Environmental Forensics (2002) **3**, 251–262
doi:10.1006/enfo.2002.0098, available online at http://www.idealibrary.com on IDEAL®

Using Multiple Criteria for Fingerprinting Unknown Oil Samples Having Very Similar Chemical Composition

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(*Received May 2002, Revised manuscript accepted July 2002*)

This paper describes a case study in which a multi-criterion approach was used to fingerprinting and identifying mystery oil samples. Three unknown oil samples were received from Quebec on March 28, 2001 for chemical analysis. The main purpose of this analysis was to determine the nature and the type of the products, detailed hydrocarbon composition of the samples, and whether these samples came from the same source. The samples were analyzed by gas chromatography with a flame ionization detector (GC-FID) and by gas chromatography coupled with mass spectrometry (GC-MS). Hydrocarbon distribution patterns of unknown oils were recognized. Multiple suites of analytes were quantified and compared. A variety of diagnostic ratios of "source-specific marker" compounds for interpreting chemical data were further determined and analyzed. The chemical fingerprinting results reveal the following: (1) These three oils are most likely a hydraulic-fluid type oil. (2) These three oils are very "pure", largely composed of saturated hydrocarbons with the total aromatics being only 4–10% of the TPH. (3) The oils are a mixture of two different hydraulic fluids. There is no clear sign indicating they had been weathered. (4) The PAH concentrations are extremely low (<10 μg/g oil) in the oil samples, while the biomarker concentration are unusually high (4700–5500 μg/g oil). (5) Three major unknown compounds in the oil samples were positively identified. They are antioxidant compounds added to oils. (6) Samples 2996 and 2997 are identical and come from the same source. (7) The sample 2998 has group hydrocarbon compositions (including the GC traces, TPH, and total saturates) very similar to samples 2996 and 2997. But, it is not identical in chemical composition to samples 2996 and 2997, and they do not come from the same source.
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Keywords: oil spill; GC-MS; GC-FID; *n*-alkane; polycyclic aromatic hydrocarbons; biomarker.

Introduction

In response to the oil spill identification and specific site investigation needs, attention has recently focussed on the development of flexible, tiered analytical approaches which facilitate the detailed compositional analysis by GC-MS, GC-FID, and other analytical techniques to determine individual petroleum hydrocarbons and their relative distribution patterns in a complex mixture of compounds (Douglas and Uhler, 1993; Nordtest Method, 1991; Page *et al.*, 1995; Sauer and Boehm, 1995; Short *et al.*, 1996; Boehm *et al.*, 1997; 2001; Bence *et al.*, 1996; Wang *et al.*, 1998; 1999). Many EPA and ASTM methods have been modified to improve specificity and sensitivity for measuring spilled oil and petroleum products in soils, waters and contaminated sites. A variety of diagnostic ratios, especially ratios of source-specific oil constituents including PAH homologous groups at different alkylation levels (such as relative distribution of alkylated chrysene series and double ratios of alkylated dibenzothiophenes over alkylated phenanthrenes), isomeric PAHs within the same alkylation levels (such as relative ratios of three methyl-dibenzothiophenes), and biomarker compounds (such as ratios of C_{29} αβ hopane to C_{30} αβ hopane, terpane C_{23}/C_{24}, and steranes C_{27} αββ/C_{29} αββ) for interpreting chemical data from oil spills have been proposed and successfully used for oil source identification and monitoring of weathering and biological degradation processes.

When crude oil or refined product enters the surface environment, it is immediately subject to a number of processes that are collectively known as weathering (Jordan and Payne, 1980). Some hydrocarbon compounds evaporate, some dissolve, some are dispersed, some are photooxidized, some adsorb onto suspended particulate materials, and the majority are eventually biodegraded. The changes in chemical composition of spilled oils due to weathering add great difficulties to the identification of the residual spilled oil in the impacted environment. Under such circumstances, characterization of high-molecular-mass PAHs and biological markers, which are highly degradation-resistant and source-specific, would be necessary for source identification and can make differentiation of oils with similar composition possible.

On March 28, 2001, three unknown oil samples were received from Montreal for hydrocarbon analysis. The main purpose of this analysis was to determine the product nature and type, chemical hydrocarbon composition of the samples, whether these samples match or are identical, and whether the oils have been weathered. In this case study, a multi-criterion approach was used to fingerprint and identify the mystery oil samples. First, we determined the product type by recognizing

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hydrocarbon distribution patterns, then compared PAH profiles, finally we went the extra mile to verify our conclusions by quantifying biomarkers, determining a variety of diagnostic ratios of "source-specific marker" compounds, and identifying additives in the oils.

Experimental

Materials and equipments

Distilled chromatographic solvents were used without further purification. Calibration standards used for the determination of individual and total petroleum hydrocarbons include *n*-alkane standards from C_8 to C_{34} including pristane and phytane, polycyclic aromatic hydrocarbon (PAH) standards (SRM 1491) from the National Institute of Standards and Technology (NIST), and biomarker standards (hopanes and steranes) from Chiron Laboratory of Norway.

Sample preparation

All three oil samples were yellow with the sample 2996 being quite cloudy, 2997 cloudy, and 2998 transparent.

After removing the custody seal number from the sample bottles, approximately 0.2 g of oils were weighed, dissolved in hexane and made up to the final volume of 5.00 mL. The final concentrations of oils were 42.30, 45.94, and 40.52 mg/mL for samples 2996, 2997, and 2998, respectively. A 300 μL of the oil solutions containing 12–14 mg of oil was spiked with appropriate surrogates (100 μL 200 ppm of *o*-terphenyl and 100 μL of mixture of deuterated acenaphthene, phenanthrene, benz[a]anthracene, and perylene, 10 ppm each), and then quantitatively transferred into a 3-g silica gel microcolumns, which was topped with about 1-cm anhydrous granular sodium sulfate and had been pre-conditioned using 20 mL of hexane, for sample cleanup and fractionation.

Hexane (12 mL) and 50% benzene in hexane (v/v, 15 mL) were used to elute the saturated and aromatic hydrocarbons, respectively. For each sample, half of the hexane fraction (labeled F1) was used for analysis of aliphatics, *n*-alkanes, and biomarker terpane and sterane compounds; half of the 50% benzene fraction (labeled F2) was used for analysis of alkylated homologous PAHs and other EPA priority unsubstituted PAHs; the remaining halves of the hexane fraction and 50% benzene fraction were combined into a fraction (labeled F3) and used for the determination of the total GC-detectable petroleum hydrocarbons (TPH) and the unresolved complex mixture of hydrocarbons (UCM). These three fractions were concentrated under a stream of nitrogen to appropriate volumes (~0.4 mL), spiked with appropriate internal standards (50 μL of 200 ppm 5-α-androstane and 50 μL of 20 ppm C_{30}-ββ-hopane, 50 μL of 10 ppm terphenyl-d_{14}, and 50 μL of 200 ppm 5-α-androstane for Fl, F2, and F3 respectively), and then adjusted to an accurate pre-injection volume of 0.50 mL for GC/FID and GC/MS analyses.

Sample analysis

Analyses for *n*-alkane distribution (*n*-C_8 through *n*-C_{41}, pristane and phytane) and TPH were performed on a Hewlett-Packard (HP) 5890 gas chromatograph equipped with a flame-ionization detector (FID) and an HP 7683 autosampler. Analyses of target PAH compounds (including five alkylated PAH homologous groups and other EPA priority PAHs) and biomarker terpanes and steranes were performed on an HP 6890 GC equipped with a HP 5973 mass selective detector (MSD). System control and data acquisition were achieved with an HP G1701 BA MSD ChemStation.

GC-FID analysis provides a baseline resolution of *n*-alkanes from *n*-C_8 to *n*-C_{41} and *n*-C_{17}/pristane and *n*-C_{18}/phytane. Quantitation of the analytes was based on the internal standard compound (5-α-androstane). GC-MS analysis was performed utilizing a selected ion monitoring mode to improve detection limits. The concentrations of the individual PAH and biomarker compounds were determined based on the internal standards d_{14}-terphenyl and C_{30}-ββ-hopane.

For detailed chromatographic conditions, analysis quality control, and quantification methodology, refer to references Wang *et al.*, 1994a, 1994b and 1994c.

Results and Discussion

Determination of hydrocarbon groups and product type

Assessment of chemical composition features of oils and refined products can be illustrated by qualitative and quantitative examination of their GC traces. Crude oil compositions vary widely. Depending on the sources of carbon from which the oils are generated and the geologic environment in which they migrated and from which reservoir, they can have dramatically varied compositions in C_5 to C_{40} carbon range such as relative amounts of paraffinic, aromatic and asphaltenic compounds, large differences in the *n*-alkane distributions and UCM, and significantly different relative ratios of isoprenoids to normal alkanes. Refined petroleum products are obtained from crude oil through a variety of distillation, blending, and catalytic processes. Light distillates are typically products in the C_3 to C_{12} carbon range. They include aviation gas, naphtha, and automotive gasoline. The GC trace of fresh light distillates are featured with dominance of light-end, resolved hydrocarbons and a minimal unresolved complex mixture of hydrocarbons (UCM). Mid-range distillates are typically products in a relative broad carbon range (C_6 to C_{26}) and include kerosene, jet fuel, and diesel products. Classic heavy refined products include fuel No. 6 and lube type oil.

Figure 1 shows the GC/FID chromatograms of F3 for *n*-alkane and TPH analysis. The saturated fractions F1 demonstrated very similar GC/FID chromatogram profiles to their corresponding Fraction 3. Figure 2 shows the GC/MS chromatograms of the *m*/*z* 85 fragment of the saturated hydrocarbons. Due to the increased resolution and higher sensitivity of the MS detector, the distribution of low-abundance *n*-alkanes and isoprenoid compounds, which may not be seen in GC/FID traces, can be more clearly distinguished. Table 1 summarizes the hydrocarbon group analysis results of the oil samples. In addition to the GC-TPH values and percentages of saturates and aromatics in the TPH, the ratios of resolved peaks/TPH and UCM/TPH are listed in Table 1 as well. The GC-TPH are defined as

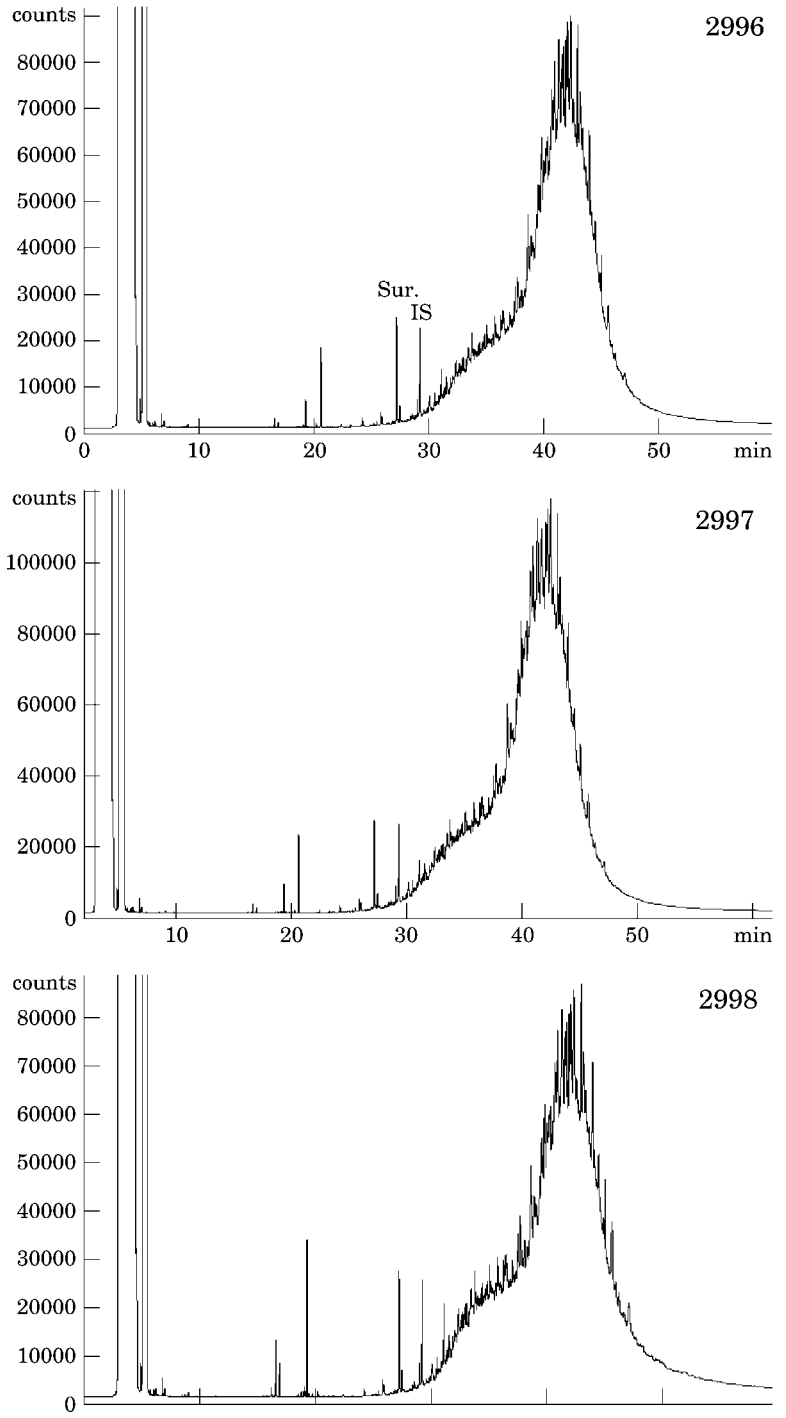

Figure 1. GC-FID chromatograms of Fraction 3 for *n*-alkane and TPH analysis. Sur and IS stand for surrogate *o*-terphenyl and internal standard 5-α-androstane, respectively. The GC traces of F3 are featured by dominance of unresolved complex mixture (UCM) of hydrocarbons with very small amount of resolved peaks being detected.

the sum of all resolved and unresolved distillable hydrocarbon detected by GC. The UCM appears as the "envelop" or hump area between the solvent baseline and the curve defining the base of resolvable peaks.

The major chemical composition features of TPH and saturate hydrocarbons in the samples are summarized as follows:

(1) GC/FID chromatograms provide a fingerprint picture of major oil components and information about weathering extent of the oil. The GC traces of F3 (Figure 1) are clearly dominated by the UCM of hydrocarbons with very small amount of resolved peaks being detected (the ratios of the GC-resolved peaks to the total GC area were determined to be only 0.04–0.05). The GC chromatographic profile and shape of the UCM "humps" are significantly different from crude oils and most refined products. The hydrocarbons of the samples are mainly distributed in a carbon range from C_{20} to C_{37} with the retention time ranging between 30 and 50 min. No hydrocarbon was detected prior to 16 min. Also, there were almost no *n*-alkanes were detected by the FID detector.

Generally, asphaletenes and polar compounds in crude oils and heavier refined products are retained on

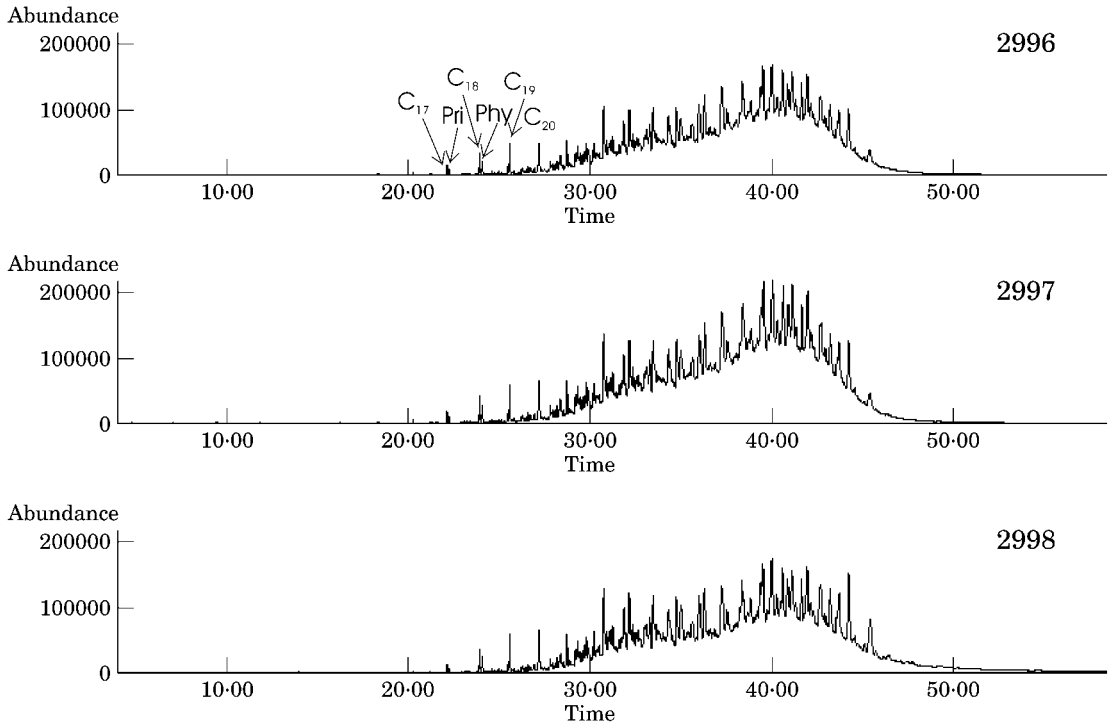

Figure 2. GC-MS chromatograms of the m/z 85 fragment of the saturated hydrocarbons, more clearly showing the distribution of the low-abundant n-C_{17} to n-C_{20} and two unresolved complex mixtures of hydrocarbon "humps".

the cleanup column, and high molecular weight saturated and aromatic hydrocarbons (called the GC-undefined hydrocarbons) are retained on the GC column and would not be detected by the FID detector. All of these result in the TPH values determined by GC being exclusively smaller than theoretical TPH values (Bragg *et al.*, 1993; Wang *et al.*, 1994c). For these three oils, however, the GC-TPH were determined to be close to 1000 mg/g oil (960–997 mg/g oil), significantly higher than the corresponding TPH values for crude oils and most refined products. The unusually high TPH values clearly indicate that the oil is "special" and very "pure".

The aromatic fractions in the TPH were determined to be only 4% for samples 2996 and 2997, and 10% for the sample 2998, significantly lower than crude oils and most refined products as well.

All these GC trace features suggest that the oil is a hydraulic fluid.

(2) GC traces, in particular the GC-MS chromatograms at m/z 85, clearly exhibit two "humps" with the maxima being around 35 and 42 min for the lighter and heavier one, respectively. This suggests that the oil is a mixture of two hydraulic fluid oils. The resolved peaks were mostly located in the second heavier component portion. There is no clear sign indicating they had been weathered.

(3) Samples 2996 and 2997 show nearly identical GC chromatographic profiles, n-alkane and isoprenoid distribution patterns (m/z 85), and the relative ratios of low abundant hydrocarbons C_{17}/C_{18} (0.51 *v.* 0.53), C_{17}/pristane (1.52 *v.* 1.54), C_{18}/phytane (1.09 *v.* 1.08), and pristane/phytane (0.37 *v.* 0.38). This implies that these two samples were probably identical and from the same source. (Note: these ratios were determined from GC/MS measurements at m/z 85).

(4) In general, the sample 2998 shows very similar GC chromatographic profiles to samples 2996 and 2997. However, the relative ratios of low abundant hydrocarbons C_{17}/C_{18}, C_{17}/pristane, C_{18}/phytane, and pristane/phytane were determined to be 0.41, 1.68, 1.14, and 0.28, respectively, slightly different from the corresponding ratios for samples 2996 and 2997. In addition, an abundant compound at the retention time of 20.61 min was detected in samples 2996 and 2997, but not found in the sample 2998. The questions which must be answered at this stage are: Do these three oils come from the same source? Is the sample 2998 really different from samples 2996 and 2997 in chemical

Table 1. Hydrocarbon group analysis results

Sample	GC-TPH (mg/g oil)	GC-saturates (mg/g oil)	Total aromatics (mg/g oil)	GC-saturates/ GC-TPH (%)	GC-aromatics/ GC-TPH (%)	Resolved peaks Total GC area	UCM GC-TPH
2996	997	956	41	96	4	0.04	0.96
2997	990	955	36	96	4	0.04	0.96
2998	959	861	98	90	10	0.05	0.95

composition, or just because of contamination or weathering? In order to unambiguously differentiate and identify these samples, the multi-criterion approach to fingerprinting must be adopted, that is, analyses of more than one suite of analytes were performed in source identification.

Distribution of target alkylated PAH homologues and other EPA priority PAHs

PAH compounds, especially the high molecular mass PAHs and their alkylated homologues are relatively stable. Therefore, the distribution patterns and the diagnostic ratios of these PAH compounds can be used as fate indicators of oil in the environment and oil source markers (Stout *et al.*, 1998; Sauer and Boehm, 1991; Short *et al.*, 1996; Fayad and Overton, 1995; Kennicutt, 1988; Teal *et al.*, 1992; Farran *et al.*, 1987; Page *et al.*, 1995; Wang *et al.*, 1997; 1999).

Figure 3 shows the total ion GC/MS chromatograms (in SIM mode) of the samples for analyses of BTEX compounds and target PAHs. The "humps" of unresolved complex mixture in 35–45 min are pronounced for samples 2996 and 2997, while the sample 2998 only shows a much smaller "hump". Table 2 summarizes quantitation results of five petroleum-characteristic alkylated PAH homologous series (alkylated naphthalene, phenanthrene, dibenzothiophene, fluorene, and chrysene series) and other EPA priority PAHs. Figure 4 shows the distribution of these target PAHs.

GC/MS measurements show that the samples only contained trace amount of BTEX and other lighter C_3-benzene compounds. The concentrations of target PAHs in the samples were found to be very low. The total of the five target alkylated PAHs and other EPA priority PAHs were determined to be 6.4, 6.5, and 6.5 μg/g of oil (Table 2) for samples 2996, 2997 and 2998 respectively, which are significantly lower than in crude oils and most refined products. Among the other EPA priority PAHs, the 2-ring compound biphenyl was the most abundant and no high molecular weight (4- to 6-ring) PAHs were detected in the sample 2998 (Table 2). Samples 2996 and 2997 show nearly identical PAH distribution patterns and fingerprints. However, sample 2998 demonstrates different PAH distribution pattern from the samples 2996 and 2997. For example, sample 2998 showed higher concentration of the total naphthalenes (5.9 *v.* 4.5 μg/g oil) but much lower concentrations of the other four alkylated PAH homologues (0.31 *v.* 0.97 μg/g oil for the alkylated phenanthrene series, 0.10 *v.* 0.52 μg/g oil for the alkylated dibenzothiophene series, and 0.02 *v.* 0.17 μg/g oil for the alkylated chrysene series).

The dominance of alkylated naphthalenes among five target alkylated PAH homologous series is pronounced for all three samples. Different from crude oils and most refined products, however, the alkylated naphthalenes demonstrate a very unique distribution pattern with the concentrations of the C_1-naphthalenes being many times higher than naphthalene and C_2- to C_4-naphthalenes.

In PAH fingerprinting, we simply do not quantify PAH alone, but determine a number of diagnostic ratios of "source-specific marker" PAH compounds as well (see Table 2). In recent years, determination of ratios of the conventional diagnostic PAH and biomarker compounds, in particular determination of relative distribution of source-specific isomers within the same alkylation levels and isomeric groups, has been used for oil source identification (Fayad and Overton, 1995; Wang *et al.*, 1995; 1999). The differences between the isomer distribution reflect the differences of the depositional environment during oil formation. Compared to PAH homologous groups at different alkylation levels, higher analytical accuracy and precision may be achieved for determination of ratios of source-specific isomers within the same alkylation levels, due to the close match of physical/chemical properties of the isomers. Also, the relative distributions of isomers at the same ratios of m/z are subject to little interference from weathering. Hence they can be more positively used for oil spill identification and differentiation. Analysis of the diagnostic ratios of "source-specific" PAHs (Table 2) clearly reveals the following:

(1) Isomeric distributions of 2-*m*-naphthalene to 1-*m*-naphthalene at m/z 142, (3- + 2-*m*-phenanthrene) to (4-/9- + l-*m*-phenanthrene) at m/z 192 were determined to be 1.83, 1.80, and 1.90; and 0.86, 0.84, and 0.80 for samples 2996, 2997, and 2998, respectively. Obviously, the differences in these isomeric ratios between samples are not significant.
(2) The double ratios $C_2D/C_2P : C_3D/C_3P$) were determined to be 0.69 : 0.15, 0.68 : 0/16, and 0.25 : 0.01 for samples 2996, 2997, and 2998, respectively.
(3) The ratios of the most abundant alkylated naphthalenes at different alkylation levels (C_0–N : C_1–N : C_2–N : C_3–N : C_4–N, normalized to the highest molecular weight C_4-naphthalene series) were determined to be 0.34 : 8.9 : 6.2 : 3.3 : 1.0, 0.33 : 8.7 : 6.0 : 3.0 : 1.0, and 0.20 : 23.0 : 11.1 : 4.1 : 1.0 for the sample 2996, 2997, and 2998, respectively. Obviously, the relative distribution of the alkylated naphthalenes for the sample 2998 is greatly different from the other two samples. Also, this difference cannot be explained by weathering effect. It has been well demonstrated that weathering causes chemical composition changes of oil and would result in development of a profile in each alkylated PAH group showing the composition changes of $C_0- > C_1- > C_2- > C_3- > C_4-$.
(4) The relative distributions of the alkylated naphthalene, dibenzothiophene, fluorene, and chrysene series to the alkylated phenanthrene series were determined to be 4.8 : 1.0 : 0.54 : 0.29 : 0.18, 4.7 : 1.0 : 0.55 : 0.29 : 0.19, and 19.1 : 1.0 : 0.32 : 0.45 : 0.06 for samples 2996, 2997, and 2998, respectively. The alkylated phenanthrene series were chosen as the normalization standard is because they are considerably abundant and more degradation-resistant than alkylated naphthalene, dibenzothiophene and fluorene series.

For most spill samples, it is preferred to determine ratios of alkylated PAH homologues normalizing to chrysene series. This is because environmental exposure will preferentially strip out lighter PAHs such as naphthalenes, while the most degradation-resistant chrysenes are not likely change much. However, this

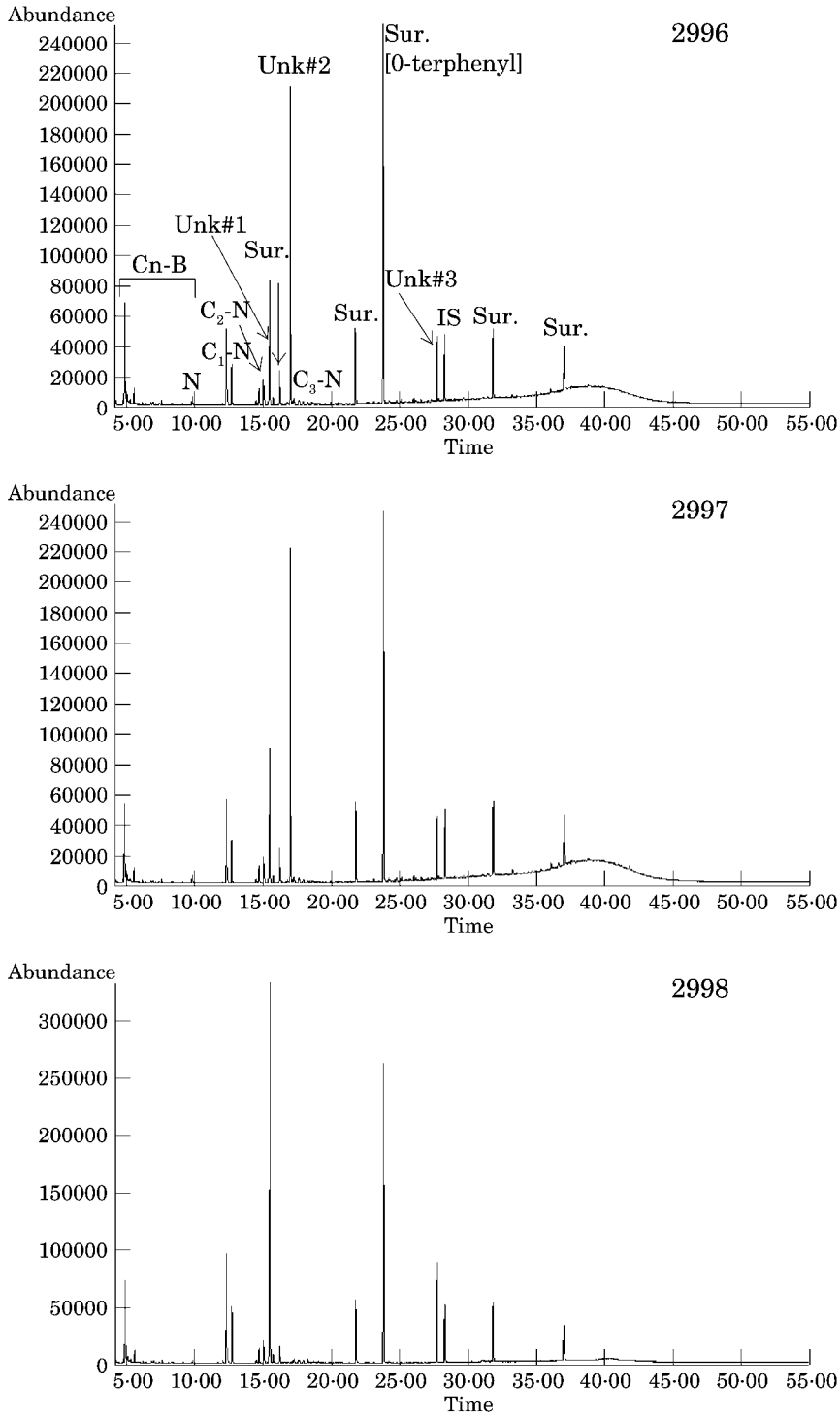

Figure 3. The GC-MS total ion chromatogram (in SIM mode) for analyses of BTEX and PAH compounds. C_n–B, N, Unk, Sur and IS represent alkyl-benzene, naphthalene, major unknown compounds, deuterated surrogates and internal standard.

situation does not allow us to determine relative ratios normalizing to chrysenes, because concentrations of chrysenes are extremely low compared to other series, determination of diagnostic ratios normalizing to chrysenes might introduce bigger uncertainty and mislead conclusions.

In summary, samples 2996 and 2997 not only show nearly identical PAH distribution, but also very matching diagnostic ratios of "source-specific" PAHs. In contrast, sample 2998 demonstrates different PAH distribution profile and diagnostic ratios from samples 2996 and 2997.

Distribution and diagnostic ratios of biomarker compounds

Figures 5 and 6 show GC/MS distribution chromatograms of the highly degradation-resistant biomarker terpane and sterane compounds at m/z 191 and at m/z 217. A wide range of terpanes are present in the samples from C_{20} to C_{35} with the C_{30} and C_{29} αβ-hopanes and 22S/22*R* C_{31} homohopanes being the most abundant. In addition, the presence of a triplet (at retention time of 35 min) in the samples is also identified. This feature is very unique and has rarely been seen in crude oils and

Table 2. PAH analysis results

Sample	2996 (μg/g oil)	2997 (μg/g oil)	2998 (μg/g oil)
Alkylated PAHs			
Naphthalene			
C0-N	0.08	0.08	0.03
C1-N	2.05	2.09	3.44
C2-N	1.43	1.43	1.66
C3-N	0.74	0.72	0.62
C4-N	0.23	0.24	0.15
Sum	4.53	4.57	5.91
Phenanthrene			
C0-P	0.03	0.03	0.02
C1-P	0.18	0.18	0.07
C2-P	0.26	0.28	0.08
C3-P	0.24	0.25	0.09
C4-P	0.22	0.24	0.05
Sum	0.94	0.97	0.31
Dibenzothiophene			
C0-D	0.02	0.02	0.00
C1-D	0.12	0.12	0.06
C2-D	0.18	0.19	0.02
C3-D	0.19	0.20	0.02
Sum	0.51	0.53	0.10
Fluorene			
C0-F	0.03	0.03	0.03
C1-F	0.05	0.05	0.04
C2-F	0.08	0.09	0.04
C3-F	0.11	0.12	0.03
Sum	0.27	0.28	0.14
Chrysene			
C0-C	0.02	0.02	0.01
C1-C	0.03	0.03	0.01
C2-C	0.06	0.07	0.00
C3-C	0.05	0.06	0.00
Sum	0.17	0.18	0.02
TOTAL	6.42	6.51	6.48
Other PAHs			
Biphenyl (Bph)	0.04	0.04	0.10
Acenaphthylene (Acl)	0.01	0.01	0.02
Acenaphthene (Ace)	0.02	0.02	0.03
Anthracene (An)	0.00	0.00	0.00
Fluoranthene (FL)	0.00	0.00	0.00
Pyrene (Py)	0.01	0.01	0.00
Benz[a]anthracene (BaA)	0.00	0.00	0.00
Benzo[b]fluoranthene (BbF)	0.00	0.00	0.00
Benzo[k]fluoranthene (BkF)	0.00	0.00	0.00
Benzo[e]pyrene (BeP)	0.01	0.01	0.00
Benzo[a]pyrene (BaP)	0.00	0.00	0.00
Perylene (Pe)	0.00	0.00	0.00
Indeno[1,2,3cd]pyrene (IP)	0.00	0.00	0.00
Dibenz[a,h]anthracene (DA)	0.00	0.00	0.00
Benzo[ghi]perylene (BgP)	0.00	0.00	0.00
TOTAL	0.09	0.08	0.16
Diagnostic ratios			
2-m-N/1-m-N	1.83	1.88	1.90
(3 + 2-m-phen)/(4-/9-+1-m-phen)	0.86	0.85	0.80
Phens/dibens	1.84	1.83	3.10
$C_2D/C_2P:C_3P/C_3D$	0.69:0.15	0.68:0.16	0.25:0.01
$C_0N:C_1N:C_2N:C3N:C_4N$(normalizing to C_4N)	0.34:8.9:6.2:3.3:1.0	0.33:8.7:6.0:3.0:1.0	0.20:23.0:11.1:4.1:1.0
Naphs:Phens:Dibens:Fluos:Chrys (normalizing to phens)	4.8:1.0:0.54:0.29:0.18	4.7:1.0:0.55:0.29:0.19	19.1:1.0:0.32:0.45:0.06

petroleum products (Kvenvolden *et al.*, 1995). As for steranes at m/z 217 and 218, in addition to the presence of diasteranes, the dominance of C_{27}, C_{28}, and C_{29} 20S/20*R* steranes, particularly the epimers of αββ-steranes, is obvious. Table 3 summarizes quantitation results for the target terpanes and two groups of αββ-steranes (C_{27} and C_{29}). These biomarker compounds have been increasingly used in recent years for the purposes of source identification and differentiation of oils, and monitoring the weathering and degradation process of oil hydrocarbons under a wide variety of conditions (Peters and Moldowan, 1993; Barakat *et al.*, 1999; Bence *et al.*, 1996; Kvenvolden *et al.*, 1995; Wang *et al.*, 1994c; 1999; 2001; Kennicutt, 1988; McKirdy *et al.*, 1994; Page *et al.*, 1996; Killops and Howell, 1991; Short *et al.*, 1999; Zakaria *et al.*, 2000).

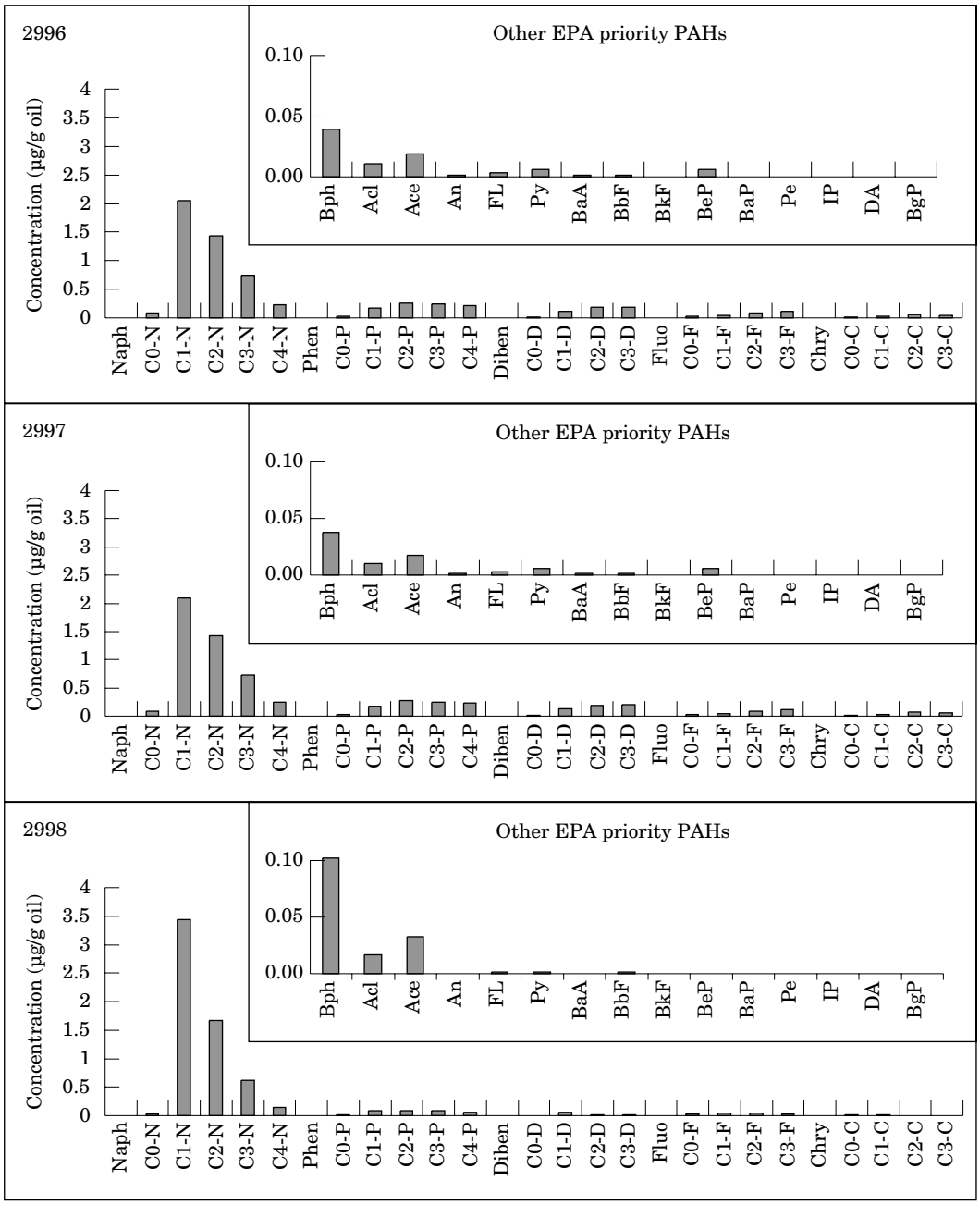

Figure 4. Alkylated PAH fingerprints of the unknown oil samples, illustrating the PAH compositional features. N, P, D, F, and C represent naphthalene, phenanthrene, dibenzothiophene, fluorene, and chrysene, respectively; 0, 1, 2, 3, and 4 represent carbon numbers of alkyl groups in alkylated PAH homologues. The fingerprints of the other EPA priority PAHs are shown in the left insets. Refer to Table 2 for the abbreviations of these PAHs.

From Figures 5 and 6 and Table 3, it can be seen that the samples 2996 and 2997 are nearly identical in distribution patterns and profiles of biomarker terpanes and steranes. The total target biomarkers were determined to be 4701, 4759, and 5464 µg per gram of oil for samples 2996, 2997, and 2998, respectively. In comparison to most crude oils, these three samples demonstrate extremely high concentrations of terpanes and steranes.

The sample 2998 shows different distribution pattern of biomarkers from the samples 2996 and 2997. The concentrations of C_{29} and C_{30} hopanes in the sample 2998 match the concentrations of C_{29} and C_{30} hopanes in samples 2996 and 2997 (Table 3), but, the concentrations of C_{23} and C_{24}, and the sum of C_{31} to C_{35} homohopanes in the sample 2998 are significantly lower (39 *v.* 145–152 µg/g oil for C_{23}, and 36 *v.* 83–87 µg/g oil for C_{24}) and higher (2072 *v.* 1358–1376 µg/g oil for C_{31-35}) than the corresponding compounds in samples 2996 and 2997, respectively.

The relative ratios of target biomarker terpanes C_{23}/C_{24}, C_{29}/C_{30}, Ts/Tm (Ts: 18α(H), 21β(H)-22,29,30-trisnorhopane; Tm: 17α(H), 21β(H)-22,29,30-trisnorhopane), C_{29}-αβ-hopane/C_{30}-αβ-hopane, C_{30}βα/C_{30}αβ-hopane, $C_{31}(22S)/C_{31}(22R)$, $C_{32}(22S)/C_{32}(22R)$, $C_{33}(22S)/C_{33}(22R)$, $C_{33}(22S)/C_{33}(22R)$, $C_{33}(22S)/C_{33}(22R)$, $C_{30}/(C_{31}+C_{32}+C_{33}+C_{34}+C_{35})$, and C_{27}αββ/C_{29} αββ are found very much the same for samples 2996 and 2997. The relative distribution ratios of the unique triplet biomarker are also nearly identical

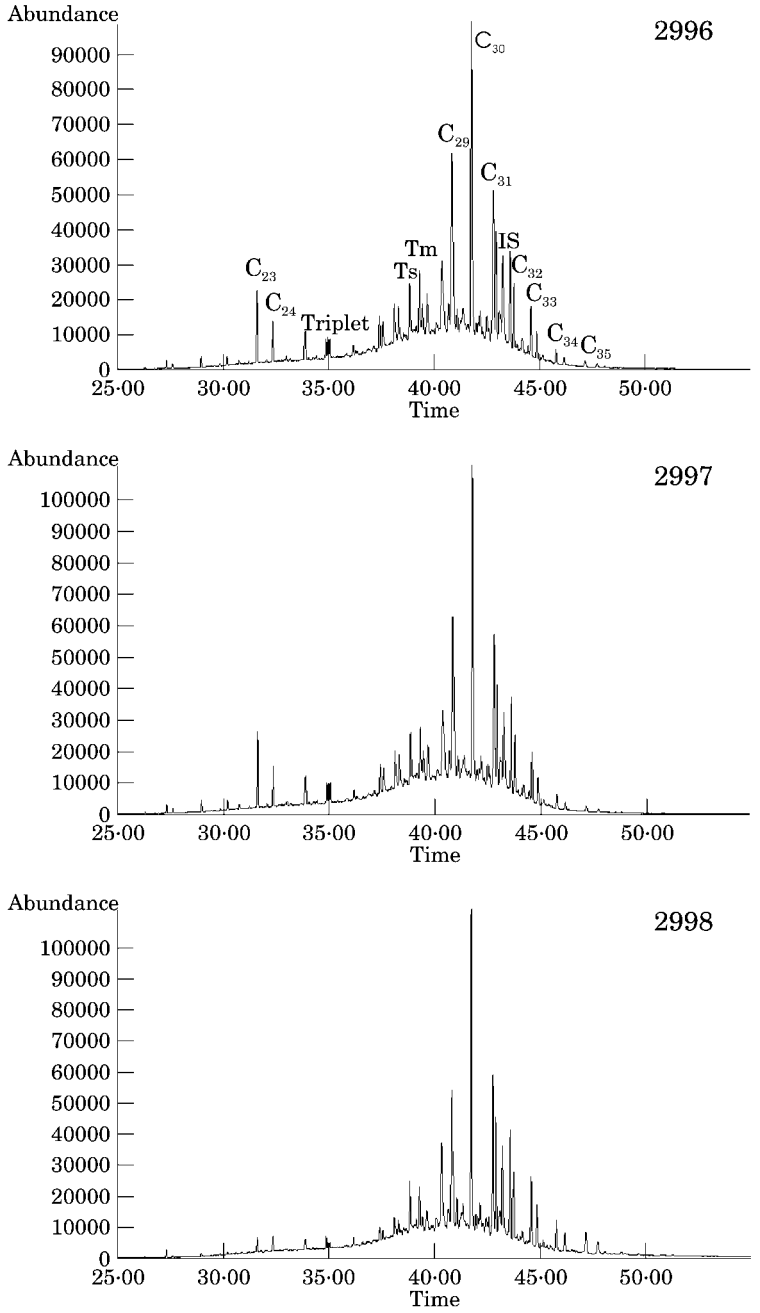

Figure 5. Comparison of distribution of biomarker terpane compounds (m/z 191). C_{23}, C_{24}, C_{29}, C_{30}, C_{31} to C_{35}, Ts, Tm represent C_{23} and C_{24} tricyclic terpanes, C_{29}- and C_{30}-αβ-hopanes, 22S/22R epimer pairs of C_{31} to C_{35} homohopanes, and 18α(H), 21β(H)-22, 29,30-trisnorhopane (Ts) and 17α(H), 21β(H)-22,29,30-trisnorhopane (Tm), respectively.

too. All these evidences, in combination with the TPH and PAH analysis results, unambiguously point toward to the conclusion that samples 2996 and 2997 are identical and from the same source.

The biomarker distribution profile of the sample 2998 looks similar to that of samples 2996 and 2997, but it is indeed different and is not from the same source as samples 2996 and 2997, evidenced by not only differences in the biomarker quantitation results, but also in diagnostic ratios of target biomarker compounds.

It is important to note that the fingerprinting results described above strongly demonstrate the necessity to analyze for more than one suite of analytes in source identification. Characterization of PAH and biomarker compounds must include determination of both concentrations and relative distributions, and should not be just measuring peak ratios alone. The quantified biomarker data show that the two set of samples have uniquely different analyte distribution, irrespective of the what the ratios might show. This is important because it is possible to have situation where a source might have similar biomarker ratio but very different actual amounts of biomarkers.

Identification of major unknown compounds

Three major unknown compounds with remarkable abundances in the aromatic fractions (F2) were positively (>96%) identified. They are 2, 6-bis

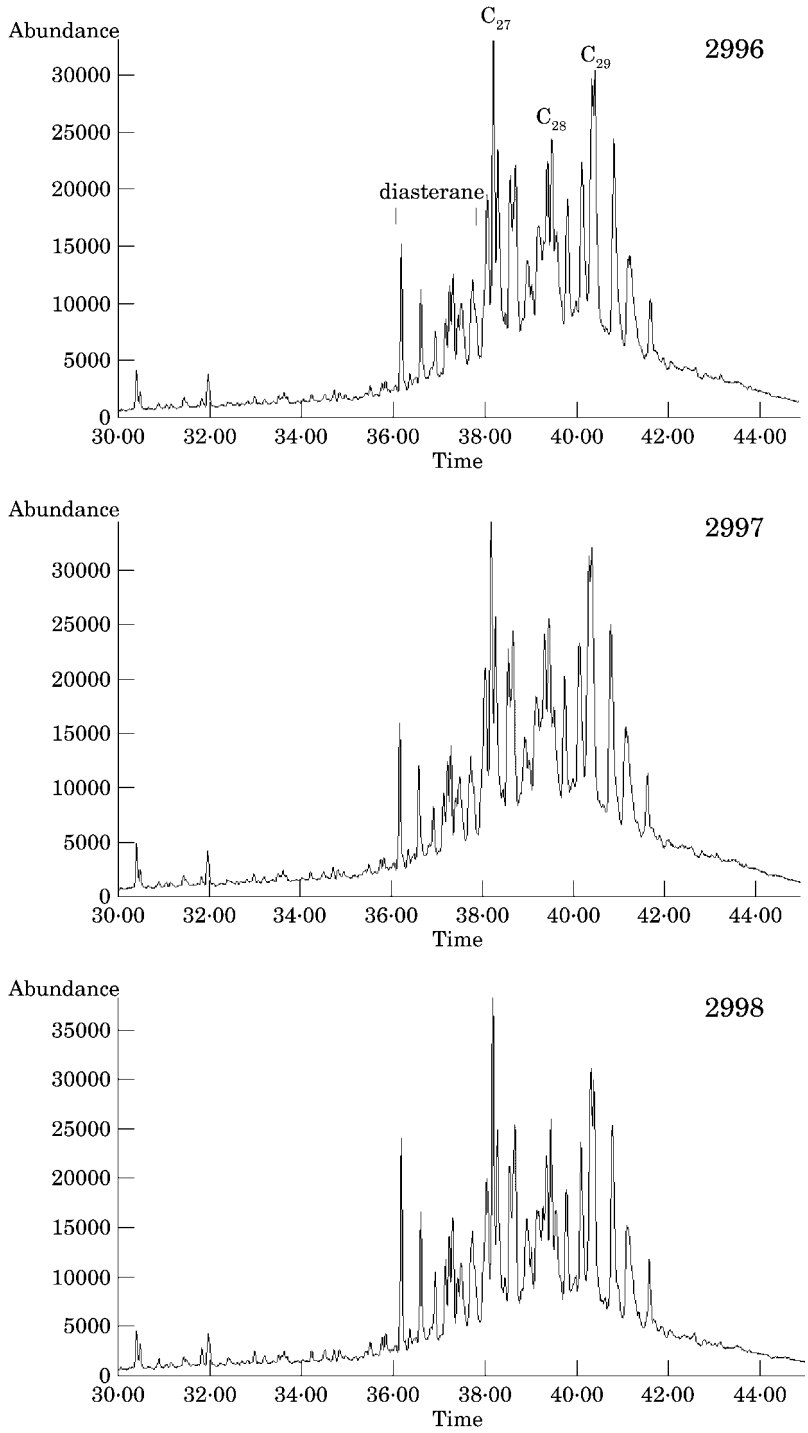

Figure 6. Comparison of distribution of biomarker sterane compounds (m/z 217). C_{27}, C_{28}, and C_{29} represent four ααα and αββ (20*S*/20*R*) epimers of C_{27}-cholestanes, C_{28}-ergostanes, and C_{29}-stigmastanes.

(1,1-dimethylethyl)-phenol at 15.49 min; butylated hydroxytoluene or 2,6-di-*tert*-butyl-4-methylphenol at 17.01 min; and *N*-phenyl-1-naphthalenamine at 27.72 min (Figure 3).

Samples 2996 and 2997 contained these three compounds with the concentrations being nearly identical. However, butylated hydroxytoluene (BHT) was not detected in the sample 2998. The sample 2998 only contained the first and the last compounds with the abundance of 2, 6-bis (1,1-dimethylethyl)-phenol being largely higher than *N*-phenyl-1-naphthalenamine.

All these three compounds identified are antioxidant compounds, also called inhibitors (Ullmann's Encyclopedia, 1985; Bland and Davidson, 1967). They are added to oxidizable organic materials (such as lubricants and gasoline) to retard autooxidation and, in general, to prolong the useful life of the substrates. There are two major classes of antioxidant compounds, or additives used for petroleum products, aromatic amines and alkyl-substituted phenols. Hydraulic oils are often stabilized with 0.5–1.0% of zinc dialkyldithiocarbamates in combination with 0.5–1.0% of a

Table 3. Quantitation results and diagnostic ratios of major biomarke compounds

	2996	2997	2998
Quantitation Results (μg/g oil)			
C_{23}	145.3	151.7	38.6
C_{24}	82.5	87.0	35.9
C_{29}	549.9	554.4	523.9
C_{30}	1049.5	1054.8	1164.9
$C_{31(S)}$	415.8	427.2	504.3
$C_{31(R)}$	259.4	262.8	333.8
$C_{32(S)}$	231.4	224.9	330.7
$C_{32(R)}$	149.0	148.0	196.4
$C_{33(S)}$	126.2	128.7	219.6
$C_{33(R)}$	73.7	75.0	136.7
$C_{34(S)}$	45.1	48.3	130.6
$C_{34(R)}$	22.0	22.1	63.8
$C_{35(S)}$	19.7	22.3	87.2
$C_{35(R)}$	15.2	16.8	68.4
Ts	133.5	136.9	137.2
Tm	148.0	148.3	128.9
C_{27}-aββ-steranes	529.2	534.0	596.5
C_{29}-aββ-steranes	705.4	715.7	766.9
Sum of C_{31} to C_{35} homohopanes	1358	1376	2072
Total	4701	4759	5464
Diagnostic Ratios			
C_{23}/C_{24}	1.76	1.74	1.08
C_{23}/C_{30}	0.14	0.14	0.03
C_{24}/C_{30}	0.08	0.08	0.03
C_{29}/C_{30}	0.52	0.53	0.45
Triplet (RT = ~35 min)	1.14:1.08:1.00	1.14:1.12:1.00	2.22:1.09:1.00
$C_{31}(S)/C_{31}(R)$	1.60	1.63	1.51
$C_{32}(S)/C_{32}(R)$	1.55	1.52	1.68
$C_{33}(S)/C_{33}(R)$	1.71	1.72	1.61
$C_{34}(S)/C_{34}(R)$	2.05	2.19	2.05
$C_{35}(S)/C_{35}(R)$	1.30	1.33	1.28
$C_{30}/(C_{31}+C_{32}+C_{33}+C_{34}+C_{35})$	0.77	0.77	0.56
Ts/Tm	0.90	0.92	1.06
C_{27}aββ-steranes/ C_{29}aßß-steranes	0.75	0.75	0.78

phenolic antioxidant (such as BHT) or with an aminic antioxidant (alkylated diphenylamine, or phenylnaphthalamine or its derivatives).

This finding clearly supports the general conclusion described above, that is, samples 2996 and 2997 are identical and come from the same source, and the sample 2998 is different from samples 2996 and 2997 and does not come from the same source as samples 2996 and 2997 do.

Summary

This paper describes an analytical approach using "source-specific marker" compounds and their diagnostic ratios for identification and differentiation of unknown oil samples with very similar but not identical chemical compositions. The chemical fingerprinting evidences and data interpretation results reveal the following:

(1) The oil are most likely hydraulic fluid type oil.
(2) The three oils are very "pure", largely composed of saturated hydrocarbons. The aromatic fractions in the TPH were determined to be only 4% for samples 2996 and 2997.
(3) The oils are a mixture of two different type of hydraulic fluids with the maxima of the lighter portion being around 35 min and of the heavier portion being around 42 min in the GC/FID chromatograms. There is no clear sign indicating they had been weathered.
(4) The PAH concentrations are extremely low (<10 μg/g oil) and the biomarker concentrations are unusually high (4700–5500 μg/g oil).
(5) Three major unknown compounds in the oil samples were positively identified as 2,6-bis (1,1-dimethylethyl)-phenol at 15.49 min, butylated hydroxytoluene at 17.01 min, and *N*-phenyl-1-naphthalenamine at 27.72 min. All these three compounds are antioxidant compounds added to oils.
(6) Samples 2996 and 2997 are identical and come from the same source.
(7) The sample 2998 is different from samples 2996 and 2997 and does not come from the same source as samples 2996 and 2997 do, but it has bulk group hydrocarbon compositions, such as the TPH and total saturates, very similar to samples 2996 and 2997. From this point of view, the sample 2998 may be used to replace samples 2996 and 2997 for certain applications.

References

Barakat, A.O., Mustafa, A.R., Rullkotter, J. and Hegazi, A.R. 1999. Application of a multimolecular marker approach to fingerprint petroleum pollution in the marine environment. *Mar. Pollut. Bull.* **38**, 535–544.

Bence, A.E., Kvenvolden, K.A. and Kennicutt II, M.C. 1996. Organic geochemistry applied to environmental assessments of Prince William Sound, Alaska, after the Exxon Valdez oil spill—a review. *Org. Geochem.* **24**, 7–42.

Bland, W.F. and Davidson, R.L. (Eds). 1967. In: *Petroleum Processing Handbook*. New York, McGraw-Hill Book Company.

Boehm, P.D., Douglas, G.S., Burns, W.A., Mankiewicz, P.J., Page, D.S. and Bence, A.E. 1997. Advances in petroleum hydrocarbon chemical fingerprinting and allocation techniques after the Exxon Valdez oil spill. *Mar. Pollut. Bull.* **34(8)**, 599–613.

Boehm, P.D., Page, D.S., Burns, W.A., Bence, A.E., Mankiewicz, P.J. and Brown, J.S. 2001. Resolving the origin of the petrogenic hydrocarbon background in Prince William Sound, Alaska. *Environ. Sci. Technol.* **35(3)**, 471–479.

Bragg, J.R., Prince, R.C., Harner, E.J. and Atlas, R.M. 1993. Bioremediation effectiveness following the Exxon Valdez Spill. In: *Proceedings of the 1993 Oil Spill Conference*, pp. 435–447. Washington, DC, American Petroleum Institute.

Douglas, G.S. and Uhler, A.D. 1993. Optimizing EPA methods for petroleum-contaminated site assessments. *Environ. Testing Anal.* **May/June**, 46–53.

Farran, A., Grimelt, J., Albaiges, J., Botello, A.V. and Macko, S.A. 1987. Assessment of petroleum pollution in a Mexican River by molecular markers and carbon isotope ratios. *Mar. Pollut. Bull.* **18**, 284–289.

Fayad, N.M. and Overton, E. 1995. A unique biodegradation pattern of the oil spilled during the 1991 Gulf War. *Mar. Pollut. Bull.* **30**, 239–246.

Jordan, R.E. and Payne, J.R. 1980. *Fate and Weathering of Petroleum Spills in the Marine Environment: A Literature Review and Synopsis*. Ann Arbor Science Publishers, Ann Arbor, Michigan.

Kennicutt II, M.C. 1988. The effect of biodegradation on crude oil bulk and molecular composition. *Oil Chem. Pollut.* **4**, 89–112.

Killops, S.D. and Howell, V.J. 1991. Complex series of pentacyclic triterpanes in a lacustrine sourced oil from Korea Bay Basin. *Chem. Geol.* **91**, 65–79.

Kvenvolden, K.A., Hostettler, F.D., Carlson, P.R., Rapp, J.B., Threlkeld, C.N. and Warden, A. 1995. Ubiquitous tarballs with a California-source signature on the shorelines of Prince William Sound, Alaska. *Environ. Sci. Technol.* **29**, 2684–2694.

McKirdy, D.M., Summons, R.E., Padley, D., Serafini, K.M., Boreham, C.J. and Struckmeyer, H.I. 1994. Molecular fossils in coastal bitumens from Southern Australia: signatures of precursor biota and source rock environments. *Org. Geochem.* **21**, 265–286.

NORDTEST, *Nordtest Method*, NT Chem 001, Ed. 2. NORDTEST. Espoo, Finland, 1991.

Page, D.S., Boehm, P.D., Douglas, G.S. and Bence, A.E. 1995. Identification of hydrocarbon sources in the benthic sediments of Prince William Sound and the Gulf of Alaska following the Exxon Valdez Spill. In: *Exxon Valdez Oil Spill: Fate and Effects in Alaska Waters*, pp. 41–83. (Wells, P.G., Butler, J.N. and Hughes, J.S., Eds). Philadelphia, PA, ASTM.

Page, D.S., Boehm, P.D., Douglas, G.S., Bence, A.E., Burns, W.A. and Mankiewiczl, P.J. 1996. The natural petroleum hydrocarbon background in subtidal sediments of Prince William Sound, Alaska, USA. *Environ. Toxicol. Chem.* **15(8)**, 1266–1281.

Peters, K.E. and Moldowan, J.W. 1993. *The Biomarker Guide: Interpreting Molecular Fossils in Petroleum and Ancient Sediments*. New Jersey, Prentice Hall.

Sauer, T.C. and Boehm, P.D. 1991. The use of defensible analytical chemical measurements for oil spill natural resource damage assessment. In: *Proceedings of the 1991 Oil Spill Conference*, pp. 363–370. Washington, DC, American Petroleum Institute.

Sauer, T.C. and Boehm, P.D. 1995. *Hydrocarbon Chemistry Analytical Methods for Oil Spill Assessments*. Technical Report 95–032. Washington, DC, Marine Spill Response Corporation.

Short, J.W., Jackson, T.J., Larsen, M.L. and Wade, T.L. 1996. Analytical methods used for the analysis of hydrocarbons in crude oil, tissues, sediments, and seawater collected for the natural resources damage assessment of the Exxon Valdez oil spill. In: *American Fisheries Society Symposium*, Vol. 18, pp. 140–148. (Rice, S.D., Spies, R.B., Wolfe, D.A. and Wright, B.A., Eds). Bethesda, Maryland, American Fisheries Society Symposium.

Short, J.W., Kvenvolden, K.A., Carlson, P.R., Hostettler, F.D., Rosenbauer, R.J. and Wright, B.A. 1999. Natural hydrocarbon background in benthic sediments of Prince William Sound, Alaska: oil vs coal. *Environ. Sci. Technol.* **33**, 34–42.

Stout, S.A., Uhler, A.D., Naymik, T.G. and McCarthy, K.J. 1998. Environmental forensics: unravelling site liability. *Environ. Sci. Technol.* **32**, 260A–264A.

Teal, J.M., Farrington, J.W., Burns, K.A., Stegeman, J.J., Tripp, B.W., Woodin, B. and Phinney, C. 1992. The west Falmouth oil spill after 20 years: fate of fuel oil compounds and effects on animals. *Mar. Pollut. Bull.* **24**, 607–614.

Campbell, F.T., Pfefferkom, R. and Rounsaville, J.F. (Eds). 1985. In: *Ullmann's Encyclopedia of Industrial Chemistry*, Volume A3, pp. 91–111. Weinheim, Germany, VCH.

Wang, Z.D. and Fingas, G. 1995. Use of methyldibenzothiophenes as markers for differentiation and source identification of crude and weathered oils. *Environ. Sci. Technol.* **29**, 2841–2849.

Wang, Z.D., Fingas, M. and Li, K. 1994a. Fractionation of ASMB oil, identification and quantitation of aliphatic aromatic and biomarker compounds by GC/FID and GC/MSD (Part I). *J. Chromatogr. Sci.* **32**, 361–366.

Wang, Z.D., Fingas, M. and Li, K. 1994b. Fractionation of ASMB oil, identification and quantitation of aliphatic aromatic and biomarker compounds by GC/FID and GC/MSD (Part II). *J. Chromatogr. Sci.* **32**, 367–382.

Wang, Z.D., Fingas, M. and Sergy, G. 1994c. Study of 22-year-old arrow oil samples using biomarker compounds by GC/MS. *Environ. Sci. Technol.* **28**, 1733–1746.

Wang, Z.D., Fingas, M., Landriault, M., Sigouin, L., Feng, Y. and Mullin, J. 1997. Using systematic and comparative analytical data to identify the source of unknown oil on contaminated birds. *J. Chromatogr.* **775**, 251–265.

Wang, Z.D., Fingas, M., Blenkinsopp, S., Sergy, G., Landriault, M., Sigouin, L. and Lambert, P. 1998. Study of the 25-year-old Nipisi oil spill: persistence of oil residues and comparisons between surface and subsurface sediments. *Environ. Sci. Technol.* **32**, 2222–2232.

Wang, Z.D., Fingas, M. and Page, D. 1999. Oil spill identification: review. *J. Chromatogr.* **843**, 369–411.

Wang, Z.D., Fingas, M., Owens, E.H., Sigouin, L. and Brown, C.E. 2001. Long-term fate and persistence of the spilled metula oil in a marine salt marsh environment: degradation of petroleum biomarkers. *J. Chromatogr.* **926**, 275–290.

Zakaria, M.P., Horinouchi, A., Tsutsumi, S., Takada, H., Tanabe, S. and Ismail, A. 2000. Oil pollution in the Straits of Malacca, Malaysia: application of molecular markers for source identification. *Environ. Sci. Technol.* **34**, 1189–1196.