Determination of Aldehydes in Diatoms by Headspace Solid-Phase Microextraction Coupled with GC–MS

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Abstract

Reactive unsaturated aldehydes produced by marine diatoms upon cell damaged interfere negatively with the reproduction success of their grazers. In this paper, a method based on headspace solid-phase microextraction (HS–SPME) coupled with gas chromatography–mass spectrometry (GC–MS) was developed for analysis of six aldehydes: [(E)-2-hexenal, heptanal, (E)-2-heptenal, (E,E)-2,4-heptadienal, (E)-2-decenal, (E,E)-2,4-decadienal] in two laboratory cultured diatoms (*Skeletonema costatum* and *Chaetoceros Muelleri*). HS–SPME experimental conditions including SPME fiber types, extraction time and temperature, desorption time and temperature were optimized. Under the optimal experimental conditions, limits of detection for six aldehydes were both in the range of 0.1–2 µg/L. The relative standard deviations ranged from 7.2 to 13.9%. Recoveries were in the range of 60.6–85.7%. The concentrations of six aldehydes in *Skeletonema costatum* were 0.359, 0.038, 0.028, 0.183, 0.023, and 0.058 ng/10^6 cells respectively. The six aldehydes were not found in Chaetoceros Muelleri. The study shown here provided a simple, fast and sensitive method for the analysis of aldehydes in diatoms.

Introduction

In recent years, a chemical defense relationship mediating the interaction of diatoms and their grazers came into the focus of ecologists and chemists (1). It was reported that some diatoms were able to form 2*E*, 4*E*/*Z* isomeric mixtures of α, β, γ, δ-unsaturated aldehydes upon cell damage (2,3). Those polyunsaturated aldehydes were produced by the enzymatic degradation of polyunsaturated fatty acids which was activated soon after cell membrane disruption (2,4), which 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 (5). A. Miralto et al. (1) reported that (E,E)-2,4-decadienal or (E,Z)-2,4-decadienal and (E,E,Z)-2,4,7-decatrienal or (E,Z,Z)-2,4,7-decatrienal from the marine diatom *Thalassiosira rotula* had the insidious effect of diatoms on copepod reproduction. In addition, (E,E)-2,4-decadienal, (E,E,E)-2,4,7-decatrienal, (E,E)-2,4-octadienal, (E,E,E)-2,4,7-octatrienal and (E,E)-2,4-heptadienal had been found in the marine diatoms *Thalassiosira rotula* (3), *Skeletonema costatum* (2), and *Skeletonema pseudocostatum* (2,6).

Because the low amounts of aldehydes present in cells were very unstable and volatile, a simple, fast, and sensitive method for analysis of aldehydes in diatoms needs to be developed. Thomas Wicharda et al. (7) had analyzed aldehydes in cultured diatoms and natural phytoplankton by in situ derivatisation with *o*-(2, 3, 4, 5, 6-pentafluorobenzyl) hydroxylamine hydrochloride (PFBHA) couple with gas chromatography-mass spectrometry (GC–MS). Espen Hansen et al. (8) extracted and purified polyunsaturated aldehydes from the marine phytoplankter *Phaeocystis pouchetii* with high-performance liquid chromatography (HPLC) and analyzed with GC–MS. Solid-phase microextraction (SPME) presents many advantages over conventional analytical methods by combining sampling, preconcentration, and direct transfer of the analytes into a standard gas chromatograph (9–11). Moreover, HS–SPME could eliminate the interference of complex sample matrix. Sampling and analysis method for aldehydes in air which combined PFBHA with SPME technique have been reported (12–14). Aldehydes in water sample derivatized with PFBHA to form oximes in solutions followed by extraction with SPME from liquid or headspace were also reported (15–18).

In the present work, without derivatization, headspace solid-phase microextraction (HS–SPME) coupled with GC–MS was developed for determination of six aldehydes [(E)-2-hexenal, heptanal, (E)-2-heptenal, (E,E)-2,4-heptadienal, (E)-2-decenal, (E,E)-2,4-decadienal] in laboratory cultured *Skeletonema costatum* and *Chaetoceros muelleri*. Some important factors affecting extraction efficiency (extraction temperature and time, desorption temperature, and time) were investigated. The method was successfully applied to determination of six aldehydes in diatoms cells.

Experimental

Reagents and SPME fibers

(E)-2-hexenal (99%), (E,E)-2,4-heptadienal (94%), (E)-2-decenal (95%), and (E,E)-2,4-decadienal (95%) were purchased from Tokyo Chemical Industry Co., Ltd. Heptanal (1000 mg/L in methanol) and (E)-2-heptenal (1000 mg/L in AcCN) were purchased from Accustandard (New Haven, CT). HPLC grade methanol was purchased from SK Chemical (Ulsan, Korea). A model Synergy 185 ultrapure water system (Millipore, Billerica,

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MA) was used to purify water. Individual stock standard solutions for each aldehyde were prepared in HPLC grade methanol at a concentration of 100 µg/L and stored at 4°C. A working standard solution containing each aldehyde at a concentration of 10 µg/L was prepared from the stock standards by appropriate dilution with a 3.5% sodium chloride solution. SPME manual holder, operation plate which allowed temperature and stirring control, the fibers of 65 µm polydimethylsiloxane–divinylbenzene (PDMS–DVB), 100 µm polydimethylsiloxane (PDMS) and 75 µm carbowax/polydimethylsiloxane (CAR–PDMB) were purchased from Supelco (St. Louis, MO).

GC–MS analysis

A Finnigan Trace GC–MS (Thermo Finnigan, San Jose, CA) equipped with a 30-m DB-5 capillary column (0.25 mm i.d., 0.25-µm film thickness, J&W Scientific, Santa Clara, CA) was used for EI-MS measurements. The inlet temperature was maintained at 230°C. The type of injection was splitless. The purge vent was opened after injecting sample 0.75 min at the flow rate of 50 mL/min. The column oven was held at 45°C for 2 min, programmed from 45°C to 100°C at 5°C/min and then to 240°C at 10°C/min. Helium was used as carrier gas at a constant flow of 1.0 mL/min and the transfer capillary was held at 250°C. Ionization energy was 70 eV with the ion source at 230°C. A mass range of *m/z* 35–350 was recorded in the full-scan mode. Peak identification of aldehydes were based on the retention times and full scan spectra of the standards. For better sensitivity, selected ion monitoring (SIM) was used to quantify the aldehydes. The retention time of each aldehyde and the quantization ions were obtained under the optimized GC–MS conditions. The characteristic ions of each aldehyde were at *m/z*: (E)-2-hexenal 41, 55, 83; heptanal 43, 55, 70; (E)-2-heptenal 41, 55, 83; (E,E)-2,4-heptadienal 53, 83, 110; (E)-2-decenal 43, 55, 70; and (E,E)-2,4-decadienal 41, 81.

Cultivation of diatoms

Cultures of *Skeletonema costatum* and *Chaetoceros muelleri* were obtained from the Institute of Oceanology, Chinese Academy of Sciences. Two diatoms grew in standing cultures at 15°C in artificial medium (F/2). The concentration of *Skeletonema costatum* was 1.2×10^6 cells/mL, and the concentration of *Chaetoceros muelleri* was $1.08 ¥ 10^5$ cells/mL. Illumination was provided on a 12:12 light: dark rhythm at 5000 lux. Cells were counted with the Leica microscope (Wetzlar, Germany). The 500 mL diatoms solution was concentrated by centrifuge, then removed the supernatant. The centrifugation sediment (diatoms) was diluted with 3 mL culture solution, and then removed it to 20 mL PTFE-capped vials for HS–SPME analysis.

HS–SPME procedure of aldehydes in diatoms

Because the diatoms samples could easily contaminate and damage the fiber if the solution contacted the fiber, headspace extraction was used instead of immersion. The SPME fiber was inserted in the headspace of the diatom sample solutions in 20 mL PTFE-capped vials with 1 cm stir bar to extract aldehydes. To allow the equilibrium of aldehydes between the aqueous phase and the headspace phase, the samples were stirred at 560 rpm for 5 min before extraction. The diatom sample solutions were treated in ultrasonic cleaner (KH-3200B, Kunshan, China) for 30 s before headspace extraction, which simulated diatom cells damage by its grazer. Headspace SPME conditions: PDMS–DVB fiber; stirring ratio 560 rpm; extraction at 75°C for 15 min. After aldehydes were extracted, fiber was inserted into the GC inlet at 230°C for 5 min for desorption.

Results and Discussion

Choice of optimal HS–SPME conditions

Before analyzing the real samples, a spiked solution at the concentration of 20 µg/L aldehydes dissolved in 3.5% sodium chloride solution (the average sea water salinity, sodium chloride dissolved in ultrapure water) was used to investigate the optimal conditions of HS–SPME. Some important factors affecting the efficiency of HS–SPME including type of SPME fibers, adsorption time and temperature, desorption time, and temperature have been investigated.

Effect of SPME fibers

The type of SPME fiber was one of the most important factors of affecting the extraction efficiency of HS–SPME. In this paper, three different SPME fibers (65 µm PDMS–DVB, 100 µm PDMS and 75 µm CAR–PDMB fiber) were used to investigate the extraction efficiencies of aldehydes. The spiked aldehyde solution was extracted at 75°C for 15 min at the stirring ratio of 560 rpm. The desorption was operated at 230°C for 5 min. Chromatographic peak areas of aldehydes obtained from different SPME fibers are shown in Figure 1. The peak areas of six aldehydes by PDMS–DVB fiber were larger than that of PDMS and CAR–PDMB fiber. It is because aldehydes are polar or weak polar volatile organic compounds, and PDMS–DVB fiber is suitable for extracting polarity volatile compounds. So PDMS–DVB fiber was selected for the next experiments. For CAR–PDMB fiber, these compounds desorbed incompletely at less than 230°C and could dramatically promote if desorption temperature is higher than approximately 280°C. However, aldehydes may breakdown at high temperature. So, desorption temperature for CAR–PDMB fiber was not optimized in this research.

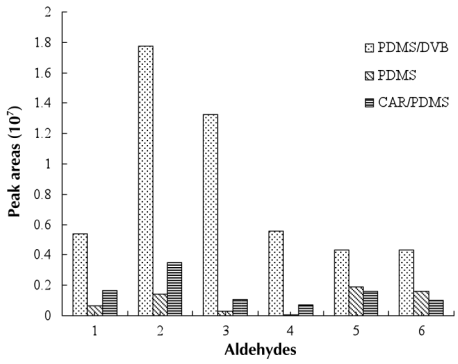

Figure 1. Peak areas using different SPME fibers: 1, (E)-2-hexenal; 2, heptanal; 3, (E)-2-heptenal; 4, (E,E)-2,4-heptadienal; 5, (E)-2-decenal; 6, (E,E)-2,4-decadienal. Conditions: 75°C for 15 min at the stirring ratio of 560 rpm for headspace extraction; desorption was at 230°C for 5 min.

Effect of extraction time and temperature

Extraction time and temperature could affect the extraction efficiency. So the choice of optimal time and temperature should be taken into account. The extraction temperature was kept at 35°C. The extraction time ranged from 5 min to 35 min. The results are shown in Figure 2. It was found that for the three aldehydes [(E,E)-2,4-heptadienal, (E)-2-decenal, and (E,E)-2,4-decadienal] the peak area did not increase after 15 min, and the extraction reached equilibrium. However, the peak areas of the other three aldehydes [heptanal, (E)-2-heptenal, and (E)-2-hexenal] increased with the increase of extraction time, and the extraction equilibrium could not be found. So the extraction temperature was increased to 75°C. Then it was found that the extraction equilibrium for heptanal, (E)-2-heptenal and (E)-2-hexenal after 15 min, and the peak areas were also higher than that at 35°C (Figure 3). According the results, extraction between temperature of 35°C and 75°C may be appropriate. Considering that lower temperatures need longer extraction time, the extraction of aldehydes was operated at 75°C for 15 min in the next experiment.

Effect of desorption time and temperature

Inadequate time and low temperature could lead to incomplete desorption of the aldehydes on fiber, and the carryover effects also disturbed next analysis. If the desorption temperature is too high and the time too long, the aldehydes might decompose. Appropriate desorption temperature and time should be determined. The peak areas were shown in Figure 4 when the desorption temperature was kept at 230°C, and desorption time was 2.5, 4, 5, and 7 min. Figure 4 shows that the highest peak area can be obtained for the six aldehydes when desorption was at 230°C for 5 min.

Linear ranges and limits of detection

Seven standard solutions at different concentrations (1, 2.5, 5, 10, 25, 50, and 100 µg/L for each aldehyde) were obtained by dilution of stock standard solution with blank cultured sea water. Under the optimal conditions mentioned above, calibration curves were plotted. The linear ranges and the correlation coefficients (r^2) obtained for each compound are shown in Table I. Linear correlation coefficients (r^2) varied between 0.9855 and 0.9993 for the aldehydes. Limits of detection (LODs) based on 3N/S for six aldehydes were in the range of 0.1–2 µg/L.

Analytical performance and application

Analytical performance of the proposed method was assessed by precision (RSD%) and accuracy (recovery) with diatom sample solutions spiked with aldehydes (final concentration of 10 µg/L). For *Skeletonema costatum,* the RSD of aldehydes ranged from 7.2% to 13.9%, and recoveries were in the range of 63.5–85.7%. For *Chaetoceros Muelleri,* RSD of aldehydes ranged from 9.8% to 13.7% and recoveries were in the range of 60.6–81.5% (Table II).

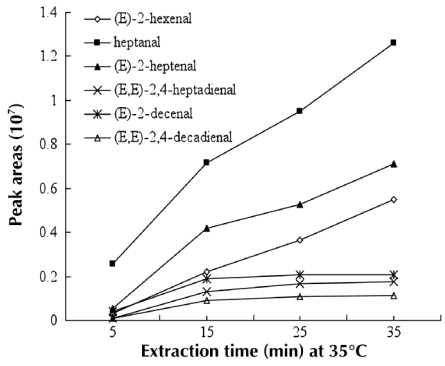

Figure 2. Peak area vs. extraction time for the six aldehydes at 35°C. Conditions: PDMS–DVB fiber, extraction temperature 35°C, desorption temperature 230°C for 5 min. Other conditions are the same as Figure 1.

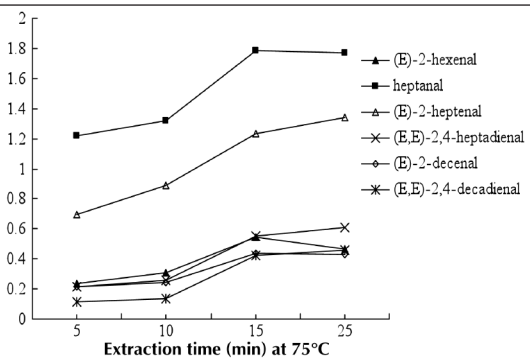

Figure 3. Peak area vs. extraction time for the six aldehydes at 75°C. Conditions: PDMS–DVB fiber, extraction temperature 75°C, desorption temperature 230°C for 5 min. Other conditions are the same as Figure 1.

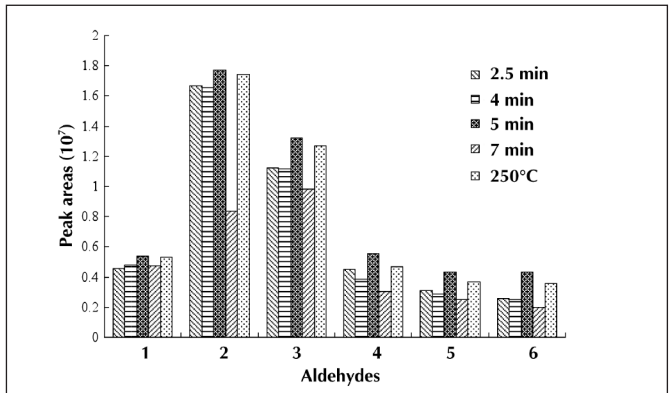

Figure 4. Peak area of the six aldehydes for different desorption times. Conditions: PDMS–DVB fiber, extraction at 75°C for 15 min, desorption temperature 230°C. Other conditions are the same as Figure 1. 1, (E)-2-hexenal; 2, heptanal; 3, (E)-2-heptenal; 4, (E,E)-2,4-heptadienal; 5, (E)-2-decenal; and 6, (E,E)-2,4-decadienal.

Table I. Linear Range and Detection Limits for the Six Aldehydes

Aldehydes	Retention time (min)	Regression equation*	Correlation coefficient	Linear range (µg/L)	LODs (µgL)
2E-hexenal	3.55	y = 81684x + 105228	0.9953	2.5–50	0.7
heptanal	5.09	y = 381421x + 508030	0.9943	1–50	0.2
2E-heptenal	7.55	y = 345840x – 206731	0.9993	1–50	0.3
2E,4E-heptadienal	9.74	y = 156974x – 526851	0.9855	5–100	2.0
2E-decenal	16.87	y = 500145x + 323624	0.9972	1–50	0.1
2E,4E-decadienal	17.89	y = 321635x – 550327	0.9922	2.5–50	0.

*x = concentration (µg/L); y = area.

The proposed analytical method has been applied to the analysis of six aldehydes in *Skeletonema costatum* and *Chaetoceros muelleri*. The aldehydes were found in *Skeletonema costatum*, while not found in *Chaetoceros muelleri*. The concentrations of six aldehydes [(E)-2-hexenal, heptanal, (E)-2-heptenal, (E,E)-2,4-heptadienal, (E)-2-decenal, (E,E)-2,4-decadienal] in *Skeletonema costatum* were: 0.359, 0.038, 0.028, 0.183, 0.023, and 0.058 ng/10^6 cells respectively. The SIM chromatogram of *Skeletonema costatum* is given in Figure 5.

Conclusions

In this paper, a simple, fast, and sensitive approach for the quantitation of six aldehydes in the cultured diatoms was developed by HS–SPME coupling with GC–MS. Profiles of headspace extraction aldehydes procedure had been optimized. This method was successfully applied in determination of six aldehydes in *Skeletonema costatum* and *Chaetoceros muelleri*. Aldehydes were detected in *Skeletonema costatum*, but in *Chaetoceros muelleri* no aldehyde was detected. Among the six aldehydes, one small molecule unsaturated aldehyde [(E)-2-hexenal] and a saturated aldehyde (heptanal) were firstly found and studied in the two diatoms. To discover whether they have negative influence on diatom grazers or not requires further research.

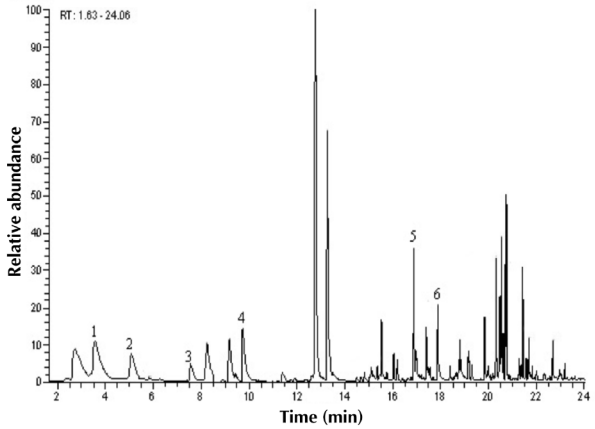

Figure 5. Chromatogram of *Skeletonema costatum* sample spiked with aldehydes (final concentration of 10 µg/L). in selected ion monitoring (SIM) mode. Peak identified: 1, (E)-2-hexenal; 2, heptanal; 3, (E)-2-heptenal; 4, (E,E)-2,4-heptadienal; 5, (E)-2-decenal; and 6, (E,E)-2,4-decadienal.

Table II. Detection Results and Recoveries of Six Aldehydes in Skeletonema Costatum and Chaetoceros Muelleri (*n* = 5)

	Skeletonema costatum			*Chaetoceros Muelleri*		
Aldehydes	Conc. (µg/L)	Recovery (%)	RSD (%)	Conc. (µg/L)	Recovery (%)	RSD (%)
2E-Hexenal	6.83	68.3	10.3	6.67	66.7	11.8
Heptanal	7.11	71.1	13.9	7.43	74.3	16.6
2E-Heptenal	7.33	73.3	10.6	6.88	68.8	10.1
2E,4E-Heptadienal	6.35	63.5	15.7	6.06	60.6	10.9
2E-Decenal	8.57	85.7	7.2	8.15	81.5	13.7
2E,4E-Decadienal	6.43	64.3	9.2	6.75	67.5	9.8

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