Journal of Chromatography, 471 (1989) 161–171
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 301

QUANTITATIVE HIGH-RESOLUTION GAS CHROMATOGRAPHY AND MASS SPECTROMETRY OF TOXAPHENE RESIDUES IN FISH SAMPLES

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SUMMARY

This report describes an analytical method which permits the determination of ppb (1 μg/kg) level of toxaphene in fish tissues. Interferences both from biogenic and from xenobiotic substances are reduced even with low-resolution mass spectrometry. The methodology has a low susceptibility to false positive determinations, which could result from the presence of a wide variety of co-contaminants. The method is based on the measurement of a signal representative of the toxaphene residue (*m/z* 158.9) relative to a known amount of an internal standard ^{37}Cl-labelled compound. A modular approach to toxaphene enrichment has permitted a moderately simple procedure, significantly reducing analytical time requirements and the number of sample manipulations, and making the procedure amenable to automation. The reliability and accuracy of the procedure are demonstrated by the results of intra- and interlaboratory studies. The methodology has been validated and the presented data indicate that the detection limit is 1 μg/kg of total toxaphene. Toxaphene recovery from fish at concentration levels between 0.1 and 10 μg/g is 84 ± 12%.

INTRODUCTION

Problems in multiresidue trace analysis represent a major proportion of research activities in environmental applications of mass spectrometry (MS) and gas chromatography–mass spectrometry (GC–MS). The quality of such analyses may be evaluated by an assessment of selectivity, sensitivity and precision. In general, the quantitative MS of environmental contaminants is performed on the basis of a real-time measurement in the selected-ion monitoring (SIM) mode. SIM permits the quantitation of picogram quantities of an analyte. Such low detection limits may not be achievable in analyses of samples containing many congeners where sensitivity may be limited by the simultaneous detection of individual congeners or their characteristic moieties formed during analysis. Improvements in selectivity during the analysis of environmental samples in addition to increasing confidence in the characterization of trace components will affect the accuracy of quantitation. One of the techniques for improving selectivity includes the use of enhanced resolution but it involves a reduction in the absolute signal intensity in MS[1].

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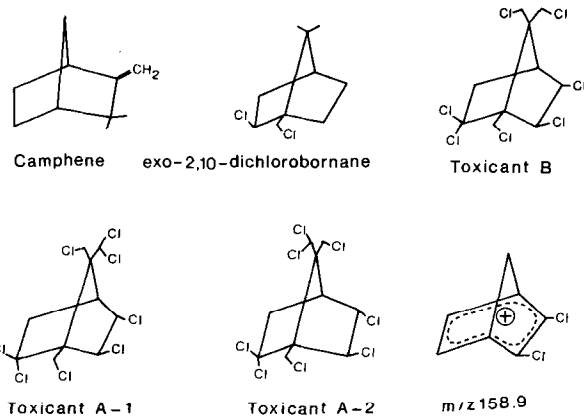


Scheme 1. Some structural moieties assumed to be present in toxaphene mixtures.

Several recent reports indicate that toxaphene is a widespread pollutant[2,3]. Many compounds of environmental interest cannot be determined by GC–MS, since they are either thermally unstable and suffer thermal decomposition at the injector or on the GC column, or else they are too involatile to pass through the system into the ion source. Toxaphene represents a complex mixture of at least 300 separated components by high-resolution gas chromatography (HRGC)[4]. It is known to be toxic to various species of fish. Toxic effects in fish include decreased viability of ova and decreased bone collagen synthesis[5]. The acute toxicity of toxaphene residues to fish is similar to that of endrin and endosulfane[6]. Quantitative determination of toxaphene has presented problems and validated methodology in environmental samples does not exist today[7].

The validity of a technique or a methodology for any environmental samples containing toxaphene depends on it having adequate sensitivity to determine the components and sufficient specificity to ensure the absence of possible interferences. Sensitivity is limited by the number of separated components when HRGC is employed.

Our laboratory has been engaged in investigations related to the development of analytical methodology for toxaphene in environmental samples for the last three years[8].

This paper reports our methodology which has been applied to fish tissue analyses and is based on the direct inlet probe (DIP) MS–SIM analysis. The DIP offers a means of quantifying toxaphene down to low levels. The simple treatment for a multicomponent mixture cannot be applied since the number of isomeric compounds is unknown. However, it is possible to choose a m/z value (158.9) to which the majority of toxaphene constituents contribute and in the SIM mode of operation, the background and possible impurities from biological extracts do not contribute significantly to cleaned-up extracts. It is the aim of this paper to show the general applicability and advantages of the proposed method by presenting an analysis of the data collected from a series of toxaphene determinations and to discuss some of the experimental aspects of the methodology.

EXPERIMENTAL

Fish samples

The collection of fish samples was performed by the Department of Fisheries and Oceans personnel in Burlington (Ontario, Canada) and homogenate fish samples (samples 3 and 4; Serial Nos. 0009, 0033, 1161 and 1154) were obtained from the United States Environmental Protection Agency-Environmental Monitoring Systems Laboratory (Cincinnati, OH, U.S.A.). Our samples were wrapped in hexane-rinsed aluminum foil, frozen, and homogenates were supplied in wide-mouthed dark glass bottles. Whole fish from a single site were then frozen into a composite sample and kept frozen at −12°C until used.

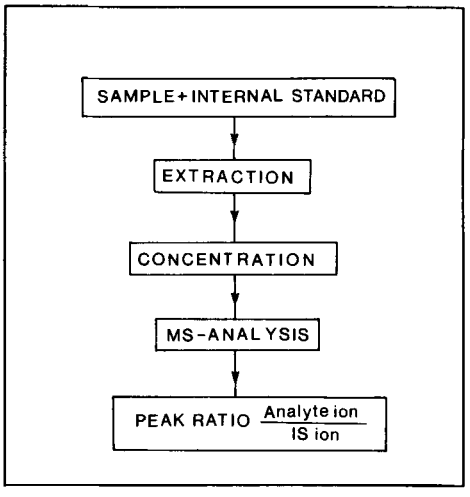
At the laboratory 10 g of fish tissue was mixed with 40 g of anhydrous sodium sulfate. The dried mixture was ground to a fine powder and packed in a chromatographic column (30 cm × 2 cm I.D.). Several basic principles are common to most quantitative MS assays, as summarized in Scheme 2.

Sample extraction

The column was equipped with a coarse-fritted plate on the bottom and a PTFE stopcock, and a 250-ml reservoir bulb at the top which flared out into a funnel shape. The samples were extracted with 200 ml of methylene chloride at a flow-rate of 3.5 ml/min[7]. The lipid extracts were collected in a 250 ml round-bottom flask and the solvent volume was reduced to 5 ml by rotary evaporation.

Cleanup and fractionation

Automated gel permeation chromatography (GPC) was employed to separate the polychlorinated biphenyls (PCBs), toxaphene and other chlorinated hydrocarbon pesticides from lipids[9]. An automated GPC system Autoprep 1001 (Analytical Biochemistry Lab., Columbia, MO, U.S.A.) was used. The solvent mixture, cyclohexane and methylene chloride was pumped through the GPC column (BioBeads SX-3) at 5 ml/min with the FMI laboratory pump, Model RRP-S4.



Scheme 2. Extraction and quantification scheme.

A volume of 5 ml or no more than 0.8 g of lipid should be placed on the GPC column. The first portion (150 ml) of the eluate was discarded and the next 120 ml were collected in a 250-ml round-bottom flask. The eluate was concentrated to 3 ml using the Rotavap.

Florisil column adsorption chromatography

A Florisil column was prepared by placing a charge of activated Florisil in a chromatographic column (30 × 1 cm I.D.) over a 1-cm layer of anhydrous Na_2SO_4. An amount of 5 g of 60–80 mesh Florisil previously activated at 130°C for 16 h was added and topped with a 1.5-cm layer of anhydrous Na_2SO_4. Each column was prewashed with 20 ml of *n*-hexane. When the solvent reached the top of the Na_2SO_4 layer, the concentrate from the GPC was quantitatively transferred to the column and allowed to drain onto the bed of Florisil. The column walls were washed with a 10-ml portion of 50 ml *n*-hexane–diethyl ether (94:6). When the solvent reached the top of the Florisil the remaining part of the eluent (40 ml) was added. The eluate was collected for further analyses. It usually contained aldrin, BHC, chlordanes, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD), DDE, and 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) isomers, heptachlor, lindane, methoxychlor, mirex, PCBs and toxaphene. More polar compounds, such as endosulfane, endrin, dieldrin and phthalates were removed from the column with 50 ml of diethyl ether–hexane (20:80) solution. PCBs can be removed from most of the pesticides by silica gel column chromatography[10]. About 20 g of silica gel (*e.g.*, silicagel 60 from E. Merck), was placed in a 100-ml beaker and activated at 130°C for 16 h. It was then transferred to a 100-ml glass-stoppered bottle. The silica gel columns was prepared by plugging a chromatographic column (30 × 1 cm I.D.) with glass wool, filling it with a 1-cm layer of anhydrous Na_2SO_4, and 5 g of activated silica gel and topping it with a second 1-cm layer of anhydrous Na_2SO_4. The column was prewashed with 20 ml *n*-hexane. The sample was added to the column and rinsed with 5 ml of the first eluent and allowed to percolate into the bed. The rest of the first eluent was added and the effluent collected in a 125-ml round-bottom flask. The first eluate (40 ml of diethyl ether–*n*-hexane, 6:94) contained the PCBs, HCB, aldrin, heptachlor, mirex and the *p,p'*-DDE. The second fraction eluate (40 ml of diethyl ether–*n*-hexane, 25:75) contained a small amount of *p,p'*-DDE, BHC isomers, toxaphene, DDT and its homologues, chlordanes, nonachlor, heptachlor epoxide and methoxychlor. The eluate volumes were reduced in volume on the Rotavap and the resulting residues adjusted to 1 ml with isooctane prior to their quantitation using HRGC or MS–SIM.

Preparation of ^{37}Cl-labelled toxaphene

^{37}Cl-labelled toxaphene was synthesized from a solution of camphene (142 mg) in CCl_4 (60 ml) in a chlorination flask. A mixture of $Na^{37}Cl$ (2 g), MnO_2 (3 g) and concentrated H_2SO_4 (3 g) diluted with about an equal volume of distilled water was placed in the chlorine generating flask and heated in an oil bath to 190°C. The chlorine generating flask was equipped with a water-cooled condensor and connected to a nitrogen gas cylinder. The camphene in CCl_4 solution was placed in the chlorination flask containing a submerged UV-lamp (254 nm, PCQ UV-Product, San Gabriel, CA, U.S.A.). The chlorination flask was connected with Tygon tubing to a water trap, which in turn was connected to an open oil-bubbler. Irradiation of the solution

began as soon as the characteristic chlorine color appeared. The heating continued until gas production ceased (*ca.* 2.5 h), at that time a barely noticeable nitrogen flow was introduced. The flow was maintained throughout the entire irradiation procedure (15 h). The chlorination mixture was then washed with saturated sodium bicarbonate solution, dried (anhydrous Na_2SO_4) and gently evaporated to dryness. The yield was 400 mg of toxaphene. The chlorine content was 66.1%. A high-resolution gas chromatogram is shown in Fig. 1. This product was used as an internal standard for the MS solid-probe quantitation and was spiked prior to this analysis.

Electron impact fragmentation of toxaphene

The electron impact ionization mass spectra of toxaphene are dominated by characteristic chlorinated clusters[11]. It has been noted that primary fragmentation characterizing the mass spectra of toxaphene congeners involved elimination of the chlorine or HCl of the toxaphene moiety. The structure and origin of the fragments of chlorinated bornenes have also been discussed in detail[11,12]. The mass spectrum of ^{37}Cl-labelled toxaphene is shown in Fig. 2.

Instrumentation

High-resolution gas chromatography with electron-capture detection (ECD). Standards and samples were analyzed on a Varian Vista 6000 gas chromatograph equipped with a 25 × 0.1 mm (d_f = 0.25 μm) bonded-phase SE-52 fused-silica column with hydrogen as the carrier gas. The chromatographic time–temperature

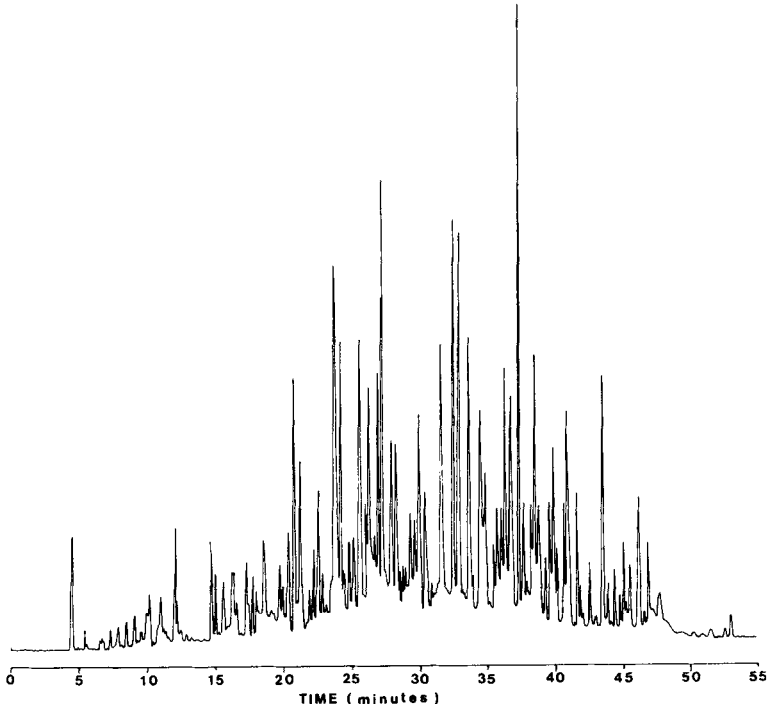


Fig. 1. Gas chromatogram of toxaphene labelled with ^{37}Cl.

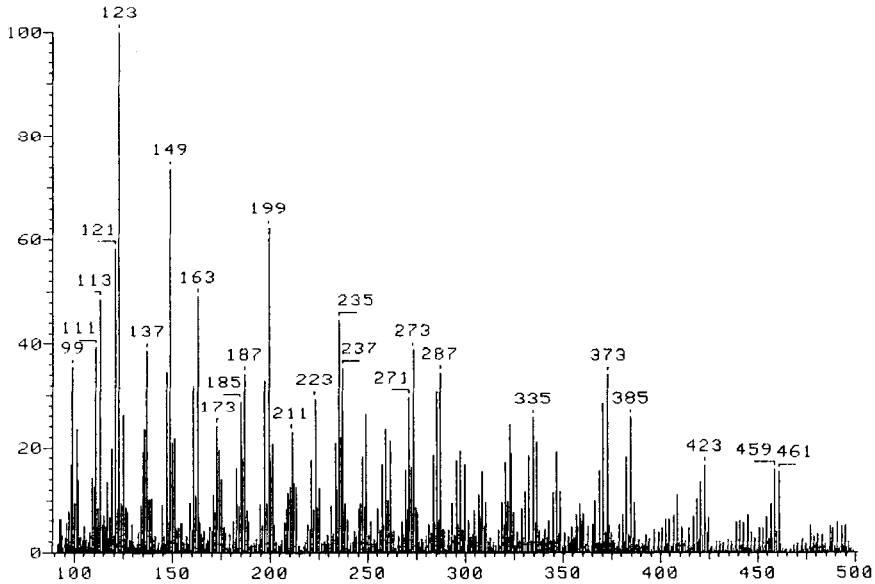


Fig. 2. The mass spectrum of ^{37}Cl-labelled toxaphene obtained under electron impact conditions.

conditions were as follows: splitless injection, 0.6 min; initial temperature 80°C, hold for 1 min; to 160°C at 20°C/min, hold for 5 min; to 230°C at 2°C/min and to 260°C at 10°C/min. The injector port was maintained at 230°C.

Mass-spectrometry. A Varian MAT 311 A Model high-resolution sector instrument, equipped with both electron impact (EI) and chemical ionization (CI) sources was used. The EI–EI detector source temperature was maintained at 170°C with an ionization voltage of 70 eV, and the combined EI–CI source at 150°C. The resolution was set at 1200 (10% valley). The mass spectrometer was equipped with a water cooled solid probe insert, which could be heated from 20 to 200°C in 5 s. The SIM recording was achieved by Varian MAT hardware kit and traces were recorded on a Spectra Physics 4100 printer–plotter. In the SIM mode m/z 158.9 and 162.9 traces were recorded.

Gas chromatography–mass spectrometry–selected ion monitoring. The GC–MS system (Finnigan MAT 311A with a Carlo Erba 4160 GC) equipped with a 30 m × 0.25 mm I.D. bonded-phase SE-54 fused-silica open tubular column was directly interfaced to a Finnigan MAT 311 A mass spectrometer ion source. Cool on-column injection was employed. The initial column temperature of 75°C was programmed to 160°C at 30°C/min and was held for 10 min and then increased to 225°C at 2°C/min. A transfer line temperature of 230°C and an ion source temperature of 150°C were maintained. Helium was used as carrier gas. The instrument was sequently set to monitor m/z 130.9 (perfluorokerosene, PFK) and 158.9. Ion dwell times of 0.01 s for 130.9 and 0.1 s for the late ion were employed.

General description of the inlet system of a mass spectrometer

The majority of organic compounds may be introduced into the ion source of a

mass spectrometer by means of a direct insertion device. This device allows the introduction of samples which have insufficient thermal stability to be heated quickly up to 350°C, or have very low vapor pressures even at this temperature. The sample is introduced directly into the ion source on the cooled end of the probe. The end of the probe can be heated fast by means of a heater in the probe. An increased vapor pressure of about 5 Torr permits a mass spectrum to be obtained using this technique. Samples of toxaphene down to 200 pg were analyzed in the SIM–EI mode. Direct probe sample vials (aluminium) with tops were obtained from Finnigan MAT.

Procedure

Reference solutions for calibration purposes were prepared by mixing a ^{37}Cl-labelled toxaphene solution (2 ng/μl) in isooctane with the native toxaphene at different concentration levels (0.2–5.0 ng/μl). Fish extracts were also analyzed with an addition of the internal standard. A 1-μl volume was brought into a crucible (10 mm × 2 mm I.D.). After the solvent was evaporated, the lid was tapped and the crucible was properly inserted to the probe tip. The probe tip was placed in an isolation chamber for 10 s. Afterwards, the probe was inserted for measurement into the ion source. When the probe was fully inserted a stop-watch was started to run. Filament, probe insertion, a probe heater and SIM hardware switching were turned on. Two masses (*m/z* 158.9 and 162.9) were recorded during a fast temperature programming of the probe tip (40°C/min). During the subsequent evaporation of a sample, the preselected ions were monitored at 45 s and the corresponding accelerating voltage

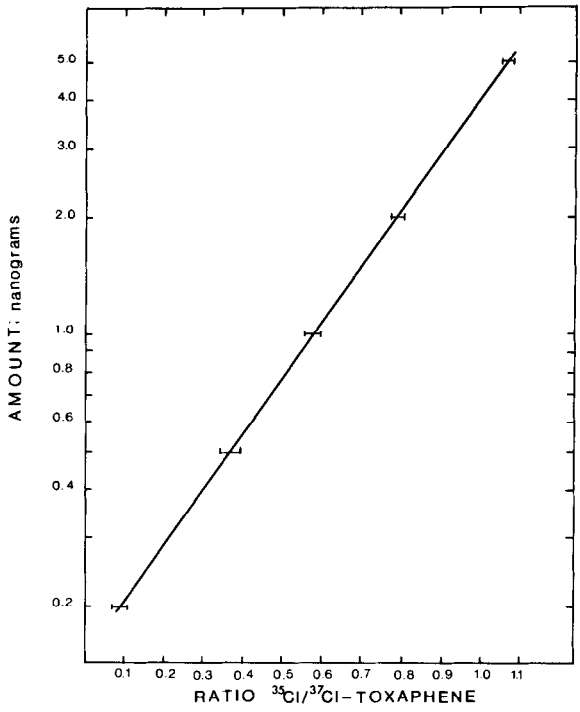


Fig. 3. Calibration curve for toxaphene using a direct insertion probe technique.

traces were recorded by two SP-4100 printer–plotter integrators. The peak profile width at half maximum current was typically smaller than 25 s. The calibration curve was designed as shown in Fig. 3. However, for high accuracy and precision measurements, calibration mixtures of accurately weighed toxaphene and its ^{37}Cl-labelled analogue were used to bracket the sample and checked with standard curves[13].

Solid probe–SIM conditions

(1) Spike 1 μl sample with 1 μl of 2 ng/μl [^{37}Cl]toxaphene in isooctane.

(2) Evaporate for 30 min at room temperature.

(3) Place in probe, evacuate in isolation chamber for 10 s, then insert probe into source.

(4) Start the timer when the probe is fully inserted, turn-on filament, probe heater and MIS hardware switching.

(5) At 30 s, start both recorders (*m/z* 158.9 and 162.9) monitoring baseline.

(6) At 45 s, start solid-probe temperature programming.

Conditions: electron multiplier, 1.5 kV; filament current, 0.6 mA; $m/\Delta m$, 1200; source temperature, 150°C. SIM: attenuation, 0.1; filter, 0.1 Hz; dwell time, 100 ms. Attenuation, 256 × (both recorders). Solid-probe programming: 25°C to 175°C in 10 s. PFK reference peak: *m/z* 130.9.

RESULTS AND DISCUSSION

Fig. 4 shows the recorder output of a low-resolution ($R = 1000$) dual SIM analysis of a native and a ^{37}Cl-labelled toxaphene sample of a fish extract. The mass spectrometer was focused to achieve a Gaussian peak shape. Following each sample introduction, the accelerating voltage, filament current and probe programmer were turned on. A reference ion of *m/z* 130.9 (derived from PFK) which was independently introduced into the ion source via a reference inlet, was used for setting individual masses to be monitored and to assure a proper focusing. Any required correction for drift (generally less than 10 ppm) was made by adjustment of the magnet current before analysis began. PFK was removed prior to running samples. The solvent blank showed no significant background contribution.

The precision for the determination of response ratios, analyte–internal standard, is influenced by fluctuations in the intensities of the selected ion currents and by

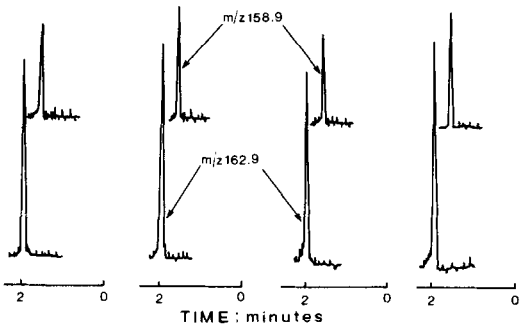


Fig. 4. The recorder-output traces of *m/z* 158.9 and 162.9 and its replicate analyses.

TABLE I

REPLICATE ANALYSES OF TOXAPHENE IN FISH SAMPLES

Sample	*Amount found (ng)*	*Response ratio*	*Total conc. (μg/g)*	*Sample*	*Amount found (ng)*	*Response ratio*	*Total conc. (μg/g)*
E-3	2.0	0.79	48.8	E-4	1.0	0.59	6.0
	2.0	0.79	48.8		1.0	0.59	6.0
	2.1	0.80	51.2		1.0	0.60	6.0
	2.3	0.84	56.1		1.0	0.60	6.0
	2.4	0.85	58.6		1.1	0.63	6.6
	2.4	0.85	58.6		1.2	0.65	7.2
$\bar{x}$	2.2	0.82	53.7		1.05	0.61	6.3
S.D.	0.1897	0.029	3.929		0.0763	0.0224	0.458
C.V.(%)	8.62	3.62	7.31		7.27	3.66	7.27

drift or noise from the peak centroid. The effect of drift is minimal at lower MS resolution. The results of replicate analyses ($n = 16$) of a single fish extract with DIP–SIM for two ions at low resolution ($R = 1200$) gave a peak area ratio of 0.84 and a coefficient of variation of 2.6%.

Fig. 4 shows the recorder output of approximately 7.0 and 60 μg/g of toxaphene and ^{37}Cl-labelled toxaphene, respectively.

Table I records the precision of the determination of response ratios for replicate analyses of toxaphene in fish samples.

Results reflecting recoveries and accuracy for the given procedure are evaluated in Table II. These data of toxaphene for fish samples provided by the supplier were

TABLE II

RECOVERIES FOR THE U.S.-EPA FISH HOMOGENATE CONTAINING TOXAPHENE BY THE TWO DIFFERENT TECHNIQUES

U.S.-EPA value[a]: fish 3, 90.8 μg/g; S.D. = 6.9; 95% confidence interval, 77–105 μg/g; fish 4, 7.2 μg/g; S.D. = 2.6; 95% confidence interval, 2–12.4 μg/g.

Batch	*HRGC–ECD*		*(%) Recovery*		*DIP–MS–SIM*		*(%) Recovery*	
	Fish 3	*Fish 4*	*Fish 3*	*Fish 4*	*Fish 3*	*Fish 4*	*Fish 3*	*Fish 4*
2	–	7.87	–	109.3	–	6.02		83.6
3	59.3	7.18	65.3	99.7	62.2	7.83	68.5	108.8
4	56.9	8.21	62.7	114.0	53.7	6.28	59.1	87.2
5	67.2	5.85	74.0	81.3	52.5	7.83	57.8	108.8
6	58.6	6.94	64.5	96.4	53.7	6.32	59.1	87.8
7	67.7	8.91	74.6	123.8	76.4	7.54	84.1	104.7
$\bar{x}$	62.0	7.49	68.2	104.0	59.7	6.97	65.7	96.8
S.D.	5.09	1.07	–	–	10.1	0.85	–	–
R.S.D.(%)	8.2	14.3	–	–	16.9	12.18	–	–

[a] True value is unknown; results obtained from the referee laboratories by packed-column gas chromatography.

TABLE III

TOXAPHENE RESULTS (μg/g) FOR SAMPLE EXTRACTS ANALYZED BY THE DIFFERENT TECHNIQUES AT TWO LABORATORIES

Sample	*HRGC–ECD*		*GC–MS–SIM*	*DIP–MS–SIM*
	Lab 1	*Lab 2*	*Lab 2*	*Lab 2*
1	0.17	0.17	[a]	0.15
2	7.87	8.54	8.84	11.40
3	1.21	1.36	1.14	1.70
4	0.47	0.52	0.72	0.67
5	1.00	1.15	1.34	1.12
6	3.09	3.01	3.36	4.10
7	15.50	16.80	17.10	17.00

[a] Below detection limit of 500 pg/μl; equivalent to 0.5 μg/g.

obtained by packed column GC. As can be seen from the results obtained by two different techniques, the data are in good agreement. Comparing the effectiveness of DIP–MS–SIM *versus* HRGC–ECD, it is evident that the former would allow up to 35 analyses per day. This is a significant improvement over the 5–6 samples per day when HRGC–ECD is used. Another advantage of this technique is that a large amount of sample can be introduced into the ion source and thus increased response and higher sensitivity.

The limit of detection in 10-g fish samples varied depending both on the toxaphene degradation and the fat content in the fish. In general, using a signal-to-noise ratio (S/N) of 3 for toxaphene as detection limit, it required at least 100 pg of toxaphene to be introduced into the ion source under the given instrumental conditions.

Using the same extraction techniques, seven fish samples were analyzed at two different laboratories. These samples were analyzed by HRGC–ECD, HRGC–MS–SIM and DIP–MS–SIM. Results of the interlaboratory quantitation are given in Table III.

It is evident that estimated values differ slightly not only between HRGC–ECD data but also between the different techniques. The HRGC–ECD results are usually lower than those of DIP–MS–SIM due to the omission of many peaks. Significant alteration of GC peaks including some interferences from other components in the fish extracts could lower these values. Considering the low toxaphene levels in the samples, it is premature to draw conclusions as to the origin of the differences.

CONCLUSIONS

The results in this paper demonstrate the feasibility of the rapid direct probe determination of toxaphene residues in fish tissue, without the attendant problems of extraction and exhaustive purification steps. Where comparisons are drawn with published data, the correlation is satisfactory. The use of sample-dependent standards has proven to be an alternative means of MS quantitation complementary to techniques based on sample-independent standards used in HRGC and GC–MS.

ACKNOWLEDGEMENTS

This work has been performed at the National Water Research Institute, Analytical Methods Division, as a part of a project requested by the Great Lakes Toxic Contaminants Committee of the Great Lakes Water Quality Program, whose financial support is gratefully acknowledged. We are grateful to Dr. J. Zajicek and Dr. D. L. Stalling from the U.S.-Fish and Wildlife Laboratory, Columbia, MO and U.S.-EPA, Quality Assurance Branch, Cincinnati, OH for providing some of the fish samples. This is National Water Research Institute contribution No. 85-113.

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