Advances in Sample Preparation 8 (2023) 100093

Contents lists available at ScienceDirect

Advances in Sample Preparation

journal homepage: www.elsevier.com/locate/sampre

Determination of chlorophylls a and b and β-carotene in environmental waters: Diminishing wastes and analysis time by in-tube solid-phase microextraction coupled on-line to nano liquid chromatography

C. Soto, R. Herráez-Hernández, P. Campíns-Falcó *

MINTOTA Research Group, Departament de Química Analítica, Facultat de Química, Universitat de València, Dr. Moliner 50, 46100-Burjassot, València, Spain

ARTICLE INFO

Keywords:
Chlorophylls
Carotenoids
Water samples
In-tube solid phase microextraction
Nano liquid chromatography
Time
Low residues
Friendly solvent

ABSTRACT

Chlorophylls and carotenoids are indicators of water quality. A new method based on in-tube solid-phase microextraction (IT-SPME) coupled on-line to nano-liquid chromatography with diode array detection (NanoLC-DAD) was developed. This paper aims to provide selectivity and sensitivity with a short analysis time of about 10 min and low residues of friendly solvents, 5 μL/run of ethanol:water 95:5 and 250 μL of ethanol for dissolving the pigments retained in a nylon filter (0.45 μm, 13 mm) from 30 mL of water. IT-SPME capillary was a fused silica capillary (10 cm length x 75 μm internal diameter, i.d.) with a coating of tetraethyl orthosilicato (TEOS), triethoximethylsilane (MTEOS) doped with SiO_2 nanoparticles (NPs) and an analytical column Zorbax 300SB-C_{18} (50 mm x 75 μm i.d., 3.5 μm particle size) was used. The recovery obtained to chlorophyll a, chlorophyll *b* and β-carotene were 93 ± 8 %, 92 ± 7 % and 94 ± 4 %, respectively. The method shows good linearity in the range up to 300 μg L^{-1} for chlorophyll a and β-carotene and up to 600 μg L^{-1} for chlorophyll *b*. The achieved limits of detection (LODs) in samples were 0.1 μg L^{-1} to chlorophyll a and β-carotene, and 0.2 μg L^{-1} to chlorophyll *b*. Values of relative standard deviations (RSD) expressed in% between 2 and 3 were obtained. Pigments were successfully determined with a short analysis time and minimal residues in real water samples.

1. Introduction

Chlorophylls and carotenoids are important compounds in photosynthesis. Among chlorophylls, chlorophyll a is the main photosynthetic pigment and it is common to all phytoplankton. Its determination in water samples allows estimating the concentration of phytoplanktonic algae and the biological activity by which eutrophication processes occur, thus being a good parameter for determining water quality [1–5]. High levels of chlorophyll a usually indicate poor water quality; legislated values in Spain are: annual average of 8 μg L^{-1} and annual maximum of 25 μg L^{-1} [6].

The main functions of carotenoids in plants include absorbing light energy for photosynthesis and providing photoprotection of chlorophylls, thus preventing the formation of reactive oxygen species [7]. Carotenoids can be divided into two main groups, those with a simple hydrocarbon structure (carotenes) or as structures that have oxygen within them (xanthophylls) [8–10]. β- Carotene is a precursor of vitamin A [7].

Spectroscopic techniques are widely used to determinate these compounds, but both underestimation and overestimation have been described [11–13]. Therefore, chromatographic separation, especially liquid chromatography (LC), is considered to be a good technique for pigment determination [1,5,14-17].

Nowadays, the development and advancement of new technologies in LC and the increasing interest in protecting the environment have focused research on miniaturized LC. In addition these techniques are easily coupled on-line with in-tube solid phase microextraction (IT-SPME) [18–23]. This coupling facilitates to increase the sample volume processed in miniaturized LC without losses in resolution and provides both, lower waste generation and greater sensitivity compared to its coupling with conventional LC [20,21].

Our research group [5] proposed in 2010 the determination of chlorophyll a in aquatic systems using IT-SPME coupled to capillary LC (Cap LC) and UV–vis diode array detection (DAD) in presence of chlorophyll *b* and their degradation products: pheophytin a and pheophytin b, respectively. Ethanol-water (95:5, v/v) as mobile phase was used and 400 μL/run was consumed. The proposed procedure improved sensitivity and analysis time with low greener organic solvent consumption

* Corresponding author.
E-mail address: pilar.campins@uv.es (P. Campíns-Falcó).

https://doi.org/10.1016/j.sampre.2023.100093
Received 25 May 2023; Received in revised form 28 August 2023; Accepted 8 September 2023
Available online 10 September 2023

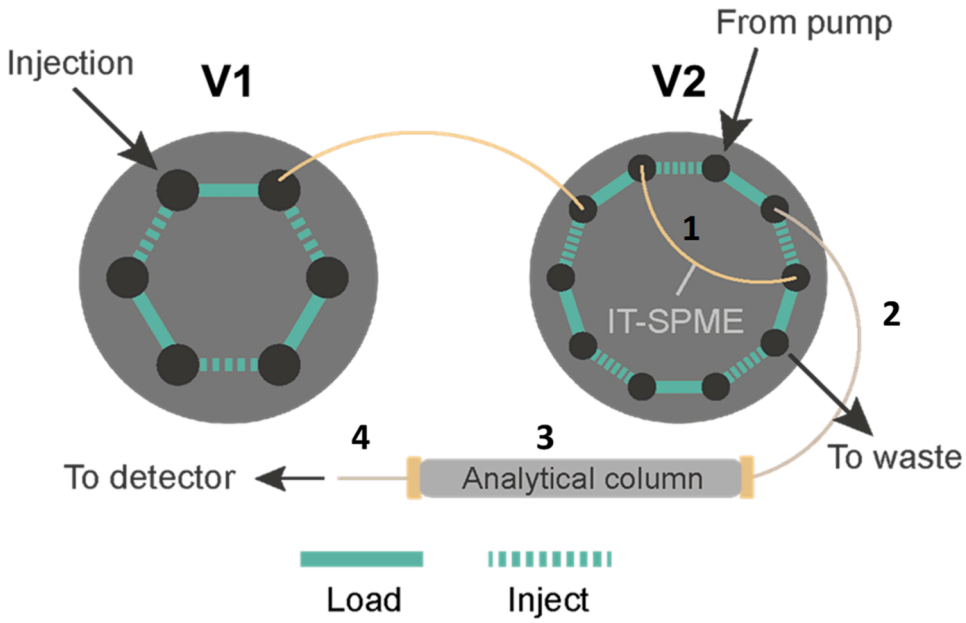

Fig. 1. Schematic representation of injection system for benchtop nanoLC. V1 and V2 are manual 6 – port and 2 – way, and automatic nano 10 – port and 2 – way injection valves, respectively. Dimensions (internal diameter, length, and volume) for the different parts are 1) 75 μm, 10 cm and 433 nL; 2) 25 μm, 10 cm and 49 nL; 3) 75 μm, 5 cm and 133 nL; 4) 25 μm, 20 cm and 98 nL. The total volume of the injection system is 703 nL. For more explanations, see text of the paper.

and waste generation with respect to conventional LC. Here the objective is to scale to lower dimensions in LC with the idea of reducing wastes and analysis time. The consumption of ethanol for dissolving the pigments retained on the filter from water samples was also diminished. Besides, in the present paper one more step was achieved by demonstrating that IT-SPME-nano LC-DAD can decrease the mobile phase consumption and can cut retention time of chlorophyll a with respect to the Cap LC method, besides β-carotenoid was included in the water analysis. If the retention time decreases, the electricity required is less and the carbon footprint is less too [24,25].

2. Materials and methods

2.1. Chemicals and solutions

Ultrapure water was obtained from an Adrona system (Riga, Latvia). Water was filtered through 0.22 μm nylon membranes purchased from GVS (Sandfor, ME, USA) before use.

All reagents used throughout out the study were of analytical grade. Chlorophyll a, chlorophyll *b* and β-carotene obtained from Sigma-Aldrich (St. Louis, MO, USA). Ethanol and acetone (both HPLC grade) were purchased from VWR Chemicals (Randnor, PA, USA). Stock solutions of the chlorophyll a and chlorophyll *b* (1000 μg mL^{-1}) were prepared by dissolving the appropriate amounts of the commercial standards in ethanol. β-Carotene (500 μg mL^{-1}) was prepared in acetone. All working solutions of the analytes and their mixtures were prepared in such a way that there was 20 % ethanol in them, the solvent used for dilution was water.

All solutions were stored at 4 °C until their use.

2.2. Chromatographic conditions

The benchtop NanoLC system was an Agilent 1260 Infinity (Waldbronn, Germany) equipped with an injection system which consists of a first Rheodyne 7725i 6 ports positions, 1/16″ manual injection valve connected to an VICI C2N 10 port-2 positions, 1/32″ automatic valve (Valco Instruments, Houston, TX, USA) and a UV–vis DAD detector (Agilent). The standard or sample is on-line preconcentrated through in-valve IT-SPME using a fused silica capillary (10 cm length x 75 μm i.d.) (Análisis Vínicos, Tomelloso, Spain) coated with a polymer of tetraethyl orthosilicate (TEOS) triethoximethylsilane (MTEOS) doped with SiO_2 nanoparticles [23]. A homogeneous coating (350 nm) of TEOS-MTEOS was formed inside the capillary column [23]. The analytical signal was recorded between 400 nm and 800 nm, and monitored at 662 and 420 nm to chlorophyll a and 662 and 450 nm to chlorophyll *b* and at 450 nm to β-carotene. The detector was coupled to a data system (Agilent, ChemStation) for data acquisition and treatment. The analytical column employed was a Zorbax 300SB-C_{18} (5 cm x 75 μm i.d., 3.5 μm particle size) analytical column (Agilent, Waldbronn, Germany). Chromatographic separation was carried out in isocratic elution mode with a mixture of ethanol-ultrapure water (EtOH/H_2O 95:5 v/v) at 0.5 μL min^{-1}. Aliquots of 100 μL of the working solutions were manually introduced in the IT-SPME capillary system (Fig. 1) by means of a 250 μL precision syringe.

2.3. Extraction procedure and analytical parameters

For the determination of pigments in environmental waters, 10 or 30 mL of sample was filtered through 0.45 μm nylon membrane filters (diameter 13 mm) from Branchia-Labbox (Barcelona, Spain). The filters were immersed in two different volumes of ethanol (250 μL and 150 μL) to define the sufficient volume for dissolving chlorophylls and carotenoids. Once the extract was obtained, it was filtered with nonsterile nylon syringe filters (Branchia-Labbox) for cleaning it and after it was properly diluted with water five times to be processed 100 μL into the 6-position valve in accordance with Fig. 1 in order to obtain the chromatograms.

Calibration curves were made by diluting the concentrated standards (chlorophyll a, chlorophyll *b* and β-carotene) with distilled water and adjusting the percentage of ethanol to 20 %.

Detection limits (LODs) and quantification limits (LOQs) were obtained by processing standards in the same form than samples. LODs were obtained by injecting gradually lower concentrations of diluted standard until achieving a signal-to-noise ratio of 3 and LOQs corresponded to concentrations that provided a signal 10 times higher than instrument noise.

To determinate the recovery percentages of each of the analytes, a sample containing concentrations below LODs was fortified with 1 μg

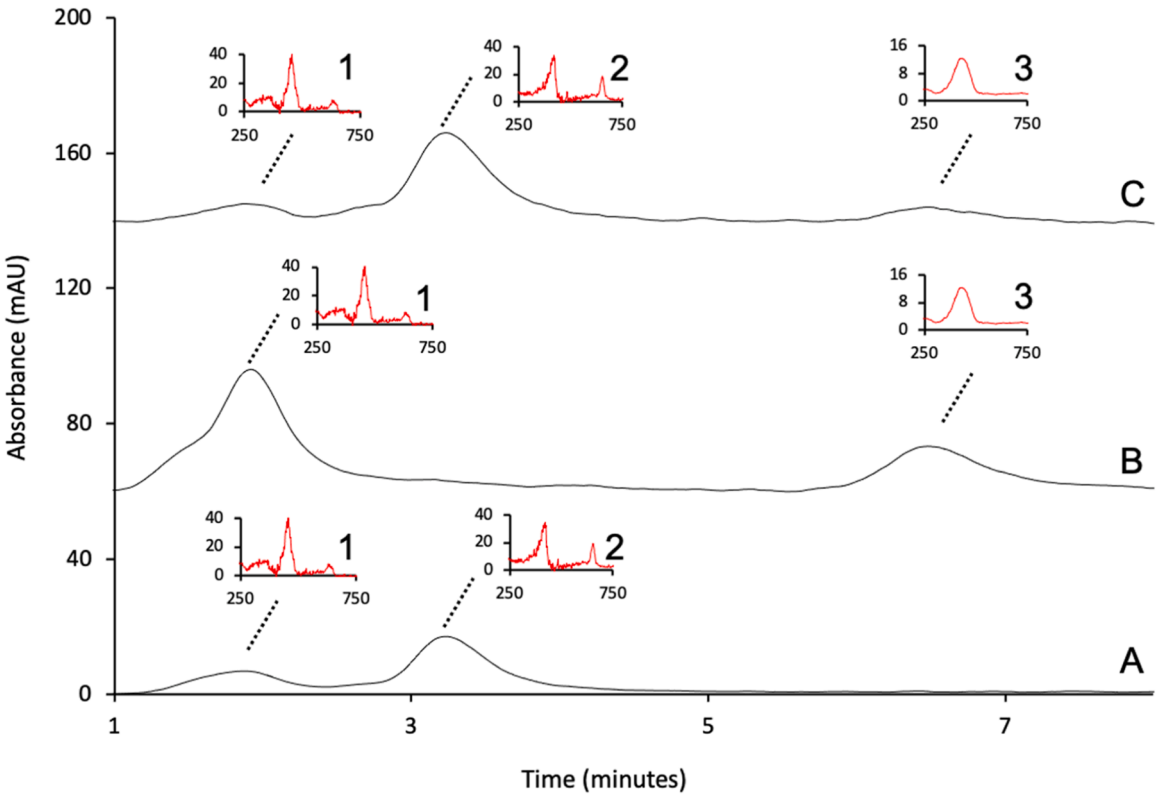

Fig. 2. Chromatogram of standard solutions of 1) chlorophyll *b*, 2) chlorophyll a (both at concentration 300 μg L^{-1}) and 3) β-carotene 200 μg L^{-1} at A) 662 nm, B) 450 nm and C) 420 nm.

L^{-1} concentration of analyte and it was processed and measured. This experiment was performed in triplicate on different days to obtain intraday and interday precision.

Both the material used for the preparation of the samples and analytical standards, their storage, and the working environment were arranged to minimize interference from external light. Amber material was used to prevent light exposure. The samples and analytical standards were prepared on the same day and injected as soon as they were ready.

2.4. Analysis of water samples

Three lake waters were collected at several points of the Albufera, Valencia (Comunidad Valenciana, Spain). Three well waters were collected in several places of the Comunidad Valenciana and three more were sea waters from Valencian Mediterranean coast.

Samples were collected in amber glass bottles (500 mL), which were previously washed with neutral detergent and abundant distilled water to eliminate all possible residues. The bottles were dried at 40 °C for 24 h. The samples were collected using gloves and stored in a cooler until the laboratory to maintain the water conditions as good as possible and once in the laboratory they were stored at 4 °C before analysis.

Analysis were performed the same day of sample collection or the following day at the latest. Water samples were processed following the procedure described before. All samples were analyzed in triplicate.

3. Results and discussion

3.1. IT-SPME coupled on line to nano LC with DAD detection

Fig. 1 illustrates the configuration of the proposed IT-SPME device, which includes the dimensions of the system (see also Section 2.2). The IT-SPME arrangement had two valves of two positions, a manual 6-port injection valve for injecting the standards and samples using only its load position and a 10-port automatic nano valve, which contained the IT-SPME capillary, the connections to the nanopump and the analytical column and allowed to transfer the preconcentrated standard or sample in the load position to the analytical nanocolumn in the injection position. Both valves are connected through a silica capillary. The total volume of the chromatographic system including the 10-port automatic nano valve, which injected standards or samples into the system was 703 nL and the volume of the IT-SPME capillary was 433 nL. The processed volume through the manual and automatic valves consisted of 100 μL of the working solutions. The 6-position valve is solely used to transport the sample to the 10-position valve where the IT-SPME capillary, connection to the pump, column and detector are located.

It has been determined that the necessary volume for dissolving the pigments from de filters is 250 μL, as at that volume, the recovery percentage has been near 95 % for studied pigments, whereas with 150 μL, it has been around 60–70 %. This extract was subsequently diluted five times with distilled water.

Fig. 2 shows the chromatograms for standards prepared in 20 % of ethanol, which were recorded at 662 nm to chlorophyll a and chlorophyll *b*, 420 nm for Chlorophyll a and 450 nm to chlorophyll *b* and β-carotene. This percentage is in accordance with the aim ensuring that both standards and samples have the same ethanol percentage and that the retention times of the analytes remain unaltered. Besides, as the mobile phase is composed of 95 % ethanol and 5 % water in an isocratic mode, the analytes cannot be retained in the ITSPME capillary and nano analytical column eluting in the dead time if the ethanol content is not optimized. Note that the IT-SPME loop is of 433 nL, a high charge for nanoLC, the peak width is good bearing in mind the analytical column dimensions, the injected volume in the analytical column, the used flow rate, the isocratic mode and the composition of the mobile phase needed for eluting the pigments. The selected values are suitable for this application, for obtaining better chromatographic peak width, a large

Table 1
Analytical parameters of the IT-SPME NanoLC-DAD. Linearity parameters were obtained from standards containing 20 % of ethanol and LODs and LOQs from extraction process proposed for samples and they are given as concentration in sample.

Linearity, $y = b_1 x + b_0$ (n = 10)							
Compound	Interval (μg L^{-1})	$b_1 \pm s_{b1}$ mg L^{-1}	$b_0 \pm s_{bo}$	R^2	LOD (μg L^{-1})	LOQ (μg L^{-1})	RSD (%)* (n=3)
Chlorophyll a	30–300	(2390 ± 40)[a]	−12 ± 6	0.998	0.1	0.3	2
	30–300	(2080 ± 40)[b]	-32 ± 6	0.998	0.1	0.3	2
Chlorophyll b	70–600	(1100 ± 40)[c]	35 ±13	0.992	0.2	0.6	3
	150–600	(344 ± 11)[b]	36 ± 5	0.991	0.5	1.5	3
β-carotene	30–300	(2610 ± 50)[c]	1 ± 8	0.997	0.1	0.3	3

* Concentration of 100 μg L^{-1} for chlorophyll a and β-carotene and 300 μg L^{-1} chlorophyll *b*. Established at [a] 420 nm, [b] 662 nm, [c] 450 nm.

analytical column can be employed, but its use will increase the analysis time.

Total analysis time for the determination of chlorophylls and carotenoid is less than 8 min, which significantly reduces the solvent consumption, here 5 μL/run, and run time for the separation of these analytes compared to other LC methods. For example, in reference [1] using ultra-high performance liquid chromatography (UHPLC) the volume of mobile phase used was 14 mL/run and in [17], which proposed conventional HPLC this volume was 20 mL/run. As indicated in the introduction section the consumptions of solvents and wastes are minimized by employing miniaturized LC. In our previous paper [5] employing CapLC the volume of mobile phase used was 400 μL/run and the retention times for chlorophylls were two times higher than those shown in Fig. 2. Besides improving the analysis time, the consumption of electricity is lowered. A parameter used to quantify the environmental impact of the analytical procedure is expressed by kilograms of CO_2 equivalent or the so called carbon footprint [24,25], whose estimation is computed by means of the following expression (Eq. (1)):

$$kg\ CO_2\ eq = \sum Instrument\ Power\ (kW) \cdot Analysis\ time\ (h) \cdot Emission\ factor\ for\ electricity\ \left(kg\frac{CO_2}{kWh}\right) \quad (1)$$

Table 2
Recovery and precision obtained in fortified with 1 μg L^{-1} of chlorophyll a, chlorophyll *b* and β-carotene in a blank water sample. Concentrations are given as in sample.

Analyte	Recovery (n = 6) %	Precision, RSD (%)	
		Intraday (n = 3)	Interday (n = 6)
Chlorophyll a	93 ± 8	8	10
Chlorophyll b	92 ± 7	7	11
β-carotene	94 ± 4	4	8

In which the electricity consumed by the instrumental equipment is multiplied by the analysis time per sample (in hours) and by a reference constant value emission factor equal to 0.247 kg CO_2/kWh [26]. Here, the most relevant improved parameters for increasing greenness and sustainability of the method are: minimization of residues and electricity consumption for comparative purpose.

Linearity was evaluated by processing in duplicate five concentrations of chlorophyll a, chlorophyll *b* and β-carotene prepared containing 20 % of ethanol. It can be seen from Table 1 that a good correlation between peaks areas and concentration of different pigments is obtained, which is reflected in the value of R^2 for all calibration lines. Working concentration range is also shown in Table 1.

LODs and LOQs were determined experimentally as the concentrations of analyte in the sample that resulted in a signal-to-noise ratio of 3 and 10, respectively for each analyte at the several wavelengths employed [5,21,23,24]. The extraction procedure of the analytes from water samples was adapted from reference [5]. Between 10 and 30 mL of water samples were passed through a 0.45 μm nylon filter and after that the analytes were dissolved with 250 μL of ethanol. Selected values were: 30 mL of sample and 250 μL of ethanol. The extract was diluted with water five times and processed in the IT-SPME- Nano LC.

Fig. 3 shows the chromatograms obtained for the three analytes at LOD level given in Table 1, which show the signals achieved for qualitative assessment of the presence of these analytes in a sample. The spectra at LOD level contained some noise, note that the signal is only three times the noise, but the profiles of the spectra are conserved. The achieved LOQ values permit monitoring if the legislation is fulfilled. Precision was also evaluated through the injection of three replicates standard solution of pigments. Values of% RSD were below 3 % as it can be seen in Table 1.

Matrix effect was assessed by spiking a blank sample, which contained the analytes below LODs, this concentration was added prior to sample filtration at 1 μg L^{-1} level of standard solution and processing the spiked sample as indicated in Section 2.3. The results show that there is

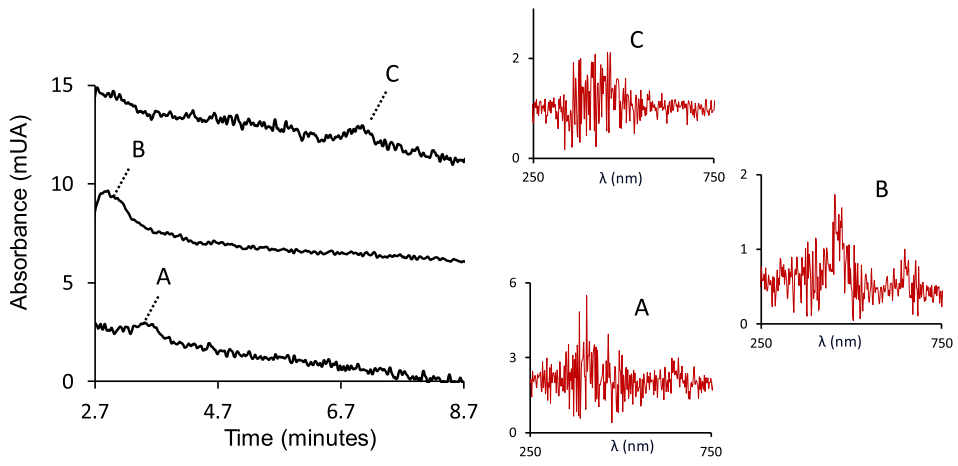

Fig. 3. Chromatograms of LOD of : A) chlorophyll a at 662 nm, B) chlorophyll *b* at 450 nm and C) β-carotene at 450 nm, which were obtained through standards treated as samples.

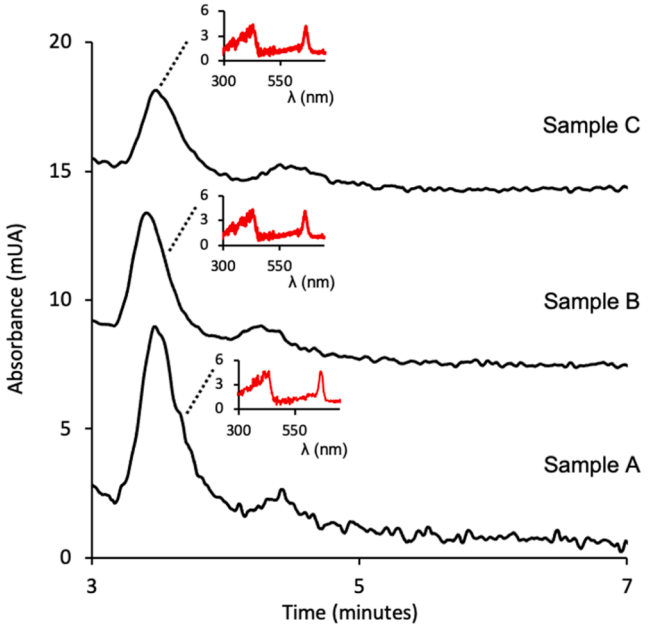

Fig. 4. Chromatogram at 662 nm for Albufera samples. (T_R Chlorophyll a: 3.5 min).

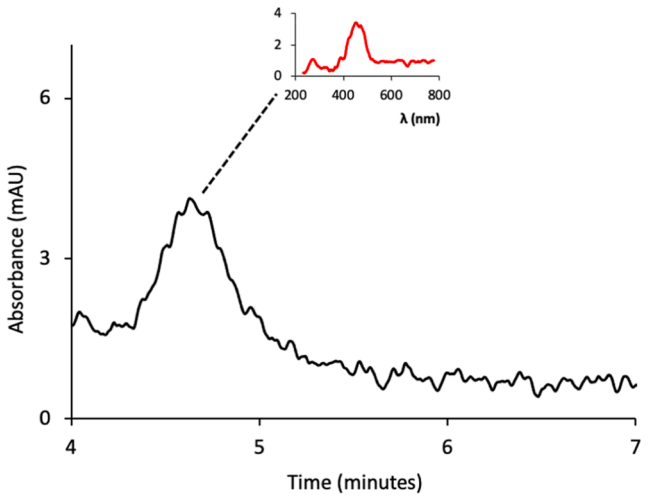

Fig. 5. Chromatogram at 450 nm of Albufera B sample with unidentified carotene.

not matrix effect so that calibration can be performed externally. Table 2

3.2. Analysis of real water samples

Several types of waters were tested: lake, well and sea as indicated in Section 2.4. Peak of chlorophyll a was present in all Albufera lake water samples analysed. The chromatograms obtained for the samples can be seen in the Fig. 4. Comparison of spectra between the sample and the chlorophyll a standard corroborated the presence of this compound in the water samples as they match completely. The results obtained for Albufera water samples were: 3.9 ± 0.1, 2.7 ± 0.1 and 2.26 ± 0.02 μg L^{-1} for chlorophyll a (Sample of Albufera A, B and C of Fig. 4, respectively). These results are within the established norm [6] for the determination of eutrophic waters and would represent non-eutrophic waters.

A carotene (inferred from UV spectrum) was found in only one of the samples, B of Albufera lake, but it could not be identified (Fig. 5). This

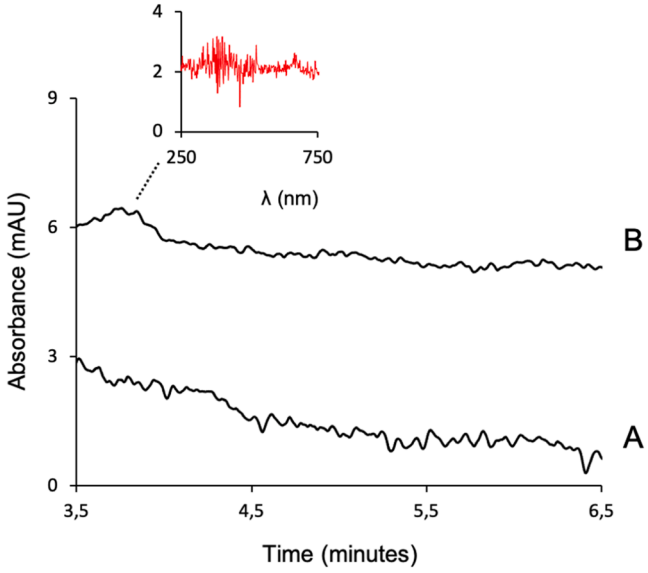

Fig. 6. Chromatogram at B) 450 nm and A) 662 nm for Albufera B sample, using 10 mL for extraction.

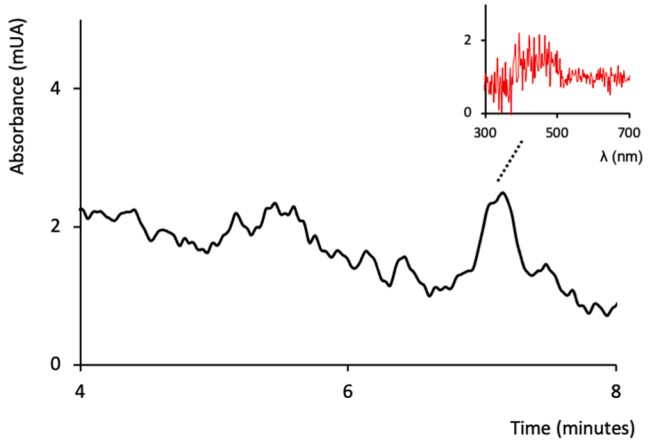

Fig. 7. Chromatogram at 450 nm for a well water sample.

Table 3
Values of pigmens found in different water samples (30 mL).

Water samples	Chlorophyll a (μg L^{-1})	Chlorophyll b (μg L^{-1})	β-carotene (μg L^{-1})
Albufera A	3.9 ± 0.1	<LOD	<LOD
Albufera B	2.7 ± 0.1	<LOD	<LOD
Albufera C	2.26 ± 0.01	<LOD	<LOD
Well water A	<LOD	<LOD	≈0.1
Well water B	<LOD	<LOD	<LOD
Well water C	<LOD	<LOD	<LOD
Sea water A	<LOD	<LOD	<LOD
Sea water B	<LOD	<LOD	<LOD
Sea water C	<LOD	<LOD	<LOD

water was tested with a sample volume of 10 mL, but when using this volume, it is observed in the chromatogram that the carotenoid is not visible (Fig. 6), although the Chlorophyll a was detected as Fig. 6 shows. For this reason, it is decided to use a volume of 30 mL to be able to extract and determine both kind of pigments in the water samples.

Only in a well water was present a small peak due to β-carotene as Fig. 7 shows, the value found in this sample is close to the detection limit. In the tested sea samples all the analytes were below LODs. As a

Table 4
Characteristics of LC-based methods proposed for analysing chlorophylls and carotenoids in environmental waters or similar matrices.

Samples	Sample volume (mL)	Filter pore size x diameter (μm x mm)	Dissolution time (min)	LC run time (min)	Flow rate (mL min^{-1})	Analytical column	Mobile phase	LOD (Chl a, Chl b, β-carotene)	Generated waste (mL)[a]	REF
Marine phytoplankton cuture and sea water	2 - 15	0.2×13	63	30	1.2	Packed C_8 (150 mm x 4.6 mm, 3.5 μm)	A: 70:30 MeOH/28 mM TBAA[c]. B: MeOH	0.26 ng and N.A.[b] for Chl b and β-carotene	36	[1]
Seawater	10	0.45×13	2	12	0.02	Packed C_{18} (150 mm × 0.5 mm, 5 μm)	95:5 EtOH/H_2O	0.05, 0.05 μg L^{-1} and N.A.[b] for β-carotene	0.24	[5]
Sediments types and sediment depths	2	N.A.[b]	N.A.[b]	20	2	Packed C_{18} (25 cm, 5 μm)	A: 80:15:5 MeOH/H_2O/ ion-pairing solution B: 80:20 MeOH/ Acetone	N.A.[b]	40	[14]
Sea, lake and river water	200	0.7×47	60 - 120	N.A.[b]	N.A.[b]	N.A.[b]	N.A.[b]	N.A.[b]	N.A.[b]	[15]
Seawater	1000 - 5000	0.7×25	10	20	1	Packed C_{18} (25 cm x 4.6 mm, 5 μm)	A: 80:20 MeOH/0.5 M AA[d] B: 90:10 ACN/H_2O C: Ethyl acetate	N.A.[b]	20	[17]
Lake water, seawater and well water	10–30	0.45×13	2	10	0.005	Packed C_{18} (50 mm x 75 μm i.d., 3.5 μm)	95:5 EtOH/H_2O	0.1, 0,2 or 0.5 and 0.1 μg L^{-1}	0.005	This work

[a]Calculated from work flow and time of analysis; [b]Not available; [c]Tetrabutylammonium acetate; [d]Ammonium acetate.

summary, Table 3 presents the analysed samples and found concentration analytes in some of them. As observed, the target analytes of this study are predominantly found in the samples from the Albufera's lake. This can be attributed to the presence of diverse vegetation surrounding the lake, where these pigments (chlorophylls and carotenoids) play a crucial role in the photosynthesis process of plants.

In Table 4, relevant data from other methods for determining chlorophylls and carotenoids in environmental water matrices can be seen. The most noteworthy aspect of the proposed method is the minimal residue generated, as it operates at low flows and achieves the separation of carotenoids and chlorophylls in less than 8 min. The proposed method is simpler, faster and more environmentally friendly.

4. Conclusions

In the present study, determination of chlorophylls and carotenoids in water samples was assessed by means of IT-SPME NanoLC. This miniaturized technique provided low wastes 5 μL/run (vs 400 μL/run in [5]) and short retention times, lesser than 8 min. A sample volume of 30 mL was filtered and the volume of ethanol used for dissolving the pigments on the filters was 250 μL of a non-hazardous solvent, so the solvent expenditure in each extraction was minimal in reference to other methods, even in front of 1 mL used in our previous paper [5]. The total analysis time for analytes and water samples was around 10 min, which is a considerable improvement compared to other methods using liquid chromatography. If the retention time decreases, the electricity required is less and the carbon footprint is less too.

The methodology resulted in good linearity and accuracy. The intraday and interday precisions were also adequate. The method is sustainable and environmentally friendly, which is reflected in the minimal use of solvent, both in the extraction of pigments and in the use of mobile phase in the equipment and minimal analysis time.

Compliance with ethical standards

Not applicable.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

Authors acknowledge EU and the Gobierno de España MCI-AEI (PID2021-124554NB-I00) and to the Generalitat Valenciana (PROMETEO Program 2020/078 and EU FEDER- Generalitat Valenciana (ID-FEDER/2018/049). C.S. thanks to Gobierno de Chile, Agencia Nacional de Investigación y Desarrollo for predoctoral grant 72210282.

References

[1] K. Suzuki, A. Kamimura, S.B. Hooker, Rapid and highly sensitive analysis of chlorophylls and carotenoids from marine phytoplankton using ultra-high performance liquid chromatography (UHPLC) with the first derivative spectrum chromatogram (FDSC) technique, Mar. Chem. 176 (2015) 96–109, https://doi.org/10.1016/j.marchem.2015.07.010.

[2] A. Wojdyło, P. Nowicka, K. Tkacz, I.P. Turkiewicz, Fruit tree leaves as unconventional and valuable source of chlorophyll and carotenoid compounds determined by liquid chromatography-photodiode-quadrupole/time of flight-electrospray ionization-mass spectrometry (LC-PDA-qTof-ESI-MS), Food Chem. 349 (2021), 129156, https://doi.org/10.1016/j.foodchem.2021.129156.

[3] M.K. Lehmann, E.M. Schütt, M. Hieronymi, J. Dare, H. Krasemann, Analysis of recurring patchiness in satellite-derived chlorophyll a to aid the selection of representative sites for lake water quality monitoring, Int. J. Appl. Earth Obs. Geoinf. 104 (2021), 102547, https://doi.org/10.1016/j.jag.2021.102547.

[4] M. Muñoz-Ortuño, P. Serra-Mora, R. Herráez-Hernández, J. Verdú-Andrés, P. Campíns-Falcó, A new tool for direct non-invasive evaluation of chlorophyll a content from diffuse reflectance measurements, Sci. Total Environ. 609 (2017) 370–376, https://doi.org/10.1016/j.scitotenv.2017.07.140.

[5] Y. Vitta, Y. Moliner-Martínez, P. Campíns-Falcó, A.F. Cuervo, An in-tube SPME device for the selective determination of chlorophyll a in aquatic systems, Talanta 82 (2010) 952–956, https://doi.org/10.1016/j.talanta.2010.05.069.

[6] RD 47/2022, Spain 2022. https://www.boe.es/boe/dias/2022/01/20/pdfs/BOE-A-2022-860.pdf (accessed April 2023).
[7] T. Maoka, Carotenoids as natural functional pigments, J. Nat. Med. 74 (2020) 1–16, https://doi.org/10.1007/s11418-019-01364-x.
[8] D. Giuffrida, M. Zoccali, L. Mondello, Recent developments in the carotenoid and carotenoid derivatives chromatography-mass spectrometry analysis in food matrices, TrAC Trends Anal. Chem. 132 (2020), 116047, https://doi.org/10.1016/j.trac.2020.116047.
[9] V. Hynstova, D. Sterbova, B. Klejdus, J. Hedbavny, D. Huska, V. Adam, Separation, identification and quantification of carotenoids and chlorophylls in dietary supplements containing chlorella vulgaris and spirulina platensis using high performance thin layer chromatography, J. Pharm. Biomed. Anal. 148 (2018) 108–118, https://doi.org/10.1016/j.jpba.2017.09.018.
[10] C.C.C.R. de Carvalho, M.J. Caramujo, Carotenoids in aquatic ecosystems and aquaculture: a colorful business with implications for human health, Front. Mar Sci. 4 (2017) 93, https://doi.org/10.3389/fmars.2017.00093, a.
[11] R.C. Thompson, M.L. Tobin, S.J. Hawkins, T.A. Norton, Problems in extraction and spectrophotometric determination of chlorophyll from epilithic microbial biofilms: towards a standard method, J. Mar. Biol. Assoc. 79 (1999) 551–558, https://doi.org/10.1017/S0025315498000678.
[12] L. Moberg, G. Robertsson, B. Karlberg, Spectrofluorimetric determination of chlorophylls and pheopigments using parallel factor analysis, Talanta 54 (2001) 161–170, https://doi.org/10.1016/S0039-9140(00)00650-0.
[13] M. Rinawati, L.A. Sari, K.T. Pursetyo, Chlorophyll and carotenoids analysis spectrophotometer using method on microalgae, IOP Conf. Ser. 441 (2020), 012056, https://doi.org/10.1088/1755-1315/441/1/012056.
[14] J. Pinckney, R. Papa, R. Zingmark, Comparison of high-performance liquid chromatographic, spectrophotometric, and fluorometric methods for determining chlorophyll a concentrations in estuarine sediments, J. Microbiol. Methods 19 (1994) 59–66, https://doi.org/10.1016/0167-7012(94)90026-4.
[15] N. Qiu, X. Wang, F. Zhou, A new method for fast extraction and determination of chlorophylls in natural water, Z. Naturforsh. Sect. C J. Biosci. 73 (2018) 77–86, https://doi.org/10.1515/znc-2017-0157.
[16] J.F. Rontani, Photodegradation of unsaturated fatty acids in senescent cells of phytoplankton: photoproduct structural identification and mechanistic aspects, J. Photochem. Photobiol. A Chem. 114 (1998) 37–44, https://doi.org/10.1016/S1010-6030(98)00201-9.
[17] S.W. Wright, S.W. Jeffrey, R.F.C. Mantoura, C.A. Llewellyn, T. Bjørnland, D. Repeta, N. Welschmeyer, Improved HPLC method for the analysis of chlorophylls and carotenoids from marine phytoplankton, Mar. Ecol. Prog. Ser. 77 (1991) 183–196, https://doi.org/10.3354/meps077183.
[18] R. Eisert, J. Pawliszyn, Automated in-tube solid-phase microextraction coupled to high-performance liquid chromatography, Anal. Chem. 69 (1997) 3140–3147, https://doi.org/10.1021/ac970319a.
[19] Y. Moliner-Martinez, R. Herráez-Hernández, J. Verdú-Andrés, C. Molins-Legua, P. Campíns-Falcó, Recent advances of in-tube solid-phase microextraction, TrAC Trends Anal. Chem. 71 (2015) 205–213, https://doi.org/10.1016/j.trac.2015.02.020.
[20] Y. Moliner-Martinez, A. Ballester-Caudet, J. Verdú-Andrés, R. Herráez-Hernández, C. Molins-Legua, P. Campíns-Falcó, In-tube solid-phase microextraction in solid-phase extraction, in: C.F. Poole (Ed.), Handbooks in Separation Science, Elsevier, Amsterdam, The Netherlands, 2020, pp. 387–427, https://doi.org/10.1016/B978-0-12-816906-3.00014-5. Chapter 14, pp.
[21] S. Cortés-Bautista, R. Navarro-Utiel, A. Ballester-Caudet, P. Campíns-Falcó, Towards in field miniaturized liquid chromatography: biocides in wastewater as a proof of concept, J. Chromatogr. A 1673 (2022), 463119, https://doi.org/10.1016/j.chroma.2022.463119.
[22] H. Kataoka, In-tube solid-phase microextraction: current trends and future perspectives, J. Chromatogr. A 1636 (2021), 461787, https://doi.org/10.1016/j.chroma.2020.461787.
[23] P. Serra-Mora, N. Jornet-Martínez, Y. Moliner-Martínez, Campíns-Falcó, In tube-solid phase microextraction-nano liquid chromatography: application to the determination of intact and degraded polar triazines in waters and recovered struvite, J. Chromatogr. A 1513 (2017) 51–58, https://doi.org/10.1016/j.chroma.2017.07.053.
[24] J. Pla-Tolós, P. Serra-Mora, L. Hakobyan, C. Molins-Legua, Y. Moliner-Martinez, P. Campins-Falcó, A sustainable on-line CapLC method for quantifying antifouling agents like irgarol-1051 and diuron in water samples: estimation of the carbon footprint, Sci. Total Environ. 569-570 (2016) 611–618, https://doi.org/10.1016/j.scitotenv.2016.06.18.
[25] A. Ballester-Caudet, P. Campíns-Falcó, B. Pérez, R. Sancho, M. Lorente, G. Sastre, C. González, A new tool for evaluating and/or selecting analytical methods: summarizing the information in a hexagon, TrAC Trends Anal. Chem. 118 (2019) 538–547, https://doi.org/10.1016/j.trac.2019.06.015.
[26] J. Jiménez, L. De la Cruz, J. Carballo, A. Domenech, Enfoques metodológicos para el cálculo de la Huella de Carbono, OSE Estudios Gráficos Europeos S.A., Spain, 2011.