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Fast quantitative analysis of *n*-alkanes, PAHs and alkenones in sediments

M. Raja [a,1], J. Villanueva [a,*], C. Moreu-Romero [a], M. Giaime [a], A. Rosell-Melé [a,b,†]

[a] Institut de Ciència i Tecnologia Ambientals (ICTA-UAB), Universitat Autònoma de Barcelona, Bellaterra, Catalonia, Spain
[b] Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Catalonia, Spain

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ABSTRACT

The study of different organic biomarker classes is essential to elucidate global Earth dynamics since different biogeochemical processes play a role in regulating environmental and climatic conditions. However, multiproxy analysis generally consists of labor-intensive and time-consuming methodologies, which hamper the study of a large number of samples. Here, we develop and validate a fast analytical method to quantify different classes of organic biomarkers (PAHs, *n*-alkanes and alkenones) in sediments. This new method sequentially extracts and fractionates the target compounds using a pressure liquid extraction (PLE) system, which allows us to selectively obtain the analytes by reducing the time of analysis, sample handling and solvent usage. We show that our method provides reproducible results and high recoveries (>70%), and can be applied to a wide range of sedimentary environments, such as oceanic basins, continental slope and shelf, and lakes. Moreover, the method provides reproducible estimates of paleoclimatic indices, such as the carbon preference index (CPI), the average chain length (ACL) and the $U^{K'}_{37}$-derived sea-surface temperature (SST). Therefore, this new method enables fast quantitative multiproxy analysis of oceanic, coastal and lake sediments.

1. Introduction

Sedimentary organic biomarkers are commonly studied in environmental and geological sciences as they provide valuable information on Earth system dynamics (Jaeschke et al., 2017; Luo et al., 2019; Francescangeli et al., 2020; López-Quirós et al., 2021). A range of compounds can potentially be analysed in the same unit of sediment to provide complementary information as environmental or climate proxies. This facilitates undertaking a paleo-multiproxy analysis, which is essential to elucidate and understand natural complex processes. However, it is common to find in the literature studies that are focused only on a few classes of compounds, such as *n*-alkanes, alkenones or PAHs (Bolton et al., 2010; Sarma et al., 2017; Martins et al., 2021; Synnott et al., 2021), rather than a wider range of available biomarker proxies. Arguably this is due to the need to generate data from a large number of samples and thus applying relatively simple methodologies.

Typically, the methodology to analyse different classes of biomarker proxies requires several procedural steps to address the different polarities of the target compounds (Jaeschke et al., 2017; Francescangeli et al., 2020; Bícego et al., 2021). Analyses begin with sediment extraction using organic solvents. The extraction is undertaken using either a Soxhlet apparatus, ultrasonic bath, microwave or PLE system. The resulting extracts require a clean-up or fractionation process using column chromatography packed with silica or alumina stationary phases, before instrumental analysis. Detection and quantification are commonly performed by gas chromatography (GC) and GC coupled to mass spectrometry (GC–MS). Consequently, conventional methodologies to analyse biomarker proxies are labor intensive, time consuming, and require high organic solvent usage.

PLE applies solvents at high pressure and temperature to efficiently extract analytes from the sample matrix. Selective pressurized liquid extraction (SPLE) combines extraction and clean-up in one step by incorporating adsorbents within the extraction cell (Runnqvist et al., 2010; Subedi et al., 2015). Under optimised conditions, the adsorbent selectively retains a fraction of matrix compounds while releasing the target analytes. Therefore, SPLE can produce extracts that do not require post-extraction purification procedures before instrumental analysis. SPLE has the potential to reduce sample handling and solvent consumption and can significantly increase laboratory productivity. There is a wealth of published SPLE methods designed to quantify chemicals at

* Corresponding author.
E-mail address: juan.villanueva@uab.cat (J. Villanueva).
[1] Present address: Business Partnership Unit, GSK Centre for Sustainable Chemistry, Triumph Road, Nottingham, UK.
[†] Prof, Antoni Rosell-Melé passed away in December, 2021.

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trace levels (pesticides, organohalide compounds, pharmaceuticals) in environmental and biological samples, and thorough reviews on PLE and SPLE methodologies are available (Runnqvist et al., 2010; Subedi et al., 2015; Alvarez-Rivera et al., 2020). In this study, we describe the development and validation of an SPLE method to simultaneously quantify different biomarker classes in marine and lacustrine sediments. We have focused our study on PAHs, *n*-alkanes and alkenones that have different polarities and yield complementary information as proxies. As part of the method validation, we evaluated its robustness to changes in the sedimentary matrix by repeatedly analysing a set of sediments from different depositional environments (open ocean, coastal and lacustrine).

2. Materials and samples

2.1. Solvents, reagents and standards

Methanol (MeOH), dichloromethane (DCM), *n*-hexane (Hex), acetone (Ac) and toluene were of GC analysis grade and purchased from Merck KGaA. Silica gel 60 for flash chromatography (0.04–0.06 mm, 230–400 mesh ASTM) was obtained from Scharlau, anhydrous Na_2SO_4 (99.5%) from VWR, and diatomaceous earth for ASE from Thermo Scientific. Prior to extraction, silica gel and anhydrous Na_2SO_4 were maintained during 24 h at 105 °C and cooled in a desiccator.

N,O-*bis*(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylsilyl (TMS) from Thermo Scientific was used to derivatize polar compounds (*n*-alcohols and sterols). Standards of PAHs (D12-perylene and D10-pyrene) were obtained from Supelco and Isotec, respectively. *n*-Alkane standards (hexatriacontane and eicosane) were purchased from Aldrich, and a ketone standard (2-nonadecanone) from Fluka. Standards of *n*-alcohol (1-heneicosanol) and sterol (5α-androstanol-3β-ol) were obtained from TCI and Sigma, respectively.

2.2. Samples

Sediment samples used to develop and validate the analytical method are listed in Table 1. Marine samples were retrieved in cruises EN651 (northern Tropical Atlantic) and MERS (Catalan Sea, Western Mediterranean). Lacustrine samples were obtained from Spanish lakes Lucenza (LUC) (Quiroga, Lugo) and Pedro Muñoz (PEM) (Mota del Cuervo, Cuenca), and Lake Hula from Israel (HUL).

Deep ocean samples EN651-ST04 and EN651-ST13 were obtained in the occidental and oriental basins of the northern tropical Atlantic Ocean, respectively, far from the direct influence of the continents. Marine coastal sediment MERS-ST10 was retrieved at 70 m from the continental shelf, close to the Spanish coast, whereas MERS-ST7 corresponds to the continental slope, and was obtained near the location of ST10.

PEM lake is located in the southeast of the Iberian Peninsula and the relatively high mean temperature (14 °C) and low mean precipitation (417 mm) of that region leads to a Mediterranean vegetation (mainly sclerophyll species). In contrast, LUC lake is located in the northeast of the Peninsula. This region presents relatively low mean temperature (9.5 °C) and high mean precipitation (1500 mm), which drives to a sub-Mediterranean vegetation (i.e. a mix of deciduous and sclerophyll species) (Ninyerola et al., 2005). The Hula valley is located in northern Israel. It is a 25 km long and 6–8 km wide valley at approximatively 70 m mean above sea level. It was occupied by a lake and marshes; the relative extent of each depending on climate and local hydrology. HUL lake, occupying the south of the valley was artificially dried in the 1950s. The region is characterized by high mean temperature (18 °C) and low mean precipitation (653 mm).

Table 1
Location of samples and type of sediment.

Sample	Type of sediment	Latitude	Longitude	Water depth (m)
EN651-ST04	Oceanic	5.00	–35.79	3993
EN651-ST13	Oceanic	10.36	–20.99	5130
MERS-ST7	Coastal	40.07	1.53	1476
MERS-ST10	Coastal	40.30	0.80	70
PEM	Lake	39.39	–2.88	–
LUC	Lake	42.59	–7.11	–
HUL	Lake	33.08	35.61	–

3. Method description

The analytical method described below corresponds to the final conditions used to test the analytical robustness. The conditions used for method development are discussed in the Results and discussion section.

Samples were freeze-dried and then homogenised with a Mixer Mill MM 400 (Retsch). PLE and clean-up were performed using an ASE-350 (Dionex Thermo) equipped with 10 mL extraction cells and 60 mL collection vials. We used activated silica as the stationary phase, and anhydrous Na_2SO_4 to remove water and filter residual particles. Thus, extraction cells were filled from bottom to top with a glass fibre filter, 3 g of activated silica, 1 g of anhydrous Na_2SO_4, 1–3 g of sediment, and diatomaceous earth to completely fill the cell (Fig. 1). On the top of the cell, 100 μL of an internal standard mixture was added for quantification. The composition of the internal standard mixture was hexatriacontane (C_{36}, 5 ng μL^{-1}), eicosane (C_{20}, 5 ng μL^{-1}), 2-nonadecanone (5 ng μL^{-1}), D12-perylene (0.2 ng μL^{-1}) and D10-pyrene (0.2 ng μL^{-1}). Blank samples were prepared similarly to sediment samples, but no sediment was packed in the cell.

Samples were extracted at 100 °C with hexane:dichloromethane (Hex:DCM) (4:3, v/v) using solvent saver mode for 10 min at 2 mL min^{-1}. This step renews the solvent mixture continuously during the extraction, at a controlled flow rate. This is different from the commonly used batch mode that executes several cycles of fill and release of solvents. This new mode can improve the capability of silica to retain compounds and also the efficacy of the clean-up process. Continuous renewal of solvents may also optimise extraction recovery. The resulting extracts were concentrated under a nitrogen stream at 30 °C using TurboVap System (Biotage) and transferred to vials with DCM. Extracts were derivatized with 50 μL of BSTFA and 50 μL of DCM at 70 °C during 1 h prior to instrumental analysis. Derivatized extracts were evaporated using a centrifugal evaporator (miVac) coupled to a cold trap (Genevac) and redissolved in 20 μL of toluene with 5% BSTFA.

For the quantification of *n*-alkanes and PAHs, the extracts were

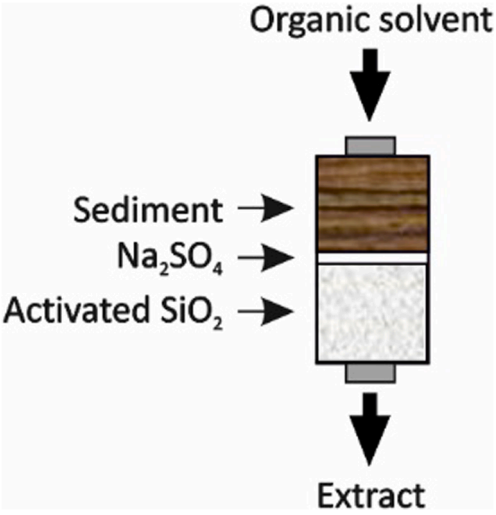

Fig. 1. Pressurized liquid extraction (PLE) packed cell.

analysed using a GC–MS system (Agilent Technologies 5975C) equipped with a DB-5 ms (30 m × 0.25 mm, 0.25 μm stationary phase). The GC carrier gas was helium at a flow of 1.5 mL min^{-1}. The injection was performed in split-less mode (50 s) at 310 °C. The oven temperature program was as follows: 1 min at 70 °C followed by a gradient of 15 °C min^{-1} to 160 °C, a second gradient to 310 °C at 6 °C min^{-1}, and isothermal elution at 310 °C during 15 min. MS acquisition was performed in single ion monitoring (SIM) mode to improve sensitivity and selectivity (Table 2). Quantitation of the different compound classes was obtained using the peak area ratios between the analyte and its associated internal standard as detailed in Table 2.

The quantitation of total C_{37} alkenones and the measurement of the alkenone unsaturation ratio $U^{K'}_{37}$ was performed using a GC-FID system equipped with a capillary column HP-1 (60 m × 0.25 mm, 0.25 μm stationary phase) and 3 mL min^{-1} of hydrogen as carrier gas. The temperature gradient used to separate the alkenones consisted of a multistep gradient: initial temperature at 80 °C during 1 min, gradient to 120 °C at 30 °C $min^{-1}$and a second ramp to 320 °C at 6 °C min^{-1}, followed by an isocratic elution at 320 °C during 21 min. Sample injection was performed at 320 °C with the split closed for 50 s. Quantification of alkenones was performed using hexatriacontane as internal standard.

4. Results and discussion

4.1. Method development and optimisation

SPLE method development aims at finding the optimum extraction conditions to achieve quantitative analyte recovery while minimizing matrix interferences in the final extract. In this study, we used long-chain *n*-alcohols and sterols as representative of polar interferences in the sedimentary matrix. The recovery of non-polar analytes (*n*-alkanes, PAHs, alkenones) and the elimination of polar interferences was investigated by spiking sediment samples with the following standards: *n*-alkanes (C_{20}, C_{32}, C_{34}, and C_{36}), PAHs (D10-pyrene, D12-perylene and retene), ketone (2-nonadecanone), *n*-alcohol (1-heneicosanol), and sterol (5α-androstan-3β-ol). Accordingly, the criteria to optimise extraction conditions were both maximum analyte recovery (*n*-alkanes, ketones, and PAHs) and minimum recovery of interfering polar compounds. We have used *n*-alcohols and sterols as tracers for polar compounds in the final extract, assuming that the removal of these lipids of medium polarity will ensure an adequate removal of other polar interfering compounds. Recoveries of the analytes of interest were measured in duplicate to assess the method reproducibility obtained using different solvent mixtures.

Optimisation of SPLE conditions in the ASE350 solvent saver mode includes the following parameters: solvent composition, temperature, solvent flow and extraction time. Several studies have investigated the retention capacity of different hydrophilic adsorbents (alumina, Florisil™ and silica gel) and concluded that silica gel has the greatest retention capacity (Lundstedt et al., 2006; Osman and Saim, 2012; Subedi et al., 2015). Hence, we assumed that silica gel is the optimum adsorbent to selectively extract compounds of interest and we focused on the optimisation of the remaining parameters.

4.1.1. Selection of SPLE solvent mixture composition

Several solvent combinations were tested to maximize analyte recoveries and minimize recoveries of polar compounds: (a) Hex:DCM, (b) Hex:Ac, and (c) DCM:MeOH. For each combination, several mixtures with variable proportions of the polar solvent component (DCM, Ac, and MeOH) were studied. The remaining experimental parameters were maintained constant (extraction temperature of 100 °C, extraction in solvent saver mode at 1 mL min^{-1} during 10 min). Extraction tests were performed with both spiked blanks and marine sediment MERS-ST7. Extraction cells were replenished with diatomaceous sand to minimize dead volume when needed.

Recoveries of spiked blank samples provided information on the retention capacity of silica gel in the absence of a sedimentary matrix. As detailed in Fig. 2a–c, both *n*-alkanes and PAHs are not retained by silica gel when using Hex:DCM and Hex:Ac mixtures. Ketones require low volumes of DCM (29%) or Ac (17%) to elute from the silica gel adsorbent. However, DCM:MeOH solvent mixtures provided inadequate and highly irreproducible results (Fig. 2). Severe reproducibility problems were observed at MeOH contents higher than 20%, where recovery of *n*-alkanes, ketones and PAHs varied randomly between 0% and 100%. In contrast, polar interfering compounds (Fig. 3a,b) display a different distribution pattern, with higher recoveries with DCM:MeOH and Hex:Ac solvent mixtures. Hex:DCM mixtures provided erratic (*n*-alcohols) or low (sterols) recoveries. Accordingly, silica gel can partially retain aliphatic *n*-alcohols and sterols when using Hex:DCM as elution solvent mixtures.

Recoveries of spiked samples containing both sediment and silica gel provided similar patterns as spiked blank samples (Fig. 2d–f and 3c,d). Thus, recoveries higher than 80% for our target compounds were obtained when using Hex:DCM and Hex:Ac solvent mixtures. Again, the use of polar DCM:MeOH solvent mixtures resulted in generally irreproducible and low recoveries of *n*-alkanes, PAHs and ketones. In contrast, polar compounds were quantitatively recovered with Hex:Ac and DCM:MeOH solvent mixtures. Quantitation in samples obtained with a high proportion of MeOH (>30%) was complicated due to the co-extraction of abundant sedimentary components. Sediment extracts obtained with DCM/MeOH contained abundant components that affected the GC–MS analysis, resulting in irreproducible and unreliable calculated recoveries (Fig. 2). An exception to the general pattern was D12-perylene, which could not be recovered from the sedimentary matrix by any of the tested solvents (dashed lines in Fig. 2). Low recoveries of D12-perylene were systematically observed in all tested sediments (data not shown) and could not be related to losses during sample processing (see Fig. 2b). Accordingly, low recoveries are

Table 2
MS parameters used in biomarkers quantification.

Biomarker class	Compound	Internal Standard (IS)	*m/z* (IS *m/z*)
n-Alkanes	C_{21}–C_{35}	C_{36}	99 (99)
PAHs	Perylene (Per)	D12-perylene	252 (264)
	Benzo[*a*]pyrene (BaP)	D10-pyrene	252 (212)
	Benzo[*e*]pyrene (BeP)		
	Benzo[*b*]fluoranthene (BbF)		
	Benzo[*k*]fluoroanthracene (BkF)		
	Pyrene (Pyr)	D10-pyrene	202 (212)
	Fluoranthracene (Flu)		
	Indenopyrene (Ind)	D10-pyrene	276 (212)
	Benzo[*g,h,i*]perylene (BghiP)		
	Dibenzoanthracene (DBA)	D10-pyrene	278 (212)
	Retene (Ret)	D10-pyrene	234 (212)
	Benz[*a*]anthracene (BaA)	D10-pyrene	228 (212)

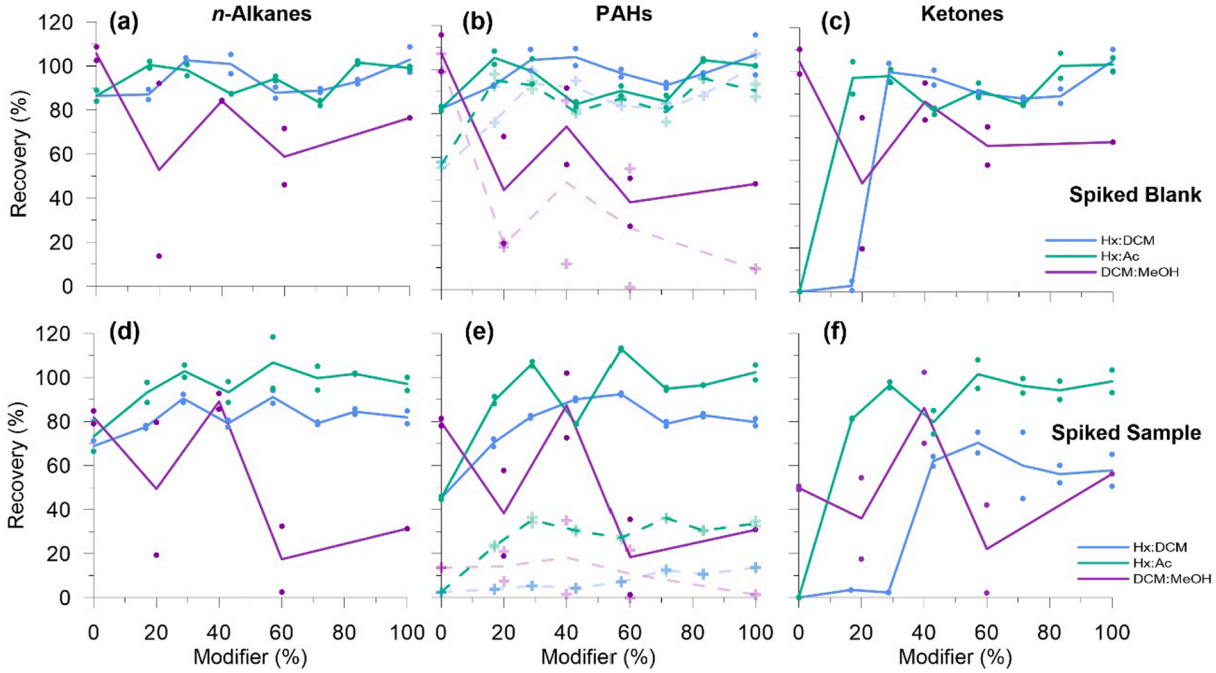

Fig. 2. Recoveries of biomarkers in spiked blank (a–c) and sediment (d–f) samples as a function of the extraction solvent composition. The x-axis indicates the percent of modifier used in each test: DCM (blue), acetone (green), and methanol (purple). Results obtained for D12-perylene were different from the other PAH standards and are represented by dotted lines. Experiments were performed in duplicate (see dots). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

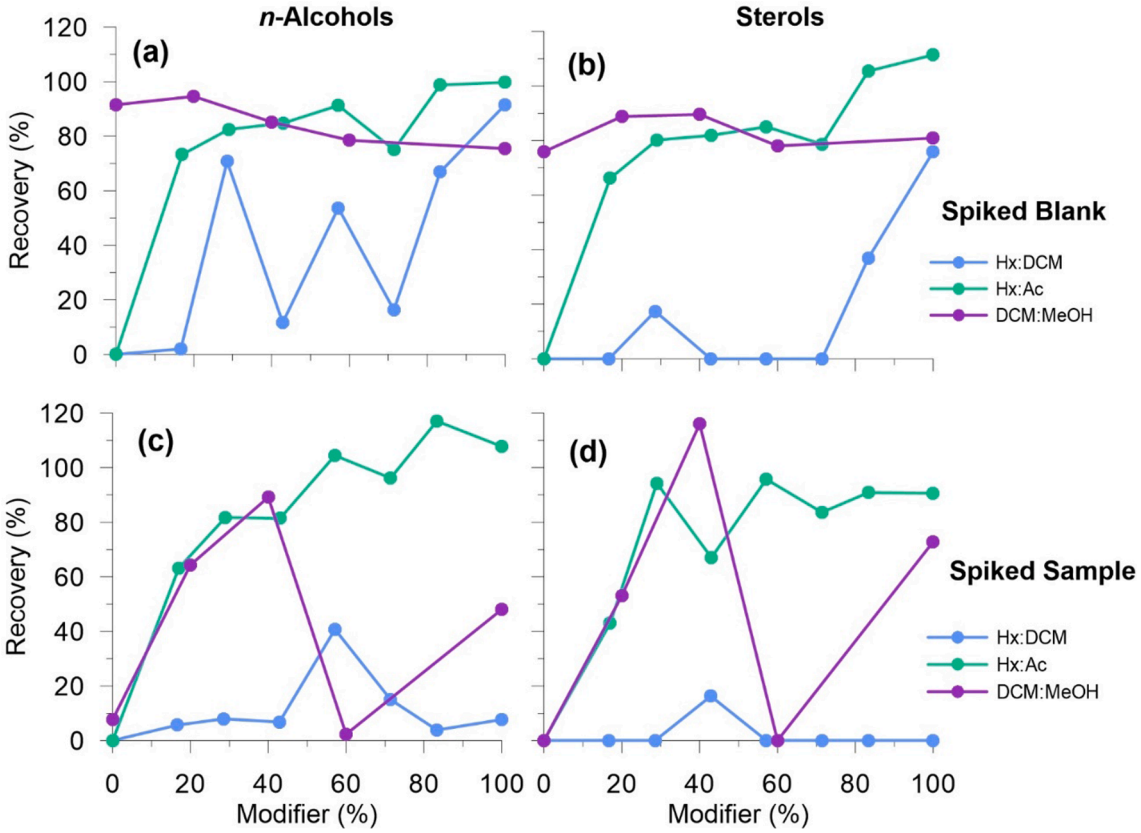

Fig. 3. Recoveries of interfering polar compounds in spiked blank (a,b) and sediment (c,d) samples.

probably caused by an unusually strong affinity of this compound to the sedimentary matrix.

Overall, most Hex:DCM and Hex:Ac solvent mixtures provide good and reproducible recoveries (80–110%) of our target compounds. However, the retention capability of silica gel is not enough to retain interferences (*n*-alcohols and sterols), when using polar Ac as a modifier

Table 3
Optimisation of SPLE conditions and concentration ratios (CR).

Test #	Temp. (°C)	Time (min)	Solvent Flow (mL min^{-1})	CR *n*-Alkanes	CR PAHs	CR Ketones	CR *n*-Alcohols	CR Sterols
1	75	5	1	56	49	36	**14**	**0**
2	75	5	2	**88**	**85**	67	30	**1**
3	75	10	1	**83**	**77**	48	**15**	**0**
4	75	10	2	**92**	**96**	**93**	60	**1**
5	100	5	1	45	43	**14**	**4**	**0**
6	100	5	2	**93**	**91**	56	**23**	**0**
7	100	10	1	**90**	**90**	59	**20**	**0**
8	100	10	2	**94**	**95**	**92**	57	**1**
9	150	5	1	71	66	**20**	**6**	**0**
10	150	5	2	**96**	**99**	**85**	74	**4**
11	150	10	1	**89**	**88**	55	49	**8**
12	150	10	2	**93**	**100**	**97**	**92**	**85**
13	200	5	1	**97**	**99**	**89**	**84**	65
14	200	5	2	**95**	**95**	**79**	**76**	48
15	200	10	1	**94**	**100**	**93**	**90**	**95**
16	200	10	2	**97**	**100**	**99**	**95**	**100**

(Fig. 3). Therefore, the optimum balance between recoveries of the compounds of interest and retention of interfering polar compounds was established at Hex:DCM (4:3, v/v). These results also indicate that moderately polar components of the sedimentary matrix, such as *n*-alcohols and sterols may be selectively recovered in a second extraction using Hex:Ac mixtures.

4.1.2. Optimisation of SPLE solvent flow rate, temperature and time

We performed a set of 16 experiments designed to identify the optimum temperature, time, and solvent flow rate to quantitatively extract the compounds of interest and minimize the presence of polar compounds in the extract (Table 3). For all tests, we extracted the sediments twice. The first fraction was obtained with Hex:DCM (4:3, v/v) to elute non-polar compounds, and the second fraction was obtained with Hex:Ac (1:1, v/v) to extract polar compounds. The separation of the five compound classes (*n*-alkanes, PAHs, ketones, *n*-alcohols and sterols) among the two fractions is expressed by the concentration ratio (CR) between the first fraction and the total recovery (CR = Fr1/(Fr1 + Fr2) × 100) and is shown in Table 3. Hence, a CR close to 100 is associated with high recoveries in the first fraction, whereas a CR close to 0 indicates high recoveries in the second fraction. Ideally, compounds of interest should be recovered in the first fraction and show high CR values, while interfering polar compounds should be eluted in the second fraction and present low CR values.

Our results show that high temperature (>150 °C) provides high CR (>75%) for all compound classes (Table 3), indicating that both polar and non-polar compounds are eluted in the first fraction. In contrast, lower temperatures generally present high CR for the target compounds (>75%) and low CR for interferences (<25%). Our data also show that increasing time and solvent flow rate enhance the CR of the analytes. Hence, we selected 100 °C, 10 min and 2 mL min^{-1} as the optimum conditions for temperature, time and solvent flow, respectively. These conditions allow us to quantitatively recover all target compounds in the first fraction and minimize the presence of polar interferences.

4.2. Method validation

The analytical procedure was developed to identify quantitative changes in the sedimentary biomarker composition, which helps to elucidate past environmental changes. In order to measure the capability to detect concentration changes, we measured the analytical reproducibility and robustness to quantitate C_{21}–C_{33} *n*-alkanes, PAHs, and C_{37} alkenones. We also studied the reproducibility of some indices commonly used to investigate past sedimentary conditions, such as the alkenone $U^{K'}_{37}$- and *n*-alkane CPI and ACL indices. To this end, we performed six analytical runs on different days, distributed within a time period of 3 months to account for temporal changes in the laboratory setup. Moreover, the analytical robustness to changes in the sample matrix was assessed by comparing reproducibility and internal standard recoveries in samples of different sedimentary environments (lacustrine, coastal marine, and pelagic ocean, see Table 1). The results of the method validation have been summarized in Table 4 and Figs. 4 and 5.

Coefficient of variation (CV) of C_{21}–C_{33} *n*-alkanes ranged between 3.4% and 12.5%, with higher values in lacustrine sediments (10.6–12.5%) compared to marine and oceanic sediments (3.4–6.5%). Similar analytical uncertainty was obtained irrespective of total C_{21}–C_{33}

Table 4
Summary of data obtained during method validation. Concentration values are expressed in ng/g and sea-surface temperatures in °C.

	Oceanic sediment (EN651_ST04)				Coastal sediment (ST7)				Lacustrine sediment (PEM)			
	n-Alkanes	PAHs	Alkenones	s.d.	*n*-Alkanes	PAHs	Alkenones	s.d.	*n*-Alkanes	PAHs	Alkenones	s.d.
Sum	1.07×10^3	0.86	39	–	1.04×10^3	126	114	–	1.84×10^3	10.1	<LOD	–
CV (%)	3.4	9.7	21.3	–	3.9	17.1	17.3	–	12.5	9.7	n.d.	–
$U^{K'}_{37}$	–	–	0.768	0.022	–	–	0.540	0.019	–	–	n.d.	n.d.
SST	–	–	16.7	0.5	–	–	11.5	0.4	–	–	n.d.	n.d.
CPI	5.7	–	–	0.34	5.8	–	–	0.45	7.0	–	–	0.68
ACL	29.9	–	–	0.05	30.1	–	–	0.03	29.6	–	–	0.21
	Oceanic sediment (EN651_ST13)				**Coastal sediment (ST10)**				**Lacustrine sediment (LUC)**			
	n-Alkanes	PAHs	Alkenones	s.d.	*n*-Alkanes	PAHs	Alkenones	s.d.	*n*-Alkanes	PAHs	Alkenones	s.d.
Sum	2.73×10^3	3.88	796	–	1.23×10^3	95.5	96.4	–	22.3×10^3	63.3	<LOD	–
CV (%)	6.5	21.1	17.2	–	4.3	8.8	16.2	–	10.6	20.4	n.d.	–
$U^{K'}_{37}$	–	–	0.897	0.009	–	–	0.587	0.064	–	–	n.d.	n.d.
SST	–	–	19.6	0.2	–	–	12,6	1.5	–	–	n.d.	n.d.
CPI	6.2	–	–	0.30	5.3	–	–	0.11	5.5	–	–	0.32
ACL	30.0	–	–	0.04	30.3	–	–	0.03	29.6	–	–	0.03

s.d.: standard deviation. LOD: limit of detection. n.d.: not detected.

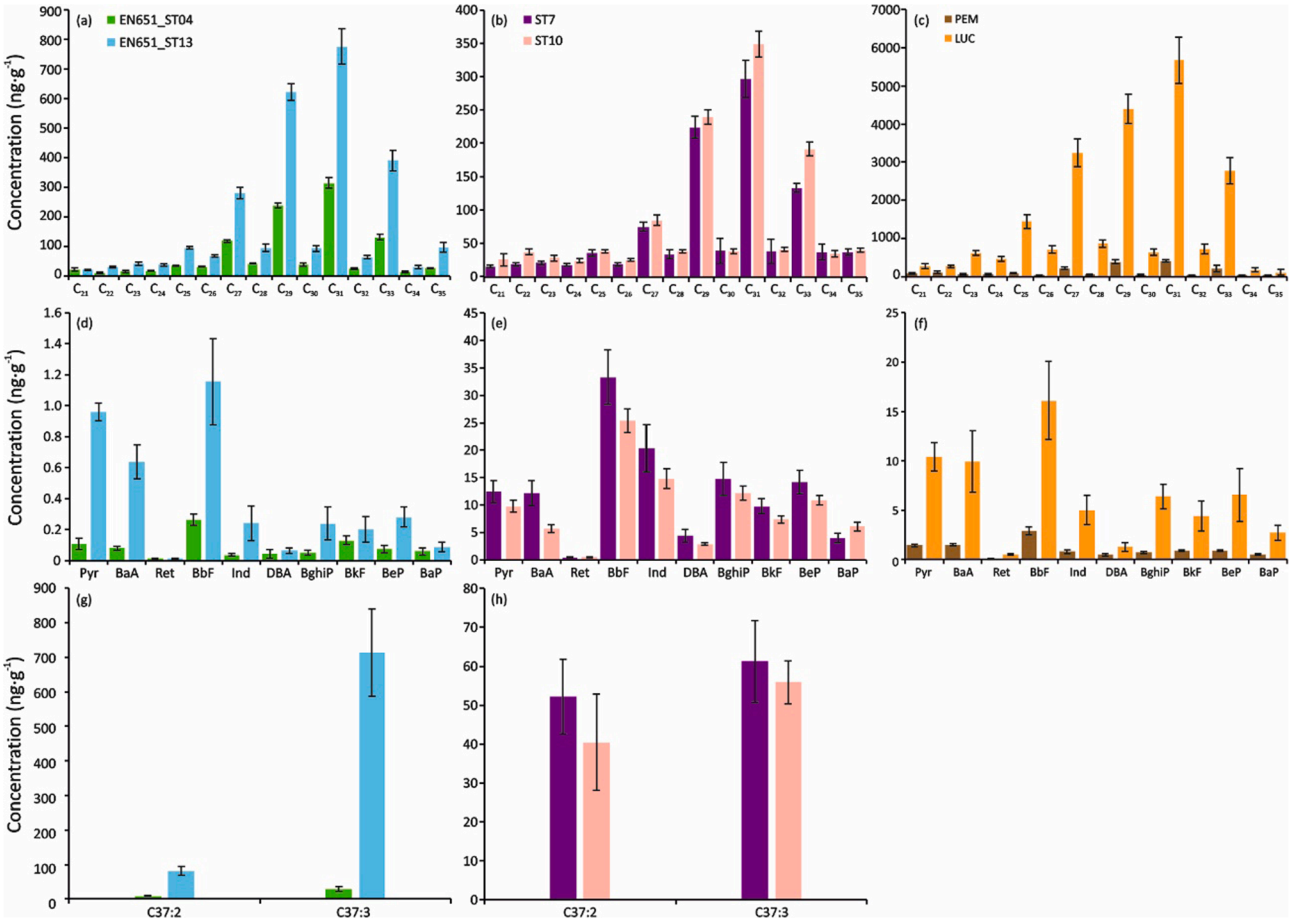

Fig. 4. Concentration of *n*-alkanes (a–c), PAHs (d–f) and alkenones (g,h) in oceanic (EN651_ST04 and EN651_ST13), coastal, (ST7 and ST10), and lake (PEM and LUC) sediments. The error bar indicates the standard deviation calculated from six replicates.

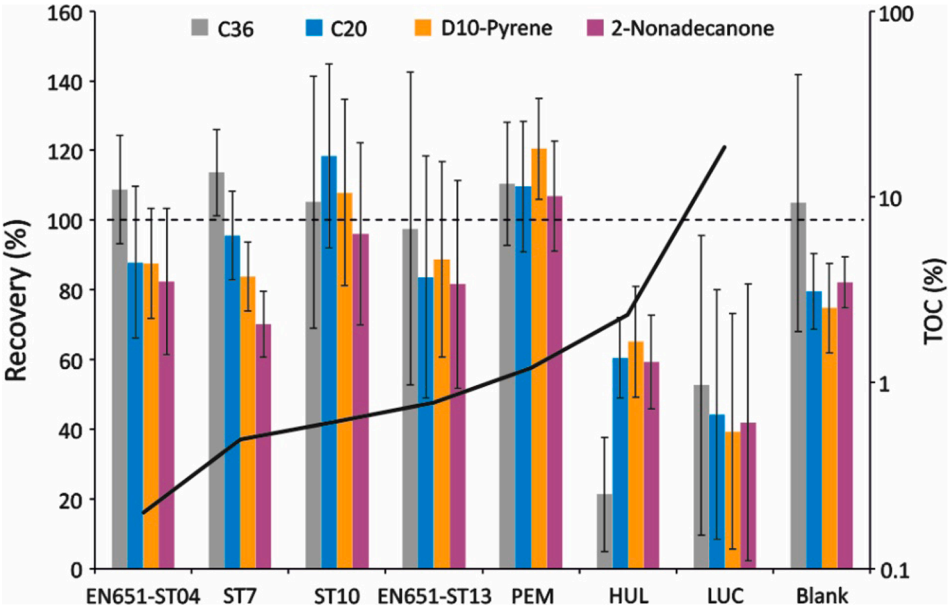

Fig. 5. Recoveries averages of internal standard of different biomarker compounds (n = 6) in oceanic (EN651_ST04 and EN651_ST13), coastal, (ST7 and ST10), and lake (PEM, HUL and LUC) sediments. The black line shows the sedimentary total organic carbon (TOC) content.

n-alkanes concentration, which ranges from 1.04 to 22.3 μg g^{-1}. Remarkably, pelagic sediment samples presented similar concentration values (1.07–2.73 μg g^{-1}) compared to coastal (1.04–1.23 μg g^{-1}) and lacustrine sediment samples (PEM lake, 1.84 μg g^{-1}). Good reproducibility was also obtained for the lacustrine sample LUC in spite of the exceptionally high *n*-alkane content (22.3 μg g^{-1}). The analytical

method also provided reproducible CPI and ACL indexes. Estimated CPI values ranged from 5.3 to 7.0 with standard deviations within 0.11–0.68. These values are representative of *n*-alkanes distributions associated with higher plants (Fig. 4). All samples provided similar ACL indices (29.6–30.3), within an analytical uncertainty of 0.03–0.21.

Obtained PAH concentrations were 3 orders of magnitude lower than *n*-alkanes (0.86–126 ng g^{-1}, Table 4). In addition, all sediments showed marked differences in both PAH concentration and the distribution of individual compounds (Fig. 4). Despite these large differences in composition, the analytical uncertainty remained within acceptable limits (CV = 8.8–21%) for all samples.

In spite of being relatively abundant in marine sediments (39–800 ng g^{-1}), the analytical uncertainty in the quantitation of total C_{37} alkenones was higher than for *n*-alkanes and PAHs (CV = 16.2–21.2%). This could be related to small differences in the recovery of the internal standard (C_{36} *n*-alkane) and the alkenones during the extraction process. However, the alkenone-derived $U^{K'}_{37}$ index provided reproducible SST estimates within 0.5 °C uncertainty, in concordance with previous studies (Villanueva and Grimalt, 1997). The high uncertainty observed in coastal sample ST10, collected at 70 m water depth, is related to the presence of interfering compounds in the GC-FID profile. Although alkenones are present in most marine sediments, they are not always detected in lacustrine sediments (Zink et al., 2001). Therefore, the absence of alkenones in PEM and LUC samples likely reflects the absence of alkenone-producing organisms in the related lacustrine environments.

Internal standards (IS) are used to correct for sample losses during the analytical procedure. In addition, the analysis of IS recoveries can provide information on the procedural efficiency and therefore, can identify potential systematic deviations in the quantitative measurements (Runnqvist et al., 2010; Subedi et al., 2015). Averaged IS recoveries for marine sediment samples ranged between 70% and 120%, indicating limited losses during sample processing (Fig. 5). Changes in analyte recoveries were adequately compensated by IS, resulting in global analytical uncertainties below 22% for all compound classes. However, lower IS recoveries (20–65%) were obtained in lake sediments with high total organic carbon concentrations (HUL 2.3% and LUC 18%, Fig. 5). These low recoveries were concurrent with the abundance of polar interfering compounds (sterols, *n*-alcohols, fatty acids and esters) in the final extract, as detected by GC–MS (data not shown).

In particular, HUL sample corresponds to shallow lake sediments that have not been subject to extensive post-depositional diagenesis. In addition, the extractable lipid composition of LUC sediment contained very high amounts of fatty acids and esters that masked *n*-alkane and PAH signals (data not shown). These results prompt the conclusion that silica gel was unable to retain and fractionate the extracted polar constituents, producing extracts that were unfit for quantitative analysis. Therefore, the analytical setup may produce reproducible, but inaccurate results when applied to shallow lake sediments with high organic carbon content (>2%). Reliable analysis of these organic-rich lake samples requires method modification that involves extensive clean-up processes to achieve successful interference removal and higher extraction recovery of the analytes.

The recovery of D12-perylene was systematically low for all tests performed with sediment samples, irrespective of the extraction conditions (solvent composition, extraction temperature and extraction time). The fact that blank samples produced good D12-perylene recoveries (Fig. 2b) indicates that perylene losses are not related to irreversible adsorption to silica nor losses during sample handling. Published analytical procedures that use PLE to extract PAH from sediments do not report recoveries of D12-perylene nor quantitate perylene (Martinez et al., 2004; Liguori et al., 2006; Choi et al., 2014; Gauchotte-Lindsay et al., 2014; Net et al., 2014; Magill et al., 2015). In contrast, good recoveries of D12-perylene have been reported for fish and soil samples (Lundstedt et al., 2006; Lund et al., 2009). Accordingly, low perylene recoveries are probably related to a strong affinity between this compound and unknown components of the sedimentary matrix.

5. Comparison with other methods

A number of studies have described significant differences in the concentration values among extraction methodologies (Saim et al., 1997; Hawthorne et al., 2000; Flotron et al., 2003; Wang et al., 2007; Lau et al., 2010; Xia et al., 2021). In particular, PLE and Soxhlet systems tend to produce relatively higher concentration values than ultrasonic-assisted (UAE) or supercritical fluid assisted (SFE) methods (Saim et al., 1997; Calvo et al., 2003; Wang et al., 2007; Lau et al., 2010), suggesting a higher extraction recovery. SFE selectively extracts apolar compounds and therefore produces cleaner extracts with a relatively lower recovery (Hawthorne et al., 2000). Differences in extraction recoveries and/or matrix effects complicate the direct comparison of quantitative values obtained using different procedures. Accordingly, we have not performed a direct comparison of the quantitative data produced by the SPLE method and other technologies. However, using spiked standards, we have demonstrated that the SPLE procedure quantitatively recovers extractable *n*-alkanes, PAHs, and aliphatic ketones. The estimated recoveries for all tested internal standards (70–120%) are similar to reported values (50–120%) using different methodologies (Berset et al., 1999; Hawthorne et al., 2000; Flotron et al., 2003; Martinez et al., 2004; Haskins et al., 2011; Choi et al., 2014; Xia et al., 2021). Also, the analytical reproducibility of the SPLE method (CV between 2% and 21%) is also comparable to other methodologies based on different extraction technologies, that report CV values between 3% and 35% (Saim et al., 1997; Berset et al., 1999; Hawthorne et al., 2000; Calvo et al., 2003; Flotron et al., 2003; Martinez et al., 2004; Lundstedt et al., 2006; Li et al., 2007; Wang et al., 2007; Lau et al., 2010; Choi et al., 2014; Magill et al., 2015; Subedi et al., 2015).

Sediment extracts produced by SPLE are free of interfering compounds and are adequate for high throughput routine quantitation of *n*-alkanes, PAHs and alkenones. The resulting extracts may contain other compounds, such as long-chain *n*-alcohols, and therefore extract derivatization with BSFTA is recommended prior to GC–MS analysis. However, selectivity of GC–MS detection in SIM mode adequately removes many potential interferences. According to our experience, other generally abundant components of the extractable lipidic fraction, such as fatty acids and sterols, are quantitatively retained in the silica gel during the extraction procedure. However, the processing method may not be adequate for samples with very high TOC content (>2%). Recoveries in organic-rich sediments dropped to 30–70%, indicating a reduced performance in samples with high TOC. These samples typically contain high concentrations of polar interfering extractable compounds (fatty acids, esters, alcohols, etc.) that can overload the silica gel adsorption capacity. These situations typically result in decreased IS recoveries, indicating incomplete sample extraction and/or purification (i.e. samples LUC and HUL). Nevertheless, this can be overcome by reducing the mass of extracted sediment or by implementing additional post-extraction purification protocols. Therefore, potential biases in the analytical accuracy can be detected by implementing systematic controls on the internal standard recovery. Alternatively, systematic detection of polar compounds, such as fatty acids, in the final extract provides another means to identify defective sample clean-up processes. Both indicators can be used to control the quality of the extraction process on a sample-to-sample basis.

6. Conclusions

We have developed and validated a fast analytical selective pressurized liquid extraction (SPLE) method for quantitative analysis of different organic biomarkers classes, including *n*-alkanes, PAHs and alkenones, in sediments. Overall, analytical reproducibility and recovery of the proposed SPLE method is similar to other analytical

technologies, such as Soxhlet, MAE (microwave-assisted extraction), UAE and SFE. The main advantage of the proposed SPLE method is that sample handling has been significantly reduced by combining extraction and clean-up processes using a SPLE system. This represents a significant reduction in solvent consumption and sample manipulation, with the consequent reduction in cost and environmental impact. The use of automatable SPLE systems also has improved the analytical reliability and diminished accidental sample losses during handling. Therefore, time required for sample processing is significantly reduced and, according to our experience, a trained user can routinely process and quantify 40–50 samples per week. The main drawback of the SPLE method is that commercial SPLE systems are expensive and require regular maintenance that make them unaffordable unless the analysis of a high number of samples is projected. This is the case of studies directed to elucidate the evolution of past environments. This methodology has been optimized to meet the requirements of high throughput, robustness, and small sample size associated with the stratigraphic analysis of sediment cores for paleoclimatic reconstructions. We have also demonstrated that the methodology provides reproducible data of paleoenvironmental indexes, such as SST, CPI and ACL.

CRediT authorship contribution statement

M. Raja: Writing – original draft, Supervision. **J. Villanueva:** Writing – original draft, Methodology, Supervision. **C. Moreu-Romero:** Investigation. **M. Giaime:** Investigation. **A. Rosell-Melé:** Funding acquisition.

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.

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