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***Research Article***

# APPLICATION OF COMPOUND-SPECIFIC ISOTOPES IN OIL-OIL CORRELATION OF TWO NORTH SEA CRUDE OILS

***Nwoko Kenneth C.**
*Department of Chemistry, School of Industrial and Applied Sciences, Federal Polytechnic Nekede, PMB 1036, Owerri, Imo State, Nigeria*
**Author for Correspondence*

## ABSTRACT
Compound specific isotope ratio analysis (CSIA) and biomarker analysis were employed in establishing a correlation between two crude oil samples obtained from fields within the UK North Sea region. One of the crude oils was un-weathered while the other sample had undergone mild to severe biodegradation, with complete removal of *n*-alkanes. Silica gel column chromatography with petroleum ether was used in eluting the saturated hydrocarbons, while asphaltene fractions were precipitated with excess *n*-hexane, and purified by Soxhlet extraction. The asphaltenes were subjected to ruthenium ion catalysed oxidation (RICO). RICO oxidizes asphaltenes, converting their aromatic subunit to carboxylic acids, with derivatisation to methyl esters. GC-MS analyses of the maltene fractions of both oils showed identical hopane distributions for both oils, while their sterane biomarkers had slightly different distribution patterns .The $^{13}C$ values of the asphaltene methyl esters of both oils did not show any significant differences, suggestive of a common source for both oils; however, the slightly altered sterane biomarkers suggest either of the following: (i) different source inputs, (ii) changes induced by microbial biodegradation or (iii) the consequence of multiple charging episodes in the reservoir. CSIA is thus more reliable in correlation studies when employed alongside other geochemical analytical tools.

***Keywords:*** *CSIA, Biomarkers, Crude Oils, Asphaltenes, Biodegradation*

## INTRODUCTION
Compound specific isotopic analysis is a powerful state of the art technique of an ever increasing importance applicable to a wide range of geochemical investigations, and is especially useful in the area of defining petroleum systems through oil-oil and oil-source correlations using *n*-alkanes and asphaltenes fractions of crude oils (Xiong and Geng, 2000). This technique becomes particularly useful when there is difficulty in establishing the source, age and organic matter input of oil accumulations; for instance to establish if two different crude oils originated from different sources or are comingled resulting from a multiple charging episode in the reservoir (Rooney *et al.*, 1998).
When crude oils are exposed to phenomena such as weathering and microbial biodegradation, they undergo compositional changes which may make it difficult to correlate various oils, determine its source, and/or the original organic matter input. Biodegraded crude oils especially are a subject of continuous study by researchers in the hydrocarbon industry because they occur in very large volumes across many of the world's petroleum provinces such as the Alberta tar sands of Canada, Orinoco belt of Venezuela and the Troll field in the North Sea.
It is estimated that in the near future, they will dominate a significant proportion of future oil discoveries made in deep water areas of the world such as the Gulf of Mexico, Atlantic margin basins of Africa, Canada, and South America, because of favourable temperature conditions for active microbial activity in reservoirs in these regions (Head *et al.*, 2003).
Biodegradation occurs in reservoirs when bacteria such as archae and prokaryotes alter crude oil by utilizing the hydrocarbons as carbon source resulting to heavy viscous oil with low API gravity, enrichment in sulphur, metal content such as vanadium and nickel; high acidic content and enrichment in polar fractions (resins, asphaltenes) (Peters and Moldowan, 1993; Peters *et al.*, 2005; Head *et al.*, 2003; Huang and Larter, 2005). The sequence of biodegradation of oils follows a seemingly regular quasi-stepwise pattern of depletion of crude oil constituents. *N*-alkanes are lost in the early stages of

biodegradation, followed by the highly branched and cyclic saturated hydrocarbons and then the aromatic compounds which are less susceptible to biodegradation relative to the *n*-alkanes (Peters *et al.*, 2005).
The asphaltene fraction of crude oils tends to display a rather different picture from the more volatile *n*-alkane fraction of crudes. They have been said to be resistant to microbial attack, rather becoming more enriched when the oil is biodegraded (Peng *et al.*, 1999a; Liao *et al.*, 2008, Hassanshahian and Capello, 2013). Even when asphaltene biodegradation does occur, it is at a very low percentage between 5 % and 35 % (Pineda-Flores and Mesta-Howard, 2001). Some explanations have been proffered for this observation such as the large micelle-like structure of the asphaltenes, which confers protection to potential reaction sites from the action of microbes, catalysts, or surface formation water flowing through the deposit; as well as their large complex molecular structure conferred by the presence of alkanes, resins and other hydrocarbons which reduces microbial availability (Peng *et al.*, 1997; Pineda-Flores and Mesta-Howard, 2001).
However, some changes due to biodegradation have been observed in the isotopic values of asphaltenes obtained from some biodegraded oils of the Liaohe Basin, China (Liao and Geng, 2007; Liao *et al.*, 2008). Liao *et al.*, (2009) reported enrichment in $\delta^{13}C$ values of specific hydrocarbons with increasing severity of biodegradation. Stahl (1980) reported increases in $^{12}C$ values of asphaltenes and a decrease in $^{12}C$ values for *n*-alkanes in Ekofisk crude oil subjected to controlled biodegradation in the laboratory while the carbon isotope values of the aromatic fraction remained unchanged.
In this study, we have used CSIA supported with biomarker analyses to attempt to establish similarities/ differences in composition and source of two North Sea crude oils; an un-weathered crude oil and biodegraded oil. The *n*-alkane $\delta^{13}C$ values of a fresh crude oil were compared with the $\delta^{13}C$ values of the asphaltene methyl esters of the biodegraded oil. The asphaltenes were used especially to evaluate the hypotheses that asphaltene fractions of crude oil are resistant to secondary alteration effects such as biodegradation as reported by Peng *et al.*, (1999a); and Liao *et al.*, (2008).

## MATERIALS AND METHODS

### *a) Geological Setting*

The two crude oil samples used for the study are a fresh oil and biodegraded crude both sourced from fields within the North Sea area. The main source rock in the UK North Sea is the Upper Jurassic Kimmeridge Clay Formation (Glennie, 1998). The reservoir is the Kimmeridgian-Volgian submarine-fan Sandstone while the regional seal is the Lower Cretaceous Cromer Knoll mudstones (Gluyas and Swarbrick, 2004). The fresh oil was obtained from the Ettrick oil field, which lies in the Moray Firth about 120 km northeast of Aberdeen in blocks 20/2a and 20/3a in the UK sector of the North Sea.
The biodegraded oil originally a North Sea black oil, was sourced from the Captain Field located in the block 13/22a about 129km northeast of Aberdeen. The reservoir is the Late Aptian (Early Cretaceous) Captain sandstone member of the Valhall /Wick Sandstone formation. It is relatively heavy oil with 19-21° API gravity.

### *b) Sample Preparation*

Two hundred grams of each sample were weighed out with a Salter ER-180A electronic weighing balance and deasphaltened with *n*-hexane in the ratio of 40:1 in favour of solvent. Column chromatography using silica and petroleum ether as solvent was used to recover the aliphatic fraction. The aliphatic fraction was subjected to GC analysis to obtain the Total aliphatic hydrocarbon chromatogram (THC) and then further GCMS analysis was carried out. Deuterated tetraeicosane was used as internal standard.
The recovered dried asphaltenes were purified using Soxhlet extraction for 72 hours after which ruthenium (iii) ion catalyzed oxidation (RICO) as described by Mojelsky *et al.*, (1992) and Ma *et al.*, (2008) was performed to obtain the alkyl esters.

### *c) Urea Adduction and GC-IRMS Analyses*

The *n*-alkanes were separated from the cyclic alkanes present in the aliphatic fraction using the urea adduction method described as follows. 5 ml of urea saturated methanol was added to 10mg of aliphatic fraction previously dissolved in 0.5$cm^3$ of dichloromethane in a 20$cm^3$ vial. The mixture was placed in a

refrigerator below 0°C for 12 hours. After crystal formation, the supernatant solution containing the cyclic and branched compounds was decanted and crystals washed with cold n-hexane ($10cm^3$, 3X) and dried under stream of nitrogen gas. Then about $20cm^3$ of deionised water was added to dissolve the crystals and the released bound linear alkanes were extracted by washing with 10cm3 of *n*-hexane (X3). The excess solvent was removed from the extract by rotary evaporation to about 2cm3 and then further dried to about 0.5cm3 under nitrogen and transferred to GC vials for IRMS analysis.

GC-IRMS analysis was performed on a Thermo-Trace Ultra GC using a split-less injector at 280°C via a combustion III interface linked to a Thermo Delta V + Isotopic Mass Spectrometer. Chromatographic separation was performed on a fused silica HP-5 capillary column (30m length, 0.32 mm internal diameter), coated with 95% dimethyl – 50% diphenyl polysiloxane film of 0.25µm thickness as the stationery phase; with helium as the carrier gas. The acquired data was processed using the Isodat Dynamic Background Integration Workspace software to give the peak retention times and isotope values as $\delta^{13}C$ values.

## RESULTS AND DISCUSSION

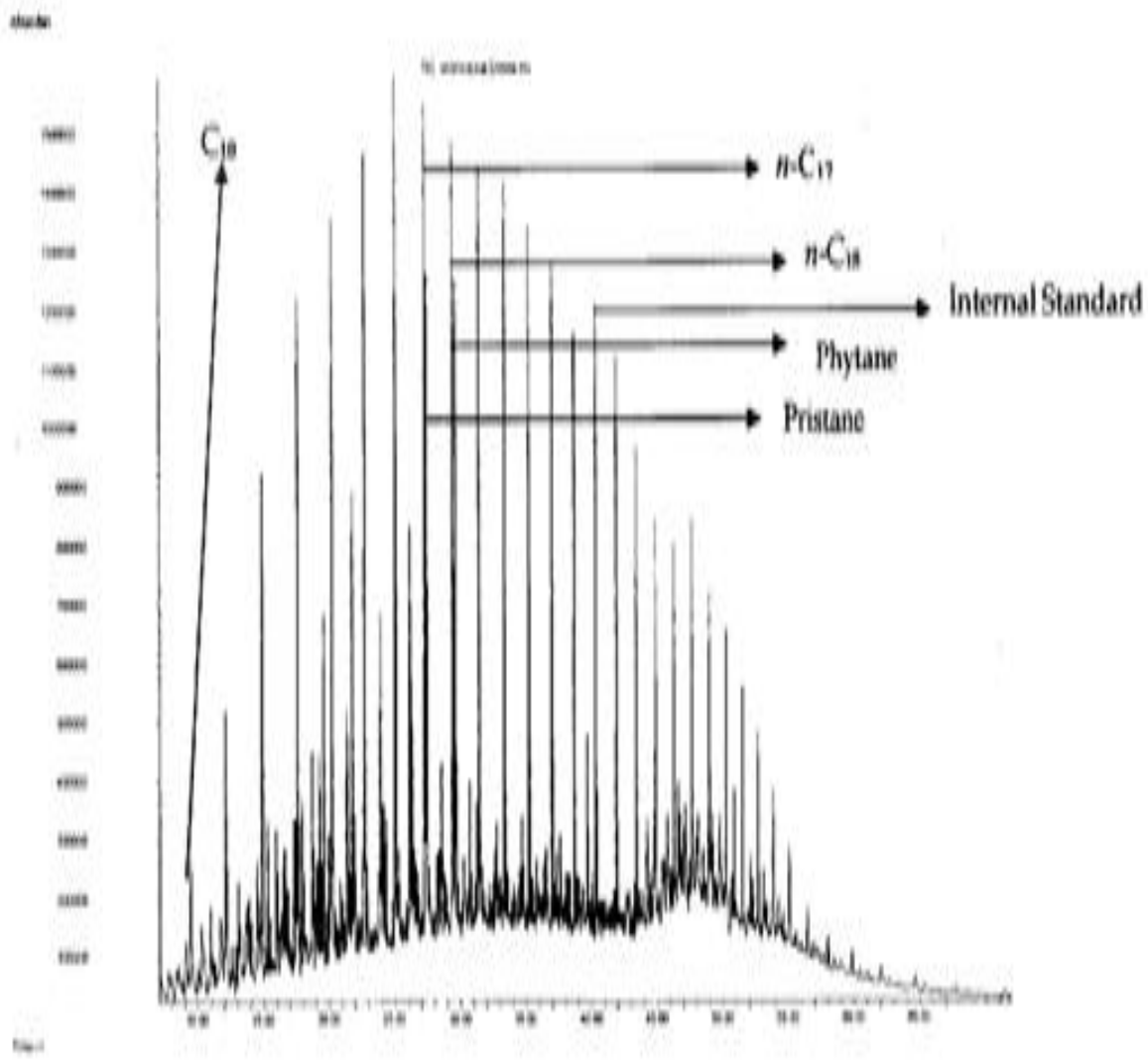


**Figure 1: Total ion chromatogram of the *n*-alkanes in the maltene fraction of the un-weathered oil showing some *n*-alkane homologues**

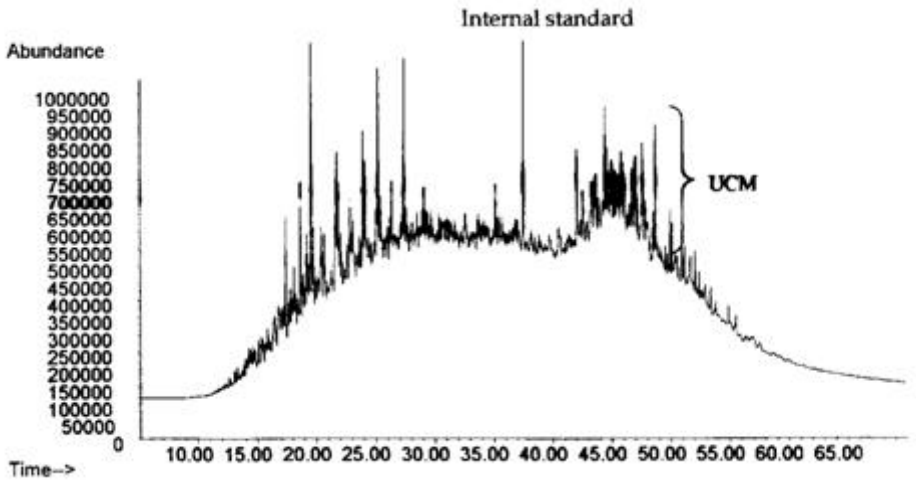


**Figure 2: Total hydrocarbon chromatogram of the maltene fraction of the biodegraded oil showing an unresolved complex mixture (UCM)**

**Table 1: Peak identification for the hopanes in Figure 3**

| Peak | Name |
|---|---|
| A | $C_{31}$ 17α (H), 21β (H) S hopane |
| B | $C_{31}$ 17α (H), 21β (H) R hopane |
| C | $C_{31}$ 17β (H) 21α (H) hopane |
| D | $C_{32}$ 17α (H), 21β (H) S hopane |
| E | $C_{32}$17α (H), 21β (H) R hopane |
| F | $C_{33}$ 17α (H), 21β (H) S hopane |
| G | $C_{33}$ 17α (H), 21β (H) R hopane |
| H | $C_{34}$ 17α (H), 21β (H) S hopane |
| I | $C_{34}$ 17α (H), 21β (H) R hopane |
| J | $C_{35}$ 17α (H), 21β (H)S hopane |
| K | $C_{35}$ 17α (H), 21β (H) R hopane |
| L | $C_{29}$ 17α (H), 21β (H) hopane |
| M | $C_{29}$ 17α (H), 21β (H) neohopane |
| N | $C_{29}$ 17α (H), 21β (H) hopane |
| O | $C_{28}$ 28, 30 bisnorhopane |
| P | $C_{30}$ rearranged hopane |
| Ts | $C_{27}$18 α (H)-22, 29,30-trisnorneohopane |
| Tm | 17α (H) 22, 29, 30-trisnorhopane |

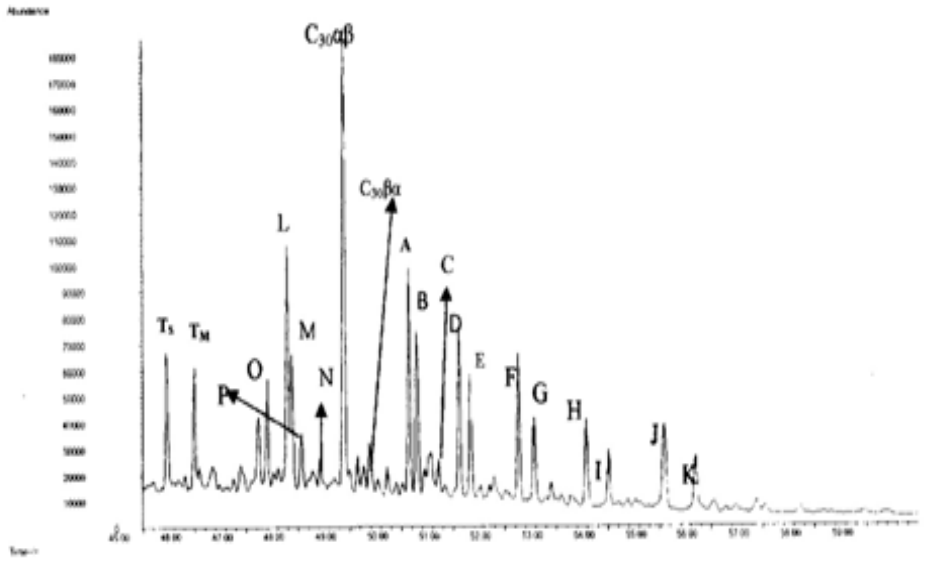


**Figure 3: M/Z 191 Hopane distributions for maltene fraction of both samples**

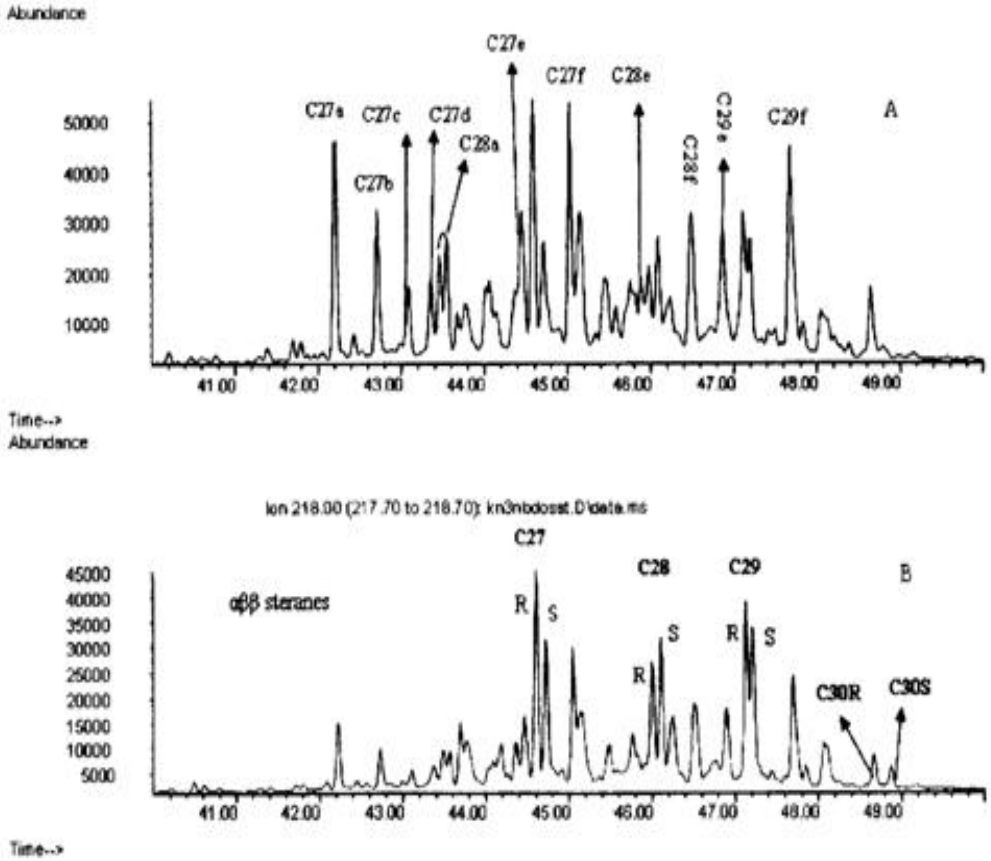


**Figure 4: M/Z 217 (A) and 218 (B) sterane biomarker distributions of the maltene fraction of the fresh oil sample**

**Table 2: Peak labels for Figure 4 (Applicable to Figure 5 for comparison)**

| Peak label | Peak name |
|---|---|
| a | 13β(H), 17α(H) –diasteranes 20S |
| b | 13β(H), 17α(H) –diasteranes 20R |
| c | 13α(H), 17β(H) –diasteranes 20S |
| d | 13α(H), 17β(H) –diasteranes 20R |
| e | 5α(H), 14α(H)17α(H) –steranes 20S |
| f | 5α(H), 14α(H)17α(H) –steranes 20R |
| $C_{27}$ R | 5α (H), 14β (H) 17β (H) –steranes 20R |
| $C_{27}$ S | 5α (H), 14β (H) 17β (H) –steranes 20S |
| $C_{28}$ R | 5α (H), 14β (H) 17β (H) –steranes 20R |
| $C_{28}$ S | 5α (H), 14β (H) 17β (H) –steranes 20S |
| $C_{29}$ R | 5α (H), 14β (H) 17β (H) –steranes 20R |
| $C_{29}$ S | 5α (H), 14α (H) 17α (H) –steranes 20S |
| $C_{30}$ R | 5α (H), 14α (H) 17α (H) –steranes 20R |
| $C_{30}$ S | 5α (H), 14α (H) 17α (H) –steranes 20S |

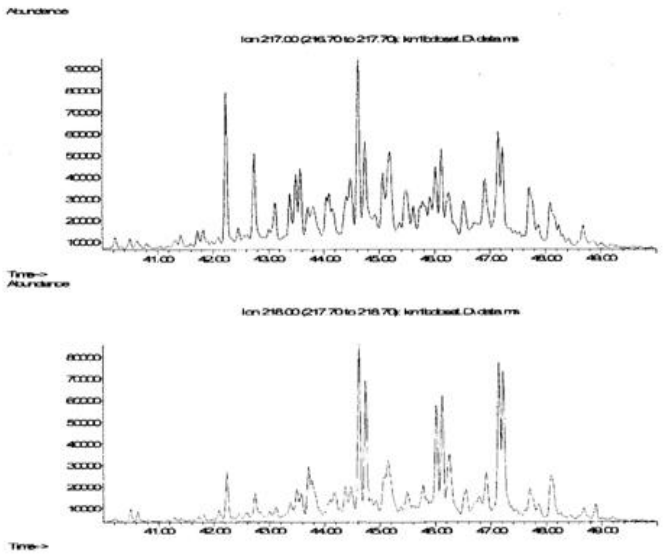


**Figure 5: M/Z 217 and 218 of the biodegraded sample. Note the near absence of $C_{27}$ ααα R isomer from its similar position compared with that of Figure 4**

**Table 3: Carbon isotopic values of *n*-alkanes in fresh oil sample**

| Carbon numbers | Isotopic values (‰) |
|---|---|
| 13 | -24.1 |
| 14 | -25.4 |
| 15 | -27.4 |
| 16 | -28.6 |
| 17 | -29.6 |
| 18 | -31.4 |
| 19 | -30.2 |
| 20 | -29.1 |
| 21 | -29.4 |
| 22 | -29.5 |
| 23 | -28.8 |
| 24 | -29.1 |
| 25 | -26.4 |
| 26 | -29.2 |
| 27 | -28.9 |
| 28 | -28.3 |
| 29 | -28.8 |
| 30 | -30.0 |
| 31 | -30.2 |

**Table 4: Carbon isotopic values of methyl esters of asphaltenes fractions**

| Carbon Numbers | Fresh oil (‰) | Biodegraded oil (‰) |
|---|---|---|
| 6 | - | -38.6 |
| 7 | - | -30.0 |
| 8 | -23.6 | -28.4 |
| 9 | -26.6 | -26.9 |
| 10 | -24.5 | -26.8 |
| 11 | -22.6 | -26.7 |
| 12 | -22.4 | -27.7 |
| 13 | -28.1 | -29.1 |
| 14 | -30.1 | -29.5 |
| 15 | -23.3 | -28.1 |
| 16 | -18.7 | -27.0 |
| 17 | -27.1 | -29.3 |
| 18 | -27.5 | -28.0 |
| 19 | -26.7 | -27.1 |
| 20 | -25.6 | -29.2 |
| 21 | -24.6 | -29.3 |

The TIC of the fresh oil maltene in figure 1 showed a fairly complete range of *n*-alkanes up to the $C_{31}$ homologue, however, it was not possible to compare it with the biodegraded oil sample as the *n*-alkanes had been removed. This is seen from the "hump" in figure 2 indicating an unresolved complex mixture which confirms the fact that the oil had undergone mild to severe biodegradation which confers a rank of 4 on the Peters and Moldowan biodegradation scale. The lower homologues $C_{10}$-$C_{12}$ and the higher homologues $C_{32}$- $C_{35}$ though identified in the initial GC-FID chromatogram were not detected by the GC-IRMS likely due to a reduced concentration after urea adduction.

Oils and petroleum products from different sources may have different distributions for biomarkers. Various biomarkers can occur in different carbon ranges; with markedly different abundances or

concentrations of individual biomarkers. This makes qualitative and quantitative comparisons of biomarkers distribution important in source identification and correlation work (Wang *et al.*, 2007). The hopane distribution of both samples was found to be similar, indicating a high likelihood of similarity of source organic matter. However, the sterane distribution of both samples was slightly different (compare Figures 4 and 5). The $C_{27}$ ααα*R* isomer in the biodegraded sample is considerably reduced, in fact almost absent. A slightly altered sterane distribution questions our earlier rank of PM=4 assigned to the biodegraded oil. However, according to the Peters and Moldowan scale, oils with slightly altered sterane distribution are assigned a PM value of 6. This observation could be a result of multiple charging of the reservoir, where some of the more biodegraded oil has already been emplaced in the reservoir before been recharged with large volumes of the less biodegraded oil (Xiong and Geng, 2000; Rooney *et al.*, 1998).

There is a progressive depletion in the heavier isotope from $C_{13}$ (-24.1‰) reaching a peak at $C_{18}$ (-31.4 ‰). The high value at $C_{18}$ may be a result of interference from phytane, of which minute traces might persist despite removal of branched saturated compounds during urea adduction. From $C_{19}$-$C_{31}$ the values are almost constant and uniform ranging between -30.2 ‰ to -30‰ respectively except at $C_{25}$ where a drop to -26.4 ‰ is recorded.

This drop might be due to interference from the deuterated $C_{24}$ alkane used as the internal standard in the analysis. On the other hand, looking at the trend of steady depletion in heavier isotope from $C_{13}$-$C_{18}$, coupled with the sharp increases at $C_{18}$ and $C_{25}$ might tempt us lead to conclude that the oil is of a mixed origin. However, the absence of homologues below $<C_{13}$ makes it more difficult to reach a conclusion on this observation. The trend is also reflective of the thermal maturity level of the oil, wherein the majority ($C_{19}$ to $C_{31}$) of the alkanes is enriched in the heavier isotope relative to the lower fraction. In the case of the non-biodegraded sample, the observed differences between individual carbon atoms are minor, or at worst fall within the range proposed by Clayton (1991) thus indicating distinct oil from a specific uniform source rock system. It has been noted by Clayton (1991) that differences of 3-4 ‰ between successive carbon atoms in the alkane fraction are normal and are due to the effects of increasing maturity of the sample.

***Conclusion***

Compound specific isotope analysis, when applied alongside other geochemical parameters such as biomarker analyses help to better assess the origins and sources of contributing organic matter to crude oils. Both crude oils show identical patterns of common source origin evidenced by their similar hopane biomarker distributions, though with slightly differing sterane biomarker distributions. The slightly differing sterane distribution in the biodegraded oil could be an indicator of mixing with a slightly more biodegraded oil. There is also some evidence as seen from the study, that the structural configuration of asphaltenes grants them more resistance to secondary alteration from biodegradation and maturity than the *n*-alkanes in the free oil, as seen in the $\delta^{13}C$ values of the asphaltene methyl esters of both oils.

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