

## GUIDANCE FOR GC/MS ANALYSIS IN SUPPORT OF OIL SPILL FORENSICS

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### Introduction:

The forensic chemistry of crude oils addresses questions regarding the source(s), age, transformations, mixtures, transport, and fate of (predominantly) hydrocarbon species that are diagnostic for releases of crude oil to the environment. A comprehensive guidance document for analytical chemistry in support of these studies was not available in literature. This need prompted a collaborative effort, conducted by laboratories and researchers experienced in the practice of hydrocarbon forensics, that resulted in the development of a method to support a single analysis by gas chromatography and mass spectrometry (GC/MS); this method provides many types of information about potential source components to a hydrocarbon mix. The rationale and development of the guidance are provided herein.

### Materials and Methods:

Example Applications of Forensic Data – Sources of hydrocarbon mixtures can be distinguished by their molecular weight range, relative abundances of individual components, and chemical classes. Consider the type of profiling offered by Iqbal, *et al.*<sup>1</sup> In this comparison, normalized histograms of alkyl PAHs, alkylated dibenzothiophenes, and hopane/stearane ratios are superimposed to provide a mixing model that would give the “best fit” to the data. See Figure 1.

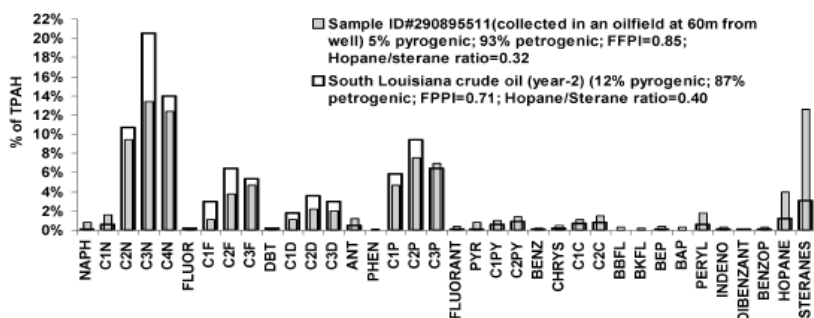


Figure 1: Iqbal, *et al.* Superimposition of PAH, Alkyl PAH, and Hopane/Sterane Ratios.

More abundance of the C2- and C3- naphthalene compounds for petrogenic samples can be seen by comparing the magnitudes of the concentrations in crude oil and diesel fuel to samples contaminated by Manufactured Gas Plant (MGP) residues. Based on data provided by Hawthorne, *et al.*<sup>2</sup>, the weight percent of example indicator compounds was calculated as a percentage of the total. The weight percents of these compounds are shown on Table 1.

Table 1. Scaled PAH Concentrations (Weight Percent)					
Analyte	Petrogenic		Pyrogenic		
	Crude Oil	Diesel Fuel	MGP (High)	MGP (Medium)	MGP (Low)
Naphthalene	1.4%	1.4%	12.3%	6.9%	2.5%
C2-Naphthalene	39.6%	35.4%	12.2%	8.5%	5.8%
C3-Naphthalene	40.9%	51.0%	5.6%	3.9%	4.8%

Obvious distinctions can be made between pyrogenic and petrogenic sources on the basis of C2 and C3 naphthalenes because the level of alkylation is much lower for pyrogenic PAHs. This distinction also holds for other homologs.

Weathering can reduce the relative abundance of alkylated PAHs, resulting in an increase in the relative abundance with each successive increment of alkylation. In the Proceedings of the 2008 International Oil Spill Conference, Wang, *et al.*<sup>3</sup> reported GC/MS data comparing profiles from petrogenic to pyrogenic-petrogenic mixtures. Differences in the proportion of parent to alkylated homologs are shown in Figures 2 and 3, below.

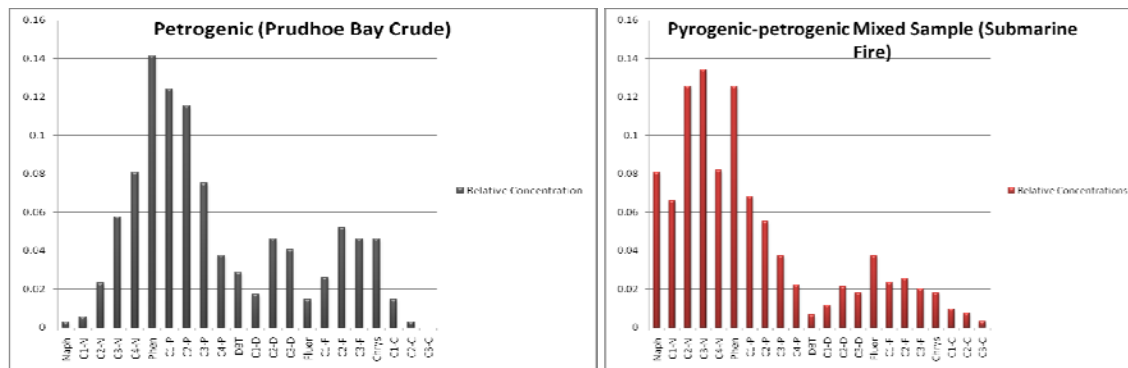


Figure 2

Figure 3

#### Weathering Indices

Weathering can be determined by the proportions of the normal alkanes to the isoprenoids and the unresolved chromatographic mixture. Figure 4 shows the chromatographic characteristics of these classes of compounds. The figure is from Daling, *et al.*<sup>5</sup>

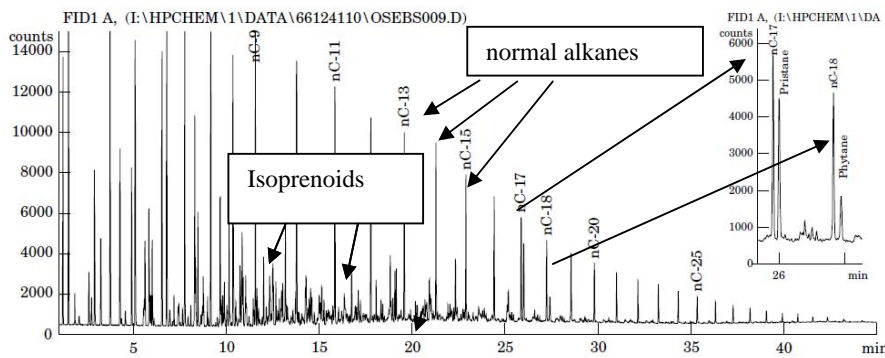


Figure 4: Chromatographic features of normal alkanes and isoprenoids under GC/MS analysis.

The pristane/nC17 and phytane/nC18 ratios provide a useful weathering index.

Geochemical Biomarkers for oil are essentially a variety of biogenic compounds (originally structural hopanoids from ancient eukaryotes and prokaryotes and sterol molecules primarily from eukaryotes) that have been transformed to their hydrocarbon analogues through diagenesis. Steranes biodegrade much more slowly than the alkanes and alkyl PAHs; the hopanes essentially do not biodegrade. They provide a way to distinguish among weathered oils that may otherwise be confused.

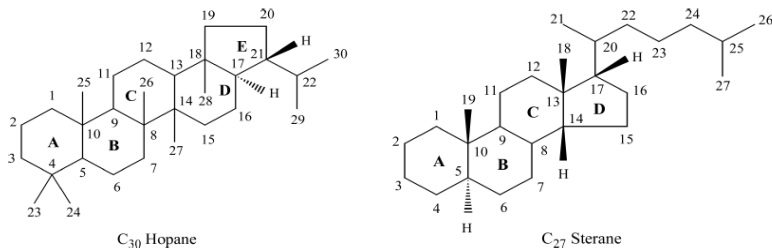


Figure 5. General structures of Hopanes and Steranes (Wang, Stout, *et al.*)<sup>6</sup>

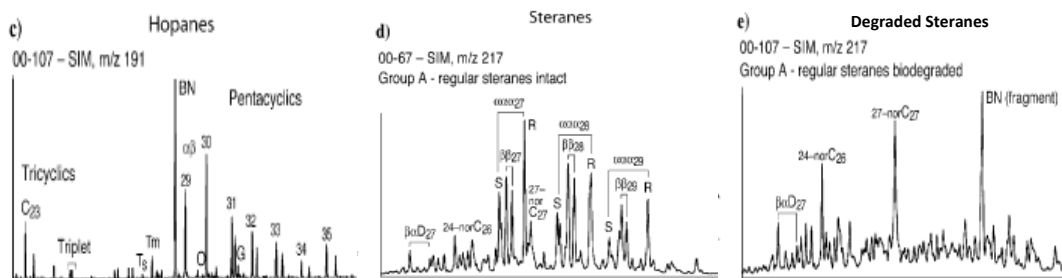


Figure 6. GC/MS profiles showing hopanes and steranes (intact and biodegraded), by Hostettler, *et al.*<sup>7</sup>

Triaromatic steroid hydrocarbons are observed as components in fossil fuels. They have been observed in natural bitumens and estuarine sediments. In some cases, their relative concentrations can increase along with sediment depth. (Pereira, *et al.*, 1999).<sup>8</sup> It is, therefore, reasonable to include this class of biomarkers in the forensics suite for oil forensics.

Considering each of these applications, a generalized method guidance document has been developed based on existing guidance in the literature, advice from laboratories practicing various aspects of the method, and personal experience in the analysis of PAHs, alkyl PAHs, and hydrocarbons in general.

#### Objective

Create a guidance document that would enable laboratories to produce useful, consistent analysis of the analytes listed on Table 2.

Classification	Number of Analytes
Normal Alkanes	32
Isoprenoids	5
Polyaromatic Hydrocarbons (PAHs)	18
Alkylated PAHs	18
Dibenzothiophene	1
Alkylated dibenzothiophenes	4
Hopanes	23
Steranes & Tricyclic Terpanes	40
Triaromatic Steroids	7
Total	148

#### Overview of Analytical Approach

- Evaluate the sample for presence of hydrocarbons using GC-FID or UV Absorption.
- If hydrocarbons are present, perform a fingerprint analysis of GC-FID data to determine if profile is a candidate (evaluating UCM, presence of isoprenes, PAHs).
- GC/MS analysis is performed. A visual inspection of key alkyl-PAH and biomarker profiles enables the investigator to select samples for quantitative and semi-quantitative workup (identification, integration, and calculation of the concentrations and relative signals of homologs and selected biomarkers).
- These values are then evaluated by the use of diagnostic ratios, diagnostic double ratios, and ANOVA techniques to provide information on relative contributions of multiple sources to a mixed sample.

#### Key steps conducted to develop the guidance

1. Review of monograph chapters in Wang, *et al.*<sup>6</sup>, Morrison *et al.*<sup>9</sup>, and Murphy, *et al.*<sup>10</sup>
2. Discussions with senior chemists at Battelle Duxbury, TestAmerica Burlington, TDI Brooks, TestAmerica Knoxville, Pace Analytical New Orleans, and Exponent.
3. Interlaboratory testing, evaluation, and feedback.

#### Key Quality Assurance Parameters

- Normal alkane marker standard (C<sub>9</sub>-C<sub>40</sub>) for GC/FID (and GC/MS if used for alkanes).
- High GC resolution of isoprenes from normal alkanes and others including alkyl PAHs (GC/FID and GC/MS).
- Demonstrated training to achieve consistent manual integrations of alkyl PAH patterns to avoid baseline noise. Daily analysis of reference oil containing all constituents of concern.
- Multipoint calibration and daily verification of parent PAHs, reference hopanes, and steranes (GC/MS).

#### Results and Discussion:

- Data for alkanes and isoprenoids can be obtained on the same run as the PAHs and alkylated PAHs - using either SIM or full scan analysis.
- SIM remains the superior technique for the trace components, including C<sub>3</sub> and C<sub>4</sub> PAH homologs and some biomarkers.
- The values obtained for biomarkers and alkylated PAHs are generally semi-quantitative and are appropriate for use in forensic applications, but not for determining absolute sample concentrations of these classes.
- A high degree of comparability for all but a few of the 150 analytes can be achieved using the guidance document to align laboratory activities.
- A significant degree of experience and training is needed for the analyst performing the integrations of chromatographic data.

#### Acknowledgements:

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