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Evaluation of ionic liquid stationary phases for one dimensional gas chromatography–mass spectrometry and comprehensive two dimensional gas chromatographic analyses of fatty acids in marine biota

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ABSTRACT

Ionic liquid stationary phases were tested for one dimensional gas chromatography–mass spectrometry (GC–MS) and comprehensive two dimensional gas chromatography (GC × GC) of fatty acid methyl esters from algae. In comparison with polyethylene glycol and cyanopropyl substituted polar stationary phases, ionic liquid stationary phases SLB-IL 82 and SLB-IL 100 showed comparable resolution, but lower column bleeding with MS detection, resulting in better sensitivity. The selectivity and polarity of the ionic liquid phases are similar to a highly polar biscyanopropyl-silicone phase (e.g. HP-88). In GC × GC, using an apolar polydimethyl siloxane × polar ionic liquid column combination, an excellent group-type separation of fatty acids with different carbon numbers and number of unsaturations was obtained, providing information that is complementary to GC–MS identification.

1. Introduction

Recently, lipids from marine biota, such as algae, are considered as a source for the third generation bio-fuels and for new food oils [1,2]. The characterization of lipids is often based on the analysis of fatty acids (FAs), which are their principal building blocks. The fatty acid profile provides interesting information for the taxonomic classification of marine species [3–6], and is mostly obtained by gas chromatography (GC) coupled with flame ionization detection (FID) or mass spectrometry (MS) [7–14]. For the separation of fatty acids, as methyl esters, polar capillary columns coated with polyethylene glycol (e.g. DB-WAX) or with bis(cyanopropyl)siloxane (e.g. BPX-70, HP-88, and SP-2380) are used. These polar stationary phases are selected since they allow differentiation between fatty acids with different carbon numbers, number of unsaturations, location and/or geometry (*cis–trans*) of the double bond(s). However, these stationary phases give substantially higher column bleeding than apolar (dimethyl siloxane) stationary phases, resulting in relatively high background, especially in mass spectrometric detection.

Moreover, the fatty acid composition of algae can be more complex than that of classical lipid sources (such as vegetable oils). Several "uncommon" fatty acids are present, with unusual number of double bonds or double bond location. The identity of these fatty acids and, especially, number, position and geometry of the double bonds are difficult to elucidate by electron ionization MS (EI-MS). Polyunsaturated fatty acids give a similar fragmentation profile with no molecular ion information. Derivatization and soft ionization are therefore used to detect the molecular ion and specific fragments. For instance, acetonitrile chemical ionization tandem mass spectrometry was used to determine double bond positions in fatty acid methyl esters (FAMEs) derived from different natural sources such as golden algae [15].

In this respect, comprehensive two dimensional gas chromatography (GC × GC) can be an interesting complementary technique to analyze fatty acids from different sources. GC × GC has already

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been applied for the analysis of FAMEs in microorganism [16–18], fish and vegetable oil [19–28], human plasma [29] and milk fat [30]. Comprehensive two dimensional gas chromatography is especially useful for group type separation, combining the carbon number separation and the separation according to the number of double bonds. Akoto et al. [16] described the analysis of fatty acids in common fresh water green algae, by methylation/direct thermal desorption combined with GC × GC–time-of-flight mass spectrometry. In most GC × GC work on fatty acid methyl esters, a combination of an apolar and a polar column is used. Often, the application is limited by the low thermal stability of the polar column. If this column is used in first dimension, column bleeding is even more problematic due to the modulation.

Recently, ionic liquid (IL) columns were introduced and their high thermal-stability was illustrated [31]. Polar ionic liquid phases were used for the analysis of fatty acid methyl esters [32–34], and their potential for the separation of eicosenoic acid (C20:1) isomers [35] and octadecenoic acid (C18:1) and octadecadienoic acid (C18:2) isomers [36] was demonstrated. For these applications, flame ionization detection (FID) was used.

In this research, commercially available polar ionic liquid stationary phases were tested in one dimensional gas chromatography combined with mass spectrometry. Polarity, selectivity and column bleeding were compared with polyethylene glycol and bis(cyanopropyl)silicone stationary phases. Two IL phases were also used as the second dimensional column for GC × GC analysis of fatty acids in the diatom algae *Cylindrotheca closterium* and *Seminavis robusta*. GC × GC analysis was done using flow modulation and a narrow bore (100 μm ID) apolar column was used in first dimension, resulting in faster analysis time and higher sensitivity, while maintaining resolution.

2. Experimental

2.1. Chemicals

Methanol (HPLC grade), sulphuric acid (ACS grade) and nonadecanoic acid (C19:0) were purchased from Sigma–Aldrich (Bornem, Belgium) and n-hexane was obtained from Fisher Scientific (Leicestershire, UK). Water was from a Milli-Q water purification system (Millipore, Bedford, MA, USA). A 37 component fatty acid methyl ester (FAME) mixture (10 mg/mL) in methylene chloride, a bacterial acid methyl ester (BAME) reference solution (10 mg/mL total concentration) in methyl caproate and polyunsaturated fatty acid mix no. 1 (PUFA No. 1, marine source) were purchased from Supelco (Bellefonte, USA). PUFA No. 1 was dissolved in isooctane, and the stock solutions of PUFA, FAMEs and BAMEs were further diluted with hexane.

FAMEs are abbreviated using the shorthand annotation according to the formula, "C*a*:*bnxz*" whereby *a* is the number of carbon atoms in the fatty acid chain (not including the methyl alcohol part), *b* is the number of double bonds, *nx* is the location of double bond on the *x*th carbon–carbon bond, counting from the terminal methyl carbon towards the carbonyl carbon and *z* is the geometrical configuration expressed as *c* for *cis* and *t* for *trans* (e.g. C18:1*n*9*c*: methyl ester of *cis*-9-octadecenoic acid).

2.2. Culture growth and harvest of algae samples

Samples for fatty acid analysis were obtained from silica-limited monoclonal cultures of the marine benthic diatoms *C. closterium* (strain F6A) and *S. robusta* (strain (BPS)F1-6). Both strains are kept cryopreserved in the culture collection of the laboratory of Protistology & Aquatic Ecology (Ghent University, Belgium). Culture conditions were 18 °C, a 12 h:12 h light:dark period, and an illumination of approximately 30 μmol photons m^{-2} s^{-1} from cool-white fluorescent tubes. Cultures were grown in 250 mL culture flasks with 100 mL of marine enrichment medium 'f/2' [37]. Both cultures were first grown exponentially for six days, with a re-inoculation to low density after the first three days, after which the medium was replaced with medium without silica source. After three (*Cylindrotheca*) or four (*Seminavis*) days under silica limitation, the cultures were harvested by gently pouring off most of the medium and suspending the cells in the remaining medium using a cell scraper. Next, the cells were concentrated in two steps into a cell pellet in a pre-weighed eppendorf tube using gentle centrifugation at 2500 rpm for 10 resp. 5 min. The supernatants were removed, and the cell pellets immediately frozen in liquid nitrogen, after which they were stored at −70 °C.

2.3. Fatty acid extraction and derivatization

Before fatty acid extraction, the cell pellets were freeze-dried for 18 h at −50 °C, and their dry weight was determined (5.6 mg for the *Seminavis* pellet, and 4.8 mg for *Cylindrotheca*). Dried cell pellets were stored again at −70 °C until fatty acid extraction. Methylation and hydrolysis of the lipids in the samples were achieved in a single step by adding 700 μL of methanol with 2.5% sulphuric acid, and homogenization of the cell suspension by shaking with a vortex for 1 min. Next, the samples were incubated in a water bath at 80 °C for 90 min, after which they were left to cool down. Fatty acids were extracted using hexane, with C19:0 as internal standard. 350 μL of hexane was added, as well as 350 μL of 0.98% NaCl to obtain a clearer separation of the 2 phases [38]. After shaking for 1 min, the samples were centrifuged at 12,000 rpm for 1 min, and 100 μL of the supernatant hexane phase was removed, concentrated to 10 μL and stored at −20 °C until analysis.

2.4. GC–MS

An Agilent 6890 GC-5973 MSD (Agilent Technologies, Wilmington, DE, USA) was used for GC–MS analysis. Separations were performed on HP-5MS, DB-WAX (30 m × 0.25 mm × 0.25 μm, Agilent Technologies, Folsom, CA, USA), SLB-IL 82, SLB-IL 100 (25 m × 0.25 mm × 0.2 μm, Supelco, Bellefonte, USA) and HP-88 (100 m × 0.25 mm × 0.2 μm, Agilent Technologies) capillary columns. The injection volume was 1 μL with a split ratio of 10:1. The inlet temperature was 230 °C. Electron ionization (EI) was used. Analyses were performed in scan mode at the range of *m*/*z* 50–430, with the MS source at 230 °C and the MS quadrupole at 150 °C. The oven temperature program was 60–175 °C at 15 °C/min, and at 2 °C/min to 240 °C (10 min) for HP-5MS, DB-WAX and SLB-IL 82. For SLB-IL 100, the oven temperature was programmed from 60 °C to 175 °C at 15 °C/min, and at 2 °C/min to 230 °C. For HP-88, the oven temperature was 50 °C (1 min) to 175 °C at 15 °C/min, and at 1 °C/min to 240 °C (5 min). Helium was used as carrier gas with the flow of 1 mL/min (constant flow mode) for HP-5MS, DB-WAX, SLB-IL 82 and SLB-IL 100. Constant pressure at 300 kPa was used for HP-88 column (2 mL/min at 50 °C, 1 mL/min at 240 °C). Data were processed by the MSD ChemStation version G1701EA (Agilent Technologies).

2.5. GC × GC with flow modulation

A DB-1MS column (10 m × 0.10 mm × 0.10 μm, Agilent Technologies, Folsom, CA, USA) was used as a first dimension column. SLB-IL 82, SLB-IL 100 and HP-88 capillary columns (4 m × 0.25 mm × 0.2 μm) were used as the second dimension columns.

An Agilent 7890A (Agilent Technologies, Wilmington, DE, USA) equipped with a flow modulator (Agilent G3486A CFT Modulator,

Wilmington, DE, USA) was used. Injections were performed in split mode with split ratio at 10:1. The injection volume was 1 μL with the inlet temperature of 230 °C. Hydrogen was used as carrier gas and performed in constant flow mode. The hydrogen-gas was from hydrogen generator (H2PD-300-220, Parker Balston, Haverhill, MA, USA) using Milli-Q water. The flow of carrier gas was 0.3 mL/min and 24 mL/min in the first and second columns, respectively. A modulation cycle of every 2 s, consisting of 1.9 s collection and 0.1 s injection time was used. Flame ionization detection (FID) was used with a sampling rate of 200 Hz. The other parameters of FID were as follows: temperature 230 °C, air 300 mL/min, hydrogen 6 mL/min and nitrogen (make-up) 40 mL/min. The oven temperature program was 100 °C (1 min), at 10 °C/min to 175 °C, and at 3 °C/min to 240 °C (5 min) when SLB-IL 82 or HP-88 was used as the second dimension. The oven temperature was programmed from 100 °C (1 min), at 10 °C/min to 175 °C, and at 3 °C/min to 230 °C (8 min) when SLB-IL 100 was used as the second dimension. GC Image software (version V 1.9b2, Zoex) was used for the construction of the GC × GC contour plots.

3. Results and discussion

3.1. GC–MS analysis using ionic liquid stationary phases

The different columns (HP-5MS, HP-88, DB-WAX, SLB-IL 82 and SLB-IL 100) were first tested for column bleeding with MS detection. After conditioning the columns according to the procedure proposed by the manufacturer (e.g. conditioning for 2 h at maximum allowable operation temperature), a blank run with temperature programming was performed. The profiles for each column (with respective end temperature) are shown in Fig. 1a. The apolar HP-5MS column obviously had the lowest column bleeding. The SLB-IL 82 column bleed was lower compared to the other polar columns. Also the SLB-IL 100 column showed significantly lower bleeding than the DB-WAX and HP-88 columns, although its maximum operating temperature is only 230 °C.

The lower column bleeding results in lower background and better detectability, as illustrated in Fig. 1b and c, showing a part of the chromatograms obtained for a reference solution of polyunsaturated FAMEs (PUFA No. 1, marine source) analyzed on, respectively, a HP-88 (Fig. 1b) and SLB-IL 82 (Fig. 1c) column. These parts of the chromatograms show the C18:4 elution window. On the (100 m) HP-88, the solutes were eluted at around 200 °C. On the (25 m) SLB-IL 82, the same solutes were eluted at around 186 °C. Separation is quite similar, but the baseline is clearly lower and less noise is observed on the IL column. Consequently, the signal to noise ratio (S/N) for the trace constituents (labeled 1, 2, 3 and 4) is higher on the IL column compared to the HP-88. Peak 3 is even not detected in the TIC trace of the HP-88 chromatogram.

Next, the separation of a 37 component FAME mixture was performed on HP-5MS, SLB-IL 82, SLB-IL 100 and HP-88 columns. The chromatograms are compared in Fig. 2. Since the DB-WAX column does not allow the separation of *cis* and *trans* fatty acid isomers, this column was not further evaluated. Peaks in the chromatograms were identified using the mass spectra, relative retention time information and literature data [39,40].

Different compound classes can be easily identified using the base peaks at *m*/*z* 74, 55, 67 and 79, characteristic for saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), di-unsaturated fatty acids (DUFA) and polyunsaturated fatty acids (PUFA), respectively [39,40]. The characteristic ion ratios "*m*/*z* 150 > *m*/*z* 149" and "*m*/*z* 80 > *m*/*z* 93" were used to confirm C18:3*n*6 (*n*6 terminal moiety) [40].

Compared to apolar columns (showing good carbon number separation but little separation between unsaturated fatty acids) the highly polar stationary phases allow the separation of unsaturated fatty acids and separation of positional and geometrical isomers. In general, the best separation of the 37 component test mixture was obtained on SLB-IL 82 within 20 min, but also excellent separation was obtained on the SLB-IL 100 column. Similar to the biscyanopropyl stationary phase (HP-88), both SLB-IL 82 and SLB-IL 100 ionic liquid phases allow the separation of *cis* and *trans* isomers. On the three polar columns, the polyunsaturated *n*3 FAME was eluted after its *n*6 counterpart (18:3*n*6 elutes before 18:3*n*3).

To compare polarity and selectivity of the ionic liquid phases with HP-88, the equivalent chain length (ECL) values for a number of main fatty acids were calculated. For ECL determination, the linear saturated fatty acid methyl esters are used as references (similar to n-alkanes in Kovats retention indices, C16:0 = 16.00, C17:0 = 17.00, C18:0 = 18.00, etc.). The calculated ECL values are listed in Table 1 for all the columns (including DB-WAX).

These data clearly demonstrate that the polarity of the IL phases is higher than a polyethylene glycol column (DB-WAX). The SLB-IL

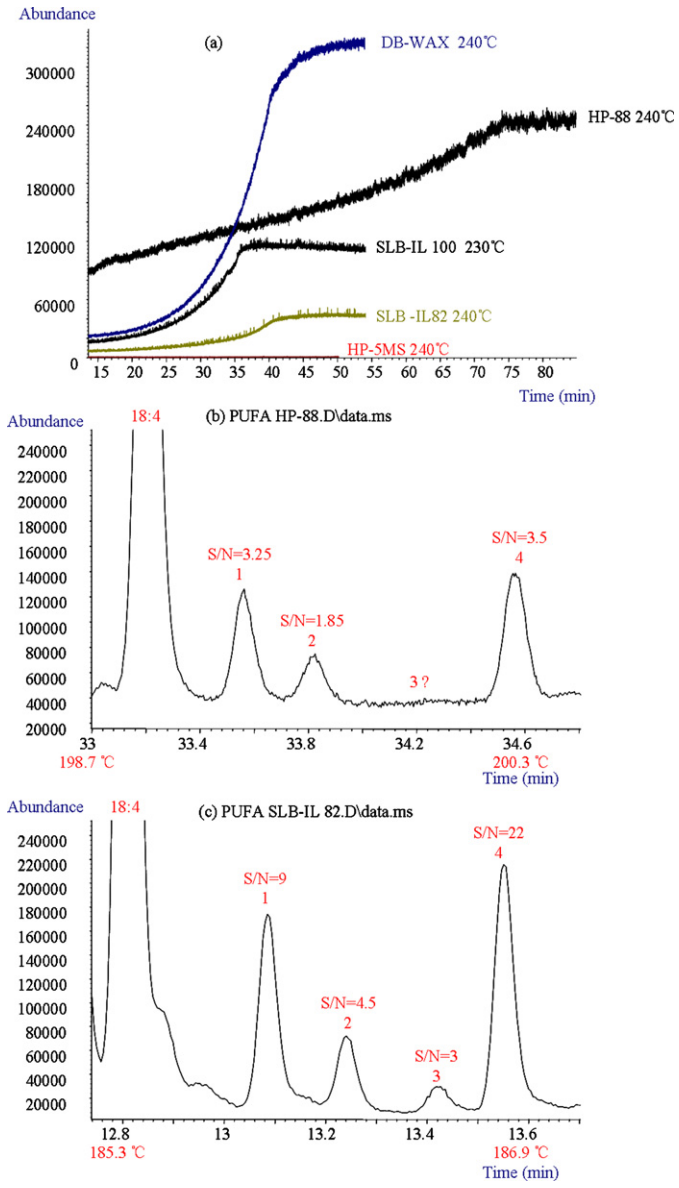

Fig. 1. Comparison of blank runs with temperature programming on different columns (HP-5MS, HP-88, DB-WAX, SLB-IL 82 and SLB-IL 100) with MS detection (end temperature indicated) (a) and part of chromatograms obtained for reference sample of polyunsaturated FAMEs (PUFA No. 1, marine source) on HP-88 (b) and on SLB-IL 82 column (c).

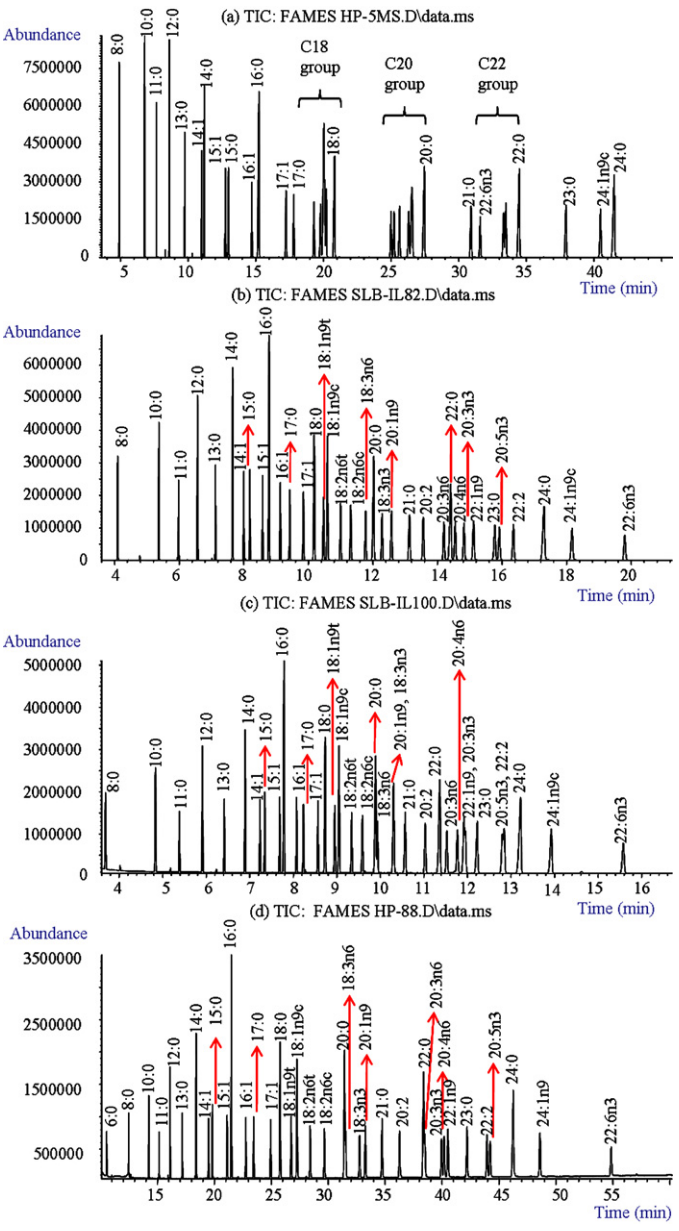

Fig. 2. Separations of 37 component FAME test mixture on (a) HP-5MS; (b) SLB-IL 82; (c) SLB-IL 100; and (d) HP-88 (temperature programs: see text).

100 is the most similar to the highly polar HP-88, while the SLB-IL 82 is only slightly less polar. Polarity and selectivity towards double bonds are also illustrated in Fig. 2 by the relative elution of C18:3*n*6 and C20:0. On the SLB-IL 82, C18:3*n*6 elutes before C20:0, on the HP-88 C18:3*n*6 and C20:0 partly overlap and on the SLB-100 C18:3*n*6 elutes after C20:0. This solute pair was previously used for the characterization of highly polar cyanopropyl stationary phase [41]. The value obtained for C20:1*n*9 (ECL = 20.62) (here, using a temperature gradient) corresponds well with the data reported by Ando and Sasaki (ECL = 20.57–20.73, depending on isothermal temperature) [35].

Recently, an even more polar IL phase (SLB-IL 111) was also evaluated for FAME analysis [36]. On this column, the unsaturated fatty acids are even more retained and saturated fatty acids (C_x:0) interfere with mono-unsaturated fatty acids with one carbon less (C_{x-1}:1).

Table 1
Equivalent chain length (ECL) of selected fatty acid methyl esters on five columns.

	HP-5MS	HP-88	IL-82	IL-100	DB-WAX
C16:1	15.77	16.65	16.54	16.65	16.25
C17:1	16.78	17.64	17.56	17.67	17.25
C18:1*n*9*c*	17.70	18.53	18.44	18.55	18.18
C18:2*n*6*c*	17.66	19.38	19.23	19.48	18.65
C18:3*n*6	17.50	20.03	19.73	20.06	18.91
C18:3*n*3	17.72	20.40	20.23	20.59	19.26
C20:1*n*9	19.71	20.55	20.50	20.62	20.18
C20:2	19.65	21.42	21.33	21.58	20.66

Calculated by interpolation using bracketing linear saturated fatty acid methyl esters, with C15:0 = 15.00, C16:0 = 16.00, etc., using ECL = $(tR_i - tR_n)/(tR_{n+1} - tR_n) + n$.

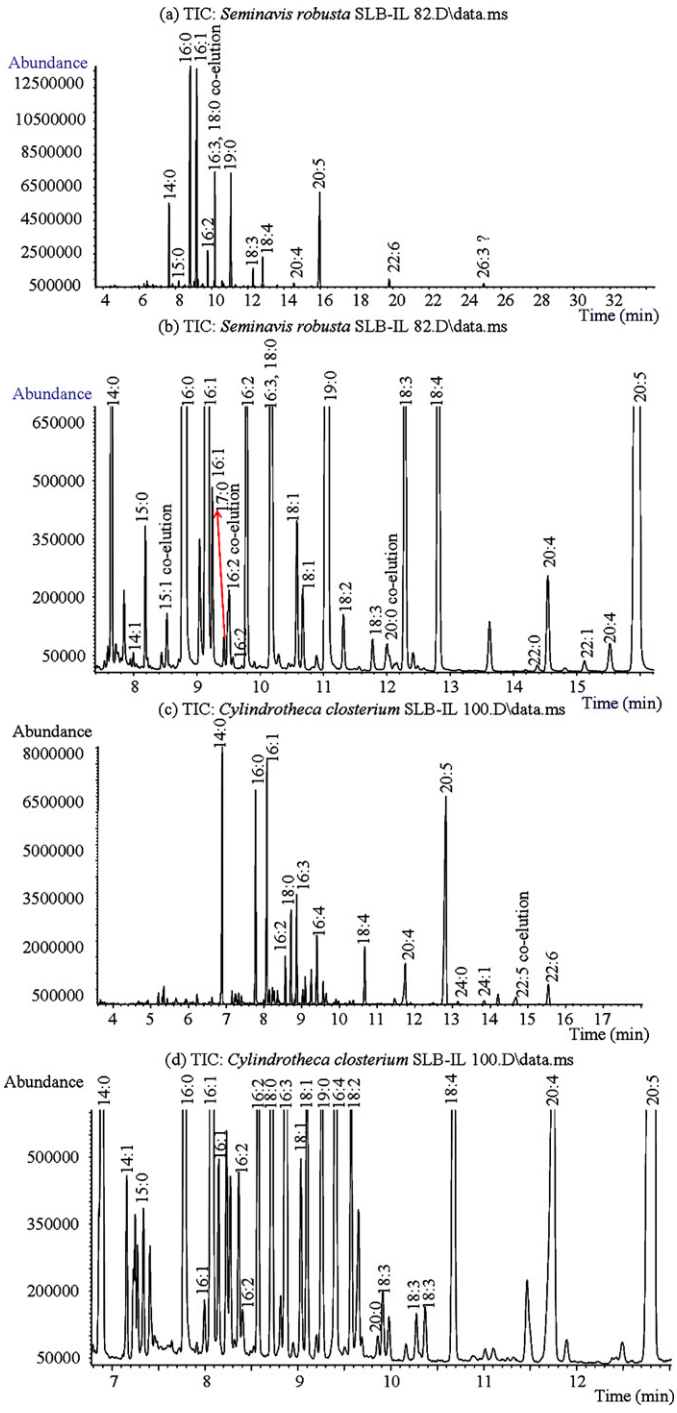

Fig. 3. FAMEs profile from *Seminavis robusta* on SLB-IL 82 (a) and a detailed chromatogram (b); FAMEs profile from *Cylindrotheca closterium* on SLB-IL 100 (c) and a detailed chromatogram (d).

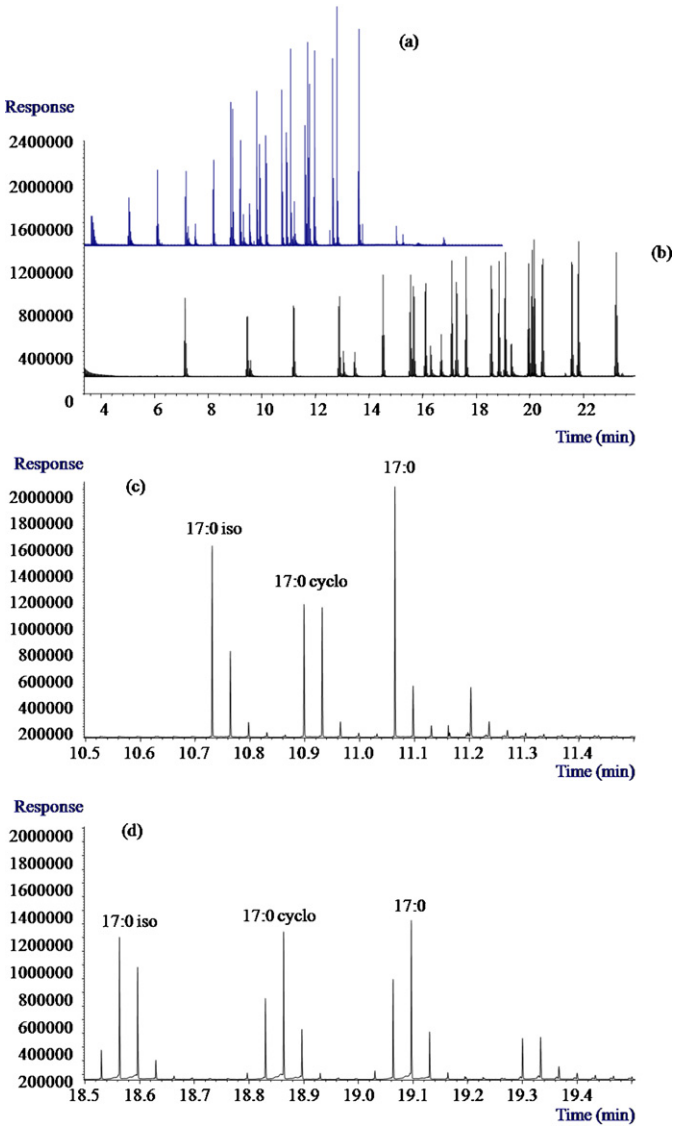

Fig. 4. GC × GC analysis of BAME test mixture using a 0.1 mm ID DB-1MS (a and c) and a 0.25 mm ID DB-1MS (b and d) in first dimension and a 4 m × 0.25 mm ID BPX-70 in the second dimension (raw modulated chromatograms). (b and d) A zoom on the C17 elution window.

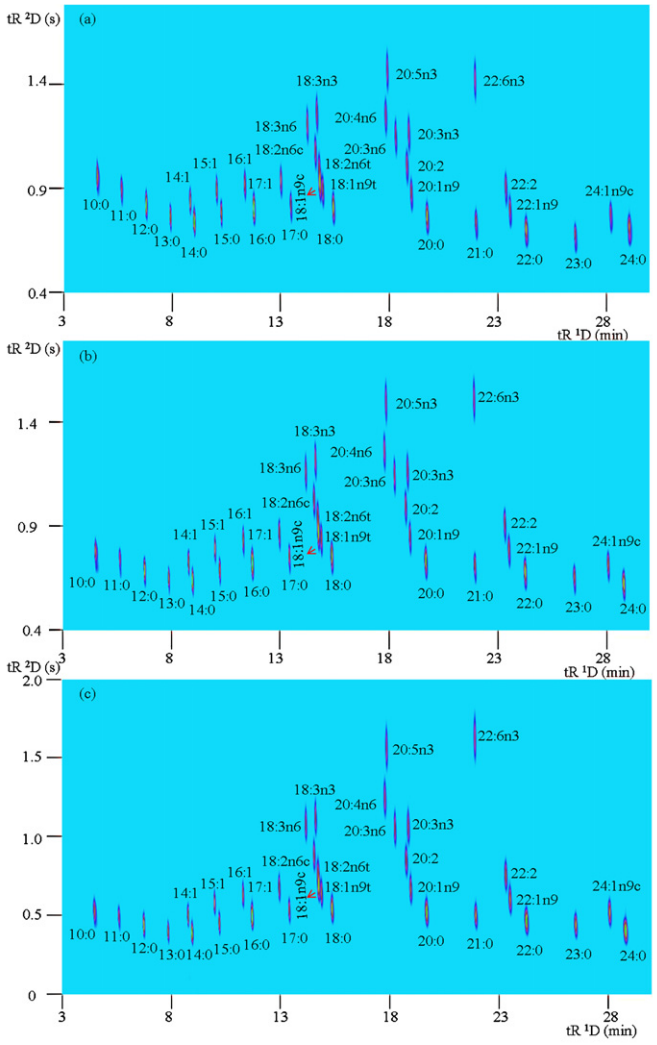

Fig. 5. GC × GC plots of the FAMEs reference in three configurations with 0.10 mm ID DB-1MS as the first dimensional column: (a) SLB-IL 100; (b) SLB-IL 82; and (c) HP-88 as the second dimensional columns.

The fatty acid methyl ester profile obtained from *S. robusta* on the SLB-IL 82 column is shown in Fig. 3a and b. The profile of FAMEs from *C. closterium* on SLB-IL 100 is shown in Fig. 3c and d. Both a full scale TIC (Fig. 3a and c) and a detailed chromatogram (Fig. 3b and d), illustrating the complexity of the samples have been shown. By one dimensional GC–MS analysis on the IL column, very interesting and detailed profiles are obtained that can be used for the characterization of the algae samples. However, during identification based on MS data, some co-elutions were detected and not all solutes could be identified with high probability.

3.2. GC × GC analysis using IL phases in second dimension

As previously reported [17], flow modulated GC × GC was used for the separation of fatty acids from bacteria (BAMEs). An apolar column (30 m × 0.25 mm × 0.25 μm HP-5MS) was combined with a 4 m × 0.25 mm × 0.25 μm BPX-70 column (stationary phase similar to HP-88). Special attention was paid to the separation of hydroxy fatty acids. Best group type separation (in the 2D space) was obtained using a relatively low (sub-optimal) column flow in the first dimension. Since these hydroxy fatty acids are not determined in the lipid fraction from algae, other conditions were applied in this work to optimize the GC × GC separation.

An apolar narrow bore column (10 m × 0.10 mm × 0.10 μm DB-1MS) was tested as first dimension column. Using a smaller ID, the first dimension column could be used closer to optimum flow rate, while the low flow is compatible with the flow modulator. A comparison of the modulated chromatograms obtained on, respectively, a 10 m × 0.25 mm × 0.25 μm and a 10 m × 0.10 mm × 0.10 μm column (both DB-1MS) is given in Fig. 4. For the 0.25 mm ID column, the ^{1}D flow was 0.6 mL/min (24 cm/s linear velocity), which is suboptimal (hydrogen is used as carrier gas). For the 0.10 mm ID column, the ^{1}D flow was 0.3 mL/min hydrogen (42 cm/s), much closer to optimum carrier gas velocity. Temperature program rate was increased from 6 °C/min on the 0.25 mm ID column to 10 °C/min on the 0.10 mm ID column. The second column flow rate was 24 mL/min. From Fig. 4, it is clear that the analysis time is reduced and the response is increased, while the resolution is maintained. This is clearly illustrated in the enlarged elution window of C17:1–C17:0 (Fig. 4c and d). Using a 2 s

Table 2
Relative composition of main FAMEs in *S. robusta* and *C. closterium*.

	tR ^{1}D (min)	tR ^{2}D[a] (s)	α[b]	*Seminavis robusta* (%)	*Cylindrotheca closterium* (%)
C14:0	8.93	0.76		3.36	14.90
C15:0	10.17	0.82		0.24	0.32
C16:0	11.80	0.80		34.84	9.95
C16:1	11.40	0.91	1.15	31.43	14.81
C16:1	11.43	0.96	1.19	–	0.24
C16:1	11.53	0.91	1.12	–	0.75
C16:2	11.03	1.04	1.28	0.28	0.82
C16:2	11.40	1.07	1.36	2.88	2.83
C16:3	11.03	1.21	1.52	5.28	6.62
C16:4	10.87	1.45	1.78	–	4.11
C18:0	15.33	0.83		2.40	3.45
C18:1*n*9*c*	14.73	0.90	1.13	0.33	0.44
C18:1	14.83	0.91	1.16	0.31	1.20
C18:2*n*6*c*	14.57	1.07	1.33	0.19	1.09
C18:3*n*6	14.20	1.19	1.46	0.19	0.32
C18:3*n*3	14.60	1.27	1.53	1.45	–
C18:4	14.23	1.42	1.72	2.41	3.62
C20:0	19.88	0.76		–	–
C20:4*n*6	17.83	1.24	1.63	0.96	3.84
C20:4	18.33	1.32	1.72	0.21	–
C20:5*n*3	17.97	1.43	1.88	12.11	27.83
C22:0	24.46	0.70		–	–
C22:5	21.80	1.19	1.71	–	0.95
C22:6*n*3	21.87	1.40	2.00	1.13	1.92

[a] The retention times of the compounds are from DB-1 × SLB-IL 100 set.
[b] Selectivity (α) in the second dimension for unsaturated fatty acid versus saturated of same carbon number (e.g. tR (C16:1)/tR (C16:0)).

modulation time (1.9 s collection–0.1 s injection), about 4–5 modulations are observed on each peak using a 0.25 mm ID column with a 0.6 mL/min flow (Fig. 4d). On the narrow bore column using a 0.3 mL/min flow, the number of modulations per peak was still 3–4 (Fig. 4c). Moreover, on the 0.25 mm ID column, a slight breakthrough was observed (fronting on peaks). This is not observed in the modulated chromatogram for the narrow bore column. In addition, all solutes eluted in the 2 s second dimension space (no "wrap-around" in different cycles). From these tests, it is clear that the 10 m × 0.10 mm ID column can successfully be applied in the first dimension for flow modulated GC × GC and therefore it was used for further work.

Next, the GC × GC separation of the 37 component FAME reference sample was performed on three column combinations: configuration 1: DB-1MS 0.10 mm ID + 4 m × 0.25 mm × 0.2 μm SLB-IL 100; configuration 2: DB-1MS 0.10 mm ID + 4 m × 0.25 mm × 0.2 μm SLB-IL 82; and configuration 3: DB-1MS 0.10 mm ID + 4 m × 0.25 mm 0.2 μm HP-88. The GC × GC plots are compared in Fig. 5. The profiles are very similar. Excellent group separation is obtained with the separation on carbon number in the first dimension, and separation according to the number of double bonds in the second dimension. Also positional isomers *n*6 and *n*3 are separated, since the *n*6 elutes before *n*3 on the first dimension. *Cis–trans* isomers are separated in both dimensions.

The linear saturated fatty acids do not elute on a straight line. Their absolute retention in the second dimension first decreases (until 8.5 min), then increases, and finally decreases again. This behavior can be explained by a combination of different oven temperature slopes and flow regimes (temperature gradient fast to 175 °C and, at about 8.5 min, slower to 230 °C or 240 °C).

In Table 2, the retention times in both dimensions (for configuration 1: DB-1 + SLB-IL 100) for several fatty acid methyl esters are given. These two retention times can be considered as two "coordinates" that are very useful for identification, providing complementary information to MS. Compared to the retention times obtained on the second dimension IL-100 column, the retention

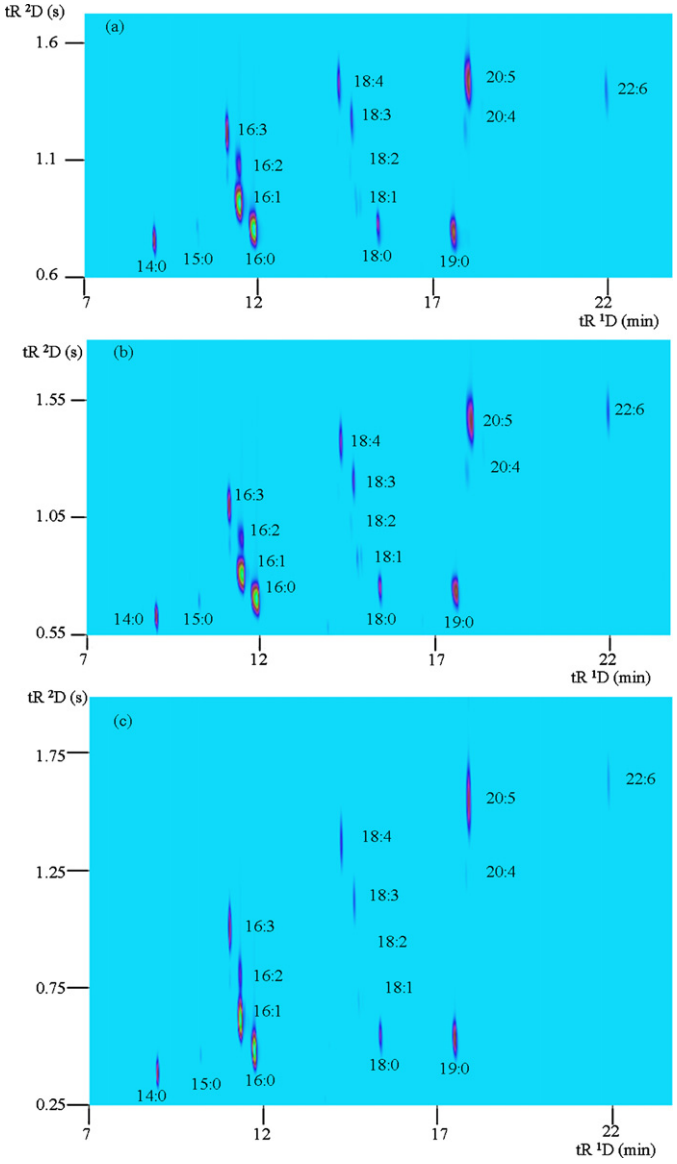

Fig. 6. GC × GC plots of the FAMEs from *Seminavis robusta* in three configurations with 0.10 mm ID DB-1MS as the first dimensional column: (a) SLB-IL 100; (b) SLB-IL 82; and (c) HP-88 as the second dimensional columns.

times obtained on the IL-82 column were slightly lower (data not shown).

For the unsaturated fatty acids also the selectivity in the second dimension was calculated (using retention times measured on the IL-100 column). The selectivity data (α = retention time of UFA/retention time of SFA of same carbon number) are also included in Table 2. These α-values are very useful to predict the number of double bonds since the values are $1.12 < \alpha < 1.19$ for MUFAs, $1.28 < \alpha < 1.36$ for DUFAs, $1.46 < \alpha < 1.53$ for FA with three double bonds and $1.63 < \alpha < 1.78$ for four double bonds. The α-value for C20:5*n*3 was 1.88 and for C22:6*n*3 $\alpha = 2.00$. Only for C22:5 a lower than expected value ($\alpha = 1.71$) was obtained.

The FAME extracts of the two species of algae samples were analyzed on the three column combinations. The GC × GC plots for *S. robusta* are shown in Fig. 6. In this sample, the "special" fatty acids C16:2, C16:3 and C18:4 are detected. These fatty acids are not typically detected in vegetable oils. The position in the GC × GC plot (and their relative position versus the saturated fatty acids) immediately enables the determination of the carbon number and

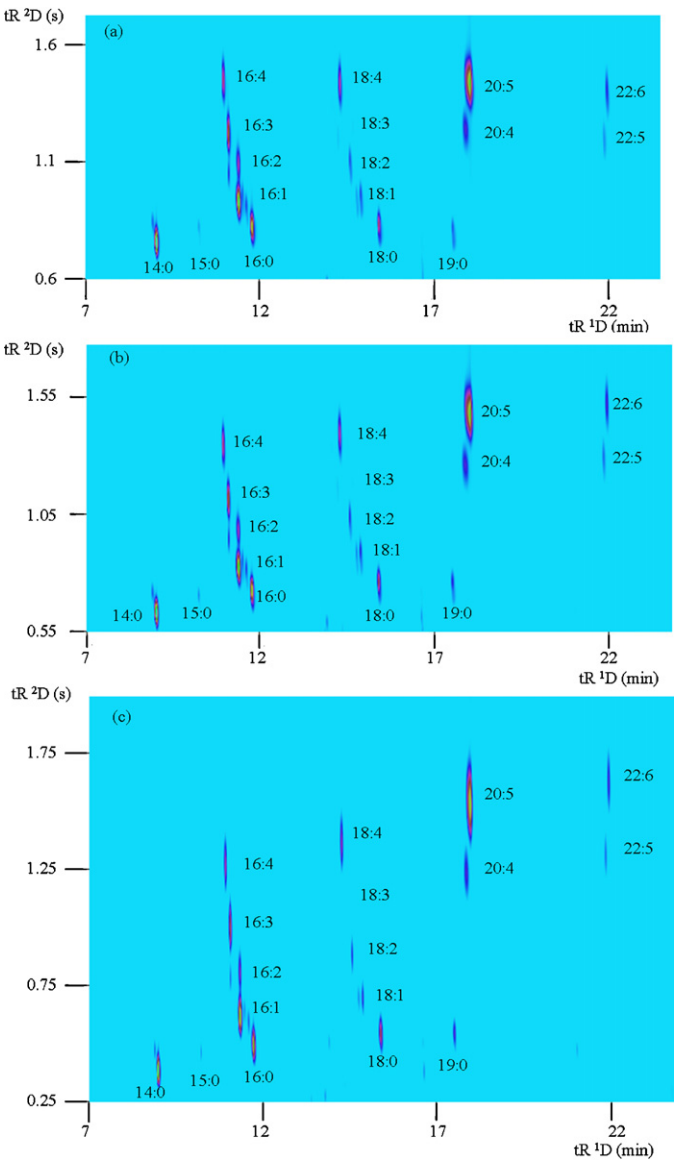

Fig. 7. GC × GC plots of the FAMEs from *Cylindrotheca closterium* in three configurations with 0.10 mm ID DB-1MS as the first dimensional column: (a) SLB-IL 100; (b) SLB-IL 82; and (c) HP-88 as the second dimensional columns.

the number of double bonds.

The GC × GC plots for the fatty acids of *C. closterium* are given in Fig. 7. Again similar profiles are obtained on the different column sets. In this sample, C16:3, C16:4, C18:4 and C22:5 are detected.

Also quantification was performed on the GC × GC data. The peak volumes were measured (using the data from the DB-1 + SLB-IL 100 column combination) and, by normalization, a relative fatty acid composition of the samples was calculated. The data are included in Table 2.

4. Conclusions

Ionic liquid stationary phases offer interesting possibilities in one dimensional GC–MS and as the second dimension in comprehensive two dimensional gas chromatography for the analysis of fatty acids in algae samples. In GC–MS, the SLB-IL 82 and SLB-IL 100 columns resulted in excellent resolution and the low column bleed enabled to obtain a very detailed fatty acid profile. The high selectivity of comprehensive GC, using the IL phases in the second dimension, combined with GC–MS identification, made it possible to detect and identify more compounds which were co-eluting in one dimension GC. Fatty acids with different double bonds and carbon numbers are group-type separated on the 2D space. Both methods can be considered as complementary tools for fatty acids analysis from different sources.

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