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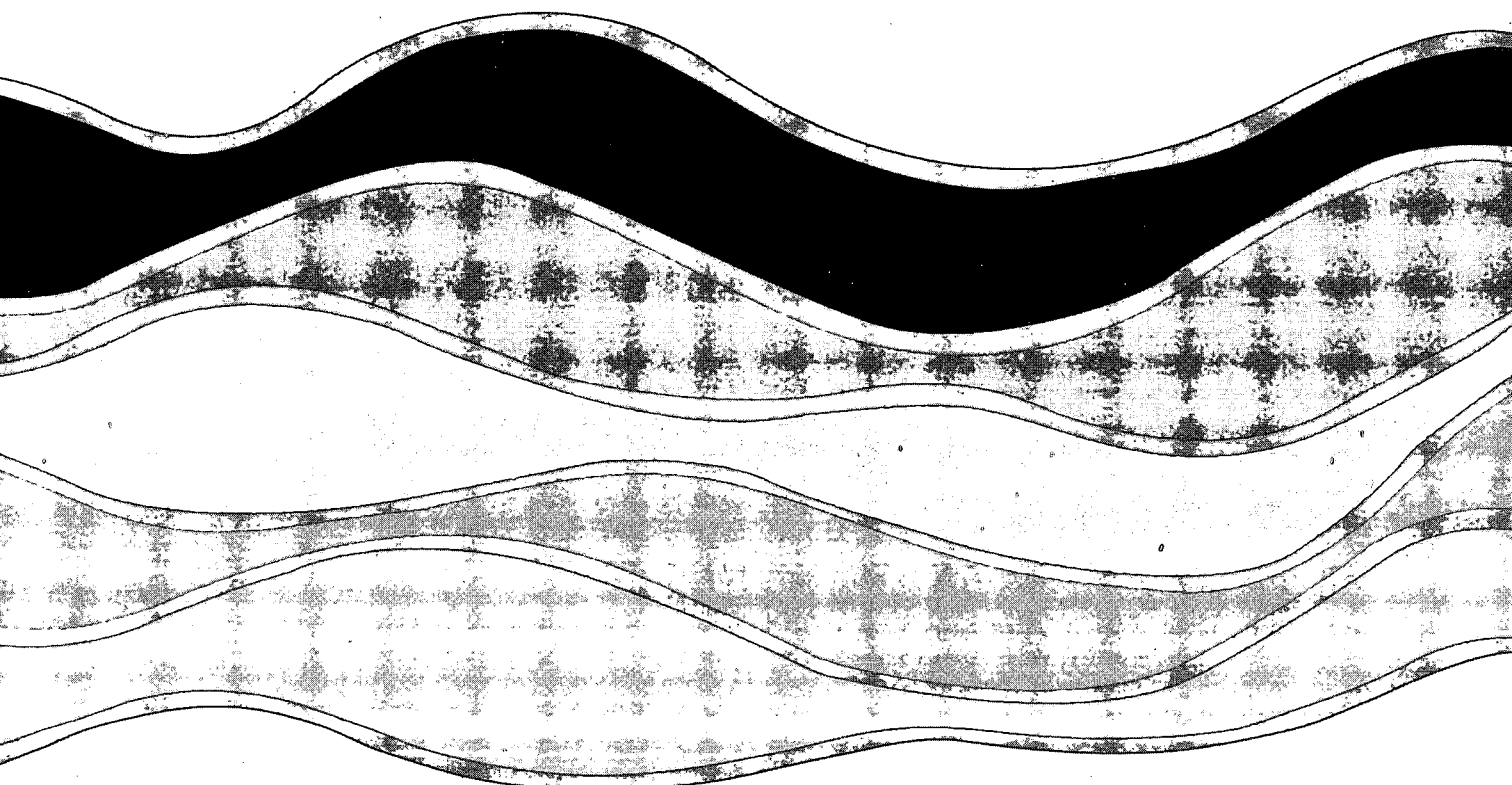
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DIBENZO-P-DIOXINS AND
CHLORINATED
DIBENZOFURANS IN FISH

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NWRI Contribution No. 92-12

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**AN MSD-BASED METHOD FOR THE DETECTION OF CHLORINATED
DIBENZO-P-DIOXINS AND CHLORINATED DIBENZOFURANS IN FISH**

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MANAGEMENT PERSPECTIVE

There is a need for more effective and efficient methods for the determination of chlorinated dioxins and furans in environmental samples. The present report describes a streamlined extraction and clean-up method which is used in combination with a Mass Selective Detector (MSD) detection system. Samples are extracted with solvent after dispersal in sodium sulphate. The liquid phase clean-up steps that were used in a previous method are replaced with a mini-acid/base silica column. The resultant method is more efficient than methods that are based on liquid phase extraction and clean-up steps. The ability of the method to detect PCDDs and PCDFs in fish samples is demonstrated. The method can be adapted for use with other matrices. The extraction and clean-up procedures can also be used with immunoassay detection techniques. A scintillation technique is described for rapidly and inexpensively trouble shooting the extraction and clean-up methods. The described method enables laboratories to screen fish for the presence of dioxins more cost effectively. Positive samples can then be confirmed using high resolution gas chromatography coupled to high resolution mass spectrometry.

SOMMAIRE À L'INTENTION DE LA DIRECTION

Il y a lieu d'améliorer la méthodologie employée dans l'analyse des dioxines et des furanes chlorés présents dans les échantillons d'origine environnementale. On décrit ici une méthode d'extraction et de purification améliorée qui s'emploie avec un système de détection à discriminateur de masse (DM). Les échantillons sont soumis à une extraction au solvant après dispersion dans du sulfate de sodium. Les étapes de la purification en phase liquide qu'on suivait auparavant sont remplacées par un passage sur une mini-colonne de silice acido-basique. La méthode améliorée est plus efficace que celles où l'extraction et la purification se font en phase liquide. On montre en outre qu'elle permet de détecter les PCDD et les PCDF dans des tissus de poisson. On peut l'adapter pour d'autres types d'analyses. Les procédures d'extraction et de purification peuvent aussi être employées avec des techniques d'immunodétection. On décrit en outre une technique de scintillation pour déceler rapidement et à peu de frais les problèmes pouvant se poser à l'extraction et à la purification. Avec la méthode améliorée, la présélection en laboratoire des échantillons de poisson contenant des dioxines est plus économique. On peut confirmer les résultats par chromatographie en phase gazeuse à haute résolution en combinaison avec la spectrométrie de masse à haute performance.

ABSTRACT

A stream-lined method for the efficient detection of polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in fish samples is described. The samples (25 g) were mechanically blended with anhydrous sodium sulphate and then packed in a glass column. Lipids and lipid soluble compounds were eluted from the column with dichloromethane. The column extraction technique recovered ^{13}C -labelled surrogate spikes from fish samples as effectively as an acidic liquid phase extraction method. Bulk lipids were removed from the extract by size exclusion chromatography. Residual lipids and biogenic molecules were then removed by chromatography on a mini acid/base silica combination column. The extract was further enriched by high performance liquid chromatography (HPLC) on basic alumina and activated carbon. The enriched analytes were separated, identified, and quantified by high performance gas chromatography combined with a mass selective detector (MSD). The method was evaluated using two sets of fish samples ($n=16$ & $n=10$). Tritiated 2,3,7,8- T_4CDD was used to conveniently trouble shoot the method.

RÉSUMÉ

On décrit une méthode améliorée permettant de détecter avec efficacité les polychlorodibenzo-p-dioxines (PCDD) et les polychlorodibenzofuranes (PCDF) présents dans des tissus de poisson. On mélange les échantillons (25 g) mécaniquement avec du sulfate de sodium anhydre, puis on verse le tout dans une colonne en verre. Les lipides et les composés liposolubles sont élués au moyen de dichlorométhane. Avec la technique d'extraction en colonne, le taux de récupération obtenu avec des échantillons de poisson enrichis au moyen d'un composé de remplacement marqué au ^{13}C de tissus de poisson est aussi bon qu'avec une extraction à l'acide en phase liquide. La majeure partie des lipides de l'extrait est éliminée par chromatographie sur gel. Les molécules lipidiques et biologiques restantes sont extraites par chromatographie sur mini-colonne de silice acido-basique. On enrichit l'extrait encore davantage par chromatographie en phase liquide à haute performance (CLHP) sur alumine basique et charbon activé. Les produits enrichis sont séparés, identifiés et quantifiés par chromatographie en phase gazeuse à haute performance avec détection au discriminateur de masse (DM). On a évalué la méthode en analysant deux groupes d'échantillons de tissus de poisson ($n=16$ et $n=10$). La 2,3,7,8- T_4CDD tritiée s'est révélée pratique pour la détection des problèmes.

INTRODUCTION

Chlorinated dibenzo-p-dioxins (PCDDs) and chlorinated dibenzo furans (PCDFs) are common contaminants of chlorinated industrial chemicals, such as chlorophenols and their derivatives (1,2), and polychlorinated biphenyls (PCBs) (3). PCDDs and PCDFs are also formed as by-products of waste incineration and other combustion processes (2); they are also inadvertently formed during the production of bleached pulp (4, 9). Contamination of the environment with PCDDs and other halogenated aromatic hydrocarbons can lead to human exposures via the food chain (2), and can also be harmful to wildlife (5).

In Canada there is particular concern about the potentially adverse effects of PCDDs and PCDFs in pulp mill effluents and wastes. Consequently a large scale surveillance study was undertaken to estimate PCDD and PCDF levels in a variety of fish species taken from locations close to several inland pulp mills (6). Time constraints meant that the analytical method previously used in our laboratories (7) would not suffice for the large number of samples involved. Although the earlier method was effective, it included a liquid phase extraction technique and several liquid phase clean-up steps that rendered it excessively time consuming and laborious (8). For that reason we explored the possibilities of a more streamlined and efficient extraction and clean-up method. Most methods for the determination of PCDDs and PCDFs rely on high resolution gas chromatographs combined with expensive high resolution mass spectrometers to isolate, identify, and quantify the individual congeners in the enriched extract. Simplified screening methods that are based on mass selective detectors can help to reduce the burden on high resolution mass spectrometers by the elimination of samples that are either analyte free or contain negligible analyte levels.

In the present method the fish tissue is mechanically dispersed on Na_2SO_4 which is then packed in a glass column and extracted with dichloromethane (DCM). This

is a modified version of an extraction method that proved successful in our laboratories in the past (8) and has also been used by others (Smith et al., 1984; Norstrom et al., 1986; Sergeant, personal communication). The clean-up method uses low and high pressure chromatographic techniques, which eliminate the emulsion problems associated with more time consuming liquid-liquid clean-up procedures. A mass selective detector (MSD) operated in the selective ion monitoring mode is used to detect and quantify the analytes. The method's performance was evaluated using a variety of fish taken from the vicinity of pulp and paper mills.

EXPERIMENTAL

Standards and Reagents

All solvents were pesticide grade and were checked for the presence of contaminants before use. The PCDD and PCDF standards were purchased from Cambridge Isotope Laboratories (Mass., USA) and were prepared in toluene. A mixture of surrogate standards was prepared using one ^{13}C labelled dioxin from each congener group (Table 2). ^{13}C -1,2,3,4-TCDD was used as an instrument performance standard: it was added to the extract immediately before the gas chromatography (GC) / MSD detection step. The MSD was calibrated at the start and end of each day using a mixture of native and ^{13}C -labelled dioxin and furan standards. The calibration standard was re-run if instrument instability was suspected. The calibration and surrogate standards were used at the following concentrations: tetra = 50 pg/ μL , penta = 100 pg/ μL , hexa = 100 pg/ μL , hepta = 100 pg/ μL , and octa = 150 pg/ μL . 2,3,7,8-tetrachloro[1,6- ^3H]dibenzo-p-dioxin (40 Ci/mmol) was purchased from Chemsyn Science Laboratories; working solutions were prepared in toluene.

Extraction

Homogenates of fish fillets were thoroughly mixed using a Polytron blender until they formed a fine uniform paste; they were then stored in screw cap teflon bottles at -20°C until use. A 25 gram portion of fish homogenate was added to a 250 mL beaker and spiked with the ^{13}C surrogate standards. The spike solution was spread over the surface of the fish tissue and allowed to soak in for 30 minutes. Anhydrous sodium sulphate (75 g) was then added to the beaker and thoroughly mixed with the sample. The beaker and its contents were then allowed to stand for about two hours. The mixture, which had by then become a hard lump, was placed in a blender jar (Osterizer Cyclo-Trol B; 215 mL) with a further portion of sodium sulphate (75 g). The homogenate and sodium sulphate were then thoroughly blended and poured into a solvent rinsed glass column (43 cm x 3.2 cm I.D.), 50 g of additional sodium sulphate was used to assist the transfer. The extract was then eluted from the column with DCM (200 mL); the solvent flow was stopped before the column bed became exposed. After the addition of a further 100 mL of DCM, the column was allowed to soak overnight, and was then eluted with a further 200 mL of DCM; the total elution volume was 500 mL.

Cleanup

The purpose of the each cleanup step is outlined in Table 1.

TABLE 1. Purpose of Individual Cleanup Steps

| Cleanup Step | Matrix Components Removed |
|---------------------------------------|---|
| GPC | lipids, biogenic molecules, fatty acids |
| Activated silica | residual polar compounds |
| Acidic silica | lipids, oxidizable compounds, polychlorinated aromatic hydrocarbons (PAHs), dehydration reactions |
| Basic silica | oxidizable compounds, acidic compounds, phenolic compounds, lipids, sulfonamides, hydroxy-PCBs, hydroxy-diphenylethers |
| Basic alumina | non-planar PCBs, non-polar compounds, biphenylenes (BPEs), chlorobenzenes, phenolic compounds, PCNs, PAHs, DDE, mirex, pesticides |
| Carbon fibre: 50% DCM:cyclohexane) | non-planar persistent organics, neutral chlorinated pesticides, malathion, parathion, ortho- substituted PCBs, |
| (50% benzene:EtAc.) | co-planar PCBs and PCBs with decreasing #s of "ortho-Cl's" |
| (toluene F1) | PCNs, PCDDs, PCDFs, PCBPE, some PAHs, |

Bulk Lipid Removal

Gel permeation chromatography (GPC) was used to remove bulk lipids from the extract. After removal of the extraction solvent, the extract was re-suspended in DCM:hexane (1:1), and then clarified by micro-filtration (5 μ m). The extract was then diluted with solvent so that lipid level was less than 0.25 g/mL. A GPC Autoprep unit (Analytical Biochemistry Laboratories Autoprep model 1002A) was used to chromatograph the extracts on Bio Beads S-X3 (60 g in a 2.5 cm x 60 cm column). The samples

were injected into 5 mL loops. The column was eluted with DCM:hexane (1:1) at a flow rate of 5 mL/min.; lipids and other biogenic molecules were eluted in the first 150 mL, which was automatically discarded; PCDDs were eluted in the second fraction (150 mL).

Silica Gel Combination Column

Silica gel (Fisher, 60-200 mesh) was activated by heating overnight at 250 °C. The acid silica was prepared by the addition of 50 g of concentrated H_2SO_4 to 100 grams of the activated silica gel. The basic silica was prepared by the addition of 35 grams of 1 N NaOH to 100 grams of activated silica gel. A mini combination column was prepared by plugging the tip of a pasteur pipette with glass wool, and then adding the following components to the column: 0.5 cm sodium sulphate, 2 cm of basic silica gel, 4 cm of acidic silica gel, and 0.5 cm of sodium sulphate. The column was prewashed with 5 ml of 50% (v/v) DCM in hexane. The eluent from the GPC step was concentrated to 0.5 mL, added to the column, and eluted with 12 ml of 50% DCM in hexane. The partially cleaned-up extract was then evaporated to near dryness and re-diluted with 0.5 mL of 1% DCM in hexane.

Alumina and Carbon Fibre Chromatography

Alumina (Fisher, 80-200 mesh) was activated overnight at 550 °C and then deactivated with 1% w/w distilled water. The deactivated alumina was packed in a stainless steel column, 30 cm x 7.8 mm I.D. (Waters PN 84175). The preparation of the carbon fibre column has been previously described (7). A programmable HPLC pump (Waters model 590) was used to deliver the solvents to the HPLC system via a six port valve.

The alumina column was prepared for use by washing with 40 ml of 1 % DCM/hexane. Similarly, the carbon fibre was prepared for use by back flushing with 40 ml of toluene followed by a forward wash with 40 ml of 50% DCM/hexane. The

partially enriched extract from the silica gel combination column was loaded onto the alumina column which was then eluted with 90 ml of 1% DCM in hexane: this fraction was discarded. The alumina column was next eluted with 75 mL of 50% DCM in hexane, and this fraction was diverted to the carbon fibre column. The alumina column was then by-passed and the carbon fibre was successively eluted with 10% benzene/ethyl acetate (40 mL) and 50% benzene/ethyl acetate (32 mL). The direction of solvent flow through the column was then reversed and the dioxins/furans were eluted with 60 ml of toluene. The enriched extract was then concentrated and transferred to a micro vial; the solvent was evaporated and the extract was re-dissolved in 25 μL of the instrument performance standard ($[^{13}\text{C}]$ -1,2,3,4-TCDD (50 pg/ μL)) in toluene.

Quantification of Tritium Spike

Extract (100 μL) that contained a $[^3\text{H}]$ 2,3,7,8-TCDD spike was added to 10 mL of Scintiverse II (Fisher Scientific) scintillation cocktail in a 20 mL vial and counted in a liquid scintillation counter (LKB RackBeta 1217).

Analyte Quantification

A Hewlett-Packard mass selective detector (MSD) (HP 5970B) coupled to a HP 5880A gas chromatograph was used to separate and quantify the PCDD/PCDF congeners and congener groups. The extracts were chromatographed on a 30 m x 0.20 mm x 0.11 μm HP Ultra 2 column. A 1.0 μL aliquot of the enriched extract was injected using the splitless mode. The injector was maintained at 250 $^{\circ}\text{C}$; helium carrier gas was used at 15 psi; the GC column was maintained at 80 $^{\circ}\text{C}$ for 3 min. and then heated to (1) 180 $^{\circ}\text{C}$ at 20 $^{\circ}\text{C}/\text{min}$, and then to (2) 260 $^{\circ}\text{C}$ at 5 $^{\circ}\text{C}/\text{min}$ where it was maintained for 30 min. The MSD was operated at 70 electron volts in the Electron Impact mode; the Electron Multiplier was operated at 2400 volts. The interface was set at 250 $^{\circ}\text{C}$; the dwell time for the target ions was 100 milli-seconds. More recently a Finnigan INCOS 50B mass spectrometer has been used with an associated improvement in the method's performance.

The presence of native PCDDs/PCDFs was confirmed in one sample (Fig. 1-3) by chromatography on a 60 m DB 5 column followed by detection and quantification on a VG Autospec MS which was operated at 10,000 resolution.

TABLE 2. Ions used for the confirmation and quantification of PCDDs/PCDFs

| Compound | Quantitation Ion | Ion | Mass Ratio | Confirmation Ion (M-COCL) | Mass Ratio | Retention time (minutes) |
|---|---------------------|-------|------------------|---------------------------------|---------------|-----------------------------|
| 2,3,7,8-T ₄ CDD | 321.9 ¹ | 319.9 | .77 ² | 258.9 | .18 | 15-18 |
| 1,2,3,7,8-P ₅ CDD | 355.9 | 353.9 | .58 | 292.9 | .19 | 18-21 |
| 1,2,3,4,7,8-H ₆ CDD | 389.8 | 391.8 | .79 | 326.8 | .24 | 21-24 |
| 1,2,3,4,6,7,8-H ₇ CDD | 423.8 | 425.8 | .97 | 360.8 | .24 | 24-28 |
| O ₈ CDD | 459.7 | 457.7 | .89 | 396.8 | .17 | 28-30 |
| ¹³ C-2,3,7,8-T ₄ CDD | 333.9 | 331.9 | .76 | | | 15-18 |
| ¹³ C-1,2,3,7,8-P ₅ CDD | 367.9 | 365.9 | .62 | | | 18-21 |
| ¹³ C-1,2,3,4,7,8-H ₆ CDD | 401.9 | 403.8 | .79 | | | 21-24 |
| ¹³ C-1,2,3,4,6,7,8-H ₇ CDD | 435.8 | 437.8 | .94 | | | 24-28 |
| ¹³ C-O ₈ CDD | 471.8 | 469.8 | .91 | | | 28-30 |
| ¹³ C-1,2,3,4-T ₄ CDD ³ | 333.9 | 332 | .76 | | | 15-18 |
| 2,3,7,8-T ₄ CDF | 305.9 | 303.9 | .78 | 243 | .09 | 15-18 |
| 1,2,3,7,8-P ₅ CDF | 339.9 | 337.9 | .59 | 277 | .10 | 18-21 |
| 1,2,3,4,7,8-H ₆ CDF | 373.8 | 376 | .79 | 311 | .13 | 21-24 |
| 1,2,3,4,6,7,8-H ₇ CDF | 407.8 | 409.8 | .93 | 345 | .16 | 24-28 |
| O ₈ CDF | 443.8 | 441.7 | .89 | 379 | .17 | 28-30 |

¹: Nominal mass²: Theoretical mass ratio of the confirmation ion to the quantitation ion.³: Internal standard

The GC/MSD was programmed to acquire the specified ions in the predicted time windows (Table 2). Congener quantification was based on the most intense ion in the molecular ion cluster. A second ion (Table 2) in the cluster was used to establish the confirming ion ratio. In addition the M-COCl ion was used to confirm the presence of native congeners. All ions were required to have the correct retention time, an acceptable peak shape, and the correct mass ratio. The calculated surrogate recoveries were based on the performance standard's response. Native isomers and homologues were automatically corrected for the recovery of the corresponding ^{13}C -PCDD surrogate, and were quantified on the basis of the relative response of the corresponding surrogate and unlabelled congener in the calibration standard. Thus, the quantification of isomers was based on one ^{13}C isomer for each congener group. The detection limit (Det. Limit) was defined as 2.5 times the background noise in the region of the ^{13}C -surrogate's quantification peak.

RESULTS AND DISCUSSION

Table 3. Recovery of ^{13}C -surrogate spikes from fish samples using Column Extraction and Liquid Phase Extraction techniques

| ^{13}C -Surrogate | <u>Column Extraction</u> | | <u>Liquid Extraction</u> | | P ¹ |
|--------------------------------------|--------------------------|--------|--------------------------|--------|----------------|
| | Mean | C.V. % | Mean | C.V. % | |
| ^{13}C -2,3,7,8-T4CDD | 67.5 | 19.6 | 74 | 14.8 | >0.05 |
| ^{13}C -1,2,3,7,8-P5TCDD | 72.8 | 17.5 | 71.2 | 22.2 | >0.05 |
| ^{13}C -1,2,3,4,7,8-H6CDD | 75.7 | 20.5 | 71.5 | 21.9 | >0.05 |
| ^{13}C -1,2,3,4,6,7,8-H7CDD | 78 | 20.5 | 65.5 | 23.6 | <0.05 |
| ^{13}C -O8CDD | 70.5 | 26.4 | 55.4 | 27.3 | <0.05 |

1: Student's t test (two sided). P is probability that the difference between the means is due to chance.

Comparison of extraction techniques

Two sets of fish samples were randomly selected from among the survey samples. The column extraction technique was used to extract one set ($n=16$) and a liquid phase acidic extraction technique (Afghan et al., 1989) was used to extract the other ($n=15$). Each sample set included the following species: northern squawfish (*Ptychocheilus oregonensis*), peamouth chub (*Mylocheilus caurinus*), largescale sucker (*Catostomus macrocheilus*) X3, white sturgeon (X1), mountain white fish (*Prosopium williamsoni*), dolly varden (*Salvelinus malma*), and lake whitefish (*Coregonus clupeaformis*). The data in Table 3 show that the $^{13}\text{C}\text{-T}_4\text{CDD} - \text{H}_6\text{CDD}$ surrogate spikes were recovered equally well by both techniques. The column extraction technique yielded significantly higher recoveries (t test; $P<0.05$) for the H_7CDD and O_8CDD spikes.

In a second experiment 6 fish samples were individually homogenised. Each sample was then divided into two 25 gram paired portions which were spiked with the ^{13}C -surrogate spikes. The paired samples were extracted by means of the column and liquid phase extraction techniques. The summarized data (Table 4) show that surrogate recoveries were comparable for both methods. The apparently higher recoveries of the $^{13}\text{C}\text{-O}_6\text{CDD} - ^{13}\text{C}\text{-O}_8\text{CDD}$ surrogates by the liquid phase extraction technique were not found to be significant (paired t test, $P>0.05$). The data for the native homologues (Table 5) suggest that the column technique is comparable to the liquid extraction technique in its ability to extract native congener groups. Indeed for some of the fish samples the recoveries of native homologues appeared to be higher when the column extraction technique (Table 5) was used. Further experiments using replicated sub-samples of a fish that is known to contain a variety of PCDD/PCDF congener groups would help to clarify the latter observation.

Table 4. Recovery of ^{13}C -surrogate spikes from paired fish samples using column extraction and liquid phase acidic extraction

| ^{13}C -Surrogate | <u>Column Extraction</u> | | <u>Liquid Extraction</u> | | P ¹ |
|--------------------------------------|--------------------------|--------|--------------------------|--------|----------------|
| | Mean | C.V. % | Mean | C.V. % | |
| ^{13}C -2,3,7,8-T4CDD | 75.5 | 14.3 | 70.4 | 16.3 | >0.05 |
| ^{13}C -1,2,3,7,8-P5TCDD | 70.2 | 13.8 | 76.7 | 20.9 | >0.05 |
| ^{13}C -1,2,3,4,7,8-H6CDD | 71.6 | 21.7 | 80.2 | 24.4 | >0.05 |
| ^{13}C -1,2,3,4,6,7,8-H7CDD | 68.8 | 16.8 | 77.7 | 25.4 | >0.05 |
| ^{13}C -O8CDD | 60.1 | 15.4 | 73.5 | 30.1 | >0.05 |

1: Paired t test.

Table 5. Comparison of PCDD/PCDF levels in extracts of paired fish samples that were prepared using the column and liquid phase techniques.

| Homologue | Fish # | | | | | | | | | | | |
|--------------------|-------------------|-------------------|------|------|------|------|------|------|------|------|------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | | | | | | |
| | Col. ¹ | Liq. ² | Col. | Liq. | Col. | Liq. | Col. | Liq. | Col. | Liq. | Col. | Liq. |
| T ₄ CDD | 137 ³ | 105 | 8.1 | 2.3 | 2.9 | 2.7 | 16.7 | 15.4 | | | | |
| P ₃ CDD | 12.8 | 10.1 | | | | | | | | | | |
| H ₆ CDD | 40.8 | 25.9 | | | | | | | | | | |
| T ₄ CDF | 1185 | 919 | 68.1 | 25.8 | 15.8 | 11.2 | 259 | 257 | 37.3 | 35.4 | | |
| P ₃ CDF | 25.1 | 18.8 | | | | | | | | | | |

1: Column extraction technique

2: Liquid extraction technique

3: pg/g

Based on the foregoing results the column extraction technique was used to determine PCDD/PCDF levels in the remainder of the survey samples (6). A representative subset of the survey data is presented in Table 5; the data are for northern squawfish (*Ptychocheilus oregonensis*) X3, peamouth chub (*Mylocheilus caurinus*) X3, largescale sucker (*Catostomus macrocheilus*) X3, and white sturgeon (X1). When compared with earlier results (Table 3), the data in Table 6 show that the recoveries of the analytical standards improved as the analytical team gained more experience with the method. The performance standard's C.V. was 7.4% which indicates that the MSD was stable during the study. The detection limit was estimated to be lowest for the T₄CDD congener and highest for O₈CDD (Table 6).

Typical mass chromatograms (Figure 1 - 3) indicated that interfering molecular species had been effectively removed from the extracts. The characteristic peaks were well defined against a reasonably low level background signal. Native 2,3,7,8-TCDD was determined in the fish samples at levels that ranged from 6 - 26 pg/g. Because of the low resolution screening criteria that were used to identify and quantify the analytes, no value was reported if there were reasonable grounds to doubt a congener's identity. Positive samples have been stored in an archive for future confirmation by high resolution mass spectrometry. A VG autospec MS was used to confirm the presence of 2,3,7,8-T₄CDD and 2,3,7,8-T₄CDF in one of the fish extracts (Fig. 4).

Table 6. Representative recoveries of ^{13}C -surrogate spikes from the fish survey

| ^{13}C -Surrogate (pg/g) | Mean | C.V. % | Det. Limit |
|--------------------------------------|-------|--------|------------|
| ^{13}C -2,3,7,8-T4CDD | 100.3 | 14.4 | 2 |
| ^{13}C -1,2,3,7,8-P5TCDD | 113.9 | 18.3 | 3 |
| ^{13}C -1,2,3,4,7,8-H6CDD | 109.5 | 22.4 | 5 |
| ^{13}C -1,2,3,4,6,7,8-H7CDD | 99.9 | 19.2 | 10 |
| ^{13}C -O8CDD | 93.6 | 22.3 | 15 |

Comparison with a second laboratory**Table 7.** Comparison of the method's performance with that of an independent method

Native Homologues (pg/g)

| Sample #Lab | | T ₄ CDD | P ₅ CDD | H ₆ CDD | H ₇ CDD | T ₄ CDF | P ₅ CDF |
|-------------|----------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
| 1 | A ¹ | 112 | 12 | 36 | 28 | 890 | 24 |
| | B ² | 137 | 13 | 41 | ND | 1185 | 25 |
| 2 | A | 50 | 10 | ND | ND | 430 | 9 |
| | B | 61 | ND | ND | ND | 387 | ND |
| 3 | A | 43 | 8 | 3 | ND | 563 | 6 |
| | B | 60 | 4 | ND | ND | 704 | 13 |

¹: present method²: B is the independent laboratory

Three fish samples that had easily detectable levels of PCDDs were also analyzed at a second laboratory. There was generally good agreement between the results produced by both laboratories (Table 7). The few observed discrepancies may result from differing interpretations of peak shapes and peak acceptance criteria by the individual analysts. High resolution mass spectrometry, which was not available to us at the time of the study, would help resolve such discrepancies.

Trouble shooting

Occasional problems with the sample preparation method were rapidly identified through the use of a [^3H]2,3,7,8-TCDD tracer. The sample was spiked with tracer (20,000 disintegrations per minute. After each step in the sample preparation procedure the extract was adjusted to 1 mL - V_r (V_r is the volume (μL) previously removed for counting purposes), and 100 μL was removed and counted. The cause of spike losses is usually apparent from these counts without the need for subsequent clean-up or MSD analysis. For example, the foregoing procedure was used to rapidly identify a defective batch of alumina that caused a 40 % loss of the ^{13}C -2,3,7,8-TCDD surrogate standard.

CONCLUSIONS

The mechanical blending technique and column extraction procedures were able to effectively recover PCDD spikes from fish tissue. Using the described procedure a single operator could easily extract 8 samples per day as opposed to 3 samples in two days when liquid extraction was used. The chromatographic clean-up procedures are less tedious than liquid phase acid and base treatments. The HPLC clean-up steps take about 2 hours per sample. The precision with which the described method can recover native PCDD/PCDF levels in fish remains to be established. This could best be done by assessing the methods ability to quantify native PCDD/PCDF congeners in

replicate (10x) portions of a fish that is known to be contaminated with a variety of congeners. Participation in a round robin study would permit an objective assessment of the method's ability to accurately recover native PCDD/PCDF congeners from fish samples whilst facilitating comparison with a broader range of analytical methods. With some minor modifications the method should be applicable to other environmental matrices. The method is intended for use as a screening technique; positive samples can be confirmed using high resolution gas chromatography coupled to high resolution mass spectrometry.

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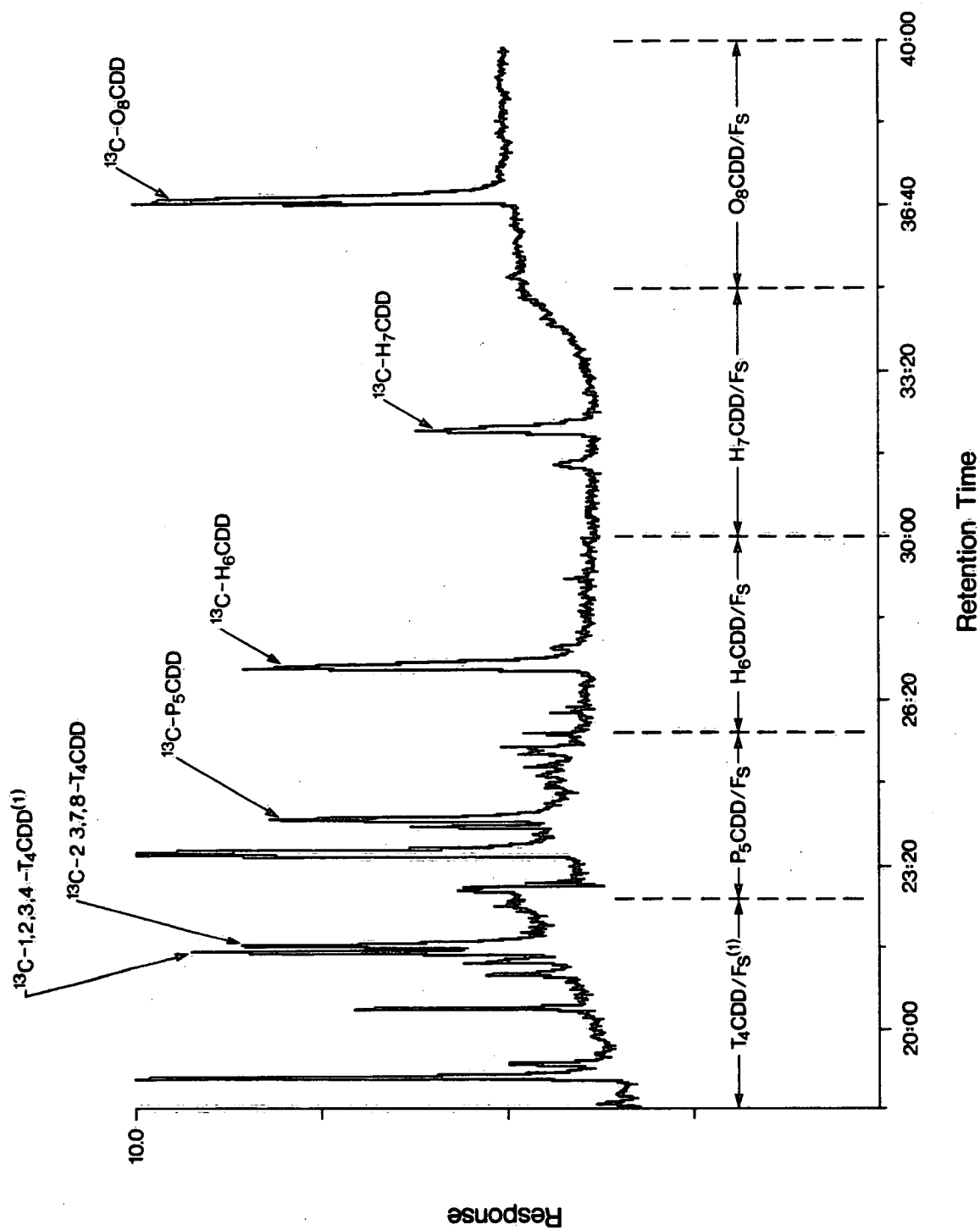


Figure 1. Mass chromatogram of potential PCDD's / PCDF's and ^{13}C -labelled surrogate standards in a typical fish extract. ⁽¹⁾All identifications are tentative.

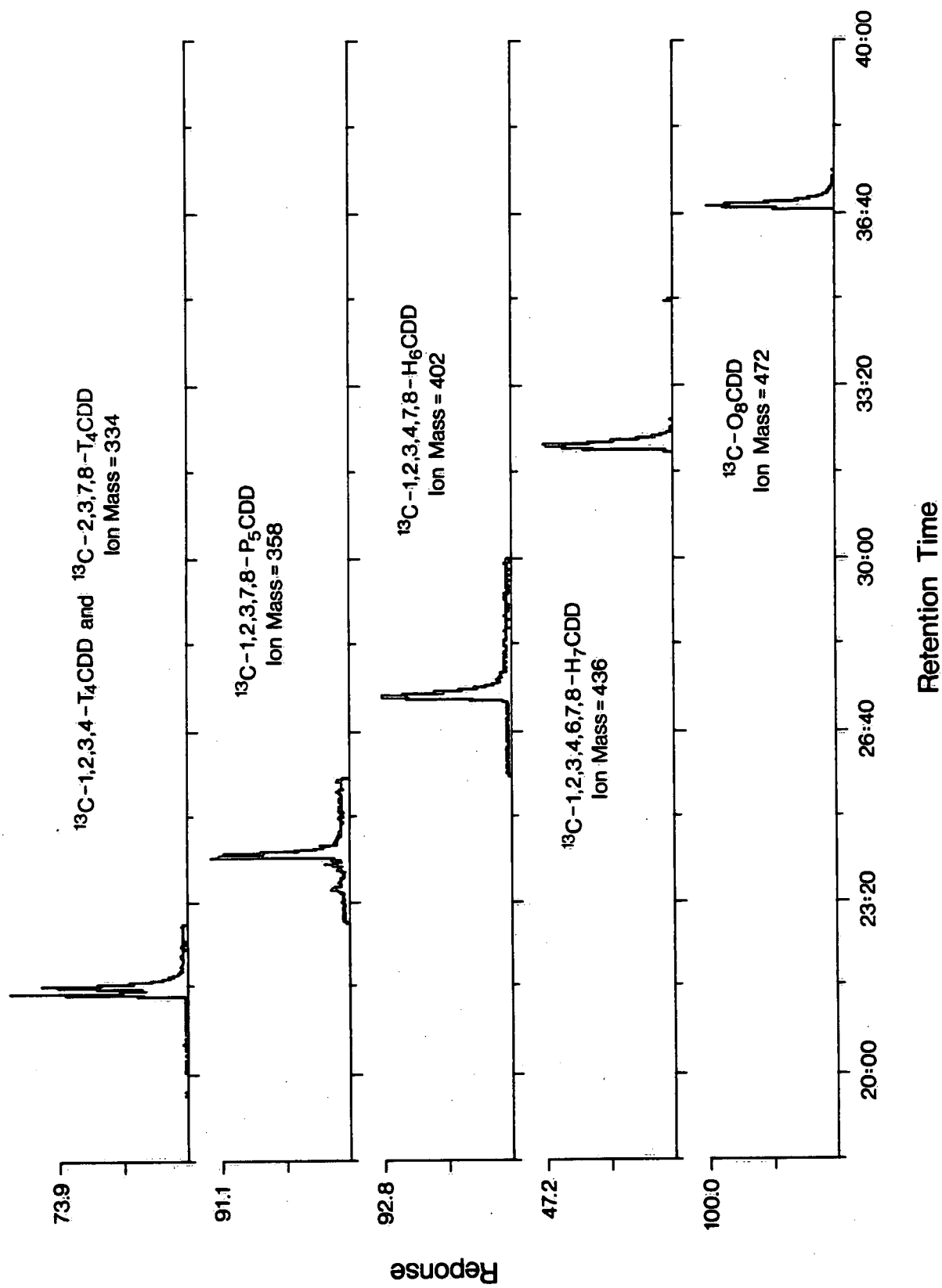


Figure 2. Chromatograms of quantitation ions for ^{13}C -labelled surrogate standards in a typical fish extract.

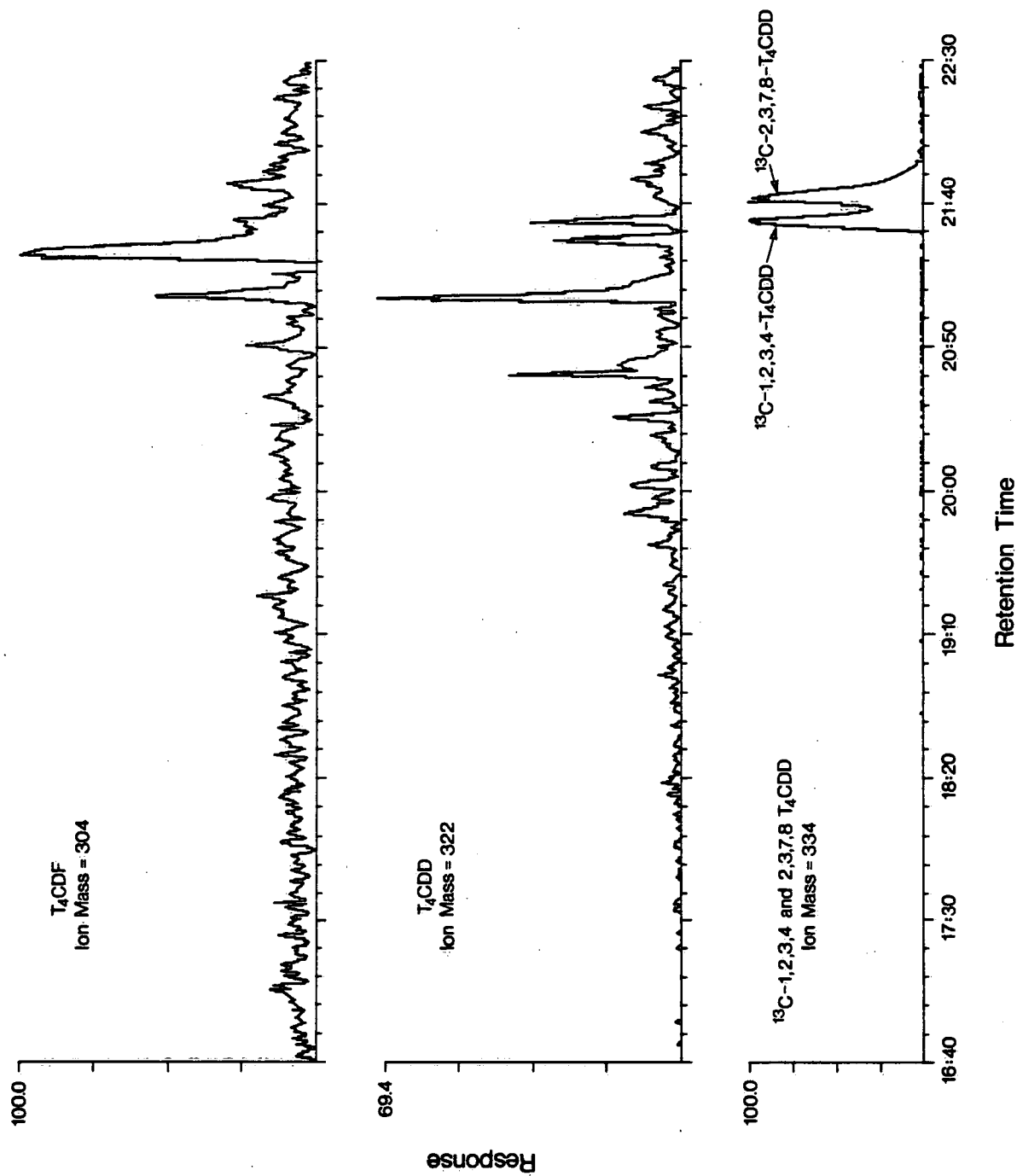


Figure 3. Low resolution selected ion chromatograms for native T_4CDD 's/ T_4CDF 's in a typical fish extract. The chromatogram of the ^{13}C -labelled T_4CDD standards is included as a reference. Peak selection and confirmation criteria are described in the Methods Section.

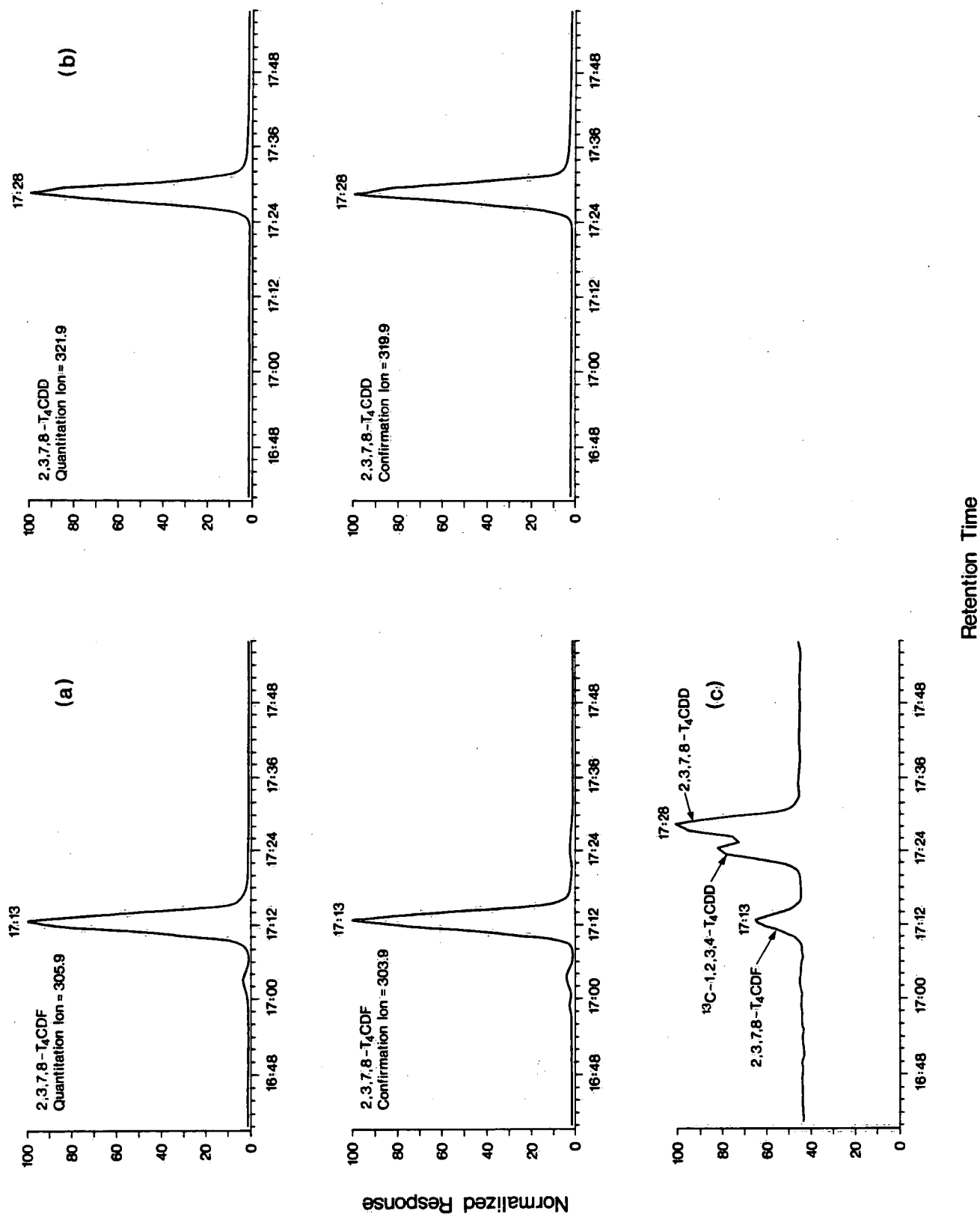


Figure 4. Confirmation chromatograms for (a) 2,3,7,8- 14 CDF's, (b) 2,3,7,8- 14 CDD's, and (c) total ion chromatogram for the TCDD/F window. The chromatograms were prepared on a VG autospec at a resolution of 10,000.

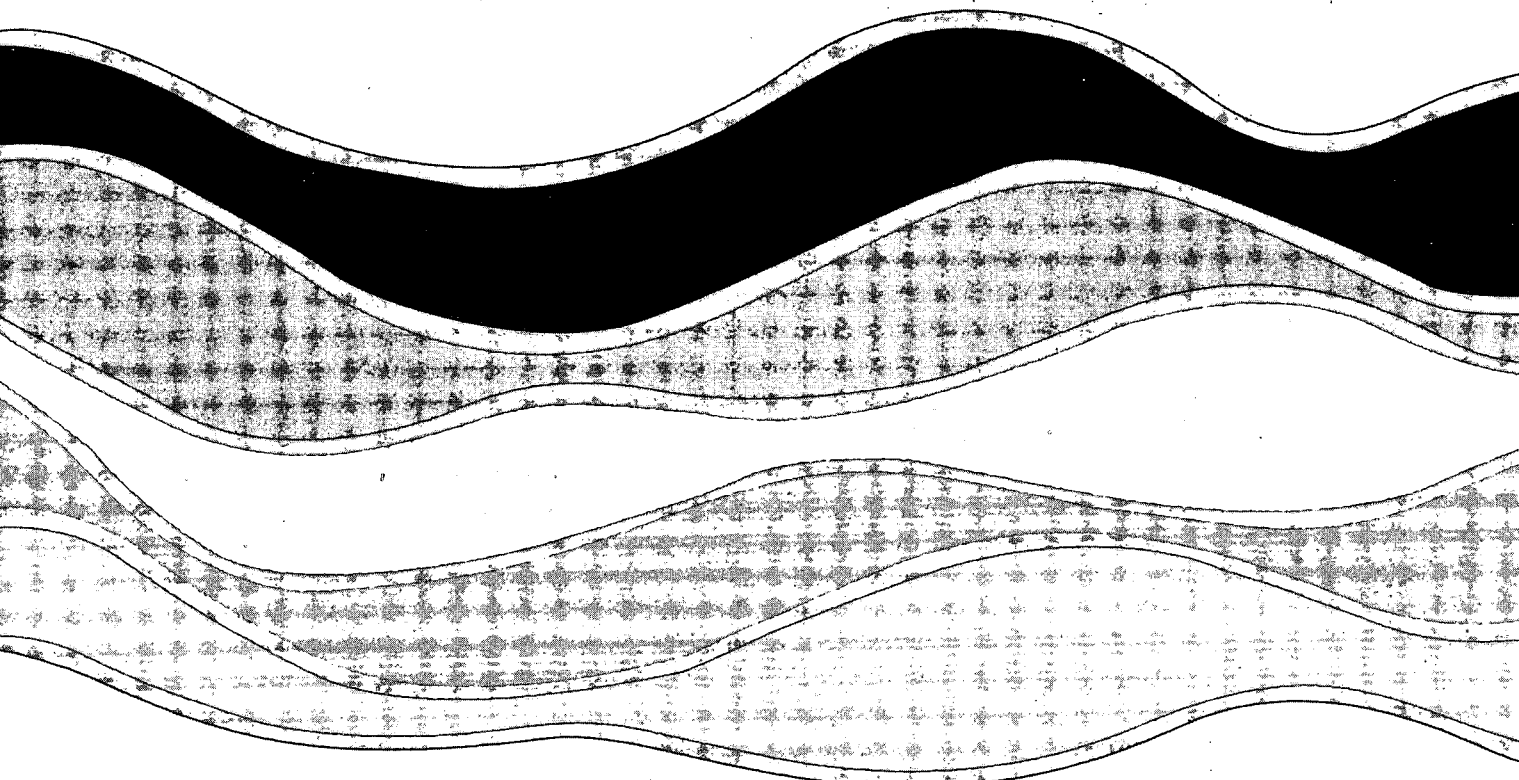


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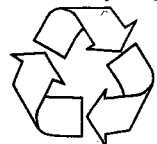
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