BS EN 15522-2:2023+A1:2025



BSI Standards Publication

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

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National foreword

This British Standard is the UK implementation of EN 15522-2:2023+A1:2025. It supersedes BS EN 15522-2:2023, which is withdrawn.

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The UK participation in its preparation was entrusted to Technical Committee PTI/13, Petroleum Testing and Terminology.

A list of organizations represented on this committee can be obtained on request to its committee manager.

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Published by BSI Standards Limited 2025

ISBN 978 0 539 29965 6

ICS 13.020.40; 75.080

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This British Standard was published under the authority of the Standards Policy and Strategy Committee on 30 April 2023.

Amendments/corrigenda issued since publication

Date	Text affected
31 May 2025	Implementation of CEN amendment A1:2025

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EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 15522-2:2023+A1

April 2025

ICS 13.020.40; 75.080

Supersedes EN 15522-2:2023

English Version

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

Identification des pollutions pétrolières - Pétrole et produits pétroliers - Partie 2 : Méthode d'analyse et interprétation des résultats sur la base des analyses par CPG-DIF et CPG-SM faible résolution

Identifizierung von Ölverschmutzungen - Mineralöl und verwandte Produkte - Teil 2: Analytische Methodik und Interpretation der Ergebnisse, basierend auf GC-FID- und GC-MS-Analysen bei niedriger Auflösung

This European Standard was approved by CEN on 25 December 2022 and includes Amendment 1 approved by CEN on 2 March 2025.

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European foreword

This document (EN 15522-2:2023+A1:2025) has been prepared by Technical Committee CEN/TC 19 “Gaseous and liquid fuels, lubricants and related products of petroleum, synthetic and biological origin”, the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by October 2025, and conflicting national standards shall be withdrawn at the latest by October 2025.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document includes Amendment 1 approved by CEN on 2 March 2025.

This document supersedes ⟩A1⟨ EN 15522-2:2023 ⟩A1⟨.

The start and finish of text introduced or altered by amendment is indicated in the text by tags ⟩A1⟨ ⟩A1⟨.

In comparison with the previous edition CEN/TR 15522-2:2012, the following technical modifications have been made:

— adding compounds to be analysed in order to include light products in the diesel range;

— adding more information about biodegradation;

— adding a Reporting and a Quality assurance chapter;

— adding Annex C with precision data;

— adding Annex D with likelihood grade conclusions;

— introduction of characterization of FAME in Annex I;

— serious revision of Annexes H, I, J and K, adding new pictures and chromatograms.

EN 15522 is composed of two parts that describe the following:

— Part 1 on sampling, describing good sampling practice, detailing sampling equipment, sampling techniques and the handling of oil samples prior to their arrival at the forensic laboratory;

— Part 2 giving the analytical method, which covers the general concepts and laboratory procedures of oil spill identification, analytical techniques, data processing, data treatment, interpretation/evaluation and reporting of results.

This document has been prepared under a standardization request addressed to CEN by the European Commission. The Standing Committee of the EFTA States subsequently approves these requests for its Member States.

A list of all parts in a series can be found on the CEN website.

Any feedback and questions on this document should be directed to the users’ national standards body. A complete listing of these bodies can be found on the CEN website.

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Introduction

This document describes a forensic method for characterizing and identifying the source of oil spills in the environment resulting from accidents or intentional discharges. By following this method, data generated can be used as evidence in support of the legal process. This method is based on the experience gained from use of the earlier revisions.

This document is composed of two parts that describe the following:

— Part 1 on sampling, describing good sampling practice, detailing sampling equipment, sampling techniques and the handling of oil samples prior to their arrival at the forensic laboratory;

— Part 2 giving the analytical method, which covers the general concepts and laboratory procedures of oil spill identification, analytical techniques, data processing, data treatment, interpretation/evaluation and reporting of results.

Oil spill source identification is a complex process due to the large variation in sample types and oil spill situations that can be encountered. Part 1 is a compilation of instructions and experiences from experts all over the world which will guide the user in sampling, storing and delivering oil samples for laboratory analysis. Part 2 will guide the reader through the analytical process. It prescribes how to prepare and analyse oil samples using Gas Chromatography with Flame Ionization Detection (GC-FID) and GC with low-resolution Mass Spectrometry (GC-MS). Any compositional difference found between samples is only relevant if this difference is larger than the variability of the method itself. Good analytical performance and strict quality assurance are therefore essential. In the Annexes of Part 2, relevant information concerning different types of oil and oil comparison techniques are presented.

In a typical standard method for testing, instructions are given on performing an “analytical” procedure. However, oil spill identification comprises both analytical and assessment components. Sample preparation is described in Chapter 6. Analytical methodology for GC-FID is provided in Annex A, while GC-MS is covered in Annexes B, E and F. Other parts of the document describe how to assess the analytical data utilizing various tools to draw a conclusion. As every case can differ in situation, size, products and weathering, the evaluation part of the method is described as a toolbox. Annexes J and K provide example documents for an oil spill case and show how the assessment tools may be applied. Further examples of specific oil spill cases are available as summary reports of the annual round robins (RR-tests) organized by Bonn-OSINet [11] and in literature.

The purpose of this document is to assist the reader in defensibly identifying the source of an oil spill by comparing the chemical composition of spill samples against suspected source oils. The basis for this method is the widely variable chemical compositions of oils, which allows oils from different sources to be distinguished by analytical techniques. The method relies upon detailed chemical characterization and statistical comparison between samples (i.e. a spilled oil and a suspected source) diagnostic features in order to determine whether they “match”. To minimize the danger of “false positive matches”, good laboratory practices are to be maintained. A “positive match” between a spilled oil and suspected source sample may not be sufficient to identify the PRP (potential responsible party) on its own. However, this result can be critical evidence in proving a case within the legal process.

It should be noted that oil spill identification methodologies have limitations and may not necessarily lead to unequivocal conclusions. In certain cases, neither the oil spill nor suspected source(s) are unique or homogeneous in nature, e.g. due to the changing/variable nature of oil in bilge tanks or due to mixing of oils spilled from several sources. For such cases there is a risk that the chemical composition of the available spill and source samples may not match. Therefore, successfully identifying a spilled oil’s source depends upon the samples available for analysis. To minimize the danger for “false positive” and in particular “false non-matches”, good sampling practice is essential (as described in Part 1: Sampling).

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When no source oil is available, the methodology within this document can be used to determine the spilled oil type and selected characteristics. The characterization of a spilled oil sample can still be useful for several reasons:

— Rapid identification of a spilled oil type can help authorities track down potential sources of a "mystery" spill before the culprit has left the area (e.g. ship) or removed additional evidence. The mere differentiation between unused refined petroleum products (e.g. diesel fuel, heavy fuel oil), crude oil or waste oil (e.g. bilge residues, sludge, slops) can provide potentially valuable information as the possible source(s);

— In some court trials, the differentiation between pure refined products and waste oil may be very important because it allows conclusions to be drawn regarding the cause of an oil discharge, e.g. technical failure, accidental discharge, intentional discharge;

— In some countries, aerial photography of a plume behind a ship, combined with evidence the plume contained mineral oil, has been enough for a conviction;

— Finally, characterisation of the spilled oil provides a baseline against which future impacts to the affected area/environment might be compared.

This document is the result of advancements in the field of oil spill identification [e.g. 22, 36, 66, 67, 78 and 85] that have been made since the Nordtest Method [54] was first introduced in 1991. These have included:

— advancements in analytical methodologies;

— improved understanding of the specific chemical compositions and diagnostic features of oils;

— improved understanding of how an oil's composition changes in the environment (e.g. due to weathering);

— improvements in the statistical and numerical analysis of chemical data.

These advancements have been made by researchers around the world and documented in a wide range of peer-reviewed literature. In addition, numerous Proficiency Testing Programs (PTPs. or round robin tests (RR)) have been conducted to evaluate and improve upon the methodology. Since 2004, in the framework of Bonn-OSINET (Bonn Agreement oil spill identification network of experts), annual interlaboratory studies have been organized jointly by RWS-lab (Rijkswaterstaat - Laboratory in the Netherlands) and BSH (Bundesamt für Seeschifffahrt und Hydrographie in Germany) in which laboratories from around the world participate. The studies have covered oil spill cases dealing with light fuel oil distillates (diesel oils), bilge water samples (a mixture of water, gas oils and lubricating oil), crude oils and heavy fuel oils. Findings from these studies have been discussed at annual meetings by the participating analysts and have been taken into account for refining the suggested methodology described herein. The final reports of the interlaboratory studies can be downloaded for free from the Bonn-OSINET part of the Bonn-Agreement website [11].

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1 Scope

This document specifies a method to identify and compare the compositional characteristics of oil samples. Specifically, it describes the detailed analytical and data processing methods for identifying the characteristics of spill samples and establishing their correlation to suspected source oils. Even when samples or data from suspected sources are not available for comparison, establishing the specific nature (e.g. refined petroleum, crude oil, waste oil, etc.) of the spilled oil still helps to constrain the possible source(s).

This methodology is restricted to petroleum related products containing a significant proportion of hydrocarbon-components with a boiling point above 150 °C. Examples are: crude oils, higher boiling condensates, diesel oils, residual bunker or heavy fuel oils, lubricants, ⟨A1⟩ sludge samples and mixtures of bilge, ⟨A1⟩ as well as distillate fuels and blends. While the specific analytical methods are perhaps not appropriate for lower boiling oils (e.g. kerosene, jet fuel, or gasoline), the general concepts described in this methodology, i.e. statistical comparison of weathering-resistant diagnostic ratios, are applicable in spills involving these kinds of oils.

Paraffin products (e.g. waxes, etc.) are outside the scope of this method because too many compounds are removed during the production process [37] to correctly distinguish them from each other. However, the method can be used to identify the type of product involved.

Although not directly intended for identifying oil recovered from groundwater, vegetation, wildlife/tissues, soil, or sediment matrices, they are not precluded. However, caution is needed as extractable compounds can be present in these matrices that alter and/or contribute additional compounds compared to the source sample. If unrecognized, the contribution from the matrix can lead to false “non-matches”. It is therefore advisable to analyse background sample(s) of the matrix that appear unoiled.

When analysing “non-oil” matrices ⟨A1⟩ (e.g. contaminated feathers, plants, rocks, sand) ⟨A1⟩ additional sample preparation (e.g. clean-up) is often required prior to analysis and the extent to which the matrix affects the correlation achieved is to be considered. Whether the method is applicable for a specific matrix depends upon the oil concentration compared to the “matrix concentration”. In matrices containing high concentrations of oil, a positive match can still be concluded. In matrices containing lower concentrations of oil, a false “non-match” or an “inconclusive match” can result from matrix effects. Evaluation of possible matrix effects is beyond the scope of this document.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 15522-1, *Oil spill identification — Petroleum and petroleum products — Part 1: Sampling*

ISO 1998-1:1998, *Petroleum industry — Terminology — Part 1: Raw materials and products*

ISO 16703:2004, *Soil quality — Determination of content of hydrocarbon in the range C10 to C40 by gas chromatography*

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3 〚A1〛 Terms, definitions and abbreviations 〚A1〛

〚A1〛 For the purposes of this document, the terms and definitions given in ISO 1998-1:1998 and the following terms, definitions and abbreviations apply. 〚A1〛

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— IEC Electropedia: available at https://www.electropedia.org/

— ISO Online browsing platform: available at https://www.iso.org/obp/

3.1 General

3.1.1
chain of custody
practice of ensuring security of the sample so that no one has an opportunity to tamper with or otherwise alter the sample or the results

Note 1 to entry: It includes chronological documentation that records the sequence of sample handling including sampling, sealing, storage, transfer, analysis and disposal to ensure that only documented sample handlers have direct access to the samples

3.1.2
mixing
mingling of sources containing or consisting of petroleum (products) before, during or after the spillage

Note 1 to entry: Can result in a heterogeneous spill composition (see 3.1.4).

3.1.3
contamination
changes in oil composition which take place during/after the spillage in either sample by addition of non-petroleum compounds from biogenic 〚A1〛 *deleted text* 〚A1〛 or anthropogenic sources 〚A1〛 *deleted text* 〚A1〛

〚A1〛 Note 1 to entry: A biogenic compound is for instance fat from feathers, where anthropogenic sources are for instance compounds from plastics. 〚A1〛

Note 2 to entry: Mixing and contamination are used to differentiate between the addition of petroleum products (mixing) and non-petroleum products (contamination).

3.1.4
sample heterogeneity
non-representative or non-homogenous character of samples 〚A1〛 *deleted text* 〚A1〛

〚A1〛 Note 1 to entry: This can be caused for example by variable degrees of stirring within a vessel, tank, pipeline or oil slick originally containing oil(product)s with different compositions. 〚A1〛

3.1.5
duplicate
second injection of the same sample extract within a sequence or within an oil case

3.1.6
replicate
second aliquot of a sample or a second sample taken from exact the same sample location and at the same time

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Note 1 to entry: A second sample taken from a different location of a larger spill is not a replicate, but a different sample.

3.1.7
waterborne oil
petroleum or petroleum product borne by water or available in the water column from marine, estuarial and aquatic environments

Note 1 to entry: Aquatic environments include lakes and rivers, but exclude groundwater.

3.1.8
weathering
〚A1〛 processes that cause changes in oil composition of source and spill samples which can take place after the spillage and processes caused by oil response techniques

Note 1 to entry: Natural processes can be evaporation, dissolution, emulsification, photo-oxidation, biodegradation, wax *redistribution*, whereas oil responses are for example chemical dispersion and burning. 〚A1〛

Note 2 to entry: Sometimes the source is more weathered than the spill. For example, when a slop tank contains a mixture of oil and water and is leaking oil into much colder water, the oil in the slop tank can be biodegraded to a higher degree than the spilled oil.

3.1.9
bilge
water which can be contaminated by oil resulting from things such as leakage or maintenance work in machinery spaces 〚A1〛 *deleted text* 〚A1〛

〚A1〛 Note 1 to entry: Any liquid entering the bilge system including bilge wells, bilge piping, tank top or bilge holding tanks is considered oily bilge water. 〚A1〛

[SOURCE: MEPC [48]]

3.1.10
slop
slop tank
mixture of water and oil residues from cargo tanks in oil tankers possibly containing crude oil, heavy fuel oil/water emulsions, wax, sediments and other tank residues

3.1.11
sludge
residual waste oil products generated during the normal operation of a ship 〚A1〛 *deleted text* 〚A1〛

〚A1〛 Note 1 to entry: Such products are those from the purification of fuel or lubricating oil from main or auxiliary machinery, of separated waste oil from oil filtering equipment, of waste oil collected in drip trays, and of waste hydraulic and lubricating oils. 〚A1〛

〚A1〛 *deleted text* 〚A1〛

3.1.12
tank washings
tank washing water containing cargo tank residues 〚A1〛 *deleted text* 〚A1〛

〚A1〛 Note 1 to entry: These tank washings include oil, wax, sediment and other foreign matter such as tank cleaning chemicals. 〚A1〛

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3.1.13
sediment
matter that settles to the bottom of a liquid, material deposited by water, wind, or glacier

3.2 Sample comparison

3.2.1
PW-plot
graph based on GC-FID or GC-MS data of two samples normalised to a non-weathered compound or group of compounds and sorted on retention time

Note 1 to entry: The name "PW-plot" is originally a reference to Per Wrang, who introduced the plot in the Nordtest method [54]. In this document, the name PW-plot is used as an abbreviation of a "Percentage Weathering" plot.

3.2.2
diagnostic ratio
DR
ratio between the peak heights of single compounds or peak areas of compound groups of the same sample selected by their diversity in chemical composition in petroleum and petroleum products, their discriminative power and on their known behaviour in weathering processes

3.2.3
likelihood ratio
LR
ratio of two probabilities

Note 1 to entry: The numerator is the probability of obtaining the evidence when the prosecutors scenario is true. The dominator is the probability of obtaining the evidence when the defence scenario is true (see Annex D).

3.2.4
critical difference
CD
14 % of the mean value of a ratio for two different samples

Note 1 to entry: The fixed value of 14 % is based on the maximum allowable RSD of 5 % for the diagnostic ratios (see 7.5.5).

Note 2 to entry: A higher variance is often seen for unsaturated and/or low abundant FAMEs, probably because of their higher polarity relative to alkanes and PAHs, see [46] and F.5. Except for the normative ratio FAME C16:0 / FAME C18:0 an RSD of 10 % and a CD of 28 % shall be used for the informative FAME ratios listed in Table E.5.

3.2.5
significant difference
difference between a property or analytical result of two samples that cannot be explained as caused by weathering, mixing or contamination

Note 1 to entry: The leaves on a broad-leaved tree changing colour and falling off in winter does not change the tree, growing pine needles the following summer would be a significant difference.

3.3 Abbreviations

For the purposes of this document, the following abbreviations apply.

CD	Critical Difference

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A1 DCM	DiChloroMethane A1
DR	Diagnostic Ratio
A1 ETFE	Ethylene-TetraFluorEthylene A1
FAMEs	Fatty Acid Methyl Esters
FID	Flame Ionisation Detection
GC	Gas Chromatography
HFO	Heavy Fuel Oil
HVO	Hydrotreated Vegetable Oil
LCO	Light Cycle Oil
LFO	Light Fuel Oil
LR	Likely Ratio
MS	Mass Spectrometry
NR	Normative Ratio
PAHs	Polycyclic Aromatic Compounds
RR-2014	Round Robin Test conducted in 2014
RSD	Relative Standard Deviation
A1 SIM	Selected Ion Monitoring A1
ULSFO	Ultra-Low Sulfur Fuel Oil
VGO	Vacuum Gas Oil

4 Strategy for the identification of oil spill sources

4.1 General

In this document the identification of spilled oils implies the comparison of specific chemical characteristics between the spilled oil and candidate source oil samples.

NOTE 1 See Annex G for further explanation on composition of oils.

The likeness of a source and spill sample is tested by analysis with GC-FID and/or GC-low resolution-MS followed by comparison of chemical composition using a suite of generic and diagnostic compounds. If no, or only insignificant differences, (i.e. differences being smaller than the analytical variability limits specified) are observed, a “positive match” should be concluded. However, if significant differences (i.e. differences not related to post-spill changes e.g. from weathering, contamination or heterogeneity) larger than the variance of the analysis are observed within these diagnostic compounds, it should be concluded that the samples are a “non-match”. Investigations may also result in conclusions intermediate to “positive match” and “non-match”, such as “probable match” or “inconclusive” (see D.2).

Likelihood ratios (see D.3), commonly used by forensic laboratories, can also be used to classify the likeliness between source and spill samples.

NOTE 2 In this document the descriptions and examples are based on the classifications of D.2.

IMPORTANT — It is practically and technically impossible to measure and compare every chemical in spilled oil and its prospective source in order to conclude a positive match exists. Therefore, in practice, two samples are considered a positive match if no statistically significant differences in diagnostic metrics

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determined by GC-FID and GC-MS analysis are present that cannot be explained by weathering, mixing or heterogeneity. This approach, i.e. looking for differences in diagnostic features instead of similarity among every possible feature, is conceptually more logical and more practically and technically achievable. As such, only distinct differences between samples can be proved. Therefore, when no statistically significant differences between samples are observed, a "positive match to a high degree of scientific certainty" should be concluded.

4.2 Basis for reliable conclusions – Numerical comparisons

The usual practice is to analyse samples qualitatively and then compare the chromatograms and ion chromatograms visually, such as described in the original Nordtest Method (1991) [54] and the ASTM methods [3] and [4]. The outcome of such qualitative comparisons is subjective and depends on the experience or bias of the analyst. Because of the high complexity of oils, and the many details that can be compared in the often very complex chromatograms, qualitative comparisons should always form an integral part of oil sample comparisons. However, in order to make conclusions more objective, reproducible, and therefore, more reliable, this document also requires the use and comparison of quantitative metrics; i.e. specific peaks or groups of peaks have to be measured and peak ratios have to be calculated and compared.

These integration values are used in two different ways:

a) Measurements of single compounds normalized to hopane – or, if hopane is not sufficiently present, to phytane bicyclic sesquiterpane 10 (BS10). If they are not sufficiently present, to any compound, preferable a compound that is robust to weathering (see also 7.4.2).

Comparing two oil samples by the normalized abundances of compounds spanning the boiling range of oil in the form of PW-plots, reveals:

— the variance of the analysis and integration, when comparing duplicates;

— the difference in concentration of compounds (e.g. biomarkers and PAHs) between the two samples;

— the effects of weathering on individual compounds and compound groups, and in turn, on prospective ratios based upon those compounds.

When the effects of weathering on a spilled oil are understood, any affected DR can be eliminated or cautiously considered when comparing a weathered spill sample to an unweathered source sample.

b) DRs: for keeping the analytical error low, measurements of compounds (peaks) that are recorded by a m/z value difference of less than 25 via GC-MS.

NOTE The term "diagnostic ratio" originates from geochemistry, and is related to the diversity in chemical composition of crude oils from different geologic provinces, oil fields, individual wells or reservoirs. DRs are characteristics, which collectively leads to a unique classification and identification of oils ("diagnosis": "sorting into categories"). The term "Diagnostic Ratio" is used here in order to pronounce the general differentiation possibilities of these ratios and thus their importance (see 3.2.2). In addition, this term is widely used in oil spill identification literature [22], [66] and [67].

Most DRs described in this document can be used for spills involving crude oil, bunker oil and bilge. However, with many DRs based upon higher boiling biomarkers they are of limited use for spills involving lighter crude oil (e.g. condensate) and lighter fuel oils (e.g. kerosene, diesel, gas oil). Further, be aware that higher boiling lubricating oils, normally rich in biomarkers, cannot contain sufficient n-alkanes or aromatic compounds for DRs based upon them to be of use.

Because of the need for some flexibility when investigating spills of different oil types, a specific list of normative diagnostic ratios (NRs) generated from diagnostic and more weathering resistant alkanes, PAH compounds and biomarkers has been developed for relevant types of petroleum (see 7.5.3).

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Normative ratios should be interpreted as being mandatory. In addition, there are also several informative ratios used at the judgment of the analyst to improve the number of features available for numerical assessment. The full set of normative ratios is sufficient for a sample comparison if at least 80 % of these ratios can be used. If the number of solid/usable ratios is lower, also all informative ratios given in Table E.5 become mandatory. Additional DRs, which may prove useful in oil spill identification, are suggested in this comprehensive review [67]. The resulting ratios are compared using a critical difference of 14 % (see 7.5.5).

Combining information derived from DRs, PW-plots, and visual comparison of ion chromatograms (see Figure 7) will provide robust understanding of the differences / similarities between two oil samples.

The current methodology is based upon semiquantitative analyses of compounds for establishing DRs from whole samples (no fractionation of aliphatic and aromatic compounds [2]). For laboratories who wish to undertake fully quantitative analysis (i.e. concentrations of individual compounds or compound groups are calculated), DRs may be established based upon absolute concentrations of the relative compounds [17], [18] and [28]. Laboratories taking this approach shall have robust QA/QC protocols for the quantitative analysis of the recommended compounds.

5 General lab instructions

5.1 Sampling and sample preparation

Once discovered, samples should be taken from the spill and any potential sources. Sampling and sample handling shall be carried out in accordance with EN 15522-1. When suspected sources are not available, the method can still be used to characterise the spilled oil.

At the laboratory the samples are visually characterised, described (see 6.2) and prepared for analysis (conservation, extraction and clean-up if needed) (see 6.3 and 6.4).

5.2 GC-FID and GC-MS analysis

Oil sample comparison by GC-FID (see Annex A) and GC-MS (see Annex B) is conducted in both cases by visual (qualitative) comparisons and by numerical (quantitative) comparisons (Figure 1).

GC-FID (or GC-MS full scan, when a GC-FID is not available) is used for the screening of all samples. Based upon the visual comparison of the gas chromatograms, source samples that are clearly a different type of petroleum than the spill sample(s), can be ruled out as “non-matching”. For example, a diesel fuel potential source sample can be eliminated from further consideration in a crude oil spill. Differences due to weathering of the spill sample are recognized and visualized by overlays of the gas chromatograms (Level 1.1). For source samples which are not clearly a different type of oil than the spill, n-alkanes and isoprenoids, normally the most abundant components of oils, are measured and compound ratios are calculated and compared. A proper visualization of the results is achieved by GC-PW-plots, where alkane-ratios of the spill sample(s) are plotted against those of the comparison sample(s) (Level 1.2).

NOTE Level 1.2 is optional and typically non-essential when a decision has been made to conduct Level 2 (GC-MS) analysis [A1] , except for light petroleum products [A1]. Its utility lies in the potential to eliminate clearly distinct source samples for analysis by GC-MS. However, if a spill involves a light petroleum product, the very low concentrations of biomarkers and often high concentrations of alkanes mean the alkane pattern, isoprenoid ratios and the GC-PW-plot can prove vital to the conclusion.

If no obvious or significant differences are evident following Level 1 (i.e. differences are below or slightly above the analytical error), conduct Level 2 (Figure 1):

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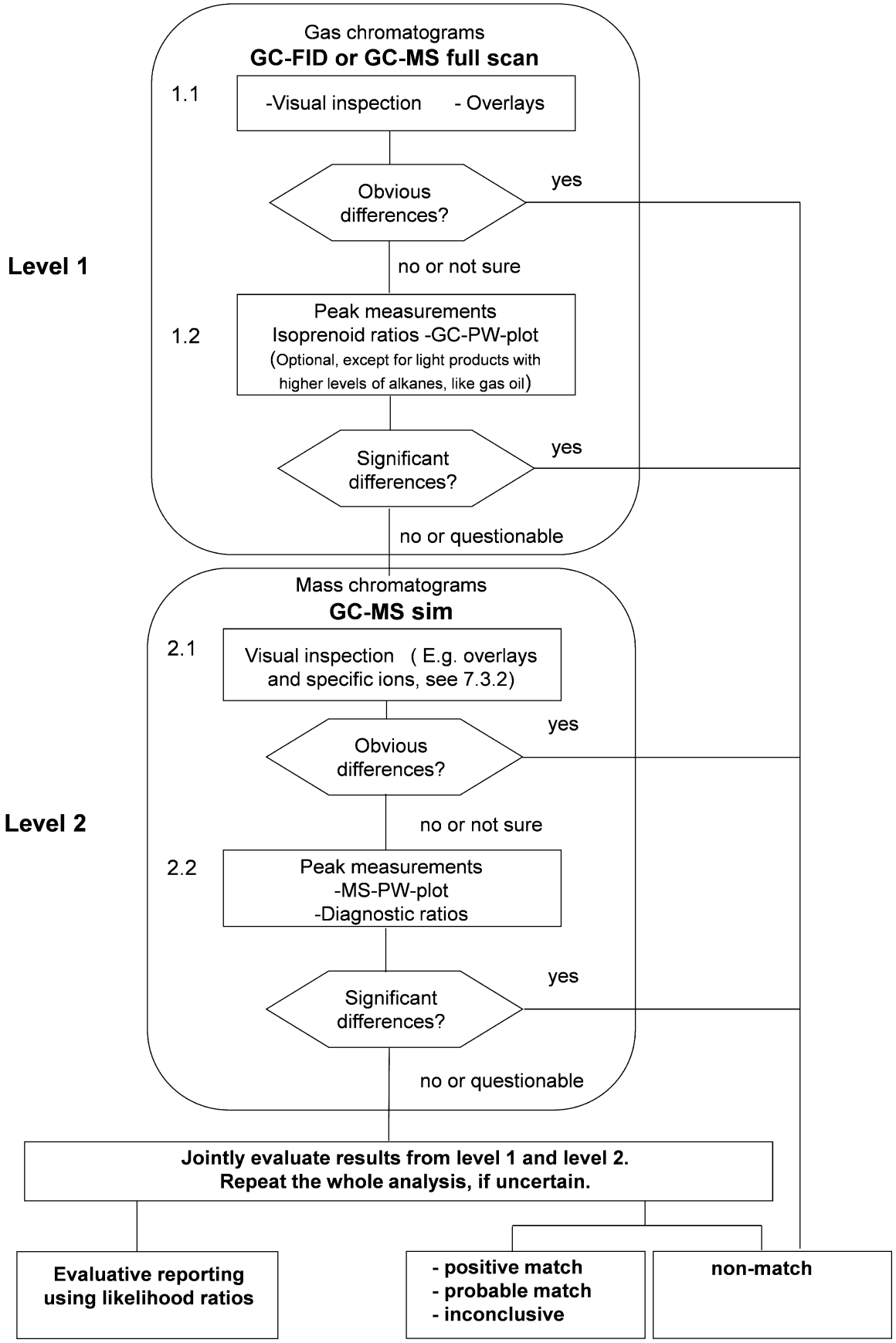


Figure 1 — Protocol/decision chart for oil spill identification

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In Level 2 detailed fingerprinting of the spill and suspected source samples are undertaken by GC-MS. While the steps followed are the same as Level 1, many more compounds are investigated and compared. Suspected source samples that are clearly different from the spill sample(s) can be ruled out as “non-matching” by visual assessment of the ion chromatograms (can be supported by overlays) (Level 2.1). For the remaining samples, results are visualized by plotting ratios of the spill sample(s) against those of the comparison sample(s) in form of MS-PW-plots (Level 2.2). At this point, “representative” target compounds are also measured and DRs calculated and compared (Level 2.2).

If no, or only smaller significant differences are observed, the conclusion of Level 2 is reached and the data from all four steps collectively evaluated (Levels 1.1 through 2.2; Figure 1).

Conclusions can be based on match definitions or on likelihood ratios (see Annex D).

5.3 Conclusions and reporting

Upon completion of the evaluation an "internal technical report" is prepared by combining and evaluating all information and all results (see 8.2 and Annex K). Final conclusions, including reasoning for those conclusions, are to be clearly stated in the “technical report”.

If requested by the client/authorities an external “identification report” shall be prepared (see 8.3 and Annex J).

6 Sample preparation

6.1 General

A single extract is prepared from an aliquot of the ‘whole oil’ and used for analysis by both GC-FID and GC-MS. This approach minimises the handling of the sample prior to analysis and reduces variability introduced during the sample preparation. Maintaining low variability/high precision is essential for small but authentic differences between samples to be recognised. Fractionation of the sample into aliphatic and aromatic hydrocarbon fractions prior to analysis [2] and [78] is neither recommended nor necessary.

6.2 Visual examination and description of samples

Sample description (e.g. an Ethylene-tetrafluorethylene (ETFE) net, see 6.3.3 and [73]), colour, odour, viscosity, content of any free water and debris shall be recorded. Any pieces of wood, fabric or other debris shall be removed from the sample with a pair of tweezers and noted in the report.

Take photos of each sample and/or samples combined to document their "as-received" condition.

Pay attention to:

— arrange good light conditions and a neutral background. E.g. a piece of white cardboard or a fume cupboard lined with e.g. Whatman Benchkote® (dull side out);

— show a scale or reference next to the sample(s), particularly in images with no clear reference point to sample size. When pots or jars of a known size are used an additional size reference is unnecessary;

— show a colour scale, when appropriate, to assist the user to check image colour balance;

— record if a bottle is sealed and what information is recorded on the label upon sample receipt;

— preserve the original image file and document it;

— save any operations on the original image, such as colour balancing or compression as separate working image file.

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6.3 Preparation

6.3.1 Sample storage

If a sample comprises a significant oil/water emulsion the sample bottle shall be stored undisturbed in a refrigerator at (4 ± 3) °C. This may allow the oil to separate from water, facilitating the oil's collection. Sample preparation shall still be carried out as quickly as possible and preferably within 7-days.

Samples of oiled sediments, soils and biota should be processed upon receipt at the laboratory. If this is not possible store in a freezer until required for processing.

When small parts of a sample (e.g. a bird or an oily cloth) are contaminated, take replicate samples because the composition can be different depending on when and where the contamination has occurred (i.e. birds can travel long distances between different locations and an oily cloth can have been used to wipe off oil from different locations).

When a sample from the freezer contains a lot of oil homogenize the entire oil sample before processing/analysis.

When a sample is primarily water and cannot be processed immediately, add dichloromethane (DCM[1]) (e.g. 20 ml to a 500 ml water sample) and shake vigorously. The sample shall be stored in a refrigerator at (4 ± 3) °C until further sample preparation is possible, preferably within 7-days.

NOTE Preferably within 7-days is an indication not to delay the analysis unnecessarily. However, it accounts for occasions where delays can occur, e.g. waiting on additional spill or source samples before the comparison can start. Any post-spill storage is a part of the weathering process a sample experiences and will therefore be considered during the assessment of the case. As such, it is not possible to state a time after which a sample is unsuitable for identification.

6.3.2 Water samples

When a water sample contains a layer of oil > 1 mm, draw off oil using a Pasteur pipette and transfer it to a vial. In this way no compounds present in the water layer are extracted. As alternative option (A) below can be used.

If samples primarily consist of water (no visual oil or less than 1 mm of oil on the surface) three options are available:

A) Extract the oil with an ETFE net (see 6.3.3).

This option shall not be used when DCM has already been added for conservation (see 6.3.1).

Take a small piece of ETFE net with a pair of tweezers and collect the oil by either moving the net up and down and around in the water or by putting the ETFE net into the sampling bottle and shake the bottle for several minutes.

After removing the net from the bottle, place it directly into a clean vial or bottle. Add an appropriate amount of DCM (e.g. 4 ml DCM in a 40 ml vial) and close the vial or bottle with a PTFE lined cap. Shake the vial vigorously for 5 to 10 s to extract the oil from the net. The extract is then diluted or concentrated to a suitable injection concentration (see 6.5, A.4.4 and B.4.6).

B) Extract the sample in the sample bottle when the sample volume is below 500 ml.

Add about 10 % of the sample volume in DCM to the sample bottle. Close the cap tightly and shake vigorously for 5 to 10 s. Let the phases separate and use a Pasteur pipette to transfer an aliquot of the DCM layer into a vial. The extract is diluted or concentrated to a suitable injection concentration (see 6.5, A.4.4 and B.4.6).

[1] If DCM is not allowed to use in the lab, use another appropriate solvent e.g. hexane.

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C) Extract the sample using a separating funnel when the volume is more than 500 ml.

The water is transferred to a separating funnel and extracted with DCM. Rinse the inside of the sample bottle with the DCM to be used for extraction, then pour into the separating funnel. Depending on the amount of oil, the sample shall be extracted once (e.g. with 60 ml when a layer of more than 1 mm of oil is present on the water), or twice (e.g. two 30 ml extractions) The combined extract is diluted or concentrated to a suitable injection concentration (see 6.5, A.4.4 and B.4.6).

IMPORTANT — If a concentration step is needed, use a gentle evaporation technique (e.g. Kuderna Danish, TurboVap®, N-Evap®).

6.3.3 Oil samples from an Ethylene-tetrafluorethylene (ETFE) net

Oil retained on ETFE[2] nets following application to spills on water, or from wiping oiled surfaces, is recovered by thoroughly rinsing with DCM or extraction in a vial using DCM. Dilute or concentrate the extract to a suitable injection concentration.

〚A1〛 *deleted text* 〚A1〛

If a sample is received that contains both water and ETFE net, but no obvious oiling, decant the water to a clean bottle/vial and start by extracting the ETFE net. Analyse the extract by GC-FID to establish if the amount of oil recovered is suitable for the analysis. If the concentration is too low to continue, extract the decanted water and combine the extracts as per 6.3.2.

Depending on batch and/or manufacturer, it is recommended to pre-clean the nets by rinsing or refluxing with DCM before use.

6.3.4 Thick oil and emulsified oil samples

Samples consisting of solid oil or sludge, e.g. high elastic/viscous heavy fuel oil (HFO), shall be equilibrated to room temperature. When the homogeneity of the sample is questionable, take two (or more) aliquots, 20 mg to 50 mg each, at different locations within the sample container and treat the aliquots as different samples.

Samples that consist primarily of thick or emulsified oil are to be equilibrated to room temperature before preparation. Decant off any free water. The thick oil/oil emulsion is gently homogenized before a 20 mg to 50 mg aliquot is weighed in a pre-weighted volumetric flask (e.g. 5 ml) and subsequently diluted with DCM to a suitable injection concentration.

Use pentane, hexane or heptane as solvent, when an asphaltenes precipitation is applied as clean-up step (see 6.4.3).

6.3.5 Tar balls and emulsified lumps

Oils weathered for a long time at sea and/or on the shoreline can form very viscous oil lumps (“tar balls”) often centimetres in diameter. The degree of weathering cannot be homogenous within the lumps, thus in such cases, it is recommended to split the lump and obtain two aliquot samples of oil (20 mg to 50 mg each), one from the middle (inside) of the lump and one from the more exposed surface of the lump. Samples shall be diluted in DCM as described in 6.3.4 before analysis.

[2] ETFE nets are recommended for sampling thin oil films/sheens [73 and 43]. ETFE is most well-known by the DuPont brand name Tefzel® and is an example of a suitable commercially available product. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product. An example is a Tefzel® net manufactured by SEFAR – Internet: www.sefar.com (Sefar Fluortex Product ref.09-150/36 or 9-250/39).

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6.3.6 Samples from oiled birds, fish and other animals and vegetation

Samples from oiled biota shall be handled with caution to reduce the risk from potentially transmissible diseases. Visible oil is collected by wiping the surface of the animal/vegetation using a piece of ETFE net. The oil on the ETFE net is recovered by rinsing with DCM. If any solids are visible in the extract (e.g. feathers, particles) filter through a glass fibre filter or a layer of glass wool.

A clean-up of the extract (as described below) may be required if only a small quantity of oil is recovered. When the oil cannot be wiped off the biota using ETFE net, the feathers, or other surfaces, may be rinsed with hexane, followed by an extract clean-up procedure as described in 6.4.2. When homogeneity is questionable, take multiple aliquots from different areas and treat the aliquots as different samples.

6.3.7 Sediment

Samples of sediment can range from sand contaminated with oil to a sample of the bank of a stream or river containing a lot of water and plant debris. Extraction depends on the oil concentration and the water content. Samples with visible oil or a strong oil smell can directly be extracted with DCM. Remaining samples shall be extracted with a mixture of acetone/n-heptane 1:1 with anhydrous Na_2SO_4 (shake or Soxhlet) followed by water back removal of the acetone, according to ISO 16703:2004, Clause 2, Clean the extracts at least by a silica or Florisil® (Magnesium silicate phase)[3] column clean-up to remove polar compounds and when biogenic compounds are suspected start with an alumina column clean-up.

6.4 Sample clean-up

6.4.1 General

Matrix interferences can be caused by contaminants that are co-extracted from the sample. Since biogenic materials and other naturally occurring materials can cause interferences, clean-up methods are needed to eliminate or minimize matrix interferences prior to GC-FID and GC-MS analysis. The materials most commonly used for cleaning samples are glass wool without or with anhydrous Na_2SO_4, alumina, Florisil® and/or silica.

High boiling compounds, not eluting from the GC column, strongly influence the performance of the GC injector, column and detector. Therefore, it is strongly recommend to clean “black oils”, such as heavy fuel oils, slops and bilges in order to remove the high amount of asphaltenes and/or soot particles, that are typically detrimental to instrumentation.

Table 1 connects sample/extract appearance with a range of clean-up methods described in 6.4.2 to 6.4.5.

IMPORTANT — All matrices (oils, sediments/soils, vegetation, wildlife, emulsions) within a given investigation shall be cleaned in the same manner in order to maintain the comparability of the samples.

6.4.2 Particle removal

Sample handling should be minimized during sample clean-up. When a simple step is enough, it should be applied. Clear, transparent samples with particles or slightly cloudy samples (Table 1 - #2 and # 3) can be centrifuged or filtered over a glass fibre filter/glass wool with or without Na_2SO_4.

A self-packed filter can be constructed from a glass Pasteur pipette (typical volume 2,5 ml). Place a small piece of glass wool into the pipette and push down to the taper using the tip of another long tip Pasteur pipette. Cut a small piece of an exchangeable plastic tip of a 10 ml piston pipette in such a way that a thin layer of anhydrous Na_2SO_4 can be guided into the Pasteur pipette until about 30 % of the bed volume, tap to settle the Na_2SO_4 layer.

[3] Florisil® is an example of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.

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Clean the loaded Pasteur pipette with three bed volumes of DCM - discard the DCM. Add 1 ml to 5 ml of the sample extract to the pipette. To compensate for dilution during the clean-up step, it is useful to do this at about four times the GC injection concentration. Extract the glass wool/Na_2SO_4 layer with three times the added sample extract volume of DCM. Adjust the final extract volume to the concentration needed for the GC injection.

Table 1 — Clean-up methods related to the sample/extract appearance

#	Appearance	Product examples	Clean-up options
1	Clear, transparent.	Condensate, diesel	No clean-up. Dilute or concentrate.
2	Clear, transparent with particles.	Diesel or bilge	Filter over glass wool with or without a layer of anhydrous Na_2SO_4
			Centrifugal separation
3	Cloudy.	Bilge, water extracts, sediment extract	Filter over glass wool with or without a layer of anhydrous Na_2SO_4
			Centrifugal separation
			Silica or Florisil® column clean-up
4	Contaminated with biogenic compounds.	Bird feather extracts, ditch water extracts, sediment extract	Alumina column clean-up.
5	Dark, black.	Crude, HFO, sludge	Asphaltenes precipitation followed by centrifugal separation
			Silica or Florisil® column clean-up
6	Brown thick foamy emulsion	Mousse	Dissolve in DCM and treat based on appearance
7	Solid	Tarball	Dissolve in DCM and treat based on appearance

6.4.3 Asphaltenes precipitation

Asphaltenes are complex large molecules that dissolve in toluene, partially in DCM, but not in pentane, hexane, or heptane [5]. Asphaltenes can be found in high concentrations in HFO. They remain on the GC liner/column after injection and will impact upon the chromatographic separation.

When a sample is expected to contain asphaltenes (6.3.4, 6.3.5. and 6.3.6) transfer an aliquot to a centrifuge tube. Add n-pentane, n-hexane, or n-heptane, cap the tube, and shake it to dissolve (most of) the sample. Place the tubes in a refrigerator for several hours then centrifuge. Dilute the supernatant with DCM to the concentration needed for the GC injection.

Proceed with a column clean-up according 6.4.5, when the supernatant is not clear and transparent.

6.4.4 Alumina column clean-up of biogenic materials

If the potential for contamination of an extract is very high, for example with lipids or fatty acids, it is recommended to pre-clean the extracts using an alumina column prior to the silica column. See [23] and [86].

The alumina column (19 mm ID glass column with stopcock) shall be prepared just prior to use: Pack the lower end of the column just above the stopcock with a small piece of glass wool. Close the stopcock and add circa 25 ml DCM to the column. Weigh 20 g of alumina into a glass beaker and add 30 ml of DCM.

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Swirl to remove any bubbles. Slowly pour the alumina into the column while rinsing the beaker with DCM. Allow to settle. Add approximately 2 g of anhydrous Na_2SO_4 (dried at 400°C for 3 h and stored in a desiccator).). Drain the DCM from the column until the head of the liquid in the column is just above the Na_2SO_4. Close the stopcock to stop solvent flow. Add 50 ml of hexane and drain to the top of the packing material.

Transfer 1 ml sample extract (in DCM) onto the column. Rinse out the extract vial with 1 ml DCM and immediately add it to the column. Allow the sample to drain to the top of the packing material. Add 100 ml of DCM and slowly drain the column (approximately 2 ml/min). Collect the eluate in a 250 ml EM-flask. Alternative clean-up procedures are described in [52].

6.4.5 Silica or Florisil® column clean-up

Use self-packed or pre-packed silica or Florisil® columns (500 mg of silica or Florisil®, e.g. Varian, Supelco or Isolute). Use PTFE or glass columns, because other column materials can leach contamination into the extract even after washing. Apply a thin layer of anhydrous Na_2SO_4 to the pre-packed columns before use.

Self-packed columns are easily prepared using glass Pasteur pipettes (typical volume 2,5 ml). Fill each pipette with a small piece of glass wool, (500 ± 50) mg of either of Florisil® or silica and a thin layer of anhydrous Na_2SO_4 on top:

— Silica: grain size in the range of 63 µm to 210 µm (230 mesh to 70 mesh). Activation by heating to (400 ± 10) °C for 4 h or to (140 ± 5) °C for 16 h and stored in a desiccator. Deactivated by adding 10 % ultrapure (Type 1) water before use.

— Florisil®: grain size in the range of 150 µm to 250 µm (100 mesh to 60 mesh), heated to (140 ± 5) °C for 16 h and stored in a desiccator.

To elute the aliphatic and aromatic oil compounds, use DCM as eluent for Florisil® columns and DCM/Hexane 1:1 for silica columns. Clean the column with three bed volumes DCM. Drain the DCM from the column until the head of the liquid in the column is just above the Na_2SO_4. Add 1 ml to 5 ml of the sample extract dissolved in DCM to the column. To compensate for dilution during the clean-up step, it is useful to prepare the sample extract about four times the GC injection concentration. Elute the column with three bed volumes of DCM. If required, a pipette pump may be utilised to increase the flow rate and purge the remaining DCM from the column. [6].

Adjust the final extract volume to the concentration needed for the GC injection.

Laboratories specialising in the analysis of hydrocarbon contaminated soils, sediments or biota may use larger self-packed columns. Glass Luer lock syringes combined with glass fibre filters and solid phase extraction (SPE) manifolds dramatically increase sample throughput. If attempting to replicate that approach increase the amount of Na_2SO_4, eluent for pre-cleaning and eluent for extraction in relation to the amount of silica or Florisil® used.

Test each batch of pre-packed columns or each batch of silica or Florisil® for the self-packed columns, when used for the first time. A1 The reference oil solution used for sequence testing (see B.4.6) may also be used for testing the clean-up column. A1

Treat the reference oil solution as a sample according to the method described above. The acceptability of the pre-packed columns, silica or Florisil® is confirmed when a “positive match” is found between reference oil samples with and without clean-up. Careful consideration is still required, even after a “positive match”. Differences in DRs close to the critical difference could indicate an effect from the clean-up that can become more pronounced in other spill/source oil extracts.

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IMPORTANT - FAMEs from biodiesel are slightly more polar than the petroleum based diesel compounds and are therefore more difficult to elute from a silica or Florisil® column. About 5 to 10 bed volumes of DCM are needed to elute the FAMEs sufficiently (See Figure F.26 for experimental results). Test the recovery of the FAMEs with diesel with 5 % to 7 % biodiesel for each new batch of pre-packed columns or new batch of silica or Florisil® for the self-packed columns. Analyse the untreated and treated diesel with GC-MS and compare the results. Samples should match according to the criteria for the MS-PW-plot and the ratio comparison. A higher variance for the informative FAMEs can be accepted, see 7.5.5. If a "positive match" is not found, or the FAMEs DRs are all close to the critical difference, additional DCM volumes is probably not sufficient to recover the FAMEs. In such cases the addition of 1 % to 2 % of acetone to the DCM is recommended to improve the extraction efficiency for the FAMEs.

6.5 Recommended injection concentration

Oil samples contain a range of compounds in a range of concentrations. For oil spill identification investigations, both high and low concentration levels of target compounds (i.e. those used in calculating DRs) have to be evaluated. It is recommended that spill and source samples have similar injection concentrations to prevent gross concentration differences from influencing the comparison. The injection concentration of an extract will ideally be around the middle of the concentration range, as determined during the method validation.

The mid-level injection concentration for GC-FID can be determined by analysing the linear range of the alkane standard mixture (see A.2). For GC-MS, a reference crude oil (see B.2) can be used to find the best peak shape for both high and low level compounds, and a sufficient S/N ratio (see 7.5.6.2) for the low level biomarkers in one injection.

Table 2 shows some recommended injection concentrations of common oil types. When the GC systems are configured as proposed in A.3 and B.3.1, it is strongly advised not to increase the injection concentration to prevent overloading of the GC column(s).

As instrument sensitivity typically improves with each new model, concentrations listed in Table 2 will need to be adjusted. Laboratories can determine the mid-level concentration as described above for one or two different oil types and reduce the recommended injection concentrations in Table 2 proportionately.

Table 2 — Recommended injection concentrations for different oil types

Oil type	Injection concentration mg/ml
Gas oil, LFO, LCO and VGO	1,5 (e.g. 20 µl → 10 ml DCM)
Lubricating oil	2
Light bunker oil	1,5
HFO very viscous to solid	5 to 10
Bilge	2
Bunker oil	2
Paraffins	0,1 (for identification, see the scope)
Crude oil	1,5 (light) to 5 (heavy, viscous) depending on composition and viscosity

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7 Characterization and evaluation of analytical data

7.1 General

Replicate analyses of two aliquots taken from a homogenous sample or the duplicate injection of an extract gives information about the performance of the analytical system for the samples involved. Replicate preparation and analysis of samples provides a measure of the handling and analytical portions of the system, while duplicate analyses of a single extract provides a measure of the analytical portion of the system.

For spill investigations involving only oils, duplicate analyses of one extract is sufficient, since the preparation or handling of pure oils prior to instrument analysis is minimal. However, for spill investigations involving matrices other than pure oils (e.g. vegetation, wildlife, soils or sediments) or oils suspected to exhibit heterogeneity, then replicate samples shall be prepared and analysed in order to monitor the precision of both the handling and analytical portions of the system.

Therefore, in order to improve the robustness of the method and to check the variance of the DRs used in an investigation, a minimum number of samples shall be analysed in duplicate as follows:

— in investigations with two samples: the extracts from both samples;

— in investigations where there are many samples, and duplicate analyses of all samples is impractical: at least 10 %, with a minimum of two samples representing the apparent variety of the samples;

— in investigations of very viscous and solid samples (e.g. tar balls): multiple aliquots of potentially heterogeneous samples shall be prepared and some of them shall be analysed in duplicate (see also 6.3.4 to 6.3.6).

All sample extracts shall be analysed under the same analytical conditions as were used in the analysis of analytical standards. All sample extracts shall be analysed first, followed by the sample extracts assigned to be analysed in duplicate in the same order (see K.3). As a result, one duplicate pair is analysed at the beginning and end of the sequence. In this way the duplicates best reflect the variance within the sequence and can indicate changes in instrument performance. E.g. a rising baseline can indicate contamination from some/all samples, indicating the need for a clean-up step, even with some diesels, or perhaps additional column conditioning.

NOTE A reverse order would put some duplicates close together (nearly back to back) while others are measured with a great time difference. Any analytical variances would affect the duplicate measurements differently.

The use and preparation of appropriate analytical standards are described in Annex A for GC-FID and Annex B for GC-MS.

In order to check instrument performance and stability during the sequence, analysis of an external standard is required at the beginning of an analytical sequence, after approximately every tenth injection, and at the end of a sequence.

It is not recommended to compare samples from different sequences, as GC system performance and mass spectrometer tuning might have been changed over time.

Special attention shall be given to maintaining the intensity of higher boiling compounds (i.e. monitoring any mass discrimination) and the resolution of closely eluting peaks. It is recommended to log the intensity of some peaks at the beginning, middle and end of the chromatogram and some of the normative ratios in, for example, a control chart to track system performance during and between sequences.

For laboratories analysing oil spill samples on a regular basis, it can be sufficient to run an external standard daily and to log the results in a control chart.

An example of the evaluation of an oil case with four samples is illustrated in Annexes J and K.

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7.2 Characterization by GC-FID – Level 1

7.2.1 General

Following the sample preparation steps described above, the chemical fingerprinting analysis in the laboratory starts with the GC-FID screening analysis of all samples (Figure 1). Annex A describes the minimum and recommended specifications for standards and instrumental conditions for GC-FID equipment used in the screening analysis. See B.3.2. if a GC-FID is not available.

The data from the GC-FID screening shall be used for:

— characterising the spill and source sample(s) by determining the overall boiling (carbon) range of the oils, i.e. the total distribution of hydrocarbons including n-alkanes from n-C10 to n-C40 if present (for an overview of boiling points of n-alkanes, see Table G.1);

— visual inspections of the chromatograms for possible characteristic features and, if possible, a classification to a type(s) of oil comprising the spilled and source oils (see Annex I);

— eliminating source samples clearly unrelated to the spilled oil from the GC-MS analysis (Level 2) if they are obvious “non-matches”;

— determining the degree of weathering, i.e. severe, moderate or slightly weathered and sort the samples by their weathering degree;

— assessing the analytical variability (as based upon duplicate analyses);

— establishing selected normative ratios (Norpri/Pri, n-C17/Pri; n-C18/Phy and Pri/Phy);

— adjusting the pre-injection concentration of the samples to the appropriate mid-level concentration (see 6.5) for the GC-MS analysis.

Annex I presents and discusses examples of characteristic GC-FID chromatograms of different oil spill sample types, including light fuel oils, heavy fuel oils, lubricating oils, ships’ bilge, sludge samples, different crude oils and a mixture of gas oil with biodiesel. Examples in Annex I can prove helpful to provide a tentative classification of the type of spilled oil.

7.2.2 Evaluation of the influence of weathering on sample comparison

7.2.2.1 General

In oil spill identification investigations, weathering is defined as all the chemical, physical, or biological processes that affect the composition of the spilled oil after the oil enters the environment. When petroleum or petroleum products are spilled onto or into water, weathering can rapidly alter the spilled oil’s “fingerprint” from that of the original oil. Differences caused by weathering shall not preclude that a match exists between an unweathered source oil and a weathered spill oil. Therefore, it is important to have a good understanding of how the various weathering processes, such as evaporation, dissolution, photo-oxidation, chemical dispersion, burning, wax-enrichment or wax-depletion and biodegradation can influence the analytical results (see Annex H and [71], [67, chapter 3 and 4] and [7], for additional information).

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7.2.2.2 Chromatogram overlay – Level 1.1

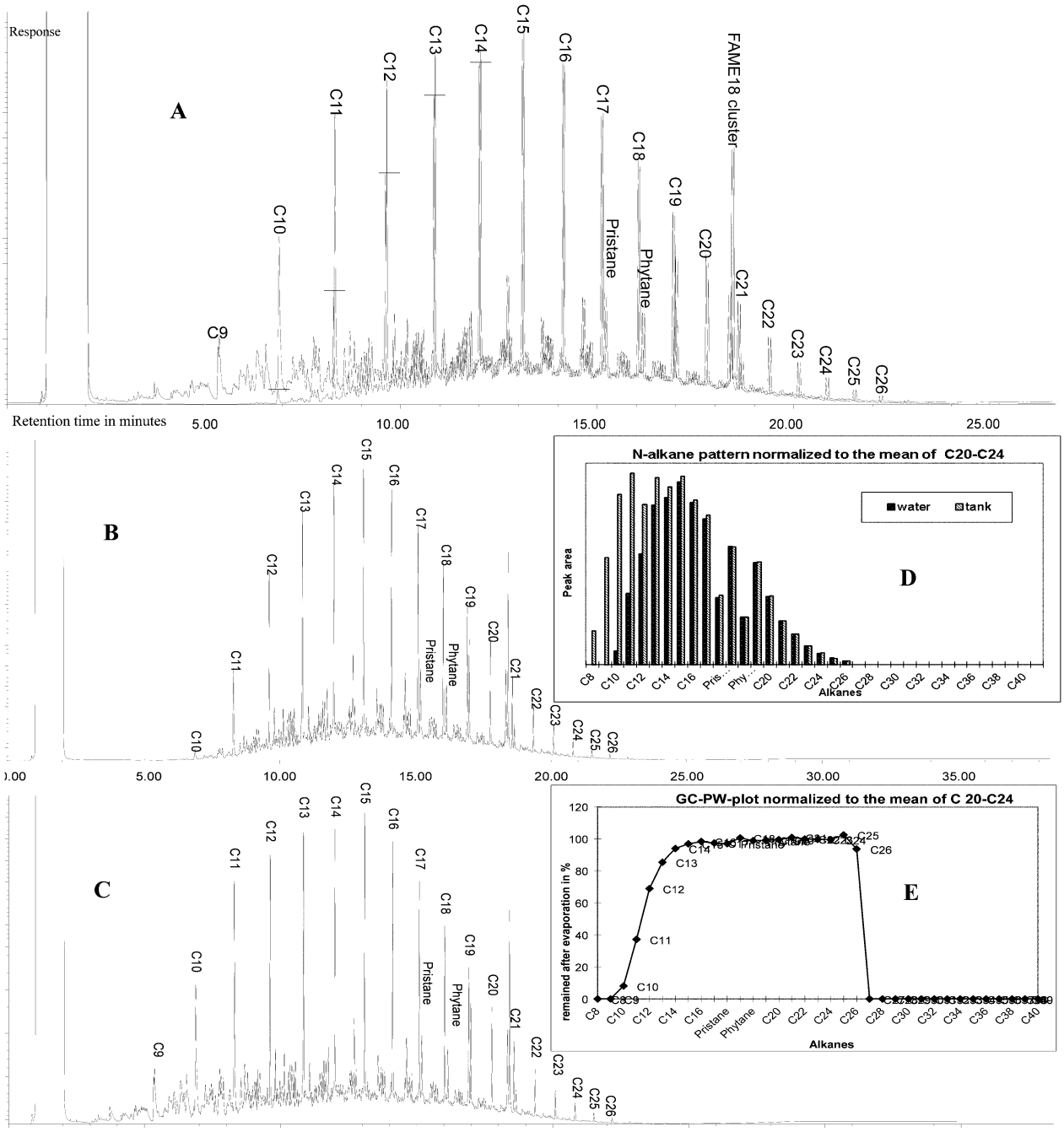
Overlays are useful to check for small differences between chromatograms. They can be generated with most data integration systems and/or with additional software tools. Figure 2 shows a sample with diesel/biodiesel spilled on surface water (B) compared with a suspected diesel/biodiesel source sample from a fuel tank (C). The upper chromatogram (A) shows an overlay of the chromatograms. The overlay was achieved by normalisation/manipulation of these samples GC-FID chromatograms to a comparable attenuation within the non-weathered portions of each, i.e. n-C16 to n-C24 range using Power Point® [4]. Retention times are slightly shifted to better recognize the individual peaks.

7.2.2.3 Bar chart (histogram) – Level 1.2

Figure 2D shows a bar chart of the n-alkane and isoprenoid abundances for the two investigated samples that was constructed using Excel®. Compound abundances were determined by integrating peaks in the GC-FID chromatograms and normalizing their areas in order to compensate for concentration differences. The n-alkanes of the two samples have been normalized to the mean areas of n-C20 to n-C24, which in this case have not been influenced by weathering. The reduced abundances of lower boiling n-alkanes in the spilled oil (solid bars) are consistent with losses attributable to evaporation affecting the spilled oil.

[4] Power Point® and Excel® are the trade names of products supplied by Microsoft. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead to the same results.

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Key

A	overlay of B and C. Lines on the peaks indicate the spill sample levels	D	bar chart
		E	GC-PW-plot
B	surface water chromatogram	x-axis A, B, C	retention time in min
C	fuel tank chromatogram	y-axis A, B, C	response

Figure 2 — Analytical results from an investigation dealing with diesel/biodiesel. The chromatogram of the surface water sample showing a gradual loss of lighter compounds

7.2.2.4 GC-PW-plot – Level 1.2

Figure 2 E shows a "percentage weathering" plot (GC-PW-plot) based on the same data as the bar chart. After normalization to the mean abundances of n-C20 to n-C24, the percentage remaining of each alkane

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in the spill sample can be calculated, when compared to the source sample (see 7.4.2). For example, in the spill sample of Figure 2 only about 1/3 of the concentration of n-C11 appears to remain, which is actually calculated to be 36 %. On the other hand, n-C20 in the spill sample is still at the same peak height and is shown as 100 % (or 0 % loss). The higher boiling n-alkanes in the GC-PW-plot show more variation, e.g. n-C26 (around 95 %), but this is because they are represented by small peaks with a higher variability in analysis and peak integration.

Except for biodegraded oil samples, the changes in the n-alkane distributions are caused mainly by evaporation, which progressively reduces the abundance of the lower boiling n-alkanes (i.e. those with higher vapour pressures) with decreasing carbon number. The GC-PW-plot shows this progressive loss of the lower boiling n-alkanes below n-C14 and therefore, it can be concluded that the differences between these two gas oil samples are caused by evaporative weathering. As such, in this example, DRs relying upon compounds boiling below n-C14 and with a retention time difference of more than 0,3 min (see Tables E.4 and E.5) are likely altered by weathering and therefore, shall not be used to compare the non-weathered source sample to the weathered spill sample.

7.2.3 Acyclic isoprenoids ratios – Level 1.2

The acyclic isoprenoids ratios of Norpri/Pri, n-C17/Pri, n-C18/Phy and Pri/Phy are potentially important DRs that can be determined from the Level 1 GC-FID chromatograms. However, if the spill sample is too weathered, or if the concentration of these compounds in a sample is low relative to the unresolved complex mixture (UCM), e.g. as in many lubricating oils or HFO's, these ratios are less valuable or unreliable. In such cases, these ratios shall be handled with care or excluded from the numerical analysis of the data at Level 1.2.

Based upon their respective boiling points/vapour pressures, Norpristane, n-C17, pristane and also n-C18 and phytane are progressively affected by evaporation. Because the retention time differences between n-C17 and pristane and between n-C18 and phytane are much smaller compared with n-C17 and n-C18, Formula (1) may be used to estimate the Pri/Phy ratio of the spill sample:

$$R_{\mathrm{pp,c}} = \frac{R_{\mathrm{pp}} \times R_{1718\ \mathrm{source}}}{R_{1718\ \mathrm{spill}}} \tag{1}$$

where

$R_{\mathrm{pp,c}}$ is the corrected Pri/Phy ratio of the spill sample

R_{pp} is the Pri/Phy ratio of the spill sample

$R_{1718\ \mathrm{source}}$ is the $\left(\frac{C17}{C18}\right)$ *ratio of the suspected source sample*

$R_{1718\ \mathrm{spill}}$ is the $\left(\frac{C17}{C18}\right)$ *ratio of the spill sample*

As the time since the spill release passes, biodegradation can happen under favourable circumstances and has a stronger effect on n-C17 and n-C18 compared to norpristane, pristane and phytane (see H.17) and therefore, this weathering process shall also be considered when reviewing Level 1 data. The comparison of samples by means of these and other ratios is shown and discussed in 7.5.

7.2.4 Level 1 criteria

Criteria to evaluate the GC-PW-plot are the same as for the GC-MS-PW-plot and described in 7.4.

Criteria to evaluate the GC-ratios are the same as for the GC-MS ratios and described in 7.5.

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7.2.5 Level 1 conclusions

The suspected source samples can be concluded to be “non-matches” to the spill and eliminated from Level 2 analysis (see Figure 1), when the GC-FID chromatograms of spill sample(s) and suspected sources show differences in

— the hydrocarbon distribution,

— the unresolved complex mixture distribution, and/or

— the acyclic isoprenoid ratios,

that obviously are not caused by weathering, mixing, or potential heterogeneities in the spill samples. If there is any doubt following the Level 1 analysis, prospective source samples shall not be eliminated based upon the Level 1 results and shall be analysed using GC-MS in accordance with Level 2.

7.3 Characterization by GC-MS – Level 2

7.3.1 General

At Level 2, the spill and suspected source samples that have not been eliminated at level 1 shall be analysed using GC-MS in selected ion monitoring (SIM) mode. Annex B describes the specifications for standards and instrumental conditions for GC-MS equipment used in the Level 2 analysis and Annex E lists the target compounds.

The GC-MS-SIM analysis is necessary for determining the content and distributions of a suite of target petroleum analytes. Data from this analysis shall be used for:

— visual inspection of the extracted ion chromatograms;

— possible determination of characteristic features of the spilled oil that can aid in describing the general nature or type of spilled oil;

— classification of the oil type of the spilled oil;

— generation of MS-PW-plots to assess the analytical variability of the method, potential effects of weathering, heterogeneity and mixing;

— calculation of a suite of DRs based on an evaluation of the abundances of relevant compounds and compound groups.

7.3.2 Visual inspection and overall characterization - Level 2.1

The extracted ion chromatograms of the biomarkers and PAHs in each suspected source oil shall be visually inspected and compared to the spilled oil in order to identify any obvious non-matching source samples. Pay special attention to the ion chromatograms of:

— m/z 74: presence of FAMEs;

— m/z 113: n-alkane and isoprenoid pattern;

— m/z 191: the presence of hopanes in light fuels can indicate the presence of lubricating oil;

— m/z 191: the hopane pattern can be used to eliminate samples;

— m/z 192: C1-phenanthrenes pattern including methyl-anthracene can indicate catalytic cracking processes;

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— m/z 217 and 218: the sterane patterns can be used to eliminate samples;

— m/z 231: Absence of the tri-aromatic steranes in combination with the presence of the hopanes indicates the presence of lubricating oil.

Inspection of the extracted ion chromatograms of the source and spill samples will also reveal any particularly diagnostic features that could be useful for the sample comparison and, if appropriate, development of new DRs to include in Level 2.2 (see informative Annexes F and I).

7.3.3 Treatment of the GC-MS results – Level 2.2

For any of the suspected source samples that cannot be obviously ruled out as possible sources following the qualitative visual inspection of extracted ion chromatograms, the biomarkers and PAH peaks necessary to establish the MS-PW-plots and to calculate the appropriate DRs shall be integrated. Integrations shall be performed in a consistent manner across all samples. Annex F gives information about retention times and integration ranges for the suite of target compounds.

The user may choose to disregard unknown peaks in e.g. an isomeric pattern if contamination is suspected (see Annex F for examples).

The integration results shall be used for:

— assessing the analytical variability (as based upon duplicate analyses), integration of compounds and the impact of weathering on the samples by means of MS-PW-plots;

— determining which, if any, samples shall be re-analysed because of apparent heterogeneity;

— deciding which DRs are appropriate for numerical comparison based on the oil type, any particular features of the spilled oil, the analytical variability and the degree of weathering of spill and sometimes source samples;

— conducting a numerical comparison of appropriate DRs in the spill samples and each source sample;

— a final visual comparison of all extracted ion chromatograms of some of the most relevant samples to check the results of the numerical comparison by means of PW-plots and DRs (ground-truth all data).

7.4 Treatment of the results using the MS-PW-plot– Level 2.2

7.4.1 General

Use the MS-PW-plot to visualize and evaluate the analytical data from the GC-MS SIM method. In 7.2.2.4, the GC-PW-plot (based on n-alkanes and isoprenoids) has been introduced as a tool to evaluate weathering effects on spill samples. The PW-plot, however, is not only proof that differences in the lower boiling part have, or have not, been caused by evaporation. The plot also gives information about the analytical error and the quality of peak integration when applied to duplicates and can also be used to evaluate mixing and contamination. It shows the (non)-similarity of oil samples and is useful in recognizing whether specific differences are significant or not (see Annexes H and K and [33] and [34] for examples).

NOTE For the MS-PW-plots shown and discussed here, more compounds and also groups of compounds have been integrated, than are needed to calculate the normative ratios of Tables 4 and 5. This has been done to be able to give information about all the compounds mentioned in Table E.2.

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7.4.2 PW-plot calculations

A data point ($\gamma_{N,spill}$) in the PW-plot represents the abundance of a compound or compound group in the spill sample ($C_{N,spill}$) relative to the abundance of the same compound or compound group in the source sample ($C_{N,source}$). The data points are calculated from the peak height of single compounds or the peak area of groups of compounds. To compensate for the total amount of oil injected, the result is normalized to a robust compound present in both samples. The calculation is expressed in Formula (2):

$$\gamma_{N,spill} = \frac{\left(C_{N,spill} / C_{H,spill}\right) \times C_{H,source} \times 100\%}{C_{N,source}} = \frac{\left(C_{N,spill} / C_{H,spill}\right)}{\left(C_{N,source} / C_{H,source}\right)} \times 100\% \quad (2)$$

where

$\gamma_{N,spill}$ is the % of the abundance of compound N in the spill sample relative to the same compound in the source sample;

$C_{N,spill}$ is the height of the peak of compound N in the spill sample;

$C_{N,source}$ is the height of the peak of compound N in the source sample;

$C_{H,spill}$ is the peak height of hopane in the spill sample;

$C_{H,source}$ is the peak height of hopane in the source sample.

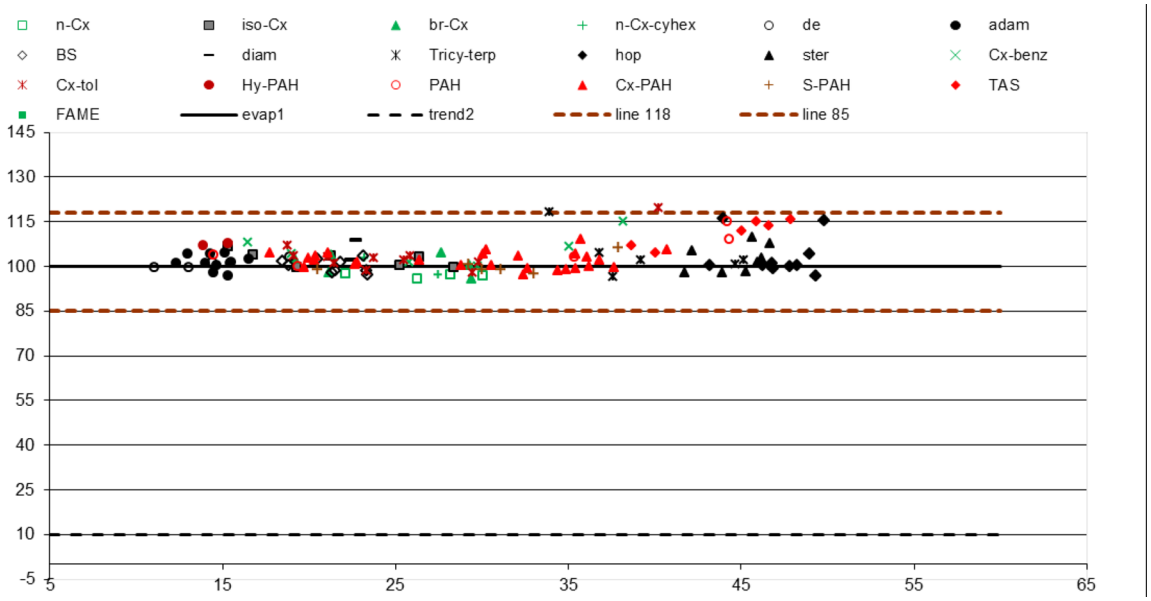
When comparing two samples by means of a PW-plot any compound can be used as a normalization compound, but in case of an oil spill it is preferable to use a compound that is robust to weathering. For this document, the peak height of hopane (C_H) has been chosen as the normalization compound for crude oil and heavy fuel oil. In lighter products, phytane (see F.2) is used as an alternative for hopane in case of a low concentration or absence of hopane. In very light products, like light diesel and condensate, BS10 (see Figure F.6) is used as an alternative for phytane in case of a low concentration or the absence of phytane.

7.4.3 Evaluation of the variability of the analysis and peak integration

In case of a duplicate analysis of an oil extract, all concentrations are the same and theoretically, all % C_{Nspill} of a PW-plot should be 100 %. In practice, the instrumentation and integration of the compounds causes some variation resulting in a range of data points around 100 % as shown in Figure 3. This MS-PW-plot is the result of a duplicate analysis of a Brent crude oil. The two dotted lines indicate the 85 % to 118 % range.

To assess the error range of the MS-PW-plot, two samples containing the same extract of a bilge sample were tested in the round robin of 2009 [34] resulting in an RSD of 7,5 %, which is since RR-2010 tested in the annual RR-tests organized by Bonn-OSINet and has been used for this document (see Annex C for the RR results and further explanation).

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Key

x-axis retention time y-axis % normalized to hopane

Figure 3 — MS-PW-plot of duplicate injections of a Brent crude oil

From a statistical point of view, the 95 % error range based on a RSD of 7,5 % should be from −2 to +2 standard deviations around the theoretical mean, i.e. 85 % to 115 %. Choosing one of the samples as 100 % and calculating the results of the other sample relative to it has however an effect on the difference to the mean of 100 %. The effect is shown in Table 3.

Table 3 — MS-PW-plot data point calculation. Influence on the error range by choosing one of the samples as reference for the other

Sample 1 peak height	100	100	100	100	100	100
Sample 2 peak height	100	95	90	85	80	70
Sample 2/Sample 1 in %	100	95	90	85	80	70
Sample 1/Sample 2 in %	100	105	111	118	125	143

The assessment to estimate the variance of the MS-PW-plot results in a range of 85 % to 118 % as indicated in Figure 3. This range is assigned as a maximum acceptable range for 95 % of the data points, when comparing duplicate analyses of sample extracts. If the comparison leads to outliers, the integration and possibly even the analytical method shall be checked and improved.

In translating this into a range to be used between source and spill samples of real cases, it should be emphasized that the range is calculated/estimated on the basis of duplicate analyses in which variations are caused exclusively by the GC-FID or GC-MS instrument used. In reality, differences in sample treatment, sample composition and injection concentration will also increase the variation. Despite that, the annual RR results show that the 85 % to 118 % range is a valid 95 % maximum acceptable range when comparing oil spill identification samples.

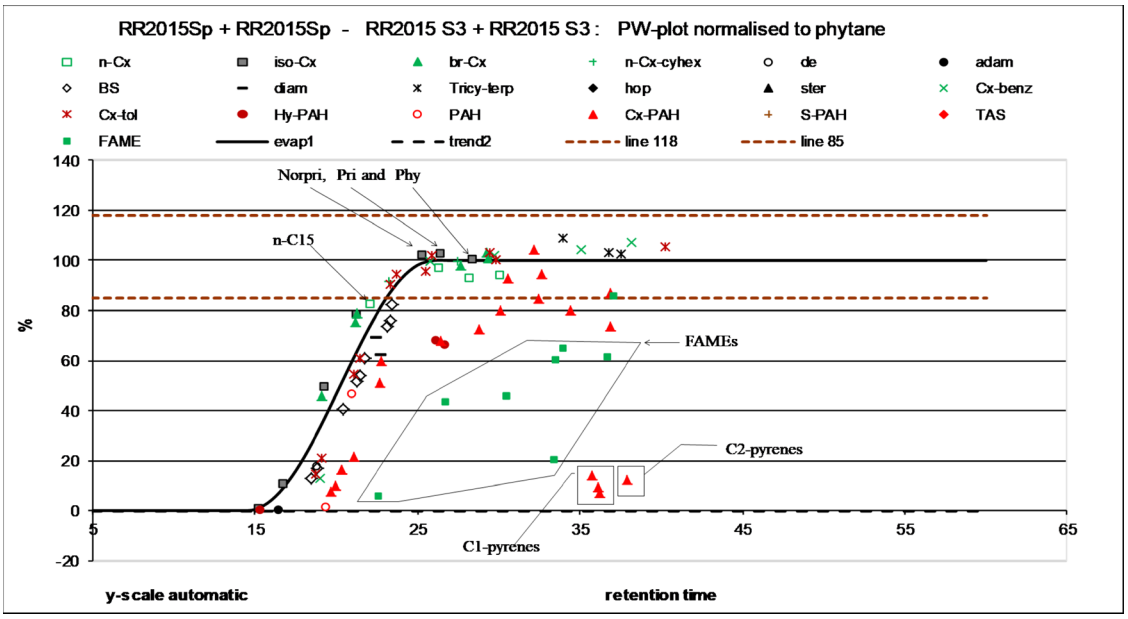
IMPORTANT — A higher variance is often seen for unsaturated and/or low abundant FAMEs, probably because of their higher polarity relative to alkanes and PAHs, see [46] and F.5. Except for FAME C16:0 and FAME C18:0 a range of 70 % to 143 % shall be used for the remaining FAMEs analysed with this method.

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7.4.4 Evaluation of weathering

Figure 4 shows the MS-PW-plot of two samples from the Bonn-OSINet round robin of 2015. The source is a diesel with biodiesel. 15,6 g of diesel with about 5 % biodiesel was added to a glass bowl filled with 10 l of river water forming a layer on top of 0,3 mm. The bowl has been outside for 1 day in shadow to half shadow, followed by 29 days covered in a shed to prevent too much evaporation and contamination from insects. The compounds listed in Annex E, Table E.2 have been analysed and integrated and each compounds' abundance has been normalized to that of phytane.

Figure 4 shows the effects of evaporation, photo-oxidation, dissolution and biodegradation.



Key

x-axis	retention time	y-axis	% normalized to phytane

Figure 4 — MS-PW-plot showing the effect of weathering on a spill sample relative to its source sample

Evaporation has removed and reduced the compounds eluting from the column before norpristane. Lighter compounds of an oil spill evaporate faster than compounds with higher boiling points. As GC separation is based mainly on boiling points of the components, a PW-plot with the retention time on the x-axis provides information about the evaporation of compounds after the spillage.

The compounds stable for weathering except evaporation follow a sinus shaped line indicated in Figure 4. The evaporation line of Figure 4 is calculated in Excel® and the formula used is shown in C.7.

The rest of the compounds are more or less reduced based on their weathering characteristics. The indicated FAMEs are mainly reduced by photo-oxidation and biodegradation, while the indicated C1- and C2-pyrenes are mainly reduced by photo-oxidation.

Based on the fact that:

— the stable compounds, eluting before norpristane, follow the evaporation line closely;

— the stable compounds, eluting after norpristane, remain present in abundances around 100 %, ranging from 85 % to 118 % relative to the source oil;

— all other compounds and compounds groups in the spill oil are reduced based on their weathering behaviour;

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it may be concluded that there are no significant differences between the abundances of the analytes in the spill and source oils and a match can be concluded.

See Annex H for detailed information about the weathering characteristics of compounds and compound groups.

7.5 Treatment of the results using diagnostic ratios – Level 2.2

7.5.1 General

If two oil samples are identical, their chemical composition is by definition the same, apart from those changes that occur in the spilled oil as a result of weathering, mixing and/or contamination. Thus, measured ratios between any pair of compounds unaffected by weathering or contamination should ideally be the same (up to a certain statistical analysis related confidence level) in identical samples. Various ratios between individual compounds or groups of compounds have been described extensively and used to compare oil samples [67]. In many cases, such ratios can be referred to as “diagnostic” if they possess the quality to genuinely discriminate between different oils. This is often the case with ratios between petroleum biomarkers, which typically provide a high degree of specificity among different crude oils [55] and [56]. Other ratios such as those based on PAHs can often be diagnostic in crude oils, but will probably not have a similar diagnostic significance in refined products in which their distributions can be affected by refinery processes (e.g. cracking, reforming, and hydro-treatment) (see I.5).

Whether genuinely diagnostic or not, any ratio not influenced by weathering or contamination shall still show the same value in any identical samples and thus be of "diagnostic" value in a specific spill case and contribute to discriminating and ruling out "non-source" oils.

Annex E includes a list of target compounds or compound groups and E.3 includes a suite of potentially DRs suitable for most oil spill investigations. It is important to realise that this list of PAH and biomarker analytes and suite of DRs are neither all-inclusive nor appropriate for all oil spill identification cases. The compounds and ratios of most use depend on the type of oil spilled (see Annex I).

In some spill cases, it can be prudent to include a certain characteristic feature of the spilled oil that is recognised as being particularly diagnostic. In other situations, the abundance of some compounds necessary to determine the suggested ratios are below analytical measurable limits or have been influenced by weathering or contamination. Thus, maintaining flexibility in the selection of the target compounds and ratios to be applied in any given oil spill identification investigation is very important. Such flexibility can be maintained by acquiring raw data for a broad range of target compounds representing various petroleum hydrocarbon groups, such as those listed in Annex F and used in Table B.2. From this suite of target compounds, potentially DRs unaffected by weathering or contamination shall be calculated and compared as described below.

The DRs and the compounds, which are used for producing the MS-PW-plots, depend on each other: in both cases the same target analytes are used. MS-PW-plots are powerful tools for the recognition of the different kinds of weathering effects. When the effects of weathering on a spilled oil are understood, any affected DRs can be eliminated or cautiously considered when comparing a weathered spill sample to an unweathered source sample.

7.5.2 Diagnostic ratios calculation

Diagnostic ratios (see definition in 3.2.2) shall be based on peak heights for individual peaks and on peak areas for compound groups such as certain alkylated PAHs. When integrating individual peaks, co-eluting peaks shall be avoided or carefully and consistently integrated. For example, if peaks of 29Ts and 29ab co-elute, it can be preferable to use the peak height of the combined peaks in calculating a combined ratio of (29Ts+29ab)/30ab.

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The DR between two distinct peaks (*A* and *B*), shall be based on the ratio formula:

$$DR = A/B; \quad (3)$$

or

$$DR = 100 \times A/B \text{ (in \%, depending on the preference of the user).} \quad (4)$$

The result of the ratio Formula A/B has a fixed RSD.

NOTE The ratio formula *DR = A/(A+B)* is also often used to calculate DRs as it has the benefit of normalizing ratios between 0 and 1. However, this ratio format yields a ratio-dependent RSD (see C.5). Because of this, DRs based upon *A/(A+B)* cannot be utilized for the statistical comparison method described in this method, which relies upon a fixed RSD.

7.5.3 Normative diagnostic ratios

7.5.3.1 General

To identify any potential source samples as "non-matches" with the spill sample(s), a distinct chromatographic pattern or just one distinct DR (unaffected by weathering or mixing) can be sufficient. However, in order to prove a "positive match" all chromatographic patterns (unaffected by weathering or mixing) shall be the same and all DRs (unaffected by weathering or mixing) shall statistically match. A minimum set of DRs is needed to represent the different "aspects" of the particular type of oil spilled to make the comparison test meaningful. To this end, normative sets of DRs for different oil types have been selected from the suite of ratios given in Annex E.

The selection of the normative DRs (NRs) for a particular oil type is based on the following criteria for the target compounds involved:

— commonly present at a higher concentration in the oil type involved;

— well separated single peaks or known co-eluting peaks (e.g. RC26TA+SC27TA);

— good discriminative power [44], [45] and [46];

— based on comparable boiling compounds within a 25 m/z extracted ion chromatogram range [64];

— first eluting compounds eluting before n-C17 are utilised as nominator, which results in a value reduction in case of evaporation (see Tables E.4 and E.5);

— with known weathering characteristics (see Annex H).

When a NR is excluded from the statistical ratio comparison (e.g. NR-30O/30ab: in many crude oils 30-O (oleanane) is not present), the reason shall be noted in the internal documentation (8.2).

The normative selection of DRs in Tables 4 and 5 can be sufficient for a sample comparison if at least 80 % of the ratios yield a robust value for that specific oil category. A1 If the number of solid/usable ratios is lower, also all informative ratios given in Table E.5 become mandatory. In addition, if the spill oil exhibits any features not represented by existing ratios it is possible to introduce new ratio(s) that reflect those features. (Examples of ratios can be found from the peer-reviewed literature.) A1

In Tables 4 and 5, the ratios based on different m/z values are indicated in bold (see Annex E for label indication and Annex F for chromatograms with compound peak indication).

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Table 4 — NRs for condensate and light and medium fuels

System		Ratio	Light fuel condensate	Medium fuel
GC		NR-GC-Norpri/Pri	X	X
		NR-GC-n-C17/Pri	X	X
		NR-GC-n-C18/Phy	X	X
		NR-GC-Pri/Phy	X	X
MS m/z	MS m/z			
135	**149**	NR-1-M-Adam/1,2-DM-Adam	X	
135	135	NR-1-M-Adam/2-E-Adam	X	
113	**104**	NR-i-C13/2-M-tetralin	X	
149	**135**	NR-c-1,3,4-TM-Adam/2-E-Adam	X	
92	92	NR-C6-/C7-Benz	X	X
135	**113**	NR-2-E-Adam/i-C14	X	X
179	179	NR-BS1/BS2	X	
180	**179**	NR-C3-de peak/BS2	X	X
154	**156**	NR-B/2-E-N	X	X
156	156	NR-2-E-N/2,6+2,7 DM-N	X	X
123	123	NR-BS4/BS5	X	X
169	169	NR-Br-Alk 169–3/n-C15	X	
123	123	NR-BS5/BS6	X	X
193	193	NR-BS8/BS9	X	X
106	106	NR-m-/o-C8-Tol	X	X
123	**113**	NR-BS10/Norpri	X	X
106	**113**	NR-Norpri/m-C9-Tol	X	X
92	**83**	NR-C10-Benz/n-C11-CyC6	X	X
85	85	NR-n-C17/Pri	X	X
85	85	NR-Pri/Phy	X	X
85	85	NR-n-C18/Phy	X	X
198	198	NR-4-M-Dbt/1-M-Dbt	X	X
225	225	NR-Br-Alk-225–3/n-C19		X
192	192	NR-2-M-Phe/1-M-Phe	X	X
74	74	NR-FAME C16:0/C18:0	X	X
212	**206**	NR-C2-dbt_s/C2-phe_s		X
216	216	NR-2-M-Fl/4-M-Py		X
216	216	NR-BaF/4-M-Py		X
216	216	NR-2-M-Py/4-M-Py		X
216	216	NR-1-M-Py/4-M-Py		X
Number of normative ratios			**4 + 24**	**4 + 24**

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Table 5 — Normative DRs for heavy fuels and crude oil

System		Ratio	Lub. oil	HFO	Sludge	Crude oil
GC		NR-GC-n-C17/Pri		X	X	X
		NR-GC-n-C18/Phy		X	X	X
		NR-GC-Pri/Phy		X	X	X
MS m/z	MS m/z					
85	85	NR-n-C17/Pri		X	X	X
85	85	NR-Pri/Phy		X	X	X
85	85	NR-n-C18/Phy		X	X	X
198	198	NR-4-M-Dbt/1-M-Dbt		X	X	X
225	225	NR-Br-Alk-225–3/n-C19		X	X	X
192	192	NR-2-M-Phe/1-M-Phe		X	X	X
212	**206**	NR-C2-dbt-s/C2-phe-s		X	X	X
216	216	NR-2-M-Fl/4-M-Py		X	X	X
92	92	C15/C17-Benz	X			
216	216	NR-BaF/4-M-Py		X	X	X
219	**218**	NR-Retene/29bb			X	X
216	216	NR-2-M-Py/4-M-Py		X	X	X
216	216	NR-1-M-Py/4-M-Py		X	X	X
191	191	NR-C23Tr/C24Tr	X			
218	218	NR-27bb/29bb	X	X	X	X
191	191	NR-27Ts/30ab	X	X	X	X
231	231	NR-SC26/RC26+SC27 TA		X	X	X
191	191	NR-27Tm/30ab	X	X	X	X
191	191	NR-28ab/30ab	X	X	X	X
231	231	NR-SC28/RC26 + SC27 TA		X	X	X
191	191	NR-29ab/30ab	X	X	X	X
191	191	NR-30O/30ab	X	X	X	X
231	231	NR-RC28/RC26+SC27 TA		X	X	X
191	191	NR-31abS/30ab	X	X	X	X
191	191	NR-30G/30ab	X	X	X	X
Number of normative ratios			**10**	**3+22**	**3+23**	**3+23**

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7.5.3.2 **Condensate** represents a very light crude oil often collected as a side product of gas wells.

7.5.3.3 **Light Fuel and Medium Fuel** represent distillation fractions of crude oils of which all hydrocarbons elute before respectively C20 (light) or C30 to C31 (medium). It includes distilled intermediates and fuels, such as gas oil, vacuum gas oil (VGO), automotive and marine diesel. Typically, the higher boiling biomarkers are not present in light fuel. However, it is quite common for refineries to add light cycle oil (LCO, also called cutter-stock) to the distilled light oil fraction. LCO is produced in a catalytic cracker and contains mainly aromatic compounds and a low concentration of biomarkers. As a result of this mixing, the chromatographic pattern and concentration of the C1-fluoranthrenes/pyrenes can be very different, while the alkane pattern is very similar. Because the concentration level of the higher boiling biomarkers is still low in such mixtures, it is advised not to calculate ratios based upon these compounds, but to visually inspect and compare this retention time area. The lower boiling biomarker compound C23-tricyclic terpane (C23Tr) elutes just behind n-C23 and is often present in light fuel oil at a higher level (see also I.3).

7.5.3.4 **Lubricating oil** is used to grease engines and needs to have a high flash point (see also I.4). Therefore, the paraffinic fraction is removed and the aromatic compounds are hydrogenated. As a result alkanes, PAHs and triaromatic steranes (m/z 231) are present in low abundance or are absent in the chromatograms of unused lubricating oil. These features can be different for used lubricating oil, which can be modified during use or mixed with other petroleum products (e.g. in bilge). Therefore, for spills involving lubricating oil it is advised to check the ion chromatograms for these features and to extend the number of DRs if possible.

7.5.3.5 **Heavy fuel oil is a residual product of a refinery (see also I.5)**

In a simple refinery HFO is the residue after vacuum distillation.

In a complex refinery the residue after vacuum distillation is been treated in a catalytic cracker [74] to produce more valuable lighter products. As a result, the aromatic compound patterns have been changed (sometimes becoming more or less standardized). As a result:

— the C1-phenanthrene pattern has changed;

— the abundance of methylanthracene has increased significantly;

— the abundance of retene reduced strongly;

— the ratios of 1-Mpy and 2-Mpy to 4-MPy are generally <0,7 in a crude oil and >0,7 in a HFO;

— the ratio B(a)F/4-MPy can vary between 0 and ~3 in crude oils, but is seldom above 1 in HFO.

7.5.3.6 **Sludge** is in general an HFO or a mixture of HFO and lubricating oil. Depending on the relative proportions of HFO and lubricating oil, the character of sludge can vary widely. Retene will probably not be present, but it is possible that other petroleum products like marine diesel are present, so the ratio DR-Retene/29bb shall be looked at (see also I.6).

7.5.3.7 **Crude oil** can vary very widely and mostly contains all normative ratios, although the compounds 28,30-bisnorhopane, oleanane and/or gammacerane can be absent (see also I.2).

7.5.3.8 **Bilge**, in the context of this document, is related to ships with a large open space of several cubic metres below older diesel engines, which use large amounts of lubricating oil. The mixture of mainly diesel and lubricating oil is collected in the bilge below the engine(s). The concentration of the biomarkers depends on the composition of the mixture. Typically, the concentration of the triaromatic steranes is low.

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Bilge is not mentioned in Tables 4 and 5 as a special product, because the ratios to be used are based on the concentration of the diesel fraction relative to the lubricating oil fraction. For bilge samples, combine the normative ratios of light or medium fuel and lubricating oil where possible (see 7.5.6 and I.3 and I.4).

NOTE The word "bilge" is a nautical term which is used to refer to several concepts. It dates from 1513, when "bilge" was first used to refer to the lowest compartment inside the hull of a ship, where the two sides meet at the bottom. However, the term is also used to discuss the matter which collects in the bilge: water drains from the decks of the ship into the bilge, dragging detritus from topside down below. The water that collects in the bilge can be brackish and also have a foul odour, so bilge has also come to be a term which refers to anything foul or unpleasant.

7.5.3.9 Bilge versus sludge, smaller ships do not use HFO, thus do not produce sludge, which consists mainly of residues from HFO precipitate produced in fuel tanks on board. Thus, with regard to the illegal discharges, the most commonly encountered oily waste is bilge discharged by smaller ships. On the other hand, it does not make much sense for bigger ships, which use HFO, to discharge oil, which is collected in the ship's bilge because the amount is much smaller than the amount of waste oil, which results from HFO-separation. Those ships have a special sludge tank (required by law), with special dimensions [48] and the relatively small amount of waste oil in the ships bilge can easily be transferred into the sludge tank and thus added to the sludge. Consequently, it can be confidently concluded that most spills consisting of diesel/lubricating oil mixtures originate from a bilge (see Annexes G and I for more information about petroleum and the different petroleum products relevant for the document).

7.5.3.10 Slop (tanks), slop tanks stores the cargo residues after cleaning. Slop can be the remnants of every product transported. Slops from tankers that transport petroleum and/or petroleum products can contain products similar as mentioned under bilge or sludge. Therefore, slop is not separately mentioned in Tables 4 and 5.

7.5.4 Analytical error

In order to test whether a difference in two measured values is significant or not, the analytical error of the method used to measure the values is needed [29], [30] and [31]:

— because of the complex and variable composition of oil samples, this error is not only different from DR to DR, but can change from case to case. Performing a validation test with a reference oil sample, a good system and careful integration can easily lead to RSDs of 3 % or lower for some of the DRs. However, as mentioned above, the composition of spill and source samples in real cases can be very different from the composition of the reference oil. For example, the concentration of single compounds can be much smaller or target peaks can be more or less overlapped by others, leading to the fact that DRs in authentic spill samples can only be determined with less precision than in a reference oil sample;

— to determine the RSD of the individual DRs in an oil case, at least eight measurements for some of the samples are needed to be able to calculate statistically sound results. As a result, it is very time consuming to determine the precision of the method for all ratios in every single case;

— working with actual error values will result in different match criteria for each oil case. For example, even another analyst in the same laboratory reanalysing an oil case can lead to different match criteria.

As a result, a default value of 5 % RSD for DRs is adopted in this document, representing the upper limit of the acceptable performance of the method (see also A.4.5 and B.4.7). The value of 5 % is based on the experience of several laboratories, on criteria used in literature [66] and has been used in many inter-calibration tests with good results [33], [34], [40] and [84].

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Laboratories shall therefore ensure that an RSD of 5 % for all relevant normative ratios of the duplicate analyses within an oil case are not exceeded. If DR values exceeding the analytical error are observed here, any oil identification analyses shall be stopped, and the reasons for those differences (e.g. integration, a leakage in the system or a dirty ion source) shall be found and corrected before resuming any oil spill sample analyses[5].

7.5.5 Match-criterion for ratios

Crucial for oil sample comparison is the consistent analytical performance of the GC-FID and GC-MS instruments during the analysis of one batch of related samples. For samples analysed in one batch, any differences in their compositions should reflect real differences between the samples and should not be caused by changes due to instrument performance e.g. the sensitivity of the detector by contamination, leaking and a reducing sensitivity for higher m/z values.

When control charts are produced by means of recurrent analysis of a standard reference oil, the difference between any two ratios of two consecutive samples shall only in one of twenty cases exceed the repeatability limit *r* 95 % [28], [29] and [30]:

$$r\ 95\ \% = 2{,}8 \times RSD \text{ (repeatability)} \quad (5)$$

Using an *RSD* of 5 % as given above leads to a repeatability limit of 14 %.

The critical difference (*CD*) (see 3.2.4) is calculated by using the following formula:

$$CD_x = ((\text{mean}_x \times r\ 95\ \%)/100) \quad (6)$$

In close analogy to this principle, ratios measured in samples from real cases originating from the same source and not influenced by weathering and/or mixing should show the same repeatability, when they are analysed within a single batch run.

Consequently, any measured difference in a DR between two samples (i.e. the absolute difference in percent based on the mean of the two ratios) higher than 14 % shall be regarded as significant, meaning that these two ratios are statistically different. This same comparison has to be evaluated for each DR being considered in any particular oil identification investigation. An example of two ratio comparisons - one showing no statistical difference and one showing a statistical difference - is given in Table 6.

Table 6 — Example of a ratio comparison based on the repeatability limit

Ratios to be compared in %		**Mean**	**Absolute difference**	**Relative difference**	**Conclusion**
Ratio sample 1	**Ratio sample 2**				
48	52	50	52 – 48 = 4	4/50 × 100 = 8 %	8 % < 14 % = > No difference
35	45	40	45 – 35 = 10	10/40 × 100 = 25 %	25 % > 14 % = > Different

[5] The use of e.g. a fast GC program to decrease the analysis time in combination with a high number of masses in one SIM section, reduces the number of scans over a peak and significantly increases the variance of the method (see B.3).

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See Figure K.11 for a presentation of the relative differences in bar graph format for comparing a large number of DRs between two duplicate samples. In that figure each ratio difference, except for the ratio FAME C20:0/C18:0, falls to the left of the 14 % line, indicating that there is no statistically significant difference between the two samples being compared.

IMPORTANT — A higher variance is often seen for unsaturated and/or low abundant FAMEs, probably because of their higher polarity relative to alkanes and PAHs, see [46] and F.5. Except for the normative ratio FAME C16:0 / FAME C18:0 an RSD of 10 % and a CD of 28 % shall be used for the informative FAME ratios listed in Table E.5.

The *RSD* of the combined variance of the DR's used for a comparison of two samples may be calculated from the differences between the individual ratios, by calculating the RSD from paired data [13, page 600]. It gives a good indication of the RSD of the ratios for the samples involved (see C.6 for an example calculation).

7.5.6 Criteria for selecting, eliminating and evaluating diagnostic ratios

7.5.6.1 General

Generally, only those DRs that can be measured with a high precision shall be evaluated by comparing suspected sources to the spilled oil [65]. Small peaks at or near the baseline have an increased variance and shall only be used for a visual, qualitative comparison and not for the quantitative DR comparison.

In order to evaluate the analytical precision of a series of samples and the precision of the individual DRs, the preparation and analysis of duplicate samples is essential (see 7.1).

Figure 5 shows a protocol/decision chart for DR evaluation.

For DRs determined to be unaffected by weathering, the chart shows two methods by which the DRs shall be evaluated in order to ensure their reliability for use in the DR comparison. Two bases exist by which DRs might be eliminated from the comparison:

— elimination by means of a signal to noise (S/N) test (see 7.5.6.2);

— elimination by means of the comparison of the duplicate analyses of extracts (see 7.5.6.3).

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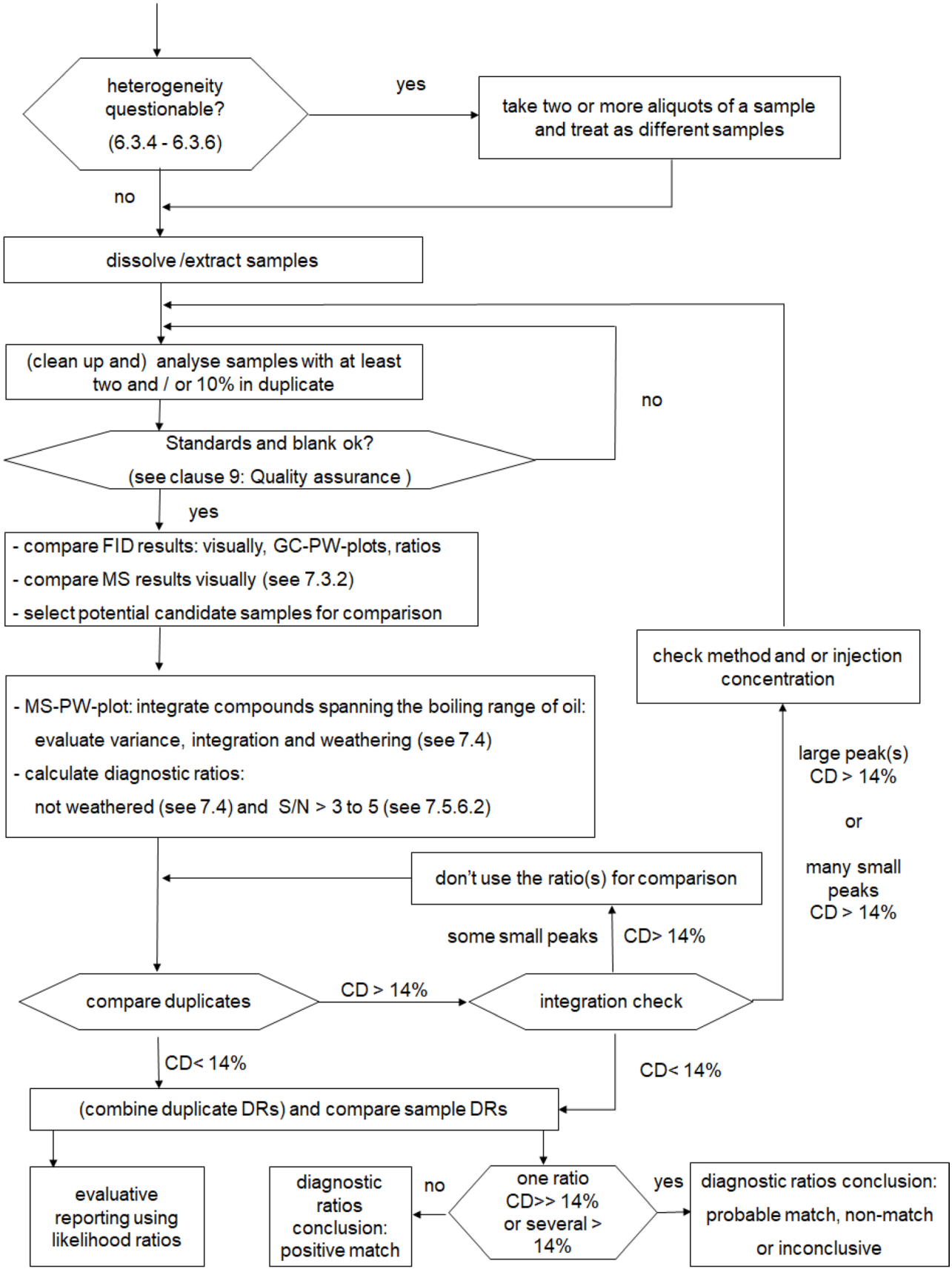


Figure 5 — Protocol/decision chart for diagnostic ratio evaluation

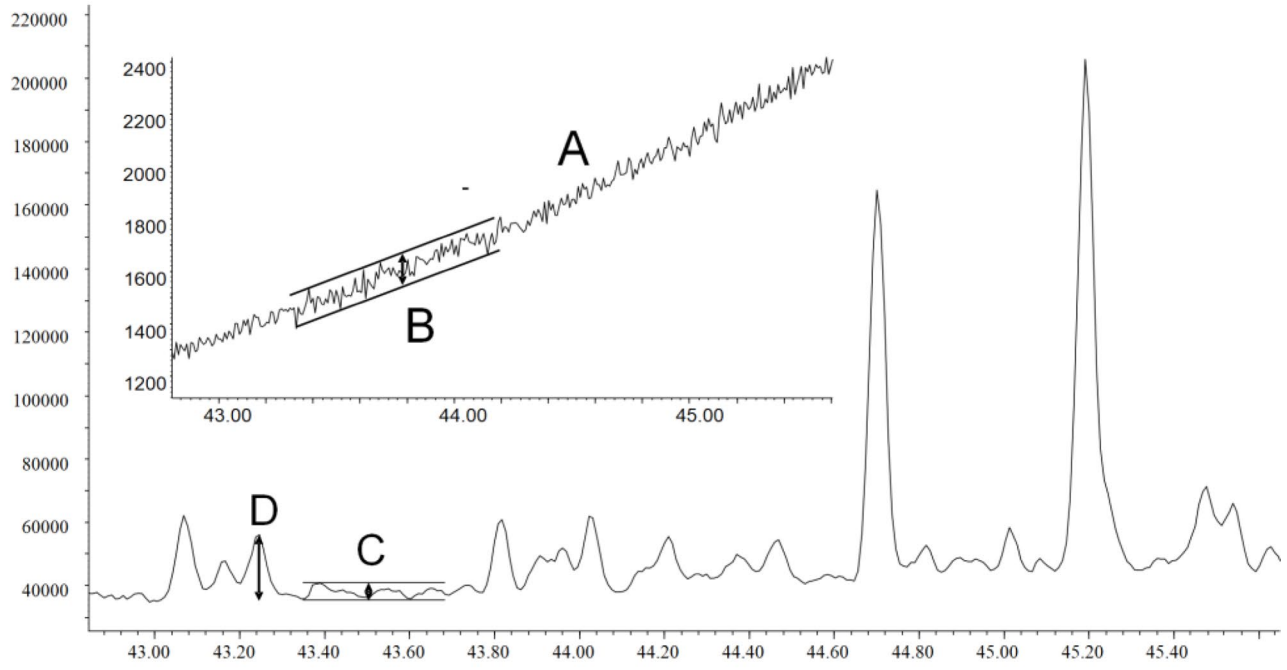
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7.5.6.2 Elimination by means of a signal to noise (S/N) test

Only peaks with a S/*N* > 3 to 5 shall be used for the DR comparison test. This S/N criterion is based on the method described by IUPAC [21] and [32] and shown in Figure 6. N is the peak-to-peak value of a part of the noise around a target peak and S the target peak. A range of 3 to 5 is given, because it is often difficult to estimate the noise, which is caused by the many peaks present in the chromatograms of oil samples.

As an example, a part of the m/z 191 ion chromatogram (C28 (22R) to 27 Tm) of the oil mixture of B.2.3. is shown in Figure 6. The inset within that Figure is a part of the ion chromatogram of an injection of clean DCM (blank injection) over the same retention time section, demonstrating the noise calculation. Normally a part around the peak should have been taken to calculate the noise. However, comparing the mainly electronic noise from the blank injection with the "chemical noise" of the oil sample, shows that the baseline in the oil sample is an ion signal of compounds.

In this example, the noise of the blank N_{bl} = 112. The "compound noise" in the oil sample is N_{sample} = 4 975 and the signal of C28 (22S) S_{sample} = 20 319 resulting in S/N_{sample} = 4,1. Compared to the decision range of 3 to 5, both decisions are valid. Smaller peaks shall not be integrated for use in DRs, but can still be useful for qualitative visual inspection. Larger peaks shall be included in the DR comparison test.



Key

A	noise after DCM injection	C	noise peak to peak of a sample injection N_{sample}	X-axis	time (min)
B	noise peak-to-peak of the blank N_{bl}	D	peak signal S_{sample}	Y-axis	response

Figure 6 — Signal to Noise calculation

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Even more difficult is the decision to integrate areas representing groups of compounds, rather than discrete peaks. For example, in propeller shaft lubricating oil many of the alkylated PAHs shown in F.4.2 are present at a low relative concentration and are represented by only the highest peaks of a group. In such cases, the pattern shall only be used for a visual comparison and can be eliminated from any DRs reliant upon alkylated PAHs.

In general, the $S/N > 3$ to 5 criterion shall be used as a rule of thumb to assess and potentially eliminate DRs based upon integrations of small single peaks from the DR statistical comparison.

7.5.6.3 Elimination by means of the comparison of the duplicate analyses of extracts

A duplicate analysis of an extract should result in statistically identical DR values. Differences are usually caused by the method, the instrumentation and the data processing (peak integration). Because of this test, several options are possible:

— ratios of large peaks differing by more than 14 %;

Check the integration of the peaks involved (retention time and baseline drawing), instrumental conditions and/or injection concentration. For example, a high injection concentration of compounds, not analysed by the SIM method, can still influence the properties of the peaks to be analysed. Eventually, perform a clean-up and reanalyse the samples.

— ratios of small peaks differing by more than 14 %;

Check the integration of the peaks involved (retention time and baseline drawing). If one or two ratios are still above the CD, the peaks involved shall only be used for a visual comparison and not for calculating ratios. If many ratios are still above the CD, act in the same way as for large peaks differing by more than 14 %.

— all ratios differing less than 14 %.

Go to the next step of the chart (Figure 5) and compare the samples involved. Use the mean value of the DRs of the samples analysed in duplicate for comparison to the other samples. The mean value has a lower variance and comes closer to the actual ratio of the sample.

NOTE Comparing the mean of a duplicate analysis makes the comparison more robust and reduces the value of the RSD by $\sqrt{n}$. Therefore, the RSD of the mean value of a duplicate analysis becomes RSD / $\sqrt{2}$. For this document however, it has been decided not to reduce the repeatability limit of 14 %, because the RSD of 5 % is a chosen limit applied as criterion for the quality of the sequence and yields no difference between samples analysed in singular or in duplicate.

7.5.6.4 Optional: Evaluation of diagnostic ratios using conventional or multivariate statistics

NOTE These methods are outside the scope of this document and therefore indicated as optional and only described in general terms.

On occasion, it is necessary to compare DRs from a large number of samples [12] or, for example, in the case of a mystery spill compared to a large database of oil samples (see for example COSIweb [14]).

In this situation, a univariate comparison of each oil sample in the database to the mystery spill can be facilitated through the use of conventional or multivariate statistics. Of course, there is the need for some caution when comparing DRs obtained over extended periods of time, such as can exist in the case of long-standing databases. Under such circumstances it can be prudent to compare DRs of laboratory reference oils analysed over time (e.g. early in the database's development versus recently in the database's development) in order to ensure that the laboratory's performance over time has remained comparable, thereby allowing comparison of the "new" mystery spill data to the database.

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Conventional statistical metrics such as various similarity indices or correlation tools (e.g. correlation coefficients) can be used to compare a spill's DRs versus ratios in a large database as a means of identifying the database's oil that are most similar to the spill oil. These most similar oils can then be the focus of purely univariate comparison to the mystery spill according to the protocol in Figure 5.

In addition, multivariate statistical methods like principal component analysis (PCA) can be used as a tool to compare DRs between multiple samples [66] instead of the purely univariate approach described in Clause 5 and Figure 1. One advantage of multivariate statistical methods compared to univariate statistical methods is the ease by which relationships between multiple samples and variables (DRs) can be resolved and visualized by scores and loading plots. Additional advantages include noise reduction, obtained by multiple measurements of the same phenomenon (i.e. inter-related variables), and their ability to detect outliers.

The fundamentals of PCA as a data treatment tool in chemical fingerprinting, is that it summarizes the information in many correlated DRs into a few so-called principal components that are weighted sums of these ratios. Hence, a model with few components (e.g. one or two) describes the most prominent trends in data. By plotting principal components, e.g. the first principal component versus the second, chemical information retained in numerous DRs for numerous samples can be compared simultaneously. Oil samples with a similar chemical composition plot closely in score plots, whereas dissimilar oils plot remotely in score plots.

The comparison of oil samples by PCA can be further refined by taking into account the uncertainty of the individual DRs. This ensures an objective matching of oil spill samples and suspected sources based on all the available information.

Conventional and multivariate statistical methods can be used to rule out potential sources, but it cannot replace the ratio comparison method and the visual inspection of the acquired ion chromatograms to conclude a positive match. Weathering effects, small differences and compounds not used in the statistical treatment could not be recognized by the multivariate method.

7.6 Conclusions

The final conclusion of an oil spill identification investigation shall be based on a total evaluation of the analytical results. This shall be a conclusion on matching definitions or on likelihood ratios (see D.2 and D.3).

A summary of the means given in Annex J and K for comparing four oil samples is shown in Figure 7. In this example, much of the necessary information is jointly at hand, permitting a final conclusion to be defensibly drawn.

In accordance with the decision charts in Figures 1 and 5, the results of a comparison between a spilled oil and a prospective source sample using this method shall be concluded using one of the four terms: positive match, probable match, inconclusive or non-match or by using likelihood ratios.

A description of the reason why a conclusion has been chosen shall be given in the internal documentation (8.2). A description of the reason why a probable match or inconclusive has been chosen shall be given in the summary report (8.3).

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