

## **Determination of Biomarkers in Geologic Materials by Gas Chromatography Mass Spectrometry**

### **1. Introduction**

Biological markers (biomarkers) are complex molecular fossils derived from biochemicals, particularly lipids, in once living organisms. Because biological markers can be measured in both crude oils and extracts of petroleum source rocks, they provide a method to relate the two (correlation) and can be used by geologists to interpret the characteristics of petroleum source rocks when only oil samples are available (Peters and others, 2005). Biomarker geochemistry is used by scientists in the Energy Resources Program to assess maturation, correlation, source input, depositional environment, and biodegradation of organic matter in petroleum source rocks, reservoirs, and the environment.

### **2. Interfaces with Other Methods**

None.

### **3. Materials and Equipment**

- a. Instrument grade iso-octane (2,2,4-trimethylpentane)
- b. 2 ml pipet (exact volume not critical)
- c. 7 ml vials and caps with Teflon<sup>TM1</sup> liners
- d. 0.1 ml conical auto sampler vials with Teflon-lined caps
- e. 100 µl pipet and disposable pipet tips
- f. JEOL GCMate Gas Chromatograph/Mass Spectrometer
- g. Standards (PFK for tuning/calibration, Duda in-house standard crude oil )
- h. Centrifuge that accommodates 7ml vials
- j. Benzene and Chloroform for rinse vials on instrument

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<sup>1</sup> Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

#### 4. Procedure

- a. Start by numbering 7 ml vials with a Sharpie™ pen. Put the numbers toward the top of each vial. If all of the samples are from the same Job, only the sample sequence number needs to be inscribed.
- b. Add three drops of crude oil (or an equivalent amount of dried rock extract, redissolved in iso-octane and sonicated) to the vials using a disposable glass pipet.
- c. Next, add 2.0 ml of iso-octane to each vial and tightly cap each vial.
- d. Place the vials in a freezer for a minimum of 20 minutes (but any longer period of time is acceptable). This will assure that most of the asphaltenes have precipitated.
- e. With the vials securely capped, place them in a centrifuge.
- f. Centrifuge the samples (at approx.. 1800 rpm) for at least 30 minutes, and then carefully remove the vials as to not distribute asphaltene into the supernatant maltene.
- g. Carefully decant the supernatant maltene into another clean, numbered 7 ml vial. Discard the vial containing asphaltenes.
- h. Withdraw 100 µl into a 0.1 ml conical auto sampler vial and tightly cap with a Teflon-lined vial cap.
- i. Place the auto sampler vials in the appropriate auto sampler position. Also include a vial of the in-house standard (Duda).
- j. On the GCMS computer, create a Sequence File for the GCMate instrument. See the JEOL Main Program Instruction Manual pages 1-35.
- k. Open the Tune Mode Tool page on the software toolbar. Select the file Biom.tuc, the tuning and calibration file. Select Tune Mode to tune the mass spectrometer's ion beam for optimal sensitivity and resolution. A peak at m/z 219 is an appropriate mass in the middle of the ions monitored. Click the Auto Tune button. If the auto tune improves the appearance of the m/z 219 peak, accept the new tuning parameters.
- l. After the auto tune completes, you will be left at the Ion Focusing page. Select the SIM Mode tool on the software toolbar. In the SIM Mode page, click the ON button. This turns on the filament, accelerating voltage, and detector. The accelerating voltage and ESA voltage will be switching between the PFK masses used for the calibration. The peaks may be shifted slightly from center, indicating that the previous calibration is no longer valid.
- m. Click the Calib button. A dialog box will open thus: A window showing 4000 iterations should be displayed.
- n. Click the Active Files tool on the software toolbar. The previously loaded sequence file should still be there. Click the OK button.
- o. Make sure your samples are in the autosampler tray and click the green arrow tool on the software toolbar. The samples will be analyzed sequentially. Analysis time is on the order of about 1 hour per sample, excluding the GC oven cool-down time (about 5 minutes). Results will be stored on the GCMS computer in the C:\Data\EJOBNUMBER folder in .LRP format.

- p. Next, copy all of the .LRP files from the GCMS computer's C:\Data folder into the newly created working job folder on the external hard drive.
- q. Start the custom application JEOL2TC4.exe. The JEOL GC/MS .LRP files contain all of the scanned or monitored ions for the entire run. The JEOL2TC4.exe application will create separate TurboChrom (version 4 <3H19>) .raw files from .LRP files for any ions that are specified. (The Visual Basic 6 source code for this application is on the CHROM computer at C:\Source Code\JEOL2TC4 Working Version – in case it needs to be modified).
- r. The .LRP files contain SIM analyses of m/z 191.1800, 217.1956, 231.1174, and 253.1956 ions, and JEOL2TC4.exe will split out these four monitored ions into separate .raw files (with the exception of ions in the “Excluded Masses” list). Check to see that the proper masses are excluded, and that the New File Creation Style is “OGDB Biomarker Style”. Press the button Convert File(s) Now. The created .raw files will be placed in the same file folder.
- s. To work with the chromatograms in TotalChrom (version 6.2.1), a Method (.mth), Report Format (.rpt), and Summary (.sum) file for each of the four masses is needed. Paste these 12 files into the working job folder from a recently completed job folder. (Files are named by mass and file type, for example; 191.1800.mth, 217.1956.mth, 231.1174.mth, and 253.1956.mth).
- t. Start the custom application GeoNav.exe by either selecting GeoNav from the Start Menu or the Office Toolbar shortcut. This program generates TurboChrom 4 Sequence (.seq) files that are used later in TotalChrom. Each ion will need a separate sequence file (same naming convention: 191.1800.seq).
- u. Single-Click the Create “Sequence from RAW Files” icon within GeoNav and use the File, Open menu option. Select the raw file(s) of the particular ion chromatogram.
- v. Click OK, examine the table of selected files (re-open if the wrong file(s) were selected). Use the “File, Save As” menu item to save the sequence file in the working job folder. Use the same naming convention, for example 191.1800.seq. Click Save to create the sequence file. The GeoNav application does not modify the individual .raw files.
- w. Open the 217.1956.mth file in the working job folder by either clicking on the file in Windows Explorer, or by pressing Build Method in TotalChrom Navigator. Press Cancel when the Instrument Selection box opens. Use the Method Editor's “Other, Graphic Editor” menu option (or Ctrl+G) to invoke the Graphic Editor.
- x. Using a house standard run at the same time (Duda), edit each of the TotalChrom Methods (191.1800.mth, 217.1956.mth, 231.1174.mth, and 253.1956.mth). Use the Graphic Method Editor to adjust retention times in the Methods.
- y. Once the Methods have been edited, use the TotalChrom Batch Reprocess to create results files (.rst) for all the chromatograms. Start Batch Reprocess from TotalChrom Navigator. Open the 191.1800.seq sequence file or the sample to process if no sequence file is made. If there is no sequence file then the method and reports associated with that sample mass will need to be selected.
- z. Set the Start analysis box to PEAK DETECTION, and the end box to COMPONENT ID, check box “Overwrite existing results files”, and click on

button for "Update existing raw file header with new sequence". Select Reprocess, Start.

- aa. Repeat the batch reprocess using 217.1956.seq, 231.1174.seq, and 253.1956.seq or each file to process if no sequence is created. The resulting .raw and .rst file pairs can be individually edited with the TotalChrom Reprocess Results application.
- bb. Once the result files are complete and have been reviewed, .cdf files are made. In TotalChrom Navigator, open the menu Apps, Convert. This launches the conversion application.
- cc. On the menu, click Convert, TC to AIA. Select the result files and click Open. The program runs and will report the success (or not) of the conversions, and place the .cdf files in the same folder. Cdf files are then reviewed for accuracy of peak identification.
- dd. The .cdf files are transferred to the appropriate folder for importing into Sample Master.

## Procedures

### Instrument Conditions

GC auto sampler conditions (HP model xx):

# sample washes	0
# pumps	3
Viscosity	1
Injection volume	2
# solvent A washes	2
# solvent B washes	3
Priority sample enabled	OFF
On-column injection	OFF

Solvent A = benzene Solvent B = chloroform

JEOL/HP7890 "Inlet" GC method:

#### Temperature

Temp (°C)	Time (min)	Rate (°C/min)
50	0	50
150	0	3
339	5	0
0	0	

  

Oven ON	ON
Max Temp (°C)	450
Equilibration Time (min)	2

Cryogenic		OFF
Run Time (min)		70
Pressure		
Flow, Initial (ml/min)		2
Column		DB-1
ID (mm)		0.32
Length (m)		60
Pressure		Off
Flow		ON
Vacuum Compensation		ON
Constant Flow		ON
Units		psi
Carrier Gas		Helium
Inlet		
Front		ON
Temp (°C)	300	
Split		OFF
Splitless		ON
purge Flow (ml/min)		30
Purge ON		ON
Purge On at (min)		1.5
Gas Saver		ON
Gas Saver Flow (ml/min)		30
Time (min)		10

## JEOL/Shrader data acquisition method:

Acquisition Mode	SIM
Analysis Time (min)	70
Delay (min)	5
Inlet	GC
Autosampler	ON
Auxiliary Measurements	OFF
SIM Definition	
Data Type	Height
Switching Field	Electric
Lock Mass	218.9856
PFK	ON
Cycle Time (sec)	0.5

## SIM Groups

Group	Start	End	# Masses
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1                      5                      70                      4

## SIM Group 1

Start Time (min)            5  
 End Time (min)            70  
 SRM ON                      OFF

Mass	Window
191.1800	0
217.1956	0
231.1174	0
253.1956	0

## SIM Calibration Setup

Scan Calibration File            BIOM  
 SIM Calibration File            BIOM

## Group 1

SIM Mass	Cal Mass	Intensity*
191.1800	192.989	11
217.1956	218.986	44.7
218.9856	218.986	44.7
231.1174	230.986	46.1
253.1956	254.986	6.3

\* Set automatically from the  
 PFK reference table.

## Tune and Calibrate

Ion Source (uA emission current)            300  
 Ionization Energy (electron volts)            70  
 Ionization Mode                                EI (electron impact)

Ion Focusing                                      Set from auto tune

## Slit Settings

Coupled    ON  
 Mass Resolution                                3000

Detector  
 Multiplier (volts)                                480

Preamp Gain	X1
Filter	1
Inlets	around 2
Vacuum	Pa
Gasses	PFK On
PFK	30%
CI	OFF
CID	OFF
FAB	OFF
Temperatures	
GC Interface (° C)	250
GC Pipe (° C)	300
Ion Chamber (° C)	200
PFK (° C)	70
Mass Selection	
Mass Range	1000 ppm in mass
<u>Tune Mode</u>	
Profile Window	1000 ppm in mass
<u>SIM Mode</u>	
Profile Window	2000 ppm in mass
<u>Scan Mode</u>	
Scan Range Lower	<191
Scan Range Upper	>253
Scan Speed (Seconds/scan)	20
Interscan Delay (seconds)	0.5
Repetitive Scanning	ON
Scan Mode	Magnet
Scan Law	Linear

## 5. Calibration and Quality Control Samples

Perfluorokerosene (PFK) is a heated inlet reference material for calibration/tuning. Blanks of iso-octane are run with each sample set for contamination checks. The in-house standard crude oil, Duda, is run with each sample set for peak identification purposes.

These ratios are:



442.97	1.20	C10F17	442.97284
454.97	0.80	C11F17	454.97284
466.97	0.50	C12F17	466.97284
480.97	1.40	C10F19	480.96964
492.97	1.10	C11F19	492.96964
504.97	0.65	C12F19	504.96964
516.97	0.50	C13F19	516.98964
530.97	0.70	C11F21	530.96645
542.97	0.60	C12F21	542.96645
554.97	0.50	C13F21	554.96645
566.97	0.60	C14F21	566.96645
580.96	0.70	C12F23	580.96325
592.96	0.65	C13F23	592.96325
604.96	0.60	C14F23	604.96325
616.96	0.50	C15F23	616.96325
630.96	0.50	C13F25	630.96006
642.96	0.50	C14F25	654.96006
654.96	0.55	C15F25	654.96006
666.96	0.50	C16F25	666.96006
680.96	0.20	C14F27	680.95686
692.96	0.25	C15F27	692.95686
704.96	0.40	C16F27	704.95686
716.96	0.25	C17F27	716.95686
730.95	0.20	C15F29	730.95366
742.95	0.25	C16F29	742.95366
754.95	0.50	C17F29	754.95366
766.95	0.20	C18F29	766.95366
780.95	0.25	C16F31	780.95047
792.95	0.30	C17F31	792.95047
804.95	0.15	C18F31	804.95047
816.95	0.05	C19F31	816.95047
830.95	0.10	C17F33	830.94727
842.95	0.10	C18F33	842.94727
854.95	0.10	C19F33	854.94727
866.95	0.05	C20F33	866.94727
880.94	0.10	C18F35	880.94408
892.94	0.10	C19F35	892.94408
904.94	0.05	C20F35	904.94408
916.94	0.05	C21F35	916.94408
930.94	0.05	C19F37	930.94088
942.94	0.05	C20F37	942.94088
954.94	0.05	C21F37	954.94088
966.94	0.05	C22F37	966.94088
980.94	0.05	C20F39	980.93768
992.94	0.05	C21F39	992.93768

1004.94	0.05	C22F39	1004.93768
1016.94	0.05	C23F39	1016.93768

Specifications:

PFK, Perfluorokerosene  
Molecular Weight: 941  
Boiling Point: 210 to 240 ° C  
d25: 1.97

## 6. Limits, Precautions, and Interferences

Required tuning of the mass spectrometer's ion beam creates optimal sensitivity and resolution. If the resolution fluctuates during tuning, then source cleaning is required. The max signal voltage is 1,000,000mv; the minimum signal is at baseline. The minimally accepted signal to noise ratio is 18 for this instrument. For this method the mass spectrometer is tuned to mass 219 because it is in the center of the SIM masses to be monitored. Masses 193, 219, 231, and 255 are monitored during the SIM calibration procedure. If the mass peaks are shifted slightly from center initially then the previous calibration is no longer valid. Interferences from n-alkanes can be seen at mass 253 when oils are a waxy consistency. Water should never be introduced as it will render the column inoperable. Personal protective equipment should be used as referenced in the laboratory's Chemical Hygiene Plan for this method.

## 7. Acceptance of Data

Quantitation of organic molecules requires the use of Deuterium or Carbon-13 internal standards representing each of the analyte compounds to be quantitated. These standards are either not available or are too expensive to be useful for the work being conducted. Internal standards closely related to these compounds of interest can be used for quantitation purposes if the submitter understands that they are only closely related and the response factor of the entire class of compounds is assumed. Quantitation of certain compounds will only be done in special request circumstances with prior approval of the Organic Section Lead. Duplicates are not necessary as this is an identification technique.

## 8. Data Handling and Transfer

Once a CDF has been created, (see procedure), the areas and heights are entered into the database where the following calculations are performed:

GAMHOP_R	RATIO Gammacerane / Hopane by Peak Height
G_GAC31R_R	RATIO [GA/C31R] Gammacerane / C31 22R Hopane by Peak Height
G_OLHOP_R	RATIO [OL/H] Oleanane / Hopane by Peak Height
G_BISHOP_R	RATIO [C28/H] Bisnorhopane / Hopane by Peak Height
C27STER_F	DECIMAL FRACTION C27 $\alpha\alpha\alpha$ 20R Sterane by Peak Height
C28STER_F	DECIMAL FRACTION C28 $\alpha\alpha\alpha$ 20R Sterane by Peak Height
C29STER_F	DECIMAL FRACTION C29 $\alpha\alpha\alpha$ 20R Sterane by Peak Height
C26TET_R	RATIO (C26 S+R Tricyclic Terpanes) / C24 Tetracyclic Terpane by Peak Height
G_TETC23_R	RATIO [Tet/C23] C24 Tetracyclic Terpane / C23 Tricyclic Terpane by Peak Height
G_C19C23_R	RATIO [C19/C23] C19 Tricyclic Terpane / C23 Tricyclic Terpane by Peak Height
G_C24C23_R	RATIO [C24/C23] C24 Tricyclic Terpane / C23 Tricyclic Terpane by Peak Height
G_C22C21_R	RATIO [C22/C21] C22 Tricyclic Terpane / C21 Tricyclic Terpane by Peak Height
G_C26C25_R	RATIO [C26/C25] C26 Tricyclic Terpane / C25 Tricyclic Terpane by Peak Area
G_C35C34_R	RATIO [C35S/C34S] C35 22S Hopane / C34 22S Hopane by Peak Height
C35C34_F	DECIMAL FRACTION (C35 22S + 22R Hopanes) / (C31-C35 22S + 22R Hopanes) by Peak Height
G_NEONOR_R	RATIO [C29D/29H] C29 18 $\alpha$ Neonorhopane / C29 Norhopane by Peak Height
G_NORHOP_R	RATIO [C29/H] C29 Norhopane / Hopane by Peak Height
G_C31RH_R	RATIO [C31R/H] C31 22R Homohopane / Hopane by Peak Height
G_XH_R	RATIO [X/H] C30 Diahopane / Hopane by Peak Height
G_S1S6_R	RATIO [S1/S6] C27 $\beta\alpha$ 20S Diasterane / C27 $\alpha\alpha\alpha$ 20R Sterane by Peak Height
DIAREG_R	RATIO C27 $\beta\alpha$ 20S Diasterane / C29 $\alpha\alpha\alpha$ 20R Sterane by Peak Height
PREGC27_R	RATIO Pregnane / C27 $\alpha\alpha\alpha$ 20R Sterane by Peak Height
G_TSTM_R	RATIO [C27 Ts/Tm] C27 18 $\alpha$ Trisnorhopane / C27 17 $\alpha$ Trisnorhopane by Peak Height

TRIHOP_R	RATIO C23 Tricyclic Terpene / Hopane by Peak Height
TRIOCR_F	DECIMAL FRACTION (C20 + C21 Triaromatic) / (C20 + C21 + Identified C26-C28 Triaromatic) Steroids by Peak Area
TRIOCR1_F	DECIMAL FRACTION (C20 Triaromatic) / (C20 + C28 20S + C28 20R Triaromatic) Steroids by Peak Area
TRIOCR2_R	RATIO (C20 + C21 Triaromatic) / (Identified C26-C28 Triaromatic) Steroids by Peak Area
TTM_F	DECIMAL FRACTION MacKenzie (Triaromatic) / (Triaromatic + Monoaromatic) Steroids by Peak Height
TRIMONO_F	DECIMAL FRACTION (C20-C28 ID'd Tri) / (C20-C28 ID'd Tri + C21-C29 ID'd Mono) Aromatic Steroids by Peak Height
MORHOP_R	RATIO (Normoretane + Moretane) / (Norhopane + Hopane) by Peak Height
C31HSR_F	DECIMAL FRACTION (C31 22S Hopane) / (C31 22S + 22R Hopane) by Peak Height
C32HSR_F	DECIMAL FRACTION (C32 22S Hopane) / (C32 22S + 22R Hopane) by Peak Height
C29SR_F	DECIMAL FRACTION (C29 $\alpha\alpha\alpha$ 20S Sterane) / (C29 $\alpha\alpha\alpha$ 20S + 20R Sterane) by Peak Height
C29BBAA_F	DECIMAL FRACTION (C29 $\alpha\beta\beta$ 20R Sterane) / (C29 $\alpha\beta\beta$ 20R Sterane + $\alpha\alpha\alpha$ 20R Sterane) by Peak Height

## 9. References

Peters, Kenneth E., Walters, Clifford C., and Moldowan, John M., 2005, Biomarker guide; Biomarkers and isotopes in petroleum systems and Earth history, 2d ed.: Cambridge, Cambridge University Press, v. 2, p. 475-1155.

JEOL GCMS Main Program Instruction Manual. JEOL ltd Japan.

<http://www.jeol.co.jp>. Copyright 2007.

## 10. Attachments

None.

## 11. History of Changes

R0: Initial Issue