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2 Chemical fingerprints and chromatographic analysis of crude oils and petroleum products

Abstract: Crude oil is an extremely complex combination of a wide range of constituents. Petroleum products are derived from crude oil by a variety of refining processes. The chemical compositions of oil released into the environment are affected by weathering and mixing with background substances. Variation among crude oils provides a basis for differentiation and identification of oils and the sources of spilled oils. Refined oil petroleum partially inherits fingerprints from the parent crude oil, and weathered oil could retain the unique characteristics of the source oil, which enables a potential to trace its origin. The most important criteria for a forensic oil analysis are the concentration, distribution profiles, and diagnostic ratios of source-specific petroleum compounds. In recent decades, the technologies of oil analysis have continually progressed due to advanced and automated instrumental techniques. This chapter overviews oil chemistry and analytical methodologies for separation, identification, and quantitative analysis of selected petroleum hydrocarbons in crude oils and various refined petroleum products. We also discuss the effect on oil chemical composition of weathering processes such as evaporation, photodegradation, and biodegradation.

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2.1 Introduction

Crude oil is a fossil fuel. It is widely accepted that crude oil originates mainly from the remains of prehistoric natural organic substances such as microscopic, photosynthetic organisms as phytoplankton, buried in the primeval mud of swamps, lakes, and oceans. The original chemistry of the organic matter, the environment of deposition, and the time and heat imposed on the organic matter dictate the type of crude oil formed [1]. Every crude oil exhibits a unique chemical fingerprint due to the variety of original organic matter, geological conditions, and ages under which it was formed. Oil composition may be altered to various extents by post-generation processes such

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https://doi.org/10.1515/9783110694529-002

as thermal alteration, migration, or biodegradation, consequently generating light, medium, or heavy crude oils.

To classify petroleum, properties such as viscosity, sulfur content, and American Petroleum Institute (API) gravity are usually used. The higher the API gravity, the lighter (less viscous) the oil, and vice versa. API values of crude oil with commercial value mostly fall within the range of 10° to 40°. Based on density, crude oils can be roughly classified into light, medium, heavy, and extra heavy oil. Light oils have an API > 31.1°. The medium oils are defined as having an API gravity between 22.3° and 31.1°. Heavy oils have a <22.3° API, and the extra heavy oils or bitumen have a gravity below 10.0° API and tend to sink if spilled on water.

Although crude oil may be used directly as an energy source, the full benefit of the different properties of the constituents may be realized only when they are separated. Distillation is the principal method for separating crude oil into valuable products. Based on the distillation pointing, petroleum products can be generally classified into light distillates, medium fuels, and heavy residuals with boiling points generally <200 °C, 200–350 °C, and >350 °C, respectively. Specific products are also generated from crude oil through a variety of refining and blending processes according to the requirement for desired end uses. Each product is comprised of different constituents. The chemical fingerprints of crudes are altered during the manufacture of refined petroleum products in the refining processes. However, the refined oil partially inherits its unique fingerprint from the parent crude oil, which enables the potential to track back its origin.

The lightest distillate of crude oil, gasoline, is the combination of a mainly lower boiling point C_4 to C_{12} hydrocarbons, including alkanes, alkenes, benzene, and alkylbenzenes, and a small portion of performance additives such as metal deactivators, corrosion inhibitors, oxygenates, and antioxidants. Now it has become universal to blend a portion of alcohols in gasoline, for example, E10 is a blend of 10% ethanol with petroleum-based gasoline.

Middle distillates generally include mineral spirits, kerosene, most jet fuels, automotive and marine diesel, and light fuel oils. These products are characterized by a predominance of C_{10} to C_{24} alkanes and polycyclic aromatics with no or little olefins. Diesel is one of the most important fuels used by automobiles due to its high efficiency. Marine diesel oil (MDO) is purposely produced for light marine and freshwater vessel fuel. MDO is formulated from mid-range distillates, typically containing a more extended carbon range than on-road diesel.

Blends of biodiesel and petroleum-derived diesel are now commonly distributed for use in the retail diesel fuel marketplace. Typically, biodiesel consists of fatty acid methyl esters (FAME) produced by transesterification of plant or animal-derived triacylglycerols. The fatty acid chains can be saturated, monounsaturated, or polyunsaturated. The distribution profiles of free fatty acids, glycerol, and monoacylglyceride congeners as well as the byproducts of biofuel could be applied for the differentiation and identification of biodiesels.

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Heavy fuel oils (HFOs) are used for off-road diesel engines, boilers, furnaces, and other combustion equipment. They are the heavy residual part of the vacuum distillation and cracking processes. These oils are often blended with lighter oils such as gas oil and kerosene streams to meet the desired product specification for the convenience of transportation and operation. These heavy fuels are considered to be less acutely toxic relative to other lighter oil types. Heavy fuels have variable acute toxicity, depending on the amount of the light fraction. Their composition is complex and varies with the feedstock of crude oil. In general, these oils contain a mixture of saturated, aromatic, and olefinic hydrocarbons with carbon numbers predominantly in the C_9 to C_{50} range. As residual distillates, these oils often contain relatively high concentrations of sulfur, oxygen, nitrogen compounds, and heavy metals (e.g., vanadium and nickel). Relative to other types of refined oil products, spills of HFOs potentially cause the most significant environmental impact with respect to their vast usage as well as their physical and chemical properties.

Lubricating oil (or simply lube oil) consists largely of base oil and a small portion of chemical additives. Mineral base oil typically originates from petroleum products; however, the use of high-performance synthetic lubricants that no longer containing petroleum base oils has become widespread. Lube oil is usually distinguished from other fractions of crude oil by its high boiling point and viscosity. Hydrogenation and hydrocracking significantly influence the chemical structures of mineral oil molecules. Unstable molecules are chemically stabilized by the removal of heteroatoms (sulfur, oxygen, and nitrogen). Severe hydrogenation can convert aromatics into saturated naphthenic and paraffinic compounds. In addition to hydrogenation, hydrocracking can break large molecules down into smaller ones.

Small and large crude oil spills occur frequently during their exploration, production, and transportation. Inland oil spills may come from pipeline ruptures, tank spills, and road transportation accidents. Most massive oil spills are usually related to accidental crude oil releases on the oceans from drilling rigs, offshore platforms, and super-tankers. The particular operating conditions and severe weather often make it more difficult to control an ocean oil spillage than an on-land incident.

Spillages of refined oils frequently occur around oil development and production facilities. Massive spills of refined petroleum products rarely occur like crude oil spills. Small and chronic spillages of refined oils also pose a great environmental problem. Unsurprisingly, fuel oils are among the mostly spilled petroleum products in waters, both by volume and by occurrences [2]. Spillages of petroleum products on open waters sometimes involve mystery illegal discharges of marine vessels and their ship bilges. The waste from the cleaning engine chambers is sometimes accidentally or deliberately released from vessels. Biomarker compounds of lubricating oil in bilges can provide a valuable clue to trace the spill source. Accurate analysis and unambiguous results are critical to identify the spill source and allocate the legal liability.

Although oil spills in the open area are unlikely to cause an immediate health threat to humans, they often have widespread damage to the ecosystem and influence

the local economy; the long-term adverse effect could last decades. The analysis of spilled oil is essential for monitoring the contamination and evaluating the recovery of the environment.

2.2 General chemical components of petroleum

The composition of crude oil is extremely complex and is highly variable from field to field, and even within a given field, it is possible to exhibit differences. Despite wide variations in the chemical composition of crudes, their elemental compositions generally fall within the following narrow ranges: carbon (84–87%) and hydrogen (10–14%), nitrogen (0.1–2.0%), oxygen (0.05–1.5%), sulfur (0.05–6.0%), and a small portion of minerals and salts [3]. Crude oil contains proportions of hydrocarbons and nonhydrocarbons including heterocyclic compounds of nitrogen, oxygen, and sulfur, organometallic compounds, inorganic sediments, and water. Although the total number of compounds in crude oil is still unknown, it is recognized that crude oil is likely comprised of thousands of compounds [4]. These petroleum hydrocarbons naturally exist in gas, liquid, or solid state. The distribution pattern and profiles of components are, in general, different from oil to oil and from oil to refined products.

Petroleum compounds are generally classified into saturates, olefins, aromatics, as well as polar resin and asphaltene fractions [3].

***Saturates*:** Saturates are hydrocarbons that only contain single bonds between carbon atoms. They are generally the predominant class of hydrocarbons in crude oil. Overall, there are two major types of saturated compounds: paraffins and naphthenes. Paraffins, also called alkanes, are saturated hydrocarbons with straight or branched chains but without any ring structure. They are one of the major constituents of crude oil and are found in refined petroleum products such as gasoline, kerosene, diesel fuel, and heating oil. The *n*-alkanes (linear alkanes) have carbon atoms arranged in a line and there are only two ends to these molecules. Branched alkanes have carbon atoms arranged similar to the *n*-alkanes; however, some of the carbon atoms are branched, thus creating many different configurations. Paraffins (mainly *n*-alkanes) ranging from C_{18} to C_{40}, which occur in solid state at room temperature, are often referred to as crude wax. Naphthenes, also known as cycloalkanes, are saturated hydrocarbons containing one or more rings, each of which may have attached paraffin side-chains. Naphthenes are also relatively stable compounds in petroleum.

A variety of petroleum saturates are composed of isoprenoid units (isoprene, C_5H_8-). The following types are the most distinguished in crude oil: (1) monoterpanes (C_{10}), both aliphatic and monocyclic; (2) sesquiterpanes (C_{15}), aliphatic, mono- and bicyclic; (3) diterpanes (C_{20}), aliphatic, bi-, tri-, and possibly tetracyclic; (4) triterpanes (C_{30}), aliphatic as well as tri-, tetra-, and pentacyclic. Among these terpenoids,

tetra- and pentacyclic terpanes and steranes are the most important compounds for petroleum chemistry and forensic oil analysis.

***Olefins*:** Olefins are also called alkenes.These unsaturated hydrocarbons have one or more pairs of carbon atoms attached by a double bond. Various types of olefins have been identified in the oils and condensates worldwide [5]. Olefins are not present in large amounts in crude oils or straight-run distillates such as kerosene, gas oil, or fuels [6]. These compounds are found in large quantities only in some refined products, produced primarily from larger molecules in cracking processes.

***Aromatics*:** Aromatics contain one or more benzene rings as structural components. A monoaromatic compound has one benzene ring with either six hydrogen groups or a combination of alkyl and hydrogen groups, attached to that six-carbon aromatic ring. Polycyclic aromatic compounds (PACs) have multiple aromatic rings such as naphthalene, phenanthrene, and pyrene.

***Resin*:** Resin includes a large group of polar compounds in crude oil. These include hetero-substituted aromatics (such as nitrogen, oxygen, and sulfur-containing polycyclic aromatic hydrocarbons (PAHs)), phenols, acids, ketones, alcohols, and monoaromatic steranes (MASs). Resins are assumed insoluble in liquid propane but soluble in *n*-pentane. Because of their polarity, these compounds are more soluble in polar solvents, including water, than the nonpolar compounds, such as waxes and aromatics, of similar molecular weight. They are largely responsible for oil adhesion. Sulfur compounds are among the most important heteroatomic constituents of petroleum and may be present in several forms, including elemental sulfur, hydrogen sulfide, mercaptans, thiophenes (thiophene, benzothiophenes, dibenzothiophenes, and naphthobenzothiophenes), and their alkylated homologues.

***Asphaltenes*:** Asphaltene is defined as the part of petroleum, coal, or oil shale, which is precipitated by the addition of a low-boiling paraffin solvent such as *n*-pentane but soluble in benzene. Asphaltene constituents are the heaviest and polar constituents in crude oil, together with other high molecular weight (HMW) content resulting in the colloidal nature of crude oil. During petroleum refining, the asphaltene constituents are nondistillable and remain in the residual fuels as the distillable fractions are removed [7]. The black color of crude oils and residues is due to the combined effect of neutral resins and asphaltenes. On heating above 300–400 °C, asphaltenes are not melted, but decompose, forming carbon and volatile products. Asphaltenes make up the largest percentage of the asphalt used to pave roads. As asphaltenes are determined as a whole, this information provides little information to distinguish an oil, and asphaltenes are not often listed as target analytes for forensic oil analysis. However, its content can reflect the oil type.

***Other components*:** Other than the main fraction of hydrocarbons, crude oil also contains compounds consisting of oxygen, sulfur, nitrogen, and trace amounts of phosphorus, and a trace amount of water. Crude oil also contains a small quantity of heavy metals such as vanadium (V) and nickel (Ni). Metals are encountered in petroleum in the form of salts of carboxylic acids or as porphyrin chelates. V and Ni predominantly occur at the highest concentration in crude oil and residual fuel oils. Refined petroleum products contain variable content of these components due to their removal or enrichment during refining processes.

In this chapter, we will mainly discuss the fingerprinting analysis of selected gas chromatography (GC)-detectable aliphatic and aromatic compounds in petroleum. Later sections will further detail the chemical fingerprints of these petroleum compounds.

2.3 Fingerprinting analysis of petroleum compounds

Database on the oil physical and chemical properties plays an important role in preparedness and response to oil spill events. In the case of an oil spill, fingerprinting analysis is essential to monitor the contamination, evaluate the damage, and overlook the environmental recovery of an oil spill. Accurate analysis and unambiguous results are critical to identify the spill source and allocate the legal liability.

The fingerprinting of oil spills can be a considerable challenge to analytical chemists due to the complexity of petroleum oil and the low concentrations of many constituents of interest. In addition, once released into the environment, oil is immediately subjected to a series of weathering processes that alter its compositional distribution, and environmental samples are often a mixture of more than one oil and background matrix, and subsequently becomes difficult to recognize its spill source. These combined factors pose significant challenges in unequivocal spill source identification [8, 9].

The most commonly used methods for oil fingerprinting include those developed by Environment and Climate Change Canada (ECCC) [10–15] and European institutes [16–20]. These analytical methods, based on GC, have been demonstrated to be efficient and suitable for the identification and quantitative characterization of petroleum hydrocarbons in both crude oils and refined products.

Figure 2.1 depicts the procedure of an oil fingerprinting analysis used in the ECCC. Forensic oil fingerprinting analysis usually involves three major approaches [11, 13, 20]:

– Characterization of hydrocarbon groups by GC-flame ionization detection (GC-FID). Information obtained from GC-FID analysis is used: to identify the oil type; to determine total saturated hydrocarbons (TSHs), total aromatic hydrocarbons (TAHs), total petroleum hydrocarbons (TPHs), and unresolved complex materials

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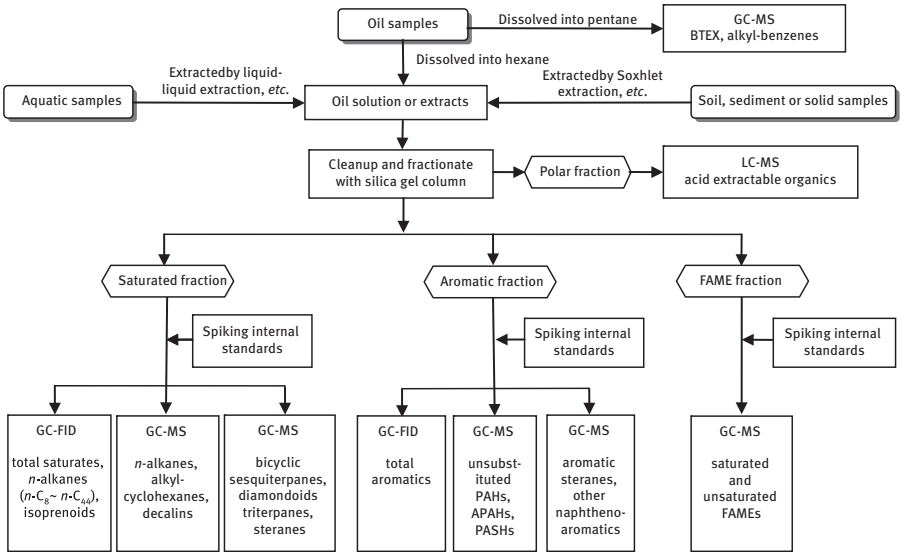

Figure 2.1: Schematic of oil analysis procedure.

(UCM); to acquire the diagnostic ratios such as UCM/TPH, Norpri/Pri, n-C_{17}/Pri; n-C_{18}/Phy, and Pri/Phy; to assess the weathering degree; and to visual preliminary examine the correlation of oils.

- Fingerprinting analysis of selected target analytes by GC-mass spectrometry (GC-MS) (selective ion monitoring, SIM). Information obtained from GC-MS analysis is used: to determine target analytes of interest including PAHs and biomarker compounds; to investigate chromatographic characteristics of source-specific compounds; and to acquire the diagnostic ratios of relevant analytes.
- Overview information from both GC-FID and GC-MS analyses; identification and characterization of major unknown peaks (e.g., additives); determination of weathering effect to the oil; and identifying the correlation of analyzed oils.

2.3.1 Sample preparation and separation

The collection of representative oil or environmental samples is not discussed in this chapter, but it is a crucial step for a successful forensic approach. Valid oil analysis must be obtained from the analysis of an aliquot representing the whole sample, which is particularly important to heavy oils and solid environmental samples. It is cautious that chemical fingerprints can be distorted or altered due to sample inhomogeneity and sample treatment prior to analysis [20].

Pure oil samples could be directly weighed and dissolved into suitable solvents for analysis. The analysis of oil-contaminated environmental samples first involves

the recovery of target compounds from their matrices such as water and soil. To select the proper extraction technique, one should consider the complex nature of petroleum. Traditional liquid–solid and liquid–liquid extractions are still used as the first choice in many laboratories due to their simplicity, relatively low cost, and proven high extraction efficiency, despite being very time-consuming. Some other techniques are used nowadays, but they may be unable to process highly oil-contaminated samples. Hexane and dichloromethane (DCM) are often used to extract oil for analysis. DCM needs to be replaced by hexane if a silica gel column is used for cleanup and fractionation. For environmental samples, if the extract appears very concentrated, it is necessary to determine the total solvent extractable materials (TSEMs) to calculate the proper aliquot of the extract to load on the chromatographic column for further sample processing and analysis and to facilitate a comparison of abundance of target compounds in samples. To determine TSEM, a 1.0 mL aliquot of extract is placed in a preweighed vial and blown to dryness under nitrogen. Alternatively, the concentration sample can be firstly evaluated by a preliminary GC-FID analysis [20].

It is not a rare practice to directly inject diluted oil solutions or extracts into an instrument, particularly for fast screening oil types. Fingerprinting analysis simply accomplished without chromatographic column fractionation could provide efficient qualitative information for oil identification but can prevent accurate quantitation of certain analytes. Typical oil concentration for direct GC injection varied from 1.5 to 10 mg/mL depending on oil types [20]. However, considering the complex nature of petroleum, proper sample cleanup, and further fractionation are essential prior to instrumental analysis in order to achieve accurate quantification of individual components and unbiased identification of the spill source, while protecting the instrument from contamination. It is strongly recommended to clean the "black oils," such as HFOs, slops, and bilges, in order to remove the high amount of asphaltenes and/or soot particles that are typically detrimental to instrumentation [20].

The most widely applied analysis scheme is to separate the petroleum compounds into aliphatic (mostly saturated hydrocarbons), aromatic, and other component groups. Silica gel and Florisil® column are often used to remove the alien objects and to separate saturated and aromatic compounds for accurate quantitative analysis without interference to each other. The chromatographic columns can be either lab-packed or commercially available SPE cartridges such as the silica gel Resprep™ Massachusetts EPH and the silica gel/cyanopropyl varieties [14, 15, 21–23].

According to ECCC's methods, an aliquot of oil solution or extract with equivalent TSEM of environmental sample is transferred to the top of a chromatographic column for sample cleanup and fractionation, with the column preconditioned using hexane. Saturated and aromatic hydrocarbons are effectively eluted with hexane and a mixture of hexane–DCM (1:1, v/v), respectively. The column can be further eluted for the analyses of other components such as FAME, nitrogen-containing polycyclic aromatic heterocycles (PANHs), and acid-extractable organics including naphthenic acids [24–26]. If these components are to be determined in the environmental samples, it is essential

to effectively recover them in the sample extraction step, which is not discussed herein. FAMEs as the primary component of biofuel are investigated where relevant. Ion of m/z 74 is used to determine saturated FAMEs, and m/z 55, 67, and 79 for monounsaturated, diunsaturated, and polyunsaturated FAMEs in biodiesel, respectively.

Saturated and aromatic fractions are carefully concentrated under a stream of nitrogen to appropriate volumes and spiked with internal standards. These fractions are finally adjusted to an accurate preinjection volume for analyses. The saturated hydrocarbon fraction (F_{sat}) is used for the analysis of TSHs, *n*-alkanes, acyclic isoprenoids, diamondoids, bicyclic sesquiterpanes, and biomarker terpanes and steranes. The aromatic hydrocarbon fraction (F_{arom}) is used for the analysis of TAHs, unsubstituted PAHs, and alkylated PAHs (APAHs), aromatic steranes, and polycyclic aromatic sulfur heterocycles (PASHs).

2.3.2 GC analysis of petroleum compounds

Petroleum contains a large number of different types of compounds. Analysis of the whole composition of petroleum can be endless and impractical; therefore, the information to be collected should be limited to those critical data. The technologies of oil analysis have been continually progressed due to advanced and automated instrumental techniques in recent decades. The methods most commonly used are to approach the major petroleum components using GC and other techniques [12, 14, 15, 17, 18, 20, 27, 28]. This chapter does not intend to provide an exhaustive list of analytical methods rather it discusses the well-established chromatographic methods. Capillary GC has been routinely used in environmental laboratories to provide a detailed analysis of most of the individual organic compounds. The characterization of individual saturated and aromatic compounds in petroleum is mainly based on GC-FID and GC-MS analyses. GC-FID is a robust, reliable, and universal technique for the detection of all species of hydrocarbons. However, this technique only produces two-dimensional data (i.e., retention time and abundance). GC-FID analysis aims to determine the oil type and to investigate if the oil is weathered through the overall distribution of hydrocarbons. These methods do not give access to detailed information of individual target compounds, and only the TPH concentration or carbon number fractions can be obtained. GC-MS instrument can provide accurate quantitation for volatile and semivolatile organic compounds in petroleum, particularly those petroleum biomarkers, which are at relatively low concentrations but of interest to forensic oil analysis.

By choosing a proper GC column based on its length, internal diameter, stationary phase, and film thickness, a wide range of petroleum hydrocarbons can be separated under optimal instrumental parameters. The most commonly used columns for petroleum hydrocarbon separation include nonpolar inert capillary columns such as DB-1 and DB-5, filmed inside with 100% methylpolysiloxane and 5% phenyl–95%

methylpolysiloxane, respectively. A conventional fused silica capillary column (30 m, 0.25 mm ID, and 0.25 μm film thickness) is used for most oil analyses. To reduce column bleeding under high temperature, some specific capillary column is used for GC-FID analysis, such as DB-5HT fused silica column (30 m, 0.25 mm ID, and 0.10 μm film thickness). Resolution of a capillary GC column is limited; it is impractical to resolve all individual oil components of interest with the chromatographic separation.

For GC-MS analysis of petroleum, electron impact ionization (EI) is most commonly used. To obtain the best sensitivity for quantitative measurement, the mass spectrometer is usually operated in SIM mode that only acquires positive ions of interest. A full scan MS is a useful mode of operation that acquires all ions formed in the ion source. This is helpful information for developing SIM acquisitions and alerts analysts to untargeted compounds other than compounds of interest. At present, the identification and characterizations of these petroleum compounds mainly rely on GC coupled to single quadrupole mass spectrometers. Low-resolution and low-mass accuracy mass spectrometers (LRMS) are sufficient for general oil analysis. LRMS analyses can face interference by other co-eluted petroleum components and external matrix material even with chromatographic separation. This is especially true to analyze target compounds in refined petroleum products and environmental samples, where their chemical fingerprints that are usually relied upon for the identification of target compounds have been significantly altered. Inaccurate and inconclusive oil analysis potentially results in misidentification and overestimation of target analytes [29].

Recently, high-resolution and high-mass accuracy mass spectrometry (HRAM-MS) such as quadrupole time-of-flight mass spectrometer (QTOF-MS) and quadrupole Orbitrap mass spectrometer has become more accessible for routine analysis with improvements in instrumentation [31]. HRAM-MS provides the simultaneous universal screening of targeted and untargeted analytes, increases detection selectivity with less sample preparation and compound optimization, and allows for retrospective analyses, which are crucial factors contributing to the increasing interest in HRAM-MS. An HRAM-MS coupled to capillary GC inherits both the advantages of both the powerful separation of GC and the rapid and accurate detection of HRAM-MS. GC-HRAM-MS can deliver higher resolution, a more accurate mass measurement, and faster full spectrum acquisition than a single quadrupole MS. Thus, it simultaneously provides good tools for solving complex analytical problems, resolving structural elucidation of unknowns, and confirming untargeted compounds. To date, there are only a few reports related to GC-HRAM-MS for oil analysis. Yang et al. have applied GC-QTOF-MS in the quantitative analysis of PAHs and biomarkers in petroleum oils and environmental samples from petroleum-impacted areas [29, 32]. The results demonstrated that accurate mass measurement by GC-QTOF-MS effectively improved characterization and differentiation of PASHs and PAHs, achieving significantly better results compared to those from the single quadrupole GC-MS method.

The improvement in quantitative analysis of one specific target compound is generally decided by many factors, such as the instrumental resolution and mass

accuracy, uniqueness of quantitation fragment, difference of monoisotopic mass between target and interfering compounds, abundance of analytes, natural complexity of samples, and so on. Practically, high-resolution and accurate MS alone cannot solve all analytical issues, particularly when the interference to a target compound is from its isomers. Therefore, according to a specific case demand, proper sample preparation, chromatographic separation, application of HRMS, and meticulous data analysis still play a critical role in reliable oil analysis.

2.3.3 Identification and quantitation of target petroleum hydrocarbons

The analysis of petroleum compositions is of particular importance in oil forensic studies since they can provide valuable information toward achieving a better characterization and identification of a petroleum sample. The determination of both concentrations and relative distributions of target petroleum hydrocarbons are equally essential to unambiguously identify an oil spill because in some cases, a source can have a similar distribution profile of analytes but significantly different amounts of these compounds [30]. Quantitative analyses of BTEX and PAHs are often required to access the health concern of an oil spill.

The data in this chapter were obtained by using our in-house methods [14, 33–38]. A large number of target analytes are identified and quantified by GC-FID (for *n*-alkanes and isoprenoids) or by characteristic ions from GC-LRMS in SIM mode. These compounds include *n*-alkanes, BTEX and alkylbenzenes, APAH series, aromatic steranes, petroleum biomarkers, diamondoids, and bicyclic sesquiterpanes, and so on. Figure 2.2 depicts the distribution of hydrocarbon groups according to their retention time (generally equivalent to boiling point order) in a typical chromatographic analysis using a nonpolar capillary column. The fingerprinting analyses of these components are discussed in more detail in the following sections.

It is unnecessary to analyze all target compounds in each oil analysis. Appropriate target analytes can be selected based on the type of oil spilled, the particular environmental compartments being assessed, and expected needs for current and future data comparison. The identification of target compounds usually relies on the application of reference oils since it is impractical to apply authentic standards for all target compounds. Reference oils processed as a normal sample also provide quality control for an oil study. For different case studies, different types of reference oils can be applied. The Emergencies Science and Technology Section of Environment and Climate Change Canada established a reference oil (13.1% artificially evaporated Prudhoe Bay crude oil). This oil consists of most of the common target compounds and at appropriate abundance. Other oils or blend oils such as NIST SRM 2779 (crude oil, Gulf of Mexico) can be used as reference oils as long as they meet the requirement as reference materials.

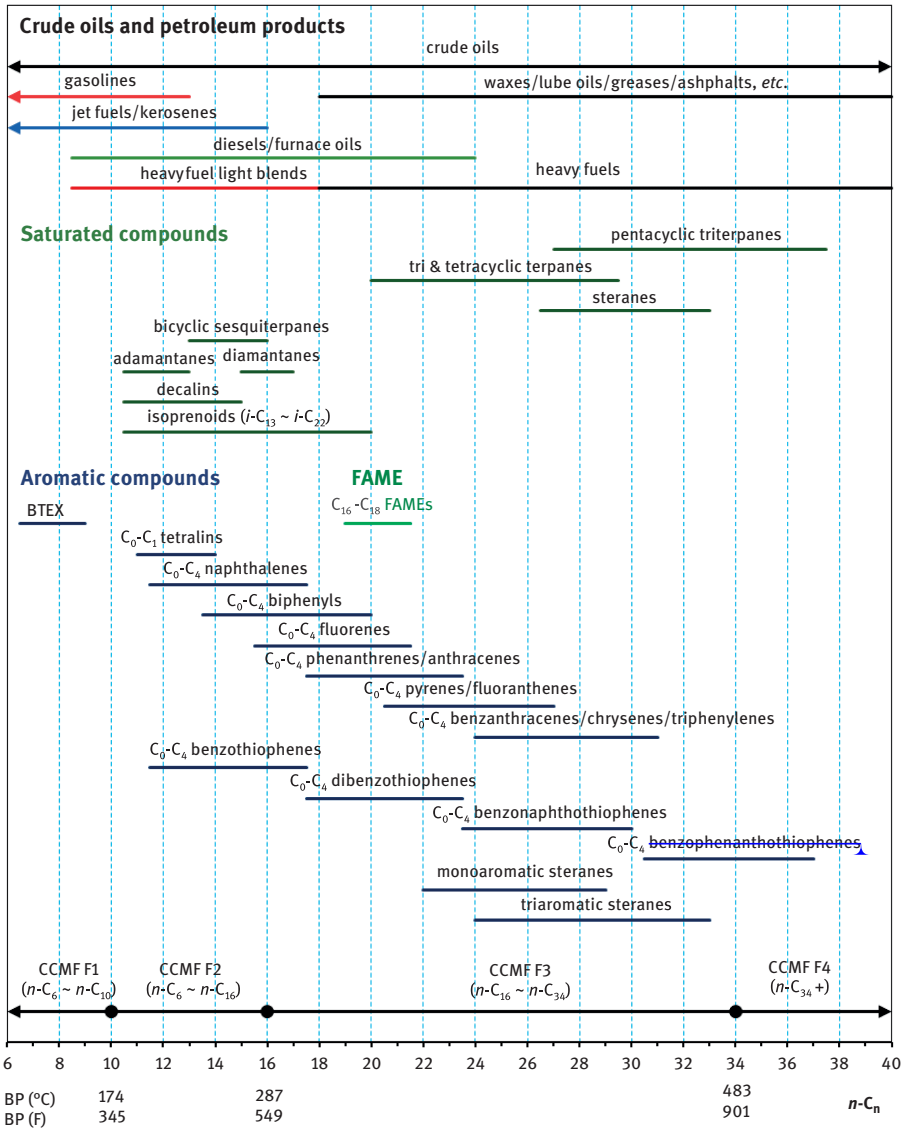

Figure 2.2: Distribution of target compounds for oil fingerprinting analysis.

Quantitative analyses of individual compounds are not always necessary and very time-consuming. Quantitative analysis requires either external or internal standard compounds. Most target compounds especially APAHs, biomarkers, and bicyclic sesquiterpanes, are not commercially available or extremely costly. Quantitation of these analytes is usually based on the relative response factor (RRFs) of their corresponding alternatives rather than authentic standards relative to the internal standards. APAHs

are usually quantified by using their parent unsubstituted PAHs instead of authentic APAH standards, except for few groups such as C_1-N, C_2-N, and C_3-N. As more alkyl-PAH are commercially available now, some are used for APAH quantitation. Yang et al. investigated the variation in RRFs of different APAH isomers and selected a suite of authentic APAH standard materials as representative compounds to accurately quantify APAHs [38]. By using the APAH standard, this could result in a 10–20% difference compared with that by using parent PAHs as quantitation standards. Many oil analytical methods are similar with subtle differences between the actual experimental procedures, while some methods differ considerably. Each lab has its specific oil analytical methodology (i.e., different quantitative standards are used), thereby quantitation discrepancy for the same sample can occur.

For forensic oil fingerprinting analysis, the relative distribution of target compounds is somewhat more important than their real-world concentration, hence quantitation using alternative standards is still meaningful for oil characterization and identification. Table 2.1 tentatively summarizes the overall distribution of these chemical components in various crude oils and refined products. It is important to note, based on our experience and knowledge from analyses of limited oil samples thus far, that the information presented may not be representative in some cases. For each petroleum fuel mixture, the table displays the approximate carbon number range of predominant hydrocarbons and the relative abundance of common target petroleum hydrocarbons in these oils. Their chemical fingerprints are described in more detail later in this chapter.

2.4 GC-FID-detectable petroleum hydrocarbons

As mentioned above, typically, the first step in forensic oil fingerprinting is screening the petroleum hydrocarbons in the sample, identifying the oil type, and evaluating the weathering extent [10, 13, 15, 17, 20]. Incorrect identification of oil type could misdirect subsequent tiered analysis, eventually leading to incorrect results. The physical appearance such as color and odor is helpful to preliminarily identify the type of oil samples. However, to unambiguously identify the oil, dedicated instrumental analysis is important, particularly to those samples that may lead to legal issues.

Generally, preliminary identification of the oil type and weathering can be readily achieved from their GC-FID traces. Measurement of TPH and other hydrocarbon groups (including the total saturates, the total aromatics, the total resolved peaks, and UCM) in oil samples, GC-FID chromatograms provide a distribution pattern of petroleum hydrocarbons (e.g., carbon range and profile of UCM).

To address the diversity of petroleum contamination types, the GC-detectable petroleum hydrocarbons are divided into four broad physicochemical subfractions

Table 2.1: Relative abundance of chemical components in crude oils and refined petroleum products.

Oil types	Approximate carbon range	Aliphatic					Aromatic			
		n-Alkanes	Branched alkanes	Bicyclic sesquiterpanes	Diamondoids	Tri- to penta-cyclic terpanes	BTEX	PAHs	PASHs	Aromatic steranes
Crude oils										
Light or medium crude oil	C_1 to C_{40}	High	High	Variable	Variable	Variable	Variable	Variable	Variable	Variable
Heavy crude/ bitumen	C_{10} to >C_{40}	Low or trace	Low	Low	Low	High	No or trace	Low in naphthalenes	High	High
Petroleum products										
Gasoline	C_4 to C_{12}	Low	High	Trace	Trace	No	High	Medium	No or trace	No or trace
Kerosene	C_6 to C_{16}	High	Lower	Low	Low	No	Low	Low	No or trace	Low
Jet A	C_8 to C_{16}	High	Low	High	High	No	Low	Low	No or trace	No or trace
Diesel no. 2 (on-road)	C_8 to C_{24}	High	Low	High	High	Trace tricyclic	Low	High	No or trace	Low
Diesel (off-road)	C_8 to C_{28}	High	Low	High	High	Low	Low	High	Low	Low
Fuel no. 5/ bunker B	C_8 to C_{40}	High	Medium	High	Medium	High	Low	High	High	Variable
Fuel no. 6/ bunker C	C_{12} to C_{34}	High	High	Low	Low	High	Low	High	High	Variable
Lubricating oil	C_{18} to C_{34}	Low	Low	Low	Low	Variable	No or trace	Trace or low	No or trace	Low

according to the Canadian Council of Ministers of the Environment (CCME) methods [39]. However, the oil analysis described herein could be very different from the CCME methodology. Fraction 1 (smaller than C_{10}) represents the volatile fraction of most hydrocarbon mixtures and consists of the aliphatic and aromatic subfractions. Fractions 2 and 3 represent the semivolatile fraction and comprise saturated and aromatic subfractions in the ranges of C_{10} to C_{16} and C_{16} to C_{34}, respectively. These two fractions make up the most proportion of GC-detectable petroleum hydrocarbons in crude oils and petroleum products. Most of the target compounds fall within these two fractions. PAHs with more than three rings are generally found in fraction 3. Fraction 4 encompasses compounds of $>C_{34}$ up to C_{50}+, having low mobility (volatility and solubility). Petroleum hydrocarbons within this range often represent a significant proportion of heavy crude oils such as Alberta oil sand bitumen (24.4%) and refined products such as lubricating oil 10W-30 (15.2%).

The GC-FID chromatograms of petroleum hydrocarbons in representative crude oils are shown in Figure 2.3, illustrating the difference of these crudes in the chromatographic profiles, carbon range, and UCM distribution patterns. The data in Table 2.2 reports TPHs at a range of ~ *n*-C_8 to *n*-C_{50} determined by GC-FID. These results do not include those components lighter than *n*-C_8, which are lost during sample preparation or co-eluted with solvent peaks (DCM and/or hexane) during GC analysis. This explains why some light oils containing high amounts of small hydrocarbons have smaller TPH values than some heavier oil samples. Heavy oils contain heavy substances, which either are retained on the silica gel fractionation column or are uneluted from GC capillary column, resulting in low TPH value for these oils.

The gross chemical composition of crude oils varies greatly, not only among geological sources but also among samples from a single deposit. In general, saturated compounds and UCM dominate GC-detectable TPHs in crude oils. The extra light Scotia crude (API = 53.2°, Nova Scotia, Canada) contains a large proportion of light components with nearly half of the resolved peaks, in which $<C_{16}$ hydrocarbons account for about 70% of TPHs while $>C_{34}$ account for only 0.6%. In addition, TSHs in this crude make up 95.3% of TPH value, obviously higher than in any other oils presented in Table 2.2. The heavy Platform Elly crude oil (API = 15.8°, US west coast, California) contains about 87% of UCM contents, and $>C_{16}$ fractions contribute to over 75% of GC-detectable TPHs. As the heaviest form of petroleum, oil sand bitumen has a pronounced chromatographic UCM hump eluting between *n*-C_{10} and *n*-C_{40}, indicating significant biodegradation of their original crudes. Normal alkanes and isoprenoid alkanes have been steadily depleted by biodegradation and/or water washing in this bitumen. A few resolved peaks eluted between *n*-C_{27} and *n*-C_{31} on the shoulder of the main UCM hump, which is contributed by high boiling point biomarker compounds. Albian Heavy Synthetic (AHS) is a partially upgraded dilbit. It is a blend of sweet Premium Albian Synthetic (API ~ 34°) upgraded from oil sand bitumen with the unconverted residue. The abundance and distribution of TPHs in AHS are significantly different from those of oil sand bitumen. Since Premium Albian Synthetic consists

AU: Table captions for Tables 2.2, 2.3, 2.5, 2.6 and 2.8 mismatch within the chapter (body) and end of the chapter. We have retained the captions in the manuscript (body). Please confirm.

Table 2.2: GC-FID-detectable petroleum hydrocarbons in representative crude oils and petroleum products.

Oil samples	Crude oils						Petroleum products					
	Scotia Light (ScL)	Bakken crude	Prudhoe Bay	Troll	Platform Elly (PlE)	Alberta oil sand bitumen	>Diesel no. 2	>Marine diesel	>IFO-180	>Bunker B (fuel no. 5)	>Bunker C (fuel no. 6)	>Lubricating oil 10W-30 (10W-30)
	(API = 53.2, Nova Scotia, Canada)	North Dakota, USA	(API = 26.7, Alaska, USA)	(API = 28.4, North Sea)	(API = 15.8, US West-Coast)	(Alberta, Canada)*	(Ottawa, Canada)	(Burnaby, BC)	(Canada, 2004)	(USA, 2002)	(Canada, 2002)	(motor oil, Ottawa, 2004)
TPH (mg/g)**	577	691	549	723	436	302	957	904	463	476	370	808
~n-C_8 to n-C_{10} (%)	9.8	9.3	8.9	6.9	5.3	0.1	6.3	2.9	1.2	0.92	1.85	ND
n-C_{10} to n-C_{16} (%)	59.5	30.4	24.5	25.0	18.1	9.5	54.5	39.5	21.8	12.8	13.3	0.1
n-C_{16} to n-C_{34} (%)	30.1	48.1	55.0	59.3	57.9	65.9	39.1	56.6	71.3	67.5	63.8	84.7
≥n-C_{34} (%)	0.6	12.2	11.6	8.8	18.7	24.4	ND	1.0	5.7	18.8	21.1	15.2
TSH/TPH (%)	95.3	77.3	68.2	71.6	51.8	57.4	89.8	82.5	52.7	63.1	47.6	94.9
TAH/TPH (%)	4.7	22.7	31.8	28.4	48.2	42.5	10.2	17.5	47.3	36.9	52.4	5.1
GC-UCM/GC-TPH (%)	52.3	73.0	77.9	84.4	87.0	97.5	77.7	74.4	80.6	74.7	72.3	95.9
Total n-alkanes (mg/g)	172	53.2	63.0	35.6	21.5	ND***	128	54.2	42.3	46.4	29.1	ND

* The concentration is based on TSEM of DCM extract from raw oil sands.

** Petroleum hydrocarbons are determined by GC-FID at a range of ~ n-C_8 to n-C_{50}.

*** ND represents nondetectable.

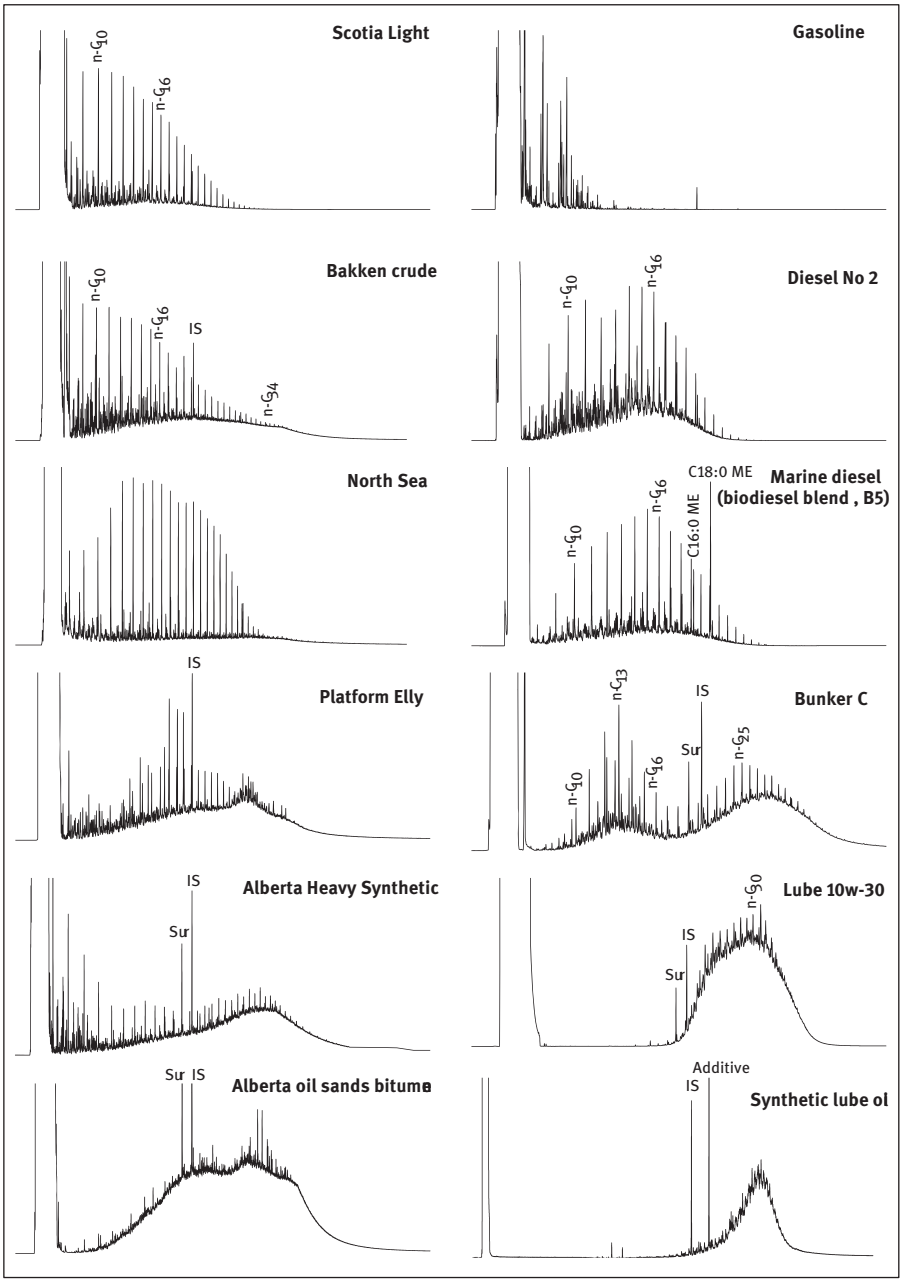

Figure 2.3: GC-FID chromatograms of petroleum hydrocarbons in crude oils and petroleum products.

mostly of light hydrocracking hydrocarbons and is absent of vacuum residue, the UCM contents are mainly constituted by the heavier portion of residues.

As observed from Figure 2.3 and Table 2.2, refined petroleum products vary significantly with type to type and oil to oil in the carbon range, hydrocarbon distribution pattern, and UCM profiles. The distinguishable characteristics for various types of products are attributed to parent crude oil feedstocks, refining processes, and the materials added in the products for specific purposes. Light distillates such as gasoline are generally products of light-end-resolved hydrocarbons nearly without any UCM content. The chemical composition of light distillate is relatively simple and has been well characterized. The composition of gasoline generally includes a majority of light PIANO compounds (paraffins, isoparaffins, aromatics, naphthenes, and olefins) and a small portion of additives (such as alcohols).

Diesel fuel, a mid-range distillate, generally has a carbon range from C_8 to C_{28}. In some regions, diesel is often blended with lighter components for winter use. Its GC-FID chromatogram presents prominent *n*-alkanes in the GC-resolved peaks over a single bell-shaped UCM hump. MDO contains a wider carbon range than on-road diesel. In the analysis of diesel blended with biofuels, additional FAME peaks eluted after *n*-C_{19} and one co-eluted with *n*-C_{21} in the GC-FID chromatograms were readily observed [24]. It is notable that in a biodiesel blend methyl palmitate (C16:0 ME) peak elutes after *n*-C_{19} and methyl stearate (C18:0 ME) and methyl oleate (*cis*-9, C18:1 ME) co-elute with *n*-C_{21} in the GC-FID chromatograms.

HFO are often burned in furnaces to generate heat or in maritime (such heavy fuel is called bunker fuel) or industrial boilers to generate power. Typical marine fuels include lighter fuel no. 5 (Bunker B) and heavier fuel no. 6 (Bunker C). These fuel oils are mainly heavy distillate residue blended by lighter distillates to reduce oil viscosity; therefore, these oils are characterized by two or more obvious chromatographic UCM humps and particular *n*-alkane distribution. HFOs could have a wide carbon range, and some HFOs have similar chromatographic features with weathered crude oils. Their unambiguous identification and differentiation need to rely on the comparison of their fingerprint details from GC-MS analysis.

Commercial lubricating oil is either mineral-based or synthetic, and mineral-based lube oils are most commonly used. The applications of lube oil are very diverse. Lube oil can be classified into various categories such as motor oil and transmission oil according to specific applications. Lubricating oil is distinguishable from other refined products by its unique chromatographic profile. They generally consist of high boiling point hydrocarbons ranging from *n*-C_{20} to *n*-C_{50} eluted as a characteristic UCM hump. GC-resolved peaks only account for a very small portion of TPHs in lubricating oil, for example, lubricating 10W-30 motor oil contains about 96% of UCM content (Table 2.2). As shown in Figure 2.3, the synthetic lube oil has typical GC-chromatographic features of regular lube oil: high UCM content but very little *n*-alkanes and resolved peaks. One high sharp peak over the UCM hump is attributed to additives such as antioxidants

(phenols and amines). It is impossible to differentiate this type of synthetic lube oil from conventional mineral-based lube oils using GC-FID analysis alone.

The analyses of oils in environmental samples can be much more difficult and complex than the analysis of pure oil. The oil in the environment is subjected to various weathering processes and mixed with background substances, which alter oil characteristics and complicate identification. Figure 2.4 shows the GC-FID chromatograms of hydrocarbons in some petroleum-impacted environmental samples. The background soil contains very little UCM, although nonpetroleum UCM content is detectable if not properly removed by a silica-gel column cleanup procedure [40]. This sample has a predominance of odd carbon numbers over even carbon number *n*-alkanes in C_{21} to C_{35} range, indicating hydrocarbon inputs from natural organic matter, plants, and bacteria. The petroleum hydrocarbons in the vessel bilge clearly consist of primarily a diesel range fuel and a slight amount of lubricating oil. More than two UCM humps are noticeable in the river sediment, indicating multiple sources of contamination. Two UCM humps ranging from *n*-C_{20} to *n*-C_{30} and *n*-C_{30} to *n*-C_{50} were detected for the waste lube oil, indicating that the waste oil is mainly a mixture of 10W-30 and 20W-50 lube oils [24]. Prominent odd *n*-alkanes were detected over the UCM hump, indicating that the main hydrocarbons in the sediment from a water treatment plant are mainly attributed to petroleum contamination and the contribution of biogenic sources. The waste lube oil also contains a small portion of *n*-alkanes between *n*-C_{12} to *n*-C_{26} and about 5.0% resolved peaks of TPH, which are certainly attributable to diesel fuel.

It is impossible to identify or quantify the petroleum biomarkers and PAHs through GC-FID analysis alone, which are at relatively low concentrations but of interest to forensic oil analysis. The GC-MS analysis provides data on the "source-specific" markers including a suit of saturated and aromatic target compounds. These analyses provide detailed information from each class of petroleum hydrocarbons to support the findings from GC-FID analysis.

2.5 Saturated hydrocarbons in petroleum

Saturated petroleum fraction consists of aliphatic hydrocarbons such as *n*-alkanes, branched alkanes, and cyclic hydrocarbons. Normal alkanes and branched alkanes are typically attributed to the major resolved peaks in gas chromatograms of the saturated fraction from light to medium crude oils and distillates.

Saturated cyclic hydrocarbons (naphthenes) comprise the most desirable petroleum hydrocarbons for the oil fingerprinting approach. Tables 2.3 summarizes the target saturated hydrocarbons frequently used for oil analysis including decalins, bicyclic sesquiterpanes, diamondoids, polycyclic terpanes, and polycyclic steranes [10, 12, 13, 15, 16, 20, 27, 29]. In brief, the most commonly used fragment ions are m/z 191 for tri- to penta- cyclic terpanes and m/z 217 and 218 for steranes. Bicyclic sesquiterpanes are

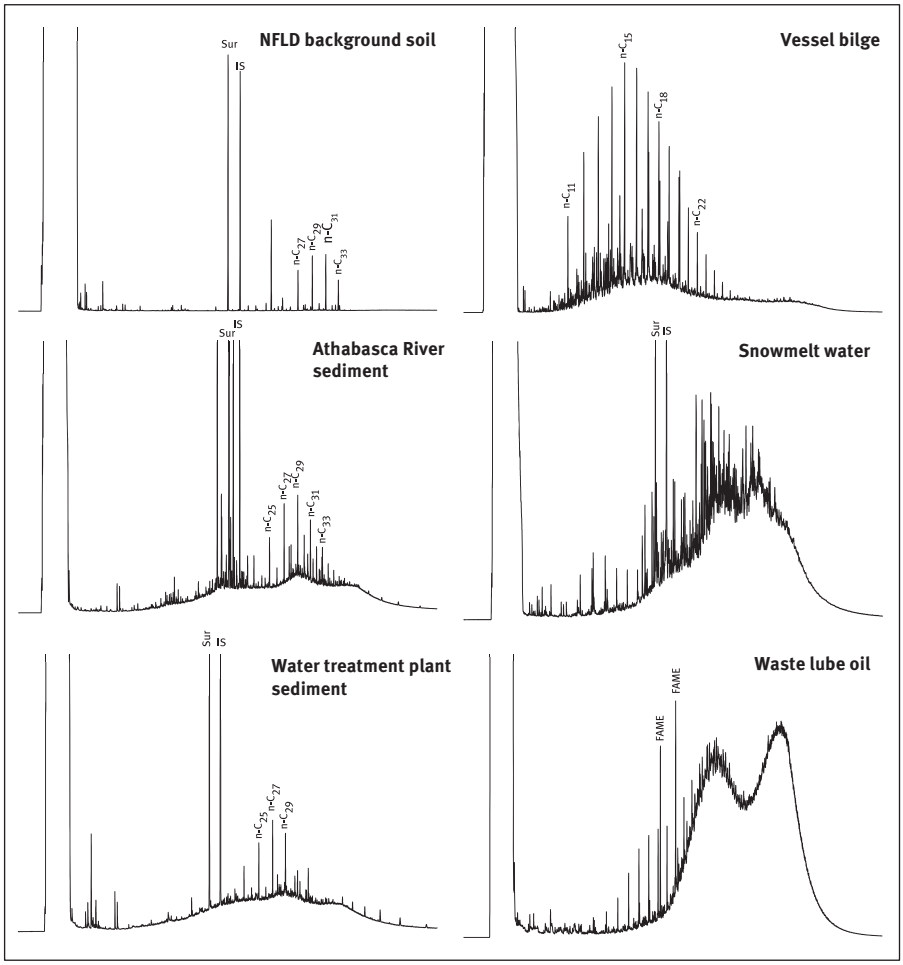

Figure 2.4: GC-FID chromatograms of hydrocarbons in environmental samples.

determined at m/z 123 and confirmed by ions of m/z 179, 193, and 207. The ions for diamondoid analysis include m/z 135, 136, 149, 163, and 177 for adamantanes and m/z 187, 188, 201, and 215 for diamantanes.

Some compounds in crude oils retain all or part of molecular structures from their original biological molecules produced by organisms. These molecular fossils are often called biological markers, or simply biomarkers. These compounds are resistant to biodegradation relative to other hydrocarbons. Their analyses have played an important role in exploring the depositional environment of crude oil and tracking the genesis, maturation, migration, and biodegradation of petroleum. The information from the analysis of backbone petrogenic compounds has been widely used to investigate the source and history of petroleum-derived environmental contaminants.

Table 2.3: Target saturated hydrocarbons frequently used for oil fingerprinting analysis.

Compounds	Code	Empirical formula	Molecular weight	Target ions
Normal alkanes				
n-Alkanes (*n*-C_{10} to *n*-C_{44})	*n*-C_n	C_nH_{2n+2}	14 n + 2	85, 71, 57
Acyclic isoprenoids				
(*i*-C_{13} to *i*-C_{20})				
Norpristane	Norpri	$C_{18}H_{38}$	254	85, 113
Pristane	Pri	$C_{19}H_{40}$	268	85, 113
Phytane	Phy	$C_{20}H_{42}$	282	85, 113, 183
Alkylated cyclopentanes		C_nH_{2n}	14 n	69, 83
Alkylated cyclohexanes		C_nH_{2n}	14 n	83, 97
Bicyclic alkanes				
Decalin	DE	$C_{10}H_{18}$	138	138
C_1-Decalins	C1DE	$C_{11}H_{20}$	152	152
C_2-Decalins	C2DE	$C_{12}H_{22}$	166	166
C_3-Decalins	C3DE	$C_{13}H_{24}$	180	180
Bicyclic sesquiterpanes				
C_4-Decalin	BS1	$C_{14}H_{26}$	194	123, 179
C_{14}-Sesquiterpane	BS2	$C_{14}H_{26}$	194	123, 179
C_{15}-Sesquiterpane	BS3	$C_{15}H_{28}$	208	123, 193
C_{15}-Sesquiterpane	BS4	$C_{15}H_{28}$	208	123, 193
8β(H)-Drimane	BS5	$C_{15}H_{28}$	208	123
C_{15}-Sesquiterpane	BS6	$C_{15}H_{28}$	208	123
C_{16}-Sesquiterpane	BS7	$C_{16}H_{30}$	222	123
C_{16}-Sesquiterpane	BS8	$C_{16}H_{30}$	222	123, 193
C_{16}-Sesquiterpane	BS9	$C_{16}H_{30}$	222	123, 193
8β(H)-Homodrimane	BS10	$C_{16}H_{30}$	222	123, 207
Adamantanes				
Adamantane	A	$C_{10}H_{16}$	136	136
1-Methyladamantane	1-MA	$C_{11}H_{18}$	150	135
1,3-Dimethyladamantane	1,3-DMA	$C_{12}H_{20}$	164	149
1,3,5-Trimethyladamantane	1,3,5-TMA	$C_{13}H_{22}$	178	163
1,3,5,7-Tetramethyladamantane	1,3,5,7-TeMA	$C_{14}H_{24}$	192	177
2-Methyladamantane	2-MA	$C_{11}H_{18}$	150	135
1,4-Dimethyladamantane, *cis*	1,4-DMA, *cis*	$C_{12}H_{20}$	164	149
1,4-Dimethyladamantane, *trans*	1,4-DMA, *trans*	$C_{12}H_{20}$	164	149
1,3,6-Trimethyladamantane	1,3,6-TMA	$C_{13}H_{22}$	178	163
1,2-Dimethyladamantane	1,2-DMA	$C_{12}H_{20}$	164	149
1,3,4-Trimethyladamantane, *cis*	1,3,4-TMA, *cis*	$C_{13}H_{22}$	178	163
1,3,4-Trimethyladamantane, *trans*	1,3,4-TMA, *trans*	$C_{13}H_{22}$	178	163
1,2,5,7-Tetramethyladamantane	1,2,5,7-TeMA	$C_{14}H_{24}$	192	177
1-Ethyladamantane	1-EA	$C_{12}H_{20}$	164	135
1-Ethyl-3-methyladamantane	1-E-3-MA	$C_{13}H_{22}$	178	149
1-Ethyl-3,5-dimethyladamantane	1-E-3,5-DMA	$C_{14}H_{24}$	192	163
2-Ethyladamantane	2-EA	$C_{12}H_{20}$	164	135

Table 2.3 (continued)

Compounds	Code	Empirical formula	Molecular weight	Target ions
Diamantanes				
Diamantane	D	$C_{14}H_{20}$	188	188
4-Methyldiamantane	4-MD	$C_{15}H_{22}$	202	187
4,9-Dimethyldiamantane	4,9-DMD	$C_{16}H_{24}$	216	201
1-Methyldiamantane	1-MD	$C_{15}H_{22}$	202	187
1,4 and 2,4-Dimethyldiamantane	1,4 and 2,4-DMD	$C_{16}H_{24}$	216	201
4,8-Dimethyldiamantane	4,8-DMD	$C_{16}H_{24}$	216	201
Trimethyldiamantane	TMD	$C_{17}H_{26}$	230	215
3-Methyldiamantane	3-MD	$C_{15}H_{22}$	202	187
3,4-Dimethyldiamantane	3,4-DMD	$C_{16}H_{24}$	216	201
Biomarker terpanes				
C_{19} tricyclic terpane	TR19	$C_{19}H_{34}$	262	191
C_{20} tricyclic terpane	TR20	$C_{20}H_{36}$	276	191
C_{21} tricyclic terpane	TR21	$C_{21}H_{38}$	290	191
C_{22} tricyclic terpane	TR22	$C_{22}H_{40}$	304	191
Biomarker terpanes				
C_{23}-Tricyclic terpane	TR23	$C_{23}H_{42}$	318	191
C_{24}-Tricyclic terpane	TR24	$C_{24}H_{44}$	332	191
C_{25}-Tricyclic terpane (a), (b)	TR25A, TR25B	$C_{25}H_{46}$	346	191
Triplet: C_{24} tetracyclic + C_{26} (*S* + *R*) tricyclic terpanes terpanes	TET24 + TR26(A + B)	$C_{24}H_{42}$ + $C_{26}H_{48}$	330, 374	191
C_{28}-Tricyclic terpane (a), (b)	TR28A, TR28B	$C_{28}H_{52}$	388	191
C_{29}-Tricyclic terpane (a), (b)	TR29A, TR29B	$C_{29}H_{54}$	402	191
18α(H)-22,29,30-Trisnorhopane	Ts	$C_{27}H_{46}$	370	191
17α(H),18α(H),21β(H)-25,28,30-Trisnorhopane	TH27	$C_{27}H_{46}$	370	191, 177
17α(H)-22,29,30-Trisnorhopane	Tm	$C_{27}H_{46}$	370	191
C_{30}-Tricyclic terpane 1 and 2	TR30A,TR30B	$C_{30}H_{56}$	416	191
17α(H),18α(H),21β(H)-28,30-bisnorhopane	H28	$C_{28}H_{48}$	384	191
17α(H),21β(H)-25-Norhopane	NOR25H	$C_{29}H_{50}$	398	191, 177
17α(H),21β(H)-30-Norhopane	H29	$C_{29}H_{50}$	398	191
18α(H),21β(H)-30-Norneohopane	C29Ts	$C_{29}H_{50}$	398	191
17α(H)-Diahopane	DH30	$C_{30}H_{52}$	412	191
17β (H),21α(H)-30-Norhopane (normoretane)	M29	$C_{29}H_{50}$	398	191
18α(H) and 18β(H)-Oleanane	OL	$C_{30}H_{52}$	412	191, 412
17α(H),21β(H)-Hopane	H30	$C_{30}H_{52}$	412	191
17α(H)-30-nor-29-Homohopane	NOR30H	$C_{30}H_{52}$	412	191
17β(H),21α(H)-Hopane (moretane)	M30	$C_{30}H_{52}$	412	191
22*S*-17α(H),21β(H)-30-Homohopane	H31S	$C_{31}H_{54}$	426	191
22 *R*-17α(H),21β(H)-30-Homohopane	H31R	$C_{31}H_{54}$	426	191
Gammacerane	G	$C_{30}H_{52}$	412	191, 412

Table 2.3 (continued)

Compounds	Code	Empirical formula	Molecular weight	Target ions
22*S*-17α(H),21β(H)-30,31-Bishomohopane	H32S	$C_{32}H_{56}$	440	191
22*R*-17α(H),21β(H)-30,31-Bishomohopane	H32R	$C_{32}H_{56}$	440	191
22*S*-17α(H),21β(H)-30,31,32-Trishomohopane	H33S	$C_{33}H_{58}$	454	191
22*R*-17α(H),21β(H)-30,31,32-Trishomohopane	H33R	$C_{33}H_{58}$	454	191
22*S*-17α(H),21β(H)-30,31,32,33-Tetrakishomohopane	H34S	$C_{34}H_{60}$	468	191
22*R*-17α(H),21β(H)-30,31,32,33-Tetrakishomohopane	H34R	$C_{34}H_{60}$	468	191
22*S*-17α(H),21β(H)-30,31,32,33,34-Pentakishomohopane	H35S	$C_{35}H_{62}$	482	191
22*R*-17α(H),21β(H)-30,31,32,33,34-Pentakishomohopane	H35R	$C_{35}H_{62}$	482	191
Biomarker steranes				
C_{20} 5α(H),14α(H),17α(H)-Sterane	S20	$C_{20}H_{34}$	274	217, 218
C_{21} 5α(H),14β(H),17β(H)-Sterane	S21	$C_{21}H_{36}$	288	217
C_{22} 5α(H),14β(H),17β(H)-Sterane	S22	$C_{22}H_{38}$	302	217
C_{27} 20*S*-13β(H),17α(H)-Diasterane	DIA27S	$C_{27}H_{48}$	372	217
C_{27} 20*R*-13β(H),17α(H)-Diasterane	DIA27R	$C_{27}H_{48}$	372	217
C_{27} 20*S*-13α (H),17β (H)-Diasterane	DIA27S2	$C_{27}H_{48}$	372	217
C_{27} 20*R*-13α (H),17β (H)-Diasterane	DIA27R2	$C_{27}H_{48}$	372	217
C_{28} 20*S*-13β(H),17α(H)-Diasterane	DIA28S	$C_{28}H_{50}$	386	217
C_{28} 20*R*-13β(H),17α(H)-Diasterane	DIA28R	$C_{28}H_{50}$	386	217
C_{29} 20*S*-13β(H),17α(H)-Diasterane	DIA29S	$C_{29}H_{52}$	400	217
C_{29} 20*R*-13α(H),17β(H)-Diasterane	DIA29R	$C_{29}H_{52}$	400	217
C_{27} 20*S*-5α(H),14α(H),17αH)-Cholestane	C27ααS	$C_{27}H_{48}$	372	217
C_{27} 20*R*-5α(H),14β(H),17β(H)-Cholestane	C27ββR	$C_{27}H_{48}$	372	217, 218
C_{27} 20*S*-5α(H),14β(H),17β(H)-Cholestane	C27ββS	$C_{27}H_{48}$	372	217, 218
C_{27} 20*R*-5α(H),14α(H),17α(H)-Cholestane	C27ααR	$C_{27}H_{48}$	372	217
C_{28} 20*S*-5α(H),14α(H),17α(H)-Ergostane	C28ααS	$C_{28}H_{50}$	386	217
C_{28} 20*R*-5α(H),14β(H),17β(H)-Ergostane	C28ββR	$C_{28}H_{50}$	386	217, 218
C_{28} 20*S*-5α(H),14β(H),17β(H)-Ergostane	C28ββS	$C_{28}H_{50}$	386	217, 218
C_{28} 20*R*-5α(H),14α(H),17α(H)-Ergostane	C28ααR	$C_{28}H_{50}$	386	217

Table 2.3 (continued)

Compounds	Code	Empirical formula	Molecular weight	Target ions
C_{29} 20*S*-5α(H),14α(H),17α(H)-Stigmastane	C29ααS	$C_{29}H_{52}$	400	217
C_{29} 20*R*-5α(H),14β(H),17β(H)-Stigmastane	C29ββR	$C_{29}H_{52}$	400	217, 218
C_{29} 20*S*-5α(H),14β(H),17β(H)-Stigmastane	C29ββS	$C_{29}H_{52}$	400	217, 218
C_{29} 20*R*-5α(H),14α(H),17α(H)-Stigmastane	C29ααR	$C_{29}H_{52}$	400	217
C_{30}-Steranes	C30ααS	$C_{30}H_{54}$	414	217

The commonly analyzed HMW petroleum biomarkers are polycyclic triterpanes and steranes. These conventional biomarker compounds are eluted after *n*-C_{21}, indicating their high boiling points, which occur within the residual range (Figure 2.2). Smaller cyclic hydrocarbons such as diamondoids and bicyclic sesquiterpanes in light to medium carbon range have attracted increasing attention due to their potential forensic applications especially for lighter oils and products [19, 33–36, 41–44]. These compounds have been detected in most crude oils and in a variety of distilled petroleum products, which makes them particularly applicable to the analysis of lighter refined products such as diesel fuels that lack suitable alternatives. In these refined products, HMW biomarkers have been removed during the refining processes.

2.5.1 Normal alkanes and acyclic isoprenoids

Normal and branched alkanes comprise a substantial portion of most crude oils and certain types of petroleum products. These compounds can be readily separated into resolved peaks by capillary GC columns. Normal alkanes in crude oil and petroleum usually occur in high concentrations, which enables them to be quantitatively analyzed by GC-FID. However, if their concentrations in heavy oils and environmental samples are too low or subject to interferences to obtain accurate analysis, GC-MS analysis can be an alternative fingerprinting technique.

Normal alkanes in crude oils often vary significantly in their concentrations and distributions (Table 2.2 and Figure 2.3). Total *n*-alkanes in Scotia Light crude oil are as high as 172 mg/g, whereas only 21.5 mg/g in the Platform Elly crude oil and almost no *n*-alkane are detected in Alberta oil sand bitumen. As shown in Figure 2.2, *n*-alkanes could account for about 25% of GC-detectable TPHs and over half of the resolved peaks in the high wax crude oil.

Refined petroleum products generally have their distinctive abundance and carbon range in their *n*-alkane profiles. Diesel consists of high levels of C_8 to C_{28} *n*-

alkanes and alkyl-cyclohexanes. The properties of a given diesel are largely a fraction of the crude oil feedstock. Diesel no. 2 has a high concentration of *n*-alkanes of 128 mg/g, and its GC chromatogram has a nearly normal distribution with maxima around *n*-C_{11} to *n*-C_{14}. The GC-TPHs are dominated by the central UCM hump, totally accounting for 77.7%. Heavy bunker fuels such as Bunker C generally contain lower *n*-alkanes, whereas lubricating oils generally contain a very trace amount of long-chain *n*-alkanes.

Normal C_{10}–C_{40} alkanes, with a marked predominance of odd-numbered hydrocarbons in the span of *n*-C_{23} to *n*-C_{35}, are identified in higher plants (concentrations of *n*-C_{27}, *n*-C_{29}, and *n*-C_{31} hydrocarbons are especially high) and soil, river, and marine sediments (see Figure 2.4). This characteristic is particularly useful to distinguish biogenic sources from petrogenic sources in environmental samples.

Some branched alkanes in petroleum are genetically related to acyclic isoprenoids and norisoprenoids. These isoprenoids are composed exclusively of "head-to-tail" links of isoprene units, whereas irregular isoprenoids have a "tail-to-tail" link. Compared to other alkanes, these hydrocarbons are characterized by their structural stability and preservation of genetic features, which are inherited from peculiarities of the original organic matter and the conditions of its transformation into petroleum hydrocarbons. Barakat et al. detected a suite of acyclic isoprenoids from C_{13} to C_{20} except C_{17} in the crude oils from the Gulf of Suez region of Egypt [45]. The most abundant acyclic isoprenoids in oil include farnesane (*i*-C_{15}: 2,6,10-trimethyl-dodecane), 2,6,10-trimethyl-tridecane (*i*-C_{16}), norpristane (*i*-C_{18}: 2,6,10-trimethyl-pentadecane), pristane (*i*-C_{19}: 2,6,10,14-tetramethyl-pentadecane), and phytane (*i*-C_{20}: 2,6,10,14-tetramethyl-hexadecane). The acyclic isoprenoids of pristane and phytane are widely assumed diagenetic products of the phytyl side chain of chlorophyll, although alternative sources of precursors have been suggested. Branched alkanes normally exhibit lower melting points and boiling points than those *n*-alkanes with the same carbon numbers; therefore, they tend to elute earlier than their normal isomers during GC analysis. Pristane and phytane are eluted closely with *n*-C_{17} and *n*-C_{18} into two pairs of characteristic peaks in chromatographic analysis. They are often investigated together with *n*-alkane analysis. Crudes with the same geological origin have similar pristane/phytane ratios.

2.5.2 Diamondoids

Diamondoids and their various substituents are widely found in crude oils, intermediate petroleum distillates, and finished petroleum products [33, 34, 46–49]. Diamondoids are a class of cage-like cyclic hydrocarbons and consist of three-dimensionally fused cyclohexane rings, resulting in a diamond-like structure. Diamondoids have the general molecular formula $C_{4n+6}H_{4n+12}$. The simplest diamondoid is adamantane ($C_{10}H_{16}$), followed by its homologues of diamantane ($C_{14}H_{20}$), triamantane, tetramantane,

pentamantane, and hexamantane. Figure 2.5 illustrates a suite of adamantanes and diamantanes identified in crude oils and petroleum products. Adamantane and its C_1- to C_4-substituents are eluted between *n*-C_{10} and *n*-C_{13} (boiling point range: 180–230 °C), while diamantane series are eluted between *n*-C_{15} and *n*-C_{17} (boiling point range: 270–300 °C) at given chromatographic conditions (Figures 2.2).

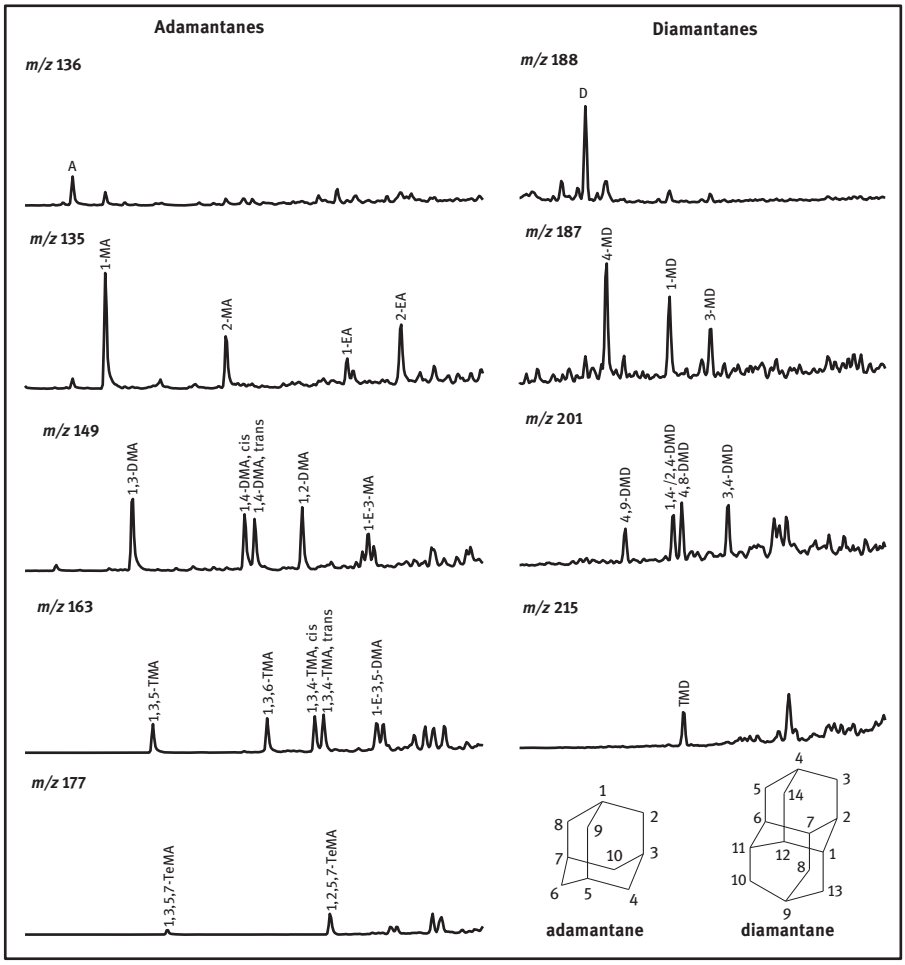

Figure 2.5: Adamantanes (at *m/z* 136, 149, 163, and 207) and diamantanes (at *m/z* 188, 187, 201, and 215) identified in crude oils and petroleum products.

A series of petroleum polymantanes were determined in a gas condensate produced from a very deep petroleum reservoir located in the U.S. Gulf Coast [50]. Dahl et al. reported successful separation of a wide variety of the higher diamondoids containing 4–11 (undecamantane) diamond-crystal cages from petroleum [51]. Diamondoid

compounds in petroleum are believed to be the result of carbonium ion rearrangements of suitable cyclic precursors (such as multiringed terpene hydrocarbons) on clay superacids in the source rock during oil generation [48, 52, 53]. The higher homologues of diamondoids are considered to be formed from lower homologues under extreme temperature and pressure [54].

Diamondoids are common in reservoir fluids and have been considered a problem due to their deposition during production and transportation of natural gas, gas condensates, and light crude oils. The naturally occurring diamondoid compounds are thermodynamically stable [49, 54, 55], and therefore, they are particularly useful in oil-source correlation and differentiation. Environmental scientists have applied the multiple criteria approach with the characterization of more than one suite of analytes, including fingerprinting the diamondoid hydrocarbons for oil spill correlation and identifications. Diamondoid analysis is particularly meaningful for those cases where the conventional tri- to pentacyclic biomarker terpanes and steranes are absent due to removal during the refining processes [34, 36, 41].

***Diamondoids in crude oils*:** GC-MS chromatograms of adamantanes and diamantanes were determined in various crude oils from light South Louisiana crude oil to extra heavy Alberta oil sand bitumen are displayed [36]. The concentration of adamantane, diamantane, and their alkyl-homologues in these crude oils are shown in Table 2.4. Overall, the one-cage adamantanes are much more abundant than the two-cage diamantanes.

As shown in Table 2.4, the concentrations of individual adamantane and its alkylated derivatives are in the range of ~1–30 μg/g for most crude oils, except ~ 1–300 μg/g for the corresponding individual diamondoids in Troll (North Sea, Norway) and South Louisiana (37.2° API, Louisiana, USA) crude oils. Among the detected adamantanes, the principal dominant adamantanes are A, 2-MA, 1-MA, 2-EA, 1,2-DMA, and 1,3-DMA, together accounting for about 50% of all detected adamantanes. Either 1-MA (bridgehead-substituted) or 2-MA is the most abundant homologue in all oil samples. Tri- and tetra-methylated diamondoids have much lower relative abundance compared to methyl- and dimethyl-homologues. Among the adamantane series, 1,3,5,7- tetramethyl-adamantane has the lowest concentration probably due to its relatively poor thermal stability; it has four methyl groups, which could spatially interfere with each other and cause the molecular structure to be strained.

The dominant diamantane compounds in crude oils are D, 4-MD, 1-MD, 3-MD, and 3,4-DMD. The concentrations of individual diamantanes range from undetected levels to 10 μg/g for most crude oil samples and up to 10–53 μg/g for the South Louisiana oil. The California Platform Elly heavy oil has the lowest concentration of adamantanes among the surveyed oils. Cook Inlet (Southern Alaska, USA) crude oil has a low concentration of diamantanes (9.10 μg/g), even though its adamantanes are at a relatively high concentration of 209 μg/g.

Table 2.4: Concentrations of diamondoids in crude oils and petroleum products.

Compounds	SoL	ANS	Troll	ArH	PlE	Oil sands*	Fed	Fed (174–287°C) 287oC)	Fed (287–481°C) 287oC)	JetA	Diesel No. 2	BkC	10W-30
A	126	19.3	48.6	6.80	0.77	3.16	8.25	31.9	0.08	181	63.3	5.19	0.08
1-MA	288	33.9	68.0	12.0	2.04	7.46	12.7	43.2	0.14	222	118	7.53	0.19
1,3-DMA	226	26.2	38.0	9.34	4.23	6.00	11.2	43.8	0.35	174	144	4.40	0.21
1,3,5-TMA	84.3	7.82	10.3	3.19	0.98	2.59	3.65	13.6	0.06	49.8	60.3	0.90	0.06
1,3,5,7-TeMA	13.7	1.28	1.50	0.90	0.19	0.52	0.72	2.7	0.02	7.44	5.85	0.03	0.01
2-MA	190	32.5	81.5	9.96	1.45	9.16	15.7	52.4	0.22	264	85.4	6.53	0.18
1,4-DMA, *cis*	109	17.1	32.2	5.97	1.81	4.84	9.57	38.7	0.33	140	54.3	2.61	0.12
1,4-DMA, *trans*	110	15.8	32.7	4.80	5.05	4.76	7.30	31.1	0.17	112	54.3	2.39	0.10
1,3,6-TMA	79.3	9.25	18.0	3.15	2.20	3.64	4.19	16.7	0.11	64.2	42.7	1.05	0.07
1,2-DMA	112	18.1	34.8	5.01	7.35	6.77	10.1	43.1	0.23	127	49.2	2.27	0.09
1,3,4-TMA, *cis*	76.7	9.36	17.5	2.69	1.33	4.99	4.74	16.3	0.16	52.5	32.0	0.92	0.06
1,3,4-TMA, *trans*	82.6	10.6	20.6	3.30	3.24	4.54	6.05	25.1	0.21	70.2	40.0	1.60	0.07
1,2,5,7-TeMA	55.5	5.28	9.50	1.88	1.65	3.33	3.25	12.0	0.16	31.5	32.3	0.47	0.05
1-EA	56.2	8.38	16.1	2.54	2.26	3.63	4.72	17.9	0.54	63.3	30.5	2.47	0.05
1-E-3-MA	94.1	14.1	20.7	4.91	3.30	6.87	6.56	22.4	0.29	75.0	71.4	2.75	0.07
1-E-3,5-DMA	89.5	10.9	21.5	2.67	1.34	7.97	7.77	26.2	0.34	76.4	31.9	1.28	0.07
2-EA	88.3	23.3	56.2	8.76	2.88	4.33	13.3	49.4	0.43	193	61.3	2.22	0.09
ΣAdamantanes (µg/g)	**1880**	**263**	**528**	**87.8**	**42.1**	**84.6**	**130**	**487**	**3.85**	**1904**	**977**	**44.6**	**1.58**
D	53.3	9.00	9.31	6.28	1.06	2.43	3.46	5.07	5.61	10.6	14.0	1.66	0.09
4-MD	36.5	5.19	4.46	5.22	4.38	1.87	2.89	5.70	6.24	4.50	9.43	1.05	0.06
4,9-DMD	11.7	1.17	0.98	1.02	0.09	ND	0.67	1.33	1.87	1.52	2.94	0.13	0.02
1-MD	22.8	3.82	5.06	2.99	0.69	ND	1.98	5.09	5.88	2.34	9.96	1.92	0.06
1,4- and 2,4-DMD	13.8	1.78	2.34	1.59	1.02	0.65	1.02	1.67	2.73	1.29	4.04	0.34	0.03
4,8-DMD	14.5	1.47	1.70	1.41	1.59	0.52	0.72	0.75	2.38	0.38	2.65	0.21	0.04
TMD	10.6	0.83	0.89	1.05	0.00	0.46	0.43	0.48	1.41	0.12	2.37	0.07	0.04

3-MD	15.4	2.13	3.53	1.44	1.08	1.22	1.03	1.40	9.52	1.14	4.43	0.59	0.04
3,4-DMD	16.3	2.13	3.38	1.65	0.46	1.85	1.26	1.88	3.84	1.14	3.78	0.39	0.04
ΣDiamantanes (µg/g)	**195**	**27.5**	**31.6**	**22.8**	**10.4**	**8.99**	**13.5**	**23.4**	**39.5**	**23.0**	**53.6**	**6.36**	**0.41**

* Concentration: µg/g of TSEM of Alberta oil sands DCM extract.

The concentrations of diamondoids do not appear to be dependent on the densities (or derived API gravities) of the crude oils. Diamondoids in oils from different sources have dissimilar signatures of both absolute concentrations and relative distribution patterns [34, 36]. These diamondoid fingerprints, as well as their molecular ratios particularly from adamantanes, may be useful for oil source identification.

***Diamondoids in petroleum products*:** The concentrations of diamondoids in refined products are significantly influenced by the crude oil feedstocks used in the production and the distillation cutpoint of the petroleum products. During the simulated laboratory distillation of crude oils, the medium distillate fraction (174–287 °C) accounted for >93% of all the adamantanes from the original crude oil. Diamantanes were found mainly in the fractions of 174–481 °C, accounting for about 90% of their total and were rarely found in the lightest fraction (initial boiling point to 174 °C) [36]. These temperature ranges for diamondoid partitioning are consistent with the observations based on GC chromatograms of *n*-alkanes and diamondoids.

Table 2.4 compares adamantanes and diamantanes measured in representative refined products including gasoline, diesel fuel, Bunker C, lubricating oil, and two distillate fractions of the Federated crude oil. The absolute concentrations and distribution patterns of diamondoids differ widely in petroleum products. As shown in Table 2.4, adamantanes were found in all fuel oil samples. As expected, little or no diamantanes were detected in light fuel and heavy-end lubricating oils. Generally, the overall distribution pattern of individual diamondoid compounds in petroleum products are comparable to that in crude oils, in which 1-MA and 2-MA, and D and 4-MD dominate the adamantanes and diamantanes, respectively.

2.5.3 Bicyclic sesquiterpanes

Bicyclic sesquiterpanes with drimane skeletons ($C_{15}H_{28}$) consist of two fused cyclohexane rings (decahydronaphthalene), with various methyl-, dimethyl-, ethyl-, or longer side chains [42, 56–58]. These compounds probably have a microbiological source and are produced from the biodegradation of bigger terpanes or are formed directly from bicyclic compounds of the same carbon framework.

Ten bicyclic sesquiterpanes commonly analyzed in petroleum fingerprinting elute between *n*-C_{13} and *n*-C_{16} (boiling points: 235–287 °C), compared with conventional polycyclic terpanes and steranes eluted between *n*-C_{21} and *n*-C_{37} (boiling points: 345–500 °C) (see Figure 2.2). These terpanes are identified as C_{14} (BS1 and BS2), C_{15} (BS3 to BS6) and C_{16} (BS7 to BS10) sesquiterpanes with molecular weights being 194, 208, and 222 atomic mass units (amu), respectively (Table 2.3). These compounds are determined at their characteristic ions at *m*/*z* 123 ($C_9H_{15}^+$), and confirmation can be conducted using other prominent ions such as *m*/*z* 179, 193, and 207 [35].

Due to the commercial unavailability of sesquiterpane standards, two hydrocarbons with a bicyclic molecular structure, *cis*-decalin and 1-methyldecalin, are applied as alternative standards for the quantitation of bicyclic sesquiterpanes [33, 35]. In our in-house method, the response factor for *cis*-decalin and 1-methyldecalin is determined relative to the internal standard of decalin-d_{18}. The average RRF of *cis*-decalin and 1-methyldecalin is then used to determine the concentrations of each target sesquiterpane compound [35]. Therefore, the quantitation results by this means do not necessarily represent real-world concentrations in the oil samples. Nevertheless, this method offers a quantitative comparison of bicyclic sesquiterpanes in various oils.

Crude oils from different sources and different petroleum products have varied signatures of both the absolute concentrations and relative distribution patterns of sesquiterpanes [33, 35]. Bicyclic sesquiterpanes are resistant against slight to medium weathering, particularly biodegradation [33, 35, 42, 59]. Early studies on sesquiterpanes have mainly focused on geological application in the maturity, depositional environment, and the origin of oils. Recently, they have become a special interest in oil-source correlation and differentiation of refined products with high ring number biomarkers removed during refining processes [20, 33, 35, 42–44]. Stout et al. have reported a successful forensic fingerprinting study of middle distillate fuels in the environment using sesquiterpanes [42]. Wang et al. have presented two real-world spill case studies using unique sesquiterpanes for fingerprinting and identifying mystery diesel spills [33]. However, it is recommended that the absence of bicyclic sesquiterpanes should be used cautiously as an indicator of biodegradation rank because there is a high potential for their alteration by water washing [59].

Bicyclic sesquiterpanes in crude oils: Bicyclic sesquiterpanes are ubiquitous components of ancient sediments, coal, and crude oils [33, 35, 56, 57, 60]. Yang et al. reported a quantitation of bicyclic sesquiterpanes in many crude oils and refined petroleum products collected from various sources [35]. Their relative concentrations in crude oils vary considerably from oil to oil. GC-MS chromatograms of sesquiterpanes for representative crude oils and petroleum products are compared in Figure 2.6. Figure 2.7 compared the total concentrations of ten target sesquiterpanes and normalized percentages of major sesquiterpanes in crude oils.

Bicyclic sesquiterpanes occur in all of the crude oils studied. Their abundance is likely independent of the densities of the crude oils [35]. In general, the GC-MS chromatograms of sesquiterpanes at m/z 123 are often characterized by the dominance of BS1, BS3, BS5, and BS10, with BS5 or BS10 being the most abundant. BS2, BS7, BS8, and BS9 have much lower relative abundance. As shown in Table 2.5, the dominant sesquiterpanes BS1, BS3, BS5, and BS10 together account for about 50–75% of the sum of ten target sesquiterpanes. Bicyclic sesquiterpanes were also detected in extremely heavy oils such as Alberta oil sand bitumen despite the depletion of *n*-alkanes by biodegradation [58, 61]. Bulk concentrations of target sesquiterpanes in oil sand bitumen are at the same level as many other crude oils. The selected ion chromatograms at m/z

123 show distribution patterns of BS10 > BS5 > BS1 for Alberta oil sand bitumen. It is evident that bicyclic sesquiterpanes have been partially biodegraded in these heavy oil samples. 8β(H)-Homodrimane is likely the most abundant homologue in oil sand bitumen, which suggests that this compound has the least degradability among all ten sesquiterpanes.

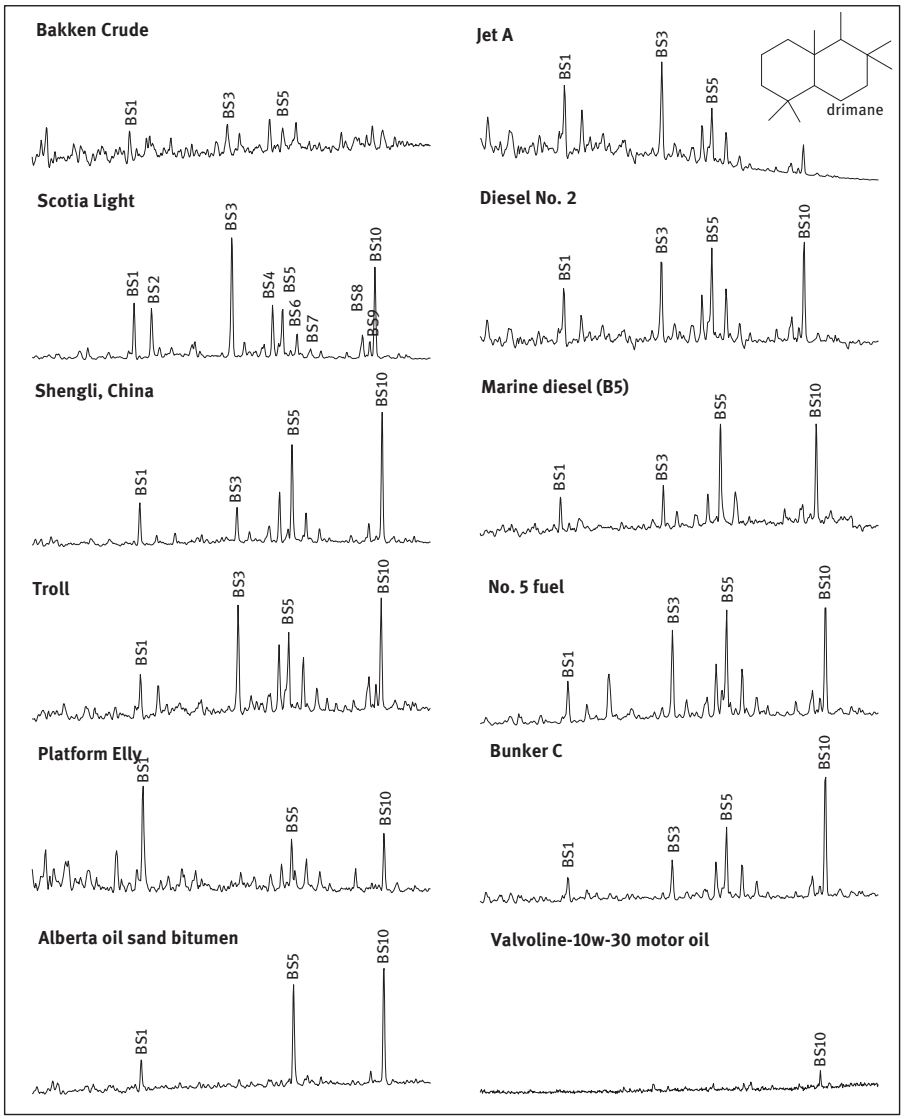

Figure 2.6: GC-MS chromatograms of bicyclic sesquiterpanes (*m*/*z* 123) in crude oils and refined petroleum products.

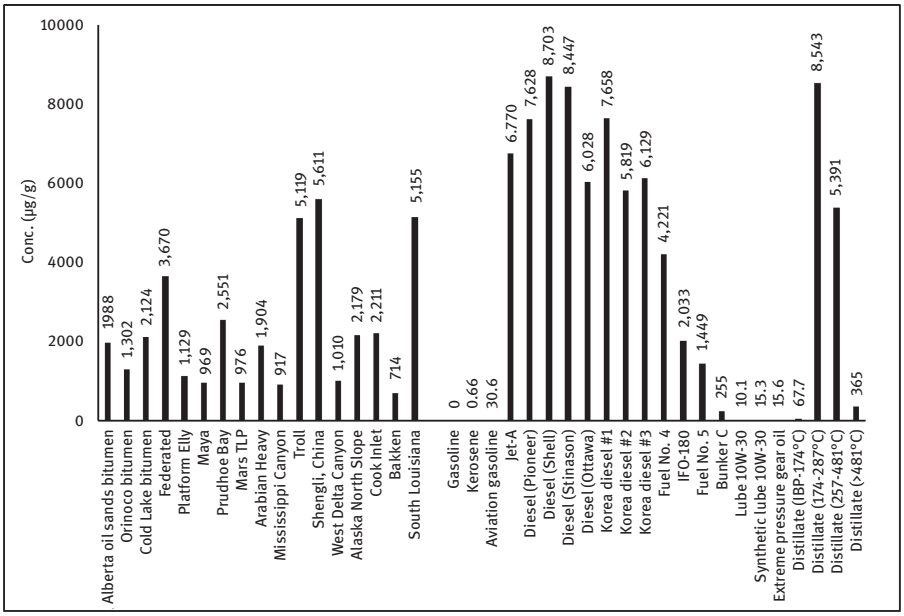

Figure 2.7: Bicyclic sesquiterpanes in crude oils and refined petroleum products.

Bicyclic sesquiterpanes in refined products: Bicyclic sesquiterpanes are widely found in intermediate petroleum distillates and finished petroleum products. The abundances and distribution patterns of sesquiterpanes differ widely in petroleum products, which are attributable to differences in the crude oil feedstocks and the refining processes. Analyses of simulated laboratory distillates of crude oils show that bicyclic sesquiterpanes were largely found in the 174–481 °C distillation fraction of the crude oil. The concentrations of sesquiterpanes in this fraction are at the same level as that found in mid-range fuels [33, 35]. Most of BS1 to BS7 from the feedstock oil entered into the 174–287 °C fraction. About 55% of BS7, to about 95% of BS1, were found in this fraction. It was also observed that in this fraction, all ten target sesquiterpanes were concentrated up to approximately fivefold compared to its parent crude oils. BS10 was found mainly in the 287–481 °C fraction, accounting for about 62% of their total amount. For this fraction, only BS4 to BS10 were concentrated to varying extent. Sesquiterpanes were in low concentration in the lightest fraction and were rarely found in the heaviest fraction.

The abundances of sesquiterpanes in various petroleum products are shown in Figure 2.7. Bicyclic sesquiterpanes were found in most fuel oil samples at various concentrations. In general, these compounds are abundant in most mid-range fuels but relatively low in heavy residual fuels. Understandably, none to trace amounts of sesquiterpanes was detected in gasoline and lubricating oils. However, light biomarkers including bicyclic sesquiterpanes and diamondoids are typically evident in

the used lube oil from diesel engine vehicles [24]. Overall, the distribution pattern of individual sesquiterpane compounds in mid-range distillates is similar to that of crude oils. BS3, BS5 and BS10 are the most dominant, while BS8 and BS9 often show the lowest abundances. It is also noted that the dominance of 8β(H)-homodrimane (BS10) is obvious in mid-range distillates and heavy residual fuels, and among ten common bicyclic sesquiterpanes, BS10 is detected in 10W-30 motor oil.

2.5.4 Biomarker terpanes and steranes

Two groups of petroleum biomarkers (i.e., polycyclic terpanes and steranes) have been extensively investigated and reported by geochemists and environmental chemists [13, 15, 59, 61–66]. These hydrocarbons have played an important role to explore the depositional environment of crude oil and track the genesis, maturation, migration, and biodegradation of petroleum. The information from biomarker analysis is also used to investigate the source and history of petroleum in the determination of petroleum-derived environmental contaminations. Peters et al. discussed the basic principles of biomarkers and their applications to studies on the origin, geological age, and environmental conditions of oil formation [59]. Compared to acyclic alkanes, those cyclic hydrocarbons are characterized by their structural stability and preservation of genetic features, which are inherited from peculiarities of the original organic matter and the conditions of its transformation into petroleum hydrocarbons. For example, the ubiquitous pentacyclic hopanes are triterpenoids derived from cell membranes of prokaryotes (heterotrophic bacteria) and phototrophic cyanobacteria [64, 66].

Biomarker abundances differ greatly from oil to oil and from oil type to type. Biomarker concentrations in crude oils largely depend on the geological source of the oils, while their concentrations in refined petroleum products vary with the feedstocks and the oil types [10, 12, 59].

***Biomarker terpanes*:** Crude oils usually have a wide distribution of triterpanes with a dominance of tricyclic and pentacyclic terpanes (Table 2.3 and Figure 2.8). These traditional biomarkers are eluted between *n*-C_{20} and *n*-C_{38} in GC analysis (equivalent to boiling points 343–500 °C) (Figure 2.2). Pentacyclic triterpanes include C_{30} hopane ($C_{30}H_{52}$) and its homologues ranging from C_{27} trinorhopane to C_{35} homohopanes. A series of hopanes beyond C_{40} were reported in crude oils and source rock extracts [67, 68]. As shown in Figure 2.8, the hopane series have a skeleton of 21 carbon atoms with four fused cyclohexane rings and one cyclopentane ring (E-ring). Gammacerane (G) and oleanane, the structural isomers of hopane, have a cyclohexane E-ring in its molecular structure. These irregular triterpanes can be very useful in oil correlation when they are detected in relatively high abundance. There are generally six methyl substituents on the ring system, of which four methyls are positioned at the ring junctions of C-8, C-10, C-14, and C-18, respectively. The molecular structure has two

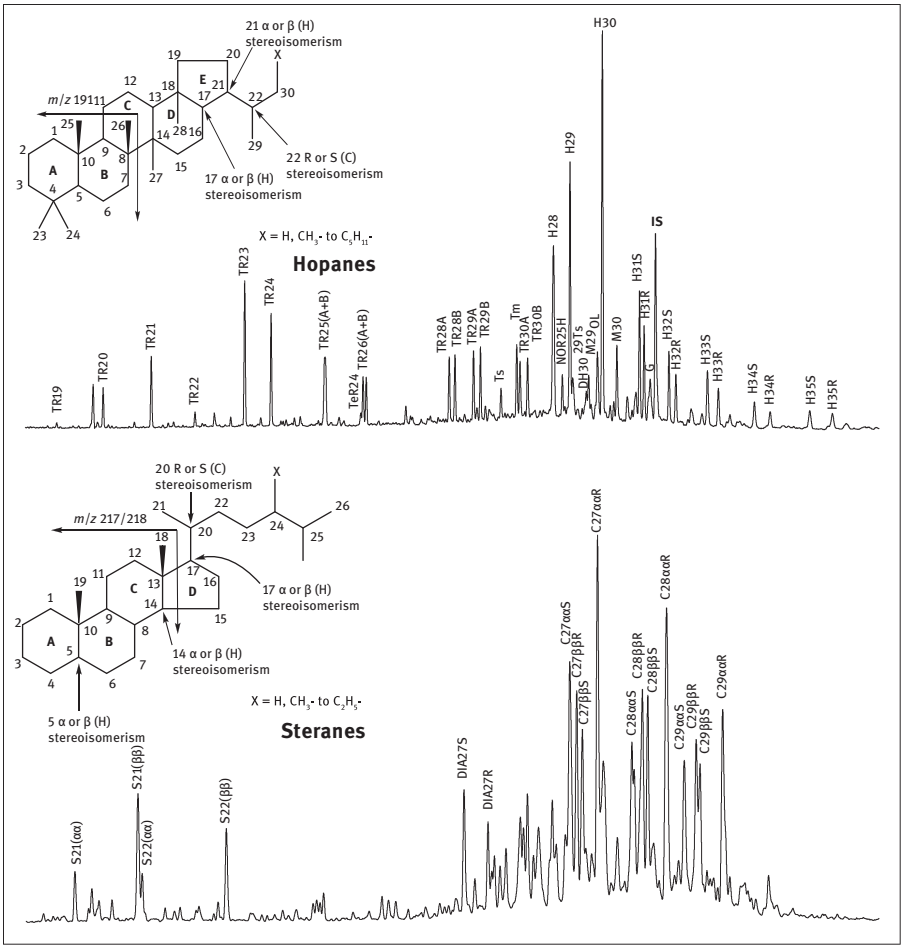

Figure 2.8: Biomarker terpanes and steranes identified in a crude oil.

asymmetric carbons at C-17 and C-21. Atoms attached to the chiral centers in the molecular structure are bonded together in the same sequence but the different orientations of the atoms in space. Common homohopanes (C_{31} to C_{35}) have an extended side chain at C-21 of the pental ring (E-ring). For cyclic biomarkers, α- and β-nomenclature is used to describe asymmetric carbons at the ring, while the R- and S-nomenclature is used for asymmetric centers out of the ring [13]. An additional asymmetric center at C-22 of the side chain results in two stereoisomers with 22R and 22S configurations.

Each biomarker terpane has many potential isomers and stereoisomers. C_{30} hopane has four stereoisomers: 17α(H),21α (H)-, 17α(H),21β(H)- (in petroleum), 17β(H),-21β(H)- (the biological configuration), and 17β(H),21α(H)-hopane (moretane). These

hopane isomers have a decreasing order of thermodynamic stability: 17α(H),21β(H) > 17β(H),21α (H) > 17α(H),21αH) > 17β(H),21β(H) [59]. Therefore, the C_{27}–C_{35} hopane series in crude oils are dominated by the 17α(H),21β(H) stereoisomers. C_{30} 17α(H),21β(H)-hopane widely occurs in crude oil at high abundance, whereas 17β(H),21α(H)-hopane occurs in relatively low concentration. It was found that C_{30} 17α(H),21α(H)-hopane occurs in low concentration in a ratio of typically 0.02–0.04 relative to C_{30} 17α(H),21β(H)-hopane in crude oils and mature sediments [69]. C_{30} 17β(H),21β(H)-hopane is thermodynamically unstable and naturally absent in petroleum, making it a desirable internal standard for quantitative biomarker analysis [10–15]. The C-22 position in the molecular structure is a chiral center resulting in R- and S-configuration epimers for the C_{31}–C_{35} homohopane series. The 22S epimers exist at a slightly higher concentration than their 22R epimers. 25-Norhopanes are a series of C_{26}–C_{34} compounds that are structurally equivalent to the regular hopanes, except for the absence of a methyl group at the A/B ring junction.

These saturated biomarker terpanes are determined at their characteristic base ions at m/z 191 ($C_{14}H_{23}^+$), which are derived from the cleavage of carbon bonds 9–11 and 8–14 in ring C of the molecule to form (A + B) ring or (D + E) fragment, respectively (Figure 2.8). The most intensive ion in the mass spectra of norhopanes, such as 17α(H),21β(H)-25-norhopane, is m/z 177 due to one less methyl group (CH_3-, 15 amu) in their molecular structures.

Biomarker steranes: Steranes represent another important group of biological marker compounds. Steranes and sterenes are believed to be principally derived from C_{27} to C_{30} sterol precursors from the cell membranes of eukaryotes, mainly algae and higher plants [70, 71]. These sterols generate a series of sterane homologs during diagenesis, which inherit the carbon skeletons from biological precursors and only differ by the addition of a sequence of CH_2- units to a certain place in the molecule.

Regular steranes have a tetracyclic androstane skeleton with a methyl at the ring junctions of the A/B ring and C/D ring and a side chain at carbon C-17 of the pental ring (D-ring). As shown in Figure 2.8, steranes have three asymmetric carbon centers at C-5, C-14, and C-17 on the ring structure, and another two asymmetric centers at the C-20 and C-24 on the extended chained attached to C-17, respectively. Chiral center at the C-20 position in the molecular structure results in R- and S-configuration epimers. Therefore, each sterane has many potential isomers. For instance, four isomers of cholestane ($C_{27}H_{48}$) including 20*S*-5α(H),14α(H),17α(H)-, 20*R*-5α(H),14β(H),17β(H)-, 20*S*-5α(H),14β(H),17β(H)-, and 20*R*-5α(H),14α(H),17α(H)-are determined from crude oils. Besides regular steranes, petroleum also contains a family of rearranged steranes of diasteranes (C_{26}–C_{30}). Diasteranes are likely formed during diagenesis and catagenesis of biological precursors.

The typical ions for biomarker steranes are m/z 217 ($C_{16}H_{25}^+$) and m/z 218 ($C_{16}H_{26}^+$) for GC-MS analysis, which are products of (A + B + C) ring due to the cleavage at junction carbon bonds 13–17 and 14–15 of the C/D ring under electron impact (Figure 2.8).

For all epimers with *cis*-C/D-connection, the intensity of the *m*/*z* 218 ions is greater than that of the *m*/*z* 217 ions. The *βαα* and *ααα* steranes have a base peak at *m*/*z* 217, whereas the base peak of *αββ* steranes is at *m*/*z* 218. The distribution profile of C_{27} to C_{29} steranes is of interest for oil exploration and oil forensic study, although it is more or less the same in most crude oils.

Biomarkers in crude oils: Biomarkers in crude oils have been extensively investigated and reported by geochemists and environmental chemists. Different crude oils often have highly distinguishable chemical fingerprints of their biomarkers, varying in both concentration and distribution patterns (Figure 2.9 and Table 2.5).

In fresh crude oils, C_{23} and C_{24} tricyclic terpanes and C_{29} αβ and C_{30} αβ hopanes are generally the most abundant triterpanes (Figure 2.9). C_{30} αβ hopane is usually at a higher concentration than C_{29} αβ norhopane in most crude oils; however, a reverse feature was observed for some crude oils such as Arabian Heavy crude (a Middle East crude oil). In Sockeye crude, a California heavy oil, H28, is even more abundant than C_{29} and C_{30} αβ-hopanes. The homohopanes in Alberta oil sand bitumen account for about 40% of the total 1,519 μg/g of target biomarker terpanes, while in the Bakken crude oil they are very low and even undetectable (Table 2.5).

Some geologically rare acyclic alkanes (i.e., C_{30} 17α(H)-diahopane, C_{30} 18α(H)-hopane, gammacerane, 4-methyl steranes, etc.) are found only in certain oils and therefore used as unique markers for specific oil spill identification. As shown in Figure 2.9, the presence of C_{28}-bisnorhopane and gammacerane is evident in oil sand bitumen and AHS. The occurrence of gammacerane may suggest a saline depositional environment of the original oil in the Alberta oil sands. The abundance of C_{31}–C_{35} homohopanes generally decrease with the increase of carbon numbers, *i.e.*, H31 > H32 > H33 > H34 > H35, and the S-configuration epimer is more common than its R-epimer. One specific feature of H34S < H35S and H34R < H35R is noticed for homohopanes in some crude oils such as Alberta oil sand bitumen, which suggests that these oils were derived from source rocks deposited under anoxic conditions [61]. C_{29} 17α(H),21β(H)-norhopane and $C_{31–35}$ homohopanes, especially C_{35} homohopanes are depleted in southeast Asian crude oils, whereas these compounds are abundant in the Middle East crude oils [72].

C_{27}, C_{28}, and C_{29} steranes are referred to as cholestane, ergostane (24-methylcholestane), and sitostane (or stigmastane, 24-ethylcholestane), respectively. The relative abundances of C_{27} to C_{29} steranes in crude oils are controlled by the types of photosynthetic organisms that contributed to the organic matter. A dominance of C_{27} steranes is usually associated with marine organisms [73]. C_{27} ββ-, C_{28} ββ-, and C_{29} ββ-steranes in crude oils generally have a relative abundance in an overall "V-shaped" distribution pattern. Unlike most of the other crude studied, the Platform Elly crude oil has a reverse V-shaped steranes distribution with a particular abundant C_{28} ββ-steranes. As observed from Figure 2.9, the Bakken crude and the Scotia Light crude oil contain very low biomarker compounds. In contrast, the California Sockeye crude oil particularly

contains a high concentration of biomarker steranes, which account for a large portion of determined biomarkers.

> ***Biomarkers in refined petroleum products***: Biomarker terpanes and steranes are found almost entirely in heavier fractions of the distillates of crude oil [15]. These biomarker compounds all exclusively come from their feedstock crude oil as they are unlikely produced in distillation and refining processes.The biomarker fingerprint of a refined oil may be totally or partially the same as that of its original feedstock crude.

Figure 2.10 presents the GC-MS chromatograms of biomarkers in representative refined petroleum products from diesel to lubricating oil. Clearly, the abundances and distribution of biomarkers vary greatly from oil to oil and from type to type (Table 2.5 and Figure 2.10). For lighter petroleum products, refining processes have removed most HMW biomarkers from the corresponding crude oil feedstocks. In general, biomarker compounds are undetectable in light fuels such as gasoline. The lower boiling C_{19}- to C_{24}-tricyclic terpanes are eluted before *n*-C_{24} during chromatographic analysis. These smaller biomarkers are within the carbon range of automobile diesel and marine diesel and are present in these medium fuel oils at a considerable level. Typically, the higher boiling point biomarkers are not present in light fuel. It is quite common for refineries to add light cycle oil (LCO) to the distilled light oil fraction. LCO is the diesel boiling range material that is produced in a catalytic cracker and contains mainly aromatic compounds and a low concentration of biomarkers [20].

Most triterpanes and steranes remain in the residual fraction during distillation. Therefore, they are detected in heavy residual fuels in equivalent or higher concentrations than their feedstock crude oil. Lubricating oils are rich in terpanes and steranes but contain relatively low lighter tricyclic terpanes (C_{21}–C_{24}). The target biomarker terpanes and steranes in 10W-30 motor oil are as high as 3,652 and 1,666 μg/g, respectively. These biomarker compounds are undetectable or at a trace amount in synthetic lube oils.

2.5.5 Diagnostic criteria of saturated hydrocarbons

Forensic oil–oil correlations are based on the concept that the target components investigated in spilled oils are source-specific and do not significantly differ from those in the source oils. Some of the petroleum compounds in spill samples, in particular those biomarker compounds, show little or no changes in their relative abundance. The abundance ratios between these target analytes serve as important diagnostic criteria for many aspects of oil studies. Diagnostic ratios have been widely applied by geochemists for oil–source rock correlation and oil–oil correlation, determination of organic input and depositional environment, assessment of thermal maturity, and evaluation of oil biodegradation. Environmental chemists frequently use them for the

Table 2.5: Concentrations of target biomarker terpanes and steranes in crude oils and petroleum products.

	Crude oils						Petroleum products					
Oil samples	**Bakken**	**SoL**	**Troll**	**PlE**	**AOS***	**Fed**	**Fed (287–481 °C)**	**Fed (>481 °C)**	**Diesel no. 2**	**Marine diesel**	**Bunker C**	**Lube 10W-30**
Biomarker terpanes												
TR21	2.46	9.43	7.81	20.1	36.2	11.5	33.5	ND	3.11	26.2	3.10	11.6
TR22	0.97	3.53	2.96	4.32	16.9	4.48	12.7	ND	1.42	9.43	1.38	15.2
TR23	5.09	14.8	11.1	41.3	109	26.1	73.8	ND	3.85	45.6	11.0	68.2
TR24	3.71	10.7	9.14	33.9	56.7	15.2	46.2	ND	1.39	18.6	5.97	25.5
Ts	1.97	20.3	34.1	13.2	27.2	22.8	48.8	27.4	ND	1.70	26.1	148
Tm	1.44	29.6	23.3	55.9	91.7	21.3	41.1	27.0	ND	2.13	2.46	215
H29	2.20	74.6	56.6	107	219	36.7	52.1	75.9	ND	3.41	31.9	864
H30	5.68	100	126	216	256	71.0	83.1	184	ND	2.71	59.0	718
H31S	1.66	26.4	44.3	64.6	114	24.6	22.1	70.2	ND	0.88	36.4	385
H31R	1.31	21.5	34.5	52.5	83.5	18.0	17.7	57.0	ND	0.69	25.2	305
H32S	1.42	15.2	30.4	43.0	72.6	18.1	14.3	60.4	ND	1.11	24.8	238
H32R	0.97	9.94	22.0	32.2	53.2	13.4	9.52	42.5	ND	0.28	19.6	164
H33S	ND	8.96	26.7	35.2	54.5	12.9	7.90	41.0	ND	0.27	17.3	140
H33R	ND	5.48	16.3	28.5	36.3	9.88	5.33	31.3	ND	ND	12.9	91.7
H34S	ND	4.65	16.4	20.0	36.7	8.14	4.29	31.0	ND	ND	10.9	77.6
H34R	ND	2.78	9.54	15.1	23.4	4.29	1.73	17.1	ND	ND	6.68	51.6
H35S	ND	3.33	12.4	22.1	39.0	5.73	1.91	22.5	ND	ND	9.94	85.7
H35R	ND	2.27	8.73	20.9	36.2	3.40	1.09	12.3	ND	ND	7.75	47.6
Biomarker steranes												
C27αββ	22.1	89.3	172	649	25.4	113	257	78.3	ND	9.16	87.8	525
C28αββ	10.6	67.4	125	754	62.6	48.6	92.4	57.0	ND	4.48	58.8	363
C29αββ	24.5	89.8	179	466	45.8	113	199	193	ND	3.95	82.9	778
Total (µg/g)	**86.1**	**610**	**968**	**2695**	**1519**	**603**	**1026**	**1028**	**9.77**	**131**	**542**	**5318**

* Concentration: µg/g of TSEM of Alberta oil sands DCM extract.

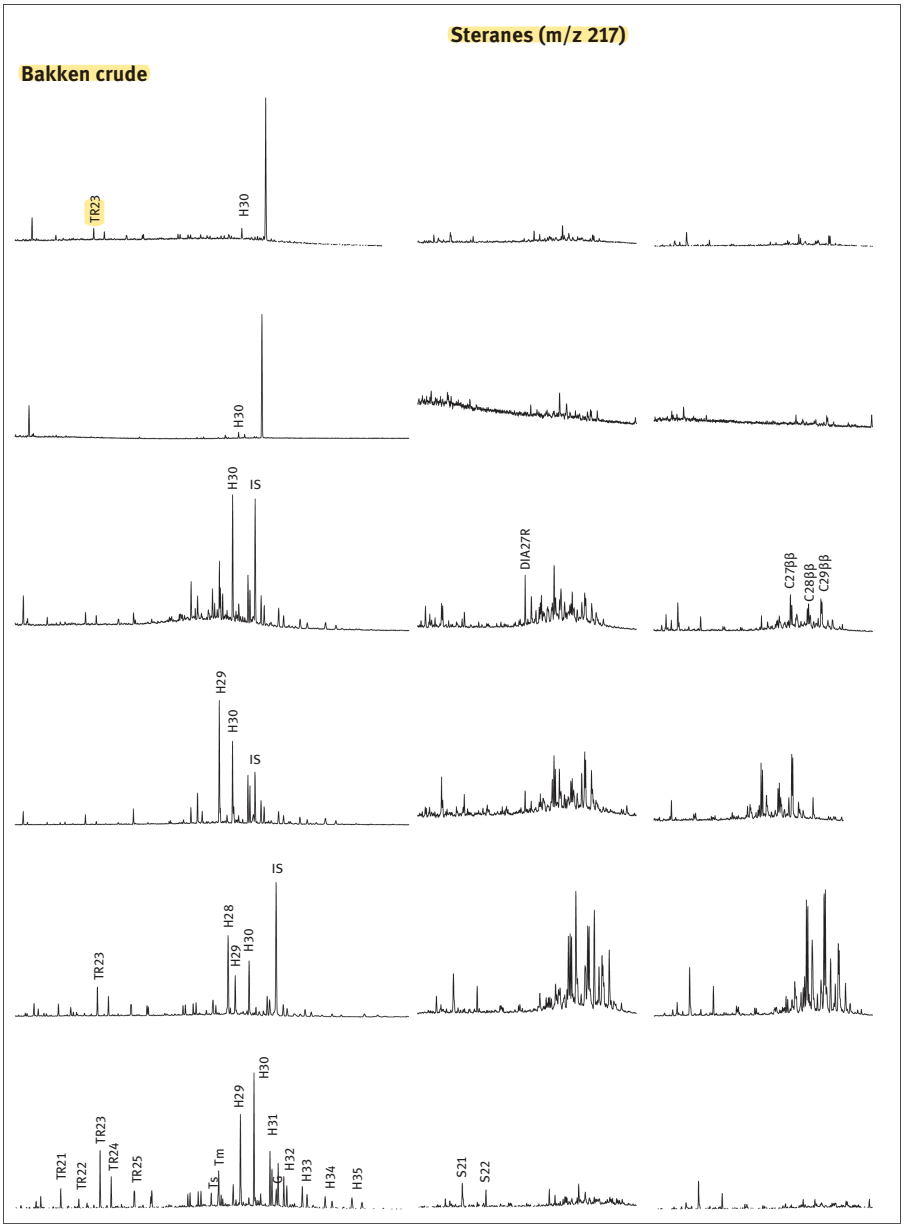

Figure 2.9: GC-MS chromatograms of biomarker terpanes and steranes in crude oils.

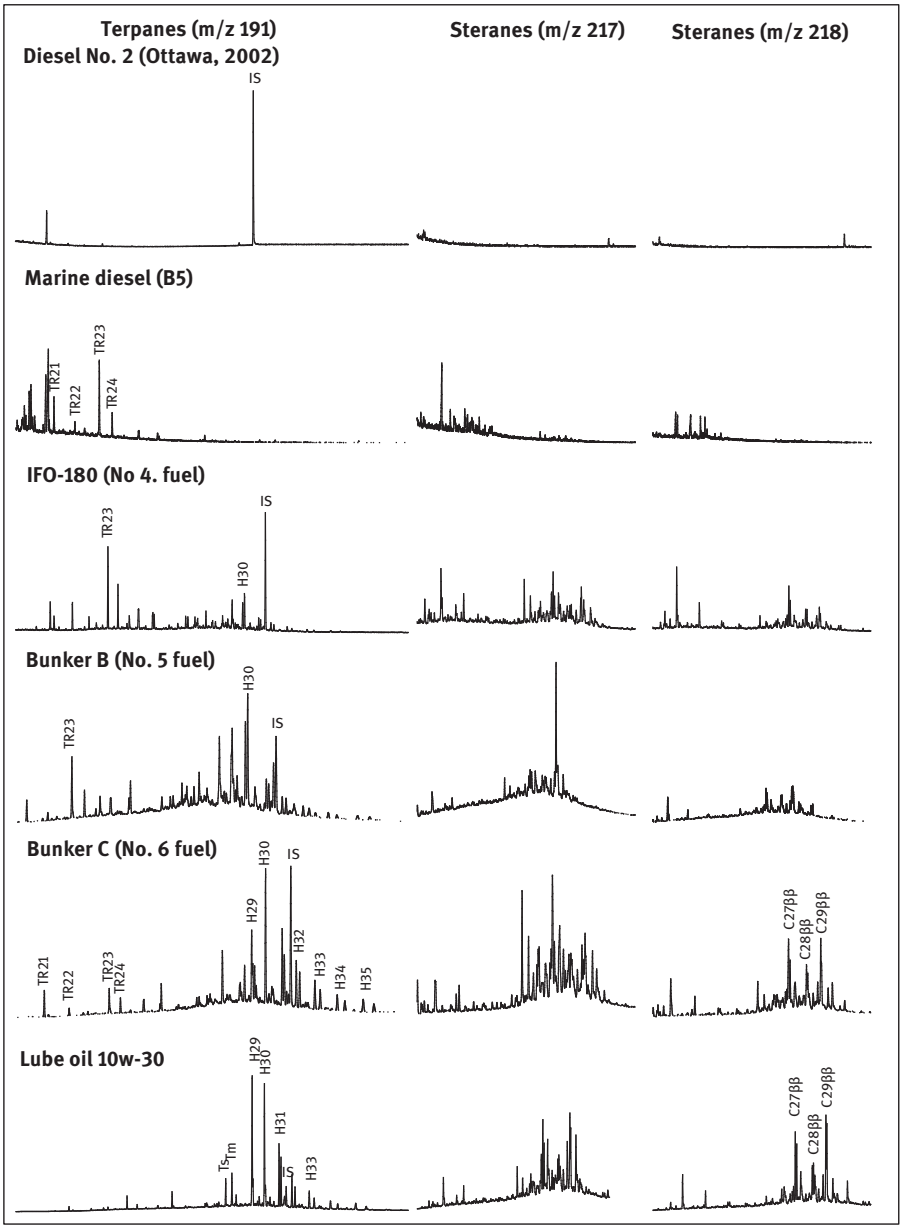

Figure 2.10: GC-MS chromatograms of biomarker terpanes and steranes in petroleum products.

identification, correlation, differentiation of spilled oil, and investigation of its spill history. An important benefit of employing diagnostic ratios in oil identification is to minimize the concentration effects. In addition, the use of ratios tends to induce a self-normalizing effect on the data since variations due to the fluctuation of day-to-day instrument operating conditions, operator, and matrix effects are minimized. Therefore, the comparison of diagnostic ratios reflects more directly differences in biomarkers of samples. However, it should be cautioned that diagnostic ratios are not only oil-dependent but also change with the method used for oil analysis; therefore, for the same oil, the ratio values could vary from different data sources. Because of low abundance or poor peak separation, the ratios of certain compounds can be heavily affected by the measurement errors.

Numerous diagnostic ratios from petroleum compounds have been proposed as molecular markers to identify the source of oil spills [12, 20, 74]. Diagnostic ratios should be used in combination to avoid erroneous conclusions. It is impossible and unnecessary to include ratios from all target analytes. It is essential to select source-specific representative and weathering-resistant diagnostic ratios for forensic oil analysis. Herein, we only select some diagnostic ratios frequently used for detailed discussion.

N-alkane and isoprenoid ratios: These ratios of *n*-alkane and isoprenoids are generally based on results from GC-FID analysis or selected ion of GC-MS analysis if these compounds are in very low concentration in weathered environmental samples. The diagnostic ratios often used from the *n*-alkane analysis include *n*-C_{16}/norpristane *n*-C_{17}/pristane, *n*-C_{18}/phytane, and pristane/phytane. These *n*-alkane and isoprenoid indices are source-specific, and the pristane/phytane ratio is widely used to assess the environmental conditions of deposition of source rock. It was discovered that crudes with the same geological origin have similar pristane/phytane ratios. In addition, *n*-C_{17}/pristane and *n*-C_{18}/phytane elute in two close pairs during chromatographic analysis. The ratios of *n*-C_{17}/pristane and *n*-C_{18}/phytane remain relatively constant through evaporation, but their values decrease remarkably in biodegraded oil due to the preferential depletion of *n*-alkanes. The ratio of pristane/phytane is relatively stable when oil is subject to a certain degree of evaporation and biodegradation.

Carbon preference index (CPI) is the ratio of odd versus even carbon numbered *n*-alkanes. The ratio of *n*-C_{17}/*n*-C_{31} is a simplified parameter indicating relative contributions from aquatic/marine and terrestrial sources. This ratio is used to estimate of the thermal maturity of crude oil and to identify the source of hydrocarbons in an environmental sample. The even and odd numbers of *n*-alkanes are generally equally abundant in high maturity petroleum resulting in a CPI of 1.0 for these oils. CPI values significantly above (odd preference) or below (even preference) 1.0 indicates low thermal maturity. A CPI value below 1.0 for low-maturity oils or bitumens suggests a typical carbonate or hypersaline environment [59]. CPI is a key diagnostic parameter

to determine the relative importance of biogenic and anthropogenic alkane sources to the ambient environment [75].

For the practice of environmental oil analysis, *n*-alkanes are examined within their full span (generally from *n*-C_8 to *n*-C_{44}). CPI is particularly useful in an environmental approach involving biogenic contribution. C_{23-33} *n*-alkanes, and especially those from *n*-C_{27} to *n*-C_{33}, originate from waxes typical of terrestrial higher plants, whereas anthropogenic sources, including fossil fuel combustion, show no enrichment of odd carbon alkanes [76]. A strong odd-to-even predominance with a CPI value ≫1 discloses predominant biogenic contribution from terrestrially derived components in an environmental sample (e.g., soil and sediment). When petroleum is the main contaminant, the CPI can be calculated from the sum of odd divided by the sum of even carbon numbered *n*-alkanes. A CPI value of near 1.0 suggests a mainly petrogenic source of hydrocarbons. The CPI values of biodiesel blends are also around 1.0 since *n*-alkanes are attributable to the petroleum components [24].

Biomarker diagnostic parameters have been long established and are widely used by geochemists for oil correlation (oil–source rock correlation and oil–oil correlation); determination of organic input and precursors, and depositional environment; assessment of thermal maturity; and evaluation of oil in-reservoir biodegradation. Many diagnostic biomarker ratios currently used in oil spill studies and environmental forensics originate from geochemistry.

Ratios of diamondoids and bicyclic sesquiterpanes are of special importance for the analysis of light petroleum. Like traditional biomarker ratios, these ratios originate from geochemical investigation. Diamondoid hydrocarbon ratios, such as methyl adamantane index and methyl diamantane index were applied to evaluate the maturation and evolution of crude oils and to determine the thermal maturity of thermogenic gas and condensate [46, 49, 77]. The ratio of methyladamantanes to adamantanes rises with increasing biodegradation. The ratio changes significantly at extreme levels, showing that diamondoids can be indicators of petroleum biodegradation especially when most other hydrocarbons have been removed. Wang et al. studied many possible diagnostic ratios and found that many diamondoids ratios, such as 1-MA/2-EA, 1-MA/1,2-DMA, 1-MA/1,3,4-TMA, and 1-MA/1,2,5,7-TeMA, are likely more sensitive and reliable parameters for correlation and differentiation of oils and petroleum products [34]. Diamantane isomers generally occur in crude oils only in relatively low abundance. High measurement uncertainty could be evident in oil analysis, particularly when column fractionation of oil sample is not performed prior to GC analysis. Therefore, diagnostic ratios associated with diamantanes should be used cautiously as criteria [32, 74].

Diagnostic ratios of selected sesquiterpanes were developed and calculated from a large number of oils and petroleum products. Diagnostic ratios vary greatly between oils from different regions. Most of these diagnostic ratios are robust for correlation and differentiation of lightly to moderately weathered oil samples. This implies that the sesquiterpane ratios, in combination with other fingerprinting data, may be

used to discriminate different oils and to identify the source of spill samples. However, for heavily weathered samples, the early-eluted lower molecular weight C_{14} sesquiterpanes could be preferentially depleted to certain degrees, resulting in some changes in their corresponding diagnostic ratios [33, 35, 41].

As shown in Figure 2.8, crude oil contains many biomarker terpanes and steranes in a wide carbon range. In theory, their analyses could derive a large number of diagnostic ratios. Diagnostic ratios of these compounds include TR21/TR22, TR23/TR24, H29/H30, Ts/Tm, H31S/H31, H32S/H32R, C27ββ/C29ββ, and C28ββ/C29ββ, as well as ratios compared H30 with other petroleum hydrocarbons. These ratios are regarded as source ratios for oil identification, among which the ratio TR21/TR22, H29/H30, Ts/Tm, and C27ββ/C29ββ are the most commonly used as source tracers [10, 12, 15, 20, 32]. Due to significant differences in boiling points, the ratios between lighter C_{21-24} tricyclic terpanes and C_{30} 17α(H),21β(H)-hopane can provide a useful tool to distinguish the refined oil type and to investigate the weathering level. Ratios of certain unique biomarker compounds (*i.e.*, C_{30} 17α(H)-diahopane, C_{30} 18α(H)-hopane, gammacerane, and 4-methyl steranes, *etc.*) relative to C_{30} 17α(H),21β(H)-hopane can provide exceptional diagnostic information in oil analysis.

Cross-plots (i.e., a plot of one diagnostic biomarker ratio vs. another ratio) are frequently used in oil geochemistry for oil–oil correlation and determination of oil source and depositional environment [72, 78]. Zakaria et al. used the cross-plots of H29/H30 ratio versus the homohopane index Σ(H31–H35)/H30 as key biomarker indicators and successfully distinguished a large number of tarball samples originated from Southeast Asian crude oil sources from those of Middle East sources [72]. Furthermore, the ratios of biomarkers with other classes of hydrocarbons can be proposed as weathering ratios. The abundances of full-range targeted compounds relative to C_{30} 17α(H),21β(H)-hopane are plotted against boiling points (in practice, GC retention time of analytes) providing a criterion where a spilled oil is weathered compared with source oil [15, 20].

2.6 Aromatic hydrocarbons in petroleum

Aromatic hydrocarbons generally make up a smaller proportion of crude oils in comparison with aliphatic hydrocarbons. However, certain refined products can contain an extremely high concentration of these substances. For example, an oil product designed for the preservation of wood products such as utility poles, cross-arms, and railway ties can possess aromatic content as high as 80% [15]. Aromatic hydrocarbons in petroleum can be classified into three main groups according to their molecular structures, including monocyclic aromatic hydrocarbons (MAHs), PAHs, and naphthenoaromatic hydrocarbons. Petrogenic aromatics are generally dominated by

the derivatives of one or more aliphatic substituents, and the alkylated homologues usually occur in significantly higher concentrations than their parent PAHs.

Tables 2.6 summarizes the target petroleum aromatic compounds frequently used for forensic oil fingerprinting analysis [10, 12, 13, 15, 16, 20, 27, 29]. PAH series can be determined by GC-MS with a suit of parent molecular ions, for example, m/z 128, 142, 156, 170, and 184 for C_0- to C_4-naphthalenes; and m/z 178, 192, 206, 220, and 234 for C_0- to C_4-henanthrenes, and so on. Aromatic steranes are determined at m/z 231 for triaromatic steranes (TASs) and at m/z 253 for ~~MASs~~.

2.6.1 BTEX and alkylbenzenes

BTEX (benzene, toluene, ethylbenzene, and *o*-, *m*-, and *p*-xylenes) are the major MAHs in petroleum. BTEX are classified as priority pollutants regulated by many environmental organizations around the world. These small aromatics are the most water-soluble and volatile fraction of petroleum. They frequently enter the air, soil, sediments, and groundwater, due to accidental oil spills, leakage of gasoline and other petroleum fuels from underground storage tanks and pipelines, and improper oil-related waste disposal [79]. Because of their potential acute toxicity and health hazard, BTEX are often monitored whenever a hydrocarbon fuel is suspected to have been spilled (especially in relatively confined areas).

Benzene and its C_7–C_{10} homologues have been thoroughly investigated in many crude oils. Wang et al. identified a large number of alkyl-substituted benzene components in Alberta Sweet Mixed Blend (ASMB) [79]. They also reported a quantitative analysis of the individual BTEX compounds and C_3-benzene isomers in various crude and weathered oils. Quantitative analyses of these highly volatile MAHs are usually separated from the analyses of other hydrocarbons. To avoid the loss of light-boiling BTEX during sample preparation, the oil sample is dissolved in *n*-pentane for direct GC analysis. The use of *n*-pentane as solvent also precipitates heavy asphaltenes content, thereby reducing column contamination. Accordingly, mass spectra of alkylated benzenes are characterized by the presence of m/z 91 for methylbenzene, m/z 105 for C_2-benzenes, and m/z 119 for C_3-benzene, similarly isomers with larger alkylation can be determined using the characteristic $(M\text{-}15)^+$ ion.

Concentrations of BTEX and selected C_3- ~ C_6-alkylbenzenes in different crude oils and refined products are presented in Table 2.7. The total concentration of monocyclic aromatics studied in crude oil is at the mg/g level, and varies between oils. Overall, BTEX and C_3-benzenes make up most of the determined monoaromatics. In general, 1,2,4-trimethylbenzene is the most abundant C_3-benzene isomer followed by 3- /4-ethyltoluene and 1,3,5-trimethylbenzene. Unlike in conventional crude oils, these light aromatics are barely detectable in extra heavy oils like Alberta oil sand bitumen.

Table 2.6: Target aromatic analytes frequently used for oil fingerprinting analysis.

Compounds	Code	Empirical formula	Molecular weight	Target ions
BTEX and alkylbenzenes				
Benzene	B	C_6H_6	78	78,77
Toluene	T	C_7H_8	92	91,92
Ethylbenzene	E	C_8H_{10}	106	91,106
Xylenes (*p*-, *m*-, *o*-)	X	C_8H_{10}	106	91,106
C_3-Benzenes	C3B	C_9H_{12}	120	105,120
C_4-Benzenes	C4B	$C_{10}H_{14}$	134	91,105,134
C_n-Benzenes	CnB	C_nH_{2n-6}		91,92,105
Unsubstituted PAHs				
Two-ring PAHs				
Naphthalene	N	$C_{10}H_8$	128	128
Biphenyl	Bph	$C_{12}H_{10}$	154	154
Three-ring PAHs				
Acenaphthylene	Acl	$C_{12}H_8$	152	152
Acenaphthene	Ace	$C_{12}H_{10}$	154	154
Fluorene	F	$C_{13}H_{10}$	166	166
Phenanthrenes	P	$C_{14}H_{10}$	178	178
Anthracene	An	$C_{14}H_{10}$	178	178
Four-ring PAHs				
Fluoranthene	Fl	$C_{16}H_{10}$	202	202
Pyrene	Py	$C_{16}H_{10}$	202	202
Benz[*a*]anthracene	BaA	$C_{18}H_{12}$	228	228
Triphenylene	TP	$C_{18}H_{12}$	228	228
Chrysene	C	$C_{18}H_{12}$	228	228
Five-ring PAHs				
Benzo[*b*]fluoranthene	BbF	$C_{20}H_{12}$	252	252
Benzo[*k*]fluoranthene	BkF	$C_{20}H_{12}$	252	252
Benzo[*e*]pyrene	BeP	$C_{20}H_{12}$	252	252
Benzo[*a*]pyrene	BaP	$C_{20}H_{12}$	252	252
Perylene	Pe	$C_{20}H_{12}$	252	252
Dibenz[*a,h*]anthracene	DA	$C_{22}H_{14}$	278	278
Six-ring PAHs				
Indeno[1,2,3-*cd*]pyrene	IP	$C_{22}H_{12}$	276	276
Dibenzo[*g,h,i*]perylene	BP	$C_{22}H_{12}$	276	276
Alkylated PAHs				
C_1-Naphthalenes	C1N	$C_{11}H_{10}$	142	142
C_2-Naphthalenes	C2N	$C_{12}H_{12}$	156	156
C_3-Naphthalenes	C3N	$C_{13}H_{14}$	170	170
C_4-Naphthalenes	C4N	$C_{14}H_{16}$	184	184
C_1-Biphenyls	C1Bph	$C_{13}H_{12}$	168	168
C_2-Biphenyls	C2Bph	$C_{14}H_{14}$	182	182
C_3-Biphenyls	C3Bph	$C_{15}H_{16}$	196	196
C_4-Biphenyls	C4Bph	$C_{16}H_{18}$	210	210
C_1-Fluorenes	C1F	$C_{14}H_{12}$	180	180

Table 2.6 (continued)

Compounds	Code	Empirical formula	Molecular weight	Target ions
C_2-Fluorenes	C2F	$C_{15}H_{14}$	194	194
C_3-Fluorenes	C3F	$C_{16}H_{16}$	208	208
C_4-Fluorenes	C4F	$C_{17}H_{18}$	222	222
C_1-Phenanthrenes/anthracenes	C1P	$C_{15}H_{12}$	192	192
C_2-Phenanthrenes/anthracenes	C2P	$C_{16}H_{14}$	206	206
C_3-Phenanthrenes/anthracenes	C3P	$C_{17}H_{16}$	220	220
C_4-Phenanthrenes/anthracenes	C4P	$C_{18}H_{18}$	234	234
C_1-Pyrenes/fluoranthenes	C1Py	$C_{17}H_{12}$	216	216
C_2-Pyrenes/fluoranthenes	C2Py	$C_{18}H_{14}$	230	230
C_3-Pyrenes/fluoranthenes	C3Py	$C_{19}H_{16}$	244	244
C_4-Pyrenes/fluoranthenes	C4Py	$C_{20}H_{18}$	258	258
C_1-Benzanthracenes/ chrysenes/triphenylenes	C1C	$C_{19}H_{14}$	242	242
C_2-Benzanthracenes/ chrysenes/triphenylenes	C2C	$C_{20}H_{16}$	256	256
C_3-Benzanthracenes/ chrysenes/triphenylenes	C3C	$C_{21}H_{18}$	270	270
C_4-Benzanthracenes/ chrysenes/triphenylenes	C4C	$C_{22}H_{20}$	284	284
Triaromatic steranes				
C_{20} TA-sterane	C20TA	$C_{20}H_{20}$	260	231
C_{21} TA-sterane	C21TA	$C_{21}H_{22}$	274	231
C_{22} TA-sterane	C22TA	$C_{22}H_{24}$	288	231
C_{26} TA-cholestane (20S)	SC26TA	$C_{26}H_{32}$	344	231
C_{26} TA-cholestane (20 R)	RC26TA	$C_{26}H_{32}$	344	231
C_{27} TA-ergostane (20S)	SC27TA	$C_{27}H_{34}$	358	231
C_{28} TA-stigmastane (20S)	SC28TA	$C_{28}H_{36}$	372	231
C_{27} TA-ergostane (20 R)	RC27TA	$C_{27}H_{34}$	358	231
C_{28} TA-stigmastane (20 R)	RC28TA	$C_{28}H_{36}$	372	231
Monoaromatic steranes				
C_{21} MA-sterane	C21MA	$C_{21}H_{30}$	282	253
C_{22} MA-sterane	C22MA	$C_{22}H_{32}$	296	253
C_{23} MA-sterane	C23MA	$C_{23}H_{34}$	310	253
C_{27} 5β(H) MA-cholestane (20S)		$C_{27}H_{42}$	366	253
C_{27} MA-diacholestane (20S)		$C_{27}H_{42}$	366	253
C_{27} 5β(H) MA-cholestane (20 R)		$C_{27}H_{42}$	366	253
C_{27} MA-diacholestane (20 R)		$C_{27}H_{42}$	366	253
C_{27} 5α(H) MA-cholestane (20S)	SC27MA	$C_{27}H_{42}$	366	253
C_{28} 5β(H) MA-ergostane (20S)		$C_{28}H_{44}$	380	253
C_{28} MA-diaergostane (20S)		$C_{28}H_{44}$	380	253
C_{27} 5α(H) MA-cholestane (20 R)		$C_{27}H_{42}$	366	253
C_{28} 5α(H) MA-ergostane (20S)		$C_{28}H_{44}$	380	253
C_{28} 5β(H) MA-ergostane (20 R)		$C_{28}H_{44}$	380	253
C_{28} MA-diaergostane (20 R)		$C_{28}H_{44}$	380	253

Table 2.6 (continued)

Compounds	Code	Empirical formula	Molecular weight	Target ions
C_{29} 5β(H)MA-stigmastane(20S)		$C_{29}H_{46}$	394	253
C_{29}MA-diastigmastane (20S)		$C_{29}H_{46}$	394	253
C_{29} 5α(H) MA-stigmastane (20S)	SC29MA	$C_{29}H_{46}$	394	253
C_{28} 5α(H) MA-ergostane (20 R)	RC28MA	$C_{28}H_{44}$	380	253
C_{29} 5β(H) MA-stigmastane (20 R)	RC29MA	$C_{29}H_{46}$	394	253
C_{29} 5α(H) MA-stigmastane (20 R)	RC29MA	$C_{29}H_{46}$	394	253
C_{30} 5β(H) MA-sterane (20S)	C30MA	$C_{30}H_{48}$	408	253
Other naphthenoaromatics				
Tetralin	Te	$C_{10}H_{12}$	132	104,132
C_1-Tetralins	C1Te	$C_{11}H_{14}$	146	104,146
Tetrahydrophenanthrene	THP	$C_{14}H_{14}$	182	182,154
PASHs				
Benzothiophene	BT	C_8H_6S	134	134
C_1-Benzothiophenes	C1BT	C_9H_8S	148	148
C_2-Benzothiophenes	C2BT	$C_{10}H_{10}S$	162	212
C_3-Benzothiophenes	C3BT	$C_{11}H_{12}S$	176	176
C_4-Benzothiophenes	C4BT	$C_{12}H_{14}S$	190	190
Dibenzothiophene	DBT	$C_{12}H_8S$	184	184
C_1-Dibenzothiophenes	C1DBT	$C_{13}H_{10}S$	198	198
C_2-Dibenzothiophenes	C2DBT	$C_{14}H_{12}S$	212	212
C_3-Dibenzothiophenes	C3DBT	$C_{15}H_{14}S$	226	226
C_4-Dibenzothiophenes	C4DBT	$C_{16}H_{16}S$	240	240
Benzo[b]naphthothiophenes	BNT	$C_{16}H_{10}S$	234	234
C_1-Benzo[b]naphthothiophenes	C1BNT	$C_{17}H_{12}S$	248	248
C_2-Benzo[b]naphthothiophenes	C2BNT	$C_{18}H_{14}S$	262	262
C_3-Benzo[b]naphthothiophenes	C3BNT	$C_{19}H_{16}S$	276	276
C_4-Benzo[b]naphthothiophenes	C4BNT	$C_{20}H_{18}S$	290	290
Dinaphthothiophenes	DPT	$C_{20}H_{12}S$	284	284
C_1-Dinaphthothiophenes	C1DPT	$C_{21}H_{14}S$	298	298
C_2-Dinaphthothiophenes	C2DPT	$C_{22}H_{16}S$	312	312
C_3-Dinaphthothiophenes	C3DPT	$C_{23}H_{18}S$	326	326
C_4-Dinaphthothiophenes	C4DPT	$C_{24}H_{20}S$	340	340

Gasoline usually consists of a high concentration of small MAHs; for example, according to Table 2.7, a fresh gasoline sample has 375 mg/g of monoaromatics. In comparison, residual fuels contain a very low concentration of these components, which are attributable to the portion of blended light fuels. MAHs barely occur in fresh lubricating oils, but these compounds are found in used lube oils from gasoline and diesel engines [24], which are attribute to unburned fuels.

The plots of the concentrations of BTEX and C_n-benzenes versus weathering percentages were used to estimate the weathering extent of weathered oil samples, especially for short-term weathered oils in which loss of BTEX and C_n-benzenes is significant [79]. For forensic oil spill analysis, these volatile aromatics are of little

Table 2.7: BTEX and alkylbenzenes in crude oils and petroleum products.

Oil samples	Bakken	Fed	Troll	PlE	Oil sand bitumen*	CLWB	Gasoline #87	Diesel no. 2	Marine diesel	Bunker C	Lube 10w-30
Benzene	3.28	2.65	0.70	0.16	ND	1.47	5.62	0.07	0.04	0.03	0.005
Toluene	4.47	6.33	2.53	0.82	0.012	4.15	140	1.03	0.62	0.19	0.009
Ethylbenzene	0.90	1.32	1.43	0.50	0.001	0.41	18.8	0.57	0.35	0.12	0.001
m- and *p*-Xylene	6.24	5.76	5.03	0.74	0.006	3.91	63.4	2.38	1.30	0.50	0.003
o-Xylene	1.99	2.31	1.30	0.55	0.002	1.01	23.3	1.13	0.59	0.24	0.001
ΣBTEX	**16.9**	**18.4**	**11.0**	**2.77**	**0.022**	**10.9**	**251**	**5.19**	**2.90**	**1.08**	**0.019**
Isopropylbenzene	0.28	0.34	0.43	0.18	ND	0.08	2.85	0.38	0.14	0.03	ND
Propylbenzene	0.44	0.40	0.61	0.39	0.001	0.13	7.28	0.62	0.38	0.18	ND
3- and 4-Ethyltoluene	1.93	1.73	1.97	0.56	0.005	0.76	35.4	2.17	1.49	1.12	0.001
1,3,5-Trimethylbenzene	1.29	1.49	1.22	0.19	0.003	0.64	12.6	1.02	0.47	0.45	0.002
2-Ethyltoluene	0.45	0.51	0.67	0.35	0.002	0.16	9.52	0.82	0.45	0.31	ND
1,2,4-Trimethylbenzene	4.14	2.12	2.09	0.53	0.008	1.31	49.1	4.13	1.86	1.99	0.002
1,2,3-Trimethylbenzene	0.24	0.17	0.37	0.14	0.001	0.34	0.46	0.72	0.67	0.04	ND
ΣC_3-Benzenes	**8.76**	**6.76**	**7.36**	**2.34**	**0.020**	**3.43**	**117**	**9.87**	**5.49**	**4.12**	**0.005**
Isobutylbenzene	0.07	0.11	0.11	0.02	0.001	0.04	0.37	0.22	0.05	0.03	ND
1-Methyl-2-isopropylbenzene	0.03	0.05	0.07	0.02	0.001	0.02	0.24	0.08	0.05	0.03	ND
1,2-Dimethyl-4-ethylbenzene	0.59	0.38	0.46	0.17	0.002	0.08	4.69	1.42	0.43	0.58	ND
Amylbenzene	0.08	0.04	0.06	0.08	0.005	0.01	1.04	1.31	0.43	0.26	ND
n-Hexylbenzene	0.04	0.03	0.05	0.06	0.006	0.02	0.06	0.45	0.24	0.09	ND
ΣC_4–C_6 alkyl benzenes	**0.80**	**0.61**	**0.75**	**0.35**	**0.014**	**0.17**	**6.39**	**3.48**	**1.19**	**0.99**	ND
Total (mg/g)	**26.4**	**25.7**	**19.1**	**5.46**	**0.056**	**14.5**	**375**	**18.5**	**9.58**	**6.18**	**0.02**

* Concentration: mg/g of TSEM of Alberta oil sands DCM extract. CLWB: Cold Lake bitumen winter blend.

importance or interest. Alkyl-benzenes in crude oil are not so source-specific informative as PAHs and biomarker compounds, and these small aromatics are highly volatile and susceptible to weathering; therefore, forensic oil identification and correlation usually do not rely on them. However, chemical fingerprints of alkylbenzenes and alkyltoluenes are still useful for a specific case involving gasoline as other forensic information are not applicable in this situation [20, 80].

2.6.2 PAHs

PAHs have two or more fused benzene rings and their structural stability results from concomitant electron pair delocalization. PAHs with three or more rings have various structural isomers due to different ring fusions, for example, phenanthrene and anthracene for $C_{14}H_{10}$; and chrysene, triphenylene, benz[*a*/*b*]anthracene, benzo[*c*] phenanthrene, naphthacene, and acepyrene for $C_{18}H_{12}$. Moreover, PAHs can have structural isomers with various alkylated groups. Some or all of the isomers and their alkylated derivatives could occur in petroleum. The occurrence of one specific isomer in petroleum can be much lower than others. PAHs are relatively persistent in the environment and are toxic, carcinogenic, and mutagenic.

PAHs have a fused ring structure and exhibit high stability due to the conjugated effect. Under typical mass spectral 70 eV collision energy (EI source), these compounds generally produce intensive molecular ions. Therefore, PAHs are normally determined by using their prominent molecular ions, although other less intensive fragmentograms can be used for confirmation [38]. Similar to unsubstituted PAH analysis, APAHs are generally determined using their prominent parent ions. Other fragmentograms such as $(M\text{-}1)^+$, $(M\text{-}15)^+$, and $(M\text{-}29)^+$ can be applied for confirmation.

PAHs are ubiquitous in the environment and generally originate from three main sources: petrogenic, pyrogenic, and biogenic. Petrogenic PAHs are related to crude oils and refined petroleum products and are generated from geochemical alteration of organic matter. Pyrogenic PAHs, particularly HMW PAHs ranging from benzo(a)anthracene to coronene, are prevalent contaminants resulting from heavy anthropogenic and industrial activities such as incomplete combustion of fuel, industrial petrochemical practices, residential wood burning, vehicular emissions, and power plant emissions. At high temperatures, the more reactive APAHs tend to be destroyed. This explains why homologue groups in pyrogenic assemblages are dominated by nonalkylated, parent compounds, with subsequently lower alkyl members as the degree of alkylation increases [81, 82]. It has been reported that the PAHs found in tire pyrolysis oil consist largely of alkylated naphthalenes, fluorenes, and phenanthrenes, meanwhile the concentrations of individual five-ring benzo(a)pyrene ranged from <10 to 600 ppm [83]. Biogenic PAHs are produced by organisms or formed during the early stage of diagenesis in sediments. Unlike petrogenic and pyrogenic PAHs, biogenic PAHs in the environment are non-anthropogenic, which are believed to be

formed during the bacteriological breakdown of organic matter in marine sediments by a process called early diagenesis. These compounds are generally found individually or in very simple mixtures, such as perylene, retene, and alkylated and partially aromatized tetra- and penta-cyclic derivatives of chrysene and picene [84, 85]. Perylene is one of the most abundant biogenic PAHs, and it is often used as an indicator to identify the biogenic contribution.

PAHs in crude oils: Crude oils and refined products generally contain significant amounts of PAHs, in particular the alkylated homologues of naphthalene, phenanthrene, fluorene, and chrysene. Although many unsubstituted PAHs occur naturally, only a selection of two- to six-ring PAHs are monitored as priority pollutants due to their relatively high toxicity and wide occurrence in the environment. The abundance and distribution profiles of these compounds in crude oils vary with the nature of the original organic matter and the geological conditions of formation. These APAHs are particularly useful for source identification because they occur in all crude oils and most the refined petroleum products in considerable concentrations. Their relative concentrations also vary significantly between different oils making them source-specific.

Table 2.8 summarizes the quantitative analytical results of petroleum-characteristic APAHs homologous series and 15 individual unsubstituted PAHs in crude oil samples. As shown in Table 2.8, the total concentration of unsubstituted PAHs in crude oils spans from tens to hundreds of ppm. It is noted that the Platform Elly heavy crude contains 30.2 μg/g of perylene, significantly more abundant than other four- to six-ring PAHs. The PAHs determined in heavily biodegraded Alberta oil sand bitumen are composed of relatively higher three- to five-ring compounds than conventional crudes, particularly light crude oils. In the market, some synthetic crude oils such as AHS (Alberta, Canada), are blended with hydrocracking residues, in which extremely high content of unsubstituted PAHs was detected with a total concentration of 624 μg/g [61].

The concentrations of target PAH alkylated homologues are roughly two orders of magnitude higher than the total concentration of the unalkylated PAHs. APAH concentrations in seven crude oils vary widely from 2,300 μg/g for Alberta oil sand bitumen to 16,670 μg/g for Troll crude. Light crude oil does not necessarily contain high concentrations of APAHs. In fresh crude oils, total APAHs are largely constituted by naphthalene and phenanthrene series. Therefore, it is understandable that the predominance of two- to three-ring PAHs including naphthalenes, fluorenes, and phenanthrenes generally implies fresh petroleum pollution.

The overall distribution of APAHs (generally refer to C_0- to C_4-) in fresh crudes usually have a characteristic bell-shaped profile, due to the different degree of alkylation. C_1- to C_3-naphthalenes are often the most abundant in fresh crude oils but are readily removed in the formation of heavy oils, resulting in a very low concentration. A distribution profile of C_0- < C_1- < C_2- < C_3- in nearly all five oil-characteristic APAH series of Alberta oil sand bitumen is very apparent. This can be explained by the fact

Table 2.8: PAHs in oils.

Samples	Bakken	SoL	Shengli	Troll	ArH	PlE	AOS*	Gasoline	Diesel no. 2	Marine diesel	Bunker C	Lube 10W-30
C0N	159	806	137	967	140	85.8	ND	1,259	933	98.0	342	0.39
C1N	1,112	2,026	566	2,900	616	377	ND	1,198	3,614	328	1,231	0.78
C2N	2,833	2,920	1,017	3,646	1,262	744	11.5	422	6,328	1,211	1,857	1.17
C3N	2,892	2,563	940	2,837	1,625	882	103	130	6,077	1,810	1,665	1.01
C4N	1,853	1,544	594	1,677	1,096	820	240	33.8	3,078	1,236	970	0.79
ΣN	**8,849**	**9,858**	**3,254**	**12,027**	**4,738**	**2,909**	**355**	**3,042**	**20,030**	**4,683**	**6,064**	**4.14**
C0P	98.6	145	104	269	55.8	27.3	10.1	5.60	359	47.3	445	0.22
C1P	396	396	332	585	169	91.4	95.5	16.7	1,081	306	1,837	2.27
C2P	651	460	399	640	280	137	214	11.6	1,041	510	2,653	10.0
C3P	619	371	332	543	224	109	274	3.96	564	454	2,297	9.51
C4P	409	229	180	410	140	95.6	198	0.78	203	226	1,672	5.27
ΣP	**2174**	**1601**	**1347**	**2448**	**868**	**460**	**791**	**38.7**	**3248**	**1544**	**8,903**	**27.2**
C0F	30.3	58.9	40.8	161	29.2	10.2	1.55	4.83	316	45.7	104	0.65
C1F	141	178	85.9	347	80.5	38.5	23.7	11.6	775	223	265	1.04
C2F	291	300	127	484	169	80.5	77.7	9.43	992	547	451	2.13
C3F	335	273	112	396	277	78.7	128	5.60	775	773	510	6.19
ΣF	**798**	**809**	**365**	**1,388**	**555**	**208**	**231**	**31.5**	**2,858**	**1,589**	**1,330**	**10.0**
C0C	13.7	8.07	26.0	40.3	10.3	10.5	15.9	0.11	2.58	2.29	277	0.13
C1C	50.3	23.3	47.6	77.3	21.9	19.7	40.3	0.27	5.65	7.90	1,017	ND
C2C	89.5	31.1	89.1	119	39.1	33.0	84.6	ND	4.72	10.9	1,372	ND
C3C	100	24.0	76.9	71.8	33.9	24.4	75.9	ND	ND	8.44	1,097	ND
ΣC	**254**	**86.6**	**240**	**309**	**105**	**87.6**	**216.6**	**0.38**	**13.0**	**29.6**	**3763**	**0.13**
ΣAPAHS (μg/g)	**12,437**	**12,844**	**5,506**	**16,670**	**8,498**	**4,226**	**2,305**	**3,112**	**26,602**	**7,846**	**21,875**	**70.8**
Bph	51.6	153	20.5	288	23.1	5.77	ND	9.96	489	142	26.1	0.11
Acl	8.11	15.9	6.78	16.4	7.65	8.56	ND	ND	40.1	6.64	12.3	ND
Ace	7.87	13.6	13.9	53.7	4.42	6.01	2.94	3.74	84.4	27.9	48.4	ND
An	1.86	3.64	2.62	2.96	1.75	0.60	ND	1.96	ND	4.09	63.1	0.03

Fl	1.66	3.27	3.60	12.6	1.89	1.57	3.02	0.66	7.05	14.6	33.8	ND
Py	9.75	4.83	15.2	16.3	4.01	3.81	10.4	0.96	29.1	86.8	160	0.08
BaA	1.32	2.67	3.71	7.75	2.11	2.43	2.03	0.20	0.45	0.46	139	0.10
B(b + k)F	1.13	2.17	4.07	10.6	2.33	2.87	4.31	ND	ND	0.30	67.8	ND
BeP	3.30	1.45	12.0	11.1	2.70	3.55	5.93	ND	ND	0.53	95.7	ND
BaP	0.38	0.59	1.43	3.15	1.33	1.32	2.22	ND	ND	0.13	59.9	ND
Pe	ND	21.2	2.41	4.70	0.97	30.2	4.83	ND	ND	0.11	26.5	ND
IP	ND	ND	1.60	1.40	0.31	ND	1.30	ND	ND	ND	12.2	ND
DA	0.68	0.23	2.09	1.50	0.37	ND	1.53	ND	ND	ND	23.2	ND
BP	ND	0.70	1.91	3.61	1.42	1.48	2.61	ND	ND	ND	11.8	ND
ΣPAHs (μg/g)	**87.7**	**223**	**91.7**	**434**	**54.4**	**68.1**	**41.1**	**17.5**	**650**	**283**	**780**	**0.32**

* AOS: Alberta oil sand bitumen, DCM extract.

that susceptibility to microbial degradation decreases as the alkylation level increases in each APAH family. Sometimes, a heavy crude like bitumen is mixed with diluent to facilitate easy transportation; therefore, it is not surprising that C_0- to C_3-naphthalene isomers can be detected in appreciable concentrations [61].

PAHs in petroleum products: Compared with crude oils, a certain types of refined oil products can contain much higher concentrations of unsubstituted PAHs, while some petroleum products such as lubricating oil only contain trace amounts. Table 2.8 summarizes the quantitative analytical results of petroleum-characteristic APAHs homologous series and 15 individual unsubstituted PAHs in representative petroleum products. In general, PAHs in light-distillate fuels are only limited to small two- and three-ring compounds; however, obvious increments of four- to six-ring PAHs attributed to refining processes are found in residual fuels. For instance, four- to six-ring PAHs in total comprise nearly 90% of parent PAHs in Bunker C, these high boiling point PAHs are low in diesel and undetectable levels in gasoline.

APAHs in refined products are partially derived from feedstock crude oil and are generated in the manufacturing processes. The distillation and refining process often alters their abundance and distribution profile. Compared to conventional crudes, many refined oil products, except for gasoline and lubricating oil, have higher contents of APAHs with the predominance of naphthalene and phenanthrene series. These compounds are extremely high in diesel fuels. For example, the concentrations of total APAHs in diesel no. 2 are as high as 26,602 μg/g. The small PAH compounds in these heavy fuels are usually attributed to blended light products. PAHs in gasoline are dominated by naphthalene and its alkyl homologues with a decreasing profile of C_0- > C_1- > C_2- > C_3-. Alkylated chrysene series are hardly detectable in these light fuels such as gasoline and diesel; in contrast, the residual fuels such as Bunker C usually contain them in relatively high amounts.

Waste or used oils are usually mixtures of different types of oils and other non-petroleum material. Used and waste lube oils are mixtures of lube oils, unburned diesel, and combustion exhaust of oils. Used lube oil from a diesel engine retains some characteristics of diesel fuel while having some characteristics of additional pyrogenic PAHs [24].

PAHs in environmental samples: The distribution of PAHs in environmental samples is very important for decoding contamination sources. Most PAHs do not dissolve easily in water and their hydrophobicity generally increases with increasing molecular mass. Consequently, these compounds tend to deposit and accumulate in the river and marine beds, resulting in long-term hazards to the environment.

Figure 2.11 shows PAH distributions in some representative environmental samples including snowmelt water from an Alberta oil sands development site, a vessel bilge, a recreational pond sediment, sediment from the oil industry affected river, and a sediment from water treatment plant. Besides five series of determined APAHs

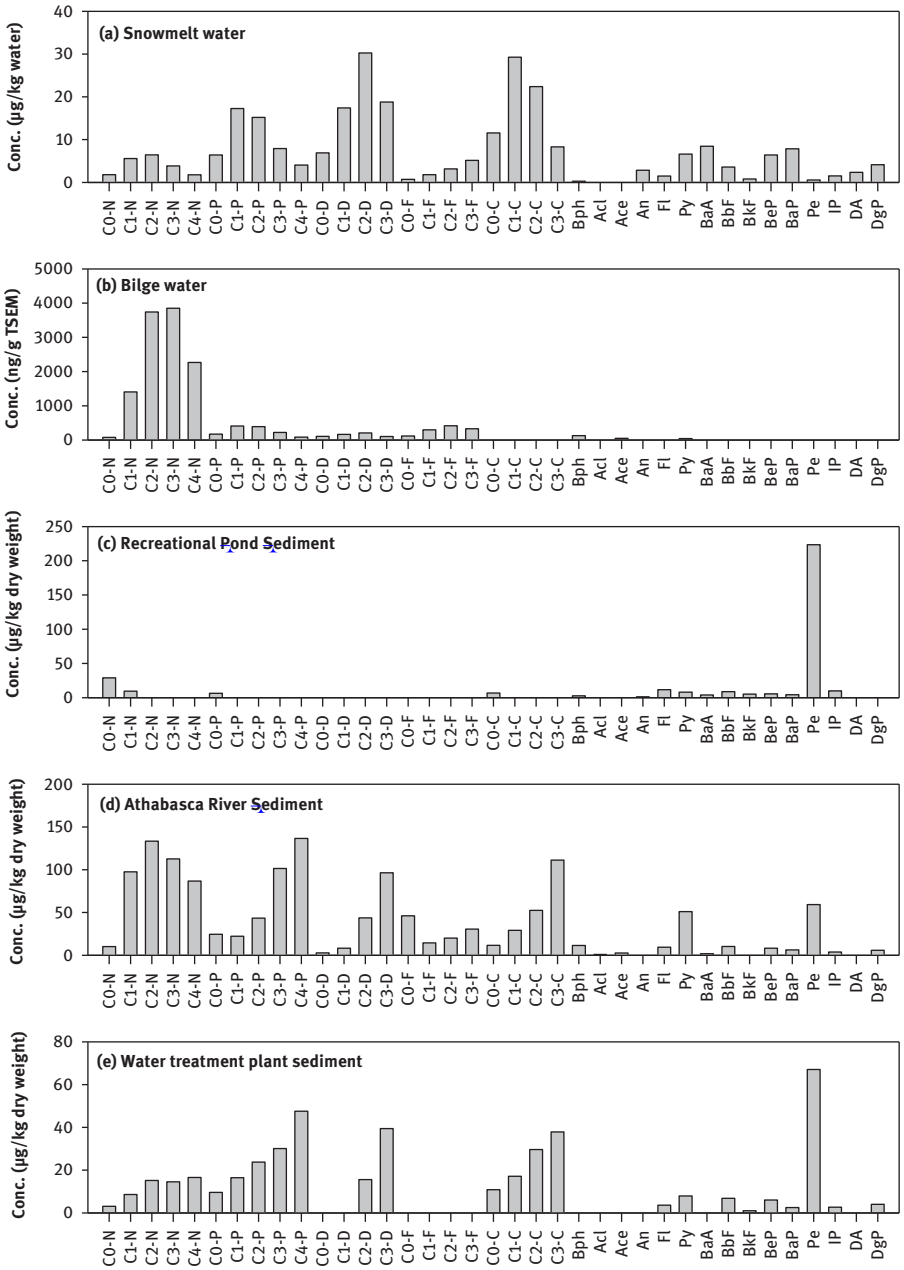

Figure 2.11: PAHs in environmental samples.

as clear evidence of petroleum contamination (oil sands particulate), the high amount of four- to six-ring parent PAHs suggests other contribution sources of contamination in the snowmelt water sample. In the filter, a high percentage of four- to six-ring parent PAHs predominate the total determined unsubstituted PAHs. This typical PAH distribution pattern indicates a contribution from pyrogenic sources, such as runoff from high levels of automobile emissions, which are known to contain a greater amount of larger molecular PAHs relative to others. Perylene only accounts for 3.0% of the total five5-ring PAHs suggesting only a minor biogenic contribution to the detected PHCs in the sample.

The total PAHs were determined to be 14,613 μg/g TSEM with a predominance of APAHs and no detection of the HMW alkylated chrysenes among target APAH homologous series in the bilge. Unsubstituted PAHs are dominated by low molecular weight (LMW) PAHs with two and three rings. This is a typical feature in the chemical composition of light diesel fuels. It has been well known that, in general, PAH concentrations are high in diesels while lube oils only contain trace amounts of APAHs [15, 24]. Therefore, it can be expected that the portion of lube oil in a mixture of diesel and lube oil would only have minimal effects on the PAH distribution pattern and profile. In addition, biogenic and pyrogenic contribution to the bilge sample was not observed from PAH fingerprinting analysis.

As shown in Figure 2.11, only traces of unsubstituted PAHs plus C_1-naphthalenes were detected without other petroleum-characteristic APAH homologues in the recreation pond sediment. These PAHs in trace amount are probably attributed to anthropogenic inputs such as runoff and atmospheric deposition. Perylene in this sample is at an unusually high concentration compared with other five-ring PAHs, accordingly resulting in a high perylene index of 90.5%, which is far higher than the pyrogenic input criteria of 10%. Therefore, the contribution of the natural biogenic sources to the hydrocarbons in the sediment is evident.

PAHs in the Athabasca River sediment in Figure 2.11 are dominated by APAHs. Except for the naphthalene series, the APAH families show an overall decreasing distribution pattern of C_0- < C_1- < C_2- < C_3-, which is consistent with the typical PAH distribution of the oil sand bitumen [61, 86]. Meanwhile, alkylated naphthalenes are highly abundant and in a bell-shaped distribution profile. This may indicate some anthropogenic contribution of oil hydrocarbons from lighter petroleum products, in addition to oil sand bitumen contribution to the sample [86]. Among target unsubstituted PAHs, perylene is the most abundant component, accounting for about 34.6% of the sum of unsubstituted PAHs and over 70% of total five-ring PAHs, clearly indicating a contribution of biogenic input to this sediment. Excluding the outstanding contribution of biogenic perylene, other unsubstituted PAHs are relatively more abundant than those in common oils and refined products. It may indicate a certain contribution of PAHs from pyrogenic sources (such as emission particulates) to the sediment sample.

2.6.3 Aromatic steranes

A series of bicyclic, tricyclic, and tetracyclic naphthenoaromatics are common in refined petroleum products but also occur in crude oil. These include indane, tetralin, tetrahydrophenanthrene, aromatic steranes, and their alkyl derivatives. These naphthenoaromatic hydrocarbons possess mixed structures of aromaticand alicyclic rings (mostly hexyl or pentyl), as well as aliphatic side chains.

Tetracyclic aromatic steroids (also known as aromatic steranes) are detected in most crude oils [37, 87, 88]. Aromatic steranes in the aromatic fraction have highly similar skeletons to saturated biomarker steranes. These compounds have 17 carbons to form a skeleton consisting of four fused rings and range from C_{20} to C_{30} homologs. MASs are composed of four fused rings in their molecular structure including two cyclohexane rings (A + B), an aromatic ring (C-ring), and one cyclopentane ring (D-ring). TASs have a similar molecular structure as MASs. The cyclohexane A and B rings in TAS are replaced by aromatic rings. Aromatic sterane isomers have a characteristic configuration of C-17 and C-20 chiral centers. Each aromatic sterane is usually represented by two epimers (20R and 20S) with different concentrations. Due to the aromatization of C-ring in monoaromatic and (A + B + C) rings in TASs, these compounds have fewer substituents on the ring skeleton and have much fewer constitutional isomers and stereoisomers than their saturated sterane analogs [37].

In a GC-MS analysis, MASs elute between *n*-C_{23} and *n*-C_{30}, while TASs elute slightly later between *n*-C_{24} and *n*-C_{34}, similar to the elution range for biomarker terpanes in GC-MS analysis (see Figure 2.2). The base peak of TASs is *m*/*z* 217 ($C_{17}H_{13}^{+}$) for the nucleus alone and *m*/*z* 231 ($C_{18}H_{15}^{+}$) if with a single methyl group. C_{26} TA-cholestane (20 R) and C_{27} TA-ergostane (20S) are co-eluted and present as the highest peaks in *m*/*z* 231 chromatograms. A cluster of TA-cholestanes (C_{26}), TA-ergostane (C_{27}), and TA-stigmastanes (C_{28}) are the most distinguishable aromatic steranes in most oil samples. It was also observed that a cluster of peaks (at *m*/*z* 231) elute in the boiling point range of C_{20} and C_{21} TASs, making it impossible to accurately identify and quantify these triaromatic compounds in heavy fuels. This cluster is probably attributed to PAHs and/or naphthenoaromatic hydrocarbons produced in refining processes and their presence could be used as evidence of cracked heavy components. MASs can be identified by their characteristic fragmentograms at *m*/*z* 253 ($C_{19}H_{25}^{+}$), due to loss of the alkyl group attached on C-17. Quantitative analysis of MASs often encounters serious interference at *m*/*z* 253 by saturated hydrocarbons if the oil sample is not fractionated prior to instrumental analysis, and sometimes insufficient abundance of these compounds can hinder reliable measurement [37].

Aromatic steranes in crude oils: These aromatic steranes widely occur in various crude oils and shales and are of utmost importance in petroleum chemistry and geochemistry. In catagenesis, MASs are transformed into triaromatic hydrocarbons via degradation of the aliphatic side chains. The aromatization of the B-ring in MASs

(aromatic C-ring) occurs at a slow pace, but once completed, subsequent A-ring aromatization proceeds with considerable speed, which results in low concentrations of diaromatic steranes (B- and C-rings) in crude oil [59, 62]. The abundance of MASs relative to TASs is used in various geochemical correlations, particularly in evaluating crude oil maturity [59, 87].

Figure 2.12 illustrates the GC-MS chromatograms of TASs in crudes and refined petroleum products. Aromatic steranes are widely detected in all of the crude oils, but their abundances are very low relative to the total concentration of five APAH series [37]. It is obvious from Figure 2.12 that crude oils from different sources and various petroleum products have unique characteristics of aromatic steranes. The abundances of aromatic steranes in crude oils vary significantly. Overall, aromatic steranes in heavier crude oils are likely more abundant than in lighter oils. These compounds occur only at low concentrations in light crude oils such as the Scotia Light and the Bakken crude oils but high concentrations in the heavy California Platform Elly crude oil [37].

Aromatic steranes in petroleum products: The abundance and distribution profiles of petroleum hydrocarbons in refined products are often altered by the distillation and refining process of their original feedstock. In general, TASs are undetectable to very low concentrations in many refined oil products, although these oils probably contain a high concentration of other APAHs. Predictably, these aromatic steranes with relatively high boiling points are barely found in light and middle distillate fuels such as volatile gasoline and light diesel. If simply based on the boiling point range of petroleum products, the heavy fuels and lubricating oils should have a high content of these aromatic steranes; however, in general, aromatic steranes are only at trace levels in these oils. The absence of TASs in combination with the presence of the hopanes confidently indicates the presence of lubricating oil [20]. This is due to the hydrogenation and hydrocracking during the refining process removing most aromatic hydrocarbons including the steranes, from the base oil [37]. As a result, aromatic steranes and PAHs are absent or at a low abundance in the unused lubricating oils (Figure 2.12).

2.6.4 Polycyclic aromatic sulfur heterocycles

Other than PAHs, petroleum also contains a large amount of heterocyclic N-, S-, O-containing analogues of PAHs. As the third most abundant element in crude oil, sulfur occurs in various forms and wide molecular weight fractions. Organosulfur compounds commonly identified in fossil fuels and crude oil include thiols, thioethers, disulfides, thiophenes, benzothiophenes, dibenzothiophenes, and benzonaphthothiophenes [89–93]. Among these organosulfur compounds, the most abundant group of compounds are analogues of PAHs, namely PASHs or thiaarenes. Similar to PAHs, three- to five-

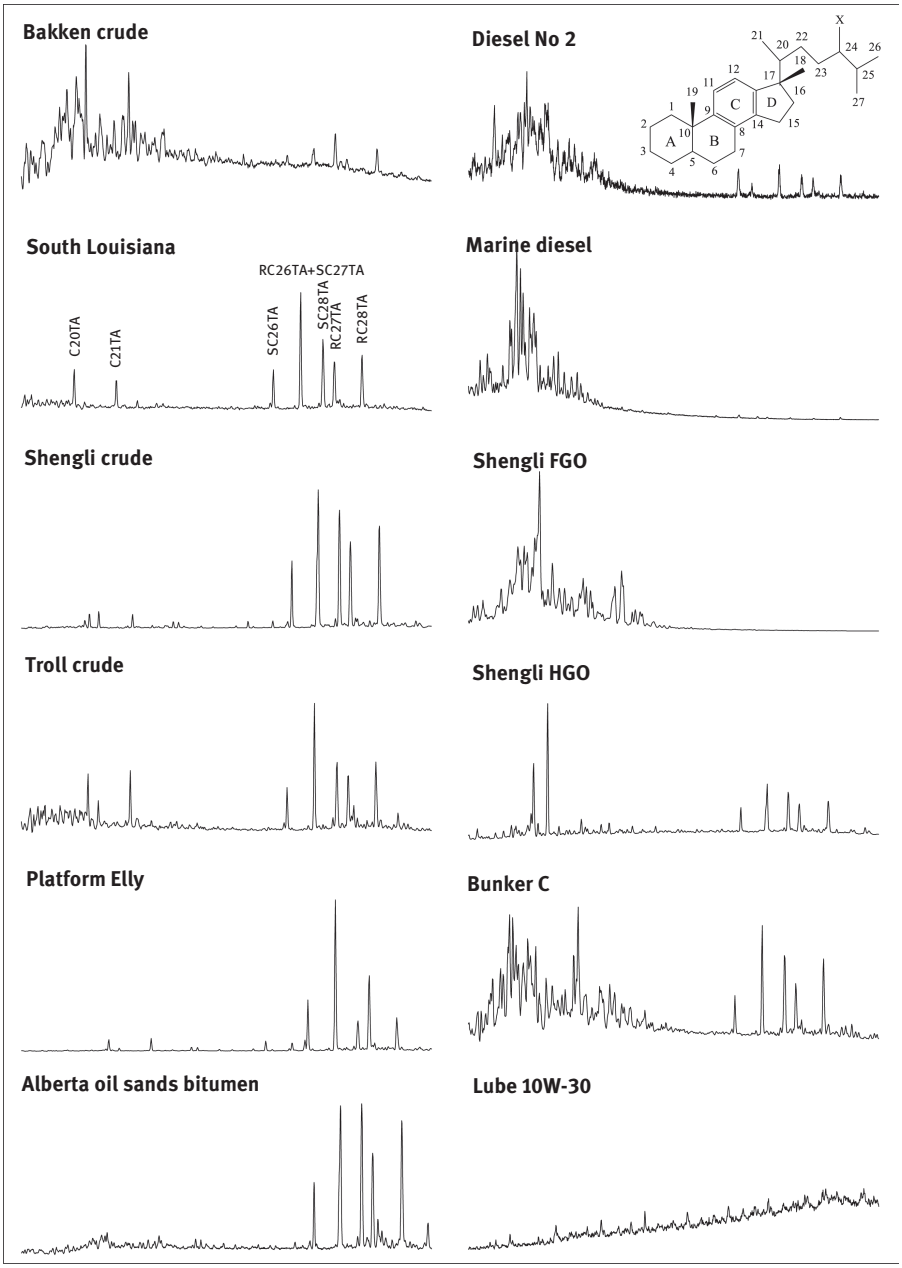

Figure 2.12: GC-MS chromatograms of triaromatic steranes in crude oils and petroleum products.

ring PASHs have various structural isomers, for example, benzo[b]naphtha[2,1-*d*] thiophene, benzo[*b*]naphtha[1,2-*d*]thiophene, and benzo[b]naphtha[2,3-*d*]thiophene for $C_{16}H_{10}S$; Some or all of these isomers and their alkylated homologues could occur in petroleum but are usually difficult to chromatographically differentiate [29].

PASHs have not been as intensively investigated as PAHs, but such studies may lead to a great deal of information not available through the study of PAHs [94]. PASHs are of great interest to environmental monitoring, especially in relation to oil spills and combustion emissions of hard coal. PASHs generally have very similar chemical properties and may exhibit similar carcinogenicity and mutagenicity as their PAH analogues. Eastmond et al. compared a series of PASH with their sterically and structurally similar PAHs for toxicity and biotransformations [95]. Benzo[b]thiophene and benzo[*b*]naphtho[2,1-*d*]-thiophene were bioconcentrated to a greater extent than naphthalene and chrysene, respectively. It was reported that benzo[2, 3] phenanthro[4,5-*bcd*]thiophene exhibited outstanding mutagenic activity exceeding even that of its homocyclic benzo[a]pyrene [96].

Accurate characterization and measurement of these sulfur species are very meaningful for unambiguous identification and differentiation of type and age of petroleum products. PASHs generally concur with other PAHs and their large number of possible alkyl-substituted isomers in crude oil [15, 92, 94]. During a chromatographic analysis, PASHs are usually present at close retention time with PAHs. Moreover, some PASHs by chance have the same nominal masses as other petroleum hydrocarbons, for example, 184 amu for both C_4-naphthalenes and dibenzothiophene, and 234 amu for both C_4-phenanthrenes/anthracenes and benzo[*b*]naphthothiophenes. PAHs and PASHs could interfere with each other's identification and quantitation. Their identification and quantitation can be improved by using high-resolution and high-mass accuracy MS [29, 32].

Sulfur-containing compounds are removed from certain refined petroleum products as undesirable content. With deep desulfurization, the sulfur concentration in refined products is lowered and the analysis of the remaining PASHs becomes increasingly difficult in the presence of the PAHs, whose concentration is unaffected by the refining processes.

PASHs in crude oils: Sulfur-containing compounds occur in the majority of crude oils and certain refined petroleum products. Table 2.9 summarizes the quantitative analytical results of four petroleum-characteristic PASH series and four PAH analogues (C_0- to C_4-naphthalenes, phenanthrenes, chrysenes, and benzopyrenes) in representative light to heavy crude oils. The distributions of APASHs (generally referring to C_0- to C_4-) in fresh crudes usually demonstrate a characteristic bell-shaped profile, which is similar to the profiles of other carbocyclic analogues.

PASH concentrations in seven crude oils vary widely from 405 μg/g for the Bakken crude to 4,825 μg/g for Alberta oil sands extract (bitumen). Among these PASH groups, tricyclic dibenzothiophenes are the most abundant, followed by tetracyclic

Table 2.9: PASHs in crude oils, refined petroleum products, and environmental samples.

Compounds	Crude oils					Petroleum products					Environmental samples		
	Bakken	Federated	South Louisiana	Shengli	Oil sands extract	Diesel (2002)	Diesel (2021)	IFO-180	Bunker C	Shengli distillated diesel	Snowmelt water	Athabasca River sediment	Pond sediment
	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/g)	(µg/kg)	(µg/kg)	(µg/kg)
C_0-BT	0.10	0.10	0.83	0.93	ND[a]	1.94	ND	37.7	32.7	8.86	2.72	0.80	1.50
C_1-BT	0.17	5.26	7.67	7.75	0.84	9.66	ND	264	142	58.8	5.83	5.90	1.07
C_2-BT	2.39	97.1	79.6	44.7	52.7	93.7	ND	621	286	304	7.88	36.6	1.63
C_3-BT	6.81	153	103	70.1	210	105	ND	457	182	452	4.15	112	1.63
C_4-BT	11.2	127	97.2	87.2	371	71.4	ND	271	108	551	1.62	145	1.41
Σ BTs	**20.7**	**382**	**288**	**211**	**635**	**282**	**ND**	**1,651**	**751**	**1,374**	**22.2**	**300**	**7.24**
C_0-DBT	7.82	95.8	42.7	16.0	7.89	42.6	ND	153	105	106	9.24	30.6	6.00
C_1-DBT	41.0	282	218	93.5	269	120	ND	573	420	585	31.36	193	7.00
C_2-DBT	87.7	384	403	148	832	106	ND	900	739	794	47.98	417	14.0
C_3-DBT	85.0	241	312	108	868	39.6	ND	834	662	256	24.67	917	13.0
C_4-DBT	45.9	129	177	83.6	657	12.7	ND	561	419	31.0	9.79	1,071	9.00
Σ DBTs	**267**	**1,132**	**1,153**	**449**	**2634**	**321**	**ND**	**3,021**	**2,345**	**1,772**	**123**	**2,629**	**49.0**
C_0-BNT	2.76	19.7	10.2	11.8	63.4	0.19	ND	206	175	ND	16.9	97.4	20.0
C_1-BNT	14.2	78.8	43.7	42.9	251	0.37	ND	783	686	ND	42.6	531	18.0
C_2-BNT	29.9	122	82.7	59.5	388	0.22	ND	1,067	901	ND	30.6	1,436	25.0
C_3-BNT	31.1	101	79.0	52.1	361	ND	ND	825	712	ND	11.5	2,130	31.0
C_4-BNT	19.3	61.0	52.6	34.4	240	ND	ND	413	388	ND	3.58	1,391	21.0
ΣBNTs	**97.3**	**383**	**268**	**201**	**1,303**	**0.78**	**ND**	**3,294**	**2,862**	**ND**	**105**	**5,585**	**115**
C_0-DNT	1.20	7.71	2.33	~~39.3~~	20.6	ND	ND	58.0	63.0	ND	32.3	148	22.0
C_1-DNT	2.56	26.8	6.29	~~20.1~~	63.1	ND	ND	185	197	ND	50.9	275	22.0
C_2-DNT	5.55	38.6	10.0	~~29.6~~	101	ND	ND	263	272	ND	46.5	462	31.0
C_3-DNT	5.60	31.3	12.0	~~34.2~~	92.7	ND	ND	214	200	ND	24.0	460	31.0

(continued)

Table 2.9 (continued)

Compounds	Crude oils					Petroleum products					Environmental samples		
	Bakken	Federated	South Louisiana	Shengli	Oil sands extract	Diesel (2002)	Diesel (2021)	IFO-180	Bunker C	Shengli distillated diesel	Snowmelt water	Athabasca River sediment	Pond sediment
C4-DNT	4.33	33.6	7.88	16.8	74.5	ND	ND	138	164	ND	8.47	341	18.0
ΣDNTs	**19.2**	**138**	**38.5**	**140**	**352**	**ND**	**ND**	**858**	**896**	**ND**	**162**	**1,686**	**124**
ΣPASHs	**405**	**2,035**	**1,747**	**1,001**	**4,924**	**603**	**ND**	**8,824**	**6,853**	**3,146**	**413**	**10,200**	**295**
ΣPASHs/ΣPACs	**0.05**	**0.14**	**0.17**	**0.18**	**0.57**	**0.02**	**0.00**	**0.21**	**0.22**	**0.14**	**0.33**	**0.57**	**0.18**

benzonaphthothiophenes. Light bicyclic benzothiophenes and heavy pentacyclic dinaphthothiophenes were both detected, but in relatively lower concentrations in all studied crude oils. PASHs concentrations are lower overall than their corresponding PAH analogues in most studied oils, while Alberta oil sand bitumen contains very high concentrations of PASHs. Four groups of PASHs collectively account for only 4.6% of eight PAC families in the Bakken crude oil, compared to 57.1% for the Alberta oil sand bitumen. Considering that PASHs have similar toxicities with their PAH analogues, the impact of oil contamination will be significantly underestimated without the determination of these sulfur-containing compounds.

PASHs in petroleum products: Sulfur oxide emissions from the combustion of fuels containing sulfur compounds pose an environmental threat. Stringent limits on sulfur levels in gasoline and vehicular diesel fuel were created to allay increasing concern on the environmental impacts. In the last few decades, sulfur levels in vehicular and marine diesel fuels dropped significantly. Marine fuel oil has been condemned for its abundance of sulfur. The International Maritime Organization has required that from January 1, 2015, fuel with less than 0.1% sulfur content is used for vessels operating in the sulfur emission control areas or the emission control areas (ECAs), unless the vessel is fitted with equipment such as scrubbers to reduce the sulfur in exhaust fumes, or is operating on alternative fuel such as liquefied natural gas, or has a dispensation conferred. From January 1, 2020 onward, the limit for sulfur in fuel oil used on ships operating outside designated ECAs will be reduced to 0.50% by weight. Low sulfur (LSFO, sulfur content ≤1%) and ultra-low sulfur ~~fuels~~ (ULSFO, sulfur content ≤ 0.1%) have become popular nowadays [97].

The abundance and distribution profile of components in oils are often altered by the distillation and refining process. The high-sulfur diesel fuel contained a larger proportion of PASHs with one or two rings [93]. PASH concentrations in selected refined petroleum products vary significantly from type to type. The higher boiling fractions contain a relatively high content of sulfur species, explaining why HFOs are usually rich in sulfur-containing compounds. Because PASHs are deliberately removed by refining processes, their profiles in refined products could be different from those of their PAH analogues, where all bi- to penta-cyclic PASHs could be very low or even undetectable in certain light petroleum products. Accurate analyses of PASHs in these fuels become very difficult, making GC-HRAM-MS more and more necessary to undertake these tasks [29]. PASHs in diesel produced from different generations probably change significantly. As given in Table 2.9, the no. 2 diesel (Ottawa, 2002) contains 282 μg/mL of benzothiophenes and 321 μg/mL of dibenzothiophenes, while PASHs are barely detectable in automobile diesel obtained in 2021 (Ottawa, ON). With implementing strict environmental regulations, it is expected that PASHs are detected at much lower concentrations or even undetectable in future marine fuel oils.

2.6.5 Diagnostic criteria of aromatic compounds

PAHs in oils have been thoroughly investigated and are widely used for forensic oil analysis. Certain PAH diagnostic ratios have been widely used in oil differentiation or contamination source identification. To reduce the influence of weathering processes, diagnostic ratios are traditionally restricted to parent PAHs with the same molecular weight and similar properties or to ratios of alkylated versus parent PAHs. Various PAH ratios between individual compounds or groups of compounds have been described extensively and used to compare oil samples [15, 20]. Some PAH ratios can often be diagnostic in crude oils, but can or may not have a similar diagnostic significance in refined products in which their distributions can be affected by refinery processes (e.g., cracking, reforming, and hydrotreatment) [20].

Excluding perylene and dibenz(*a*,*h*)anthracene, nine four- to six-ring higher molecular PAHs (refer to Table 2.6) were classified as combustion PAHs. The ratios derived from less stable versus thermodynamically stable PAH isomers, such as fluoranthene to pyrene (Fl/Py), phenanthrene to anthracene (Ph/An), and methylphenanthrenes to phenanthrene (C_1P/C_0P), are often used to investigate petrogenic contamination against pyrogenic input in environmental samples [98–103]. In contrast to pyrogenic sources, petrogenic sources are characterized by high ratios of Ph/An > 15 and $C_1P/C_0P > 2$ in association with lower ratios of fluoranthene/pyrene (Fl/Py < 1) and benzo(a)anthracene/chrysene (BaA/CHR < 0.4) [102]. Yunker et al. reported that the ratio of IP/(IP + BgP) < 0.20 likely implies petroleum; between 0.20 and 0.50 liquid fossil fuel (vehicle and crude oil) combustion; and over 0.50 imply grass, wood, and coal combustion [101, 102]. Refining processes often altered the profile of aromatic compounds in the stock crudes, particularly resulting in less standardized patterns of C_1-phenanthrenes and C_1-pyrene in heavy fuels [20].

Wang et al. proposed the "Pyrogenic Index," which is defined as the sum of three- to six-ring unsubstituted PAHs (see Table 2.8) over the sum of the five APAH homologues (Σ3– 6-ring EPA priority PAHs/Σ5 series APAHs) [99]. The unsubstituted PAHs used PI measurement, including three-ring (acenaphthylene, acenaphthene, anthracene), four-ring (fluoranthene, benz(a)anthracene, pyrene), five-ring (benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*e*)pyrene, benzo(*a*)pyrene, perylene, dibenz(*ah*)anthracene), and six-ring (indeno(1,2,3-cd)pyrene, benzo(ghi)perylene). The APAH series include C_0–C_4 naphthalenes, C_0–C_4 phenanthrenes, C_0–C_3 fluorenes, C_0–C_3 dibenzothiophenes, and C_0–C_3 chrysenes. This ratio is a robust quantitative indicator for the identification of pyrogenic PAHs and for differentiating pyrogenic and petrogenic PAHs. Lighter petroleum products and most crude oils show ratios smaller than 0.01, while heavy oils and heavy fuels show significantly higher ratios in the range of 0.01–0.05. The ratios for the oil-burn soot can be as high as 2.0 [99].

Perylene comes primarily from diagenesis of biogenic precursors and partially from petroleum or pyrolytic processes [84, 99]. Perylene index, defined as the

concentration of perylene divided by the total of five-ring PAHs, is a very useful tool to distinguish biogenic from pyrogenic hydrocarbons. This ratio is effective in differentiating and characterizing biogenic sources from others in environmental samples. Perylene indices greater than 10% often indicate diagenetic (biogenic) inputs, whereas those <10% indicate a pyrogenic or petrogenic origin.

Ratios derived from APAHs are of special use for forensic oil identification. Methylated PAHs are present in crude oils and refined products in high concentrations. These C_1-PAHs are well separated in the chromatographic analysis due to their small number of isomers. The ratios among their isomers are often employed for oil analysis, including 2-naphthalene and 1-naphthalene at *m*/*z* 142, 4-, 2-/3-, and 1-methyl dibenzothiophene, and (3- + 2-methyl-phenanthrene) to (4-/9- + 1-methyl-phenanthrene). Relative abundance of five APAHs, ΣN:ΣP:ΣD:ΣF:ΣC, is an important ratio to distinguish oil from oil and type from type. The relative distribution profile C_0-:C_1-:C_2-:C_3-:C_4- of each APAH family is often used to differentiate petrogenic source from other sources, and to investigate the weathering effect.

Among the five APAH series, tricyclic phenanthrenes are frequently applied for forensic oil analysis. These compounds are detected in considerable abundance in crude oils; in addition, they remain relatively stable under weathering and therefore are widely applied to distinguish oils and investigate weathering. As an isomer of phenanthrene, anthracene has three fused benzene rings in a straight linear arrangement. Methylanthracenes are generally absent in most conventional crude oils, with some present in low concentrations relative to methylphenanthrenes. 2-Methylanthracene (2-MAn) is eluted between two pairs of methylphenthrarene isomers (2- and 1-, 4-/9- and 3-, *m*/*z* 192) in variable concentration in different types of oils. A relatively high presence of 2-MAn in the oil sample generally indicates cracking. Based on our experience in analyzing a large number of crude oils and petroleum products, the ratio of 2-MAn relative to the total of methyl-phenanthrenes ranges from 0 to 0.03 for most crude oils, diesels, and lube oils studied, while this ratio is generally greater than 0.03 for heavier refined petroleum products such as Bunker C. In addition, another aromatic cluster at *m*/*z* 216 (methyl-fluoranthenes and methyl-pyrenes) has proven to be relatively stable and especially suitable for comparing light fuel oil samples.

Although aromatic steranes are in relatively low concentrations in oils, their specific fingerprints and high weathering resistance make them desirable biomarkers for forensic investigation [20, 37, 59, 88]. Most parent PAHs and APAHs are susceptible to microbial degradation; however, aromatic steranes are highly resistant to physical weathering and biodegradation, other than the usual biomarker terpanes and steranes. These features make them suitable candidates for forensic oil analysis, particularly when the oils involved are heavily weathered. The relevant diagnostic ratios associated with aromatic steranes are robust for oil-to-oil correlation and oil source tracking.

PASHs have been extensively used as critical criteria for forensic oil analysis, particularly for oil-type recognition. These compounds are particularly useful in oil

spill source identification in a wide variety of weathering conditions such as evaporation, photo-oxidation, and biodegradation [9, 94, 96, 104, 105]. It has been established that, among the PASH markers, the ratios of methylated dibenzothiophenes (C_1-DBTs) vary widely with the source of petroleum. C_1-DBT isomers are present in crude oils at relatively high concentrations, and their distribution profiles vary significantly. Their relative distributions are subject to little interference from evaporative weathering in a short term but are altered by biodegradation. Thereby, the chemical profile of PASHs can be distinctively used as an indication of microbial degradation of oils. For instance, bunker fuels have a 2-/3- to 4-methyldibenzothiophene (MDBT) ratio around 1.0, which is unusually high compared to most crude oils [104]. The presence of a clear V-pattern (4-methyl > 2- + 3-methyl < 1-methyl) for the MDBTs is generally associated with oils from predominantly carbonate source rocks, while a stair-step pattern (4-methyl > 2 + 3-methyl > 1-methyl) is associated with predominantly siliciclastic source rocks or advanced maturity (late- to post-oil window) oils from carbonate sources. The MDBTs are also used as biodegradation indicators. 2- and 3-MDBT are biodegraded at a higher rate, as shown by the strong decrease of their ratio to 4-MDBT, while 1-MDBT is slightly more resistant to biodegradation than 4-MDBT, indicated by an increase of the ratio 1-MDBT/4-MDBT [106].

2.7 Weathering effect on oil chemical composition

Once oil enters the environment, it is immediately subject to natural weathering. Weathering is termed as the combination of a series of physical, chemical, or biological processes that affect the composition and change the physicochemical properties of spilled oil in the environment. Oil weathering processes have an extensive influence on the behavior, effects, and ultimate fate of an oil spill and have therefore been the topic of much research to inform spill-response activities. These processes highly depend on the nature of the oil spilled and the weather conditions during and after the spill (e.g., temperature, wave movement, wind speed, and sun incidence).

Numerous studies have been conducted to study the effects of weathering on oil components [8, 20, 30, 35–37, 107–110]. These weathering processes to spilled oil include spreading, evaporation, dispersion, dissolution, and emulsification in the early stage, following the longer processes of sedimentation, oxidation, and biodegradation. Multiple weathering processes can occur simultaneously and influence each other. Herein we only selectively stress three weathering processes of evaporation, photodegradation, and biodegradation. These processes often significantly alter the physical and chemical properties of spilled oil, which bring challenges to oil identification. However, weathered oil could retain the unique characteristics of some weathering-resistant components, which enables the potential to identify the source oil. Variations of certain analytes that are susceptible to specific weathering processes

could be used as particular indicators of these weatherings, for instance, depletion of *n*-alkanes suggesting oil biodegradation.

2.7.1 Evaporation

In the short term after an oil spill, among the potential physical weathering processes, evaporation is usually one of the dominant weathering processes [8]. The evaporation effect is particularly significant on the oil remaining on land or water surface after a spill. Evaporation simply transports part or all oil mass from its bulk to the surrounding air. The evaporation speed of a specific oil mainly depends on its composition and weathering conditions such as the ambient temperature.

Evaporation in sequence removes components from an oil according to their boiling points, but in fact, the molecular interaction of the complex hydrocarbon mixtures could affect their evaporation. In theory, the oil loses lighter compounds with low boiling points first. As GC separation is based mainly on boiling points of the components, a plot of relative abundance of target compounds in evaporated oil and source oil against the retention time on the *x*-axis shows a sine-curve shape [20]. For lightly evaporated petroleum, most mass loss is attributable to low-boiling point compounds such as BTEX, alkylated benzenes, and alkanes. By this means, evaporation reduces the acute toxicity of the remained oil. An oil with a high amount of light compounds evaporates faster than one with a large proportion of heavier components. Gasoline evaporates completely in a few hours, while diesel range oil evaporates more gradually. Since heavy oils have generally been severely weathered in their formation, evaporation has little effect on their chemical composition. Fingas indicated that, on the land, ongoing oil evaporation could be considerably slowed down due to the "crust" on the oil surface formed by resin and waxes [8]. Evaporation of crude oil leaves heavier and viscous components, resulting in the formation of floating patches of oil and tarballs (or tarmats) or sinking in freshwater [8, 20, 111]. Tarballs could be eventually found on shorelines hundreds of miles away. Stranded tarballs are very persistent in the marine environment and could preserve essential information to determine the extent of oil spillages and to trace the source of an oil spill [112–114].

Slight to medium evaporative weathering has little effect on distribution patterns of most target petroleum hydrocarbons. Most target hydrocarbons were concentrated in proportion with the increase of the weathered percentages [15]. Heavier weathering could lead to a significant reduction in abundances of light *n*-alkanes, all adamantanes, and light sesquiterpanes in some oils [35, 36]. If evaporation continues and has affected the *n*-alkanes beyond *n*-C_{13}, then a relative depletion of the lower boiling $C_{14}H_{26}$ sesquiterpanes would be expected [42]. In a forensic investigation, the concentration reduction of the low boiling components and the buildup of the high boiling components relative to the smaller *n*-alkanes could indicate that the oil has been significantly weathered, even though its *n*-alkanes are not completely

lost [78]. The distributions of target hydrocarbons, particularly biomarker terpanes and steranes, high PAH compounds, and TASs, remain consistent through the evaporation process. In addition, evaporation does not remove significant UCM content, resulting in an increased UCM proportion for evaporated oils.

In summary, the main chemical composition changes caused by evaporative weathering include [8, 13, 20, 104]:

- Evaporation is a physical weathering process. It does not cause preferential loss of one isomer over another.
- Evaporation progressively reduces the abundances of the lower boiling compounds then to heavier components.
- The concentrations of HMW components relatively increase due to the loss of LMW components.
- Evaporation results in an increased UCM proportion in weathered oils
- The CPI values remain virtually unchanged through the entire evaporation process.
- The distribution profiles of HMW terpanes, steranes, and aromatic steranes are unaffected while they are concentrated during evaporative weathering. Relative ratios of these compounds demonstrate great consistency.

2.7.2 Photodegradation

Besides physical weathering processes, oil spilled is also subjected to other destructive chemical weatherings such as oxidative photodegradation or photooxidation. It is established that solar irradiation is an important effect for the alteration or removal of petroleum from water surface and on the land, especially in tropical and subtropical latitudes under conditions of intensive solar radiation and in oligotrophic waters when nutrients needed for biodegradation may be limited [115–122]. Photodegradation is generally not considered as an important process for the degradation of oils in soils or deep-water sediments.

Photooxidation is a slow chemical process and only gradually breaks down spilled oil. The speed and extent of photooxidation are dependent on the thickness of the oil slick as well as sun exposure. Sunlight (particularly short wavelength light <300 nm) directly or indirectly oxidizes petroleum compounds into more polar ketones, aldehydes, carboxylic acids, esters, and so on. Because these products are more soluble in water, photooxidation enhances the overall solubilization of intact petroleum. In contrast, photooxidation may also result in HMW products through the condensation of peroxide intermediates, ultimately leading to tar and gum-like residues [111, 115]. Moreover, photochemical processes are probably also important to oil dissolution and the subsequent biological consumption of oil [117].

Oil photodegradation was not thoroughly studied and less understood by environmental scientists in comparison with evaporation and biodegradation. The studies

on photodegradation of petroleum mainly focused on polycyclic compounds in petroleum due to their high concentrations and potential high environmental impact. Nicodem et al. reported the photochemical weathering of crude oil as a film over seawater by sunlight [118]. The fluorescence emission intensity of the crude decreases rapidly and is only two-fifths of its initial intensity after 100 h of irradiation. It is believed that the reduction of fluorescence intensity is due to the formation of oxygenated derivatives, which are less fluorescent. Ali et al. used a laboratory photodegradation apparatus, incorporating a calibrated xenon lamp, controlled temperature unit, and quartz reaction cells to simulate natural irradiation by sunlight [120]. They reported a pseudo-first-order photodegradation constant (k) of phenanthrene in seawater at 25 °C.

Photooxidation affects different groups of petroleum composition in different degrees. Andersson reported lower losses of sulfur heterocycles than phenanthrene series when oil was exposed to sunlight irradiation [119]. Their photodegradation follows a declining order: phenanthrene > 1-methylphenanthrene = DBT > 2-methylphenanthrene > 4-methyl-DBT > l-methyl-DBT > 2- + 3-methyl-DBT > 3,4-dimethyl-DBT > 1, 7-dimethyl-DBT > 3,7-dimethyl-DBT. Jacquot et al. [115] found that naphthalene and its alkylated homologues in petroleum spilled in the marine environment were severely altered by photooxidation whereas, phenanthrenes, dibenzothiophenes, and their alkylated homologues were rather recalcitrant. Furthermore, methyl derivatives were more degraded than their parent molecules. The degradation of methylated phenanthrenes (MP) follows a decreasing order of 2-MP < 1-MP < 3-MP < 9-MP. It was reported that the photosensitivity of petroleum hydrocarbons increased with increasing aromaticity and alkylation [122]. Photooxidation significantly affects a series of PAHs, that is, methyl anthracene, methyl pyrenes, C_4-phenanthrenes, and methyl chrysenes. Pronounced photodegradation of TASs was also observed although these compounds are traditionally considered robust at medium- to long-term environmental exposure. In particular, the chromatographic patterns of C_1-pyrenes and C_2-pyrenes are easily affected by sunlight, which can serve as markers to disclose photooxidation. Observed from many real oil spills, the reduction of methyl-pyrenes by photodegradation follows an order of 1-MPy > 4-MPy > 2-MPy [20, 123].

It was also reported a preferential alteration of branched alkanes rather than straight alkanes [115]. The distribution of biomarkers such as drimanes, hopanes, and steranes remain stable in photodegradation. Therefore, the diagnostic ratios of these biomarker compounds are still applied to oil identification when oil is exposed to photodegradation.

In summary, the main effect of photodegradation on spilled oil [13, 115, 116]:

- Photochemical degradation yields a variety of oxidized compounds that are more soluble in water than the starting compounds, enhancing the overall solubilization of intact petroleum.
- Generation of the persistent residue by photooxidation may also result in the formation of tarballs.

- Photooxidation affects different groups of petroleum composition in different degrees.
- APAHs are oxidized faster than their parent molecules.

2.7.3 Biodegradation

Oil biodegradation occurs during the formation of crude oils under geological conditions. Natural biodegradation plays an important role in the cleanup of oil spilled into the environment and recovery of the impacted ecosystem. Biodegradation is a long-term oil weathering process, while its effect is not significant in the immediate aftermath of a spill. Besides the physical and chemical properties of the oils, many other environmental factors including the nature of the environmental media (e.g., oxygen concentrations, temperature, salinity, and pressure), the content of nutrient and hazardous contaminant, the characteristics of the microbial population, and so on could greatly affect the rate and extent of oil biodegradation [111]. The biodegradation of petroleum can also be simulated in the laboratory under controllable conditions; however, the process in a laboratory environment could be significantly different from a spill site. Many oil spill incidents around the world have offered scientists great opportunities to study oil biodegradation.

Biodegradation can considerably affect the composition of oil released into the environment. One of the significant differences between biodegradation and physical weathering processes is that the former selectively reduces an individual compound or a group of petroleum compounds. Initial or mild biodegradation readily removes LMW *n*-alkanes, which can be easily observed on whole-oil gas chromatograms. Moderate biodegradation is marked by a nearly total loss of *n*-alkanes and the reduction of alkylcyclohexanes, alkylbenzenes, and acyclic isoprenoid alkanes.

Different classes of petroleum hydrocarbons have different susceptibilities to biodegradation. There are many discussions in the literature about the biodegradation order of petroleum hydrocarbons [15, 30, 59, 124, 125]. Some researchers tried to rank oil biodegradation based on the depletion of petroleum hydrocarbon classes [59, 124, 125]. In general, the biodegradation effects on the oil composition can be summarized in the following patterns [126, 127]: 1) smaller hydrocarbons are degraded faster than larger hydrocarbons; 2) straight-chain *n*-alkanes are degraded faster than branched alkanes; 3) GC-resolved compounds are degraded more than GC-unresolved complex hydrocarbons; 4) small aromatics are degraded faster than HMW aromatics; 5) increase in alkylation level within their alkylated homologous families significantly decreases susceptibility to microbial attack; 6) microbial degradation is often isomer specific. Biodegradation of specific petroleum hydrocarbons generally follows an overall declining order of *n*-alkanes > benzene > toluene > isoalkanes and anteisoalkanes > cyclohexylalkanes and/or methylcyclopentylalkanes > acyclic isoprenoids ≫ naphthalene > phenanthrene ≫ PAHs > C_{27-29} steranes > C_{30-35} hopanes > diasteranes

> C_{27-29} hopanes > C_{21-22} steranes > tricyclic terpanes [30, 59, 124, 128]. However, this sequence does not imply complete removal of one class before another is degraded and does not necessarily always apply to all oil biodegradation.

Biodegradation of biomarkers: Small biomarker compounds such as diamondoids and bicyclic sesquiterpanes are resistant to slight to medium weathering, particularly from biodegradation [33, 35, 59]. These compounds are determined in significant abundance in extra-heavy oil sand bitumen despite the depletion of *n*-alkanes by biodegradation [58, 61, 129]. It was reported that adamantanes are more susceptible to biodegradation than diamantane analogues [36]. Williams et al. found that a series of diamondoid compounds demonstrated resistance to biodegradation in a severely degraded crude oil, in which pentacyclic triterpanes were almost completely demethylated [47]. The degradation resistance of adamantanes is at least as strong as that of tricyclic terpanes and that the adamantine series should therefore be useful for correlating severely biodegraded oils. Grice et al. investigated the effect of biodegradation on diamondoid distribution in a series of crude oil reservoirs in two Australian sedimentary basins [54]. They reported variable susceptibility to microbial degradation for different diamondoids. Wei et al. reported that the concentration of total diamondoids tends to decrease as the biodegradation rank of oil deposits increases [130].

High boiling point biomarkers of steranes and triterpanes, which are relatively resistant to biodegradation, have been used extensively for the correlation of biodegraded crudes [128]. 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) [124, 131]. Studies on oil spills have demonstrated the degradation of C_{23} and C_{24} tricyclic terpanes in intensive weathering [78, 104]. Microbial alteration and removal of the regular steranes and 4α-methylsteranes from petroleum occur after the complete removal of C_{15}–C_{20} isoprenoids, and before or after the hopanes depending on the circumstance.

As mentioned above, terpanes and steranes have many constitutional isomers and stereoisomers. Certain molecular configurations could obstruct microbial degradation. Wang et al. found that 17α(H),21β(H)-22,29,30-trisnorhopane (Tm) is degraded faster relative to 18α(H),21β(H)-22,29,30-trisnorhopane (Ts), resulting in an increase of the Ts/Tm ratio for heavily degraded oil samples [78]. In general, sterane susceptibility to microbial degradation is as follows: ααα 20R > αββ 20R > ααα 20S > αββ 20S > diasteranes. The susceptibility of steranes to biodegradation typically decreases with increasing carbon number for each isomeric configuration $C_{27} > C_{28} > C_{29} > C_{30}$. Diasteranes are particularly resistant to biodegradation. Evidence suggests that the C_{27}–C_{29} steranes are depleted before diasterane alteration. Pregnane and homopregnane have high resistance to biodegradation, comparable to diasteranes [59].

Biodegradation of aromatics: aromatic hydrocarbons comprise a large percentage of crude oil and refined oil products. Many studies have been conducted to investigate their biodegradation. George et al. studied the biodegradation of a suite of oils. The breakdown of aromatic compounds by ring cleavage is an essential biochemical step in the natural carbon cycle and is performed by several kinds of microorganisms [132]. There are three main controls on the susceptibility to biodegradation of cyclic, branched, and aromatic LMW hydrocarbons: carbon skeleton, degree of alkylation, and position of alkylation. The rate of degradation of PAHs decreases with the increase of the number of rings in the PAH molecules. Among APAH homologues, two-ring naphthalene homologues were the most susceptible to biodegradation, while the alkyl homologues of four-ring chrysene were the most resistant to biodegradation. In addition, within each APAH class, the degradation rate tends to decrease with the increase of alkylation level, resulting in a trend of $C_1 \sim > C_2 > C_3 > C_n$ [78, 126].

The positions of alkyl substituents also strongly affect the rate of biodegradation. The biodegradation of methylphenanthrenes is in a decreasing order of 3-MP or 2-MP > 1-MP ≫ 9-/4-MP [61, 126]. 2-/3-Methyl dibenzothiophene biodegrades at the fastest rate within all four isomeric series. In heavy oil sand bitumen, the most refractory isomers of the *m*/*z* 216 cluster appear to be 4-methyl-pyrene (4-MPy) and 1-methyl-pyrene (1-MPy), while other isomers were degraded in different degrees. The C_{26-28} isomers of TASs are very resistant to biodegradation and only degraded under extreme conditions [124]. Preferential depletion of C_{20}–C_{22} TAS isomers is found for the severely biodegraded oils. Aromatic steranes changed slightly in their abundance and distribution pattern in the *m*/*z* 231 fragmentograms, even though most aromatics including methylphenanthrenes and methyldibenzothiophenes were nearly depleted.

In summary, biodegradation affects the oil composition in the following patterns [13, 59, 126, 127, 131]:

- small hydrocarbons degrade faster than large hydrocarbons;
- straight-chain *n*-alkanes degrade faster than branched alkanes;
- GC-UCM in petroleum oil is more resistant to biodegradation than the GC-resolved compounds;
- small aromatics are more susceptible to biodegradation than HMW aromatics;
- increased alkylation level of PAHs significantly decreases susceptibility to microbial degradation;
- microbial degradation is often isomer-specific, and the positions of the alkyl substituents also strongly affect the rate of biodegradation;
- biomarker compounds such as diamondoids, bicyclic sesquiterpanes, triterpanes, steranes, and aromatic steranes are resistant to biodegradation.

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