Trace determination of lower volatile fatty acids in sediments by gas chromatography with chemically bonded FFAP columns

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ABSTRACT

A capillary gas–liquid chromatography method was developed for the quantification of free lower volatile fatty acids (LVFA) in freshwater sediments. The method is based on the application of water-resistant FFAP (free fatty acid phase) columns and splitless injection. An important feature is the ability to determine LVFA directly at picomole levels in 1–3 μl of water without sample extraction, clean-up or derivatization. Continuous saturation of the carrier gas with formic acid is superfluous, making this method compatible with mass-selective detection. Detection limits of 0.2 pmol can be obtained for propionate with mass-selective detection and 1 pmol for acetate with flame ionization detection. The ability to study LVFA metabolism using stable isotope tracers is discussed. The method allows the measurement of well defined concentration profiles (4–70 μM) in sediment pore waters and is a good alternative to existing techniques for determining trace amounts of LVFA in very small volumes of organic-rich matrices.

INTRODUCTION

Lower volatile fatty acids (LVFA) such as acetate and propionate, generated during diagenesis of organic matter, play a key function in aquatic ecosystems as precursors of methanogenesis and respiration[1–3]. Respiration in meso-eutrophic lakes is limited mainly to the top few centimeters of the sediment–water interface[4] while methanogenesis occurs in deeper strata, making spatial differences in LVFA metabolism with sediment depth expected. A study of natural concentration profiles of LVFA and their actual turnover rates will lead to a better understanding of complex interactions between fermentative and respiratory processes. A knowledge about the actual conversion of LVFA to carbon dioxide and methane in freshwaters is still limited, however, owing to the lack of analytical methods[2] for measuring low LVFA concentrations (4–70 μM) routinely in very small sample volumes of porewater.

Gas–liquid chromatography (GLC) is routinely applied to the determination of LVFA of food[5], clinical[6], atmospheric[7] and waste-water samples[8]. Ion-exclusion chromatography (IEC) is a alternative for determining LVFA in relatively clean matrices such as Antarctic ice water[9], but trace measurements in sediments can be complicated by interfering peaks[10]. To analyse sediments by IEC requires sample clean-up methods such as vacuum distillation[11] or ultrafiltration[12]. In an earlier study we determined LVFA, including formate and lactate, after derivatization to a fluorophore[2]. Detection limits below 100 fmol can be achieved but complex sample pretreatment is required[2]. For determining volatile acids only, GLC without derivatization or sample purification is preferable for its speed and simplicity[13].

Recently, FFAP-CB (free fatty acid phase chemical bond; Varian) capillary columns have been introduced[15]. These columns tolerate aqueous injections, making them very suitable for the determination of hydrophilic LVFA. Capillary columns facilitate the application of mass-selective detectors and consequently the use of stable isotopes to trace metabolic processes as discussed in this paper.

EXPERIMENTAL

Apparatus

A Hewlett-Packard Model 5890 gas chromatograph with a split–splitless injection port, a 90 × 4 mm I.D. glass liner and a flame ionization detector was used. The capillary columns were a 10 m × 0.53 mm I.D. column coated with 1-μm FFAP-CB wax (Hewlett-Packard) and a 25 m × 0.32 mm I.D. column coated with 0.33-μm FFAP-CB wax (Chrompack, Middelburg, The Netherlands). Chromatographic conditions are given in Table I. The column was primed daily by ten injections of 1.0% formic acid (Suprapur; Merck, Darmstadt, F.R.G.) in Milli-Q water.

TABLE I

CHROMATOGRAPHIC CONDITIONS FOR THE DETERMINATION OF LVFA USING 25 m × 0.32 mm I.D. AND 10 m × 0.53 mm I.D. CAPILLARY FFAP-CB COLUMNS

Programme	*Column I.D. (mm)*		
	0.32 (FID)	*0.32 (MS)*	*0.53 (FID)*
Injector temperature	225°C	150°C	150°C
Detector temperature	260°C	250°C	200°C
Isothermal period	80°C (1.10 min)	80°C (1.10 min)	70°C (1.10 min)
First ramp rate	15°C min⁻¹	15°C min⁻¹	10°C min⁻¹
Isothermal period	105°C (1.00 min)	105°C (1.00 min)	105°C (0.25 min)
Second ramp rate	10°C min⁻¹	15°C min⁻¹	n.d.
Isothermal period	140°C (1.00 min)	140°C (1.00 min)	n.d.
Purge on time	1.1 min	1.1 min	1.1 min
Purge off time	2.5 min	2.5 min	4.8 min
Gases (ml min⁻¹)			
Carrier gas (He)	4	4.5	20
Make up gas (He)	35	27	20
Septum vent	1	1	1
Purge vent	30	30	30

For peak identification, a C_1–C_{10} fatty acid mixture (Supelco, Bellefonte, PA, U.S.A.) and gravimetric standards (Suprapur; Merck) were analysed with an HP 5970 mass-selective detector, to which a 25 m × 0.32 mm FFAP (Chrompack) column was linked. Samples (0.5 μl) were introduced by splitless injection. Chromatographic conditions are included in Table I; the vacuum in the mass-selective detector was below $8 \cdot 10^{-5}$ Torr and the ionization energy was 70 eV. During elution, continuous scans were made from m/z 44 to 550. The mass spectral data were processed with an HP-300 computer to trace molecular ions (M^+) or specific fragments originating from LVFA. Sediment and stable isotope analysis were performed by selective ion monitoring (SIM).

Sediment analysis

Undisturbed sediment cores were taken with a Jenkin sampler from Vechten, a 10-m deep lake with a clay-rich anoxic sediment[1]. Samples of 0.35 g were drawn by syringe through 2.8-mm holes (covered with Scotch tape No. 471) in the acrylic glass sampling core. The samples were centrifuged at 1000 g for 5 min in 0.4-ml polypropylene sampling tubes (Emergo, The Netherlands). The supernatant was separated and frozen for storage. Before injection, 100 μl of supernatant were adjusted with 1 μl of 50% formic acid (Suprapur; Merck). A volume of 3 μl was injected in duplicate or triplicate into the gas chromatograph. Calibration was done by external standardization with gravimetric standards (0–50 μM) of acetate (Suprapur; Merck), [$^2H_3$]acetate (99.5% pure; Aldrich, Beerse, Belgium) and propionate (analytical-reagent grade; J. T. Baker, Philipsburgh, NY, U.S.A.) in Milli-Q water, acidified with 0.5% (v/v) formic acid. The glass insert in the injector needs regular cleaning; pushing a wetted ball of crumpled paper though the insert is usually sufficient to pick up deposits.

RESULTS

With FFAP-CB, good separation of acetate and propionate from other LVFA was achieved (Figs. 1 and 2). Only intense molecular ion peaks of acetate (m/z 60 and 61), propionate (m/z 73–77) and butyrate (m/z 88) were recovered in scans of the fatty acid mixture. Other LVFA were identified by their retention times and interpretation of related spectra. Formate (m/z 44–46) was not recovered in the ion chromatograms. [$^2H_3$]Acetate signals (m/z 63) were of same intensity as those of unlabelled acetate.

Sediment samples (natural pH 7.5–8; 5–10 mequiv l^{-1} HCO_3^-) require acidification (pH < 4.5) to improve volatilization and suppress adsorption in the instrument. At pH 4.5–5.5 the peak heights were already 70% lower. New columns require 0.5% formic acid acidification, but contaminated columns need a higher concentration. Only the acetate blank was affected by formic acid addition; an increase in formic acid concentration by 0.5% leads to a *ca.* 2 μM rise in the acetate blank. With 0.5% formic acid, the acetate blanks for Milli-Q water (1.5–4 μM) were similar to those previously found (2–5 μM[2]). The reproducibility improved with increasing concentration (Table II), column diameter and with injection volume [0.53 mm I.D. column and 25 μM: 0.5 μl, 10.5%; 3 μl, 1.2%; 5 μl, 0.6% ($n = 5$)]. With the 0.32 mm I.D. column the relationship between injection volume and peak height was linear up to 8 μl ($r = 0.993$) and with the 0.53 mm I.D. column until at least 5 μl ($r = 0.997$) for acetate.
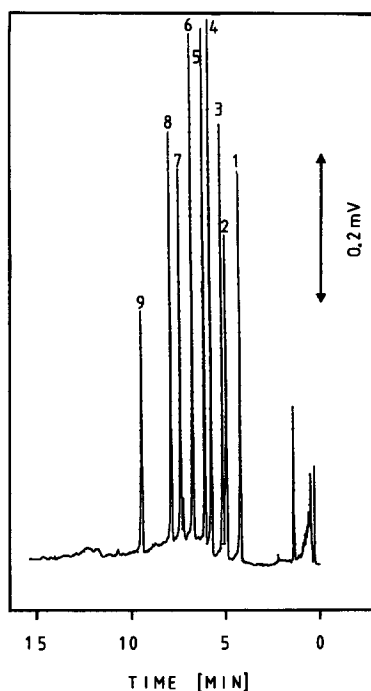
Fig. 1. Chromatogram of a mixture of volatile fatty acids (Supelco; 3 μl 100 μ*M* or 300 pmol each) by GLC with a 25 m × 0.32 mm I.D. FFAP column and FID. Peaks: 1 = acetic acid; 2 = propionic acid; 3 = isobutyric acid; 4 = butyric acid; 5 = valeric acid; 6 = isovaleric acid; 7 = capronic acid; 8 = isocapronic acid; 9 = heptanoic acid.
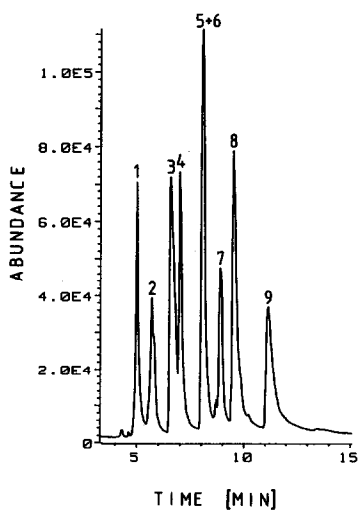

Fig. 2. Composed ion chromatogram of a mixture of volatile fatty acids (0.6 μl, 100 μ*M* or 60 pmol each) by GLC on a 25 m × 0.32 mm I.D. FFAP column and MS processed in the SIM mode. The ion chromatogram was composed by summation of intensities of ions of *m/z* 45 and 60. Peaks as in Fig. 1.

TABLE II

RELATIONSHIP BETWEEN RELATIVE STANDARD DEVIATION, CONCENTRATION ($n = 10$) AND COLUMN DIAMETER FOR ACETATE ANALYSIS WITH SPLITLESS INJECTION (3 μl) AND FID

Standards were made in Milli-Q water (blank 3 μM) which was acidified with 0.5% formic acid.

Concentration (μM)	Relative standard deviation (%)	
	0.32 mm I.D. column	0.53 mm I.D. column
0	19	7
5	14	9
10	8	4
25–100	8	3

The detection limits, defined as a signal change equal to three times the signal-to-noise ratio, were 1–3 pmol for flame ionization detection (FID), and 0.2–0.7 pmol for mass-selective detection (MS) (Table III). Acetate calibration graphs [signal obtained by summation of ABU (abundance units or relative intensity) for m/z 45 and 60) were linear from 20 to 400 pmol ($r = 0.947$). Below 20 pmol the curves flatten. The [^{2}H$_3$]acetate (m/z 63; $r = 0.998$) and propionate calibration graphs (m/z 45 and 75; $r = 0.957$) were linear from 0.2 to 400 pmol.

DISCUSSION

Capillary FFAP-CB columns can be applied to the trace determination of LVFA (<200 pmol) in aqueous samples. Gross sediment sample sizes of 300 μl can be processed with ease as only 3 μl of centrifugate are needed for GLC. Continuous

TABLE III

COMPARISON OF DETECTION LIMIT, LINEAR RANGE AND SENSITIVITY OF THE DETERMINATION OF ACETATE AND PROPIONATE BY GLC

The signal of acetate was composed of m/z 45 and 60 and that of propionate m/z 45 and 74.

Detection	Column I.D. (mm)	Detection limit (pmol)	Linear range		Sensitivity (attenuation 32)[a]
			pmol	r	
Acetate					
FID	0.32	1	0–150	0.990	5.7 μV pmol^{-1}
FID	0.53	1	0–125	1.000	6.7 μV pmol^{-1}
MS	0.32	0.2	30–400	0.947	261 ABU pmol^{-1}
Propionate					
FID	0.32	3	n.d.	n.d.	4.7 μV pmol^{-1}
FID	0.53	2.5	0–125	0.999	5.8 μV pmol^{-1}
MS	0.32	0.7	0–400	0.957	86 ABU pmol^{-1}

[a] ABU, abundance units.

saturation of the carrier gas with formic acid vapour to suppress ghosting[2,13,14] was unnecessary. Sample pretreatment requires no purification or concentration step, thereby avoiding losses due to ineffective extraction or freeze-drying[2]. Derivatization of LVFA in pore water requires excess of reagent, as part of the reagent will be neutralized by dissolved organic carbon[2]. Addition of an excess of reagent implies that reproducibility of the reagent blanks rather than the detection limit of the derivative determines the accuracy at trace levels. Lactate, for example, does not appear as an impurity in reagents and can be well determined as a derivative. Acetate, however, is a common contaminant in reagents and solvents[2]. Using the FFAP-CB column, reagent interference is circumvented and only the instrument blank limits the linear dynamic range in which acetate can be accurate determined (Table II). The absence of a reagent label has for MS a second advantage that stable isotope signals originate only from isotope in the LVFA molecules themselves. Note that ^{13}C in aromatic reagent labels can contribute considerably to "blank" signals.

The practical advantages of using stable isotope labelling instead of radioactive isotopes[3] are obvious. Stable isotope tracers can be used for fieldwork or in ordinary laboratories without special licenses. We previously applied on-line gas proportional counting of radioactively labelled LVFA[3]. In the proportional counter most of the chromatographic resolution was lost, which is not the case with MS (Fig. 2). Similar MS chromatograms of free LVFA (Fig. 2) have, to our knowledge, not yet been published. Of the LVFA, only formate (m/z 44–46[16]) could not be detected by MS or FID and derivatization or other techniques[2,10,17] may be required for this compound.

Sediment analysis

Profiles of free acetate concentrations measured in 1988 and 1989 in sediment porewater from Lake Vechten (Fig. 3) regularly showed similarly shaped concentration profiles to those observed in 1978[4] and 1983[2], but the peaks in the profiles
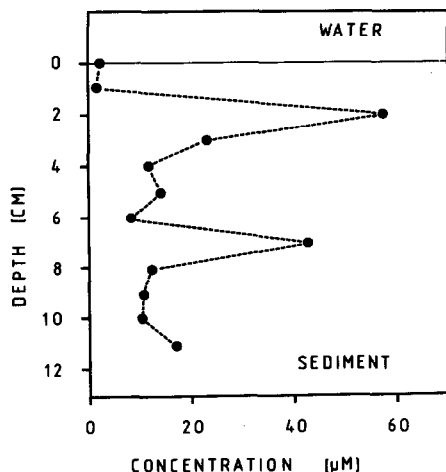

Fig. 3. Profile of free acetate concentration with sediment depth in pore water collected in Lake Vechten on April 24th, 1989.

measured with the FFAP-CB column were much sharper. Although changes in LVFA metabolism in the lake cannot be excluded, the lower concentrations found in 1978 and 1983 can be ascribed to dilution effects due to the larger sample sizes (5–10 ml instead of 0.35 ml) used in previously applied techniques. The sharp sediment acetate peaks obviously reflect intense acetogenesis under well defined metabolic conditions. The acetate peak just below the sediment–water interface can be explained by respiratory and/or diffusion processes[2,3]; the peak at − 6 cm, however, hints at as yet unexplained fermentative processes. To unravel these processes, [^{2}H$_3$]acetate can be used as a tracer.

In conclusion, GLC on capillary FFAP columns is a good alternative to existing techniques for the determination of trace amounts of acetate and higher homologues in small sample volumes of organic-rich matrices such as sedimental porewater.

ACKNOWLEDGEMENTS

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