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

M. Olivares[a], M. Irazola[a], A. Vallejo[a], X. Murelaga[b], O. Zuloaga[a], N. Etxebarria[a,*]

[a] University of the Basque Country, Department of Analytical Chemistry, P.O. Box 644 E-48080, Bilbao, Basque Country, Spain
[b] University of the Basque Country, Department of Stratigraphy and Paleontology, P.O. Box 644 E-48080, Bilbao, Basque Country, Spain

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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-

* Corresponding author. Tel.: +34 94 601 5530; fax: +34 94 601 3500.
E-mail address: nestor.etxebarria@ehu.es (N. Etxebarria).

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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.