Petroleum Geochemistry Research Laboratory Method for Qualitative Biomarker Analysis of Crude Oil and Rock Extracts by Gas Chromatography-Single Quadrupole Mass Spectrometry

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1. Method Summary

This method identifies biomarkers in sedimentary organic matter, including rock extracts and petroleum, to provide insight into thermal maturity, depositional environment, reservoir characterization, oil-to-oil and oil-to-rock correlation, and source rock characterization (Peters and others, 2005). The biomarkers are chromatographically separated and detected using gas chromatography (GC) interfaced with a single quadrupole mass spectrometer (MS), which is also known as a mass selective detector (MSD). The MSD collects mass spectra using full scan and selected ion monitoring (SIM) modes in parallel. The instrument parameters described herein are based on previously published GC-MS biomarker methods (for example, Wang and others, 2006; Hays and others, 2012; French and others, 2019; American Society for Testing and Materials [ASTM] Method D5739, 2020). Compounds are identified as peaks in the total ion chromatogram (TIC) from GC-MS full scan analysis and in extracted ion chromatograms (EIC) from GC-MS SIM analysis. The results of this method are qualitative, and the method does not consider differences in analyte response factors. Parameters are calculated from the peak areas and/or heights of specific compounds to determine their relative abundance in a sample. This biomarker screening tool is intended to provide additional context to a sample and may inform subsequent in-depth analysis with complimentary methods, such as GC–triple quadrupole mass spectrometry or stable isotope analysis.

2. Scope and Application

This method details the identification of biomarkers from saturated and aromatic hydrocarbon fractions that are isolated via silica-alumina gel column chromatography of whole oil, solvent extractable organic matter (EOM), or the deasphaltened maltene fractions of either whole oil or EOM. GC-MS analysis in full scan mode provides a TIC while parallel GC-MS analysis in SIM mode provides EICs for ions of interest, the latter of which is used to identify specific biomarkers. In analyses of saturated fractions, tricyclic, tetracyclic, and pentacyclic terpanes, which include hopanes, are analyzed in the EIC mass-to-charge ratio (*m/z)* of 191, and steranes are analyzed in the EIC *m/z* of 217. Similarly, phenanthrene, dibenzothiophene, methylphenanthrenes, and triaromatic steroids are analyzed in aromatic fractions within the EICs *m/z* 178, 184, 192, and 231, respectively. These compound classes were selected as they are commonly used to distinguish and characterize petroleum systems (Peters and others, 2005; Peters, 2017; Luo and others, 2019 and references therein).

3. Interferences

3.1. The saturated and aromatic fractions are analyzed separately in this method to minimize mass fragment interferences and carry-over issues. The use of SIM mode and EICs reduces co-elution and interference issues.

3.2. Rubber lined septum caps should not be used as their exposure to sample solvent may leach heavy hydrocarbons that are identified in this analysis. Recommended caps have PTFE/red silicone septa (Agilent Part No 5185-5820 or equivalent).

3.3. The use of plastic containers should be avoided as they can contaminate the sample with hydrocarbons.

3.4. Carry-over issues may occur if large compounds (resins or asphaltenes for example) were not removed during whole oil separation or if highly concentrated samples were previously injected. The solvent in the autosampler wash vials is replaced prior to every batch of samples analyzed to maintain a clean syringe, and the wash vials are cleaned and combusted on an as needed basis. A solvent blank is run after every 10 analyses to determine if carryover from one sample to the next is present. If biomarker peaks (carryover) are observed in the blanks, they can usually be eliminated by cleaning or replacing the injection needle, front inlet septum, liner, o-ring and/or gold seal, baking the GC column for 30 minutes at the isothermal temperature limit specified by the manufacturer, or cutting 1–2 feet off the front of the GC column. Samples potentially affected by carryover should be reanalyzed after carryover is eliminated.

3.5. Samples should be free of water to avoid damage to the stationary phase of the GC column.

3.6. Hydrocarbon contamination from the carrier gas or sample injection is to be avoided, which may be accomplished by using clean glassware, ultra-high purity (UHP) gases, and hydrocarbon traps in the gas lines between the tank and instrument. Glass autosampler vials and inserts are always combusted (425 ± 25°C) overnight before use. Previously used glass autosampler vials are cleaned via soaking with water and Alconox and rinsing with deionized water prior to combustion. Glass GC vial inserts are discarded after a single use.

3.7. The mass spectrometer operates under vacuum ($\leq 1x10^{-5}$ Torr) and exposure to air can damage the instrument, particularly when it is operating at high temperatures. Oxygen can damage the column and shorten the life of the filament, while nitrogen leads to ineffective compound ionization in the MSD source. Water and oxygen traps are installed between the carrier gas tank and the GC to remove oxygen and water before they enter the instrument. The abundance of nitrogen, water, and oxygen are monitored in the tune and air/water reports. If one or more of these components are high, either more time is required to pump down the mass spectrometer or the system has a leak. Sources of the leaks are checked and resolved through instrument maintenance, which may include replacing or reseating the o-rings around the analyzer, tightening the column nuts, or cutting or replacing the GC column.

3.8. Petroleum and EOM samples, including saturated and aromatic fractions, are partly composed of an unresolved complex mixture (UCM), which appears in the gas chromatogram to various degrees depending on the sample as an elevated baseline or hump(s) in the baseline.

4. Safety Precautions

This method does not attempt to address health and safety concerns. Adherence to appropriate health, safety, and regulatory practices are the responsibility of the end user.

5. Sample Handling, Preservation, Storage and Holding Times

Samples are received in a variety of containers, though it is important that containers are intact to avoid sample contamination. Glass containers are preferred to store samples. Preservation, storage of samples, and holding times are not a concern for this method as samples are typically geologically stable.

6. Trademark disclaimer

The use of trade, product, or firm names in this method is for descriptive purposes only and does not imply endorsement by the U.S. Government.

7. Apparatus and Reagents

7.1. Agilent 8890 GC or equivalent with an Agilent 5977B MSD or equivalent single quadrupole mass spectrometer
7.2. Agilent 7650 autosampler or equivalent automatic liquid sampler
7.3. Helium gas, hydrogen gas, and nitrogen gas; ultra-high purity (UHP)
7.4. Organic solvents (all high purity high pressure liquid chromatography (HPLC) grade, optima or equivalent) for sample preparation, solvent blanks, and syringe wash for example *iso*-octane, benzene, *n*-hexane, or chloroform.
7.5. Solvent evaporation system such as a nitrogen evaporator, Organomation N-evap, or equivalent
7.6. 2–20 µL BrandTech Transferpette pipette (or equivalent) used for non-quantitative transfer purposes only
7.7. 20–200 µL BrandTech Transferpette pipette (or equivalent) used for non-quantitative transfer purposes only
7.8. Explosion proof refrigerator/freezer
7.9. Clean and combusted glass GC vials (Agilent Part No 5182-0715 or equivalent)
7.10. GC caps that are compatible with GC vials (Agilent Part No 5185-5820 or equivalent). Screw caps are preferred over crimp caps to reduce sample evaporation.
7.11. Optional new combusted glass GC vial inserts for small-volume samples (≤500 µL; Agilent Part No 5181-3377 or equivalent)
7.12. Clean and combusted glass solvent rinse and waste vials and caps for liquid autosampler (Agilent Part No 5182-0551 or equivalent)
7.13. Computer hardware and software: OpenLab CDS Software Version 2.5, Windows 10 (or equivalent), and an HP desktop computer (or equivalent)

8. Procedure

This method details the analysis of saturated and aromatic hydrocarbon fractions to separate and identify biomarker compounds of interest in reference oils as well as unknown samples. The fractions are obtained through a series of steps (Lowry, 2020) that include asphaltene removal and liquid column chromatography. Specifically, the saturated and aromatic hydrocarbons are isolated from the deasphaltened fraction known as the maltene fraction by chromatographic column separation using a

mixed silica-alumina solid phase and successive elution with *iso*-octane and benzene, respectively.

All fractions are diluted or concentrated, if necessary, to bring the compounds of interest within the detection range of the MSD. Ideally, the tallest peaks in the TIC should have peak heights on the order of 10^6 to 10^7 counts. Samples are diluted with the solvent appropriate to the sample type (such as *iso*-octane for saturated fractions, benzene for aromatic fractions) when the peak height of the tallest peak exceeds a response greater than ~10^8 counts in the TIC or if peak shape shows signs of overloading, such as fronting, asymmetry, and/or broadening. If the height of the tallest peak in the TIC is less than ~10^6 counts, the sample is concentrated to reduce the sample volume. Concentration or dilution is performed with the consideration that samples are analyzed within the calibration range of the instrument to ensure that the signal is directly proportional to analyte concentration. For example, dilution of a sample by a factor of two will reduce the signal by half.

All saturated hydrocarbon fractions of reference oils and unknowns are analyzed sequentially in one group and all aromatic fractions of reference oils and unknowns are analyzed as a separate group. The saturated or aromatic fractions of the reference oils are analyzed at the beginning and end of each group of saturated or aromatic fraction unknowns, respectively, and should contain most or all compounds of interest. In cases where a reference oil does not contain all targeted analytes, multiple reference oils may be analyzed. Examples of such reference oils include in-house oils such as Duda and San Emidio Monterey and external oils such as Norwegian Petroleum Directorate North Sea Oil (NSO-1 herein) and NIST 1582. A blank is analyzed twice at the start of each run, after the first group of reference oils, minimally after analysis of every 10 samples, and at the end of the sequence. Therefore, a generalized sequence may be Blank, Blank, Reference Oils, Blank, 10 samples, Blank, 10 samples, Reference Oils, Blank.

8.1. Instrument Data Acquisition Parameters

All samples are analyzed by an Agilent 8890 GC coupled to an Agilent 5977B MSD using Agilent OpenLab CDS v. 2.5 Acquisition Software. An equivalent GC-MS capable of operating under the parameters listed in table 1 may be used.

Table 1. Example instrument parameters utilized to analyze saturated and aromatic fractions in parallel full scan and selected ion monitoring (SIM) modes. MSD=mass selective detector.

Injector	Injection Volume	1 μL
	# Sample Pumps	3
	Viscosity Delay	2
	Injection Dispense Speed	6000 μL/min
	Sample Washes	0
	Solvent A	Chloroform
	Solvent B	Solvent matching the solvent in sample vial
	Solvent Wash Volumes	8 μL
	# Solvent A pre-washes	3
	# Solvent B pre-washes	3
	# Solvent A post-washes	3
	# Solvent B post-washes	3
Inlet	Injection Mode	Splitless
	Inlet liner	Ultra-inert splitless single taper with glass wool
	Temperature	300°C
	Pressure	20.8 psi
	Purge time	1 min
	Purge flow	100 mL/min
	Septum Purge Flow Mode	Standard
	Septum Purge Flow	3 mL/min
	Gas saver	On
	Saver flow	25 mL/min
	Saver time	2 min
Oven	Equilibration Time	0.25 min
	See tables 2 and 3 for sample-specific oven temperature programs	
Column[†]	Type	100% dimethyl polysiloxane (DB-1ms or equivalent) or (5%-Phenyl)-methylpolysiloxane (DB-5ms or equivalent)
	Dimensions	60m × 0.25mm × 0.25μm
	Pressure	20.8 psi
	Flow	1.2 mL/min
	Average Velocity	28.4 cm/min
	Mode	Constant Flow
Detector (MSD)	Source Temperature	280°C
	Quadrupole Temperature	150°C
	Transfer Line Temperature	300°C
	Trace Ion Detection	On
	Gain Factor	1
	EM Saver	On
	Ionization Mode	Positive Electron Impact (EI)
	Ionization Energy	70 eV
	Tune method	Autotune (atune)
	Solvent delay	6.5 min
	Mode	Scan/SIM

	Scan Resolution	Low
	Scan Threshold	100 counts
	Full Scan Speed	1.562 Hz
	SIM Dwell Time	60 ms/ion

Gases	Carrier	Helium or Hydrogen‡
	Sleep Mode*	Nitrogen
	Jet Clean**	Hydrogen

†Noted parameters are initial column conditions, which may be adjusted as needed

‡All methods and results presented herein use helium as a carrier gas. The use of hydrogen as a carrier gas would require modification to the acquisition settings and results should be verified as comparable to those obtained using helium prior to their publication.

*Sleep mode loads at the end of a sequence.

**Jet Clean is used as needed to clean the source without venting the MSD

8.1.1. Saturated Fraction Analysis

The saturated fraction of at least one reference oil is analyzed at the beginning and end of each group of saturated fractions to assess instrument performance (see Section 11 for quality control metrics). More than one reference oil may need to be analyzed in cases where a reference oil does not contain all target compounds. In addition to the parameters listed in Section 8.1, the parameters noted in table 2 are used for analysis of the saturated fractions.

Table 2. Additional example instrument parameters specific to analysis of saturated fractions. MSD=mass selective detector.

Oven	Total Run Time	85.5 min	
	Rate (°C/min)	**Temperature (°C)**	**Hold Time (min)**
	NA	60	2
	20	150	0
	3	315	24

Detector (MSD)*	Full Scan Mass Range	50–570 amu
	SIM ions	191, 217 *m/z*
	Full Scan Cycle Time	355 ms/cycle

*Full scan and SIM (Selected Ion Monitoring) are run in parallel.

8.1.2. Aromatic Fraction Analysis

The aromatic fractions of at least one reference oil are analyzed at the beginning and end of each group of aromatic fractions to assess instrument performance (see Section 11 for quality control metrics). More than one reference oil may need to be analyzed in cases where a reference oil does not contain all target compounds. In addition to the parameters listed in Section 8.1, the example parameters noted in table 3 may be used for analysis of the aromatic fractions.

Table 3. Additional example instrument parameters specific to analysis of aromatic fractions. SIM=selected ion monitoring, MSD=mass selective detector.

Oven	Total Run Time	90.25 min	
	Rate (°C/min)	**Temperature (°C)**	**Hold Time (min)**
	NA	60	2
	20	160	0
	4	325	42
Detector (MSD)	Full Scan Mass Range	50–580 amu	
	SIM Ions	178, 184, 192, 231 *m/z*	
	Full Cycle Time	362 ms/cycle	
*Full scan and SIM are run in parallel.			

9. Data Processing

The chromatographic and mass spectral data obtained for the saturated and aromatic fractions are processed using Agilent OpenLab CDS v. 2.5 Data Analysis Software. The software performs data processing using a method file referred to herein as the Data Processing Method (DPM). There is a DPM for each respective sample type (saturated or aromatic fraction).

In the DPM, peak identifications and integration parameters are defined for a single analysis of a reference oil that is referred to as the Master Analysis. During data processing, the DPM compares all other analyses to the Master Analysis in order to output EICs, auto-integrate the chromatograms, identify peaks, and generate sample reports according to the sample-specific metrics detailed in Sections 9.1 and 9.2. The DPM uses absolute retention time windows to identify compound peaks, and it uses the ChemStation integrator or equivalent to determine the local baseline and auto-integrate the identified peaks. The analyst reviews peak and baseline selections and revises the autointegration as needed using manual integration. Manual integrations are automatically electronically recorded.

The software accommodates for temporal peak shifts between runs by calculating the difference in the retention time of the time-reference peak between the sample and Master Analysis chromatograms. This is accomplished by setting the expected retention times to never update within the DPM so that the retention times of identified peaks on any given chromatogram are compared to the expected retention times of identified peaks in the Master Analysis. Ideal time-reference peaks are abundant in all petroleum samples and have a retention time that does not interfere with peaks of interest. The software is set to identify the time-reference peak within a wide retention time window, such as 1.5 minutes, to accommodate for any significant retention time shifts. In this method, pristane is identified in the TIC and is used as the time-reference peak for saturated fraction analyses, while phenanthrene is identified in EIC m/z 178 and is used as the time-reference peak for aromatic fraction analyses.

Modifications to the data processing parameters may be required to ensure proper peak integration and identification for unknowns. Such modifications (if present) are recorded.

The final processed data for each analysis (table(s) of the peak areas and heights of identified biomarker compounds and calculated parameters defined in Section 10) are provided in Wycech and French (2023).

9.1 Saturated Fraction Data Processing

Table 4 lists examples of terpane and sterane biomarkers identified in saturated fraction EICs 191 and 217 (*m/z*). Table 5 notes examples of EIC-specific integration parameters, which have been optimized from reference oil analyses. Example chromatograms of the North Sea Oil (NSO-1) saturated fraction are illustrated in figures 1 and 2 which include the compound peaks noted in table 4 and peak integrations that resulted from the parameters noted in table 5.

Table 4. Example of common compound identifications and data processing parameters for saturated fractions. TIC=total ion chromatogram, EIC=extracted ion chromatogram.

Chromatogram	Compound
TIC	Pristane*
EIC *m/z* 191	C19 Tricyclic Terpane
	C20 Tricyclic Terpane
	C21 Tricyclic Terpane
	C22 Tricyclic Terpane
	C23 Tricyclic Terpane
	C24 Tricyclic Terpane
	C25 22S Tricyclic Terpane
	C25 22R Tricyclic Terpane
	C24 Tetracyclic Terpane
	C26 22S Tricyclic Terpane
	C26 22R Tricyclic Terpane
	C28 22S Tricyclic Terpane
	C28 22R Tricyclic Terpane
	C29 22S Tricyclic Terpane
	C29 22R Tricyclic Terpane
	C27 18a Trisnorneohopane
	C27 17a Trisnorhopane
	C30 22S Tricyclic Terpane
	C30 22R Tricyclic Terpane
	C28 Bisnorhopane
	C29 Norhopane
	C29 18a Neonorhopane
	C30 Diahopane
	C29 17B21a Normoretane
	C30 Oleanane
	C30 Hopane
	C30 17B21a Moretane
	C31 22S Hopane
	C31 22R Hopane
	C30 Gammacerane
	C32 22S Hopane
	C32 22R Hopane
	C33 22S Hopane
	C33 22R Hopane
	C34 22S Hopane
	C34 22R Hopane
	C35 22S Hopane
	C35 22R Hopane

Chromatogram	Compound
EIC *m/z* 217	Pregnane
	Homopregnane
	C27 20S Ba Diasterane
	C27 20R Ba Diasterane
	C27 20R aaa Sterane
	C28 20R aaa Sterane
	C29 20S aaa Sterane
	C29 20R aBB Sterane
	C29 20S aBB Sterane
	C29 20R aaa Sterane
	C30 20R aaa Sterane
*Time-reference peak for all other compounds identified in saturated fraction	

Table 5. Suggested initial integration settings for the saturated fraction data processing method. EIC=extracted ion chromatogram.

EIC (*m/z*)	Time (min)	Event	Value
191	0	Slope sensitivity	500
	0	Peak width	0.1
	0	Area reject	5000
	0	Height reject	1500
	0	Shoulders mode	Tangential
	0	Area% reject	0
	41.4	Area reject	11000
	45.0	Area reject	5000
	51.0	Shoulders mode*	Drop
	55.5	Shoulders mode*	Tangential
	62.0	Shoulders mode*	Drop
	64.0	Shoulders mode*	Tangential
217	0	Slope sensitivity	500
	0	Peak width	0.1
	0	Area reject	3000
	0	Height reject	500
	0	Shoulders mode	Tangential
	0	Area% reject	0
	30.0	Baseline hold	On
	37.4	Area reject	6500
	41.0	Area reject	3000
	43.0	Baseline hold	Off
	46.0	Shoulders mode	Drop
	51.0	Baseline hold	On
	55.3	Area reject	30000
	57.8	Area reject	3000
	57.8	Shoulders mode*	Tangential
	59.0	Baseline hold	Off

* The frequent changes to the shoulders mode provides for a more consistent and improved peak integration in the noted time intervals.

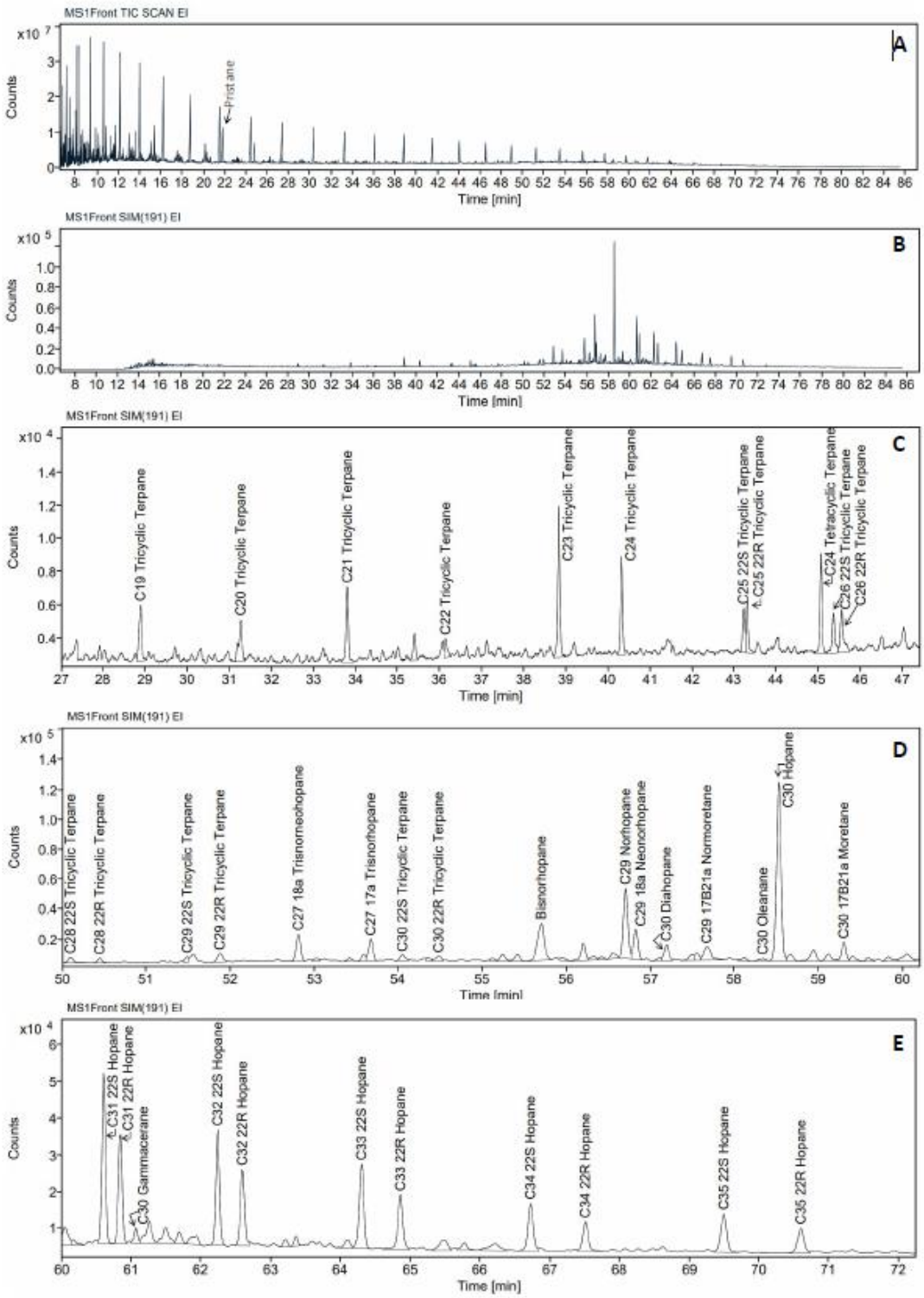

Figure 1. Example chromatograms of the North Sea Oil (NSO-1) saturated fraction. Note variable scales of axes x (time) and y (counts). **A.** Total ion chromatogram (TIC) obtained from full scan mode noting the time-reference peak (pristane), **B.** Extracted ion chromatogram (EIC) 191 (mass-to-charge) obtained from Selected Ion Monitoring (SIM) mode. **C–E.** Magnified regions of the chromatogram in B showing integrated peaks of interest (see table 4).

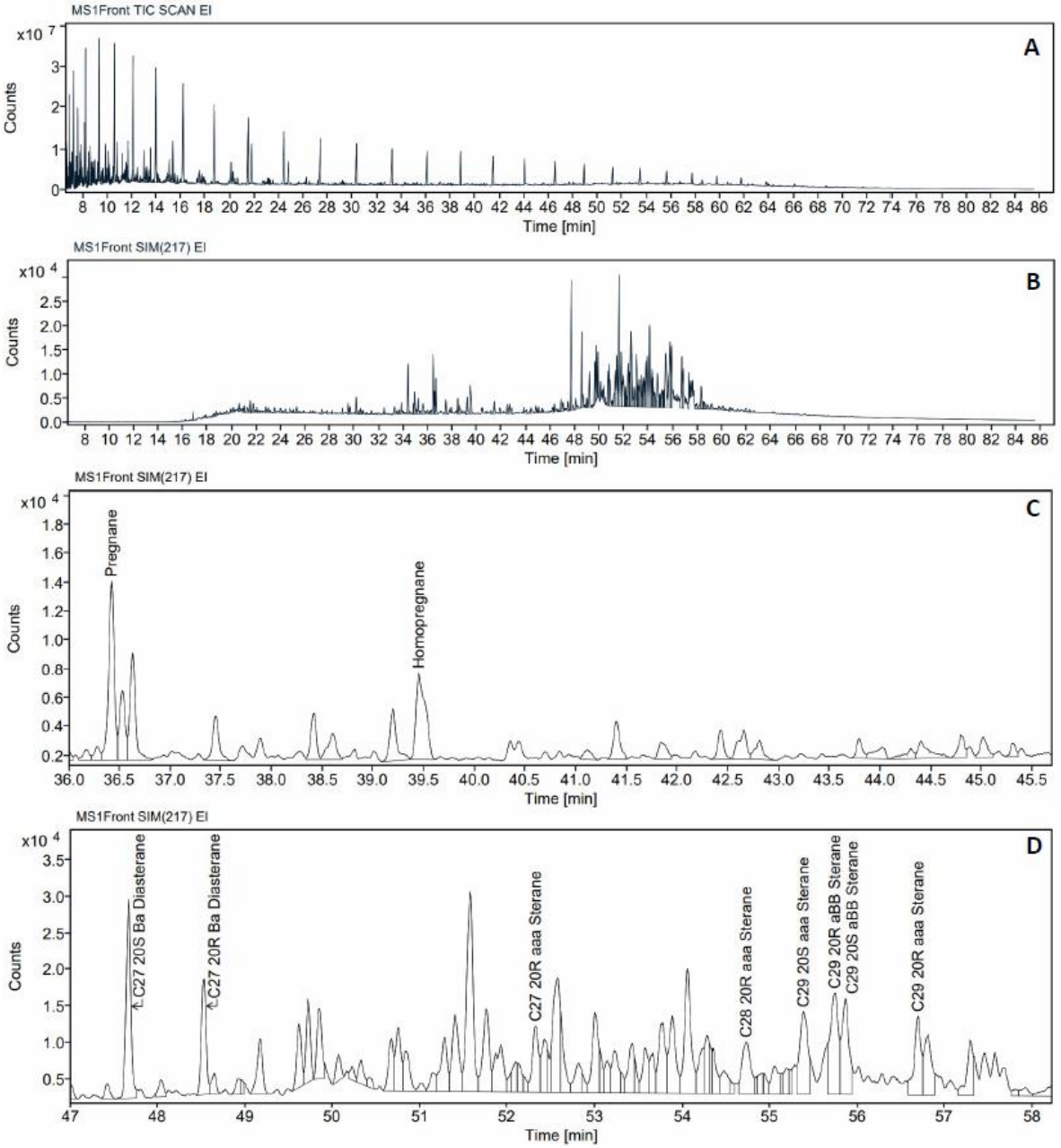

Figure 2. Example chromatograms of the North Sea Oil (NSO-1) saturated fraction. Note variable scales of axes x (time) and y (counts). **A.** Total ion chromatogram (TIC) obtained from full scan mode, **B.** Extracted ion chromatogram (EIC) 217 (mass-to-charge) obtained from Selected Ion Monitoring (SIM) mode. **C–D.** Magnified regions of the chromatogram in B showing integrated peaks of interest (see table 4).

9.2. Aromatic Fraction Data Processing

Table 6 lists examples of phenanthrene, dibenzothiophene, methylphenanthrenes, and triaromatic steroid biomarkers identified in EICs 178, 184, 192, and 231 (*m/z)*, respectively. Table 7 notes examples of EIC-specific integration parameters, which have been optimized from analyses of reference oil aromatic fractions. Run-to-run shifts in retention times are accounted for by the time reference peak of

phenanthrene in EIC *m/z* 178. Example chromatograms of the North Sea Oil (NSO-1) aromatic fraction are illustrated in figure 3 which includes the compound peaks noted in table 6 and peak integrations that resulted from the parameters noted in table 7.

Table 6. Example of common compound identifications and data processing parameters for aromatic fractions. EIC=extracted ion chromatogram.

EIC (*m/z*)	Compound
178	Phenanthrene*
184	Dibenzothiophene
192	3-methylphenanthrene
	2-methylphenanthrene
	9-methylphenanthrene
	1-methylphenanthrene
231	C20 Triaromatic Steroid
	C21 Triaromatic Steroid
	C26 20S Triaromatic Steroid
	C26 20R+C27 20S Triaromatic Steroid
	C28 20S Triaromatic Steroid
	C27 20R Triaromatic Steroid
	C28 20R Triaromatic Steroid
*Time reference peak for all other compounds identified in aromatic fraction	

Table 7. Suggested integration settings for the aromatic fraction data processing method.

Time (min)	Event	Value
0	Slope sensitivity	500
0	Peak width	0.1
0	Area reject	5000
0	Height reject	5000
0	Shoulders mode	Drop
0	Area% reject	0

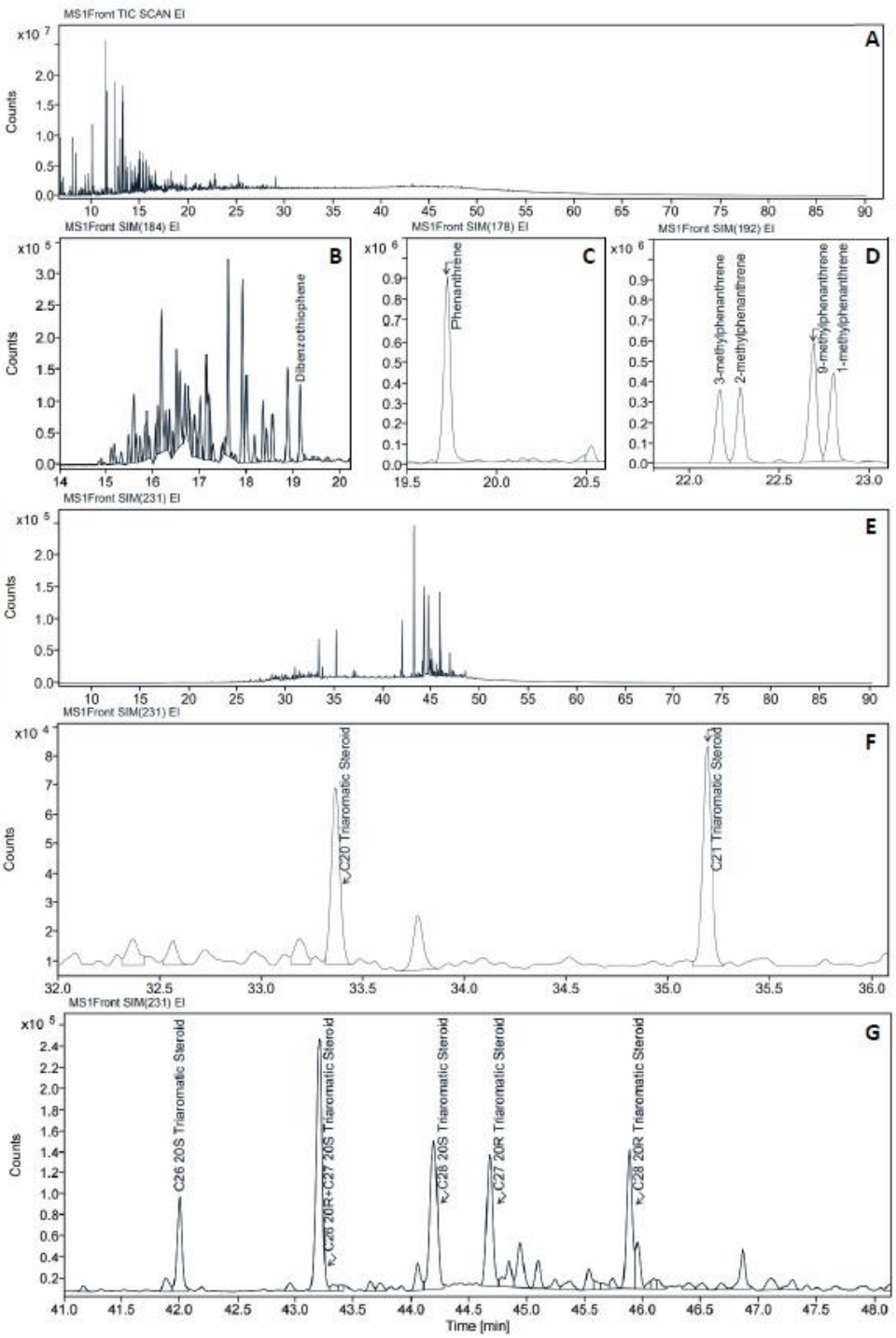

Figure 3. Example chromatograms of the North Sea Oil (NSO-1) aromatic fraction. Note variable scales of axes x (time) and y (counts). **A.** Total ion chromatogram (TIC) obtained from full scan mode. **B–G.** Extracted ion chromatograms (EICs) obtained from Selected Ion Monitoring (SIM) mode. **B.** EIC 184 (mass-to-charge) showing dibenzothiophene peak. **C.** EIC 178 (mass-to-charge) noting time-reference peak (phenanthrene). **D.** EIC 192 (mass-to-charge) showing methylphenanthrene peaks. **E.** EIC 231 (mass-to-charge). **F–G.** Magnified regions of the chromatogram in E showing triaromatic steroid peaks of interest (see table 6).

10. Data Reduction, Analysis and Calculations

Peak heights and peak areas are measured for the biomarkers of interest (see tables 4 and 6) and are used to calculate the parameters defined below. These parameters supplement geochemical data obtained from the same sample to provide additional information.

The example parameters shown below may be calculated using peak heights or peak areas.

$$BISHOP = \frac{C28\ Bisnorhopane}{C30\ Hopane}$$

$$C19C23 = \frac{C19\ Tricyclic\ Terpane}{C23\ Tricyclic\ Terpane}$$

$$C22C21 = \frac{C22\ Tricyclic\ Terpane}{C21\ Tricyclic\ Terpane}$$

$$C24C23 = \frac{C24\ Tricyclic\ Terpane}{C23\ Tricyclic\ Terpane}$$

$$C26C25 = \frac{C26\ 22R + 22S\ Tricyclic\ Terpanes}{C25\ 22R + 22S\ Tricyclic\ Terpanes}$$

$$C26TET = \frac{C26\ 22S + 22R\ Tricyclic\ Terpanes}{C24\ Tetracyclic\ Terpane}$$

$$C27STER = \frac{C27\ 20R\ aaa\ Sterane}{(C27\ 20R\ aaa + C28\ 20R\ aaa + C29\ 20R\ aaa\ Steranes)}$$

$$C28STER = \frac{C28\ 20R\ aaa\ Sterane}{(C27\ 20R\ aaa + C28\ 20R\ aaa + C29\ 20R\ aaa\ Steranes)}$$

$$C29BBAA = \frac{C29\ 20R\ aBB\ Sterane}{C29\ 20R\ aBB\ Sterane + C29\ 20R\ aaa\ Sterane}$$

$$C29SR = \frac{C29\ 20S\ aaa\ Sterane}{C29\ 20S\ aaa + C29\ 20R\ aaa\ Steranes}$$

$$C29STER = \frac{C29\ 20R\ aaa\ Sterane}{(C27\ 20R\ aaa + C28\ 20R\ aaa + C29\ 20R\ aaa\ Steranes)}$$

$$C31HSR = \frac{C31\ 22S\ Hopane}{C31\ 22S + 22R\ Hopanes}$$

$$C31RH = \frac{C31\ 22R\ Hopane}{C30\ Hopane}$$

$$C32HSR = \frac{C32\ 22S\ Hopane}{C32\ 22S + 22R\ Hopanes}$$

$$C35HOP = \frac{C35\ 22S + 22R\ Hopanes}{C31\ through\ C35\ 22S + 22R\ Hopanes}$$

$$C35C34 = \frac{C35\ 22S\ Hopane}{C34\ 22S\ Hopane}$$

$$DBTPhen = \frac{Dibenzothiophene}{Phenanthrene}$$

$$DIAREG = \frac{C27\ 20S\ Ba\ Diasterane}{C29\ 20R\ aaa\ Sterane}$$

$$F1 = \frac{2\text{-}MePhen + 3\text{-}MePhen}{2\text{-}MePhen + 3\text{-}MePhen + 1\text{-}MePhen + 9\text{-}MePhen}\ \dagger$$

$$F2 = \frac{2\text{-}MePhen}{2\text{-}MePhen + 3\text{-}MePhen + 1\text{-}MePhen + 9\text{-}MePhen}\ \dagger$$

$$GAC31R = \frac{C30\ Gammacerane}{C31\ 22R\ Hopane}$$

$$GAMHOP = \frac{C30\ Gammacerane}{C30\ Hopane}$$

$$MORHOP = \frac{C29\ 17B21a\ Normoretane + C30\ 17B21a\ Moretane}{C29\ Norhopane + C30\ Hopane}$$

$$MPI1 = \frac{1.5 \times (2\text{-}MePhen + 3\text{-}MePhen)}{(Phenanthrene + 1\text{-}MePhen + 9\text{-}MePhen)}\ \dagger$$

$$NEONOR = \frac{C29\ 18a\ Neonorhopane}{C29\ Norhopane}$$

$$NORHOP = \frac{C29\ Norhopane}{C30\ Hopane}$$

† MePhen = methylphenanthrene

$$OLHOP = \frac{C30\ Oleanane}{C30\ Hopane}$$

$$PREGC27 = \frac{Pregnane}{C27\ 20R\ aaa\ Sterane}$$

$$S1S6 = \frac{C27\ 20S\ Ba\ Diasterane}{C27\ 20R\ aaa\ Sterane}$$

$$TETC23 = \frac{C24\ Tetracycic\ Terpane}{C23\ Tricyclic\ Terpane}$$

$$TRIHOP = \frac{C23\ Tricyclic\ Terpane}{C30\ Hopane}$$

$$TSTM = \frac{C27\ 18a\ Trisnorneohopane}{C27\ 17a\ Trisnorhopane}$$

$$XH = \frac{C30\ Diahopane}{C30\ Hopane}$$

$$TRIOCR = \frac{C20 + C21\ Triaromatic\ Steroids}{\text{C20 + C21 + C26 20S + (C26 20R \& C27 20S) + C28 20R + C28 20S + C27 20R TAS}}\ ‡$$

$$TRIOCR1 = \frac{C20\ Triaromatic\ Steroid}{C20 + C28\ 20S + C28\ 20R\ Triaromatic\ Steroids}$$

$$TRIOCR2 = \frac{C20 + C21\ Triaromatic\ Steroids}{\text{(C26 20R\&C27 20S) + C26 20S + C27 20R + C28 20R + C28 20S Triaromatic Steroids}}$$

11. Method Performance

Saturated and aromatic fractions of reference oils were analyzed at least 30 times on separate days by a single analyst to determine the initial dynamic quality control acceptance criteria. The acceptance criteria constrain the reproducibility and repeatability of analyses and are initially defined as ± 3 standard deviations (SD) of the average for three parameters: C29SR, TSTM, and TRIOCR (see Section 10 for definitions).

If the reference oil parameters are not within the acceptance criteria during runs with unknown samples, the reason for the failure is addressed and the unknown samples are either reanalyzed or the data is qualified.

‡ TAS=Triaromatic steroid

11.1. Method Validation

This method was validated by analysis of the saturated and aromatic fractions of NSO-1 reference oil. NSO-1 was selected for method validation because it was originally developed by the Norwegian Petroleum Directorate for qualitative biomarker identifications and parameter calculations (Weiss and others, 2000), similar to the objectives outlined herein. The saturated fraction of NSO-1 was analyzed 43 times by one analyst (Jody Wycech) on 42 separate days spanning 9 months on a single instrument. The aromatic fraction of NSO-1 was analyzed 57 times by the same analyst on 56 separate days spanning 9 months on a single instrument.

Table 8 and figure 4 summarize the statistics on the parameters C29SR, TSTM, and TRIOCR measured by the PGRL and reported by the Norwegian Petroleum Directorate (NPD; Dahlgreen and others, 1998). The NPD parameters reflect a compilation of results from numerous laboratories of which 60% calculated the parameters from peak heights and 40% calculated the parameters from peak areas. For this reason, both peak areas and peak heights measured in the PGRL were separately used to calculate parameters for the purpose of comparison. The parameters calculated from peak areas or peak heights measured by the PGRL are nearly identical to one another. Regardless of the selected peak metric, the average values of these biomarker parameters measured by the PGRL are within 3 standard deviations of the average parameters reported by the Norwegian Petroleum Directorate and are therefore considered to agree with one another. These acceptance criteria are valid over a range of dilutions. Subsequent method validation experiments showed that NSO-1 diluted by a factor of 20 had biomarker parameters that were still within these accepted ranges.

Table 8. Comparison of acceptance criteria measured by the Petroleum Geochemistry Research Laboratory (PGRL) and the Norwegian Petroleum Directorate (NPD) for the C29SR, TSTM, and TRIOCR biomarker parameters. SD=standard deviation, n=number of analyses.

		C29SR	**TSTM**	**TRIOCR**
NPD	Comparable Reported Parameter Name	20S	Ts/Tm	Cracking
	Average	0.51	1.21	0.18
	SD	0.06	0.16	0.04
	n	24	23	20
	Permissible Range*	0.45–0.59	1.00–1.30	0.13–0.19
PGRL	**Calculated from Peak Areas**			
	Average	0.58	1.34	0.16
	3 SD	0.09	0.20	0.06
	n	43	43	57
	Acceptable Range**	0.49–0.67	1.14–1.54	0.10–0.22
	Calculated from Peak Heights			
	Average	0.51	1.34	0.19
	3 SD	0.05	0.20	0.07
	n	43	43	57
	Acceptable Range**	0.46–0.56	1.14–1.54	0.12–0.26
* Defined as the 75% Bayesian confidence interval				
** Defined as the average ± 3 SD				

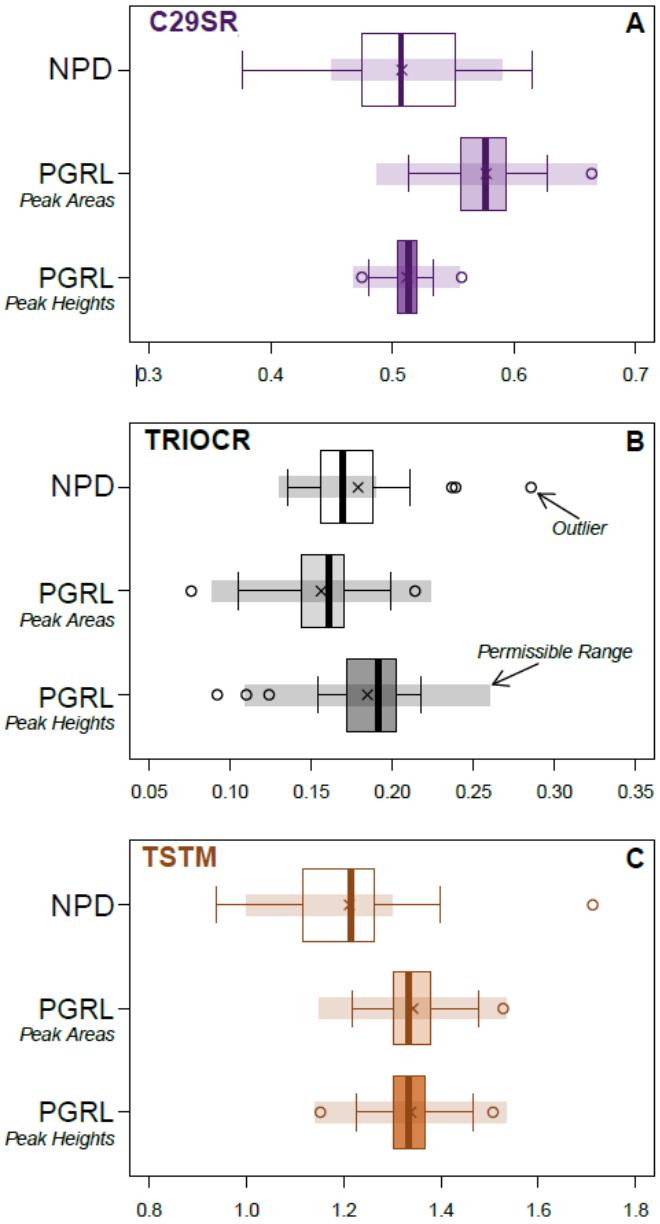

Figure 4. Statistical comparison of the acceptance criteria on three biomarker parameters measured by the Petroleum Geochemistry Research Laboratory (PGRL) and the Norwegian Petroleum Directorate (NPD) for North Sea Oil (NSO-1): **A.** C29SR, **B.** TRIOCR, and **C.** TSTM. PGRL parameters were calculated using peak areas (middle boxplot per panel) and peak heights (lower boxplot per panel). Boxes=interquartile range (IQR), whiskers=1.5 times the IQR, circles=outliers, X=mean, bold line=median, transparent squares over boxplots=permissible ranges (see table 8 for definitions). Note that depicted outliers are still included in the summary statistics (table 8).

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