

# Analysis of Molecular Fossils: Crude Oil Steroid Biomarker Characterization Using Triple Quadrupole GC-MS/MS

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## Introduction

Due to the variety of geological conditions and ages under which oil was formed, every crude oil exhibits a unique biomarker fingerprint. Because of this, biological biomarkers are the most important hydrocarbon groups in petroleum because they can be used for chemical fingerprinting. Biomarkers reveal all or most of the original carbon skeleton of the original natural products.<sup>1,2</sup> Relative to other hydrocarbon components petroleum biomarkers are more resistant to biodegradation but concentrations steadily decrease as petroleum matures. The information from biomarker analysis is used to determine the migration pathways from a source rock to the reservoir, for the correlation of oils in terms of oil-to-oil and oil-to-source rock, the source potential, ranking of the relative thermal maturity, as well as possible secondary alteration processes. Decisions for the commercial exploitation of a prospect are based on that analytical background. Economics ultimately determine if a petroleum reservoir is further developed and finally brought to the market.<sup>3</sup> Refinery chemists are mostly interested in how oil behaves when feeding the processes into marketable products. Analyses of petrochemical biomarkers also have been proven useful in the determination of petroleum-derived environmental contaminations.<sup>2</sup>

Biomarkers found in crude oils, rocks and sediments, also referenced as “molecular fossils” in the literature, demonstrate few or even no changes from their former precursor compounds: terpenoids (isoprenoids) and steroids found in the cells of the originating living organisms.<sup>2,4</sup> Biomarker concentrations in oils are low, typically in the low ppm and sub-ppm level in the presence of a highly complex petroleum hydrocarbon matrix. As the concentrations of biomarkers in petroleum decrease with thermal maturity, oils of high maturity exhibit particular analytical challenges with only low biomarker concentrations. Highly selective, fast and sensitive mass analyzers as high resolution or triple quadrupole GC/MS instruments are common and required for meaningful biomarker analysis.



## Experimental Conditions

Triple quadrupole mass spectrometry allows the determination of the structure related precursor-product ion relationships with significantly less matrix interference than single stage quadrupole MS. Based on the selected reaction monitoring process (SRM) triple quadrupole technique provides a unique selectivity for biomarker studies. The analysis of sterane, tricyclic and pentacyclic terpanes (hopanes) biomarker have been subject to the described application.

The homologous series of sterane compounds in the range of C<sub>27</sub>-C<sub>30</sub> produces in the collision cell of a triple stage quadrupole mass spectrometer under soft CID conditions common product ions at  $m/z$  217 from the individual molecular ions ranging from  $m/z$  274 to 428. Precursor ions of the hopane series in the range of C<sub>27</sub>-C<sub>35</sub> cover the molecular ion range of  $m/z$  370 to 482. Each of these precursor ions produces an intense product ion at  $m/z$  191.

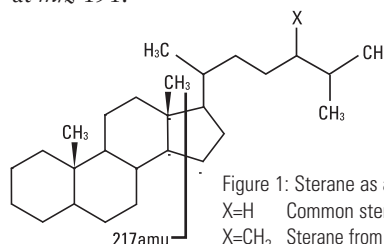


Figure 1: Sterane as a molecular fossil<sup>4</sup>  
X=H Common sterane, found in all sources  
X=CH<sub>3</sub> Sterane from algae origin (marine or limnic)  
X=C<sub>2</sub>H<sub>5</sub> Sterane from plant origin (terrestrial)  
X=C<sub>3</sub>H<sub>7</sub> Sterane from from algae origin (marine)

For this application, chromatographic separation was achieved using a 60 m apolar fused silica column, as is standard in most laboratories. The temperature program is characterized with a low heating profile, which offers increased chromatographic resolution especially for the series of biomarker isomers.

## Key Words

- TSQ Quantum XLS
- Crude Oil
- GC-MS/MS
- Hopanes
- SRM
- Steranes
- Steroid Biomarker

## Sample Measurements

In high resolution and triple quadrupole mass spectrometers, highest selectivity for biomarker targets is achieved by monitoring the structure specific decay after electron impact ionization (EI) into group-specific fragments. Due to the low activation potential of the initially generated metastable ions, only low collision energies are required for a collision induced dissociation (CID) in a triple quadrupole MS. Within a series of initial measurements the optimum collision energy of 10 eV offers the best compound response (Figure 2).

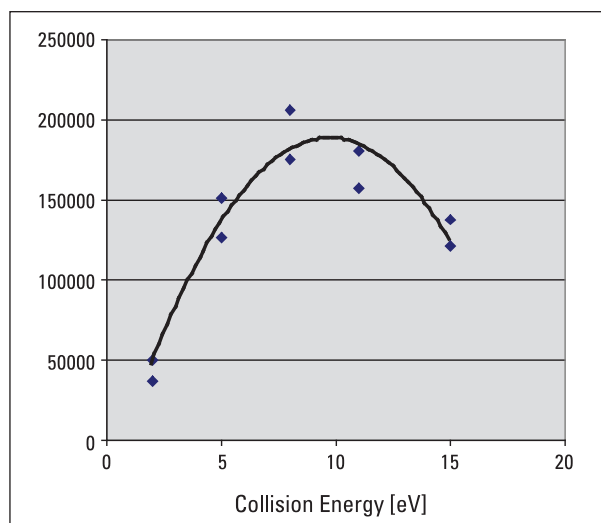


Figure 2: CID Optimization for Steranes on  $m/z$  217.10

All described analyses were performed using the Thermo Scientific TSQ Quantum XLS with a Thermo Scientific TRACE GC Ultra gas chromatograph and a Thermo Scientific TriPlus AS autosampler. Chromatographic separation was achieved using a Thermo Scientific TRACE TR-1MS capillary column (60 m, 0.25 mm I.D. and 0.25  $\mu$ m film thickness). The GC parameters are given in Table 1.

Table 3: SRM Transitions for TSQ Quantum XLS

### Steranes

Carbon Number	Precursor Mass $m/z$	Product Mass $m/z$
C20	274.3	217.2
C21	288.3	217.2
C22	302.3	217.2
C23	316.3	217.2
C24	330.3	217.2
C25	344.3	217.2
C26	358.4	217.2
C27	372.4	217.2
C28	386.4	217.2
C29	400.4	217.2
C30	414.4	217.2
C31	428.4	217.2

### Tricyclic Terpanes

Carbon Number	Precursor Mass $m/z$	Product Mass $m/z$
C28	388.3	191.2
C29	402.4	191.2
C30	412.4	191.2
C31	416.4	191.2
C32	430.4	191.2
C33	444.4	191.2
C34	472.4	191.2
C35	486.4	191.2
C36	500.4	191.2

### Pentacyclic Terpanes

Carbon Number	Precursor Mass $m/z$	Product Mass $m/z$
C27	370.3	191.2
C28	384.3	191.2
C29	398.4	191.2
C30	412.4	191.2
C31	426.4	191.2
C32	440.4	191.2
C33	454.4	191.2
C34	468.4	191.2
C35	482.4	191.2

Injector	split/splitless
Injector Temp.	260 °C
Carrier Program	constant flow 1.0 mL/min
Injection	splitless injection, split flow 70 mL/min
Pre-/Post Inj. Time	1 s each
Oven Temp. Program	50 °C, 2 min 20 °C/min to 150 °C 1.5 °C/min to 310 °C 310 °C, 17 min
Transfer Line Temp.	250 °C

Table 1: Selected Method Parameters for TRACE GC Ultra™ with TriPlus™ AS

The MS method was set up for the given crude oil sample to perform a target compound analysis on steranes, tri- and penta-cyclic terpanes with common product ions at  $m/z$  217.20 and  $m/z$  191.20 respectively (Table 2). The resolution of the Q1 quadrupole of the TSQ Quantum XLS™ was set to 0.7 Da to provide high selectivity typical of the Thermo Scientific hyperbolic quadrupole rod technology. This enables selection of the steroid precursor ions from the complex hydrocarbon matrix. For data acquisition by selected reaction monitoring (SRM) the masses according to Table 3 were used for the three compound groups under investigation.

Samples (1.6 mg) were taken from a native crude oil sample and dissolved in 320  $\mu$ L hexane. *i*-Octane would work well as alternative solvent.

Ionization	70 eV, EI
Selectivity Resolution Q1	0.7 Da FWHM
Mass Scan Width	2 mDa
Collision Energy	8 eV
Collision Gas	Ar, 1 mTorr
Scan Cycle Time	1 s

Table 2: Data Acquisition Method for TSQ Quantum XLS

## Results and Discussion

The TSQ Quantum XLS was used to successfully analyze biomarkers in a native crude oil sample. Use of MS/MS offered excellent selectivity and sensitivity, allowing the biomarker information to be accurately determined despite the complex hydrocarbon matrix. The chromatograms demonstrate the desired hopane and sterane compound distribution with highest certainty even at the lowest levels. The individual peaks appear very well separated in remarkably intensity without interference from the intense background.

Of the total GC runtime of about 120 min., the sterane and hopane elution window for the homologous series of C20 to C35 compounds is approximately 35 min. The compounds elute as homologous series with increasing carbon number within the typical distribution curve. Individual compounds are well separated with excellent peak shape. Extracted ion chromatograms are shown in Figures 3, 4, and 5, which depict steranes, tricyclic terpanes and pentacyclic terpanes in native crude oil.

Sterane biomarkers are found in low abundance with increased concentration relative to the higher molecular weight compounds. Even at lower levels the tricyclic terpanes are found in the given sample. Hopane compounds in contrast are detected with high abundance over the complete molecular range.

## Conclusion

Because biomarker concentrations decrease with increasing thermal maturity, light oils contain only a low concentration of detectable biomarkers, and in consequence require instrumentation of highest selectivity and sensitivity. The information of source, thermal maturity, and secondary alteration processes are furthermore often revealed by only subtle variations in the isomeric distribution of such trace components. GC-MS/MS using the Thermo Scientific TSQ Quantum XLS is the premier analytical method for the sensitive characterization of “molecular fossil” biomarkers in crude oil.

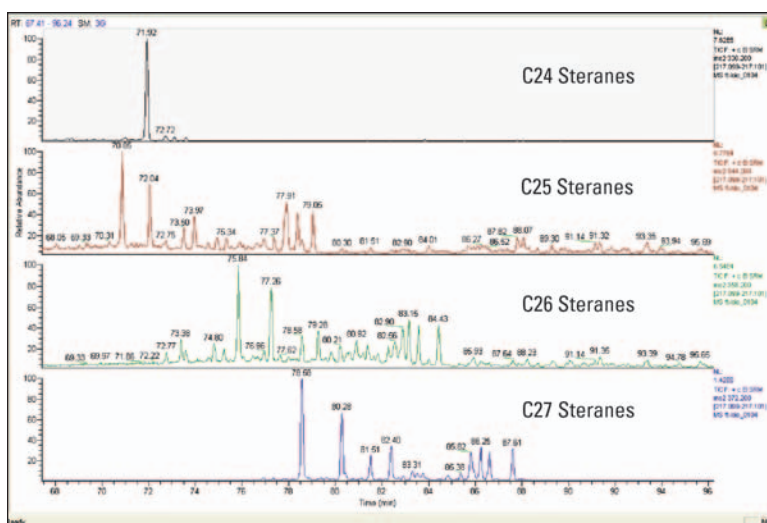


Figure 3: Sterane profiles in the elution range C24–C27

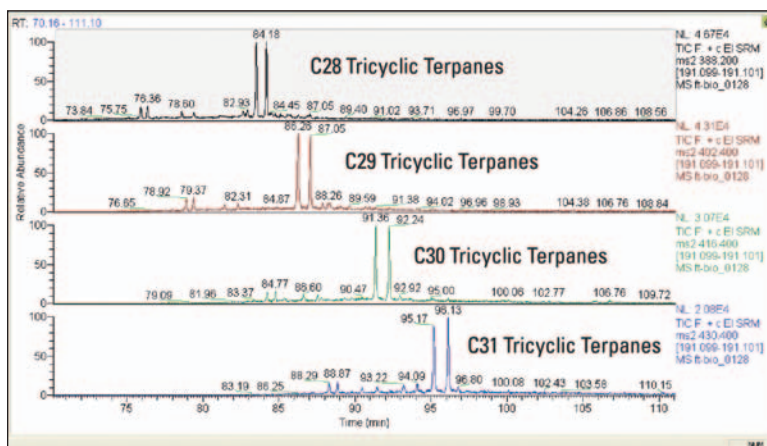


Figure 4: Tricyclic terpanes profiles in the elution range C28–C31

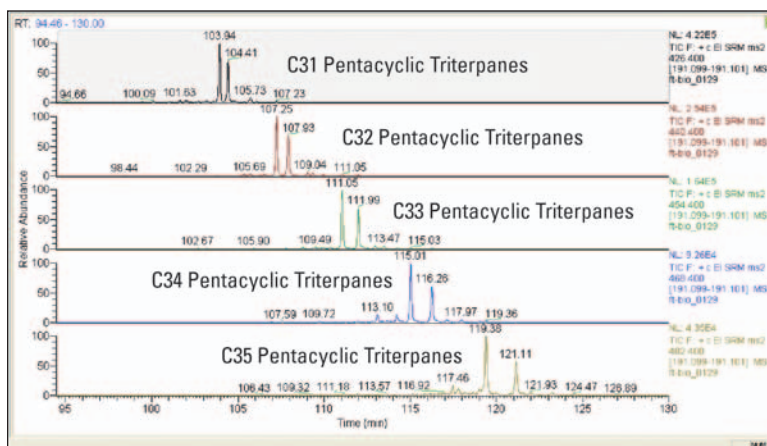


Figure 5: Pentacyclic terpanes (hopanes) profiles in the elution range C31–C35

## References

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Original data acquired using the Thermo Scientific TSQ Quantum GC. Performance of the Thermo Scientific Quantum XLS typically meets or exceeds these results.

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