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Comprehensive two-dimensional gas chromatography to characterize hydrocarbon mixtures in lithic materials

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ABSTRACT

The analysis of organic biomarkers in chert samples offers key information about the environmental conditions in which these samples were formed, and this information can be used to track the lithic materials of many archaeological artifacts. Since the content of the organic fraction is very low and the complexity of the organic extracts is quite high, we have optimized the GC × GC separation of these mixtures. Making use of mixture of $C_{16}H_{34}$–$C_{44}H_{90}$ *n*-alkane standards, a central composite design was carried out taking into account the carrier flow in the first and second columns, the modulation period and the discharge time. Regarding the measured responses, though the initially considered one was the peak volume, we have also evaluated the effects on the number of modulated peaks per analyte, the symmetry of the modulated peaks and the number of detected compounds. The final optimum conditions were defined as follows: a hydrogen flow of 1.2 mL/min in the first column and 18 mL/min in the second one, a modulation period of 1.4 s and a discharge time of 0.1 s and under these conditions all the response variables showed optimum values. Based on this optimized method several chert samples obtained from different stratigraphic levels in an ancient quarry were studied and we were able to distinguish them on the basis of the different constituents of organic biomarkers, such as mono-methylated alkanes, cyclic *n*-alkanes, branched alkanes, steranes and hopanes.

1. Introduction

The suitability of organic biomarker analysis for geochemical interpretation purposes has been widely demonstrated by several works in the literature [1–3]. Most of the interest in geochemical analysis lies in the fact that to capture subtle differences in many closely related materials it is required a deeper knowledge of the geological material composition.

Carbonaceous chert samples contain a wide variety of organic biomarkers which are resistant to degradation. In fact, it is well known that the distribution and features of biomarkers are closely linked to the original source of organic matter and this may provide clues about the palaeoenvironmental conditions in which siliceous deposits were formed [4].

The identification of molecular constituents within complex organic mixtures is typically achieved by a series of chemical and chromatographic separations [5–7]. The most common technique is the gas chromatography (GC), typically coupled to a mass spectrometer, in order to make possible the separation and identification of individual molecular components in complex mixtures. However, many analytical applications still require a resolution power much higher than that provided by a single dimension which does not allow the separation of complex mixtures [8]. Owing to this requirement, it is possible to extend the instrumental dimension either in the detection axis (i.e. tandem MS) or in the separation one. In the latter case, comprehensive two-dimensional gas chromatography (GC × GC or 2D comprehensive gas chromatography) is currently receiving widespread attention for the analysis of complex samples [9]. Several review articles have been published in the literature both as refinements in the analytical methodology and as new applications in diverse areas such as petrochemical, environmental or food analysis [10–12].

The power of comprehensive gas chromatography, developed by Phillips and Liu in 1991, lies in the possibility to separate organic complex mixtures using two capillary gas chromatographic columns of different polarities installed in series and coupled by a flow modulator [13]. The growth of GC × GC gave rise to the development of a wide variety of modulators (i.e. cryogenic modulators or pulsed flow modulators) suitable for an increasing range of applications [14,15]. In this sense, very recently, capillary flow technology (CFT) was introduced by Agilent Technologies for a several chromatographic applications [16]. This modulator uses a low thermal mass deactivated stainless steel hydrogen flow device with no moving parts for switching purposes. Due to this, cryo-

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gen devices are not needed and pneumatic device is controlled by a micro three-way solenoid valve. Within the flow modulator, analyte bands eluting from the first column according to their volatilities are collected in a fixed-volume channel. Then, a group of co-eluting components are successfully launched quickly into the short second column, where the mixture will be separated based on the different polarities. Although all types of stationary phases can be used, most of the reported applications are performed using a non-polar stationary phase in the first column, and a polar stationary phase in the second column [17]. Any separation that occurs on the first column due to a different volatility of analytes is preserved during the transfer to the second column where there is a new separation based on differences on polarity. In this way, compared to one-dimensional separations, 2D GC can increase peak resolution resulting in a greater number of individual compounds being separated.

Regarding the detection, commonly GC × GC is coupled to a flame ionization detector (FID), at least for routine analysis [18]. However, mass spectrometer (MS), as time of flight (TOF) or fast quadrupole MS, is required in order to obtain structural data of unknown compounds [19–21]. In the field of petroleum chemistry, the GC × GC–MSD configuration has been widely applied for several applications with promising results [22,23].

In spite of the enhanced advantages provided by GC × GC, optimization of the analytical separation is more difficult than in ordinary one dimensional gas chromatography, regardless of the detection system. Attending to the optimization requirements, multivariate and computer modeling have been widely applied in the literature in order to build easy models and templates to identify target compounds and to quantify them [24]. In the same way, experimental designs can be applied to study how different experimental settings affect the resultant chromatographic separation [25].

The goal of the optimized method in two-dimensional gas chromatography is to achieve maximum peak capacity and display the maximum number of well-separated discrete compounds. For this purpose, it is known that several chromatographic variables can show a large influence in the width and shape of modulated peaks, and thus, in the separation of analytes. In this sense, the optimization of the stationary phase, gas flow in both capillary columns, and modulation period should be considered.

In the framework of the analysis of biomarkers in some chert samples of archaeological use, we found the opportunity to get a closer view of the diversity of organic compounds. Therefore, the initial purpose was to get the optimum instrumental variables through an experimental design and then, we wanted to apply this method to reveal the distribution of organic biomarkers in the different chert extracts. In a further step, this methodology could be useful to analyze real geological samples of archaeological interest in a routine basis.

2. Experimental

2.1. Material and reagents

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 μg/g each) was purchased from Supelco (Walton-on-Thames, UK). In order to carry out the optimization step of the GC × GC chromatographic method intermediate dilution of the above-mentioned stock (10 μg/g) was prepared in *n*-hexane (HPLC grade, LabScan, 95%). Other intermediate dilutions were also prepared in *n*-hexane between 0.5 and 10 μg/g in order to build the calibration curves.

2.2. Studied samples

Studied geological chert samples were collected from lacustrine–palustrine carbonate sediments near the locality of Cucho (Trebiño County, Burgos, Northern Spain). The host formation, informally known as the Cucho Limestone, is an up to 85-m thick succession of alternating limestones, marlstones and clays that was deposited during the Miocene. We have selected the chert of the Cucho Limestone among other geological cherts in the region 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 since they have been heavily compacted. Different types of cherts can be identified in the Cucho lacustrine–palustrine carbonates but we have analyzed two of them: *laminar* and *massive-brechoid*. The first one is represented by finely laminated microcrystalline silica nodules occurring within limestones with fine planar to slightly contorted microbial lamination. The *massive-brechoid* chert occurs forming m-thick irregular stratiform levels of dark-grey chert with a characteristic brechoid texture, which contain plant remains and root traces, vadose cements, fracturation and subsequent stages of silica remobilization.

2.3. Sample pre-treatment

The procedure of organic matter extraction from chert samples has been already optimized and explained in a previous work [26]. Briefly, 10 subsamples of 2 g of chert were treated with 15 mL of extractant solvent mixture consisting of 60% dichloromethane (HPLC grade, 99.8%), 30% hexane (HPLC grade, 99.8%) and 10% acetone (HPLC grade, 99.8%). The extractable organic fraction (bitumen) was isolated by means of microwave digestion system (Mars 5, CEM) in the optimal extraction conditions (at 110 °C for 20 min). The extracts were centrifuged and the supernatants were combined and concentrated to dryness using nitrogen blow-down evaporation and re-dissolved in 200 μL *n*-hexane (HPLC grade, LabScan, Dublin, Ireland).

2.4. GC × GC–FID/MS equipment

The GC × GC–FID/MS analyses were performed using a GC7890A (Agilent Technologies, Avondale, PA, USA) equipped with a FID detector and a 5975C MS detector and an Agilent G-3486A capillary flow plate. The control of the second pressure source was handled with a pressure control module (PCM). A three-way solenoid, Fluid Automation System Valve, was used for flow switching.

The column set used for bidimensional gas chromatography was a HP-5ms (30 m × 0.25 mm, 0.25 μm, Agilent) capillary column coupled to a DB-17ms (5 m × 0.25 mm, 0.25 μm, Agilent) capillary column. Two deactivated but not coated fused silica tubes (restrictor) were used in order to divide the flow to FID and MS detectors, a 0.70 m, 0.32 mm i.d. restrictor connected to FID and a 0.45 m, 0.10 mm i.d. connected to MS detector.

The oven temperature program started at 60 °C was held for 0.5 min, raised to 140 °C at 20 °C/min and a second ramp to 300 °C at 6 °C/min and held for 15 min. The carrier gas was hydrogen (AD-1020 Hydrogen Generator, Cinel Strumenti Scientifici, Padova, Italy) and the flow rate in both the columns was optimized. Two microliters of the sample were injected in the splitless mode at 300 °C using a 7683 Agilent autosampler.

The flame ionization detector (FID) was operated at a data collection frequency of 100 Hz at 300 °C. The mass spectrometer detector (MSD) worked in full scan mode from *m*/*z* 50 to 450, in an acquisition frequency of 20 scan/s and temperatures of quadrupole and source were 150 °C and 230 °C, respectively.

Table 1
The design matrix and the responses (as chromatographic peak volumes × 10^5) obtained for CCD.

Exp	Studied variables				Responses (as peak volume × 10^5)						
	A Flow 1st column	B Flow 2nd column	C Modulation period (s)	D Discharge time (s)	$C_{16}H_{34}$	$C_{18}H_{38}$	$C_{20}H_{42}$	$C_{22}H_{46}$	$C_{24}H_{50}$	$C_{26}H_{54}$	$C_{30}H_{62}$
1	0.82	20.5	1.45	0.1	5.07	5.21	4.90	3.89	2.90	1.99	0.92
2	1.325	20.5	1.45	0.1	8.72	8.93	8.63	7.89	6.28	4.69	1.27
3	1.075	17.5	1.45	0.1	8.70	9.10	8.52	7.48	5.51	3.84	0.78
4	1.075	23.5	1.45	0.1	6.40	6.60	6.44	5.21	4.41	2.71	0.97
5	1.075	20.5	1.35	0.1	7.67	7.06	7.35	5.99	4.37	2.98	1.05
6	1.075	20.5	1.55	0.1	7.32	6.88	6.42	6.75	5.42	3.16	0.92
7	1.075	20.5	1.45	0.001	2.56	2.87	3.22	2.58	2.01	1.81	0.64
8	1.075	20.5	1.45	0.2	1.80	1.89	2.65	2.13	2.54	1.28	0.76
9	0.95	19	1.4	0.05	6.48	5.53	4.78	5.81	3.59	1.88	0.54
10	1.2	19	1.4	0.05	4.34	3.98	3.67	3.22	2.69	2.04	0.74
11	0.95	22	1.4	0.05	5.59	4.90	5.30	4.32	3.28	1.85	0.70
12	1.2	22	1.4	0.05	3.85	3.82	3.12	2.78	2.84	2.07	0.81
13	0.95	19	1.5	0.05	5.50	5.32	4.40	4.11	2.78	1.77	0.81
14	1.2	19	1.5	0.05	3.07	2.55	2.62	2.72	2.02	1.18	0.49
15	0.95	22	1.5	0.05	4.78	4.54	3.45	4.35	2.22	1.42	0.66
16	1.2	22	1.5	0.05	2.80	2.60	2.43	2.46	2.22	1.88	0.71
17	0.95	19	1.4	0.15	6.51	6.23	5.74	5.65	4.69	3.22	1.17
18	1.2	19	1.4	0.15	8.95	8.52	7.39	6.85	4.50	2.96	1.09
19	0.95	22	1.4	0.15	6.32	6.03	5.40	4.44	3.11	2.26	0.71
20	1.2	22	1.4	0.15	7.52	5.37	5.33	4.57	3.76	2.84	0.83
21	0.95	19	1.5	0.15	6.67	6.14	6.16	5.42	3.49	2.56	0.60
22	1.2	19	1.5	0.15	7.20	7.84	6.02	6.67	4.23	2.97	0.86
23	0.95	22	1.5	0.15	5.37	4.62	4.01	3.49	3.89	2.18	1.03
24	1.2	22	1.5	0.15	5.03	4.99	4.50	3.96	3.10	2.24	0.75
25	1.075	20.5	1.45	0.1	7.98	7.37	6.87	6.17	4.63	3.02	0.82
26	1.075	20.5	1.45	0.1	7.28	7.81	6.97	6.49	5.12	3.66	0.83
27	1.075	20.5	1.45	0.1	7.51	6.95	6.45	6.59	5.16	3.50	1.07

Chemstation software (Agilent Technologies) was used for chromatographic raw data acquisition. The monodimensional chromatogram was transformed into one bidimensional plot and GC Image and GC project software (Zoex Corporation, Houston, USA) were used for the data analysis in the 2D plots in which the intensity of the signal was represented by colours (from the light blue of the background to the top of the most intense peaks in the pale green–yellow–orange–red colour range).

3. Results and discussion

The optimization of the best chromatographic method was performed in order to separate the major number of compounds. As it has been mentioned before, several chromatographic variables can largely affect the resolution of the modulated peaks, the symmetry of the modulated peaks as well as the sensitivity of analytical method, making necessary the right choice of the chromatographic variables. To accomplish this work a standard mixture of hydrocarbons was used because the chromatographic outcomes were easy to treat compared to the results that would have been obtained from a real sample [26], though the final aim of this work is precisely to apply in unknown samples.

Central composite design (CCD) was performed using the Unscrambler® program (v.7.5, Camo, Trondheim, Norway) in order to establish the best working conditions as well as to detect the influential variables for this purpose. Four variables were taken into account in the optimization procedure: carrier flow in the first and second columns and modulation parameters (modulation period and discharge time). All the experiments were performed with the same standard solution (n-$C_{16}H_{34}$–$C_{44}H_{90}$) and the same oven temperature program was used in all the proposed experiments.

When one dimensional GC method is optimized, we can select several parameters related to the resolution or selectivity, but the chromatographic peak area for each analyte is one of the responses typically taken into account. However, in GC × GC analyses, though the peak volume of each analyte could be the most important response variable to be analyzed (since it is directly related to the sensibility to resolve different peaks), other response variables can also be studied such as the number of modulated peaks of a pure analyte (ideally, between 3 and 5), the symmetry of modulated peaks (which can be calculated with asymmetry factor (ASF) parameter and ideally should be close to the unity) and the number of detected compounds (referred as *blob* number). Therefore, we should consider as many responses as we can to adequate the optimization process.

The central composite design (CCD) proposed (including three replicates of the central point) by the Unscrambler® program and the responses (as chromatographic peak volumes) obtained for each analyte are summarized in Table 1. The precision of the measures was estimated from the three replicates of the central point (the RSD % values for all the analytes were between 4% and 6%).

In order to study the effect of each variable, both multiple linear regression (MLR) and partial least squares regression (PLS2) were used to build the response surface models. In the case of MLR, variables with a p-value coefficient lower than 0.05 were considered as significant. Using PLS2 and when both the X and the Y matrices are normalized, the variables will be considered significant if the absolute values of regression coefficients are higher than 0.2 [27]. In this last case, cross-validation was used to validate and identify the significant variables. For most of the analytes a 4 PC model explaining up to 85% of the response variance was built.

Table 2 summarizes the regression coefficients obtained by means of PLS2 as well as those variables with p-values minor than 0.05 obtained by means of MLR for the studied analytes. As can be observed both data treatments yielded equivalent models. Consequently, from the values of the regression coefficients shown in Table 2, it can be concluded that the carrier flow in the first (A) and second columns (B) as well as the sample discharge time (D) to the second column has a significant influence on the chromatographic separation. Additionally, the interaction between A and D variables seems to be a parameter to take into account. Finally, analyzing the response surface obtained from significant variables it

Table 2
Values of the regression coefficients obtained through PLS2 in the composite central design. Bold numbers are those parameters that were significant (p-value < 0.05) in MLR analysis (A: flow in the first column (mL/min), B: flow in the second column (mL/min), C: modulation period (s), D: discharge time (s)).

	Regression coefficients (β)			
	$C_{16}H_{34}$	$C_{20}H_{42}$	$C_{24}H_{50}$	$C_{26}H_{54}$
A	−0.003	−0.003	−0.003	−0.003
B	**−0.223**	**−0.232**	**−0.223**	**−0.218**
C	**−0.157**	**−0.163**	−0.157	−0.153
D	**0.405**	**0.420**	**0.405**	**0.395**
AB	−0.020	−0.020	−0.020	−0.019
AC	−0.063	−0.065	−0.063	−0.061
AD	**0.217**	**0.225**	0.216	0.211
BC	0.026	0.027	0.026	0.025
BD	−0.133	−0.138	−0.133	−0.130
CD	−0.001	−0.001	−0.001	−0.001
AA	**−0.399**	**−0.414**	**−0.399**	**−0.389**
BB	0.051	0.053	0.051	0.050
CC	0.031	0.032	0.031	0.030
DD	**−0.471**	**−0.489**	**−0.471**	**−0.460**

can be concluded that lower carrier flow in the first column and higher carrier flow in the second column provide higher peak volumes. Thus, taking into account the obtained results the following standard working conditions were proposed: hydrogen flow in the first apolar column (1.2 mL/min), hydrogen flow in the second polar column (18 mL/min), modulation period (1.4 s) and discharge time (0.1 s).

As has been mentioned previously, among the responses to be analyzed in a GC × GC chromatographic method, we should include the symmetry of the peak in both first and second columns, the modulated peaks and the detected compound number. Thus, although standard working conditions were proposed just considering the peak volume results, these new responses were also taken into account afterwards. In order to get the best separation model, we built the PLS2 regression model for each response from the experimental results and we tested the response of the other variables at the fixed optimum conditions. In this sense, in order to confirm the adequacy of the already defined working conditions we should get peak symmetries close to the unity, the modulated peak of pure analyte between 3 and 5, and the maximum number of detected compounds (blob).

As it can be seen in the response surfaces illustrated in Fig. 1, the resulting response surfaces were in good agreement with the previously suggested optimum GC × GC chromatographic conditions. Thus, the peak symmetry in the first dimension as well as in the second dimension is near to unity at the optimum chromatographic conditions. According to the modulation, in all the experiments proposed by the Unscrambler®, the number of modulation was between 3 and 5. Finally, all the compounds can be easily identified because the maximum sensitivity was achieved in the selected chromatographic conditions.

In spite of the broad optimization and the satisfactory results, one of the main drawbacks of GC × GC–FID/MS is the low sensitivity, essentially when it is compared with the typical GC–MS (SIM mode), yielding poor detection limits. This is basically because the flow is split into two outlets, one to the FID and the other to the MS detectors.

In order to estimate the detection limits as well as the precision of the analyses, a calibration curve was built from 0.5 to 10 μg/g and, as in the case of the optimization step, the volume of the peak was taken as a response. Good determination coefficients were obtained ($r^2 > 0.995$) for the analytes studied. The detection limits were calculated from the calibration parameters using the intercept value plus three times its standard deviation. The detection limits calculated in this way were above 0.2 μg/g for light n-alkanes and 0.4 μg/g for the heavier ones. Nevertheless, no problems were found in the repeatability of the chromatographic method as the RSD % were within 3–8% range for almost all the analytes except for heavier n-alkanes whose RSD % value rises up to 10–20% range for concentrations close to the detection limit (0.5 μg/g).

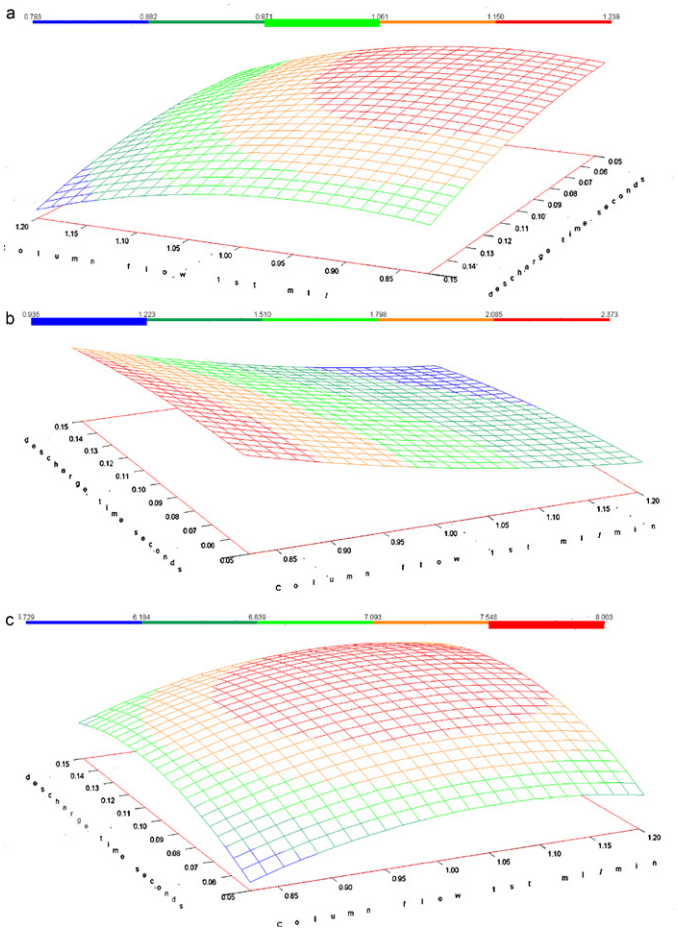

Fig. 1. (a) Peak symmetry in the first dimension (values close to the unity coloured in green), (b) peak symmetry in the second dimension (values close to the unity coloured in blue) and (c) number of detected compounds (mayor number of detected compounds coloured in red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Application to real samples

Although organic biomarkers are presented in most sedimentary rock types, the study of their distribution patterns can provide reliable information about the source of the organic matter and thus, can provide information about the environment of deposition [4]. In order to apply the optimized methodology to real samples, the organic fraction of two different cherts (called *laminar* and *massive-brechoid* and collected from the same sedimentary formation) was extracted and analyzed by means of GC × GC–FID/MS.

The GC–MS total ion current (TIC) chromatogram of *laminar* chert (see Fig. 2) displays some well resolved compounds (i.e. n-alkanes) but some unresolved organic mixtures (UCMs) can also be observed. GC × GC–MS has allowed the identification of the UCMs (covering the range of n-C_{15} to n-C_{22}) which are mainly associated with monomethylated alkanes and cyclic n-alkanes. The UCMs covering the higher hydrocarbon homologues range comprises biomarkers of higher molecular weight.

Fig. 3 displays the 2D chromatogram of a bitumen fraction associated with algal origin organic matter extracted from *laminar*

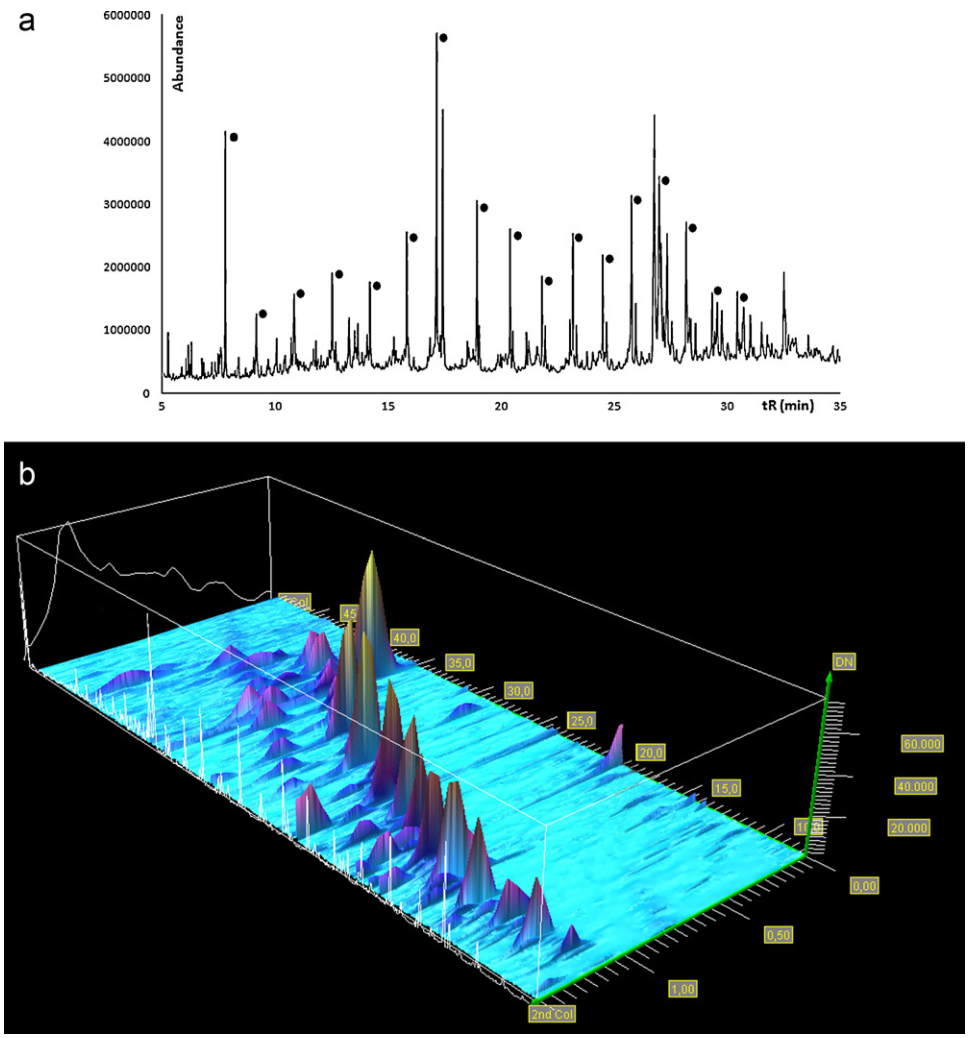

Fig. 2. (a) Total ion current (TIC) chromatogram from the extractable hydrocarbon fraction of *Cucho laminar* chert. *n*-Alkanes marked with dots. (b) 3D image of GC × GC–MS fragment pattern of *Cucho laminar* sample. The larger peaks correspond to *n*-alkanes.

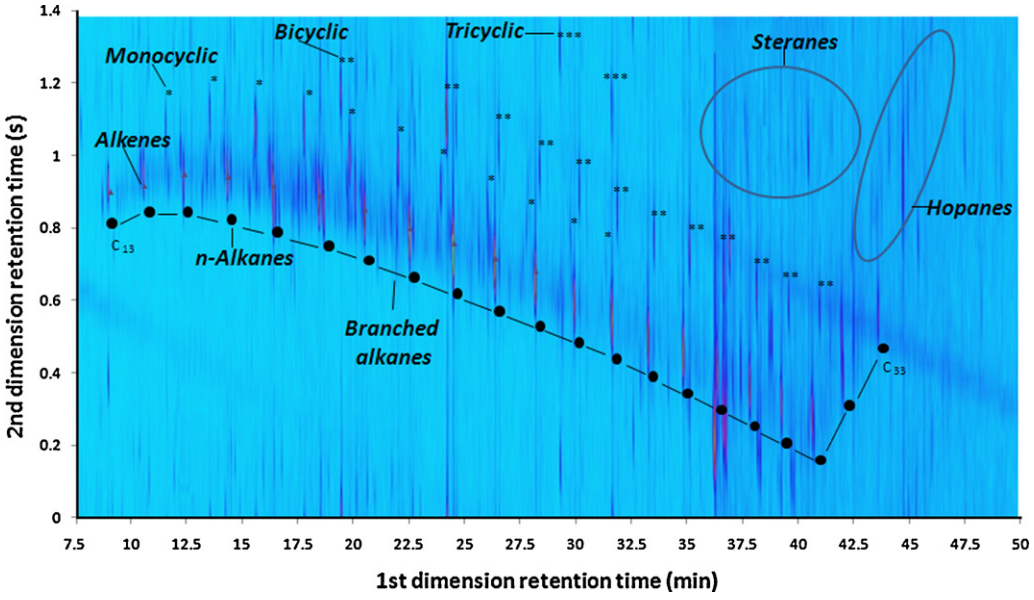

Fig. 3. 2D representation of GC × GC–MS fragment pattern of *Cucho laminar* sample. The different detected compounds are represented with dots of different sizes.

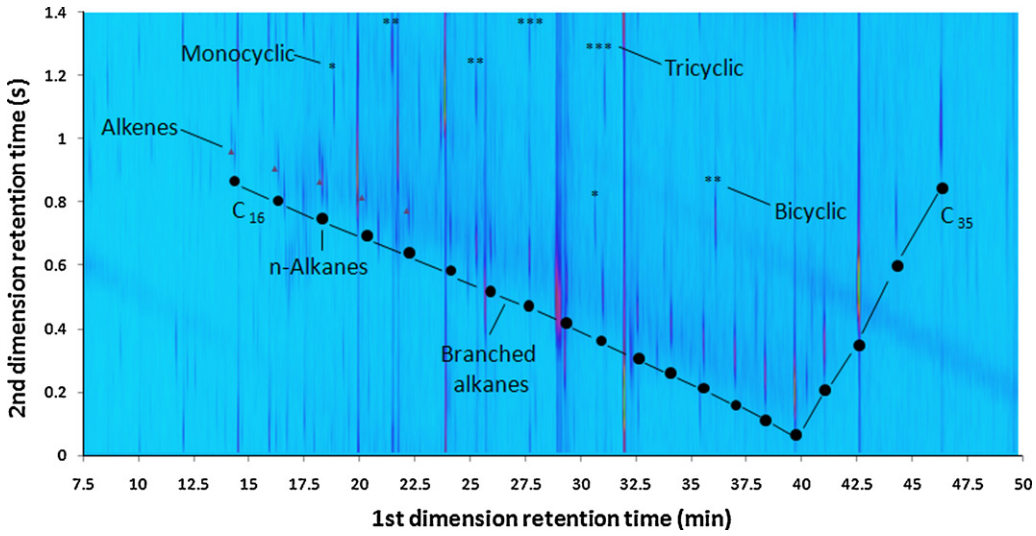

Fig. 4. 2D representation of GC × GC–MS fragment pattern of *Cucho massive-brechoid* sample. The different detected compounds are represented with dots of different sizes.

chert. The first dimension of GC × GC separation has volatility-based selectivity to separate the bitumen components by carbon number. The major peaks attributed from left to right along the *x*-axis of the GC × GC chromatogram are the $C_{13}H_{28}$ to $C_{30}H_{62}$ *n*-alkanes (see Fig. 3) identified with their characteristic *m*/*z* fragmentograms: 57, 71, 85 and represented as black dots in the first line of 2D chromatogram. The second dimension has polarity-based selectivity to separate components by chemical groups. Peaks separated from bottom to top on the *y*-axis are the *n*-alkanes followed by single and multi-ring cycloalkanes. The tentative identification of the separated compounds was carried out using their *m*/*z* fragmentograms and literature data [28,29], and they have been marked with different dots in the 2D chromatogram plotted in Fig. 3.

The monounsaturated *n*-alkanes co-elute close to their homologues *n*-alkanes and they have been characterized using their characteristic ion *m*/*z* 97. Light mono-unsaturated *n*-alkanes have been widely detected whereas the relative abundance of higher homologues decreases progressively (see Fig. 3).

In the first dimension, branched *n*-alkanes have been also detected together with unsaturated and mono-saturated alkanes. Although they share the same *m*/*z* 57 fragment as *n*-alkanes, GC × GC has allowed their identification since these compounds co-elute between two consecutive *n*-alkanes. In this case also low molecular weight branched *n*-alkanes are abundant compared to the concentration of higher molecular weight homologues which decreases rapidly with increasing carbon number. Specific short-chain (C_{15}–C_{20}) branched *n*-alkanes, such as monomethyl- and dimethylalkanes, are thought to be cyanobacterial biomarkers [4]. The information obtained can provide clues to know the origin of the analyzed archaeological chert samples. Thus, it was possible to associate the analyzed sample to a lacustrine arid environment with an algae organic biomass input.

Cyclic alkanes contribute also to unresolved complex mixtures. The identification of these compounds by means of their characteristic fragment ions, i.e. *m*/*z* 69, *m*/*z* 83, *m*/*z* 97 and *m*/*z* 123, does not provide enough information to resolve the diversification of these compounds. Nevertheless, based on the number of carbon atoms and number of rings, these compounds can be separated in the second dimension showing a sub-parallel alignment of peaks in the 2D GC × GC plot (see Fig. 3).

In this sense, although the overall distribution of cyclic alkanes has been monitorized by their characteristic fragmentations *m*/*z* 96 and *m*/*z* 110, the molecular ion was taken into account for their clear identification. Thus, for the identification of C_{16} alkylcycloalkanes the *m*/*z* 224 was followed. The higher homologues of monocyclic alkanes co-elutes slightly later in both first and second dimensions. That is, C_{16} bicyclic alkanes were monitorized using the *m*/*z* 222 fragment and appear slightly later in the second dimension than cycloalkanes (see Fig. 3). Similarly, although tricyclic compounds contribute less to unresolved complex mixtures than monocyclic and bicyclic compounds, tiny amounts of these compounds have been also detected. The molecular ion *m*/*z* 220 was used to identify the first tricyclic *n*-alkane (*n*-C_{16}), and the homologue compounds elute slightly later in the first dimension.

The unresolved complex mixtures of higher molecular weight homologues comprise systematically steranes and above all hopanes biomarkers. These biomarkers are frequently used to reveal the nature and thermal maturity of organic matter biomass and to provide information about the depositional environment in which the organic matter was trapped [4]. Both sterane and hopane compounds elute close to higher molecular weight *n*-alkanes in the first dimension but their higher polarity compared to *n*-alkanes allows their separation in second dimension. The 14α(H) steranes have been identified using *m*/*z* 217 fragment ion whereas few compounds of 14α(H) methylsteranes have been also detected using the *m*/*z* 231 fragment in their mass spectrum. On the other hand, hopanes are slightly more polar than steranes (pentacyclic vs. tetracyclic), so they are more retained in second column and thus they have been identified in the later zone of GC × GC 2D plot. The *m*/*z* 191 has been used for the identification of hopane biomarkers in GC × GC pattern. Although the assignment of each peak to a hopane compound requires further studies, the relative high proportion of hopane biomarkers compared to steranes is indicative of a bacterial input, which is in agreement with the information provided by the other detected organic biomarker compounds.

In order to test the separation and classification possibilities of 2D gas chromatography, another type of chert, *massive-brechoid*, collected from the same stratigraphic section in Cucho was also analyzed in the same conditions. Fig. 4 shows the 2D plot obtained for the bitumen fraction extracted from this sample, and in this case also, almost all the compounds were largely resolved.

GC × GC–MS analysis allows an easy analytical comparison of different bitumen fractions. In this sense, the higher homologues of *n*-alkanes ($n > C_{25}$) have been identified whereas the lighter *n*-alkanes are present in lower concentration. However, GC × GC–MS analysis allows going deeper in the characterization due to, a difference of the bitumen fraction analyzed before, in this fraction

branched *n*-alkanes and homohopane compounds have not been identified. These geochemical markers can be indicative of the presence of organic compounds related to a terrestrial organic matter input in the analyzed archaeological chert [4].

5. Conclusions

The enhancement of resolution associated with the high peak resolution and the structure of the chromatograms are the main advantages of comprehensive GC × GC in order to get more detailed analysis of organic compounds present in cherts bitumen fraction and thus, leading to obtain reliable information about geochemical data.

GC × GC–FID/MS proved to be a powerful analytical tool to get complementary information to that obtained previously by means of conventional GC–MS. In this sense, visual interpretation of biomarkers makes GC × GC a highlighted analytical tool for organic geochemistry investigations especially when GC × GC is coupled to a MS system. Capillary flow modulators allow this reproducible compound separation easier than using cryogenic modulators in which a rigorous control of temperature is required. However, sensitivity is lost when this configuration is coupled to MS detector. Therefore, in order to improve the sensitivity of the chromatographic method, an optimization of chromatographic method was carried out yielding a robust model in which all the responses came to the same agreement.

Finally, the optimized method was successfully applied in the analysis of organic matter extracted from cherts, and many organic biomarkers have been identified. Based on the distribution of the biomarkers we were able to complete the provenance of some of the studied samples according to the geological formation environment. This information will be highly valuable to support the origin studies of archaeological cherts.

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