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Research Article

Headspace solid-phase microextraction with on-fiber derivatization for the determination of aldehydes in algae by gas chromatography–mass spectrometry

A simple, fast, sensitive and cost-effective method based on headspace solid-phase microextraction (HS-SPME) with on-fiber derivatization coupled with gas chromatography–mass spectrometry was developed for the determination of six typical aldehydes, 2*E*-hexenal, heptanal, 2*E*-heptenal, 2*E*,4*E*-heptadienal, 2*E*-decenal and 2*E*,4*E*-decadienal in laboratory algae cultures. As derivatization reagent, *O*-2,3,4,5,6-(pentafluorobenzyl) hydroxylamine hydrochloride, was loaded onto the poly(dimethylsiloxane)/divinylbenzene fiber for aldehydes on-fiber derivatization prior to HS-SPME. Various influence factors of extraction efficiency were systematically investigated. Under optimized extraction conditions, excellent method performances for all the six aldehydes were attained, such as satisfactory extraction recoveries ranging from 67.1 to 117%, with the precision (relative standard deviation) within 5.3–11.1%, and low detection limits in the range of 0.026–0.044 μg/L. The validated method was successfully applied for the analysis of the aldehydes in two diatoms (*Skeletonema costatum* and *Chaetoceros muelleri*), two pyrrophytas (*Prorocentrum micans* and *Scrippsiella trochoidea*) and *Calanus sinicus* eggs (feeding on the two diatoms above).

Keywords: Aldehyde / Alga / Derivatization / GC-MS / HS-SPME
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1 Introduction

Aldehydes receive an increasingly considerable concern due to their potential adverse health effects and environmental prevalence [1–6]. Various aldehydes can be recognized as biomarkers of cancer diseases [7]; as a result, the analysis of aldehydes in biological samples (mainly blood) has obtained much attention due to their directly being related to the internal activities [7–9]. A number of studies have focused on the presence of aldehydes (especially unsaturated ones) produced by algae (mainly diatoms) upon cell damage, which seriously disrupt the reproductive systems of their grazers [10]. A negative influence of diatom-derived unsaturated aldehydes on the reproductive success of copepods and invertebrates has been suggested [11–13]. Some diatom species were able to form 2*E*,4*E*/*Z* isomeric mixtures of unsaturated aldehydes upon cell damage [14, 15]. The compounds responsible for abortive and teratogenic effects in copepods were identified in the diatom *Thalassiosira rotula* as a mixture of polyunsaturated C10 aldehydes [16]. 2,4-Decadienal, 2,4,7-decatrienal, 2,4-octadienal, 2,4,7-octatrienal and 2,4-heptadienal have been found in the marine diatoms such as *T. rotula*, *Skeletonema costatum* and *Skeletonema pseudocostatum* [17]. The analysis of aldehydes can provide useful information about diatom states. Therefore, because of the central importance of diatoms as the dominant primary producers and key players in the marine food web [18], the determination of aldehydes in the diatoms expects to an alternative and assistant approach in the early monitoring of eco-environmental problems of the marine and coastal zone.

The most widely employed methods for aldehyde determination include gas chromatography–mass spectrometry (GC-MS) [7–10, 15, 19], high-performance liquid chromatography (HPLC) [6] and spectrophotometry [20]. However, generally, the instrumental sensitivity and selectivity are insufficient for the trace determination of aldehydes in diverse samples without enrichment procedures.

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Abbreviations: **DVB**, divinylbenzene; **GC-MS**, gas chromatography–mass spectrometry; **HS-SDME**, headspace single-drop microextraction; **PFBHA**, *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine; **SPME**, solid-phase microextraction

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Direct headspace extraction [21] and solid-phase microextraction (SPME) [22, 23] were previously applied to diatom cultures for the determination of the aldehydes. Headspace extraction can avoid possible contamination and damage to the fiber that might occur through direct liquid contact. And SPME presents many advantages over conventional sample pretreatment methods by combining sampling, preconcentration and direct transfer of the analytes into a standard gas chromatograph. However, it is still difficult to accurately measure these aldehydes by using the previous methods if without significant concentration efforts, since they are high volatile and active, and typically present at trace but with hundreds of volatile compounds in complicated matrices.

Derivatization of aldehydes is required prior to analysis to solve this problem. Because of the reactivity of the carbonyl group, oximes formed by reaction with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine (PFBHA) hydrochloride, the most widely employed derivatization reagent, exhibit excellent chromatographic properties and allow selective detection with GC-MS [4, 6–10, 24–27]. Zhang's research group has made great efforts [4, 7, 8, 24, 25] via PFBHA *in situ* derivatization, such as headspace solid-phase microextraction (HS-SPME) with on-fiber derivatization for GC-MS determination of hexanal, heptanal and other aldehydes in human blood [4, 7], headspace single-drop microextraction (HS-SDME) with in-drop derivatization of aldehydes in water [8, 24], and rapid determination of C6-aldehydes in tomato plant emission by SPME with on-fiber derivatization [25]. Wichard et al. realized the determination and quantification of unsaturated aldehydes in diatom cultures and natural phytoplankton populations, by using SPME with on-fiber derivatization [10, 26]. Schmarr et al. developed HS-SPME with on-fiber derivatization method for the analysis of aldehydes in diverse matrices, such as biological samples and foodstuffs [9] and grape seed oil [27]. Besides the above PFBHA derivatization with GC-MS detection, latest in 2010, a novel derivatization reagent 2,4-dinitrophenylhydrazine (DNPH) was employed, and dispersive liquid–liquid microextraction based on solidification of floating organic droplet method (DLLME-SFO) was developed, for the HPLC-UV detection of volatile aldehyde biomarkers in human blood [6].

In the present work, PFBHA on-fiber derivatization combined with HS-SPME and GC-MS was developed for the determination of six typical aldehydes in several algae samples, including 2*E*-hexenal, heptanal, 2*E*-heptenal, 2*E*,4*E*-heptadienal, 2*E*-decenal and 2*E*,4*E*-decadienal. The aldehydes were headspace extracted and reacted with PFBHA adsorbed on the SPME fiber, and then the resultant oximes were subjected to HS-SPME, followed by desorption and analysis using GC-MS. The HS-SPME conditions were systematically optimized for excellent efficiency. The method was validated and applied for the analysis of six aldehydes in two kinds of diatoms (*S. costatum* and *Chaetoceros muelleri*) cultured in laboratory, two pyrrophytas (*Prorocentrum micans* and *Scrippsiella trochoidea*) and *Calanus sinicus* eggs (feeding on the two diatoms above).

2 Material and methods

2.1 Reagents and SPME fibers

2*E*-Hexenal (99%), 2*E*,4*E*-heptadienal (94%), 2*E*-decenal (95%) and 2*E*,4*E*-decadienal (95%) were purchased from Tokyo Chemical Industry (Japan). Individual stock standard solutions for these aldehydes were prepared in HPLC-grade methanol at a concentration of 1000 mg/L and stored at 4°C. Heptanal (1000 mg/L in methanol) and 2*E*-heptenal (1000 mg/L in acetonitrile) were purchased from Accustandard (New Haven, USA). HPLC-grade methanol was purchased from SK Chemical (UIsan, Korea). PFBHA hydrochloride (97%) was obtained from Sigma-Aldrich (Milwaukee, WI, USA).

SPME manual holder, operation plate which allowed temperature and stir control, and the fibers of 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB), 100 μm PDMS and 75 μm carbowax (CAR)/PDMS were all purchased from Supelco (St. Louis, MO, USA).

2.2 GC-MS analysis

A Finnigan Trace GC-MS (Thermo Finnigan, CA, USA) equipped with a 30-m DB-5 capillary column (0.25 mm internal diameter, 0.25 μm film thickness, J&W Scientific, USA) was used for EI-MS measurements. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The sample injection was performed in a splitless mode for 0.75 min. The injector temperature was maintained at 250°C. The column temperature was held at 60°C for 1 min, programmed from 60 to 150°C at 15°C/min and then to 230°C at 5°C/min. The transfer capillary was held at 280°C. Ionization energy was 70 eV with the ion source at 230°C. A mass range of m/z 50–500 was recorded in the full-scan mode. Peak identification of objects was based on the retention times and full-scan spectra of the standards. For better sensitivity, selected ion monitoring (SIM) mode was used to quantify the analytes. The total ion chromatogram (TIC) of six aldehyde PFBHA oximes is shown in Fig. 1. It is observed that there are *syn* and *anti* isomers of the oximes because aldehydes are asymmetrical carbonyl compounds. The sum of isomers peak areas was used for the quantification. PFBHA derivative characteristic ions were at m/z: 2*E*-hexenal, 181 and 253; heptanal, 181 and 239; 2*E*-heptenal, 181 and 250; 2*E*,4*E*-heptadienal, 181 and 276; 2*E*-decenal, 181 and 250; 2*E*,4*E*-decadienal, 181 and 276, which were all employed for quantification.

2.3 Cultivation and sampling

Cultures of *S. costatum*, *C. muelleri*, *P. micans*, *S. trochoidea* and *C. sinicus* were all obtained from the Institute of Oceanology, Chinese Academy of Sciences (China). Four algae were grown in standing cultures at 15°C in artificial

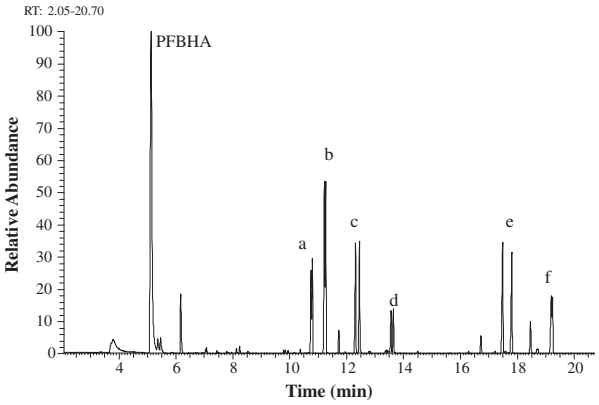

Figure 1. Total ion chromatogram (TIC) of six aldehyde PFBHA oximes for 10 μg/L solutions. Peak identification: (a) 2*E*-hexenal, 10.75 and 10.80 min, (b) heptanal, 11.22 and 11.27 min, (c) 2*E*-heptenal, 12.31 and 12.45 min, (d) 2*E*,4*E*-heptadienal, 13.56 and 13.65 min, (e) 2*E*-decenal, 17.48 and 17.81 min, (f) 2*E*,4*E*-decadienal, 19.19 and 19.24 min.

medium (F/2) to the final concentrations, namely *S. trochoidea* at 8.8×10^5 cells/mL, *P. micans* at 8.0×10^5 cells/mL, *C. muelleri* at 5.0×10^5 cells/mL and *S. costatum* at 1.2×10^6 cells/mL. Illumination was provided on a 12:12 (light/dark) rhythm at 5000 lux. Cells were counted with the Leica microscope (Germany). The alga solution of 500 mL was concentrated by centrifugation, the supernatant was removed and the concentrated liquid was remained for 3 mL, and then the liquid was transferred into a 20 mL PTFE-capped vial for HS-SPME procedure. *C. sinicus* fed on the four algae, and then its eggs were collected under the microscope, that is, 66 eggs were collected when fed on *S. costatum*, 126 eggs on *C. muelleri*, 160 eggs on *S. trochoidea* and 44 eggs on *P. micans*. All the pretreated egg samples were put into 3 mL blank culture sea water and then transferred into the 20 mL PTFE-capped vials for analysis.

2.4 HS-SPME of aldehydes

2.4.1 Loading of PFBHA on SPME fiber

PDMS/DVB fiber was selected to adsorb PFBHA. A PFBHA solution of 2 mL at 17 mg/mL in aldehyde-free water was placed in 4 mL PTFE-capped vials with a 1-cm stir bar. PFBHA in the vial was first loaded on the PDMS/DVB fiber at 75°C for 3.5 min with the stirring ratio of 560 rpm for on-fiber derivatization, and then the resultant oximes were subjected to HS-SPME.

It is notable that the fibers need to be derivatized every time before use and they cannot be used for multiple analyses after one-time derivatization. The fibers must be entirely covered by excess PFBHA, which can avoid the aldehydes to be adsorbed by the exposed fibers and thereby result in inaccurate quantitation. If the derivatized fibers are used for multiple adsorption and analyses of the aldehydes, it might cause the derivatization reagents to be insufficient and undermine the determination results.

2.4.2 Headspace SPME procedures

After loading with PFBHA, the PDMS/DVB fiber was inserted into the headspace of the 20 mL PTFE-capped vial with 3 mL alga sample solution or *C. sinicus* egg sample solution (headspace volume 17 mL) at 75°C for 25 min to extract aldehydes. From earlier work, it is known that diatoms produce unsaturated aldehyde only after cell damage [22, 23]. Unsaturated aldehydes were determined by damaging the cells with treatment in seawater before the addition of the derivatization reagent. To simulate alga cell damage by its grazer, the alga sample solutions were treated with ultrasound for 30 s before the headspace extraction. Finally, the formed oximes on the fiber were desorbed in GC injector at the temperature of 250°C for 5 min.

3 Results and discussion

3.1 Optimization of HS-SPME conditions

HS-SPME extraction efficiency can be influenced by many factors, such as extraction fiber type, headspace volume, adsorption time and temperature, desorption time and temperature [4, 8]. A spiked diatom solution at 10 μg/L of each aldehyde dissolved in 3.5% sodium chloride solution (average sea water salinity) served as a sample in the following HS-SPME condition testing experiments. Peak area of the extracted oximes formed by aldehyde-PFBHA reaction was adopted as the indicator of HS-SPME method.

3.1.1 Selection of fiber coating

Selection of suitable fibers is crucial for extraction efficiency. Three different SPME fibers (65 μm PDMS/DVB, 100 μm PDMS and 75 μm CAR/PDMS) were investigated. Loading PFBHA (17 mg/mL) on fibers was controlled at 60°C for 3.5 min with the stirring rate of 560 rpm. Then the 10 μg/L of spiked aldehyde solutions were headspace extracted with SPME fibers at 60°C for 30 min with the same stirring rate. The formed oximes on the fibers were desorbed in GC injector at 250°C for 5 min. As shown in Fig. 2, the best extraction efficiency was achieved with PDMS/DVB as the extraction fiber.

3.1.2 Effect of headspace volume

Headspace volume is also one of the most important factors influencing the efficiency of headspace extraction process. The PTFE-capped vials of 10, 15 and 20 mL containing 3 mL of sample solution were used to investigate the effect of different headspace volumes on the extraction efficiency. As can be seen in Fig. 3 the peak areas of oximes from four aldehydes increase while the other two ones decrease

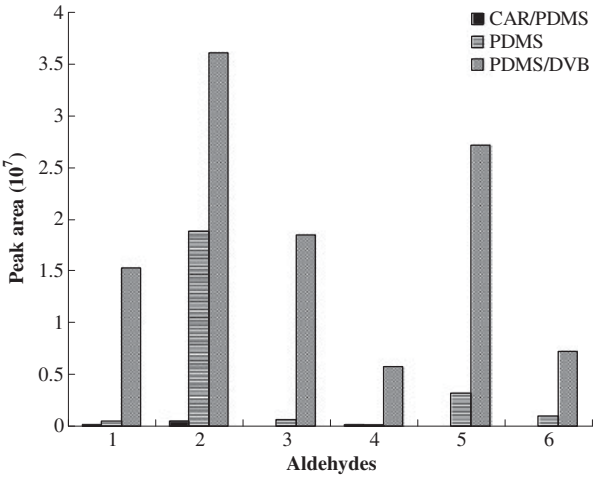

Figure 2. Peak areas of extracted oximes formed from aldehyde-PFBHA reaction using different SPME fibers. (1) 2*E*-Hexenal, (2) heptanal, (3) 2*E*-heptenal, (4) 2*E*,4*E*-heptadienal, (5) 2*E*-decenal and (6) 2*E*,4*E*-decadienal. PFBHA on-fiber derivatization procedure: 60°C for 3.5 min at the stirring ratio of 560 rpm for loading PFBHA on fiber; 60°C for 30 min at 560 rpm for headspace extraction. Desorption condition: 250°C for 5 min.

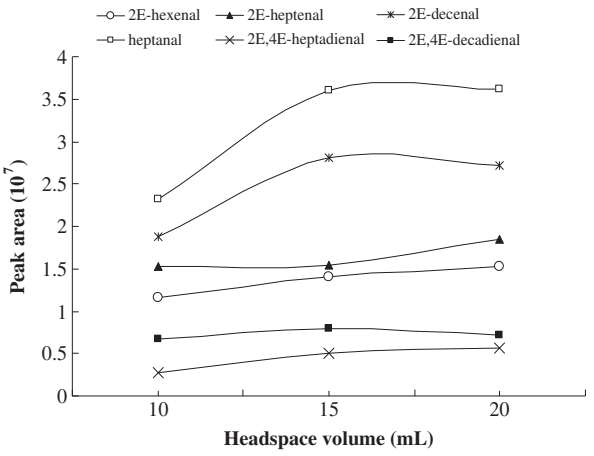

Figure 3. Effect of headspace volume on peak areas of aldehyde PFBHA oximes. PDMS/DVB fiber was used and other experimental conditions were the same as those described in Fig. 2.

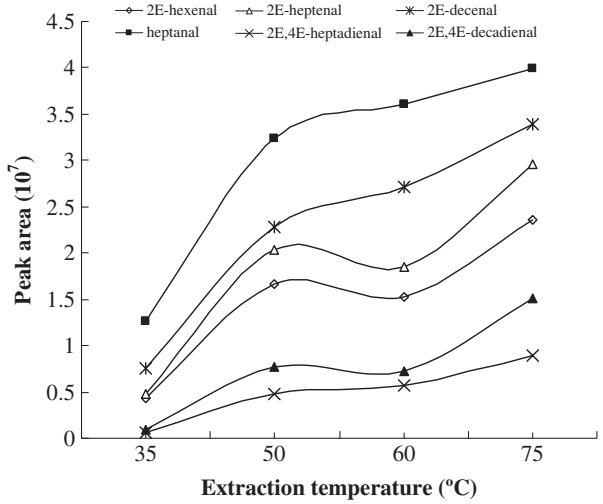

Figure 4. Effect of extraction temperature on peak areas of aldehyde PFBHA oximes. Twenty milliliters of PTFE-capped vials for the extraction for 30 min and other experimental conditions were the same as those described in Fig. 3.

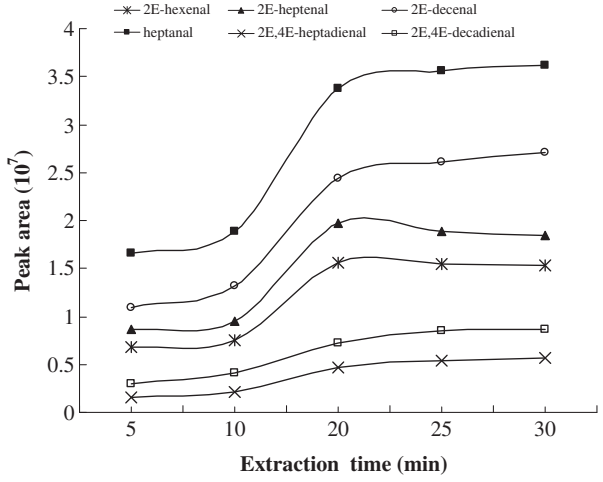

Figure 5. Effect of extraction time on peak areas of aldehyde PFBHA oximes. Extraction temperature was 75°C and other experimental conditions were the same as those described in Fig. 4.

slightly with the headspace volumes increase. Thereby, 20 mL PTFE-capped vial, namely the headspace volume of 17 mL, as a compromise was chosen for the following experiments.

3.1.3 Effect of extraction temperature and time

The choice of optimal extraction temperature and time should be taken into account for the highest efficiency. Extraction profiles were studied from 5 to 30 min and from 35 to 75°C. Figure 4 shows the extraction temperature profiles for six aldehydes, which indicated that higher amounts of oximes were obtained at 75°C than at other temperatures. For HS-SPME, higher temperature might result in the increase in the volatile analytes vapor pressure in the headspace owing to the temperature dependence of Henry's constant. However, too high temperature may also have negative effect of less favorable coating headspace partition coefficients. Under non-equilibrium conditions increasing the temperature can give more rapid transfer of analytes from liquid-to-vapor phase, and thus shorten the extraction time for HS-SPME.

Extraction time profiles at 75°C are shown in Fig. 5 for the six aldehydes. All aldehydes demonstrated similar trends of extraction efficiency, suggesting a rapid increase after 10 min, then the oxime amounts being changed very slowly after 20 min and beginning to level off at 25 min. The equilibrium was attained for all tested aldehydes within

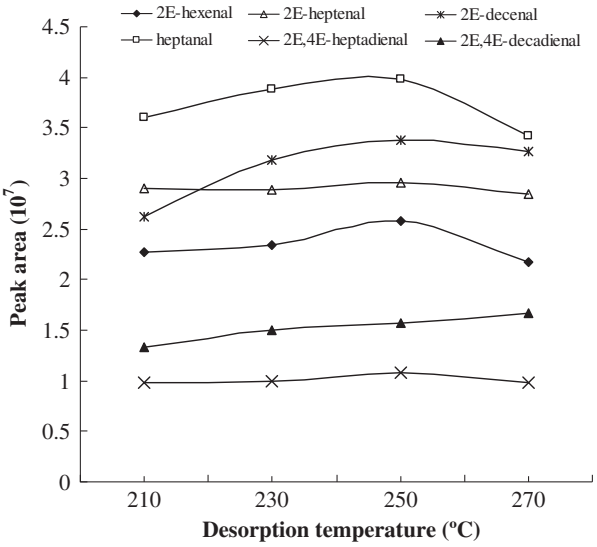

Figure 6. Effect of desorption temperature on peak areas of aldehyde PFBHA oximes. Extraction at 75°C for 25 min and other experimental conditions were the same as those described in Fig. 5.

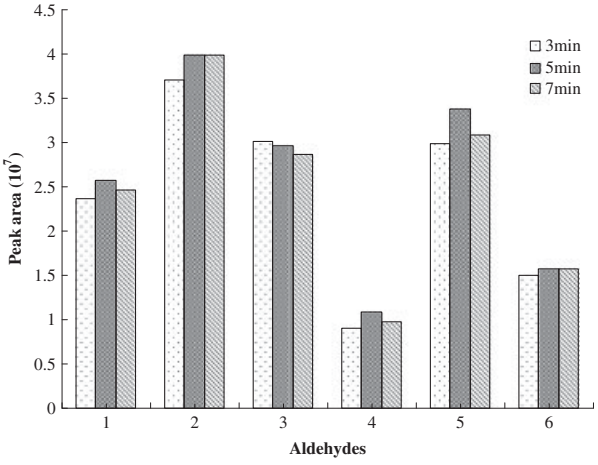

Figure 7. Effect of desorption time on peak areas of aldehyde PFBHA oximes. (1) 2*E*-Hexenal, (2) heptanal, (3) 2*E*-heptenal, (4) 2*E*,4*E*-heptadienal, (5) 2*E*-decenal and (6) 2*E*,4*E*-decadienal. Desorption at 250°C and other experimental conditions were the same as those described in Fig. 6.

30 min. Therefore, 25 min was selected as the optimum extraction time for the six aldehydes.

3.1.4 Effect of desorption temperature and time

Inadequate desorption time and low temperature could lead to incomplete desorption for the oximes on fibers, and the carryover effects also could disturb next analysis. While if the desorption time was too long and/or temperature was too high, the oximes might be decomposed. As seen from Figs. 6 and 7, 250°C at 5 min for oxime desorption temperature and time were selected.

Table 1. Regression results and method detection limits for the six aldehydes

Aldehydes	Regression equation[a)]	R^2	Linear range (μg/L)	LOD (μg/L)
2*E*-Hexenal	$y = 2 \times 10^6\ x + 731\,039$[b)]	0.9954	0.1–25	0.031
	$y = 2 \times 10^6\ x + 273\,966$[c)]	0.9985		
Heptanal	$y = 3 \times 10^6\ x + 1 \times 10^6$	0.9986	0.1–25	0.044
	$y = 4 \times 10^6\ x + 1 \times 10^6$	0.9995		
2*E*-Heptenal	$y = 2 \times 10^6\ x + 521\,157$	0.9972	0.1–25	0.026
	$y = 3 \times 10^6\ x + 853\,008$	0.9972		
2*E*,4*E*-Heptadienal	$y = 732\,892\ x + 112\,696$	0.9985	0.1–25	0.027
	$y = 941\,373\ x + 39\,824$	0.9995		
2*E*-Decenal	$y = 2 \times 10^6\ x + 88\,2437$	0.9967	0.1–25	0.032
	$y = 3 \times 10^6\ x + 64\,544$	0.9981		
2*E*,4*E*-Decadienal	$y = 2 \times 10^6\ x - 273\,563$	0.9980	0.1–25	0.027
	$y = 1 \times 10^6\ x - 393\,780$	0.9998		

a) *x*, concentration (μg/L) of the PFBHA-derivative oximes; *y*, peak area.
b) Cultured free blank sea water samples.
c) Standard sodium chloride solution.

3.2 Method validation

Under the optimum conditions mentioned above, calibration curves were plotted for concentrations ranging from 0.1 to 25 μg/L. The regression results and method detection limits obtained for each compound are shown in Table 1. The calibration coefficients (R^2) of linearity for aldehyde compounds were 0.9954–0.9986. The limit of detection (LOD) was calculated based on the signal-to-noise ratio of 3 ($S/N = 3$) at low concentrations, namely between 0.026 and 0.044 μg/L for all the six compounds. Compared with the reported PFBHA method without SPME [10], whose limit of quantification for aldehydes in concentrated diatom cultures was 5 μg/L, the proposed method obtained remarkably high sensitivity.

3.3 Analysis of aldehydes in algae samples

The validated method was applied to analyze the six aldehydes in algae. Table 2 shows the recovery and repeatability (relative standard deviation (RSD)), and Table 3 lists the endogenous contents. The recoveries for the four algae (two diatoms and two pyrrophytas) were 67.1–117% and for the eggs of *C. sinicus* feeding on *S. costatum* were 76.7–98.4%, with the RSD $<11.1\%$ (Table 2). The contents of aldehydes present in diatoms (*S. costatum* and *C. muelleri*) were at much higher levels than those in pyrrophytas (*P. micans* and *S. trochoidea*) (Table 3). Figure 8 shows the SIM chromatograms of the six aldehydes from *S. costatum*. As seen from Table 3, only heptanal as a saturated aldehyde was detected in *C. sinicus* eggs feeding on *S. costatum*, whereas no aldehydes were detected in *C. sinicus* eggs feeding on other algae (data not shown). Therefore, whether

Table 2. Method RSD and recoveries for the determination of aldehydes

Aldehydes	RSD[a] (%)	RSD[b] (%)	Recovery[c] (%)				
			SC[d]	CM[e]	PM[f]	ST[g]	Eggs[h]
2*E*-Hexenal	5.4	7.5	94.7	107.5	101.1	86.7	93.1
Heptanal	6.3	5.3	85.5	84.2	90.7	95.9	86.6
2*E*-Heptenal	9.2	6.8	86.4	95.1	84.6	74.3	81.5
2*E*,4*E*-Heptadienal	7.4	9.7	117.0	88.5	90.1	108.9	98.4
2*E*-Decenal	10.5	10.5	81.2	77.0	69.1	67.1	76.7
2*E*,4*E*-Decadienal	7.5	11.1	68.5	71.2	78.6	73.4	79.5

a) 1 μg/L of spiked culture alga-free blank sea water sample ($n = 8$).
b) 10 μg/L of spiked culture alga-free blank sea water sample ($n = 8$).
c) Spiked concentrations: 10 μg/L of each aldehyde in SC; 5 μg/L in other four samples ($n = 3$).
d) *S. costatum*.
e) *C. muelleri*.
f) *P. micans*.
g) *S. trochoidea*.
h) *C. sinicus* eggs (feeding on *S. costatum*).

Table 3. Endogenous contents of aldehydes in real samples (μg/L)

Aldehydes	SC	CM	PM	ST	Eggs[a]
2*E*-Hexenal	2.052	0.250	0.038	0.044	ND[b]
Heptanal	0.216	0.820	ND	ND	0.051
2*E*-Heptenal	0.228	0.040	ND	ND	ND
2*E*,4*E*-Heptadienal	1.068	0.380	0.04	0.088	ND
2*E*-Decenal	0.084	ND	ND	ND	ND
2*E*,4*E*-Decadienal	0.276	0.125	ND	ND	ND

a) Eggs of *C. sinicus* feeding on *S. costatum*.
b) Not detected.

heptanal negatively influences diatom grazers or not deserves further investigation.

4 Concluding remarks

A new method based on HS-SPME on-fiber PFBHA derivatization coupled with GC-MS was developed for the determination of six aldehydes in alga matrices, by virtue of the selectivity and enrichment property of HS-SPME and stability of oxime derivates. This procedure is sensitive and specific, since only molecules containing aldehydic groups react satisfactorily with the derivatizing agent. Extraction and derivatization of the aldehydes were simultaneously performed, which led to simple sample preparation and short analysis time. This method was successfully applied in the determination of six aldehydes in the cultured algae (diatom and pyrrophyta) and copepod eggs. Good recovery, repeatability and low detection limits were presented. This method proves great potential for routine monitoring of toxic algae.

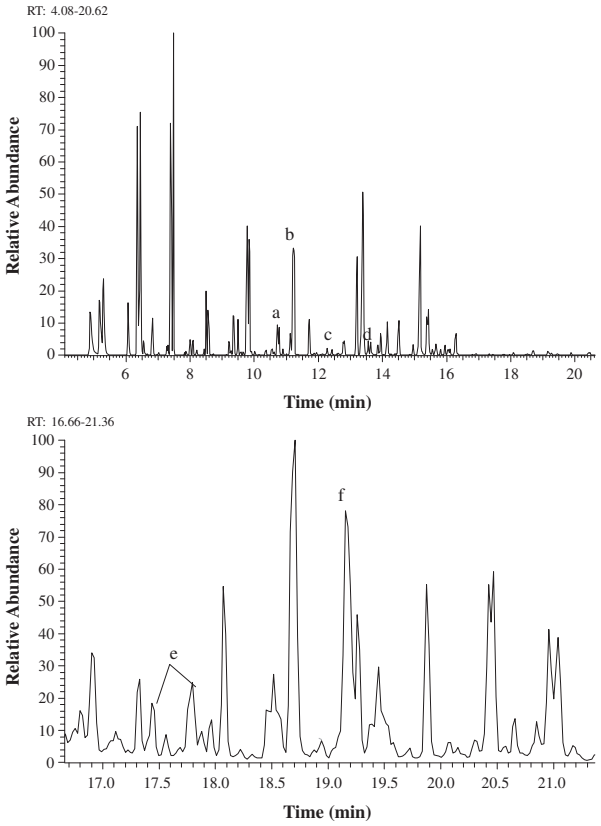

Figure 8. GC-MS chromatograms of aldehyde PFBHA oximes from *S. costatum* under the optimal experimental conditions. Peak identification: (a) 2*E*-hexenal, (b) heptanal, (c) 2*E*-heptenal, (d) 2*E*,4*E*-heptadienal, (e) 2*E*-decenal and (f) 2*E*,4*E*-decadienal.

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The authors have declared no conflict of interest.

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