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Analysis of Kazakhstan Crude Oil Biomarkers by Gas Chromatography in Combination with Mass Spectrometry

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Abstract: Kazakhstan ranks as the 12th largest oil producer globally and boasts a diverse range of crude oils. This research introduces a method for distinguishing between the different types of crude oils based on biomarker analysis of 28 crude oils from Western and Southern Kazakhstan using gas chromatography-mass spectrometry. Biomarkers serve as valuable tools, especially in forensic investigations of oil spills. These biomarkers effectively retain a significant portion of the original natural product's carbon structure, providing crucial evidence regarding the origin and identity of the oils under examination. This study identifies a set of biomarkers, including pristine, phytane, n-C_{17} and n-C_{18} alkanes, hopanes, bisnorhopanes, iso-copalanes, pregnane, androstane, allopregnane, homopregnane, cholestane, and stigmastane. By examining ratios such as pristane/phytane, pristane/n-C_{17} alkane, tricyclic/pentacyclic terpanes, and hopane, as well as the distribution of steranes, it was deduced that crude oils from West Kazakhstan exhibited resilience to biodegradation. These findings showed that gas chromatography-mass spectrometry is an effective method for oil biomarkers determination, especially because it provides efficient separation and identification. Additionally, this study delved into the origin conditions and maturity of these oils, contributing to a deeper understanding of their characteristics and analysis that is simple to use and available worldwide.

Keywords: gas chromatography; mass spectrometry; crude oil; biomarkers; GC-MS; environmental pollution; fingerprinting

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1. Introduction

Kazakhstan is a major oil producer with the second-largest oil reserves and oil production among the former Soviet republics, after Russia, and is 12th on a global scale among oil-producing countries based on production volume [1]. Kazakhstan has produced crude oil since 1911. Throughout the country, 169 hydrocarbon deposits have been discovered comprising 87 oil fields, 17 gas fields, 30 gas and oil, 25 oil-and-gas condensate, and 10 oil condensate fields [2]. The production of crude oil reached a total of 1.77 million barrels/day in 2017. The three main oil fields passing through the Caspian Sea are the Tengiz, Karachaganak, and Kashagan fields, respectively [3].

It is proved that crude oil reserves in Kazakhstan have 30 billion barrels, the 2nd largest endowment in Eurasia after Russia, and the 12th largest in the world after the United States [3].

The Caspian Sea, in addition to Western and Southern Kazakhstan borders, in the Southeast to Turkmenistan, in the South to Iran, in the Southwest to Azerbaijan, and in the Northwest to Russia, constitutes the main transport route for crude oil to other countries. To prove the quality of crude oil knowledge of the origin of the product, as well as information on its chemical composition and physical properties, is crucial [4].

The objective of the present study is to identify crude oil origin partly by studying inherent biomarkers. The decline in the physical properties of crude oil observed in numerous basins is commonly associated with biodegradation, and the extent of this degradation is often identifiable through the features exhibited by the crude oil biomarkers [5]. Further, the results of the present study gain relevance since the transport of crude oils obviously leads to unwanted situations of an oil spill, which calls for analytical methods to identify the origin of the spilled oil and, thus, not least, to make the polluter accountable.

Biomarkers stand as crucial hydrocarbon components within crude oil for chemical fingerprinting. These molecules possess intricate molecular structures inherited from previously living organisms, which seemingly endured without alteration until the present time. Leveraging biomarkers for identifying spilled oils enables the determination of a specific crude oil's origin. Crude oil fingerprinting technology is the main forensic method for oil spill identification. In comparison with other hydrocarbons, biomarkers have shown to be highly resistant to degradation and may thus disclose the specific origin of the oil due to its unique biomarker fingerprint [6] and then possibly pinpoint the actual polluter.

From an environmental point of view, there is a great complexity in establishing polluters of oil spill accidents [7].

Identifying crude oils through biomarker analysis has significance in the characterization of crude oils. Biomarkers are organic compounds found in crude oil that provide information about its origin, thermal history, and the type of organic matter from which it was formed. These molecular fossils are useful in determining the source rock, maturity, and age of the oil and simple in-use.

Gas chromatography-mass spectrometry in the determination of biomarkers was carried out in the following steps:

1. Sampling: Crude oil samples are collected from different wells or sources for analysis.
2. Extraction and Separation: The crude oil is processed to isolate the organic compounds from the sample with the gas chromatography technique.
3. Identification of Biomarkers: Various biomarkers are identified and analyzed including pristane (Pr), phytane (Ph), n-C_{17} alkane, n-C_{18} alkane, terpanes, pregnane, androstane, allopregnane, homopregnane, cholestane, and stigmastane.
4. Analysis and Interpretation: Examining the ratios and distributions of the biomarkers determines the type of source rock (marine, terrestrial), the thermal maturity, and the age of the oil [8,9].
5. Comparative Analysis: The obtained biomarker data are compared to a database of known biomarker profiles of different crude oils to infer the possible origin and characteristics of the analyzed oil sample [10].

This analysis helps in understanding the oil's characteristics, which is valuable for oil exploration, reservoir management, and production strategies. By determining the source and maturity of crude oil, companies can make informed decisions regarding drilling locations and extraction methods.

So over time, biomarker analyses have developed as the main techniques used in petroleum exploration to study crude oils, their origin, and maturity. In this context, gas chromatography-mass spectrometry (GC–MS) has been widely used as the method of choice for disclosing biomarkers [11–26].

Mass spectrometry has been long recognized as the most powerful detecting method for gas chromatography [27]. Thus, GC-MS is one of the most valuable tools for the identification of unknown compounds. In recent years two-dimensional gas chromatography (GC $\times$ GC) has proved its importance for the analyses of complex samples [28–31]. Hence, GC $\times$ GC has also found its application for oil fingerprinting purposes [32]. However, the limitation of this approach, i.e., the excessive dependence on a relatively small number of biomarkers for the characterization of complex fluids such as crude oil, should be emphasized [32,33].

In the literature review, available information on biomarkers in various crude oils determined by gas chromatography-mass spectrometry is summarized. Thus, initial information on the parameters of the GC-MS analyses may be found here [33–64].

Despite the long history of oil production in Kazakhstan, petroleum biomarkers with modern methods of analysis have not yet been conducted. Hence, the main objective of the present study is to disclose the biomarker fingerprints for a series of Kazakhstan crude oils applying a GC-MS-based method.

2. Materials and Methods

2.1. Selected Samples

The present study included 28 Kazakh crude oils, each originating from one of the four oil-producing areas in West and South Kazakhstan (Table 1). It should be noted that two samples, Nuraly and Kosshagyl, were chosen to optimize GC-MS parameters.

Table 1. List of crude oil studies and the deposits' location.

No	Crude Oil Field	Year of Discovery	Location
1	Akingen	1980	Atyrau region (West Kazakhstan)
2	Akkudyk	1981	
3	Baichunas	1931	
4	Balgimbaev	1978	
5	Kosshagyl	1926	
6	Prorva	1964	
7	Tengiz	1979	
8	Zhanatalap	1964	
9	Dossor	1911	
10	Kashagan	2000	
11	Akshabulak	1988	Kyzylorda region (South Kazakhstan)
12	Aryskum	1985	
13	Aschysai	2005	
14	Konys and Bektas	1989 and 1987	
15	Nuraly	1987	
16	Sarybulak	1975	
17	Kyzylorda	1986	
18	Beineu	1966	Mangystau region (West Kazakhstan)
19	Kalamkas	1976	
20	Karamandybas	1988	
21	Karazhanbas	1984	
22	Zhanaozen	1961	
23	Zhangurshi	1981	
24	Zhetybai	1961	
25	Buzachi	1975	
26	Atasu	1939	Karagandy region (South Kazakhstan)
27	Kumkol	1984	
28	Kyzylkiya	1986	

2.2. Sample Separation

According to the literature review (Appendix A), accurate sample preparation is a crucial component for successful chromatography to ensure the integrity of the sample and removal of impurities that otherwise may be detrimental to the analyses. The sample preparation in the present study included dissolution of the petroleum samples in n-hexane (SupraSolv®, ≥95%; Sigma-Aldrich, Burlington, MA, USA) to remove asphaltenes followed by a column chromatographic fractionation to separate the sample into subsamples of aliphatic and aromatic hydrocarbons, respectively. Based on the literature review (Appendix A), observations used a chromatographic column (length 200 mm × 10.5 mm i.d.) applying silica gel (Sigma-Aldrich, USA), aluminum oxide, and anhydrous granular sodium sulfate oxide (purchased from LLP (Laborpharma), Almaty, Kazakhstan) in a

proportion of 2:1:1 was used for the fractionation. Before fractionation aluminum oxide was activated with distilled water (1:1) and dried at 360 °C for 5 h or overnight. The silica gel was washed with acetone (≥96%; LLP (Laborpharma), Almaty, Kazakhstan) and *n*-hexane and dichloromethane for GC (≥99.9%; Sigma-Aldrich, USA), and was subsequently completely dried in a fume hood at 160–180 °C for 20 h. Glass wool was used as stopper, washed with acetone, hexane, and dichloromethane and dried. Sodium sulfate was calcined and subsequently cooled in a desiccator. A total of 0.1 g of crude oil was weighed and diluted with 10 mL of hexane. Samples (10 mL) of petroleum in n-hexane were applied to the column. The eluents were collected as follows: 12 mL of *n*-hexane expected to contain the aliphatic hydrocarbons (Fraction 1), followed by 15 mL of *n*-hexane, and dichloromethane (1:1) to elute aromatic hydrocarbons (Fraction 2).

However, GC MS has limitations in analyzing high molecular weight compounds. It also has highly sensitive capability and is effective in identifying and quantifying the number of compounds. Moreover, the method is simple in use and relatively faster in comparison with other separation techniques.

2.3. GC-MS Parameters for Biomarker Analysis

Determination of biomarkers was carried out by using GC with an MS detector (6890N/5973N; Agilent, Santa Clara, CA, USA) applying a DB-35ms coated capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent, USA). Three oven programs were initially used for the optimization of GC-MS parameters:

1. 60 °C (held for 4 min) to 300 °C by a rate of 10 °C/min and held for 15 min.
2. 50 °C (held for 5 min) to 250 °C by a rate of 10 °C/min, from 250 °C to 300 °C by a rate of 5 °C/min.
3. 50 °C (held for 5 min) to 300 °C by a rate of 20 °C/min and held for 20 min.

Three injector temperatures at 200, 240, and 280 °C, respectively, were used for further optimization of the GC analyses. With an injection volume of 1 μL, the GC was operated in splitless mode. Helium was used as carrier gas with a flow rate of 1 mL/min. The obtained chromatograms were processed using both single ion monitoring (SIM) and scan mode, respectively. The analyses were performed in duplicate, each comprising five replicates. A comparison of the diagnostic ratios was applied to show the most specific biomarker distribution differences between samples.

Eventually, the optimization process pointed at the 3rd oven program and injection temperature 280 °C as being optimal.

3. Results and Discussion

3.1. Optimal GC Parameters

Scrutinizing the literature review (Appendix A) indicated optimal GC parameters for injection, column, and oven temperature programming. Thus, according to the data (Appendix A), splitless injections (1 μL) at 240 °C, 280 °C, and 290 °C, are commonly used by several scientists [32,33,42,48,50]. Further, it not surprisingly appeared that the choice of column apparently is a crucial parameter (for details vide supra).

Figure 1A shows that for Kosshagyl crude oil (Atyrau region) the total peak area of Terpanes (*m*/*z* 191) was virtually unaffected by variation in injection temperatures, whereas for Nuraly crude oil (Kyzylorda region) a clear preference for an injection temperature at 280 °C was seen. A further increase in injection temperature may lead to the decomposition of organic substances and as such should be avoided.

Further, the oven temperature program is also an important parameter. According to the literature review (Appendix A), a wide variety of oven programs have been applied in the analyses of biomarkers. Typically, oven programs like (1) 50 °C (2 min)-300 °C, υ = 6 °C/min (15 min); (2) 50 °C (2 min)-310 °C, υ = 6 °C/min (18 min); and (3) 50 °C (1 min)-320 °C, υ = 10 °C/min (8 min), are commonly used for analysis [31,38,53] and appear as illustrative examples.

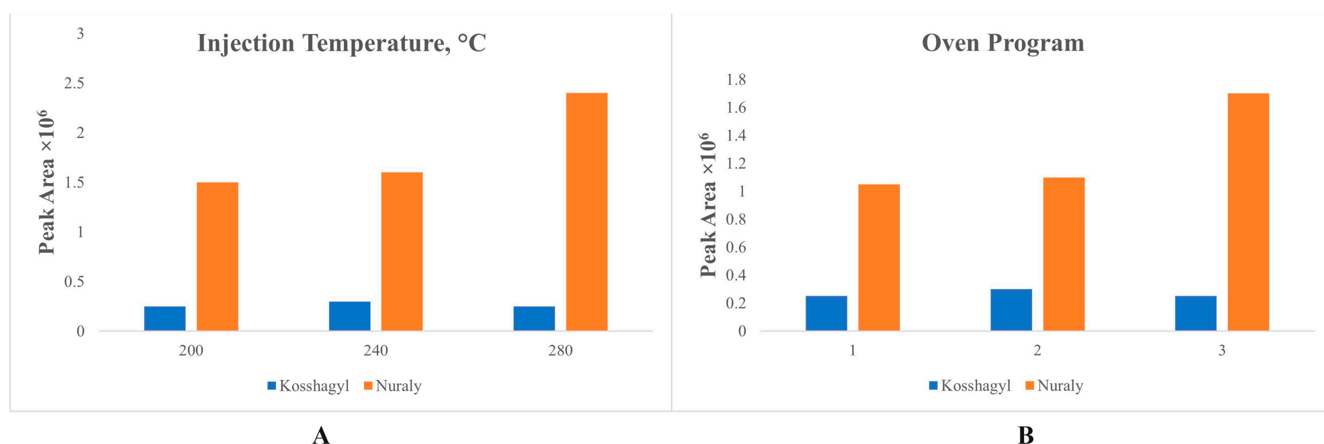


Figure 1. Total peak area of terpanes of the function of injection temperature (**A**) and oven temperature program (**B**).

In the case of the present paper, three different oven programs (cf. Section 2.3.) were used to optimize GC-MS parameters. In Figure 1B, the results of three different oven temperature programs are shown. Again, only minor variations in the terpanes in the Kosshagyl oil were seen as a function of the oven program, while program No. 3 obviously appeared as optimal for the Nuraly oil.

In summary, oven program No. 3 can be considered optimal as all biomarkers were visible at 40 min, whereas programs 1 and 2 apparently do not allow the biomarkers to elude.

3.2. Diagnostic Ratios of Biomarkers

Absolute peak heights of individual biomarkers are typically of limited use as diagnostic tools. Hence, advantageously, the ratios between selected biomarkers are preferred in this respect. The primary advantage of comparing biomarker ratios from different spilled oils and possible suspected source oils is the minimization of concentration effects. Further, this procedure tends to exhibit a self-normalizing effect, thus, minimizing day-to-day, operator, and matrix effects.

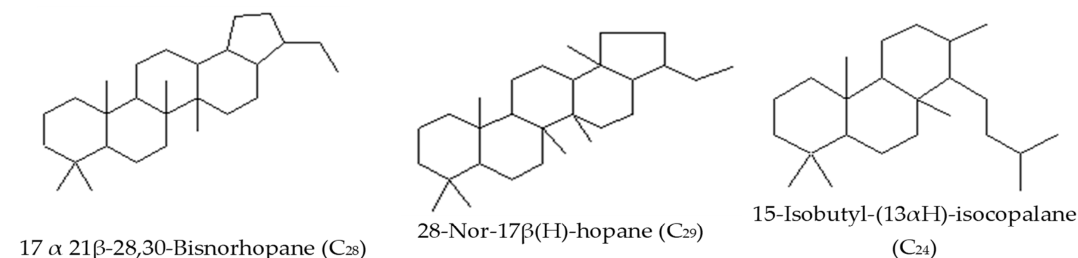
Diagnostic ratios can be obtained either from quantitative, i.e., compound concentrations, or semi-quantitative data, i.e., peak areas or heights. It should be emphasized that such diagnostic biomarker ratios constitute defensible indices, e.g., used by environmental chemists for source identification of oil spills [6,65,66].

Based on the literature review (Appendix A), the 28 Kazakhstan crude oil-specific biomarkers, determined by GC-MS, comprised pristane (Pr), phytane (Ph), n-C_{17} alkane, n-C_{18} alkane, terpanes, pregnane, androstane, allopregnane, homopregnane, cholestane, and stigmastane. In Table 2 and Figure 2, the MS parameters and molecular structure of selected biomarkers are given.

Table 2. MS parameters of selected biomarkers.

No	Biomarkers	Main Ion *m*/*z* (Dwell)	Additional Ions *m*/*z* (Dwell)	Formula	CAS
1	Pristane	57	71, 43, 85, 41, 113	$C_{19}H_{40}$	1921-70-6
2	Phytane	57	71, 43, 85, 41, 55	$C_{20}H_{42}$	638-36-8
3	n-C_{17} alkane	57	43, 71, 85, 41, 55	$C_{17}H_{36}$	628-78-7
4	n-C_{18} alkane	57	43, 71, 41, 85, 29	$C_{18}H_{38}$	593-45-3
5	Bisnorhopane	191	95, 81, 69, 163, 55	$C_{28}H_{48}$	65636-26-2
6	Hopane	191	109, 192, 123, 135, 137	$C_{29}H_{50}$	36728-72-0
7	Isobutyl-isocopalane	191	69, 95, 81, 55	$C_{24}H_{44}$	228729-94-0
8	Pregnane	55	41, 81, 67, 67, 217	$C_{21}H_{36}$	481-26-5
9	Androstane, (5α)-(C_{19})	260	245, 95, 203, 81	$C_{19}H_{32}$	438-22-2
10	Androstane, (5β)-(C_{19})	245	260, 41, 95, 55, 81	$C_{19}H_{32}$	24887-75-0
11	Allopregnane	217	218, 149, 288, 109, 81	$C_{21}H_{36}$	641-85-0
12	Homopregnane	217	302, 55, 95, 81, 67	$C_{22}H_{38}$	35575-28-1
13	Cholestane	217	372, 218, 149, 95, 109	$C_{27}H_{48}$	481-21-0
14	Stigmastane	217	43, 218, 55, 149, 41	$C_{29}H_{52}$	601-58-1

Terpanes (*m*/*z* 191):



Steranes (*m*/*z* 217):

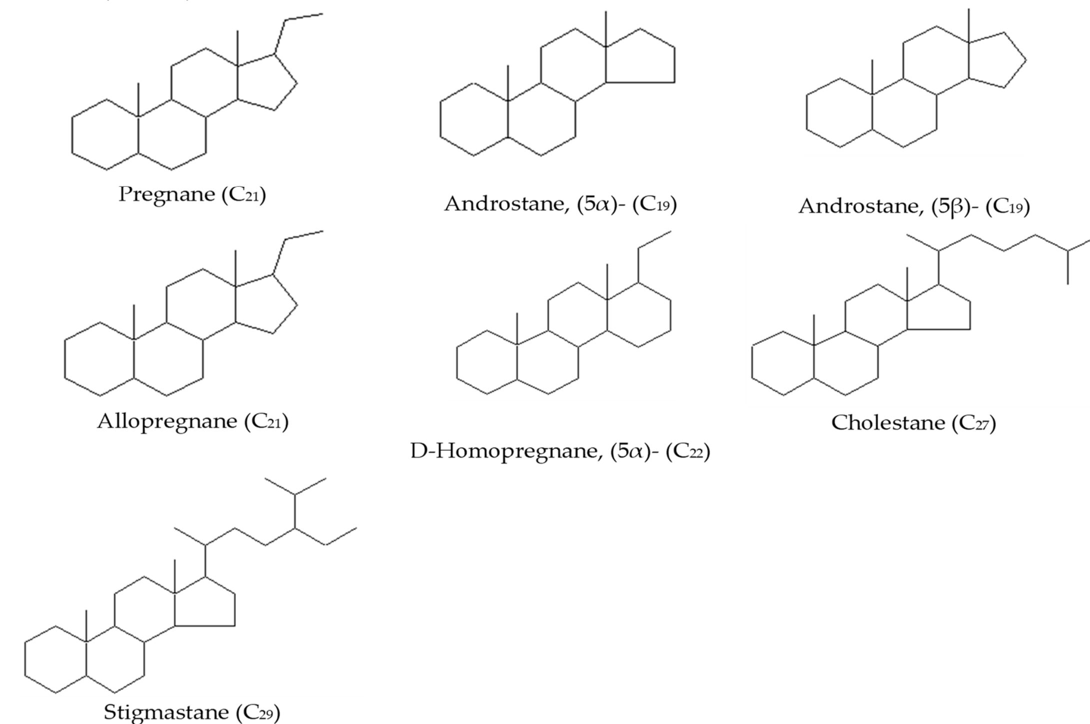


Figure 2. Molecular chemical structures for the biomarkers.

3.2.1. Pristane/Phytane (Pr/Ph)

The most abundant source of Pr and Ph is the pythyl side chain of chlorophyll. A redox reaction of phytol leads to the formation of Pr and Ph. Thus, cleavage of a phytol side chain to yield phytol is promoted by reducing conditions, which leads to dihydrophytol and then Ph. Oxidic conditions, on the other hand promote the competing conversion of phytol to Pr by the oxidation of phytol to phytenic acid and the decarboxylation to pristine, followed by the reduction to Pr (Figure 3). Hence, by identifying the Pr and Ph, it is possible to indicate the conditions of the deposition environment of where crude oil forms.

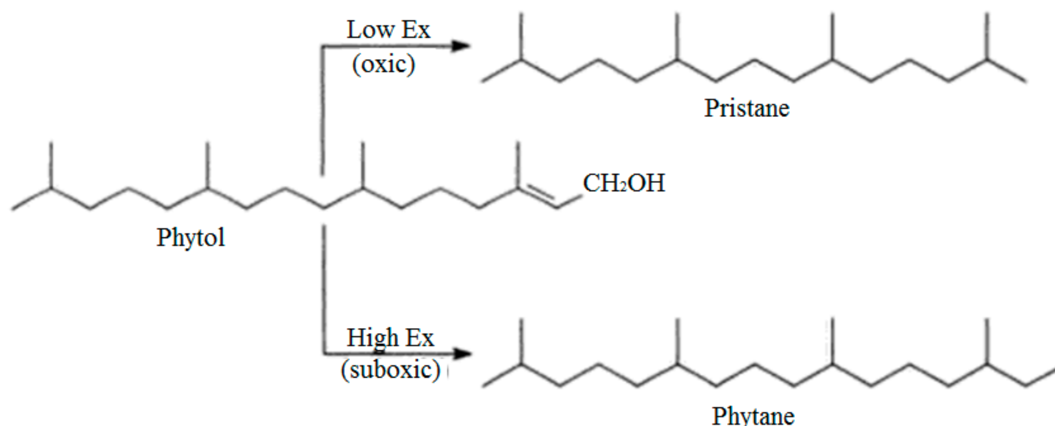


Figure 3. Oxidation and reduction reaction of phytol.

Under conditions with low oxygen (reducing or anoxic) in sediments, the phytol side chain tends to break down, resulting in the formation of phytol, which then undergoes a reduction to dehydrophytal and Ph. Conversely, in oxidic conditions, phytol can transform into pristine through a competing process, involving the oxidation of phytol to phytenic acid, the decarboxylation to pristene, and a subsequent reduction to Pr. [67].

In Figure 4, chromatograms of Nuraly and Kosshagyl crude oils are shown, with Pr and Ph being identified using retention time. The detection was conducted in SIM mode at *m*/*z* 57.

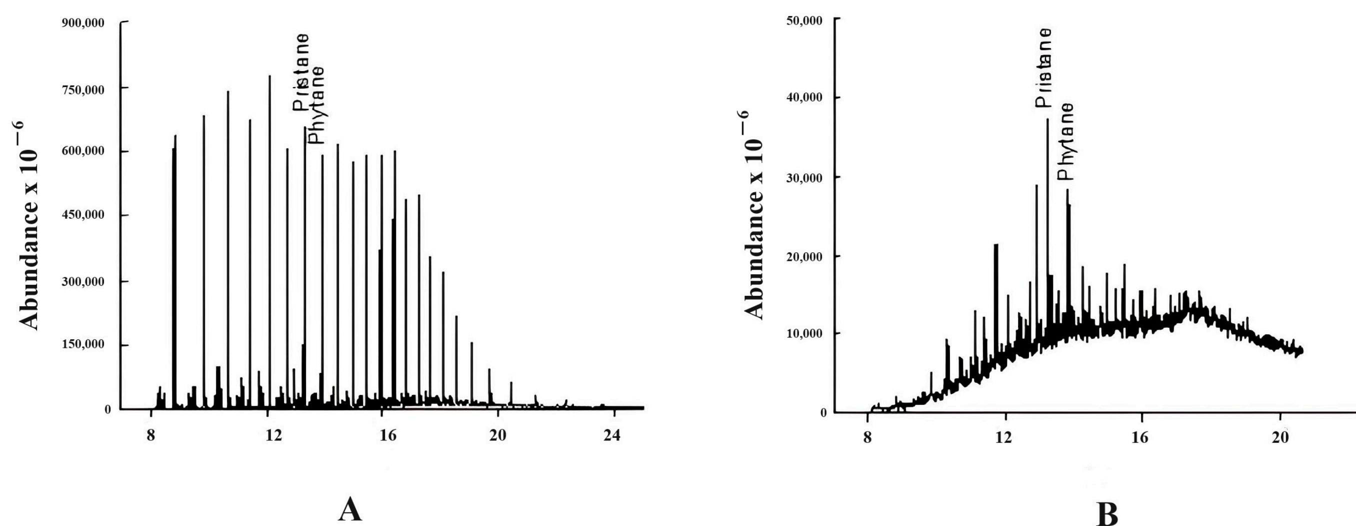


Figure 4. Chromatograms of Nuraly (**A**) and Kosshagyl (**B**) crude oils at *m/z* 57.

The Pr/Ph ratio was obtained from the chromatograms (cf. Figure 4A,B) and is for the present study summarized in Table 3. This ratio is one of the most used correlation parameters, which has been used as an indicator for the degree of maturity and deposition

environment. Further, Ph is often one of the most abundant isoprenoids in oils and has thus been widely used for estimation of the degree of oil biodegradation in the environment [68].

Table 3. Pr/Ph, Pr/n-C_{17} and Ph/n-C_{18} ratios for 28 crude oils.

No	Location	Crude Oil Field	Ph/n-C_{18}	Pr/n-C_{17}	Pr/Ph
1	Atyrau region (West Kazakhstan)	Akingen	1.1	1.4	1.7
2		Akkudyk	0.4	0.6	1.9
3		Baichunas	1.2	3.0	1.4
4		Balgimbaev	1.8	2.9	1.7
5		Kosshagyl	2.3	3.1	1.7
6		Prorva	0.3	0.3	1.3
7		Tengiz	0.4	0.3	0.9
8		Zhanatalap	3.5	2.5	1.6
9		Dossor	0.7	0.6	1.4
10		Kashagan	0.8	0.7	1.3
11	Kyzylorda region (South Kazakhstan)	Akshabulak	0.2	0.3	2.0
12		Aryskum	0.2	0.4	2.0
13		Aschysai	0.2	0.4	1.9
14		Konys and Bektas	0.5	0.6	1.4
15		Nuraly	0.2	0.2	1.9
16		Sarybulak	0.1	0.4	3.3
17		Kyzylorda	0.1	0.2	1.7
18	Mangystau region (West Kazakhstan)	Beineu	0.5	0.6	1.2
19		Kalamkas	0.8	0.8	1.1
20		Karamandybas	1.0	1.1	1.2
21		Karazhanbas	6.3	4.1	0.9
22		Zhanaozen	0.3	0.3	1.5
23		Zhangurshi	0.2	0.3	1.8
24		Zhetybai	0.1	0.2	1.9
25		Buzachi	1.8	1.7	1.1
26	Karagandy region (South Kazakhstan)	Atasu	0.4	0.5	1.4
27		Kumkol	0.2	0.4	2.0
28		Kyzylkiya	0.1	0.3	2.6

Pr/Ph ratio is an indicator of the deposition environment. Thus, low Pr/Ph values (<2) indicate aquatic deposition environments including marine, fresh, and brackish water (reducing conditions), whereas intermediate values (2–4) indicate fluviomarine and coastal swamp environments, and high values (up to 10) are related to peat swamp deposition environments (oxidizing conditions) [69]. According to some research [67,70], a Pr/Ph ratio lower than 0.8 in crude oil suggests deposition from anoxic source rocks. Conversely, a Pr/Ph ratio higher than 0.8 implies deposition in oxidic environments. When the Pr/Ph ratio exceeds 3.0, it signifies the presence of terrigenous plant material deposited under oxygen-rich to moderately oxidic conditions. From Table 3 it can be noted that the diagnostic ratios for virtually all Kazakh crude oils were less than 2, strongly indicative of aquatic depositional environments. Crude oils with a somewhat higher Pr/Ph ratio than 2, such as Sarybulak with 3.3, Kyzylkiya with 2.6, and Kumkol, Akshabulak, Aryskum with 2.0, indicated fluviomarine and coastal swamp environments. The ratios of the Pr/Ph for most petroleum samples discussed before in this study were typically high and varied within the range of 0.9 to 2.6 (higher than 0.8) (Table 3) indicating oxidic deposition. Only one sample from the Sorbulak field had a value of 3.3, which indicated that this petroleum was due to terrigenous plant input deposited under oxidic to suboxidic conditions.

3.2.2. Isoprenoides/n-Alkanes Ratios (Pr/n-C_{17} and Ph/n-C_{18})

Isopreniodes/n-alkanes (Pr/n-C_{17} and Ph/n-C_{18}) ratios provide valuable information about the biodegradation properties and maturation of crude oils [70]. Isoprenoid hydrocarbons are generally more resistant to biodegradation than normal alkanes. Thus,

the higher the ratio of the Pr to n-alkane $C_{17,}$ or the ratio of Ph to n-alkane C_{18} is a rough indicator of the relative state of biodegradation.

The Pr/n-C_{17} ratio serves as a method to distinguish organic matter originating from swamp environments (with values higher than 0.1) and those formed within marine settings (typically less than 0.5). However, it is important to note that this ratio can be influenced by both the maturity level of the material and the extent of biodegradation [71]. The ratio of Pr/n-C_{17} (Table 3) for the samples ranged from 0.2 to 0.5 for South Kazakhstan region samples (more than 0.1 and less than 0.5), indicating organic matter from a swamp and marine environment of deposition also (unless Konys—0.6).

According to the analysis (Table 3) of oils selected from West Kazakhstan, the origin of the oil is significantly different, with a ratio of Pr/n-C_{17} from 0.2 to 4.1. The high Pr/n-C_{17} ratio (>1.0) in crude oil is evidence that terrigenous plant contribution played a major role in its origin [72]. The ratio of Pr/n-C_{17} (Table 3, Figure 5) for the crude oils from Akkudyk, Prorva, Tengiz, Dossori, and Kashagan (less than 1) indicated a marine environment of deposition, but other oils from Atyrau region (Samples—№ 4, 5) originated from a typical type III (terrigenous).

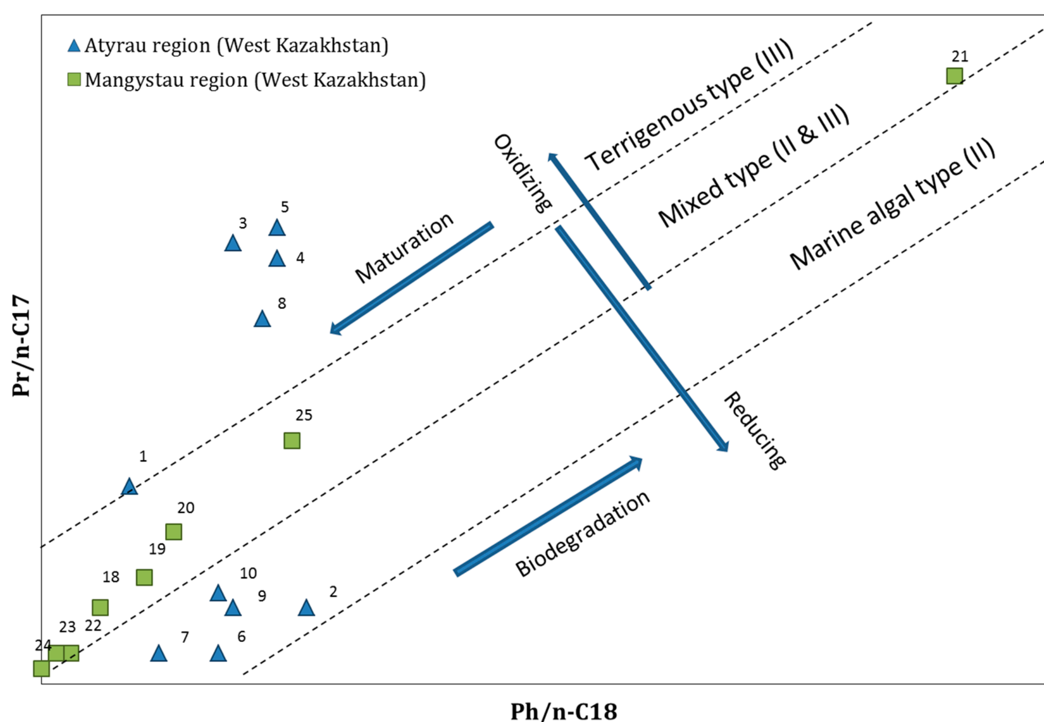


Figure 5. Plot of Pr/n-C_{17} vs. Ph/n-C_{18} showing the sources from the West Kazakhstan crude oils.

Ph/n-C_{18} values less than 1.0 are indicative of non-biodegraded oils [67]. From the results (Table 3), Ph/n-C_{18} ratios found in the range from 0.10 to 6.3 were seen. Most crude oils (20 samples) were recorded with a Ph/n-C_{18} less than one (<1.0), suggesting that these samples were non-biodegraded.

The diagram (Figure 5) shows that West Kazakhstan region crude oils have different maturation and biodegradation. The cross-plot of Pr/n-C_{17} against Ph/n-C_{18} for the Atyrau region oils samples showed that part of the samples (1, 3–5, 8) consisted of terrestrial organic matter inputs and other parts of samples showed clear marine source organic matters deposited. The cross-plot of Pr/n-C_{17} against Ph/n-C_{18} for the crude oils from the Mangystau (Figure 5) and South Kazakhstan (Figure 6) regions showed mixed organic matter (source or transitional environment).

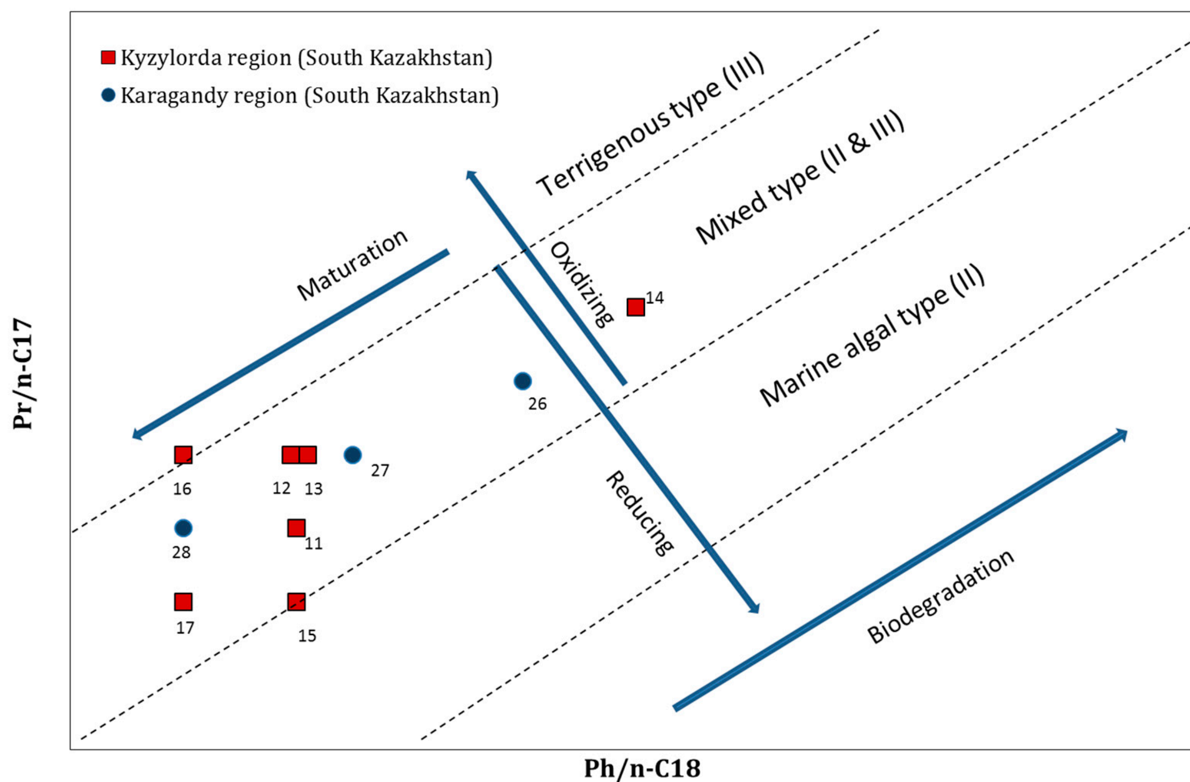


Figure 6. Plot of Pr/n-C_{17} vs. Ph/n-C_{18} showing the sources from the South Kazakhstan crude oils.

Figures 5 and 6 display plots of Pr/n-C_{17} vs. Ph/n-C_{18}, disclosing the depositional environment of the oils. Thus, comparing to the data in Table 3, it can be concluded that 8 samples of crude oils from West Kazakhstan, i.e., Akingen, Baichunas, Balgimbaev, Buzachi, Karamandybas, Karazhambas, Kosshagyl, and Zhanatalap, were less resistant to biodegradation in contrast to crude oils from South Kazakhstan that apparently were the most resistant.

3.2.3. Sterane Distribution (*m/z* 217 and 218)

The mass chromatograms of *m*/*z* 217 and 218 ions display the distribution of steranes in crude oil samples, with steranes being the preferred biomarkers for assessing maturity. [71]. Ratios based on varying carbon numbers within the C_{27}–C_{29} steranes range were employed to identify distinctions in sources. It is widely accepted that the proportions of C_{27}–C_{29} steranes serve as indicators of source disparities. Elevated levels of C_{29} steranes are associated with organic matter primarily influenced by higher plant inputs, whereas greater proportions of C_{27} steranes are characteristic of marine-derived organic matter [71,73].

As it is shown in Figure 7, the Akingen, Baichunas, Prorva, and Zhanatalap crude oils displayed insignificant dominance of C_{27}, only indicative of the source of these crude oils being terrestrial plants mixed with marine microorganisms. Several West crude oils showed a prominence of C_{29}, which indicated more input of organic matter with higher plant inputs. Figure 5 indicates that the crude oils from Karazhanbas and Zhanatalap originated between mixed terrestrial and marine organic sources, while Figure 6 has a higher terrestrial plant sources input. In the case of crude oils from South Kazakhstan, all crude oils showed dominance of C_{29} indicative of organic matter with higher plant inputs (Figure 8). These results of sterane analysis were similar with the data gained from the ratios of Pr/n-C_{17} and Ph/n-C_{18} (Figure 6).

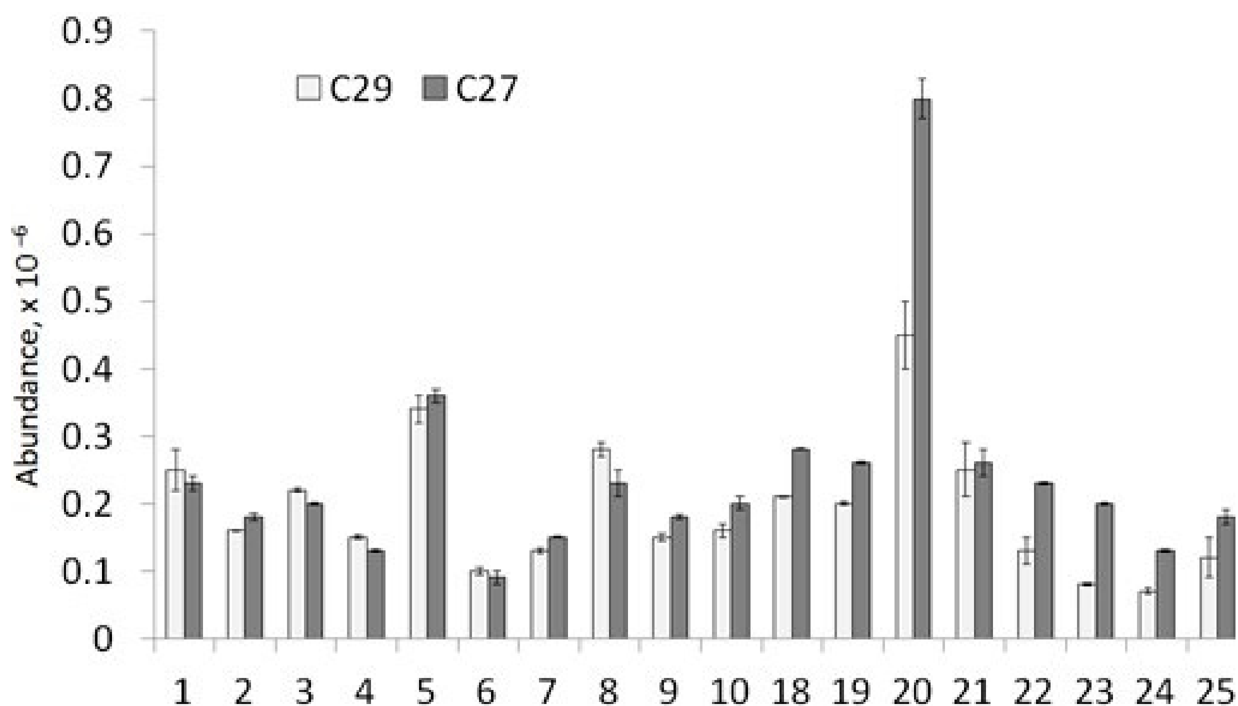


Figure 7. C_{27} and C_{29} Steranes (*m/z* 217) distribution of West Kazakhstan crude oils (numbering according to the Table 1).

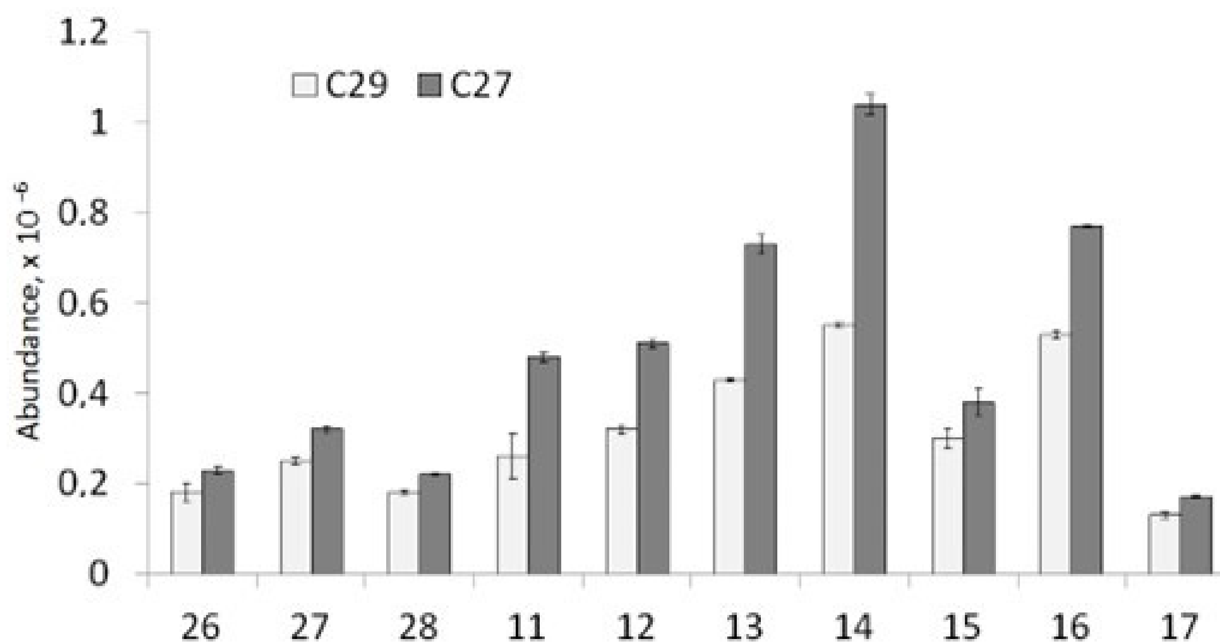


Figure 8. C_{27} and C_{29} Steranes (*m/z* 217) distribution of South Kazakhstan crude oils (numbering according to the Table 1).

3.2.4. Tricyclic/Pentacyclic Terpanes (*m/z* 191)

Tricyclic terpanes are commonly found in marine sources and are used as a maturity indicator. The origin of tricyclic terpanes is from algae and bacteria or higher plants. They are present in oils in different concentrations relative to pentacyclic terpanes [67,68]. Their presence in oils varies in terms of concentration compared to pentacyclic terpanes. Various types of deposition environments have shown that C_{23} tricyclic terpanes are often dominant in marine-sourced oils while C_{19} and C_{20} members are more abundant in oils of terrestrial

origin. In highly matured oils, the distribution of tricyclic terpanes is dominated more than in oils of low maturation [67,68,74]. Figure 9 contains information about the maturity of Kazakhstan crude oils. The higher the signal of the ratio of tricyclic/pentacyclic terpanes, the more mature a crude oil will be considered.

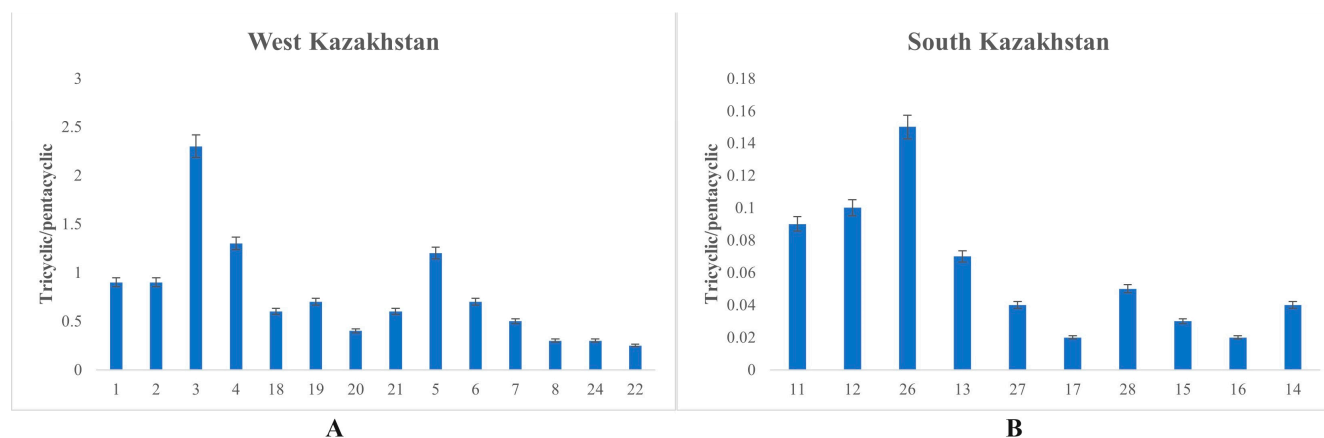


Figure 9. Distribution of ratio tricyclic/pentacyclic terpanes as maturity indicator for West (**A**) and South (**B**) Kazakhstan crude oils (numbering according to the Table 1).

The studied crude oils sampled from Western Kazakhstan showed the highest tricyclic/pentacyclic terpanes ratios from 0.2 to 2.3. Thus, Western Kazakh oils are more mature than South Kazakh oils where ratios in the range from 0.02 to 0.15 were found.

4. Conclusions

For the first time, the petroleum biomarkers in Kazakhstan crude oils have been determined by applying optimized chromatographic parameters for sample injection and oven temperature programming. In addition, the sample preparation method was optimized.

Based on biomarker ratios it was concluded that virtually all Kazakh crude oils were formed under reducing conditions; only two oils, Kyzylkiya and Sarybulak, apparently were formed in an oxidizing environment. Crude oils from South Kazakhstan deposits are more resistant to biodegradation and weathering conditions than oils from West Kazakhstan deposits.

An indicator of source difference is shown in the Akingen, Baichunas, Balgimbaev, and Prorva crude oils where the C_{29} steranes dominate, strongly indicating that the source of these crude oils is terrestrial plants mixing with marine microorganisms. Far West crude oils showed a prominence of C_{29}, indicating an increased input of plant organic origin, while crude oils from South Kazakhstan showed a prominence of C_{29} steranes.

Crude oils from West Kazakhstan are more mature and, thus, older than oils from South Kazakhstan.

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Appendix A

Table A1. Literature review of related topics.

№	Analytes	Sample Preparation	Method	Parameters of GC	Country	Ref.
1	Hopanes, nor-hopanes, and steranes	Deasphalte samples in n-heptane. Saturated fraction by LC (alumina/silica + n-heptane).	GC-QMS	DB-5ms (60 m × 0.25 mm × 0.25 µm); Oven program: 70–110 °C, υ = 50 °C/min; υ = 5 °C/min to 295 °C.	France	[33]
2	Bicyclic alkanes, pentacyclic terpanes, and steranes	Deasphalte in n-heptane (reflux for 2 h with 100 mL of n-heptane for 2.5 g of crude oil). Column chromatography "aliphatic + aromatic" fraction.	GC-QMS	DBI (60 m × 0.32 mm × 0.25 µm); Oven program: for hopanes; 70–295 °C υ = 5 °C/min (15 min); Oven program: for steranes: 70–180 °C, υ = 10 °C/min, υ = 2 °C/min to 295 °C (25 min)	Brazil	[34]
3	Terpanes and Steranes	Crude oil (0.5 g) in 5 mL of n-pentane. Filter the supernatant using a syringe-operated (0.5 mm) Silica column + 2–3 mL of n-pentane. Concentrate the n-pentane fraction under a dry nitrogen jet to 0.5 mL and store for further analysis.	GC-MS	DB-5 (30 m × 0.32 mm × 0.25 µm); T(inj) 290 °C; Oven program: 50 °C (2 min)-300 °C, υ = 60 °C/min, 300 °C (18 min)	Kuwait	[35]
4	Terpanes and steranes	Alumina + n-hexane (saturated fraction).	GC-MS	SE-52 (60 m × 0.25 mm); T(inj) = 250 °C; Oven program: 40–300 °C =υ = 3 °C/min	Spain	[36]
5	Stigmastane and hopanes	10 g soil sample + silica column. Extract acetone + hexane (1:1) and a ratio of 1:2 (wt/vol) of solid to solvent. Shake 5 min, sonicate 15 min, shake 5 min, centrifuge 5 min. Fractionation (silica + hexane).	GC-MS	HP-5 (30 m × 0.25 mm × 0.25 µm); T(Inj) = 290 °C; Oven program: 40 °C (2 min)-140 °C, υ = 5 °C/min, υ = 10 °C/min to 300 °C (10 min)	Italy	[37]
6	Hopanes and steranes	Column chromatography 24–32 mg crude oil. The aliphatic fraction (n-hexane). The non-aliphatic fraction (dichloromethane and methanol (3:1, *v:v*). Concentrate in a stream of nitrogen, re-dissolve in 1 mL of n-hexane.	GC-QMS	ZB-5 (30 m × 0.25 mm × 0.1 µm); T(inj)-50 °C (0.2 min)-320 °C, υ = 150 °C/min (5 min); Oven program: for aliphatic fraction: 60 °C (4 min)-300 °C υ = 10 °C/min, 300 °C (15 min); Oven program: for non-aliphatic fractions: 80 °C (5 min)-370 °C υ = 10 °C/min, 370 °C (10 min)	Sweden	[38]
7	Hopanes and steranes	LC (saturated and aromatic hydrocarbons).	GC-FID GC-MS	SPB-1 (60 m × 0.53 mm); Oven program: 100–320 °C (20 min), υ = 3 °C/min. SE-54 (50 m × 0.25 mm); Oven program: 100–310 °C, υ = 4 °C/min.	Egypt	[39]
8	Hopanes, steranes, diasteranes, and triaromatic steroids	Water and sediment extracted of crude oil samples. Light-protected and stored at 4 °C until analysis 20–50 mg in 5 mL of CH_2Cl_2.	GC-MS	HP-5MS (60 m × 0.25 mm × 0.25 µm); T(Inj) = 300 °C; Oven program: 40 °C (1 min) to 300 °C, υ = 6 °C/min (30 min)	Spain	[40]
9	Sterane and tricyclic and pentacyclic terpanes (hopanes) biomarkers	Dissolve samples (1.6 mg) in 320 µL hexane.	GC-MS-MS	TR-1MS (60 m × 0.25 mm × 0.25 µm); T(inj) = 260 °C; Oven program: 50 °C (2 min)-150 °C υ = 20 °C/min, υ = 1.5 °C/min to 310 °C (17 min)	Germany	[41]
10	Dibenzothiophene, and hopanes, steranes	Dissolve in hexane 10 times Extract crude oil 100 mg in 10 mL of hexane using sonication. Centrifuge Dilute 10 times (1 mg/mL)	GC-QTOF	DB-5ms (30 m × 0.25 mm × 0.25 µm); Oven program: 50 °C (1 min)-320 °C (8 min) υ = 10 °C/min	Belgium, USA	[42]
11	Terpanes and steranes	Precipitate asphaltenes with n-heptane in a 1:40 *v*/*v* ratio. Separate into saturated, aromatic, and resin fractions by LC. Elute using a column filled with silica-alumina (aliphatics/n-hexane; aromat-ics/toluene;resins/toluene/methanol (70:30 *v/v*)).	GC-MS	HP-5ms (30 m × 0.25 mm); Oven program: 260–280 °C υ = 4 °C/min	Venezuela	[43]

Table A1. *Cont.*

№	Analytes	Sample Preparation	Method	Parameters of GC	Country	Ref.
12	Adamantanes and their derivatives	Oils were applied on a platinum tape and were subjected to thermal desorption at 350 °C for 20 s.	GC-MS	HP-5ms (30 m × 0.25 mm × 0.25 µm); Oven program: 40 °C (4 min)-290 °C (10 min) υ = 5 °C/min.	Russia	[44]
13	Hopane and sterane	Place samples in 40 mL clear vials. 25 mg oil in 10 mL hexane. Add 0.5 g of Chem-Tube-Hydromatrix and vortex the samples for 5 min and allow to settle at room temperature for 4 h. Filter and separate in silica gel. Vortex for 2 min and allow to settle for 2 min.	GC-MS	Hopane analysis: DB-EUPAH (20 m × 0.18 mm × 0.14 µm); Oven program: 50 °C (2 min)-310 °C (15 min) υ = 6 C/min. T(inj) = 280 °C; *m*/*z* 191. Sterane analysis: HP-5ms (60 m × 0,25 mm × 0.25 µm); Oven program: 50 °C (1 min)-150 °C (2 min) υ = 70 °C/min, υ = 5 °C/min to 310 °C (15 min).	USA	[45]
14	Saturated hydrocarbons, Steranes and terpanes, 25-Norhopanes, Aromatic hydrocarbons, and Triaromatic steroid hydrocarbons	Remove asphaltenes with n-hexane followed by filtration. Separate into saturate, aromatic, and polar fractions (silica and alumina (4:1, *v*/*v*) + n-hexane, Dichloromethane, and methanol, respectively).	GC-MS	HP-5 (30 m × 0.25 mm × 0.25 µm); Oven program: 80 °C (1 min)-280 °C (30 min) υ = 3 °C/min	China	[46]
15	Triterpanes oleananes, bicadinanes, hopanes, and steranes	Precipitate asphaltenes with n-hexane 50 times. Separate maltenes into saturated hydrocarbons, aromatic hydrocarbons, and resins fraction Remove n-alkanes from saturated fraction	GC-QMS	Rtx-5 (30 m × 0.25 mm × 0.25 µm); T(inj) = 300 °C; Oven program: 100 °C (3 min)-200 °C υ = 25 °C/min, υ = 2 °C/min to 300 °C (3 min)	India	[47]
16	Hopanes, steranes and diasteranes, and triaromatic steroids biomarkers	Oil samples were water and sediment extracted following an ASTM D2709—16 guide, light-protected, and stored at 4 C until analysis.	GC-MS	HP-5ms (60 m × 0.25 mm × 0.25 µm); Oven program: 40 °C (1 min)-300 °C, υ = 6 °C /min (30 min), $T_{inj.}$ = 300 °C.	Spain	[48]
17	Hopanes and steranes and terpanes	Bitumen extractions were performed on 56 samples using a Soxhlet apparatus for 72 h with a dichloromethane/methanol mixture (93:7 *v/v*).	GC-MS	HP-5ms (30 m × 0.25 mm × 0.25 µm); Oven program: 50 °C (1 min)-100 °C, υ = 20 °C/min, 100-310 °C, υ = 3 °C/min (18 min)	China	[49]
18	Hopanes, steranes	The oil samples were deasphaltened by hexane. Then fractionated on a silica: alumina column using hexane, benzene, and methanol.	GC-MS	HP-5ms (60 m × 0.25 mm × 0.25 mm); Oven program: 50 °C (1 min)-120 °C, υ = 20 °C/min, 120-250 °C, υ = 4 °C/min, to 310 °C (3 °C/min, 30 min)	China	[50]
19	Hopanes, steranes	Extract by chloroform for 72 h by means of Soxhlet extraction.	GC-MS	HP-5 (30 m × 0.25 mm × 0.25 µm); T(inj) = 280 °C; Oven program: 80–290 °C at 4 °C/min (30 min)	China	[51]
20	Isoprenoid, Moretanes, Bisnorhopanes, Gammacerane, Pentacyclic extended hopane	Separated into saturate, aromatics and resins by column chromatography (1:1 alumina:silica gel). Elution with n-heptane, toluene, and chloroform.	GC-FID GC-QMS	SPB-1 (60 m × 0.53 mm); Oven program: 100–320 °C at 3 °C/min (20 min). SE 54 (50 m × 0.25 mm) Oven program: 100–310 °C at 4 °C/min	Egypt	[52]
21	Phenanthrene, anthracene, methyl-phenanthrene, methyl-anthracene	Extract 0.15 g oil samples with 10.0 mL n-hexane/dichlormethane (1:1, *v/v*), add about 1.00 g of anhydrous sodium sulfate. Vortexed for 30 s. Centrifuge at 3000 r.p.m. 5 min. Transfer 1.0 mL of the supernatant to a vial (silica gel. + n-hexane/dichloromethane (1:1, *v/v*). Vortex and centrifuge an aliquot of 1.0 mL analysis.	GC-MS	HP-5MS (60 m × 0.25 mm × 0.25 µm); T(inj)= 290 °C; Oven program: 60–300 °C υ = 6 °C/min (30 min).	China	[53]

Table A1. *Cont.*

№	Analytes	Sample Preparation	Method	Parameters of GC	Country	Ref.
22	n-alkanes, isoprenoids, and steranes and triterpanes	The samples were fractionated into saturated hydrocarbons, aromatic hydrocarbons, and polar compounds by column chromatography. For aliphatic fraction: hexane. For aromatic fraction: 1:1 (*v/v*) hexane/dichloromethane.	GC-MS-MS	ZB-5 (30 m × 0.25 mm × 0.10 µm); Oven program: 70–100 °C (30 °C/min) 100–308 °C (4 °C/min, 8 min)	Serbia	[54]
23	Pentacyclic terpanes	Oil samples were mixed with a solution of dichlormethane.	GC-MS	DB-5 (30 m × 0.25 mm × 0.25 µm); Oven program: 50–300 °C, (5 °C/min, 20 min)	Germany	[55]
24	Steranes, diasteranes, and pentacyclic triterpanes	The crude oils were diluted in dichloromethane prior to analysis.	GC-MS	MXT-5 (60 m × 0.25 mm × 0.25 µm); $T_{inj.}$ = 300 °C; Oven program: 50 °C (3 min)-150 °C υ = 20 °C/min 150–350 °C, (2 °C/min, 25 min)	Mexico	[56]
25	Terpanes and steranes, bicyclic sesquiterpanes, and diamondoids	16 mg of each oil in hexane. The oil solution was mixed with 100 mL of o-terphenyl and d50-tetracosane (200 mg/mL each) and 100 mL of mixture of deuterated naphthalene, acenaphthene, phenanthrene, benz[a]anthracene, and perylene (10 mg/mL each).	GC-MS	DB-5ms (30 m × 0.25 mm × 0.25 µm); Oven program: 50 °C (2 min)-310 °C υ = 6 °C/min (18 min)	Canada	[57]
26	Steranes and terpanes	Oil samples were extracted in a Soxhlet extractor using dichloromethane (DCM): methanol (93:7, *v:v*) for 72 h. Separated into maltene and asphaltene fractions using a deasphaltening procedure.	GC-MS	HP-1 (30 m × 0.25 mm × 0.25 µm); $T_{inj.}$ = 300 °C; Oven program: 40–300 °C, υ = 4 °C/min (20 min)	China	[58]
27	Pristanes and phytanes	A total of 461 samples were subjected to bulk geochemical analysis.	GC-MS	RTX-1 (30 m × 0.32 mm × 0.25 µm); Oven program: 60-320 °C, υ = 4 °C/min	Colombia	[59]
28	Sterane, terpane, and aromatic biomarker distributions	Oil samples were subjected to asphaltene precipitation using excess n-hexane. The maltene was fractionated into saturated and aromatic hydrocarbons by column chromatography with activated silica gel using hexane, dichloromethane, and dichloromethane/methanol (50:50).	GC-MS	J&W DB5 (50 m × 0.2 mm × 0.11 µm); Oven program: 150–325 °C, υ = 2 °C/min	Egypt	[60]
29	Tricyclic terpanes, gammacerane, dibenzothio-phene, steranes, and diasteranes,	The oils were deasphalted using n-hexane, and fractionated using column chromatography.	GC-MS	HP-5MS (30 m × 0.25 mm × 0.25 µm); Oven program: 50 °C (1 min)-120 °C, υ = 20 °C/min, 120–310, υ = 3 °C/min (25 min)	China	[61]
30	17 adamantanes, 10 bicyclic sesquiterpanes, 37 terpanes, and 17 steranes	2 g soil sample was spiked with acenaphthene-d_{10} the extracted sample solution was filtered and eluted with n-hexane and then concentrated to 1 mL. Eluted with n-hexane, mixed n-hexane/dichloromethane, dichloromethane. Concentrated and refreshed with cyclohexane.	GC-MS	HP-5 (30 m × 0.25 mm × 0.25 µm); Oven program: 50 °C (2 min)-300 °C, υ = 6 °C/min (15 min)	China	[62]

Table A1. *Cont.*

№	Analytes	Sample Preparation	Method	Parameters of GC	Country	Ref.
31	High C26/C25 tricyclic terpanes, low C31 homohopane, 4α-methyl-24-ethylcholestanes, and C30 tetracyclic polyprenoids	The oil samples were spiked with standard compound 5α-androstane and n-Hexane—to remove asphaltenes by ultrasound and centrifugation. Saturate and aromatic fractions were separated by activated silica gel/alumina column chromatography using n-hexane and n-hexane: dichloromethane (2:1, *v/v*).	GC-MS	HP-5MS (30 m × 0.25 mm × 0.25 μm); T_{inj}. = 300 °C; Oven program: 50 °C (2 min)-200 °C, υ = 4 °C/min, 200–310 °C υ = 2 °C/min (10 min)	China	[63]
32	17.alfa., 21β-28, 30-Bisnorhopane, 28-Nor-17β(H)-hopane, 15-Isobutyl-(13αH)-isocopalane, Pregnane, Androstane, (5α)-, Androstane, (5β)-, Allopregnane, D-Homopregnane, (5α)-, Cholestane, and Stigmastane	Crude oil samples dissolved in *n*-hexane by column chromatography to the fractions of saturated and aromatic hydrocarbons. The silica gel is washed with acetone, hexane, and dichloromethane, completely dried, and activated at 160–180 °C for 20 h. The glass wool is washed with acetone, hexane, and dichloromethane. Sodium sulfate is calcined and cooled. 10 mL of crude oil. The eluents were collected as follows: 12 mL of hexane for saturated hydrocarbons (Fraction 1), 15 mL of hexane: dichloromethane for aromatic hydrocarbons (*v/v*, 1:1, Fraction 2).	GC-MS	DB-5ms (30 m × 0.25 mm × 0.25 μm); T_{inj} = 280 °C; Oven program: 50 °C (held for 5 min) to 300 °C by a rate of 20 °C/min and held for 20 min.	Kazakhstan	[this article]

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