3 Petroleum Biomarker Fingerprinting for Oil Spill Characterization and Source Identification

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3.1 Introduction

Biological markers, or biomarkers, are one of the most important hydrocarbon groups in petroleum used for chemical fingerprinting. They are complex molecules derived from formerly living organisms. Biomarkers found in crude oils, rocks, and sediments have little or no changes in structures from their parent biochemicals, or so-called biogenic precursors (e.g., terpanoids and steroids), found in living organisms. In comparison with the concentrations of the biogenic precursors in sediments, biomarker concentrations in oil are low, often in the range of several to less than a hundred parts per million (ppm).

Biomarkers are useful for chemical fingerprinting of spilled oils because they retain all or most of the original carbon skeleton of the original natural product, and thereby testify to the specific conditions for oil generation (see Chapter 1 herein). Excellent reviews on the fundamentals of biomarker characterization, their application in petroleum geochemistry, and interpretation of biomarker data for oil exploration and production were published in 1993 (Peters and Moldowan, 1993). A fully updated and expanded edition provides a comprehensive account of the role that biomarker technology plays both in petroleum exploration and in understanding earth history and processes, including environmental applications (Peters et al., 2005). More recently, Wang et al. (2006) have reviewed the environmental applications of biomarker fingerprinting.

Biomarker fingerprinting has historically been used by petroleum geochemists in characterization of oils in terms of (1) oil-to-oil and oil-to-source rock correlation, (2) the type(s) of precursor organic matter present in the source rock, (3) effective ranking of the relative thermal maturity of petroleum, (4) evaluation of migration and the degree of in-reservoir biodegradation based on the loss of *n*-alkanes, isoprenoids, aromatics, terpanes, and steranes during biodegradation, (5) determination of depositional environmental conditions, and (6) providing information on the age of the source rock for petroleum.

Biomarkers can be detected in low quantities (ppm and sub-ppm level) in the presence of a wide variety of other types of petroleum

hydrocarbons by the use of the gas chromatography-mass spectrometry (GC-MS). Relative to other hydrocarbon groups such as alkanes and most aromatic compounds, biomarkers are highly resistant to degradation in the environment (see Chapter 11 herein). Furthermore, due to the wide variety of geological conditions and ages under which oil has formed, every crude oil may exhibit an essentially unique biomarker fingerprint. Therefore, chemical analysis of biomarkers can generate highly specific "source" information of great importance to environmental forensic investigations in terms of determining the source of spilled oil, differentiating and correlating oils, studying the fate and behavior of hydrocarbons in the environment, and monitoring the degradation process and weathering state of oils under a wide variety of environmental conditions. They have also proven useful in identification of petroleum-derived contaminants in the marine and aquatic environments (Stout et al., 2002; Kvenvolden et al., 1995, 2002; Hostettler et al., 1999a; Boehm et al., 1997; Bence et al., 1996; Volkman et al., 1997; Zakaria et al., 2000; Wang et al., 1994a, 1994b, 1999a) and in indicating chronic industrial and urban releases (Stout et al., 1998; Volkman et al., 1992a; Kaplan et al., 1997).

In this chapter we will focus our discussion on a brief description of biomarker chemistry, an overview of analytical methodologies for biomarker separation and analysis, the identification of biomarkers, biomarker distributions in crude oils and various petroleum products, sesquiterpane and diamondoid biomarkers in oils and lighter petroleum products, diagnostic ratios and cross-plots of biomarkers, source-specific biomarkers, weathering effects on oil and biomarker fingerprinting, and an application of biomarkers for oil spill source identification, oil correlation, and differentiation.

3.2 Analytical Methodologies for Petroleum Biomarker Fingerprinting

3.2.1 Petroleum Biomarker Families

Oil consists of complex mixtures of hydrocarbons and nonhydrocarbons that range from small, volatile compounds to large, nonvolatile ones. For example, recently, ultrahigh-resolution Fourier transform ion cyclotron resonance mass spectrometry (Marshall, 2004) revealed that crude oil contains heteroatom-containing (N, O, S) organic components having more than 20,000 distinct elemental compositions ($C_cH_hN_nO_oS_s$). In general, petroleum hydrocarbons are characterized and classified chemically by their structures, including saturates (including straight-chain and branched chain saturates, cycloalkanes, terpanes, and steranes); olefins; aromatics (including the monoaromatic hydrocarbons such as BTEX and other alkyl-substituted benzene compounds, and oil-characteristic alkylated C_0- to C_4-PAH homologous series and other U.S. EPA priority PAHs ranging from 2-ring up through 6-rings); and polar resins (including heterocyclic S, N, and O containing compounds, phenols, acids, alcohols, and monoaromatic steroids) and very high-molecular-weight asphaltenes (Speight, 1999; Berkowitz, 1997).

In 1887, German chemist Otto Wallach determined the structures of several terpenes and discovered that all of them are composed of two or more five-carbon units of isoprene [2-methyl-1,3-butadiene, $CH_2{=}C(CH_3){-}CH{=}CH_2$]. The isoprene unit maintains its isopentyl structure in a terpene, usually with modification of the isoprene double bonds. The isoprene molecule and the isoprene unit are said to have a "head" (the branched end) and a "tail" (the unbranched ethyl group). Organic chemists and geochemists have long realized that *isoprene* is the basic structural unit of many natural products and all oil biomarker compounds (Peters and Moldowan, 1993; Wade, 2003). Compounds composed of isoprene subunits (that is, obeying the "isoprene rule") are called terpenoids or isoprenoids. The triterpenoids constitute a large diverse group of natural products (Connolly and Hill, 1991). Terpenoids are ubiquitous in microorganisms and in higher and lower plants, and have been characterized to an increasing extent within the animal kingdom. Few have been known for centuries, but in recent decades the level of research and activity in isolating and studying

new substances has shown no sign of abating, and the discovery of completely new carbon skeletons among the naturally occurring plant and animal terpenoids is a frequent occurrence.

Terpenoids are grouped according to the number of isoprene units from which they are biogenetically derived, even though some carbons may have been added or lost (Connolly and Hill, 1991). The isoprene rule states that biosynthesis of these compounds occurs by polymerization of appropriately functionized C_5-isoprene subunits. Unlike other biopolymers such as proteins, terpenoids are not readily depolymerized because they are joined together by covalent carbon–carbon bonds. As for the oil-saturated terpenoids, they are generally categorized into families based on the approximate number of isoprene subunits they contain. Terpenoids containing 1 to 8 isoprene subunits are termed as hemi-, mono-, sesqui-, di-, sester-, tri-, and tetraterpanes. The various oil terpane families are composed of a wide variety of acyclic and cyclic structures (Peters and Moldowan, 1993).

3.2.1.1 Acyclic Terpenoids or Isoprenoids

One of the most important discoveries in petroleum chemistry and organic geochemistry was the detection of a large number of aliphatic isoprenoid hydrocarbons in oils, coals, shales, and dispersed organic materials. The variety of isoprenoid compounds is incomparably large. The linkages between isoprene subunits can be regular (head-to-tail) or irregular (differing in the order of attachment of the isoprene subunits, such as head-to-head or tail-to-tail) linkages. Phytane ($C_{20}H_{42}$), which is one of the most abundant isoprenoids in oil and has been widely used for estimation of the degree of oil biodegradation in the environment, is a typical example of a regular, acyclic isoprenoid consisting of four head-to-tail linked isoprene units. Squalane ($C_{30}H_{62}$) and Botryococcane ($C_{34}H_{70}$) are examples of irregular isoprenoids. Squalane contains six isoprene subunits with one tail-to-tail linkage, while irregular Botryococcane is a highly specific biomarker for lacustrine sedimentation.

Degraded, rearranged, or homologous structures can be categorized into their corresponding parent terpenoid family. The precise number of carbon atoms in a given terpenoid family varies due to differences in source materials, diagenesis, thermal maturity, and in-reservoir biodegradation. For example, pristane ($C_{19}H_{40}$), another isoprenoid compound widely used for environmental oil biodegradation studies, contains one less methylene group (—CH_2—) than phytane ($C_{20}H_{42}$), but it is still classified as an acyclic diterpane. Other examples include pseudohomologous series of regular isoprenoids from C_{15} (farnesane) through C_{16} (trimethyl-C_{13}) and C_{18} (norpristane), which are also quite abundant in oil.

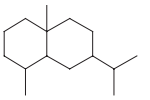
3.2.1.2 Cyclic Terpenoids

The most common cyclic terpenoids in oil are terpanes, steranes (irregular cyclic terpernoid compounds), and aromatic steranes. Although cyclic terpenoids containing almost any number of carbons can occur in theory, only those containing combinations of five or six carbons (cyclopentyl or cyclohexyl) occur commonly in petroleum.

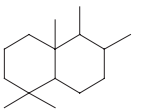
As mentioned above, the terpanes include sesqui- (C_{15}, bicyclic), di- (C_{20}, largely tricyclic), and triterpanes (C_{30}, mainly pentacyclic, and some tricyclic and tetracyclic), which are found in most crude oils. The terpanes comprise several homologous series, including bicyclic, tricyclic, tetracyclic, and pentacyclic compounds. Hopanes are pentacyclic triterpanes commonly containing 27 to 35 carbon atoms in a naphthenic structure composed of four six-membered rings and one five-membered ring (Figure 3-1). Hopanes with the 17α(H), 21β(H)-configuration in the range of C_{27} to C_{35} are characteristic of petroleum because of their large abundance and thermodynamic stability compared to other epimeric ($\beta\beta$ and $\beta\alpha$) series.

The four-ringed steranes are a class of biomarkers containing 21 to 30 carbons,

Sesquiterpanes (C_{15})

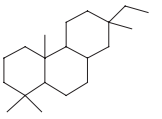


Eudesmane (bicyclic)

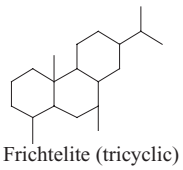


Drimane (bicyclic)

Diterpanes (C_{20})



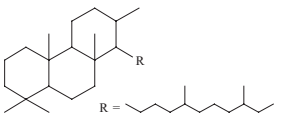
Pimarane (tricyclic)



Frichtelite (tricyclic)

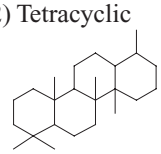
Triterpane (C_{30})

(1) Tricyclic



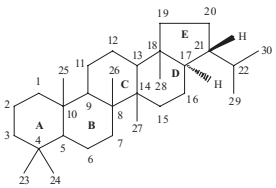
C_{30} tricyclic terpane
(R=$C_{12}H_{25}$, an extended cheillanthane)

(2) Tetracyclic

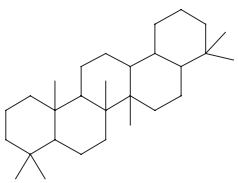


C_{24} 17,21-secohopane

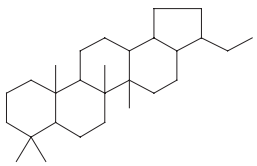
(3) Pentacyclic (C_{30})



Hopane

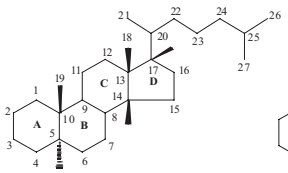


Gamacerane

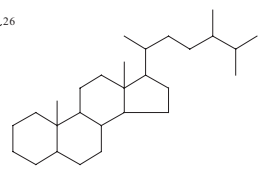


28,30-Bisnorhopane

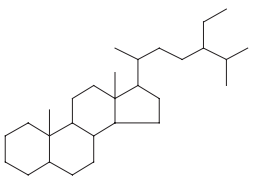
Steranes



Cholestane (C_{27})

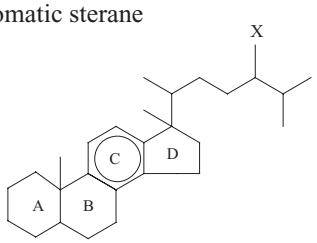


Ergostane (C_{28})

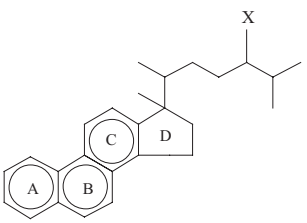


Stigmastane (C_{29})

Aromatic sterane



Monoaromatic sterane



Triaromatic sterane

Figure 3-1 Molecular structures of example cyclic terpenoid compounds in oil.

including regular steranes, rearranged diasteranes, and mono- and triaromatic steranes. Among them, the regular C_{27}—C_{28}—C_{29} homologous sterane series (cholestane, ergostane, and stigmastane) are the most common steranes and are useful for chemical fingerprinting because of their high source specificity. These sterane homologue series do not contain an integral number of isoprene subunits, and thus only approximate the isoprene rule. However, they still show some terpenoid character and can be categorized into the corresponding cyclic terpenoid families.

Aromatic steranes are another group of biomarker compounds found in the oil aromatic hydrocarbon fraction. These compounds can also provide valuable information for forensic investigations on oil-to-oil correlation, differentiation, and source identification. The C-ring monoaromatic (MA) steranes are characterized by a series of 20R and 20S C_{27}—C_{28}—C_{29} 5α- and 5β-cholestanes, ergostanes, and stigmastanes. The ABC-ring triaromatic steranes are formed from aromatization of C-ring monoaromatic steranes involving the loss of a methyl group at the A/B ring junction. This fraction is composed mainly of C_{20} and C_{21}, and C_{26}—C_{27}—C_{28} homologous triaromatic steranes. Examples of monoaromatic and triaromatic steranes are also shown in Figure 3-1. As a summary, Table 3-1 lists important biomarker terpane, sterane, and aromatic sterane compounds, used frequently for forensic oil spill studies.

3.2.2 Labeling and Nomenclature of Biomarkers

The chemical structures of terpenoids are more complicated than that of normal alkanes and isoalkanes (Peters and Moldowan, 1993; Morrison et al., 1992; Wade, 2003). The system used for the nomenclature of terpenoids has evolved over many years. For many terpenoid classes, several names have been proposed for the carbon skeleton, but the basic rules of the IUPAC (International Union of Pure and Applied Chemistry) system are used for nomenclature of biomarkers. For example, the acyclic isoprenoids pristane ($C_{19}H_{40}$) and phytane ($C_{20}H_{42}$) are named as 2,6,10, 14-tetramethylpentadecane and 2,6,10,14-tetramethylhexadecane, respectively.

Cyclic triterpanes and steranes (Figure 3-1) are labeled according to the following rules (Peters and Moldowan, 1993; Wade, 2003): (1) each carbon atom and the rings in biomarker molecules are labeled systematically. Rings are specified in succession from left to right as the A-ring, B-ring, C-ring, D-ring, and so on. (2) A capital "C" followed immediately by a subscript number refers to the number of carbon atoms in a particular compound (e.g., C_{30} hopane and C_{27} sterane mean that they contain 30 and 27 carbon atoms, respectively). (3) A capital "C" followed by a dash and numbers refers to a particular position within the compound [e.g., C-17 and C-21 in the 17α(H), 21β(H)-hopane is the carbon atoms at positions 17 and 21]. (4) Prefixes are used to indicate the changes to the normal biomarker carbon skeleton, which include the prefixes *nor-*, *seco-*, *neo-*, and others. Table 3-2 summarizes the nomenclature used to modify the structural specification of cyclic biomarkers. The prefix *nor-* is used to indicate loss of carbons from a carbon skeleton. For example, 17α(H), 21β(H)-30-norhopane is identical to C_{30} 17α(H), 21β(H)-hopane except that a methyl group at the C-30 position has been lost from its point of attachment at the C-22 position. Similarly, 25-norhopanes are identical to C_{30} hopane except that a methyl group (at C-25) has been removed from its point of attachment at the C-10 position. If two or three carbons are lost, the prefix "*bisnor-*" or "*trisnor-*" is used, respectively. Thus, 28-, 30-bisnorhopanes have two methyl groups (at C-28 and C-30) removed from their parent C_{30} hopane. The prefix "*seco-*" is used to indicate cleavage of a bond, with the locants for both ends of the broken bond given, e.g., 3,4-secoeudesmane. The 17, 21-secohopane indicates that the bond between carbon number 17 and 21 in the E-ring of C_{30} hopane has been broken, resulting in the formation of a new tetracyclic terpane. The prefix "*homo-*" is used

Table 3-1 Petroleum Biomarkers Frequently used for Forensic Oil Spill Studies

Peak	*Compound*	*Code*	*Empirical formula*	*Target ions*
	Sesquiterpanes (Bicyclic terpanes)			
	C_{14} sesquiterpanes		$C_{14}H_{26}$	123, 179
	C_{15} sesquiterpanes		$C_{15}H_{28}$	123, 193
	C_{16} sesquiterpanes		$C_{16}H_{30}$	123, 193, 207
	Diamondoids			
	Adamantanes		$C_{10}H_{16}$, alkyl-$C_{10}H_{15}$	136, 135, 149, 163, 177
	Diamantanes		$C_{14}H_{20}$, alkyl-$C_{14}H_{19}$	188, 187, 201, 215, 229
	Terpanes			
1	C_{19} tricyclic terpane	TR19	$C_{19}H_{34}$	191
2	C_{20} tricyclic terpane	TR20	$C_{20}H_{36}$	191
3	C_{21} tricyclic terpane	TR21	$C_{21}H_{38}$	191
4	C_{22} tricyclic terpane	TR22	$C_{22}H_{40}$	191
5	C_{23} tricyclic terpane	TR23	$C_{23}H_{42}$	191
6	C_{24} tricyclic terpane	TR24	$C_{24}H_{44}$	191
7	C_{25} tricyclic terpane (a)	TR25A	$C_{25}H_{46}$	191
8	C_{25} tricyclic terpane (b)	TR25B	$C_{25}H_{46}$	191
9	triplet: C_{24} tetracyclic terpane + C_{26} (S + R) tricyclic terpanes	TET24 + TR26A + TR26B	$C_{24}H_{42}$ + $C_{26}H_{48}$	191
10	C_{28} tricyclic terpane (a)	TR28A	$C_{28}H_{52}$	191
11	C_{28} tricyclic terpane (b)	TR28B	$C_{28}H_{52}$	191
12	C_{29} tricyclic terpane (a)	TR29A	$C_{29}H_{54}$	191
13	C_{29} tricyclic terpane (b)	TR29B	$C_{29}H_{54}$	191
14	Ts: 18α(H),21β(H)-22,29,30-trisnorhopane	Ts	$C_{27}H_{46}$	191
15	17α(H),18α(H),21β(H)-25,28,30-trisnorhopane	TH27	$C_{27}H_{46}$	191, 177
16	Tm: 17α(H),21β(H)-22,29,30-trisnorhopane	Tm	$C_{27}H_{46}$	191
17	C_{30} tricyclic terpane 1	TR30A	$C_{30}H_{56}$	191
18	C_{30} tricyclic terpane 2	TR30B	$C_{30}H_{56}$	191
19	17α(H),18α(H),21β(H)-28,30-bisnorhopane	H28	$C_{28}H_{48}$	191, 163
20	17α(H),21β(H)-25-norhopane	NOR25H	$C_{29}H_{50}$	191, 177
21	17α(H),21β(H)-30-norhopane	H29	$C_{29}H_{50}$	191
22	18α(H),21β(H)-30-norneohopane (C_{29}Ts)	C29Ts	$C_{29}H_{50}$	191
23	17α(H)-diahopane	DH30	$C_{30}H_{52}$	191,
24	17α(H),21β(H)-30-norhopane (normoretane)	M29	$C_{29}H_{50}$	191
25	18α(H) and 18β(H)-oleanane	OL	$C_{30}H_{52}$	191, 412
26	17α(H),21β(H)-hopane	H30	$C_{30}H_{52}$	191
27	17α(H)-30-nor-29-homohopane	NOR30H	$C_{30}H_{52}$	191
28	17β(H),21α(H)-hopane (moretane)	M30	$C_{30}H_{52}$	191
29	22*S*-17α(H),21β(H)-30-homohopane	H31S	$C_{31}H_{54}$	191
30	22*R*-17α(H),21β(H)-30-homohopane	H31R	$C_{31}H_{54}$	191
31	Gammacerane	GAM	$C_{30}H_{52}$	191, 412
32	17β(H),21β(H)-hopane	(IS)	(Internal standard)	191
33	22*S*-17α(H),21β(H)-30,31-bishomohopane	H32S	$C_{32}H_{56}$	191
34	22*R*-17α(H),21β(H)-30,31-bishomohopane	H32R	$C_{32}H_{56}$	191
35	22*S*-17α(H),21β(H)-30,31,32-trishomohopane	H33S	$C_{33}H_{58}$	191
36	22*R*-17α(H),21β(H)-30,31,32-trishomohopane	H33R	$C_{33}H_{58}$	191
37	22*S*-17α(H),21β(H)-30,31,32,33-tetrakishomohopane	H314S	$C_{34}H_{60}$	191
38	22*R*-17α(H),21β(H)-30,31,32,33-tetrakishomohopane	H34R	$C_{34}H_{60}$	191
39	22S-17α(H),21β(H)-30,31,32,33,34-pentakishomohopane	H35S	$C_{35}H_{62}$	191
40	22*R*-17α(H),21β(H)-30,31,32,33,34-pentakishomohopane	H35R	$C_{35}H_{62}$	191
	Steranes			
41	C_{20} 5α(H),14α(H),17α(H)-sterane	S20	$C_{20}H_{34}$	217 & 218
42	C_{21} 5α(H),14β(H),17β(H)-sterane	S21	$C_{21}H_{36}$	217 & 218
43	C_{22} 5α(H),14β(H),17β(H)-sterane	S22	$C_{22}H_{38}$	217 & 218
44	C_{27} 20*S*-13β(H),17α(H)-diasterane	DIA27S	$C_{27}H_{48}$	217 & 218, 259
45	C_{27} 20*R*-13β(H),17α(H)-diasterane	DIA27R	$C_{27}H_{48}$	217 & 218, 259
46	C_{27} 20*S*-13α(H),17β(H)-diasterane	DIA27S2	$C_{27}H_{48}$	217 & 218, 259
47	C_{27} 20*R*-13α(H),17β(H)-diasterane	DIA27R2	$C_{27}H_{48}$	217 & 218, 259
48	C_{28} 20*S*-13β(H),17α(H)-diasterane	DIA28S	$C_{28}H_{50}$	217 & 218, 259
49	C_{28} 20*R*-13β(H),17α(H)-diasterane	DIA28R	$C_{28}H_{50}$	217 & 218, 259
50	C_{29} 20*S*-13β(H),17α(H)-diasterane	DIA29S	$C_{29}H_{52}$	217 & 218, 259
51	C_{29} 20*R*-13α(H),17β(H)-diasterane	DIA29R	$C_{29}H_{52}$	217 & 218, 259
52	C_{27} 20*S*-5α(H),14α(H),17α(H)-cholestane	C27S	$C_{27}H_{48}$	217 & 218
53	C_{27} 20*R*-5α(H),14β(H),17β(H)-cholestane	C27ββR	$C_{27}H_{48}$	217 & 218
54	C_{27} 20*S*-5α(H),14β(H),17β(H)-cholestane	C27ββS	$C_{27}H_{48}$	217 & 218
55	C_{27} 20*R*-5α(H),14α(H),17α(H)-cholestane	C27R	$C_{27}H_{48}$	217 & 218
56	C_{28} 20*S*-5α(H),14α(H),17α(H)-ergostane	C28S	$C_{28}H_{50}$	217 & 218
57	C_{28} 20*R*-5α(H),14β(H),17β(H)-ergostane	C28ββR	$C_{28}H_{50}$	217 & 218
58	C_{28} 20*S*-5α(H),14β(H),17β(H)-ergostane	C28ββS	$C_{28}H_{50}$	217 & 218
59	C_{28} 20*R*-5α(H),14α(H),17α(H)-ergostane	C28R	$C_{28}H_{50}$	217 & 218
60	C_{29} 20*S*-5α(H),14α(H),17α(H)-stigmastane	C29S	$C_{29}H_{52}$	217 & 218
61	C_{29} 20*R*-5α(H),14β(H),17β(H)-stigmastane	C29ββR	$C_{29}H_{52}$	217 & 218
62	C_{29} 20*S*-5α(H),14β(H),17β(H)-stigmastane	C29ββS	$C_{29}H_{52}$	217 & 218
63	C_{29} 20*R*-5α(H),14α(H),17α(H)-stigmastane	C29R	$C_{29}H_{52}$	217 & 218
64	C30 steranes	C30S	$C_{30}H_{54}$	217 & 218
	Monoaromatic steranes			253
	Triaromatic steranes			231

Table 3-2 Common Modifiers and Nomenclatures Used to Modify the Structural Specification of Cyclic Biomarkers

Modifier	*Description*	*Example Biomarker*
homo-	one additional carbon on the parent molecular structure	C_{31} 17α(H),21β(H)-30-homohopane
bis-, tris-, tetrakis-, pentakis- (also di-, tri-, tetra-, and penta-)	two to five additional carbons on the parent molecular structure	C_{32} 17α,21β-30,31-bishomohopane C_{33} 17α,21β-30,31,32-trishomohopane C_{34} 17α,21β-30,31,32,33-tetrakishomohopane C_{35} 17α,21β-30,31,32,33,34-pentakishomohopane
seco-	cleaved C-C bond	C_{24} 17,21-secohopane (tetracyclic)
nor-	one less carbon on the parent molecular structure	25-norhopane
bisnor-	two less carbons on the parent molecular structure	28,30-bisnorhopane
trisnor-	three less carbons on the parent molecular structure	25,28,30-trisnorhopane
neo-	methyl group shifted from C-18 to C-17 position on hopanes	C_{29}Ts: 30-norneohopane
α	asymmetric carbon in ring with "H" down	17α(H),21β(H)-hopane
β	asymmetric carbon in ring with "H" up	17β(H),21β(H)-hopane
R	asymmetric carbon in acyclic moiety of biomarkers obeying convention in a clockwise direction	C_{27} 20R cholestane
S	asymmetric carbon in acyclic moiety of biomarkers obeying convention in a clockwise direction	C_{27} 20S cholestane

*Modified from Peters and Moldowan (1993).

to indicate addition of a carbon from the parent carbon skeleton, for example, 30-homohopanes are identical to C_{30} hopane except that a methyl group has been added at C-30 position. If two to five carbons are added on the parent molecular structure, the prefixes "*bis*-", "*tris*-", "*tetrakis*-", and "*pentakis*-" are used, respectively. For more information on compound-naming protocols, see *Appendix IV* in the current *Chemical Abstract Index Guide* (CAS, 2002). *Chemical Abstract* uses these prefixes extensively for classes of terpenoids.

3.2.2.1 Stereoisomers

Isomers are different compounds that have the same molecular formula but the atoms are attached in different ways. There are two classes of isomers (Figure 3-2): (1) *constitutional isomers* and (2) *stereoisomers*. Constitutional isomers (or structural isomers) differ in their bonding sequence, and their atoms are connected differently and the number of *constitutional* isomers increases dramatically with the increase of carbon atoms in each compound. For example, there are two constitutional isomers of butane (C_4H_{10}: *n*-butane and isobutane), three isomers of pentane (C_5H_{12}: *n*-pentane, isopentane, and neopentane), respectively, five isomers of hexane, 18 isomers of octane, 75 possible isomers of decane, and 355 possible isomers of eicosane ($C_{20}H_{42}$), respectively.

Stereoisomers are isomers whose atoms are bonded together in the same sequence but differ from each other in the orientation of the atoms in space. Stereoisomers that are mirror images of each other (i.e., differing in the same manner as right and left hands) are called *enantiomers*; while all other stereoisomers, which are not mirror images, are *diaste-*

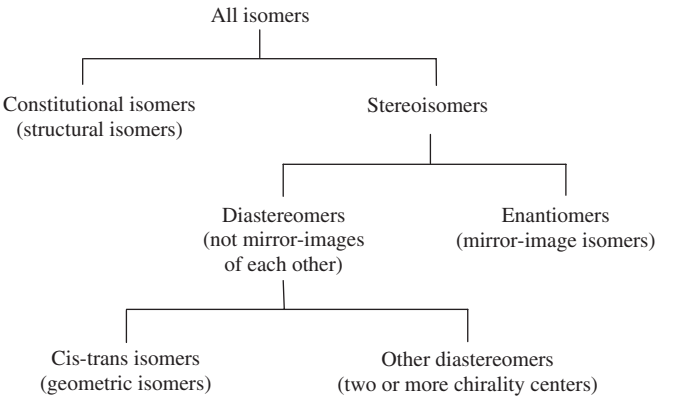


Figure 3-2 Types of isomers.

reomers. The *cis-trans* geometric isomers (such as *cis*- and *trans*-1,2-dimethylcyclopentane) are special types of *diastereomers*. Enantiomer molecules are not superimposable. Many pairs of biomarkers with the same molecular formula (such as 22R and 22S homohopane homologous series in C_{31} to C_{35} range) are *enantiomers*. Differences in special orientation might seem unimportant, but stereoisomers often have remarkably different physical, chemical, and biological properties.

3.2.2.2 Asymmetric (or Chiral) Carbons and α and β Stereoisomers

A carbon atom bonded to four different groups is called *asymmetric* carbon or a *chiral* carbon atom and is often designed by a *. For example, the possible chiral centers for steranes are at C-5, C-14, C-17, C-20, and C-24 (for C_{28} and C_{29} steranes).

As described in Table 3-2, hydrogen atoms attached to an *asymmetric* or *chiral* carbon in a ring structure and are below the plane of the molecule are called α hydrogens, and the bond is drawn with a dashed line and designated as having the α-configuration. Conversely, hydrogen atoms located above the plane of the molecule are called β hydrogens, and the bond is drawn with a wedge bond and designated as having β-configuration. In many common ring systems the α hydrogen atoms found at ring junctions are generally omitted for clarity. For example, in 17α(H), 21β(H)-hopane ($C_{30}H_{52}$, Figure 3-1) the hydrogens at carbon numbers 17 and 21 are down and up; while in 5α(H), 14β(H), 17β(H)-cholestane ($C_{27}H_{48}$, Figure 3-1) the hydrogens attached to carbon numbers 5, 14, and 17 are down, up, and up.

Previously, hopanes were considered to exist as three stereoisomers: 17α(H), 21β(H)-hopane, 17β(H), 21β(H)-hopane, and 17β(H), 21α(H)-hopane (Peters and Moldowan, 1993; Waples and Machihara, 1991). Hopanes in the βα series are also called moretanes. Hopanes with the αβ-configuration in the range of C_{27} to C_{35} are characteristic of petroleum because of their greater thermodynamic stability compared to other epimeric series (ββ and βα). Hopanoids produced by living organisms have generally a ββ-configuration. With increasing maturity the thermodynamically less stable ββ-hopanes are lost or converted to αβ- and βα-hopanes. The ββ series are, generally, not found in petroleum because it is thermally unstable. It was considered that the αα series were not natural products, and it is unlikely that they occur in more than trace levels in petroleum. However, mechanics calculations have shown that the αα-hopanes should be less stable than αβ- and βα-hopanes, but more stable than ββ-hopanes. Recently, Nytoft and Bojesen-Koefoed (2001) found that moderate quantities of 17α(H), 21α(H)-hopanes are present in several sediments and oils. The ratios of C_{30} 17α(H), 21α(H)-hopane to C_{30} 17α(H), 21β(H)-hopane are typically 0.02–0.04 in crude oils and mature sediments,

but ratios up to 0.10 have been found in immature sediments.

3.2.2.3 R and S Stereoisomers of Cyclic Biomarkers

Since chiral molecules are not superimposable on their *mirror images*, chirality is a necessary and sufficient condition for existence of enantiomers. Thus, a compound with at least one chiral carbon atom can exist as enantiomers, whereas a compound without chirality cannot exist as enantiomers. The Cahn–Ingold–Prelog convention procedure proposed by R. S. Cahn, C. Ingold, and V. Prelog (Cahn et al., 1966) is the most widely accepted system for naming the *configurations* (the arrangement of atoms that characterizes a particular stereoisomer of chiral centers). Each asymmetric carbon atom is assigned a letter (R) or (S) based on its three-dimensional configuration. To determine the stereoisomeric (R or S) configuration, two steps are involved: (1) following the *sequence rules* (Cahn et al., 1966), the *sequence of priority* is assigned to the four atoms or groups of atoms bonded to the asymmetric carbon atom. In the case of bromochloroiodomethane (CHClBrI), the four atoms attached to the chiral center are all different and priority depends on the atomic number, the atom of higher number having higher priority, thus, the sequence of priority is I, Br, Cl, H. (2) The molecule is oriented so that the group of lowest priority is directed away from the viewer. Subsequently, the remaining groups are arranged. If proceeding the remaining groups in a clockwise direction, that is, from the group of the highest priority to the group of second priority and then to the third, the configuration is specified **R** (Latin: *rectus*, meaning right); if counterclockwise, the configuration is specified **S** (Latin: *sinister*, meaning left). Thus, the compound CHClBrI has two stereoisomers and specified as the R and S enantiomers (their mirror images are nonsuperimposable), respectively.

For cyclic biomarkers, the use of R and S nomenclature is generally restricted to carbon atoms that are not part of a ring, while the use of α versus β nomenclature is used to describe asymmetric configurations at ring carbons. The steranes including the ones most abundant in oils: cholestanes ($C_{27}H_{48}$), ergostanes ($C_{28}H_{50}$), stigmastanes ($C_{29}H_{52}$) can have R- and S-configuration at the acyclic (chain position) carbon atom C-20, resulting in two homologue series with 20R (20R ααα and 20R αββ) and 20S (20S ααα and 20S αββ) configurations. Hopanes with 30 carbons or less show asymmetric centers at C-21 and all ring-juncture carbons including C-5, C-8, C-9, C-10, C-13, C-14, C-17, and C-18. Common homohopanes (C_{31} to C_{35}) have an extended side chain with an additional asymmetric center at C-22, resulting in two homologues with 22R and 22S configurations. These two homologous homohopanes (22R and 22S) can be well separated by GC-MS as well-resolved double peaks, prominent in gas chromatograms. The R and S and α versus β designations are a useful means of describing the relative configuration of biomarker compounds. It should, however, be noted that these designations are determined strictly on the basis of the convention as described by Cahn et al. (1966) without reference to optical rotation.

3.2.3 Analysis Methods for Biomarker Fingerprinting

In the last two decades, a wide variety of instrumental techniques have been developed and used for fingerprinting petroleum hydrocarbons including biomarkers (Wang et al., 1994a, 1995a; ETC Method, 2002; Wang and Fingas, 2003; Uhler et al., 1998–1999; Stout et al., 2002; Dimandja, 2004; Gains et al., 1999; Reddy et al., 2002; Frysinger et al., 2003). A variety of diagnostic ratios, especially ratios of PAH and biomarker compounds, for interpreting chemical data from oil spills have been proposed (Wang et al., 1999a; Stout et al., 2001, 2002; Daling et al., 2002). Many EPA and ASTM methods have been modified (such as the modified EPA Method 8015, 8260, 8270; and the modified ASTM Methods D3328, D5037, and D5739) in recent years to allow flexibility in the deployment of the "standard" analytical methods and to improve specificity

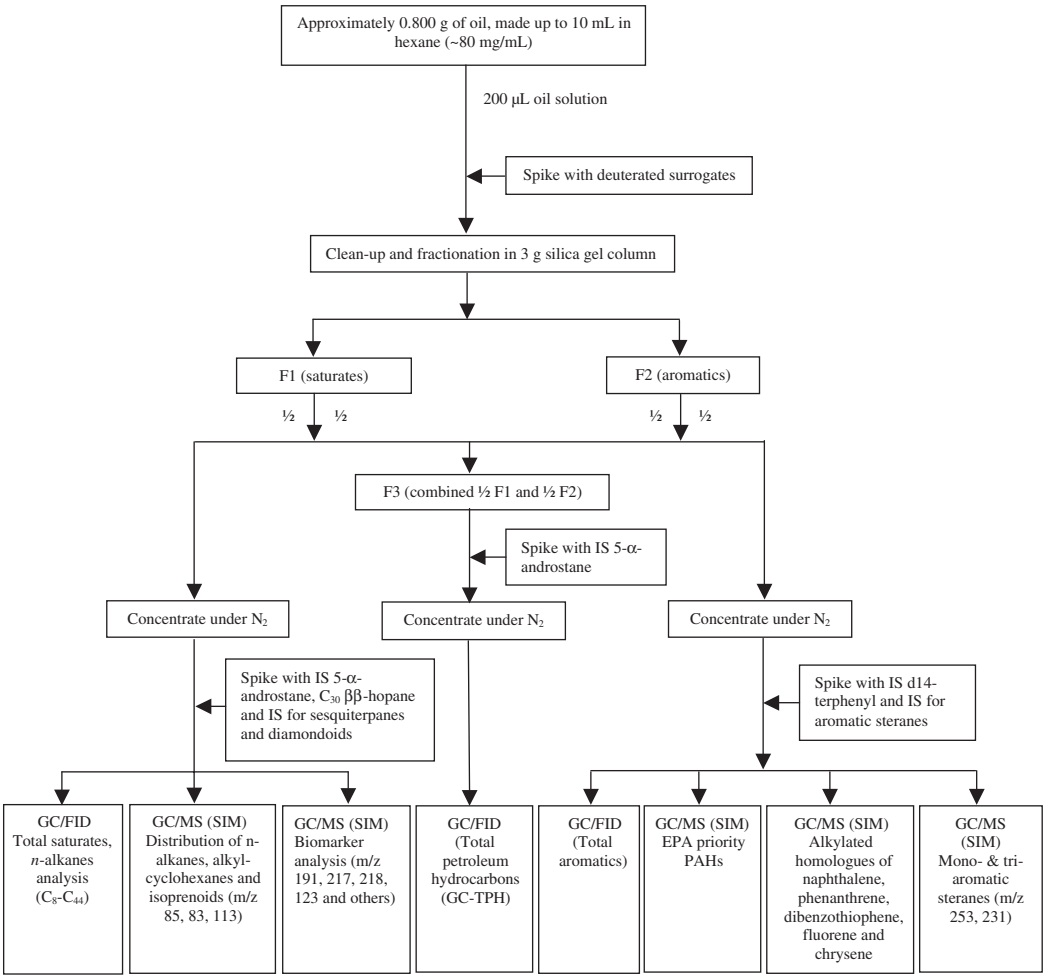


Figure 3-3 Oil sample preparation flowchart.

and sensitivity for measuring spilled oil and petroleum products in soil and water. As an example, Figure 3-3 shows a flowchart of sample preparation procedures used by the Environment Canada Oil Spill Research Laboratory. Table 3-3 summarizes the surrogate and internal standard compounds used for oil and biomarker fingerprinting at the same lab.

Silica gel is used frequently for cleanup and fractionation of oil extracts. The column cleanup procedure used by the Environment Canada Oil Research Laboratory is the following: a chromatographic column with a Teflon stopcock (200 × 10.5 mm i.d.) is plugged with Pyrex glass wool at the bottom, serially rinsed with methanol, hexane, and dichrolomethane, and allowed to dry. The column is dry-packed with 3 g of activated silica gel and topped with about 1-cm anhydrous granular sodium sulfate. Columns are then preconditioned using 20 mL of hexane. Just prior to exposure of the sodium sulfate layer to air, appropriate volumes of the oil solutions or concentrated oil extracts are transferred quantitatively to the column (Wang et al., 1994a; ETC Method, 2002). Saturated hydrocarbons are eluted with 12 mL of hexane (Fraction 1, labeled F1). Aromatic hydrocarbons are eluted with 15 mL of hexane: dichloromethane (v/v, 1 : 1, Fraction 2, labeled

Table 3-3 Surrogate and Internal Standard Used for Oil and Biomarker Fingerprinting

Compounds	*Chemical Names*	*Target Ions (m/z)*
Surrogates	*o*-terphenyl (for TPH determination by GC/FID)	
	mixture of d_{10}-acenaphthalene, d_{10}-phenanthrene, d_{12}-benz[a]anthracene, and d_{12}-perylene	164, 188, 240, 264
Internal standards	5-α-androstane (for TPH determination by GC/FID)	
	d_{14}-terphenyl (for quantitation of PAHs)	244
	C_{30} ββ-hopane (for quantitation of terpanes and steranes)	191
	d_3-monoaromatic steranes [5α(H)/5β(H), $C_{21}H_{27}D_3$] (for quantitation of mono- and tri-aromatic steranes)	285
	d_{18}-decahydronaphthalene (cis-) (for quantitation of sesquiterpanes)	156
	d_{16}-adamantane (for quantitation of diamondoids)	152

F2). Saturated biomarkers are eluted with other saturates in F1. Aromatic steranes are eluted in the aromatic fraction, F2. Polar compounds are eluted with 15 mL of methanol (labeled F4). For each sample, half of F1 is used for analysis of the total GC-detectable saturates, *n*-alkanes and isoprenoids, and biomarker compounds; and half of F2 is used for analysis of alkylated PAH homologues and other EPA priority parent PAHs, and aromatic steranes. The remaining halves of the F1 and F2 are combined into one fraction (Fraction 3, labeled F3) and used for the determination of TPH and UCM. The three fractions are concentrated under a stream of nitrogen to appropriate volumes, spiked with appropriate internal standards, and then adjusted to an accurate pre-injection volume (1.00 mL) for GC-FID and GC-MS analyses.

In accordance of the quality assurance (QA) and quality-control (QC) programs (Page et al., 1995; Douglas et al., 2004; Wang et al., 1999a; Stout et al., 2002; Faksness et al., 2002; EPA, 1997, 1998a, 1998b, 2001; ASTM, 1997a, 1997b), the GC-MS must be calibrated using the terpane standards prior to quantification of the biomarkers in oil. In the Environment Canada Oil Research Laboratory the terpane standards (Table 3-3) include C_{27} 17α(H)-22,29,30-trisnorhopane, C_{29} 17β(H), 21α(H)-30-norhopane, and C_{30} 17β(H), 21α(H)-hopane. The sterane standards include C_{21} 5β(H)-pregnane, C_{22} 20-methyl-5α(H)-pregnane, and the series of C_{27}, C_{28}, and C_{29} steranes. The C_{30} 17β(H), 21β(H)-hopane is used as the internal standard for quantification of tri- to pentacylic biomarkers. The response factors (RRF) are determined relative to the internal standard C_{30} 17β(H), 21β(H)-hopane. In most cases, the average RRF for C_{30} 17β(H), 21α(H)-hopane at *m/z* 191 are used for quantification of C_{30} 17α(H), 21β(H)-hopane, and other terpanes (in the range of C_{19} to C_{35}). For steranes, the average RRF of C_{29} 20R-ααα-ethylcholestane at *m/z* 217 relative to the internal standard are used to calculate the concentrations of sterane compounds. The deuterated d_3 monoaromatic steranes [5α(H)/5β(H), $C_{21}H_{27}D_3$] are used as internal standards for quantification of monoaromatic and triaromatic steranes. Certified sesquiterpane standards are not commercially available. The average response factors of *cis*-decahydronaphthalene ($C_{10}H_{18}$, *m/z* 138) and 1-methyldecaline ($C_{11}H_{20}$, *m/z* 152), which have similar molecular structures to those of the sesquiterpanes, relative to the internal standard *cis*-decahydronaphthalene-d_{18} (*m/z* 156) were used for quantitation of sesquiterpanes. The d_{16}-adamantane is used as the internal standard for quantification of diamondoid compounds.

3.2.4 Capillary Gas Chromatography — Mass Spectrometry (GC-MS)

GC-MS is the principal instrument used for characterizing biomarkers. Early use of mass chromatograms in organic geochemistry was pioneered at Chevron and led to a stereochemical understanding of steroids and the

first practical method of oil fingerprinting based on terpanes and steranes (Seifert, 1977). Today, computerized GC-MS (e.g., benchtop quadrupole GC-MS, high-resolution GC-MS, GC-ion trap MS, and GC-MS-MS) has become the routine technique used in most oil and environmental forensics laboratories to analyze a wide range of petroleum hydrocarbons.

3.2.4.1 Benchtop Quadrupole GC-MS

The quadrupole is the most common mass separator in use today. The benchtop quadrupole GC-MS systems, although lacking the high-resolution capabilities of larger and more expensive magnetic-sector instruments, have sufficient sensitivity and selectivity for most purposes of biomarker analysis. Most benchtop GC-MS use a quadrupole mass filter to separate ions produced from gaseous neutral molecules or species in the ionization chamber. In a high vacuum, ions pass down the lengths of four parallel metal rods to which are applied both a constant voltage and a radio-frequency oscillating voltage. The electric field deflects ions in complex trajectories as they migrate from the ionization chamber toward the detector, allowing only ions with one particular mass-to-charge (*m/z*) ratio to reach the detector at any instant. Other nonresonant ions collide with the rods and are lost before they reach the detector. By rapidly varying the applied voltages, ions of different masses are selected to reach the detector. A wide range of masses can be recorded in less than 1 second. In this way many mass spectra are taken and stored on a computer as the components of the sample pass from the chromatographic column into the mass spectrometer. Benchtop quadrupole GC-MS can be operated in various modes including (full) scan and selected ion monitoring (SIM).

3.2.4.1.1 Scan Mode. In scan mode, sometimes called *full scan mode*, the mass spectrometer is used to scan (that is, to measure) the entire range of ions generated in the ion source. As the MS detector scans through a predefined mass range (e.g., 50–700 amu), a mass spectrum is generated. Full scan records hundreds of ions per scan (typically, greater than 500 ions per scan are recorded in 3 seconds; the larger the mass range, the fewer scans per second), but with lower sensitivity due to shorter dwell time in comparison with the SIM mode. Each peak that elutes from the GC yields a particular distribution of fragment ion masses. Among these ions generated from the scan, there are always several ions being the most characteristic and diagnostic of the molecule or the compound type, and the most abundant ion in the mass spectrum is called the *base peak*. The magnitude of the total ion current for all mass spectra in an oil sample is generally plotted versus the GC retention time on a total ion chromatogram (TIC) to show a series of peaks that represent relative amounts of components in the sample. Identification and characterization of petroleum hydrocarbons are largely based on the full mass spectral data for structural elucidation, comparison of GC retention data with that of reference standards, recognition of distribution pattern, calculation of retention indexes (RI), and comparison with literature RI values.

3.2.4.1.2 Selected Ion Monitoring (SIM) Mode. In the SIM mode, only a limited number of characteristic ions (for example, the *base peaks* 191, 217, and 218, and those *m/z* values diagnostic for molecule structural elucidation for target terpanes and steranes) are monitored. For quantification of individual target compounds, the SIM mode is used most frequently, since it shows several advantages in comparison to the scan mode: (1) SIM only records a few selected *m/z* per scan, resulting in a much longer dwell time for each monitored ion (usually between 25 and 100 milliseconds, depending on the number of *m/z* selected) than in the scan mode; (2) method detection limits for target analytes are generally lower by almost an order of magnitude than those produced by the full scan GC-MS; (3) the use of the SIM mode is often less noisy and the linear quantification range is increased for trace analytes. As examples, the following

briefly describe the analytical GC-MS conditions used by the Environment Canada Oil Spill Research Lab and Petrobras Geochemistry Laboratory (Barbanti, 2004), respectively.

3.2.4.1.3 Example Benchtop GC-MS Conditions (EC Oil Spill Research Laboratory). Analyses of biomarkers are performed on an Agilent 6890 GC coupled with an Agilent 5973 mass selective detector (MSD). System control and data acquisitions are achieved with the Agilent G1701 BA MSD ChemStation. A 30 m × 0.25 mm i.d. (0.25-µm film thickness) HP-5MS fused-silica capillary column is used. The chromatographic conditions are as follows: carrier gas, helium (1.0 mL/min); injection mode, splitless; injector and detector temperature, 280 and 300°C, respectively. The temperature program employed for biomarkers and alkylated PAHs is 50°C hold for 2 min, then ramp at 6°C/min to 300°C and hold for 20 minutes. Prior to sample analysis, the GC-MS is tuned with perfluorotributylamine (PFTBA). The total run time is 60 minutes.

3.2.4.1.4 Example Benchtop GC-MS Conditions (Petrobras Geochemistry Laboratory). A 60 m × 0.25 mm i.d. (0.25-µm film thickness) HP-5MS or equivalent 60-m capillary column is used to achieve improved resolution for biomarkers (Barbanti, 2004). The temperature program is as follows: 55°C hold for 2 min, ramp at 20°C/min to 150°C and then 1.5°C to 310°C and hold for 15 minutes. The total run time is 128 minutes.

The 30-m capillary column is used in many environmental forensic labs for most oil spill work. However, the 60-m capillary column with a slow temperature rate and longer running time offers further improved resolution for some paired biomarker isomers, which may not be well resolved by the use of a 30-m column.

3.2.4.2 Triple Quadrupole GC-MS-MS

The combination of two or more MS analyzers, commonly known as MS-MS or tandem mass spectrometry, is a highly specific means of separating mixtures and studying molecular fragments. In the first MS, one ion is isolated and subsequently in the second MS, reactions of that ion are studied further. GC-MS-MS includes linked and de-linked double focusing and triple quadrupole mass spectrometry. Triple quadrupole mass spectrometers are the most common type of tandem mass spectrometers. The first (or parent) and the third (or daughter) quadrupole are MS-1 and MS-2, whereas the second quadrupole in the middle acts as the collision cell. In the collision cell, the transmitted ions formed in the ion source and selected by or passed through MS-1 undergo low-energy collision with an inert gas such as argon. The fragment ions or daughter ions formed in the collision cell are selectively monitored by the daughter quadrupole and recorded using an electron multiplier. Because of the use of three linked quadrupoles, triple quadrupole mass spectrometry allows determination of specific parent–daughter relationships with less interference from other reactions and their related ions and, therefore, increases signal-to-noise ratios and offers improved selectivity for biomarker analysis. Triple quadrupole mass spectrometers can be operated in three GC-MS-MS modes (Linscheid, 2001): (1) precursor (parent) ion scan mode; (2) product (daughter) ion scan mode; and (3) neutral loss scan mode.

In the precursor (parent) ion scan mode, the first quadrupole is scanned and only one or more product ions (daughter ions) are selected and recorded. For example, parent ions of the C_{30}—C_{35} hopanes consist of 412, 426, 440, 454, 468, and 482, respectively. Each of these parent ions produces a major daughter ion at *m/z* 191 following collision with the inert gas in the collision cell. By the same mechanism, parent ions of common sterane homologous compounds (C_{27}—C_{28}—C_{29}—C_{30} steranes) produce major daughter ions at *m/z* 217 and 218. Both parent and daughter ions can be selectively monitored to improve signal-to-noise ratio. In comparison with the routine benchtop GC-MS, the GC-MS-MS technique offers a significant refinement for biomarker separation. For example, monitoring the frag-

ment ions at *m/z* 217 by benchtop GC-MS in SIM mode provides a single mass chromatogram for all steranes (from C_{27} to C_{30}) in an oil sample, many of which co-elute. However, by specifying parent and daughter ions (such as from *m/z* 386 to *m/z* 217 for C_{28} steranes), triple quadrupole GC-MS-MS can provide nearly complete separation of an individual sterane family by carbon number. This approach has been successfully used for identification of a biomarker and a biomarker family, product screening, and distribution pattern recognition of biomarkers.

In the product (daughter) ion scan mode, only one precursor (parent) ion is selected and enters the collision cell. The second MS analyzer scans for all product (daughter) ions produced in the collision cell. This type of scan is often used to analyze the fragmentation pattern of a component with specific molecular weight in a complex mixture without interference from any co-eluting compound of different molecular mass.

In the neutral loss scan mode, both analyzers are scanned with the selected mass difference, and for reaction monitoring only one precursor and one product ion species are permitted to travel through MS-1 and MS-2, respectively. This approach can be particularly useful in the search for specific compounds derived from a certain precursor compound.

3.2.5 Mass Spectra and Identification of Biomarkers

Mass spectra produced by GC-MS are one of the most valuable tools for identification of unknown compounds. In addition to molecular formula, the mass spectrum provides structural information of a given molecule. An electron with typical energy of 70 eV (1610 kcal/mol or 6740 kJ/mol) has far more energy than needed to ionize a molecule. Much work on the isolation and identification of individual biomarker components in oils and sediment extracts has been done by petroleum geochemists. The Chevron Biomarker Laboratory developed a *coinjection and mass spectra matching* technique, in combination with other analytical techniques, for provisional identification of unknown biomarker compounds (Peters and Moldowan, 1993).

As an example, Figure 3-4 shows mass spectra for several common petroleum biomarkers used in environmental forensic studies. These figures show that common features of the mass spectra of terpanes, steranes, monoaromatic steranes, and triaromatic steranes: a large parent ion (M^+), an important parent minus a methyl ion ($M^+ - 15$), and a base peak at *m/z* 191, 217 and 218, 253, and 231, respectively. C_{30} 17β(H), 21β(H)-hopane has, for example, a characteristic parent ion, parent minus methyl ion, and base peak at 412, 397, and 191, respectively. C_{27} 20R ααα-cholastane has a characteristic parent ion, parent minus methyl ion, and base peak at 372, 357, and 217, respectively; while C_{27} 20R αββ-cholastane has a characteristic parent ion, parent minus methyl ion, sterane-characteristic ion, and base peak at 372, 357, 217, and 218, respectively. Adamantane, which is very stable under typical electron impact ionization conditions, has both its base peak and parent ion at *m/z* 136.

The *m/z* 191 fragment is often the base peak of mass spectra of cyclic terpanes. It is derived from rings (A+B) of the molecule, but rings (D+E) may also be the source. The *m/z* 177 fragment is most likely derived from rings (A+B) of triterpane molecules that have lost a methyl group from position 10, that is, 25-norhopanes (Volkman et al., 1983a, 1983b; Grahl-Nielsen and Lygre, 1990). The notable feature of mass spectra for 25-demethylated hopanes is that the *m/z* 177 fragment has higher intensity than the *m/z* 191 fragment. Demethylated triterpanes contain different information than the triterpanes and have been suggested as markers for biodegradation (Volkman et al., 1983a). The other triterpanes do also give the *m/z* 177 fragment upon electron impact in the mass spectrometer, but in lower abundance than the *m/z* 191 fragment. The fragment is formed by the loss of CH_2 from the *m/z* 191 fragment and can be seen in all mass spectra of triterpanes. The biomarker

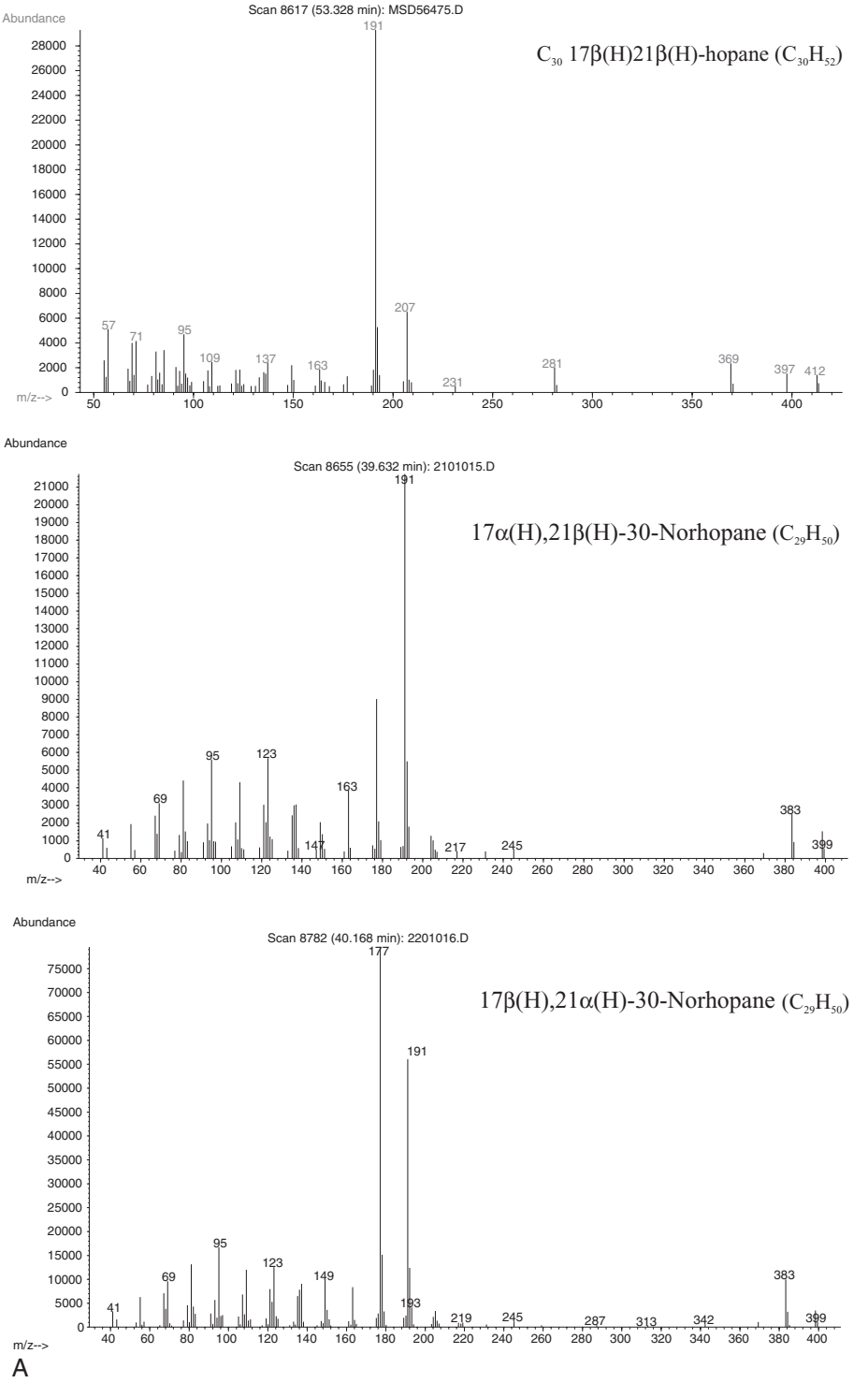


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Figure 3-4 Mass spectra of some common biomarkers used in environmental forensic studies.

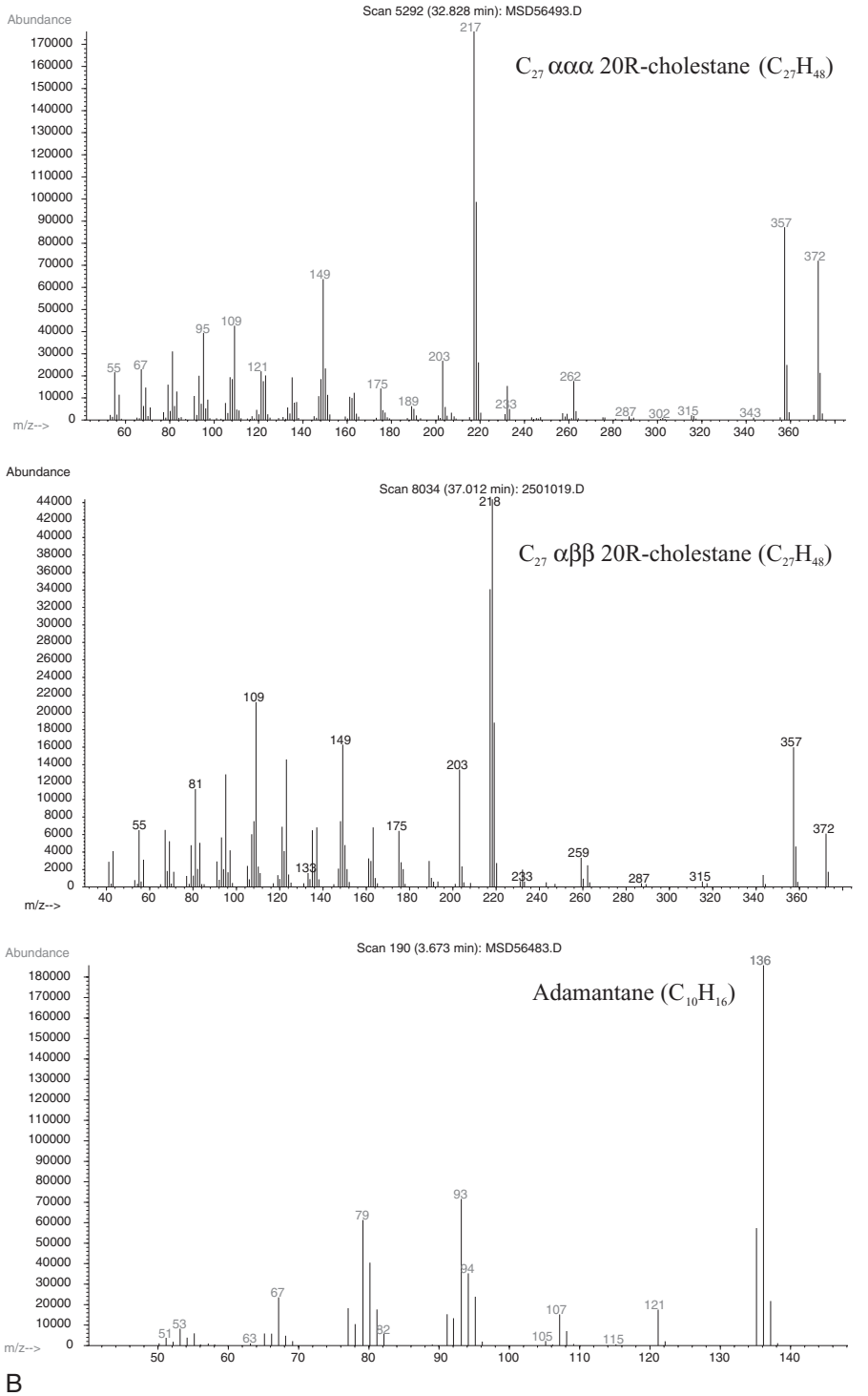


Figure 3-4, continued

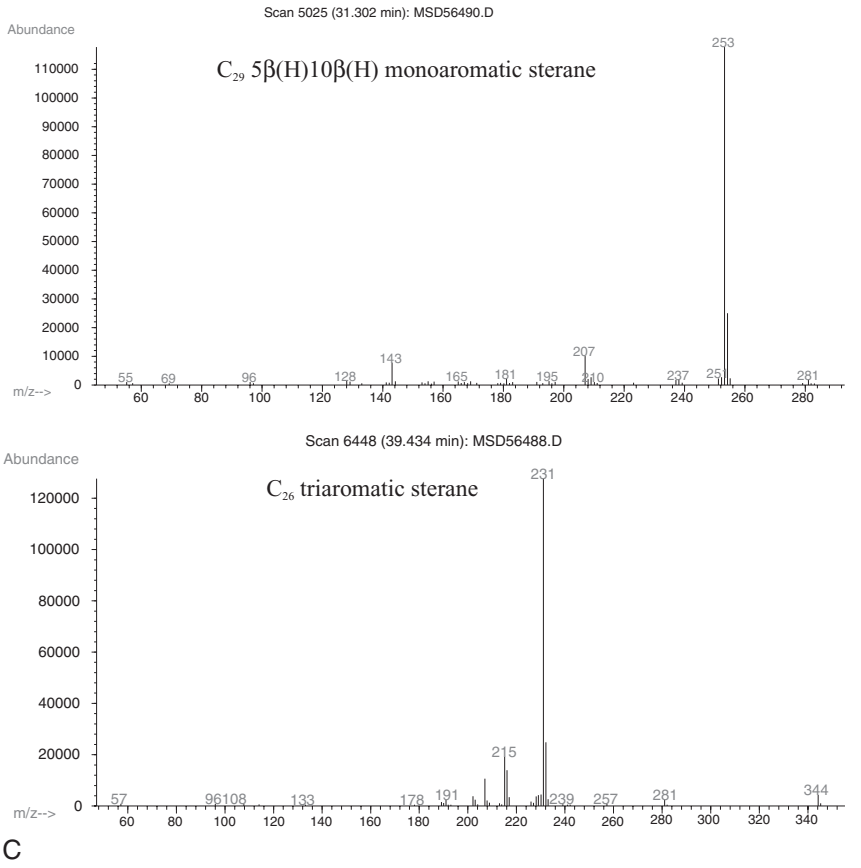


Figure 3-4, continued

C_{29} 18α(H), 21β(H)-30-norneohopane (or C_{29}Ts), which elutes immediately after C_{29} αβ-hopane, has a greater abundance of the *m/z* 177 ion than of the *m/z* 191 ion. The proposed mechanism is that the *m/z* 177 ion is derived from the (D+E) ring fragment and stabilized by the methyl group at position 14 (Gallegos, 1971; Killops and Howell, 1991). Increased stability of the (D+E) ring fragment producing the *m/z* 177 ion relative to that generating the *m/z* 191 ion may relate to ring junction configuration or to methyl substitution position in the (D+E) ring. For the similar mechanism, C_{29} 17β(H), 21α(H)-30-norhopane (normoretane) also shows significantly greater abundance of the *m/z* 177 ion than of the *m/z* 191 ion [see the mass spectrum of C_{29} 17β(H), 21α(H)-30-norhopane in Figure 3-4].

The *m/z* 217 and 218 fragment ions are derived from rings (A+B+C) of most 14α(H)- and 14β(H)-steranes. The βαα and ααα steranes have a base peak at *m/z* 217, while the base peak of αββ steranes is at *m/z* 218. The relative intensities of *m/z* 149 to *m/z* 151 fragment ions in the mass spectra of steranes have been used to distinguish between 5α- and 5β-stereoisomers (Gallegos, 1971). Note that the only significant difference between the mass spectra of 5α- and 5β-epimers is that the *m/z* 149 fragment is more abundant than the *m/z* 151 moiety for the 5α-epimer (e.g., 5α-cholestane versus 5β-cholestane, $C_{27}H_{48}$). Furthermore, the GC retention time of the 5β epimer is shorter than that of the 5α isomer. Hence, the stereo configuration of 5α- and 5β-steranes having the same parent ion, a parent

ion minus a methyl ion, and a base peak at *m/z* 217 can be determined from the peak ratio at *m/z* 149 to *m/z* 151. If the ratio of the *m/z* 149 ion to the *m/z* 151 ion is greater than 1, it is 5α-sterane; otherwise, it is 5β-sterane.

The SIM chromatogram of one ion of given *m/z* with the GC retention time is often diagnostic of a class of homologous compounds with similar structures but different carbon numbers and isomerism and can be used for identification. As an example, Figure 3-5 shows the SIM chromatograms for common biomarker classes (terpanes at 191 and steranes at 217) in a Kuwait crude oil obtained by using a 60-m column (Barbanti, 2004). Thirty-eight terpanes from C_{19} tricyclic terpane to C_{35} homohopanes (*m/z* 191) and 19 steranes from C_{21} to C_{29} steranes (*m/z* 217) in total have been unambiguously identified and characterized in this Kuwait oil (refer to Table 3-1 for peak identity). Paired biomarker isomers (H29 and C29Ts, H30 and NOR30H) and triplet (TET24 + TR26A + TR26B) are well resolved. Less abundant C_{30} steranes can be clearly recognized as well.

Identification of Vegetation Biomarkers. In addition to petroleum biomarkers, oil-contaminated sediment samples may contain modern plant biomarker compounds that represent oxygenated or unsaturated equivalents of biomarkers found in oil. Identification of these biogenic biomarkers of sediment extracts can often provide valuable information about the nature and source of samples. For example, in the Nipisi spill study (Wang et al., 1998a), three unknown vegetation biomarker compounds with significant abundances were detected. They were positively identified as 12-oleanene ($C_{30}H_{50}$, MW = 410.7, RT = 42.27 min), 12-ursene ($C_{30}H_{50}$, MW = 410.7, RT = 42.74 min), and 3-friedelene ($C_{30}H_{50}$, MW = 410.7, RT = 44.26 min). Formation of a six-membered ring E from the baccharane precursor leads to the oleanane group. Oleananes and their derivatives form the largest group of triterpenoids and occur widely in the plant kingdom (Connolly and Hill, 1991). The friedelene-type triterpenoids arise by increasing degrees of backbone rearrangement of the oleanene skeleton. Methyl migration in ring E of the oleanene precursor leads to the ursene skeleton (Connolly and Hill, 1991).

3.3 Fingerprinting Petroleum Biomarkers

Characterization of *n*-alkanes is achieved using GC-FID and GC-MS at *m/z* 85, 71, and 57, while characterization of major biomarker groups is achieved using GC-MS at their diagnostic fragment ions (Table 3-4). Various biomarkers occur in different carbon ranges of crude oils (Figure 3-6).

3.3.1 Biomarkers in Crude Oils

Depending on the oil sources and the geological migration conditions, crude oils can have (1) large differences in distribution patterns of the *n*-alkane and cyclic-alkanes as well as UCM profiles, (2) significantly different relative ratios of isoprenoids to normal alkanes, and (3) large differences in distribution patterns and concentrations of alkylated PAH homologues and biomarkers. For many oils, their GC-MS chromatograms of terpanes at *m/z* 191 are characterized by the terpane distribution in a wide range from C_{19} to C_{35} often with C_{29} αβ- and C_{30} αβ-pentacyclic hopanes and C_{23} and C_{24} tricyclic terpanes being often the most abundant. As for steranes (at *m/z* 217 and 218), the dominance of C_{27}, C_{28}, and C_{29} 20S/20R homologues, particularly the epimers of αββ-steranes, among the C_{20} to C_{30} steranes is often apparent. As examples, Figures 3-7 and 3-8 show GC-MS chromatograms at *m/z* 191, 217, and 218 for five light (API >35) to medium (API: 25–35) crude oils. For comparison, Figures 3-9 and 3-10 present biomarker fingerprints for five heavy oils including California API 15, Sockeye and Platform Elly from California, and Boscan and Orinoco from Venezuela. Table 3-5 summarizes the quantification results of major target biomarkers in these light, medium, and heavy oils.

In addition to composition, the concentrations of biomarkers can vary widely with the

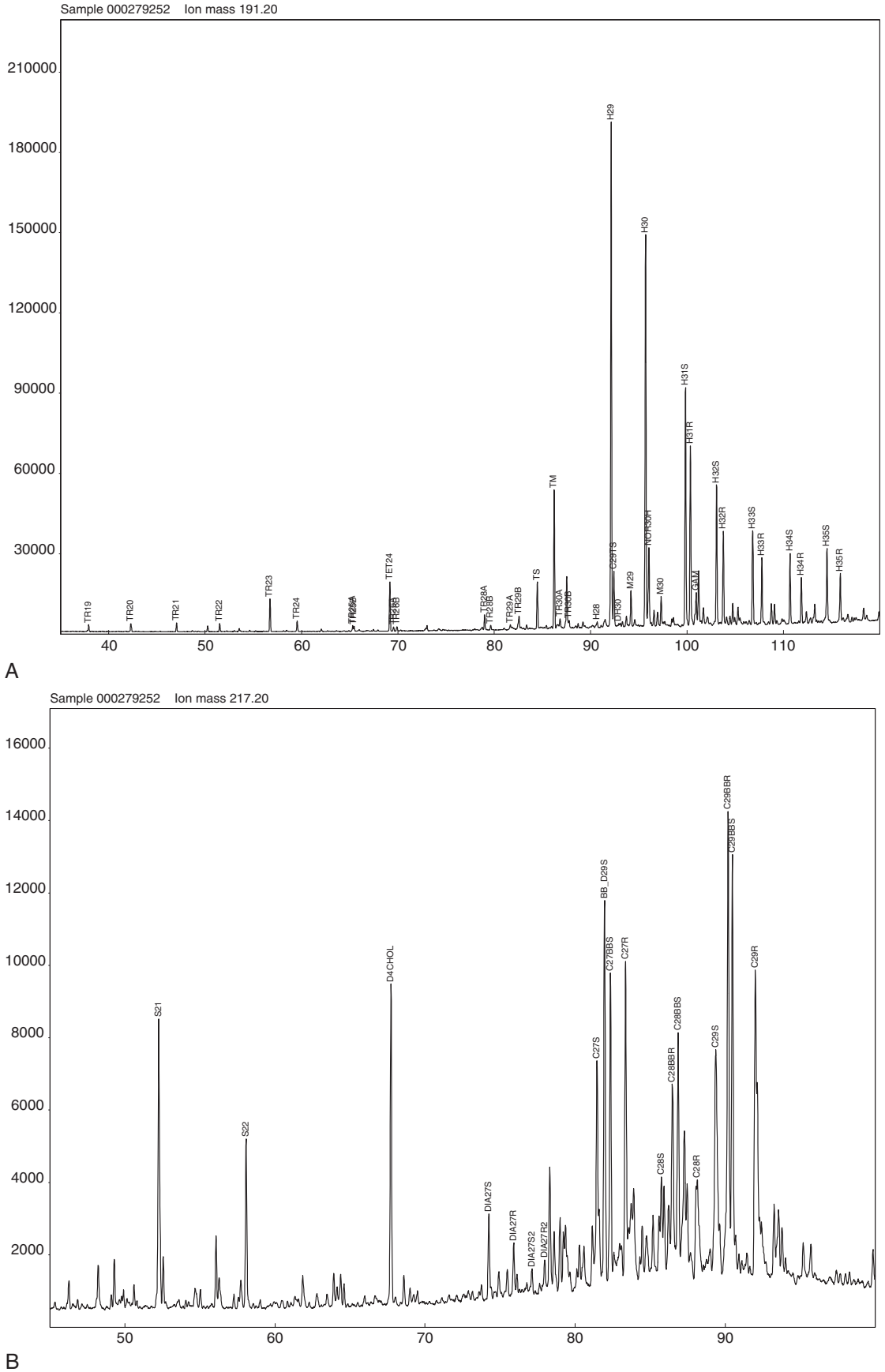


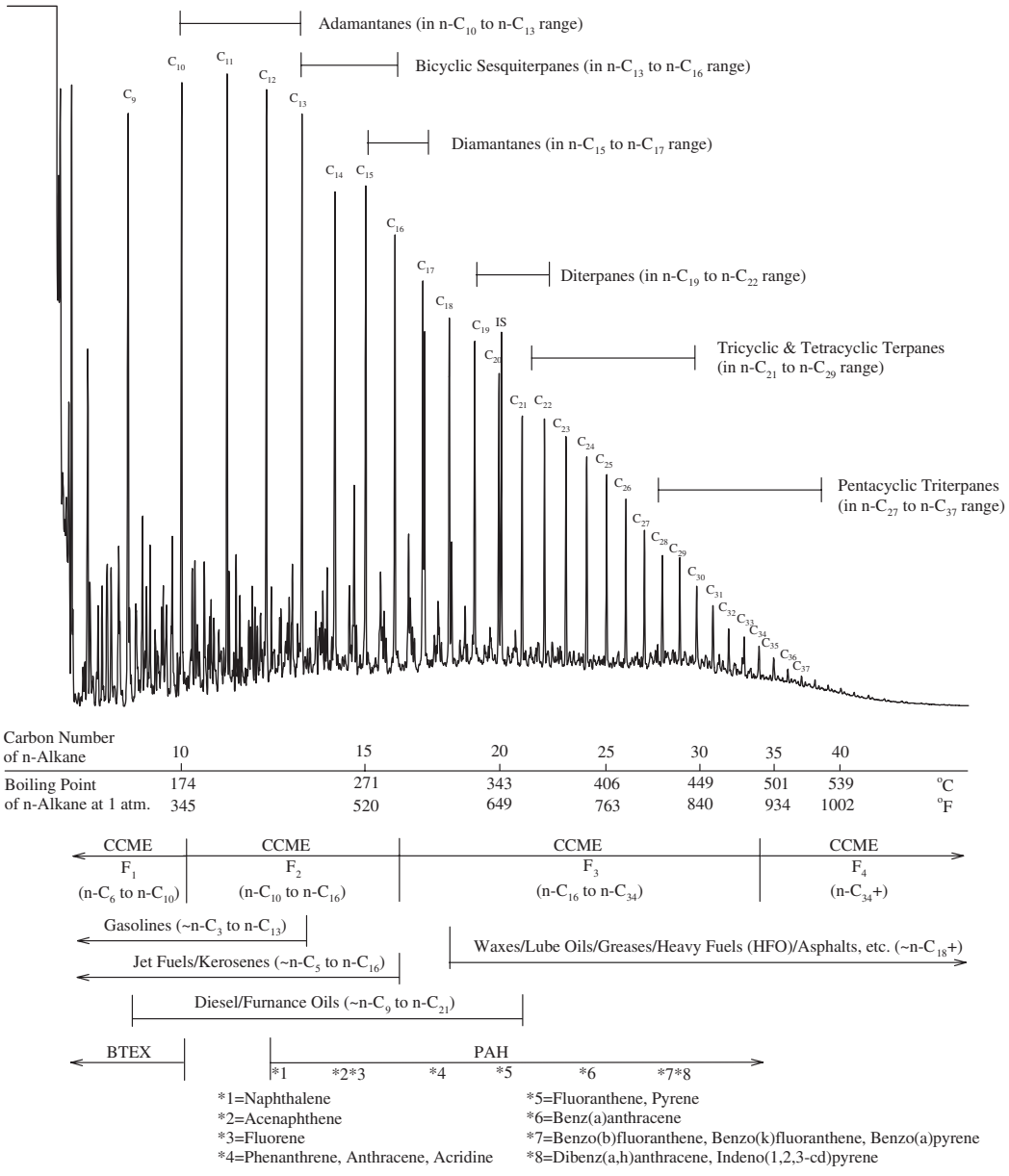
Figure 3-5 The SIM chromatograms at *m/z* (a) 191 and (b) 217 of a Kuwait crude oil for common terpane and sterane biomarker classes.

Table 3-4 Characteristic Fragment Ions for Various Biomarkers

Biomarkers	*Diagnostic Fragment Ions*
Acyclic terpenoids	
alkyl-cyclohexanes	83
methyl-alkyl-cyclohexanes	97
isoprenoids	113, 127, 183, M^+
Cyclic terpenoids	
sesquiterpanes with drimane structure	123
adamantanes	135, 136, 149, 163, 177, 191
diamantanes	187, 201, 215, 229
tri-, tetra-, penta-cyclic terpanes	191, M^+
25-norhopanes	177, 191
28, 30-bisnorhopanes	163, 191
steranes	217, 218
5α(H)-steranes	149, 217, 218
5β(H)-steranes	151, 217, 218
diasteranes	217, 218, 259
methyl-steranes	217, 218, 231, 232
Aromatic steranes	
monoaromatic steranes	253, 267
triaromatic steranes	231, 245

type of depositional environment (noxic/anoxic, freshwater/marine/hypersaline), type of organic matter (e.g., terrigenous origin or marine origin), maturity and biodegradation as well (see Chapter 1 herein). For a given type of organic material, the biomarker concentrations generally decrease with increasing thermal maturity. Very light oils or condensates (e.g., the Scotia Light) typically contain low concentrations of detectable biomarkers. In most cases, characterization of biomarkers should include determination of both absolute concentrations and relative fingerprinting distributions, and should not be just measuring peak ratio alone. This is important because it is possible to have a situation where a source might have a similar biomarker ratio but very different actual amounts of biomarkers. Quantitative determination of biomarkers is also critical in oil spill studies involving recognition and/or allocation of mixtures of different oils (e.g., see Chapter 8).

Figures 3-7 to 3-10 and Table 3-5 qualitatively and quantitatively demonstrate differences in biomarker distributions between 10 oils. Different from most crude oils, the Scotia Light (API = 59) only contains trace amounts of biomarkers [the total concentration of target biomarkers (i.e., terpanes and steranes) is only 29 μg/g oil], far lower than the corresponding values for other crude oils. The Alaska North Slope (ANS) oil contains a wide range of terpanes from C_{20} tricyclic terpane to C_{35} pentacyclic terpanes with the C_{30} αβ hopane as the most abundant, followed by C_{29} αβ hopane. The triplet C_{24} tetracyclic + C_{26} (S + R) tricyclic terpanes are highly abundant as well. In contrast, the Arabian Light, South Louisiana, and Troll oils have terpanes largely located in the C_{27} to C_{35} pentacyclic hopane range, and only contain small amounts of C_{20} to C_{24} tricyclic terpanes. In addition, the abundance of C_{29} αβ hopane is higher than that of C_{30} αβ hopane in Arabian Light crude oil. The steranes are present in all five light to medium crude oils but with different distribution patterns. The characteristic V-shaped C_{27}—C_{28}—C_{29} regular αββ sterane (*m/z* 218) distribution is clearly demonstrated, which indicates high thermal maturity. The relative abundances of C_{27}—C_{28}—C_{29} steranes in oils reflect the carbon number distribution of the sterols in the precursor organic matter in the source rocks for these oils. In general, a dominance of



Note: The carbon ranges of the four fractions, F1 to F4, are defined in the Tier 1 TPH analytical method by the Canadian Council of Ministers of the Environment (CCME).

Figure 3-6 Carbon range of common cyclic biomarker classes in crude oil and petroleum products.

C_{27} over C_{29} steranes specifies marine algae organic matter input, while a predominance of C_{29} steranes over C_{27} steranes may indicate a preferential higher plant input (Peters and Moldowan, 1993; Gürgey, 2002). The ANS, South Louisiana, and Troll oils contain higher amounts of diasteranes as well as C_{21} and C_{22} regular steranes. By contrast, the Arabian Light has much lower concentrations of steranes in total (the total of C_{27}—C_{28}—C_{29} αββ steranes is only 110 μg/g oil) but displays significantly higher concentration of C_{29} αββ

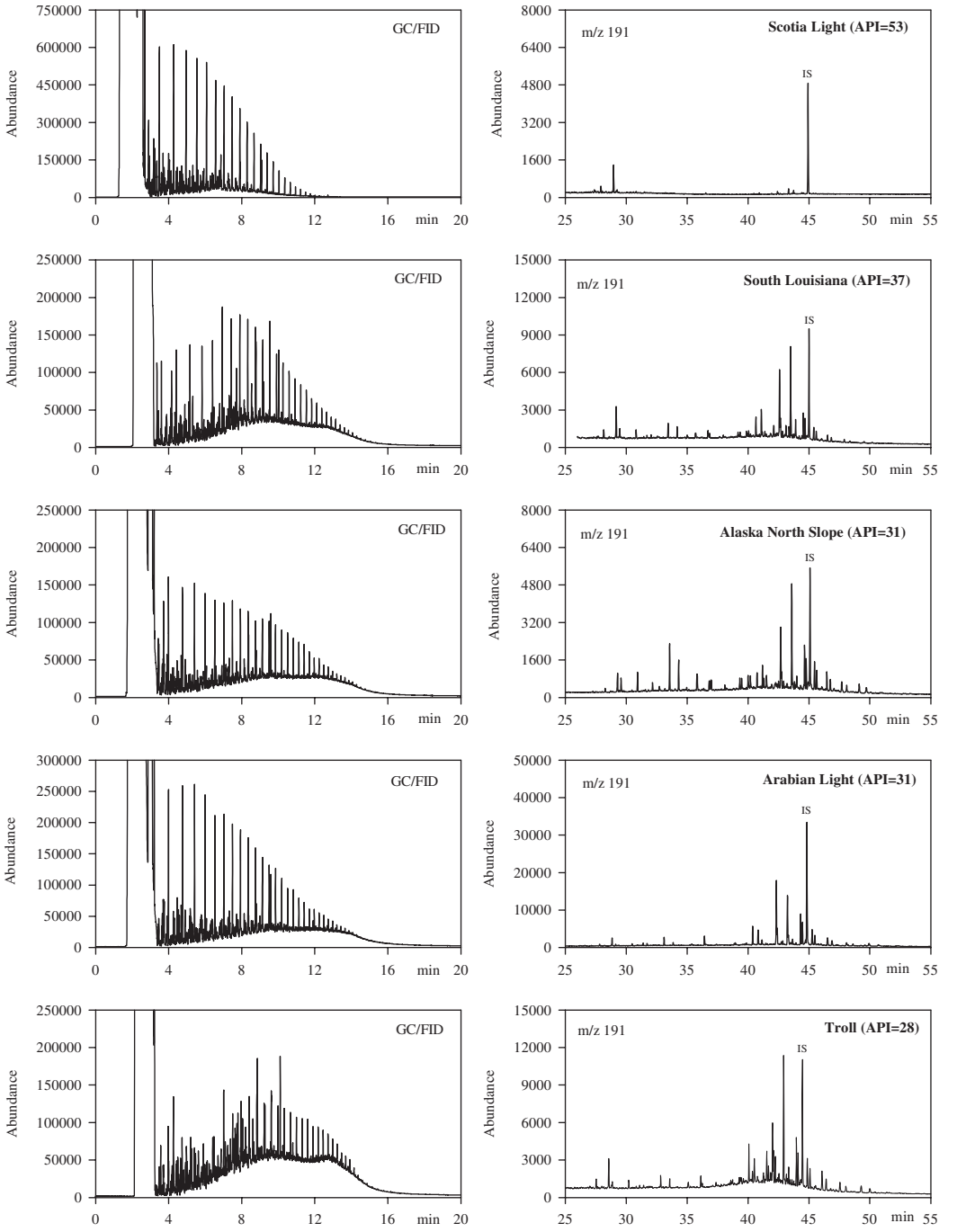


Figure 3-7 GC-FID (left panel) and GC-MS (at *m/z* 191, right panel) chromatograms of five different oils (light to medium) to illustrate differences in the *n*-alkane and tri-, tetra-, and pentacyclic terpane distributions between oils.

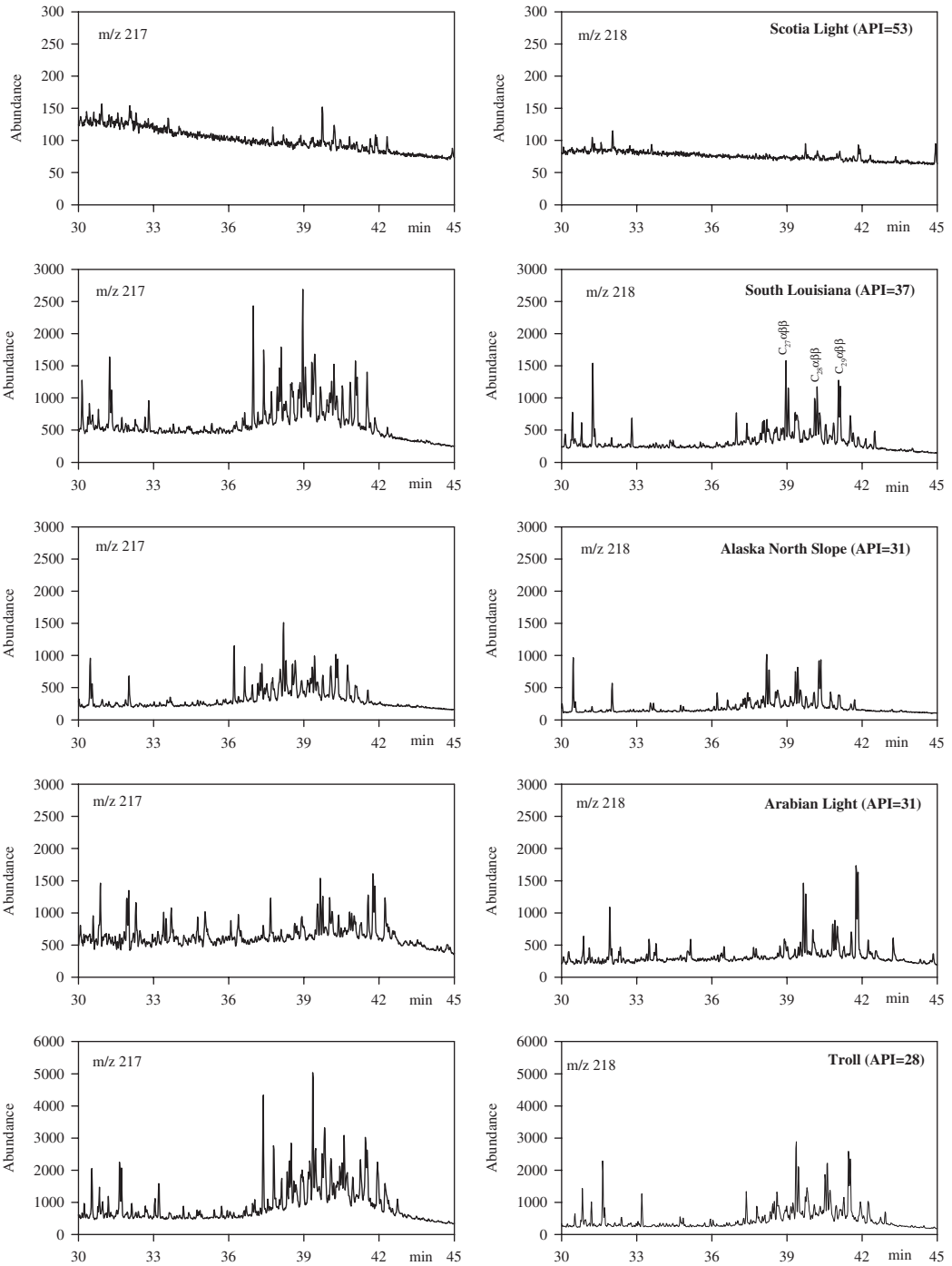


Figure 3-8 GC-MS chromatograms at *m/z* 217 and 218 for five different oils (light to medium) to illustrate differences in sterane distributions between oils.

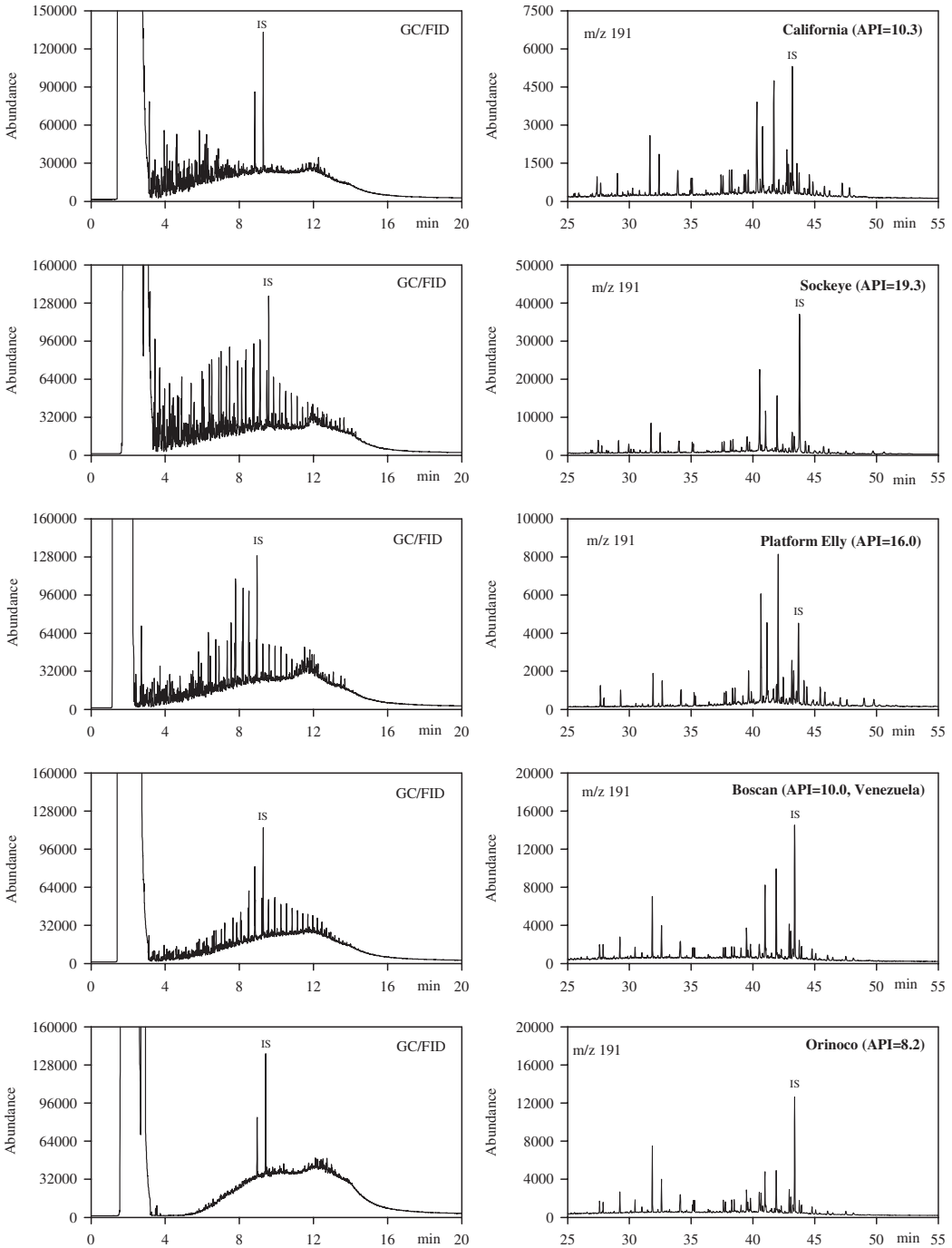


Figure 3-9 GC-FID (left panel) and GC-MS (at *m/z* 191, right panel) chromatograms of five heavy oils from different regions.

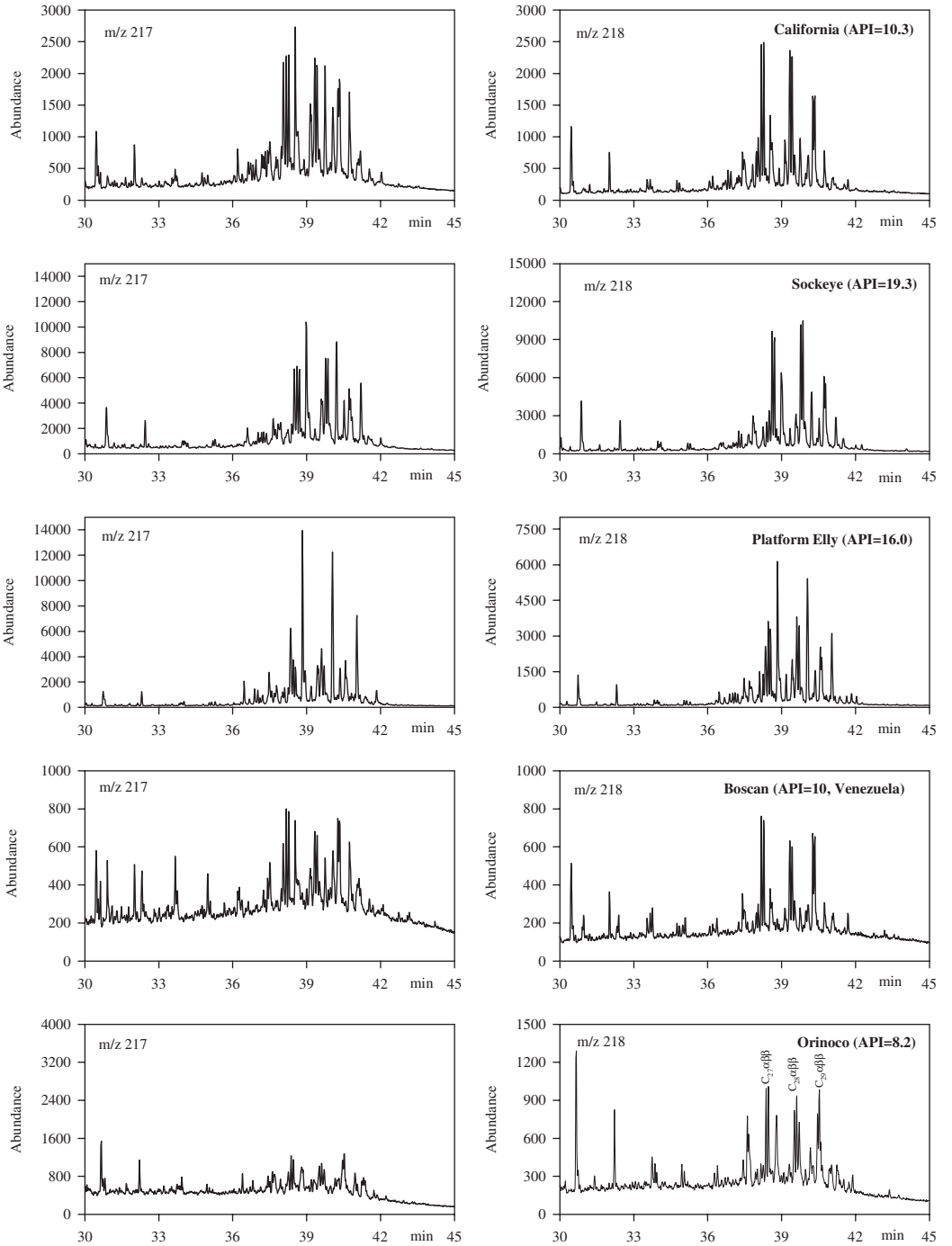


Figure 3-10 GC-MS chromatograms at *m/z* 217 and 218 of five heavy oils from different regions.

Table 3-5 Quantitation results of Major Target Biomarkers in Example Crude Oils and Petroleum Products

Oil Samples	*Scotia Light*	*South Louisiana*	*Alaska North Slope*	*Arabian Light*	*Troll*	*California (API = 11)*	*Sockeye*	*Platform Elly*	*Used Air Compressor Oil*	*Valvoline 10W-30 Motor Oil*
Biomarker compounds (μg/g oil)										
C_{21}	0.00	9.43	18.7	4.47	7.81	22.5	19.1	20.1	17.1	11.6
C_{22}	0.00	3.53	8.65	4.73	2.96	8.86	17.7	4.32	14.3	15.2
C_{23}	0.87	14.8	49.6	17.7	11.1	56.5	46.2	41.3	86.7	68.2
C_{24}	0.61	10.7	31.6	6.60	9.14	39.3	31.3	33.9	45.0	25.5
C_{29} αβ	3.32	74.6	69.3	152	56.6	69.3	61.2	107	190	864
C_{30} αβ	5.79	100	112	125	126	109	99.5	216	414	718
C_{31} (S)	1.74	26.4	48.9	79.9	44.3	46.1	38.7	64.6	180	385
C_{31} (R)	1.24	21.5	35.8	65.7	34.5	32.7	40.6	52.5	148	305
C_{32} (S)	0.95	15.2	37.4	48.1	30.4	32.5	27.5	43.0	142	238
C_{32} (R)	0.79	9.94	24.6	29.8	22.0	22.0	18.9	32.2	96.1	164
C_{33} (S)	0.00	8.96	24.2	27.0	26.7	25.1	18.8	35.2	104	140
C_{33} (R)	0.00	5.48	16.1	17.8	16.3	17.6	12.8	28.5	69.5	91.7
C_{34} (S)	0.00	4.65	19.1	14.4	16.4	17.9	8.40	20.0	78.3	77.6
C_{34} (R)	0.00	2.78	11.2	8.80	9.54	11.6	5.70	15.1	43.1	51.6
C_{35} (S)	0.00	3.33	17.7	14.7	12.4	23.0	12.1	22.1	72.5	85.7
C_{35} (R)	0.00	2.27	15.0	7.80	8.73	20.8	9.15	20.9	46.5	47.6
Ts	1.40	20.3	16.2	42.6	34.1	9.08	6.90	13.2	61.9	148
Tm	1.66	29.6	25.2	36.5	23.3	20.7	35.4	55.9	74.8	215
C_{27} αββ-steranes	2.84	89.3	124	35.1	172	438	208	649	437	525
C_{28} αββ-steranes	2.77	67.4	121	20.1	125	427	260	754	384	363
C_{29} αββ-steranes	5.20	89.8	152	55.1	179	289	152	466	761	778
Total	**29.2**	**610**	**979**	**814**	**968**	**1738**	**1129**	**2695**	**3466**	**5318**
Diagnostic ratios										
C_{23}/C_{24}	1.42	1.39	1.58	2.68	1.22	1.44	1.48	1.22	1.93	2.68
C_{23}/C_{30} αβ	0.15	0.15	0.45	0.14	0.09	0.52	0.46	0.19	0.21	0.09
C_{24}/C_{30} αβ	0.11	0.11	0.28	0.05	0.07	0.36	0.31	0.16	0.11	0.04
C_{29} αβ/C_{30} αβ	0.57	0.75	0.62	1.22	0.45	0.64	0.62	0.49	0.46	1.20
$C_{31}(S)/C_{31}(S + R)$	1.40	1.23	1.36	1.22	1.28	1.41	0.95	1.23	1.22	1.26
$C_{32}(S)/C_{32}(S + R)$	1.20	1.53	1.52	1.61	1.38	1.48	1.46	1.33	1.48	1.45
Ts/Tm	0.84	0.69	0.64	1.17	1.46	0.44	0.19	0.24	0.83	0.69
C_{27} αββ-steranes/C_{29} αββ-steranes	0.55	0.99	0.82	0.64	0.96	1.52	1.37	1.39	0.57	0.67
$C_{30}/(C_{31} + C_{32} + C_{33} + C_{34} + C_{35})$	1.23	0.99	0.45	0.40	0.57	0.44	0.52	0.65	0.42	0.45

steranes than C_{27} αββ cholestane and C_{28} αββ ergostane series (Table 3-5).

The dominance of C_{28} 17α(H), 18α(H), 21β(H)-28,30-bisnorhopane (BHN28) is particularly prominent in California API-11, Sockeye, and Platform Elly (all three oils are from California), and its abundance is even higher than C_{30} and/or C_{29} 17α(H), 21β(H)-hopane (Figure 3-9). A high concentration of C_{28} 17α(H), 18α(H), 21β(H)-28,30-bisnorhopane is typical of petroleum from highly reducing to anoxic depositional environments (Mello et al., 1990). The California API-11 and Platform Elly demonstrate higher concentration of C_{31} to C_{35} homohopanes than the Sockeye oil. Also, the California API-11 has a significantly higher concentration of C_{35} homohopanes (22S + 22R) than C_{34} homohopanes (22S + 22R), further indicating a highly reducing marine environment of deposition with no available free oxygen (Peters and Moldowan, 1993). For the Orinoco Bitumen, C_{23} terpane is the most abundant, followed by the C_{30} and C_{29} hopane; while the Boscan oil demonstrates higher concentrations of C_{29} and C_{30} terpanes than C_{23} terpane. The presence of triplets with different relative distributions is apparent for most heavy oils. Orinoco and Boscan oils have somewhat the V-shaped C_{27}—C_{28}—C_{29} regular αββ sterane (*m/z* 218) distribution. Three California oils have very high concentrations of steranes (Table 3-5), with a more abundant C_{28} ergostane than C_{27} and C_{29} sterane series. This is also the case for several other heavy California oils including California API-15 and Platform Irene (data not shown here). The high relative levels of C_{28} ergostane may be related to increased diversification of phytoplankton assemblages in the Jurassic and Cretaceous oils.

3.3.2 Biomarkers in Petroleum Products

Petroleum products are refined from crude oils through a variety of refining processes including distillation, cracking, catalytic reforming, isomerization, alkylation, and blending (Olah and Molnar, 1995; Speight, 2002; Simanzhenkov and Idem, 2003). Depending on the chemical composition of their "parent" crude oil feedstocks, varying refining approach and conditions, wide range of applications, regulatory requirements, and economic requirements, refined products can have a wide variety in chemical compositions.

Light distillates are typically products in the C_4 to C_{13} carbon range. They include aviation gas (gasoline-type jet fuel), naphtha, and automotive gasoline. The GC traces of fresh light distillates are featured with dominance of light-end, resolved hydrocarbons and a minimal UCM. Gasoline is a complex mixture of hundreds of different hydrocarbons predominantly in the C_4 to C_{13} boiling range. Additives are often added to gasoline to improve some specific properties and antiknock properties. The major components of gasoline that are of environmental concern include MTBE, BTEX, C_3-benzenes, and naphthalene. Gasoline and other light distillates do not contain any terpane and sterane biomarker compounds. But, it has been recently reported that gas condensates and some gas-derived nonaqueous phase liquids (NAPLs) contain diamondoid compounds, which can have potential applications in distinguishing natural gas condensate from automotive gasoline (Stout and Douglas, 2004).

Mid-range distillates are typically products in a relatively broader carbon range (C_6 to C_{26}) and include kerosene, aviation jet fuels, and lighter diesel products. Jet fuel is kerosene-based aviation fuel. Jet fuel is used for aviation turbine power units and usually has the same distillation characteristics and flash point as kerosene. Jet fuels are similar in gross composition, and compositional differences are attributable to additives designed to control some fuel parameters such as freeze and pour point characteristics. As Figure 3-11 shows, the chromatogram of a commercial jet fuel (Jet A) is dominated by GC-resolved *n*-alkanes in a narrow range of *n*-C_7 to *n*-C_{18} with maximum around *n*-C_{11} and a well-defined UCM. Diesel fuels were originally straight-run products obtained from the distillation of crude oil. Currently, diesel fuel may also contain varying amounts of selected cracked distillates to increase the available volume. The boiling range of diesel fuel is approximately

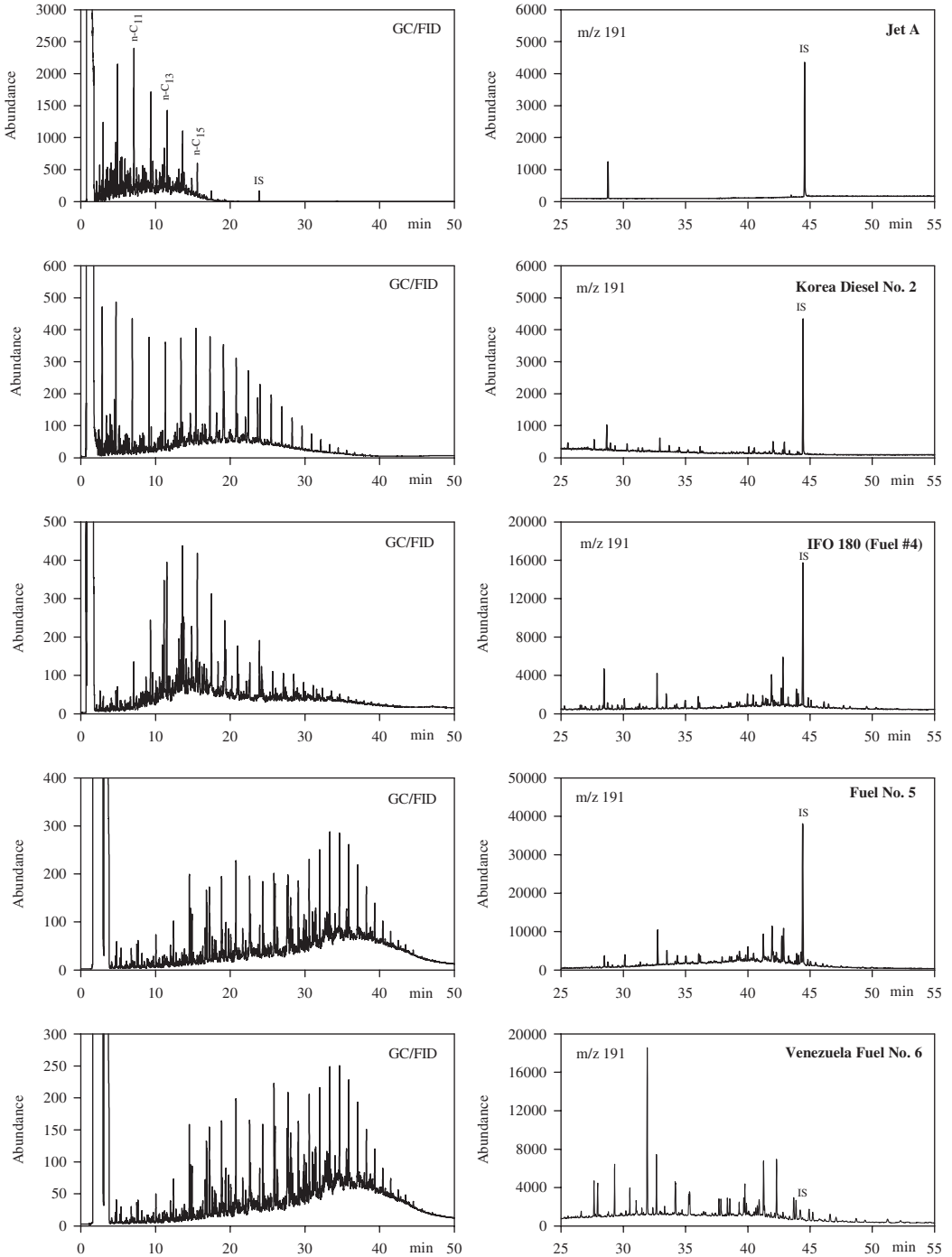


Figure 3-11 GC-FID (left panel) and GC-MS (at *m/z* 191, right panel) chromatograms of five common petroleum products (light to heavy) to illustrate differences in the *n*-alkane and tri-, tetra-, and pentacyclic terpane distributions between oils.

125–380°C. One of the most widely used specifications (ASTM D-975) covers three grades of diesel fuel oils: diesel fuel No. 1, diesel fuel No. 2, and diesel fuel No. 4. The marine fuel specifications have 4 categories of distillate fuels and 15 categories of fuels containing residual components (ASTM D-2069). Diesels consist of hydrocarbons in a carbon range of C_8 to C_{28} and contain high levels of *n*-alkanes, alkyl-cyclohexane, and PAHs. The properties of a given diesel are largely a function of the crude oil feedstock and any blending of various distillate stocks. The GC chromatogram of the Korean diesel fuel No. 2 (Figure 3-11) is dominated by a nearly normal distribution of *n*-alkanes with maxima around *n*-C_{11} to *n*-C_{14}. Also, a central UCM hump is obvious.

Heavy residual fuels. Heavy fuel oils (HFO) are blended products manufactured from residues of various refinery processes. The heavy residual fuels are largely used in marine applications and industrial power generation. Classic heavy fuel types include fuel No. 5 and No. 6 (also known as Bunker C) fuel. For years the term "Bunker C fuel oil" has been widely used to designate the most viscous residual fuels for general land and marine use. The chemical composition of Bunker C (or IFO 380) can vary widely, depending on production oil fields, production years, and processes it has undergone (see Chapter 10 herein for more details). Currently, many Bunker-type fuels are produced by blending residual oils with diesel fuels or other lighter fuels in various ratios to produce residual fuel oil of acceptable viscosity for marine or power plant use. The GC chromatograms of IFO 180, a lighter residual fuel No. 5 (also called Bunker B), and a Bunker C from Venezuela are also shown in Figure 3-11. The differences in the chromatographic profiles, carbon range, shapes of UCM, *n*-alkane, and isoprenoid distributions among these products are obvious. GC-MS chromatograms of *m/z* 191, 217, and 218, for example, jet fuel, diesel, IFO 180, Fuel No. 5 (Bunker B), and Venezuela Fuel No. 6 (Bunker C) are shown in Figures 3-11 and 3-12, respectively.

The differences in the concentrations and relative distributions of tri-, tetra-, and pentacyclic terpanes and steranes between refined products are apparent. No target terpane and sterane compounds are detected in the Jet A fuel. Generally, most diesels contain none or only a trace of terpanes and steranes. However, the Korean diesel No. 2 demonstrates abundant biomarkers in a much wider carbon number range, indicating that diesels from different manufacturers may have correspondingly varying biomarker fingerprints. The GC-MS chromatogram of terpanes for the Venezuela Fuel No. 6 is characterized by a distribution in a wide range from C_{19} to C_{35} with C_{23} tricyclic triterpanes being the most prominent. As for steranes, the dominance of C_{27}, C_{28}, and C_{29} 20S/20R homologues is apparent. The relative proportion of C_{27}—C_{28}—C_{29} αββ steranes shows a consistent decrease with increasing carbon number ($C_{27} > C_{28} > C_{29}$). For the IFO-180, the presence of diasteranes is significantly higher than in other heavy fuel oils. As for Fuel No. 5, the dominance of C_{29} sterane peaks, in particular the 20S C_{29} ααα sterane, in SIM chromatogram (*m/z* 217) is pronounced.

3.3.3 Biomarkers in Lubricating Oils

Petroleum-derived lubricating oils are the most commonly used for both automotive and industrial applications. Small-scale spills and contamination by lubricating oil are quite common due to their wide application. Lubricating oils have broad GC profiles in the carbon range of C_{20} to C_{40} with boiling points greater than 340°C. Lubricating oil does not, generally, contain the low boiling fraction of petroleum hydrocarbons. Lubricating oil is largely composed of saturated hydrocarbons, and its GC trace is often dominated by a large UCM with few resolved peaks. In lubricating oil such as hydraulic fluid, for example, the PAH concentrations can be very low, while the biomarker concentrations are, generally, high. As examples, Figure 3-13 shows the GC-FID chromatograms and GC-MS fingerprints of five common lubricating oils.

Significant features of the biomarker distribution in petroleum-derived lubricating oils include the following: (1) biomarkers are

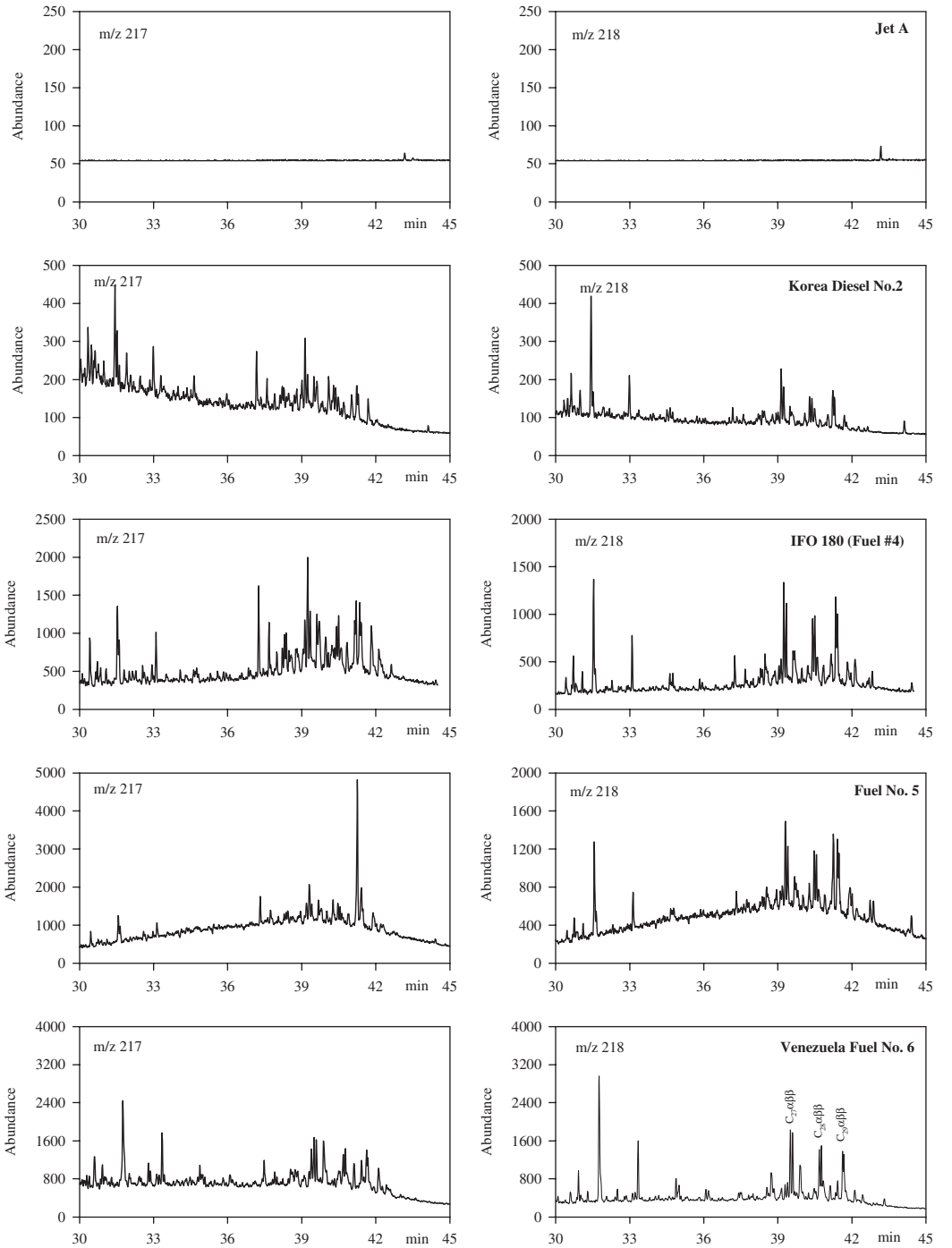


Figure 3-12 GC-MS chromatograms at *m/z* 217 and 218 for common petroleum products (light to heavy) to illustrate differences in sterane distributions between oils.

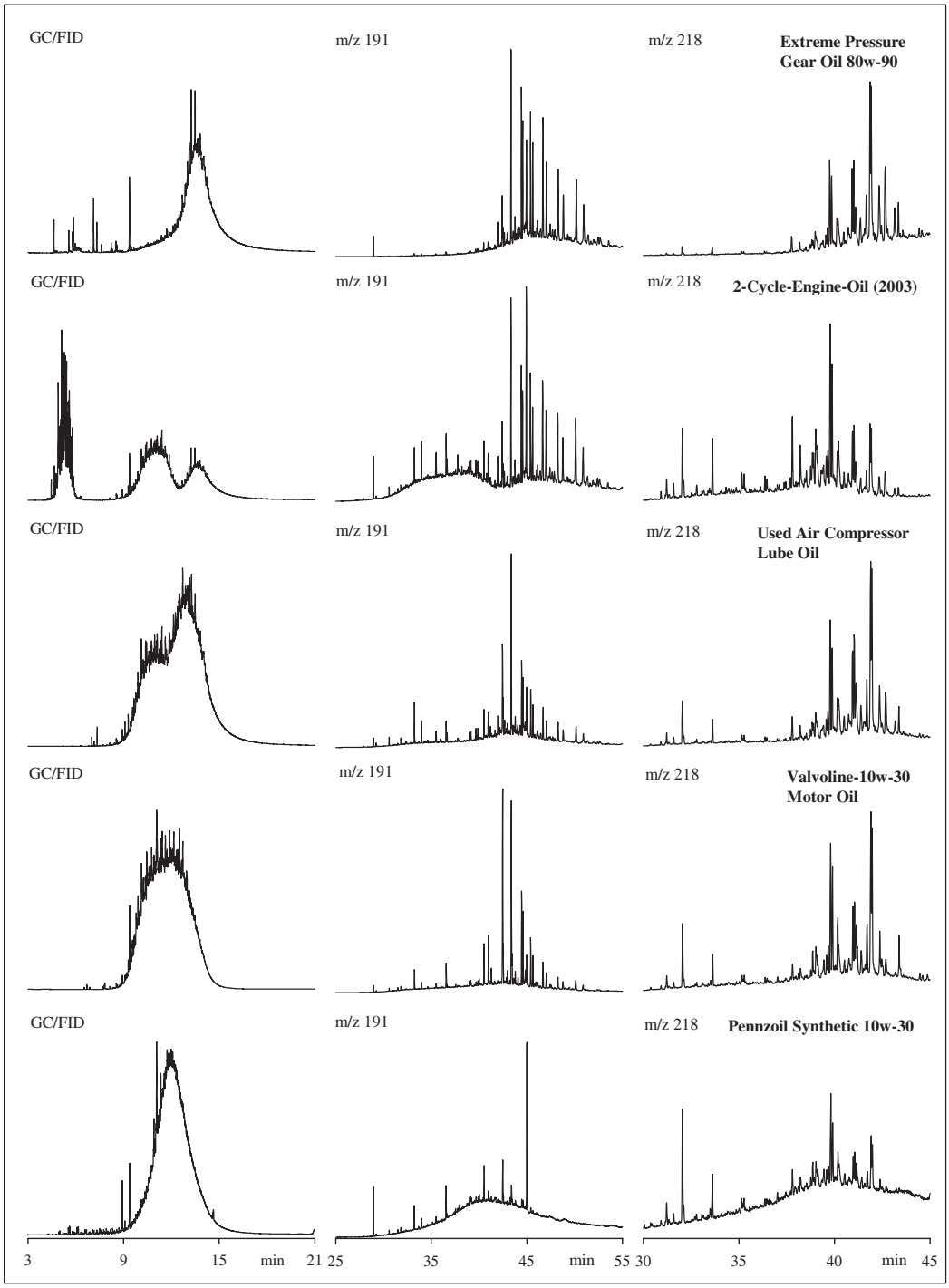


Figure 3-13 GC-FID (left panel) and GC-MS (at *m/z* 191, middle panel; *m/z* 218, right panel) chromatograms of five lubricating oils.

predominantly located in the high carbon number end, because the refining processes have removed low MW biomarkers and concentrated high MW biomarkers from the corresponding crude oil feed stocks; (2) lubricating oils, in general, have high concentrations of target terpane and sterane compounds (2000–6000 μg/g oil) in comparison with most crude oils and petroleum products (Table 3-5); (3) the dominance of characteristic pentacyclic C_{31} to C_{35} homohopanes is particularly prominent; (4) the dominance of C_{27}, C_{28}, and C_{29} 20S/20R homologues is apparent, too; (5) the concentrations of biomarkers are very low in the chemically synthetic lube oil, and the unresolved hump is pronounced.

Lubricating oil contamination through engine exhaust and through leakage and spillage occurs everywhere (Stout et al., 2001; Kaplan et al., 2001). Bieger et al. (1996) have reported the use of terpane biomarker fingerprints of refined oils and motor exhausts to indicate the presence of and trace the origin of diffuse lubricating oil contamination in plankton and sediments around St. John's, Newfoundland, Eastern Canada.

3.3.4 Biomarkers in Oil Fractions with Different Carbon Number Range

A research project to study the relative toxicity of oil fractions to fish (Khan et al., 2004; Clarke et al., 2004) was launched in 2004. The overall aim of this project was to generate useful and relevant data on the mechanism of hydrocarbon toxicity to fish to provide a strong foundation for ecological risk assessment of crude oil. Four oils (Federated crude oil, ANS oil, Scotia Light, and MESA oil) were selected for this research and fractionated into four oil fractions each using a distillation method. The nominal carbon number and boiling point ranges of these fraction are: Fraction 1, C_6 to C_{10}, 50–173°C; Fraction 2, C_{10} to C_{16}, 174–287°C; Fraction 3, C_{17} to C_{34}, 288–481°C; and Fraction 4, >C_{34}, >481°C. Each distillation fraction was quantitatively characterized. Characterization results clearly show that four oil fractions are significantly different from each other in their chemical composition including the carbon range and molecular-weight range, diagnostic ratios of target individual compounds and compound classes, and distribution patterns and profiles of *n*-alkanes, BTEX and alkyl benzenes, PAHs, and biomarkers. The left panel of Figure 3-14 shows the GC chromatograms of the MESA oil (a medium South American crude, API gravity of 29.7) and four fractions of the oil to illustrate major chemical composition features of each fraction; and the right panel graphically compares quantitative distribution of target alkylated PAHs and other EPA priority PAHs in the oil and its four fractions. Figure 3-15 presents GC-MS chromatograms at *m/z* 191 and 218 to illustrate differences in biomarker distributions of four fractions. Table 3-6 summarizes the quantitation results of target biomarkers in the MESA oil and its four fractions.

Figure 3-15 clearly reveals that the distribution patterns and profiles of biomarkers in four fractions are significantly different from each other. No biomarkers were present in Fraction 1, and only several smaller biomarker compounds (C_{21} to C_{24} terpanes) with very low abundances were detected in Fraction 2 (2.14 μg/g oil in total). The totals of the target biomarkers were determined to be 2523 and 2045 μg/g oil for Fractions 3 and 4, respectively, much higher than that in the original oil (1771 μg/g oil). Obviously, as the lower-molecular-weight hydrocarbons were removed from the crude oil by distillation, the higher-molecular-weight biomarkers were correspondingly concentrated, resulting in higher concentration of biomarkers in Fractions 3 and 4. In Fraction 4, no biomarkers with a carbon number smaller than C_{27} were detected. The terpanes (pentacyclic hopanes) were confined to a much narrower range of C_{29} to C_{35}, and the C_{29} 20S/20R steranes were significantly more abundant than the C_{27} and C_{28} group steranes.

3.3.5 Aromatic Steranes in Oils and Petroleum Products

Aromatic steranes are another group of biomarker compounds that are highly resistant to

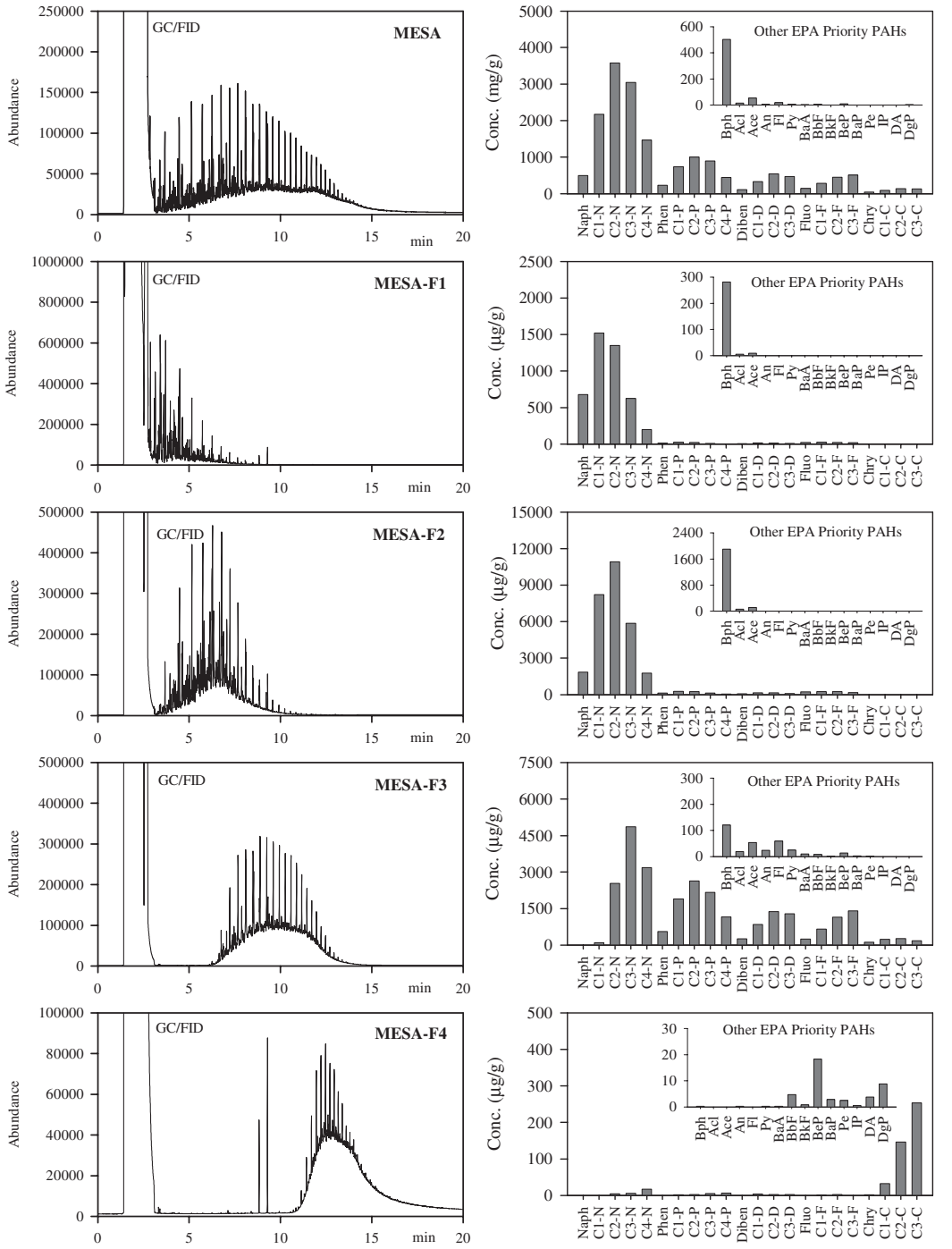


Figure 3-14 GC-FID chromatograms (left panel) and PAH distributions (right panel) of the MESA oil and its four fractions.

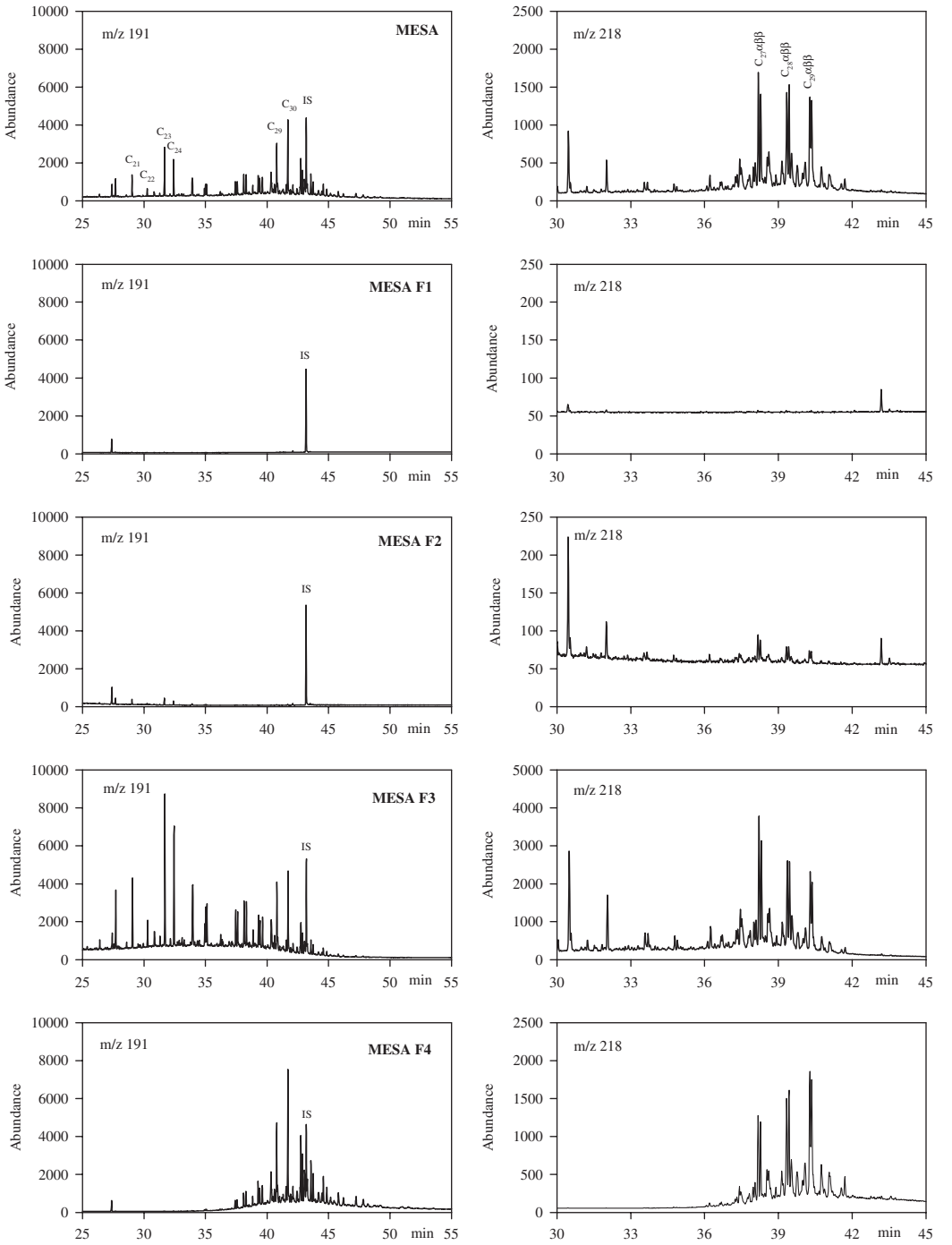


Figure 3-15 GC-MS chromatograms of the MESA oil and its four fractions at *m/z* 191 (left panel) and 218 (right panel) to illustrate differences in biomarker distributions.

Table 3-6 Quantitation Results of Target Biomarkers in the MESA Oil and Its Four Fractions

Oil Samples	*MESA*	*MESA-F1*	*MESA-F2*	*MESA-F3*	*MESA-F4*
Biomarker compounds (μg/g oil)					
C_{21}	35.8	0.76	7.73	95.0	0.00
C_{22}	13.2	0.20	2.24	35.4	0.00
C_{23}	77.3	0.76	8.62	211	0.32
C_{24}	59.8	0.42	5.97	161	0.26
C_{29} αβ	92.1	0.00	1.02	98.9	150
C_{30} αβ	148	0.00	1.62	124	262
C_{31} (S)	65.9	0.00	0.51	44.6	122
C_{31} (R)	46.8	0.00	0.21	31.5	84.2
C_{32} (S)	40.3	0.00	2.35	22.7	72.9
C_{32} (R)	25.4	0.00	0.00	15.2	50.2
C_{33} (S)	26.5	0.00	0.00	13.3	58.0
C_{33} (R)	16.7	0.00	0.00	8.12	38.4
C_{34} (S)	16.9	0.00	0.00	6.97	39.2
C_{34} (R)	9.05	0.00	0.00	3.76	24.8
C_{35} (S)	12.7	0.00	0.00	4.51	34.9
C_{35} (R)	14.0	0.00	0.00	4.13	35.4
Ts	17.9	0.00	0.39	27.4	17.5
Tm	37.6	0.00	0.66	47.4	40.2
C_{27} αββ-steranes	366	0.00	6.65	649	236
C_{28} αββ-steranes	308	0.00	5.19	506	352
C_{29} αββ-steranes	340	0.00	4.06	412	428
Total	**1771**	**2.14**	**47.2**	**2523**	**2045**
Diagnostic ratios					
C_{23}/C_{24}	1.29	1.81	1.44	1.31	1.21
C_{23}/C_{30} αβ	0.52	NA	5.32	1.70	0.00
C_{24}/C_{30} αβ	0.40	NA	3.68	1.29	0.00
C_{29} αβ/C_{30} αβ	0.62	NA	0.63	0.79	0.57
C_{31}(S)/C_{31}(S + R)	1.41	NA	2.43	1.42	1.44
C_{32}(S)/C_{32}(S + R)	1.59	NA	NA	1.50	1.45
Ts/Tm	0.48	NA	0.60	0.58	0.44
C_{27} αββ-steranes/C_{29} αββ-steranes	1.08	NA	1.64	1.57	0.55
$C_{30}/(C_{31} + C_{32} + C_{33} + C_{34} + C_{35})$	0.54	NA	0.53	0.80	0.47

biodegradation and can be used for oil-to-oil correlation and oil source tracking. Aromatic steranes are monitored using *m/z* 231 and 253 for triaromatic (TA) and monoaromatic (MA) steranes, respectively. The *m/z* 231 mass chromatograms of crude oil are characterized by series of 20R and 20S C_{26}-C_{27}-C_{28} triaromatic a steranes (TA-cholestanes, TA-ergostanes, and TA-stigmastanes) plus C_{20} to C_{22} TA-steranes. The *m/z* 253 mass chromatograms are featured by series of 20R and 20S C_{27}-C_{28}-C_{29} 5β(H) and 5α(H) MA steranes as well as rearranged ring-C 20S and 20R MA-diasteranes. Peak identification of TA- and MA-steranes in the Platform Elly oil is summarized in Figure 3-16 and Table 3-7. As Figure 3-16 shows, all target TA-steranes are well separated under the present GC conditions except that the C_{26} 20R isomer co-elutes with the C_{27} 20S isomer (Peak 5). The structures of rearranged MA steranes have been established as 10-desmethyl 5α- and 5β-methyl (20S and 20R) MA-diasterane isomers (Riolo et al., 1985; Moldowan and Fago, 1986).

To illustrate the differences in TA- and MA-steranes between oils and refined products, Figure 3-17 compares the mass chromatograms of the TA- and MA-steranes in the aromatic hydrocarbon fractions of the NIST 1582 oil and refined products (IFO-180, a lubricating oil, and a Diesel No. 2 from Korea).

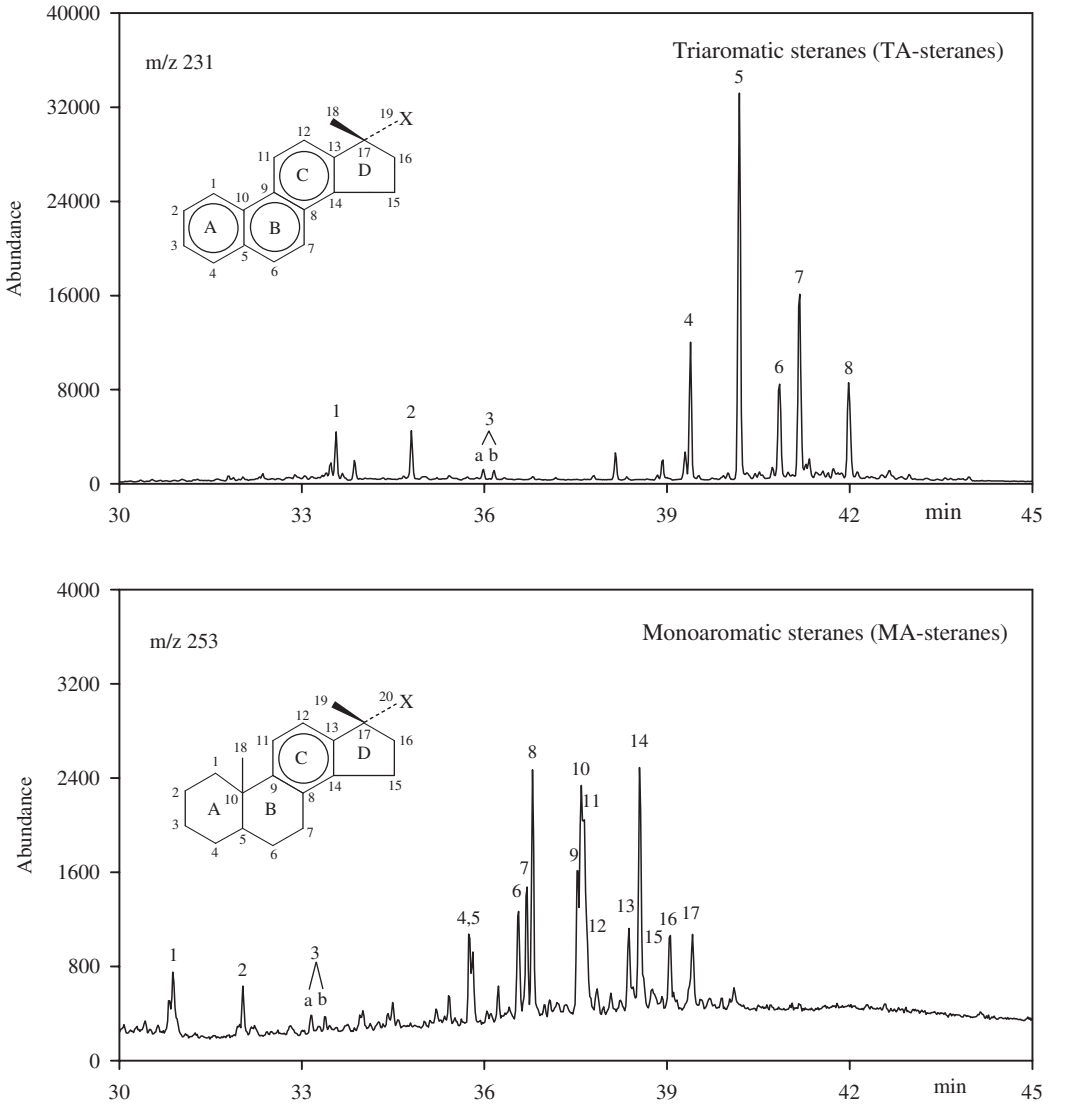


Figure 3-16 Peak identification of the triaromatic (*m/z* 231) and monoaromatic (MA, *m/z* 253) steranes in the Platform Elly oil.

Figure 3-17 shows apparent differences in the relative distributions and absolute concentrations of TA- and MA-steranes between oils and refined products. Generally, triaromatic steranes are much more abundant than monoaromatic steranes for all oils studied. In many lighter oils such as Cook Inlet, Federated, West Texas, and Scotia Light, only trace MA-steranes are detected. This implies that TA-steranes are more valuable marker compounds than MA-steranes for environmental forensic investigations. Unlike most Canadian diesels, the Korean Diesel No. 2 still contains a relatively large quantity of high carbon number TA-steranes. Similarly, lubricating oils do not or only contain trace levels of MA-sterane compounds. Synthetic lubricants should not contain any TA- or MA-sterane compound if they are purely chemically synthesized. However, GC-MS analyses show that

Table 3-7 Peak Identification of the Triaromatic and Monoaromatic Steranes in the Platform Elly Oil

Peak No.	*Compounds*	*Code*	*Molecular Formula*
Triaromatic steranes (TA-steranes, m/z 231)			
1	C_{20} TA-sterane (X = ethyl)	C20TA	$C_{20}H_{20}$
2	C_{21} TA-sterane (X = 2-propyl)	C21TA	$C_{21}H_{22}$
3	C_{22} TA-sterane (X = 2-butyl) (a and b are epimers at C-19)	C22TA	$C_{22}H_{24}$
4	C_{26} TA-chloestane (20S)	SC26TA	$C_{26}H_{32}$
5	C_{26} TA-chloestane (20R) + C_{27} TA-ergostane (20S)	RC26TA + SC27TA	$C_{26}H_{32}$ $C_{27}H_{34}$
6	C_{28} TA-stigmastane (20S)	SC28TA	$C_{28}H_{36}$
7	C_{27} TA-ergostane (20R)	RC27TA	$C_{27}H_{34}$
8	C_{28} TA-stigmastane (20R)	RC28TA	$C_{28}H_{36}$
Monoaromatic steranes (MA-steranes, m/z 253)			
1	C_{21} MA-sterane (X = ethyl)		$C_{21}H_{30}$
2	C_{22} MA-sterane (X = 2-propyl)		$C_{22}H_{32}$
3	C_{23} MA-sterane (X = 2-butyl) (a and b are epimers at C-20)		$C_{23}H_{34}$
4	C_{27} 5β(H) MA-cholestane (20S)		$C_{27}H_{42}$
5	C_{27} MA-diacholestane (20S)		$C_{27}H_{42}$
6	C_{27} 5β(H) MA-cholestane (20R) + C_{27} MA-diacholestane (20R)		$C_{27}H_{42}$
7	C_{27} 5α(H) MA-cholestane (20S)		$C_{27}H_{42}$
8	C_{28} 5β(H) MA-ergostane (20S) + C_{28} MA-diaergostane (20S)		$C_{28}H_{44}$
9	C_{27} 5α(H) MA-cholestane (20R)		$C_{27}H_{42}$
10	C_{28} 5α(H) MA-ergostane (20S)		$C_{28}H_{44}$
11	C_{28} 5β(H) MA-ergostane (20R) + C_{28} MA-diaergostane (20R)		$C_{28}H_{44}$
12	C_{29} 5β(H) MA-stigmastane (20S) + C_{29} MA-diastigmastane (20S)		$C_{29}H_{46}$
13	C_{29} 5α(H) MA-stigmastane (20S)		$C_{29}H_{46}$
14	C_{28} 5α(H) MA-ergostane (20R)		$C_{28}H_{44}$
15	C_{29} 5β(H) MA-stigmastane (20R)		$C_{29}H_{46}$
16	C_{29} 5α(H) MA-stigmastane (20R)		$C_{29}H_{46}$
17	C_{30} 5β(H) MA-sterane (20S)		$C_{30}H_{48}$

TA-steranes are present in the *Synthetic 10W-30* lubricating oil. This fact indicates that this lubricating oil may not be 100% synthesized and may be composed of a portion of petroleum-derived hydrocarbons. Barakat et al. (2002) have recently reported a case study in which oil residues were correlated to a fresh crude oil sample of the Egyptian Western Desert-sourced oil by fingerprinting monoaromatic and triaromatic steranes and by determination and comparison of molecular ratios of the target MA- and TA-sterane compounds.

3.3.6 Sesquiterpanes in Oils and Petroleum Products

Polymethyl-substituted decalins or decahydronaphthalenes (i.e., C_{14}–C_{16} bicyclic alkanes), commonly known as sesquiterpanes, were first reported in 1974 (Bendoraitis, 1974) and later discovered in crude oils of the Loma Novia and Anastasievsko-Troyitskoe deposits (Petrov, 1987). Alexander et al. (1983) identified and confirmed the existence of 8β(H)-drimane and 4β(H)-eudesmane in most Australian oils. Noble (Noble, 1986) identified a series of C_{14} to C_{16} sesquiterpane isomers using synthesized standards and mass spectral studies. Various sesquiterpanes, with the greatest enrichment in condensate, were also identified by Simoneit et al. from fossil resins, sediments, and crude oils (Simoneit et al., 1986), and by Chen and He from a great offshore condensate field of Liaodong Bay, Northern China (Chen and He, 1990).

Bicyclic biomarker sesquiterpanes with the drimane skeleton are ubiquitous components of crude oils and ancient sediments. Most

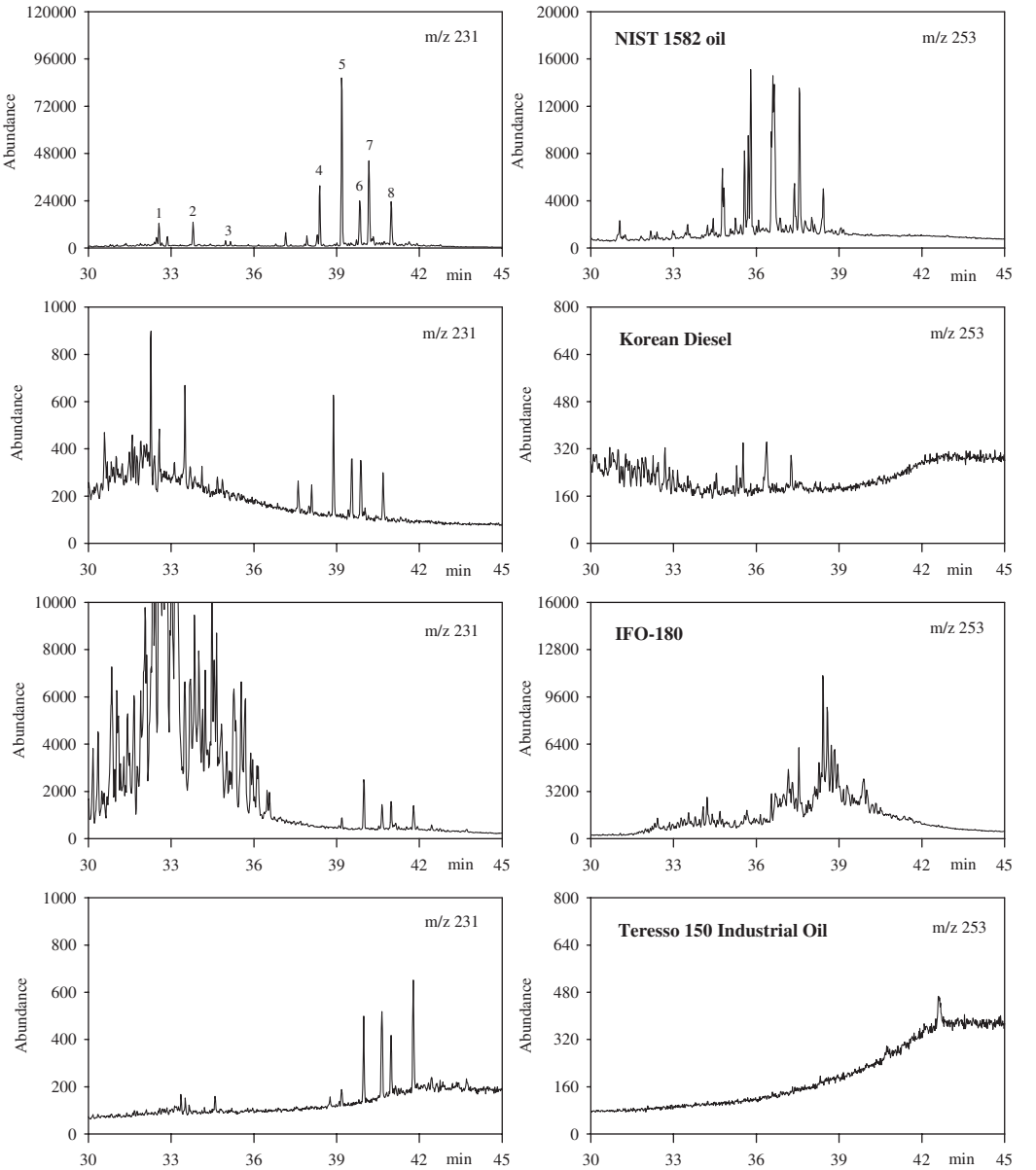


Figure 3-17 Mass chromatograms of the TA- (*m/z* 231) and MA-steranes (253) in the aromatic hydrocarbon fractions of the NIST 1582 oil, a Diesel No. 2 from Korea, IFO-180, and a lubricating oil.

sesquiterpanes probably originate from higher plants and also from algae or bacteria (Alexander et al., 1984; Philp, 1985; Fan et al., 1991). During the thermal evolution, the relative concentration of C_{14} sesquiterpanes decreases with increasing maturation of organic matters. The concentration of C_{14} bicyclic sesquiterpanes is higher at the immature stage, while those of C_{15} drimane and C_{16} homodrimane are relatively lower. As a result of the dehydroxylation and chemodynamics of their higher-molecular-weight precursors, the concentrations of drimane (C_{15}) and homodrimanes (C_{16}) gradually increase, and the

concentrations of C_{14} sesquiterpanes decline (Cheng et al., 1991).

Though biomarker sesquiterpanes have found increasing application in petroleum exploration in recent years, there have been few reports of use of these compounds for forensic oil spill identification (Stout et al., 2005; Wang et al., 2005a). For lighter petroleum products, refining processes have removed most high MW biomarkers from the original crude oil feedstock. Thus, the pentacyclic terpanes and steranes are generally absent or in low abundance in lighter petroleum products (e.g., jet fuels and diesels), while the sesquiterpanes are concentrated in these distillates. The sesquiterpanes with the drimane skeleton are monitored at *m/z* 123 (a base fragment ion common to all sesquiterpanes). Confirmation ions include 179 (the ion after sesquiterpane $C_{14}H_{26}$ loses $-CH_3$), 193 (the ion after $C_{15}H_{28}$ loses $-CH_3$ and after $C_{16}H_{30}$ loses $-C_2H_5$), and 207 (the ion after $C_{16}H_{30}$ loses $-CH_3$). Examination of GC-MS chromatograms of these characteristic ions of sesquiterpanes provides a highly diagnostic means for correlation, differentiation, and source identification of light to middle-range petroleum products, in comparison with the use of other hydrocarbon groups.

The sesquiterpanes ranging from C_{14} to C_{16} elute between *n*-C_{13} and *n*-C_{16} (boiling point 235–287°C) in the SIM chromatogram of the saturated hydrocarbon fraction. Peaks 1 and 2, 3 to 6, and 7 to 10 (Figure 3-18) are identified as C_{14}, C_{15}, and C_{16} sesquiterpanes, respectively. Of 10 identified sesquiterpanes, peaks 5 and 10 are identified to be 8β(H)-drimane and 8β(H)-homodrimane, respectively. GC-MS analyses demonstrate different distribution patterns of sesquiterpanes in crude oils and refined products of different origins. The left panel of Figure 3-19 shows SIM chromatograms of sesquiterpanes at *m/z* 123 for light (API >35), medium (API: 25–35), and heavy (API <25) crude oils, including Alaska North Slope (ANS), Arabian Light, Scotia Light oil (Nova Scotia), West Texas, and California API 11, while the right panel compares sesquiterpane distributions in common petroleum products from light kerosene to heavy fuel oil.

Ten sesquiterpanes are present in all oils studied. However, distributions and concentrations of sesquiterpanes vary between oils of different origins. Lighter oils ANS, Arabian Light, and Scotia Light have high concentrations of sesquiterpanes, with Peak 10 (C_{16} homodrimane) being the most abundant for the ANS and Arabian Light, and Peak 3 (C_{15} sesquiterpane) for Scotia Light, respectively. The Arabian Light has the lowest concentration of C_{14} sesquiterpanes (Peaks 1 and 2), indicating that this oil is highly mature. On the contrary, the heavy California API 11 oil has the highest concentration of C_{14} sesquiterpane, indicating that this oil is relatively immature.

Sesquiterpanes are absent in light kerosene and heavy lubricating oils. However, refined products IFO-180 and HFO 6303 (Bunker C type) contain high concentrations of sesquiterpanes. It is also noticed that an unknown bicyclic biomarker compound (between Peaks 2 and 3) is abundant in these two products. Jet A is characterized with Peaks 1, 3, and 5 being the most abundant, while Peak 10 is the most abundant in middle-distillates such as diesel due to concentration of lower-MW biomarkers from the original crude oil feedstock. The differences in distribution patterns and concentrations are often apparent between diesels.

Oil spills were reported and sampled on March 17 and 23, 1998, at a sewer outlet flowing into the Lachine Canal in Quebec. Following the accident, a diesel fuel, which was suspected to be the source of the spill, was collected from a reservoir at a pumping station located in Lachine, Quebec. Biomarker fingerprinting of the samples revealed that only trace amounts (<10 μg/g oil) of C_{19}–C_{24} tricyclic terpanes, regular C_{20}–C_{22} steranes, and diasteranes were detected. However, the spill samples contained significant amounts of sesquiterpanes. The GC-MS/SIM chromatogram (at *m/z* 123) and diagnostic ratios of target sesquiterpanes of the spill samples were found to be nearly identical to that of the suspected-source diesel. The only noticeable difference is that, compared to the suspected-source-diesel, the

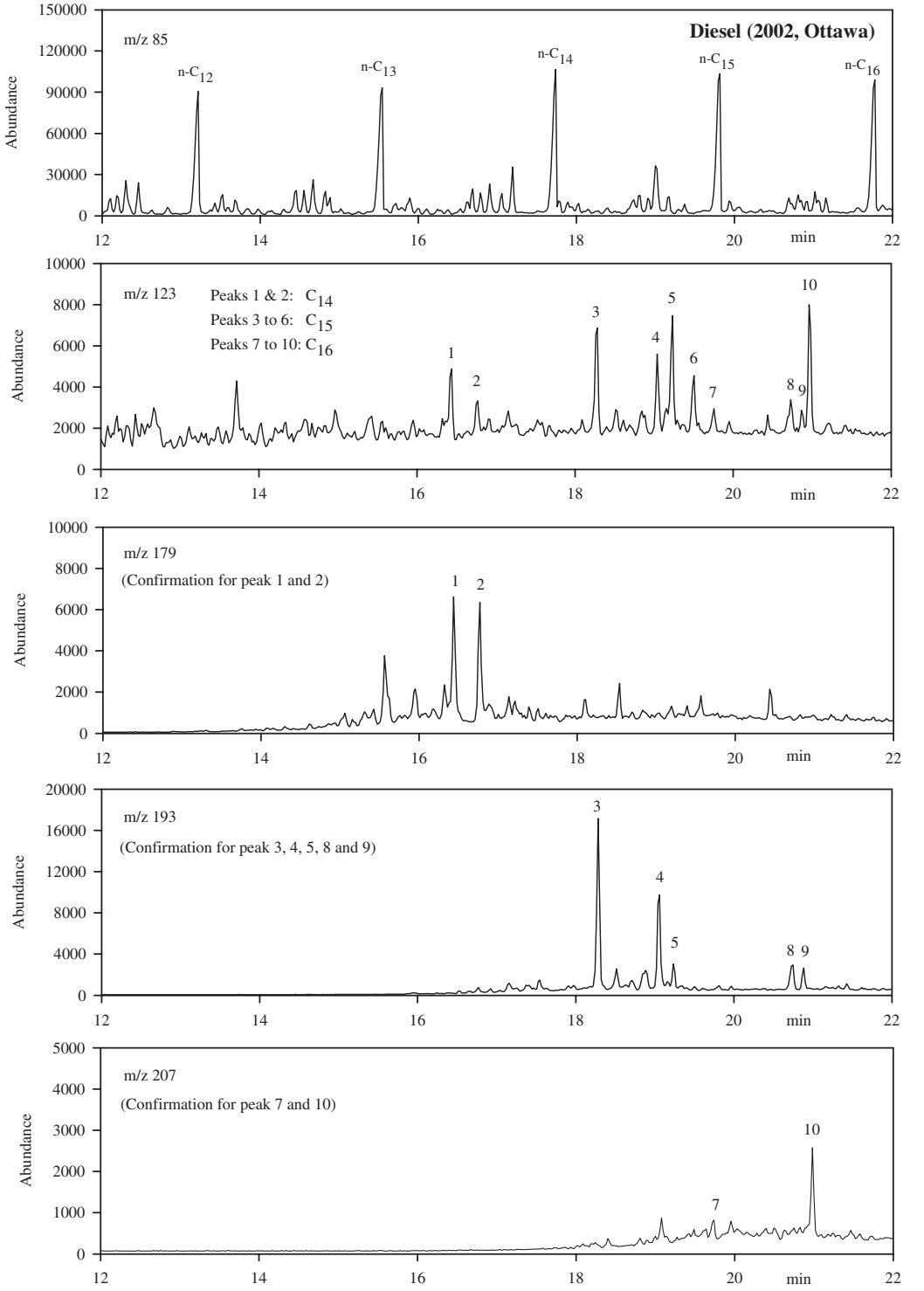


Figure 3-18 GC-MS (SIM) chromatograms of sesquiterpanes eluting in the *n*-C_{13} and *n*-C_{16} range.

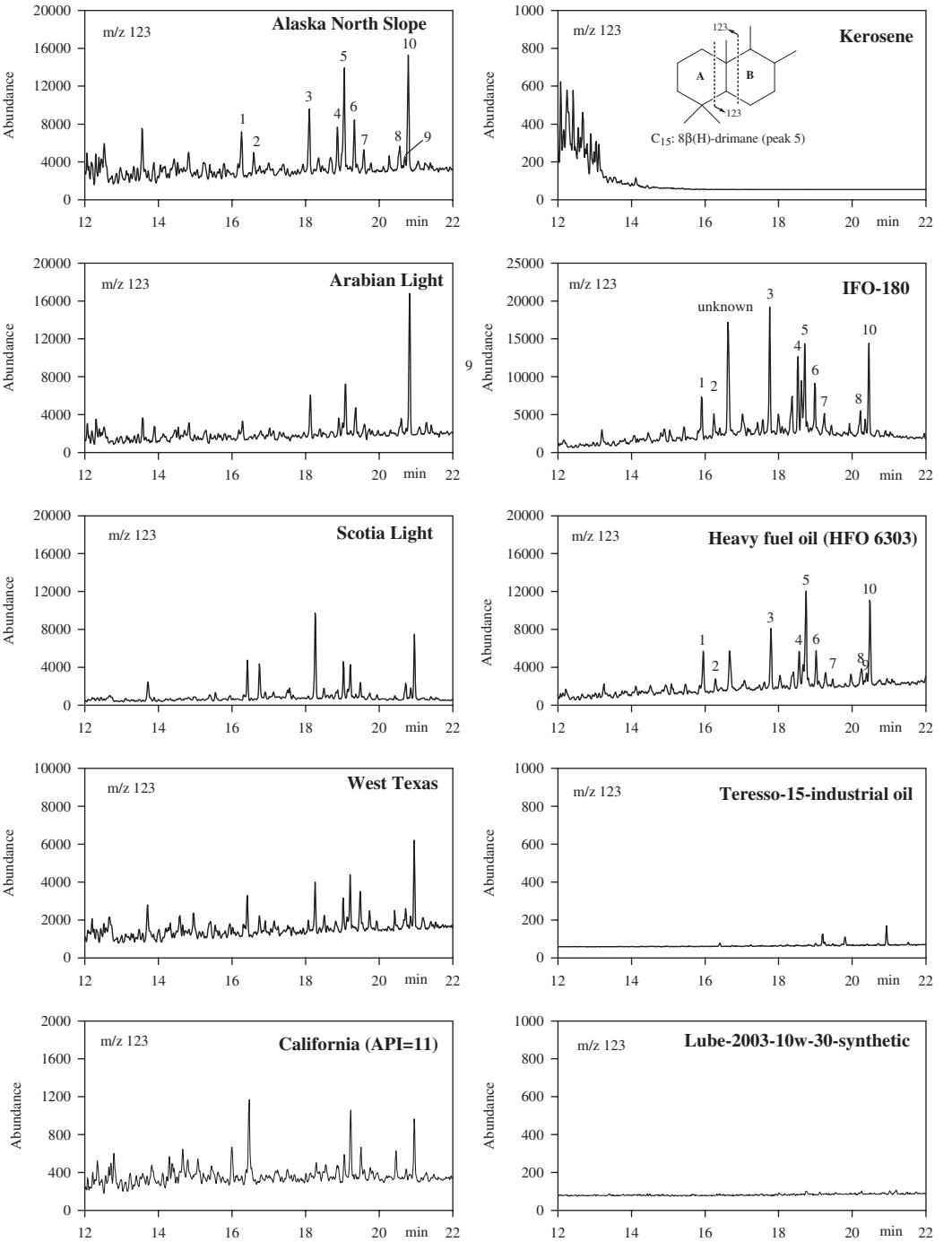


Figure 3-19 *Left panel*: GC-MS chromatograms of sesquiterpanes at *m/z* 123 for light (API > 35), medium (API: 25–35), and heavy (API < 25) crude oils, including Alaska North Slope (ANS), Arabian Light, Scotia Light oil (Nova Scotia), West Texas, and California API 11. *Right panel*: Comparison of SIM chromatograms of sesquiterpanes at *m/z* 123 for common petroleum products.

spilled sample had slightly higher abundances due to weathering. These similarities, in combination with other hydrocarbon quantification results such as bulk hydrocarbon groups, *n*-alkane distribution, and fingerprints of alkylated PAHs and diagnostic ratios of source-specific PAH compounds (Wang et al., 2000), argued strongly that the suspected diesel collected from the pumping station close to the spill site was the source of the spilled diesel.

3.3.7 Diamondoid Compounds in Oils and Lighter Petroleum Products

The group of *diamondoids* (collective term for adamantane, diamantane, and their alkyl homologous series) is another group of low-boiling cyclic biomarkers of interest to environmental forensics. Diamondoids are rigid, three-dimensionally fused cyclohexane-ring alkane compounds that have a diamondlike cage structure (Chen et al., 1996; Dahl et al., 1999; Grice et al., 2000). They consist of pseudo-homologous series with the general formula, $C_{4n+6}H_{4n+12}$, including adamantane (C_{10}), dia- (C_{14}), tria-, tetra-, and pentamantane (n = 1–5, respectively) and higher polymantanes, and their alkylated homologues. Adamantane was first discovered and isolated from a Czechoslovakian petroleum in 1933. Since then, more diamondoids have been found in crude oils (Petrov, 1987; Williams et al., 1986; Wingert, 1992; Grice et al., 2000; Lin and Wilkes, 1995). Adamantane and diamantane found in petroleum are thought to be formed from rearrangements of suitable organic precursors including multiring terpene hydrocarbons under thermal stress with strong Lewis acids (typically clays) acting as catalysts during oil generation (Chen et al., 1996; Dahl et al., 1999). The higher homologues of diamondoids are thought to be formed from lower homologues under extreme temperature and pressure conditions (Grice et al., 2000). The diamond structure endows these molecules with a high kinetic and thermodynamic stability. Laboratory thermal cracking experiments (Dahl et al., 1999) have shown that diamondoids have a higher thermal stability than most other hydrocarbons during thermal cracking of oil; therefore, diamondoids become increasingly enriched in the residual oil or condensate. The increase in methyldiamantane (C_{15}) concentration is directly proportional to the extent of cracking, indicating that under the conditions of the experiments, diamondoids are neither destroyed nor created. Instead, they are conserved and concentrated, and hence can be considered a naturally occurring *internal standard* by which the extent of oil loss can be determined.

Adamantanes and diamantanes elute in the ranges of n-C_{10} and n-C_{13} and n-C_{15} and n-C_{17}, respectively, in the GC-MS chromatogram of a saturated hydrocarbon fraction. Adamantanes are monitored at their characteristic ions at *m/z* 136 for adamantane, 135 for methyl- and ethyladamantanes, 149 for dimethyladamantanes, 163 trimethyladamantanes, and 179 for tetramethyladamantanes; while diamantanes are monitored at *m/z* 188, 187, 201, and 215 for diamantane, methyldiamantanes, dimethyl-diamantanes, and trimethyldiamantanes, respectively. Figure 3-20 shows the GC-MS-SIM chromatograms for analysis of diamondoids in Prudhoe Bay oil. Twenty-six diamondoid compounds were identified, and among these 17 are adamantanes and 9 diamantanes. Peak assignments are presented in Table 3-8. Identification of diamondoid hydrocarbons as based on mass spectra, comparison of GC retention data with reference standards, and calculation of reference index (IR) and comparison with literature RI values (Wingert, 1992; Chen et al., 1996).

Figure 3-20 reveals the following: (1) the differences in concentrations and relative distributions of adamantanes are apparent. 1,3,5,7-tetramethyladamantane (Peak 5) has the lowest concentration among the adamantane series. This is most likely due to the fact that 1,3,5,7-tetramethyladamantane has four methyl groups that could affect each other and cause the molecule structure to be thermally unstable. (2) The group of methyladamantanes contains only two isomers (Peak 2: 1-methyladamantane and Peak 6: 2-methyladamantane)

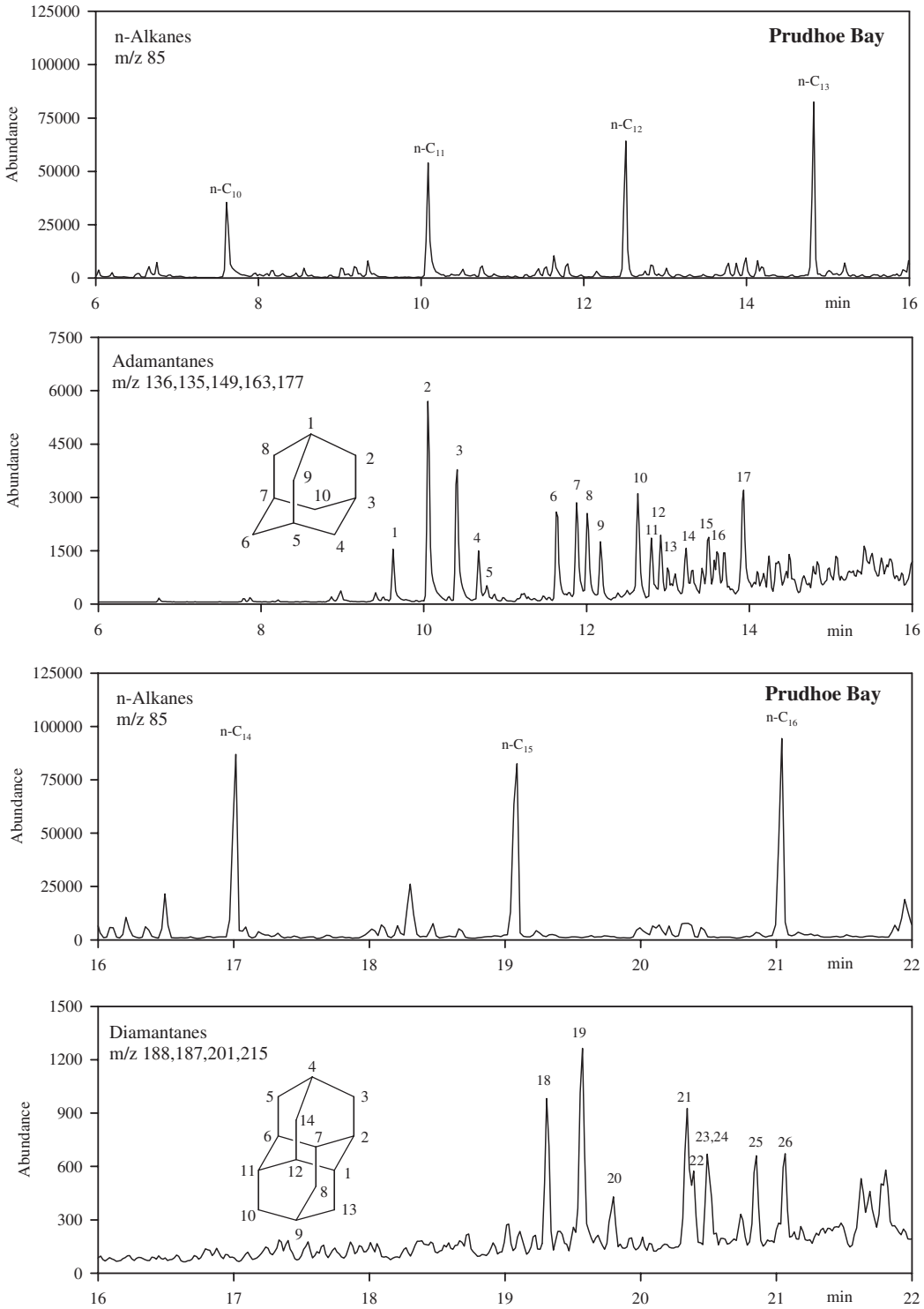


Figure 3-20 GC-MS chromatograms of diamondoids in Prudhoe Bay oil for peak identification.

Table 3-8 Peak Identification of Diamondoid Compounds in Prudhoe Bay Oil

Peak No.	*Compounds*	*Abbreviations*	*Base Peak*	M^+ *(m/z)*	*Formula*
Adamantanes					
1	Adamantane	A	136	136	$C_{10}H_{16}$
2	1-Methyladamantane	1-MA	135	150	$C_{11}H_{18}$
3	1,3-Dimethyladamantane	1,3-DMA	149	164	$C_{12}H_{20}$
4	1,3,5-Trimethyladamantane	1,3,5-TMA	163	178	$C_{13}H_{22}$
5	1,3,5,7-Tetramethyladamantane	1,3,5,7-TeMA	177	192	$C_{14}H_{24}$
6	2-Methyladamantane	2-MA	135	150	$C_{11}H_{18}$
7	1,4-Dimethyladamantane, *cis*	1,4-DMA, *cis*	149	164	$C_{12}H_{20}$
8	1,4-Dimethyladamantane, *trans*	1,4-DMA, *trans*	149	164	$C_{12}H_{20}$
9	1,3,6-Trimethyladamantane	1,3,6-TMA	163	178	$C_{13}H_{22}$
10	1,2-Dimethyladamantane	1,2-DMA	149	164	$C_{12}H_{20}$
11	1,3,4-Trimethyladamantane, *cis*	1,3,4-TMA, *cis*	163	178	$C_{13}H_{22}$
12	1,3,4-Trimethyladamantane, *trans*	1,3,4-TMA, *trans*	163	178	$C_{13}H_{22}$
13	1,2,5,7-Tetramethyladamantane	1,2,5,7-TeMA	177	192	$C_{14}H_{24}$
14	1-Ethyladamantane	1-EA	135	164	$C_{12}H_{20}$
15	1-Ethyl-3-methyladamantane	1-E-3-MA	149	178	$C_{13}H_{22}$
16	1-Ethyl-3,5-dimethyladamantane	1-E-3,5-DMA	163	192	$C_{14}H_{24}$
17	2-Ethyladamantane	2-EA	135	164	$C_{12}H_{20}$
Diamantanes					
18	Diamantane	D	188	188	$C_{14}H_{20}$
19	4-Methyldiamantane	4-MD	187	202	$C_{15}H_{22}$
20	4,9-Dimethyldiamantane	4,9-DMD	201	216	$C_{16}H_{24}$
21	1-Methyldiamantane	1-MD	187	202	$C_{15}H_{22}$
22	1,4 & 2,4-Dimethyldiamantane	1,4 & 2,4-DMD	201	216	$C_{16}H_{24}$
23	4,8-Dimethyldiamantane	4,8-DMD	201	216	$C_{16}H_{24}$
24	Trimethyldiamantane	TMD	215	230	$C_{17}H_{26}$
25	3-Methyldiamantane	3-MD	187	202	$C_{15}H_{22}$
26	3,4-Dimethyldiamantane	3,4-DMD	201	216	$C_{16}H_{24}$

due to their structural symmetry. 1-methyladamantanes that have only one methyl group attached to the bridgehead position (that is, at carbon position 1) are the most abundant. Similarly, 4-methyldiamantane (Peak 19), also a bridgehead methylated compound, is the most abundant compound in the diamantane series. The reason is that the methyl substitution in adamantane or diamantane at a bridgehead position (i.e., position of a tertiary carbon in the ring structure) creates a more stable molecule than substitution at a secondary carbon atom (carbon position 2) as the latter produces additional skew-butane repulsions that are not imposed by the bridgehead attachment (Wingert, 1992). Therefore, 1-methyladamantane has a higher thermal stability than 2-methyladamantane. Likewise, 4-methyldiamantane has a higher thermal stability than 1-methyldiamantane (Peak 21) and 3-methyldiamantane (Peak 25). Stable hydrocarbons will gradually increase in relative abundance over the less stable isomeric ones with increasing thermal stress. Hence, the relative distribution of alkyl-substituted diamondoid hydrocarbons may be used for assessing the maturity, especially for highly mature petroleum. Two diamondoid hydrocarbon ratios [methyladamantane index (MAI) and methyldiamantane index (MDI), defined as 1-MA/(1 + 2-MA) and 4-MD/(1 + 3 + 4-MD), respectively] have been developed and used as novel high-maturity indices to evaluate the maturation and evolution of crude oils, and to determine the thermal maturity of thermogenic gas and condensate in several Chinese basins, the maturity of which may be difficult to assess using routine geochemical techniques (Chen

et al., 1996). (3) The elution of alkyladamantanes (i.e., the sequence of their boiling points) is quite peculiar. All methyladamantanes substituted at the bridgehead (that is, at position 1) have much lower boiling points than adamantanes with at least one of the methyl groups not situated at the bridgehead (such as 2-methyladamantane, 1,2-dimethyladamantane, 1,4-dimethyladamantane, and 1,3,4-trimethyladamantane). The difference in the boiling points of these adamantanes is so large that 2-methyladamantane (C_{11}) elutes later than 1,3,5,7-tetramethyladamantane (C_{14}).

Experimental results demonstrate that both concentrations and relative distributions of diamondoids vary significantly between crude oils and refined products of different origins. Figure 3-21 diplays the GC-MS chromatograms of adamantanes and diamantanes for five representative crude oils from Cold Lake Bitumen to the South Louisiana oil. Both adamantanes and diamantanes occur in detectable quantities in all of the crude oils studied. Overall, the one-cage adamantanes are much more abundant than two-cage diamantanes. Based on quantitation data, the principal dominant adamantane hydrocarbons are A, 2-MA, 1-MA, 2-EA, 1,2-DMA, and, 1,3-DMA, together accounting for about 50% of all detected adamantanes; and the dominant diamantane compounds are D, 4-MD, 1-MD, 3-MD, and 3,4-DMD. Figure 3-22 compares the GC-MS chromatograms of adamantanes and diamantanes for five representative refined petroleum products including Jet A, two diesel fuels, a fuel No. 4, and a lubricating oil. Adamantanes were found in all fuel oil samples and were detected in most lubricating oils at a trace level. As expected, however, only quite low or no diamantane compounds were detected in light kerosene and heavy-end lubricating oils. Generally, the overall distribution pattern of individual diamondoid compounds in petroleum products is similar to that in crude oils, in which 1-MA and 2-MA, and D and 4-MD, dominated the adamantanes and diamantanes, respectively. The absolute concentrations and distribution patterns of diamondoids differ widely in the petroleum products studied. These differences are attributed to the differences in the crude oil feedstocks used in the production and to the distillation cut point of the petroleum products.

The unique molecular structures of diamondoids imply that their distributions and relative ratios have the potential to differentiate spilled oils, particularly to correlate and differentiate spilled lighter refined products in which high-molecular-weight tri- to pentacyclic biomarkers are present, if at all, in only trace amounts. Although diamondoids have found increasing application in petroleum exploration and refining in recent years, there have been few reports of use of these compounds for forensic oil spill investigations. Recently, Stout and Douglas (2004) reported application of diamondoid hydrocarbons in the chemical fingerprinting of natural gas condensates and gasoline. In this laboratory, diamondoids in over a hundred crude oils and refined products have been quantitatively characterized, and distributions of diamondoids in different oils, oil distillation fractions, and various refined products have been qualitatively and quantitatively compared. A number of diagnostic indices of diamondoids have been developed for forensic oil correlation and differentiation (Wang et al., 2005b).

3.3.8 Application of Biomarker Fingerprintings to Oil Spill Studies

The fingerprints of petroleum biomarkers have been applied to investigations of oil spill accidents (e.g., Barakat et al., 1999; Bence et al., 1996; Kvenvolden et al., 1993; Page et al., 1988; Wang et al., 1994b, 1995b, 1998b; Zakaria et al., 2000, 2001; Stout et al., 2001) and to trace the record of hydrocarbon input to the San Francisco Bay (Hostettler, 1999a). In recent years, biomarkers, together with PAHs and other hydrocarbon characterization results, have been extensively applied to assess the origin of the petrogenic hydrocarbon background in Prince William Sound (PWS) of Alaska, the site of the 1989 *Exxon Valdez* oil spill (i.e., is the petrogenic hydrocarbon background mainly from eroding Tertiary shales

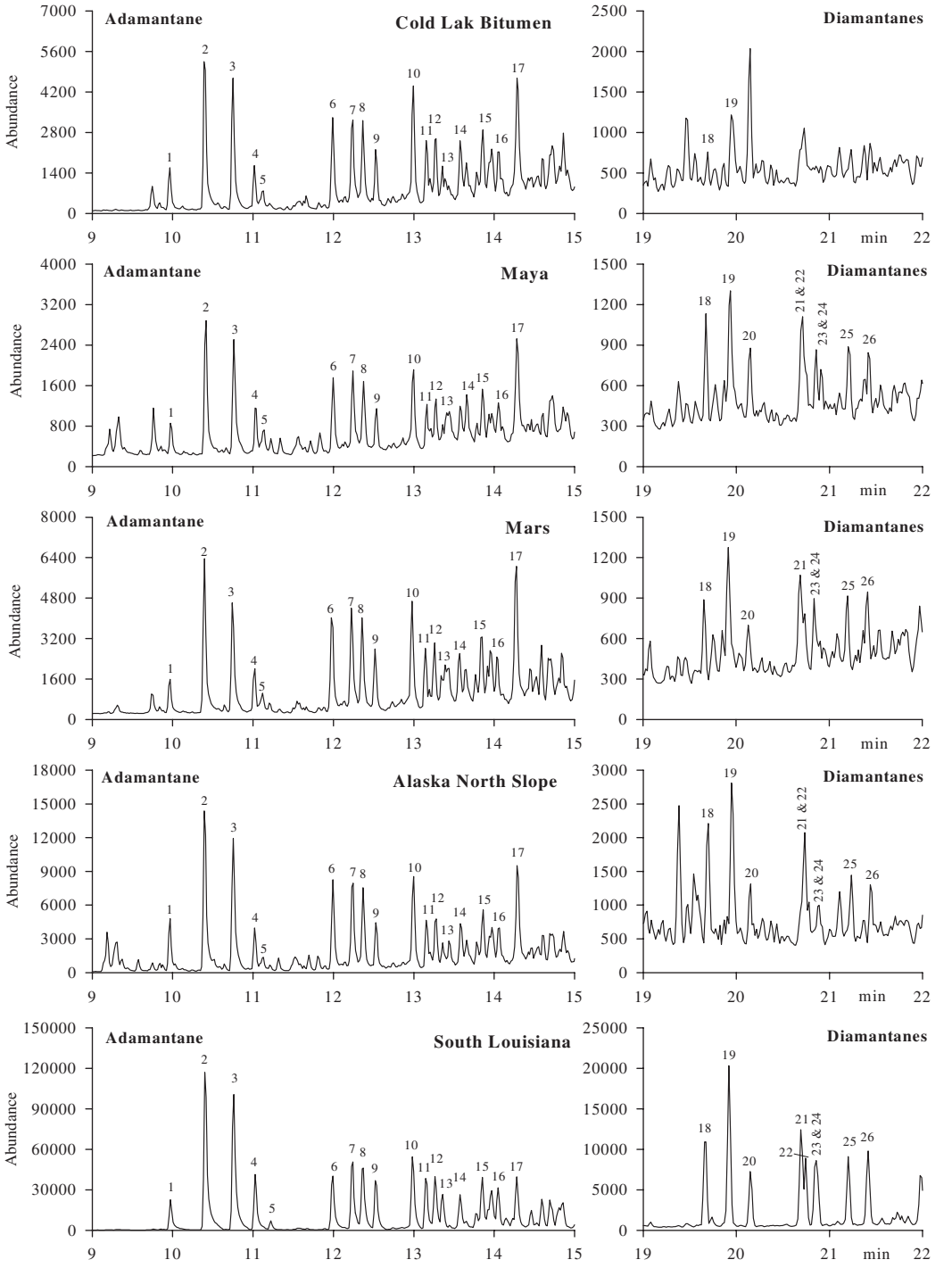


Figure 3-21 GC-MS total ion chromatograms of adamantanes (left) and diamantanes (right) of five representative crude oils including Cold Lake Bitumen, Maya, Mars, Alaska North Slope, and South Louisiana crude oil.

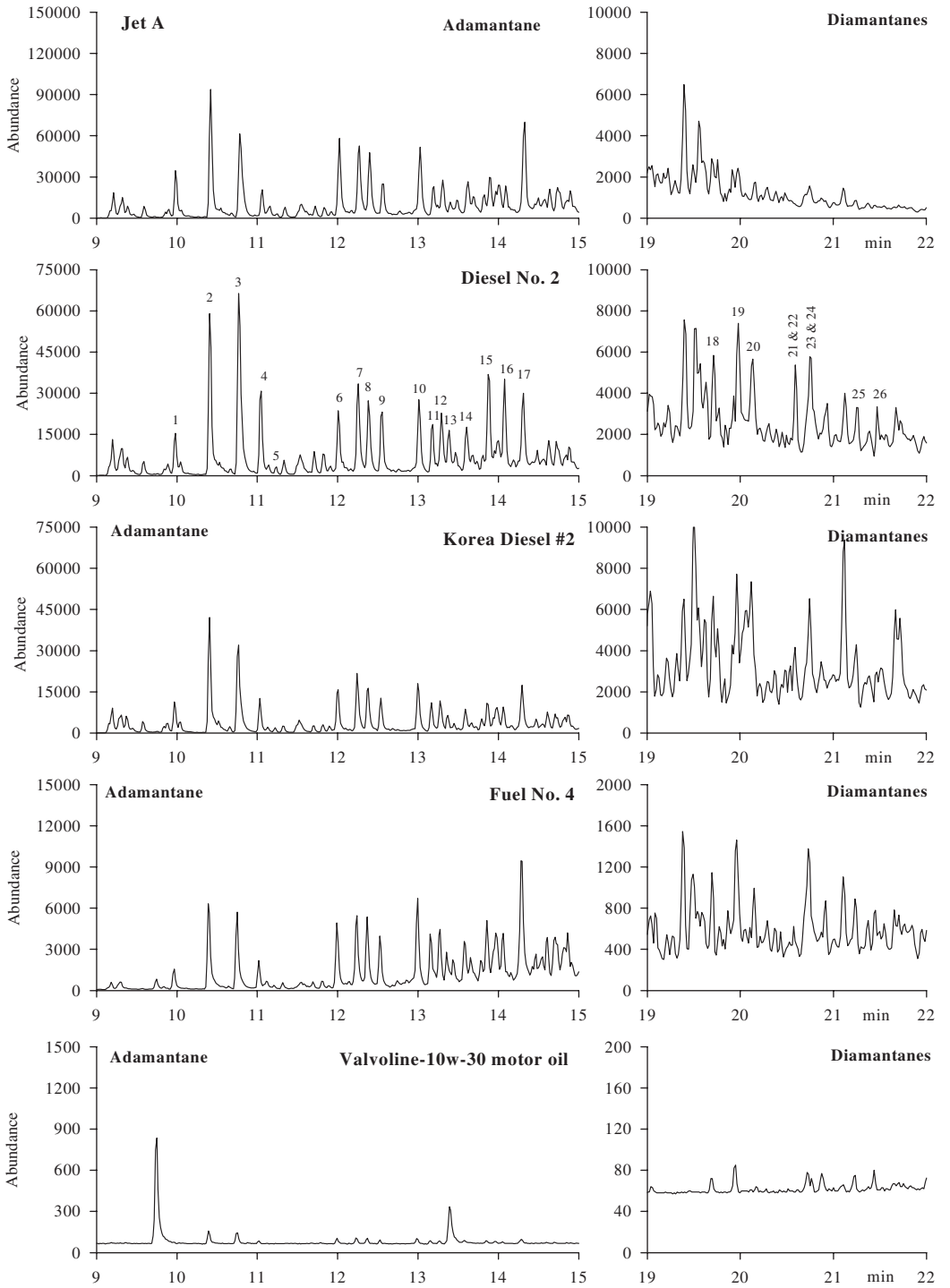


Figure 3-22 GC-MS total ion chromatograms of adamantanes and diamantanes of five representative refined petroleum products including Jet A, Diesel No. 2, Korea Diesel, Fuel No. 4, and Valvoline-10W-30 lube oil.

and residues of natural oil seepage, or mainly from Berling River coals and oil from the Katalla area?) by two groups of scientists (Boehm et al., 2001, 2002; Page et al., 1995, 1996, 2002; Bence et al., 1996; Short et al., 1997, 1999; Hostettler et al., 1999b). (See Chapter 15 herein.)

Examination and comparison of biomarker fingerprinting patterns and profiles are widely used for oil correlation and differentiation in environmental forensic studies. As described above, the distribution pattern and profiles of biomarkers are, in general, different from oil to oil and from oil to refined products of different origins. Various biomarkers can occur in different carbon ranges. Also, concentrations of individual biomarkers could be markedly different. Therefore, qualitative and quantitative comparisons of biomarker distribution are important for spill/source identification: (1) whether target biomarkers detected in spill samples can be found in suspected source candidates; (2) whether the distribution patterns and profiles of biomarkers match; (3) whether the abundances of target biomarkers match; (4) whether there are any "source-specific" or unknown biomarker compounds; (5) whether the diagnostic ratios of major biomarkers match. In most cases, disparity (no matching) of biomarker distribution is strong evidence for lack of correlation between spill sample(s) and suspected source(s). Matching may be an indication of a correlation of spill sample(s) and suspected source(s), but under certain spill scenarios is not necessarily "proof" that samples are from the same source.

Based on analysis of triterpane distribution patterns and determination of two pentacyclic C_{27} triterpanes, Shen (1984) distinguished four Arabian crudes, which in their weathered forms were extremely similar. Volkman et al. (1992b) determined the distribution of various biomarker compounds in a range of aquatic sediment samples to confirm the presence of oil contamination and identify possible oil sources. Among a number of pollution sources, lubricating oils were identified as a major source of hydrocarbon pollution in many estuaries and coastal areas around Australia. Currie et al. (1992) proposed utilization of triterpanes to distinguish tar balls originated from Southeast Asia from those of Australian petroleum sources. Mello et al. (1988) studied the geological and biomarker features of a wide selection of oils from the major Brazilian offshore basins. The study results reveal significant differences in chemical features of various oils, which enable them to be divided into five groups. The diagnostic features used for this classification include the absolute concentrations and distributions of hopanes and steranes, their abundances relative to 4-methylsteranes, and the occurrence and abundance of several specific biomarkers including 18α-oleanane, gammacerane, β-carotane, higher acyclic isoprenoids, 28,30-bisnorhopane, and 25,28,30-trisnorhopane. Barakat et al. (1997) studied the biomarker distribution within five crude oils from the Gulf of Suez, Egypt. The results revealed significant differences in biomarker distribution within the oils, and the oils can be categorized into three groups. Type 1 oils show a high relative abundance of gammacerane indicating a marine saline-source depositional environment. Furthermore, these oils have a predominance of C_{35} over C_{34} 17α(H)-homohopanes. Type 2 oils have an oleanane content of more than 20% of the concentration of C_{30} αβ hopane, indicating they originated from an angiosperm-rich, Tertiary source rock. Type 3 oil has geochemical characteristics intermediate between type 1 and 2 oils. Lu and Kaplan (1992) studied biomarker distribution in natural bitumen extracted from four coals: Rocky Mountain coal (RMC), Australian Gippsland Latrobe Eocene coal (GEC), Australian Gippsland Latrobe Cretaceous coal (GCC), and Texas Wilcox lignite (WL). They found there is a significant difference in the distribution of terpanes among these coals. Whereas pentacyclic triterpanes are dominant in GEC, GCC, and WL, diterpanes strongly predominate in the bitumen of RMC. Furthermore, the composition of triterpanes is also different. For example, tricyclic diterpanes are the only diterpanes present in RMC, whereas tetracyclic and tricyclic diterpanes are both

present in GEC and GCC, and tetracyclic diterpanes are most abundant in GEC. However, diterpanes are nearly absent in WL.

Spill Samples Are Different in General Chemical Composition. Wang et al. (1999b) studied oil spilled after a fire broke out at a carpet factory in Acton Vale, Quebec, on June 29, 1998. The GC-FID chromatograms of the spill samples were markedly different from the suspected-source sample. Spill samples were highly weathered (e.g., the *n*-alkanes were nearly completely lost with the abundances of pristane and phytane greatly reduced, and only a hump of UCM was seen in the chromatograms). However, the GC-MS biomarker analysis demonstrated that the distribution pattern of biomarker terpanes and steranes were nearly identical for the highly weathered spill and the relatively fresh suspected-source samples. In addition to the presence of the regular biomarkers from C_{21} to C_{35}, C_{30}-$\beta\alpha$ hopane in high abundance was also observed. It was concluded that the spilled oil was a Bunker C-type fuel, and it matched with the oil in the heat exchange equipment near the boiler, suggesting the oil spilled in the river came from the burned factory.

Spill Samples Are Very Similar in General Chemical Composition. In some cases, unknown oil samples may have very similar bulk chemical composition but markedly different biomarker distribution. On March 28, 2001, three unknown oil samples were received from Montreal for product characterization, correlation, and differentiation. The GC-FID screening results show that the three samples are hydraulic fluid-type products. The samples have very similar GC profiles (Figure 3-23). However, biomarker characterization results demonstrated that samples 1 and 2 are nearly identical in biomarker distribution patterns and concentrations, but sample 3 shows significantly different biomarker distribution from samples 1 and 2 (Figure 3-23). Concentrations of C_{29} and C_{30} hopanes in sample 3 match those in samples 1 and 2. Conversely, concentrations of C_{23} and C_{24}, and the sum of C_{31} to C_{35} homohopanes, are markedly lower and higher than those of the corresponding compounds in samples 1 and 2, respectively. Consequently, the diagnostic ratios of target biomarkers are very similar for samples 1 and 2, but apparently different from sample 3. All these observations point toward the conclusion that samples 1 and 2 are identical, while sample 3 comes from another source (Wang et al., 2002). This case study illustrates that successful forensic investigation will require fingerprinting not only common *n*-alkanes and isoprenoids but also biomarkers and determining their diagnostic ratios of spill samples, in particular for oils and products exhibiting very similar *n*-alkane and isoprenoid distributions.

3.3.9 Source-Specific Biomarkers

Biomarker terpanes with a hopane skeleton and steranes are common constituents of crude oils. However, certain oils may also contain some "source-specific" biomarker compounds including several geologically rarer acyclic alkanes. These biomarkers and their ratios can furthermore provide additional diagnostic information on the types of organic matter that give rise to the crude oil. For example, the geologically rare acyclic alkane botryococcane ($C_{34}H_{70}$) was used to identify a new class of Australian nonmarine crude oils (McKirdy et al., 1986). The presence of botryococcane indicates that the source rock contains remains of the algae *Botryococcus braunii*. The broad platform area of the northern North Sea, including Statford, Gullfacs, Brent, Oseberg, Troll, etc., seems to be specially featured by relative high abundances of *C_{28}-bisnorhopane* (Dahlmann, 2003). Thus, C_{28}-bisnorhopane can be regarded as a "source-specific" parameter. Dahlmann (2003) also found that oils from the Niger Delta (Nigeria) and oils from Africa (in Angola Cabinda and Nemba crudes and in Kongo and Gabon crudes) are characterized by the presence of highly abundant *oleanane* and *gammacerane*, respectively. The presence of 18α(H)-oleanane in benthic sediments in PWS, coupled with its absence in

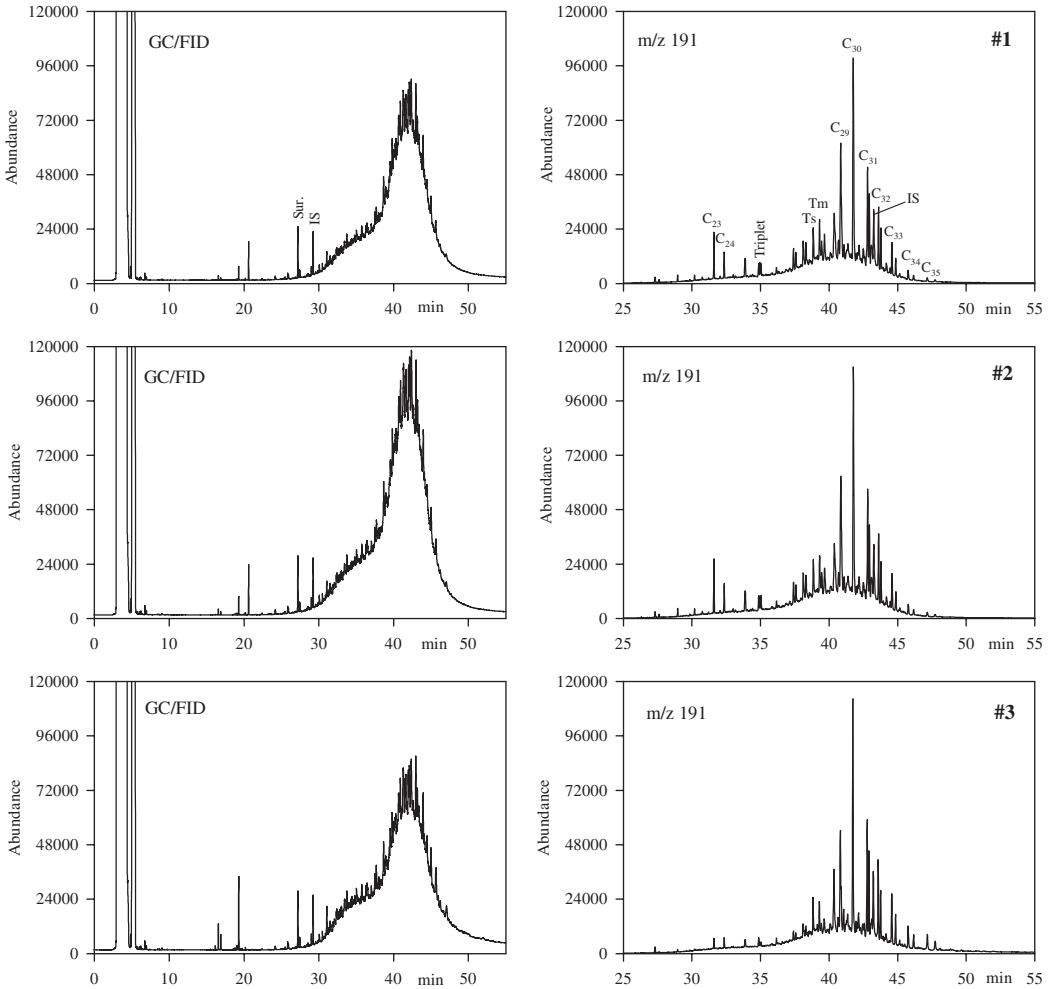


Figure 3-23 Comparison of GC-FID and GC-MS (*m/z* 191) of three unknown oil samples.

Alaska North Slope crude and specifically in *Exxon Valdez* cargo oil and its residues, confirmed another petrogenic source (Bence et al., 1996; see also Chapter 15). Characterization of 18α(H)-oleanane in oils from the Anaco area and Maturin subbasin, Venezuela, has been used for organic type and age indicator for assessment of the Venezuelan petroleum system (Alberdi and Lopez, 2000). Other "source-specific" petroleum biomarkers (Figure 3-24) include:

1. *C_{30} 17α(H)-diahopane*: C_{30} 17α(H)-diahopane (C_{30}*) elutes right after C_{29} αβ-norhopane and 18α(H), 21β(H)-30-norneohopane (C_{29}Ts) in the *m/z* 191 mass chromatogram. C_{30} 17α(H)-diahopane has been regarded as a possible terrestrial marker (Moldowan et al., 1991). El-Gayar et al. (2002) characterized seven oils representing the different petroleum-bearing basins in the Western Desert, Egypt. The characterization indicated that Type 2 and Type 3 oils are similar and show relative high pristane/phytane ratios, paucity of C_{30} steranes, and high relative abundance of C_{30}*, suggesting that they probably originated from source rocks containing

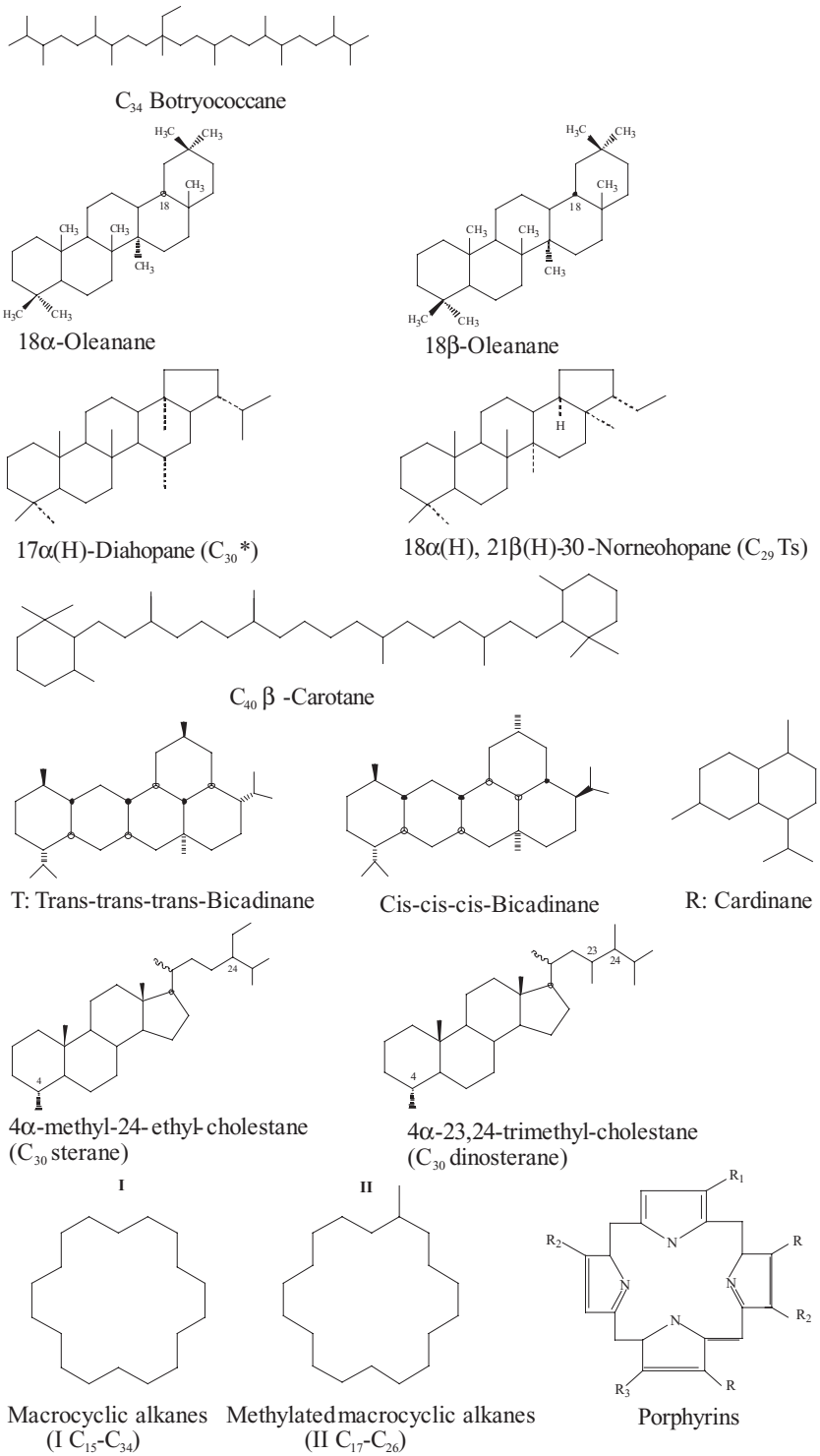


Figure 3-24 Molecular structures of a selection of "source-specific" biomarkers.

significant proportions of higher plant material.

2. β-*carotane*: This compound is a fully saturated C_{40}-dicyclane. It elutes after C_{35} homohopane in the oil saturate fraction. It is highly specific for anoxic, saline, and lacustrine deposition of algal organic matter. It is measured at fragment *m/z* 125 and/or at molecular ion *m/z* 558. β-carotane has been detected in several Chinese oils and the Mississippian Alberta shale. The presence of a significant amount of β-carotane and gammacerane relative to the hopanes has recently been detected, suggesting that the source rocks of the oil from the Liaohe Basin of China were probably deposited in a highly stratified, strongly reducing environment (Wang et al., 1996).
3. *Extended hopanes beyond C_{40}*: A series of side-chain extended 17α(H), 21β(H)-hopanes and 17β(H), 21α(H)-hopanes up to C_{44} have been identified in crude oils and source rock extracts in the Liaohe Basin, Northeast China (Wang et al., 1996). These compounds may be viewed as the representatives of a new class of molecules and may find applications in forensic fingerprinting of unknown spill oils.
4. *Bicadinanes*: Bicadinanes are C_{30}-pentacyclic biomarker compounds and have three configurations, labeled as W (cis-cis-trans-bicadinane), T (trans-trans-trans-bicadinane), and R (cardinane). The mass spectra of bicadinane contain prominent *m/z* 191 and 217 fragments, while peaks can appear in corresponding chromatograms of both hopanes and steranes. All three bicadinanes (W, T, and R) form elute prior to C_{29} hopane in the *m/z* 191 chromatogram. But it can be conveniently monitored with little interference using the *m/z* 412 mass chromatogram. They are highly specific for resinous input from certain higher plants that commonly contributed to source rocks for Tertiary oils from the Far East (van Aarssen et al., 1990). Based on biomarker composition, crude oils from the North, Central, and South Sumatra basins, Indonesia, were classified into three types (Sosrowidjojo et al., 1994), and Group II oils were further distinguished from Group 1 oils by their high abundance of bicadinanes relative to C_{30} hopane on the *m/z* 412 mass chromatogram.
5. *4-methyl steranes*: The 4-methyl steranes can be divided into two major classes: (1) C_{28}–C_{30} analogues of the steranes at positions 4 and 24 (e.g., the C_{30} sterane is 4α-methyl-24-ethylcholestane), and (2) C_{30} dinosteranes (e.g., 4α,23,24-trimethylcholestanes). 4α-Methyl-24-ethylcholestanes often occur in relatively high abundance in Tertiary source rocks and related oils from China (Fu et al., 1992). For example, almost all of the oils from the eastern Pearl River Mouth Basin contain significant amounts of 4-methylsteranes (Zhang et al., 2003). Hu (1991) found that 4-methylsteranes in the range of C_{28}–C_{30} are unusually rich (which comes up to 20–40% of the total steranes) in certain oils from terrestrial facies within the South China Sea. C_{30} 4-methylsterane ($M^+ = 414$) is particularly abundant among the 4-methylsteranes. Dinosterane has only been reported in petroleum younger than Triassic age (Summons et al., 1992). The presence of dinosterane in relatively high concentrations in asphaltic bitumens from southern Australia (Mckirdy et al., 1994) suggests that their source is no older than mid-Triassic. In a study to re-evaluate the petroleum prospective potential in southeast Australia, Volkman et al. (1992b) examined 10 bitumen samples collected between 1880 and 1915. The high proportions of C_{27} steranes and the presence of C_{30} steranes including dinosteranes suggested that the bitumens were derived from a marine source rock containing mainly marine organic matter.
6. *Macrocyclic alkanes*: Murrisepp et al. (1994) first reported the presence of non-isoprenoidal macrocyclic alkanes in sedimentary material and tentatively identified these cyclic hydrocarbons of the cyclododecane and cyclohexadecane series in the

nonaromatic hydrocarbon fractions of the semicoking oil from an Estonian oil shale. Audino et al. (2001, 2002) have unambiguously identified for the first time a new class of cyclic hydrocarbon biomarker, macrocyclic alkanes and their methylated analogues in a *Botryococcus braunii*-rich sediment (torbanite) of Late Carboniferous age (Audino et al., 2001) and in two Indonesian crude oils (Audino et al., 2002). The compounds consist of a homologous series of macrocyclic alkanes in a wide range from C_{15} to C_{34} and their methylated derivatives (ranging from C_{17} to C_{26}). The distribution of macrocyclic alkanes was measured at the characteristic ion *m/z* 111. The macrocyclic alkanes appear to be novel markers of *B. braunii* and add to the catalogue of the characteristic hydrocarbons derived from this alga. More importantly, these compounds could be original markers specific to highly resistant algaenan of *B. braunii* in sediments and crude oils.

7. *Porphyrins*: Porphyrins are a special class of N-containing compounds. They are complex derivatives of the basic material porphine. Porphine consists of four pyrrole [(CH═CH)$_2$═NH] units joined by methine, –C═, bridges; the methine bridges establish conjugated linkages between the component pyrrole nuclei, forming a more extended resonance system. Although the resulting structure retains much of the inherent character of the pyrrole components, the larger conjugated system gives increased aromatic character to the porphine molecule. The porphyrin compounds are degradation products of the chlorophyll (photosynthetic pigments of plants and some bacteria). Most of the porphyrin material in crude oils is chelated with metal, of which vanadium is the most important, followed by nickel. Iron and copper-porphyrin chelates may also be present in oil. Porphyrins are not usually considered among the usual nitrogen-containing constituents of petroleum, nor a metallo-containing organic material. Conversely, they are often classified as a unique class of biomarker compounds because they may establish a link between compounds found in the geosphere and their corresponding biological precursors. Crude oils and bitumens contain small amounts of vanadyl and nickel porphyrins. In general, mature, lighter oils contain less of these compounds, whereas heavy oils may contain larger amounts of vanadyl and nickel porphyrins. Chen et al. (1999) have successfully separated nine free petroporphyrin compounds from a Chinese crude oil by reversed-phase HPLC. These were further identified by mass spectrometry as C_{27}E (*m/z* 408), C_{28}E (*m/z* 422), C_{29}E (*m/z* 436), C_{30}E (*m/z* 450), C_{31}E (*m/z* 464), C_{29}D (*m/z* 434), C_{30}D (*m/z* 448), C_{31}D (*m/z* 462), and C_{32}D (*m/z* 476) porphyrins.

The search for source-specific geochemical biomarkers continues to be a fertile area of research for fingerprinting similar sources of petroleum. If an oil shows any additional characteristic compositional features (such as "extra" biomarker peaks), these should of course always be included in the characterization and considered in the identification and correlation. It should be noted, however, that reliable biomarker interpretation is usually based on a whole biomarker distribution chromatogram and a series of biomarker parameters. No single parameter can be exclusively used for unambiguous source identification of unknown spills. Individual unique biomarker parameters only become valuable and meaningful when used together and they agree with other biomarker parameters.

3.3.10 Using Diagnostic Ratios and Cross-Plots of Biomarkers for Source Identification of Oil Spills

Biomarker diagnostic parameters have been long established and are widely used by geochemists for oil correlation, determination of organic input and precursors, depositional environment, assessment of thermal maturity, and evaluation of in-reservoir oil biodegradation (Peters and Moldowan, 1993). Many

diagnostic ratios currently used in oil spill studies and environmental forensics originate from the petroleum geochemistry literature.

3.3.10.1 Diagnostic Ratios of Biomarkers

Most biomarkers in spill samples and source oils, in particular those homologous series of biomarkers with similar structure, show little or no changes in their diagnostic ratios. 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 the data since variations due to the fluctuation of instrument operating conditions day-to-day, operator, and matrix effects are minimized. Therefore, comparison of diagnostic ratios reflects more directly differences of the target biomarker distribution between samples.

Diagnostic ratios can either be calculated from quantitative (i.e., compound concentrations) or semiquantitative data (i.e., peak areas or heights). Diagnostic biomarker ratios frequently used as defensible indices by the environmental chemists for identification, correlation, and differentiation of spilled oils are summarized in Table 3-9. These ratios consist of alkanes, terpanes, steranes, sesquiterpanes, and diamondoids. Ratios are generally defined from (biomarker 1)/(biomarker 2) for simplicity, but can readily be redefined in other forms such as (biomarker 1)/(biomarker 1 + biomarker 2). Selection of diagnostic ratios employed in oil spill studies is mainly based on source-specific variables (e.g., specificity, diversity, and analytical precision). It is important to realize that the suite of diagnostic ratios as listed in Table 3-9 is neither inclusive nor appropriate for all oil spill identification cases. In some spill cases, it may be prudent to include some particularly characteristic ratios. In other situations, the abundance of some biomarkers may be too low to obtain reliable diagnostic ratios. Thus, maintaining flexibility in the selection of diagnostic ratios to be used in specific cases is important.

For diamondoid compounds, numerous diagnostic indices based upon concentrations of target adamantanes have been developed and calculated for the crude oil samples as well as the refined petroleum products. In principle, a large number of diagnostic ratios from 26 identified adamantanes and diamantanes can be produced. However, some ratios are heavily affected by measurement errors due to low peak abundances and poor peak separation; thus, a proper selection of diagnostic ratios of diamondoids is important in order to keep the uncertainties to a minimum and yield reliable results. For this purpose, the diagnostic power (DP) is used for selection of diagnostic ratios (Christensen et al., 2004). DP is defined as the relative standard deviation (RSD_V) of a diagnostic ratio for oils of different origins (~100 oil samples in total) divided by the relative standard deviation (RSD_A) of the same ratios calculated from six measurements of the ESTD reference oil (Prudhoe Bay crude oil, 13.1% weathered). Based on the determined DP values for oil in the Environmental Canada Oil Research Laboratory, diagnostic ratios of 1-MA/2-EA, 1-MA/1,3,4-TMA, 1-MA-1,2-DMA, 1-MA/1,2,5,7-TeMA, 1,3,5,7-TeMA/1,2,5,7-TeMA, 1,3,5-TMA,/1,2,5,7-TeMA, 1,3,5-TMA,/1,3,6-TMA, and 1,4-DMA/1,3,4-TMA with high DP values are selected from more than 50 possible diagnostic ratios as more sensitive and reliable parameters for source correlation and differentiation of oils and petroleum products. It should be noted that the ratios with low DP values, particularly those developed from low abundant diamantanes, are not recommended as reliable distinguishing tools and may be used only as supplementary diagnostic information for certain case studies. Otherwise, higher analytical uncertainties related to these indices could lead to erroneous conclusions for oil source identification.

The triplet ratio, if present, generally varies in oils from different sources and is dependent upon sources, depositional environment, and maturity. The ratio was first used by Kvenvolden et al. (1985) to study a North Slope crude, in which the ratio is ~2. The

Table 3-9 Diagnostic Biomarker Ratios Frequently used for Identification, Correlation, and Differentiation of Spilled Oils

Biomarker classes	*Diagnostic ratios*	*Code*
Acyclic isoprenoids	pristane/phytane	pri/phy
	pristane/n-C_{17}	pri/C_{17}
	phytane/n-C_{18}	phy/C_{18}
Terpanes (*m/z* 191)	C_{21}/C_{23} tricyclic terpane	TR21/TR23
	C_{23}/C_{24} tricyclic terpane,	TR23/TR24
	C_{23} tricyclic terpane/C_{30} αβ hopane	TR23/H30
	C_{24} tricyclic terpane/C_{30} αβ hopane	TR24/H30
	C_{24} tertracyclic/C_{26} tricyclic (S)/C_{26} tricyclic (R) terpane	triplet ratio
	C_{27} 18α,21β-trisnorhopane/C_{27} 17α,21β-trisnorhopane	Ts/Tm
	C_{28} bisnorhopane/C_{30} αβ hopane	H28/H30
	C_{29} αβ-25-norhopane/C_{30} αβ hopane	NOR25H/H30
	C_{29} αβ-30-norhopane/C_{30} αβ hopane	H29/H30
	oleanane/C_{30} αβ hopane	OL/H30
	moretane(C_{30} βα hopane)/C_{30} αβ hopane	M30/H30
	gammacerane/C_{30} αβ hopane	GAM/H30
	tricyclic terpanes (C_{19}–C_{26})/C_{30} αβ hopane	Σ(TR19-TR26)/H30
	C_{31} homohopane (22S)/C_{31} homohopane (22R)	H31S/H31R
	C_{32} bishomohopane (22S)/C_{32} bishomohopane (22R)	H32S/H32R
	C_{33} trishomohopane (22S)/C_{33} trishomohopane (22R)	H33S/H33R
	relative homohopane distribution	H31 : H32 : H33 : H34 : H35
	$\Sigma(C_{31} - C_{35})/C_{30}$ αβ hopane	Σ(H31 − H35)/ H30
	homohopane index	H31/Σ(H31 − H35) to H35/Σ(H31 − H35)
Steranes and diasteranes (*m/z* 217 & 218)	C_{27} 20S-13β(H), 17α(H)-diasterane/ C_{27} 20R-13β(H), 17α(H)-diasterane	DIA 27S/DIA 27R
	relative distribution of regular C_{27}-C_{28}-C_{29} steranes	C27 : C28 : C29 steranes
	C_{27} αββ/C_{29} αββ steranes (at m/z 218)	C27ββ(S + R)/C29ββ(S + R)
	C_{28} αββ/C_{29} αββ steranes (at m/z 218)	C28ββ(S + R)/C29ββ(S + R)
	C_{27} αββ/(C_{27} αββ + C_{28} αββ + C_{29} αββ) (at m/z 218)	C27ββ/(C27 + C28 + C29)ββ
	C_{28} αββ/(C_{27} αββ + C_{28} αββ + C_{29} αββ) (at m/z 218)	C28ββ/(C27 + C28 + C29)ββ
	C_{29} αββ/(C_{27} αββ + C_{28} αββ + C_{29} αββ) (at m/z 218)	C29ββ/(C27 + C28 + C29)ββ
	C_{27}, C_{28}, and C_{29} ααα/αββ epimers (at m/z 217)	C27αα/C27ββ C28αα/C28ββ C29αα/C29ββ
	C_{27}, C_{28}, and C_{29} 20S/(20S + 20R) steranes (at m/z 217)	C27 (20S)/C27 (20R) C28 (20S)/C28 (20R) C29 (20S)/C29 (20R)
	C_{30} sterane index: C_{30}/(C_{27} to C_{30}) steranes	C30/(C27 to C30) steranes
	selected diasteranes/regular steranes	C27-C28-C29 steranes/H30
	regular C_{27}-C_{28}-C_{29} steranes/C_{30} αβ-hopanes	
Sesquiterpanes (*m/z* 123)	relative distribution of sesquiterpanes	
	C_{14} group: Peak 1/Peak 2	P1/P2
	C_{15} group: Peak 3/Peak 5, Peak 4/Peak 5, Peak 6/Peak 5	P3/P5, P4/P5, P6/P5
	C_{16} group: Peak 8/Peak 10	P8/P10
	inter-group: Peak 1/Peak 3, Peak 1/Peak 5,Peak 3/Peak 10, Peak 5/Peak 10	P1/P3, P1/P5, P3/P10, P5/P10
Adamantanes (*m/z* 135, 149, 163, 177)	methyl adamantane index: 1-MA/(1- + 2-MA)	MAI
	1,4-DMA, cis/1,4-DMA, trans	DMAI
	dimethyl admantane index: 1,3-DMA/(1,3- + 1,4- + 1,2-DMA)	TMAI
	1,3,4-TMA, cis/1,3,4-TMA, trans	
	trimethyl adamantane index: 1,3,4-DMA, cis/(1,3,4-DMA, cis + 1,3,4-DMA, trans)	
	ethyl adamantane index: 1-EA/(1- + 2-EA)	EAI
Diamantanes (*m/z* 187, 201, 215)	methyl-diamantane index: 4-MD/(1- + 3- + 4-MD)	MDI
	relative distribution of diamantanes: C_0-D : C_1-D : C_2-D : C_3-D	
Triaromatic steranes (*m/z* 231)	C_{20} TA/(C_{20} TA + C_{21} TA)	
	C_{26} TA (20S)/sum of C_{26} TA (20S) through C_{28} TA (20R)	
	C_{27} TA (20R)/C_{28} TA (20R)	
	C_{28} TA (20R)/C_{28} TA (20S)	
	C_{26} TA (20S)/[C_{26} TA (20S) + C_{28} TA (20S)]	
	C_{28} TA (20S)/[C_{26} TA (20S) + C_{28} TA (20S)]	
Monoaromatic steranes (*m/z* 253)	C_{27}-C_{28}-C_{29} monoaromatic steranes (MA) distribution.	

*Ratios are defined for simplicity, but can be readily redefined in other forms. For example, the ratio of C_{29} αβ-30-norhopane/C_{30} αβ hopane (H29/H30) can be readily redefined as H29/(H29 + H30) × 100%.

spilled *Exxon Valdez* oil (an Alaska North Slope crude) and its residues also have triplet ratios of ~2. Conversely, many tar balls and residues collected from the shorelines of the Prince William Sound were similar to each other but chemically distinct from the spilled *Exxon Valdez* oil with triplet ratios of ~5. The triplet ratio, combined with other diagnostic biomarker ratios and isotopic compositions, revealed that these non-*Valdez* tar balls originated from California with a likely source being the Monterey Formation (Kvenvolden et al., 1995). During the Arrow oil spill work, the ratio of the most abundant C_{29} to C_{30} hopane as well as C_{23}/C_{24}, Ts/Tm, and $\alpha\beta\beta/(\alpha\beta\beta + \alpha\alpha\alpha)$ of C_{27}, C_{28}, and C_{29} steranes as defined and used by Wang et al. (1994b) as reliable source indicators. Similar approaches, combined with determinations of a number of other "source-specific marker" ratios, were applied to characterize oil samples from the Arctic Baffin Island spill (Wang et al., 1995b), oil on birds (Wang et al., 1997), the 25-year-old wetland Nipisi spills (Wang et al., 1998a), a mystery spill in Quebec (Wang et al., 2001a), and the Detroit River oil spill (Wang et al., 2004). Barakat et al. (2002) have proven the molecular ratios of triaromatic steranes including C_{28}TA 20R/C_{28}TA 20S, C_{27}TA 20R/C_{28}TA 20R, and C_{28}TA 20S/(C_{26}TA 20R + C_{27}TA 20S) were useful source indicators for correlating naturally weathered oil residues in the Egyptian Western Desert to a fresh crude oil sample of the Western Desert-sourced oil.

Use Diagnostic Ratios of Biomarkers in Combination with PAH Ratios for Source Identification. In January/February 1996, a significant number of tar ball incidents occurred along the coasts of Vancouver Island, Washington, Oregon, and California. Samples of the tar balls were collected from the affected beaches and characterized by GC-FID and GC-MS, and further analyzed using a carbon isotopic technique (Wang et al., 1998b). Biomarker characterization revealed that the BC and CA samples have similar diagnostic ratios of most biomarkers, but the CA samples show lower ratios of C_{23}/C_{30} and C_{24}/C_{30} than the BC samples. Only after in combination with characterization results of PAHs and PAH diagnostic ratios, was it defensively concluded that (1) CA/Oregon samples were chemically similar and consistent with the same source of a Bunker-type fuel. (2) BC tar ball samples were chemically similar and consistent with the same source (also Bunker-type fuel). They were similar to the CA/Oregon samples but may have a different source. (3) The spill samples had been highly weathered since release, and the CA samples were more heavily weathered than the BC samples. (4) The source of the tar ball samples was neither ANS nor California Monterey Miocene oil.

In application of diagnostic ratios of biomarkers for spill studies, it is important to acknowledge that regardless of diagnostic parameters used, a basic rule applied to all correlations and differentiations should be

- poorly matching biomarker distribution and/or diagnostic ratios are strong evidence for lack of a correlation between a spill sample(s) and suspected source(s),
- matching may be an indication of a correlation of a spill sample(s) and suspected source(s), but is not necessarily "proof" for identity.

Hence, in order to make more reliable and defensible correlations, the use of a "multicriteria approach" is often a prerequisite. In a multicriteria approach, the final conclusion is based on analysis and evaluation of the distribution of more than one suite of petroleum compounds (Peters et al., 2005; Stout et al., 2002; Wang et al., 1999a; Christensen et al., 2004; Daling et al., 2002).

3.3.10.2 Cross-Plots of Biomarkers

Cross-plots (i.e., plot of one diagnostic biomarker ratio versus another ratio) are another diagnostic means frequently used in oil geochemistry for oil–oil correlation and determination of oil source and depositional environment (Peters and Moldowan, 1993). Gürgey (2002) analyzed 56 rock and 28 crude oil samples from the sub-salt and supra-salt section of the southern Pre-Caspian Basin.

Based on plots of $C_{24}/C_{26}T$ (C_{24} tetracyclic/C_{26} tricyclic terpanes) versus C_{29}/C_{30} hopane, the author illustrates a clear separation between two populations: Population 1 (1A and 1B) and Population 2. Seifert and Moldowan (1986) applied cross-plots of C_{29} $\alpha\beta\beta/(\alpha\beta\beta+\alpha\alpha\alpha)$ sterane versus C_{29} 20S/(20S+20R) steranes as a particularly effective measure in describing the thermal maturity of source rocks or oils. Zhang et al. (2003) classified crude oils from the eastern Pearl River Mouth Basin into groups based on cross-plots of relative abundance (at *m*/*z* 123) of various isomeric sesquiterpanes versus relative abundances of bicadinanes to C_{30} hopane on the *m*/*z* 412 mass chromatogram (bicadinane-T/C_{30}-hopane).

Cross-Plots of Biomarkers for Spill Source Identification. Malaysian coasts are subjected to various threats of petroleum pollution including deliberate and accidental oil spills from various sources. The identification of detailed sources of the oil pollution, therefore, is essential to reduce the oil pollution through effective regulation. Based on chemical evidence that Middle East crude oils were characterized by C_{29} 17α,21β-norhopane and C_{31}–C_{35} homohopanes, whereas these compounds were depleted in South East Asian crude oils, Zakaria et al. (2000, 2001) proposed utility of the cross-plots of C_{29} αβ/C_{30} αβ hopane ratio versus the homohopane index $\Sigma(C_{31}$–$C_{35})/C_{30}$ hopane as key biomarker indicators and successfully distinguished a large number tar ball samples that originated from South East Asian crude oil sources from those of Middle East sources.

Cross-Plots of Sesquiterpane Isomers for Distinguishing Oils and Petroleum Products. Wang et al. (2005a) depict the cross-plots of sesquiterpanes (Peak 4/Peak 5 versus Peak 3/Peak 5) for more than 50 crude oils and refined products (Figure 3-25, left panel). There is large scatter in this set of oils in the cross-plot data: P4 : P5 and P3 : P5 fall in the

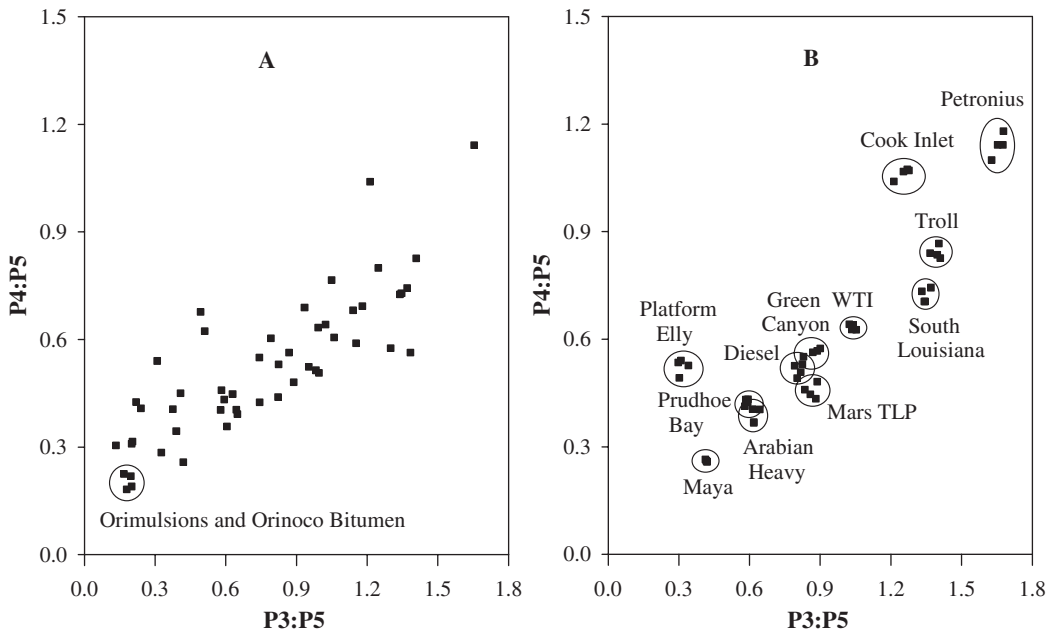


Figure 3-25 **A**: Cross-plots of the double ratios of Peak 4/Peak 5 versus Peak 3/Peak 5 for over 50 different oils and refined products. The circle indicates related samples from the same origin. **B**: Cross-plots of the double ratios of Peak 4/Peak 5 versus Peak 3/Peak 5 for 11 weathering oil series and 1 diesel weathering series. Each weathering oil series produces a tight cluster.

ranges of 0.2–1.2 and 0.1–2.1, respectively. Furthermore, related oils, such as the circle for Orimulsion samples from different batches and for the original Orinoco bitumen, produce tight clusters on the plot. This implies that sesquiterpane ratios, in combination with other fingerprinting data, may be used to discriminate different oils and to identify the source of spill samples. A double ratio plot of P4 : P5 versus P3 : P5 for 11 weathered crude oil and 1 weathered diesel series is shown in the right panel of Figure 3-25. The four weathered samples for each oil series form tight clusters, indicating that moderate weathering would not be expected to alter sesquiterpane distributions. For example, no depletion of sesquiterpanes, relative to the most abundant Peak 3, was observed for the weathered diesel samples (an Ottawa diesel, 2002) at four weathering percentages of 0, 7.2, 14.2, and 22%.

3.4 Effects of Weathering on Biomarker Fingerprinting

3.4.1 Processes Affecting the Fate and Behavior of Spilled Oil

When oils and petroleum products are released into the environment — water or land — they undergo a series of changes in chemical compositions and physical properties that in combination are termed "weathering." Weathering can strongly influence how oils move and behave in the environment (Jordan and Payne, 1980; Wang et al., 1995c; NRC, 2002). Weathering processes could include evaporation, emulsification, natural dispersion, dissolution, microbial degradation, photooxidation, and other processes (such as sedimentation, adhesion onto the surface of suspended particulate materials, and oil-fine interaction). Each of the weathering processes affects the hydrocarbon family differently. For example, aromatics tend to be more water soluble than aliphatics. Weathering processes occur at very different rates, depending on both the oil type and environmental conditions.

Evaporation. In the short term after an oil spill, evaporation is usually the single most important and dominant weathering process, in particular for the light petroleum products such as gasoline. Evaporation has the greatest effect on the amount of oil remaining on water or land after a spill. In the first few days following a spill, the loss can be up to 70 and 40% of the volume of light crudes and petroleum products, and gasoline can evaporate completely above zero degrees. For heavy or residual oils such as Bunker C oil, the losses due to evaporation comprise only a few percentages of the total volume. The rate at which oil evaporates depends primarily on the oil composition. The more volatile components an oil or fuel contains, the greater the extent and rate of its evaporation. The extent of evaporation is often the most important factor for determining oil properties at a given time after the spill and for changing the behavior of the oil.

Emulsification. Emulsification is the process by which water is dispersed into oil in the form of small droplets. Water droplets can remain in an oil layer in a stable form, and the properties of the emulsified oil are very different from the starting oil. The mechanism of water-in-oil emulsion formation is not yet fully understood, but most likely it starts with sea energy forcing the entry of small water droplets, about 10 to 25 μm in size, into the oil. Emulsions contain about 70% water, and thus, when emulsions are formed, the volume of spilled oil more than triples. In general, water can be present in oil in four ways (Fingas and Fieldhouse, 2003): (1) soluble; (2) unstable emulsion; (3) semi- or meso-stable emulsion; and (4) stable emulsions. Stable emulsions are reddish-brown in color and appear to be nearly solid. These emulsions do not spread and tend to remain in lumps or mats on the sea or shore. It has been noted that when oil forms stable or meso-stable emulsions, the rate of evaporation slows down considerably. Microbial degradation also appears to slow down. The dissolution of soluble components from oil may also cease once emulsification has occurred.

Natural Dispersion. Natural dispersion occurs when fine droplets of oil are transferred

into the water column by wave action or turbulence. Small droplets (<20 μm) are relatively stable in water and will remain so for long periods of time. Large droplets tend to rise and larger droplets (>100 μm) will not stay in the water column for more than a few seconds. Natural dispersion is dependent on both the oil type and weather conditions of sea (such as wave action and sea energy). Heavy oils such as Bunker C or a heavy crude will not disperse naturally to any significant extent, whereas light crudes and diesel fuel can disperse significantly. Dispersed oil may also rise to form another surface slick or it may become associated with sediment and be precipitated to the bottom. Dispersant, a chemical spill-treating agent, may be applied to promote the formation of small droplets of oil that "disperse" throughout the top layer of the water column.

Dissolution. Dissolution occurs immediately after the spill. Through the process of dissolution, some of the most soluble components of the oil are lost to the water under the slick. The amount of an individual compound dissolving in the water phase from oil slicks in a given time largely depends on kinetic and equilibrium conditions affected by molecular structure and polarity. In general, (1) the aromatic hydrocarbons are more soluble than aliphatic hydrocarbons, (2) the solubility increases as the degree of alkylation of benzenes and PAHs decrease, (3) the lower-molecular-weight hydrocarbons are more soluble than the high-molecular-weight hydrocarbons in each class of petroleum compounds, and (4) the more polar S-, N-, and O-containing compounds are more soluble than hydrocarbons. Hence BTEX, lighter alkyl-benzene compounds, and PAHs with fewer rings such as naphthalene are particularly susceptible to dissolution or *water-washing*. As only a small amount of oil components actually enters the water column, dissolution does not measurably change the mass balance of the oil. The significance of dissolution is that the soluble aromatic compounds are particularly toxic to fish and other aquatic life. If a spill of oil containing a large amount of soluble aromatic components occurs in shallow water and creates high localized concentrations, then significant numbers of aquatic organisms can be at risk and killed.

Biodegradation. Biodegradation of hydrocarbons by natural populations of microorganisms represents the primary mechanisms by which petroleum and other hydrocarbon pollutants are eliminated from the environment (Prince, 1993; Leahy and Colwell, 1990). The quantitative and qualitative aspects of biodegradation depend on the composition of the microbial community (for example, indigenous bacteria and other microorganisms are often the best adapted and more effective at degrading oil as they are acclimatized to the temperature and other conditions of the area); the type, nature, and amount of oil; and the ambient and seasonal environmental conditions (such as temperature, oxygen, nutrients, salinity, and pH). Petroleum hydrocarbons differ in their susceptibility to microbial attack. Transformations of petroleum hydrocarbons by biodegradation occur stepwise, producing oxidized compounds including alcohols, phenols, aldehydes, and carboxylic acids in sequence by phase 1 and phase 2 metabolic pathways. The compounds may eventually be completely metabolized to carbon dioxide and water, or the polar metabolites may be spread to the surrounding water or accumulated in the residual oil.

Photooxidation. Photooxidation is a potentially significant process in degradation of crude oil spilled at sea, but the effects of photooxidation on the oil composition following oil spills are not yet well understood. In general, photooxidation is considered to be a factor involved in the transformation of crude oil or its products released into the marine environment (Garrett et al., 1998). The photooxidation is dependent on the thickness of the oil slick as well as sun incidence. The photochemical degradation yields a variety of oxidized compounds including alcohols, aldehydes, ketones, and acids, which are more soluble in water than the starting compounds.

Photodegradation affects the oil composition differently than is the case for microbial degradation and can hence complicate the observed weathering patterns for spills in areas with large sun incidence. For most oils, photooxidation is not an important process in terms of changing their fate or mass balance after a spill.

Sedimentation and Oil–Mineral Aggregation. Sedimentation is the process by which oil is deposited on the bottom of the sea or other water body. Once oil is on the bottom, it is usually covered by additional sedimentation and degraded very slowly. Oil–mineral aggregates (OMA) result from interactions among the oil residues, fine mineral particles, and seawater. OMA formation has been identified as an important process that facilitates the natural removal of oil stranded in coastal sediments (Bragg and Owens, 1994; Owens and Lee, 2003). OMA formation is enhanced by physical processes such as wave, energy, tides, or currents. It has recently been noted that oil biodegradation may be enhanced by OMA formation.

3.4.2 Weathering Effects on Biomarkers Fingerprinting

Weathering causes considerable changes in the physical properties and the chemical composition of spilled oil. For severely weathered oils, not only *n*-alkanes but also branched and cyclo-alkanes are heavily or completely lost, and the UCM becomes pronounced; the BTEX and alkyl benzene compounds can be completely lost, and the PAHs and their alkylated homologous series could also be highly degraded, resulting in the development of a profile in each alkylated PAH family with the distribution of C_0- < C_1- < C_2- < C_3-. Hence it is difficult and often impossible to identify severely weathered oil samples through recognition of *n*-alkane and PAH fingerprinting patterns. However, the biomarker fingerprinting patterns are often unaltered even for some severely weathered oil samples. Thus, biomarker fingerprints could provide a powerful tool for tracking the source and correlation and differentiation of weathered oils.

The laboratory evaporative weathering (Wang et al., 1995c; Wang and Fingas, 2003; EPA report, 2003) reveals that biomarker terpanes and steranes are not depleted during evaporative weathering; all target biomarker compounds from the C_{19} to C_{35} range are concentrated in proportion with the increase of the weathered percentages; and both terpanes and steranes show a great consistency in the relative ratios of paired biomarker compounds and biomarker compound classes. A number of the laboratory biodegradation studies (Wang et al., 1998c; Blenkinsopp et al., 1996; Swannel et al., 1996; Atlas and Bartha, 1992; Foght et al., 1998) also demonstrate that no sign of alteration in the composition of biomarkers was observed, regardless of the oil type (light, middle, or heavy), incubation times (7, 14, and 28 days), incubation conditions (incubated at 4, 10, 15, and 22°C), with and without the presence of nutrients. The concentrations of terpanes and steranes in the tested oils were consistent, and the diagnostic ratios of paired terpanes and steranes remained constant. For example, the average of the sum of eight target diagnostic biomarker ratios [including C_{23}/C_{24}, Tm/Ts, C_{29}/C_{30}, C_{32}(S)/C_{32}(R), C_{33}(S)/C_{33}(R), C_{23}/C_{30}, C_{24}/C_{30}, and $C_{27}\alpha\beta\beta/C_{29}\alpha\beta\beta$ steranes] from 70 biodegradation samples of the ASMB oil inoculated under various inoculum conditions during 1994 was 8.2 ± 0.2 with relative standard deviations less than 4%. Contrary to the biomarker compounds, *n*-alkanes, pristine, and phytane were greatly reduced in the positive controls, and *n*-C_{17}/pristane, *n*-C_{18}/phytane, and pristane/phytane ratios were significantly altered, indicating degradation of pristane and phytane had also occurred.

Compared to the laboratory-controlled evaporative weathering and biodegradation, the field biodegradation of contaminated petroleum in the environment is generally a long-term and complex process. The study of the 25-year-old Nipisi spill (Wang et al., 1998a) indicates that the surface oil (0–2 cm) has been heavily weathered, evidenced by nearly complete depletion of *n*-alkanes and

isoprenoids and by complete loss of BTEX compounds, striking decreases in the abundances of alkylated naphthalene series, and development of a profile of C_0- < C_1- < C_2- < C_3- in each alkylated PAH group. Conversely, the subsurface residual oil (>30–40 cm) from the same location is still almost unaffected by weathering, with GC chromatographic profiles similar to the reference oil. In contrast to alkane and PAH groups, the biomarker composition of the Nipisi spilled oil is nearly unaffected. The accumulation of terpanes relative to the reference oil during the 25-year period of weathering is apparent, especially for the severely weathered surface sample N2–1A: the concentration of C_{30}-αβ hopane was approximately 1.8 times that found in the reference oil, and five diagnostic biomarker ratios (C_{23}/C_{24}, Ts/Tm, C_{29}/C_{30}, C_{32} 22S/22R, and C_{33} 22S/22R) were found to be consistent between samples as well.

3.4.3 Biodegradation of Biomarkers in Spilled Oil

Although terpanes and steranes are highly resistant to biodegradation, several studies have shown that they can be degraded to a certain degree under severe weathering conditions (i.e., extensive microbial degradation) (Seifert et al., 1984; Chosson et al., 1991). Based on several geochemical studies, Peters and Moldowan (1993) have created a "quasi-stepwise" sequence for assessing the extent to which biomarkers are degraded in the reservoir. The Arrow (Wang et al., 1994b) and BIOS oil spill studies (Wang et al., 1995b; Prince et al., 2002) have demonstrated degradation of C_{23} and C_{24} tricyclic terpanes. In addition, Tm is degraded faster relative to Ts, even though Ts chromatographically elutes earlier than Tm. In March 1986, sections of peaty mangrove in a tropical ecosystem were polluted by Arabian Light crude oil. Eight years later, Munoz et al. (Munoz et al., 1997) found that isoprenoids were severely degraded and the biomarker distribution altered as well. Norhopanes were found to be the most biodegradation-resistant among the studied terpane and sterane groups, and the C_{30}-αβ hopane appeared more sensitive to weathering than its higher homologues. Frontera-Suau et al. (2002) examined degradation of petroleum biomarkers using mixed cultures of microorganisms enriched from surface soils at four different hydrocarbon-contaminated sites. They found that these cultures degraded C_{30} 17α, 21β-hopane and the C_{31}–C_{34} extended hopanes in Bonny Light crude oil after 21 days of incubation at 30°C.

Three coastal sites, heavily oiled from the 1974 Metula oil spill in the Strait of Magellan, Chile, were examined in May 1998 to determine the long-term fate and persistence of Metula oil in a marine marsh environment (Wang et al., 2001b). Among the characterized samples, the asphalt pavement samples were the most heavily weathered, evidenced by a complete loss of *n*-alkanes from *n*-C_8 to *n*-C_{41} and by depletion of more than 98% of the alkylated PAHs. Even the most refractory biomarker compounds were affected to varying degrees. Biomarkers showed degradation in the following sequences:

- Biomarkers were altered in the declining order of importance as: diasteranes > C_{27} steranes > tricyclic terpanes > pentacyclic terpanes > norhopanes (C_{29}Ts) ~ C_{29} αββ steranes.
- Steranes degraded in the order of C_{27} > C_{28} > C_{29} with the stereochemical degradation sequence 20R ααα steranes > 20(R+S) αββ steranes > 20S ααα steranes.
- Degradation of terpane C_{35} > C_{34} > C_{33} > C_{32} > C_{31} was apparent with a significantly preferential degradation of the 22R epimers over the 22S epimers.
- C_{30}-αβ-hopane appeared more degradable than the 22S epimers of C_{31} and C_{32} homohopanes, but had roughly the same biodegradation rate as the 22R epimers of C_{31} and C_{32} homohopanes and was significantly more resistant to degradation than the 22S and 22R epimers of C_{34} and C_{35} homohopanes.
- C_{29}-18α(H), 21β(H)-30-norneohopane, and C_{29}-αββ 20R and 20S stigmastanes were

found to be the most degradation-resistant terpane and sterane, respectively, among the studied target biomarkers.

3.4.4 Determination of Weathered Percentages Using Biomarkers

Highly degradation-resistant oil components such as C_{30} αβ hopane or C_{29} αβ norhopane have been applied as conserved "internal standards" for more precise estimation of the weathering degree and extent of the spilled residual oil (Butler et al., 1991; Douglas et al., 1994; Prince et al., 1994; Wang et al., 1995b):

$$P(\%) = (1 - C_s/C_w) \times 100\% \qquad (1)$$

where P is the weathered percentages of the weathered samples, and C_s and C_w are the concentrations of C_{30} αβ-hopane in the source oil and weathered samples, respectively. It should be noted, however, that the weathered percentages can still be underestimated by using C_{30}-αβ-hopane as an internal oil reference for extremely degraded oil samples because C_{30}-αβ-hopane under such circumstances is itself partially depleted, such as in the case of the Metula oil spill (Wang et al., 2001b). However, in most cases, C_{30}-αβ-hopane is the preferred choice and used as "internal standards" for estimating weathered percentages, because C_{30}-αβ-hopane is often the most abundant among C_{19} to C_{35} biomarkers and can thus be quantified more accurately. For lighter refined products, such as diesel samples, which generally do not contain high-molecular-weight terpane and sterane compounds, the bicyclic sesquiterpanes (Wang et al., 2005a) as well as a selection of the more conservative PAHs with a high degree of alkylation such as C_3- or C_4-phenanthrenes can be used as an alternative internal standard for estimating the degree of weathering.

3.4.5 Case Study: Source Identification of a Harbor Spill by Forensic Fingerprinting of Biomarkers

A harbor spill occurred in the Netherlands in 2004. A thick layer of oil (sample 2) was found between a bunker boat and the quay next to the bunker center, and it was suspected that something had gone wrong during bunkering of the vessel. Fuel oils from the bunker boat (sample 1) and the bunker center (sample 3) were collected as suspected sources for comparison with the spill sample. A multi-criterion approach was applied to fingerprint and identify these oil samples and to determine the source of the spill.

3.4.5.1 Product Type-Screening

The samples were type-screened from their GC traces: (1) all have similar GC-FID and GC-MS chromatographic profiles at m/z 83 and 85 for alkyl cyclo-hexanes and *n*-alkanes, respectively; (2) hydrocarbons ranged between *n*-C_8 and *n*-C_{32} with maximal abundances between *n*-C_{15} to *n*-C_{17}, and no hydrocarbons heavier than C_{32} were detected; (3) a nearly symmetrical UCM (unresolved complex mixtures of hydrocarbons) of middle-range distillate was apparent; (4) GC-detectable total-petroleum-hydrocarbons (GC-TPH) ranged from 870 to 920 mg/g oil, typical of lighter distillate fuels, significantly higher than most crude oils; (5) total *n*-alkanes including pristane and phytane were 142, 142, and 145 mg/g oil for the three samples, typical for diesel fuels; (6) all three samples had similar ratios of *n*-C_{17}/pristane, *n*-C_{18}/phytane, and pristane/phytane with sample 1 (Bunker boat) being closer to the spill sample 2 than sample 3 (Bunker center); (7) spill sample 2 had been slightly weathered, having considerably lower concentrations of *n*-C_8, *n*-C_9, and *n*-C_{10} than the suspected source samples 1 and 3. All the chromatographic evidence suggests that the spilled oil (sample 2) was a diesel-type fuel and the spill sample was slightly weathered. In this case, two questions remain after the product type-screening: (1) Did these three samples come from the same source? (2) Were the minor differences in chemical composition between samples caused by weathering or mixing with other (pre-existing) contamination? To unambiguously answer these ques-

tions, characterization of more than one suite of analytes was performed.

3.4.5.2 Characterization of Bicyclic Sesquiterpanes

Figure 3-26 compares the GC-MS chromatograms of sesquiterpanes at 123 and their corresponding GC-FID chromatograms. Three samples contain significant amounts of sesquiterpanes (Table 3-10). To aid comparison, the diagnostic ratios of paired sesquiterpane isomers with the same carbon number and between groups (with different carbon number) for three samples are presented in Table 3-10 as well. Figure 3-26 and Table 3-10 reveal that (1) sesquiterpanes are extremely abundant in three oil samples. The total concentrations of 10 sesquiterpanes were determined to be as high as 7986, 8255, and 7384 μg/g oil (n = 3). (2) Samples 1 and 2 have nearly identical distribution patterns of sesquiterpanes. (3) More importantly, the diagnostic ratios of eight sesquiterpane isomeric pairs were nearly identical for samples 1 and 2 as well. (4) Sample 3 is distinctly different from samples 1 and 2 not only in the diagnostic ratios but also in the concentrations of target sesquiterpanes. In particular, the abundances of Peaks 2, 3, 4, and 8 of sample 3 are much lower than the corresponding peaks of samples 1 and 2. (5) Furthermore, the diagnostic ratios of P3/P5 and P4/P5 for sample 3 are considerably lower than the corresponding ratio values for samples 1 and 2. Conversely, sample 3 has a much higher ratio of P1/P2 than samples 1 and 2. (6) Note that, because of weathering, most probably due to evaporation of the spill sample, the spill sample 2 had slightly higher concentrations of all the observed sesquiterpanes compared to sample 1. Based on the sesquiterpane concentrations, the evaporative mass-loss of sample 2 relative to sample 1 is estimated to be between 4 and 6%.

The diagnostic ratios of sesquiterpanes are compared in double-ratio plots at the 95% confidence limits (Figure 3-27). Specifically, the spill sample (oil sample 2) is compared to both suspected source oil samples 1 and 3, respectively. Based on the criteria described in the revised Nordtest method (Daling et al., 2002), there is a perfectly "positive match" between the spill sample (sample 2) and the spill source candidate (sample 1), while sample 3 is a "nonmatch" to the spill.

3.4.5.3 Confirmation of Source Identification by Quantitative Evaluation of Alkylated PAHs and Pentacyclic Terpanes and Steranes

The source identification by characterizing sesquiterpanes is further validated by quantitative evaluation of five petroleum-characteristic alkylated PAH homologous series (naphthalene, phenanthrene, dibenzothiophene, fluorene, and chrysene) and the pentacyclic biomarker terpanes and steranes. PAH fingerprinting results show that (1) the totals of alkylated PAHs were 24,902, 25,870, and 21,528 μg/g oil for samples 1, 2, and 3, respectively; (2) sample 2 and sample 1 have nearly identical distribution patterns of target alkylated PAHs and other EPA priority PAHs. The pattern for sample 3, however, is noticeably different; (3) diagnostic ratios of target PAH groups and paired PAH isomers are all very similar for all samples, but the ratios for sample 1 and 2 were more similar to each other than to sample 3.

GC-MS analysis found that, in this case, all three oil samples contain detectable amounts of high molecular-weight terpanes and steranes (Table 3-10). The extracted ion chromatograms at m/z 191 and 218 for terpane and sterane characterization are shown in Figure 3-28. The concentrations of target terpanes (C_{21} to C_{31}) and three groups of αββ-steranes (C_{27}, C_{28}, and C_{29}) were determined, and the relative ratios of target biomarker terpanes C_{23}/C_{24}, C_{29}/C_{30}, Ts/Tm, C_{29}-αβ-hopane/C_{30}-αβ-hopane, C_{31}(22S)/C_{31}(22R), and C_{27}αββ/C_{29}αββ steranes were also calculated. Terpane and sterane fingerprinting results reveal that (1) only traces of terpanes and steranes were detected in the samples (157, 181, and 101 μg/g oil for samples 1, 2, and 3, respectively),

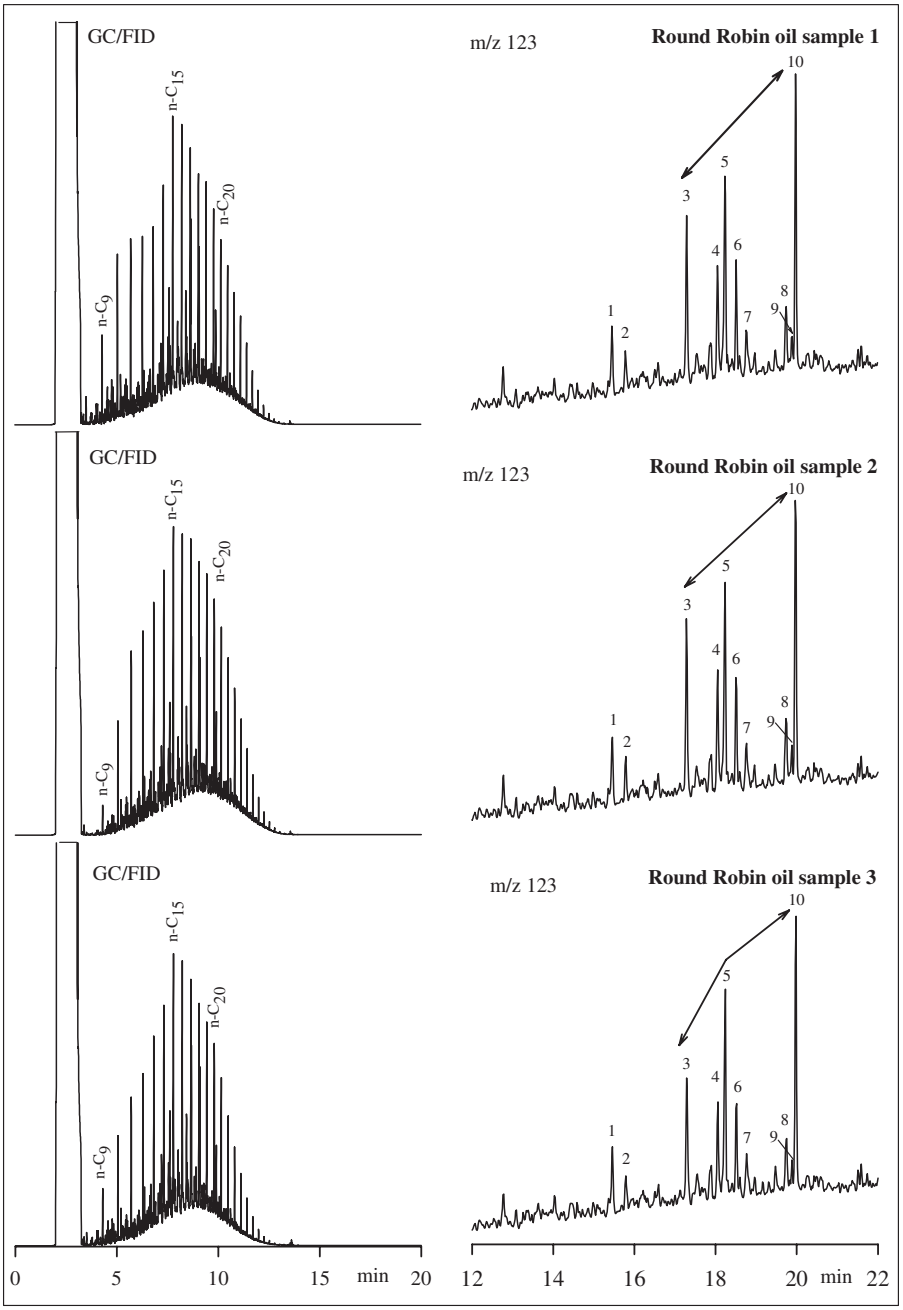


Figure 3-26 Extracted ion chromatograms at *m/z* 123, sesquiterpanes, for three Round Robin samples (right) and their corresponding GC-FID chromatograms for *n*-alkane analysis (left).

Table 3-10 Quantitation Results and Diagnostic Ratios of Sesquiterpanes in Three Oil Samples

Oil Samples	*Sample 1*	*Sample 2*	*Sample 3*
Sesquiterpanes (μg/g oil)			
Peak 1	481 (1.2)*	518 (0.2)	527 (0.6)
Peak 2	334 (0.5)	355 (2.3)	283 (2.6)
Peak 3	1163 (0.9)	1212 (1.4)	965 (2.4)
Peak 4	805 (1.0)	836 (1.4)	666 (2.2)
Peak 5	1349 (2.4)	1392 (1.5)	1370 (1.0)
Peak 6	722 (1.4)	750 (1.2)	658 (2.3)
Peak 7	368 (4.8)	377 (2.4)	384 (4.1)
Peak 8	625 (2.1)	640 (1.4)	507 (2.6)
Peak 9	251 (4.1)	259 (4.6)	220 (4.2)
Peak 10	1889 (0.7)	1916 (0.3)	1803 (1.2)
Total	7986 (0.7)	8255 (0.5)	7384 (0.6)
Sesquiterpane diagnostic ratios			
C_{14} group			
P1:P2	1.44 (1.6)	1.46 (2.3)	1.87 (3.0)
C_{15} group			
P3:P5	0.86 (3.3)	0.87 (2.5)	0.70 (1.5)
P4:P5	0.60 (1.4)	0.60 (2.7)	0.49 (1.5)
P6:P5	0.54 (1.0)	0.54 (2.7)	0.48 (3.2)
C_{16} group			
P8:P10	0.33 (2.7)	0.33 (1.4)	0.28 (1.5)
Intergroup			
P1:P5	0.36 (3.2)	0.37 (1.3)	0.38 (0.6)
P3:P10	0.62 (0.4)	0.63 (1.3)	0.54 (1.9)
P5:P10	0.71 (3.0)	0.73 (1.3)	0.76 (0.8)
Terpanes and steranes (μg/g oil)			
C_{21}	16.2 (2.9)	14.7 (3.6)	15.9 (5.2)
C_{22}	8.47 (2.4)	7.80 (2.7)	7.16 (4.9)
C_{23}	25.7 (0.7)	24.5 (0.9)	22.4 (4.9)
C_{24}	13.1 (1.2)	12.9 (3.7)	11.6 (5.6)
C_{29}	6.61 (1.2)	8.17 (7.0)	3.03 (2.4)
C_{30}	6.39 (0.8)	7.13 (7.0)	2.75 (5.6)
Ts	4.88 (5.0)	6.43 (2.0)	2.14 (2.0)
Tm	4.53 (7.0)	5.67 (2.7)	2.27 (7.3)
C_{27} αββ	37.0 (2.4)	48.5 (4.3)	17.8 (3.8)
C_{28} αββ	17.1 (3.7)	22.7 (5.4)	8.13 (8.2)
C_{29} αββ	17.3 (2.5)	22.1 (6.1)	7.91 (6.4)
Total	157 (1.2)	181 (3.6)	101 (3.7)
Diagnostic ratios of target terpanes and steranes			
C_{21}/C_{22}	1.92 (4.6)	1.89 (1.8)	2.23 (1.1)
C_23/C_{24}	1.97 (1.3)	1.90 (3.9)	1.94 (4.2)
C_{23}/C_{30}	4.03 (0.9)	3.44 (6.8)	8.17 (3.6)
C_{24}/C_{30}	2.05 (2.0)	1.81 (4.0)	4.23 (7.5)
C_{29}/C_{30}	1.03 (0.4)	1.15 (0.3)	1.11 (4.4)
Ts/Tm	1.08 (2.0)	1.13 (3.8)	0.95 (8.7)
C_{27} αββ/C_{29} αββ	2.13 (3.7)	2.20 (5.3)	2.25 (8.7)

*The concentrations and diagnostic ratios were determined from three measurements. The values in parentheses are relative standard deviation (% RSD) of three measurements.

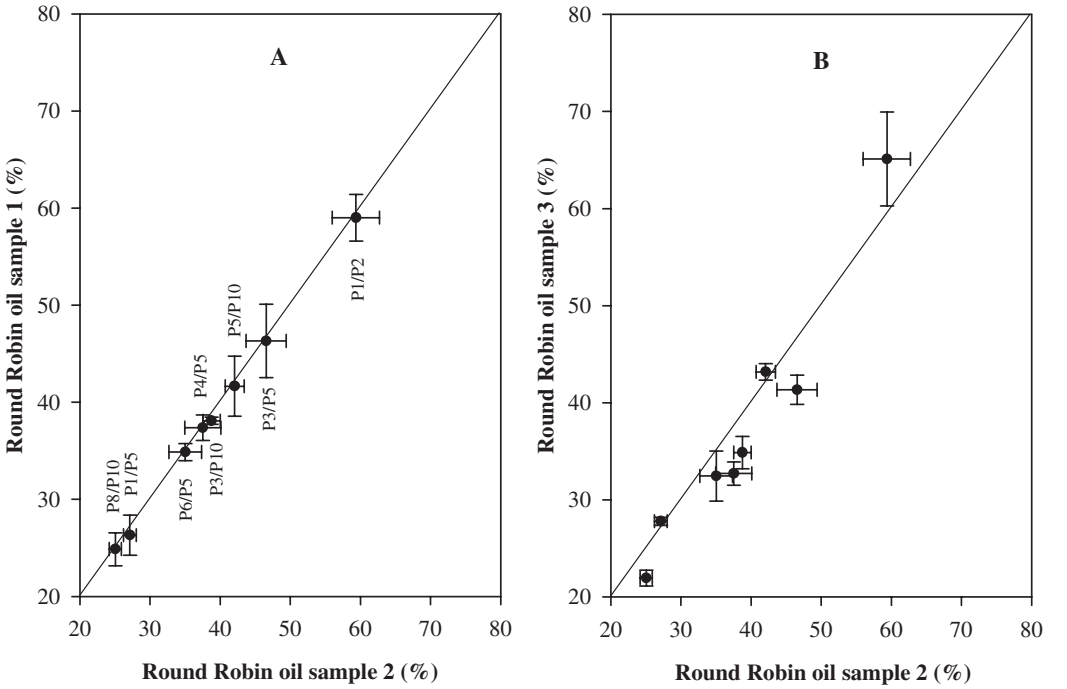


Figure 3-27 Correlation of diagnostic ratios (normalized to %) of sesquiterpanes between spill sample 2 and suspected source samples 1 (left) and 3 (right) at 95% confidence. All the data (A: left panel) overlap the 1 : 1 line at 95% confidence, representing a "perfect" match between samples 2 and 1. Conversely, most data points (B: right panel) between samples 2 and 3 do not overlapping the line at 95% confidence, representing a "nonmatch."

mostly lower-MW C_{19}–C_{24} terpanes, diasteranes, and C_{27}–C_{29} steranes. No C_{33}–C_{35} pentacyclic hopanes were detected. (2) Samples 2 and 1 have nearly identical terpane and sterane distribution patterns. (3) Sample 3 shows the distribution pattern different from that of samples 1 and 2. The tricyclic terpanes (C_{21} to C_{24}) in sample 3 are similar to samples 1 and 2, but the pentacyclic terpanes (C_{29}–C_{32}) and C_{27}–C_{29} steranes have much lower concentrations than samples 1 and 2. (4) The diagnostic ratios of target hopanes and steranes are similar for samples 1 and 2, while the diagnostic ratios of sample 3, however, are significantly different from either. Clearly, the fingerprinting and quantitation data of PAH and biomarker terpanes and steranes further confirm the conclusion obtained from the fingerprinting results of sesquiterpanes, that is, sample 1 (Bunker boat) is a positive match to the spill sample 2 (spill oil on the water surface), while sample 3 (Bunker center) is a nonmatch to the spill.

The fingerprinting results described above strongly demonstrate that for defensive forensic investigation and unambiguous spill source identification, the use of the "multicriteria" analytical approach must be followed. In many cases, characterization of biomarker and PAH compounds should include determination of both concentrations and diagnostic ratios/relative distributions.

3.5 Conclusions

Biomarkers retain all or most of the original carbon skeleton of the original natural product, and this structural similarity reveals highly specific information about a spilled oil's source than do other compound groups present in oil. Therefore, chemical fingerprinting of source-characteristic and environmentally

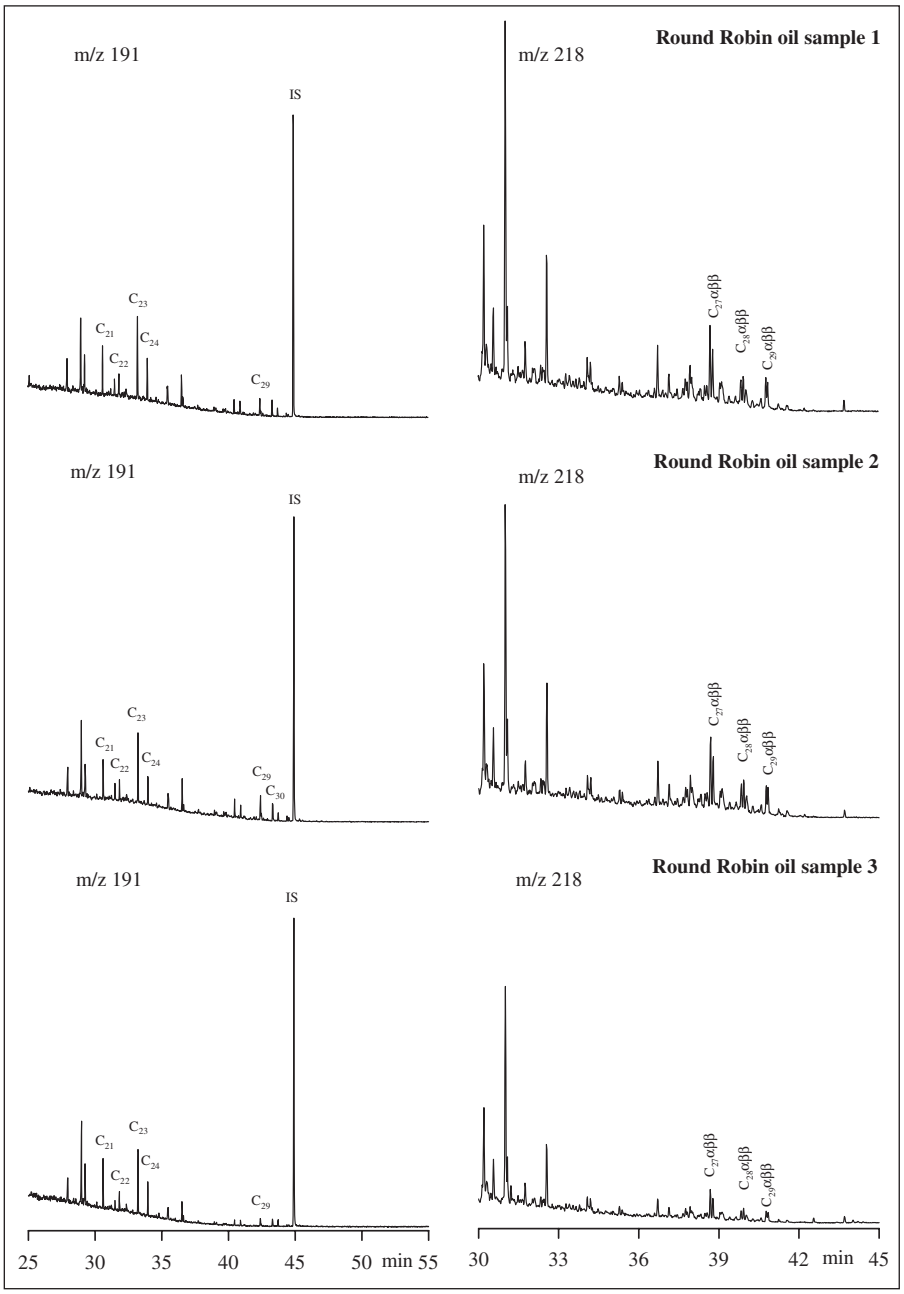


Figure 3-28 Comparison of GC-MS chromatograms of terpanes (*m/z* 191) and steranes (*m/z* 218) in three oil samples.

persistent biomarkers generates information of great importance to environmental forensic investigations in terms of determining the source of spilled oil, differentiating and correlating oils, and monitoring the degradation process and weathering state of oils under a wide variety of conditions. Advancements in spilled oil fingerprinting techniques will continue and these advancements will further enhance the utility and defensibility of oil

hydrocarbon fingerprinting and spill source identification.

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