ANALYSIS OF THREE SPECIFIC TOXAPHENE CONGENERS IN FISH AND FEEDINGSTUFFS BY ION TRAP HRGC-EI-MS/MS

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Introduction

Toxaphene is the term used to refer to a complex mixture primarily consisting of chlorinated bornanes (CHBs). This widely used insecticide was mainly applied to cotton, cereal grains, fruits, nuts and vegetables and was used to control ticks and mites in livestock [1]. It has been one of the most heavily used pesticides in the world [2]. Although toxaphene was banned in Western Europe and North America in the seventies and eighties because of its environmental peristence and toxicity, it is still applied in many countries [3].

Toxaphene is among the most dominant organochlorine contaminants in fish and especially marine mammals compared to other organochlorine pollutants such as PCBs and DDTs [4]. It has a high potential to bioaccumulate in lipid rich tissues of aquatic organisms and it is biomagnified through marine food chains [1]. Although there are no data regarding long term biological effects of toxaphene in wildlife species [5], laboratory experiments on rodents have shown neurotoxic, nephrotoxic and hepatotoxic properties [5, 6]. *In vitro* studies also suggest endocrine toxicity and carcinogenic and genotoxic effects [6]. Recently, it was found that toxaphene may negatively affect soil organisms [7].

Regarding EU legislation, Commission Directive 2005/86/EC on undesirable substances in animal feed, establish a total toxaphene residue limit as sum of indicator congeners (P#26, P#50 and P#62) in fish (and their products and by-products), fish oil and feedingstuffs of 0.02, 0.20 and 0.05 mg/Kg, respectively [8].

In this study, a methodology for the analysis of the three indicator toxaphene congeners in fish and feedingstuffs based on the use of HRGC-MS/MS is presented. The different parameters affecting the MS/MS detection have been optimized and discussed for each individual congener. The application of the method to both fish and feedingstuffs matrices is also shown.

Material and methods

Chemicals and standards

A standard mixture of three toxaphene congeners containing Parlar#26, Parlar#50 and Parlar#62 in iso-octane at a concentration of 4.79, 4.87 and 4.80 ng/µL, respectively was purchased from LGC Promochem (Wesel, Germany). 4,4'-DDT-D8 (100 ng/µL in acetone) was provided by Dr. Ehrenstorfer GmbH (Augsburg, Germany). It was adequately diluted to 4,4 ng/µL with iso-octane. This solution was kept refrigerated and in the dark until used as internal recovery standard. $^{13}C_{12}$-PCB#101 was obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Dilution of 4 ng/µL was prepared in iso-octane and kept refrigerated until used as internal injection standard.

All HRGC grade solvents were provided by Merck (Darmstadt, Germany). Sulphuric acid (Merck) was pro analysis quality. Anhydrous sodium sulphate was obtained from Sigma-Aldrich (Steinheim, Germany). Silica gel 60 from Panreac (Barcelona, Spain) was extracted with dichloromethane and methanol prior to its use.

Sample preparation

Sample preparation for the determination of toxaphene consisted of a three steps procedure. 5 g of homogenized muscle fish or feedingstuff sample was chemically dried with 10 g of anhydrous sodium sulphate for 24h. The sample and sodium sulphate mixture was ground with 1,5 g of silica gel in a ceramic mortar until a free flowing mixture was obtained. The sample was then packed into a glass column and spiked with 5 µL of the labelled 4,4'-DDT-D8 (4,4 ng/µL). Contaminants and lipids were extracted with 150 mL of a dichloromethane:hexane (1:1) solution. Sample was rotary evaporated until a volume about 10 mL for fish matrices and 25 mL for feedingstuff samples. Following, in the case of fish samples, the extract was cleaned-up on a three layer column chromatography, consecutively containing one layer of neuter silica and two layers of sulphuric acid modified silica at 22% and 44% (w:w). The column was topped and bottomed with 1 cm of anhydrous sodium sulphate and conditioned with 150 mL of hexane. Sample was loaded and then eluted with 150 mL of hexane and collected in round bottom flasks. Eluate was concentrated on a rotary evaporator to about 5 mL. In the case of a feedingstuff sample, the hexane extract was repeatedly treated with sulphuric acid (25 mL) with the aim of remove lipids. Clean hexane layer was then concentrated to 5 mL.

In both cases, the latter extract was fractionated on 5 g of neutral silica previously deactivated with 2% of water. A first 10 mL hexane fraction was discarded. The second 40 mL dichloromethane:hexane (1:1) fraction containing toxaphene congeners of interest and the internal standard was evaporated to a volume of about 1 mL. Then it was transferred to a conical bottom injection vial and evaporated to dryness under a gentle nitrogen stream. Finally, the sample was reconstituted with 40 µL of $^{13}C_{12}$-PCB#101 (100 pg/µL) for instrument performance control and analyzed by GC-MS/MS.

Instrumentation

Analyses were performed on a Varian gas chromatograph CP-3800 (Palo Alto, CA, USA) equipped with a Varian CP-8200 autosampler and a programmable split/splitless injector. Samples were injected in the splitless mode (1 µL; 250ºC; split ratio: 30) and separations were carried out in a capillary BPX-5 column (60 m, 0.25 mm i.d., 0.25 µm film thickness) obtained from SGE (Griesheim, Germany). The oven temperature was programmed from 60ºC (3 min) to 200ºC (3 min) at a rate of 30ºC/min, then to 270ºC at 5ºC/min and held for 20 min. Helium was used as the carrier gas at a constant flow rate of 1 mL/min.

The GC was coupled to a Varian Saturn 2000 ion trap mass spectrometer. The temperature of the transfer line and the ion trap were set at 280ºC and 230ºC, respectively. The ionization was performed by electron impact at 70 eV and the ion trap was operated in MS/MS mode.

Results and discussion

MS/MS detection of toxaphene congeners

A solution containing the three toxaphene congeners at 0.50 ng/µL was used for optimizing all the parameters affecting the detection. Two possible dissociation pathways were studied. The first one, which can be observed in the three congeners, is the dissociation of the m/z 125 as parent ion (I_p) into the m/z 89 as daughter ion (I_d) (general dissociation). The second one involves the dissociation of a high mass I_p into another I_d, being both different for each congener (specific dissociation). In this last case, the selection of the I_p was made according to ions observed in EI mass spectra. With the aim of studying the influence of the voltage applied in collision induced dissociation (CID) mode on the fragmentation of the I_p, the voltage was varied from 0.1 to 1.9 V, applied for 20 ms and in 0.01 V steps. This study was carried out for both general and specific dissociations approaches. The selected parent and daughter ions as well as the suitable voltages obtained are listed in Table 1. Finally, in order to select the best dissociation of I_p into I_d for monitoring and quantifying purposes, chromatograms monitoring each daughter ion were compared in terms of signal-to-noise ratio. In the case of Parlar 26 the best results were obtained using the general dissociation. For Parlars 50 and 62, the specific dissociation using the fragments m/z 279 and m/z 303, as parent ions respectively, significantly enhanced sensitivity. However, when real samples were analyzed, the selection of m/z 279 as parent ion for Parlar 50 produces some disturbances maybe related to sample matrix. This fact was also reported by Skopp *et al.* [9]. The selection of the fragment m/z 303 as parent ion allowed to overcome this limitation for the analysis of Parlar 50 in real matrices. The influence of the CID time on the dissociation of I_p into I_d was also studied. For this purpose selected parent ions for each compound were isolated and stabilized in the resonant mode for 5 ms. The effect of the applied voltage was investigated, but in this case Collision induced dissociation was made during 5 ms. Results were compared with those obtained for 20 ms of CID time in terms of signal-to-noise ratio. Finally, optimized MS/MS parameters for monitoring toxaphene congeners and internal standards are listed in Table 2.

Method evaluation

Method detection limit (LOD) and quantification limit (LOQ) were based in a signal-to-noise ratio of 3 and 10, respectively. Thus, the LODs ranged from 8 to 50 pg/g, whereas the LOQs ranged from 0.48 to 0.96 ng/g wet weight.

The concentration of each congener was calculated using its relative response factor to the one of 4,4'-DDT-D8 in the same sample. Relative response factor (RRF) for each congener was determined using standard solutions where the 4,4'-DDT-D8 concentration was kept constant at 0.55 ng/µL while the toxaphene concentrations varied from 0.06 to 2.40 ng/µL.

Recoveries were determined from fish tissue and feedingstuff spiked with three different concentrations of each indicator compounds in order to obtain concentrations ranging from 0.96 to 20 ng/g wet weight. Original concentrations determined in no spiked samples were taken into account. Recoveries of the three indicator compounds varied between 80 and 116% in fish tissue and between 80 and 107% in feedingstuff. Reproducibility of the method was

acceptable with RSD values between 12 and 14% for the three studied compounds. Figure 1 shows an example of the analysis of a fish sample spiked with 20 ng/g of each congener.

References

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Table 1. Selected voltages for the dissociation of different I_p into I_d for each congener.

P#26			P#50			P#62		
I_p (m/z)	I_d (m/z)	Voltage (V)	I_p (m/z)	I_d (m/z)	Voltage (V)	I_p (m/z)	I_d (m/z)	Voltage (V)
125	89	0.40	125	89	1.01	125	89	1.01
305	269	0.33	279	243	0.85	303	267	0.71
303	267	0.43	303	267	0.70			

Table 2. MS/MS parameters selected for the toxaphene congeners and internal standards.

	P#26	P#50	P#62	PCB#101	DDT-D8
I_p (m/z)	125	303	303	338	243
I_d (m/z)	89	267	267	266	-
Quantification masses (m/z)	89	265+267	265+267	266+268	243
Excitation storage level (m/z)	48	151	151	168	121
Voltage (V)	0.40	0.70	0.71	1.19	0
CID time (ms)	20	5	5	20	-

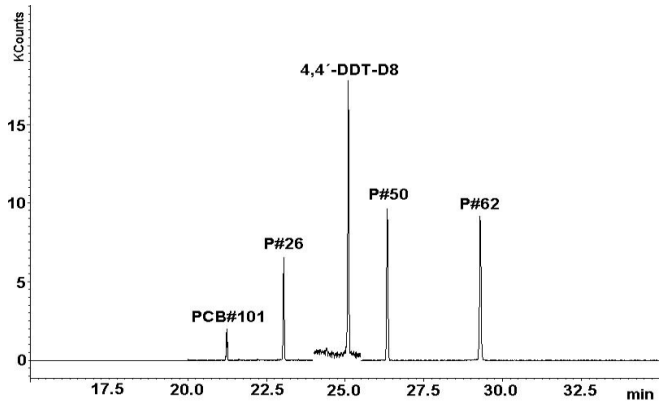

Figure 1. Chromatogram corresponding to the analysis of a fish sample spiked with 20 ng/g of each congener and the internal and injection standards under the optimized conditions.