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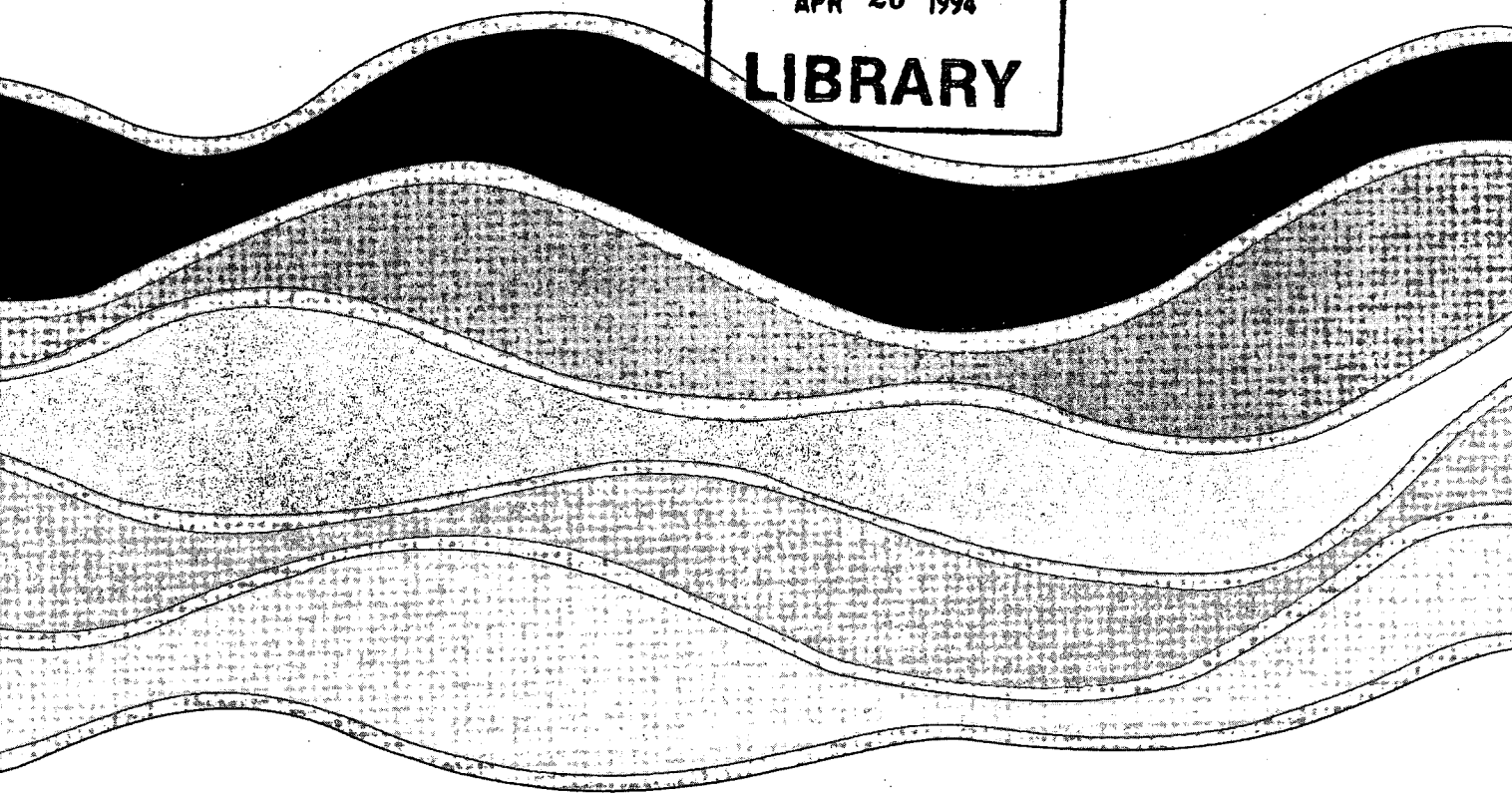

A RAPID SUPERCRITICAL CARBON DIOXIDE EXTRACTION METHOD FOR THE DETERMINATION OF POLYCHLORINATED BIPHENYLS IN FISH

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A RAPID SUPERCRITICAL CARBON DIOXIDE EXTRACTION METHOD FOR THE DETERMINATION OF POLYCHLORINATED BIPHENYLS IN FISH

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MANAGEMENT PERSPECTIVE

Determination of polychlorinated biphenyls (PCBs) in fish by conventional methods require as much as 0.5 litre of dichloromethane per sample in the tedious extraction and cleanup steps. Using supercritical carbon dioxide, we have developed an efficient method that can completely eliminate the use the chlorinated solvent for the extraction of PCBs in fish. The other advantages of this new method are the short extraction time, near lipid-free extract, and automated operation.

SOMMAIRE À L'INTENTION DE LA DIRECTION

Le dosage des biphényles polychlorés (BPC) dans le poisson par des méthodes conventionnelles nécessite jusqu'à 0,5 litre de dichlorométhane par échantillon au cours d'étapes laborieuses d'extraction et de nettoyage. Nous avons mis au point une méthode efficace utilisant le dioxyde de carbone supercritique qui permet d'éliminer complètement l'utilisation de solvants chlorés pour l'extraction des BPC du poisson. L'autre avantage de cette nouvelle méthode est un temps d'extraction plus court, un extrait presque exempt de lipides et des opérations automatisées.

ABSTRACT

An efficient and environmentally friendly supercritical fluid extraction (SFE) method for the determination of native polychlorinated biphenyls (PCBs) in fish tissues is described. The effect of various SFE conditions on lipid content and PCB recovery in fish extracts was studied. For best results, PCBs were extracted from fish using non-modified carbon dioxide at 100°C, a pressure of 5000 psi (35 MPa) and a flow rate of 2.5 mL/min. Recoveries of PCBs were ≥ 95% for both naturally contaminated and spiked fish and this automated procedure required less than 40 min per sample. By incorporating activated basic alumina with the sample in the extraction process, fish lipid was selectively retained by the adsorbent. The lipid content in the SFE extract varied from < 0.1 % to 2.2 % for the fish samples tested including those with over 30 % lipid by weight. In most cases, the remaining lipid was conveniently removed from PCBs by a down-sized Florisil column cleanup. Three commonly found chlorinated insecticides, namely, hexachlorobenzene, *p,p'*-DDE and mirex, were also extracted and determined simultaneously with PCBs.

RÉSUMÉ

On décrit une méthode efficace et écocompatible d'extraction par fluide supercritique (EFS) pour le dosage des biphényles polychlorés (BPC) contenus dans les tissus de poisson. L'effet de diverses conditions d'EFS sur la teneur en lipides et la récupération des BPC dans les extraits de poisson a été étudié. Les meilleurs résultats sont obtenus quand on extrait les BPC des poissons à l'aide de dioxyde de carbone non modifié à 100 °C, à une pression de 5 000 lb/po^2 (35 MPa) à un débit de 2,5 mL/min. Les récupérations des BPC étaient inférieures ou égales à 95 % tant avec les échantillons naturellement contaminés qu'avec les échantillons enrichis, et ce processus automatisé ne demandait que 40 min par échantillon. En incorporant de l'alumine basique activée à l'échantillon au cours du processus d'extraction, on a retenu sélectivement les lipides des poissons à l'aide de l'adsorbant. La teneur en lipides de l'extrait EFS était comprise entre <0,1 et 2,2 % avec les échantillons de poisson à l'essai, y compris ceux contenant plus de 30 % de lipides en poids. Dans la plupart des cas, le reste des lipides a été séparé facilement des BPC à l'aide d'une colonne de nettoyage de dimensions réduites garnie de Florisil. On a également extrait et dosé simultanément avec les BPC trois insecticides chlorés communs, soit l'hexachlorobenzène, le *p,p'*-DDE et le mirex.

INTRODUCTION

The wide-spread use and improper storage and disposal of the 1 to 1.5 million tonnes of polychlorinated biphenyls (PCBs) produced earlier in this century resulted in ubiquitous contamination of the ecosystem by these pollutants [1,2]. Due to their deleterious and persistent properties as well as their tendency to bioaccumulate in fish and other aquatic organisms, PCBs in environmental samples are analyzed in nearly all water quality monitoring programs. As a result of biomagnification, PCB concentrations exceeding 100 μg/g have been found in fish near point source discharges, while concentrations in the low μg/g range are generally reported in biota near large urban areas [3]. Regulatory guidelines have been established for the protection of fish and human health. While maximum residue limits for PCBs in various food and feed products range from 0.2 to 3 μg/g in different countries, Canada, the United States and Sweden have set a guideline of 2 μg/g for commercial fish [4].

Numerous methods for the determination of PCBs in a wide variety of sample matrices have been reported. In the past two decades, significant advances in PCB analysis resulted from the application of high resolution capillary columns [5] and the availability of individual PCB congeners [6]. Collectively, they enable the analytical chemists to report the results not only as Aroclors or total PCBs but also as individual congeners. The latter aspect is more intriguing to the researchers in toxicology, since a few PCB congeners, namely the coplanar or non-ortho substituted ones, are known to exhibit much greater toxicity than the others [7]. At the same time, the use of sensitive and selective mass spectrometric techniques for the quantification and confirmation of PCBs [8,9] was also pivotal in the improvement of data quality in complex environmental samples.

In the same period of time, relatively few breakthroughs have been reported for the extraction of PCBs from solid samples and most established methods are still

based on the use of organic solvents. For instance, the official AOAC method 983.21 for organochlorine insecticides and PCBs in fish involves blending of the sample twice with petroleum ether followed by Florisil cleanup [10]. For samples of high lipid content, additional cleanup is often necessary. In the service laboratories of Environment Canada and Fisheries and Oceans Canada, fish tissues are extracted with a 1:1 mixture of dichloromethane and hexane in a Soxhlet apparatus or by a sonicator. The organic extract is then cleaned up by gel permeation chromatography (GPC) followed by Florisil column fractionation. The above procedures have been used routinely since the 1970's and are known to produce reliable results, although this dated technique requires a lengthy sample turn-around time, has high solvent consumption and produces large amount of coextracted lipid.

In recent years, supercritical fluids, particularly carbon dioxide, have been successfully applied to the extraction of organic pollutants in solid samples. Some of the reported examples include the extraction and determination of PCBs [11-13], polynuclear aromatic hydrocarbons (PAH) [11,12,14,15], chlorinated furans and dioxins [16,17], total petroleum hydrocarbons (TPH) [18], organotin [19], chlorobenzenes [12], acidic herbicides [20], chlorinated phenols [21], guaiacols and catechols [22] as well as resin and fatty acids [23] from sediment, soil and other matrices. The short extraction times, near solventless operation and more selective extraction provided by supercritical fluid extraction (SFE) eliminate the intrinsic drawbacks created by solvent extraction. In a previous publication, removal of PCB from fish oil using supercritical carbon dioxide has been demonstrated [24]. In this publication, we describe an efficient SFE technique for the quantitative extraction of native PCBs and several chlorinated insecticides from homogenized fish with minimal amounts of coextracted lipid.

EXPERIMENTAL

Reagents and Materials

(a) Solvents.--All organic solvents of distilled-in-glass grade were supplied by Burdick and Jackson and were used without purification.

(b) Standards.--PCB congener standard solutions (200 μg/mL in hexane) were purchased from Ultra Scientific Co. Hexachlorobenzene, *p,p'*-DDE and mirex were obtained either from Aldrich Chemical Co. or Ultra Scientific Co. Chrysene-d_{12} was a product of MSD Isotopes.

(c) Sodium Sulfate.--Anhydrous, granular, analytical grade (BDH). Heated at 500°C overnight, cooled and kept in a tightly capped bottle inside a desiccator.

(d) Basic alumina.--80 to 200 mesh, Brockman activity I (Fisher Scientific). Reactivated at 200°C overnight and kept as described above.

(e) Florisil.--60 to 100 mesh (Supelco). Reactivated at 130°C and kept as described above for alumina.

(f) Carbon dioxide.--SFE/SFC grade (Air Products) without helium head pressure.

Preparation of fish samples

Except for a small proportion of samples, all extractions in this work were performed on selected fish archives with native PCBs. The edible part of each contaminated fish was previously homogenized in a Waring blender until the sample was in a slurry form. The tissues were then subsampled into 50 mL glass jars and kept at -20°C until extraction. The storage time of these samples varies from a few months to a few years. In the spike recovery experiments, each 1 g subsample of an uncontaminated and freshly homogenized trout in a 50 mL beaker was fortified with 500 μL of a 1:1 mixture of Aroclors 1254 and 1260 in acetone to a predetermined level. The sample was mixed and equilibrated for 30 min inside a fume hood before it was processed.

SFE of fish tissue

All extractions were performed with a Hewlett-Packard (Little Falls site, Wilmington, DE 19808) 7680T SFE module equipped with standard 7-mL stainless steel thimbles and an octadecylsilane (ODS) trap. Before extraction, a layer of Whatman GFC filter paper cut to the diameter of the thimble was placed just above the bottom thimble cap. In a typical experiment, a 1 g fish tissue sample was mixed with 3 g of anhydrous sodium sulfate before it was transferred to the thimble with the aid of an adapter funnel which came with the module. The thimble was then filled with 4 g of the activated basic alumina and sealed with another cap at the top. Extraction was performed at 100°C with carbon dioxide at a density of 0.71 g/mL (5000 psi or 35 MPa). Extraction times were 0.5 min static and 20 min dynamic and the flow rate was 2.5 mL/min. The variable diameter restrictor nozzle, used to depressurize carbon dioxide and to provide constant flow at the prescribed pressure, was kept at 45°C. During the extraction, the ODS trap was maintained at 15°C for the efficient adsorption of the organics. At the end of extraction, the trap was heated to 40°C before the analytes were eluted with 1.2 and 0.8 mL of *iso*-octane in two fractions. The rinse solvent flow rate was 2 mL/min. The time required for the entire extraction cycle was ca. 38 min.

Cleanup of fish extract

A Florisil cleanup column was prepared by packing 5 cm of activated Florisil with 5 mm of anhydrous sodium sulfate on top in a disposable Pasteur pipet with a glasswool plug. Prior to use, the column was first rinsed with 3 mL of petroleum ether (b.p. 30-60°C) and the rinsing was discarded. Just before the rinse solvent sank into the sodium sulfate layer, the SFE extracts were quantitatively transferred, with three 1 mL rinses of petroleum ether to the Florisil column. The column was further eluted with the same solvent until a total of 10 mL of eluate was collected in a graduated test tube. The solvent was then exchanged into *iso*-octane and the final volume was adjusted to 1 mL. One hundred μL of concentrated sulfuric acid was added to the concentrated extract and

the mixture was vigorously shaken on a vortex mixer for 1 minute. After the acid settled, the top (organic) layer was ready for GC analysis.

Determination of lipid content in sample extract

For lipid determination, the SFE extracts were combined, with hexane rinsings, in a tared test tube and evaporated under nitrogen in a 45°C water bath until the solvent was completely removed. The percentage lipid content in the extract was calculated by multiplying the ratio of the oily residue weight to the sample weight before SFE by 100.

Analysis of PCBs by GC-ECD

To determine the relative recovery of PCBs, samples extracted by Soxhlet and SFE techniques were analyzed by a Hewlett-Packard (HP) 5890 Series II GC equipped with an electron capture detector (ECD) and a 30 m x 0.25 mm I.D. J&W DB-5 column of 0.25μ film thickness. The GC conditions were: injection port, 250°C, detector, 300°C, initial oven temperature, 70°C with a 1 min hold, programming rates, 30°C/min (from 70 to 160°C) and 2°C/min (from 160 to 260°C), and a 5 min hold at the final temperature of 260°C. Constant carrier (hydrogen) flow at 1.5 mL/min was maintained by an electronic pressure controller. One μL splitless injections were made by a HP 7673 autosampler with a splitless time of 1 min. Areas of all peaks, with the exception of hexachlorobenzene, *p,p'*-DDE and mirex, in the ECD chromatogram of a SFE sample were summed and compared to the areas of the corresponding Soxhlet extracted sample for the calculation of the relative PCB recovery. Concentrations of the above three insecticides in the extracts were determined by ECD using authentic external standards.

Analysis of PCBs by GC-MSD

For the determination of absolute PCB concentrations, the method developed by Alford-Stevens et al. [25] was adopted. Briefly, a chlorobiphenyl congener which has

the average mass spectrometric response factor from each level of chlorination was selected to measure the total concentration of all isomers in the group. The congeners used were: 2-chlorobiphenyl, 2,3-dichlorobiphenyl, 2,4,5-trichlorobiphenyl, 2,2',4,6-tetrachlorobiphenyl, 2,2',3,4,5'-pentachlorobiphenyl, 2,2',4,4',5,6'-hexachlorobiphenyl, 2,2',3,4',5,6,6'-heptachlorobiphenyl, 2,2',3,3',4,5'6,6'-octachlorobiphenyl and decachlorobiphenyl. The last congener was selected to quantitate both the nona- and deca- chlorobiphenyls. Mixtures of the above nine congeners at 250 and 25 pg/μL with chrysene-d_{12} (internal standard) at 500 pg/μL were prepared in *iso*-octane for the generation of response factors. PCBs in sample extracts were quantitated in selected ion monitoring mode using a Hewlett-Packard 5972A Mass Selective Detector. The same GC conditions described for the ECD work above were used except that helium was the carrier gas and the flow rate was 0.85 mL/min. The electron energy and electron multiplier voltage were 70 eV and 2000 V, respectively. The quantitation and confirmation ions monitored were, respectively, m/z 188 and 190 for the mono's, m/z 222 and 224 for the di's, m/z 256 and 258 for the tri's, m/z 292 and 290 for the tetra's, m/z 326 and 328 for the penta's, m/z 360 and 362 for the hexa's, m/z 394 and 396 for the hepta's, m/z 430 and 432 for the octa's, m/z 464 and 466 for the nona's and m/z 498 and 500 for the deca.

RESULTS AND DISCUSSION

Effect of SFE condition on the lipid content in fish extracts

One of the major drawbacks in conventional solvent extraction for fish tissues of high fat content is the presence of a large amount of coextracted lipid which has to be separated from other organics otherwise chromatographic performance will seriously deteriorate. Fish oils and lipids can be effectively removed by gel permeation chromatography yet the process is time consuming and it also consumes a large amount of solvent including dichloromethane. In an earlier study, Krukonis developed a process to remove PCBs from fish oils using supercritical carbon dioxide under modest

temperature and pressure [24]. David et al. also showed that PCBs could be selectively extracted from seagull eggs with carbon dioxide and the extracts were nearly fat-free [26]. Research at Hewlett-Packard on the SFE of cholesterol and Vitamin A from fatty food also indicated that fats are largely unextracted when lower density (or pressure) carbon dioxide was used [27,28]. Using trout and carp samples with high percentages of lipid (from 23 to 38% w/w), the lipid content in sample extract obtained under various SFE conditions were determined. In these experiments, 1 g of homogenized fish tissue was mixed with 3 g of granular anhydrous sodium sulfate before SFE since the salt improved extraction efficiency by increasing the effective surface area of the sample. The sample was then extracted with carbon dioxide for 20 minutes at a flow rate of 3 mL/min under low ($\leq$ 2250 psi) and high (ca. 5000 psi) pressure conditions at 60°, 80° and 100°C. The results (Figure 1) indicated that, for all three samples, less than 3% by weight of lipid was extracted by carbon dioxide at a pressure of 2250 psi or lower, regardless of the extraction temperature. In contrast, extractions carried out with carbon dioxide at ca. 5000 psi all yielded much higher lipid content (from 13 to 28%) in the SFE extracts at temperatures of either 60°, 80° or 100°C. It should be noted that, even under the more drastic SFE conditions, the amounts of lipid in the extracts were less than 70% of the values determined by Soxhlet extraction. The key to low lipid contents in the fish extracts, however, is to carry out the extraction at low carbon dioxide pressure.

Effect of SFE condition on the recovery of native PCBs from fish

Whole fish homogenates of a lake trout and a carp with native PCBs were employed to evaluate the SFE recoveries using non-modified carbon dioxide. For comparison, the same samples were extracted by a 1:1 (v/v) mixture of dichloromethane and hexane for 7 h in a Soxhlet apparatus and the extracts were cleaned up by GPC and Florisil column chromatography. At the beginning, a mild SFE condition (60°C and 1872 psi) was used since this condition was shown to coextract very little lipid from the sample. However, incomplete extraction of PCBs was experienced in both samples since less than 35% of the PCBs was extracted from the fish when compared to Soxhlet

extraction (Figure 2). Raising the extraction temperature to 100°C while keeping the carbon dioxide pressure at a relatively low 2253 psi only produced a slight improvement in the recovery. In contrast, raising the fluid pressure to 5000 psi while keeping the extraction temperature at 60°C generated a PCB recovery as much as 85%. Soxhlet-like recoveries were only obtained at 100°C and 5000 psi, a condition which had also been shown to produce quantitative results for PCBs in sediments [13]. However, this condition also recovered high percentages of lipid which necessitated more than a simple column cleanup step for the complete removal of the coextractives. In an attempt to simplify the cleanup procedure, further optimization of the SFE procedure was attempted to achieve quantitative and yet selective (i.e. lipid-free) extraction of PCB in fish tissues.

Reduction of lipid in SFE extract of fish

Adsorbents such as alumina and Florisil have long been used for the removal of lipids in the column cleanup of PCBs and organochlorine insecticides in fatty food extract. In the first experiment we tried, a small amount (1 g) of activated basic alumina was incorporated in the extraction thimble prior to SFE. Since the adsorbent was placed downstream of the sample in the flow path, this experimental setup mimicked a SFE followed immediately by an *in situ* and concurrent column chromatographic cleanup of the SFE extract using supercritical carbon dioxide in a one-step process. The results (Table 1) indicated that there was a drastic (ca. 50%) reduction of the lipid content of the SFE extract and at the same time, no losses of PCB recovery. Increases in the amount of alumina from 1 g to 2 or 4 g further reduced the lipid levels in the extracts. For example, the amount of lipid in the extract was reduced from 27.1 to 2.2 % when 4 g of alumina were incorporated in the SFE step for a carp sample having a lipid content as high as 38%. For other less fatty fish under similar SFE conditions, the amount of coextracted lipid was typically 0.5 % or less. Because of the physical dimension of the thimble, the amount of alumina used could not exceed 4 g by a great extent. There was no significant difference in the amount of coextracted lipid when 5% deactivated alumina was used in place of activated alumina. The lipid removal efficiency of activated Florisil

was also similar. Since Florisil has a lower density than alumina, the latter was chosen for the selective adsorption of lipid in the SFE process so that a larger amount of the adsorbent could be used.

Another way to reduce lipid in the SFE extracts was to optimize the amount of carbon dioxide used (i.e. the product of flow rate and extraction time) in the extraction, an aspect similar to the calibration of chromatographic column for the proper elution of different polarity fractions. The recovery of PCBs and lipid content in the extract were evaluated by varying the amount of carbon dioxide (80, 60, 50 and 40 mL) used in the extraction. As indicated in Table 1, optimal results (i.e. highest recovery of PCBs together with the least amount of lipid) were obtained by extractions using 50 mL of carbon dioxide. The use of more extraction fluid would only increase the lipid content with no improvement in the recovery of PCB. Although the amount of lipid could be further minimized with less carbon dioxide (40 mL or less), such conditions were deemed unsatisfactory since they all resulted in less than quantitative recovery for PCBs.

The combined SFE extract was cleaned up on a miniature Florisil column as described above to remove the remaining lipids. After solvent exchange and volume adjustment, two drops (ca. 100 μL) of concentrated sulfuric acid were shaken with the concentrated extract to remove the last trace of lipid before GC analysis. In our experience, sulfuric acid treatment was only required for samples with extremely high lipid content such as carp and fish liver.

Further evaluation and application of the SFE technique

Subsamples of an uncontaminated rainbow trout homogenate were spiked with a 1:1 mixture of Aroclors 1254 and 1260 to total PCB levels of 10 and 1 μg/g. Replicate extraction and analyses of the samples were performed to determine the precision and accuracy of the present procedure. The results in Table 2 indicate acceptable precision and no significant losses in the entire analytical process. Based on a sample size of 1 g,

the detection limit using the Mass Selective Detector was 10 ng/g for all chlorobiphenyls. This detection limit easily exceeds the requirement established by the guideline of 2 μg/g of PCBs for fish products. For lower detection limits of PCBs, methods employing negative ion chemical ionization mass spectrometry can be used [8,29].

The SFE/GC-MSD procedure has been applied to the determination of native PCBs in fish homogenates of various species, origin and lipid content. The results of three samples are summarized in Table 3. Again, the SFE results are in good agreement with the Soxhlet data for the same sample. In all three cases, the levels of mono-, di-, nona- and deca- chlorobiphenyls were below our detection limit. The majority of the chlorobiphenyls found were the penta's and hexa's, followed closely by the tetra's and hepta's, which is consistent with our previous findings on Lake Ontario salmonids [30]. Reconstructed single ion chromatograms for the tri- through octa- chlorobiphenyls in a trout are shown in Figure 3. Three commonly found organochlorines in fish, namely, hexachlorobenzene, *p,p'*-DDE and mirex, were also determined by the present procedure. As indicated by the results listed in Table 3, the SFE results of these three chemicals were nearly identical to the Soxhlet results, suggesting quantitative recoveries of these insecticides by the SFE procedure.

CONCLUSION

Supercritical carbon dioxide can replace organic solvents for the quantitative extraction of native PCBs, hexachlorobenzene, *p,p'*-DDE, and mirex in fish tissues. The incorporation of activated basic alumina in the SFE procedure reduces the amount of lipid in the extract to 2% or less, thereby eliminating the need of any GPC cleanup. The sample preparation, extraction and cleanup time for this procedure is less than 90 minutes and only 15 mL of non-chlorinated solvent are required for a sample. Unattended extraction of a series of samples can easily be accommodated by the automation features of supercritical fluid extractors. Soxhlet-like recoveries, near lipid-free extracts, environmental friendliness, rapid and automated extraction are the major advantages

which make this SFE approach a worthy consideration for the replacement of the conventional solvent extraction procedure for the determination of PCBs in fish.

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Table 1. % Lipid and PCB recoveries from fish under various SFE conditions. All extractions were performed with 1 g of fish tissue premixed with 3 g of anhydrous sodium sulfate at 100°C and 5000 psi with various amounts of alumina and carbon dioxide as indicated in the table. Lipid and PCB results were average of two determinations and the PCB results were relative to the Soxhlet values.

		Carp		Trout	
Adsorbent	CO_2 vol. (mL)	% Lipid	% PCB	% Lipid	% PCB
None	60	27.1	84	13.9	87
1 g basic alumina	60	14.6	89	6.7	92
2 g basic alumina	60	9.5	93	2.8	91
4 g basic alumina	60	5.0	99	0.4	93
4 g basic alumina	80	7.7	94	1.8	96
4 g basic alumina	50	2.2	97	<0.1	95
4 g basic alumina	40	0.4	72	<0.1	78

Table 2. Precision and accuracy of the SFE procedure for PCB from spiked trout homogenate (number of replicates = 6).

Spiking level (μg/g)	% Recovery	S.D.
10	97.9	2.3
1	98.6	3.8

Table 3. Levels of chlorobiphenyls and selected chlorinated insecticides (ng/g) in fish as determined by SFE. Soxhlet values (in parentheses) are included for comparison. All results are the average of two determinations.

Parameter	Carp	Pickerel	Trout
Chlorobiphenyls	<10 (<10)	<10 (<10)	<10 (<10)
Dichlorobiphenyls	<10 (<10)	<10 (<10)	<10 (<10)
Trichlorobiphenyls	68 (71)	10 (11)	72 (77)
Tetrachlorobiphenyls	1146 (1199)	136 (141)	886 (923)
Pentachlorobiphenyls	2725 (2854)	484 (467)	2658 (2710)
Hexachlorobiphenyls	1868 (1920)	510 (497)	2203 (2263)
Heptachlorobiphenyls	326 (321)	199 (193)	583 (597)
Octachlorobiphenyls	74 (71)	60 (65)	142 (143)
Nonachlorobiphenyls	<10 (<10)	<10 (<10)	<10 (<10)
Decachlorobiphenyl	<10 (<10)	<10 (<10)	<10 (<10)
Total PCBs	6207 (6436)	1399 (1373)	6544 (6712)
Hexachlorobenzene	13 (15)	<1 (<1)	7 (6)
p,p'-DDE	458 (477)	127 (122)	353 (368)
Mirex	151 (152)	137 (140)	127 (125)

LIST OF FIGURES

Figure 1

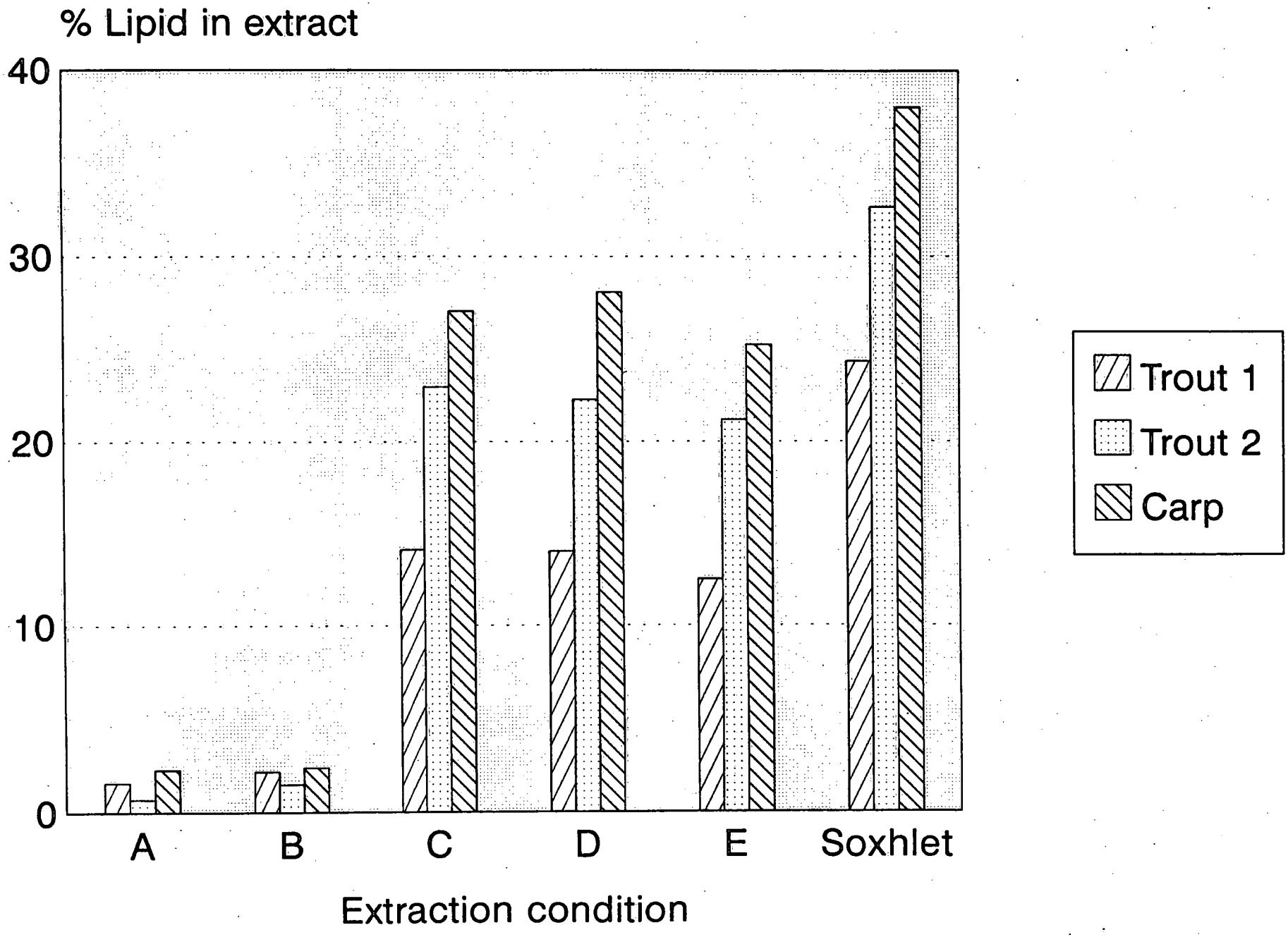

% Lipid in extract
40
30
20
10
0
A
B
C
D
E
Soxhlet
Extraction condition
Trout 1
Trout 2
Carp

Figure 2

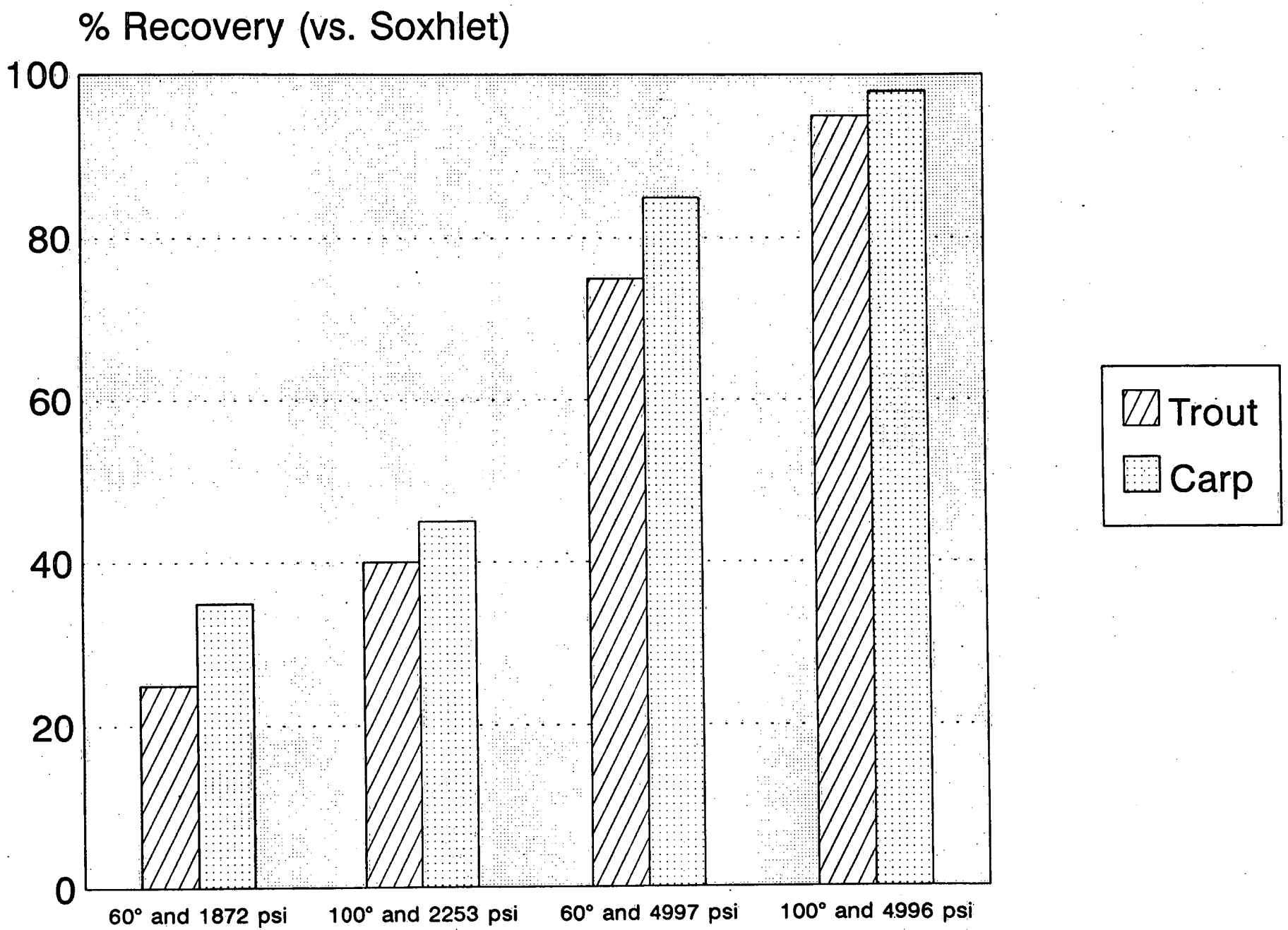

% Recovery (vs. Soxhlet)
100
80
60
40
20
0
60° and 1872 psi
100° and 2253 psi
60° and 4997 psi
100° and 4996 psi
Trout
Carp

Figure 3

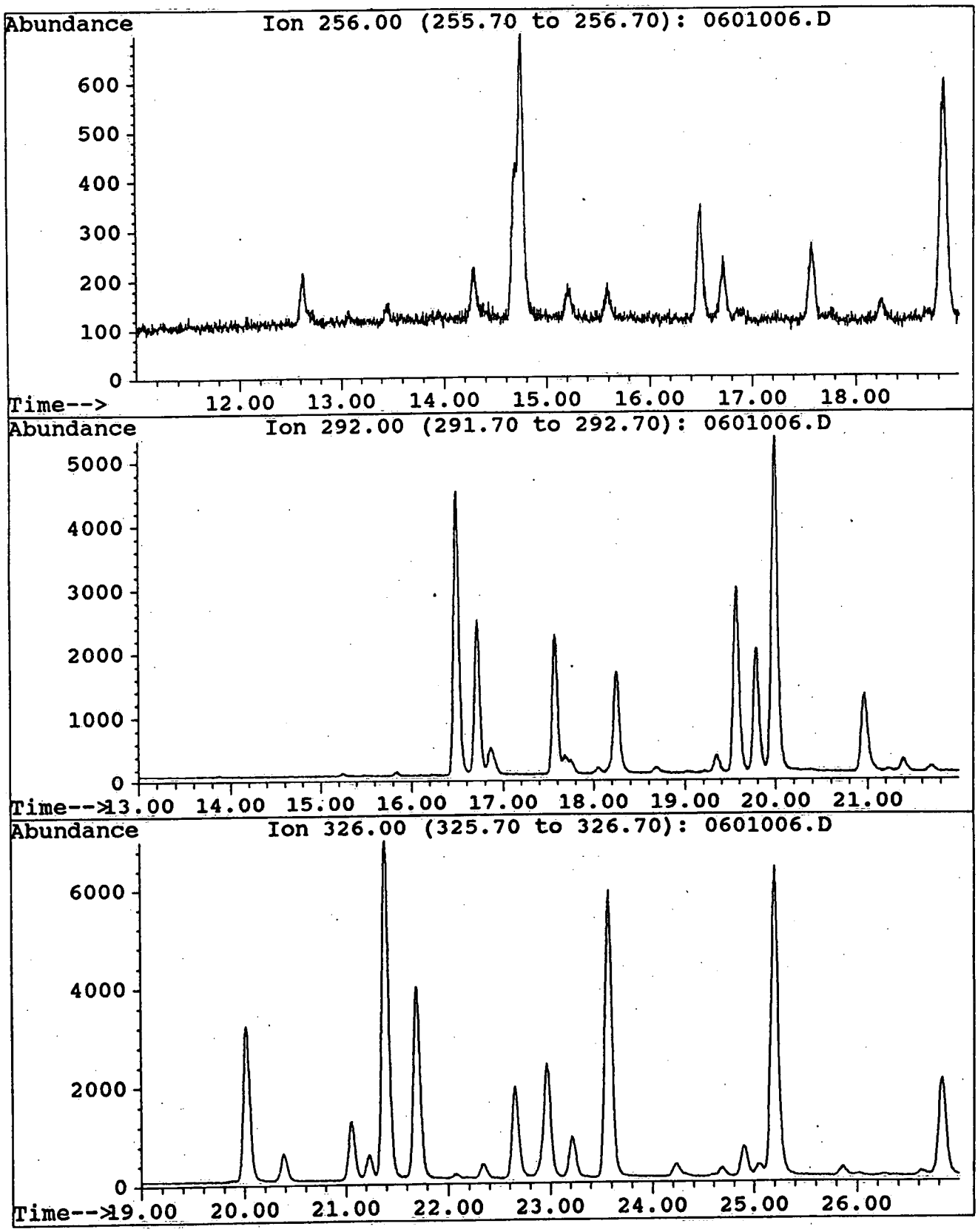
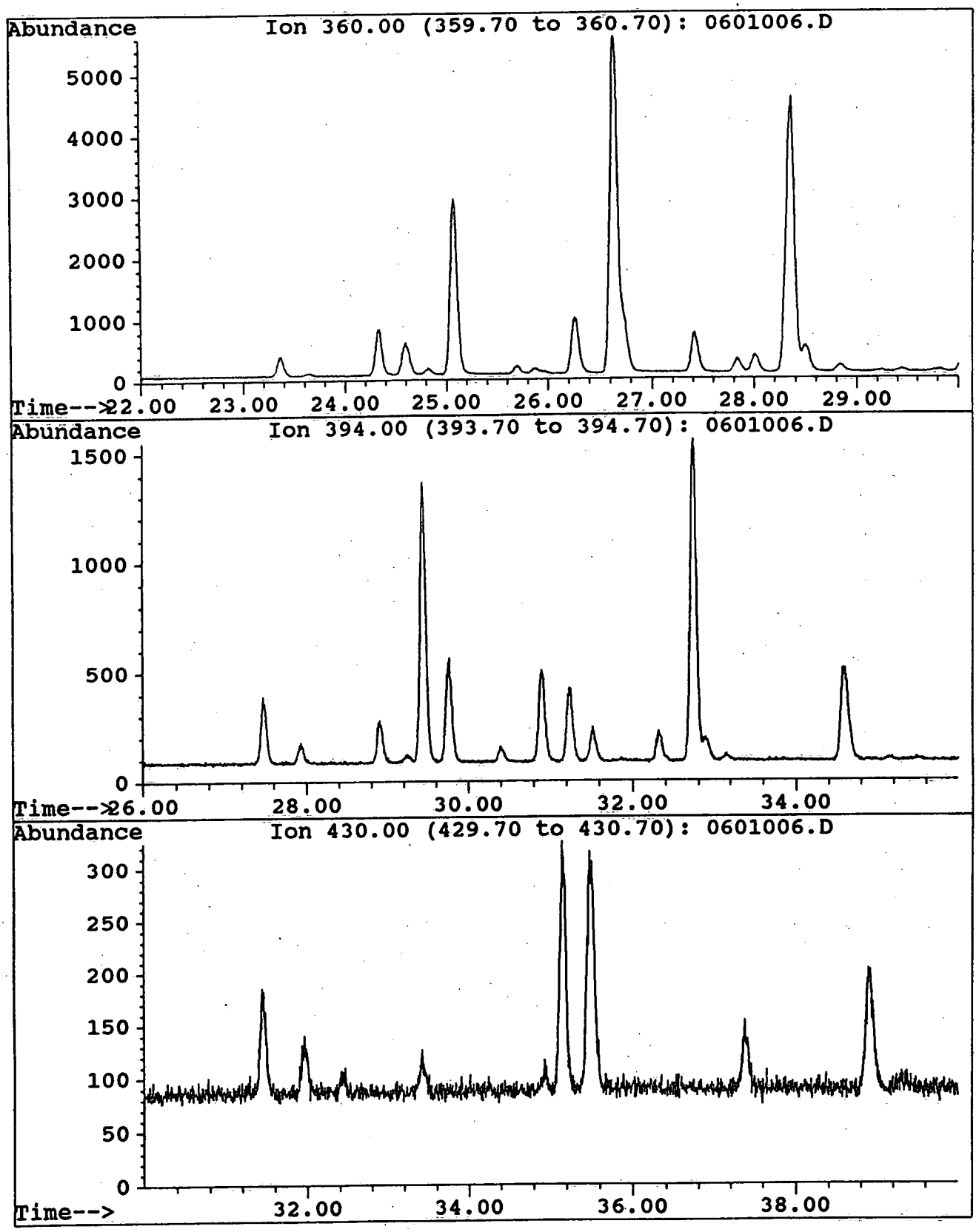
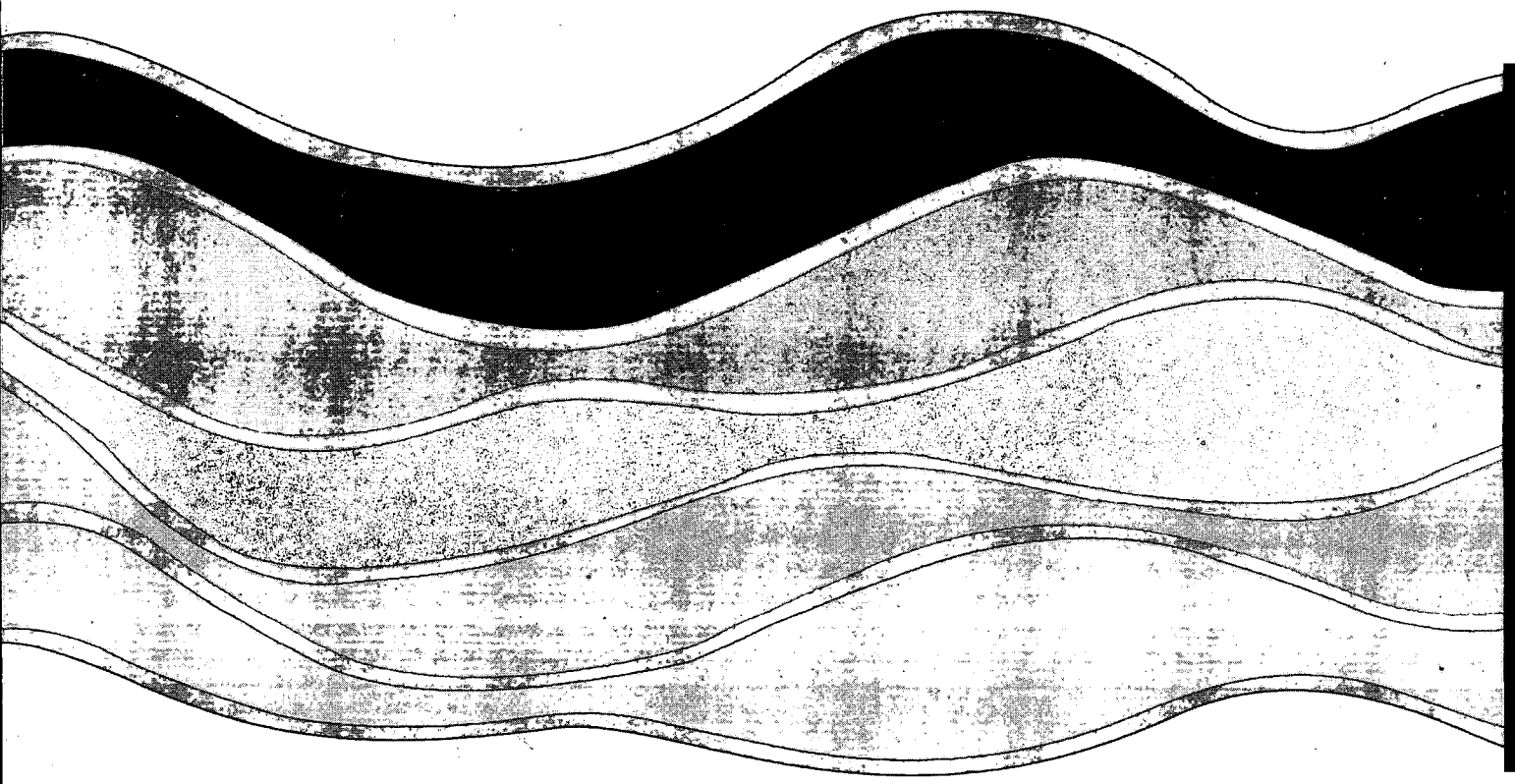

Abundance
Ion 256.00 (255.70 to 256.70): 0601006.D
600
500
400
300
200
100
0
Time-->
12.00 13.00 14.00 15.00 16.00 17.00 18.00
Abundance
Ion 292.00 (291.70 to 292.70): 0601006.D
5000
4000
3000
2000
1000
0
Time-->13.00 14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00
Abundance
Ion 326.00 (325.70 to 326.70): 0601006.D
6000
4000
2000
0
Time-->19.00 20.00 21.00 22.00 23.00 24.00 25.00 26.00

Figure 3 (cont'd)





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