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Rapid spilled oil analysis using direct analysis in real time time-of-flight mass spectrometry

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Abstract

Background The biomarker diagnostic ratio analysis outlined by the European Committee for Standardization is considered the current gold standard in oil forensic analysis. However, it has a major limitation as an emergency response procedure in the case of a large scale oil spill due to the high number of samples collected, long GC/MS instrument run time, and the time-consuming data processing required. This current study utilized direct analysis in real time time-of-flight mass spectrometry to develop a rapid spilled oil screening method. An exploratory search of biomarkers and synthetic additives was conducted on reference oil samples of various types. To build a robust yet swift procedure for oil typing, specific heat maps were built with extensive reference sample modelling. These heat maps were then used to select relevant ions from which principal component analysis and discriminant analysis of principal component models were constructed to result in defensible oil classifications.

Results The initial exploratory search of biomarkers and additives in the various reference oil samples resulted in promising preliminary matches. The heat map and multivariate statistical analysis oil typing method was applied to three unknown samples, all of which were classified accurately.

Conclusion The merit of direct analysis in real time time-of-flight mass spectrometry on oil forensic was confirmed with the detected biomarkers compound class starting members and lubricating additives along with the successful application of heat maps and multivariate statistical analysis, providing a swift yet reliable screening tool for oil spill environmental monitoring and impact surveying.

Keywords Oil Spill, Oil forensics, Direct analysis in real time, Mass spectrometry, Multivariate statistical analysis

Introduction

In the face of climate change, there is an urgent need to transition to renewable resources as the primary means of energy. Unfortunately, oil is continuing to be widely used as a primary energy source due to its current perceived cost advantage, established infrastructure and wide versatility (U.S. Energy Information Administration (EIA): Oil and petroleum products explained: Use of Oil, 2022). The consequent high demand for oil leads to extensive extraction of crude oil and its transportation (more common usage in North America) to other locations. In Canada, over four million barrels of oil are transported annually via various means, including pipelines, transport trucks, railway, and cargo ships (Government of Canada: Impact Canada—Oil Spill Response Challenge, 2022). With such a high volume of oil cargo, the chance of a disastrous oil spill within Canadian boundaries is elevated. In cases such as the port of Vancouver, British Columbia, the marine gateway is located in a region of pristine natural waters alive with exotic

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marine animals and wetland marshes that support migratory birds and other wildlife (British Columbia, Ministry of Environment: Estuaries in British Columbia 2006). An oil spill in this, and other similar regions, could have profound and even irreversible ecological consequences on the environment, which are only exacerbated by delays in identification and on-going monitoring (Brody et al. 2012; ClearSeas: Oil Spills in Canada 2022; Deepwater Horizon's Impact on Wildlife 2022). When spilled oil is exposed to the environment, several types of weathering could occur, further hampering the ability of environmental chemists to monitor and biologists to determine long-term effects (Chua et. al. 2021; Filewood et. al. 2022a). Therefore, frequent oil spills of small and large scale require the development of rapid and reliable oil forensics tools to survey the scale, monitor the impact, and locate the source of the contamination.

Current oil spill forensic studies include several qualitative screening methods (Chua et al. 2020a; Filewood et. al. 2022c). Currently, the method described in the European Committee for Standardization (CEN) EN 15522–2 Oil Spill Identification guidelines is considered the gold standard for oil spill identification (Chua et. al. 2020a; Chua et. al. 2020b; Chua et. al. 2021; Filewood et. al. 2022a; Filewood et. al. 2022b; Filewood et. al. 2022c; McCallum et. al. 2023; Yang et. al. 2022; Yang et. al. 2017). The CEN method relies on biomarker ion ratio comparison between environmental samples and potential source oils. Biomarkers are compounds contained in oils that are particularly resistant to weathering (CEN 2021; Filewood et. al. 2022a; McCallum et. al. 2023; Yang et. al. 2022; Yang et. al. 2017). Diagnostic ratios are ratios between biomarkers, and the CEN method contains 78 standard ratios. For the spilled oil to be considered a match to source oil, a high number of those diagnostic ratios should exhibit a difference of less than 14% compared to the same diagnostic ratios measured in the source oils. The CEN method, an internationally accepted technique, has demonstrated its ability to source spilled oil using gas chromatography mass spectrometry (GC–MS) and, more recently, gas chromatography quadrupole time-of-flight (GC-QToF) instruments (CEN 2021; Filewood et. al. 2022b; McCallum et. al. 2023). Enhancements of the diagnostic ratio analysis have incrementally increased its robustness through addition of biomarkers such as adamantanes, APAHs, and APASHs, APANHs and employing the use of multivariate statistics (Chua et. al. 2020b; Filewood et. al. 2022a; McCallum et. al. 2023). However, the diagnostic ratio methodology still has some limitations as an emergency response method (CEN 2021; Wang and Stout 2007). Data processing is highly complex and involved, often requiring a level of expertise that can only be found in a senior analyst (Chua et.

al. 2020a; CEN 2021; Filewood et. al. 2022a, b; McCallum et al. 2023). Sample preparation is extensive and data collection itself is slow with normally one hour GC–MS run time for each sample, making the diagnostic ratio analysis a labour intensive and time-consuming process (Chua et. al. 2021; CEN 2021; Filewood et. al. 2022a; McCallum et. al. 2023). Therefore, research into alternative fast and reliable screening techniques is urgently needed to supplement the current biomarker diagnostic ratio analysis method.

Direct analysis in real time time-of-flight mass spectrometry (DART-ToF MS) offers a promising solution to this challenge. In contrast to diagnostic ratio analysis, DART-ToF MS characterization requires little to no sample preparation and is capable of screening multiple samples in a short amount of time, allowing for instantaneous data results (Easter and Steiner 2014; Ezpinoza et. al. 2014; Lancaster and Espinoza 2012, Espinoza et. al. 2015; Finch et. al. 2017; Lian et. al. 2017). In support of DART-ToF MS, the procedure has been used forensically to quickly screen and identify different wood species, drugs, and even sea turtle oil (Easter and Steiner 2014; Ezpinoza et. al. 2014; Lancaster & Espinoza 2012, Espinoza et. al. 2015; Espinoza et. al. 2021; Finch et. al. 2017; Lian et. al. 2017). DART-ToF MS compares two-dimensional sample spectra to those of reference materials that can be used to form the basis of spectral library. Heat maps are used to visually show the compound presence and relative abundance of ions for samples in comparison to potential comparable references, in the present case, source oils (Price et. al. 2022). Heat maps generated using DART-ToF MS are typically coupled with machine learning algorithms and multivariate statistics to corroborate visual conclusions (Brunswick et. al. 2021; Price et. al. 2022). For example, the wood identification protocol consists of three steps: (1) a database match against the ForeST (Forensic Spectra of Trees) database to identify the top matching species, (2) creation of a heat map followed by Principal Component Analysis for the top matching species, and (3) Discriminant Analysis of Principal Components to determine the best assignment. Cluster analysis may provide confirming information (Price et. al. 2022). Similar machine learning algorithms and statistical software have been used to assess mass spectral data by accurate unbiased selection of relevant species, for example, in differentiating chemotypically similar wood species (Price et. al. 2022). In wood identification, the value of the speed of DART-ToF MS analysis together with the ability to collate a library for different chemotypes, has proven an invaluable tool in forensic analysis (McClure et. al. 2015; Musah et. al. 2015; Paredes-Villanueva et. al. 2018; Price et. al. 2022). Considering the existence of large number of biomarkers in the crude oil and timber

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products which DART/ToF MS wood species identification is based on, we believe that DART/ToF MS could be used for oil forensics. Nevertheless, extensive literature search has yielded no result on this subject. The potential of this approach was a stimulating factor in the development of an analogous procedure in the current study.

Herein, we proposed a novel approach to oil forensics screening employing DART-ToF MS. The current work involved extensive reference sample screening and building of oil type-specific heat maps to visually compare and identify oil types. Additionally, efforts were made for the identification of important biomarkers in the mass spectra from DART-ToF MS. The current method development was conducted to achieve three goals. The first goal was to use heat maps to develop a robust method for oil typing. The next goal was to do an exploratory search for biomarkers and additives present in various types of oils. The final aim was to confirm if the developed method could be used to match unknown samples to the correct type using heat map oil typing and the use of statistical analysis methods.

Materials and methods
Reagents and sample preparation
Dichloromethane (DCM) and methanol, OmniSolv grade, were purchased from VWR, (Mississauga, Canada). Closed end borosilicate glass melting point capillary tubes were purchased from Fisher Scientific (New Hampshire, United States).

Forty reference oil samples (Additional file 1: Table SI) were used for analysis in the current study. To simulate unknown samples, three oil samples were transferred to vials as quality assurance (QA) samples by a separate chemist. These samples were labelled QA1, QA2, and QA3 with the identities unknown to the primary analyst. All oil samples were diluted in DCM. For all crude oil, diluted bitumen, intermediate fuel oil, and heavy fuel oil, 0.1 mL of each oil was diluted with 10 mL of DCM and used directly. For diesel, jet fuel and lubricating oil, 0.1 mL of each oil was diluted with 1 mL of DCM.

Exploratory search for biomarkers and additives
Select sample spectra of several compound classes (Table 1) were searched for potential distinguishing characteristics including differences between biomarkers, molecular weight distributions, compound class compositions and lubricant additives. These compound searches were conducted using Mass Mountaineer and curated compound lists containing masses and compositions of each of the compounds. The lists containing biomarker compound classes and lubricant additives were uploaded to the program. The software was given parameters to account for H^+ adducts, a tolerance of 5 mmu and a threshold of 1%. The algorithm parsed the sample spectra to glean compounds of interest and then reported the relative abundance of the compounds present.

DART/ToF MS data acquisition
For DART-ToF MS oil sample analysis, closed-end capillary tubes (1.5–1.8 × 90 mm) were dipped in oil sample and then held in the helium gas stream. Polyethylene glycol 600 (PEG 600) from Tokyo Chemical Industry (Tokyo, Japan) was used as the accurate-mass calibration standard and run before each sample to ensure correct peak reading. A DART-SVP ion source (IonSense, Saugus MA USA) along with the AccuTOF-DART 4G mass spectrometer (JEOL USA, Inc., Peabody MA USA) were used to acquire spectra in positive ion mode. The DART source heater temperature was set at 400 °C. The mass spectrometer settings included are listed in Tables 2. The spectra were obtained over the mass range of *m/z* 70 to 1000 at a spectral acquisition rate of one scan per second. The factory-preset helium flow rate for the DART-SVP source was used. Eight replicates for each oil sample were collected.

Table 1 Oils selected for exploratory search of biomarker compound classes and lubricant additives

Oil label	Oil type	Search conducted
QSPP	Lubricating oil	Biomarker compound classes; Lubricating additives
MD	Diesel	Biomarker compound classes
JET A1	Jet fuel	Biomarker compound classes
IFO-180	Intermediate fuel oil	Biomarker compound classes
ANS	Crude oil/Diluted Bitumen	Biomarker compound classes
HFO6303	Heavy fuel oil	Biomarker compound classes
AWB	Crude oil/Diluted Bitumen	Biomarker compound classes
PVG	Lubricating oil	Biomarker compound classes; Lubricating additives
UNI	Lubricating oil	Biomarker compound classes; Lubricating additives

Table 2 AccuTOF-DART 4G mass spectrometer parameters

Parameter Description	Set Value
Ring lens voltage	5 V
Orifice 1 voltage	20 V
Orifice 2 voltage	5 V
Orifice 1 temperature	120 ℃
Ion guide voltage [RF]	500

Heat maps for oil typing

The extensive reference sampling consisted of 40 different oil samples of seven distinct types of oils obtained from various sources (Additional file 1: Table S1). Spectra were collected from eight replicates of each oil sample and used to build the heatmaps. The msAxel@LP Data Processing software was used to extract chromatograms and apply mass calibration to the spectra. From the msAxel@LP Data Processing software, the mass spectra were exported as text files for heat map generation. Heat maps are a graphical representation of raw data using colour intensity to depict relative abundance of the ions present (McClure et. al. 2015; Musah et. al. 2015; Paredes-Villanueva et. al. 2018; Price et. al. 2022). The x-axis represents the *m/z* values and along the y-axis, each row displays a different spectrum. As the relative abundance of an ion increases, the colour of the point representing the ion proportionally intensifies. Mass Mountaineer (massmountaineer.com) was employed to generate heat maps from multiple data sets. Heat maps for each oil type were collated and contained different sources of the oil type in an effort to assess the full character and variability of the oil type. Heat maps were used to select relevant ions and build statistical models for further analysis.

Multivariate statistical analysis

Based on the heat maps generated, ions were selected to build statistical analysis models in Mass Mountaineer. Number of ions used for each statistical model of the different data sets is listed in Additional file 1: Table S3. Mass Mountaineer was used to discriminate oil samples by type using principal component analysis (PCA) scatterplots and discriminant analysis of principal components (DAPC) modelling. Principal component analysis was applied to determine whether there are innate class-dependent differences in the DART mass spectra. Qualitative identification of QA1, QA2, and QA3 was accomplished by using Discriminant Analysis of Principal Components (DAPC) with 50 principal components that covered 76.89% of the variance. Each model had a tolerance of 15 mmu. The accuracy of each model was tested with external validation. External validation was conducted by randomly reserving 30% of the reference sample files from the training set, recalculating the model, and treating the removed spectra as unknown samples. The assessments derived from DAPC were compared to classifications of the QA samples assigned by the discriminant analysis of principal components models.

Results and discussion

Exploratory search of biomarkers and additives

The classic biomarker diagnostic ratio method is considered the gold standard in oil forensics due to its robustness and repeated success (Chua et. al. 2020a, b; Chua et. al. 2021; CEN 2021; Filewood et. al. 2022a, b, c; McCallum et. al. 2023). However, this procedure requires time-consuming GC-MS analysis followed by visual assessment of chromatography and application of forensic diagnostic ratio analysis. The process would be enhanced by the ability to perform a preliminary rapid, qualitative analysis to confirm the presence of recognized biomarkers. The presence or absence of biomarkers constitutes the foundation of the diagnostic forensic ratio approach, with ability to not only distinguish between oil types, but also as an indication of weathering. The current study was able to explore the use of DART/ToF MS and Mass Mountaineer to search biomarker compounds from the reference oil samples.

To perform this exploratory search, a list of common biomarker compound classes was curated (Additional file 1: Table S2b) and used by the Mass Mountaineer software to identify these classes in sample spectra from their exact masses. In Fig. 1, the search of a MD reference sample yielded biomarker classes diamantane, adamantane, anthracene or phenanthrene, and pyrene or fluoranthene starter compounds. Additional file 1: Figs. S10, S11, S12, S13, S14, S15, S16, S17, S18 contain a list of all the oils searched for biomarker compound classes as well as the relative abundances of the compounds found in each sample spectrum. Overall, several biomarker groups were identified across oil types successfully in this exploratory search. A similar approach was applied to lubricating oils to identify common lubricant additives in three lubricating oils (Additional file 1: Figs. S10, S17, 18) to explore the possibility of using additives to help identify spilled lubricating oils. Like the exploratory search for biomarkers, the exploratory search for lubricant additives was successful as additives were identified in all three of the lubricating oil spectra that were subjected to this process.

Oil type-specific heat map comparison

Figure 2 summarizes the observed chemotypes for each oil type. Heat maps, as visual representations, provide a

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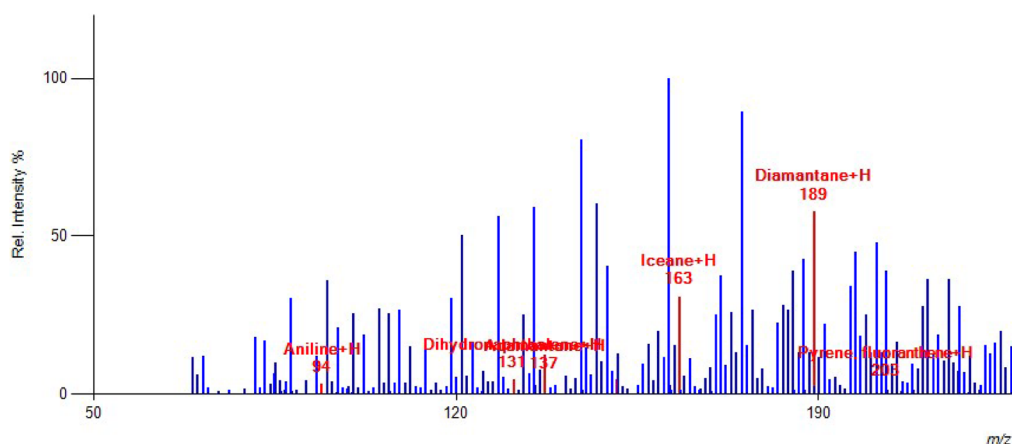
Fig. 1 Spectra of marine diesel with identified compound classes highlighted in red (Ions identified from left to right: 94: Aniline + H; 131: Dihydronaphthalene + H; 137: Adamantane + H; 163: Iceane + H; 189: Diamantane + H; 203: Pyrene, fluoranthene + H)

swift means of determining patterns and differences that exist within the same and different oil types. For instance, heat maps can visually confirm the reproducibility of chemical fingerprints collected from various reference materials (Easter and Steiner 2014; Ezpinoza et. al. 2014; Lancaster and Espinoza 2012, Espinoza et. al. 2015; Finch et. al. 2017; Lian et. al. 2017). Further heat maps are provided in Additional file 1: Figs. S19, S20, S21, S22, S23, S24, S25 showing spectra obtained from duplicates of the same oil reference with similar relative abundances.

From the collated heat map, lubricating oils were shown to be most unique and its chemotypes were
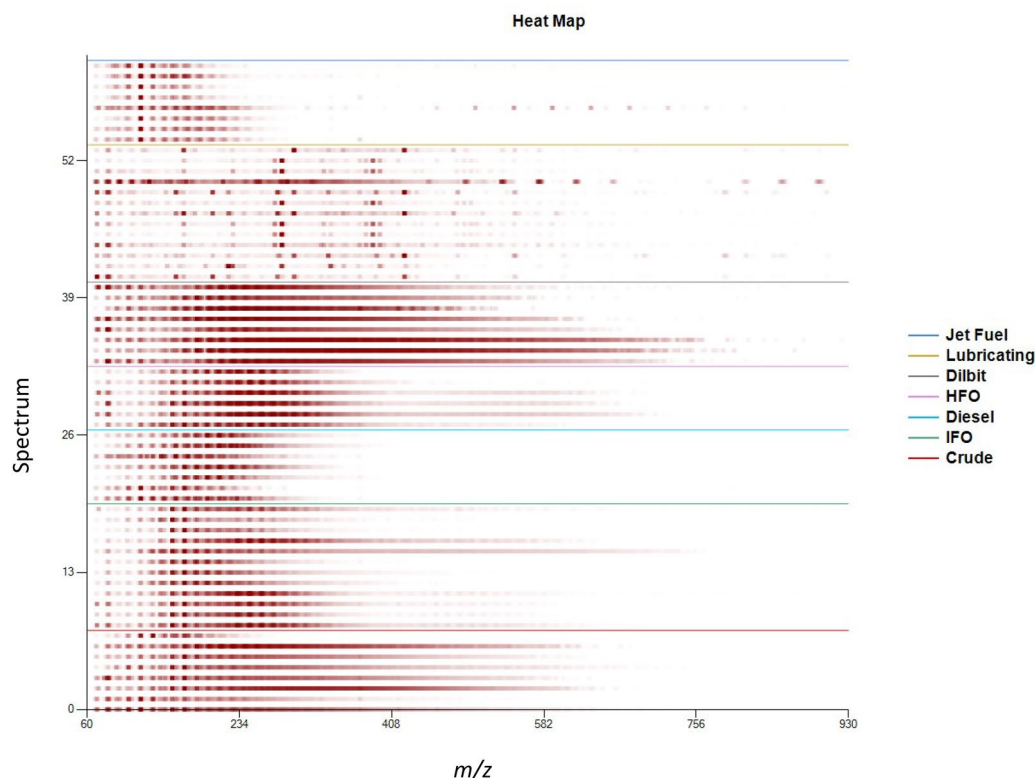
Fig. 2 Positive ion heatmap of different types of oils shows the similarities of chemotypes of oil references within the same type. Heatmap also provides a tool to differentiate oils based on type, though some oil types are difficult to distinguish visually. Oil spectra are organized by type, indicated by the coloured lines above them and the legend

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clearly distinct from all other types of oil (Fig. 2). The shared characteristic pattern of lubricating oils may be due DART-ToF MS being more sensitive to the lubricant additives in the base oils (Easter and Steiner 2014; Ezpinoza et. al. 2014; Lancaster and Espinoza 2012, Espinoza et. al. 2015; Finch et. al. 2017; Lian et. al. 2017). Similarly, jet fuel and diesel were also fairly distinct from the other oil types though they populated the same *m/z* range as a type. The distinctness of jet fuel and diesel is likely due to the extensive refining process that they are subject to. Of interest was that diluted bitumen (dilbit) was not as obviously visually distinct from crude oil (Fig. 2). These similarities are expected given that the components of crude oil and diluted bitumen are the same (The science of diluted bitumen (Ask NRCan), 2023). Due to these compositional similarities, dilbit and crude oil were treated as the same oil type for the purposes of this study (referred to as crude/dilbit). The chemotypes obtained from heavy fuel oil (HFO) and intermediate fuel oil (IFO) were similar and difficult to distinguish based on visual comparison alone (Figs. 2).

Spectra of the quality assurance samples are displayed in Fig. 3. A heat map of the three QA samples (Fig. 4) was visually compared to the oil type heat maps before any further analysis. The first quality assurance sample, QA1, contained ions with a similar range and relative abundances as the heavy fuel oil and intermediate fuel oil reference samples (Additional file 1: Figs. S19, S24). It also had a range similar to diesel, though the relative abundances of most of the diesel spectra for the higher *m/z* values were lower in diesel than they appear to be in QA1, making diesel a less likely type match. QA2's chemotype shared the most similarity with the crude/dilbit spectra in terms of range of ions and relative abundance (Additional file 1: Figs. S20, S25). In the case of QA3, the *m/z* range of ions and the observed relative abundances in QA3 were only observed in the crude/dilbit chemotypes and in none of the other oil types included in this study. In this case, QA2 and QA3 could be classified based on the type-specific heat maps alone. While QA1 could not be definitively classified based on the heat map alone, the use of heat maps narrowed down its type, thus demonstrating the screening potential of heat map comparisons. As seen with the QA samples, results using the rapid visual assessment by heat maps are intuitive but classifying certain oils by type may require more complex analysis i.e. the visual comparison may not be legally defensible in some cases. To overcome this limitation, multivariate statistical modelling methods were applied.

Multivariate statistical analysis

Principal component analysis (PCA) and discriminant analysis of principal components (DAPC) has been extensively used in forensic analysis for oil data obtained from GC-QToF MS (Filewood et. al. 2022a, b, c; McCallum et. al. 2023) and DART-ToF MS wood identification (Easter and Steiner 2014; Ezpinoza et. al. 2014; Lancaster & Espinoza 2012, Espinoza et. al. 2015; Finch et. al. 2017; Lian et. al. 2017) with promising results. PCA is an unsupervised classification method (Abdi and Williams 2010) that reduces the complexity of multidimensional data by transforming the measured values into a smaller number of "principal components" that cover the largest amount of variance in the data (Abdi and Williams 2010). The first principal component accounts for the most variance and the subsequent principal components are chosen to be orthogonal to the first principal component (Abdi and Williams 2010). DAPC relies on variables generated during PCA, i.e., the principal components, to maximize the separation of elements from the data set (Jombart et. al. 2010). In the current study both tools were used as second-tier investigation for oil typing identification.

The statistical approach employed in this study was based on the pattern recognition observed from the oil type-specific heat maps. Oil typing using multivariate statistical models was conducted in two levels. The first level involved differentiating oils into the following classes: HFO/IFO, Crude/Dilbit, Diesel/Jet and Lubricating. Lubricating oils were clearly distinguished by their characteristic additive compositions and would be considered a final classification. Similarly, if an oil was predicted to be crude/dilbit, the oil-typing would be considered complete as well. Otherwise, the sample would undergo the second level of statistical analysis. For example, if an unknown sample was classified as HFO/IFO during the first level of analysis, the second level of analysis would consist of building a model using only HFO and IFO reference samples.

Principal component analysis (PCA) scatter plots of seven types of oil (Fig. 5) display clustering that corroborates the visual comparison of type-specific heat maps. The PCA scatter plot in Fig. 5 of the four classes of oils exhibit four separated clusters. This separation demonstrated that this method can differentiate among these four classes of oil types. Most of the lubricating (lube) oils along with the diesel oil/jet oil class form especially compact clusters. Though still separated from the other oil type classes, the crude/dilbit class and heavy fuel oil/ intermediate fuel oil display relatively large variance within each data set. A 3D version of this PCA plot can be found in the SI. Discriminant analysis was carried out with 74 principal components which contained most of the variance at 78.93% (the number of principal
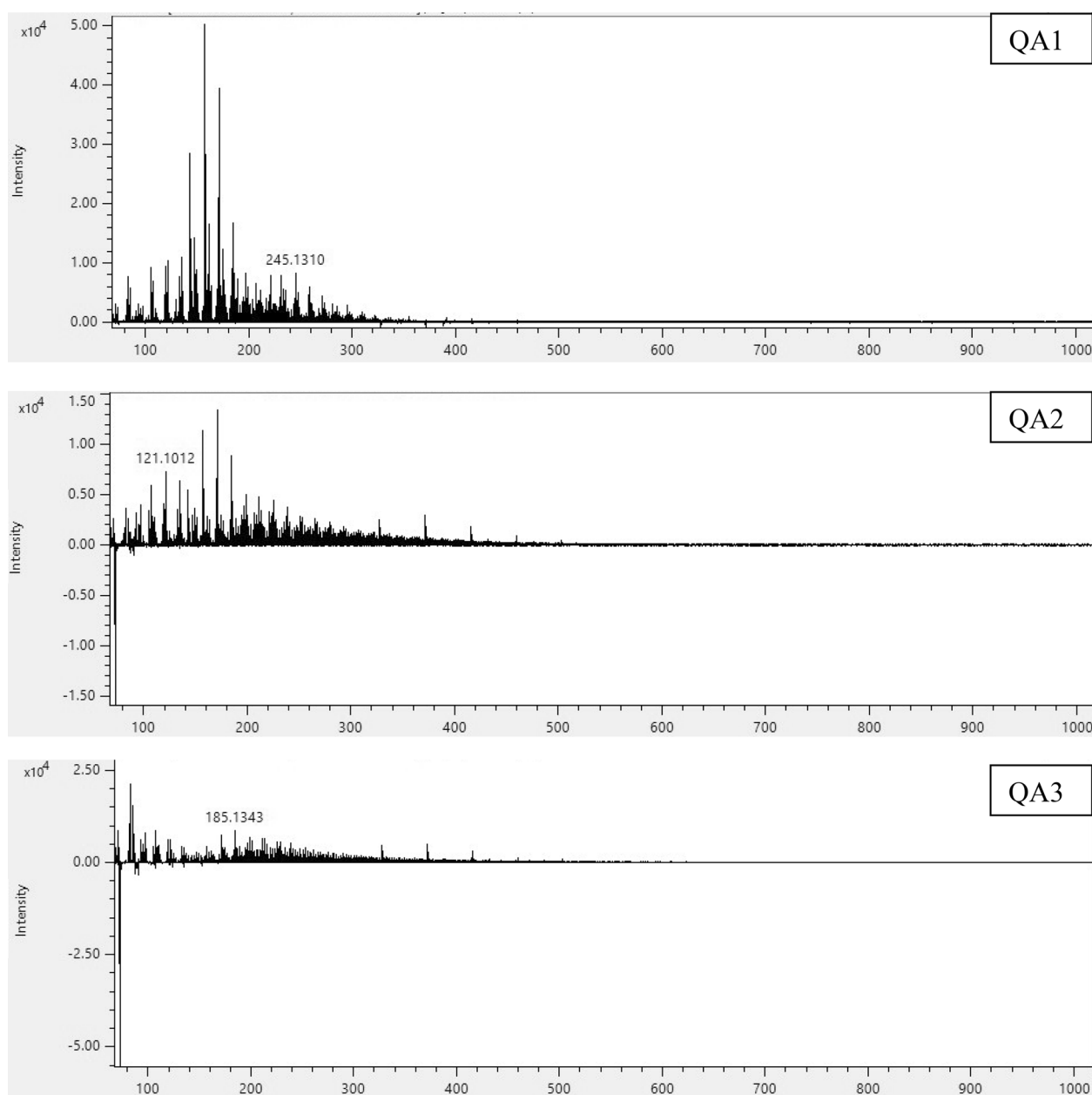
Fig. 3 Spectra of QA1, QA2 and QA3

components was chosen to cover > = 70% of the variance but less than 100% to avoid overfitting). Additional file 1: Table S3 shows the principal components and variance covered by all models. The scatterplot generated by DAPC also exhibited four distinct clusters representing the type oil type classes (Fig. 6) with improved separation relative to the PCA plot.

The DAPC model was externally validated to measure its accuracy for oil classification. The accuracy of the DAPC model was 96.90%. This score suggested that the

model predicted sample types with high accuracy and was highly reliable for classifying oils into one of the four oil types, i.e., diesel/jet, crude/dilbit, HFO/IFO, and lube oils.

To conduct the initial oil typing classifications of the QAs, a PCA plot was first established to determine the relation of the QAs to the reference oils (Fig. 7). Visual inspection of the PCA plot indicates the likely classification for each QA sample oil. Clearly, QA1 was clustered with HFO/IFO, making it likely to be the correct
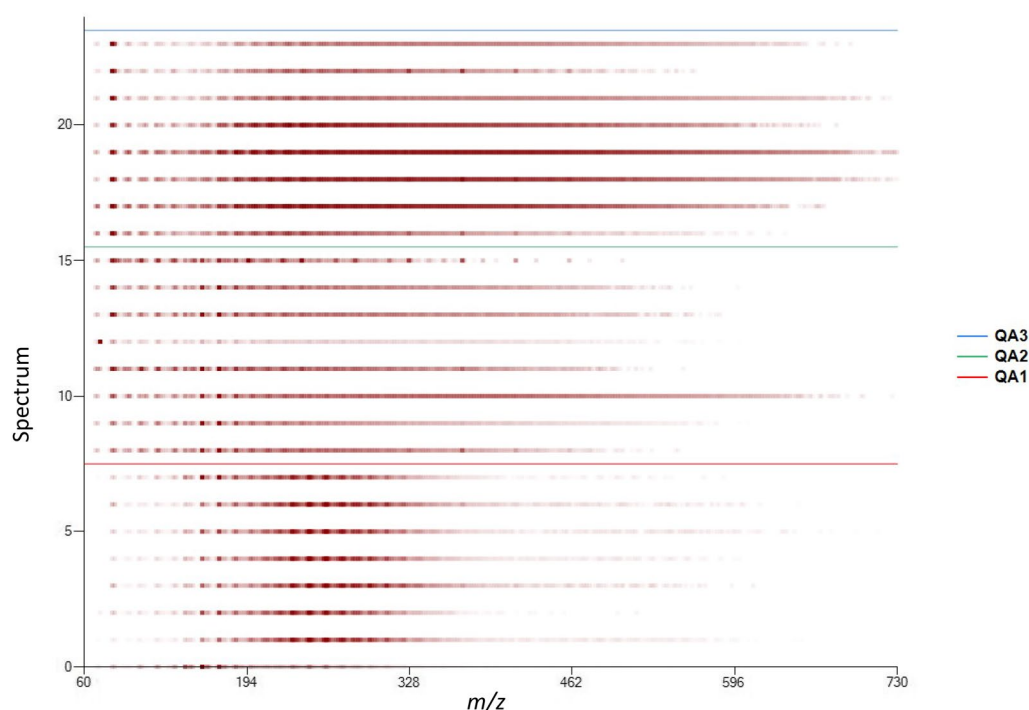
Fig. 4 Positive ion heat map containing chemotypes of QA1, QA2 and QA3 replicates
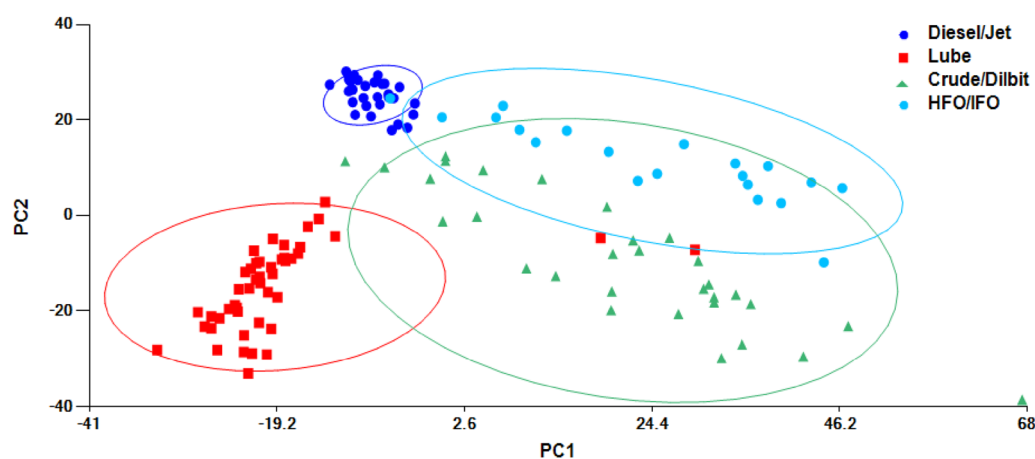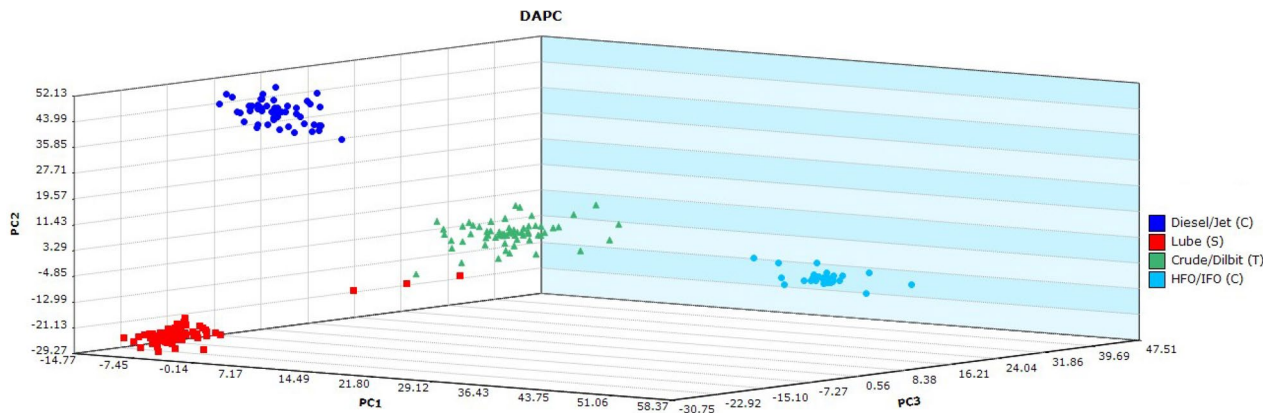

Fig. 5 Two-dimensional principal component analysis scatterplot of reference oil samples. Each reference sample is represented by a point

classification. From Fig. 7, it was also apparent that both QA2 and QA3 were crude oils/diluted bitumen. Figure 8 also shows that the plot generated with DAPC produced five clearly distinct clusters. Similar to the PCA plot, QA1 was clustered with the HFO/IFO date set and QA2 clustered with the crude/dilbit data set. QA3 formed its own cluster, but it was closest in proximity to the crude oil/dilbit cluster. The subsequent automatic predictions by the DAPC model confirmed these visual classifications for the unknown oil samples.

The discriminant analysis of principal component models was validated (Additional file 1: Table S4) and automatically assigned each QA to one of the crude/dilbit, diesel/jet, HFO/IFO and lube oil classes. QA1 was classified as HFO/IFO while QA2 and QA3 were classified as crude/dilbit. The results for each the classification for each replicate are available in Additional file 1: Tables S5, S6. For the DAPC model, the average confidence for the QA1, QA2 and QA3 classifications were 100%, 100%, and 100% respectively. This confirmed the conclusion
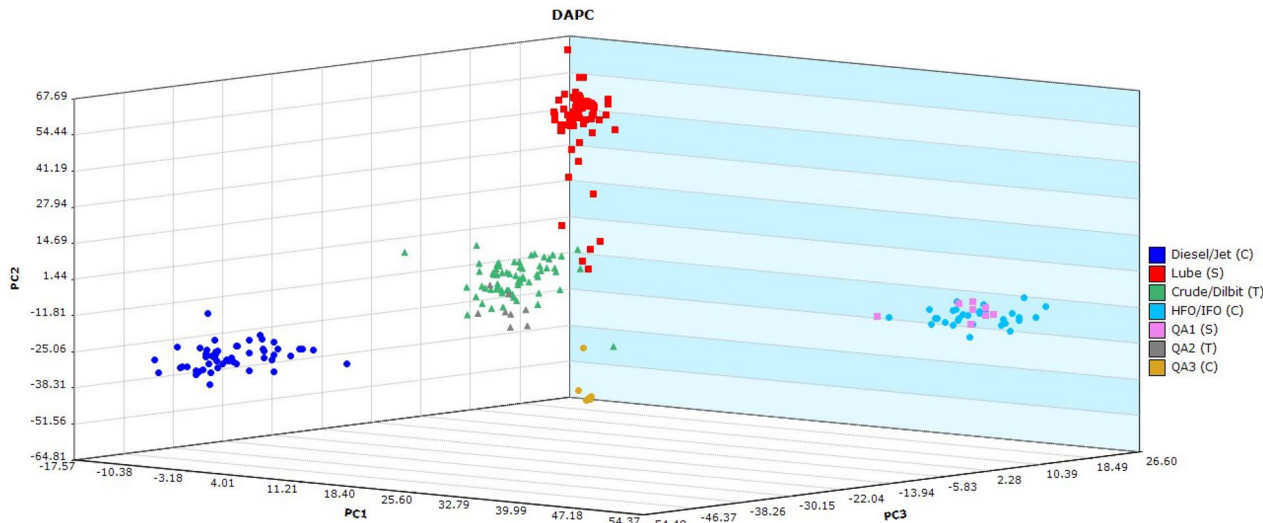
Fig. 6 Discriminant analysis of principal components of the reference oils
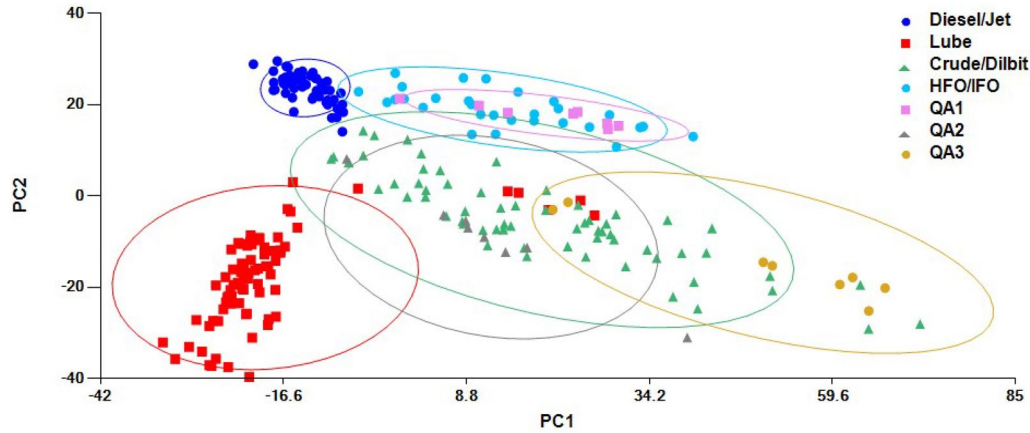
Fig. 7 Two-dimensional PCA plot with QAs with 2-sigma ovals

Fig. 8 DAPC plot of QAs in relation to the diesel/jet, lube, crude/dilbit and HFO/IFO clusters

Table 3 Summary of final oil type classifications of quality assurance samples

QA Label	Predicted type	Actual type
QA1	HFO	HFO
QA2	Crude/Dilbit	Crude/Dilbit
QA3	Crude/Dilbit	Crude/Dilbit

from the inspection of the PCA plot generated (Fig. 7). Since QA2 and QA3 both classified as crude/dilbit, their classification was complete. With these initial classifications complete, QA1 could undergo final typing with the next level of multivariate statistical analysis.

Diesel/jet and HFO/IFO PCA plots (Additional file 1: Figs. S26, S27, S28) showed recognizable separation between the oil types and indicate that these models can distinguish between oil types, even they were relatively closely related. According to the PCA plot in Figure S35, QA1 was a heavy fuel oil contained within the HFO cluster.

The automatic DAPC modelling were used to assign the final consensus oil type prediction for the QA1 samples. The DAPC model classified 87.5% of QA1 samples as a heavy fuel oil. This classification was treated as the final assignment for the oil typing of QA1. A summary of the QA sample oil type classifications are shown in Table 3. The confidence of the heavy fuel oil assignment for QA1 was averaged to be 94.12%, 100% for the crude/dilbit QA2 assignment, and 100% for the QA3 crude/dilbit assignment. The full prediction results are shown in Additional file 1: Table S6. In general, these confidence scores were very high and, coupled with the principal component analysis plot inspection and the heat map analysis, allowed for reliable typing of the unknown samples. The oil typing of QA1, QA2, and QA3 were confirmed to be accurate by the secondary chemist who prepared the samples once the analysis was complete.

Conclusion

The potential for direct analysis in real time time-of-flight mass spectrometry as an oil forensics tool was demonstrated. DART/ToF MS data was automatically searched for biomarkers and additives, demonstrating its ability to identify these compounds. A systematic approach to oil typing using DART/ToF MS was also developed. Three unknown samples were visually compared to oil type-specific heat maps that were built using extensive reference sampling. To provide conclusive oil type classifications, multivariate statistical analysis was applied in two stages. First, discriminant analysis of principal components was used to assign blind QA1, QA2 and QA3 to one of four classes: heavy fuel oil/intermediate fuel oil,

diesel/jet fuel, crude oil/ diluted bitumen and lubricating oil. At this stage, QA2 and QA3 were both confirmed to be crude oil/diluted bitumen. The second stage of statistical analysis involved using DAPC to accurately identify QA1 as a heavy fuel oil.

A DART/ToF MS oil forensics approach was developed to provide a rapid technique to supplement the reliable but time-consuming classic CEN biomarker diagnostic ratio method. Overall, this study successfully achieved three main goals: (1) to perform an exploratory search of biomarkers and lubricant additives on reference oil samples (2) to build oil type-specific heat maps for initial oil screening and statistical modelling and (3) to apply heat map-based oil typing and statistical tools to three unknown samples.

In the case of oil spills, prompt analysis is critical for minimizing long-term ecological damage. Oil typing is the important first step in oil forensic analysis to identifying the source of the spilled oil. The development of a novel, rapid approach to oil typing using DART/ToF MS and its successful application in this study reveals the potential of DART/ToF MS to fill a current gap in oil forensics. Further research is being carried out by the current authors on using DART/ToF MS to rapidly analyse large number of oil spill environmental water samples.

Supplementary Information

Additional file 1: Table S1. Specific details for each oil used for heat map building. **Table S2.** Excel documents used for Exploratory Search of Biomarker Class Compounds and Lubricant Additives. **Table S3.** Ions used to construct Principal Component Analysis and Discriminant Analysis of Principal Component. **Table S4.** Discriminant Analysis of Principal Components External Validation Scores. **Table S5.** Discriminant Analysis of Principal Components classifications of QAs into lubricating oil, crude oil/diluted bitumen, heavy fuel oil/intermediate fuel oil and diesel/jet fuel. **Table S6.** Final Oil typing results. **Figure S1.** Spectra of QSPP (Lubricating oil). **Figure S2.** Spectra of MD (Diesel). **Figure S3.** Spectra of JET A1 (Jet Fuel). **Figure S4.** Spectra of IFO-180 (Intermediate Fuel Oil). **Figure S5.** Spectra of WCS (Crude Oil/Bitumen). **Figure S6.** Spectra of HFO6303 (Heavy Fuel Oil). **Figure S7.** Spectra of AWB (Crude Oil/ Diluted Bitumen). **Figure S8.** Spectra of PVG (Lubricating Oil). **Figure S9.** Spectra of UNI (Lubricating Oil). **Figure S10.** Spectra of QSPP with identified compound classes highlighted in red. Relative abundances of all extracted compounds are listed. **Figure S11.** Spectra of MD with identified compound classes highlighted in red. Relative abundances of all extracted compounds are listed. **Figure S12.** Spectra of IFO with identified compound classes highlighted in red. Relative abundances of all extracted compounds are listed. **Figure S13.** Spectra of WCS with identified compound classes highlighted in red. Relative abundances of all extracted compounds are listed. **Figure S14.** Spectra of HFO with identified compound classes highlighted in red. Relative abundances of all extracted compounds are listed. **Figure S15.** Spectra of AWB with identified compound classes highlighted in red. Relative abundances of all extracted compounds are listed. **Figure S16.** Spectra of PVG with identified compound classes highlighted in red. Relative abundances of all extracted compounds are listed. **Figure S17.** Spectra of UNI with

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identified compound classes highlighted in red. Relative abundances of all extracted compounds are listed. **Figure S18.** Intermediate Fuel Oil Heatmap. **Figure S19.** Crude Oil Heatmap. **Figure S20.** Jet Fuel Oil Heatmap. **Figure S21.** Lubricating Oil Heatmap. **Figure S22.** Diesel Heatmap. **Figure S23.** Heavy Fuel Oil Heatmap. **Figure S24.** Diluted Bitumen Heatmap. **Figure S25.** Three dimensional PCA plot for classes: Diesel/Jet, Lube, Crude/Dilbit and HFO/IFO. **Figure S26.** Two-dimensional discriminant analysis of principal components plot for classes: Diesel/Jet, Lube, Crude/Dilbit and HFO/IFO. **Figure S27.** PCA plot of dilbit and crude reference data. **Figure S28.** PCA plot of HFO and IFO reference data. **Figure S29.** DAPC plot of dilbit and crude oil reference data. **Figure S30.** DAPC plot of jet fuel and diesel reference data. **Figure S31.** DAPC plot of heavy fuel oil and intermediate fuel oil reference data. **Figure S32.** Positive ion heat map of QA1 compared to intermediate fuel oil and heavy fuel oil reference data. **Figure S33.** Positive ion heat map of QA2 compared to crude oil and diluted bitumen reference data. **Figure S34.** Positive ion heat map of QA3 compared to crude oil and diluted bitumen reference data. **Figure S35.** PCA of QA1 compared to intermediate fuel oil and heavy fuel oil reference data. **Figure S36.** PCA of QA1 compared to intermediate fuel oil and heavy fuel oil reference data.

Author contributions
KT contributed by writing the original draft, and completing formal analysis, methodology, and visualization. PM contributed by reviewing and editing the various drafts, and completing formal analysis, and visualization. TF contributed by reviewing and editing the various drafts, and completing formal analysis, and visualization. HK contributed by reviewing and editing the various drafts, validation, and investigation. JY contributed by reviewing and editing the various drafts, and investigation. PB contributed by reviewing and editing the various drafts, validation, and investigation. RC contributed by reviewing and editing the various drafts, methodology, providing instrument resources and completing investigation. DS contributed by reviewing and editing the various drafts and through supervision, project administration, methodology, resources, and conceptualization. KT, PM and TF equally contributed to this work. All authors read and approved the final manuscript.

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There are no competing interests to declare.

References
Abdi H, Williams LJ (2010) Principal component analysis. WIREs Comp. Stat 2:433–459. https://doi.org/10.1002/wics.101

Brody TM, Di Bianca P, Krysa J (2012) Analysis of inland crude oil spill threats, vulnerabilities and emergency response in the midwest United States. Risk Anal 10:1741–1749. https://doi.org/10.1111/j.1539-6924.2012.01813.x

Brunswick P, Cuthbertson D, Yan J, Chua C, Duchesne I, Isabel N, Evans P, Gasson P, Kite G, Bruno J, van Aggelen G, Shang D (2021) A practical study of CITES wood species identification by untargeted DART/QTOF, GC/QTOF and LC/QTOF together with machine learning processes and statistical analysis. Environ Adv 5:100089. https://doi.org/10.1016/j.envadv.2021.100089

CEN (2021) Oil spill identification–Petroleum and petroleum related products–Part 2: analytical method and interpretation of results based on GC-FID and GC-low resolution-MS analyses. J Hazard Mater 435:129027

Chua CC, Brunswick P, Kwok H, Yan J, Cuthbertson D, van Aggelen G, Helbing CC, Shang D (2020) Enhanced analysis of weathered crude oils by gas chromatography-flame ionization detection, gas chromatography-mass spectrometry diagnostic ratios, and multivariate statistics. J Chromatogr 1634:461689. https://doi.org/10.1016/j.chroma.2020.461689

Chua CC, Brunswick P, Kwok H, Yan J, Cuthbertson D, van Aggelen G, Shang D (2020b) Tiered approach to long-term weathered lubricating oil analysis: GC/FID, GC/MS diagnostic ratios, and multivariate statistics. Anal Methods 12(43):5236–5246. https://doi.org/10.1039/D0AY01510E

Chua CC, Kwok H, Yan J, Cuthbertson D, van Aggelen G, Brunswick P, Shang D (2021) Development of a tiered analytical method for forensic investigation of mixed lubricating oil samples. Environ Forens 23(5–6):511–523. https://doi.org/10.1080/15275922.2021.1907821

ClearSeas: Oil Spills in Canada. https://clearseas.org/en/responding-to-oil-spills/#:~:text=Mechanical%20recovery%20is%20the%20most,oil%20out%20of%20the%20water. Accessed Dec 2022

Deepwater Horizon's Impact on Wildlife. https://www.nwf.org/oilspill. Accessed Dec 2022.

Easter JL, Steiner RR (2014) Pharmaceutical identifier confirmation via DART-TOF. Forensic Sci Int 240:9–20. https://doi.org/10.1016/j.forsciint.2014.03.009

Espinoza EO, Wiemann MC, Barajas-Morales J, Chavarria GD, McClure PJ (2015) Forensic analysis of CITES-protected *Dalbergia* timber from the Americas. IAWA J 36(3):311–325. https://doi.org/10.1163/22941932-20150102

Espinoza EO, Moore MK, Hamlin BC, Baker BW, Espinoza AJ (2021) Forensic characterization of sea turtle oil by ambient ionization mass spectrometry: *Caretta caretta, Chelonia mydas, Dermochelys coriacea, Eretmochelys imbricata, Lepidochelys kempii, and Lepidochelys olivacea*. Forens Sci Int Anim Environ 1:100008. https://doi.org/10.1016/j.fsiae.2021.100008

Ezpinoza EO, Lancaster CA, Kreitals NM, Hata M, Cody RB, Blanchette RA (2014) Distinguishing wild from cultivated agarwood (*Aquilaria* spp) using direct analysis in real time and time of-flight mass spectrometry. Rapid Commun Mass Spectrom 28(3):281–289. https://doi.org/10.1002/rcm.6779

Filewood T, Kwok H, Brunswick P, Yan J, Ollinik JE, Cote C, Kim M, van Aggelen G, Helbing CC, Shang D (2022) Advancement in oil forensics through the addition of polycyclic aromatic sulfur heterocycles as biomarkers in diagnostic ratios. J Hazard Mater 435:129027. https://doi.org/10.1016/j.jhazmat.2022.129027

Filewood T, Kwok H, Brunswick P, Yan J, Ollinik JE, Cote C, Kim M, van Aggelen G, Helbing CC, Shang D (2022b) A rapid gas chromatography quadrupole time-of-flight mass spectrometry method for the determination of polycyclic aromatic hydrocarbons and sulfur heterocycles in spilled crude oils. Anal Methods 14(7):717–725. https://doi.org/10.1039/D1AY02216D

Filewood T, Kwok H, Brunswick P, Yan J, Ollinik JE, Cote C, Kim M, van Aggelen G, Helbing CC, Shang D (2022c) Investigating the fate of polycyclic aromatic sulfur heterocycle compounds in spilled oils with a microcosm weathering experiment. Environmental Systems Research 11(1):1–11. https://doi.org/10.1186/s40068-022-00252-w

Finch K, Espinoza E, Jones FA, Cronn R (2017) Source identification of western oregon douglas-fir wood cores using mass spectrometry and random forest classification. Appl Plant Sci 5(5):1600158. https://doi.org/10.3732/apps.1600158

Government of Canada: impact canada–oil spill response challenge. https://www.nrcan.gc.ca/science-and-data/funding-partnerships/funding-opportunities/funding-grants-incentives/impact-canada-oil-spill-response-challenge/24681. Accessed Dec 2022

Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet 11:94. https://doi.org/10.1186/1471-2156-11-94

Lancaster C, Espinoza E (2012) Evaluating agarwood products for 2-(2-phenylethyl) chromones using direct analysis in real time time-of-flight mass spectrometry. Rapid Commun Mass Spectrom 26(23):2649–2656. https://doi.org/10.1002/rcm.6388

Lian R, Wu Z, Lv X, Rao Y, Li H, Li J, Wang R, Ni C, Zhang Y (2017) Rapid screening of abused drugs by direct analysis in real time (DART) coupled to time-of-flight mass spectrometry (TOF-MS) combined with ion mobility spectrometry (IMS). Forensic Sci Int 279:268–280. https://doi.org/10.1016/j.forsciint.2017.07.010

McCallum P, Filewood T, Sawitsky J, Kwok H, Brunswick P, Yan J, Chibwe L, Tikkisetty K, Shang D (2023) Enhancement of oil forensic methodology through the addition of polycyclic aromatic nitrogen heterocycle biomarkers for diagnostic ratios. Environ Monit Assess 195:416. https://doi.org/10.1007/s10661-023-10941-3

McClure PJ, Chavarria GD, Espinoza E (2015) Metabolic chemotypes of CITES protected *Dalbergia* timbers from Africa, Madagascar, and Asia. Rapid Commun Mass Spectrom 29:738–788. https://doi.org/10.1002/rcm.7163

Ministry of Environment, British Columbia. Estuaries in British Columbia. Habitat Conservation Trust Fund (2006) ISBN 0–7726–7723–9.

Musah R, Espinoza EO, Cody R, Lesiak AD, Christensen ED, Moore HE, Maleknia S, Drijfhout FP (2015) A High throughput ambient mass spectrometric approach to species identification and classification from chemical fingerprint signatures. Sci Rep 5:11520. https://doi.org/10.1038/srep11520

Paredes-Villanueva K, Espinoza E, Ottenburghs J, Sterken MG, Bongers F, Zuidema PA (2018) Chemical differentiation of Bolivian Cedrela species as a tool to trace illegal timber trade. For Int J Forest Res 91(5):603–613

Price ER, McClure PJ, Huffman AN, Voin D, Espinoza EO (2022) Reliability of wood identification using DART-TOFMS and the ForeST© database: a validation study. Forens Sci Int Anim Environ 2:100045. https://doi.org/10.1016/j.fsiae.2022.100045

The science of diluted bitumen (Ask NRCan). https://www.nrcan.gc.ca/simply-science/the-science-diluted-bitumen-ask-nrcan/21288. Accessed Jan 2022

U.S. energy information administration (EIA): Oil and petroleum products explained: use of oil. https://www.eia.gov/energyexplained/oil-and-petroleum-products/use-of-oil.php. Accessed Dec 2022

Wang Z, Stout SA (2007) Chemical fingerprinting of spilled or discharged petroleum— methods and factors affecting petroleum fingerprints in the environment. Oil Spill Environ Forens. https://doi.org/10.1016/B978-012369523-9.50005-7

Yang C, Lambert P, Zhang G, Yang Z, Landriault M, Hollebone B, Fieldhouse B, Mirnaghi F, Brown CE (2017) Characterization of chemical fingerprints of unconventional bakken crude oil. Environ Pollut 230:609–620. https://doi.org/10.1016/j.envpol.2017.07.011

Yang C, Fieldhouse B, Waldie A, Yang Z, Hollebone B, Lambert P, Beaulac V (2022) Parallel quantitation of salt dioctyl sodium sulfosuccinate (DOSS) and fingerprinting analysis of dispersed oil in aqueous samples. J Hazard Mater 435:129046. https://doi.org/10.1016/j.jhazmat.2022.129046

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