Analytical pyrolysis and thermal analysis to chemically characterise bitumen from Italian geological deposits and Neolithic stone tools

Federica Nardella*, Celia Duce, Erika Ribechini

Department of Chemistry and Industrial Chemistry, University of Pisa, Via Moruzzi 13, 56124 Pisa, Italy

Abstract

The chemical study of bitumen from stone tools from Italian Neolithic sites was carried out using analytical pyrolysis-based techniques, EGA-MS (evolved gas analysis mass spectrometry) and DSPy-GC/MS (double shot pyrolysis-gas chromatography/mass spectrometry). The study was mainly aimed at demonstrating the suitability of analytical pyrolysis for studying archaeological bitumen and for obtaining information regarding its origin. EGA-MS was employed to obtain information on the thermal complexity of the samples and on their thermal degradation areas and DSPy-GC/MS along with Principal Component Analysis (PCA) were tested for biomarker analysis to assess bitumen source in archaeological objects. Geological bituminous rocks from Central-Southern Italy were selected and used as reference materials to both optimize experimental parameters and to support data interpretation for archaeological samples.
Geological samples were also preliminary characterised by thermogravimetric analysis coupled with FTIR spectroscopy (TG-FTIR) under nitrogen and by TG analysis under oxygen to quantify their relative content of organic and inorganic species.

The combination of thermal analysis and analytical pyrolysis-based techniques allowed us to quantify the organic content of the bitumen samples and to obtain information on both soluble and insoluble organic fractions. In addition, the proposed approach highlighted the main degradative patterns and the main differences among samples coming from different geographical areas as well as differences between geological and archaeological bitumen. Finally, DSPy-GC/MS associated with PCA proved to be successful in assessing the bitumen source in archaeological objects by the detection of terpanes, distinctive biomarkers.

Keywords: Archaeological bitumen; biomarker analysis; analytical pyrolysis; thermal analysis

1. Introduction

Bitumen belongs to the class of fossil materials originated from crude oil by evaporation, polymerization and maturation reactions over geological timescale [1]. Natural bitumen deposits are widespread in the Middle-Eastern region although bitumen can be also found all over Europe [2–4]. The accessibility and distinctive chemical-physical properties of this ubiquitous natural resource have made it one of the materials of choice since the Middle Palaeolithic for many purposes (adhesive, hydro-repellent, coating and sealing agents) [5–7].

Bitumen is a complex mixture of hydrocarbons and its chemical composition changes according to bitumen origin because each natural deposit has a different genesis. This means that the chemical characterization can allow us to establish the source of bitumen [8]. Two distinct classes of compounds can be mainly identified, maltenes and asphaltenes. Maltenes are the most studied because they contain polycyclic hydrocarbons such as terpanes and steranes, used as biomarkers for the chemical fingerprinting of bitumen. Asphaltenes correspond to the more complex and heavy fraction of bitumen [9,10]. The investigation of both maltenes and asphaltenes provides several information for bitumen source identification and has been widely used for many application such as oil-oil and oil-source rock correlation, evaluation of thermal maturity and degree of degradation and identification of the depositional environmental conditions and type of biologic precursor [9,11]. The characterization of the samples at molecular level is useful even in archaeological field because of the numerous information achievable. It can provide criteria for correlation between archaeological bitumen and geological reference materials to hypothesize the origin of the archaeological objects from which bitumen is collected [12].

In the last decade of the last century, several studies mainly based on GC/MS, GC-FID and IRMS have been focused on bituminous materials characterization, in the field of archaeology and petroleum geochemistry [13–16]. Procedures based on GC/MS, GC-FID and IRMS normally require sample quantities that are often unavailable in the case of archaeological finds. In addition, such procedures entail several wet-chemical sample pre-treatments leading to possible loss of analytes and contamination [17–19]. Thus, optimisation of classical analytical protocols along with the employment of innovative and more efficient extraction methods have been proposed [20]. In this framework, methods based on analytical pyrolysis (EGA-MS, Py-GC/MS) have also demonstrated their suitability and versatility for studying samples from cultural heritage [21,22]. They require significantly lower amounts of sample than those needed for gas chromatographic analyses and they do not need any sample pre-treatment. In addition, analytical pyrolysis allows us to obtain information simultaneously both on solvent soluble/hydrolysable and macromolecular fractions of the same sample [23–25]. In particular, EGA-MS can be used to establish thermal degradation regions and DSPy-GC/MS (double shot pyrolysis-gas chromatography/mass spectrometry) is able to accomplish thermal desorption and high-temperature pyrolysis in two separate steps on the same sample. Thermogravimetric analysis coupled to Fourier transform infrared spectroscopy (TG-FTIR) is also widely used to study archaeological and artistic objects [26]. TG-FTIR is complementary to EGA-MS or DSPy-GC/MS allowing us to quantify the mass loss associated to a pyrolytic decomposition, combustion and evaporation of a sample and to estimate the relative content of inorganic and organic species [27–29]. The analysis of the evolved gases by FTIR reveals the evolution of small molecules such as CO_2 and water that are not generally acquired when using a mass spectrometer as a detector of pyrolysis products. However, despite all the positive aspects of both thermal analysis and analytical pyrolysis, such techniques are rarely reported in the study of bitumen and archaeological bitumen [30,31].

In this paper, we assess the appropriateness of TG-FTIR, EGA-MS and DSPy-GC/MS for the study of bitumen and its biomarkers in both geological rocks from Central-Southern Italy and archaeological objects. The geological bitumen samples were selected and used as reference materials to both optimize experimental parameters and to support data interpretation for archaeological samples. In addition, principal component analysis (PCA) was used to find out if a classification based on bitumen source can be obtained with the information inferred from DSPy-GC/MS.

2. Materials and method

2.1. Samples

Table 1 lists the geological samples used as reference materials and the archaeological samples studied in this work.

Natural bitumen was collected from caves located in Central – Southern Italy [32,33]; in particular in Abruzzo (4 samples from Pescara and 2 samples from L'Aquila), Sicily (3 samples from Ragusa) and Lazio (1 sample from Frosinone). The samples occur as rocks in which bitumen is solid or semi-solid.

Archaeological bitumen was sampled from Neolithic flint flakes (about two centimetres long) dated back to 5800 – 5000 b.C. that showed spots of a thin layer of black organic material. The stone tools were collected from archaeological excavations in Abruzzo in the Neolithic villages of Colle Cera and Catignano (Pescara) [34,35]. All the samples studied were formerly analysed by an optimized method based on GC/MS [20].

Table 1 – Geological and archaeological samples used in this study.

	Sample	**Location**
Geological bitumen	A1	Abruzzo (PE)
	A2	Abruzzo (PE)
	A3	Abruzzo (PE)
	A4	Abruzzo (AQ)
	A5	Abruzzo (AQ)
	A6	Abruzzo (PE)
	R1	Sicily
	R2	Sicily
	R3	Sicily
	F	Lazio
Archaeological bitumen	CC6	Colle Cera - Abruzzo (PE)
	CC7	Colle Cera - Abruzzo (PE)
	CC9	Colle Cera - Abruzzo (PE)
	CT1	Catignano - Abruzzo (PE)

2.2. Analytical procedures and instrumentation

2.2.1. TG-FTIR

Thermogravimetric analysis was performed using a TA Instruments Thermobalance Q500IR equipped with a spectrophotometer FTIR Agilent Technologies Cary 640 for evolved gas analysis. TG measurements were performed at a rate of 10 °C/min under nitrogen and air from 50 °C to 800 °C. TG-FTIR measurements were performed in the same temperature range at a rate of 20 °C/min, under nitrogen flow (70 mL/min), from 500 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. To reduce the strong background absorption from water and carbon dioxide in the atmosphere, the optical bench was purged with nitrogen. In addition, a background spectrum was taken before each analysis in order to zero the signal in the gas cell and to eliminate the contribution due to the amount of ambient water and carbon dioxide. For each experiment, approximately 5 mg of sample were used.

2.2.2. EGA-MS

The instrumentation consists of a micro-furnace Multi-Shot Pyrolyzer EGA/PY-3030D (Frontier Lab Ltd. Koriyama, JP) coupled with a 5973 Agilent Technologies mass spectrometer equipped with a deactivated and uncoated stainless steel transfer tube (UADTM-2.5N, 0.15 mm i.d. x 2.5 m length, Frontier Lab) kept at 300 °C as well as the oven and the injector. Injector was used in split mode (20:1). A program temperature was chosen for the micro-furnace chamber: initial temperature 50 °C; 15 °C/min up to 900 °C. The mass spectrometer was operated in EI positive mode (70 eV, scanning m/z 50-800). The MS transfer line temperature was 300 °C. The MS ion source temperature was kept at 230 °C and the MS quadrupole temperature at 150 °C. For each experiment, approximately 500 µg of sample were placed into a stainless steel cup and inserted into the micro-furnace.

2.2.3. DSPy-GC/MS

The instrumentation consists of a micro-furnace Multi-Shot Pyrolyzer PY-3030D (Frontier Lab Ltd. Koriyama, JP) coupled to a gas chromatograph 6890 Agilent Technologies (Palo Alto, USA) equipped with an HP-5MS fused silica capillary column (stationary phase 5% diphenyl-95% dimethyl-polysiloxane, 30 m x 0.25 mm i.d., Hewlett Packard, USA) and with a deactivated silica pre-column (2 m x 0.32 mm i.d., Agilent J&W, USA). The GC was coupled with an Agilent 5973 Mass Selective Detector operating in electron impact mode (EI) at 70 eV. Double shot pyrolysis entails the pyrolysis of the same sample at two different temperatures. Each sample (about 500 µg) was placed into a cup connected with the sample holder through a long stainless steel stick and inserted into the micro-furnace. This enabled the sample to be retrieved from the pyrolysis chamber after the first shot. DSPy temperatures were selected on the basis of maximum temperature peaks in the EGA-MS profiles. The first shot temperature was a value between 210 – 280 °C (Thermal Desorption duration 1 min; TD). The second shot temperature was a value between 445 – 460 °C (Pyrolysis duration 1min; Py). Gases evolved at the two different temperatures were eluted in the chromatographic column and detected by mass spectrometry. Chromatographic conditions of both shots were as follows: initial temperature 50 °C, 2 min isothermal; 10 °C/min up to 300°C, 20 min isothermal. Carrier gas: He (purity 99,995%), constant flow 1,1 mL/min.

2.3. Multivariate statistical analysis

Principal component analysis (PCA) was performed via XLSTAT software using the covariance matrix. Data matrix for the PCA was built using the relative percentages of the sum of the peak areas of principal biomarkers detected in first and second shot of DSPy-GC/MS chromatograms. The biomarkers were obtained from Extract Ion Chromatograms (m/z 191) characteristic of terpanes and are listed in Table 7.

3. Results and discussion

3.1 TG-FTIR

Three of the geological samples (one for each geographic area considered) were analysed by TG-FTIR to obtain a preliminary characterization. Figure 1 shows the TG and DTG curves obtained under nitrogen for samples A4, from Abruzzo (AQ), F, from Lazio, and R1, from Sicily. Peak DTG temperatures and mass loss percentage of each step under nitrogen are given in Table 2.

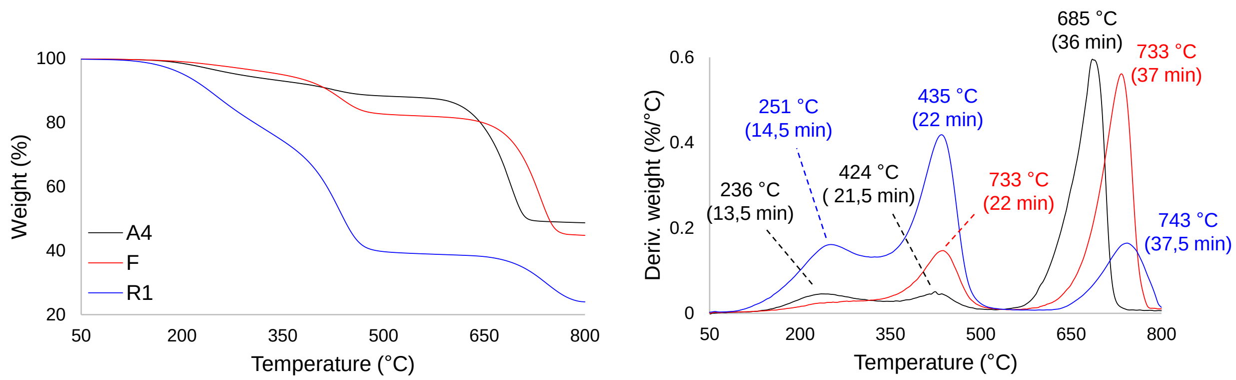


Figure 1 - TG (left) and DTG (right) curves obtained for sample A4, F and R1 under a stream of nitrogen of 10 °C/min.

Table 2 - DTG peak temperatures and mass loss percentages for all the steps of the TG curves.

	DTG peak temperature (Weight loss %)		
	A4 (Abruzzo)	F (Lazio)	R1 (Sicily)
Step 1	236 °C (6,5%)	- -	251 °C (22,7%)
Step 2	424 °C (5,3%)	437 °C (18,0%)	435 °C (38,4%)
Step 3	685 °C (39,3%)	733 °C (37,2%)	743 °C (24,0%)
Residue at 800 °C	(48,7%)	(44,8%)	(14,8%)

The thermal curves of samples A4 and R1 show three main degradation steps, the first in the temperature range 100-300 °C, the second between 350-500 °C, and the last at about 700°C. Sample F has only two mass losses in the same temperature ranges of second and third step of A4 and R1. The gaseous species evolved by thermal degradation of the samples were analysed with FTIR spectroscopy. Figure 2 shows the FTIR spectra of the gases evolved in the decomposition steps for sample A4, F and R1. The evolution profiles of the main gaseous compounds were monitored based on their strongest infrared bands. The profiles are shown in Figure 3 for the three samples.

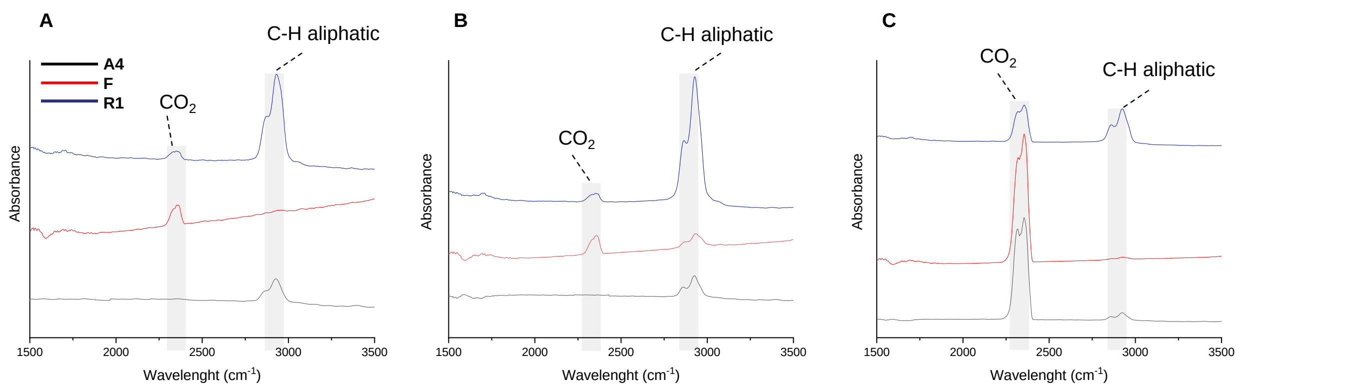


Figure 2 - FTIR spectra of the evolved gas in the range (A) 100 - 300 °C, (B) 350 - 500 °C and (C) 550 - 800 °C for sample A4, F and R1 under nitrogen flow.

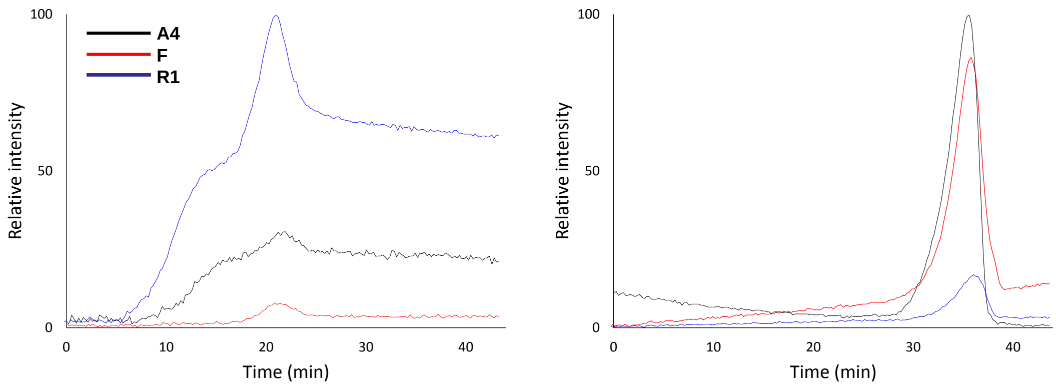


Figure 3 - Evolution profiles of (left) aliphatic compounds and (right) CO_2 in sample A4, F and R1.

The spectra show the bands of the aliphatic C-H stretching at 2968-2883 cm^{-1} in the temperature ranges of the three degradative steps of samples A4 and R1 even though the intensity of the bands in sample R1 is much higher. Sample F presents these bands just in the second degradative step (300 – 500 °C). In addition, we identify very strong bands of CO_2 (2400-2300 cm^{-1}) at about 700°C. The presence of C-H stretching band can be related to aliphatic compounds that are evolved first at lower temperatures suggesting the presence of volatile fraction, and then at higher temperature maybe as decomposition compounds of higher molecular weight molecules. Sample F did not show evolution of aliphatic compounds at lower temperatures. This can be confirmed by examining the evolution profiles in Figure 3. The CO_2 evolved at 700 °C can be ascribed to the degradation of inorganic carbonates of the rock on which bitumen is absorbed [29]. On this respect, samples A4 and F have a residue at 900 °C higher than 45% weight indicating a high content of inorganic material, while R1 has a residue of about 15%. In order to quantify the organic fraction of the bitumen respect to the inorganic ones, we repeated the TG measurements under oxygen, and we measured the mass loss of the samples up to 600°C ascribable to the combustion of the organic material and before the degradation of the rock fragments. The organic and inorganic percent of the bitumen rocks calculated in that way are reported in Table 3 and the TG curves under oxygen are reported in Figure S1 of Supplementary Material.

Sample A4 and F have a lower quantity of organic material respect to R1, (15% vs 88%). The high quantity of organic material in R1 (up to 500°C) under nitrogen is also revealed as a cue in the spectra at 700°C not really evolved by the sample at 700°C but coming from the evolution at lower temperature.

Table 3 – Relative amount of organic and inorganic material.

	T (°C)	% organic fraction	% inorganic fraction
A4	595	15,9	84,1
F	600	14,2	85,8
R1	560	88,3	11,7

3.2 EGA-MS analysis

The geological samples were subjected to evolved gas analysis (EGA-MS) in order to study their thermochemistry and select the experimental temperatures to perform DSPy-GC/MS analyses.

All samples give very similar results. The total ion thermogram (TIT) profile and the average mass spectra obtained by EGA-MS of bitumen A4, F and R1 are shown in Figure 4. Table 4 summarizes the maximum temperature of each degradation step.

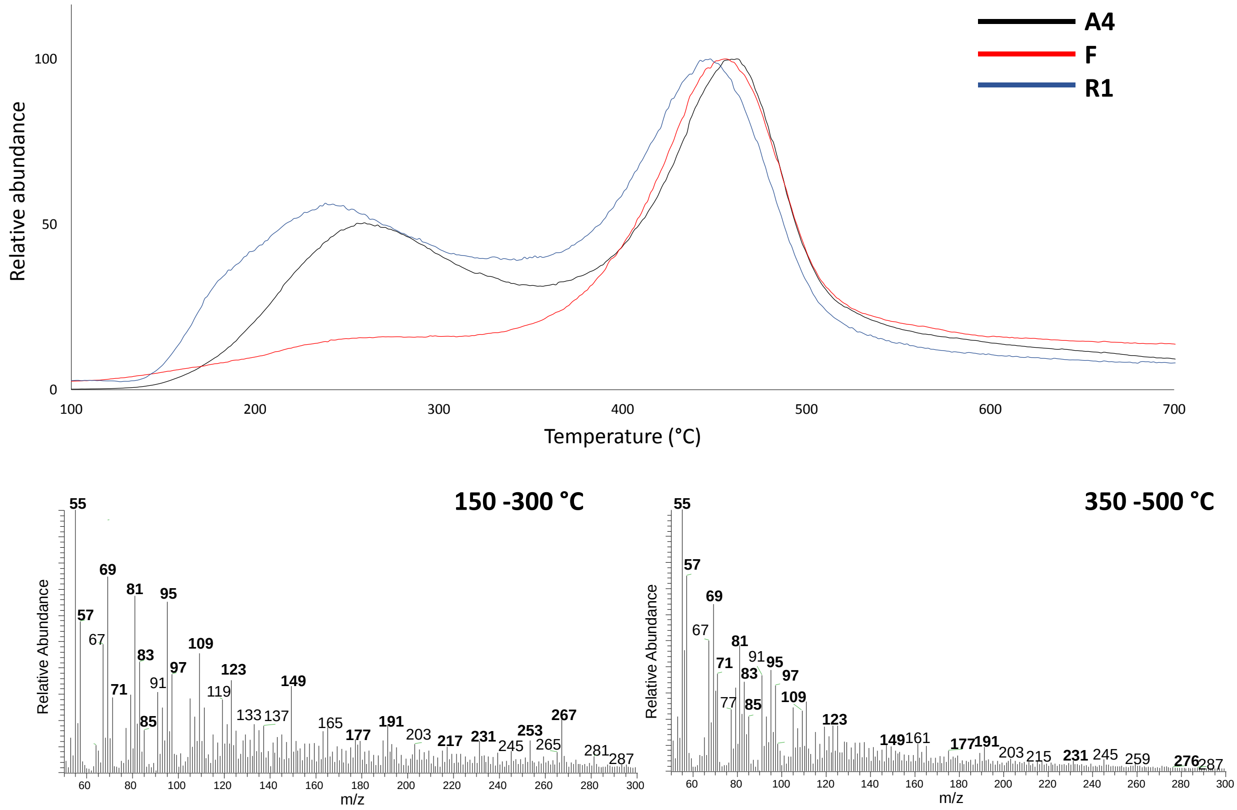


Figure 4 - Total Ion Thermogram (TIT) and average mass spectra associated of geological sample A4, F and R1.

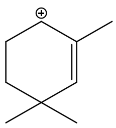
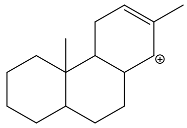
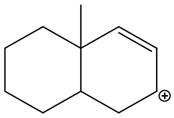
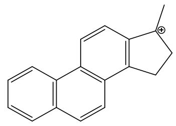
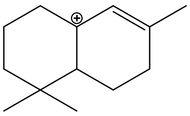
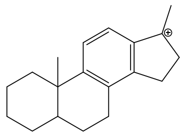
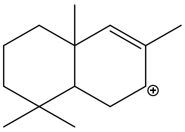
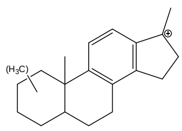
Table 4 - Experimental temperatures of the degradation steps obtained for geological samples.

Sample	Temperature of the maximum	
	Step #1	Step #2
A1	232 °C	461 °C
A2	220 °C	457 °C
A3	228 °C	460 °C
A4	260 °C	460 °C
A5	265 °C	465 °C
A6	224 °C	459 °C
R1	245 °C	448 °C
R2	240 °C	448 °C
R3	230 °C	458 °C
F	247 °C	456 °C

The thermograms of the geological bitumen exhibit two peaks, ascribable to two main degradation steps, in good agreement with the mass losses revealed by TG analysis. The first peak has a maximum at temperatures ranging from 220 to 265 °C and the second one from 445 to 465 °C. Given that the first step takes place at relatively low temperature, it is considered a thermal desorption process in which the smaller and more volatile compounds are released. The average mass spectrum associated to the first peak in the thermogram is dominated by the peaks due to the fragmentation of hydrocarbons as also suggested by TG-FTIR analysis. In particular, peaks at *m/z* 57, 71, 85 from alkanes, at *m/z* 55, 69, 83, 97 from alkenes and at *m/z* 67, 81, 95, 109 from dienes are present as well as peaks at *m/z* 91 and 105 from aromatic rings obtained by the rearrangements of aliphatic chains at high temperature [36]. In addition, peaks at *m/z* 123, 149, 177, 191, 217, 231, 253 and 267, characteristics of terpanes and steranes, can be observed. Table 5 lists the main peaks present in the mass spectra along with the structure of the corresponding ions and fragment ions and class of compounds from which they derive.

The second step takes place at higher temperature and it is considered as an actual pyrolytic process in which the polymeric network is decomposed. Nevertheless, the average mass spectrum associated to the second peak is quite similar to first one and shows peaks at the same *m/z* ratios. The analogies between the mass spectra corresponding to the two thermal degradation steps suggest that similar species are released during the two steps. We believe that free and more volatile compounds are desorbed during the first step (at about 250 °C), while at higher temperatures (at about 450 °C) the decomposition of the asphaltenes, consisting in complex polycyclic structure including alkyl-substituted structures [37], is occurring. In addition, similar species could be trapped into the asphaltenic matrix and can be released when the asphaltenes decomposes.

Table 5 - List of the most abundant peaks identified in the average mass spectra of the two degradation steps along with the structure of the corresponding fragment ions and class of compounds from which they derive.

m/z	Structure	Class	*m/z*	Structure	Class
55	$C_4H_7^{\bullet+}$	Unsaturated hydrocarbons	83	$C_6H_{11}^{\bullet+}$	Unsaturated hydrocarbons
57	$C_4H_9^{\bullet+}$	Saturated hydrocarbons	85	$C_6H_{13}^{\bullet+}$	Saturated hydrocarbons
69	$C_5H_9^{\bullet+}$	Unsaturated hydrocarbons	95	$C_7H_{11}^{\bullet+}$	Unsaturated hydrocarbons
71	$C_5H_{11}^{\bullet+}$	Saturated hydrocarbons	97	$C_7H_{13}^{\bullet+}$	Unsaturated hydrocarbons
81	$C_6H_9^{\bullet+}$	Unsaturated hydrocarbons	109	$C_8H_{13}^{\bullet+}$	Unsaturated hydrocarbons
123		Terpanes	217		Steranes
149		Steranes	231		Triaromatic steranes
177		Norhopanes	253		Monoaromatic steranes
191		Hopanes	267		Monoaromatic steranes

EGA-MS analysis was performed on four archaeological samples, three from Colle Cera excavation (CC6, CC7 and CC9) and one from Catignano excavation (CT1).

Compared to geological samples thermograms, the profiles of these four samples show a single degradation step with a maximum between 445 and 460 °C (Table 6). The thermogram and the mass spectra associated to sample CC6 are shown in Figure 5 as an example. The thermograms of the other archaeological samples are reported in Figure S2 of Supplementary Material.

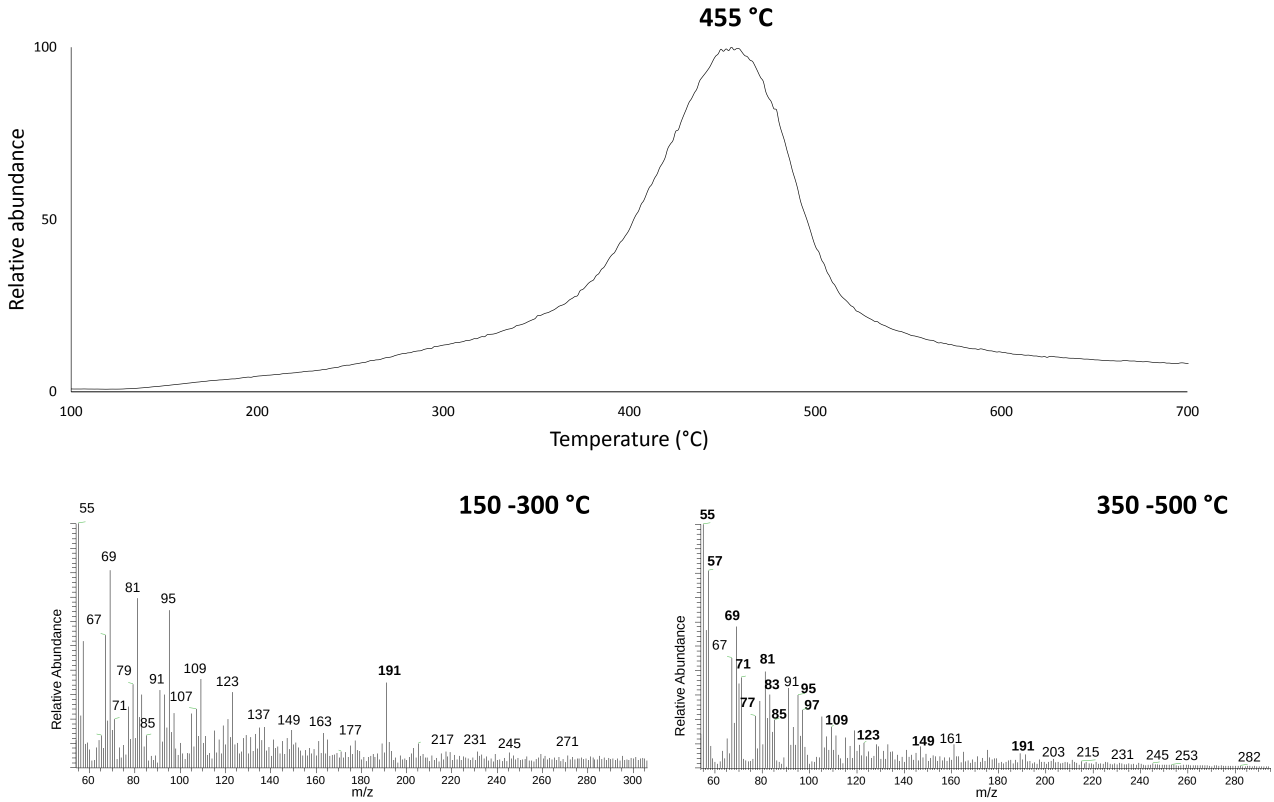


Figure 5 - Total Ion Thermogram (TIT) and average mass spectra associated of archaeological sample CC6.

The temperature range is the same than that of the second degradation step of the reference materials, suggesting the only presence of species derived from the decomposition of asphaltenic fraction. This is confirmed by the average mass spectra shown in Figure 5: the spectrum relative to the first area shows an increasing in the abundance of the peaks at *m/z* 191 probably due to the decreasing of the fragment ions of alkanes, alkenes, dienes and aromatic rings. On the other hand, the spectrum associated to the degradation peak has the same profile of that obtained in second step degradation of geological samples.

We assume that the depletion of the more volatile fraction of bitumen could have been induced by the anthropogenic treatments performed on bitumen in the interest of extracting it from the rocks and making it suitable for several applications. In particular, heating is thought to be responsible for the evaporation of more volatile compounds.

Table 6 - Experimental temperatures of the degradation steps obtained for archaeological samples.

Sample	Temperature of the maximum
CC6	455 °C
CC7	455 °C
CC9	440 °C
CT1	460 °C

3.3 DSPy-GC/MS analysis

To further investigate the main products of thermal decomposition at the different temperatures, and thus to better understand the results obtained by TG and EGA, samples were analysed by double shot pyrolysis gas chromatography/mass spectrometry (DSPy-GC/MS). The temperatures of the two shots were selected for each sample basing on EGA-MS results: a first thermal desorption (TD) was selected in a range of 220 – 260 °C and subsequently an actual pyrolysis (Py) was performed in a range of 445 – 465 °C. As suggested by previous analysis, both the thermal desorption chromatogram and the pyrogram of the geological samples are dominated by the peaks of alkanes, alkenes and dienes. In particular, extracting the signal at *m/z* 85, *n*-alkanes ranging from 11 to 34 carbon atoms can be identified. Figure 6 reports the result obtained for geological sample A4 as an example, same results were obtained for other samples investigated.

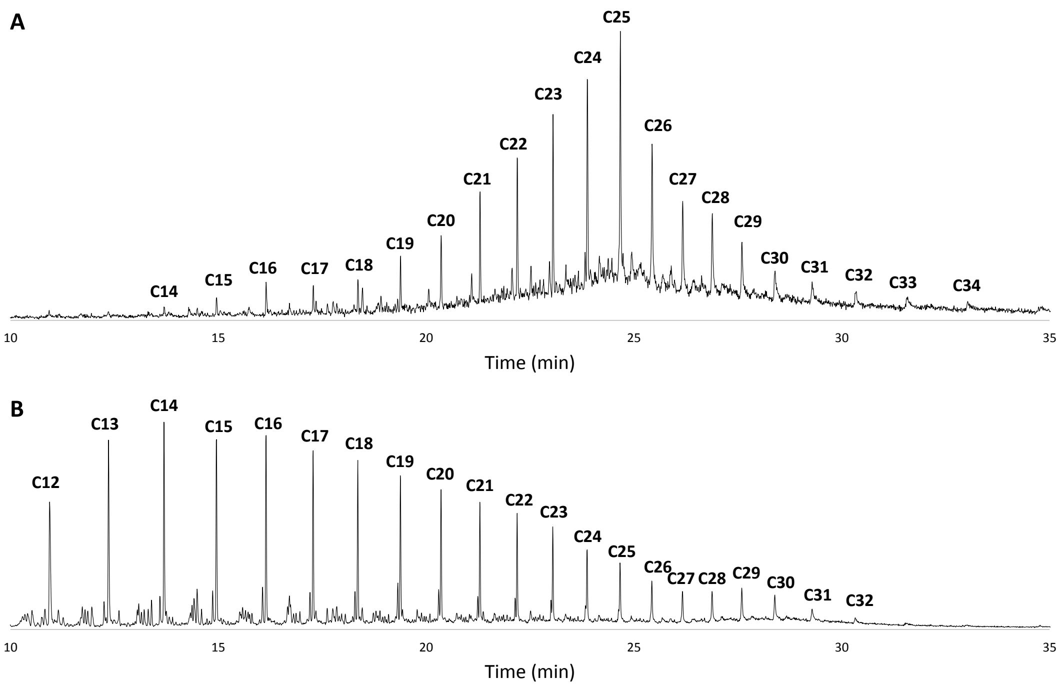


Figure 6 - DSPy-GC/MS extract ion chromatograms (m/z 85) at (A) 260 °C and (B) 460 °C of geological sample A4. Cn: linear alkane with n carbon atoms.

For archaeological samples, analyses were performed in double shot mode, despite the lack of the first degradation step, showing that alkanes are absent in the chromatogram of thermal desorption temperature range (Figure 7) supporting the results obtained from EGA-MS analysis.

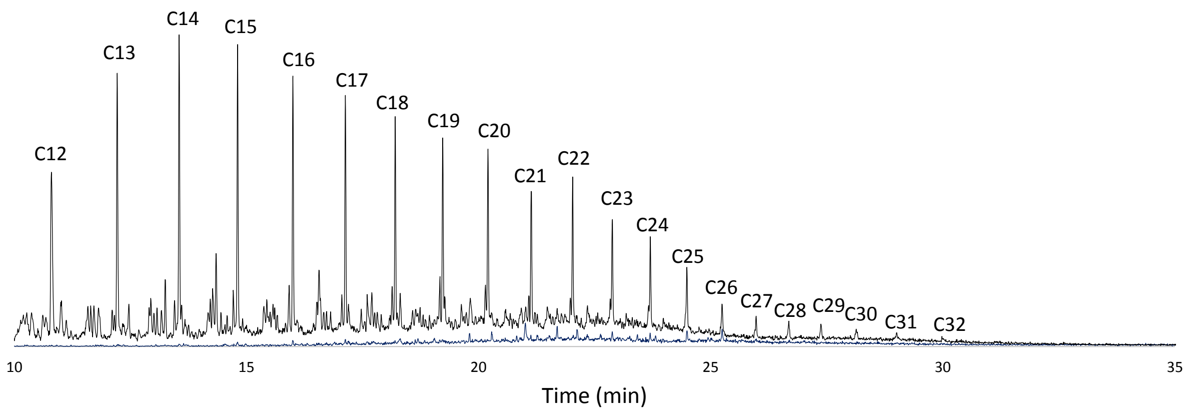


Figure 7 - Double shot Py-GC/MS extract ion chromatograms (m/z 85) of the archaeological sample CC6 at 250 °C (blue line) and at 460 °C (black line). Cn: linear alkane with n carbon atoms.

Given that analytical pyrolysis requires small amount of sample which does not need any pre-treatments we decided to test this technique to establish if it is suitable for biomarker analysis. To this purpose, terpane distribution pattern (*m/z* 191) was used. Chromatographic profiles obtained for the geological and archaeological samples at the temperatures of 250 °C and 460 °C are shown in Figure 8 and Figure 9, respectively. Table 7 lists the identified biomarkers.

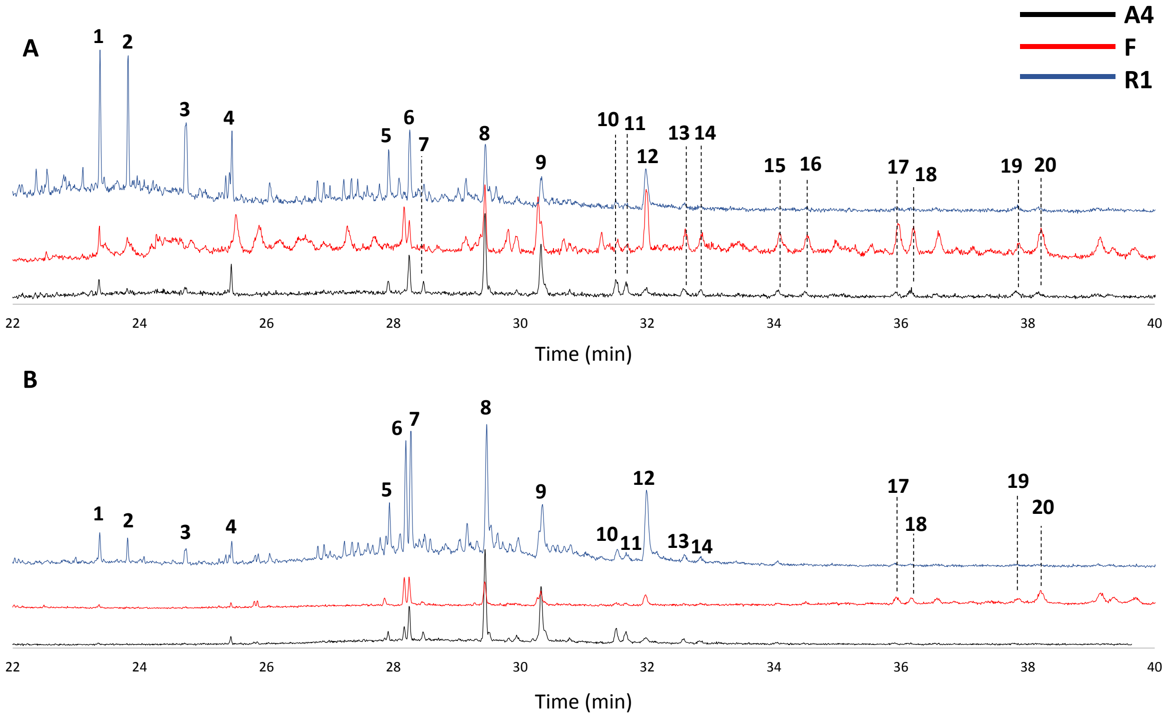


Figure 8 - Double shot Py-GC/MS extract ion chromatograms (m/z 191) at (A) 250 °C and (B) 460 °C of geological sample A4, F and R1. Peaks are labelled according to Table 7.

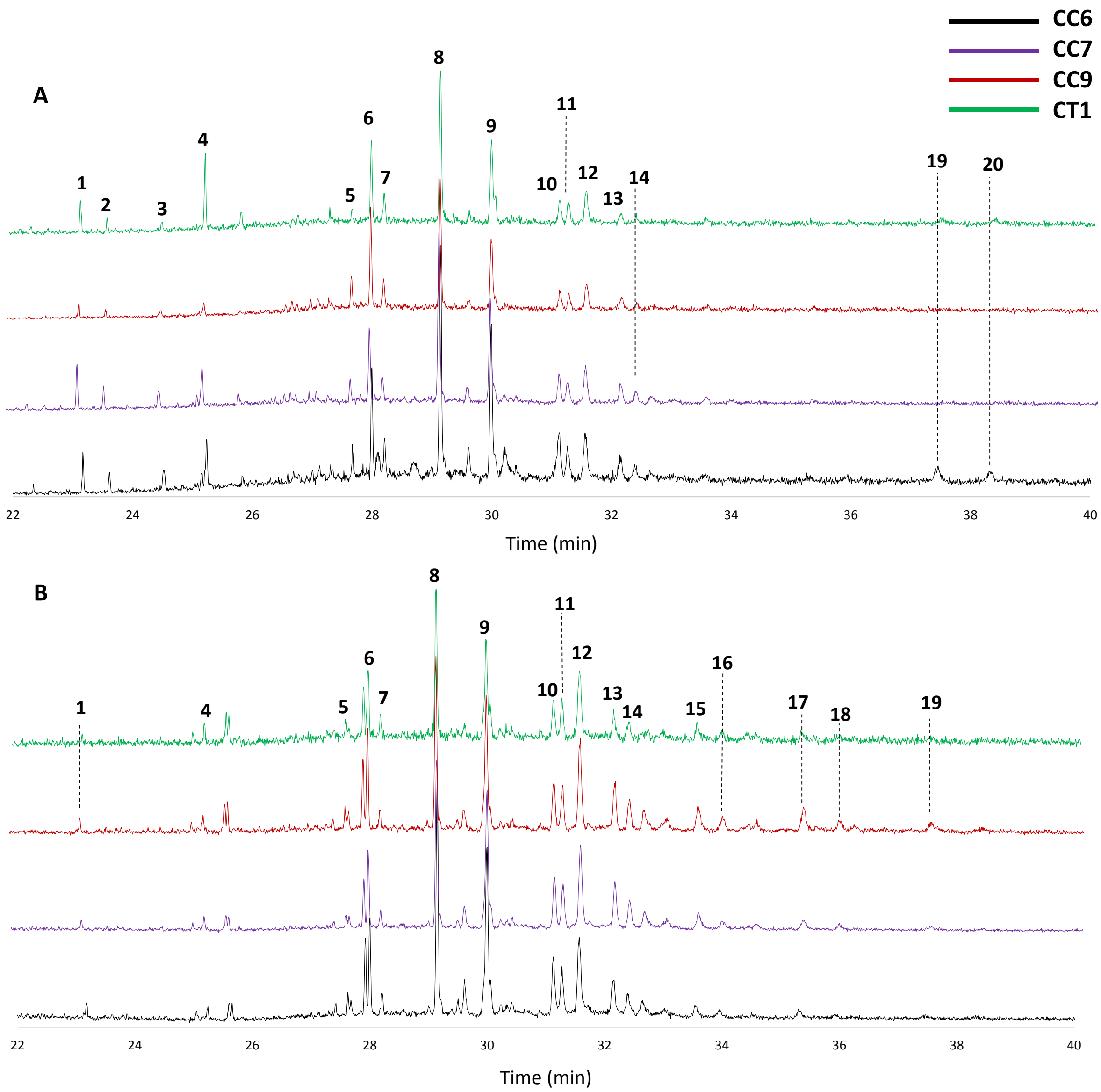


Figure 9 - Double shot Py-GC/MS extract ion chromatograms (m/z 191) at (A) 250 °C and (B) 460 °C of archaeological sample CC6, CC7, CC9 and CT1. Peaks are labelled according to Table 7.

Table 7 - Principal compounds detected in thermal desorption chromatograms and pyrograms of geological and archaeological samples.

Peak number	Compound	Abbreviation
1	C23 tricylic terpane	TR23
2	C24 tricylic terpane	TR24
3	C25 tricylic terpane	TR25
4	C24 tetracyclic terpane	TET24
5	18α(H),21β(H)-22,29,30-trisnorhopane	Ts
6	17α(H),18α(H),21β(H)-22,29,30-trisnorhopane	Tm
7	17α(H),18α(H),21β(H)-28,30-bisnorhopane	H28

8	17α(H),21β(H)-30-norhopane	H29
9	17α(H),21β(H)-hopane	H30
10	22S-17α(H),21β(H)-30-homohopane	H31S
11	22R-17α(H),21β(H)-30-homohopane	H31R
12	Gammacerane	GAM
13	22S-17α(H),21β(H)-30,31-bishomohopane	H32S
14	22R-17α(H),21β(H)-30,31-bishomohopane	H32R
15	22S-17α(H),21β(H)-30,31,32-trishomohopane	H33S
16	22R-17α(H),21β(H)-30,31,32-trishomohopane	H33R
17	22S-17α(H),21β(H)-30,31,32,33-tetrakishomohopane	H34S
18	22R-17α(H),21β(H)-30,31,32,33-tetrakishomohopane	H34R
19	22S-17α(H),21β(H)-30,31,32,33,34-pentakishomohopane	H35S
20	22R-17α(H),21β(H)-30,31,32,33,34-pentakishomohopane	H35R

Several biomarkers were identified in the chromatographic profiles despite the low resolution and the small abundance of the peaks. Table S1 of Supplementary Material reports the relative abundance of the biomarker identified in the first and second shot of DSPy-GC/MS of the investigated samples.

To highlight the compositional differences, data obtained were submitted to multivariate statistical analysis by principal component analysis (PCA). The resulting scatter and loading plots for the first two principal components are shown in Figure 10.

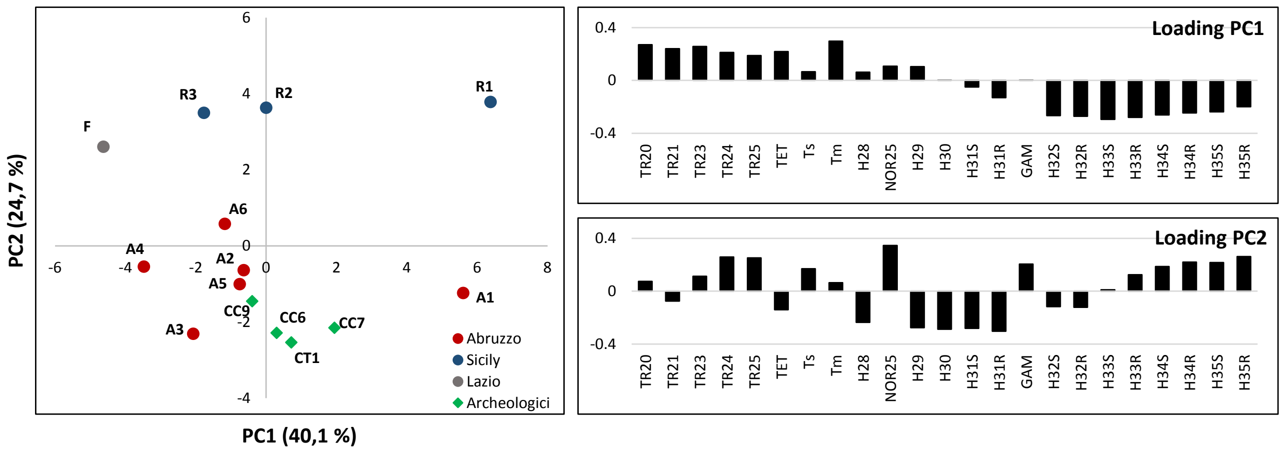


Figure 10 - PCA scatter plot and loading plot of the chromatographic data obtained by DSPy-GC/MS for the geological and archaeological samples analysed.

PC1 and PC2 account for 64,8% of the variance. The samples in the plot get separated on PC2: samples from Sicily and Lazio are located at positive values of PC2, while archaeological and geological samples from Abruzzo are located at values of PC2 lower than 1. The abundance of gammacerane (GAM) and trisorhopanes (Ts and Tm) characterises the samples from Sicily and Lazio, while samples from Abruzzo are enriched in hopane and homohopane homologs (H28 – H31) and are characterized by the lack of 25-norhopane (NOR25H). Although the separation is not so pronounced, pyrolysis data allow us to discriminate the different bitumen source and to state that archaeological samples share the origin with bitumen from Abruzzo. These results are consistent with those achieved by GC/MS [20], thus proving the suitability of the proposed method based on analytical pyrolysis to study bitumen and its origin identification.

4. Conclusions

Data and results obtained within this work show how the use of the combination of thermal analysis and analytical pyrolysis, used into two different configurations, EGA-MS and DSPy-GC/MS, is an extremely effective way to chemically characterize bituminous samples. Above all, it has been proven the suitability of such approach for the study of bitumen, in particular of archaeological bitumen, since the techniques used requires significantly low amounts of sample and no sample pre-treatment.

The thermochemistry and the thermal-complexity of bitumen samples were investigated by TG-FTIR and EGA-MS, allowing us to highlight the main degradative patterns and the main differences among samples coming from different geographical zones and to emphasize the differences between geological and archaeological samples. The data show that geological bitumen decomposes with two main degradation steps peaked in the temperature range 100-300 °C and 350-500 °C, respectively during which the same kind of compounds is evolved. These compounds are present in the samples as free species, which are desorbed at lower temperature, and either can derive from asphaltenes decomposition at higher temperatures. Archaeological samples show a single degradation step peaked at 445-465 °C. The lack of the more volatile fraction of bitumen is probably due to the evaporation caused by the anthropogenic treatments performed on bitumen in the interest of extracting it from the rocks and making it suitable for its several applications. Double shot pyrolysis gas chromatography/mass spectrometry (DSPy-GC/MS) was used to deepen the TGA-FTIR and EGA-MS data and to outline the chemical composition of the samples. The lack of the more volatile fraction in the archaeological bitumen was confirmed analysing alkanes distribution pattern (m/z

85). In addition, examining the distribution profile (m/z 191) of the terpane compounds, we were able to identify more than 20 biomarkers, whose chromatographic areas were used as variables for PCA allowing us to discriminate among bitumen from different Italian geological area and to assess the origin of the archaeological bitumen.

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Supplementary Material

Analytical pyrolysis and thermal analysis to chemically characterise bitumen from Italian geological deposits and Neolithic stone tools

Figure S1 - TG (left) and DTG (right) curves obtained for sample A4 (Abruzzo, AQ), F (Lazio) and R1 (Sicily) under a stream of air of 20 °C/min.

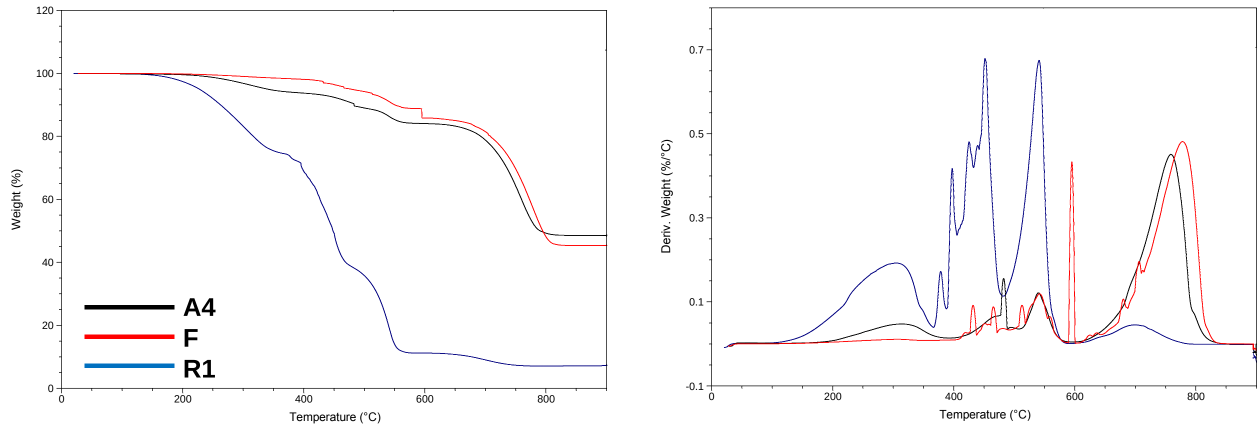

Figure S2 - Thermograms of archaeological samples (A) CC7, (B) CC9 and (C) CT1.

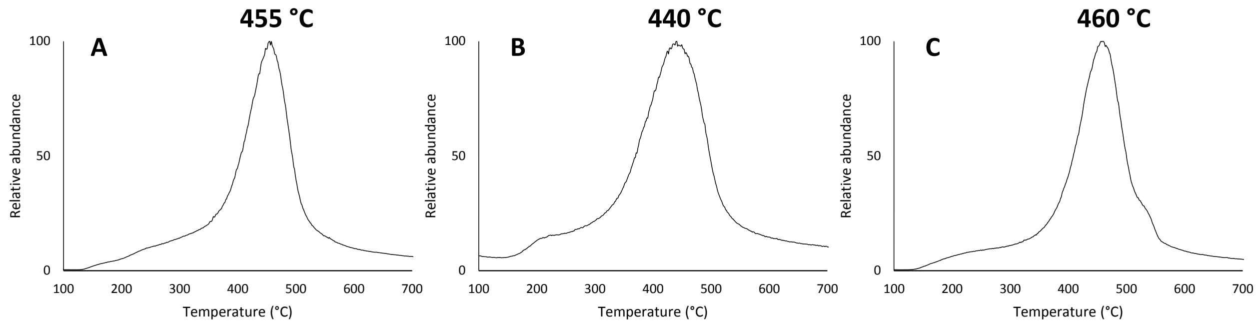

Table S1 - Relative abundance of biomarker identified in first and second shot of DSPy-GC/MS of the samples investigated.

	A1	A2	A3	A4	A5	A6	R1	R2	R3	F	CT1	CC7	CC6	CC9
TR20	6.2	0.0	0.0	0.0	0.0	0.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TR21	1.7	0.0	0.0	0.0	0.0	0.7	3.6	0.0	0.0	0.0	2.5	1.8	2.3	2.5
TR23	6.9	4.5	0.3	1.3	1.9	3.1	5.3	3.9	2.6	0.5	2.3	2.4	1.4	1.2
TR24	1.0	0.0	0.0	0.0	0.0	0.6	4.8	1.8	2.6	0.0	0.9	1.5	0.5	0.3
TR25	0.0	0.0	0.0	0.0	0.0	0.0	4.7	1.7	1.7	0.0	0.0	1.5	0.7	0.4
TET	8.3	3.7	0.4	2.9	1.2	2.2	3.1	2.0	1.2	0.6	6.0	4.1	2.3	2.1
Ts	2.5	1.7	0.9	1.9	4.7	1.0	3.8	4.0	4.0	3.6	1.9	5.1	1.7	2.8
Tm	18.0	14.2	7.1	8.7	13.5	12.5	20.5	13.1	10.5	8.8	11.8	16.3	10.9	13.2
H28	5.3	4.0	2.3	2.2	3.4	3.1	0.0	0.0	0.0	2.5	3.6	13.4	2.6	2.5
NOR25	0.0	0.0	0.0	0.0	0.0	0.0	3.2	2.9	2.5	0.0	0.0	0.0	0.0	0.0
H29	24.9	20.4	21.1	15.7	24.7	18.8	15.2	13.3	8.2	9.2	20.1	12.6	20.9	17.4
H30	15.6	10.1	21.7	11.8	15.3	8.3	9.8	7.9	10.2	9.9	14.9	9.7	19.8	16.1
H31S	2.8	4.8	7.0	5.7	4.4	3.9	2.2	4.3	4.1	2.9	6.1	10.2	7.1	5.7
H31R	2.2	5.0	6.5	6.0	4.1	4.4	1.5	3.5	3.6	2.1	5.8	6.0	5.1	4.9
GAM	2.6	4.7	9.0	4.2	2.7	9.8	13.0	11.2	10.9	9.5	10.3	5.6	9.5	10.5
H32S	1.0	2.9	4.1	4.5	3.0	3.3	1.5	2.6	3.8	4.6	4.3	3.5	3.9	4.7
H32R	0.9	2.4	3.2	4.1	3.7	2.9	1.0	1.7	3.3	4.1	4.0	2.3	3.3	3.6
H33S	0.0	5.5	3.5	6.3	3.7	3.4	0.0	2.2	4.5	5.4	2.3	2.2	1.8	3.5
H33R	0.0	3.8	2.2	5.3	3.1	2.3	0.0	3.0	3.9	7.8	1.7	1.8	1.1	2.1
H34S	0.0	4.6	2.9	3.5	2.1	3.8	0.0	5.0	4.4	8.8	1.6	0.0	1.4	3.2
H34R	0.0	4.0	2.4	3.9	4.3	6.1	0.0	4.4	6.3	7.2	0.0	0.0	0.8	1.3
H35S	0.0	4.0	2.6	7.3	2.7	6.6	0.8	4.8	7.9	5.9	0.0	0.0	2.2	1.9

H35R	0.0	0.0	2.8	4.5	1.6	3.0	0.8	6.7	3.6	6.6	0.0	0.0	0.7	0.0