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Isolation of chlorinated fatty acid methyl esters derived from cell-culture medium and from fish lipids by using an aminopropyl solid-phase extraction column

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Abstract

An aminopropyl-based solid-phase extraction technique was used for isolation of chlorinated fatty acids in lipids. A range of different chlorinated fatty acids was eluted in a small volume of solvent (4 ml) and the recoveries of the different species and isomers were quantitative. Only 1% of the vastly dominating unchlorinated fatty acid methyl esters were recovered in the fractions containing the chlorinated fatty acid methyl esters. This method makes it possible to isolate and detect ≥1 μg of a chlorinated fatty acid methyl ester in 1 g of lipid.

Keywords: Fatty acid methyl esters; Lipids; Organochlorine compounds

1. Introduction

Chlorinated fatty acids (ClFAs) have been found to be the major contributors to the extractable organochlorine compounds in marine biota, making up to 90% of extractable organically bound chlorine (EOCl) [1–7]. The presence of ClFAs in human tissue has not been reported, but can be regarded as possible because ClFAs have been found to be assimilated in the food chain in much the same way as unchlorinated fatty acids [8–10]. Further, in a recent study it was shown that human cell lines can incorporate and metabolise chlorinated fatty acids [11]. High concentrations of ClFAs in biota have been connected to anthropogenic inputs such as effluents from chlorine bleached pulp production [3,7,12,13]. A comprehensive review on ClFAs has recently been published [14]. Methods for the determination of ClFAs have relatively newly been developed [2,4,5], which may explain why these compounds only recently have come to attention. The methods were developed primarily for analysis of ClFAs originated from effluents of chlorine bleached pulp production. On account of their relatively low natural abundance, the analysis of ClFAs demands the use of extensive isolation methods and

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very selective and sensitive detection methods, such as an electrolytic conductivity detection (ELCD) [2] or halogen-specific detection (XSD) [15], when determined by gas chromatography (GC) as the corresponding fatty acid methyl esters (FAMEs). The concentration of EOCl in most fish samples ranges from 20 to 60 μg/g [7,16,17], but concentrations up to 2000 μg/g lipid have been reported [18]. The separation of chlorinated (ClFAMEs) and unchlorinated FAMEs is important, because the latter will otherwise overload the GC capillary column, when samples containing detectable amounts of ClFAMEs are injected. Moreover, in the GC–mass spectrometry (MS) system, some of the common FAMEs and the ClFAMEs have similar retention times, resulting in problems of identification of the ClFAMEs, again calling for better methods for isolation of ClFAMEs to be developed.

In FAMEs released from lipid extracts, polyunsaturated and saturated species can to a large degree be removed by silver ion and urea complexation [4], thus increasing the concentration of ClFAMEs. Additional removal of unchlorinated FAMEs can be achieved by thin-layer chromatography (TLC) [4]. Such a procedure was developed for an eel lipid sample containing an EOCl concentration of 1200 μg/g lipid. On analysing samples with very low concentrations of ClFAMEs (<<20 μg/g), however, the silver and urea complexation methods are not satisfactory in removing unchlorinated FAMEs, and TLC is too laborious. Gel permeation chromatography (Sephadex LH-20) has been used for the enrichment of methyl ester of dichlorotetradecanoic acid in transesterified lipids of lobster digestive glands with EOCl concentrations of about 100 μg/g lipid [5], but this method demands several hours for fractionation [5].

Solid-phase extraction (SPE), on an aminopropyl column, has been shown by Kalunzy et al. [19] to be an efficient technique in the separation of different lipid classes. Therefore, the purpose of this work was to study the separation of ClFAMEs from unchlorinated FAMEs by using SPE. Standards of FAMEs and ClFAMEs, and complex samples, a cell-culture medium, containing ClFAME metabolites, and a well-examined eel sample containing several different isomers and species of ClFAMEs, were used in the study. The aim was to further improve the isolation of ClFAs.

2. Materials and methods

2.1. Chemicals

The following chemicals were used in this study: *cis*-9-octadecenoic acid (99%, Sigma, St. Louis, MO, USA), *cis*-9-tetradecenoic acid (99%, Sigma), methyl esters of hexadecanoic acid, octadecanoic acid, *cis*-9-octadecenoic acid, *cis*,*cis*-9,12-octadecadienoic acid, *cis*,*cis*,*cis*-9,12,15-octadecatrienoic acid (99%, Sigma), borontrifluoride (20% solution in methanol, Merck, Hohenbrunn, Germany), *n*-hexane (>99% Merck, Drabant, Germany), cyclohexane (>99.5%, Riedel-de Haën, Seelze, Germany), chloroform (99.8%, J.T. Baker, Deventer, The Netherlands), diethyl ether (>99.5%, Merck, Darmstadt, Germany), methanol (J.T. Baker, >99.8%), ethyl acetate (>99.5%, Merck), methylene chloride (>99.5%, Riedel-de Haën), toluene (>99.5%, J.T. Baker), and acetone (>99.5%, J.T. Baker). The purity was controlled in blank procedures. After conventional cleaning; all glassware was rinsed with acetone (>99.5%) and heated at 250 °C for 15 h before use.

2.2. Sample preparation

threo-9-Dichlorooctadecanoic acid and *threo*-9-dichlorotetradecanoic acid were prepared from *cis*-9-octadecenoic acid and *cis*-9-tetradecenoic acid, respectively, according to the procedure of Mu et al. [20]. The acids were esterified with 20% BF_3 in methanol and toluene, according to Morrison and Smith [21]. The purities of methyl dichlorooctadecanoate ($C_{18}Cl_2$) and methyl dichlorotetradecanoate was studied by GC–MS with electron ionisation (EI-MS) [20] and were found to be 85 and 90%, respectively (based on assessment of the total ion chromatogram, TIC). Two complex samples containing ClFAs were prepared. One of the samples was derived from lipids extracted from a cell growth (Intestine-405, a human cell line) medium where the cells had been incubated with dichlorooctadecanoic acid [11]. The other sample was an earlier well-examined eel lipid extract [20]. The cell growth medium containing chlorinated metabolites (dichlorotetradecanoic acid and dichlorohexadecanoic acid) of dichlorooctadecanoic acid was prepared according to procedure described by Gustafson-Svärd et al. [11]. The lipids were extracted according

to a modified version of the procedure described by Bligh and Dyer [22], where addition of KCl was used to ensure the complete extraction of polar lipids [23] and transesterified with BF_3 in methanol and toluene. The eel lipids were transesterified and enriched by Wesén according to Wesén et al. [2] and Mu et al. [4].

2.3. GC analysis

A GC system (Shimadzu, Model 17 A, Kyoto, Japan) was equipped with two BPX5 (5% phenyl polysilphenylene–siloxane, 25 m×0.22 mm I.D., film thickness=0.25 μm) fused-silica capillary columns (SGE, Sydney, Australia) fitted to two separate injectors. One column was connected to an XSD system (Model 5360, OI Analytical, College Station, TX, USA). The other column was connected to a quadrupole EI-MS system (Shimadzu, Model QP-5000, Kyoto, Japan). Helium (99.997%, Air Liquide, Malmö, Sweden) was used as carrier gas. The injector temperatures were 280 °C and the carrier gas flow was 1 ml/min. The column oven temperature program was 90 °C (held for 2 min) to 300 °C at 15 °C/min (held for 5 min). In the XSD system, a manual wide-bore injection technique was used. Medical air (Air Liquide) was used, as combustion gas at a flow of 12–20 ml/min, and the detector temperature was 1000 °C. In the EI-MS system, a split injection technique was used (split ratio 1:70), the interface temperature was 300 °C, the electron energy was 70 eV, and the m/z range 34–500 was scanned at a rate of 2 scans/s. The GC column, used in this system, could not resolve $C_{18:1}$ and $C_{18:2}$, which therefore are quantified together.

2.4. Solid-phase extraction procedure

A 500-mg aminopropyl (LC-NH_2) column (Supelco, No. 57014, Bellefonte, PA, USA), with a capacity of about 25 mg, and a bed volume of 0.6 ml, was connected to a vacuum manifold (Visiprep, Supelco). Three different columns from different lots were tested. The solvent flow through the column was 0.5–1 ml/min. All the columns were conditioned with 2 ml of *n*-hexane. In a first attempt to separate FAMEs from ClFAMEs, a mixture of 1 mg of $C_{18:1}$ and 10 μg of $C_{18}Cl_2$ were eluted from the LC-NH_2 columns according to Kalunzy et al. [19], and the different fractions were analysed for their content of $C_{18:1}$ and $C_{18}Cl_2$ by using GC–MS and GC–XSD, respectively. The isolation of ClFAMEs in a complex sample was studied by applying 4.0 mg of transesterified sample, liberated from the cell-

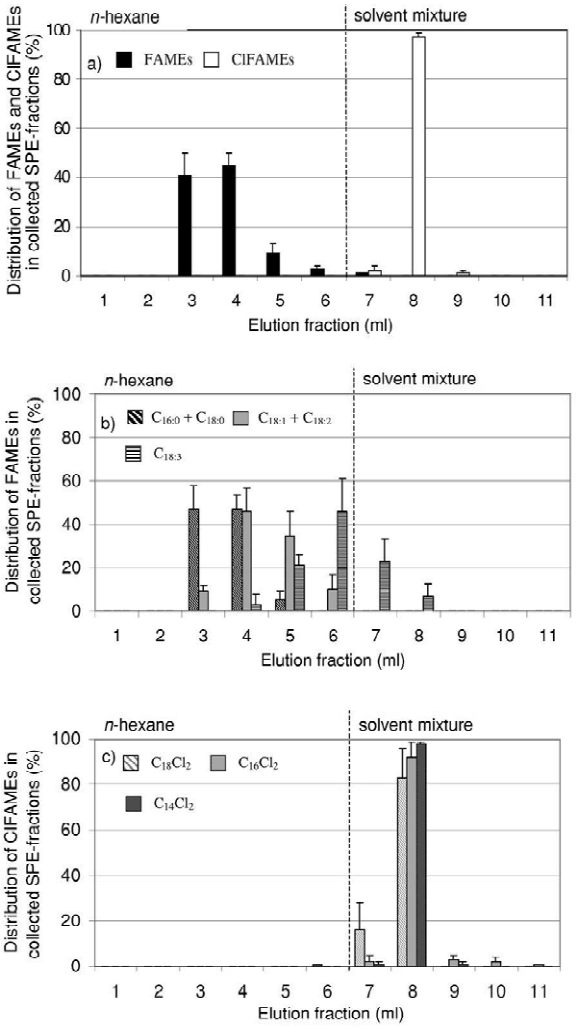

Fig. 1. Distribution of eluted FAMEs and ClFAMEs (MS- and XSD-based), liberated from cell-culture medium containing chlorinated fatty acid metabolites, collected from a 500-mg LC-NH_2 column. In (a) the distributions of total FAMEs and ClFAMEs are shown, in (b) and (c) the distributions of FAME and ClFAME species, respectively, are shown. Fractions 1–6 ml are *n*-hexane, and fractions 7–11 consists of a solvent mixture of *n*-hexane–diethyl ether–methylene chloride (89:1:10, v/v). Fractions 12–14 are excluded from the figure, because no FAMEs and ClFAMEs were detected in these fractions. Values in the figure are distribution means±SD of four samples run in parallel.

culture medium, in 200 μl of *n*-hexane onto the column. Six 1-ml fractions of *n*-hexane and eight 1-ml fractions of a solvent mixture (*n*-hexane–diethyl ether–dichloromethane, 89:1:10, v/v) were pulled through (the elution was stopped when the solvent was at level with the phase surface) and collected separately. Each of the collected samples was evaporated to dryness at 40 °C under a gentle flow of nitrogen and then dissolved in 200 μl of *n*-hexane. Four identical experiments were made. The mass of every fraction was determined gravimetrically (Sartorius, Model BP211D, Goettinger, Germany). The recoveries of FAMEs and ClFAMEs were determined by GC–MS and GC–XSD, respectively, and 1 μl was injected in each system. In accordance with the result of the experiments above, ClFAMEs in a sample of natural origin were isolated. An eel lipid sample, transesterified to methyl esters and treated with silver nitrate and urea (0.34 mg in 200 μl of *n*-hexane) [4,20] was applied to the column and 6 ml of *n*-hexane, and 4 ml of the solvent mixture were used for the fractionation. Each fraction was collected separately and evaporated to dryness and then dissolved in 200 μl of *n*-hexane, and the mass of each fraction was determined gravimetrically. The recoveries of FAMEs and ClFAMEs were determined by GC–MS and GC–XSD as above.

3. Results and discussion

3.1. SPE fractionation of ClFAMEs and common FAMEs in extracts of cell-culture medium

The reference compounds, $C_{18:1}$ and $C_{18}Cl_2$, were not completely separated when applying the original SPE method [19], which recommends 4 ml of *n*-hexane to collect unpolar fatty acid cholesterol esters. $C_{18:1}$ was partly co-eluted with $C_{18}Cl_2$. In order to improve the resolution, the volume of *n*-hexane was increased to 6 ml. $C_{18:1}$ was recovered in *n*-hexane and $C_{18}Cl_2$ was recovered in the following solvent mixture, which is more polar than *n*-hexane. If more than 6 ml of *n*-hexane was pulled through, part of the $C_{18}Cl_2$ was eluted in the *n*-hexane fraction (data not shown). When studying extracts of the cell-culture medium, it was found that the recovery of ClFAMEs was almost 100% in 4 ml of the polar solvent mixture, fractions 7–11 (Fig. 1 and Table 1). Most of the ClFAMEs were recovered in only one fraction, fraction 8 (Fig. 1), but up to 30% of the eluted $C_{18}Cl_2$ could be found in fraction 7 (Table 1). Neither methyl dichlorotetradecanoate ($C_{14}Cl_2$) nor methyl dichlorohexadecanoate ($C_{16}Cl_2$) was co-eluted with the FAMEs, but a small part of $C_{18}Cl_2$ was co-eluted with the FAMEs (Fig. 1). Most of the FAMEs were eluted in fractions 3

Table 1
Distribution of FAMEs and ClFAMEs in SPE fractions

Fraction	$C_{18.0}+C_{16:0}$		$C_{18:1}+C_{18:2}$		$C_{18:3}$		$C_{18}C_{12}$		$C_{16}Cl_2$		$C_{14}Cl_2$	
	%	SD	%	SD	%	SD	%	SD	%	SD	%	SD
3[a]	47	11	9	3								
4[a]	47	7	46	11	3	5						
5[a]	5	4	35	11	21	5						
6[a]	0	0	10	7	46	15	1	0				
7[b]	<1	<1	<1	<1	24	10	16	12	2	3	1	1
8[b]	<1	<1	<1	<1	7	6	83	13	92	7	98	1
9[b]									3	2	1	1
10[b]									2	2		
11[b]									<1	<1		

Lipids liberated from cultured cell medium containing chlorinated fatty acids metabolites. Values are distribution means±SD of four samples run in parallel.

[a] *n*-Hexane.

[b] Solvent mixture.

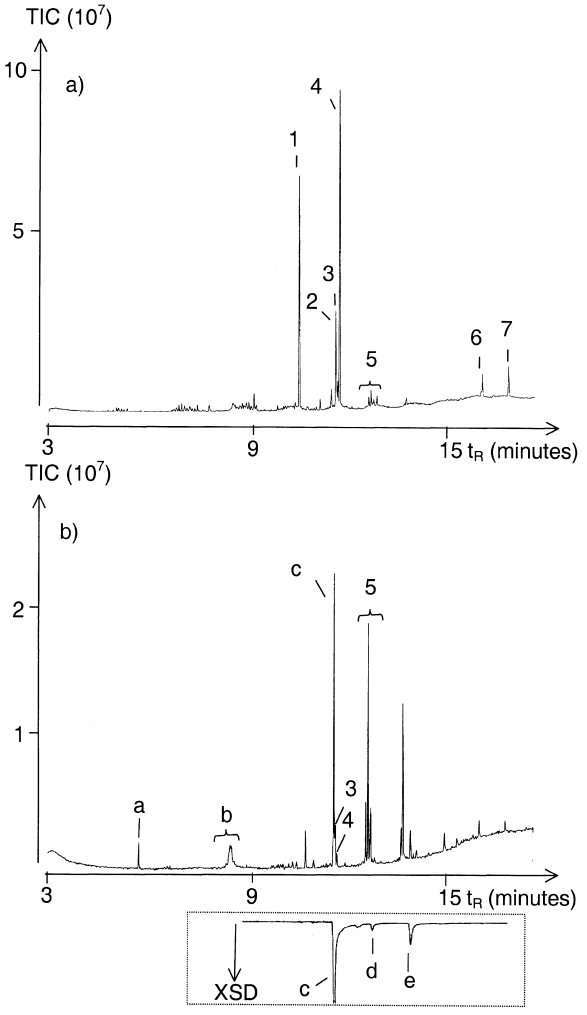

Fig. 2. TIC of transesterified lipids, liberated from cell-culture medium containing chlorinated fatty acid metabolites (a) before SPE isolation (the sample is diluted five times and 4 μg dry mass was injected), and (b) after SPE isolation (the sample is not diluted and <0.5 μg dry mass was injected). The following peaks are identified as: (1) methyl hexadecanoate, (2) methyl octadecadienoate+methyl octadecenoate, (3) methyl octadecatrienoate, (4) methyl octadecanoate, (5) a cluster of methyl esters of FAMEs of different fatty acids containing 20 carbon atoms, (6 and 7) possible cholesterol derivatives, (a) bicyclohexyl, (b) phthalates, (c) methyl dichlorotetradecanoate. The other peaks are unidentified. (d) Methyl dichlorohexadecanoate and (e) methyl dichlorooctadecanoate can only be obtained in the XSD chromatogram (lower chromatogram), not in the TIC. The other peaks are unidentified.

and 4 (Fig. 1), but some of the polyunsaturated FAMEs such as $C_{18:3}$ and $C_{20:n}$ (FAMEs from fatty acids containing 20 carbon atoms and different numbers of double bonds) were also found to be co-eluted with the ClFAMEs (Fig. 2). According to the TIC peak area, about 40% of eluted $C_{20:n}$ were co-eluted with the ClFAMEs (data not shown). The separation of different species of FAMEs (Fig. 1) might be explained by saturated and monounsaturated FAMEs being preferentially eluted with *n*-hexane, whereas polyunsaturated FAMEs need a more polar solvent such as dichloromethane to be eluted [24]. Saturated FAMEs are supposed to form weaker dipolar interactions with the stationary phase than polyunsaturated FAMEs [24], resulting in a larger retention of the polyunsaturated FAMEs. A similar reasoning is probably also applicable to the ClFAMEs, which need a solvent mixture containing some dichloromethane. $C_{16}Cl_2$ and $C_{14}Cl_2$ were found to elute later than $C_{18}Cl_2$ (Fig. 1). Thus, small differences in polarities between $C_{18}Cl_2$, $C_{16}Cl_2$ and $C_{14}Cl_2$ might be enough to be used in future isolation of individual ClFAMEs.

The recoveries of $C_{18}Cl_2$, $C_{16}Cl_2$, and $C_{14}Cl_2$ and of the saturated and monounsaturated FAMEs were high (Table 2), but the exceedingly high value for $C_{16}Cl_2$ might be explained by its concentration being close to the detection limit. A small absolute error can result in a large relative error, giving a high uncertainty in the recovery calculation. The recovery of polyunsaturated FAMEs, such as $C_{20:n}$ was only 50–60% (data not shown), might be explained by the solvent mixture, not being polar enough to elute the polyunsaturated FAMEs.

3.2. Isolation of ClFAMEs from a cell-culture medium

By using this SPE method modified from Kalunzy et al. it was possible to eliminate the largest portion of common FAMEs from a mixture containing ClFAMEs (Fig. 2 and Table 3), but for isolation ClFAMEs it is also of interest to remove other types of compounds that can be present in a chloroform extract [25]. Most of the compounds in the cell-culture sample do not generate any signal in a GC–MS system, only 25% of the extract applied on the SPE column was calculated to generate signal in

Table 2
FAMEs and ClFAMES before and after SPE fractionation

Compound	Applied[a] (μg)	Eluted[b] (μg)	SD	Recovery (%)	SD
$C_{16:0}$	210	180	10	83	4
$C_{18:0}$	320	290	10	89	4
$C_{18:1}+C_{18:2}$	80	60	10	78	8
$C_{18:3}$	30	20	0	68	8
$C_{18}Cl_2$	2.46	1.85	0.21	75	9
$C_{16}Cl_2$	0.22	0.43	0.08	192	37
$C_{14}Cl_2$	20.00	22.29	2.20	111	11

Lipids are liberated from cultures cell medium containing ClFA metabolites.
[a] Based on one sample, the instrumental relative SD previously being determined to about 10%.
[b] Values are means±SD of our four samples run in parallel, and are from the same experiment.

Table 3
Relative peak areas of methyl esters in TIC before and after SPE fractionation

Compound	Applied sample		Fraction 8	
	Distribution (%)	SD	Distribution (%)	SD
$C_{14}Cl_2$	0.6[a]	0.4	23	2
$C_{16:0}$	19.3[b]		0	0
$C_{18:1}+C_{18:2}$	11.1[b]		0	0
$C_{18:3}$	1.5[b]		3	3
$C_{18:0}$	31.5[b]		1	1
$C_{20:n}$	3.7[b]		22	7
Total	67[b]		48	9

Lipids liberated from cultured cell medium containing ClFA metabolites. Values are means±SD of four samples run in parallel.
[a] A theoretical value calculated by using the peak area obtained in fraction 8.
[b] Based on one sample.

Table 4
Gravimetrically determined masses after SPE isolation of 4.0 mg transesterified cell sample

Fraction	Mass (mg)	SD	Recovery (%)	SD
1	1.5	0.1	36	3
2	0.1	0.1	<5	
3	0.2	0.1	6	3
4	0.3	0.1	6	3
5	<0.1		<2	
6	<0.1		<2	
7–14	<0.1		<2	
Total	<2.4		<59	

Lipids liberated from cultured cell medium. Values in the table are mean±SD of four samples run in parallel.

the GC–MS system. About 0.6–0.7 mg of the applied sample (4 mg) was identified as $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$ and $C_{16:0}$ and by using XSD, about 20 μg was identified as ClFAMEs (Table 2). It was found that <1% of $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, and $C_{16:0}$ (Table 1) and <0.1 mg (gravimetrically determined) of the 4 mg applied masses (Table 4) were recovered in fraction 8. This agrees with the total mass, 0.07–0.09 mg, estimated from the TIC and XSD chromatogram in comparison with known external standards. This means that most of the compounds that do not generate signal in the GC–MS system had been removed. By assuming that; (i) only 2% of the compounds applied on the column are recovered in the fraction 8, (ii) the detection limit of a ClFAME in the GC–XSD system is 0.5 ng (five times the noise), (iii) a maximum of 5 μg can be injected into the GC–XSD system, a lower limit for detecting ClFAMEs in cell-culture medium lipids was estimated at 1 μg/g lipid.

As the unchlorinated $C_{18:3}$ can interfere with $C_{14}Cl_2$ (peak 3 and c in Fig. 2) on the GC column used, only fraction 8 was collected for mass spectrometric analysis. When using a halogen selective detector, interference from unchlorinated compounds is of minor importance, however, a more correct value for the recovery of ClFAMEs would be obtained by including fraction 7.

3.3. Isolation of ClFAMEs from an earlier silver nitrate and urea treated eel sample

Methyl esters of saturated and unsaturated, dichlorinated fatty acids, as well as saturated tetrachlori-

nated fatty acids were previously identified in the eel sample. These ClFAMEs were now detected with XSD when using a slow temperature gradient in the GC column oven (Fig. 3a). The XSD chromatograms obtained when using the steep temperature gradient, before (Fig. 3b) and after SPE treatment (Fig. 3c), showed an unchanged pattern of ClFAMEs. Thus, the SPE procedure has no negative impact to the multitude of ClFAMEs in the eel sample.

Only about 1 μg of ClFAMEs was present in the eel sample (340 μg) before its being fractionated by SPE and the recovery of ClFAMEs was 70–80% (data not shown). Although the eel sample had been enriched 30-fold with respect to ClFAMEs, most of the compounds in the extract that were applied to the SPE column were recovered in the *n*-hexane fraction (about 300 μg of gravimetrically determined dry mass out of 340 μg applied). The compounds generating mass spectrometric signal corresponded to about 100 μg and almost 100% of these compounds were recovered with *n*-hexane. In the mixed solvent fraction (4 ml) containing the ClFAMEs, only 2–3% of the common FAMEs were co-eluted (the removal of the FAMEs can be studied in Fig. 4). Thus, a combination of $AgNO_3$ for removal of polyunsaturated FAMEs and SPE for removal of saturated

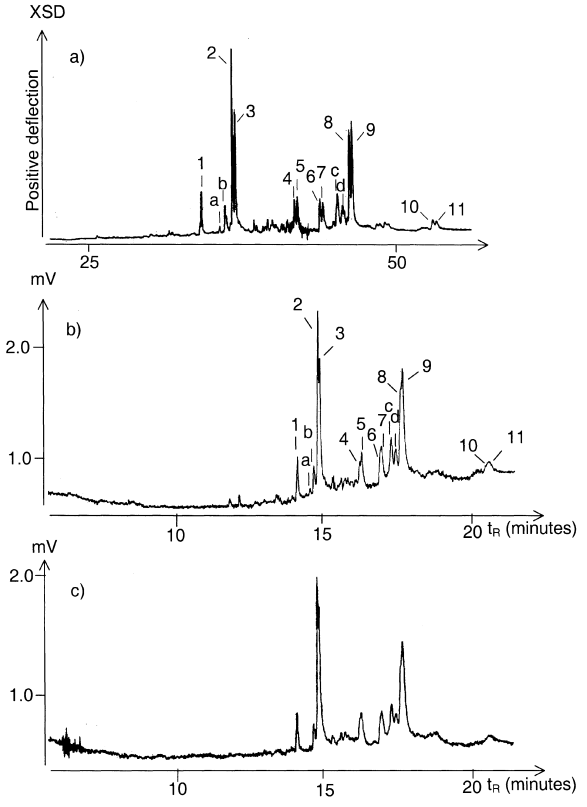

Fig. 3. XSD chromatograms obtained of transesterified lipids and silver nitrate and urea treated eel sample. (a) Using a slow temperature gradient [90 °C (held for 2 min) to 280 °C at 4 °C/min]. Using a steep temperature gradient [90 °C (held for 2 min) to 300 °C at 15 °C/min (held for 5 min)] (b) before SPE isolation, (c) after SPE isolation. By comparison with a similarly obtained XSD gas chromatogram [15], the SPE isolated ClFAMEs are assigned the following identities: (1) methyl dichlorotridecanoate, diastereomeric (*erythro*- and *threo*-) forms of (2, 3) methyl dichlorotetradecanoate, (4, 5) methyl dichlorohexadecanoate, (8, 9) and methyl dichlorooctadecanoate and (6, 7) isomers of methyl tetrachlorotetradecanoate (10, 11) isomers of methyl tetrachlorooctadecanoate. Unsaturated compounds: (a, b) isomers of methyl dichlorotetradecenoate, and (c, d) isomers of methyl dichlorooctadecenoate.

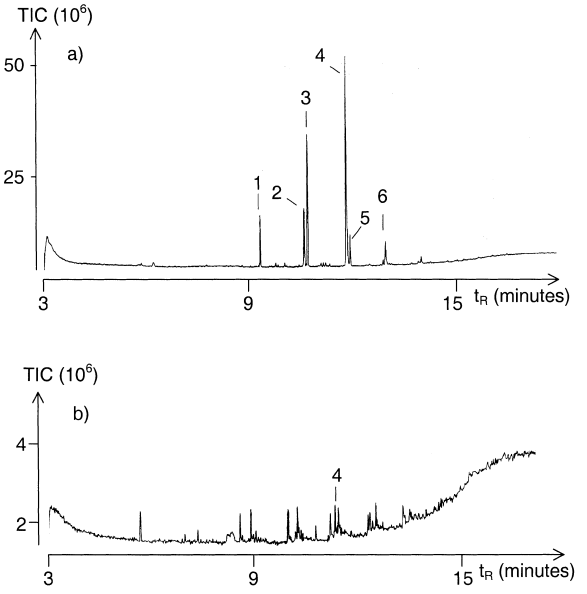

Fig. 4. TIC of transesterified lipids and silver nitrate and urea treated eel sample (a) before SPE isolation (17 μg dry mass was injected), (b) after SPE isolation (<<5 μg dry mass was injected). The following peaks are identified as: (1) methyl tetradecanoate, (2) methyl hexadecenoate, (3) methyl hexadecanoate, (4) methyl octadecenoate, (5) methyl octadecanoate, (6) a possible cluster of methyl esters of FAMEs of different fatty acids containing 20 carbon atoms. The other peaks are unidentified.

FAMEs, monounsaturated FAMEs and compounds that do not generate MS signal, seems to be a useful technique for isolation of trace amounts of ClFAMEs. Furthermore, the use of silver salt-treated SPE columns for separating FAMEs on the basis of the their degree of unsaturation [24,26], might be considered in a future isolation of chlorinated, unsaturated FAMEs. The lowest concentration of ClFAMEs possible to detect was estimated at about 1 μg/g of eel lipid, which agrees with the estimated lowest concentration allowing for isolating ClFAMEs from cell-culture medium lipid. The estimated lowest value of a ClFAME corresponds to about 0.2 μg EOCl/g lipid, which seems to be an appropriate sensitivity because EOCl in biota from remote waters is about 30 μg/g lipid [17].

4. Conclusions

The SPE-based analysis procedure described here appears to be promising for use in studies of trace amounts of ClFAs in lipids of natural origin; about 1 μg of a ClFAMEs in 1 g of lipid can be isolated and detected, which means that it should be possible to study ClFAs in environmental samples from remote areas. Further, independent of the character of the ClFAMEs studied, all ClFAMEs are eluted to almost the same degree using 4 ml of the solvent mixture and the recoveries of the different species and isomers are 70–110%.

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References

[1] C. Wesén, G.E. Carlberg, K. Martinsen, AMBIO 19 (1990) 36.
[2] C. Wesén, H. Mu, A. Lund Kvernheim, P. Larsson, J. Chromatogr. 625 (1992) 257.
[3] C. Wesén, K. Martinsen, G.E. Carlberg, H. Mu, in: Swedish Environmental Protection Agency Report, No. 4031, 1992, p. 207.
[4] H. Mu, C. Wesén, P. Sundin, J. Skramstad, G. Odham, J. Chromatogr. A 731 (1996) 225.
[5] J.E. Milley, R.K. Boyd, J.M. Curtis, C. Musial, J.F. Uthe, Environ. Sci. Technol. 31 (1997) 535.
[6] H. Mu, Ph.D. Thesis, Lund University, Lund, 1996.
[7] C. Wesén, Ph.D. Thesis, Lund University, Lund, 1995.
[8] H.M. Cunningham, G.A. Lawrence, Food Cosmet. Toxicol. 14 (1976) 283.
[9] G. Vereskuns, Ph.D. Thesis, Acta Universitatis Agriculturae Sueciae, Agria 203, 1999.
[10] H. Björn, Ph.D. Thesis, Lund University, Lund, 1999.
[11] C. Gustafson-Svärd, G. Åkesson-Nilsson, M. Mattsson, P. Sundin, C. Wesén, Pharmacol. Toxicol. 89 (2001) 56.
[12] H. Björn, P. Sundin, C. Wesén, H. Mu, K. Martinsen, A. Lund Kvernheim, G. Odham, Naturwissenschaften 85 (1998) 229.
[13] J.M. Leach, A.N. Thakore, Prog. Water Technol. 9 (1977) 787.
[14] V.M. Dembitsky, M. Srebnik, Prog. Lipid Res. 41 (2002) 315.
[15] G.Å. Nilsson, O. Nilsson, I. Odenbrand, C. Wesén, J. Chromatogr. A 912 (2001) 99.
[16] H. Håkansson, P. Sundin, T. Andersson, B. Brunström, L. Dencker, M. Engwall, G. Ewald, M. Gilek, G. Holm, S. Honkasalo, J. Idestam-Almquist, P. Jonsson, N. Kautsky, G. Lundberg, A. Lund Kvernheim, K. Martinsen, L. Norrgren, M. Personen, M. Rundgren, M. Stålberg, M. Tarkpea, C. Wesén, Pharmacol. Toxicol. 69 (1991) 459.
[17] G. Lunde, J. Gether, E. Steinnes, AMBIO 5 (1976) 180.
[18] G.E. Carlberg, A. Kringstad, K. Martinsen, O. Nashaug, Pap Puu 69 (1987) 337.
[19] M.A. Kalunzy, L.A. Duncan, M.V. Merritt, D.E. Epps, J. Lipid Res. 26 (1985) 135.
[20] H. Mu, C. Wesén, P. Sundin, E. Nilsson, J. Mass Spectrom. 31 (1996) 517.
[21] W. Morrison, L.M. Smith, J. Lipid Res. 5 (1964) 600.
[22] E.G. Bligh, W.J. Dyer, Can. J. Biochem. Physiol. 37 (1959) 911.
[23] J. Folch, M. Lees, G.H. Sloane-Stanley, J. Biol. Chem. 226 (1957) 497.
[24] R. Wilson, R.J. Henderson, I.C. Burkow, J.R. Sargent, Lipids 28 (1993) 51.
[25] A.V. Zhukov, A.G. Vereshchagin, in: Advances in Lipid Research, Academic Press, New York, 1981, p. 247.
[26] W.W. Christie, J. Lipids 30 (1989) 1471.