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Determination of Toxaphene Residues in Fish Foodstuff by GC–MS

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Abstract A method for the determination of toxaphene residues in fish and fish-based baby foods has been developed. The cleanup of the fatty matrices was performed using an acid treatment on an Extrelut-NT3 and ENVI-Florisil SPE cartridges system, using light petroleum as eluent. Instrumental analysis was carried out by gaschromatography with mass spectrometry detector (GC/MS) in SIM mode. Recoveries from spiked samples were tested at 0.005 and 0.01 mg/kg per single congener and were in the range 82–104% while relative standard deviations (RSDs) were in the range 3.7–10.9%. Nineteen samples of both frozen fish food and fish-based baby foods representative of the Italian market were collected from local dealers and analysed.

Keywords Toxaphene · Pesticides · Residues analysis · Mass spectrometry · Food analysis · Fish analysis

Toxaphene is a complex mixture of at least 177 chlorinated bornanes containing 67%–69% chlorine; the ISO term for this product is Camphechlor (Andrews et al. 1995).

Toxaphene entered the environment as an insecticide. Before 1982 it has been widely used in the US and in many other countries (Voldnen and Li 1995). It was primarily used on cotton, soybeans, peanuts and maize. In fact, it replaced dichlorodiphenyltrichloroethane (DDT) as a major agricultural insecticide when it was withdrawn from the market (Saleh 1991).

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Toxicological studies confirmed the carcinogenic and mutagenic properties of the technical mixture. In the United States, Toxaphene was banned for most uses in 1982 and all uses were banned in 1990.

The use of Camphechlor was similarly banned in Italy since 1985.

Toxaphene has recently been observed to have also estrogenic effects and it may acts as an endocrine disruptor (Saleh 1991). In addiction, toxaphene is classified as a probable human carcinogen (US EPA 1999).

Since Toxaphene is relatively persistent and highly mobile it can accumulate in the environment far from application places (Saleh 1991). It is now realized that Toxaphene represents a global menace like polychlorinated biphenyls (PCB) and organochlorine (OC) pesticides.

Residues of Toxaphene have been found in products of vegetal and animal origin, human milk and in the environment (Bidleman et al. 1995; Vaz and Blomkvist 1985). Freshwater and marine fishes are among the most remarkable species for their relatively high content of Toxaphene (Alder et al. 1997; Bidleman et al. 1993).

General population, children and other sensitive subpopulations may be exposed at great risk for toxic effects due to Toxaphene residues in food (Mazur 2003) so it is very important to monitor the foodstuffs and baby foods for this class of compounds.

Italian legislation, in agreement with the EU legislation, allows a maximum residue limit (MRL) for toxaphene in fruits, vegetables and cereals of 0.1 mg/kg, regarded as the limit of quantification. MRLs have not been set for products of animal origin and then a conventional "zero" value has been established at 0.01 mg/kg.

Special attention has been paid to baby food by national and European authority: each pesticide is tolerated to be

present in baby foods at a level not exceeding 0.01 mg/kg (European Commission Directive 99/39/EC).

Capillary GC coupled to electron capture detector (GC-ECD) or to a mass spectrometer detector (GC-MSD) are the most commonly used detection tools for the analysis of toxaphene in biological and environmental samples at residue levels (Chan et al. 1998; De Boer and De Geus 1997; Krock et al. 1997).

We have developed a MS detection-based method coupled with fast, easy extraction and cleanup steps, suitable for monitoring of toxaphene residues in fish foodstuffs.

Materials and Methods

Thirteen different fish samples of frozen foodstuff and six fish-based baby foods, representative of the Italian market, were collected from local retailers (Table 1).

The frozen samples were stored at −20°C until the analysis. The samples of baby food were stored at ambient temperature. Each sample was homogenised with a Buchi Mixer B–400 apparatus.

Technical toxaphene mixture and toxaphene reference standards (cyclohexane solutions at 1 μg/mL of congeners Parlar 26, 32, 39, 44 and 50) were provided by Dr. Ehrenstorfer (Augsburg, Germany).

Pesticide grade solvents were used and obtained by Carlo Erba (Milan, Italy). Concentrated sulphuric acid (95%–97%) was supplied by Riedel–de Häen (Germany). Anhydrous sodium sulphate (p.a.) was supplied by Merck (Darmstadt, Germany).

Ready-to-use Extrelut-NT3 cartridges (code no. 1.15095.0001) were supplied by Merck (Darmstadt, Germany), while ready-to-use ENVI-Florisil SPE cartridge (0.5 g, cat. No. 5-7046) were obtained from Supelco (Bellefonte, USA).

Twenty grams of homogenate were weighed in a 250 mL centrifuge tube and then extracted with light petroleum 40–60°C + acetone (100 mL; 1+1, v+v) homogenizing with the Ultra Turrax T25 apparatus (IKA, Janke and Kunkel, equipped with an S25 dispersing tool) for 2 min at about 9,500 rpm. The mixture was centrifuged at about 1,500 rpm for 10 min.

The organic phase was passed through a column of sodium sulphate (25 g in a glass tube, 200 × 20 mm i.d.) and the eluate was collected into a 250 mL Erlenmeyer flask (weighed at ±0.01 g). The extraction was repeated with 2 × 50 mL portions of light petroleum.

The extracts were concentrated under vacuum to a small volume (1–2 mL) by rotary evaporator and to dryness by manually rotating the flask in order to avoid the loss of volatile compounds. The lipidic residue was weighed and then redissolved in light petroleum. The analytes were purified by a combination of a chemical treatment with concentrated sulphuric acid (H_2SO_4) and Florisil chromatography.

Table 1 List of various fish samples analysed

Frozen fish foodstuff (fishing area)	Fish-based baby food
Hake (Atlantic Ocean, South West)	*Hake*
Tattler (Atlantic Ocean, South West)	*Salmon*
*Mussel (*Pacific Ocean)	*Plaice*
Cuttlefish (Pacific Ocean)	*Cod*
Clam (Mediterranean Sea)	*Trout mark a*
Shrimp (Indian Ocean)	*Trout mark b*
Sole (Atlantic Ocean, North East)	
Grouper	
Cod mark a (Pacific Ocean, South East)	
Cod mark b (Atlantic ocean, South East)	
Plaice mark a (Atlantic Ocean, North East)	
Plaice mark b	
Swordfish (Indian Ocean)	

The acid treatment was carried out by loading an Extrelut–NT3 cartridge with 3 mL of concentrated sulphuric acid solution. The acid solution was allowed to drain into the cartridge waiting 10 min to obtain an even distribution into the filling material.

A 2 mL light petroleum solution containing up to 1 g of the fatty residue was then applied to the cartridge.

A florisil cartridge, previously rinsed with 2 × 2 mL of *n*-hexane + toluene (80 + 20 v/v) and activated with 2 × 2 mL of light petroleum, was connected to the end of the Extrelut-NT3 and the system of the combined cartridges was eluted with 10x2 mL portions of light petroleum. The eluate was collected in a 50 mL flask and concentrated to a small volume (1–2 mL) by rotary evaporator (bath temperature 40°C, reduced pressure) and then to dryness by manually rotating the flask.

The final extract was dissolved in 1 mL of iso-octane and analysed by GC–MS.

Gas chromatographic analysis was performed with a GC Agilent System 6890 Series Plus. Split/splitless injector operating in pulsed-splitless mode. Fused silica capillary column: HP-5MS, 30 m × 0.25 mm × 0.25 μm film thickness. Carrier gas: helium; flow rate 1.5 mL/min. Electronic pressure control (EPC): constant flow mode. Oven temperature program: 60°C (initial isotherm: 2 min); up to 160°C at a rate of 10°C/min and then up to 260°C at a rate of 3°C/min (final isotherm: 20 min).

The gas chromatograph was coupled with a quadrupole Agilent 5973 Mass Selective Detector (MSD) set at 70 eV. Ion source temperature: 200°C.

The instrument was operated in selected ion monitoring (SIM) mode. The selected m/z values for each congener are reported in Table 2.

Table 2 List of the investigated toxaphene congeners

Parlar's N	IUPAC Name	Formula	M.W.	Selected ions (m/z)
# 26	2-exo, 3-endo, 5-exo, 6-endo, 8b, 8c, 10a, 10b-Octachlorbornan	$C_{10}Cl_8H_{10}$	410	159, 161, 1 95
# 32	2,2,5-endo, 6-exo, 8b, 9c, 10a- Heptachlorbornan	$C_{10}Cl_7H_{11}$	376	159, 161, 195
# 39	2, 2, 3-exo, 5-endo, 6-exo, 8b, 9c, 10a-Octachlorbornan	$C_{10}Cl_8H_{10}$	410	159, 161, 195
# 44	2-exo, 5, 5, 8b, 8c, 9c, 10a, 10b- Octachlorbornan	$C_{10}Cl_8H_{10}$	410	195, 375, 377, 379
# 50	2-exo, 3-endo, 5-exo, 6-endo, 8b, 8c, 9c, 10a, 10b-Nonachlorbornan	$C_{10}Cl_9H_9$	444	195, 375, 377, 379

Blank fish samples were fortified at 0.005 mg/kg and 0.01 mg/kg with a mixture of the single congeners Parlar 26, Parlar 32, Parlar 39, Parlar 44 and Parlar 50. For total commercial camphechlor, the blank samples were fortified at 0.25 and 0.5 mg/kg as total toxaphene. Recoveries were performed in six replicates at each fortification level. Table 3 shows the results of these experiments reporting the average recoveries and the standard deviations (s.d.) at each spiking level. The external standard quantitation method was chosen for quantitative analysis.

The calibration curves were obtained by plotting peak area versus concentration. In Table 4 are reported the calibration data (equation of the curves and linear correlation coefficients) and the limit of detection for each congener.

Results and Discussion

Analysis of fish samples for Toxaphene residues requires an efficient cleanup method and a selective method of detection because of the presence of contaminants of similar analytical behaviour. In this methodology, the fatty residue (up to 1 g) was purified in less than 30 min by a simple procedure based on the use of concentrated sulphuric acid, light petroleum and a system of Extrelut-NT3 and ENVI–Florisil SPE cartridges.

Table 3 Recoveries (%) of specific toxaphene congeners spiked in 10 g (baby food) homogenized fish at 0.005 and 0.01 mg/kg

Compound	Spiked level (mg/kg)	Recovery (%) (mean, n = 6)	RSD (%)
Parlar 26	0.005	92.4	3.7
	0.01	102.5	8.9
Parlar 32	0.005	86.0	8.4
	0.01	102.5	9.0
Parlar 39	0.005	81.6	10.9
	0.01	101.4	8.3
Parlar 44	0.005	90.2	6.5
	0.01	104.0	10.4
Parlar 50	0.005	97.5	5.3
	0.01	102.3	7.5

Table 4 Calibration data (in the range 0.05–0.2 μg/ml) and limits of detection of each specific congeners

Compound	Calibration equation	r^2	Limits of determination (mg/kg)
Parlar 26	$y = 579881x + 4732.5$	0.9995	0.0016
Parlar 32	$y = 407829x + 3930$	0.9997	0.003
Parlar 39	$y = 507324x + 2022.5$	0.9991	0.003
Parlar 44	$y = 625906x + 3789$	0.9999	0.0016
Parlar 50	$y = 282279x + 3270.5$	0.9989	0.003

Extrelut-NT3 columns are ready-to-use, disposable glass cartridges filled with a macro porous diatomaceous earth, already extensively tested in our laboratory as a solid support to carry out the cleanup step of fatty matrices (Di Muccio et al. 1997, 2002).

In this procedure, the cartridges were used as a solid support to perform the acid treatment. The role of concentrated sulphuric acid was to digest the fat and to degrade organic substances while SPE Florisil cartridge was used in order to achieve a good cleanup in terms of removal of the interferences.

In an early stage of method development, technical Toxaphene mixture was used to investigate the method; two spiking levels were studied with good results: at 0.25 mg/kg the average recovery was 87.7% while at 0.5 mg/kg was 91.9%.

With up to 1g of fatty matrix loaded into the Extrelut cartridge, the amount of fat residue after the cleanup was 2.1 ± 0.8 mg (mean ± SD, n = 15), giving a good average efficiency (about 99.6%) in removing the fat. The final solution was colourless and clear, ready to GC/MS analysis without the need for further purification step.

For the monitoring and confirmatory analysis, the selected ion monitoring (SIM) mode was chosen, using two acquisition groups with different selected m/z values in the same GC run.

In the first group we have selected m/z 159, 161 and 195 ions, common ions for all congeners. This group is used to monitor Parlar 26, 32, 39 congeners.

In the second group we chose to select a different set of ions at m/z 375, 377 and 379 together with the ion at m/z

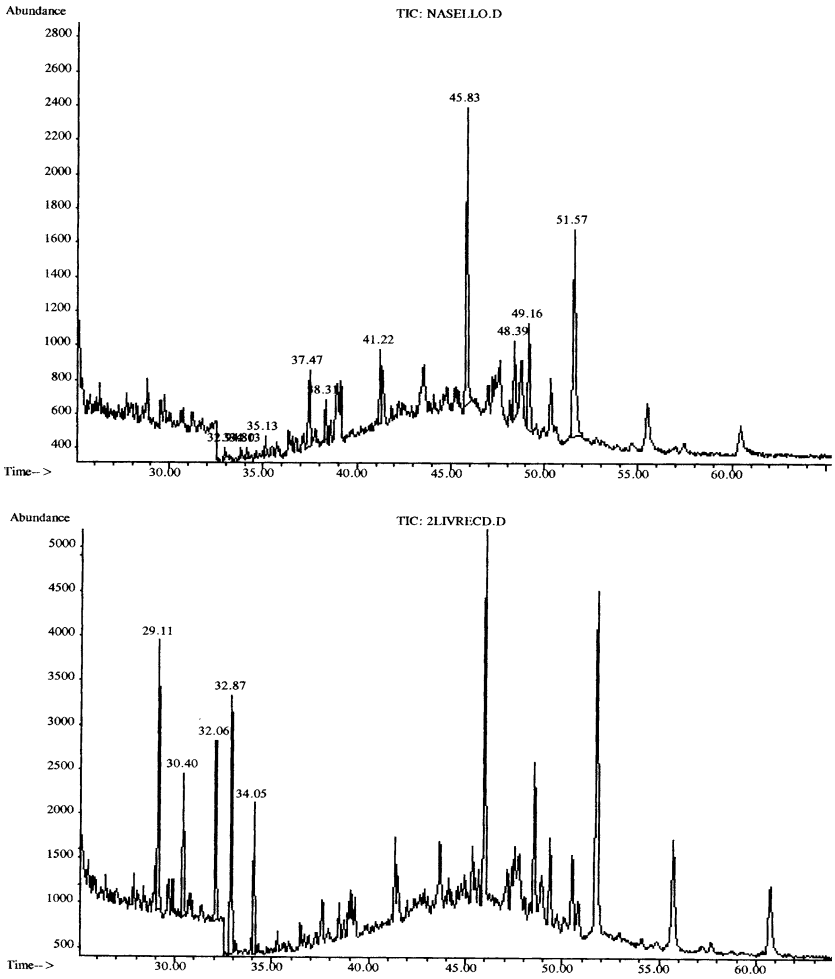

Fig. 1 GC–MS chromatogram of Hake sample (found blank) and GC–MS chromatogram of the Hake sample spiked with mixture of 5 Toxaphene congeners at level 0.005 mg/kg. Elution order: Parlar 26, Parlar 32, Parlar 39, Parlar 44, Parlar 50

195 always present in any of the analysed congener. This second group is used to monitor Parlar 44 and 50 that show a high abundance of the m/z 375, 377, 379 ions. In that way we reduced the chromatographic noise and the sensitivity was increased.

Figure 1 shows a representative chromatogram of an extracted Hake blank sample and a chromatogram of the same sample spiked with five specific toxaphene congeners at 0.005 mg/kg each. The blank sample chromatogram shows no interfering peaks in the areas of interest, so the specificity of the method is assured.

The recovery and relative standard deviation (RSD) results reported in Table 3 provide that the method is accurate and repeatable. Average recoveries (n = 6) at the investigated spiking levels (0.005 and 0.01 mg/kg) were respectively: 92.4% and 102.5% for Parlar 26; 86.0% and 102.5% for Parlar 32; 81.6% and 101.4% for Parlar 39; 90.2% and 104% for Parlar 44; 97.5% and 102.3% for Parlar 50. These values fall in the range 70%–110% that is commonly accepted for pesticide residues analysis.

The repeatability expressed as RSD was found out in the range 3.7%–10.9% and 7.5%–10.4% for specific congener at 0.005 and 0.01 mg/kg, respectively. A good precision was indicated by these results.

A linearity check was performed for all the congeners in the range of 0.05–0.2 μg/mL; the correlation coefficients (r^2) show a good linearity for all investigated congeners.

The limit of detection can be defined as the lowest concentration giving a signal to noise ratio of 3. Limits of detection for investigated specific congeners were estimated in the range 0.0016–0.003 mg/kg.

The limit of quantification (LOQ) for this method was defined as the lowest concentration of compounds in a sample that could be quantitatively determined with suitable precision and accuracy: LOQ was assumed to be 0.005 mg/kg for every congener, corresponding the lowest investigated spiking level. Our method shows enough sensitivity to guarantee the analysis of fish-based foodstuff at the residue limits set by EU and Italian regulation.

No detectable residues for any toxaphene congener were found in the investigated samples, indicating the positive, favourable long term effect of regulatory measures.

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