Oil Source Identification Using Diagnostic Biomarker Ratio Analyses

Buffy M. Meyer[†*], Edward B. Overton[†] and R. Eugene Turner [§]

[†] Department of Environmental Sciences, Louisiana State University, Baton Rouge, LA 70803

[§] Department of Oceanography and Coastal Sciences, Louisiana State University, Baton Rouge, LA 70803

[*]To whom correspondence should be addressed:

Buffy M. Meyer
Louisiana State University
Department of Environmental Sciences
1263 Energy, Coast & Environment Building
Baton Rouge, LA 70803
Tel.: (225) 578-4293
Fax: (225) 578-4286
Email: bashton@lsu.edu

ABSTRACT 299977:

The foremost questions asked about spilled oil are its source, quantity in various compartments of the environment, and the risk and consequences associated with various levels of oil within these compartments. The heterogeneous distribution of oil, with a continually changing composition due to weathering, causes considerable uncertainty in determining the source of a spilled oil and whether or not any notable impacts are due to the presence of that particular oil. Oil source-fingerprinting, an environmental forensics technique, is one way to determine the origin of oil in an unknown sample by comparison to a known oil source. Oil source-fingerprinting utilizes oil biomarkers that are naturally occurring in crude oils and most petroleum products which tend to be more resistant to environmental weathering processes compared to most other oil components. More importantly, vast amounts of geochemical research has established that distributions of oil biomarkers are unique for different types and blends of petroleum products and represent an oil-specific fingerprint to which samples can be correlated.

In order to determine whether oil detected in coastal Louisiana marsh sediments originates from the *Deepwater Horizon* incident, an oil source-fingerprinting methodology using GC/MS and specific hopane, sterane, and triaromatic steroid ratios in Macondo 252 (MC252) source oil was developed and tested. A final suite of 15 diagnostic biomarker ratios were determined that will allow for the statistical comparison of diagnostic biomarker ratios of an "unknown" sample to the 15 diagnostic biomarker ratios of MC252 source oil. Unknown samples can then be classified into one of four operational and technically defensible categories:

positive match, probable match, inconclusive, or non-match based on their diagnostic biomarker ratio score.

INTRODUCTION:

Oil source-fingerprinting is an environmental forensics technique that utilizes analytical chemistry to compare samples containing spilled oil to a suspected source. Oil biomarkers, for environmental forensic purposes, are naturally occurring, ubiquitous, and stable hydrocarbons that are present in crude oils and most petroleum products (Daling *et al.*, 2002; Wang *et al.*, 2006; Hansen *et al.*, 2007). Since biomarker compounds are more resistant to environmental weathering processes, compared to most other oil compounds, they can be utilized as conserved reference compounds against to which the loss of less stable oil components can be quantitatively estimated by calculating certain ratios (Prince *et al.*, 1994; Wang and Fingas, 2003; Hansen *et al.*, 2007). Furthermore, the distributions of oil biomarkers is unique for different types and blends of petroleum products (Peters and Moldowan, 1993; Wang and Fingas, 1995; Stout *et al.*, 2002; Wang and Fingas, 2003); therefore, they can represent an oil-specific fingerprint to which distinct oil samples can be correlated.

Analytical chemistry and instrumentation provides environmental scientists with the ability to identify and track the fate of spilled oil residues in the environment. The objective of this research was to utilize high performance capillary gas chromatography/mass spectrometry (GC/MS) to determine a suite of diagnostic biomarker ratios with statistical limitations that can determine whether or not oil residues in environmental samples are a match to Macondo 252 (MC252) oil that was released during the *Deepwater Horizon* tragedy in April 2010.

Since oil biomarker compounds are typically more resistant to environmental weathering, calculated diagnostic ratios will show little or no change over time (Wang and Fingas, 1995; Wang *et al.*, 2006; Hansen *et al.*, 2007). An important benefit of comparing diagnostic ratios of spilled oil and suspected source oils is that concentration effects are minimized. In addition, the use of ratios tends to induce a self-normalizing effect on data (Wang and Fingas, 2003; Hansen *et al.*, 2007). As instrument conditions may change as a result of matrix effects, column degradation, sensitivity, or tune degradation, both integers used to calculate the ratio (assuming they are similar in molecular weight, chemistry and quantitation ion) will be affected by the same relative degree of instrumental change; therefore, the index or ratio of the two integers should remain constant. Accordingly, measured diagnostic ratios between any pair of compounds should also match – up to a certain statistical confidence level – in identical samples.

MATERIALS AND METHODS:

Source Oil Preparation for GC/MS Analysis

MC252 source oil was collected by BP from a riser pipe from the damaged wellhead of *Deepwater Horizon* drilling rig in the Gulf of Mexico on May 20, 2010. The source oil was weighed and a proportional amount of hexane was added (e.g. 0.10 grams of oil in 10 milliliters of hexane). A 1-milliliter aliquot of the extract was analyzed daily in the laboratory as a quality control mechanism and a total of 32 of these analyses were used to determine the final suite of diagnostic biomarker ratios.

GC/MS instrumentation

Chemical analyses of the MC252 oil extract were performed using an Agilent 7890A Gas Chromatograph (GC) equipped with an Agilent 5975C inert XL mass selective detector (MSD) and fitted with a 5% diphenyl/95% dimethyl polysiloxane high resolution capillary column (Phenomenex ZB-5MSi, 30 m x .25 mm ID x 0.25 micron thick film). The GC injection temperature was set at 280°C and only high-temperature, low thermal-bleed septa were used in the GC inlet. The carrier gas was ultrahigh purity helium at a constant flow rate of 1 ml min^{-1}. The injection port was set at 280 °C, run in splitless mode, and was fitted with a deactivated borosilicate liner. The GC was operated in the temperature program mode with an initial column temperature of 60°C for 3 minutes then increased to 280°C at a rate of 5°C/minute and held for 3 minutes. The oven was then heated from 280°C to 300°C at a rate of 1.5°C/min and held at 300°C for two minutes. Total run time is 65.33 minutes per sample. The interface to the MS was maintained at 300°C. The MSD was operated in the selective ion monitoring (SIM) mode to ensure low level detection of the targeted oil analytes.

Diagnostic Biomarker Ratio Calculations

In order to have the capability to determine whether oil detected in coastal Louisiana marsh sediments originated from the *Deepwater Horizon* disaster, an oil source-fingerprinting methodology using GC/MS and specific hopane, sterane, and triaromatic steroid ratios in MC252 source oil was developed and tested. The foundation and statistical criteria of the methodology was adapted from the 2007 edition of *Oil Spill Environmental Forensics: Fingerprinting and Source Identification*, Chapter 7, "Emerging CEN Methodology for Oil Spill Identification", by A.B Hansen, P.S. Daling, L. Faksness, K.R. Sorheim, P. Kienhuis, and R. Duus, and edited by Zhendi Wang and Scott Stout.

The first step of the process was to isolate specific diagnostic biomarker ratios from the chromatographic profiles of the hopanes; the steranes (diasteranes and regular steranes, and 14β(H) steranes); and the triaromatic steroids. A number of MC252 source oil analyses (n=32) were used to determine the diagnostic biomarker ratios to be tested. The MC252 diagnostic biomarker ratios tested were chosen by using many already published combinations of ratios and a few new ratios using similar guidelines as the other ratios. Diagnostic ratios presented in this paper were calculated by using the ratio of peak heights of compounds within the same mass to charge ratio. Hansen *et al.* (2007) recommends integration of peak heights for diagnostic biomarker ratios since peak heights tend to be more robust than area responses for peaks that may be poorly resolved and have noisy baselines. After a corrected base line value and peak heights have been determined, the diagnostic ratios were calculated by dividing peak height "A" by peak height "B" within an ion group (e.g. *m/z* 191, 217, 218, 231) to exclude the mass spectrometer's varying response for different ions. In addition to diagnostic ratios calculated as A/B, some diagnostic ratios were calculated using the sums of peak heights within the ion group (e.g. A/(A+B)).

To determine the final suite of diagnostic biomarker ratios, a fixed coefficient of variation or relative standard deviation (RSD) of 5% was applied to overcome the variation in critical differences as described in Hansen et al.'s chapter. Each MC252 ratio chosen was averaged, the standard deviation calculated, and the %RSD determined (%RSD = standard deviation/ ratio μ *

100%). Ratios producing RSD values higher than 5% should not be used in an environmental forensics context; therefore, any MC252 diagnostic biomarker exceeding this limit were excluded.

After excluding highly variable ratios, the %RSD of each remaining ratio can then be combined with the expected normal distribution variance, with the assumption that the sample diagnostic biomarker ratios and the MC252 diagnostic biomarker ratios are not statistically different, to determine a difference limit of each unknown sample and MC252 diagnostic ratio pair (not to exceed a 95% probability level).

RESULTS AND DISCUSSION:

A total of 15 diagnostic biomarker ratios calculated from 32 separate analyses of MC252 source oil GC/MS runs were determined. Table 1 provides the compound name and abbreviation for biomarkers chosen for diagnostic ratios and information regarding peak labels in Figures 1-4. Figures 1-4 display the MC252 GC/MS fingerprints for each ion group (e.g. *m/z* 191, 217, 218, 231). Table 2 gives the 15 specific diagnostic ratios chosen and Table 3 shows the average (n=32) MC252 source oil ratios and their corresponding %RSD. All diagnostic ratios chosen had %RSDs less than 5%. The fixed 5% RSD limit was applied as a quality criterion, because methods producing higher RSD values should not be used to analyze and compare oil samples in a forensic context (Hansen *et al*., 2007).

By statistical treatment of the ratios, spill samples compared to a source sample can then be classified into one of four operational and technically defensible terms: positive match, probable match, inconclusive, or non-match (Hansen *et al.*, 2007). Classification will depend on the final diagnostic ratio score (the # of "matching" ratios / 15 * 100%) and can be grouped as so: 93-100 = Match; 80-92 = Probable Match; 50-79 = Inconclusive; and <50 = Non-match.

CONCLUSIONS:

In conclusion, the statistical comparison of diagnostic ratios will provide a quantitative evaluation of data; however, it is not to be considered as all conclusive. It is important that other quantitative and qualitative evaluations corroborate with the statistical evaluations; therefore, visual inspection of all the chromatograms should be done before any final conclusions are made. Low petrogenic content in environmental samples may affect the calculation of diagnostic ratios and is an important consideration when making all conclusions. Thus, the presence of petrogenic hydrocarbons does not necessarily mean that MC252 oil is present in the samples. Another important factor to consider is the eventual weathering of the biomarker compounds themselves. This has been documented by Wang *et al.* (2001) to occur and depends greatly on the environmental conditions and whether or not oil residues are buried or remain at the surface.

The diagnostic biomarker ratios determined in this paper will be applied to sediment samples collected throughout Louisiana's coastal marshes from 2010 to 2013. The samples have been collected from areas that were documented as impacted by the *Deepwater Horizon* catastrophe and other sites that were unimpacted. The statistical comparison of diagnostic biomarker ratios of an unknown sample to the same diagnostic biomarker ratios of MC252

source oil will allow each unknown sample to be classified into one of four operational and technically defensible categories: positive match, probable match, inconclusive, or non-match to MC252. Furthermore, using diagnostic biomarker ratios as outlined in this paper will provide a statistically rigorous evaluation of detecting and characterizing oil in the environment after oil spill events.

ACKNOWLEDGEMENTS:

This analysis was made possible by funding from the U.S. Geological Survey, National Wetlands Research Center, Lafayette, LA. The financial sources had no role in the design or execution of the study, data analysis, decision to publish, or manuscript preparation. Additional funding was provided by the LUMCON Coastal Waters Consortium through the Gulf of Mexico Research Initiative.

REFERENCES:

Daling, P.S., L. Faksness, A.B. Hansen, and S.A. Stout. 2002. Improved and standardized methodology for oil spill fingerprinting. Environmental Forensics, 3:263-278.

Hansen, A.B, P.S. Daling, L. Faksness, K.R. Sorheim, P. Kienhuis, and R. Duus. 2007. Emerging CEN Methodology for Oil Spill Identification. In: Zhendi Wang and Scott Stout, eds., *Oil Spill Environmental Forensics: Fingerprinting and Source Identification*. Burlington, MA: Academic Press, pp. 229-256.

Peters, K.E., C.C. Walters, and J.M. Moldowan. 2005. *The Biomarker Guide, 2nd Edition*. Cambridge, UK: Cambridge University Press.

Prince, R.C., D.L. Elmendorf, J.R. Lute, C.S. Hsu, C.E. Haith, J.D. Senius, G.J. Dechert, G.S. Douglas, and E.L. Butler. 1994. 17*α(H),*21*β(H)*-hopane as a conserved internal marker for estimating the biodegradation of crude oil. Environmental Science and Technology, 28:142-145.

Stout, S.A., A.D. Uhler, K.J. McCarthy, S. Emsbo-Mattingly. 2002. Chemical fingerprinting of hydrocarbons. In: B.L. Murphy and R.D. Morrison, eds., *Introduction to Environmental Forensics*. London, UK: Academic Press, pp. 137–260.

Wang, Z.D. and M. Fingas. 1995. Differentiation of the source of spilled oil and monitoring of the oil weathering process using gas chromatography-mass spectrometry. Journal of Chromatography, 712:321-343.

Wang, Z., M.F. Fingas, L.Sigouin, and E.H. Owens. 2001. Fate and persistence of long-term spilled *Metula* oil in the marine salt marsh environment: degradation of petroleum biomarkers. International Oil Spill Conference Proceedings, 2001:115-125.

Wang, Z. and M. Fingas. 2003. Development of oil hydrocarbon fingerprinting and identification techniques. Marine Pollution Bulletin, 47:423-452.

Wang, Z., S.A. Stout, and M. Fingas. 2006. Forensic fingerprinting of biomarkers for oil spill characterization and source identification. Environmental Forensics, 7:105-146.

Table 1. Petroleum Biomarkers Used For Calculating MC 252 Diagnostic Ratios

Abbreviation	Compound Name	*m/z* Value	Figure Reference
C27 Ts	C27 18α(H)-22,29,30-trisnorneohopane	191	1, a
C27 Tm	C27 17α(H)-22,29,30-trisnorhopane	191	1, b
C29 aB	C29 17α(H),21β(H)-30-norhopane	191	1, c
C29 Ts	C29 18α(H)-30-norneohopane	191	1, d
C30 aB	C30 17α(H),21β(H)-hopane	191	1, e
C31 aB (S+R)	C31 17α(H),21β(H)-22(S+R)-homohopane	191	1, f+g
C32 aB (S+R)	C32 17α(H),21β(H)-22(S+R)-bishomohopane	191	1, h+i
C33 aB (S+R)	C32 17α(H),21β(H)-22(S+R)-trishomohopane	191	1, j+k
C27D Ba-S	C27 13β(H),17α(H),20S-diasterane	217	2, a
C27D Ba-R	C27 13β(H),17α(H),20R-diasterane	217	2, b
C29D Ba-S	C29 24-ethyl-13β(H),17α(H),20S-diacholestane	217	2, c
C29D Ba-R	C29 24-ethyl-13β(H),17α(H),20R-diacholestane	217	2, d
C28 aaa-R	C28 24-methyl-5α(H),14α(H),17α(H), 20R-cholestane	217	2, e
C29 aaa-R	C29 24-ethyl-5α(H),14α(H),17α(H), 20R-cholestane	217	2, f
C27 BB (R+S)	C27 5α(H),14β(H),17β(H)-cholestane (20R+20S)	218	3, a+b
C28 BB (R+S)	C28 24-methyl-5α(H),14β(H),17β(H)-cholestane (20R+20S)	218	3, c+d
C29 BB (R+S)	C29 24-ethyl-5α(H),14β(H),17β(H)-cholestane (20R+20S)	218	3, e+f
C20 TA	C20-triaromatic steroid (pregnane derivative)	231	4, a
C21 TA	C21-triaromatic steroid (homopregnane derivative)	231	4, b
C26 TA-S	C26-triaromatic steroid,20S (cholestane derivative)	231	4, c
C28 TA-S	C28-triaromatic steroid,20S (ethylcholestane derivative)	231	4, d
C27 TA-R	C27-triaromatic steroid,20R (methylcholestane derivative)	231	4, e
C28 TA-R	C28-triaromatic steroid,20R (ethylcholestane derivative)	231	4, f

Table 2. Diagnostic Ratios Chosen for MC 252 Source Oil

Ratio	*m/z* Value	Ratio	*m/z* Value
C27 Ts/ C27 Tm	191	C27 BB(R+S)/C28 BB(R+S) + C29 BB(R+S)	218
C29 aB/C29 Ts	191	C28 BB(R+S)/C27 BB(R+S) + C29 BB(R+S)	218
C29 aB/C30 aB	191	C29 BB(R+S)/C27 BB(R+S) + C28 BB(R+S)	218
C31 aB(S+R)/C32 aB(S+R) + C33 aB(S+R)	191	C20 TA/C21 TA	231
C32 aB(S+R)/C31 aB(S+R) + C33 aB(S+R)	191	C26 TA-S/C28 TA-S	231
C33 aB(S+R)/C31 aB(S+R) + C32 aB(S+R)	191	C27 TA-R/C28 TA-R	231
C27D Ba-S/C27D Ba-R	217		
C29D Ba-S/C29D Ba-R	217		
C28 aaa-R/C29 aaa-R	217		

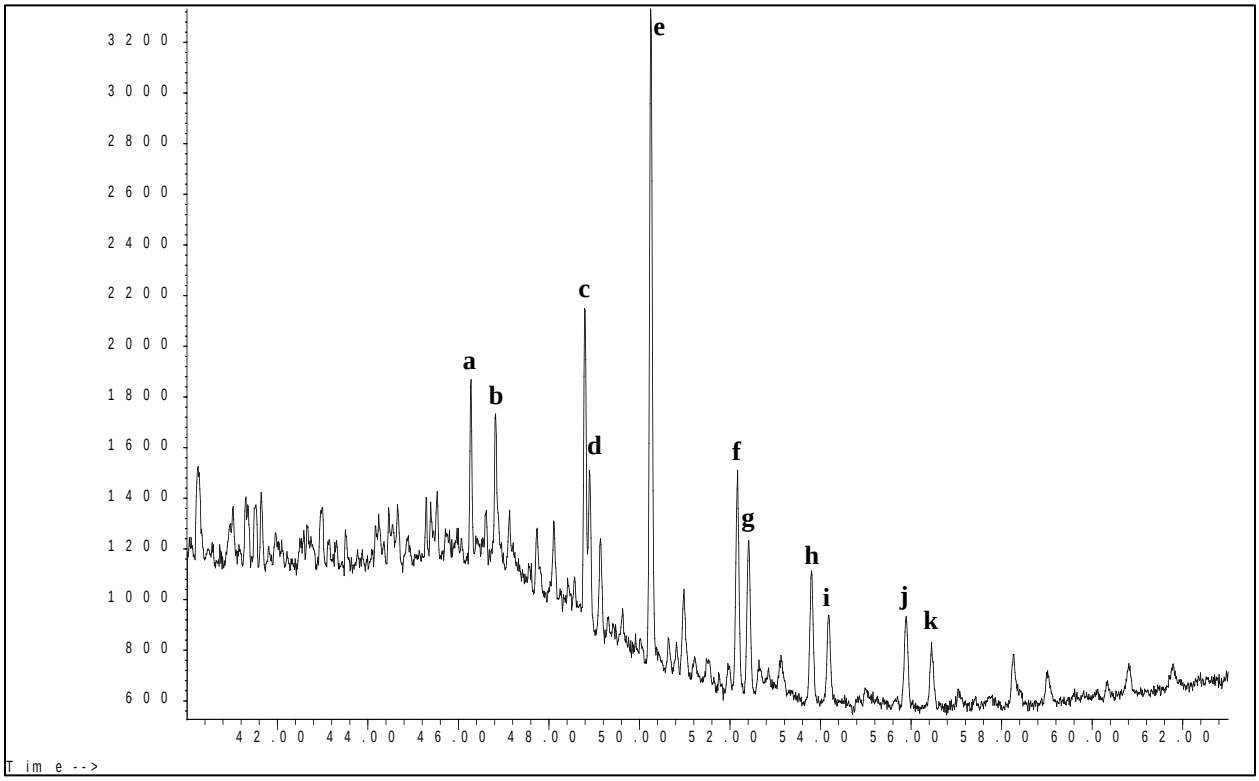

Figure 1. GC/MS fingerprint of hopanes recorded at *m/z* 191 in MC 252 oil.

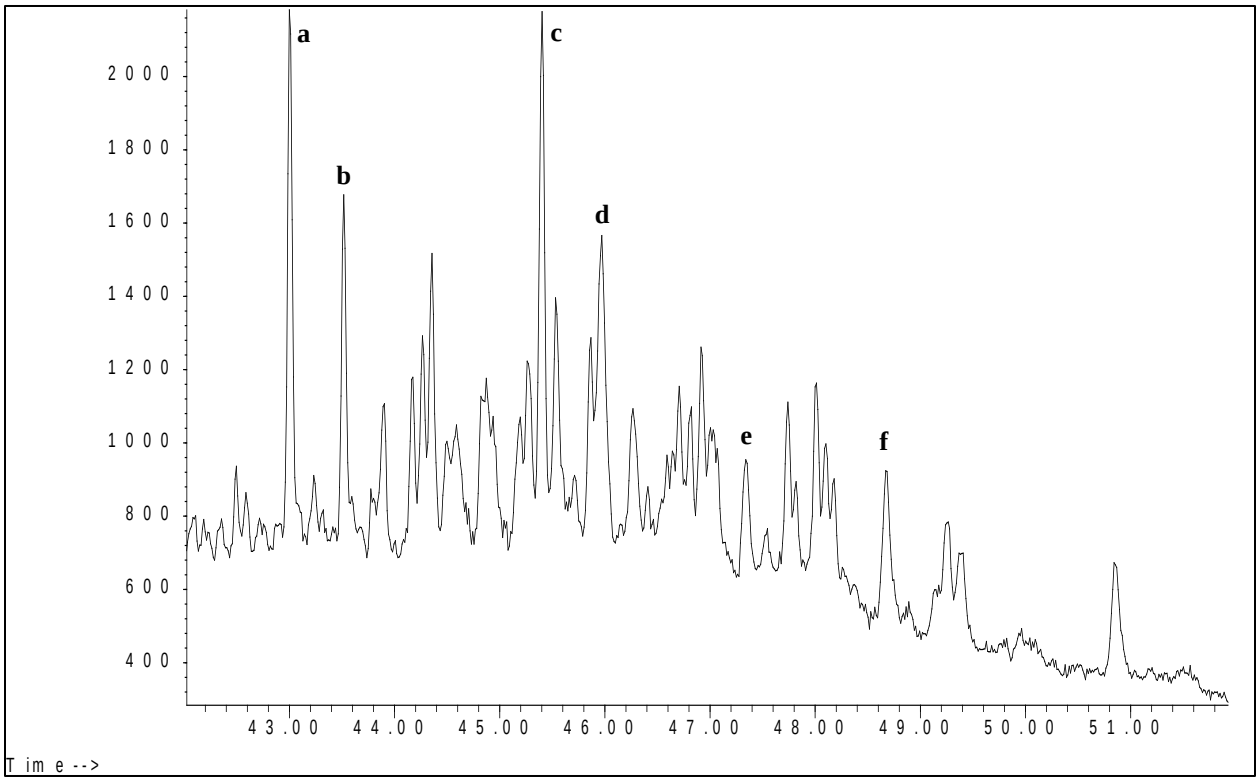

Figure 2. GC/MS fingerprint of diasteranes and regular steranes recorded at *m/z* 217 in MC 252 oil.

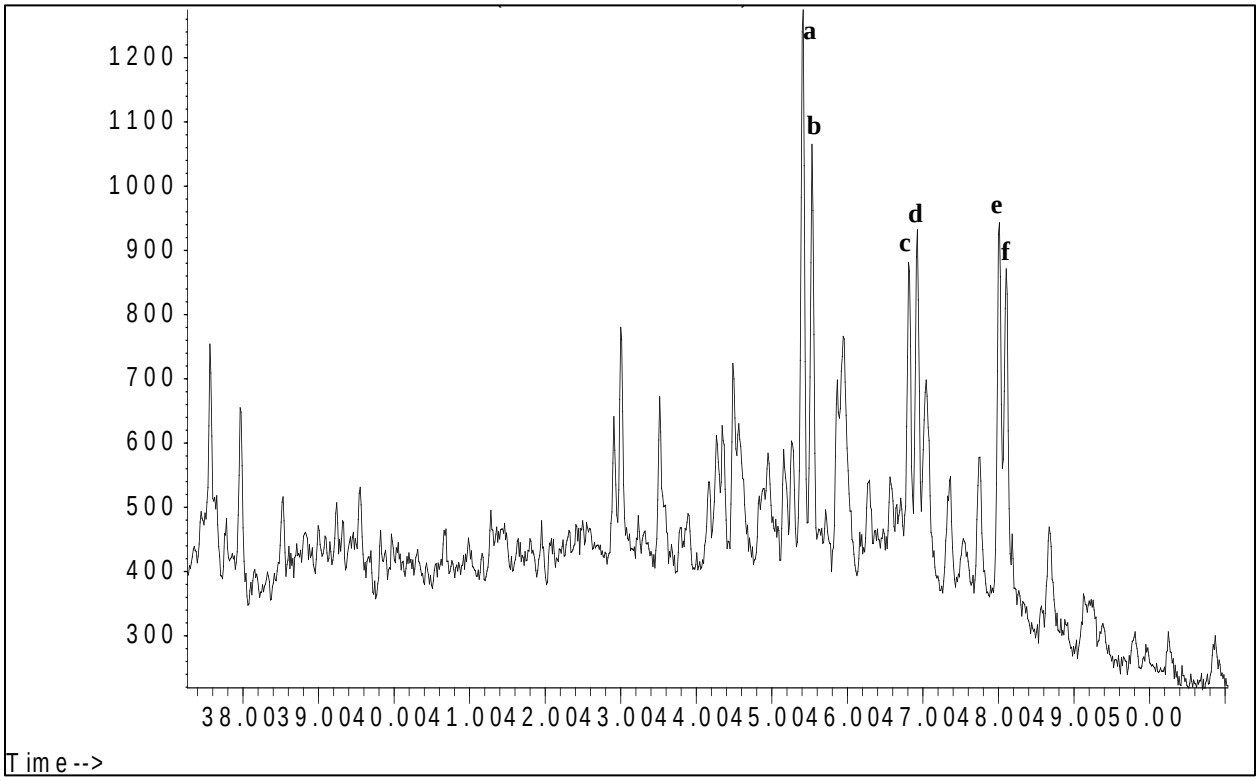

Figure 3. GC/MS fingerprint of 14β(H)-steranes recorded at *m/z* 218 in MC 252 oil.

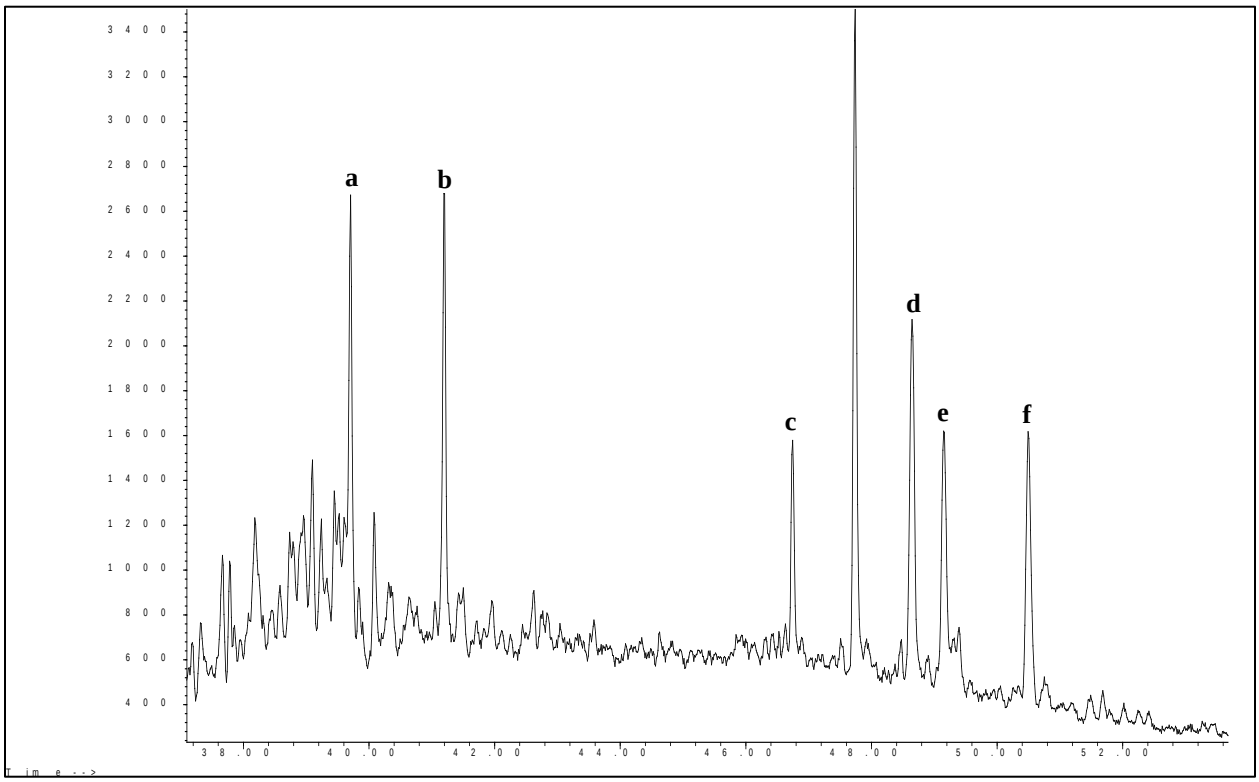

Figure 4. GC/MS fingerprint of triaromatic steroids recorded at *m/z* 231 in MC 252 oil.

Table 3. Average Diagnostic Ratios for MC 252 Source Oil (n=32)

Hopanes (m/z 191)	**AVG**	**STDEV**	**%RSD**
C27 Ts/ C27 Tm	1.26	0.043	3.43
C29 aB/C29 Ts	2.17	0.053	2.46
C29 aB/C30 aB	0.48	0.017	3.61
C31 aB(S+R)/C32 aB(S+R) + C33 aB(S+R)	0.93	0.021	2.22
C32 aB(S+R)/C31 aB(S+R) + C33 aB(S+R)	0.43	0.015	3.41
C33 aB(S+R)/C31 aB(S+R) + C32 aB(S+R)	0.28	0.010	3.60
Diasteranes and Regular 14a(H)-Steranes (m/z 217)	**AVG**	**STDEV**	**%RSD**
C27D Ba-S/C27D Ba-R	1.61	0.035	2.15
C29D Ba-S/C29D Ba-R	1.63	0.052	3.17
C28 aaa-R/C29 aaa-R	0.74	0.017	2.24
14B(H)-Steranes (m/z 218)	**AVG**	**STDEV**	**%RSD**
C27 BB(R+S)/C28 BB(R+S) + C29 BB(R+S)	0.69	0.021	3.03
C28 BB(R+S)/C27 BB(R+S) + C29 BB(R+S)	0.38	0.013	3.48
C29 BB(R+S)/C27 BB(R+S) + C28 BB(R+S)	0.46	0.018	3.86
Triaromatic Steriods (m/z 231)	**AVG**	**STDEV**	**%RSD**
C20 TA/C21 TA	1.07	0.026	2.38
C26 TA-S/C28 TA-S	0.63	0.016	2.62
C27 TA-R/C28 TA-R	0.92	0.023	2.45