QUANTIFICATION OF TOXAPHENE IN FISH OILS

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Abstract

Toxaphene[R], one of the most important chlorinated hydrocarbon insecticides, is very difficult to determine, because it is a complex mixture of more than 200 substances, primarily polychlorinated bornanes [1-5]. In the GC-experiments interferences with other chlorinated hydrocarbons cannot be excluded [6-8]. Some of them can be separated by column chromatography [9,10]. Unfortunately, any additional clean-up procedure reduces recoveries and sensitivities. A better way is to use the negative ion mass spectrometry - selected ion monitoring (NICI-SIM) [11-14]. The GC analyses of fish samples are furthermore complicated by changes in the original composition of the chlorobornanes due to biotic and abiotic conversions [9]. Differences in the degree of chlorination or in positional isomerization influence NICI-SIM response significantly [8]. Quantification using a technical standard can therefore lead to erroneous results. Considering conversion and accumulation processes of toxaphene components in the environment, a chlorobornane mixture was prepared, yielding a GC-NICI-SIM chromatogram, which resembles very much that of the residues obtained from different fish tissues. It was demonstrated, that this mixture can be used as representative standard for the exact quantification of toxaphene residues in fish and fish products.

Due to the biotic and abiotic transformation processes in the environment, chlorinated bornanes are converted to partly dehalogenated or dehydrohalogenated chlorobornanes, which can be bioaccumulated by organisms [5,15].

The GC-NICI-SIM chromatograms of the extracts of representative fish samples confirm these results and show, that the peak patterns of the extracts are completely different from those of the original mixture [12-14] (Fig. 1, A and C). Most of the transformation products are monodechlorinated chlorobornanes or chlorobornenes with shorter retention times than those of their parent compounds.

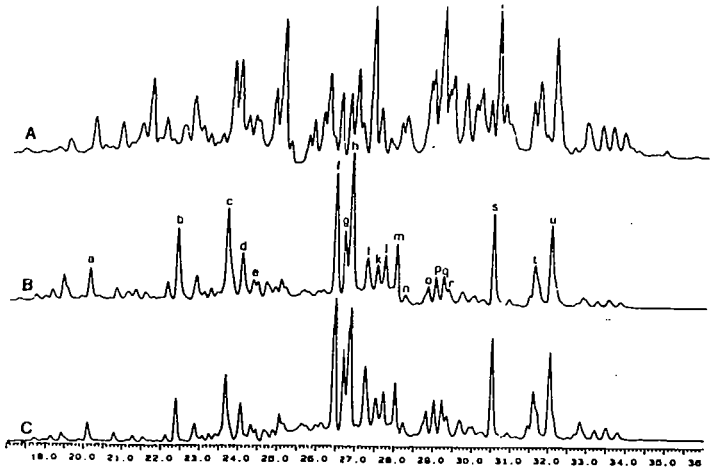

Fig. 1: GC-MS/NICI-SIM spectra of a technical toxaphene standard (A), the new analytical standard (B) and an extract of a cod liver oil (C) (clean-up procedures[10])
[HPS988A, HP-1 column, 25 m x 0,2 mm x 0,3 µm; 140 °C/4 °C min - 250 °C (15 min); splitless injector (1,5 min) 230 °C; transfer line temp: 280 °C; ion source temp: 100 °C; CH_4 as reactant gas]

Unfortunately, the conversion products usually are accompanied by decreased responses when using the NICI–SIM method. Because of these significant differences in the detector sensitivities an exact quantification by technical toxaphene mixture as a standard is not possible.
These problems can easily be overcome by using analytical standards with the same peak pattern as found in fish or fish oil extracts. Environmental transformation pathways and accumulation tendencies of toxaphene components can be simulated under laboratory conditions. UV radiation of a solution of technical mixture in oxygen free n-hexane with wavelengths at 254 nm for 2 hours leads to a partly dechlorinated and dehydrochlorinated mixture, whose chromatographical separation by a silica gel column and n-hexane as eluant gives a main fraction [Fig. 1, B]. The GC-NICI-SIM chromatogram of this fraction resembles that of toxaphene residues found in the lipid-free fish extracts [Fig. 1, C], thus fulfilling all requirements for an analytical standard.

The ions 340/342; 341/343; 375/377 and 411/413 are used for the quantification, which are common to both families of compounds. While many of the chlorinated hydrocarbon interferences are not a problem, when monitoring for selected fragments, there are several substances that give ions common to those of toxaphene residues. These especially include dieldrin, heptachlor and technical chlordane which contains cis- and trans-chlordane, respectively cis- and trans-nonachlor as major components[12].

These interferences however yield a small molecular ion cluster and almost no $(M-Cl)^-$ ion cluster in NICI. As a result, the ions that cyclodieninsecticides do produce are different from those arising from toxaphene residues, and there are no mutual interferences. Other potential interferences, including p-p'-DDT, p,p'-DDD and p,p'-DDE, can be satisfactoraly eliminated by selected retention time windows for each of the 20 main peaks (see Table 1).

Table 1. GC-MS/NICI characterization of the new standard and selected cod liver oil extracts (Rt_1 = absolute retention time in min; Rt_2 = relative retention time to DDE = 1.00; Rt_W = retention time windows for NICI/SIM experiments)

Peak	Rt_1	Rt_2	Rt_W	new standard	% in oil 1	% in oil 2	main product
a)	20.10	0.779	20.00 - 20.20	1.08	0.35	0.28	Hexachlorobornene
b)	22.35	0.889	22.25 - 22.50	5.64	1.79	2.00	Heptachlorobornene
c)	23.64	0.940	23.50 - 23.90	5.23	2.15	2.10	Heptachlorobornene
d)	24.03	0.955	23.90 - 24.10	3.38	2.50	2.35	Heptachlorobornane
e)	24.30	0.966	24.20 - 24.50	1.35	1.00	1.20	Heptachlorobornene
f)	26.40	1.050	26.20 - 26.50	9.84	11.07	10.50	Heptachlorobornene
g)	26.66	1.060	26.50 - 26.70	2.24	3.90	3.95	Octachlorobornene
h)	26.80	1.066	26.70 - 27.00	13.59	12.19	12.00	Octachlorobornane
j)	27.22	1.082	27.10 - 27.30	3.69	4.50	5.05	Octachlorobornene
k)	27.46	1.092	27.40 - 27.60	1.34	1.57	1.47	Octachlorobornene
l)	27.68	1.101	27.60 - 27.80	3.38	2.25	2.75	Octachlorobornane
m	27.98	1.113	27.90 - 28.10	2.16	1.75	1.95	Octachlorobornane
n)	28.19	1.121	28.10 - 28.30	0.38	0.25	0.30	Octachlorobornane
o)	28.79	1.145	28.70 - 28.90	1.68	1.60	1.60	Octachlorobornane
p)	28.97	1.152	28.90 - 29.10	1.93	1.70	1.85	Octachlorobornane
q)	29.17	1.160	29.10 - 29.20	2.09	2.00	2.05	Octachlorobornane
r)	29.32	1.166	29.20 - 29.40	1.70	2.06	2.17	Octachlorobornane
s)	30.47	1.212	30.30 - 30.60	7.05	8.08	7.75	Nonachlorobornane
t)	31.56	1.255	31.40 - 31.70	7.81	8.00	8.35	Nonachlorobornane
u)	31.98	1.272	31.80 - 32.20	7.79	6.90	10.80	Nonachlorobornane

Preparation of the analytical standard

100 mg Toxaphene (Merck Ag, FRG) in 100 ml oxygen free n-hexane were irradiated in 10 quarz tubes (l = 35 cn, Ø = 0,5 cm, thickness = 1 mm) with wavelengths at 254 nm (Vycor 250 mA/500 V, Fa. Graentzel, FRG). Each of the irradiated solutions was freed from solvent, redissolved in 1 ml n-hexane and separated with the help of column chromatography (Silica gel 60 (Merck AG, FRG), achieved at 140 °C and subsequently desactivated with 5 % water; l = 29 cm, Ø = 0,8 cm; n-hexane as solvent, eluation speed: 7.1 ml/min at low pressure (0,3 bar)) into three 9 ml fractions. The collected fast fractions were freed from solvent under reduced pressure (10^{-2} torr) and gave 40 mg of the new standard.

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