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Selected ion monitoring in quantitative gas–liquid chromatographic – mass spectrometric detection of fatty acid methyl esters from environmental samples

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Abstract

To calculate selected ion monitoring (SIM) gas–liquid chromatography (GLC)–mass spectrometry (MS) results of phospholipid fatty acids (PLFAs) from environmental samples, coefficients were calculated for each fatty acid by dividing the sum of ion intensities in SCAN with that of ions followed in SIM. The SIM chromatogram areas were multiplied with the coefficients, and then processed as in SCAN. The results were compared to those obtained using calibration curves and SCAN. The calibration curve and coefficient based results had the greatest errors of 7.8 and 6.7%, respectively, outside standard deviations of SCAN percentages. The PLFA contents calculated using calibration curves and coefficients were $104.9 \pm 7.3\%$ and $101.5 \pm 8.6\%$, respectively, of SCAN values. SIM increased sensitivity approximately 10-fold from SCAN, and the smallest detectable injected amount was approximately 50 ng (0.18 nmol) for 20 fatty acids, corresponding to 4×10^6 cells.

Keywords: GLC–MS; SIM; SCAN; Fatty acids; Environment

1. Introduction

The phospholipid fatty acid (PLFA) analysis gives reproducible qualitative and quantitative information on viable microbial community structure in the environment, and has been used in studies on environments ranging from aquatic sediments to terrestrial soil and water container. In the PLFA analysis, lipids are first extracted from an environmental sample, and then fractionated to phospholipids (PLs), glycolipids (GLs) and neutral lipids (NLs). Fatty acids of fractions can be analysed [e.g. Refs. 1–7]. The quantification of PLFAs from lipid phosphate or the fatty acid content has been used to estimate microbial biomass [8]. For analyses of environmental samples containing a wide variety of compounds in small concentrations, mass spectrometry (MS) detection in gas–liquid chromatography (GLC) enables the reliable identification of fatty acids from mixtures of approximately 30 acids with variable concentrations, in contrast to the flame ionisation detection giving a similar response from fatty acids and impurities. The selected ion monitoring (SIM) in MS detection of fatty acids generally increases sensitivity from that in total ion monitoring (SCAN) [9,10]. However, processing of SIM GLC–MS areas of individual fatty acids using mixtures of approximately 30 calibration standards may be troublesome. Therefore, an alternative for processing of SIM fatty acid areas was developed, based on the hypothesis that ions followed in SIM represent a constant proportion of all fragmentation ions. For each fatty acid, the sum of ion intensities in SCAN was divided with that of two ions followed in SIM. The coefficients obtained were used to multiply the SIM GLC–MS areas, and the percentage profiles were calculated. The reliability of the method was determined by analysing PLFAs, GLFAs and NLFAs from drinking water pipeline sediments.

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2. Experimental

2.1. Materials

Solvents were from Rathburn Ltd. (Peeblesshire, United Kingdom), standards from Sigma (St. Louis, MO, USA), HCl from Riedel-de Haën (Seelze, Germany), and other reagents from Merck (Darmstadt, Germany).

2.2. Drinking water pipeline sediments

The sediments were collected in December 1996 during the swab cleaning of the drinking water distribution network connected to the waterworks 8B in Finland. The surface layer was removed from pipe walls with the first swab pushed by the water pressure through the pipeline 2170 m in length. The samples representing deeper sediment layers were removed during outcomes of the second and third swabs. The pipeline sediment samples were filtered through 0.45 μm filters (Pall Europe Ltd., Portsmouth, England) in a Sartorius SM 16274 filtration equipment (Sartorius GmbH, Goettingen, Germany), frozen at −20 °C, and lyophilised (Edwards 4 K Modulyo freeze dryer, Crawley, England).

2.3. Lipid extraction and fractionation

The lipid extractions and storage of samples were done under a nitrogen atmosphere. The duplicate pipeline sediment samples of 4.0–4.2 g were extracted in 47.5 ml of chloroform: methanol: 0.15 M citrate buffer, pH 4 (1:2:0.8, v/v/v) [3,10–12] by shaking overnight at the temperature of 21 ± 2 °C. The internal standard, 375 μl of 1 mM dipentadecanoylphosphatidylcholine in methanol was added, and samples were shaken for further 5 min. The solvent phase was separated by centrifuging (2000 × *g*), the volume was measured, and chloroform and citrate buffer were added to the solvent phase to obtain final solvent ratios of chloroform:methanol:0.15 M citrate buffer (pH 4) of 1:1:0.9 (v/v/v). The samples were centrifuged for 10 min (2000 × *g*), the solvent layer was separated, and evaporated into dryness in a centrifugal evaporator (Jouan RC10.10, Jouan Inc., Saint-Herblain, France). The dry lipid extract was dissolved in chloroform (3 × 100 μl), and applied on the top of a glass column (height 100 mm, inner diameter 6 mm) containing 0.75 g of silicic acid (100–200 mesh size, Unisil, Clarkson Chemical, Williamsport, Pennsylvania, USA). The silicic acid was activated at 120 °C for 2 h, and washed with chloroform. NLs were eluted from the column with 20 ml of chloroform, GLs with 25 ml of acetone, and PLs with 15 ml of methanol [6]. The fractions were evaporated into dryness in a centrifugal evaporator.

2.4. Fatty acid analyses

Internal standards, 50 μl of approximately 0.1 μM tridecanoic and nonadecanoid acid methyl esters in hexane were added to PL, GL and NL fractions, and fatty acids were saponified, methylated, and extracted as methylesters as described [13]. The fatty acids were analysed with a Hewlett-Packard G1800A GCD system having gas chromatograph equipped with a mass selective detector and a HP-5 capillary column (30 m, 0.32 mm, 0.25 μm). Helium (1.0 ml/min) was used as a carrier gas. The injector temperature was 250 °C, and that of detector transfer interface 270 °C. The oven temperature was programmed to hold 50 °C for 1 min, and then increase 30 °C/min up to 160 °C, and thereafter 5 °C/min up to 270 °C. The mass spectra were recorded at an electron energy of 70 eV and a trap current of 300 μA. The ion source temperature was 180 °C, and that of molecular separator 135 °C. The fatty acid profiles were analysed using SCAN, and SIM (Table 1). The peaks were identified according to the mass spectra of fatty acid methyl esters. To compare differences in sensitivity between SIM and SCAN analyses of fatty acids, 10^{-1} dilution series of PLFA samples from water pipeline sediments were made, and analysed by GLC–MS.

2.5. Calculations

Internal standard, dipentadecanoylphosphatidylcholine, was used to calculate the PLFA content. The ratio of PLFAs to GLFAs, or to NLFAs was calculated using tridecanoic and nonadecanoic acid methyl esters as internal standards in each fraction obtained from the silica column. The fatty acid content in SIM analyses was calculated using calibration curves, for which calibration standards were made with known ratios of bacterial fatty acids relative to the internal standard methyl nonadecanoate (19:0) as has been presented [9,10]. The standards contained fatty acids at four to five concentrations ranging from 0.045 to 4.5 nmol/μl for 16:0 with an internal standard amount of 50 pmol/μl, and the responses were linear with correlation coefficients of 0.990 ± 0.015 (R^2 ± S.D.).

Alternatively, the sum of ion intensities in SCAN mass spectrum was divided with that of two ions followed in SIM. The coefficients obtained for each fatty acid (Table 1) were used to multiply SIM areas of fatty acids in the GLC–MS chromatogram, and then percentage fatty acid profiles and contents were calculated. Results are presented as mean ± S.D. The analysis of variance (ANOVA) followed by Dunnett two-sided test at an alpha level of 0.05 using SPSS for Windows version 10.0 [14] were used to estimate differences between results obtained using SCAN and SIM GLC–MS analyses. To convert the PLFA content to the cell density, bacteria were calculated to contain 100 μmol PLFA/g dry wt, and 1 g of bacteria (dry wt) is equivalent to 2.0×10^{12} cells [15].

3. Results and discussion

3.1. GLC–MS analyses of PLFAs, GLFAs, and NLFAs

In SIM of fatty acids, the first of two ions followed was the McLafferty rearrangement product $H_3COCOHCH_2^+$ at *m*/*z* 74, which is formed in the 2,3-cleavage with the simultaneous migration of one hydrogen atom from the fragment lost [16]. The ion is characteristic of fatty acid methyl esters and often the base peak of saturated fatty acids (Table 1). The other ion monitored was the mass peak (*m*/*z* 268, 294, 298, 310, 312, and 326), or the cleavage fragment of *m*/*z* 199, 250, 264, or 278,

Table 1
Relative GLC–MS retention times of fatty acid methyl esters (T_R is the retention time of a fatty acid methyl ester divided by that of myristic acid methyl ester); ions followed in SIM; coefficients calculated as the intensity ratio of all ions sum to that of ions followed in SIM for each fatty acid methyl esters; the intensity ratio of ions (m/z x_2/x_1)

Fatty acid	T_R	Ions in SIM (m/z)		Coefficient (±S.D.)	Intensity ratio of ions, m/z (x_2/x_1)
		x_1	x_2		
13:0	0.88 ± 0.01	74	199	3.17 ± 0.25	0.045 ± 0.006
i-14:0	0.95 ± 0.01	74	199	3.19 ± 0.03	0.267 ± 0.041
14:1	0.97 ± 0.01	74	199	17.23 ± 0.12	0.019 ± 0.014
14:0	1.000	74	199	3.23 ± 0.03	0.124 ± 0.013
br-15:1	1.06 ± 0.01	74	199	11.69 ± 0.41	0.123 ± 0.032
br-15:0	1.07 ± 0.01	74	199	9.36 ± 0.47	0.041 ± 0.008
i-15:0	1.09 ± 0.01	74	199	3.89 ± 0.03	0.047 ± 0.013
a-15:0	1.10 ± 0.01	74	199	4.22 ± 0.07	0.229 ± 0.055
15:1	1.12 ± 0.01	74	199	14.57 ± 0.19	0.019 ± 0.007
15:0	1.14 ± 0.01	74	199	3.50 ± 0.02	0.044 ± 0.010
i-16:0	1.24 ± 0.01	74	199	6.70 ± 1.31	0.056 ± 0.016
16:1ω9c	1.26 ± 0.01	74	268	11.88 ± 1.14	0.063 ± 0.050
16:1ω7c	1.27 ± 0.01	74	268	18.36 ± 0.04	0.078 ± 0.041
16:0	1.31 ± 0.01	74	199	3.88 ± 0.04	0.046 ± 0.012
br-17:1	1.36 ± 0.01	74	199	16.33 ± 0.09	0.024 ± 0.011
10Me-16:0	1.39 ± 0.02	74	199	6.64 ± 0.05	0.060 ± 0.070
i-17:0	1.41 ± 0.01	74	199	5.05 ± 0.37	0.073 ± 0.019
a-17:0	1.42 ± 0.01	74	199	5.24 ± 0.15	0.091 ± 0.027
cy-17:0	1.45 ± 0.01	74	250	14.85 ± 0.27	0.304 ± 0.099
17:0	1.47 ± 0.01	74	199	4.64 ± 0.12	0.070 ± 0.022
i-18:0	1.55 ± 0.01	74	298	7.13 ± 1.14	0.043 ± 0.006
18:2ω6c	1.58 ± 0.01	74	294	36.67 ± 2.33	0.140 ± 0.074
18:1ω9c	1.60 ± 0.01	74	264	13.86 ± 0.95	0.398 ± 0.165
18:1ω7c	1.61 ± 0.01	74	264	14.86 ± 0.18	0.415 ± 0.174
18:1	1.64 ± 0.03	74	264	15.23 ± 0.66	0.357 ± 0.110
18:0	1.65 ± 0.01	74	298	3.83 ± 0.01	0.132 ± 0.065
br-19:1	1.66 ± 0.01	74	310	37.28 ± 2.83	0.091 ± 0.075
cy-19:0	1.79 ± 0.01	74	278	15.33 ± 0.46	0.368 ± 0.155
19:0	1.81 ± 0.02	74	312	5.22 ± 0.23	0.165 ± 0.087
20:0	1.98 ± 0.02	74	326	4.22 ± 0.08	0.171 ± 0.093

and their intensity was between 1.9 and 41.5% from that of the base peak. The ratio of two ions was used to control possible impurities with same ion(s). SIM was a more reliable detection method than SCAN for NLFAs without further fractionation, due to a high content of waxes, hydrocarbon chains, and other non-fatty acid lipids, which could overlap with fatty acids in SCAN and even made them undetectable. Similarly, non-fatty acid impurities may disturb GLC–MS analyses of GLFAs by SCAN.

The PLFA percentages calculated from SIM chromatograms differed significantly from those obtained by SCAN, based both on S.D.s and ANOVA followed by Dunnett two-sided test (Table 2, Fig. 1). To produce same percentage and quantitative results from SIM chromatograms as were obtained by SCAN, coefficients presented in Table 1 were used for calculations. As a result, according to ANOVA followed by Dunnett two-sided test ($p > 0.05$) one, eight and six fatty acid percentages differed from SCAN profiles in the 1st, 2nd and 3rd swab PLFA results, respectively. Thirteen of these acids were present in small amounts below 1%, while the percentages of 16:1ω7c (2nd swab), 16:1ω9c and 10Me-16:0 (3rd swab) were greater than 1%. Based on the error evaluation by S.D.s, the percentage difference of four, ten and six fatty acids was less than 0.24 (16:1ω9c), 0.63 (18:1ω7c) and 0.45 (10Me-16:0) % outside S.D.s of SCAN values in the 1st, 2nd, and 3rd swab results, the error being 0.7, 5.5 and 2.0%, respectively. However, the difference of 0.34% outside S.D. was measured for 16:1ω7c, which represented the greatest error of 6.7% from the SCAN value.

Alternatively, the dose-response calibration curves for each fatty acids were constructed from pipeline sediment PLFAs using nonadecanoate as an internal standard for the quantification, and subsequent calculation of the percentage profiles (Table 2). Based on ANOVA followed by Dunnett two-sided test ($p > 0.05$), two, seven and ten fatty acid percentages in the 1st, 2nd and 3rd swab results, respectively, had a statistically significant difference from SCAN profiles. Thirteen of these acids were detected in amounts below 1%, while six fatty acids (2nd swab, 18:2ω6c; 3rd swab, 16:1ω9c, 10Me16:0, cy-17:0, 18:1ω7c and cy-19:0) had greater than 1% proportions. Further, the S.D.s did not cover differences from SCAN results of six, fourteen and eighteen fatty acids in the 1st, 2nd and 3rd swab results, respectively. However, these differences were smaller than 0.53 (10Me-16:0), 0.28 (18:1ω7c) and 0.40 (cy-19:0, small percentage of 2.50) % in the 1st, 2nd and 3rd swab results, representing the error of 3.6, 2.4 and 16.0%, respectively. However, differences outside S.D. of cy-17:0 (2nd swab), 16:1ω9c, 10Me-16:0, cy-17:0 and 18:1ω7c (3rd swab) were 1.32, 1.00, 1.57, 1.54, and

Table 2
Phospholipid fatty acid profiles (±S.D., $n = 2$) of water pipeline sediments released with the 1st, 2nd, and 3rd swab

Sample	1st swab (% ± S.E.)				2nd swab (% ± S.E.)				3rd swab (% ± S.E.)			
	SCAN	COEF	CALIBR	SIM	SCAN	COEF	CALIBR	SIM	SCAN	COEF	CALIBR	SIM
i-14:0	0.15 ± 0.03	0.16 ± 0.02	0.18 ± 0.02	0.50 ± 0.05***	0.12 ± 0.01	0.10 ± 0.04	0.11 ± 0.05	0.30 ± 0.13	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.32 ± 0.01***
14:1	0.11 ± 0.02	0.12 ± 0.02	0.12 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.05 ± 0.01*	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.04 ± 0.01**
14:0	0.48 ± 0.09	0.51 ± 0.05	0.56 ± 0.05	1.60 ± 0.16***	0.45 ± 0.06	0.49 ± 0.01	0.52 ± 0.01	1.46 ± 0.01***	0.54 ± 0.03	0.57 ± 0.01	0.59 ± 0.01	1.62 ± 0.01***
br-15:1	0.53 ± 0.07	0.52 ± 0.07	0.59 ± 0.06	0.46 ± 0.05	0.55 ± 0.17	0.48 ± 0.08	0.52 ± 0.06	0.42 ± 0.05	0.50 ± 0.03	0.54 ± 0.05	0.57 ± 0.03	0.43 ± 0.01
br-15:0	0.30 ± 0.04	0.34 ± 0.04	0.43 ± 0.01*	0.35 ± 0.01	0.22 ± 0.14	0.35 ± 0.03	0.42 ± 0.01*	0.33 ± 0.01	0.29 ± 0.05	0.33 ± 0.05	0.39 ± 0.04	0.31 ± 0.03
i-15:0	2.74 ± 0.41	2.90 ± 0.13	2.91 ± 0.13	7.54 ± 0.36***	2.62 ± 0.35	2.93 ± 0.09	2.86 ± 0.08	7.24 ± 0.16***	3.03 ± 0.04	3.25 ± 0.15	3.10 ± 0.12	7.71 ± 0.22***
a-15:0	1.50 ± 0.08	1.56 ± 0.09	1.51 ± 0.07	3.73 ± 0.16***	1.31 ± 0.15	1.48 ± 0.06	1.40 ± 0.06	3.37 ± 0.12***	1.27 ± 0.11	1.34 ± 0.04	1.24 ± 0.04	2.94 ± 0.06***
15:1	0.15 ± 0.01	0.15 ± 0.02	0.18 ± 0.02	0.10 ± 0.01	0.14 ± 0.05	0.13 ± 0.01	0.15 ± 0.01	0.08 ± 0.01	0.17 ± 0.02	0.17 ± 0.01	0.19 ± 0.01	0.11 ± 0.01**
i-16:0	0.56 ± 0.51	0.38 ± 0.53	0.23 ± 0.27	0.58 ± 0.55	0.20 ± 0.02	0.06 ± 0.02**	0.04 ± 0.01**	0.09 ± 0.01**	0.33 ± 0.10	0.16 ± 0.14	0.10 ± 0.06	0.22 ± 0.13
16:1ω9c	36.85 ± 0.42	38.65 ± 1.14	35.77 ± 0.74	21.29 ± 0.50***	26.24 ± 1.31	27.92 ± 1.43	25.18 ± 0.99	14.61 ± 0.51***	16.35 ± 0.09	16.88 ± 0.71	14.91 ± 0.35*	8.51 ± 0.28***
16:1ω7c	6.84 ± 1.05	7.64 ± 0.74	7.22 ± 0.63	4.23 ± 0.38*	5.11 ± 0.33	6.02 ± 0.24*	5.55 ± 0.16	3.17 ± 0.08**	4.74 ± 0.35	4.88 ± 0.09	4.41 ± 0.04	2.47 ± 0.01***
16:0	7.87 ± 0.24	7.38 ± 0.48	7.03 ± 0.40	19.23 ± 1.04***	7.76 ± 0.45	7.61 ± 0.21	7.07 ± 0.19	18.86 ± 0.41***	6.62 ± 0.31	6.55 ± 0.16	5.95 ± 0.11	15.62 ± 0.15***
br-17:1	0.33 ± 0.20	0.15 ± 0.05	0.01 ± 0.01	0.10 ± 0.03	0.11 ± 0.06	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.20 ± 0.07	0.01 ± 0.01*	0.01 ± 0.01*	0.01 ± 0.01**
10Me-16:0	14.83 ± 0.35	14.67 ± 0.51	13.62 ± 0.33	22.34 ± 0.48***	19.22 ± 1.76	18.74 ± 0.48	16.96 ± 0.27	27.13 ± 0.56**	22.72 ± 0.07	23.61 ± 0.37**	20.92 ± 0.16**	32.90 ± 0.06***
i-17:0	0.31 ± 0.02	0.32 ± 0.05	0.26 ± 0.02	0.65 ± 0.05***	0.38 ± 0.01	0.43 ± 0.04**	0.33 ± 0.01**	0.82 ± 0.01***	0.63 ± 0.07	0.50 ± 0.08	0.38 ± 0.03*	0.92 ± 0.07*
a-17:0	0.17 ± 0.06	0.17 ± 0.03	0.16 ± 0.02	0.34 ± 0.05*	0.07 ± 0.01	0.14 ± 0.01***	0.12 ± 0.01***	0.25 ± 0.01***	0.06 ± 0.05	0.10 ± 0.04	0.09 ± 0.03	0.18 ± 0.07
cy-17:0	12.79 ± 1.49	12.39 ± 1.33	15.10 ± 1.28	8.43 ± 0.76*	17.17 ± 0.65	16.85 ± 0.94	20.02 ± 0.88	10.91 ± 0.53**	21.12 ± 0.72	20.58 ± 0.63	23.95 ± 0.57*	12.83 ± 0.42***
17:0	0.20 ± 0.03	0.12 ± 0.01*	0.10 ± 0.01*	0.26 ± 0.02	0.49 ± 0.37	0.21 ± 0.05	0.17 ± 0.03	0.44 ± 0.09	0.40 ± 0.05	0.20 ± 0.01**	0.16 ± 0.01**	0.39 ± 0.01
18:2ω6c	1.60 ± 0.13	1.54 ± 0.20	1.66 ± 0.10	0.42 ± 0.03***	1.87 ± 0.02	1.89 ± 0.16	1.99 ± 0.03*	0.50 ± 0.01***	2.38 ± 0.01	2.72 ± 0.41	2.80 ± 0.23	0.69 ± 0.05***
18:1ω9c	0.72 ± 0.41	0.41 ± 0.39	0.47 ± 0.45	0.30 ± 0.29	0.45 ± 0.01	0.32 ± 0.06*	0.35 ± 0.04	0.22 ± 0.02**	0.72 ± 0.04	0.43 ± 0.05**	0.47 ± 0.02**	0.28 ± 0.01***
18:1ω7c	8.30 ± 0.72	7.48 ± 0.44	9.09 ± 0.48	5.09 ± 0.26**	11.53 ± 0.13	10.41 ± 0.36	12.32 ± 0.38	6.73 ± 0.24***	13.46 ± 0.36	12.85 ± 0.05	14.89 ± 0.02**	8.00 ± 0.06***
18:1	0.47 ± 0.04	0.39 ± 0.09	0.43 ± 0.08	0.26 ± 0.05	0.64 ± 0.01	0.57 ± 0.04*	0.61 ± 0.02	0.36 ± 0.01***	0.57 ± 0.02	0.47 ± 0.04*	0.50 ± 0.02	0.29 ± 0.02***
18:0	0.28 ± 0.01	0.25 ± 0.04	0.26 ± 0.04	0.65 ± 0.10**	0.41 ± 0.01	0.32 ± 0.02*	0.33 ± 0.02*	0.81 ± 0.05***	0.51 ± 0.04	0.41 ± 0.01*	0.41 ± 0.01*	0.99 ± 0.01***
br-19:1	0.24 ± 0.01	0.27 ± 0.11	0.35 ± 0.11	0.07 ± 0.02	0.35 ± 0.04	0.15 ± 0.06*	0.19 ± 0.06	0.04 ± 0.01**	0.51 ± 0.04	0.50 ± 0.05	0.61 ± 0.01	0.12 ± 0.01***
cy-19:0	1.48 ± 0.03	1.33 ± 0.08	1.63 ± 0.06	0.88 ± 0.03***	2.30 ± 0.05	2.13 ± 0.09	2.53 ± 0.04	1.34 ± 0.03***	2.50 ± 0.06	2.56 ± 0.08	2.98 ± 0.02**	1.54 ± 0.02***
20:0	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.02 ± 0.01*	0.05 ± 0.01**	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.07 ± 0.01*

The percentage fatty acid profiles were calculated directly from total ion (SCAN) or selected ion (SIM) monitoring results. In addition, SIM results were converted to correspond SCAN fatty acid profiles using calibration curves for each fatty acids (CALIBR), or SIM areas were multiplied with coefficients calculated for each fatty acids as the intensity ratio of all ions sum in SCAN to selected ions sum in SIM (COEF). Asterisks indicate significant differences from SCAN results (ANOVA, Dunnett two-sided *t*-test, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

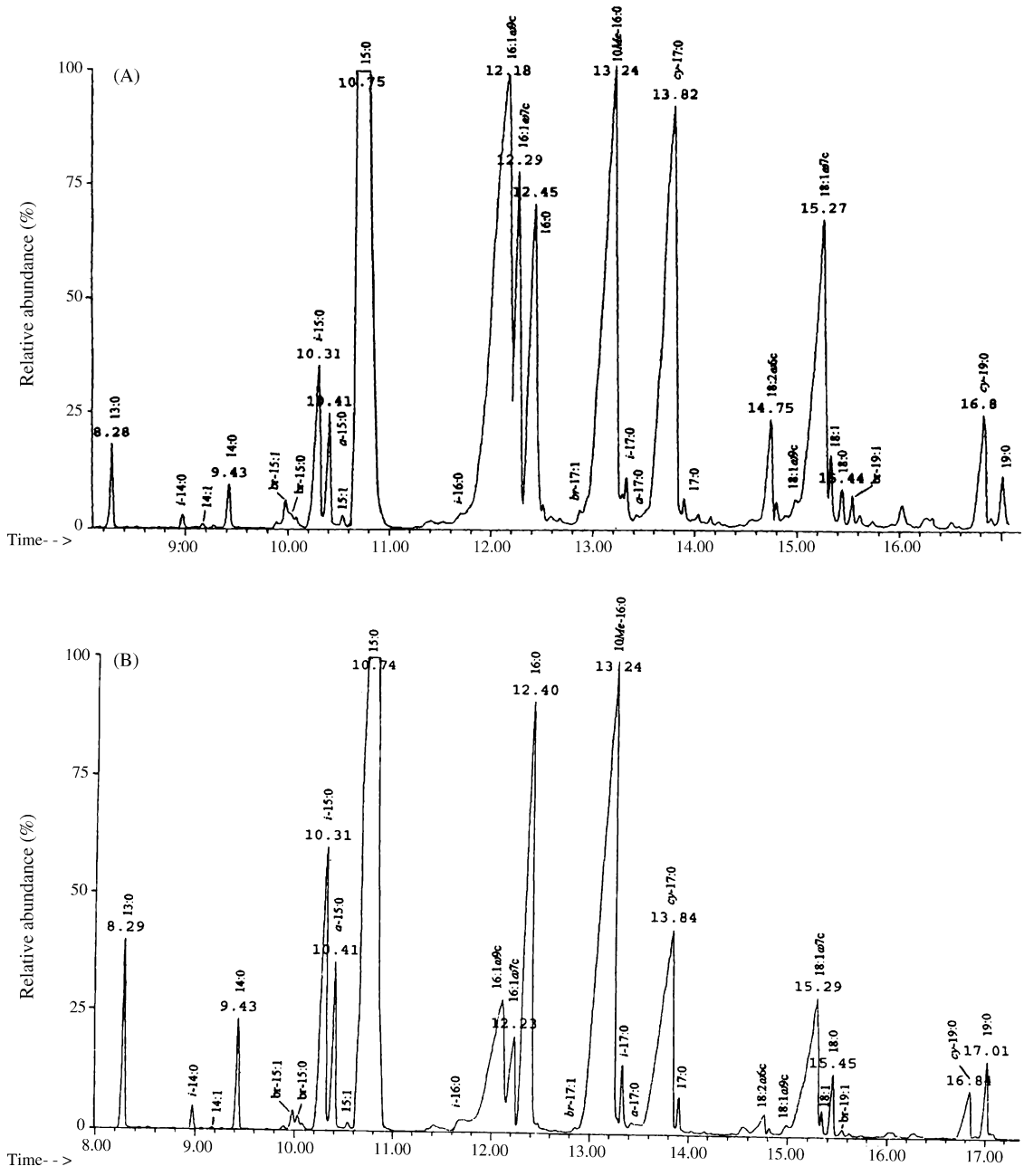

Fig. 1. Representative GLC–MS chromatogram of (A) total ion and (B) selected ion monitoring of phospholipids fatty acids from the pipeline sediment released with the 3rd swab.

1.05%, the error from SCAN proportions being 7.7, 6.1, 6.9, 7.3 and 7.8%, respectively.

Altogether, statistically significant differences were observed between SCAN, and SIM combined with coefficient-based calculations; as well as between SCAN, and calibration curve based calculations (Table 2). These errors might result from factors like problems in handling of minor fatty acids from pipeline sediment PLFAs as calibration standards. Further, the errors could be due to the partial overlap of, e.g. br-C_{15} and 16:1 acids in both standard and sample GLC–MS chromatograms produced with the HP-5 column, widely used in detection of microbial fatty acids from the environment [e.g. 1,2,5,12]. The careful examination of GLC–MS SCAN chromatograms revealed that especially in case of fatty acids with low abundance below 1%, several ions not included in their mass spectra often co-eluted. The ions could either increase the percentage areas of fatty acids or hide them into the background, both problems being avoided by SIM. All the problems presented may cause small errors especially in the percentages of minor fatty acids, which then cumulate to great errors in major fatty acids.

3.2. Quantification

Two different processing methods for SIM chromatograms both gave quantitative and qualitative amounts of PLFAs close to those obtained by SCAN monitoring, although PLFA

percentages calculated directly from SIM areas differed significantly. The SIM chromatogram areas could not be used for the quantification of microbial communities. The PLFA content calculated using calibration curves and coefficients was 104.9 ± 7.3% and 101.5 ± 8.6% ($n = 6$), respectively, of that in SCAN analyses. The use of SIM increased the sensitivity in fatty acid analyses approximately 10-fold. The minimum detectable injected amount of PLFAs in SIM analyses was approximately 50 ng for 20 fatty acids, so that the higher the number of fatty acids in the sample, the higher the amount of PLFAs needed. The smallest detected injected amount for one fatty acid was approximately 0.1 ng. The PLFA amount of 50 ng is approximately 0.18 nmol, which corresponds to 4×10^6 cells [15].

The odd-numbered fatty acids are quite rare in the environment due to biosynthesis of fatty acids via adding two carbons from acetyl-CoA [17], and can be used for the quantification. In this work, dipentadecanoylphosphatidylcholine was added as an internal PLFA standard, the other possibility being diheptadecanoylphosphatidylcholine. The addition of different internal standards to parallel samples would also enable the concomitant estimation of the background levels of pentadecanoic and heptadecanoic acids. The amount of pentadecanoic acid added with internal PL standard was evaluated to be at least 50 times greater than the environmental background level, which caused the error of less than 2%. To avoid the lipolytic degradation of standard, the preferential point for its addition was in the end of extraction, after which the sample was properly mixed and the solvent phase separated. One of the greatest advantages of PL standard was its identical behaviour with PLFAs, which compensated in calculations, e.g. for the incomplete recovery of lipids in extractions and other reactions. The additional analyses for quantification, like phosphorus [3,14,15] were avoided, and small PL amounts could be analysed without need to separate part of the sample for the quantification.

3.3. Microbial community structures in drinking water pipeline sediments

The major fatty acids in PLs, GLs and NLs were 16:1ω9c, 16:1ω7c, 18:1ω7c, cy-17:0, 10Me-16:0 and 16:0 (Tables 2 and 3). However, in GLFAs the percentage of *i*-15:0, *i*-17:0 and br-17:1 was greater than that of 16:1ω7c, and the sum of *iso*- and *anteiso*-branched acids in GLFAs (16.73 ± 1.32%) was greater than that in PLFAs (5.21 ± 0.28%) and NLFAs (7.45 ± 0.75%). Indeed, glycolipids are common to gram-positive bacteria, which also typically contain *iso*- and *anteiso*-branched acids [18]. Thus, the major microbial groups in pipeline sediments of this drinking water network consisted of gram-negative bacteria, and sulphate reducing bacteria with 10Me-16:0 [8,19]. In addition, *iso*- and *anteiso*-branched acids indicative of gram-positive bacteria, and linoleic acid characteristic of fungi were detected in pipeline sediments [18,20].

Table 3
Glycolipid and neutral lipid fatty acid profiles (±S.D., $n = 2$) of water pipeline sediments released with the 1st, 2nd and 3rd swab, and analysed using SIM combined with coefficient-based calculations

	Glycolipid fatty acids (% ± S.D.)			Neutral lipid fatty acids (% ± S.D.)		
	Swab 1	Swab 2	Swab 3	Swab 1	Swab 2	Swab 3
i-14:0	0.27 ± 0.03	0.20 ± 0.01	0.10 ± 0.01	0.34 ± 0.02	0.29 ± 0.01	0.23 ± 0.01
14:1	0.23 ± 0.09	0.16 ± 0.01	0.05 ± 0.01	0.53 ± 0.09	0.66 ± 0.08	0.54 ± 0.02
14:0	1.22 ± 0.21	0.96 ± 0.08	0.63 ± 0.06	2.75 ± 2.16	1.50 ± 0.07	1.67 ± 0.04
br-15:1	1.42 ± 0.44	1.30 ± 0.03	0.71 ± 0.08	0.74 ± 0.02	0.65 ± 0.01	0.58 ± 0.01
br-15:0	0.60 ± 0.27	0.92 ± 0.03	1.12 ± 0.80	0.80 ± 0.09	0.85 ± 0.09	0.93 ± 0.42
i-15:0	8.44 ± 0.62	9.93 ± 0.14	8.39 ± 0.03	3.94 ± 0.28	3.78 ± 0.24	3.52 ± 0.26
a-15:0	2.04 ± 0.27	1.79 ± 0.02	1.20 ± 0.01	2.03 ± 0.11	1.83 ± 0.14	1.46 ± 0.14
15:1	0.06 ± 0.09	nd	nd	0.41 ± 0.09	0.64 ± 0.06	0.64 ± 0.02
15:0	0.58 ± 0.01	0.52 ± 0.01	0.40 ± 0.03	0.51 ± 0.01	0.53 ± 0.03	0.54 ± 0.01
i-16:0	1.73 ± 0.10	1.88 ± 0.01	1.33 ± 0.05	0.87 ± 0.10	1.00 ± 0.01	0.76 ± 0.02
16:1ω9c	10.15 ± 0.69	8.71 ± 0.04	7.94 ± 0.66	28.44 ± 2.66	24.45 ± 1.14	16.59 ± 0.41
16:1ω7c	2.17 ± 0.53	1.54 ± 0.02	1.16 ± 0.05	7.62 ± 0.34	5.68 ± 0.18	4.15 ± 0.16
16:0	13.13 ± 1.15	11.12 ± 0.15	9.59 ± 0.26	11.33 ± 1.94	8.33 ± 0.25	7.72 ± 0.02
br-17:1	5.28 ± 0.11	6.35 ± 0.04	6.34 ± 0.24	0.50 ± 0.03	0.95 ± 0.03	0.57 ± 0.10
10Me-16:0	18.81 ± 0.67	22.14 ± 0.01	27.82 ± 0.60	15.15 ± 1.43	18.89 ± 0.73	24.87 ± 0.38
i-17:0	3.37 ± 0.04	3.91 ± 0.04	4.03 ± 0.06	0.71 ± 0.17	0.82 ± 0.06	0.85 ± 0.10
a-17:0	0.93 ± 0.01	0.93 ± 0.01	0.90 ± 0.01	0.31 ± 0.04	0.29 ± 0.01	0.24 ± 0.02
cy-17:0	5.96 ± 0.84	7.54 ± 0.13	8.68 ± 0.17	11.35 ± 0.37	15.58 ± 0.12	19.40 ± 0.52
17:0	0.68 ± 0.09	0.60 ± 0.01	0.53 ± 0.04	0.29 ± 0.01	0.31 ± 0.01	0.54 ± 0.20
18:2ω6c	0.78 ± 0.09	0.76 ± 0.04	0.72 ± 0.09	1.30 ± 0.15	1.84 ± 0.11	1.95 ± 0.16
18:1ω9c	1.96 ± 0.70	1.14 ± 0.04	0.79 ± 0.08	1.62 ± 1.00	0.80 ± 0.06	0.86 ± 0.04
18:1ω7c	13.04 ± 0.18	11.66 ± 0.09	12.35 ± 0.08	5.16 ± 0.40	7.11 ± 0.61	7.99 ± 0.09
18:1	0.35 ± 0.03	0.32 ± 0.01	0.26 ± 0.01	0.24 ± 0.06	0.22 ± 0.01	0.13 ± 0.01
18:0	3.57 ± 2.44	1.91 ± 0.16	1.56 ± 0.23	1.60 ± 1.02	0.82 ± 0.03	0.97 ± 0.01
br-19:1	1.81 ± 0.17	1.74 ± 0.01	1.71 ± 0.05	0.52 ± 0.34	0.31 ± 0.07	0.32 ± 0.05
cy-19:0	1.18 ± 0.05	1.80 ± 0.03	1.55 ± 0.01	0.83 ± 0.20	1.76 ± 0.09	1.87 ± 0.04
20:0	0.25 ± 0.02	0.16 ± 0.01	0.12 ± 0.03	0.12 ± 0.01	0.12 ± 0.02	0.11 ± 0.01

nd, not detected.

Table 4
The amounts of phospholipid (PLFA), glycolipid (GLFA) and neutral lipid (NLFA) fatty acids, and the cell number calculated from the PLFA content in drinking water pipeline sediments released with the 1st, 2nd, and 3rd swabs

Sample	PLFA	GLFA	NLFA
mg/g dry wt			
1st swab	1.925 ± 0.039	0.141 ± 0.027	0.556 ± 0.066
2nd swab	1.590 ± 0.011	0.185 ± 0.030	0.576 ± 0.041
3rd swab	2.228 ± 0.057	0.520 ± 0.001	0.912 ± 0.055
μmol/g dry wt			
1st swab	7.048 ± 0.102	0.545 ± 0.069	2.020 ± 0.173
2nd swab	5.760 ± 0.029	0.669 ± 0.076	2.100 ± 0.106
3rd swab	8.039 ± 0.144	1.890 ± 0.004	3.323 ± 0.142
cells/g dry wt			
1st swab	$(1.4 \pm 0.1) \times 10^{11}$		
2nd swab	$(1.2 \pm 0.1) \times 10^{11}$		
3rd swab	$(1.6 \pm 0.3) \times 10^{11}$		

In PLFAs, GLFAs and NLFAs the proportions of 10Me-16:0 and cy-17:0 increased from the surface layer released with the first swab towards the deepest sediment obtained with the third swab, and concomitantly the amounts of 16:1ω9c and 16:1ω7c decreased. Thus, the amounts of sulphate reducing bacteria of *Desulfobacter* type, and gram-negative bacteria having cyclopropane acids [8,19] increased towards the deepest sediment. The cyclopropane fatty acids have also been related with stress conditions and stationary growth phase [8].

The amount of PLFAs in pipeline sediments was the greatest in the deepest sediment released with the 3rd swab, intermediate in the surface sediment obtained with the 1st swab, and the smallest in the middle sediment, although differences in calculated cell densities were small (Table 4). The sediment cell numbers ($1.2–1.6 \times 10^{11}$ cells/g dry wt) were greater than those common to soil [8]. The amount of GLFAs and NLFAs was the greatest in the deepest layer of pipeline sediments. The high amount of GLFAs is characteristic of gram-positive bacteria [18]. NLFAs could originate from, e.g. triacylglycerols accumulated by fungi and gram-positive streptomycetes [20,21], fatty acids of dead microorganisms, and also from degradation of lipids during analyses.

3.4. Concluding remarks

In this study, coefficients were calculated for each fatty acid by dividing the sum of ion intensities in SCAN mass spectrum with the intensity sum of two ions followed in SIM, based on the hypothesis that this ratio is constant in a well-calibrated mass spectrometer. PLFAs, GLFAs and NLFAs of pipeline sediments were analysed using SCAN monitoring, and SIM combined with calibration curve and coefficient-based calculations. The quantitative and qualitative results obtained with these different methods had errors of the same order of magnitude, and were approximately equal with respect to precision and accuracy. The microbial community structures evaluated from PLFAs processed with the three methods were also similar, and agreed with those known to be present in drinking water pipelines [7,22]. GLFAs and NLFAs gave additional information on the microbiology of samples. The SIM increased the sensitivity 10-fold from SCAN to the minimum detectable injected amount of approximately 50 ng for 20 fatty acids. The SIM was the only reliable method for the detection of GLFAs and NLFAs without further fractionation. In conclusion, the coefficient-based calculation of SIM GLC–MS areas enabled the processing of fatty acid SIM chromatograms without use of over 30, often easily oxidising fatty acids in standard mixture for the preparation of calibration curves. The coefficient-based calculation method for SIM detection could also be appropriate for quantitative detection of other, non-commercially available compounds difficult to separate as pure.

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