# The Use of Tandem Quadrupole GC/MS/MS for the Selective Identification of Sterane Biomarkers in Crude Oils as Age and Facie Diagnostic Indicators.

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## OBJECTIVE

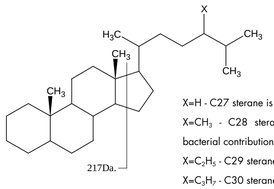
Through the use of the Waters Quattro micro™ GC tandem quadrupole mass spectrometer and its high sensitivity and high selectivity we have been able to specifically identify C27-C30 steranes and use the information to accurately identify the facie of the rock oil under investigation. In addition, the use of C26 steranes and their associated 24-norcholestane and nordiachloestane ratios as age-diagnostic indicators are also shown for a number of sample extracts.

## INTRODUCTION

Molecular biological markers, or biomarkers, are natural products that can be traced to a particular biological origin. They are structurally similar to, and are diagenetic alteration products of, specific natural compounds (chemicals produced by living organisms) and have a wide variety of applications, e.g. in the study of ancient environments and in petroleum exploration. Specifically, biomarkers in an oil can reveal the age of the source rock, the environment of deposition as marine, lacustrine, fluvio-deltaic or hypersaline, the lithology of the source rock (carbonate vs. shale), and the thermal maturity of the source rock during generation. Examples of biological markers include acyclic isoprenoids and terpanes, steranes (tetracyclic triterpanes) and hopanes (pentacyclic triterpanes). The tetracyclic steranes originate from naturally occurring sterols. Sterols are widespread in animals and plants, where they are important in cell membranes.

Diagenesis of sterols in sediments leads to sterenes and, ultimately, steranes. Diagenesis is a low temperature process prior to deep burial and thermal maturation. During this process the reduction of oxygen moieties and unsaturated regions result in the production of non native, stable, saturated hydrocarbon biomarkers in crude oil. These biomarkers can therefore be used to identify the original organic molecule and hence identify the original source organism. This ultimately results in the identification of the environment (facies) from which the oil was produced. Not surprisingly, steranes are one of the largest classes of biomarkers.

Figure 1 shows the structural backbone and the respective side groups for C27-C30 steranes. These have been identified as biomarkers a number of specific facies including higher plants (terrigenous) and algae (sea and fresh water associated). The associated key shows the variation in carbon chain length which is used to determine the organism and hence environment from which the oil was produced (1,2).




X=H - C27 sterane is ubiquitous in all organic matter

X=$CH_3$ - C28 sterane algal (fresh water or marine)/ bacterial contribution

X=$C_2H_5$ - C29 sterane Terrigenous (higher plant) material

X=$C_3H_7$ - C30 sterane algae from a marine environment

**Figure 1: The Sterane structure, relevant side chain length, the associated organism and hence environment from which the compound was produced.**

### Facie Identification

A South East Asian sample was prepared for analysis from a terrigenous (higher land plant) ecosystem. The source rock was solvent extracted and the resulting residue was fractionated by polarity to produce a non-polar hexane fraction.

Gas Chromatography/Mass Spectrometry (GC/MS) is the principal method used to detect and identify sterane isomers and other biological markers. Where the concentration of biomarkers of interest is relatively high, analysis of characteristic fragment ions in single ion recording (SIR) mode, e.g. using a single quadrupole instrument provides adequate results. However, some oils have very low concentrations of the biomarkers of interest and abundant interfering compounds. SIR of the 217 Da fragment ion can be used to identify the steranes of interest. However, this type of analysis is prone to interferences from other families of compounds found in rock oil extracts including hopanes, methyl steranes and bicadinanes. In this case, tandem quadrupole instruments (GC/MS/MS) in multiple Reaction Monitoring (MRM) mode provides a significant advantage over single quadrupole instruments, offering greater selectivity and sensitivity.

### Use of 24-norcholestanes as age-diagnostic indicators.

24-Norcholestanes are C26 steranes that show promise as indicators of geologic age and depositional environment (3). However, C26 steranes are generally present in oils and sediments in low concentrations giving GC/MS signals an order of magnitude lower than observed with the more common C27, C28, C29 and C30 steranes; therefore, GC/MS/MS analysis is required for greater specificity.

In 1998, Holba *et al.* (3) observed that elevated 24-nordiacholestanes and 24-norcholestanes in Cretaceous or younger oils and sediments relative to their 27-norcholestane analogues may be related to geologic age. Two C26 sterane ratios, the 24 nordiacholestane ratio (NDR) and the 24-norcholestane ratio (NCR), may be defined respectively by equations (a) and (b) using peaks designated by the numbers in Figure 2.

### GC/MS/MS analysis of C26 steranes (norcholestanes)

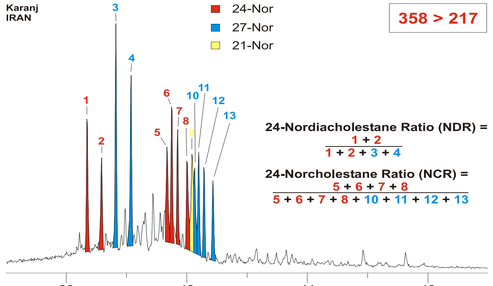



**Figure 2. Calculation of NDR and NCR ratios.**

The initial elevation of both the NCR and NDR ration occurs in the Jurassic (4,5)(NDR > 0.20, NCR > 0.30). In the Cretaceous, a second and more significant increase in 24-norcholestane abundance occurs (4,5,6) (NDR>0.25, NCR>0.40). There is strong circumstantial evidence that diatoms maybe the source of the 24-nor moiety. Indeed, in the Cretaceous, diatoms experienced a rapid expansion and species diversification (4,5,6).

We will therefore also explore the use of the NDR ratio in identifying ages of oils derived from two different sources, including the Beaufort Mackenzie Delta, Canada and Greenland.

## METHODS AND MATERIALS

The samples were analysed on an Agilent 6890 GC attached to a Waters Micromass Quattro micro GC triple quadrupole mass spectrometer operated in EI+ mode.

**Figure 3. Quattro micro tandem quadrupole mass spectrometer.**

The GC capillary column employed for all analysis was a J&W Scientific DB-5MS column, 30m x 0.25 mm i.d. x 0.25mm film thickness.

The facie analysis was completed using a 1µl splitless injection. The temperature program employed was 120 °C (1min hold) to 300 °C (8mins hold) at 15 °C/min, (Total run time of 38mins).

The age diagnostic analyses were completed using a 2 µl splitless injection. The temperature program employed was: 70 °C (2 mins hold), to 100 °C at 30°C/min (no hold), to 308 °C (8mins hold), at 4 °C/min (Total run time of 63 mins).

Argon was used as the collision gas for all MRM experiments.

## RESULTS

### Facie Identification

The SIR analyses of the South East Asian sample is shown in Figure 4 and is an oil produced from a terrigenous environment. The selected ions are described on the figure. The 85Da channel shows the oil sample containing a high concentration of alkane compounds while the 191Da and 369Da channels show the presence of a number of bicadinane and hopane components. The bicadinanes are significant in the terrigenous sample (369 m/z) due to the fact that the compounds originate from a resin exuded from a higher plants (7). The 217 channel shows the presence of not only C27-C30 steranes but also of the significant concentration of bicadinanes, in addition to hopane and methyl sterane interferences.

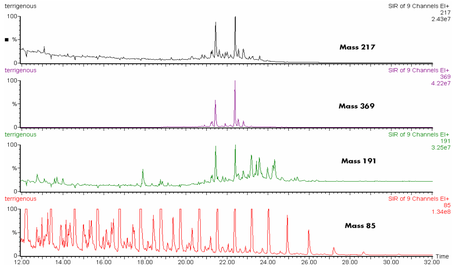



**Figure 4. SIR of an oil formed from a terrigenous environment.**

Figure 5 shows the MRM analysis of the terrigenous oil with the five MRM transitions described below. The presence of the four steranes (their associated isomers) are confirmed from the relevant transitions displayed. Figure 5 shows a greater intensity of C29 steranes compared to the C28 sterane (possibly from freshwater algae or bacteria) which indicates a terrigenous environment rather than a marine environment. The lack of any C30 steranes and the presence of a significant quantity of bicadinane material further confirm the terrigenous hypothesis.

The bicadinane interferences seen in the SIR experiments are clearly identified and shown to cause no interference when the same sample is analysed by MRM.

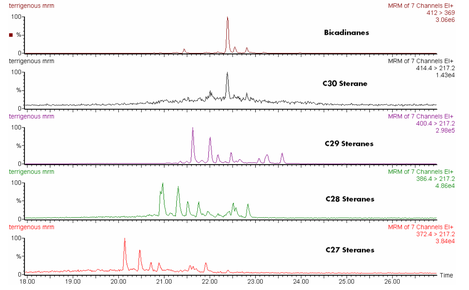



**Figure 5. MRM analysis of a terrigenous oil.**

### NDR ratios, used to assess petroleum source age: Two case studies

The C26 steranes in oils from two different sources of known age have been identified by monitoring the MRM transition 358>217 and the calculated 24-Nordiacholestane ratios . The samples selected for this study include a Late Ordovician oil (8) extract derived from Greenland, and an extract of Tertiary Amauligak oil (9) from Canada.

Figure 6 shows the C26 steranes in an extract of Amauligak oil from the Mackenzie Delta in Canada (9). The NDR ratio = 0.50, reflecting the relatively

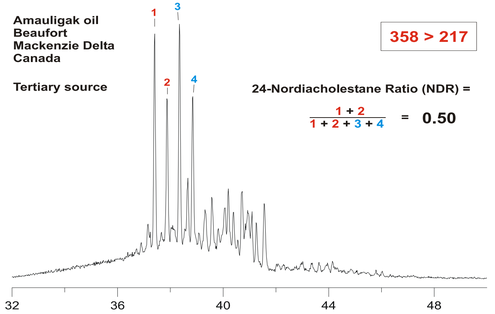



**Figure 6. GC-MS/MS analysis of the C26 steranes of Amauligak oil (Tertiary) from the Mackenzie Delta in Canada along with its NDR ratio (9).**

A source rock from North Greenland (8) (Figure 7) was calculated to have an NDR ratio of 0.12 this identifies the extract to be of the Late Ordovician origin. The 24-Nordiacholestanes (peaks 1 and 2) are very low in abundance in this sample.

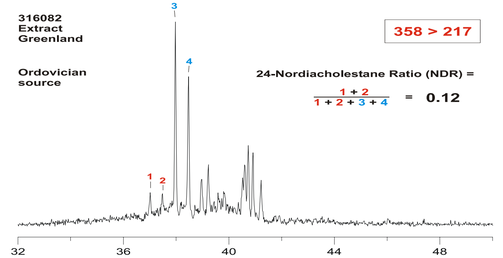



**Figure 7. GC-MS/MS analysis of the C26 steranes of 316082, a Late Ordovician source rock from North Greenland (8), along with its NDR ratio.**

## CONCLUSIONS

Through the use of the Waters Quattro micro™ GC tandem quadrupole mass spectrometer we have been able to specifically identify C27-C30 steranes and use the information to accurately identify the facie of the rock oil under investigation.

Comparison with the SIR analyses clearly identifies the difficulties encountered when undertaking such an investigation on a single quadrupole instrument and that for rapid unambiguous routine analysis a triple quadrupole mass analyser is required. The bicadinane interferences seen in the SIR experiments are clearly identified and shown to cause no interference when the same samples are analysed by MRM.

NDR ratios provide an extremely useful tool in assessing petroleum source age for oils from a range of different sources. As illustrated in this study, oils derived from Greenland appear to have low diatom input showing the age of these oil sources to be significantly older to those from the Mackenzie Delta in Canada.

The combination of high sensitivity and high selectivity offered by tandem quadrupole GC/MS/MS in MRM mode has provided significant advantages over the single quadrupole technique when targeting trace compounds in complex matrices.

The ability to accurately quantify over a wide dynamic range is essential in studies of this nature where precise ratios of abundance are required.

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