# Application Note

## *Instrument: Pegasus® BT 4D*

# Petroleum Forensics: Identifying Biomarkers in Crude Oil

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Key Words: Petroleum, GCxGC, Flow Modulation, Biomarkers, FLUX

## *Introduction*

Petroleum forensics is the art of tracing the geochemistry of crude oil using petroleum biomarkers. Ratios of chemical "biomarker" compounds such as hopanoids, diasteranes, and steranes that are resistant to weathering and other forms of degradation, often called "molecular fossils", are crucial for differentiating various sources of crude oil. Depending on thermal maturity, depositional environment, and geographic location/age, the type and concentration of different hopanoid and sterane structures can provide unique identifiers for specific sources of oil. Used extensively in geochemical and biomarker analysis of the volatile and semi-volatile portions of petroleum samples, gas chromatography (GC) provides the foundation for petroleum forensics. However, the use of comprehensive two-dimensional gas chromatography (GCxGC) to characterize crude oil has been widely adopted because of its utility in resolving complex mixtures that prove difficult to analyze with GC alone. GCxGC separations are achieved by connecting two columns of complementary stationary phases in series through a modulating device, which injects effluent from the primary column into the secondary column at frequent intervals throughout the primary separation, effectively separating the analytes in both dimensions. The benefits of GCxGC are not only increased chromatographic resolution, but also easily distinguishable speciation of compound types due to structurally-grouped elution bands or "fairways". In addition, the higher resolution affords cleaner mass spectra of compounds in a complex mixture. In this application note, various biomarkers from samples collected in different geographic regions worldwide are identified, using a robust and easy-to-use GCxGC system that incorporates a flow-based modulator paired with a time-of-flight mass spectrometer (TOFMS) to assist in providing insight on the age, origins, and forensic fingerprint of the petroleum samples analyzed.

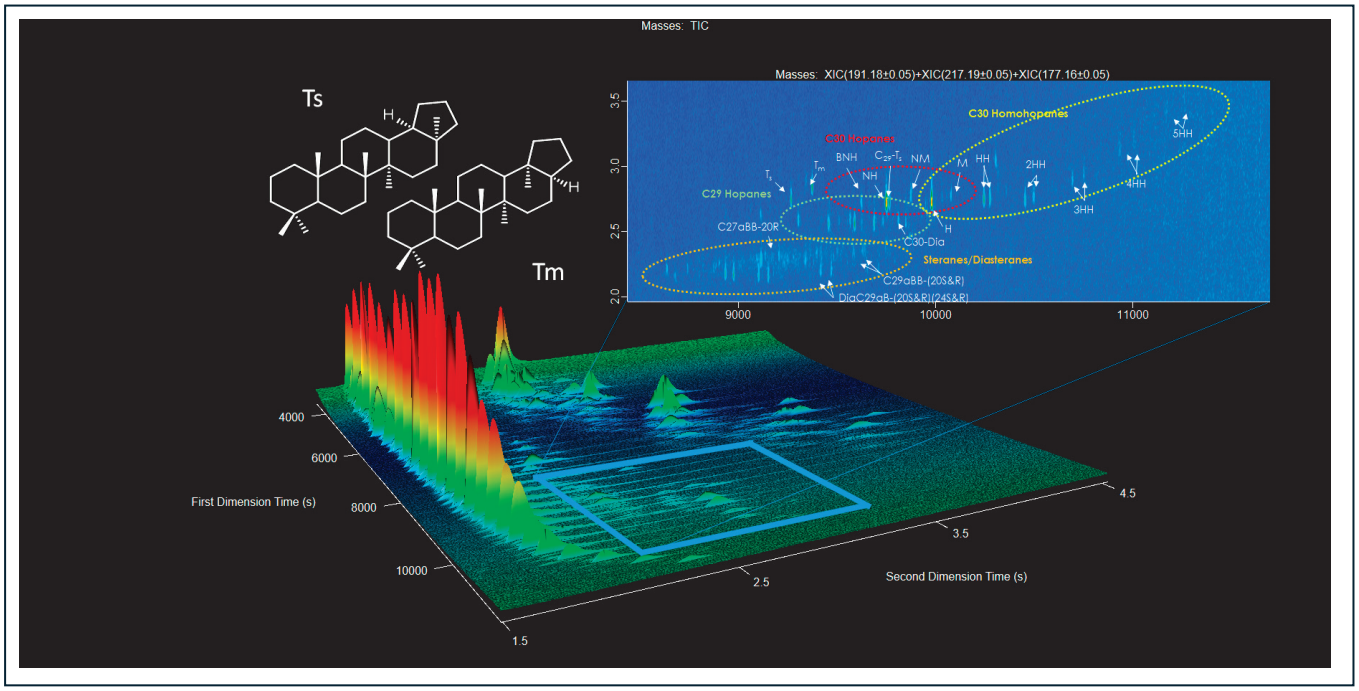


***Figure 1. Surface Plot of Intermediate Fuel Oil with zoomed-in contour plot for biomarker region labelled with hopanes and steranes of interest. Structures for the Ts and Tm hopanes are also shown.***

## *Experimental*

Petroleum samples were obtained from various environmental sources and diluted to 5 mg/mL in hexane. Analytical parameters for the GCxGC-TOFMS system equipped with the FLUX™ flow-based modulator are shown in Table 1 below. Data processing was performed using ChromaTOF® brand software, with automatic peak find and library search. Mathematically deconvoluted peaks were compared to both a spectral library of biomarkers generated at the Woods Hole Oceanographic Institution (WHOI) and the NIST17 spectral library. Method parameters were developed using a sample of intermediate fuel oil from the oil tanker *Kirby*. Second-dimension separation time and secondary oven temperature offset were determined by finding the balance between elution of the most-highly retained peak in the second dimension, ensuring each first-dimension peak was sampled at least three times, and maintaining sensitivity requirements for observation of the naturally low-concentration biomarkers.

**Table 1: Instrument parameters for data acquisition**

| **Gas Chromatograph** | **LECO *FLUX* GC×GC** |
|---|---|
| Injection | 2 µL liquid injection, splitless @ 310 °C |
| Carrier Gas | He @ 1.0 mL/min, constant flow |
| Column One | Rxi-1ms, 60 m x 0.25 mm i.d. x 0.25 µm coating (Restek, Bellefonte, PA, USA) |
| Column Two | BPX-50, 1.91* m x 0.10 mm x 0.10 µm coating<br>*1.6 m coiled in 2nd oven and 0.31 m in transfer line |
| Temperature Program | 15 min at 80 °C, ramped 1.5 °C/min to 335 °C, hold for 10 min |
| Secondary Oven | +40 °C relative to primary oven |
| 2nd Dimension Separation Time | 3.5 s, injection duration of 0.08 s |
| Transfer Line | 320 °C |
| **Mass Spectrometer** | **LECO *Pegasus* BT** |
| Ion Source Temperature | 250 °C |
| Mass Range | 40-600 m/z |
| Acquisition Rate | 200 spectra/s |

## *Results and Discussion*

The advantage of using GCxGC is the ability to separate compounds of interest from each other in two dimensions of chromatographic space. Figure 2 below shows a 3D surface plot representation of the intermediate fuel oil GCxGC chromatogram, where over 3,000 peaks were identified.

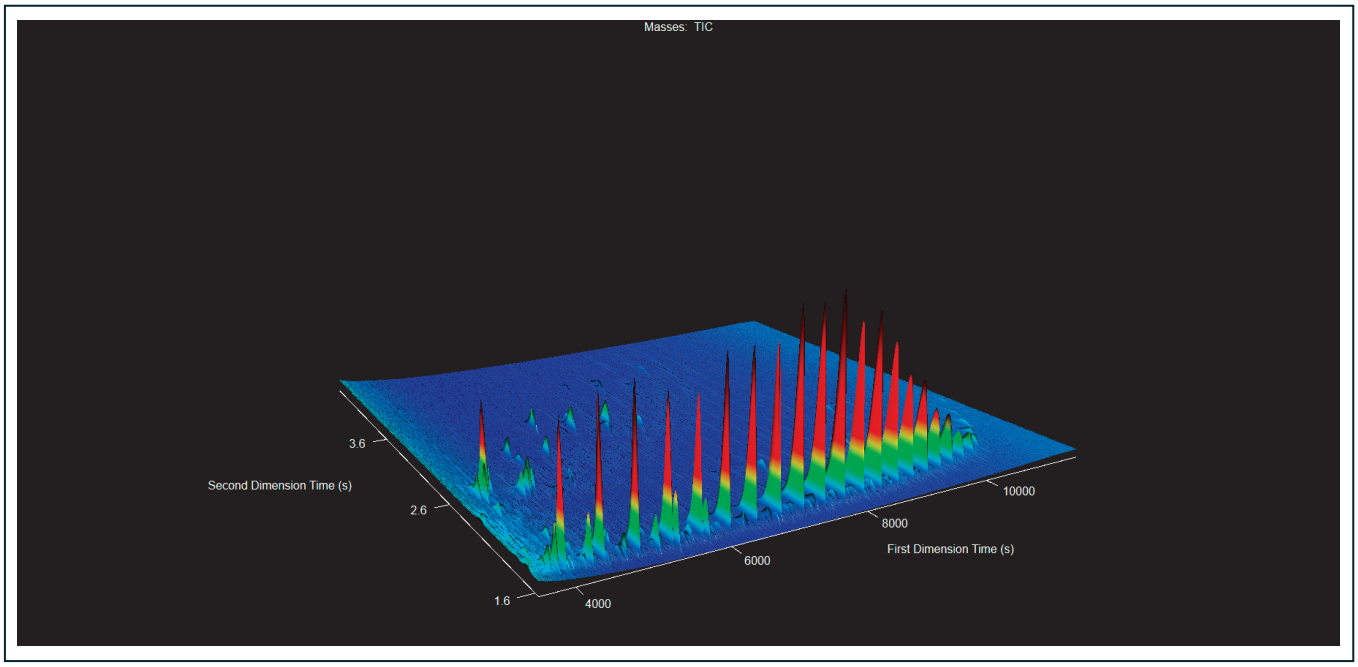


***Figure 2: GCxGC Surface plot of intermediate fuel oil from the oil tanker* Kirby. *The X and Y axes correspond to the first and second dimension separation times, respectively, with the Z-axis representing relative signal intensity for the TIC.***

This high level of sample complexity leads to situations where coelutions among the biomarkers and other relevant species would be abundant in one-dimensional GC, as can be observed in the linear reconstruction of the chromatogram below the full two-dimensional TIC chromatogram (contour plot) in Figure 3. With GCxGC, it is clear that the additional chromatographic space in the second dimension allows separation of compounds that would otherwise coelute with only a single dimension of separation, as any peaks that occupy the same vertical space in the contour plot would have eluted at the same time in a standard one-dimensional GC run.

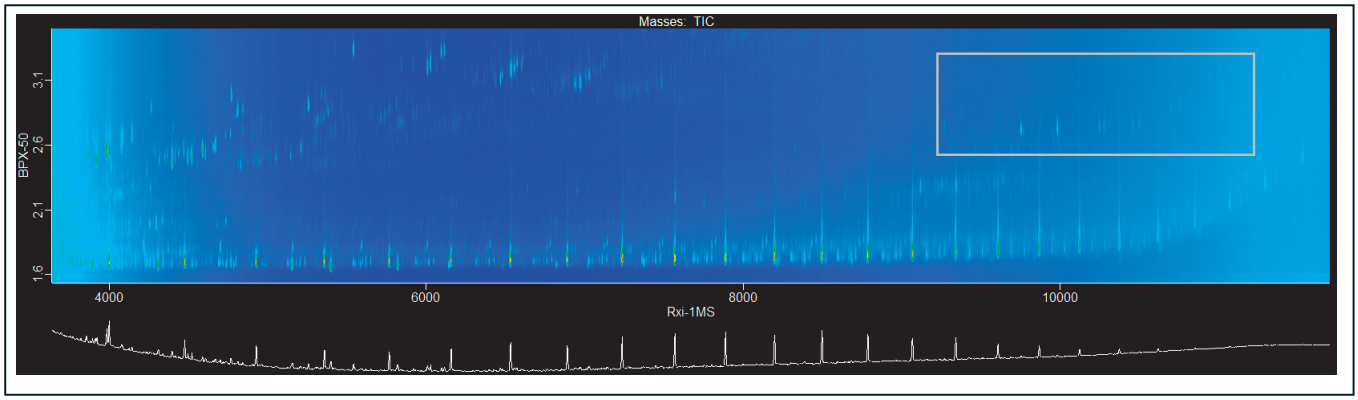


***Figure 3: GCxGC Contour plot and reconstructed linear chromatogram of intermediate fuel oil from the oil tanker* Kirby. *The elution region for hopanes and steranes is indicated with a gray box, which shows that these higher RDBE structures elute higher on the contour plot, away from lower RDBE compounds such as the linear alkanes, which elute in a band near the bottom of the contour plot.***

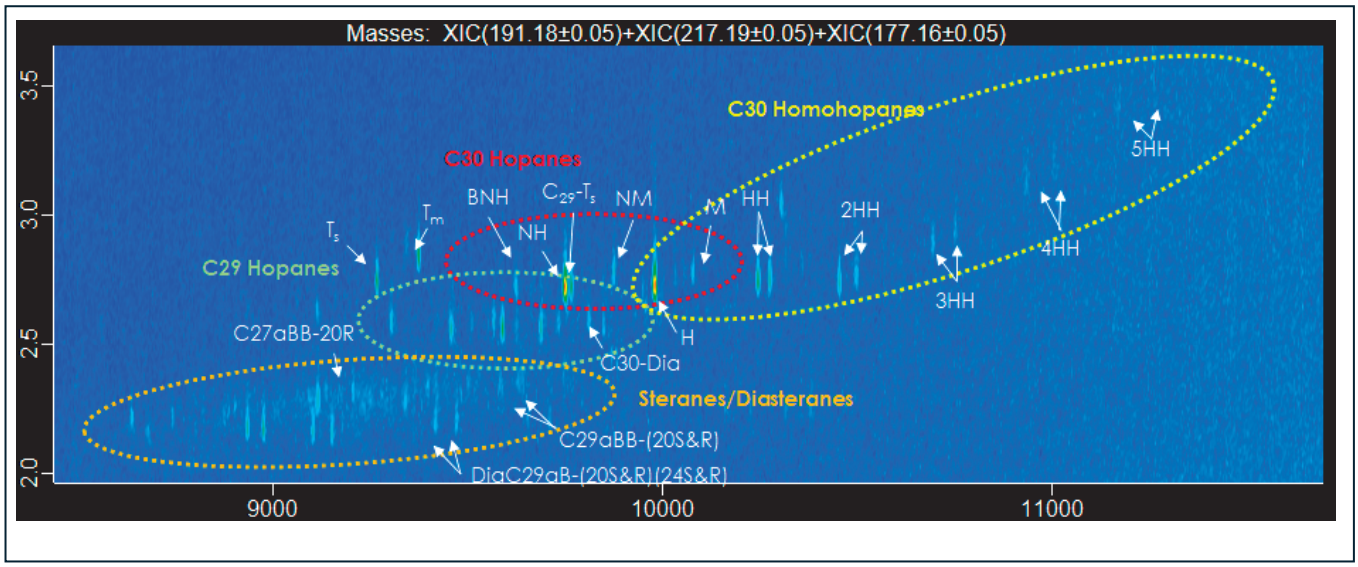


***Figure 4: Zoomed-in biomarker region on contour plot of intermediate fuel oil sample, with the summed signal of masses 191.18, 217.19, and 177.16 displayed. Hopane and sterane biomarkers of interest are labelled.***

Figure 4 shows the zoomed-in section where classic petroleum biomarkers elute with clear chromatographic resolution in GCxGC space, with the characteristic fragment masses for common hopanes and steranes extracted. Several important biomarkers are noted, with Table 2 providing a key to the compound names.

**Table 2: List of Hopanes and Steranes Labelled in Contour Plots**

| Abbreviation | Compound Name |
|---|---|
| $T_s$ | 18α(H)-22,29,30-trinorneohopane |
| $T_m$ | 17α(H)-22,29,30-trinorhopane |
| NH | 17α(H),21β(H)-30-norhopane |
| $C_{29}$-$T_s$ | 18α(H),21β(H)-30-norneohopane |
| C30-Dia | 17β(H),21β(H)-diahopane |
| NM | 17β(H),21α(H)-30-norhopane |
| H | 17α(H),21β(H)-30-hopane |
| M | 17β(H),21α(H)-30-hopane |
| HH | 17α(H),21β(H)-22-homohopane |
| 2HH | 17α(H),21β(H)-22-bishomohopane |
| 3HH | 17α(H),21β(H)-22-trishomohopane |
| 4HH | 17α(H),21β(H)-22-tetrakishomohopane |
| 5HH | 17α(H),21β(H)-22-pentakishomohopane |
| C27aBB-20R | 5α(H),14β(H),17β(H)-20R-cholestane |
| C28aaa-20R | 24-methyl-5α(H),14α(H),17α(H)-20R-cholestane |
| C29aaa-20S | 24-ethyl-5α(H),14α(H),17α(H)-20S-cholestane |
| C29aaa-20R | 24-ethyl-5α(H),14α(H),17α(H)-20R-cholestane |
| C29aBB-(20S&R) | 24-ethyl-5α(H),14β(H),17β(H)-20S&R-cholestane |
| DiaC29aB-(20S&R)(24S&R) | 24S&R-ethyl-13α(H),17β(H)-20S&R-diacholestane |

An example of one WHOI library matched peak is shown in Figure 5, with a high spectral similarity score of 858/1000.

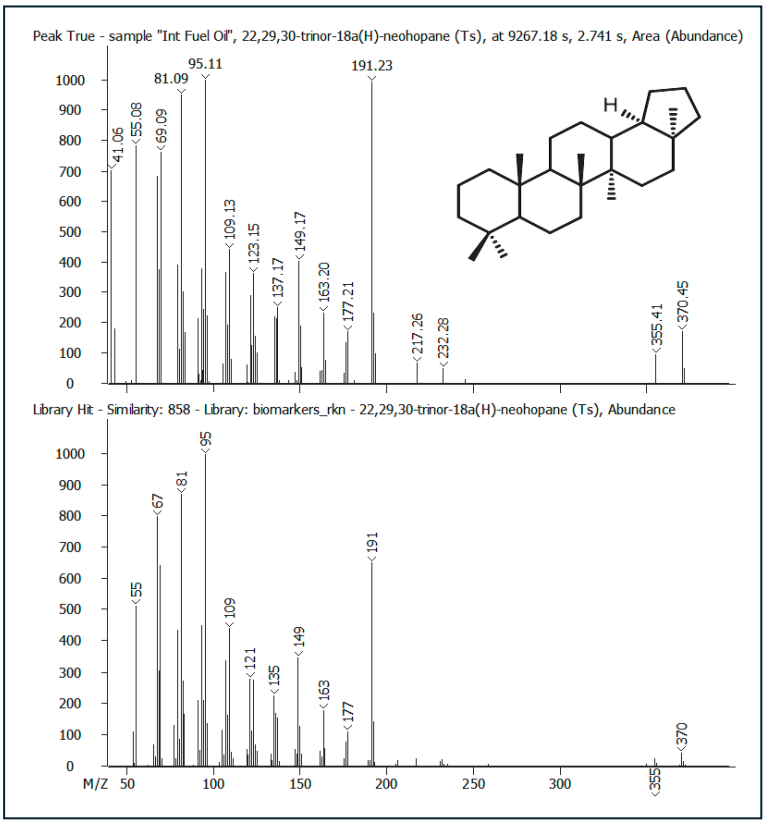


***Figure 5: Comparison of Peak True and User-generated Library Spectra for Ts.***

While quantitative work has previously been done on these samples using GCxGC-FID, some of which can be found in Chapter 5 of *Oil Spill Environmental Forensics: Fingerprinting and Source Identification (2007),* Table 3 shows the ratios of important biomarkers in various crude oil samples from different regions of the world, using information from quantitative masses characteristic to each set of biomarkers. One commonly used ratio for determination of thermal maturity is (Ts/Ts+Tm) because of the ubiquitous presence of these hopane biomarkers in oil samples. While Ts and Tm are commonly present in fairly high concentrations, M is a biomarker that is usually seen in relatively low concentrations, due to its lower stability with increased thermal maturity. Another hopane biomarker, BNH, reflects the possibility of anoxic depositional environments when it is present in high concentrations. In addition to the hopane biomarker ratios, differences in the pristane/phytane ratio are also used as an indicator of depositional environment based on high or low oxidation levels of starting material.

**Table 3: Biomarker Ratios for Oil Samples**

| Sample | NM/M | Pris/Phy | C29-Ts/H | Ts/(Ts+Tm) | BNH/NH | Region |
|---|---|---|---|---|---|---|
| Exxon Valdez | 0.429 | 1.711 | 0.289 | 0.425 | 0.039 | Northern America |
| Kalamazoo | 0.599 | 1.765 | 0.105 | 0.274 | 0.216 | Northern America |
| Jackpot Seep | 0.312 | 0.757 | 0.073 | 0.200 | 2.095 | California Coast |
| Refugio 901 | 0.381 | 0.863 | 0.036 | 0.196 | 2.288 | California Coast |
| Kuwait HS | 0.926 | 0.816 | 0.107 | 0.237 | 0.006 | Middle East |
| UAE Zakum HS | 0.869 | 0.887 | 0.249 | 0.550 | 0.035 | Middle East |
| Iranian Heavy HS | 0.447 | 1.187 | 0.130 | 0.352 | 0.017 | Middle East |
| Permian Basin | 0.279 | 1.478 | 0.099 | 0.414 | 0.020 | Gulf of Mexico |
| Ixtoc I | 0.494 | 1.077 | 0.146 | 0.397 | 0.037 | Gulf of Mexico |
| Platform Marlin | 0.340 | 2.115 | 0.209 | 0.562 | 0.083 | Gulf of Mexico |

Visual comparisons of the biomarker ratios from different regions of the world can be seen in the ratio plots in Figure 6. Samples from the similar geographic regions exhibit similar ratio values, as expected.

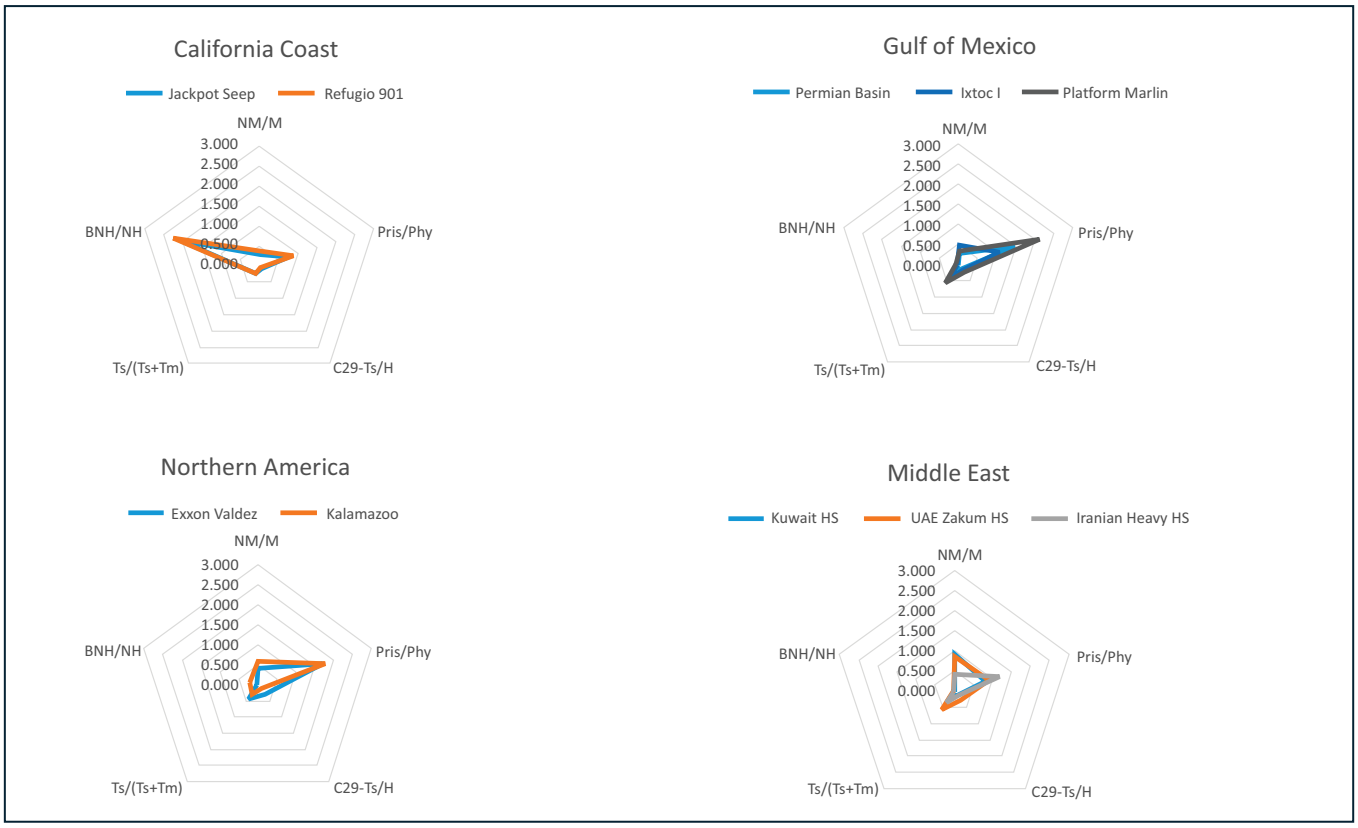


***Figure 6: Ratio plots generated from the biomarker ratios shown in Table 3 are shown above, with samples grouped by geographic region.***

## *Conclusion*

Analysis of biomarkers in oil using a *Pegasus* BT 4D equipped with the robust, and easy-to-use LECO *FLUX* flow modulator allowed for acquisition of high-quality chromatograms, confirming the identity of various conformations of hopanes and steranes from multiple complex samples in one method and enabling analysis that distinguished sources of petroleum products from around the world.


## *Acknowledgements*

The LECO Applications team would like to thank the Woods Hole Oceanographic Institute (WHOI) for providing samples and expert guidance on petroleum forensics. For more information on how GCxGC helps advance the mission of WHOI, visit https://www2.whoi.edu/site/gcxgcfacility/.




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