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Journal of Chromatography B, 814 (2005) 155–161

JOURNAL OF
CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

Determination and quantification of α,β,γ,δ-unsaturated aldehydes as pentafluorobenzyl-oxime derivates in diatom cultures and natural phytoplankton populations: application in marine field studies

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Received 30 June 2004; accepted 7 October 2004
Available online 11 November 2004

Abstract

Reactive α,β,γ,δ-unsaturated aldehydes and oxo-acids produced by marine diatoms upon cell damage interfere negatively with the reproduction success of their grazers. A simple, sensitive and specific method based on gas-chromatography coupled to mass spectrometry (EI or CI/EC) was developed for the quantification of these deleterious substances in laboratory diatom cultures and in natural phytoplankton populations. For aldehyde quantification, diatom containing samples are damaged in the presence of *O*-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA·HCl) which leads to an in situ derivatisation without inhibition of the biosynthesis of the aldehydes. The oxime derivates of oxo-acids were in addition reacted with *N*-*tert*-butyldimethylsilyl-*N*-methyl-trifluoracetamide (MTBSTFA).

Keywords: Decadienal; Oxo-acids; Marine plankton; Chemical defence; GC/MS

1. Introduction

In the last decade, a chemical defence relationship mediating the interaction of diatoms and their grazers came into the focus of ecologists and chemists [1–6]. Some diatom species are able to form 2*E*, 4*E*/*Z* isomeric mixtures of α,β,γ,δ-unsaturated aldehydes like 2,4-decadienal, 2,4,7-decatrienal, 2,4-octadienal, 2,4,7-octatrienal, and 2,4-heptadienal upon cell damage [7–9]. This wound activated defensive reaction is under the control of a phospholipase A_2/lipoxygenase/hydroperoxide lyase enzyme cascade [10–12]. The fatty acid derived polyunsaturated aldehydes (PUA) were implicated in numerous deleterious effects on herbivorous crustaceans including the interference with their reproductive success by inhibiting egg hatching and the reduction of their survival [3,6]. Diatoms are dominant primary producers and key players in the marine food web [13,14]. Because of this central importance, the influence of these unicellular algae on higher trophic levels has to be investigated with emphasis on PUA-presence and function in further studies. Up to now, the metabolites in question were only determined in cultivated diatom strains [3,7–9,15], and a direct proof of the aldehyde formation in natural phytoplankton was not obtained. Nevertheless, several field studies address the relationship between diatom blooms and copepod reproduction success without giving any information about the actual occurrence of deleterious aldehydes in the plankton [3,6,16]. Phytoplankton samples contain numerous diatom species and a prediction of their PUA-production

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Abbreviations: PFBHA·HCl, *O*-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride; PFBO, *O*-(2,3,4,5,6-pentafluorobenzyl) oxime; MTBSTFA, *N*-*tert*-butyldimethylsilyl-*N*-methyl-trifluoracetamide; 12-ODTE, (5*Z*,8*Z*,10*E*)-12-oxo-dodeca-5,8,10-trienoic acid; PUA, polyunsaturated aldehydes; CI/EC, chemical ionisation electron capture; TRR, *Thalassiosira rotula*; SC, *Skeletonema costatum*; PM, *Prorocentrum minimum*; AF, *Asterionella formosa*; TPA, *Thalassiosira pseudonana*

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doi:10.1016/j.jchromb.2004.10.021

is impossible. This is due to the fact that not all diatom species are able to produce PUA upon cell damage and that even different isolates from the same species have varying PUA-producing capability [7]. Investigation of phytoplankton field samples is mainly hampered by the fact that only few producing cells are present in a litre of seawater and that the sensitivity of the established methods for aldehyde detection is often not sufficient. Headspace extraction [15], solid phase microextraction (SPME) [10,17] and a Wittig-based-derivatisation approach of volatile aldehydes [9] were previously applied to diatom cultures for determination of these aldehydes. Given the low effective concentration of PUA causing the malfunctions in grazers [18], the Wittig-derivatisation which allows the stabilisation of reactive PUA and enables NMR analysis [9] seems to be too insensitive for the determination of PUA in phytoplankton field samples without significant concentration efforts. The more sensitive SPME approach implicates an immediate GC/MS analysis, which is often not feasible during field studies. In order to overcome the problems of low sensitivity or of low practical value during field studies, we developed an in situ derivatisation of PUA in phytoplankton matrices. The fast and sensitive method for the determination of aldehydes is based on the treatment of algal samples with *O*-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA·HCl) before wounding of the cells. In previous studies, the analysis of PFB-oxime derivatives in different biological matrices was successfully applied and provided a sensitive detection method for labile aldehydes [19–22]. The GC/MS identification of these derivates and their fragmentation by EI [23] or CI [20] is well established. We have extended the use of this derivatisation method for PUA-determination during marine field studies and for screening of diatom cultures in algal collections. The introduced protocol allows enzymatic reactions in the presence of the derivatisation reagent and thus enables an in situ trapping of aldehydes upon cell disruption of diatoms. An enzyme cascade is activated that releases PUA, which are directly converted to *O*-pentafluorobenzyl-oxime derivates (PFB-oximes). Our method combines the advantages of sensitivity of detection and stability of oxime derivates with mild derivatisation conditions that allow parallel enzymatic reactions.

2. Experimental

2.1. Reagents

2*E*,4*E*-octadienal (96%), 2*E*,4*E*-decadienal (89%), tridecanal (95%) and H_2SO_4 were purchased from Sigma (Taufkirchen, Germany). 2*E*,4*E*-Heptadienal (97%), *O*-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA·HCl, 99%) and *N*-*tert*-butyldimethylsilyl-*N*-methyl-trifluoracetamide (MTBSTFA, 97%) were obtained from Fluka (Taufkirchen, Germany). Benzaldehyde (99%), methanol and hexane were obtained from Merck (Darmstadt, Germany).

2.2. Equipment

A Finnigan Trace GC/MS (Thermo Finnigan, CA, USA) equipped with a 15 m EC-5 capillary column (0.25 mm internal diameter, 0.25 μM film thickness, Alltech, USA) was used for EI-MS measurements. The inlet temperature was maintained at 250 °C and samples were injected in splitless mode. The column oven was held at 60 °C for 2 min, programmed from 60 to 300 °C at 8 °C/min and finally held at this temperature for 3 min. Helium was used as carrier gas at a constant flow of 1.5 ml/min and the transfer capillary was held at 270 °C. Ionisation energy was 70 eV with the ion source at 200 °C. The mass detector was operated in the TIC mode at a scan rate of 2.4 scans/s. To increase sensitivity negative ion chemical ionisation electron-capture mass spectrometry with methane as reagent gas (70 mTorr pre-vacuum) was used. This was performed on a Finnigan GC Q GC/MS (Thermo Finnigan, CA, USA) equipped with a 30 m RTX-200 column (i.d. = 0.25 mm, 0.25 μm film thickness) (Restek, USA).

2.3. Cultivation and sampling

Cultures of *Thalassiosira rotula* (TRR, CCMP 1647) and *Thalassiosira pseudonana* (TPA, CCMP 1335) were obtained from the Provasoli-Guillard National Centre for Culture of Marine Phytoplankton (Maine, USA). *Skeletonema costatum* (SC, RCC 75) and *Prorocentrum minimum* (PRO, RCC 291) were obtained from the Station Biologique, Roscoff in France. The fresh water diatom *Asterionella formosa* (AF, SAG 8.95) was purchased from the Culture Collection of Algae (SAG) at the University of Göttingen (Germany).

Marine diatoms were grown in standing cultures at 16 °C in artificial medium [24] to the final concentration of 7×10^4 to 23×10^4 cells/ml (TRR). Illumination was provided on a 14:10 light:dark rhythm. *A. formosa* was cultured as described in [10]. Cells were counted with the Neubauer improved chamber (Marienfeld, Germany). Phytoplankton was sampled using a phytoplankton net (mesh size, 20 μm) in the coastal area off Roscoff (Brittany, France) during a weekly ship cruise in spring 2003 and spring 2004. Samples were gently concentrated by filtration. A filter with a mesh size of 315 μm was used to remove larger particles and zooplankton. The phytoplankton containing flow through was concentrated on an 11 μm filter. This was rinsed with 60 ml filtered seawater and residual herbivores were removed by pipetting. For cell counting, 51 ml of the initially collected seawater samples were filled into a graduated cylinder. After addition of a few drops of Lugol solution (10 g KI and 5 g I_2 in 100 ml water) the cylinder was covered and the samples were left undisturbed for 2 days before evaluation by counting sedimented cells on the grid [25].

2.4. Derivatisation and standards

Freshly sampled and prepared phytoplankton samples or cultivated diatoms were further concentrated onto a GF/C filter with a diameter of 21 or 47 mm (Whatman, GB) under reduced pressure (700 mbar). The filter was rinsed with 1 ml derivatisation reagent (25 mM PFBHA·HCl in 100 mM Tris/HCl, pH 7.0) and the solution was transferred to a 4 ml vial, which can be sealed air tight with a Teflon septum. After addition of 5 μl of internal standard (1 mM benzaldehyde in methanol), the sample was cooled to 4 °C and treated with ultra sound for 1 min (B. Braun Sonicator 1000l, Germany). The vial was sealed and incubated at room temperature for 30 min. For extraction, 0.5 ml methanol and 1 ml hexane were added [19] and the sample was vortexed for 1 min. The mixture was acidified by addition of a few drops of sulphuric acid and vortexed again. The hexane upper layer was removed by pipetting, dried over sodium sulphate and evaporated under a stream of argon. The residue was taken up in 50 μl hexane. The obtained samples can be directly used for GC/MS determination of unsaturated aldehydes with aliphatic side chains. For additional derivatisation of carboxylic groups, 10 μl MTBSTFA are added and after incubation at room temperature for 1 h, these samples can be directly analysed by GC/MS [26,27]. Derivates were identified by comparison with commercial and synthetic standards [10,28]. A one pot reductive *bis*-Wittig-olefination was applied for the synthesis of decatrienal. Therefore, propyl-1,3-*bis*-triphenylphosphonium bromide was reacted in a sequential one pot synthesis with THP-protected (*E*)-4-hydroxy-but-2-enal and propionaldehyde according to [29] the protection group of the obtained THP ether was removed using PPTS and the resulting alcohol oxidized with activated MnO_2 (Adolph, unpublished results).

2.5. Wound activation of aldehyde production and in situ derivatisation

In order to evaluate the influence of the derivatisation reagent on the enzymatic production of aldehydes and the influence of different cell damage protocols, a series of extraction protocols was tested. Three replicates of four samples of a TRR-culture (each 50 ml, 7.4×10^4 cells/ml) were filtered as described above and cell damage to activate aldehyde production [10] was performed by different approaches. Two sets of filters were rinsed either with seawater or directly with the derivatisation reagent (25 mM). After addition of the internal standard, both mixtures were treated with ultrasound. After incubation for 15 min, derivatisation reagent was added to the seawater rinsed sample (final concentration: 25 mM) and the samples were incubated at room temperature for 30 min. One further sample was rinsed with derivatisation reagent and frozen to stimulate aldehyde production [15]. After thawing, the sample was incubated at room temperature for 30 min. One filter was only rinsed with derivatisation reagent and incubated without any further treatment for 30 min.

2.6. Calibration curves and quantification

Calibration curves were determined for the major unsaturated aldehydes in the range from 0.1 up to 8.0 μM in triplicates. Therefore, different amounts of a stock solution of an aldehyde mixture (1 mM, 2E,4*E*-heptadienal, 2*E*,4*E*-octadienal and 2*E*,4*E*-decadienal, respectively, in MeOH) were added to 4 ml glass vials with 1 ml derivatisation reagent and 5 μl of internal standard (1 mM benzaldehyde in methanol). Extractions and GC EI/MS measurements were performed as described above. The calibration curves were constructed by plotting the area ratios of each analyte relative to the internal standard against the concentration of the analyte. For quantification of 2,4,7-octatrienal and 2,4,7-decatrienal, the calibration curve of the corresponding commercially available 2,4-dienals were used, which were assumed to behave similarly during derivatisation and GC/MS.

Linearity between diatom cell density and PUA-detection was verified with 50 ml of a TRR-culture, which was filtered as described above and rinsed with seawater. Samples (1 ml) of a dilution series from 10^4 to 10^6 cells/ml were directly treated with 1 ml of derivatisation reagent before sonication. The linear regression of diatom cells against aldehyde production was calculated.

2.7. Stability of the derivates

Stability of the derivates was determined by splitting a hexane extract. After immediate GC/MS analyses, one part was stored for 11 days at room temperature, the other sample was stored for the same time at −80 °C. The detected derivates in the three samples were compared.

2.8. Recovery of the derivates from phytoplankton matrices

Recovery of the derivates was determined by analysing triplicates of a phytoplankton sample spiked with the aldehyde mixture (see Section 2.6) at a final concentration of 1 μM. The measured concentrations were corrected by the natural amount of these aldehydes in the same sample. For comparison, identical amounts of aldehydes were directly derivatised.

3. Results and discussion

3.1. Derivatisation and cell damage procedures

A strain of the diatom *T. rotula* which produces 2,4-octadienal and 2,4,7-decatrienal as dominant PUA besides minor amounts of 2,4-hepatdienal, 2,4,7-octatrienal and 2,4-decadienal [7] was used for method development. From earlier work, it is known that *T. rotula* produces PUA only after cell damage [10]. In order to determine this PUA-production upon cell disruption, we have conducted a se-

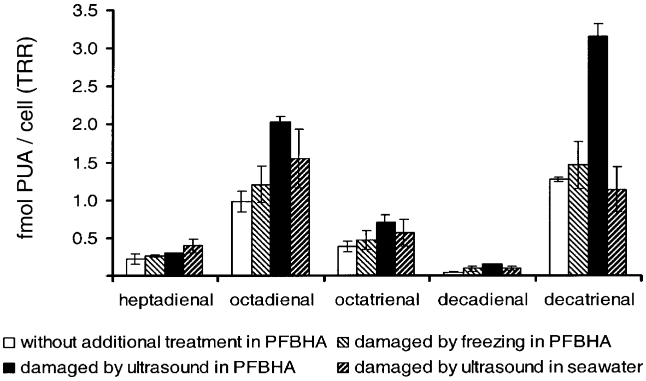

Fig. 1. Validation of different cell damage treatments. The sum of isomers are given, error bars are based on S.D. ($n = 3$). Open bars: addition of derivatisation reagent to filtered diatoms; white hatched bars: addition of derivatisation reagent to filtered diatoms with subsequent freezing and thawing; black bars: addition of derivatisation reagent to filtered diatoms with subsequent ultrasound treatment; black hatched bars: derivatisation reagent added 15 min after sonication of filtered diatom cells in seawater.

ries of different cell damage and derivatisation procedures (Fig. 1). First, we determined PUA-release by damaging the cells with ultrasound treatment in seawater before addition of the derivatisation reagent. This showed clearly that the released aldehydes can be derivatised in the seawater matrix. The yields of oxime derivates increased significantly if sonication was performed in the presence of the derivatisation reagent (Fig. 1). The increased amount of PUA-derivatives detected in these experiments demonstrates that PFBHA·HCl in Tris/HCl does not inhibit the involved enzymatic activities. Therefore, the in situ-treatment with PFBHA·HCl allows the direct trapping of the products released by a phospholipase/lipoxygenase/hydroperoxide lyase enzymatic cascade. The lower yields obtained from cells damaged before derivatisation compared to those from cells damaged in the presence of the derivatisation reagent can be attributed to the high reactivity of PUA. These aldehydes might form covalent adducts to other cell constituents like DNA or proteins [19,30] if the trapping reagent PFBHA·HCl is not present. Since even simple treatment of filtered samples with the derivatisation reagent leads to detectable amounts of PUA, it can be concluded that the osmotic stress to the cells caused by the assay reagent already initiates the formation of the aldehydes. The cell damage caused by shock freezing in liquid nitrogen and thawing of cells embedded in derivatisation reagent is convenient, but the yield of oxime derivates reaches only 55% of the total amount of PUA detected after ultrasound treatment in derivatisation reagent. To obtain best yields, we suggest to perform cell damage preferably by ultrasound directly in the derivatisation reagent under near physiological conditions at pH 7.

For development of GC/MS methods for the detection of oxo-acids, we used the 12-ODTE producing diatom *A. formosa*. Additional derivatisation of the acidic head group is required to render the analytes volatile and suitable for GC/MS analysis. For safe use in field studies, we suggest the use of the silylation reagent MTBSTFA, which is less toxic, compared to known methylation reagents. The oxo-acid containing samples obtained by the filtration/derivatisation/extraction approach as described above can be silylated and used directly for GC/MS. The silylated acids bear the additional advantage over methylated acids that the ion with a mass of M-57 ($(H_3C)_3C^\bullet$) can be used as analytical fragment [27].

3.2. Application in field studies and recovery of PFB-oxime derivates

During field studies the derivatisation and extraction has to be conducted under standard laboratory conditions following a simple and reproducible protocol. The applied methods should enable a safe handling without additional security precautions, which is fulfilled for the PFBO-derivatisation and the silylation. To monitor the stability of the oxime derivates we compared the detectable derivatives directly after sample preparation with those from samples that have been stored at room temperature or frozen. The derivates proved to be relatively stable (80–90% recovery after 11 days) at room temperature, which is essential for temporally not temperature-controlled storage during a field trip. Moreover, the oxime derivates did not undergo degradation when stored at −80 °C.

Due to the possible reactions of PUA with other components in the phytoplankton extract, the recovery from this matrix was determined. Therefore, defined amounts of PUA were added after filtration to phytoplankton samples. The recovery for 2,4-hepatdienal, 2,4-octadienal and 2,4-decadienal was in the range from 43 to 62%. The loss of detected PUA is probably due to binding of these reactive 2,4-dienals to nucleophiles like amino acid residues of proteins [19] or DNA [30] in the plankton sample.

3.3. GC/MS analysis of aldehyde-PFB-oxime derivatives

In order to validate the derivatisation procedure, we have chosen two important bloom forming marine diatom species *T. rotula* and *S. costatum* as well as the fresh water diatom *A. formosa*. Furthermore, we have investigated *T. pseudonana*, the first marine algae with a complete sequenced genome. *P. minimum* (PM), a dinoflagellate which does not produce any unsaturated aldehydes, served as a negative control [7]. Aldehyde-PFBO-derivates of a natural phytoplankton population, dominated by diatoms during the spring bloom in the coastal waters off Roscoff (Brittany, France) was investigated as well. In this sample, about 5500 *Thalassiosira* cells/l seawater were present and the total diatom cell count was 1.5×10^4 cells/l (Fig. 2A). The ion trace chromatograms of *m/z* 181 (GC EI/MS) can be used for the detection of all derivatised aldehydes in this study (Fig. 2B–E). Interestingly, the diatom TPA does not produce any of the hitherto known diatom-PUA upon cell disruption, and besides an unidentified carbonyl, GC/MS profiles were comparable to those from the negative control PM. With the cultivated diatoms TRR and SC, we could identify and quantify all PUA described in two

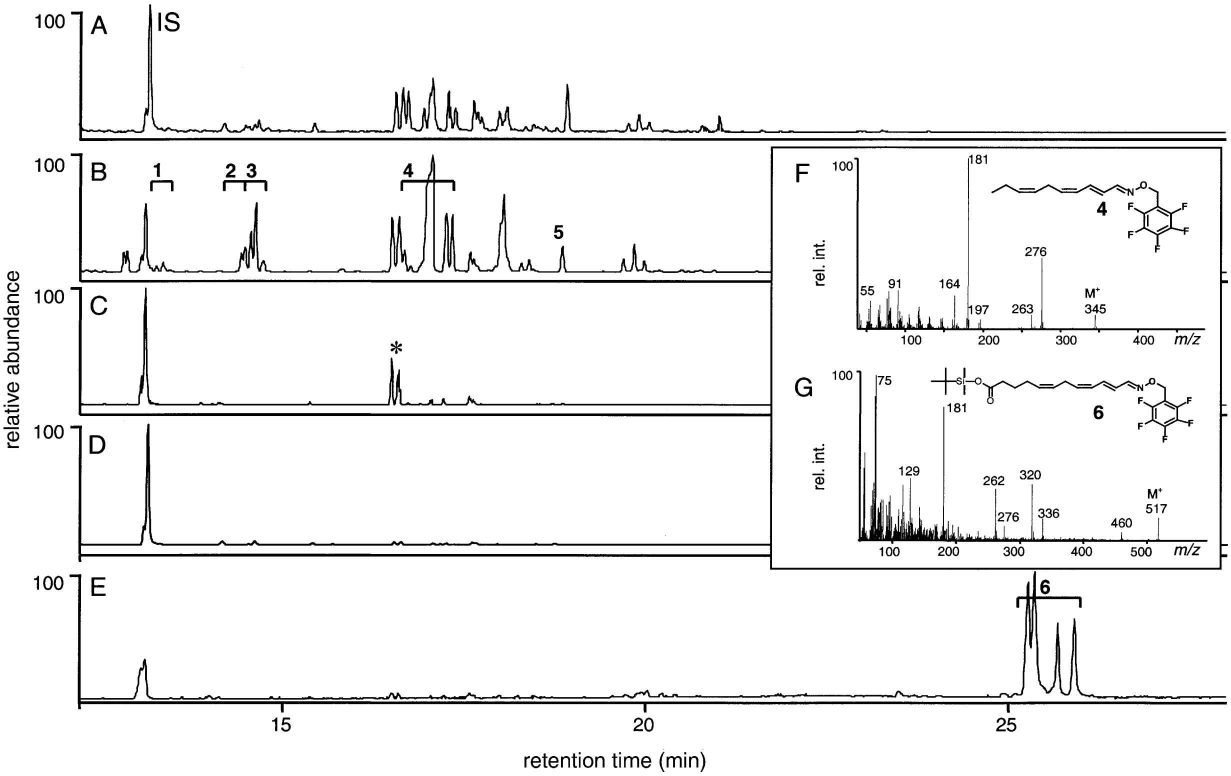

Fig. 2. Ion trace chromatograms (*m/z* 181) of phytoplankton and culture extracts: (A) phytoplankton; (B) *Thalassiosira rotula*; (C) *Thalassiosira pseudonana*; (D) *Prorocentrum minimum*; (E) *Asterionella formosa*. The numbers 1–6 correspond to the oxime derivates of the identified substances: (1) 2,4-heptadienal; (2) 2,4,7-octatrienal; (3) 2,4-octadienal; (4) 2,4,7-decatrienal and 2,4-decadienal; (5) tridecanal and (6) 12-ODTE. The insets (F and G) show mass spectra of the peak at 17.33 (4) and 25.90 (6). Analytical fragments for the PFBO-derivatives are 181 and 276 as well as the molecular ion (F and G). *tert*-Butyl-dimethylsilyl derivates prepared from acidic aldehydes show the additional characteristic fragment (M-57) due to loss of $(H_3C)_3C^\bullet$ (G); (*) unidentified oximes.

previous studies [7,8] as oxime derivates. We also found the derivatised saturated longer chain aldehyde tridecanal (detectable by $M–OH^\bullet$ and by the McLafferty fragment *m/z* 239) in phytoplankton and diatom cultures. Besides AF, neither the phytoplankton nor the other cultured species contained detectable amounts of oxo-acids.

Commercial available compounds were used to identify most of the derivates. Mass spectra of 2,4,7-decatrienal and 12-ODTE are given in Fig. 2F and G, the identity of these compounds has been proven by comparison with synthetic standards. Most of the derivates cause two or more peaks due to the syn- and anti-stereoisomers of the oximes [20]. Chromatograms are further complicated since diatoms release 2*E*, 4*E*/*Z* isomeric mixtures of α,β,γ,δ-unsaturated aldehydes [3]. Nevertheless, groups of signals, which are caused from the respective isomers of the derivatives, can be unambiguously defined by their molecular ions and fragmentation patterns. Molecular ions were also used for the quantification of the oxime derivates (Fig. 3). Standard curves were constructed for 2*E*, 4*E*-heptadienal, 2*E*,4*E*-octadienal and 2*E*,4*E*-decadienal, respectively (slopes/S.D.: 0.103 ± 0.016, $r > 0.989$; 0.120 ± 0.007, $r > 0.994$; 0.119 ± 0.011, $r > 0.991$). Linearity range of calibration curves was from 0.1 to 8.0 μM, which makes the method suitable for the quantification of PUA in phytoplankton samples as well as in few ml of laboratory cultures. Linearity was also proven between 2,4-octadienal and 2,4,7-decatrienal formation and the cell number of TRR in the range of 5×10^4 to 2.7×10^6 diatom cells (slope/S.D.: 0.117 ± 0.004, $r > 0.993$ and 0.099 ± 0.012, $r > 0.992$). The limit of quantification for the standard was 11 ng/ml medium, corresponding to less than 2000 cells of aldehyde-producing-species in 1 l seawater before filtration. For comparison, the phytoplankton blooms in French Brittany coastal waters reach 10^5 to 10^6 diatom cells per litre of seawater [31]. The detection limit of this assay can be increased by negative ion chemical ionisation electron capture mass spectrometry and detection of the $(M–HF–NO)^{\bullet-}$ ion down to 4.4 pg on column. This corresponds to less than 10 PUA-producing cells in 1 l seawater before filtration.

3.4. Quantification in cultures and phytoplankton samples

For interpretation of bioassays on the deleterious effect of diatoms on their grazers, it is essential to quantify the potential aldehyde production in the algal diets. We performed quantification in stationary phase cultures of TRR, SC and in a diatom dominated phytoplankton sample. The determined amounts of PUA from TRR fit previous reported values ob-

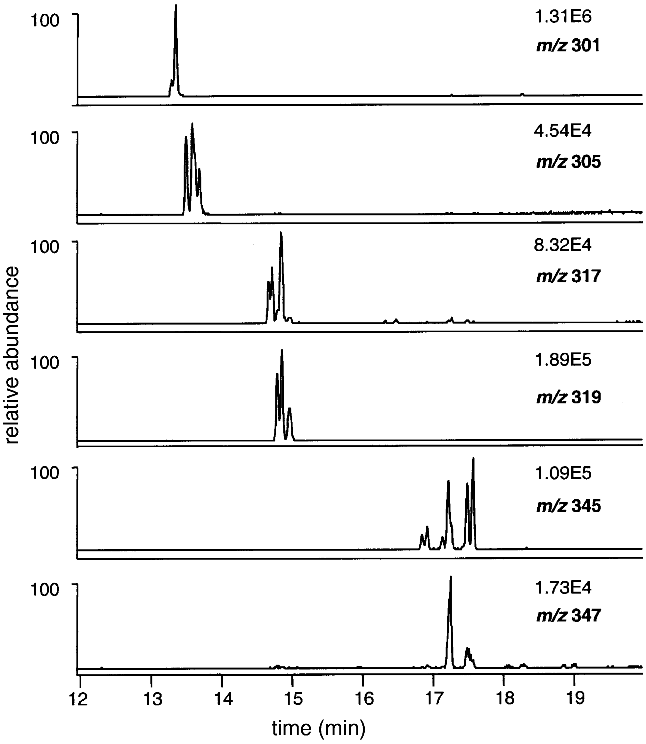

Fig. 3. Molecular ion trace chromatogram of derivatised TRR-culture extracts used for quantification. From top to bottom: the ion trace chromatograms of benzaldehyde, 2,4-heptadienal, 2,4,7-octatrienal, 2,4-octadienal, 2,4,7-decatrienal and 2,4-decadienal are shown.

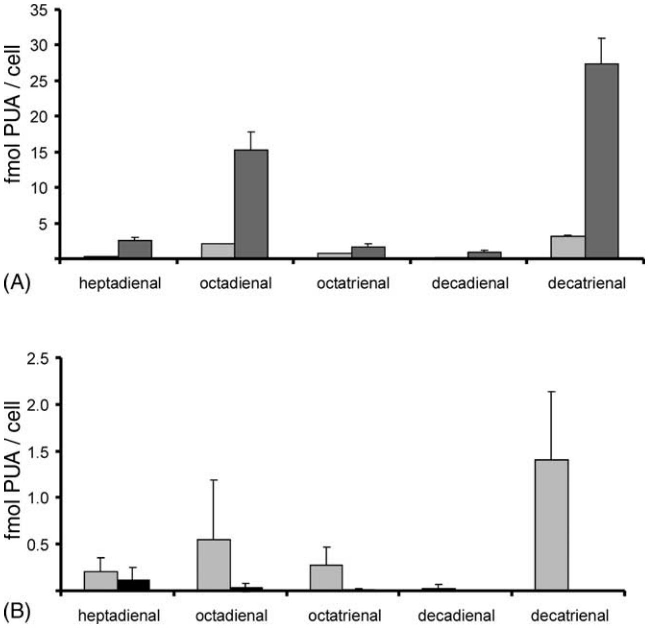

Fig. 4. Quantification of PUA in diatom cultures and phytoplankton samples. Repeated derivatisation of (A) the same TRR-culture (lighter grey, $n = 3$) and phytoplankton sample (dark grey, $n = 3$); (B) different cultures TRR (lighter grey, $n = 10$) and SC (black, $n = 4$). The error bars are based on S.D.

tained by solid phase microextraction [7]. TRR produces major amounts of 2,4-octadienal and 2,4,7-decatrienal and minor amounts of 2,4-hepatdienal, 2,4,7-octatrienal and 2,4-decadienal upon cell damage, whereas SC only forms major amounts of 2,4-hepatdienal and 2,4-octadienal [8] (Fig. 4). For estimation of the deleterious potential of a phytoplankton population, we calculate the sum of all α,β,γ,δ-unsaturated aldehydes, thus including all compounds in the sample containing the structural motive, which can be made responsible for antiproliferative effects [28]. The determination of PUA in triplicates of the same TRR-culture shows a low standard deviation and high reproducibility (Fig. 4A). The PUA-quantification from different cultures of the same strain show a higher standard deviation probably due to the varying ability of PUA-production depending on the age of the culture and to errors related to cell counts (Fig. 4B). Interestingly, the natural phytoplankton is a stronger producer than the cultivated TRR (total amount of PUA/S.D.; natural phytoplankton samples: 47.7 ± 5.9 fmol/cell, $n = 3$, compared to TRR: 6.35 ± 0.29 fmol/cell, $n = 3$). This might be due to optimum growth conditions of the field population, the bigger cell size in natural samples or increased intrinsic toxicity of field diatoms compared to the same species cultured in the laboratory. The pattern of aldehyde production formed by the diatom-dominated phytoplankton resembles that obtained from the TRR-culture. It is most likely caused by *Thalassiosira* spp. dominating the natural phytoplankton population (∼35% of total diatom population) in the coastal area of Roscoff (Brittany) during the sampling period in April 2004.

4. Conclusion

We have applied a simple and sensitive approach for the quantification of deleterious aldehydes in marine phytoplankton matrices using standard GC/MS equipment. The method provides an excellent tool for field studies addressing the interaction of phytoplankton and the planktonic grazers. Since PUA are also considered as off flavours, monitoring of PUA-content in drinking water using the introduced method is a potential application as well [32]. Moreover, PFBO-based protocols can also be used for the determination of saturated aldehydes in drinking water samples [33] and thus different analytes might be surveyed using a single protocol. The method is fast and allows the screening for PUA and oxo-acids in algae collections as well as the monitoring during different growth phases of diatoms. The high sensitivity of GC EI/MS allows monitoring of the production of PUA from less than 2000 cells/l seawater following a well-established filtration procedure. The detection limit can be increased by more than two orders of magnitude if negative ion CI/EC/MS equipment is available. Derivatisation with PFBHA·HCl and MTBSTFA enables to detect both, the volatile PUA, and the acidic aldehydes harbouring a Michael-acceptor structure element. The aliphatic α,β,γ,δ-unsaturated aldehydes can be identified by three characteristic peaks in EI/MS (*m*/*z* 181, *m*/*z* 276 and the molecular ion), whereas oxo-acids are easily detectable by the fragments *m*/*z* 181 and (M-57). This approach is now routinely used for the determination of the toxic potential of diatom populations in Roscoff.

Acknowledgements

We are grateful to the sailors at the Roscoff Marine Station for providing the phytoplankton samples. Sven Adolph is acknowledged for synthetic standards. The authors acknowledge the financial funding of the Deutsche Forschungsgemeinschaft (TW, GP), DAAD–PROCOPE and funding from the French Institute of Biodiversity (SP). Prof W. Boland is acknowledged for helpful support and discussion during the preparation of this manuscript.

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