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Optimisation of focused ultrasound extraction (FUSE) and microwave-assisted extraction (MAE) of hydrocarbons in geological chert samples

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ABSTRACT

The analysis of hydrocarbons in chert rocks provides a worthwhile source of information regarding the geochemical features of a depositional setting. Since the typical analytical procedure requires long Soxhlet extractions and the use of large quantities of sample (30–50 g), in this work we have optimised the focused ultrasound extraction (FUSE) and the microwave-assisted extraction (MAE) to make available a less severe procedure. In both cases a full experimental design including solvent mixture composition (Dichloromethane/Hexane/Acetone) and process variables (sonication time and cycles, and extraction temperature and time) by means of D-optimal designs. In the extracted fractions hydrocarbons (C_{16}–C_{40}) were analysed by gas-chromatography-mass spectrometry. In the case of FUSE the process variables were the most sensitive variables and the optimum conditions were defined at 60:40 DCM/Hex mixture and a sonication time of 30 min and 9 cycles. In the case of MAE all the variables shown a significant effect on the extraction yield and the most adequate conditions (60:30:10 DCM/Hex/Ace mixture and an irradiation time of 15 min at 110 °C) were established from the analysis of the response surface. Both methods were systematically applied with different chert samples collected in Cucho (Trebiño County, Burgos, Spain) and we were able to assure quantitative extractions (>85%) in the first extraction. Additionally, from the distribution patterns of n-alkanes obtained in the different chert samples (nodular chert, laminar chert and massive-brechoid chert) collected in Cucho, we were able to distinguish different origins and diagenetic history.

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1. Introduction

The origin of organic matter in cherts, one of the oldest siliceous sediments, is still an ongoing debate [1,2]. Cherts are amongst the oldest sedimentary rocks on Earth and offer a remarkable record of well-preserved microfossils which gives clues to understand the origins of life on our planet [3]. Besides the information achieved from the study of microfossils, the composition and distribution of organic carbon preserved in sedimentary rocks can provide powerful information about the geochemical characteristics of the depositional setting [4]. In this sense, organic geochemistry is focused on the identification of aliphatic hydrocarbons and especially on molecular biomarkers which provide valuable information about the original organic matter and the transformations that took place during sedimentation (oxidation, biodegradation and transport) and diagenesis [5,6,7]. Geochemical analyses provide the tools to distinguish between different geological cherts and, by analogy, the geochemical features of archaeological chert items can be predicted.

Although the analysis of preserved organic matter presents unique challenges, cherts generally exhibit low contents of organic matter due to the intense oxidation processes associated with chert formation [8]. In those cases in which organic matter have been preserved as syndepositional organic matter, very little is present as extractable organic matter, basically consisted of n-alkanes, methylalkanes and complex polycyclic biomarkers such as hopanes and steranes [4,7]. The extractable organic matter in such trace concentration implies a careful extraction process and accurate analytical procedure due to that even slight contamination would compromise the obtained results. In this sense, some authors have focused their work mainly in the study of cross-contamination during the organic extraction procedure [9]. Furthermore, extractable organic compounds tend to get trapped in mineral clay matrix or to be adsorbed in the complex macromolecular insoluble kerogen, thus, the efficient solvent extraction is highly demanding.

The characterisation of many geological and particularly in archaeological samples is typically accomplished through the use of non-invasive methods such as X-ray fluorescence, FT-IR or Raman

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spectroscopy, often combined with microscopic methods [10,11]. However, owing to the required sensitivity and detection limits we have found these options rather limited. The use of destructive or invasive methods implies the isolation of organic traces from such hard matrix, which definitely implies a long process with several iterative steps. According to the literature, classically long extraction procedures using conventional Soxhlet extraction are used. This extraction method requires a large amount of sample (100–150 g) and long time of extraction (48–72 h) [12,13]. Extraction based on sonication has been used by Sherman et al. but also requires 50 g of sample [9]. The use of such large amount of sample makes these methods not suitable for archaeological purposes and thus, the development of highly effective extraction methods and sensitive measurement techniques are required. In this sense, several approaches such, supercritical fluid extraction (SFE) [14], pressurised liquid extraction (PLE) [15,16] ultrasound-assisted extraction (USE) or focused ultrasonic-assisted extraction (FUSE) [17] and microwave-assisted extractions (MAE) [18] have been investigated to enhance the extraction process. By far, the last option has been the most widespread one over the last 10 years in order to extract organic and inorganic compounds in several matrices due to its highlighted features [19–21].

In addition to the microwave radiation, ultrasound energy has been also used to accelerate the extraction of both organic and inorganic compounds from several matrices. In contrast to the poor irradiation power of ultrasound bands, focused ultrasound system provides 100 time higher irradiation power and also higher reproducibility and efficiency. The ultrasound energy is focused in the tip of a probe, typically made in titanium, which is immersed in the solution to be irradiated. Use of focused ultrasound produces relatively short, quantitative and reproducible extraction of organic compounds from different solid samples [22,23].

The aim of this work was the approach of a methodology for the extraction of organic compounds from chert samples in order to reduce the extraction time and the amount of sample required by classical Soxhlet extraction. For this purpose, microwave-assisted extraction (MAE) and focused ultrasound extraction (FUSE) were carried out. The results obtained have allowed us to propose another organic isolation method which requires lower extraction times with the minimal sample amount. These characteristics make the extraction method to be suitable also for the analysis of archaeological chert remains.

2. Materials and methods

2.1. Studied material

The rock samples used to test the new technique of organic extraction are geological cherts collected from lacustrine–palustrine carbonate sediments near the locality of Cucho (*Trebiño County*, *Burgos*, *N Spain*). The host formation is an up to 85 m-thick succession of alternating limestones, marlstones and clays that was deposited during the Miocene. The sedimentological features and internal stratigraphic arrangement of the lacustrine succession were established by Tarriño [24]. According to this work, the system is interpreted as a shallow lake with margins largely characterised by marshes, grass-covered areas and local stagnant ponds exemplified by the presence of thin organic-rich clay intercalations. The vertical arrangement of the unit suggests several superimposed cycles of expansion and ulterior retraction of the lake system which is in agreement with the regional climatic regime established for Western Europe during the middle Miocene, a time with sub-tropical temperatures [25].

We have selected the chert of the Cucho Limestone because of its high variability in shape, textures and organic matter content. In addition, this unit was not deeply buried after deposition, a situation that has prevented significant late mineralogical transformations due to strong compaction. According to sedimentological features, three different types of chert can be distinguished. *Nodular chert* is the most common type and corresponds to microcrystalline massive silica nodules. *Massive-brechoid chert* is also relatively common, particularly at the upper parts of the internal depositional sequences of the unit. It occurs forming m-thick irregular stratiform levels of dark-grey chert which contain plant remains and root traces, vadose cements, fracturation and subsequent stages of silica remobilisation and cementation. Finally, *laminar chert*, the less abundant type, is represented by finely-laminated microcrystalline silica nodules occurring within limestones with fine planar microbial lamination (stromatolites).

There is not much information about the organic content in these samples essentially because it has been overlooked. However, in comparison with the literature the presence of aliphatic hydrocarbons is expected [12,13]. The optimisation and validation of the extraction by means of MAE and FUSE was carried out using a massive-brechoid chert sample.

2.2. Reagents and materials

Hydrocarbon mix (n-hexadexane, n-octadecane, n-eicosane, docosane, n-tetradecane, n-hexacosane, octacosane, n-triacontane, dotriacontane, n-tetratetracontane, n-hexatriacontane and n-tetracontane at 100 $\mu g\,g^{-1}$ each) was purchased from Supelco (Walton-on-Thames, UK). The deuterated internal standards n-docosane (D_{46}) and n-dotriacontane (D_{66}) (CDS Isotopes Incs, Sainte-Foy-La-Grande, France) were individually dissolved in n-hexane (HPLC grade, LabScan, 95%) at ~1000 $\mu g\,g^{-1}$. Intermediate dilutions of above mentioned stocks were prepared in n-hexane (HPLC grade, LabScan, 95%) in order to build the calibration curves between 0.1 $\mu g\,g^{-1}$ and 5 $\mu g\,g^{-1}$. Finally, the extractant reagents were dichloromethane (HPLC grade, 99.8%), n-hexane (HPLC grade, 99.8%) and acetone (HPLC grade, 99.8%) all of them obtained from LabScan (Dublin, Ireland).

Special care was taken in all analytical steps, from sample cleaning and crushing to extraction and fractionation, to minimise cross-contamination sources. Chert samples were ground to <100 mesh grain size using an agate ball mill (Pulverisette 6, Fritsch, Germany) and 2 g of the homogenised sample were extracted using focused ultrasound extraction and microwave-assisted extraction methods.

On the one hand, the focused ultrasound extractions were carried out using an HD 2070 Sonopuls Ultrasonic Homogenizer (Bandelin, Germany) equipped with a GM 2070 generator (70 W, 20 KHz), an UW 2070 ultrasonic converter and either a SH 70G horn and MS 73 titanium probe (3 mm). A variable power setting (0–100%) allows controlling amplitude of the delivered ultrasound. On the other hand, the microwave-assisted extractions were performed in a Mars X (CEM, Matthews, NC, USA) microwave oven, making use of special PTFE vessels prepared for supporting high pressures. During the extraction both temperature and pressure were measured in one vessel.

2.3. Optimisation of FUSE

In order to perform the focused ultrasound extraction, both process variables and solvent mixture composition were taken into account. Since the composition of the solvent mixture is constrained (i.e. the sum of solvents is 100%), this introduces a linear dependency in the composition that makes not viable the composite designs or the linear regression techniques. Instead of this classical approach, D-optimal designs and biased regression methods such as partial least squares (PLS) are very suitable

Table 1
Extraction conditions and responses for the ultrasound focused extraction.

Exp.	D-optimal mixture design matrix (FUSE)					Response
	DCM (%)	Hex (%)	Ace (%)	Time (min)	Cycles	
1	60	40	0	10	3	142,825,104
2	40	40	20	10	3	116,000,904
3	40	40	20	40	3	124,832,040
4	40	40	20	10	9	132,033,880
5	60	20	20	10	3	121,171,304
6	60	20	20	10	9	59,199,968
7	60	30	10	10	3	147,077,984
8	60	30	10	10	9	150,369,696
9	50	30	20	10	9	134,082,448
10	60	30	10	40	3	138,442,144
11	50	40	10	40	3	69,467,680
12	60	30	10	40	9	157,887,520
13	50	40	10	40	9	187,396,832
14	60	40	0	40	6	177,019,872
15	60	40	0	25	9	185,449,312
16	40	40	20	25	9	171,145,792
17	50	40	10	10	6	135,255,584
18	50	30	20	40	6	161,210,176
19	50	30	20	25	3	151,069,600
20	60	20	20	25	6	149,031,024
21	53.33	33.33	13.33	25	6	162,070,672
22	53.33	33.33	13.33	25	6	157,256,320
23	53.33	33.33	13.33	25	6	170,325,680

Table 2
Extraction conditions and responses for the microwave-assisted extraction.

Exp.	D-optimal mixture design matrix (MAE)					Response
	DCM (%)	Hex (%)	Ace (%)	T (°C)	t (min)	
1	40	40	20	130	5	7,800,000
2	60	20	20	90	20	12,355,002
3	60	30	10	90	5	11,516,131
4	50	40	10	90	20	11,623,153
5	50	40	10	130	20	11,038,740
6	45	40	15	90	5	8,290,832
7	50	30	20	90	5	7,251,525
8	60	25	15	90	20	–
9	40	40	20	90	12.5	7,717,014
10	45	40	15	130	5	7,840,100
11	50	30	20	130	5	7,954,695
12	60	25	15	130	20	11,595,260
13	50	30	20	130	20	8,044,710
14	60	20	20	130	12.5	8,901,739
15	60	30	10	130	12.5	11,091,780
16	60	20	20	110	5	14,401,269
17	60	30	10	110	5	13,415,000
18	40	40	20	110	20	12,499,877
19	50	40	10	110	12.5	14,512,729
20	52.5	32.5	15	110	12.5	13,442,544
21	52.5	32.5	15	110	12.5	12,681,775
22	52.5	32.5	15	110	12.5	13,115,816

in these cases [26–28]. The D-optimal design was built by The Unscrambler® programme (v.7.5, Camo Asa, Trondheim, Norway) considering all the interactions within process and mixtures variables (but not between them) and the squared terms as well.

Approximately 2 g of the geological chert sample were accurately weighed in a glass vessel. 15 mL of the extraction solvent mixture (dichloromethane, n-hexane and acetone) was added to the sample according to the experiments proposed for the D-optimal design (see Table 1). The ranges of the solvents (dichloromethane: 40–60%; acetone: 0–20%; hexane: 20–40%) were fixed taking into account both the mixtures typically used in the literature and the features of the hydrocarbons [11]. Regarding the process variables, in this work sonication time (10–40 min) and sonication cycles (1–9, where the sonication cycle is the 1/10 of a second that the ultrasound is sonicating) were studied whereas the sonication power was fixed at 50% in order to extend the good efficiency of the titanium probe.

Once the extraction procedure was completed, the liquid fraction was separated from the residual solid by centrifugation at 2000 rpm during 15 min. The supernatant was concentrated to dryness using nitrogen blow-down evaporation at the Turbovap LV Evaporator (Zymark, Hopkinton, MA, USA) and re-dissolved in 200 μL n-hexane.

2.4. Optimisation of MAE

A D-optimal design was also followed to optimise microwave-assisted extraction. In the case of the mixture solvent composition the presence of a minimum amount of acetone was required to assure the absorption of microwave radiation and to achieve higher extraction temperatures. Therefore, the ranges of solvents were as follows: dichloromethane: 40–60%, hexane: 20–40%, acetone: 10–20%. In the case of the process variables, the extraction temperature (90–130 °C) and the time in which the maximum temperature was maintained (5–20 min) were studied (see Table 2).

Approximately 2 g of the geological chert sample were accurately weighed in a PTFE lined extraction vessel and 15 mL of solvent mixture was added. When the irradiation was completed, samples were removed from the microwave cavity and were allowed to cool to room temperature before opening. As in the case of FUSE extracts, the liquid fraction was recuperated after a centrifugation process at 2000 rpm during 15 min. The supernatant was concentrated to dryness using nitrogen blow-down evaporation and re-dissolved in 200 μL n-hexane.

2.5. GC–MS analysis

The extracted organic fractions were analysed on a 6890N Agilent gas chromatograph coupled to an Agilent 5973N electron impact ionisation mass spectrometer and a 7683 Agilent autosampler. An aliquot of 2 μL of the extract was injected in the splitless mode at 300 °C into a HP-5ms (30 m × 0.25 mm, 0.25 μm, Agilent) capillary column. The temperature program used for the chromatographic separation was as follows: 60 °C (0.5 min), temperature increase at 20 °C min^{-1} to 120 °C and a second ramp of 6 °C min^{-1} up to 300 °C, where it was finally held for 15 min. Helium (99.9995%, Carburos Metálicos, Barcelona, Spain) was used as a carried gas at a constant pressure of 13.4 psi. The transfer line temperature was maintained at 310 °C, and the ion source and quadrupole at 230 and 150 °C respectively. All the analyses were performed initially in SCAN mode and then in the selected ion monitoring (SIM) mode using the most abundant m/z fragment of n-alkanes (m/z: 57) as quantifier and m/z: 71 and 85 as qualifiers.

3. Results and discussion

3.1. Optimisation of FUSE

The data analysis of the experimental designs could be done in several ways: making use of the individual signal of each compound (several responses) or summing up all the signals in one combined response. In the later case, as the principal aim of the optimisation is the extraction of all organic matter present in the sample, we process the data summing up all the detected signals. Table 1 summaries the sum of the peak area of all n-alkanes ($C_{27}H_{56}$–$C_{35}H_{72}$) extracted using focalised ultrasounds under the different experimental conditions. The experimental precision was estimated from the three replicates of the central point (RSD 4%, $n = 3$).

Based on the data shown in Table 1, a PLS1 regression of the scaled data was performed using The Unscrambler® programme. As mentioned before, besides the design variables (extraction sol-

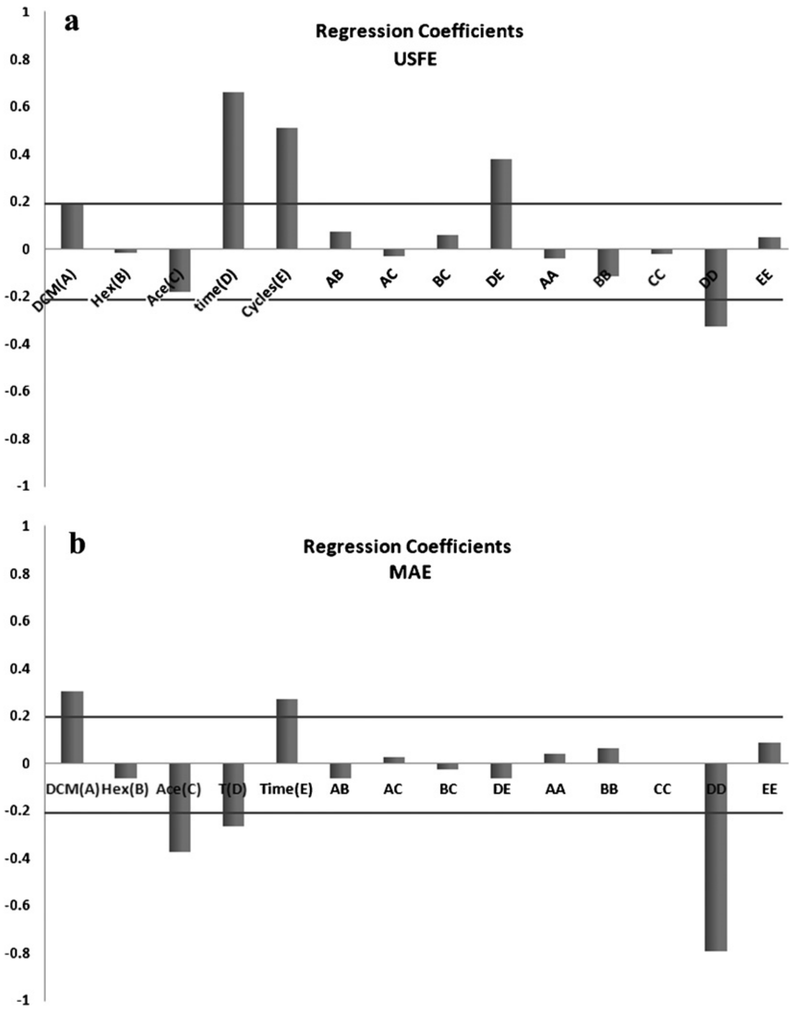

Fig. 1. Regression coefficients obtained for the different variables and variable interactions studied in the (a) FUSE and (b) MAE after PLS1 regression.

vent mixture and process variables), the interaction and square terms of mixture and process variables were included in the regression model. Cross-validation was used to validate the regression model and to identify the most significant variables. Finally, a 3 PC model explaining up to 86% of the response variance was built.

As it is typically considered based on scale data regression (both *X* and *Y* matrices), when the absolute values of the regression coefficients are higher than 0.2 the variables will be considered significant, whereas values smaller than 0.1 will be considered as not significant [26]. Consequently, from the values of the regression coefficients shown in Fig. 1a, it can be concluded that the process variables (cycles and time) have a significant influence on the extraction of n-alkanes, and the solvent mixture composition seem to be not so important. Thus it was decided to fix the solvent mixture at 60% dichloromethane and 40% hexane (without acetone). According to the process variables, as the coefficients are positive, longer extraction periods using higher sonication cycles yielded better recoveries. Thus,as can be seen in the response surface given in Fig. 2a, the process conditions were optimised at 9 cycles during 30 min.

3.2. *Optimisation of MAE*

Following the same procedure we studied the influence of the extraction solvent composition, extraction time and temperature on the microwave-assisted extraction for the sum of n-alkanes. The results (as peak area) obtained for all n-alkanes ($C_{27}H_{56}$–$C_{35}H_{72}$) detected at different experimental conditions are shown in Table 2. From the replicates of the central point the relative standard deviation was 3%.

The PLS1 regression of the scaled data and cross-validation required 6 PCs to explain up to 93% of the experimental variance. Based on the values of the regression coefficients the solvent mixture composition and the process variables are significant for the extraction step, i.e. the regression coefficients were higher than 0.2 (see Fig. 1b). On the one hand, the response surface of the process variables showed that intermediate temperatures (105–115 °C) were the most convenient, and that the effect time was not so powerful. Therefore we decided to fix those variables at 110 °C and 15 min. On the other hand, according to the response surface of the solvent mixture composition shown in Fig. 2b, the best extraction solvent mixture is 60% dichloromethane, 30% hexane and 10% acetone.

In both FUSE and MAE, we have built the PLS1 model for each analyte and technique and they were cross-validated. The resulting models confirmed the general conclusions described above since the most significant variables were the same and the shape of the response surfaces pointed roughly to the given conditions.

3.3. *Comparison of FUSE and MAE*

As no certified reference material was available for the analysis of n-alkanes in siliceous chert samples, the results of both methods were compared using the same real sample. In order to study

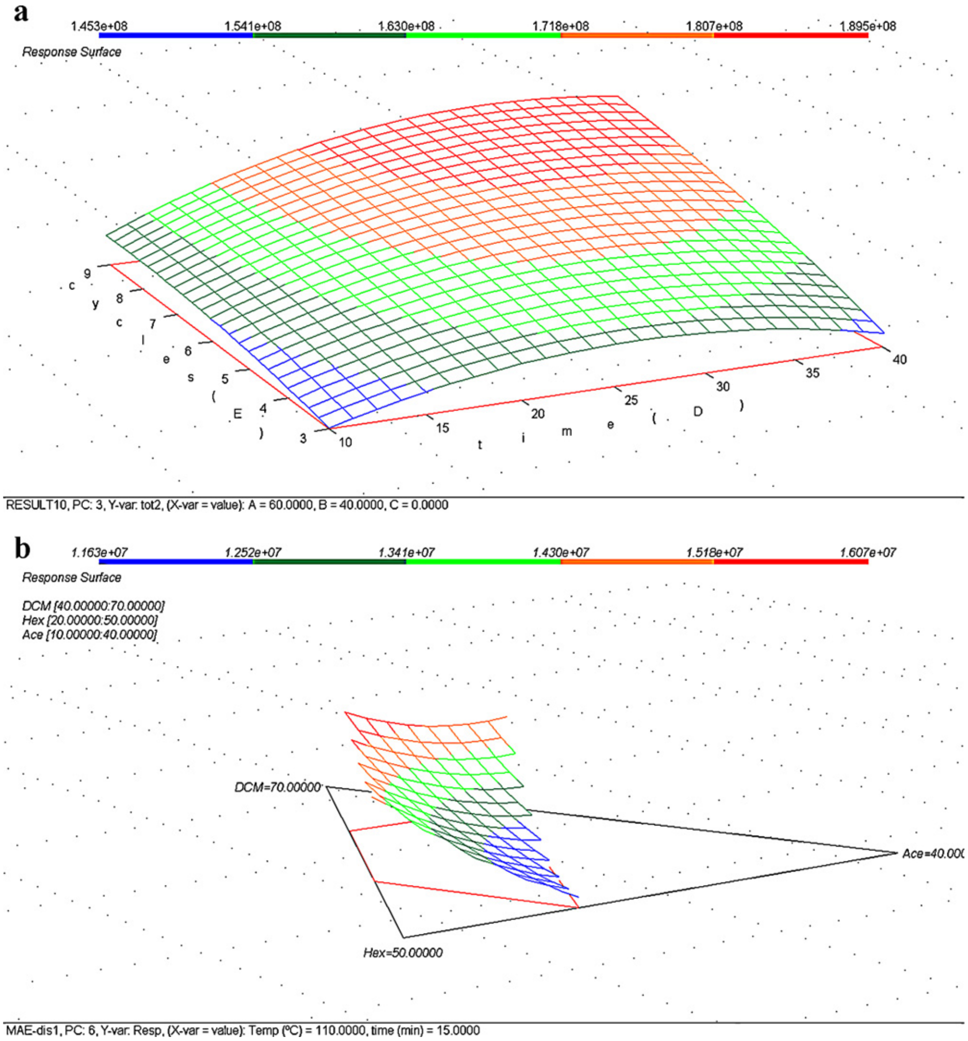

Fig. 2. Influence of the extraction solvent of n-alkanes from chert samples on different extraction methods: (a) ultrasound focused extraction and (b) microwave-assisted extraction.

the recovery of both methods, successive extractions of the same sample aliquots were carried out. Another strategy for this purpose would have been the extraction of spiked samples, but it was very problematical to find a blank sample without any hydrocarbon and, above all, the efficiency of this method to estimate the apparent recovery in such hard material does not seem too high.

Approximately 2 g of chert sample collected from Cucho were extracted under the optimal conditions for FUSE (60% dichloromethane and 40% hexane at 9 cycles during 30 min) and for MAE (60% dichloromethane, 30% hexane and 10% acetone at 110 °C, which is maintained for 15 min). Three successive extractions were performed in the same extraction conditions and the extracts were separately pre-treated before the GC–MS analysis as reported above. The total concentration of n-alkanes ($C_{27}H_{56}$–$C_{35}H_{72}$) obtained for the three extracts after MAE and FUSE extraction procedures are shown in Fig. 3. According to these results, concentrations obtained in the second extraction using both extraction methods were lower than 15% of the total amount extracted, therefore it can be concluded that almost a quantitative extraction of analytes was obtained in the first extraction.

The analysis of variance (ANOVA) of the concentrations obtained for geological chert by means of MAE-GC–MS and FUSE-GC–MS were highly comparable for the $C_{27}H_{56}$–$C_{35}H_{72}$ n-alkanes (p-level > 0.1). However, the relative standard deviation values (RDS) obtained using microwave-assisted extraction were slighter lower (<11%, for the first extraction) than those obtained using ultrasound focused extraction (<18%, for the first extraction). These results together with the fact that MAE device allows the simultaneous extractions of up to 8 samples make microwave-assisted extraction a very convenient option to extract organic matter from chert samples.

Moreover, the detection limits provided by this analytical method are low enough to detect n-alkanes in many chert samples.

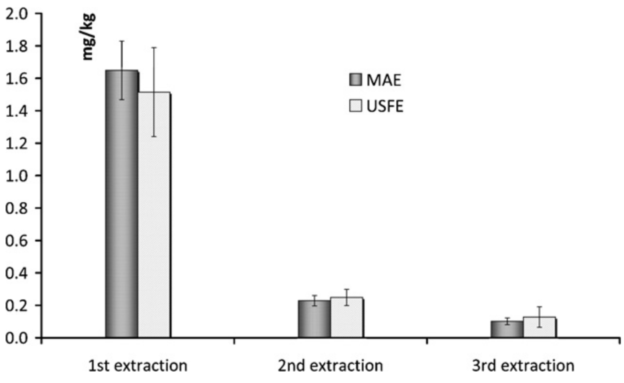

Fig. 3. Comparison of the concentration for the sum of n-alkanes ($C_{27}H_{56}$–$C_{35}H_{72}$) by MAE-GC–MS and FUSE-GC–MS.

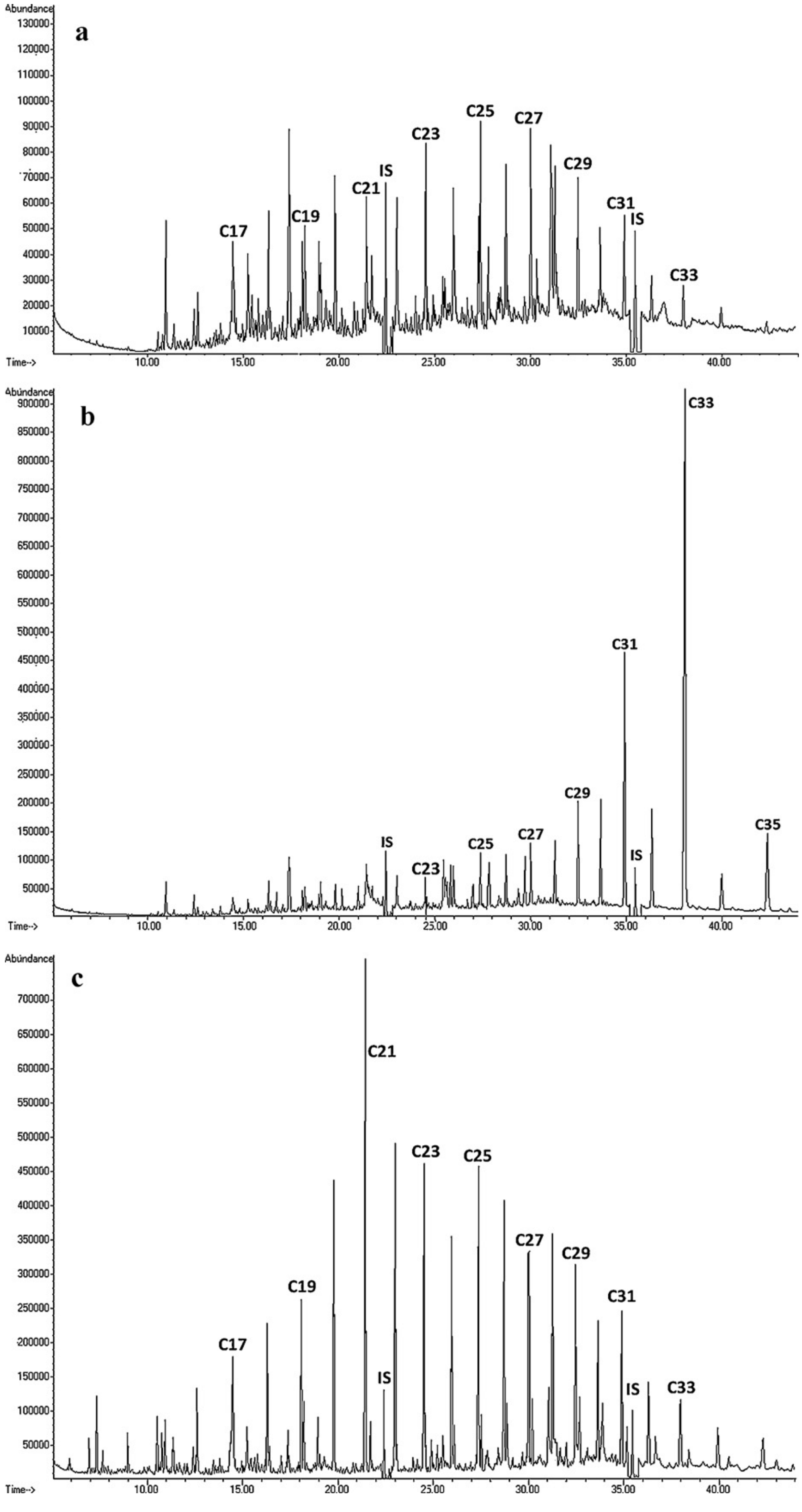

Fig. 4. Examples of typical n-alkanes distributions obtained after MAE-GC–MS analyses and determined by SIM at ions *m*/*z*: 57, 71, 85. n-docosane and n-dotriacontane were used as internal standards (IS).(a) Nodular cherts (b) Silcrete cherts and (c) Massive-brechoid cherts.

Table 3
Concentrations of n-alkanes (ng g^{-1}) observed in three different chert samples for each chert type collected in Cucho. The RSD% values are lower than 12% for all the compounds.

	Silcrete			Massive-brechoid			Nodular		
	CU-1	CU-2	CU-3	CU-4	CU-5	CU-6	CU-12	CU-13	CU-14
$C_{16}H_{34}$	12	18	16	66	69	125	33	19	8
$C_{17}H_{36}$	25	26	31	118	110	225	41	24	19
$C_{18}H_{38}$	36	34	39	137	136	308	44	35	24
$C_{19}H_{40}$	29	25	26	147	122	387	35	32	26
$C_{20}H_{42}$	34	30	33	219	196	658	51	52	35
$C_{21}H_{44}$	78	4	84	382	274	1257	42	41	38
$C_{22}H_{46}$	43	33	42	221	200	687	49	46	34
$C_{23}H_{48}$	51	30	57	208	178	684	51	50	43
$C_{24}H_{50}$	45	29	69	155	137	474	47	42	33
$C_{25}H_{52}$	50	25	83	196	175	609	58	44	42
$C_{26}H_{54}$	52	24	104	181	147	552	53	30	23
$C_{27}H_{56}$	62	35	107	245	196	838	67	42	35
$C_{28}H_{58}$	73	36	119	228	198	1087	48	27	28
$C_{29}H_{60}$	117	63	145	171	148	490	55	28	21
$C_{30}H_{62}$	121	64	154	127	106	347	36	22	17
$C_{31}H_{64}$	368	184	446	151	127	386	48	37	28
$C_{32}H_{66}$	188	91	201	94	80	274	26	23	15
$C_{33}H_{68}$	1216	555	1245	99	89	261	30	27	20
$C_{34}H_{70}$	100	49	103	64	53	180	16	18	12
$C_{35}H_{72}$	347	157	342	101	72	332	11	8	4

The detection limits were calculated as the signal of the blank plus three times the standard deviation of the three blank extractions and they were in the range of 30–80 ng g^{-1}.

3.4. Application of the analytical method to the extraction of hydrocarbons from geological chert samples

Although n-alkanes are ubiquitous in most sedimentary rock types they are usually difficult to be correlated to a particular biological source. However, the distribution patterns of n-alkanes can provide reliable interpretation about the possible organic matter sources and thus give inferences about the environment of deposition [7]. In this sense, the analysis of three different chert types was performed following the optimal extraction conditions mentioned above.

The concentration of n-alkanes obtained after the analysis of each chert type collected (nodular, laminar and massive-brechoid) are summarised in Table 3. The analysis of each sample was carried out three times and the %RSD values were lower than 12% in almost all the cases. Additionally, the chromatograms obtained for samples from each chert type are plotted in Fig. 4. Based on these results, quite different distribution patterns of n-alkanes can be distinguished depending on the predominance of low or high molecular weight n-alkanes is compared.

Briefly, the n-alkanes distribution observed for the *nodular chert* type samples don't provide enough information as they present low concentration and a poorly-defined distribution pattern of n-alkanes (see Fig. 4a). The scarce preservation of hydrocarbons in this chert type can be associated with the physico-chemical conditions during the formation of chert nodules. On the contrary, both *massive-brechoid* and *laminar* cherts and contain well-preserved organic matter at a concentration of 2 μg g^{-1} but marked differences can be observed between them. In the case of *massive-brechoid cherts* a distribution dominated by homologous series of heavy n-alkanes, ranging from $C_{29}H_{60}$ to $C_{35}H_{72}$ and odd over even carbon number predominance is systematically observed (see Fig. 4b). Such distribution is generally considered as reflecting a strong organic terrestrial input with the long-chain n-alkanes derived from plants [7]. Laminar cherts, otherwise, show a distribution of n-alkanes dominated by compounds in the n-C_{18}–n-C_{28} range, and the concentration of higher homologues decreases rapidly with increasing carbon number (see Fig. 4c). Spores and algal cell walls commonly found in Carboniferous deposits contain highly aliphatic macromolecules which can contribute to the presence of n-C_{18}–n-C_{28} in sedimentary cherts [29]. Although all the chert analysed occur in similar lithologies and are thus linked to a similar depositional setting, the differences in hydrocarbon content among different types point to significant differences in their origin and diagenetic history. Briefly, on the one hand, massive-brechoid and laminar cherts are closely related to biological activity whereas nodular cherts were formed as a depletion of quartz in sedimentary rocks improved by the presence of organic matter.

4. Conclusions

Microwave-assisted and ultrasound focused extraction of n-alkanes from chert samples turned out to be suitable extraction procedures in order to replace the classical Soxhlet extraction which requires large amount of sample. In this sense, approximately 50 g of sample are required for Soxhlet extraction whereas MAE and FUSE have allowed obtain good results using approximately 2–3 g. This characteristic allows carrying out analyses with geological and also archaeological purposes. The capability of the microwave-oven to handle more than one sample simultaneously is very helpful when the analysis of tenths of samples is required. This advantage together with better repeatability obtained using microwave-assisted extraction allows performing successfully the analytical method for the determination of hydrocarbons in geological cherts. In this sense, different chert samples have been identified in the same local area using their hydrocarbon distribution.

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