Development and application of an analytical method using gas chromatography/triple quadrupole mass spectrometry for characterizing alkylated chrysenes in crude oil samples

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RATIONALITY: Recent advances in analytical techniques have led to the development of gas chromatography/triple quadrupole mass spectrometry methods that allow the identification of target analytes in complex environmental samples. We have employed this technology to develop a method for characterizing alkylated chrysenes, which are environmental toxins that are resistant to weathering.

METHODS: An Agilent 7890 gas chromatograph coupled to an Agilent 7000B triple quadrupole mass spectrometer was used. The mass spectral fragmentation of seven commercially available alkylated chrysenes standards was studied under full-scan and product-ion scan conditions. The calibration curves used were in the linear range with r^2 values greater than 0.99. The recovery and limit of detection of target analytes in the samples were in the range of 80–120% and 0.11–1.09 ng/mL, respectively.

RESULTS: The information inferred from full-scan and product-ion scan data was combined with literature data to develop a GC/MS/MS method for the identification and quantification of C1-, C2-, C3-, and C4-chrysenes homologues. The method was employed to characterize MC252 crude oil which was released during the 2010 Deepwater Horizon accident. The results showed that the chrysen concentration estimated by the proposed method were well within the range of previously reported values.

CONCLUSIONS: The proposed method is useful for analyzing chrysenes and its alkylated homologues in crude oil samples. Copyright © 2014 John Wiley & Sons, Ltd.

Polycyclic aromatic hydrocarbons (PAHs) and their alkylated homologues are a class of common organic environmental contaminants that contain two or more aromatic rings. They are naturally found in organic substances such as crude oil, coal and creosote. They are also formed during the incomplete combustion of coal, oil, gas, or other organic substances. It is well established that alkylated homologues of certain PAHs (known as alkylated PAHs) can be present in high abundance in environmental samples, and, in some cases, can be more toxic than their parent PAHs. In addition, certain groups of alkylated PAHs in crude oil released into the marine environment (e.g., alkylated chrysenes) are highly resistant to natural weathering processes such as volatilization, photoreoxidation, and biodegradation. Furthermore, due to their low aqueous solubility, the relative concentrations of some of these PAHs can increase over time under certain environmental conditions. These properties make chrysenes and alkylated chrysenes excellent indicator compounds for assessing the impacts of large-scale environmental events such as oil spills.

In this study we focus on four homologues of alkylated chrysenes (C1- to C4-chrysenes). In general, alkylated chrysenes are commonly estimated using gas chromatography/mass spectrometry (GC/MS). The efficiency of GC/MS methods depends on the level of interference from the sample matrix, and lower detection limits can be achieved only after extensive sample clean-up procedures prior to analysis. Recent advances in mass spectrometry have led to the development of triple quadrupole mass spectrometry (GC/MS/MS) that allows the identification of low concentration target analytes in complex samples with greater certainty by minimizing or removing matrix interferences. This is achieved by monitoring multiple reactions between precursor ions from the electron ionization process and characteristic product ions from collision cell reactions of these precursor ions. A comparison study carried out by Fernández-González et al. has shown that GC/MS/MS methods can avoid interferences in biota samples, and can also yield lower detection limits. Pitarch et al. quantified various semi-volatile organic compounds in water samples and concluded that GC/MS/MS methods can lead to excellent selectivity and sensitivity.

Currently, there are no published GC/MS/MS methods available for accurately identifying and quantifying different types of alkylated chrysenes present in environmental samples. The objective of this project was to develop a robust analytical method using gas chromatography/triple quadrupole mass spectrometry to characterize alkylated chrysenes in crude oil and/or weathered oil spill samples. A new GC/MS/MS instrument was first used to study the mass
spectral fragmentation patterns of several commercially available alkylated chrysenes standards using full-scan and product-ion scan modes. These experimental data were then used to predict the fragmentation patterns of other isomers of chrysene homologues for which standards were not available. The experimental and theoretical fragmentation data were then combined to develop a GC/MS/MS method for identifying and quantifying the total concentrations of C_{17}, C_{18}, C_{19}, and C_{20} chrysenes homologues in crude oil samples. The developed method was tested in the characterization of alkylated chrysenes in MC252 crude oil which was spilled into the Gulf of Mexico waters during the 2010 Deepwater Horizon oil spill event.

**EXPERIMENTAL**

**Materials**

High-purity standards of chrysene (purity >98%), C_{17}-chrysenes (1-methylchrysene, 2-methylchrysene, 3-methylchrysene), C_{18}-chrysenes (6-ethylchrysene), C_{19}-chrysenes (6-n-propylchrysene, 1,3,6-trimethylchrysenes) and C_{20}-chrysenes (6-n-butylchrysenes) were purchased from Chiron AS (Trondheim, Norway). For structures, see Fig. 1. All alkylated chrysenes homologue standards were of purity greater than 99%. Chrysenes-d_{12} (purity >99.9%) was purchased from Supelco (Bellefonte, PA, USA). p-Terphenyl-d_{14} (purity >98.5%) and pyrene-d_{10} (purity >98.6%) were purchased from AccuStandard (New Haven, CT, USA). Hexane and dichloromethane were purchased from VWR International (Suwanee, GA, USA). All organic solvents used in this study were of reagent grade. GC capillary columns (J&W DB-EUPAH, 20 m x 0.18 mm x 0.14 μm, p/n 121-9627) and deactivated GC liners (splitless tapered glass wool) were purchased from Agilent Technologies (Wilmington, DE, USA). MC252 crude oil was provided by British Petroleum (BP).

**Preparation of weathered oil under laboratory conditions**

MC252 crude oil (1 mL) was transferred into an aluminum pan (5 cm diameter and 1.5 cm deep) and was evaporated for 7 days within a laboratory fume hood (Mott Manufacturing Ltd, Brantford, Ontario, Canada) at a face velocity of 200 feet per minute (fpm). The weathering process was completed at room temperature (22 °C) and the resulting evaporated crude oil was designated as ‘Weathered MC252 oil’.

**Column chromatographic fractionation**

Fractionation of oil samples was conducted using methods similar to those discussed in earlier research. Activated silica gel was prepared using the methods outlined in Wang et al. Anhydrous sodium sulfate was purified by heating at 400 °C. A glass column (10.1 mm diameter and 200 mm length) was plugged with glass wool, and about 3 g of activated silica gel was added to the column, followed by 1 g of anhydrous sodium sulfate. Then 20 mL of hexane was added to the column. About 25 mg of an oil sample was weighed in a 12 mL glass vial and spiked with surrogates p-terphenyl-d_{14} and pyrene-d_{10}. The contents were then extracted with 1 mL of hexane and transferred to the column. The vial was then sequentially washed with 2 mL of hexane, with 1 mL in each step, and the contents were transferred to the column. All solvents eluted from the column prior to this step were discarded. Then 12 mL of hexane was used to elute aliphatic hydrocarbon fractions from the sample. The hexane-eluted fraction was labeled as F1. Next, a 50% hexane and 50% dichloromethane solvent mixture (15 mL) was used to elute all the aromatic hydrocarbons, and this fraction was labeled as F2. The F1 and F2 fractions were concentrated under a gentle stream of nitrogen gas and then reconstituted in 10 mL of a hexane and hexane/dichloromethane solvent mixture (ratio of 1:1), respectively. The samples were filtered through 0.2 μm PTFE filters, spiked with internal standard (IS) chrysenes-d_{12}, and analyzed for alkylated chrysenes. All the samples were prepared in duplicate and analyzed in triplicate.

**Instrumentation**

An Agilent 7890 gas chromatograph coupled with an Agilent 7000B triple quadrupole (QqQ) mass spectrometer, fitted with an electron ionization (EI) source and a collision cell was used. Various analytical experiments were performed using this instrument running under different conditions including full-scan, product-ion scan, and multiple reaction monitoring (MRM) modes. The GC and MS conditions used are summarized in Table 1.

**Full-scan analysis**

To identify the appropriate precursor ions, high concentrations of target analytes (5 mg/L) were prepared by diluting the standards in a solvent mixture of hexane and dichloromethane (ratio of 1:1). These samples were then run in the full-scan mode, scanning from m/z 50 to 300. Results of the full-scan analysis were used to select the precursor ion for each homologue.

**Product ion scan analysis**

The product-ion (PI) spectra of selected precursor ions were acquired at various collision energies (ranging from 5 to 40 eV). To facilitate the collision-induced dissociation (CID) of the precursor ion, ultra high purity nitrogen was delivered to the collision cell at a flow rate of 1.5 mL/min, and helium was delivered at a flow rate of 2.3 mL/min to quench the reactions. From the product-ion spectrum of a precursor ion, the most abundant product ions and the optimal collision energy required to obtain these ions were selected.

**Multiple reaction monitoring (MRM) analysis**

The combination of precursor ion, the most intense product ions and their corresponding optimal collision energies were employed to form the MRM transitions for each analyte; two different MRM transitions were monitored – one as a quantitative transition and the other as a confirmatory transition.
RESULTS AND DISCUSSION

Full-scan analysis

The electron ionization (EI) mass spectra of the alkylated chrysene standards are shown in Fig. 2. For C1-chrysenes, the base peak is the molecular ion \([M]^+\) at \(m/z\) 242, as cleavage of the aromatic ring requires much higher energy.\(^{15}\) Fragment ions were observed at \(m/z\) 241 and 239. All three C1-chrysene isomers exhibit similar EI mass spectra.

For the C2-chrysene, 6-ethylchrysene, the base peak is the \([M – CH_3]^+\) ion at \(m/z\) 241 (Fig. 3), formed by cleavage of the benzylic bond of the ethyl group.\(^{15}\) Other abundant ions were the molecular ion at \(m/z\) 256 and a fragment ion at \(m/z\) 239.

For the C3-chrysene, 1,3,6-trimethylchrysene, the base peak is the molecular ion at \(m/z\) 270 with major fragment ions at \(m/z\) 255 and 239. The base peak for 6-n-propylchrysene, however, is the \([M – C_2H_5]^+\) ion at \(m/z\) 241 formed by cleavage of the benzylic bond of the propyl group\(^{15}\); other abundant ions were the molecular ion at \(m/z\) 270 and a fragment ion at \(m/z\) 239.

For 6-n-butylchrysene, (a C4-chrysene) the base peak is the \([M – C_3H_7]^+\) ion at \(m/z\) 241, formed by cleavage of the benzylic bond of the butyl group\(^{15}\); other abundant ions were the molecular ion at \(m/z\) 284 and a fragment ion at \(m/z\) 239.

Analysis of quantitative MRM transitions from experimental data

The parent chrysene molecule, chrysene-\(d_{12}\), and the surrogates, \(p\)-terphenyl-\(d_{14}\) and pyrene-\(d_{10}\), have been studied extensively by others, and the expected MRM transitions can be inferred from the literature data.\(^{16,17}\) However, product-
ion (PI) information is not available for any of the alkylated chrysenes. In all our PI scan experiments, we selected the molecular ions as the precursor ions since abundant molecular ion peaks were obtained for all four alkylated chrysenes homologues (Fig. 2). The range in the PI scan experiment for each pure alkylated chrysenes standard was from m/z 50 to the mass of the molecular ion.

Typical PI scans collected at 20 eV for all pure alkylated chrysenes standards are shown in Fig. 3. For all three C1-chrysenes, the predominant product ion is the [M–H]+ ion at m/z 241, with its maximum intensity occurring at a collision energy of 20 eV (Fig. 4). Therefore, the optimal quantitative MRM transition for C1-chrysenes is m/z 242 → m/z 241 at a collision energy of 20 eV.

For 6-ethylchrysenes, the predominant product ion was the [M–CH3]+ ion at m/z 241, with its maximum intensity occurring at a collision energy of 15 eV (Fig. 4). Therefore, the optimal MRM transition for this type of C2-chrysenes was m/z 256 → m/z 241 at a collision energy of 15 eV.

The most significant product ion from the molecular ion of 1,3,6-trimethylchrysenes is the [M–H]+ ion at m/z 255 (Fig. 3), while the most significant product ion for 6-n-propylchrysenes is the [M–C2H5]+ ion at m/z 241. Based on these results, we selected m/z 270 → m/z 255 as the quantitative MRM transition for monitoring C3-chrysenes that have three methyl groups and m/z 270 → m/z 241 as the quantitative MRM transition for monitoring C3-chrysenes with a propyl group. Figure 4 shows that the optimal collision energies for monitoring these transitions to m/z 255 and 241 are 20 and 15 eV, respectively. Therefore, the transitions of m/z 270 → m/z 255 and m/z 270 → m/z 241 were monitored at collision energies of 20 and 15 eV, respectively.

For 6-n-butylchrysenes, a C4-chrysenes, the most significant product ion (Fig. 3) is the [M–C3H7]+ ion at m/z 241 whose maximum intensity occurred at a collision energy of 15 eV (Fig. 4). Therefore, the optimal quantitative MRM transition for this type of C4-chrysenes is m/z 284 → m/z 241 at a collision energy of 15 eV.

Analysis of quantitative MRM transitions from literature data

For C2-chrysenes, the PI scan data (of the 6-ethylchrysenes standard) indicated that the m/z 256 → m/z 241 transition would capture all ethyl-type C2-chrysenes. However, C2-chrysenes can also have two methyl groups, and McLafferty and Tureček[15] showed that an abundant fragment ion would be yielded by removing one of the methyl groups. Therefore, the m/z 256 → m/z 241 transition, originally intended to capture ethyl-type C2-chrysenes, would also naturally identify the presence of C2-chrysenes with two methyl groups.

From the C3-chrysenes dataset, we know that multiple MRM transitions are needed to capture compounds having three different types of alkylations. Based on the PI scan data of the C3-chrysenes standards, we have already assigned the m/z 270 → m/z 255 transition for monitoring C3-chrysenes having three methyl groups, and the m/z 270 → m/z 241 transition for monitoring C3-chrysenes having a propyl group. However, C3-chrysenes compounds can also have an ethyl and methyl group. Based on our PI scan analysis of the other chrysenes, we expect the most significant product ion for these chrysenes to be the [M–CH3]+ ion at m/z 255.

For i-propylchrysenes we again expect the predominant

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**Figure 3.** Product-ion scan mass spectra for the seven alkylated chrysenes standards at a collision energy of 20 eV (precursor ions selected are reported in the respective figures).
product ion to be the [M–CH₃]⁺ ion at m/z 255. Therefore, the m/z 270 → m/z 255 transition, originally intended to monitor C₃-chrysenes having three methyl groups, would also capture C₂-chrysenes having an ethyl group and a methyl group and the i-propylchrysenes.

For the C₄-chrysenes, the PI scan data of 6-n-butylchrysene indicated that the m/z 284 → m/z 241 transition would capture all the butyl-type C₄-chrysenes. However, C₄-chrysenes can also have other types of alkyl substitution with compounds involving: (a) four methyl groups, (b) two ethyl groups, (c) a methyl and a propyl group, (d) i-butylchrysenes, and (e) t-butylchrysenes. For C₄-chrysenes containing four methyl groups, we expect there to be an abundant [M–CH₃]⁺ product ion at m/z 269, as observed for 1,3,6-trimethylchrysene. Therefore, the m/z 284 → m/z 269 transition can be used to capture all the C₄-chrysenes containing four methyl groups. This transition to the [M–CH₃]⁺ product ion at m/z 269 would also be suitable for the detection of C₄-chrysenes containing two ethyl groups and t-butylchrysenes. Therefore, the m/z 284 → m/z 269 transition, used to capture C₄-chrysenes having four methyl groups, would also detect the C₄-chrysenes containing two ethyl groups and t-butylchrysenes. We selected a collision energy of 20 eV to monitor this transition.

For C₄-chrysenes containing a methyl and a propyl group the most abundant product ion would be expected to be the [M–C₂H₅]⁺ ion at m/z 255, as found for 6-n-propylchrysene. For i-butylchrysenes, the predominant product ion would be the [M–C₃H₇]⁺ ion at m/z 241, as found for i-butylbenzene. Therefore, the m/z 284 → m/z 269 transition is used to capture all the C₄-chrysenes having a methyl and a propyl group, and t-butylchrysenes, with the selected collision energy for this transition being 15 eV.

Selection of confirmatory MRM transitions from experimental data

Confirmatory MRM transitions are chosen to improve the selectivity and sensitivity of GC/MS/MS MRM methods. Full-scan EI mass spectra of all seven alkylated chrysene standards yielded a fragment ion at m/z 239, although the intensities differed. Furthermore, PI spectra of the molecular ions [M⁺] of all the compounds yielded a product ion at m/z 239, whose abundance increased as the collision energy was increased. Therefore, we used the following four confirmatory MRM transitions: m/z 242 → m/z 239, m/z 256 → m/z 239, m/z 270 → m/z 239 and m/z 284 → m/z 239 to verify the C₁-, C₂-, C₃- and C₄-chrysenes homologues, respectively. The collision energy for all these confirmatory transitions was set at 40 eV. A detailed summary of the proposed MRM method is presented in Table 2. The relative intensities of the quantitative transition to that of the confirmatory transition for p-terphenyl-d₄, pyrene-d₁₀, chrysene, 3-methylchrysene, 2-methylchrysene, 1-methylchrysene, 6-ethylchrysene, 6-n-propylchrysene, 1,3,6-trimethylchrysene and 6-n-butylchrysene are 1.30, 1.14, 76.44, 1.48, 1.49, 1.53, 2.67, 3.85, 0.89 and 4.19, respectively.

Application of the MRM method for the characterization of MC252 crude oil

The developed MRM method was used to characterize a complex environmental sample, MC252 crude oil which was spilled during the 2010 Deepwater Horizon oil spill event.

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Figure 4. Collision energy optimization data for the seven alkylated chrysene standards (precursor and product ions selected are reported in the respective figures).
One of the major unknowns of this oil spill is the fate of toxic PAHs such as chrysene and its alkylated homologues. Therefore, detailed characterization of various components of chrysene in MC252 crude oil would help to provide the preliminary data required to assess the potential human and ecological risks resulting from the oil spill. Furthermore, Mulabagal et al.\[21\] reported hopane levels in MC252 oil spill residue recovered along the Alabama shoreline and showed that the relatively fresh looking, sticky brownish oil spill samples are not degrading and hence have the potential to remain in the environment for an extended period of time\[22\]. These studies support the importance of understanding the fate of various forms of chrysene in oil spill samples.

To accurately quantitate the concentration of alkylated chrysenes in environmental samples, pure standards of various forms of alkylated chrysenes are required; however, several of these compounds might not be available commercially. Therefore, United States Environmental Protection Agency (USEPA) methods typically use relative response factors (RRFs), which are estimated based on the response of the parent chrysene, to make semi-quantitative estimates of the alkylated chrysenes.\[23\] It has been reported that methods that employ a parent PAH to quantify alkylated homologues can introduce large quantitation errors.\[24\] Other simpler methods have also been proposed to make highly approximate estimates for alkylated compounds. For example, during the Deepwater Horizon oil spill event, the USEPA recommended using a constant (factor of 5 with respect to parent chrysene) to estimate the total amount of the alkylated chrysenes in oil spill samples.\[25\] In this study, we used seven different alkylated chrysenes standards. The MRM responses of these standards (at the concentration level of 100 ng/mL) are given in Fig. 5. Their fragmentation patterns in full-scan and PI scan spectra are presented in Figs. 2 and 3, respectively. The data show that all three \(C_1\)-chrysenes standards (3-methylchrysene, 2-methylchrysene and 1-methylchrysene) have similar fragmentation patterns, and they also show similar levels of response. Therefore, any one of these pure compounds can be used as the standard to quantify \(C_1\)-chrysenes. In this study, we selected 3-methylchrysene, which is a standard compound used in previous research.\[11]\]

### Table 2. Summary of optimized MRM transitions used for quantifying various target compounds

<table>
<thead>
<tr>
<th>Homologue/Compound</th>
<th>Quantitative transitions</th>
<th>Confirmary transitions</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Ions (m/z)</td>
<td>Collision energy (eV)</td>
</tr>
<tr>
<td>(^1p)-terphenyl-(d_{14})</td>
<td>212 → 208</td>
<td>40</td>
</tr>
<tr>
<td>(^1)Pyrene-(d_{10})</td>
<td>244 → 212</td>
<td>40</td>
</tr>
<tr>
<td>(^1)Chrylene-(d_{12})</td>
<td>240 → 236</td>
<td>40</td>
</tr>
<tr>
<td>(^1)Chrylene</td>
<td>228 → 226</td>
<td>38</td>
</tr>
<tr>
<td>C(_1)-chrysenes</td>
<td>242 → 241</td>
<td>20</td>
</tr>
<tr>
<td>C(_2)-chrysenes</td>
<td>256 → 241</td>
<td>15</td>
</tr>
<tr>
<td>C(_3)-chrysenes</td>
<td>270 → 241</td>
<td>15</td>
</tr>
<tr>
<td>C(_4)-chrysenes</td>
<td>284 → 241</td>
<td>20</td>
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<tr>
<td></td>
<td>284 → 255</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>284 → 269</td>
<td>20</td>
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</table>

\(^1\)These MRM transitions are selected from published literature.

Since only one isomer is commercially available for the \(C_2\) and \(C_4\)-chrysenes homologues (6-ethylchrysen for \(C_2\) and 6-n-butylichrysen for \(C_4\)), these two compounds were used as representative calibration standards for these two sets of homologues. In the case of \(C_3\)-chrysenes, two standards were available that represent two distinct types of alkylation with two different MRM transitions. We used 6-n-propylchrysen to quantify those \(C_3\)-chrysenes that had the MRM transition \(m/z\) 270 → \(m/z\) 241, and 1,3,6-trimethylchrysen to quantify those that had the MRM transition \(m/z\) 270 → \(m/z\) 255.

The quantitation approach used here (which uses a distinct calibration standard for each homologue) is a robust approach for quantifying different types of chrysen homologues. A calibration stock solution consisting of 10 mg/mL of the parent chrysene, 3-methylchrysen, 6-ethylchrysen, 6-n-propylchrysen, 1,3,6-trimethylchrysen, 6-n-butylichrysen, and two surrogate standards \(p\)-terphenyl-\(d_{14}\) and pyrene-\(d_{10}\) was prepared in a solvent mixture of hexane and dichloromethane in a ratio of 1:1. The solution was then diluted to prepare calibration standards having concentrations of 1, 2, 5, 10, 20, 50, 100, 200 and 500 ng/mL. The calibration curves were constructed using the standard solutions. The linearity of the calibration curve was confirmed by ensuring that the \(r^2\) values were greater than 0.99.\[26\]

### LOD/LOQ and recovery data

The limit of detection (LOD) and limit of quantitation (LOQ) for the representative compounds were determined by measuring a series of blanks with no analyte. The blank mean value and the standard deviation (SD) were calculated for each
representative compound. The LOD is estimated as the mean blank value plus three times the SD. The LOQ is estimated as the mean blank value plus ten times the SD. The LOD and LOQ values for the representative compounds are presented in Table 3.

The MC252 crude oil extract was spiked to increase the concentration by 10 and 20 ng/mL (using the following pure standards: chrysene, 3-methylchrysene, 2-methylchrysene, 1-methylchrysene, 6-ethylchrysene, 6-n-propylchrysene, 1,3,6-trimethylchrysene and 6-n-butylchrysene) prior to GC/MS/MS analysis. The recovery levels were computed using the following equation:

$$%R = \left( \frac{C_S - C_U}{C_n} \right) \times 100$$

where %R is the measured percentage recovery level, $C_S$ and $C_U$ are the concentration levels of the target analyte in the spiked and non-spiked samples, and $C_n$ is the spiked concentration level (10 and 20 ng/mL). The recovery levels are summarized in Table 3. The overall spiked target recovery levels observed ranged from 85 to 120% and these values are well within typical spiked compounds recovery levels.

Results for MC252 crude oil sample

The crude oil fractions F1 and F2 (discussed in the Experimental section) were analyzed and alkylated chrysenes were found only in the F2 fraction. In order to enhance sensitivity, within the MRM method, we set distinct time segments to monitor each homologue. The total ion chromatogram for the crude oil and the extracted ion chromatograms for each MRM transition are given in Fig. 6.

To quantify the concentrations, the extracted chromatographic peaks that satisfied both the quantitative and the confirmatory MRM transitions of a homologue were selected and quantified using the respective calibration standard. The estimated concentration values of chrysene and its alkylated homologues in MC252 crude oil are summarized in Table 4. The two surrogate compounds, p-terphenyl-d14 and pyrene-d10, were also quantified; the relative recovery of the surrogate compounds ranged between 80% and 120%, which is well within the USEPA recommended levels for surrogate recovery in environmental samples.

The National Institute of Standards and Testing (NIST, Gaithersburg, MD, USA) compiled the analytical results of the MC252 oil characterization studies completed by 26 different laboratories and released a summary report. Table 4 compares the values of the different types of chrysenes estimated in our study against the reference dataset presented in the NIST report. The data show that the concentration estimates for chrysene and its homologues evaluated in our study are well within the range reported by the NIST.

<table>
<thead>
<tr>
<th>Table 3. LOD and LOQ values, and percentage recovery values from spiking studies</th>
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<tr>
<td><strong>Compound</strong></td>
</tr>
<tr>
<td>Chrysene</td>
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<tr>
<td>3-methylchrysene</td>
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<tr>
<td>2-methylchrysene</td>
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<tr>
<td>1-methylchrysene</td>
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<tr>
<td>6-ethylchrysene</td>
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<tr>
<td>6-n-propylchrysene</td>
</tr>
<tr>
<td>1,3,6-trimethylchrysene</td>
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<tr>
<td>6-n-butylchrysene</td>
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Figure 6. Total ion chromatogram and extracted ion chromatograms for various MRM transitions used for quantifying chrysene and alkylated-chrysene homologues in the MC252 crude oil sample.
was developed based on full-scan and product-ion scan data collected for seven different alkylated standards using a novel MS/MS instrument (Agilent 7000B MS/MS system). The full-scan data, product-ion scan data, and the collision cell energy optimization data for all seven standards are provided. Based on these experimental data, coupled with literature-derived information, the fragmentation patterns of other isomers of chrysene homologues for which standards are not commercially available were proposed. This information was used to develop a GC/MS/MS method for identifying and quantifying the total concentrations of C1-, C2-, C3-, and C4-chrysenes in crude oil samples. The developed method was employed to characterize MC252 crude oil, released into the Gulf of Mexico during the Deepwater Horizon oil spill event. The results show that the concentrations of chrysene and its alkylated homologue estimated by the proposed method are mostly well within the range of previously reported values. The proposed method is a promising approach for quantifying alkylated chrysenes in more complex environmental samples such as crude oils and weathered oil-spill-waste samples.

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