ASTM INTERNATIONAL

Designation: D5739 – 06 (Reapproved 2020)

Standard Practice for
Oil Spill Source Identification by Gas Chromatography and Positive Ion Electron Impact Low Resolution Mass Spectrometry[1]

This standard is issued under the fixed designation D5739; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice covers the use of gas chromatography and mass spectrometry to analyze and compare petroleum oil spills and suspected sources.

1.2 The probable source for a spill can be ascertained by the examination of certain unique compound classes that also demonstrate the most weathering stability. To a greater or lesser degree, certain chemical classes can be anticipated to chemically alter in proportion to the weathering exposure time and severity, and subsequent analytical changes can be predicted. This practice recommends various classes to be analyzed and also provides a guide to expected weathering-induced analytical changes.

1.3 This practice is applicable for moderately to severely degraded petroleum oils in the distillate range from diesel through Bunker C; it is also applicable for all crude oils with comparable distillation ranges. This practice may have limited applicability for some kerosenes, but it is not useful for gasolines.

1.4 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1129 Terminology Relating to Water
D3325 Practice for Preservation of Waterborne Oil Samples
D3326 Practice for Preparation of Samples for Identification of Waterborne Oils
D3328 Test Methods for Comparison of Waterborne Petroleum Oils by Gas Chromatography
D3414 Test Method for Comparison of Waterborne Petroleum Oils by Infrared Spectroscopy (Withdrawn 2018)[3]
D3415 Practice for Identification of Waterborne Oils
D3650 Test Method for Comparison of Waterborne Petroleum Oils By Fluorescence Analysis (Withdrawn 2018)[3]
D5037 Test Method for Comparison of Waterborne Petroleum Oils by High Performance Liquid Chromatography (Withdrawn 2002)[3]
E355 Practice for Gas Chromatography Terms and Relationships

3. Summary of Practice

3.1 The recommended chromatography column is a capillary directly interfaced to the mass spectrometer (either quadrupole or magnetic).

3.2 The low-resolution mass spectrometer is operated in the positive ion electron impact mode, 70 eV nominal.

3.3 Mass spectral data are acquired, stored, and processed with the aid of commercially available computer-based data systems.

4. Significance and Use

4.1 This practice is useful for assessing the source for an oil spill. Other less complex analytical procedures (Test Methods D3328, D3414, D3650, and D5037) may provide all of the necessary information for ascertaining an oil spill source;

---

however, the use of a more complex analytical strategy may be necessary in certain difficult cases, particularly for significantly weathered oils. This practice provides the user with a means to this end.

4.1.1 This practice presumes that a "screening" of possible suspect sources has already occurred using less intensive techniques. As a result, this practice focuses directly on the generation of data using preselected targeted compound classes. These targets are both petrogenic and pyrogenic and can constitute both major and minor fractions of petroleum oils; they were chosen in order to develop a practice that is universally applicable to petroleum oil identification in general and is also easy to handle and apply. This practice can accommodate light oils and cracked products (exclusive of gasoline) on the one hand, as well as residual oils on the other.

4.1.2 This practice provides analytical characterizations of petroleum oils for comparison purposes. Certain classes of source-specific chemical compounds are targeted in this qualitative comparison; these target compounds are both unique descriptors of an oil and chemically resistant to environmental degradation. Spilled oil can be assessed in this way as being similar or different from potential source samples by the direct visual comparison of specific extracted ion chromatograms (EICs). In addition, other, more weathering-sensitive chemical compound classes can also be examined in order to crudely assess the degree of weathering undergone by an oil spill sample.

4.2 This practice simply provides a means of making qualitative comparisons between petroleum samples; quantitation of the various chemical components is not addressed.

5. Apparatus

5.1 *Gas Chromatograph Interfaced to a Mass Spectrometer,* with a 70-eV electron impact ionization source. The system shall include a computer for the control of data acquisition and reduction.

5.2 *Capillary Column,* with a high-resolution, 30 m by 0.25-mm or 0.32-mm inside diameter (0.25-μm d_f) (such as J & W DB-5 or Supelco PTE-5), interfaced directly to the mass spectrometer.

6. Reagents and Materials

6.1 *Purity of Reagents*—Only pesticide grade, nanograde, or distilled in glass grade solvents will be used.

6.2 *Purity of Reference Compounds*—All must be certified to be at least 95 % pure.

6.3 *Septa*—Only high-temperature, low-bleed (such as Thermogreen[4]) shall be used.

6.4 *Vials,* glass, polytetrafluoroethylene-lined screw cap, 10-mL capacity.

6.5 *Syringes,* 10 μL.

6.6 *Perfluorotributylamine,* used for tuning the mass spectrometer.

6.7 *Resolution Mixture*—Pristane, phytane, n-heptadecane, and n-octadecane in equal concentration in cyclohexane (50 to 150 ng/μL).

6.8 *Mass Discrimination Mixture*—Naphthalene, fluoranthene, and benzo (g, h, i) perylene in equal concentration in cyclohexane (50 to 150 ng/μL).

6.9 Reference oil, possibly a crude oil, used for generation of the extracted ion chromatograms (EICs) listed in Table 1 and validation of system performance for oil sample comparison purposes. (See Appendix X1 for representative EICs produced using the conditions stated in Section 8.)

7. Preparation of Instrumentation

7.1 Set an initial head pressure of between 5 and 20 psi using helium as the carrier at 250°C (for either a 30-m by 0.25-mm inside diameter column or a 30-m by 0.32-mm inside

TABLE 1 Extracted Ion Chromatograms (EICs)

Compound Type		Ion	Approximate Time Interval, min
Naphthalenes	C_2	156	18 to 23
	C_3	170	20 to 25
	C_4	184[A]	22 to 27
Dibenzothiophenes	C_0	184[A]	23 to 28
	C_1	198	27 to 32
	C_2	212	29 to 34
	C_3	226	31 to 35
Phenanthrenes/ anthracenes	C_0	178[B]	27 to 28
	C_1	192	28 to 33
	C_2	206	30 to 35
	C_3	220	32 to 37
Steranes	14a(H)	217	40 to 60
	14b(H)	218	40 to 60
Triterpanes		191	40 to 60
Alkanes		85	4 to 60
Alkanes		113	4 to 60
Alkanes and Acyclic isoprenoids		183	4 to 60
Benzonaphthothiophene		234	30 to 34
Tri-aromatic steranes		231	39 to 45
Norhopanes		177	33 to 47
Methylhopanes		205	41 to 46
Pyrene/fluoranthene		202	24 to 32
Methylpyrenes		216	30 to 32
Fluorenes		166	16 to 21
Bicyclonaphthalenes		208	15 to 22

[A] An authentic standard of dibenzothiophene can be chromatographed to ascertain its actual retention time.
[B] Phenanthrene is both pyrogenic and petrogenic. Consequently, m/e 178 may demonstrate an increase relative to its source in spill cases in which arson or other combustion processes have occurred. This can result in a significant distortion in the C_0 anthracene/phenanthrene distribution, which is, generally speaking, counter to expected weathering processes.

---

[4] A trademark of Sigma-Aldrich Co., LLC, St. Louis, MO..

diameter column). Adjust a final head pressure (for either column) such that the linear velocity is in the range from 30 to 40 cm/s.

7.2 *Mass Spectrometric Tuning:*

7.2.1 Tune the mass spectrometer to the following perfluorotributylamine (PFTBA) specification, addressing both mass scale calibration and peak-to-peak ratios:

(m/e 69 at 100 % of base peak)
(m/e 219 at 35 to 40 % [A] of base peak)[B]
(m/e 502 at 1 to 2 % of base peak)[C]

[A] The sensitivity for almost all of the ions monitored (Table 1) can be improved somewhat by adjusting this percentage to between 60 and 65; however, the resulting mass spectra may be distorted significantly so that MS computer search routines for the identification of unknowns by comparison to conventionally acquired mass spectral libraries may be impaired significantly.
[B] Adjust the entrance lens voltage.
[C] Adjust the ion focus voltage.

7.2.2 Retune every 12 h of mass spectrometer operation.

7.3 *Resolution Check*—Under the instrumental conditions listed (7.1), pristane and phytane usually display 80 % or greater resolution from C_{17} and C_{18}, respectively. If the resolution is less than 50 %, take corrective action such as replacement of the injector liner and seals and removal of the front of the analytical column. Report the degree of resolution in Section 10. Refer to Practice E355 for calculation of resolution values.

7.4 *Mass Discrimination Check:*

7.4.1 Use the gas chromatographic instrumental parameters enumerated in 8.3.1; operate the mass spectrometer, but in the linear scan mode from m/e 45 to 360 in 1 s.

7.4.2 Inject a 1-µL solution of naphthalene, fluoranthene, and benzo (g, h, i) perylene in equal concentrations (from 50 to 150 ng/µL) in cyclohexane.

7.4.3 Integrate the total ion chromatogram (TIC).

7.4.4 Calculate the following ratios:

(1) Area of naphthalene to area of fluoranthene, and

(2) Area of benzo (g, h, i) perylene to area of fluoranthene.

7.4.5 The ratio of *(1)* must be less than or equal to 2, and the ratio of *(2)* must be greater than or equal to 0.2. Report this value in Section 10.

7.4.6 A high molecular weight response can sometimes be improved by changing the penetration of the chromatographic column into the injector body or using silanized glass wool or quartz as injector packing material, or both. Electronic flow control (instead of constant column head pressure) has recently become available for Capillary GC. It can be used to provide a high molecular weight response by increased flow during splitless injection.

7.5 *Retention Time Check*—The absolute retention times for the mass discrimination check compounds (7.4.2) must be recorded. The batch-to-batch retention time reproducibility can be documented in this way. Report these retention times in Section 10.

8. Procedure

8.1 Refer to Terminology D1129 for terms relating to water and Practice D3415 for identification of waterborne oils. Refer to Practice D3325 for the preservation of oil samples and

Practice D3326 for preparation of the neat oil sample. (Practice D3326 includes Procedure F for recovering oil from thin films on water and Procedure G for recovering oil from sand and debris.) It is the responsibility of the user to validate this method for use with these types of matrices since oil recovered from them may contain contamination derived from the substrate material.

8.2 *Sample Preparation*—Weigh 100 to 200 mg of oil into a screw-cap glass vial, and add 10 mL cyclohexane. Sonication may be necessary, as well as centrifugation, to remove particulates if the sample does not dissolve completely.

8.3 *Instrumental Parameters:*

8.3.1 *Gas Chromatograph*—Use the following parameters: 1-µL splitless injection for 45 s; an initial column temperature of 55°C for 2 min; a temperature ramp at 6°C/min to 270°C; a temperature ramp of 3°C/min to 300°C; a final column temperature of 300°C for 17 min; an injection temperature of 290°C; and a mass spectrometer (MS) interface temperature of 300°C. A total run time of approximately 65 min will be achieved using these parameters.

8.3.2 *Mass Spectrometer Data Acquisition Parameters*—Operate the mass spectrometer in selected ion monitoring (SIM) for the 24 ions listed in Table 2. Since all of the ions will be scanned every second, the dwell time for each is 70 ms. Allow a solvent delay time of 4 min before the start of MS scanning.

NOTE 1—It is recognized that the different monitored classes of analytes elute only in certain regions of the chromatogram; consequently, not all ions need be monitored continuously. However, no effort has been made to segment the chromatogram by using different SIM masses at different times for the sake of maintaining simplicity. It is also recognized that the signal-to-noise ratio is improved by an increase in the dwell time; however, this improvement is directly proportional to the square root of the proportional dwell time increase. A signal-to-noise ratio increase of only two would thus result from a four-fold increase in the dwell (from 70 to 280 ms). This increased dwell time would permit only 3 ions/s to be monitored. Nevertheless, the experienced analyst who is working with a

TABLE 2 SIM Acquisition

m/e	Dwell/ms	Elution Range/min
85	70	4 to 60
113	70	4 to 60
156	70	4 to 60
166	70	4 to 60
170	70	4 to 60
177	70	4 to 60
178	70	4 to 60
183	70	4 to 60
184	70	4 to 60
191	70	4 to 60
192	70	4 to 60
198	70	4 to 60
202	70	4 to 60
205	70	4 to 60
206	70	4 to 60
208	70	4 to 60
212	70	4 to 60
216	70	4 to 60
217	70	4 to 60
218	70	4 to 60
220	70	4 to 60
226	70	4 to 60
231	70	4 to 60
234	70	4 to 60

well-characterized oil source, such as monitoring degradation over time, may choose to monitor fewer ions in order to maximize the signal-to-noise ratios and consequently improve the sensitivity for a subset of the ions listed in the table. Similarly, users of certain older model mass spectrometers may also choose to modify SIM acquisition by monitoring fewer ions simultaneously in order to offset lowered MS sensitivity.

8.4 *Sample Analysis Batching Requirements*—Every time the mass spectrometer is used, bracket all samples by a duplicate analysis, and specifically prepare an oil sample in duplicate (8.2). Also, the first and last samples to be analyzed must be these duplicates. Generate the resulting EICs in accordance with 9.1.1, and compare them visually in accordance with 9.1.2; any variations observed will serve to define the analytical error for the entire batch.

9. Interpretation

9.1 *Evaluation of EICs:*

9.1.1 *Data Presentation*—EICs will be generated for each oil sample. These EICs are as follows: (*1*) C_2 through C_4 homologs of naphthalene, (*2*) dibenzothiophene and its C_1–C_3 homologs, (*3*) anthracene and phenanthrene and their C_1–C_3 homologs, (*4*) triterpanes, (*5*) steranes, and (*6*) alkanes, (*7*) benzonaphthothiophene, (*8*) tri-aromatic steranes, (*9*) hopanes, (*10*) pyrene/fluoranthene, (*11*) fluorene and (*12*) bicyclonaphthalenes. The EICs and their approximate time intervals are summarized in Table 1. The method can be extended to include other suitable ions, if necessary. (With this in mind, the user may desire to include naphthalene and C_1 naphthalene homologs for light, minimally weathered spills or chrysene and its C_1 to C_2 homologous series for heavily weathered residual oils, or both.)

9.1.2 *Direct Visual Comparison of EICs*—The EICs for each suspect source oil will be compared to the appropriate EICs of the spilled oil; evaluation of the patterns (EICs) will be performed as a peak-to-peak comparison simply by placing the EICs one over the other. Since the *y* axis will be normalized at 100 %, automatically, the EICs from identical oils will have identical plots (although not necessarily identical scale, which is dependent on the absolute weight of the injected sample), and they will therefore overlay each other completely (within the confines of analytical error defined in 8.4). This uniform presentation of the EICs makes visual comparison by overlay a straightforward procedure, regardless of differences in injection amounts.

9.1.3 *Weathering Stability:*

9.1.3.1 The more highly alkylated homologs are preferred for characterization purposes over the unsubstituted parent compound, or even its monomethylated forms, since both solubility in water and biodegradation are related inversely to the degree of alkylation.

9.1.3.2 In similar fashion, biodegradation and water solubility are also related inversely to the number of fused rings. Dibenzothiophene and anthracene/phenanthrene are therefore inherently more resistant than naphthalenes.

9.1.3.3 Steranes and triterpanes are relatively water insoluble and are extraordinarily resistant to biodegradation.

9.1.3.4 The most stable EICs should be examined first, progressing toward the less stable ones. This order from more weathering stable to less weathering stable is shown in Fig. 1.

9.1.4 *Susceptibility of the Various Compound Classes to Weathering Exposure*—It may be best to first examine the highest molecular weight homologous series with the greatest degree of substitution, since weathering results in progressive losses greatest for the lowest molecular weight homologous series with the least degree of substitution, progressing toward the highest molecular weight series with the greatest degree of substitution. In those cases in which weathering has not progressed sufficiently to eradicate an entire substituted series completely, the remnants will continue to reflect the original ratios of the unweathered oil. The EICs for a weathered oil versus its unweathered source will thus remain qualitatively the same, that is, the EICs will not change.

NOTE 2—A signal-to-noise ratio of 3:1 is used to ascertain the remnant presence of a peak for those weathered oil EICs displaying drastic loss. It may be helpful to auto-range sections of such an EIC in order to examine such a low-level signal in greater detail. This same procedure may be used to step around a dominant peak in an EIC in order to auto-range on other, less dominant peaks and consequently examine them in greater detail.

9.1.4.1 *m/e 85*—This m/e serves to provide a chromatographic trace for the alkanes. It is included to assess gross weathering effects since this compound class is very weathering sensitive. Losses in the rapidly eluting, early part of the spill EIC relative to the source are to be expected due to various weathering phenomena (evaporation, dissolution, and biodegradation). An example of how pronounced this is, is shown in Fig. 2.

NOTE 3—Differences in the amounts of oil injected cause differences in the full-scale *y* axis, as indicated in Fig. 2 and discussed in 9.1.2.
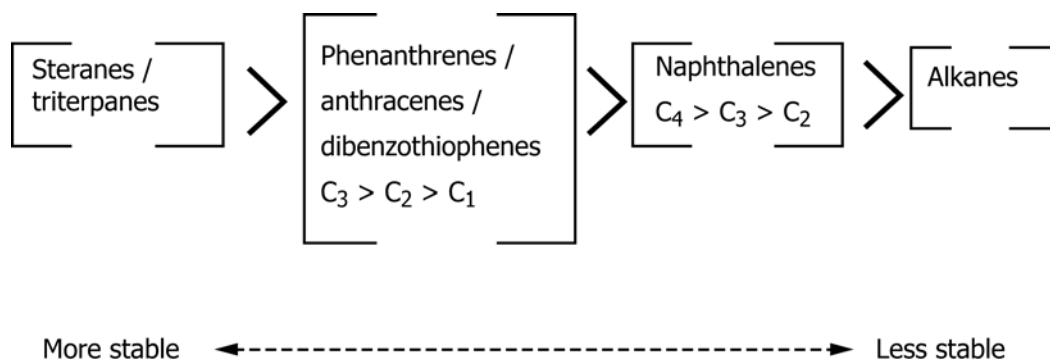

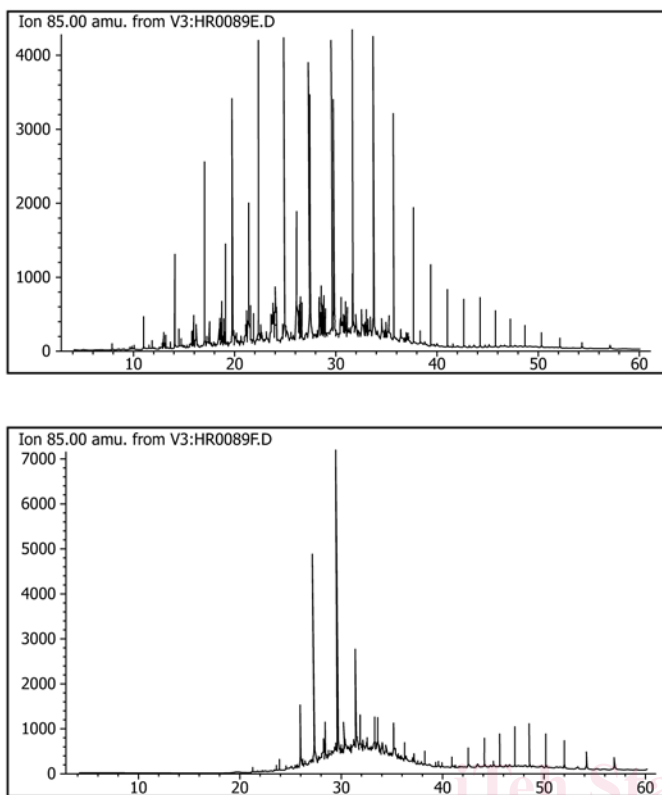FIG. 1 Weathering Stability for the Target Compound Classes

FIG. 2 EIC (m/e 85) for a Slightly Weathered (Upper) and Heavily Weathered (Lower) Oil Sample

9.1.4.2 *m/e 113*—This m/e serves to provide a chromatographic trace for the saturated alkanes as does m/e 85, but more pronounced if the sample is weathered.

9.1.4.3 *m/e 183*—This m/e serves to provide a chromatographic trace for the alkanes and acyclic isoprenoids as does m/e 85, but accentuates the acyclic isoprenoids (that is, pristane and phytane) in the sample.

9.1.4.4 *m/e 156 and 170*—These m/e's represent the homologous series of C_2 and C_3 substituted naphthalenes, respectively. These are included since they are important components in low-boiling oil products and cracked oils, although they are generally inferior choices for examination since the entire naphthenic series is relatively weathering sensitive. (Naphthalene and C_1-substituted naphthalenes are not included due to their extremely poor weathering stabilities.)

9.1.4.5 *m/e 184*—This m/e represents both the C_4-substituted naphthalene series and dibenzothiophene, and it is more weathering stable than m/e 156 and 170.

9.1.4.6 *m/e 177, 191, and 205*—These m/e serve to provide a chromatographic trace for the basic structures for three variants of hopanes (that is, m/e 177 norhopanes, m/e 191 sterioisomers of hopane A and B and m/e 205 methylhopanes) present in the sample and are important due to their resistivity to weathering.

9.1.4.7 *m/e 178, 192, 206, 220*—The m/e 178 represents the anthracene/phenanthrene content of an oil; their C_1, C_2, and C_3 homologous series are represented by m/e 192, 206, and 220, respectively. The C_2 and C_3-substituted phenanthrenes/ anthracenes provide the most weathering impervious chro-

matographic patterns. Fig. 3 shows the 206 EIC for the same oil sample slightly weathered (less than 5 % depletion) and heavily weathered (40 to 60 % depletion). Virtual overlays of these EICs demonstrate the very good stability to degradation for these compounds. Consequent examination of the most highly substituted members would be a prudent analytical choice.

9.1.4.8 *m/e 198, 212, and 226*—These m/e's represent the sulfur content of an oil as demonstrated in the dibenzothiophene fingerprint of the C_1, C_2, and C_3 homologous series, respectively.

9.1.4.9 *m/e 191, 217, 218*—The m/e 191 represents the triterpane content of an oil; m/e 217 and 218 represent stereoisomeric steranes. All of these compounds reflect different processes of maturation undergone by the source crude oils. Both triterpanes and steranes are highly resistant to biodegradation and weathering since they are both virtually nonvolatile and water insoluble; their chromatographic profiles remain unaltered effectively. These traces remain as virtual overlays despite severe weathering. The stability of the triterpanes is shown in Fig. 4 (m/e 191) for the same crude oil sample slightly weathered (less than 5 % depletion) and heavily weathered (50 to 70 % depletion). The use of these EICs would be most desirable since they demonstrate exceptional stability to weather while generally providing unique chromatograms for identification purposes.

9.1.4.10 *m/e 166, 202, and 216*—These m/e serve to provide a chromatographic trace for the fluorenes, pyrene fluoranthene and methylpyrene present in the sample. These make good
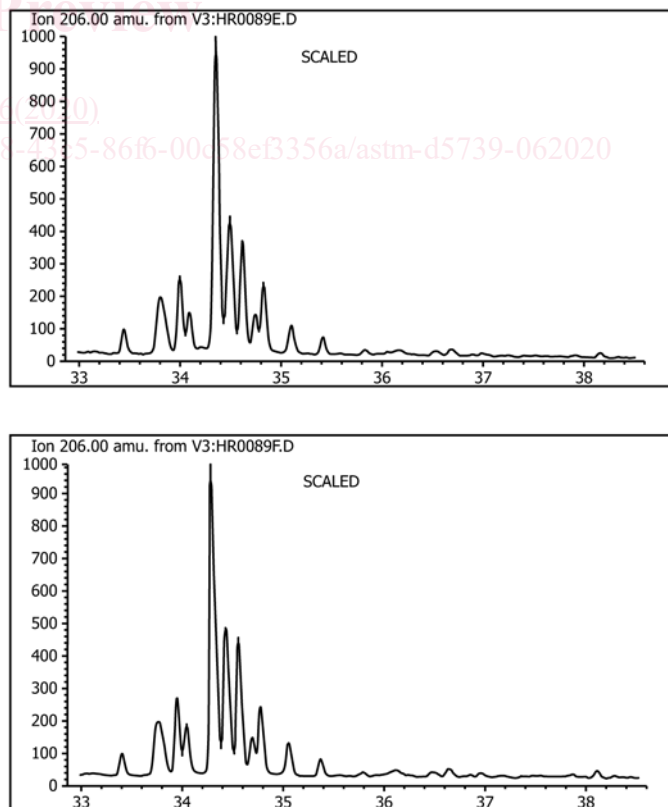

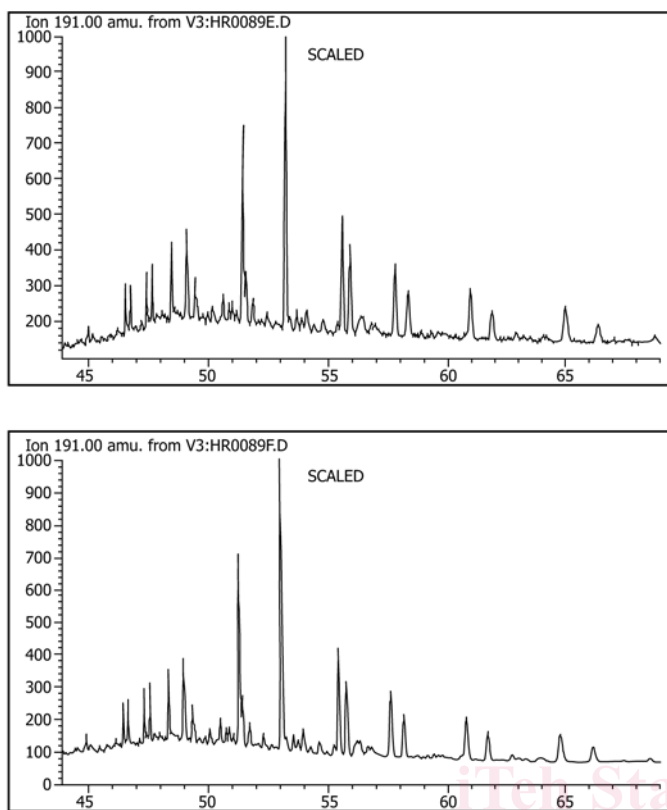**FIG. 3 EIC (m/e 206) for a Slightly Weathered (Upper) and Heavily Weathered (Lower) Oil Sample**

FIG. 4 EIC (m/e 191) for a Slightly Weathered (Upper) and Heavily Weathered (Lower) Oil Sample

supplementary ions useful for evaluating the origin of the hydrocarbons in the oil sample (that is, pyrogenic versus petrogenic).

9.1.4.11 *m/e 208*—This m/e produces a chromatographic trace for the bicyclonaphthalene content present in the sample. These make good supplementary ions useful for evaluating the weathering process for the oil sample.

9.1.4.12 *m/e 231*—This m/e indicates the tri-aromatic sterane content present in the sample. These make good supplementary ions useful for evaluating the maturation process for the oil sample, since these ions are transitional in the formation of phenanthranes.

9.1.4.13 *m/e 234*—This m/e serves to provide a chromatographic trace for the benzonaphthiophene content present in the sample. This is a good supplementary ion useful for evaluating sulfur content for a more weathered oil sample.

9.2 *Assessment of the Degree of Weathering Undergone by an Environmental Sample:*

9.2.1 *Introduction*—In addition to qualitative assessment of the EICs discussed previously, spill oil samples may also be assessed for the degree of weathering they have undergone by examining the relative changes in distributions of the various homologous series. Furthermore, environmental samples are affected by weathering phenomena in certain predictable ways, and the extent of these changes is a gross measure of the weathering severity.

NOTE 4—Examination of the relative distributions of the various homologous series described as follows is meant only to assist in ascertaining the identity of a spill oil sample relative to its unweathered source. By careful examination of the EICs, using visual overlay as specified in 9.1, the identity or nonidentity should have been established previously. Comparison of the various distributions provides only additional confirmatory data by verification of weathering-expected losses.

9.2.2 *Procedure for Graphic Analysis*—Integrate each of the various EICs as a whole for both the spill sample(s) and the identified source(s). Use manual straight baseline integration from the first to the last peak in each homologous series. (This results in the summation of all peak areas between 33 and 36 min for the case of the 206 EIC shown in Fig. 3.)

9.2.2.1 The total integrated areas of the EICs from the spill (weathered) and its source are then normalized to the most weathering-stable homologous series and plotted graphically in the form of a histogram. This results in the graphic plot shown in Fig. 5 for the relative distributions for an unweathered and severely weathered Iranian crude oil, which had spent over six months on a rocky shore and had lost over 40 % by weight. This results in normalizing the less weathering stable to the most weathering stable because the various distributions are normalized to C_3 dibenzothiophenes. Expected weathering-induced differences can be tracked in this way for an environmental sample relative to its unweathered source by examination of the relative area count differences as reflected in readily apparent graphic differences.

9.2.2.2 This process simply results in a relative comparison of the various analyte distributions between two samples since only the area counts are compared. Minor differences between injection amounts will then have no consequences because this procedure involves normalizing.

9.2.3 *Expected Changes in the Relative Distributions Resulting from Weathering*—The bacterial degradation rate within a homologous series and degree of water solubility are inversely proportional to the degree of alkylation. This results in the more highly substituted homologs being depleted more slowly than the less highly substituted ones (as mentioned in 9.1.4). The severity of weathering is reflected in the normalized area differences for the less highly substituted members within each homologous series; the relative loss increases with decreasing alkylation. The expected weathering trends are indicated in Fig. 5, as shown by a progressively greater loss with decreasing alkylation within each series in addition to a greater overall loss for the naphthalenes (two fused rings) relative to phenanthrenes/anthracenes (three fused rings). The relative losses of the various homologous series can be estimated by displaying the weathering data in a manner similar to that shown in Fig. 5. For the naphthalene series, C_2 shows the greatest relative loss with only approximately 7 % remaining; approximately 15 % C_3 remains; and approximately 20 % C_4 remains. For the phenanthrene/anthracene series, approximately 40 % C_1 remains; approximately 80 % C_2 remains; and approximately 100 % C_3 remains. In a similar fashion, approximately 25 % dibenzothiophene remains; approximately 50 % C_1 remains; and approximately 75 % C_2 remains.