

Geochemical Study of Crude Oil Samples to Evaluate Extent and Effect of Secondary Alteration Process (i.e. Biodegradation).

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Abstract- A mild chemical treatment i.e. Ruthenium ion catalysed oxidation (RICO) has been employed to examine the asphaltenes of two oils (fresh and slightly degraded) chosen from four oils samples after bulk geochemical analysis. The oils were from same geographical location but unknown oilfield in North America. The distributions of hopanoic acids released from the asphaltenes were investigated and compared with that of hopanes in the maltenes. The distributions were dissimilar e.g. distribution of carbon number maximized at C32 in the terpenoic acids which are not consistent with that of terpanes (hopanes) in the maltenes. However, C29 and C30 hopanes were the prominent in the maltenes where as C32 hopanoic acids homologues in the released products. Comparatively, biodegradation was inferred to have no effect on the bonded biomarkers due to their unaltered nature after RICO treatment. Concomitantly, the difference and level of degradation between the samples were also investigated and found to be small (insignificant) with minor or no loss of n-alkanes or any other biomarkers in the samples. Although, samples 6661 and 8673 were slightly degraded and ranked "1" in the Peters and Moldowan (1993) scale, still none of the sample appeared to have lost their isoprenoids, hopanes and steranes. This indicates that the samples were slightly biodegraded. On the other hand, oils (8673 and 8676) were ranked "0" on the same scale indicating that they are not degraded.

Index Terms- Asphaltenes, Biodegradation, Hopanes and Hopanoic acids, Maltenes, RICO, Whole oils.

I. INTRODUCTION

Crude oil is a mixture of organic compounds containing diverse chemical, physical and compositional behaviours. This mixture consists of many thousands of different components. Most of these are hydrocarbons i.e. carbon and hydrogen atoms arranged in chains or rings [1]. However, molecular weight (i.e. the weight of each hydrocarbon molecule) primarily depends on the number of carbon atoms, which can vary from one (1) to more than hundred (100). The overall properties of crude oils are dependent upon their chemical composition and structure [2]

Light oil has a greater proportion of the low molecular weight components, while heavy oil has more of the higher molecular weight components. Other components of this complex mixture contain heteroatoms and metals. In general,

crude oil mixture can be classified into different groups such as alkanes, branched alkanes, alkenes, branched alkenes, aromatics, monocyclic-aromatics etc. [3]; [1].

Petroleum study reveals that solubilities of compounds in particular solvent varied from one compound to another and molecular weights of these compounds is always useful for separating different type of compounds from one another [2]

Asphaltenes are one of the four main classes of petroleum mixture. The other three main classes of compounds in the latter are saturated hydrocarbons, aromatic hydrocarbons and resins making the acronym SARA. Similarly, asphaltenes are the largest molecules in oil, they contain aromatic rings system containing one to four aromatic rings, usually bonded to linear or cyclic saturated hydrocarbon structures and polar functional groups. They are a dark brown to black, friable, infusible solid component of crude oil [4]. [5] characterized this infusible solid component of crude oil (asphaltene) by a C:H ratio close to one and specific gravities near to one and reported that they are extremely aromatic [5].

The percentage compositions of SARA in the crude oil samples were determined in this work using Iatroscan analysis with special consideration given to asphaltene content in each of the sample for selection purposes.

[6] and [7] revealed that asphaltenes, like many compounds found in crude oil fraction, contains chemically bound biomarkers which hold important information about the origin, biological source material, and diagenetic history of the oil. These features and others made them of economic importance for geochemical studies.

In spite of an undisputed argument about their structures and molecular weights [8] they have been shown to contain bound biomarkers that are normally present in the deasphaltened (maltene) fraction from oils and bitumen [7]; [6].

[9] examined the bound biomarkers from asphaltenes of a range of different oils, released in the form of acids by ruthenium ion catalysed oxidation (RICO) and analysed in form of methyl esters. All the samples show high proportion of n-alkanoic acids and isoalkanoic acids up to C₃₅ among the oxidation products. In addition, hopanoic and steranoic acids were also detected [9].

II. MATERIALS AND METHODS

2.1 Precipitation of Asphaltenes:

For crude oils, deasphalting of the samples is usually the first step prior to further analysis. This is usually done by a standard asphaltene precipitation by alkanes like pentane, hexane or heptane. In this work, the asphaltene precipitation was conducted using *n*-hexane for cost reason. The method was adopted based on the procedure carried out by [10] although *n*-hexane was used as a precipitation solvent instead of *n*-heptane for the stated reason above.

About 200 ml of *n*-hexane was added into a 250 ml conical flask containing about 5 g of the crude oil sample with regular swirling. The mixture was stirred for 2 hours and was allowed to equilibrate for 24 hours. It was centrifuged at 3500 rpm per minute for 15 minutes and the asphaltene recovered. The supernatant (maltenes) is filtered without disturbing the whole mixture and kept in a vial. The asphaltene recovered was re-precipitated with another 40 fold of *n*-hexane per gram of the sample. The mixture was sonicated for about 30 minutes and the asphaltene recovered by centrifugation. The former and the latter procedures were repeated twice to enhance recovery. The recovered asphaltene was transferred into a pre-weighed vial with minimal amount of DCM. The excess solvent was removed under stream of nitrogen and was allowed to stand at ambient temperature until evaporation completed. The amounts recovered were weighed respectively. The same precipitation procedure was repeated for another new 5 g of the same sample.

2.2 Cleaning of the Asphaltenes:

Due to possibility of “trapped” maltenes and other impurities, the dried asphaltene residue recovered by precipitation was thoroughly cleaned by Soxhlet extraction using *n*-hexane in order to remove the co-precipitated maltenes. The procedure was carried out by transferring the asphaltene in to a cellulose thimble and placed in a Soxhlet apparatus and refluxed with *n*-hexane for 72 hours. The cellulose thimbles were pre-extracted with DCM prior to usage. The heating rate, condenser cooling rate, and fluid level of the solvent in the Soxhlet apparatus was adjusted to maintain a temperature of 30 (3 °C in the sample chamber). Contact between the asphaltenes and the solvent (*n*-hexane), the Soxhlet was enhanced by stopping the procedure once a day and the asphaltene sample crushed and mixed (Alboudwarej *et al.*, 2002).

After the extraction, the asphaltenes were allowed to dry inside the thimble and 50 mg was taken for RICO analysis.

2.3 Thin Layer Chromatography (TLC) for whole oils and maltenes:

About 15-20 mg of each of the four crude oil samples and maltenes were separated into saturated hydrocarbon fraction using thin layer chromatography (TLC). The maltenes were further separated in to aromatic fraction.

The TLC plates were prepared using a 20 cm square glass plates coated with 0.5 mm thick silica gel 60G as the stationary phase. The plates were activated in an oven at a temperature of 100°C for 24 hours and then pre-eluted with DCM and oven dried.

The crude oil samples and the maltenes were dissolved into small amounts of DCM and then spotted onto the silica plate using small pipettes. A standard was used along the side of plate in order to recognise different hydrocarbon fractions.

Afterwards, the plates were immersed in the TLC tank containing 200 ml petroleum ether. It was kept monitored until the solvent (mobile phase) migrated to the top of the plate.

The plates were removed and air dried and sprayed with Rhodamine-6G dye and viewed under ultra violet light (UV). The bands of saturate in the whole and saturate and aromatic fractions in the maltenes were marked while viewing under the ultra violet light (UV). It was scraped off and transferred in to short columns where it was eluted out with 15 ml petroleum ether and 15 ml DCM, evaporated using a rotary evaporator and then analysed by GC and GC-MS.

2.4 Ruthenium Ion Catalysed Oxidation (RICO):

This is a chemical technique that selectively oxidises aromatic ring structures into carbon dioxide (CO₂) with aliphatic moieties transformed to fatty acids. This technique has been commonly used to illuminate the aliphatic composition of geopolymers [7]. In this work, it has been carried out as follows. About 50 mg of asphaltene transferred into a 100 ml conical flask, it was followed by 4 ml of DCM and stirred until the asphaltene dissolves.

Afterwards, 4 ml of acetonitrile was added followed by 5 ml of 12% aqueous sodium periodate (NaIO₄). About 5 mg RuCl₃ was soon added and stirred for 24 hours at room temperature. Subsequently, 10 ml of DCM was added followed by 10 ml methanol and swirled.

It was then centrifuged at 3500 rpm for 15 minutes and the supernatant decanted. The residue was washed with 15 ml DCM and 15 ml of de-ionised water and was repeated twice.

After that, the washings were combined with the supernatant, and the organic phase was recovered using separating funnel. The aqueous phase was washed with 20 ml DCM (3x). Then the solvent was removed using rotary evaporator to about 10 ml. concurrently, 5 ml of 1M NaOH solution was added in a separating funnel and shaken rigorously.

Afterward, the organic phase was removed and discarded, and the aqueous phase washed with 20 ml DCM (3x). It was followed with 5 ml of 2 M HCl (50% HCl) solution (i.e. added) and shaken rigorously. The acid was extracted with 20 ml DCM (3x) and the excess solvent was removed using rotary evaporator and nitrogen below down [7].

However, to derivitised the acids to methyl esters before analysis esterification came in to place as follows;

Acid Esterification; About 1 ml DCM was used to transfer the acids into a boiling tube. It was followed by addition of 50 µl of internal standard (1 mg/ml C₁₆D₃₁COOH). This was followed by addition of about 5 ml of 2% concentrated sulphuric acid in methanol. Some (3 to 4) anti-bumping granules were added. The mixture was refluxed on a test tube heater for 3 hours. It was allowed to cool and 10 ml of deionised water added. The methyl esters were extracted with DCM (10 ml, x3). The DCM extract was washed with 4 ml of 2% NaHCO₃. The esters were dried over stream of nitrogen. Excess solvent was removed using a rotary evaporator at room temperature. The esters were transferred into a GC vial using 1 ml DCM and submitted for GC & GC-MS analyses.

2.5 Gas Chromatography:

This was done using an Agilent 5890 gas chromatograph (GC) instrument, which was equipped with a split/ split less inlet and an Agilent HP-5 fused silica capillary column (30m x 0.25mm I.D. x 0.25µm film thickness) to analyse the aliphatic hydrocarbon fractions. The saturates were injected on to the GC column in split/split less mode (1 min. Split less, then 30 ml/min split) by an Agilent 6890 automatic injector. The inlet temperature was 300 °C and the detector temperature was 310 °C. The carrier gas used (hydrogen gas) was applied at constant pressure of 50 kPa and an internal flow rate of 1.5 ml/ minute. Flame ionisation detector (FID) was used as detector. However, In order to arrive at 300°C (final hold time 20 min), the GC oven was heated to 40°C (initial hold time 2 min) at 4°C ramp per minute. The data were collected and processed using a Thermo Atlas software system.

2.6 Gas Chromatography-Mass Spectroscopy:

GC-MS analysis was carried out on an Agilent 7890A gas chromatograph (GC) equipped to an Agilent 5975C mass selective detector (MSD). The separation of dissimilar components was achieved by using an Agilent HP-5 fused silica capillary column (30 m x 0.25 mm I.D. x 0.25 µm film thickness). An Agilent 7683B auto sampler was used as sample

injector. The GC inlet was operated in pulsed split less mode (inlet pressure = 150 kPa for 1 min split less, then inlet pressure = 50 kPa with a split flow rate of 30 ml/min). For the saturated hydrocarbons, the temperature of GC oven was programmed from 50°C (initial hold time 5 min) to 310 °C at 5 °C/min, with a final hold time of 10 min. The GC inlet temperature was 280 °C and the GC/MSD interface temperature was 300 °C. Helium gas was used as carrier at a constant flow of 1 ml/ min.

The MSD was operated in complete scan mode (40-550 amu/s) or in combined selected ion monitoring (SIM)/scan mode by following the conditions: electron voltage 70 eV, filament current 220 µA, source temperature 230 °C, quad temperature 150 °C, multiplier voltage 2247 V. Agilent Chemstation software was used to processed the data.

III. RESULTS

3.1 Thin Layer Chromatography (TLC) of the whole oils:

The saturate fraction after the TLC of the whole oils were analysed by GC and GC-MS with the results demonstrated in the subsequent figures below.

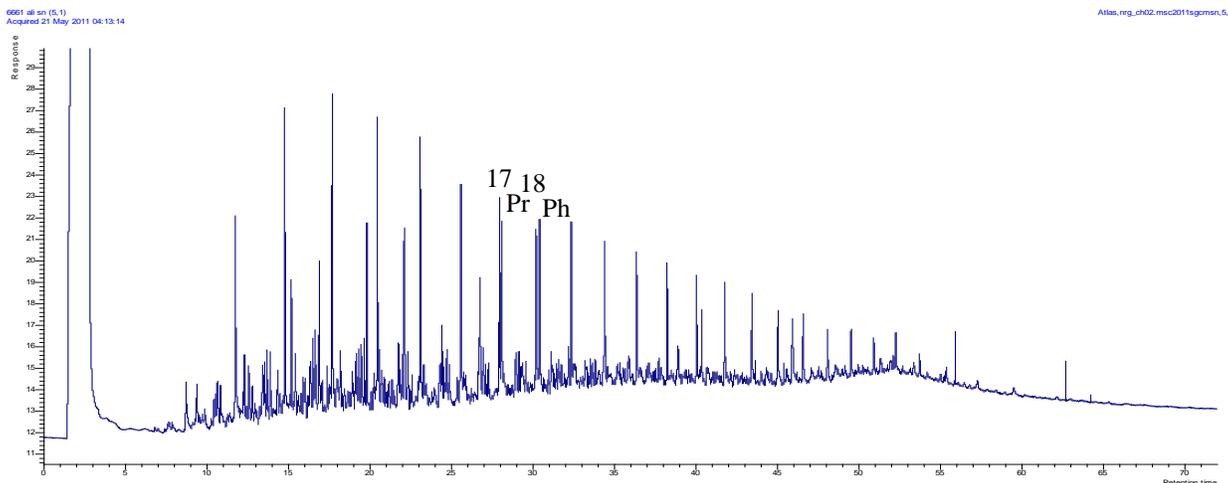


Figure 3.1 GC traces demonstrating the distribution of hydrocarbon in the saturate fraction of sample 6661. Pr=Pristane and Ph=Phytane coming immediately after nC₁₇ and nC₁₈ respectively.

8672_08_en (6,1)
Acquired 21 May 2011 05:32:24

Atlas_rmg_ch02_msc2011sgmsn,6,1,1

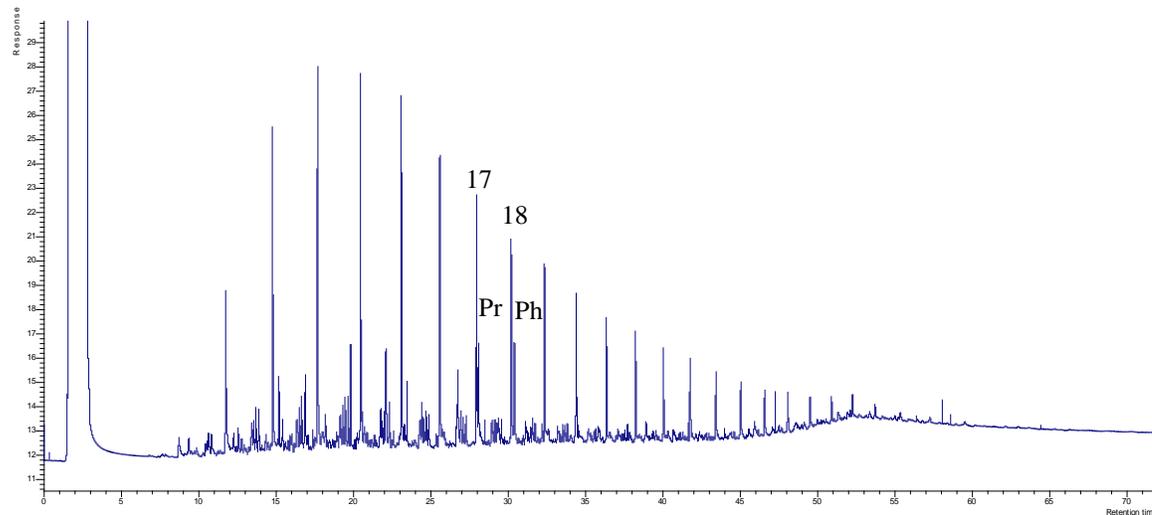


Figure 3.2 GC traces demonstrating the distribution of hydrocarbon in the saturate fraction of sample 8672. Pr=Pristane and Ph=Phytane coming immediately after nC_{17} and nC_{18} respectively

8673_01 (13,1)
Acquired 25 May 2011 16:06:13

Atlas_rmg_ch02_msc2011sgmsn,13,1,1

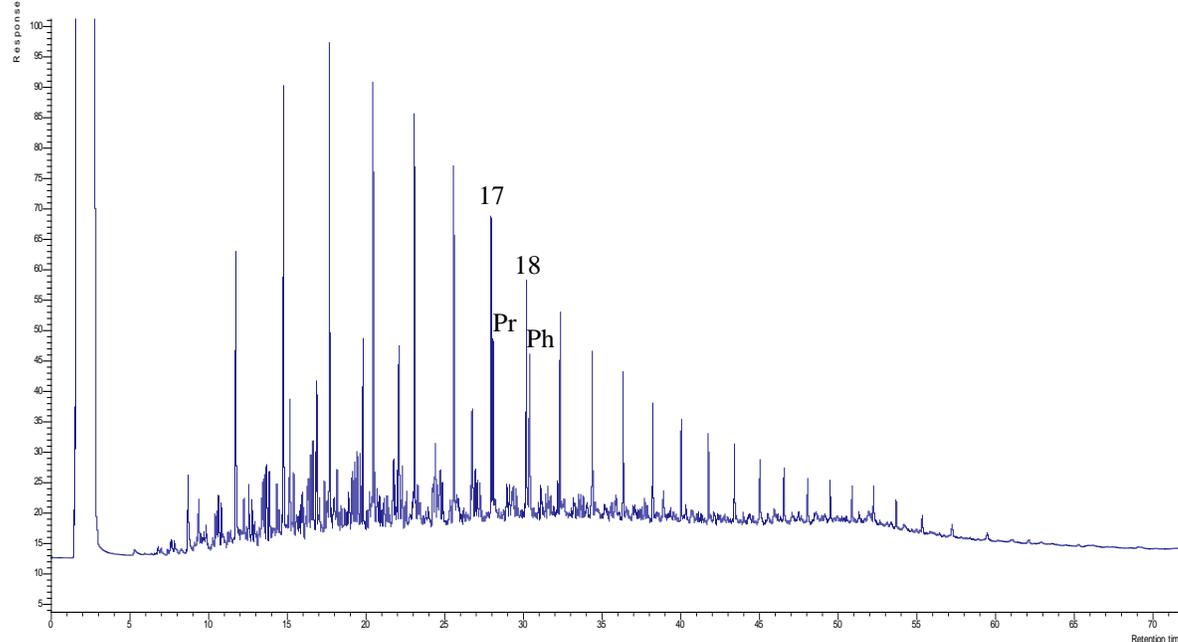


Figure 3.3 GC traces demonstrating the distribution of hydrocarbon in the saturate fraction of sample 8673. Pr=Pristane and Ph=Phytane coming immediately after nC_{17} and nC_{18} respectively.

8676-88 (8,1)
Acquired 25 May 2011 09:29:13

Atlas.org_ch02_msc2011sgmen.8.1.1

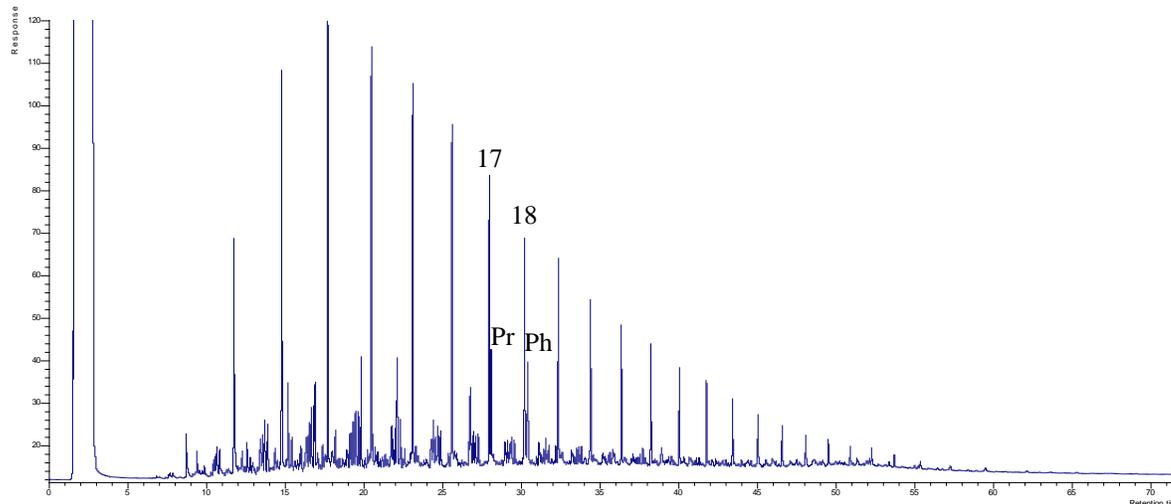


Figure 3.4 GC traces demonstrating the distribution of hydrocarbon in the saturate fraction of sample 8676. Pr=Pristane and Ph=Phytane coming immediately after nC_{17} and nC_{18} respectively.

The early stages of oil biodegradation (loss of *n*-paraffins) can be readily identified by gas chromatography (GC) analysis of the oil as did in this study using Pr/ nC_{17} and Ph/ nC_{18} ratios [11] modified by [12].

The *n*-alkanes in Fig. 3.1 to 3.4 exhibited a unimodal distribution for all the oils indicating similarities of the oils. There were slight systematic changes with increasing biodegradation as seen in the gas chromatograms e.g.

concentration of lower *n*-alkanes were not prominent in oil 6661 when compared to its counterpart 8676 in which the *n*-alkanes were relatively intact Fig 3.4. Based on this reason, and additionally by considering the relatively very lower UCM in sample 8676 and relatively higher UCM in 6661, the two samples were categorised as slightly degraded and non-degraded using [11] biodegradation Scale as computed in Table 3.1 and Fig. 3.5 and 3.6 below.

Table 3.1 Ranking of biodegradation in the crude oil samples. It was done in the light of [11] Biodegradation Scale.

S/N	Sample	P&M (1993) Ranking	Chemical composition
1	6661	1	Lower homologous <i>n</i> -alkanes depleted
2	8676	0	Presence of abundant <i>n</i> -alkanes
3	8673	1	Lower homologous <i>n</i> -alkanes depleted
4	8672	0	Presence of abundant <i>n</i> -alkanes

From the scale above, two of the samples (6661 & 8673) were categorised as slightly degraded whereas the other two samples (8676 & 8672) as fresh non-degraded oils.

Table 3.2 Ratios of some selected parameter (Straight chain alkanes and acyclic isoprenoids) in the saturate (aliphatic fraction) obtained after the TLC of the whole oil fraction. The ratios were calculated from the GC traces in Fig. 3.1 to 3.4 above.

S/N	Sample	Pr/Ph ratio	Pr/ nC_{17} ratio	Ph/ nC_{18} ratio
1	6661	1.03	0.89	1.06
2	8676	1.14	0.39	0.44

3	8673	1.12	0.59	0.68
4	8672	1.00	0.40	0.49

From Table 3.2 above, all the pristane/phytane ratios of the oils are almost similar with very little variation. In considering the Pr/nC_{17} and Ph/nC_{18} ratios, oil 6661 has relatively higher of these ratios compared to its counterparts. On the other hand, oil 8676 has the least of the ratios.

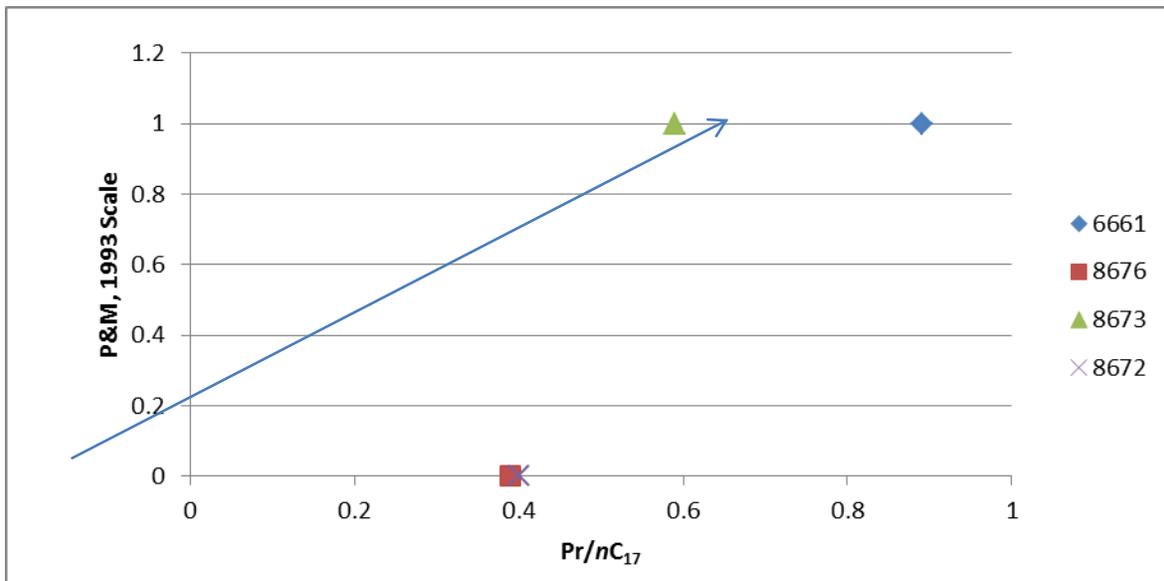


Figure 3.5 Ratio Pristane/ nC_{17} against the level of biodegradation on [11] Biodegradation Scale.

There was systematic increase of Pristane/ nC_{17} ratio among the oils with increasing biodegradation. Oils 6661 and 8673 were having higher values of this ratios and corresponds to level '1' on the vertical axis which is the biodegradation assessment scale. However, the other two samples were having relatively lower ratios and received '0' on the vertical axis.

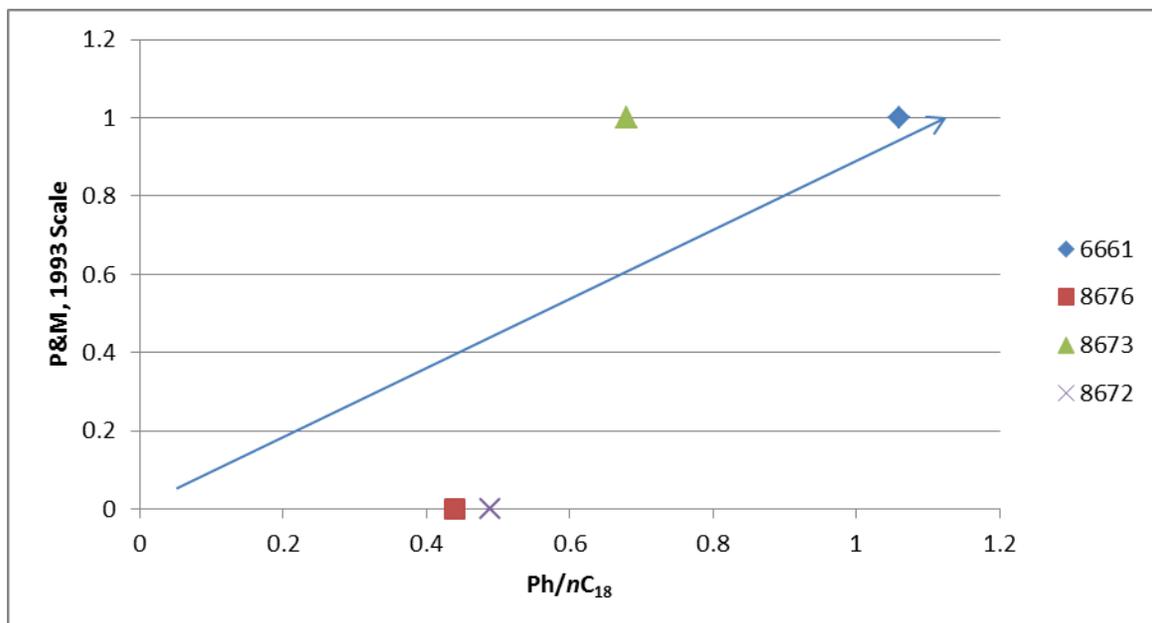


Figure 3.6 Level of biodegradation against Phytane/ nC_{18} ratio. There was systematic increase of Pristane/ nC_{18} ratio among the oils with increasing biodegradation.

Relatively, large UCM observed in Fig. 3.1 coupled with higher Pr/nC_{17} and Ph/nC_{18} ratios supported the view that sample 6661 is most biodegraded. Relative small UCM observed in Fig. 3.4 and lower Pr/nC_{17} and Ph/nC_{18} ratios as in Table 3.2 indicate that sample (8676) is least biodegraded compared to its counterparts.

After considering the *n*-alkanes and acyclic isoprenoids as demonstrated in the previous figures above, the hopanes and steranes distributions in the oils were also considered with *m/z* 191 and 217 chromatograms displayed in the subsequent figures below;

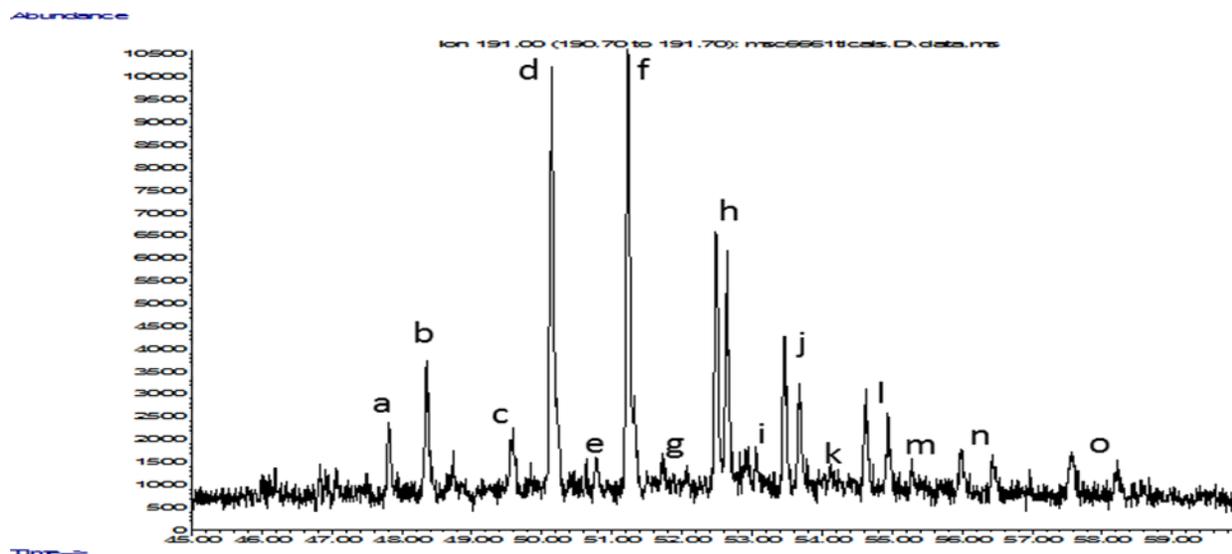


Figure 3.7 Chromatogram (ion 191) of the saturate fraction of sample 6661 obtained after the TLC of the whole oil fraction of the sample and analysed by GC-MS. The Biomarkers (Hopanes) are marked with alphabets above the peaks. The interpretation of the alphabets (acronyms) for each peak is given below.

a \equiv C₂₇ Ts, b \equiv C₂₇ Tm, c \equiv C₂₈, d \equiv C₂₉ $\alpha\beta$ hopane, e \equiv C₂₉ $\beta\alpha$ hopane, f \equiv C₃₀ $\alpha\beta$ hopane,
 g \equiv C₃₀ $\beta\alpha$ hopane, h \equiv C₃₁ $\alpha\beta$ hopane 22S & 22R isomers, i \equiv C₃₁ $\beta\alpha$ hopane, j \equiv C₃₂ $\alpha\beta$ hopane 22S & 22R isomers, k \equiv C₃₂ $\beta\alpha$ hopane,
 l \equiv C₃₃ $\alpha\beta$ hopane 22S & 22R isomers, m \equiv C₃₃ $\beta\alpha$ hopane, n \equiv C₃₄ $\alpha\beta$ hopane 22S & 22R isomers, o \equiv C₃₅ $\alpha\beta$ hopane 22S & 22R isomers

From Fig. 3.7 above, the distribution indicate dominance of C₂₉ $\alpha\beta$ hopane and C₃₀ $\alpha\beta$ hopane and decrease as molecular weight increase i.e. from C₃₁ $\alpha\beta$ hopanes (peak h) to C₃₅ $\alpha\beta$ hopanes (peak o)

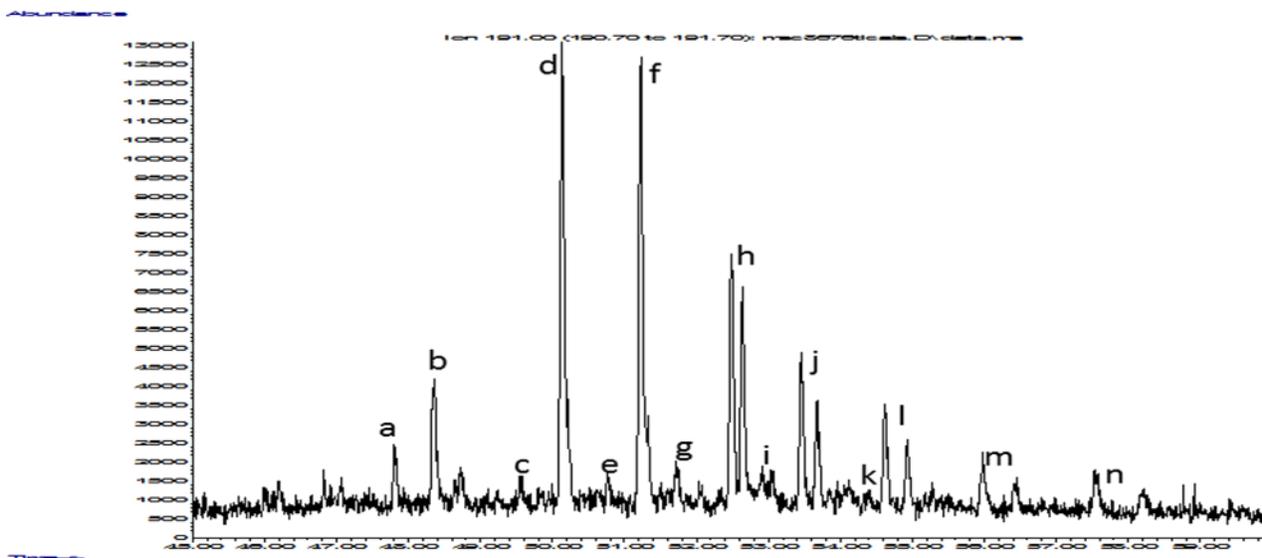


Figure 3.8 Chromatogram (ion 191) for the saturate fraction of sample 8676 obtained after the TLC of the whole oil fraction of this sample and analysed by GC-MS. The Biomarkers (Hopanes) are marked with alphabets above the peaks. The interpretation of the alphabets (acronyms) for each peak is given below.

a ≡ C₂₇ Ts, b ≡ C₂₇ Tm, c ≡ C₂₈, d ≡ C₂₉αβ hopane, e ≡ C₂₉βα hopane, f ≡ C₃₀αβ hopane,
 g ≡ C₃₀βα hopane, h ≡ C₃₁αβ hopane 22S & 22R isomers, i ≡ C₃₁βα hopane, j ≡ C₃₂αβ hopane 22S & 22R isomers, k ≡ C₃₂βα
 hopane, l ≡ C₃₃αβ hopane 22S & 22R isomers, m ≡ C₃₃βα hopane, n ≡ C₃₄αβ hopane 22S & 22R isomers.

From Fig. 3.8 above, the distribution indicate dominance of C₂₉αβ hopane and C₃₀αβ hopane and decrease as molecular weight increase from C₃₁αβ hopanes (peak h) to C₃₅αβ hopanes (peak o). The distribution is similar to sample 6661 in Fig. 3.7

3.2 Thin Layer Chromatography (TLC) of the Maltenes:

This section demonstrates the distribution of hopanes in the maltene fractions obtained after the TLC of the maltenes

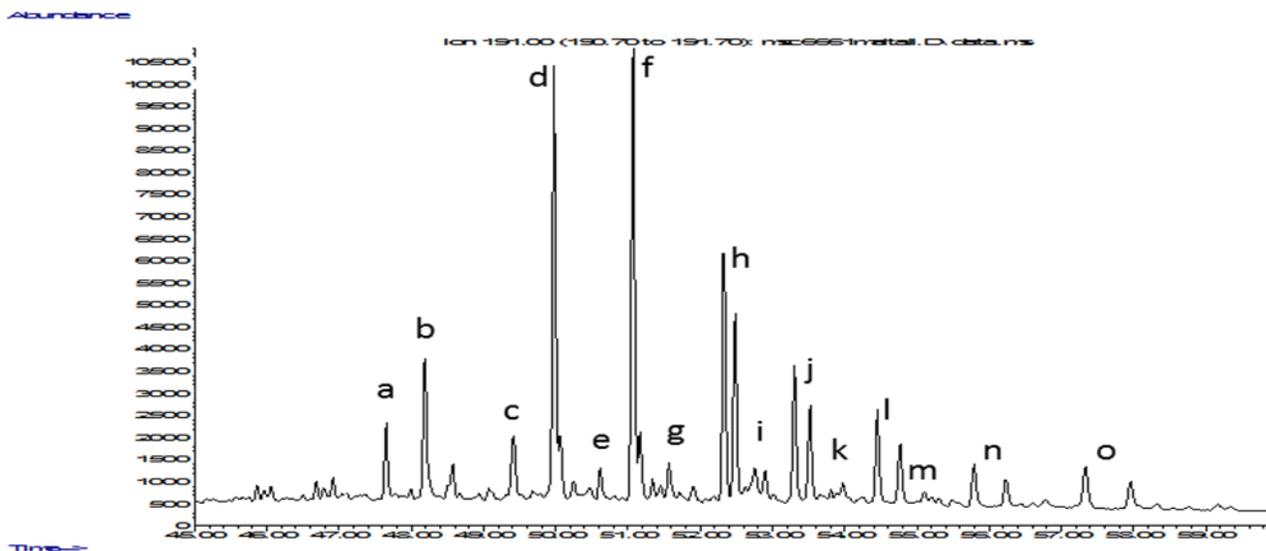


Figure 3.9 Chromatogram (ion 191) of sample 6661 obtained after the TLC of the maltene fraction of this sample and analysed by GC-MS. The biomarkers (hopanes) are marked with alphabets above the peaks. The interpretation of the alphabets (acronyms) for each peak is given below.

a ≡ C₂₇ Ts, b ≡ C₂₇ Tm, c ≡ C₂₈, d ≡ C₂₉αβ hopane, e ≡ C₂₉βα hopane, f ≡ C₃₀αβ hopane,
 g ≡ C₃₀βα hopane, h ≡ C₃₁αβ hopane 22S & 22R isomers, i ≡ C₃₁βα hopane, j ≡ C₃₂αβ hopane 22S & 22R isomers, k ≡ C₃₂βα
 hopane, l ≡ C₃₃αβ hopane 22S & 22R isomers, m ≡ C₃₃βα hopane, n ≡ C₃₄αβ hopane 22S & 22R isomers, o ≡ C₃₅αβ hopane 22S & 22R isomers

From Fig. 3.9 above, it is clearly observed that C₂₉ and C₃₀ hopanes (i.e. peak d and f) are more prominent than their counterparts. Similarly, concentrations of C₃₁ to C₃₅ were decreasing with increasing molecular weights (i.e. peak h to peak o)

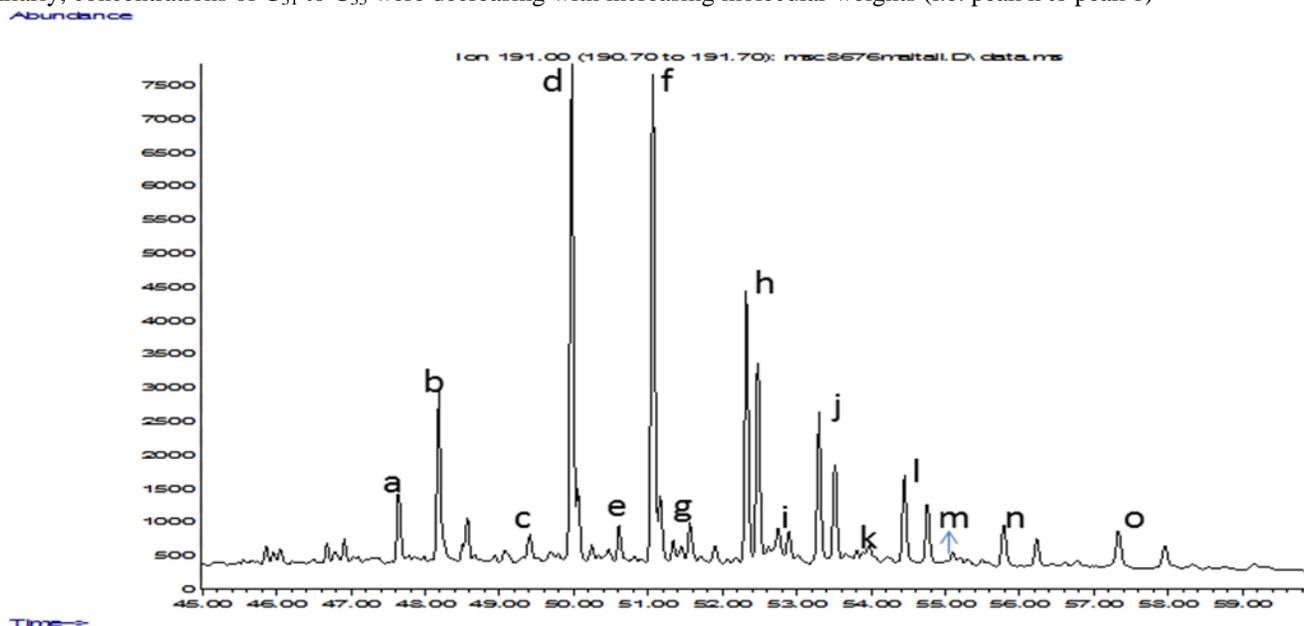


Figure 3.10 Chromatogram (ion 191) of sample 8676 obtained after the TLC of its maltene fraction and analysed by GC-MS. The biomarkers (hopanes) are marked with alphabets above the peaks. The interpretation of the alphabets (acronyms) for each peak is given below.

a \equiv C₂₇ Ts, b \equiv C₂₇ Tm, c \equiv C₂₈, d \equiv C₂₉ $\alpha\beta$ hopane, e \equiv C₂₉ $\beta\alpha$ hopane, f \equiv C₃₀ $\alpha\beta$ hopane,

g \equiv C₃₀ $\beta\alpha$ hopane, h \equiv C₃₁ $\alpha\beta$ hopane 22S & 22R isomers, i \equiv C₃₁ $\beta\alpha$ hopane, j \equiv C₃₂ $\alpha\beta$ hopane 22S & 22R isomers, k \equiv C₃₂ $\beta\alpha$ hopane, l \equiv C₃₃ $\alpha\beta$ hopane 22S & 22R isomers, m \equiv C₃₃ $\beta\alpha$ hopane, n \equiv C₃₄ $\alpha\beta$ hopane 22S & 22R isomers, o \equiv C₃₅ $\alpha\beta$ hopane 22S & 22R isomers

From Fig. 3.10 above, it is clearly observed that C₂₉ and C₃₀ (i.e. peak d and f) hopanes are more prominent than their counterparts. Similarly, concentrations of C₃₁ to C₃₅ were decreasing with increasing molecular weights (i.e. peak h to o). However, the distributions of all the hopanes were similar to oil 6661 in Fig. 3.9 above reaffirming their similar source.

3.3 Results of the RICO generated products:

The results displayed in this section are the hopanoic acids and *n*-alkanoic acid methyl esters released by RICO. The hopanoic acids were used for comparison study with distribution of hopanes in the maltenes. Whereas *n*-alkanoic acids with *n*-alkanes in whole oil.

(a) Distribution of terpanoic acids methyl esters from the RICO of asphaltene (sample 6661)

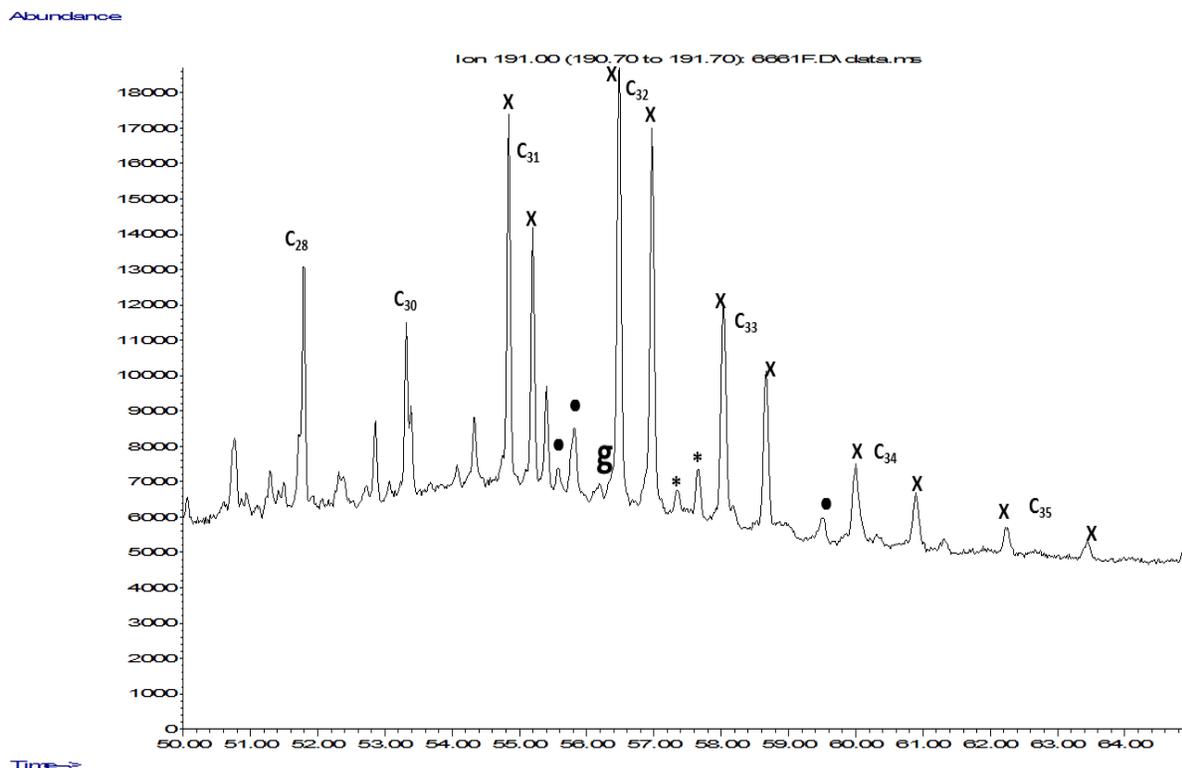
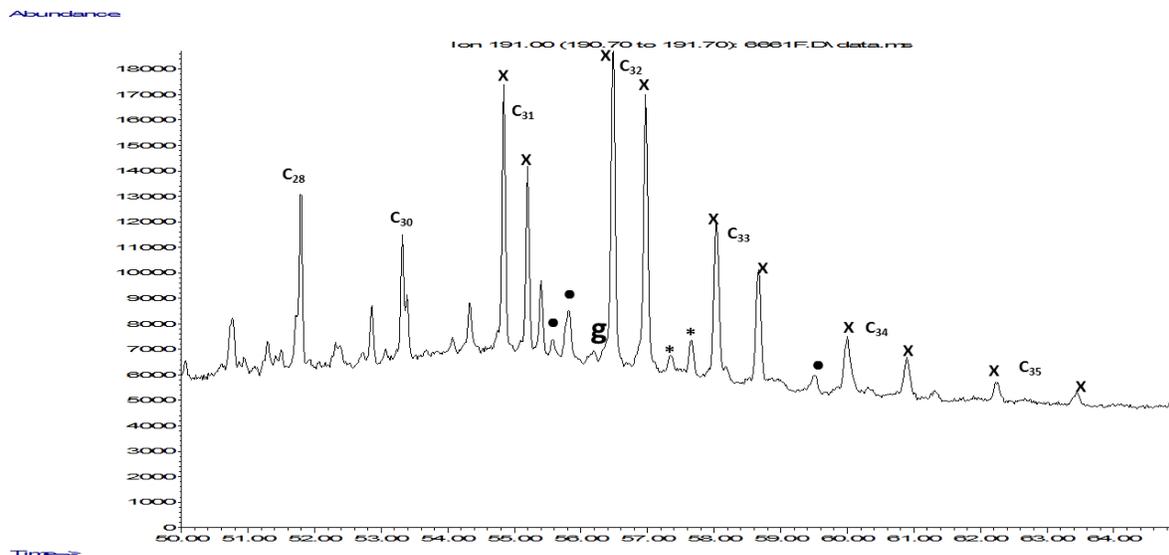


Figure 3.11 *m/z* 191 demonstrating the terpanoic acid methyl esters in asphaltene released after RICO treatment, in oil sample 6661 from North America. G refers to gammaceranoic acid. C₃₁ refers to C₃₁ hopanoic acid methyl ester, x to 17 α (H), 21 β (H) hopanoic acid methyl ester, the former is 22S, the latter is 22R. •, 17 β (H), 21 α (H) moretanoic acid methyl ester, the former is 22S, the latter is 22R, others are the same, peak assignment after [13].

(a) Distribution of terpanoic acids methyl esters from the RICO of asphaltene (sample 6661)



(b) Distribution of terpane in saturate of the maltene (sample 6661)

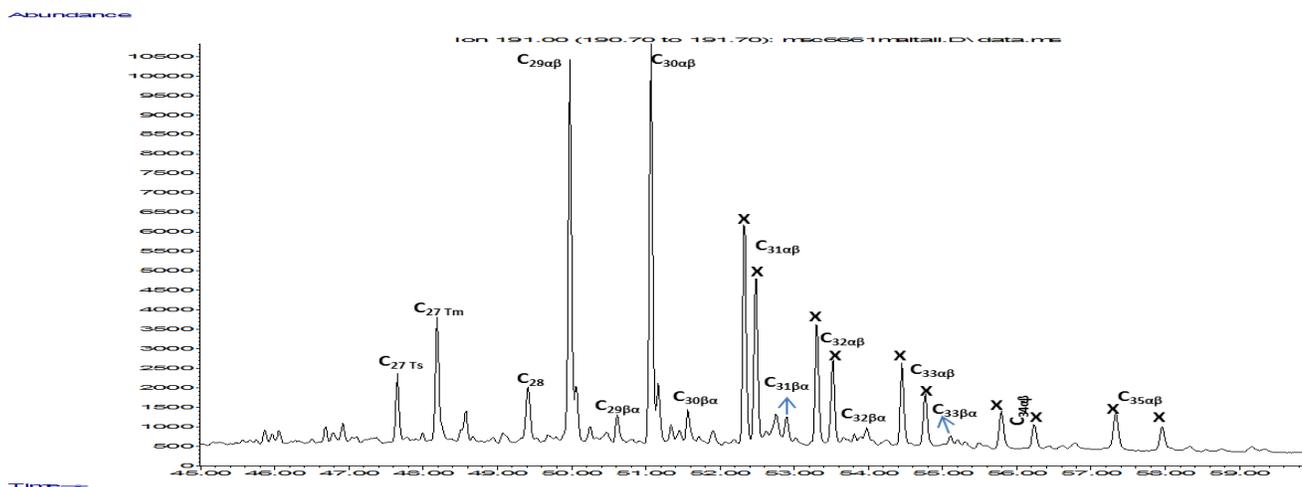


Figure 3.12 *m/z* 191 demonstrating the comparison of the terpanes in saturate of the maltene (deasphaltene fraction) and the terpanoic acid methyl esters in asphaltene released after RICO treatment, in the oil sample 6661 from North America. G in terpanoic refers to gammacerane. C₃₁ in asphaltene refers to C₃₁ hopanoic acid methyl ester, x to 17 α(H), 21 β(H) hopanoic acid methyl ester, the former is 22S, the latter is 22R, •, 17 β(H), 21 α(H) moretanoic acid methyl ester, the former is 22S, the latter is 22R, others are the same, peak assignment after [13].

From Fig. 3.11 demonstrated above, there was no C₃₆ hopanoic acid in the RICO products and this could be due to none addition of extra carbon from oxidative degradation of the asphaltene aromatic moiety. Similarly, no C₃₆ hopane was observed in the maltene.

IV. DISCUSSION

4.1 Distributions of Biomarker e.g. *n*-alkanes, isoprenoids, hopanes and steranes in the whole oils and maltenes fractions-TLC:

According to [14] *n*-alkanes are the saturated hydrocarbon that are easily destroyed by bacteria, hence biodegraded oil can be characterised by absence or very low concentration of these compounds. Several indices can be used to determine extent of biodegradation e.g. C₃₀αβ hopane/(Pr+Ph), C₂₉αβ-25-norhopane/C₃₀αβ hopane, Pr/*n*C₁₇ and Ph/*n*C₁₈. In this work, the extent of biodegradation was determined using ratios involving *n*-alkanes and isoprenoids compounds e.g. Pr/*n*C₁₇ and Ph/*n*C₁₈. This is because the latter are more useful when determining early stage of oil biodegradation [15]; [16]. The ratios are based on the reason that *n*-alkanes are more prone to biodegradation than isoprenoids. In this study, the ratios were observed to increase with increasing biodegradation as presented in Table 3.2 and Fig. 3.5 to 3.6.

The gas chromatograms of the saturate fractions of the four oils from North America obtained after the TLC of the whole oil fraction Fig. 3.1 to 3.4 are dominated by *n*-alkanes in the range of nC_{11} – nC_{35} , maximizing at nC_{13} or nC_{14} and in some cases extend up to nC_{37} as equally shown in Fig. 3.4.

After exhaustive analysis, all of the four oil samples showed slight to no biodegradation on a lately proposed biodegradation ranking scale based on considering the biomarkers [11]. This was demonstrated in Table 3.1 and Fig. 3.5 to 3.6. According to the scale, non-degraded oils are ranked “0” where *n*-alkanes, isoprenoids, steranes, diasteranes, and hopanes are all unaltered and were all clearly identifiable in the extract with no UCM observed Fig. 3.2 and 3.4 and Table 3.1. These features correspond to sample 8676 and 8672 respectively. On the other hand, two of the samples (6661 and 8673) were ranked “1” in the scale as presented in Table 3.1, indicating slight or early stage of degradation due to partial depletion of fewer lower *n*-paraffins. Similarly, presence of relatively large unresolved complex mixture (UCM) in sample 6661 supports the notion of its biodegradation whereas absence of UCM in 8676 and presence of *n*-alkanes and acyclic isoprenoids that were recorded in abundance in gas chromatograms of oil 8676 indicate that the latter oil was not biodegraded. Generally speaking, non-biodegraded oil can be found in reservoirs with a very recent oil charge and can also occur in sediments which are rapidly deposited [17]. Concurrently, the Pr/nC_{17} and Ph/nC_{18} ratios for the oils computed in Table 3.2 also confirmed that oil 6661 is relatively the most biodegraded due to relatively higher values of these ratios whereas 8676 least degraded due to lower ratios of Pr/nC_{17} and Ph/nC_{18} .

Evidently, as presented in Fig. 3.1, biodegradation was shown to have been very mild and not adequately severe to have affected isoprenoids, hopanes and steranes distributions. Likewise, demethylated hopanes (i.e. 25-norhopanes), considered to be either the result of hopanes biodegradation or their unmasking through their resistance to microbial attack [15]; [18]; [19]; [20]; [21] were not identified. Therefore, absence of 25-norhopane in sample 6661 which is believed to be present in severely degraded crude oils [15]; [22] indicate that sample 6661 is slightly degraded.

Furthermore, in comparison of abundance and distribution of *n*-alkanes in the saturated hydrocarbons of the whole oils Fig. 3.1 to 3.4 with *n*-alkanoic acid methyl esters in the asphaltene after RICO Fig. 3.11 it could be seen that the short chain *n*-alkanoic acids were more abundant and ranges from nC_7 – nC_{31} compared to their counterparts *n*-alkanes in the whole oils. This implies that the asphaltene within its structure has protected the bonded biomarkers from secondary alteration process i.e. biodegradation.

V. CONCLUSION

Whole oils GCs analysis showed a progressive increase in biodegradation in two of the oils (6661 & 8673) and the decline of their quality. The difference in level of biodegradation amongst the oils was small. However, increasing pristane / nC_{17} and phytane/ nC_{18} observed in sample 6661 and 8673 demonstrate the preference for *n*-alkanes over isoprenoids in biodegradation. The other two oils (8672 & 8676) were not biodegraded based on

Peters and Moldowan (1993) biodegradation scale. Moreover, the abundance of hopanes in the maltenes indicates a contribution from bacteria to the oil shale organic matter. Similarly, the calculated biomarker indices indicate that the oils trail from mature oil source and 6661 and 8673 have undergone a very low biodegradation.

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