Improved Resolution of Hydrocarbon Structures and Constitutional Isomers in Complex Mixtures Using Gas Chromatography-Vacuum Ultraviolet-Mass Spectrometry

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S *Supporting Information*

ABSTRACT: Understanding the composition of complex hydrocarbon mixtures is important for environmental studies in a variety of fields, but many prevalent compounds cannot be confidently identified using traditional gas chromatography/mass spectrometry (GC/MS) techniques. This work uses vacuum-ultraviolet (VUV) ionization to elucidate the structures of a traditionally "unresolved complex mixture" by separating components by GC retention time, t_R, and mass-to-charge ratio, m/z, which are used to determine

carbon number, N_C, and the number of rings and double bonds, N_{DBE}. Constitutional isomers are resolved on the basis of t_R, enabling the most complete quantitative analysis to date of structural isomers in an environmentally relevant hydrocarbon mixture. Unknown compounds are classified in this work by carbon number, degree of saturation, presence of rings, and degree of branching, providing structural constraints. The capabilities of this analysis are explored using diesel fuel, in which constitutional isomer distribution patterns are shown to be reproducible between carbon numbers and follow predictable rules. Nearly half of the aliphatic hydrocarbon mass is shown to be branched, suggesting branching is more important in diesel fuel than previously shown. The classification of unknown hydrocarbons and the resolution of constitutional isomers significantly improves resolution capabilities for any complex hydrocarbon mixture.
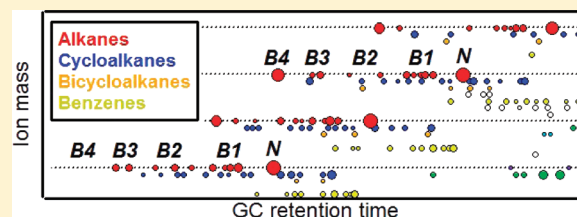
Chemical analyses of environmental contamination often rely on chemical speciation of complex organic mixtures. Quantifying known tracer compounds in these environments can provide insight into biological and chemical degradation processes[1,2] and contaminant sources.[3−6] Over the past few decades, a variety of liquid and gas chromatography (LC and GC) techniques have been used with great success to improve understanding of pollutants as well as transformations in intricate natural systems. One of the newest promising techniques is vacuum-ultraviolet (VUV) single-photon ionization mass spectrometry (SPI-MS).[7,8]

Traditional GC/MS employs electron impact (EI) ionization, where a molecule is ionized by an electron at an energy near the peak of ionization efficiency (usually 70 eV). This technique is broadly applicable, as nearly all organic compounds can be ionized at this energy, and generation of electrons is easily achieved using a filament in a vacuum chamber. However, because the ionization energy (IE) of most large organic

compounds lies below 11 eV,[9] EI imparts considerable excess energy to the ionized molecule, causing dissociation into smaller fragment ions. For EI-MS analysis of pure compounds, parent ions are sometimes visible and/or the fragmentation pattern can be used to deduce molecular structure, but because EI of different organic compounds often yields similar fragment ions, interpretation of EI-MS spectra of organic mixtures can be ambiguous. Alternatively, using photons with energy of 11 eV or less, organic compounds can be ionized with minimal excess internal energy and therefore fragmentation, thus facilitating the identification of a compound by its molecular formula. This "soft" ionization is achieved in SPI-MS with ultraviolet photons in a vacuum (hence "vacuum-ultraviolet" or VUV) generated by a laser,[10] excitation of a rare gas,[11] or in the case of this work, synchrotron radiation.[12] Soft ionization mass spectrometry has proven to be a valuable tool for analysis of complex mixtures because parent ions can be used for identification. For this reason, GC/VUV-MS analysis is being applied to a wide array of environmental and public health fields.[8]

Chromatography of many environmental samples yields a large "unresolved complex mixture" that has yet to be well quantified or described. This is because aliphatic hydrocarbons, a significant source of environmental contamination, fragment heavily upon EI ionization, yielding many smaller fragments that are indistinguishable. Consequently, large straight-chain alkanes do not yield significantly different mass spectra nor are they distinguishable from branched isomers. Though there is a small parent ion contribution to the mass spectra of many n-alkanes, it is not typically large enough to be used for unambiguous identification in a complex mixture. This parent ion is even smaller for branched alkanes, significantly hindering identification of branched hydrocarbons using EI ionization, though such compounds are common in environmental samples. To address coelution of compounds, gas chromatography can be coupled with a second gas chromatographic separation based on polarity (GCxGC), providing better separation and identification of compound classes in many environmentally relevant mixtures.[13,14] Though useful, this technique typically does not significantly improve resolution of the aliphatic unresolved complex mixture because aliphatic hydrocarbons are not well retained on the second column (demonstrated by figures by Schnelle-Kreis et al.,[13] Hamilton,[14] and Worton et al.[15]). However, VUV ionization allows improved identification of hydrocarbons because branched and straight-chain hydrocarbons both display a significant parent ion peak in photoionization mass spectra (see examples in the Supporting Information). This work uses the capabilities of GC/VUV-MS to develop methods for resolving the "unresolved complex mixture" by classifying compounds according to molecular mass and structure, including separation of structural isomers.

Applications of GC/VUV-MS analyses have often focused on petroleum fuels as sample organic mixtures[7,8,10,16,17] because of their environmental importance and daunting complexity. Diesel fuel serves as a useful example for typical pollution while its complexity provides an additional test of the resolution and capabilities of a separation technique. Diesel has also been analyzed in great detail using traditional GC/EI-MS techniques,[18] which is useful in comparing results from traditional methods to VUV ionization techniques.

GC/VUV-MS allows separation in the two-dimensional plane of chromatographic retention time (t_R, typically a function of volatility) and ion mass-to-charge ratio (m/z).

Previous work[7,16] has effectively used this GCxMS plane to identify classes of compounds in complex mixtures by parent ion. Using this method, the volatility distribution of petroleum fuels was shown to approximately agree with GC/MS analysis of diesel fuel,[18] consisting primarily of saturated aliphatic hydrocarbons and polycyclic aromatic hydrocarbons (PAHs). However, the dependence of retention time on chemical structure allows GCxMS analysis to separate not only classes of compounds but also many isomers. While this has been shown previously,[17] no attempt has been made to explore this capability in depth or determine the structures of unknown compounds. Owing to the limitations of GC/EI-MS in resolving complex hydrocarbon mixtures, only the most dominant branched hydrocarbons, such as pristane and phytane in diesel fuel, have been able to be quantified in past work.[18] Therefore, no complete picture of isomer content of petroleum mixtures is currently available. Isomer separation is a potentially important step forward in understanding fates of fossil fuel contaminants in the environment because structure of a compound strongly affects degradation and oxidation pathways,[19,20] which has implications for applications ranging from bioremediation to atmospheric aerosol modeling, as well as providing insights into chemical ecology such as insect interactions.[21−24]

Using GC/VUV-MS, we improve the identification of typically unresolved compounds in complex hydrocarbon mixtures and demonstrate the quantitative resolution of structural isomers. Determination of carbon number, compound class, and branching provides structural information about any unknown compound in a mixture. These concepts are shown in the context of diesel fuel, as it is a useful surrogate for many environmental systems, but are intended to be applicable for a wide variety of systems. Distribution of isomers in a diesel sample is shown to be more predictable and constrained than might be expected by the number of potential isomers.

EXPERIMENTAL SECTION

Instrumentation. Samples and standards were analyzed by a GC (6890 Series, Agilent) coupled to a time-of-flight mass spectrometer (TOFMS; HTOF model, Tofwerk). Helium (with splitless flow) was used as a GC carrier gas, with a temperature ramp of 15 °C per minute from 80 to 320 °C. The effluent from the GC column (Rxi-5 ms, 30 m × 0.25 mm × 0.25 μm, Restek Corporation) entered the TOFMS ionization chamber at vacuum. Molecules in this gas stream were then ionized either by electron impact (EI) ionization using an internal filament or vacuum-ultraviolet (VUV) photoionization at the Chemical Dynamics Beamline of the Advanced Light Source (ALS) at Lawrence Berkeley National Lab. The photon beam passes through a gas filter and MgF$_2$ window to remove unwanted undulator harmonics, resulting in photons that are tunable between approximately 8 and 11 eV with a flux of $\sim 10^{15}$ photons/s.[25] The heated region through which the GC column entered the TOFMS was varied between 150 and 275 °C, as was the temperature of the ionization chamber (see Parameter Optimization below for detailed discussion). The photon beam entered the ionization chamber perpendicular to the column outflow and the EI filament, allowing photoionization without removing the filament.

All standards were purchased from Sigma-Aldrich and AccuStandard. Diesel sample was purchased from AccuStan-
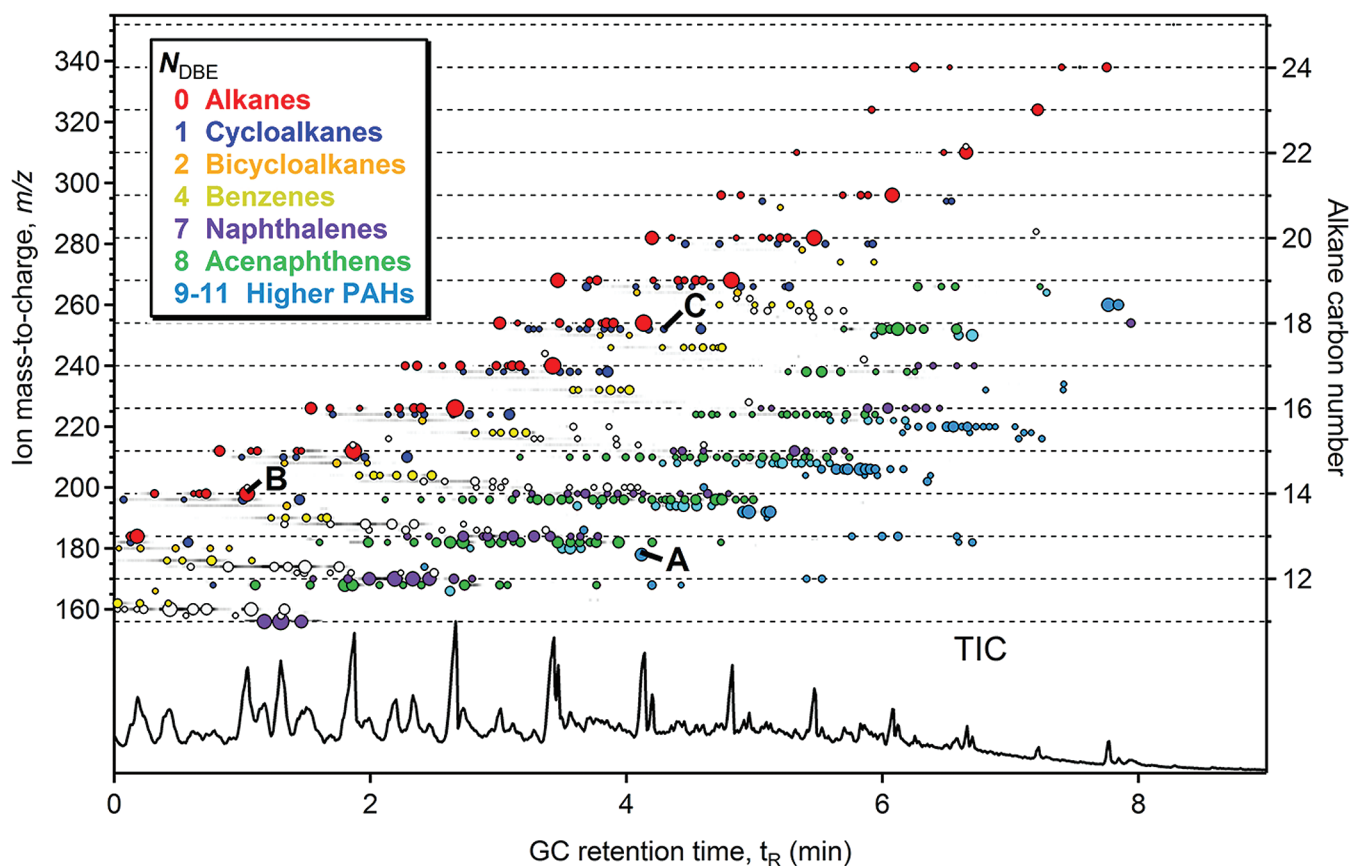
Figure 1. GCxMS plot of diesel fuel at a transfer temperature of 150 °C and a photon energy of 10.5 eV. Circles represent peaks and are approximately sized by logarithmic peak height and colored by (N_{DBE}); red: alkanes (0); blue: cycloalkanes (1); orange: bicycloalkanes (2); yellow: benzenes (4); purple: naphtalenes (7); green: acenaphthenes (8); blues: higher order PAHs (9−11); and white: unassigned class (3, 5, 6). Gray areas in background are ion abundance and dashed horizontal lines correspond to the mass of alkanes (N_{DBE} = 0). The total ion chromatogram (TIC) at the bottom shows total signal intensity. The blue circle labeled "A" and the red circle labeled "B" are phenanthrene ($C_{14}H_{10}$) and tetradecane ($C_{14}H_{30}$), respectively, and are discussed in Classification by Carbon Number. The compounds represented by the blue circle labeled "C" is an unknown compound identified in Separation of Structural Isomers as a C_{18} cycloalkane with a single methyl branch.

dard. Data analysis was performed using custom processing code written in Igor Pro 6.0.4 (Wavemetrics).

Parameter Optimization. Operating conditions can be adjusted by varying temperature and photon energy to minimize fragmentation; lowering these parameters increases the ion fraction observed as the parent ion (f_P) in a mass spectrum by decreasing internal energy.[26] Experimental conditions were optimized to find a balance between chromatographic separation and minimal fragmentation. This optimization should be performed for all GC/VUV-MS applications and is therefore discussed in the Supporting Information. A temperature of 150 °C for both the transfer line between the GC and the TOFMS, and the ionization chamber in the TOFMS was used in this work as these operating conditions were found to yield little fragmentation without significantly adversely affecting the chromatography in the volatility range of interest. Briefly, fragmentation was observed to be strongly dependent on transfer temperature and not significantly affected by the energy of the ionizing photons (Figure S-1, Supporting Information). However, varying photon energy is an effective means of identifying unresolved compounds by selectively ionizing compounds that differ in ionization energies by more than 0.2−0.3 eV, as this is the width of the energy distribution of photons generated at the ALS.[25] Photons of 10.5 eV were used for most purposes in this

work to ionize all compounds of interest, but photons of 10.0 eV were used to selectively ionize unsaturated hydrocarbons (which have a lower ionization energy[26,27]), resolving unsaturated and cyclic compounds of identical mass.

Classification by Double Bond Equivalents. In this work, the mass of a given hydrocarbon molecule, i, will be used to classify compounds according to carbon number, N_C, and number of double bonds and rings in their structure, N_{DBE}. Each ring or double bond in the molecule i removes two H atoms compared to the alkane $C_{N_C(i)}H_{2N_C(i)+2}$, reducing the mass of the hydrocarbon, $m(i)$, by twice the mass of hydrogen, $2*m(H)$. $N_C(i)$ can reliably be estimated by the GCxMS characteristics of the molecule as discussed in Classification by Carbon Number. The mass of the alkane therefore equals $m(C_{N_C(i)}H_{2N_C(i)+2})$. The number of double bonds and rings, called double bond equivalent number, N_{DBE}, becomes

$$N_{DBE}(i) = \frac{m(C_{N_C(i)}H_{2N_C(i)+2}) - m(i)}{2 \cdot m(H)} \tag{1}$$

Geissler et al.[16] have shown that each N_{DBE} (adaptation of "class residue" from reference) is expected to represent a different class of compounds common in diesel fuel.

The assignment of DBE classes in this work is based on fuel analysis performed by Schauer et al.,[18] Geissler et al.,[16] and

Mitschke et al.[17] DBE classes are expected to contain saturated and cyclic aliphatic compounds (N_{DBE} = 0 to 3), alkylated benzenes (N_{DBE} = 4), and polycyclic aromatic hydrocarbons ($N_{DBE} \geq 7$). Unsaturated aliphatic hydrocarbons are not expected to be significant components of diesel fuel. For instance, N_{DBE} = 1 is presumed in this and previous work to consist mostly of cycloalkanes, not alkenes. Accurate DBE classification relies on GC separation to distinguish between alkyl and PAH compounds of equal mass (e.g., N_{DBE} = 0 vs N_{DBE} = 7), which is possible because chromatographic separation is a function not only of molecular formula but also of chemical structure such as the presence of rings and branches.

■ RESULTS AND DISCUSSION

Spectra of Known Compounds. In the Supporting Information, we provide the first compilation of VUV mass spectra of large organic compounds, with molecular masses primarily in the range of 150 to 400 Da. Spectra of 84 known compounds are shown, representing a wide range of functional groups: saturated and unsaturated aliphatic hydrocarbons including hopanes and steranes, polycyclic aromatic hydrocarbons, aliphatic and aromatic acids and esters, aliphatic ketones and aldehydes, oxygenated and multifunctional aromatics, and chloro- and nitro-aromatics. Spectra are collected using VUV ionization at 10.5 eV with transfer temperatures of 150 °C (for a subset of 38 compounds) and 275 °C (for all compounds). All spectra are collected by GC/VUV-MS of known compound mixtures.

From the spectra, it is clear that transfer temperature has a significant impact on fragmentation. A transfer temperature of 150 °C increases f_P for all compounds, while higher transfer temperatures enhance fragment peaks. Low temperature operating conditions can therefore be used to focus on resolution by molecular mass as is done in this work. However, using high temperature operating conditions provides some structural information, potentially facilitating identification, as is done in EI, while still providing the molecular mass in most cases; these capabilities may be preferable for identification of compounds in some mixtures.

Classification by Carbon Number. An injection of diesel fuel (7.5 μg diluted to 1.5 μL with chloroform) was analyzed by GC/VUV-MS to explore resolution capabilities. The sample is shown in the GCxMS plane in Figure 1, where each chromatographic peak is shown as a circle, while the gray areas in the background represent ion abundance. Note that some DBE classes that do not appear to have a significant number of peaks contain long gray areas, indicating a rising background with no identifiable chromatographic peaks. Such areas are likely composed of many small chromatographic peaks that are not well resolved but in some cases account for a non-negligible fraction of total detector response. It is clear from Figure 1 that certain DBE classes (colored circles in Figure 1) are more highly populated with peaks than others. This work will focus primarily on the aliphatic DBE classes, as this best utilizes the soft ionization capabilities of VUV-MS; aromatics and PAHs tend not to fragment heavily even using EI ionization owing to resonance stabilization.

The presence of rings and double bonds causes compounds in higher DBE classes to shift toward longer retention times, with aromatics eluting later than aliphatics with the same mass due to interaction with aromatic groups in the phase of the chromatographic column. Compounds of the same carbon

number therefore fall in diagonal regions in the GCxMS plane. A clear example of this phenomenon is illustrated by phenanthrene and tetradecane (compounds A and B, respectively) in Figure 1. Though phenanthrene, a C_{14} PAH with N_{DBE} = 10, has a retention time similar to octadecane ($C_{18}H_{38}$) and a mass equal to a C_{13} compound with N_{DBE} = 3, it falls into a diagonal band of peaks containing tetradecane ($C_{14}H_{30}$). These compounds therefore define an area of C_{14} compounds spanning DBE classes of 0 to approximately 11 (beyond which there are few peaks). There is an analogous band for all observed carbon numbers, so any peak or unknown compound can be classified by N_C and N_{DBE}.

Quantitation of the composition of diesel fuel by carbon number and DBE classifications (Figure 2) using VUV
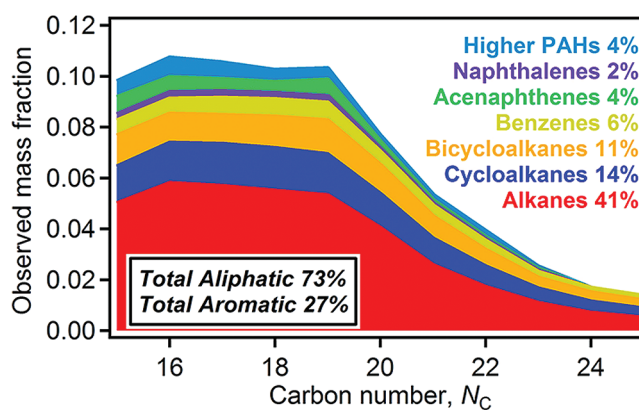
Figure 2. Composition of analyzed diesel fuel sample as fraction of total observed mass of each DBE class at each carbon number in the range of C_{15} to C_{25}. DBE classes shown (colored as in Figure 1: N_{DBE} = 0, 1, 2, 4, 7, 8, 9–11) account for 82% of total observed mass. Calibration is approximate and based on measured responses of hexadecane for N_{DBE} = 0 through 3, dodecylbenzene for N_{DBE} = 4 through 6, and phenanthrene for N_{DBE} = 7 through 11.

ionization is performed here as a validation against previous analyses of petroleum fuels. Signal intensity at each DBE and carbon number was corrected for transfer efficiency through the GC (dependent on t_R) and was calibrated using one of three standards: hexadecane for aliphatic compounds (N_{DBE} = 0 through 3), dodecylbenzene for alkyl aromatics (N_{DBE} = 4 through 6), and phenanthrene for PAHs ($N_{DBE} \geq 7$). Absorption cross-section and detector response are expected to be structure and functional group dependent[10] so are neglected here by calibrating to compounds of similar structure. Compounds of N_C below 14 eluted during the chromatographic solvent delay so were not quantified in this work. Consequently, smaller aromatics (such as C_2-alkylnaphthalenes and short-chain alkylbenzenes) as well as short-chain alkanes were not measured. In this sample, alkanes account for nearly half (41%), while total aliphatic compounds constitute approximately three-quarters (73%) of the observed mass fraction. Cycloalkanes comprise a more significant mass fraction (14%) of diesel fuel than has been shown in past work, though this may be a function of the specific fuel sample analyzed. The compound distribution shown in Figure 2 approximately agrees with previous diesel fuel characterization[18] and suggests a volatility distribution similar to that measured by VUV-MS of crude oil.[16] Classification by carbon number therefore yields similar results to past work using other methods, demonstrating that quantitation using GC/VUV-MS is an effective method for

composition analysis of hydrocarbon mixtures. Characterization by N_C and N_{DBE} may be useful in parametrization of chemically explicit models. Further study of the effects of branching on fragmentation, as well as calibrating with authentic standards for a wider variety of observed compounds, will improve future quantitation by GCxMS analysis.

Though photon energy does not significantly affect fragmentation patterns, it is potentially useful in the identification of unknowns. Comparison of diesel fuel ionization at 10.5 and 10.0 eV supports the assumption that most aliphatic hydrocarbons in the sample are saturated and that $N_{DBE} = 1$ is likely comprised primarily of cycloalkanes, not alkenes. This information on saturation, coupled with N_{DBE} and N_C obtained by GCxMS analysis, provides critical constraints for elucidating the structure of an unknown compound.

Separation of Structural Isomers. GC/VUV-MS was used to separate and identify structural isomers that are unresolvable using GC/EI-MS. Alkanes are typically quantified by EI-MS using ions with m/z 57 (the $C_4H_9^+$ fragment), while VUV-MS allows quantitation based on parent ions; an example of this is shown in Figure 3, which compares two separately
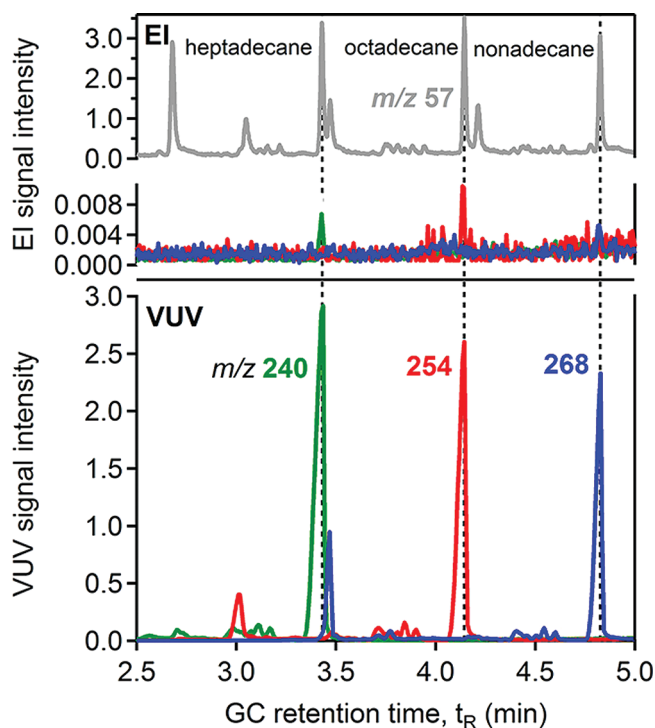
Figure 3. Chromatograms of diesel fuel using GC/EI-MS (top) and GC/VUV-MS (bottom). Ions shown are the parent ions of C_{17} (green line, m/z 240), C_{18} (red line, m/z 254), and C_{19} (blue line, m/z 268) alkanes using both EI and VUV, and m/z 57 (gray line) using EI, the ion typically used for the quantitation and identification of alkanes. Dashed lines show n-alkanes. Branched isomers can be identified by parent ion using VUV but not EI ionization.

collected VUV and EI chromatograms of diesel fuel in the C_{16} to C_{19} retention time window. The large peaks of m/z 57 can be identified as n-alkanes by retention time matching to standards as well as the presence of parent ions, though these are 2 orders of magnitude smaller than the quantitation ion. However, the interspersed smaller peaks representing branched isomers are undetectable on their parent ions and therefore cannot be identified without authentic standards. Using GC/

VUV-MS, each chromatographic peak can be identified by parent ion, allowing identification and quantitation of branched isomers. In many cases, the branched regions observed on m/z 57 using GC/EI-MS are shown by GC/VUV-MS to be composed of alkanes of different carbon numbers.

The GCxMS plot (Figure 1) shows several structural isomers in each DBE class in diesel fuel. Alkanes ($N_{DBE} = 0$, red circles in Figure 1) will be used to elucidate isomer separation as they are the largest fraction of diesel, have the clearest isomer patterns, and are not well resolved using EI-MS. Isomers of a given N_C shift toward shorter retention times as branching increases, so alkane isomers can be compared (Figure 4a) by a relative retention index: the difference (ΔI_K) between the Kovats index[28] of the isomer and of the n-alkane of the same carbon number; by definition, $\Delta I_K = 0$ for n-alkanes while the earliest-eluting peak with a given N_C has the highest ΔI_K value.

Isomer structure can be determined from ΔI_K. The branched endmembers in the C_{19} and C_{20} alkane bands are identified using authentic standards as tetramethyl isomers: pristane and phytane (structures shown in Figure 4a), the two most prevalent branched isomers in diesel fuel. The range of C_{19} and C_{20} alkane isomers is therefore bracketed by the n-alkane (N) and a C_4-branched isomer (B4), with less-branched isomers in between. Due to a lack of available standards, the dependence of retention time shifts on branching location cannot be determined, so an isomer with 4 methyl groups cannot be distinguished from an isomer with a single branch containing a butyl group; therefore, this work will refer to C_n-branched (as Bn) isomers in place of specific identification. Intermediately branched isomers appear to fall into approximately evenly spaced regions separated by natural gaps, suggesting C_1-, C_2-, and C_3-branching. Pristane has a ΔI_K significantly larger than the most branched C_{18} alkane, thus defining the boundary with the B3 region. A similar increase in ΔI_K of the most branched alkane occurs at C_{24}, defining a region of C_5-branched alkanes (B5). Squalane, a hexamethyl C_{30} alkane (blue square in Figure 4a), displays another significant increase in ΔI_K, suggesting a region of C_6-branched (B6) isomers that is unpopulated in the diesel sample analyzed in this work. Boundaries of the regions of C_1- through C_3-branching are inferred from gaps in peaks, though regions B2 and B3 do not have a clear separation point in this sample. Resolved B1 through B3 alkanes are below detection limits for larger alkanes, though some unresolved mass is often present.

As carbon number increases, the degree of possible branching increases correspondingly, so an increase in the width of the alkane band is expected. However, ΔI_K exhibits not a smooth upward trend but rather a step function punctuated by increases of 30 to 50 every 5 carbon numbers, suggesting a theoretical maximum of branching, with a branch on every fifth carbon such as in the case of pristane. These compounds are thought to be polymers of isoprene (2-methyl-1,3-butadiene, C_5H_8), a biological carbon backbone commonly observed in plants,[29] biogenic emissions,[30] and insects.[18] Only one B4 C_{19} isomer is observed, though the presence of ethyl or propyl branches would allow for multiple possible B4 C_{19} isomers while maintaining five carbons between branches. This preference for methyl groups and absence of more complex branching patterns would be expected for compounds with an isoprenoid origin, though structural changes could also occur during refining processes. These rules allow fewer isomers as branching increases, supported by the large number of B1 peaks at each carbon number compared to relatively fewer isomers of
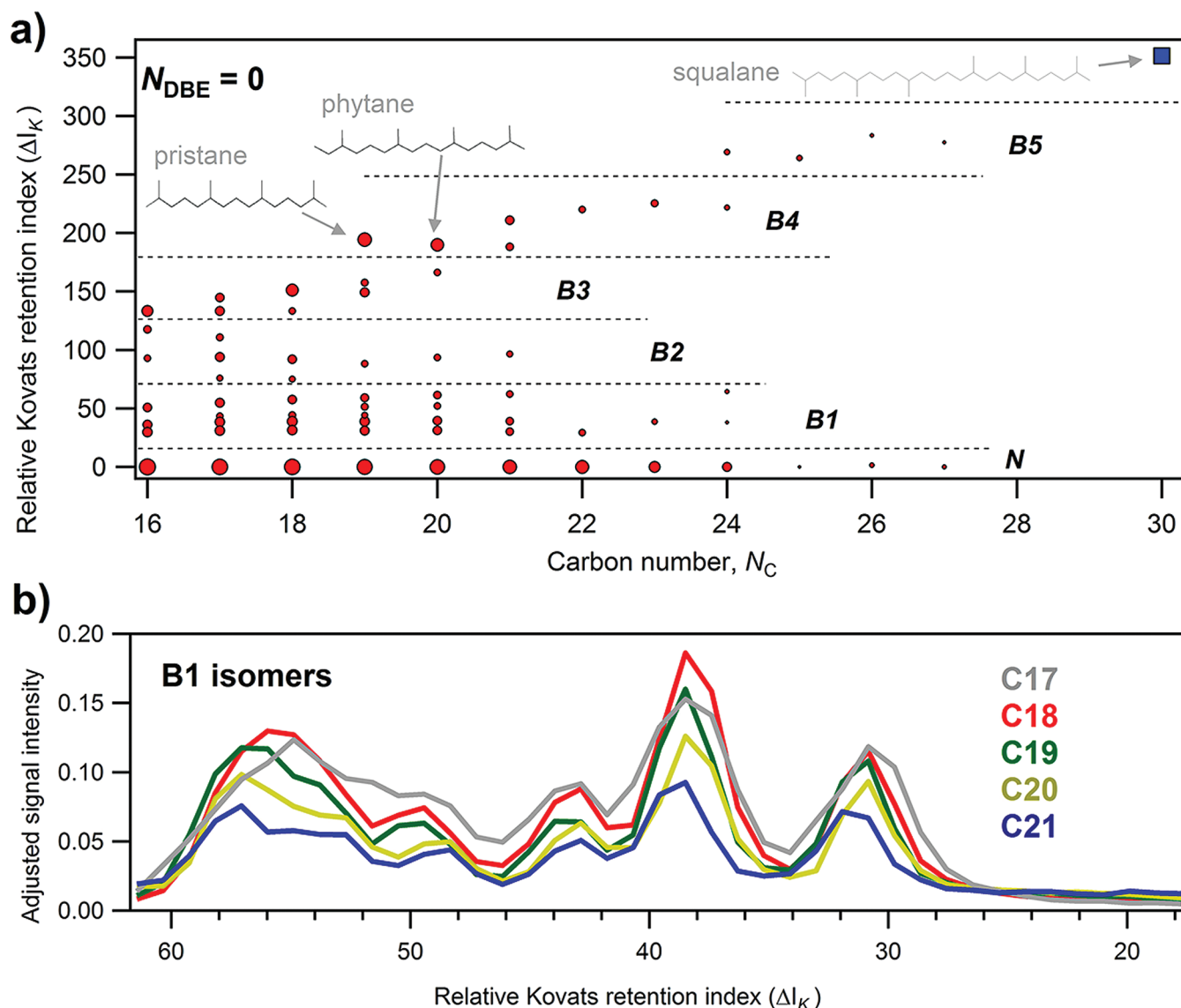
Figure 4. (a) Isomer separation of alkanes demonstrated by relative Kovats retention index, measured as the difference between the Kovats index (I_K) of a given peak and that of the n-alkane (N) of the same carbon number ($\Delta I_K = 0$). Red circles are peaks found from GCxMS analysis of diesel fuel while the blue square represents squalane, a C_{30} hexamethyl alkane (shown). Pristane and phytane, C_{19} and C_{20} tetramethyl alkanes, respectively (shown), are identified by authentic standards. Peaks fall into naturally separated regions shown by dashed lines through the gaps, defining areas of increasing branching from methyl (B1) through C_5-branching (B5). Squalane is in a B6 region that is unpopulated by alkanes observed in this diesel sample. Further discussion of these characterizations can be found in Separation of Structural Isomers. (b) Reproducibility of single methyl group isomer (B1) patterns of alkanes ($N_{DBE} = 0$) from C_{17} through C_{21}. Detector response has been adjusted for GC transfer efficiency so all peaks are on equivalent scales, assuming approximately equivalent detector responses and absorption cross sections.

higher branching. In this diesel sample, alkanes typically have 8−10 major isomers, despite the tens or even hundreds of thousands of isomers that are theoretically possible.[31]

These isomer regions are reproducible between carbon numbers, as demonstrated by the comparison of C_{17} through C_{21} methyl isomers (Figure 4b). Each carbon number consists of four well-resolved methyl isomers and a broad peak centered at $\Delta I_K \approx 56$ that is likely a coelution of at least two isomers. The number of methyl isomers observed is a significant fraction of the number of possible isomers; a C_{18} alkane, for instance, has 8 possible B1 isomers, of which 5 or 6 are observed. The same isomers have similar relative intensities at each carbon number. The similarity of the distribution of B1 isomers across all carbon numbers suggests a process either in formation or in petroleum refining that constrains isomer composition.

Quantification of isomers provides an estimate of the importance of branched alkanes in this diesel fuel sample (Figure 5). B4 isomers are quantified on the basis of pristane and phytane, which have similar response factors but fragment approximately twice as heavily as n-alkanes. This fragmentation calibration factor is unknown for B1 through B3 isomers but is expected to fall between n-alkanes and B4 isomers, providing upper and lower bounds. Figure 5 shows that, as alkane carbon number increases, the total mass fraction of branching increases from approximately 0.4 to 0.6, which may be due to the increase in number of possible isomers. The B4 isomer is largest at the lowest carbon numbers for which it is present, a pattern that appears to apply to B3 and B5 isomers and may be related to being polymers of isoprene. Though B5 isomers are a large fraction of C_{24} through C_{26} alkanes, these low-volatility
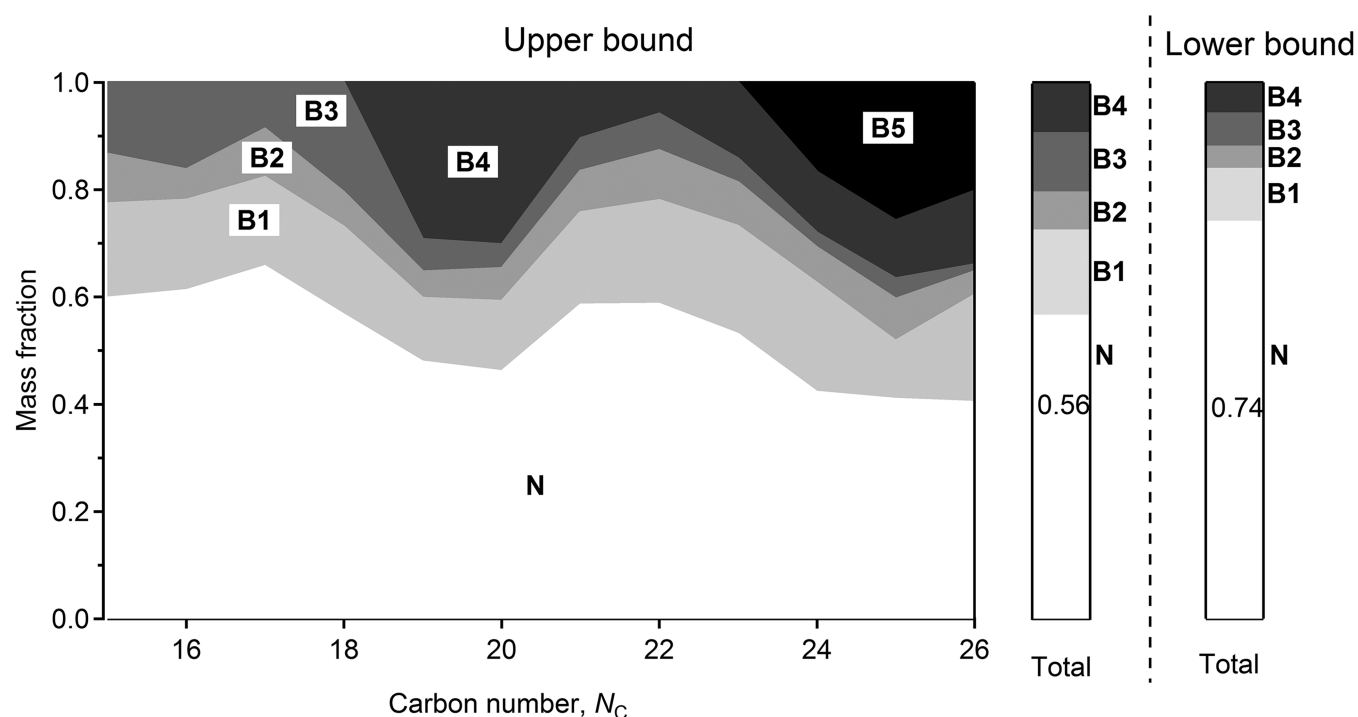
Figure 5. Mass fraction of each alkane ($N_{DBE} = 0$) carbon number composed of branched isomers of type B1 (methyl), B2 (C_2-branched, e.g., dimethyl),...B5 (C_5-branched, e.g., pentamethyl). Branched isomers have been normalized to *n*-alkanes (N) by f_P. Upper bound calibration assumes f_P for B1 through B3 is similar to B4 and B5 (half that of the *n*-alkane), while lower bound calibration assumes f_P for B1 through B3 is similar to that of the *n*-alkane. Total fraction on the right is the fraction at each carbon number weighted by the distribution of alkanes (Figure 2), showing branched isomers account for between 26 and 44% of total alkane mass.

alkanes are a small fraction of total observed alkane mass. In total, branched isomers account for 26 to 44% of alkane mass in this diesel fuel sample. Methyl isomers, which are difficult to resolve by GC/EI-MS and therefore not often quantified, are the largest fraction, comprising 10 to 16% of all alkane mass. B4 isomers are a significant fraction of alkanes (5 to 9%), but quantifying only pristane, phytane, and a few other prevalent branched alkanes underestimates branching in diesel fuel by approximately half. Furthermore, understanding not just the mass of branched isomers but also identifying and classifying them by N_C extends the utility of this analysis into understanding compound-specific reactions and interactions.

Branched isomers of higher aliphatic DBE classes are also uniquely resolvable using GC/VUV-MS. Cycloalkanes ($N_{DBE} = 1$) are shown in Figure 1 to be composed of 10 to 12 different isomers. Quantitative analysis shows these isomers to account for approximately half of the mass of cycloalkanes, though isomer distribution patterns are not as reproducible or clear as those of alkanes. Analysis of complex mixtures by GC/EI-MS would therefore be likely to underestimate the importance of branching for all aliphatic hydrocarbons. Furthermore, GC/VUV-MS allows the branching of previously unknown compounds to be estimated from ΔI_K, further elucidating structure. The utility of the classification scheme developed in this work can be demonstrated on the unknown peak labeled "C" in Figure 1 that is unidentified by GC/EI-MS and lies in the middle of the "unresolved complex mixture". From carbon number separation and DBE class, this compound is expected to be a C_{18} cycloalkane, while its shift in t_R suggests a single methyl branch; the structure of an otherwise unresolved compound can therefore be determined.

■ CONCLUSIONS

Soft ionization coupled to chromatographic separation allows characterization of compounds based on structural characteristics and mass. GCxMS analysis of complex mixtures is used here to classify unknown compounds by carbon number (N_C), number of rings and double bonds (N_{DBE}), and degree of branching based on retention time shifts (ΔI_K). Classification by these parameters provides structural information about unknown compounds in environmentally relevant hydrocarbon mixtures, allowing identification of aliphatic hydrocarbons that cannot be differentiated using traditional MS techniques. The characterization scheme described in this work therefore provides significantly improved resolution of the "unresolved complex mixture" often present in GC analysis of environmental samples. The structure of any unknown compound in a complex mixture can be well-constrained by N_C, N_{DBE}, and ΔI_K, allowing estimation of reactivity, vapor pressure, and other physical properties that can be useful across a variety of scientific fields. Demonstration of these classification parameters on diesel fuel highlights the importance of isomer analysis. Quantitative analysis of branching using ΔI_K is useful in estimating the prevalence of branched isomers in environmental samples and is expected to provide significant insight into the formation and degradation of contaminants. Furthermore, the mass spectra of known compounds compiled in the Supporting Information provides a useful reference for other VUV-MS applications.

Improved resolution and isomer separation capabilities provided by GC/VUV-MS and GCxMS analysis are useful to any scientific fields that routinely characterize complex hydrocarbon mixtures, e.g., atmospheric chemistry, petroleum refining, microbial and chemical ecology, and bioremediation.

Due to differences in chemical decomposition pathways of constitutional isomers, characterization of hydrocarbon branching has potential applications to the modeling of petroleum degradation in atmospheric and biological systems. Furthermore, some biological systems have been shown to be sensitive to isomer composition and branching.[22,23] The ability to quantitatively separate isomers and constrain the structure of unknown compounds in complex hydrocarbon mixtures will be useful in the analysis of a variety of samples from wide-ranging disciplines.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Megharaj, M.; Ramakrishnan, B.; Venkateswarlu, K.; Sethunathan, N.; Naidu, R. *Environ. Int.* **2011**, *37*, 1362−1375.

(2) Simoneit, B. R. *Mass Spectrom. Rev.* **2005**, *24*, 719−765.

(3) Fraser, M. P.; Lakshmanan, K. *Environ. Sci. Technol.* **2000**, *34*, 4560−4564.

(4) Mazurek, M. A. *Environ. Health Perspect.* **2002**, *110*, 995−1003.

(5) Schauer, J. J.; Rogge, W. F.; Hildemann, L. M.; Mazurek, M. A.; Cass, G. R. *Atmos. Environ.* **1996**, *30*, 3837−3855.

(6) Williams, B. J.; Goldstein, A. H.; Kreisberg, N. M.; Hering, S. V.; Worsnop, D. R.; Ulbrich, I. M.; Docherty, K. S.; Jimenez, J. L. *Atmos. Chem. Phys.* **2010**, *10*, 11577−11603.

(7) Eschner, M. S.; Welthagen, W.; Gröger, T. M.; Gonin, M.; Fuhrer, K.; Zimmermann, R. *Anal. Bioanal. Chem.* **2010**, *398*, 1435−1445.

(8) Hanley, L.; Zimmermann, R. *Anal. Chem.* **2009**, 4174−4182.

(9) Sieck, L. W. *Anal. Chem.* **1983**, *55*, 38−41.

(10) Adam, T.; Zimmermann, R. *Anal. Bioanal. Chem.* **2007**, *389*, 1941−1951.

(11) Zimmermann, R.; Mühlberger, F.; Fuhrer, K.; Gonin, M.; Welthagen, W. *J. Mater. Cycles Waste Manag.* **2008**, *10*, 24−31.

(12) Mysak, E. R.; Wilson, K. R.; Jimenez-Cruz, M.; Ahmed, M.; Baer, T. *Anal. Chem.* **2005**, *77*, 5953−5960.

(13) Schnelle-Kreis, J.; Welthagen, W.; Sklorz, M.; Zimmermann, R. *J. Sep. Sci.* **2005**, *28*, 1648−1657.

(14) Hamilton, J. F. *J. Chromatogr. Sci.* **2010**, *48*, 274−82.

(15) Worton, D. R.; Kreisberg, N. M.; Isaacman, G.; Teng, A. P.; McNeish, C.; Górecki, T.; Hering, S. V; Goldstein, A. H. *Aerosol. Sci. Technol.* **2012**, *46*, 380−393.

(16) Geissler, R.; Saraji-bozorgzad, M. R.; Gro, T.; Fendt, A.; Streibel, T.; Sklorz, M.; Krooss, B. M.; Fuhrer, K.; Gonin, M.; Kaisersberger, E.; Denner, T.; Zimmermann, R. *Anal. Chem.* **2009**, *81*, 6038−6048.

(17) Mitschke, S.; Welthagen, W.; Zimmermann, R. *Anal. Chem.* **2006**, *78*, 6364−6375.

(18) Schauer, J. J.; Kleeman, M. J.; Cass, G. R.; Simoneit, B. R. *Environ. Sci. Technol.* **1999**, *33*, 1578−1587.

(19) Kroll, J. H.; Seinfeld, J. H. *Atmos. Environ.* **2008**, *42*, 3593−3624.

(20) Lim, Y. B.; Ziemann, P. J. *Environ. Sci. Technol.* **2009**, *43*, 2328−2334.

(21) Blomquist, G. J., Bagneres, A., Eds. *Insect Hydrocarbons*; Cambridge University Press: Cambridge, UK, 2010.

(22) Martin, S. J.; Helanterä, H.; Drijfhout, F. *Proc. R. Soc. B* **2011**, *278*, 496−503.

(23) van Wilgenburg, E.; Sulc, R.; Shea, K. J.; Tsutsui, N. D. *J. Chem. Ecol.* **2010**, *36*, 751−788.

(24) Youngsteadt, E.; Guerra Bustios, P.; Schal, C. *PloS One* **2010**, *5*, e15822.

(25) Heimann, P. A.; Koike, M.; Hsu, C. W.; Blank, D.; Yang, X. M.; Suits, A. G.; Lee, Y. T.; Evans, M.; Ng, C. Y.; Flaim, C.; Padmore, H. A. *Rev. Sci. Instrum.* **1997**, *68*, 1945−1951.

(26) Gloaguen, E.; Mysak, E.; Leone, S.; Ahmed, M.; Wilson, K. *Int. J. Mass Spectrom.* **2006**, *258*, 74−85.

(27) NIST Mass Spec Data Center, S.E. Stein, director. Mass Spectra. In *NIST Chemistry WebBook, NIST Standard Reference Database Number 69*; Linstrom, P. J., Mallard, W. G., Eds.; National Institute of Standards and Technology: Gaithersburg, MD, 2011.

(28) Kovats, E. *Helv. Chim. Acta* **1958**, *41*, 1915−1932.

(29) Langenheim, J. H. *J. Chem. Ecol.* **1994**, *20*, 1223−1280.

(30) Guenther, A.; Hewitt, C. N.; Erickson, D.; Fall, R.; Geron, C.; Graedel, T.; Harley, P.; Klinger, L.; Lerdau, M.; Mckay, W. A.; Pierce, T.; Scholes, B.; Steinbrecher, R.; Tallamraju, R.; Taylor, J.; Zimmerman, P. *J. Geophys. Res.* **1995**, *100*, 8873−8892.

(31) Goldstein, A. H.; Galbally, I. *Environ. Sci. Technol.* **2007**, *41*, 1514−1521.