heptachlorobornane
25	2,2,3-exo,8,8,9,10 heptachlorocamphene
26	**2-endo,3-exo,5-endo,6-exo,8,8,10,10 octachlorobornane**
31	2,2,3-exo,8,8,9,9,10 octachlorocamphene
32	**2,2,5-endo,6-exo,8,9,10 heptachlorobornane**
38	2,2,5,5,9,9,10,10 octachlorobornane
39	2,2,3-exo,5-endo,6-exo,8,9,10 octachlorobornane
40	2-endo,3-exo,5-endo,6-exo,8,9,10,10 octachlorobornane
41	2-exo,3-endo,5-exo,8,9,9,10,10 octachlorobornane
42*	2,2,5-endo,6-exo,8,9,9,10 octachlorobornane
42*	2,2,5-endo,6-exo,8,8,9,10 octachlorobornane
44	2-exo,5,5,8,9,9,10,10 octachlorobornane
50	**2-endo,3-exo,5-endo,6-exo,8,8,9,10,10 nonachlorobornane**
51	2,2,5,5,8,9,10,10 octachlorobornane
56	2,2,5-endo,6-exo,8,8,9,10,10 nonachlorobornane
58	2,2,3-exo,5,5,8,9,10,10 nonachlorobornane
59	2,2,3-endo,6-exo,8,9,9,10,10 nonachlorobornane
62	**2,2,5,5,8,9,9,10,10 nonachlorobornane**
63	2-exo,3-endo,5-exo,6-exo,8,8,9,10,10 nonachlorobornane
69	**2,2,5,5,6-exo,8,9,9,10,10 decachlorobornane**

Table 1. Contents of the 22 component toxaphene mixture. The items highlighted in bold indicate the contents of the 5 component mixture. *- these two components co-elute on DB-5 columns, and hence have been given the same number by Parlar, which is based on elution order.

Results and Discussion

In most laboratories, one of three MS methods have been used to characterise toxaphene congeners, namely negative ion chemical ionisation (also called electron capture negative ionisation), in which the $[M-Cl]^-$ or $[M]^-$ species are monitored, positive EI in which $[M-Cl]^+$ or $[M-Cl_2H]^+$ and related high mass fragment ions are monitored, and positive EI monitoring low mass fragment ions. For a recent review of these three methods, refer to Lau et al., ref [7]. A fourth MS method described in the literature involves MS/MS quantitation [8], but we have not evaluated this approach.

Both methods for monitoring the high mass ions suffer from the disadvantage that the various toxaphene congeners exhibit markedly different relative responses; an approximately 250-fold difference has been reported [9]. In agreement with this finding, in our hands Parlar 69 produced almost no negative ion signal by methane CI, although it gave an intense spectrum when analysed by positive EI. One of the main criticisms of the low mass EI method in which the ubiquitous dichlorotropyllium ions at 159 and 161 are monitored has been the relatively low sensitivity of this approach [7], with detection limits of around 10ppb in fish [10], for example.

Most authors report using PFK as the mass calibrant / lockmass in high resolution analyses of the toxaphene 159 and 161 fragment ions (the PFK being introduced continuously to the mass spectrometer ion source, to provide a real-time correction for instrumental drift during the analyses). However, the use of hexadecane as an alternative lockmass compound is at least in part responsible for the significantly improved instrumental detection limit obtained in our studies, of around 2 to 5 pg on column for Parlar 26, 32, 50, 62 and 69 standards in the 5 congener mixture (Table 1).

At present our method detection limit is around 25 parts per trillion for Parlar 26, 32 and 50 when spiked in human serum, as shown in Figure 1, and experiments to further improve the sample clean up procedure are continuing. Figure 2 shows the analysis of the 22-congener standard mixture under identical conditions.

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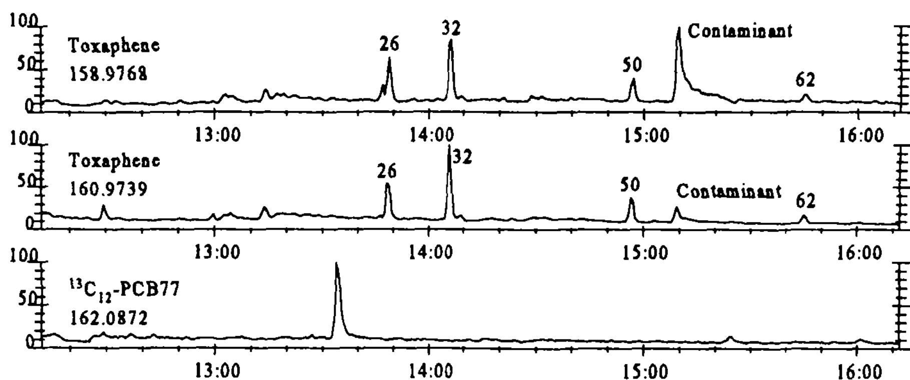

Figure 1. Ion chromatograms from the 10,000 resolution SIM GC/MS analysis of human serum spiked with 50 parts per trillion of toxaphene 5-congener mixture. Upper and middle traces: toxaphene m/z 159 and 161 fragment ion channels. Note that the peaks labelled 'contaminant' are not derived from a toxaphene congener because the 159/161 ratio is incorrect. Bottom trace: labelled PCB m/z 162 fragment ion used as a recovery standard for quantitation.

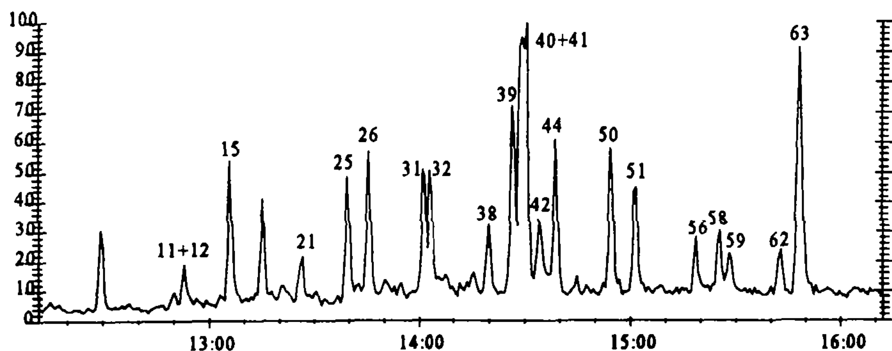

Figure 2. Ion chromatogram from the 10,000 resolution SIM GC/MS analysis of the 22-congener toxaphene standard mixture (only the m/z 161 fragment ion channel is shown).

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