

Class separation of the trace polar species present in diesel using hydrophilic interaction chromatography and high resolution mass spectrometry

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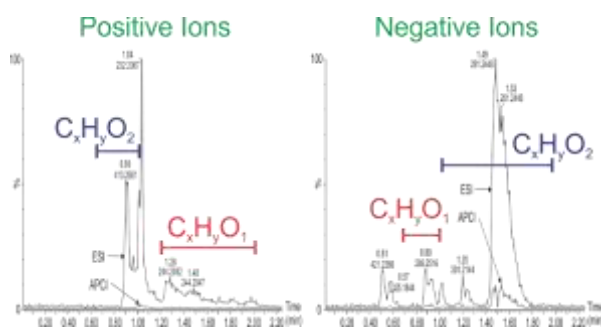
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Abstract

The analysis of fuels is not straightforward, due to its complex nature, and many different techniques have been developed to address this complexity. This paper reports on the use of hydrophilic interaction liquid chromatography (HILIC) as a complementary and green method for classifying diesel fuel composition. For comparative reasons, analyses with and without chromatographic pre-separation were performed. Selective ionization of polar species was achieved with electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). High resolution time of flight mass spectrometry (TOFMS) was used to summarize the fuel composition in terms of compound classes based on accurate mass based elemental compositions. These compound classes showed different retention characteristics and were separated to a certain extent under hydrophilic interaction conditions. In normal phase liquid chromatography, the separation is based on heteroatom content, whereas alkylation (chain length) and degree of unsaturation (or double bond equivalents, DBE) play an important role in reversed phase liquid chromatography. The separation achieved with the proposed HILIC method depended on heteroatom content, DBE as well as hydrogen bonding, whilst alkylation did not influence retention significantly. This work therefore presents an alternative and complementary method for fuel analysis, compared to traditional reverse and normal phase liquid chromatography, by considering specific advantages and limitations.

Introduction

Due to the complex nature of crude-derived fuels, its chemical composition is often investigated by considering classes of compounds rather than individual chemical species. In terms of middle-distillate fuels, such as diesel and jet fuel (kerosene), the most important compound classes are saturated alkanes (also referred to as paraffins and isoparaffins), unsaturated alkanes (also referred to as olefins), aromatic species and heteroatom containing species (e.g. O/N/S-containing compounds). The non-polar species (i.e. alkanes) make up the bulk of the fuel (major constituents), whilst polar species (O/N/S-containing compounds) are present at very low levels (< 0.1% by mass).¹ Nonetheless, the characterization of these polar species is important due to their influence on the fuel's physical properties, and this has given rise to the field of petroleomics.^{2,3} For commercial fuels, additives are often required to improve specific physical properties (e.g. cold flow, lubricity etc.), and it is important to note that many of these additives contain oxygen, nitrogen and sulfur.⁴ Additive levels may vary significantly depending on the type of additive, quality of the unadditized fuel as well as region and/or season where the fuel will be sold commercially. It is however possible to differentiate between additives and species that are part of the inherent fuel composition. Species that occur in the fuel naturally will most likely be part of a group of compounds with similar functionality (i.e. chemical functional group) and various chain lengths, whereas additives will be present as single components (or a limited mixture of components).

Since it can be reasonably expected that compounds with similar chemical properties will influence the fuel's physical properties in the same way, the composition of the polar fraction of fuels can also be considered in terms of compound classes. Compound classes can be defined in

terms of heteroatom distribution and double bond equivalents (DBE) when using high resolution mass spectrometry, e.g. Fourier Transform Ion Cyclotron Resonance (FT-ICRMS)³ and time of flight mass spectrometry (TOFMS).⁵ Compound classes can also be defined in terms of chemical structure and functional groups (e.g. alcohols vs. ethers or alkenes vs. cyclic alkanes), when using chromatographic techniques, e.g. comprehensive two-dimensional gas chromatography (GC×GC-TOFMS)^{6,7} and high performance liquid chromatography (HPLC),⁸ to separate and/or fractionate crude-derived fuels.

Literature indicates that reverse phase HPLC has been used for the analysis of diesel,⁹ jet fuel¹⁰ and crude-oil,¹¹ but group-type separations are most often achieved with normal phase liquid chromatography.^{8,12} Standard methods, i.e. IP 391, EN-12916 and ASTM D 6591, are available for the group-type separation (based on the number of aromatic rings) of diesel with normal phase HPLC combined with refractive index detection.^{8,13} Polar silica, aminosilane or dinitroanilinopropyl-based stationary phases are employed, together with non-polar solvents (e.g. dichloromethane and hexane) as the mobile phase. The mobile phases employed in normal phase HPLC present two important disadvantages: (i) in terms of health, safety and environmental impact, these chemicals are classified as “hazardous”,¹⁴ and (ii) ionization suppression is a common phenomenon when normal phase HPLC is hyphenated with electrospray ionization (ESI-MS) and several strategies have been developed to deal with this challenge.¹⁵

Hydrophilic interaction liquid chromatography (HILIC) is a form of HPLC that is similar to normal phase HPLC in the sense that a polar stationary phase (typically underivatized silica or silica derivatized with polar/ionic groups¹⁶) is utilized, but these methods differ in terms of the solvents used as mobile phase. HILIC is sometimes referred to as reversed-reverse phase LC,¹⁶ and is based on a polar stationary phase which is hydrated, forming an adsorbed liquid layer on

the surface.¹⁷ Analytes partition between the mobile phase (e.g. acetonitrile) and this adsorbed liquid layer, leading to separation based on polarity and/or hydrophilicity. It is important to keep in mind that specific solvent ratios are required in order to achieve hydrophilic interaction conditions, e.g. a minimum amount of water is required at all times to establish the adsorbed liquid layer on the surface of the stationary phase.^{16,17} Sample solvent composition is also a common limitation in HILIC.¹⁸ The retention mechanism of HILIC has been discussed broadly, and is considered to be based on the partitioning of analytes as well as hydrogen bonding.¹⁶

HILIC utilizes “greener” solvents,¹⁹ e.g. methanol, acetonitrile and water, and these solvents are also more compatible with ESI-MS²⁰ compared to the non-polar solvents employed in normal phase HPLC. Literature also suggests that HILIC, in combination with evaporative light scattering detection, is applicable to the analysis of oil-biodiesel mixtures and that problems with reproducibility of normal phase HPLC (for these mixtures) can be overcome.²¹ Recently, HILIC (combined with UV and ESI-MS detection) was used to investigate a metal deactivator additive in jet and diesel fuels.²² This was achieved with an amide column and acidic mobile phase. However, the fuel composition in itself was not analyzed in depth, since the aim was to detect and quantify a specific additive.

The aim of this paper was to develop a chromatographic separation method, based on hydrophilic interactions, for the analysis of polar compound classes in diesel fuel extracts, as a complementary and greener method compared to traditional normal phase HPLC. Sensitive and selective atmospheric ionization techniques that do not produce ions from the bulk alkanes in the fuel²³ were utilized, i.e. electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) in a combined ion source (ESCI), allowing quick analysis without excessive sample clean-up or pre-concentration. By performing analyses with and without chromatographic pre-separation, the

effect of chromatographic separation on matrix effects and ionization suppression (due to the presence of a more/less complex mixture in the ion source) could be evaluated. High resolution time of flight mass spectrometry (TOFMS) was used to summarize the fuel composition in terms of compound classes based on accurate mass based elemental compositions, as is commonly done with data obtained from high resolution FT-ICRMS analysis of crude oil and crude-derived fuels.³

Experimental setup

Sample preparation. Four reference fuels were obtained from Haltermann GmbH (Germany) and Petrochem Carless (Belgium). These fuels were produced by blending refining streams to comply with the standard specifications of Europe (EN590), Sweden (Swedish MK1), USA (US 2-D) and California (California), respectively. Some properties of the reference fuels (as obtained from suppliers) are provided in Table S1 (see Supporting Information). Note that the Swedish diesel contained fatty acid methyl esters (FAMES) at low levels (< 0.1 %), although this is significantly lower than regular biodiesel blending levels (up to 7 %).²⁴ Nine commercial diesels were bought from fuel stations in and around the Pretoria area in South Africa, i.e. from different suppliers in the same region. These commercial diesels will be referred to as commercial diesel X (CDX), where $1 \leq X \leq 9$. Two different diesel fuel grades were available in the trade at this time, referred to as 50 ppm diesel or 500 ppm diesel (containing a maximum of 50 mg/kg or 500 mg/kg Sulphur, respectively). All the commercial diesels were 50 ppm fuels except for CD8 and CD9 which were 500 ppm fuels. Solvents used were methanol, acetonitrile and water (Romil-UpSTM Ultra Gradient for HPLC-MS, > 99.9 %). Note that diesel containing biodiesel was not yet mandated in South Africa when the commercial diesel samples were collected.

The polar fraction of each of the diesel fuels was extracted by mixing 1 ml of fuel with 2 ml of methanol. The mixture was shaken vigorously and then centrifuged for 10 minutes at ± 2500 g in order to facilitate phase separation. The methanol phase (top layer) was removed and diluted 1:3 with acetonitrile. These *fuel extracts* were analyzed by ESCI-TOFMS as well as HPLC-ESCI-TOFMS.

Direct Mass Spectrometric Analysis. A Synapt G2 HDMS system (Waters Inc., Milford, Massachusetts, USA) was used to analyze the samples. MassLynxTM (version 4.1) software (Waters Inc., Milford, Massachusetts, USA) was used to acquire and process data. Selective ionization of polar species was achieved with a combined ion source (ESCI),²⁵ which allows for quasi-simultaneous analysis alternating between electrospray (ESI) and atmospheric pressure chemical ionization (APCI). The fuel extracts were infused at a flow rate of 5 μ L/min. Capillary voltages of +4 kV and -2.5 kV were used to collect positive and negative ions, respectively. The sampling cone was set to 40 V whilst the extraction cone was set to 5 V. The source temperature was 120 °C and the desolvation temperature was 300 °C. A current of 20 μ A was applied to the corona needle during APCI experiments.

Mass to charge ratios (m/z) between 50 and 1200 Da were recorded although only peaks with m/z values below 600 Da were considered during data processing. The ESI and APCI data was acquired in separate functions and processed separately. The raw data was collected in the form of a continuous profile by combining 200 primary mass spectral acquisitions (scans). An automatic peak detection algorithm within the software was used to integrate mass spectral peaks and obtain data where the area of each peak is represented by its height. Automated background subtraction was applied prior to integration. An external calibration of the m/z scale was performed using

sodium formate (mass range 112.936 – 1132.688 Da). A 200 pg/ μ L solution of leucine enkephalin (m/z 555.2693) was infused into the ion source throughout the analysis and internal calibration was performed automatically by the software based on the measured and exact mass of this peptide. Resolution of 20 000 at m/z 200 (FWHM) was obtained.

Elemental compositions were assigned to mass spectral peaks with an absolute intensity above 3000 and 300 for ESI and APCI, respectively. These arbitrary values were chosen based on close inspection of noise levels in the raw mass spectra. Both odd and even electron configurations were allowed, with a double bond equivalent (DBE) range of -1.5 to 30 and mass accuracy was specified to be within 5 ppm. The following limits were applied to the range of atoms allowed: C: 0 – 50, H: 0 – 100, N: 0 – 2, O: 0 – 5 and S: 0 – 5. For positive ion mass spectra, Na atoms (0 – 1) were also included to account for the formation of adducts.

Chromatographic Separation and Mass Spectrometric Analysis. A Waters Acquity UPLC® system was used to perform hydrophilic interaction liquid chromatography (HILIC). Initially a mixture of equal amounts of all fuel extracts were analyzed for method development, followed by the analysis of individual fuel extracts. An XBridge BEH HILIC XP column (2.5 μ m \times 2.1 mm \times 100 mm) was used together with an in-line pre-column filter (Phenomenex, KrudKatcher, 0.5 μ m depth filter \times 0.004 inch ID).

During initial experiments, several parameters were varied to determine their influence on the chromatographic separation. The mobile phase composition had the most significant effect on analyte retention. As the amount of acetonitrile increased, analytes were more retained. To maintain HILIC conditions, a minimum of 3 % water had to be present in the mobile phase. Therefore, an isocratic mobile phase of 97:3 acetonitrile:water containing 10 mM ammonium

acetate (Merck, univAR, 97 %) as buffer was used with a flow rate of 0.4 mL/min. The column temperature was kept constant at 25 °C and the injection volume was 5 µL. All the (ionizable) compounds eluted within 3 minutes.

The same parameters were used for ESCI-TOFMS as discussed above. Mass spectral scans (primary acquisitions) were collected every 0.2 seconds. Elemental compositions were assigned to mass spectral peaks with an absolute intensity above 300 for both ionization methods and mass accuracy was specified to be within 10 ppm. The other parameters used for elemental composition assignment were the same as discussed previously.

To evaluate the separation achieved by employing HILIC, two different approaches were followed. Firstly, mass spectral scans were combined in order to obtain combined mass spectral scans for five retention windows for each of the chromatograms, i.e. for each window 30 and 40 scans were combined into a single spectrum for the positive and negative ion modes, respectively. Ideally individual chromatographic peaks or mass spectral scans at specific retention times should be considered, but this was not possible due to low signal intensities (this will be discussed in depth later). Secondly, extracted ion chromatograms of selected species that represent prominent ion classes were investigated.

To investigate the effect of chromatographic pre-separation on the observable (ionizable) species, mass spectral scans collected throughout the chromatographic run (where sample peaks eluted) were combined to obtain a single mass spectrum and this was compared to results obtained from direct mass spectrometric analysis (ESCI-TOFMS). In this case the separated chromatographic peaks were not considered individually, i.e. chromatographic resolution was not taken into account. The positive and negative ion mass spectra were collected in separate chromatographic runs (employing the same separation conditions) and consisted of a combination

of 150 and 200 mass spectral scans, respectively. The combination of mass spectral scans was done specifically to obtain data that is directly comparable to the results obtained from analyses without chromatographic separation (i.e. direct infusion into the ion source). The ionization efficiency of an analyte is determined by its chemical nature as well as the presence of other (ionizable) compounds, i.e. matrix effects.³ Therefore, by comparing (combined) mass spectral data obtained with and without chromatographic separation, the effect of the chromatographic separation on ionization efficiency can be evaluated. In principle, if the chromatographic separation had no effect on the observable (ionizable) species, similar results would be obtained when chromatographic separation was not considered, i.e. by combining all the mass spectral scans.

Data processing. The MassLynx™ software (version 4.1) was used to assign elemental compositions to mass spectral peaks, obtained from the various ionization modes and analyses with and without chromatographic separation, as discussed above. Each set of mass spectral peaks represent a combination of mass spectral scans for the entire analysis or for specific retention windows. The resulting data sets were treated in the same way for all samples and techniques utilized (although the way in which the data sets were obtained differed depending on the technique as discussed above, e.g. different intensity thresholds were used). The observed mass spectral peaks represented quasi-molecular ions for both positive and negative ion modes, i.e. $[M+H^+]$ and $[M-H^-]$, respectively. This resulted in non-integer DBE values where $DBE = C - (H - N - 2)/2$. The mass spectral data (peak lists) were processed using a previously described method⁵ that is based on the use of Kendrick mass defects (KMD)²⁶ and nominal mass series values (z^*).²⁷ Briefly, the observed ions were grouped together into clusters of species with nominal masses differing

with exactly 14 Da. For each of these clusters, several homologous series of ions differing from one another with a CH₂ unit was present and Kendrick mass defects were used to distinguish between these homologous series. The preceding clustering based on the nominal mass series values ($z^* = \text{modulus}(\text{Nominal Kendrick Mass}/14) - 14$) allowed for a larger variation in KMD to be acceptable; peaks with KMD values within 1 mDa were considered to be part of the same homologous series, i.e. ions differing from one another with a CH₂ unit (also referred to as an ion subclass). A general formula obtained from the accurate mass based elemental compositions and corresponding DBE values (e.g. 2.5CHO₂ represents species containing two O-atoms with a DBE of 2.5) was assigned to each subclass, based on the most frequent general formula assigned to each of the peaks in the subclass. To a certain extent, this process allowed for the elimination of incorrect assignments (especially at higher masses). The intensity of each ion subclass was determined as the sum of intensities of the peaks assigned to that subclass. The species were then further grouped together into ion classes based on heteroatom content, e.g. CHO₂ represents species containing two O-atoms. The intensity of each ion class was determined as the sum of intensities of the peaks assigned to that class. The relative intensity of each ion class was determined as a percentage of the total intensity of all ion classes.

Molecular masses (together with KMD and DBE values) of hydrocarbons and heteroatomic species commonly found in crude-oil have been reported.²⁸ Similar species, with limited chain lengths and heteroatom complexity, can be expected to be present in diesel since it is a distillation fraction of crude oil. Therefore, the species reported in literature²⁸ were used as an indication of the identity of observed species in the diesel fuel extracts studied here.

During chromatographic separations, mass spectral scans were collected at regular intervals. For each of these recorded mass spectral scans, the absolute intensity of the base peak (i.e. the most

intense peak in the mass spectrum) was used to construct the chromatogram, as an alternative to the traditional total ion chromatogram, i.e. TIC (often used in GC). These chromatograms are referred to as base peak ion chromatograms and are presented with a y-axis indicating relative intensity (0 – 100%), where the absolute intensity of the most intense base peak (i.e. at 100 %) is given in the top right-hand corner of the chromatogram. Additionally, extracted ion chromatograms (EIC) were also considered where the intensity of a specific m/z value (representing selected quasi-molecular ions) was used to construct the chromatogram.

Results and Discussion

ESI- vs. APCI-TOFMS. To establish which species in the fuel extracts are observable (ionizable) with ESCI-TOFMS, an in-depth discussion of results obtained without chromatographic separation follows, with comparisons between results obtained with ESI and APCI as well as positive and negative ion mode.

In general, the positive ion mass spectra were much more complex than the negative ion mass spectra, reflecting the sample composition in terms of species that readily form positive or negative ions (see Figure S1 in Supporting Information). The most prominent ion classes were CHN_1 and CHO_2 in positive and negative ion mode, respectively, which corresponds to previously reported results obtained with ESI-FT-ICRMS.⁵ Interestingly, similar ion classes were observed with ESI and APCI but in general more peaks were observed during ESI-TOFMS (absolute intensities of signals differed with approximately two orders of magnitude). The relative intensities of selected ion classes were determined for each of the diesel samples (see Figure 1). These results confirm that similar species were ionized with ESI and APCI albeit to a different extent.

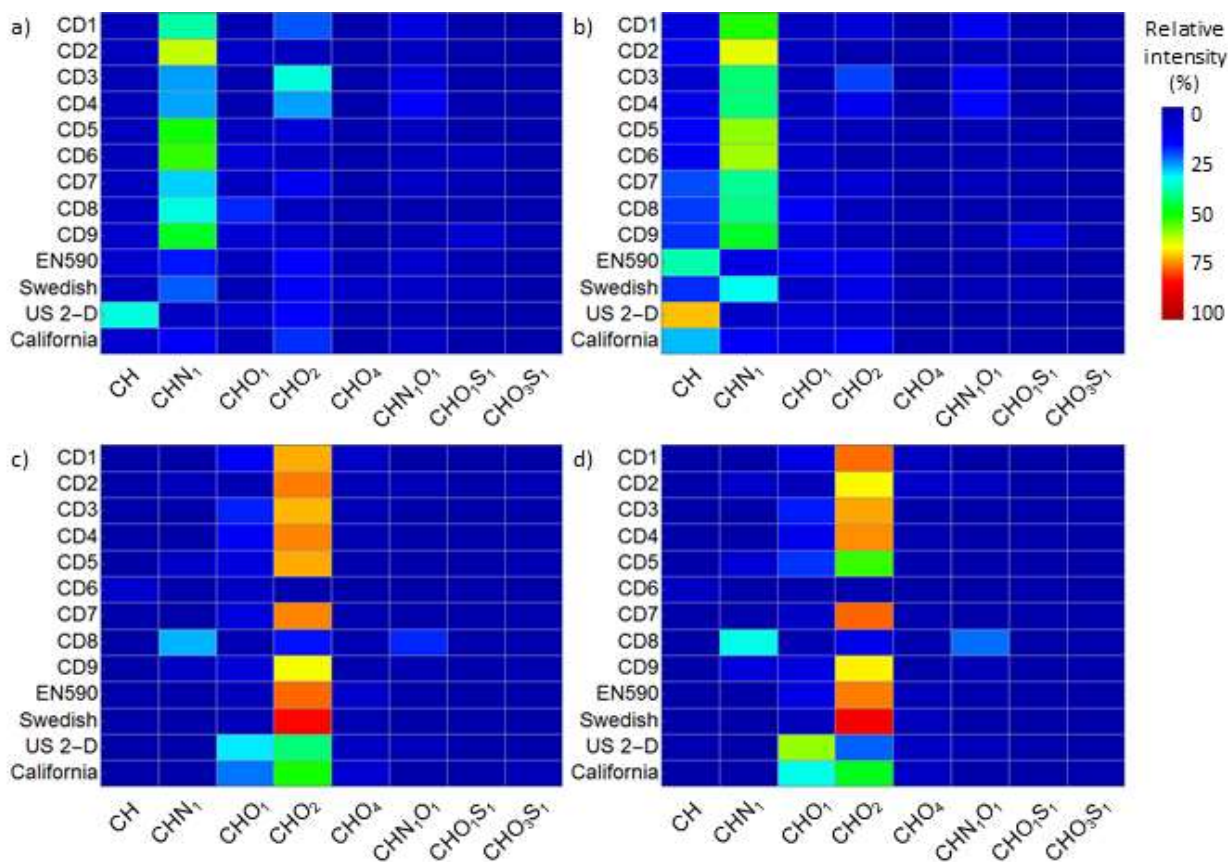


Figure 1. Comparison of relative intensities of selected ion classes obtained for methanol extracts of the reference and commercial diesels. Results based on positive ion mass spectra detected by ESI-TOFMS (a) and APCI-TOFMS (b) as well as negative ion mass spectra detected by ESI-TOFMS (c) and APCI-TOFMS (d) are shown.

The most abundant ion class in the positive ion mass spectra was CHN₁ for most of the commercial diesels (Figure 1 a and b), reflecting the ease of protonation of the N lone pair. The reference diesels contained relatively smaller amounts of these ions. CD3 and CD4 contained relatively large amounts of CHO₂ and closer inspection of results revealed that these classes consist of many ions with varying DBE values. It is possible that these species represent diols or esters, since carboxylic acids would form negative ions more readily. Hydrocarbons with no heteroatoms

(CH class, reflecting aromatic compounds as discussed below) were ionized more readily during APCI compared to ESI. The reference diesels contained relatively higher amounts of the CH class compared to the commercial diesels. Note that the CHN_1O_1 and CHO_1S_1 classes may represent oxidation products of N- and S-containing species, respectively, formed due to biodegradation during fuel storage.²⁹⁻³¹

The relative intensities of the ion classes detected in the negative ion mass spectra echo the similarity between ESI and APCI (Figure 1 c and d). The major difference for these ionization techniques is that the CHO_1 class was slightly more pronounced for APCI. The most abundant compound class for most of the samples was CHO_2 , consisting mainly of a base peak with m/z value of either 281 or 279 which represent quasi-molecular ions of $\text{C}_{18}\text{H}_{34}\text{O}_2$ and $\text{C}_{18}\text{H}_{32}\text{O}_2$, respectively (based on observed accurate m/z values). These species may be long chain carboxylic acids such as oleic and linoleic acid, respectively. Long chain carboxylic acids are often used as additives in diesel, such as lubricity improvers and demulsifiers.^{4,32} Salts of carboxylic acids may also be used as corrosion inhibitors.⁴ It is unlikely that these species represent fatty acid methyl esters (FAMES) from biodiesel additives, since the molecular ions would contain an uneven number of carbons. Furthermore, the certificates of analysis of the reference diesels indicated that these fuels did not contain any biodiesel, except for Swedish diesel which contained less than 0.1 % (vol) FAMES, likely responsible for the much higher CHO_2 value from this source.

Based on the results in Figure 1, there were four samples in which the CHO_2 class was not very abundant: US 2-D, California, CD6 and CD8. Closer inspection of individual mass spectra revealed that US 2-D, California and CD8 did not contain large amounts of ions with m/z 279 or 281. The base peak of CD6 was at m/z 279, but the quasi-molecular ion of the infused calibrator leucine enkephalin was not detected during the analysis (the reason for this was not clear) and

therefore internal calibration could not be performed by the software. This resulted in incorrect elemental composition assignments and consequently ion class distributions that do not follow the general trends observed for the other fuel extracts, illustrating the importance of internal calibration for accurate mass measurement. The negative ion mass spectra (for both ESI and APCI) obtained from direct infusion of CD6 will therefore be excluded in further discussions.

ESI-TOFMS: DBE-distributions. The DBE distributions for selected ion classes in CD5 are shown in Figure 2. CD5 was chosen as it is representative of the samples considered in this study. In the positive ion mass spectra (Figure 2 a and b) the CH subclasses are relatively more abundant for APCI compared to ESI (see also Figure 1), with the most intense subclass having a DBE value of 5.5, most likely representing quasi-molecular ions of aromatic compounds. This corresponds to the fact that APCI is well known to be more selective towards aromatic species compared to ESI which is more selective towards acidic and/or basic species.³³ The CHN₁ class shows a wide distribution of DBE values with the most intense subclasses having DBE values of 4.5 and 5.5, most likely representing quasi-molecular ions of alkylated indoles and indolines, respectively.²⁸ The DBE distributions of O-containing species differ considerably for the different ionization methods. For ESI (Figure 2 a), the CHO₁ species tend to have very high DBE values, whilst the CHO₂ subclasses tend to have lower DBE values. The CHO₁ subclass with DBE of 8.5 may represent quasi-molecular ions of alkylated dibenzofurans, whilst subclasses with higher DBE values may represent hydroxylated polycyclic aromatic hydrocarbons or other furan derivatives.²⁸ For APCI (Figure 2 b), the CHO₁ subclasses with DBE of 5.5 and 6.5 may represent quasi-molecular ions of alkylated benzofurans and alkylated naphthols, respectively.²⁸

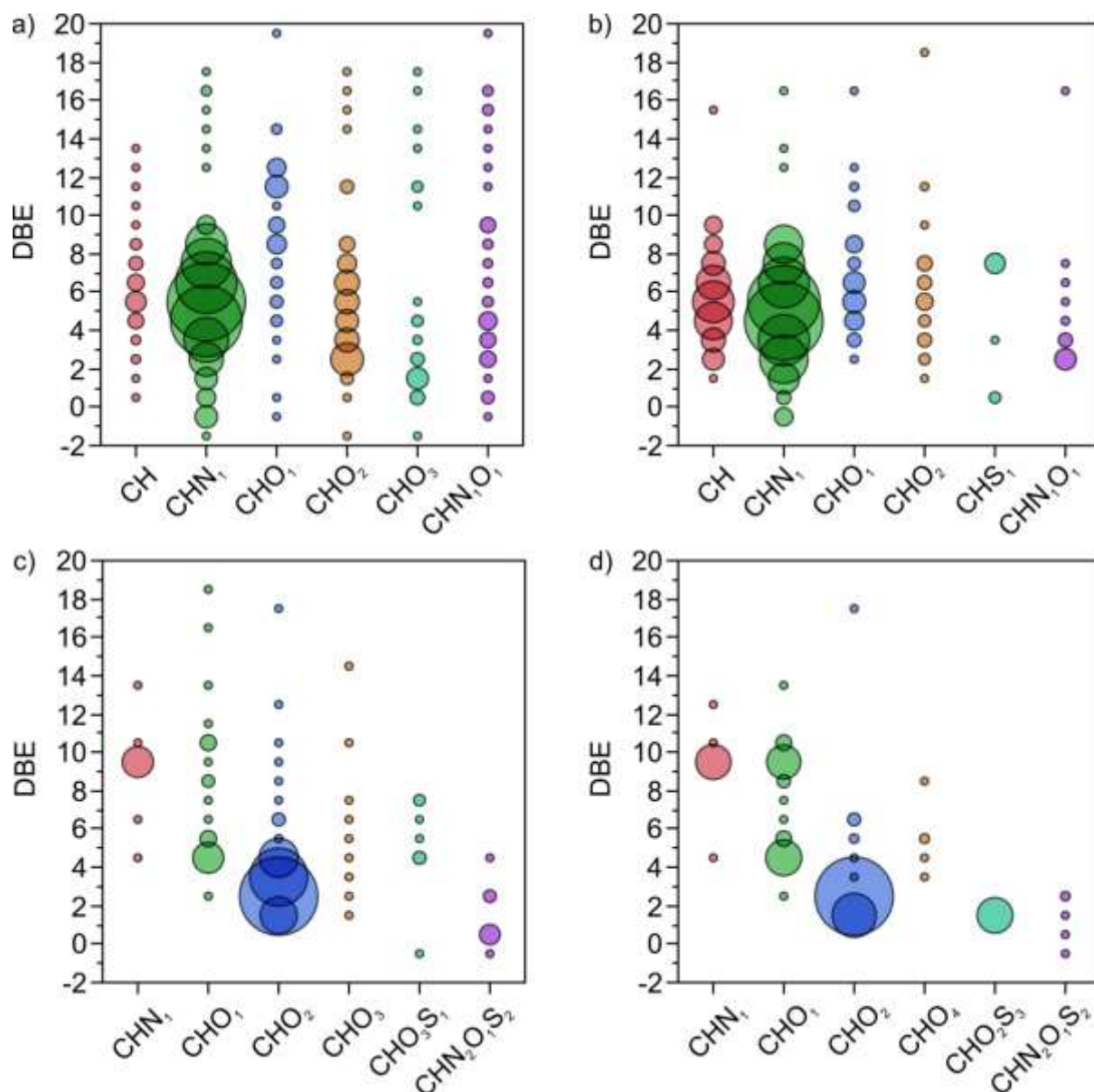


Figure 2. Bubble plots of DBE distributions of some ion classes for CD5 (extracted fuel) analyzed with (a) ESI-TOFMS (positive ions), (b) APCI-TOFMS (positive ions), (c) ESI-TOFMS (negative ions) and (d) APCI-TOFMS (negative ions). Each circle represents a subclass (homologous series), whilst the size of each circle represents its relative abundance.

The DBE distributions of ions observed in the negative ion mass spectra of CD5 are similar for ESI and APCI (Figure 2 c and d). Even though the CHO_2 class was the most abundant for both

ionization methods (Figure 1), this was mainly due to the presence of a single subclass with a DBE of 2.5 corresponding to the base peaks with m/z 281. The most abundant subclass in the CHN_1 class had a DBE of 9.5 and most likely represents quasi-molecular ions of alkylated carbazoles.²⁸ These species have previously been detected in biodegraded oil³⁴ as well as in jet fuel¹⁰ with ESI-FT-ICRMS. The most abundant subclasses observed for the CHO_1 class had DBE values of 4.5 (for both ESI and APCI) and 9.5 (for APCI only). The former most likely represents alkylated phenols,²⁸ whilst there are several structural possibilities for the latter, including alkylated dibenzofurans.²⁸ The most abundant subclasses in the CHO_3S_1 class have DBE values of 4.5 and 7.5, which may represent alkylated benzenesulfonates and naphthalenesulfonates²⁸, respectively. Even though sulfonates are not commonly used as diesel fuel additives, they play an important role in oil recovery processes.³⁵

It is interesting to note that CHN_1 and CHO_2 classes (among others) were observed in both positive and negative ion mass spectra, but their DBE distributions are not necessarily the same. This may indicate that these species are amphiprotic to a certain extent (if similar DBE distributions were observed). In cases where the DBE distributions differ significantly (for positive and negative ion mass spectra) it is possible that compounds with different functional groups were detected. Structural differences may cause compounds with the same general formula to form either positive or negative ions, e.g. carboxylic acids will readily form negative ions whilst esters will more likely form positive ions³⁶ even though both species belong to the CHO_2 class.

HPLC-ESCI-TOFMS: Mixture Analysis. The base peak ion chromatograms showing the positive and negative ion base peaks obtained for the analysis of the mixture of fuel extracts are

shown in Figure 3. The absolute intensity of the base peak (i.e. the most intense peak in each mass spectral acquisition/scan) was used to construct these chromatograms.

Some chromatographic separation of components is evident but a lot of co-elution was observed, which was mainly due to the limited retention of analytes. Chromatographic separations are based on the partitioning of analytes between the mobile phase and stationary phase (or adsorbed liquid layer in terms of HILIC). Different analytes will be more/less retained by the stationary phase (based on this partitioning) and result in different retention times. Analytes that interact strongly with the stationary phase will be more retained (and elute later during the chromatographic run), whilst those that are less retained will elute earlier. If analytes are retained to a limited extent, limited chromatographic separation is achieved, as observed here since all the (ionizable) compounds eluted within 3 minutes. However, the limited partitioning of polar analytes into the adsorbed water layer, may have facilitated the separation into ion classes. If all the polar analytes interact with the stationary phase to a limited extent, the differentiation between polar species with similar chemical structures would be even more limited (or even negligible). If species from the same class or subclass is observed at different retention times, i.e. they were more/less retained, this is an indication of significant structural differences which cannot be detected by mass spectrometry alone. The advantage of employing HILIC is not directly evident from the resulting chromatograms, but closer inspection of the accompanying mass spectral data, reveals interesting characteristics of this method.

Similar chromatographic profiles (relative responses) were obtained for ESI and APCI, but since very low ion intensities were observed for APCI, only the ESI results will be discussed in detail. Due to the extent of co-elution, individual chromatographic peaks were not considered, and specific retention time windows were used instead to evaluate the chromatographic separation, i.e.

fractions of eluting compounds were investigated by combining mass spectra from retention time windows. The relative intensities of selected ion classes observed for each of these chromatographic fractions are shown in the inserts of Figure 3. The last bin represents a combination of all the mass spectral scans that produced ions in the given mass window (150 and 200 acquisitions/scans for positive and negative mode, respectively).

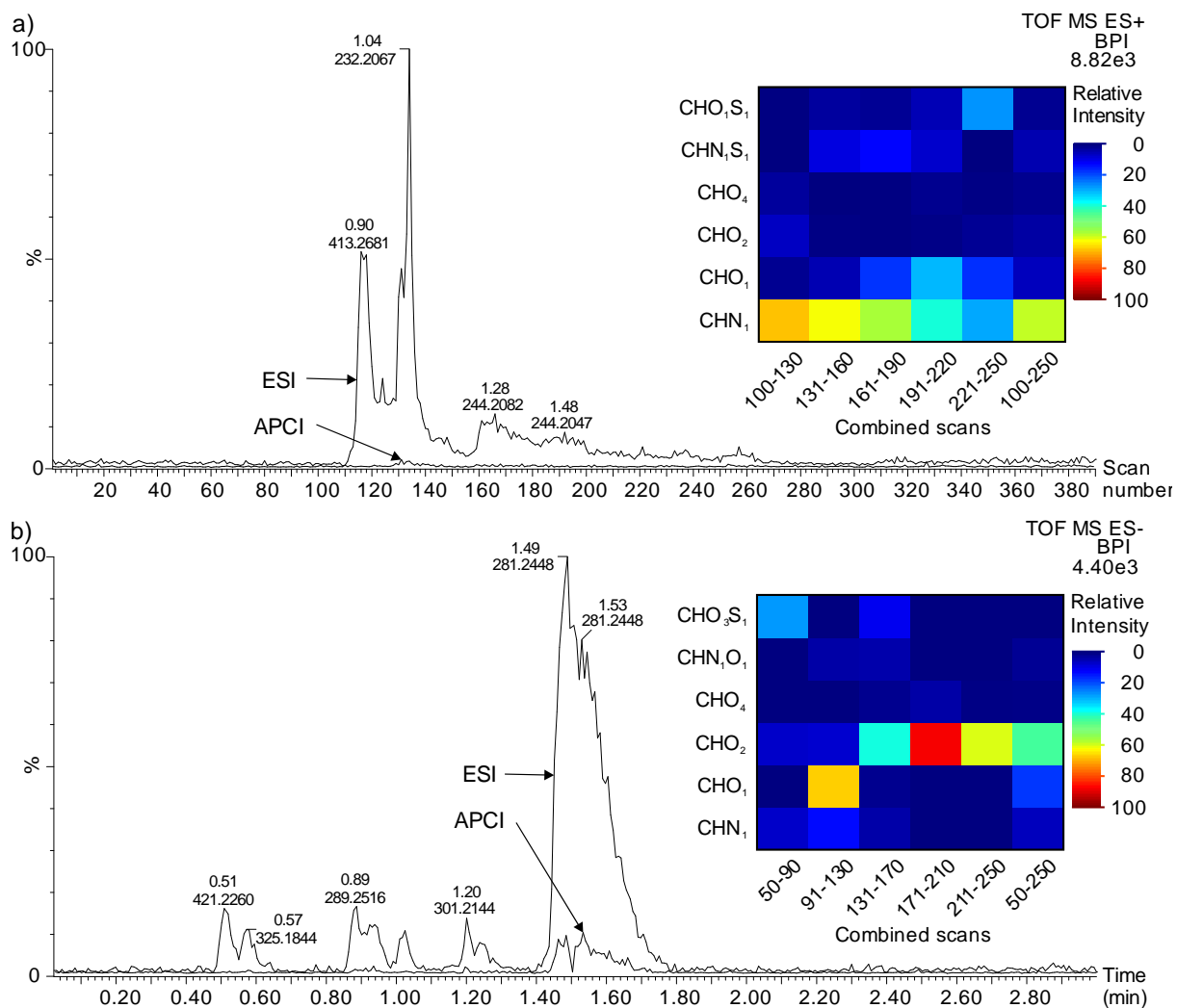


Figure 3. Base peak ion chromatograms showing the separation obtained for a mixture of methanol extracts of 13 diesel samples using hydrophilic interaction liquid chromatography. The positive (a) and negative (b) base peak ions of each mass spectral acquisition/scan are plotted against scan number and retention time, respectively (note that the x-axes are interchangeable for a and b). Signals obtained from ESI-TOFMS and APCI-TOFMS are shown for both positive and negative ion mode. The annotated peaks show the retention time in minutes together with the m/z value

of the most abundant ion to give the base peak of that specific mass spectral acquisition/scan. The inserts show the relative intensity of selected ion classes obtained when mass spectra from retention time windows (fractions) were combined and indicate that class separation was achieved to a certain extent.

For the positive ion mass spectra, the chromatographic fractions (Figure 3 a) are dominated by the CHN_1 class, which correlates with results obtained for the fuel extracts that were analyzed without chromatographic pre-separation. The ions in the CHN_1 class are present in all the chromatographic fractions, indicating that this class may consist of species with different functional groups which experience different retention mechanisms. This corresponds to literature where different N-containing compounds in gas oil (distillation fraction of crude oil with boiling point range of 204 – 524 °C) were separated/fractionated with normal phase HPLC.³⁷

The other (less prominent) classes reveal interesting characteristics of the chromatographic separation. The CHO_2 class was less retained than the CHO_1 class. This corresponds to previous observations that the CHO_1 class consists mostly of ions representing highly unsaturated species, whilst the CHO_2 class most likely consist of ions representing more saturated species, specifically esters. The CHO_1S_1 class was highly retained although ions assigned to CHN_1 and CHO_1 classes were also observed in this fraction. Interestingly, when reversed phase HPLC is employed (for the analysis of crude oil), the CHO_1S_1 class elutes earlier than the CHN_1 and CHO_1 classes.¹¹ The results indicate that separation of species that readily form positive ions (during ESI) occurs based on heteroatom content as well as degree of unsaturation (or DBE).

For the negative ion mass spectra, chromatographic fractions (Figure 3 b) reveal a different retention mechanism for more acidic species (compounds that will preferentially form negative ions during ESI). The first chromatographic fraction corresponds to the first group of co-eluting peaks in the chromatogram. In this fraction, the CHO_3S_1 class was very prominent and a possible

explanation for this may be that these ions are present in the fuel as a sulfonate together with a counter-ion, therefore it may not interact with the polar adsorbed layer on the stationary phase and reaches the ESI source as a preformed anion.

A clear separation between the CHO₁ and CHO₂ classes are observed although the order of elution is reversed from what was observed in the positive ion mass spectra. This suggests that different functional groups are represented by the CHO₁ and CHO₂ ion classes observed in the positive and negative ion mass spectra, indicative of their tendency to accept and donate protons. This supports previous discussions (based on DBE distributions, Figure 2) where the most prominent species in the CHO₁ class were proposed to be alkylated dibenzofurans and alkylated phenols for the positive and negative ion mass spectra, respectively.

In the negative ion mass spectra, the CHN₁ class co-elutes with the CHO₁ class and in previous discussions it was suggested that the most prominent ions in these classes may represent alkylated carbazoles and phenols, respectively. This indicates that the aromatic rings (in the CHO₁ and CHN₁ classes) interact less with the polar adsorbed layer on the stationary phase compared to the (proposed) carboxylic acid group of the CHO₂ class. This may be explained by suggestions in literature¹⁶ that the HILIC retention mechanism is especially influenced by hydrogen-bonding at low water levels (as employed here).

Extracted ion chromatograms of positive quasi-molecular ions having the same heteroatom content (one N-atom) but differing DBE values are shown in Figure 4. Chromatographic peaks can be observed around 0.95 and 1.04 minutes together with a low intensity tailing peak around 1.31 minutes. This confirms that the CHN₁ class consists of ions with different functional groups and/or polarities. For the ions with lower DBE values (more saturated) (Figure 4 a and b) negligible amounts of ions were detected at 0.95 minutes, with most of the ions eluting at 1.04 and 1.02

minutes, respectively. For the ions with higher DBE values (less saturated) (Figure 4 c and d) the first two peaks were not completely resolved, but most of the ions seem to elute around 0.95 minutes.

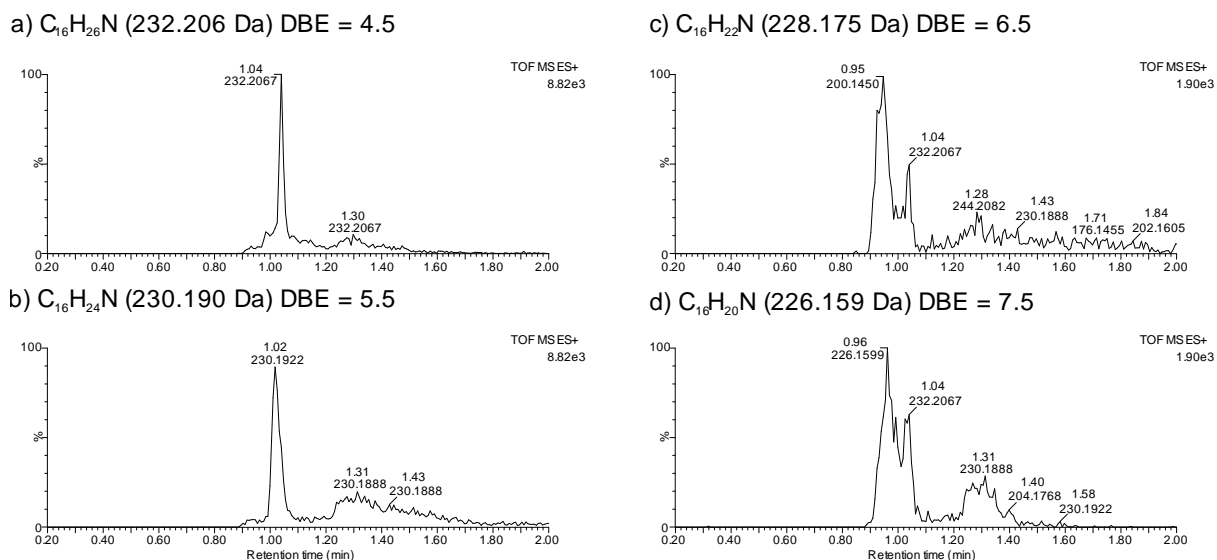


Figure 4. Extracted ion chromatograms of selected positive quasi-molecular ions, $[M+H]^+$, detected by ESI-TOFMS. The most intense ions elute between 0.95 and 1.04 minutes whilst a small amount of ions elute around 1.31 minutes indicating that chromatographic separation of at least two different polarities (functional groups) of CHN_1 class compounds was obtained. Note that for (a and b) a relative intensity of 100 % is equivalent to an absolute intensity of 8.82×10^3 , whilst for (c and d) a relative intensity of 100% is equivalent to an absolute intensity of 1.90×10^3 .

The tailing chromatographic peak eluting at 1.31 minutes most likely represents compounds with a primary amine group, which will more readily partition into and/or interact with the polar adsorbed layer on the stationary phase. The tailing is most likely caused by strong hydrogen bonding interactions between the lone pair on the N-atom and the exposed silanol groups of the stationary phase and/or the water molecules in the adsorbed layer. Secondary and tertiary amines (which include cyclic amines) are more sterically hindered. This is also true for structures where

the N-atom forms part of an aromatic ring, such as pyridines and pyrroles. Therefore, these compounds will not interact as strongly with the adsorbed layer on the stationary phase and be less retained. It is therefore suspected that the peaks eluting at 0.95 and 1.04 originated from a mixture of cyclic amines (e.g. indolines), pyridine or pyrrole derivatives. This corresponds to literature that indicates that separations under HILIC conditions occur based on hydrogen-bonding interactions.¹⁶

The separation of species that readily formed positive ions was influenced by heteroatom content, degree of unsaturation (or DBE) as well as hydrogen bonding. In comparison, the separation achieved with reversed phase HPLC in previous studies depended on DBE and alkylation, whilst heteroatom content did not influence the elution order.¹¹

Extracted ion chromatograms of selected negative quasi-molecular ions are shown in Figure 5. When the base peak ions are considered (Figure 5 a) at least four completely resolved chromatographic peaks can be observed at 0.51, 0.89, 1.20 and 1.49 minutes. Partially resolved shoulder peaks can also be observed.

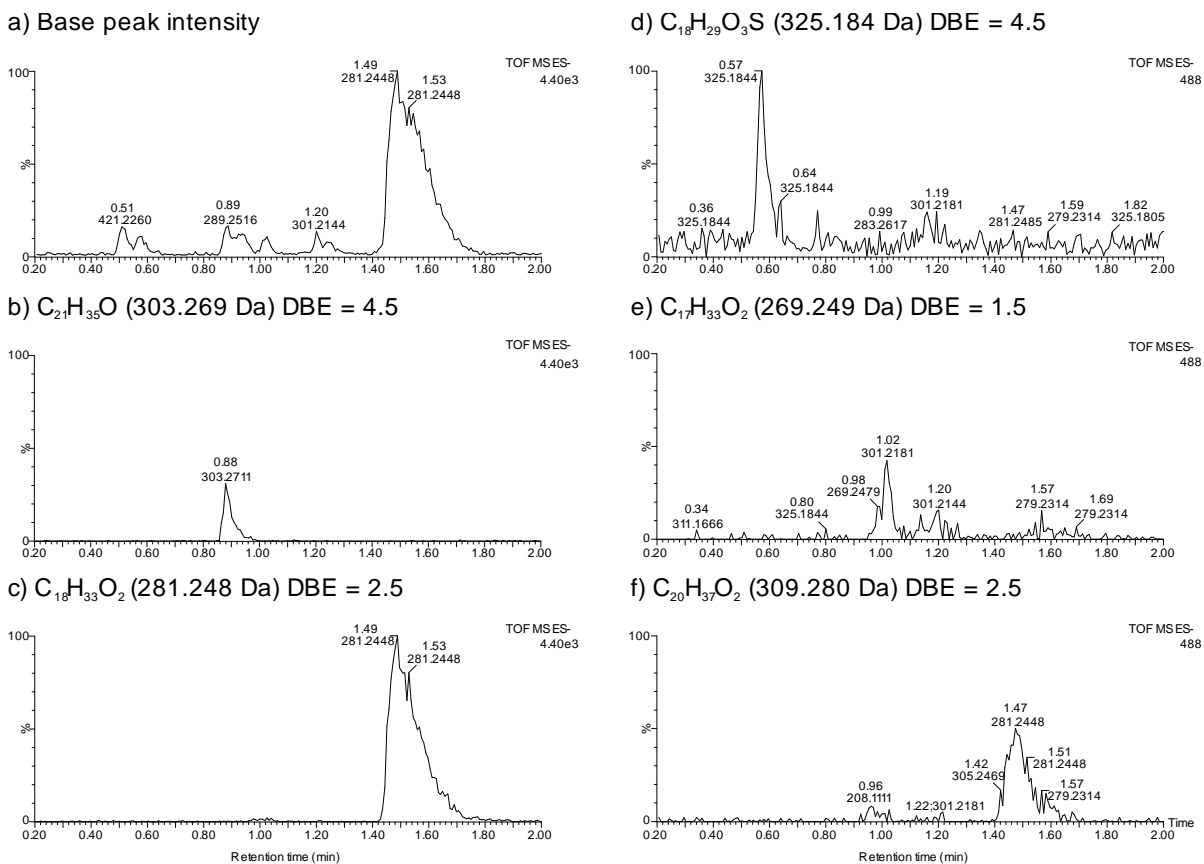


Figure 5. The base peak ion chromatogram (a) and extracted ion chromatograms of selected negative quasi-molecular ions, $[M-H]^-$, (b – f) detected by ESI-TOFMS. The results show the clear separation between the ions representing the CHO_1 (b) and CHO_2 (c and f) classes. Ions representing other subclasses (with lower relative intensities) show different retention times (d and e). Note that for (a - c) a relative intensity of 100 % is equivalent to an absolute intensity of 4.40×10^3 , whilst for (d – f) a relative intensity of 100% is equivalent to an absolute intensity of 488.

Ions representative of the detected classes were extracted and the separation obtained for CHO_1 and CHO_2 classes can be seen in Figure 5 b and c. The CHO_2 class ions have different retention times based on their DBE (Figure 5 c, e and f). The quasi-molecular ion assigned as $C_{17}H_{33}O_2$ most likely represents a carboxylic acid with a saturated hydrocarbon chain. At least two peaks can be observed in this extracted ion chromatogram (Figure 5 e), indicating that isomeric species (with different functional groups and/or degrees of branching) were originally present. Comparison of

Figure 5 c and e also reveals that DBE influences retention during HILIC. The extracted ion chromatogram of $C_{20}H_{37}O_2$ (Figure 5 f) indicates that alkylation (chain length) does not influence retention significantly when compared to Figure 5 c. Literature suggests that alkylation influences retention during reversed phase HPLC,¹¹ indicating (as can be expected) that HILIC offers a different separation mechanism. The chromatogram of an ion representing the CHO_3S_1 class is shown in Figure 5 d.

HPLC-ESCI-TOFMS: Individual fuel extracts. The chromatograms (in terms of base peak intensity) obtained from the analysis of individual fuel extracts are shown in Figure 6. Different elution profiles were obtained for the different samples which accentuates differences in sample composition in terms of ionizable species. Elution trends are very similar (and directly related) to those observed for the mixture analysis. The individual fuel extract chromatograms are discussed in conjunction with results obtained from mass spectral data that was combined to represent the overall composition (in terms of ion classes) of observed (ionized) species in each fuel extract (see Figure 7). To evaluate the advantages and limitations of the proposed method, the results are compared to those obtained without chromatographic pre-separation (Figure 1).

A major concern was the low ion signal intensities that were observed (compared to mass spectra collected without chromatographic pre-separation). Low ion intensities may result in the inaccurate measurement of ion masses, and therefore incorrect elemental compositions may be assigned to ions. Unfortunately, a different dilution ratio could not be used to overcome this limitation, due to the composition of the mobile phase employed, i.e. 97:3 acetonitrile:water. In order to prevent peak broadening and improve chromatographic separation, the sample solvent composition should match that of the mobile phase as much as possible. The manufacturer of the

HILIC column suggests that a dilution ratio of 1:3 of methanol (containing the sample) and acetonitrile be used, as employed here. The mobile phase composition could also not be altered as discussed previously. It was also not possible to inject a larger sample volume, and therefore the effect of slower flow rates combined with various scan rates should be investigated in future with the aim of optimizing ion intensities.

Despite the limited chromatographic separation achieved, the benefit of the HILIC method becomes evident when taking a closer look at the resulting mass spectral data. Certain aspects need to be considered when comparing results for individual fuels obtained with and without chromatographic pre-separation. A larger error in mass accuracy was allowed when chromatographic pre-separation was performed (due to low ion signal intensities) and therefore the presence of ion classes that were not observed without pre-separation should be interpreted with care. Furthermore, the presence of an ammonium acetate buffer in the mobile phase (which was not present when samples were infused directly into the ion source) may influence the ionization efficiencies of certain species slightly.

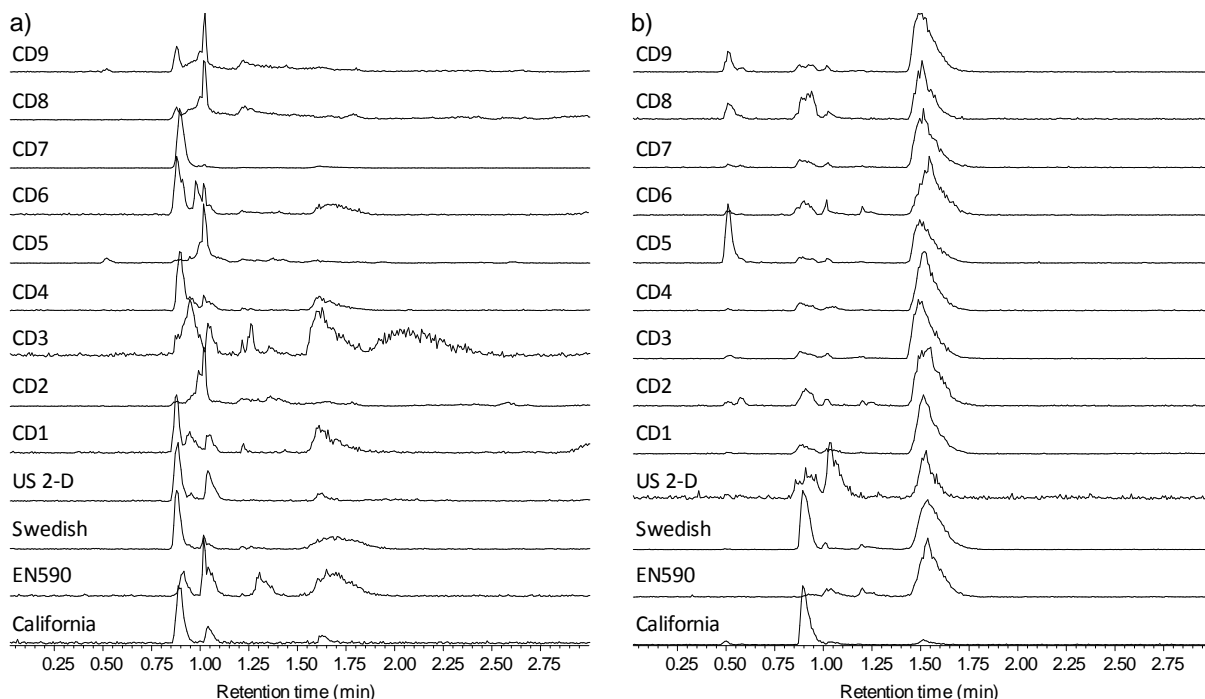


Figure 6. Base peak ion chromatograms showing the separation obtained for methanol extracts of 13 diesel samples using hydrophilic interaction liquid chromatography. Note that each base peak chromatogram is separately normalized to its largest base peak signal, the relative intensities between the different samples not being of interest here. The positive (a) and negative (b) base peak ions are plotted against retention time. Only results from ESI-TOFMS are shown, since similar elution profiles (with lower signal intensity) were observed for APCI-TOFMS.

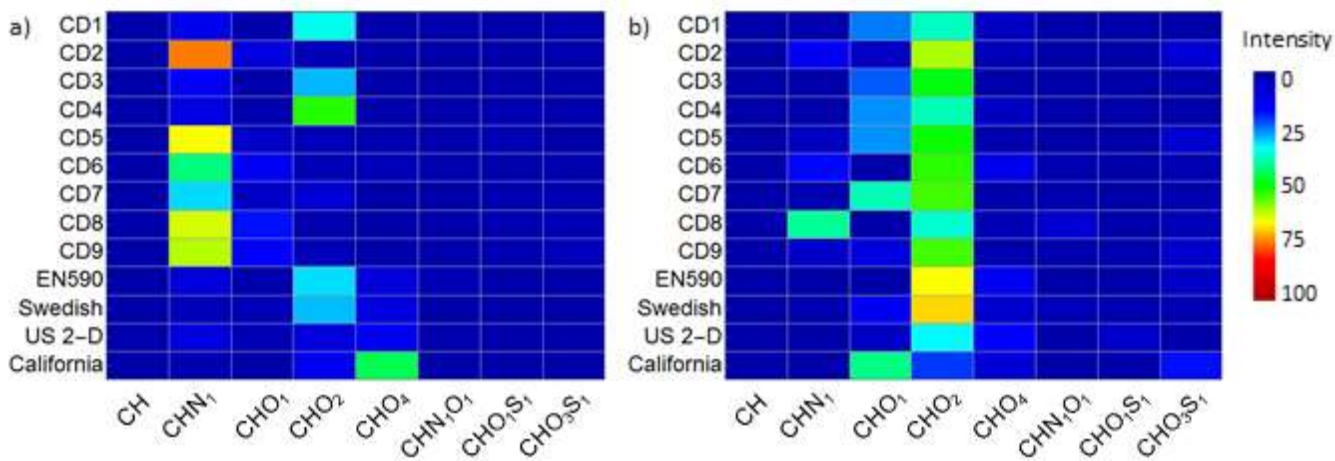


Figure 7. Comparison of relative intensities of selected ion classes obtained for methanol extracts of the reference and commercial diesels separated by HPLC. Results based on positive (a) and negative (b) ion mass spectra detected by ESI-TOFMS are shown and represent a combination of 150 and 200 mass spectral scans, respectively.

By comparing results shown in Figure 1 and Figure 7 it is clear that the relative intensities of the ion classes were influenced significantly by the HPLC pre-separation. If sufficient separation is achieved between highly acidic or basic compounds (that suppress the ionization of less polar compounds) and other compounds, the ionization efficiency and therefore relative intensities of ion classes are influenced. This reduction in matrix effects may improve the detection of species with lower ionization efficiencies or those that were present at lower concentrations. In theory, this may also result in higher mass accuracy (and assigned elemental compositions) due to less overlap of mass spectral peaks (since they are separated in time), but in practice the lower peak intensities resulted in lower mass accuracies.

For the positive ion classes, the CHO₂ class was more prominent when pre-separation was employed (Figure 1a and Figure 7a). For the California sample however, the CHO₄ class is the most prominent. The base peak ion chromatogram for California diesel (Figure 6a) shows that the species eluting between 0.85 and 0.95 minutes were the most prominent, and together with results from Figure 3, indicate that this peak represents the CHO₄ class in this sample. Even though chromatographic peaks were observed in the same region for other samples (where the CHO₄ class was not as prominent), previous results indicate that other ion classes including CHO₂ and CHN₁ have similar retention times (Figure 3).

For US 2-D, the CH class was originally detected as the most prominent (positive ion) class when the methanol extract was infused directly into the ion source (Figure 1 a), but this was not observed after chromatographic pre-separation (Figure 7 a). This may indicate that these species only tend to form positive ions in the presence of other compounds which may act as proton donors (e.g. carboxylic acids). It is unlikely that these species were highly retained and did not elute from

the column. It is interesting to note that the elution profiles of US 2-D and California are very similar (Figure 6 a).

CD3 has the most distinct elution profile for positive ions (Figure 6 a) due to the relatively broad and intense chromatographic peak between 1.90 and 2.50 minutes, indicating the presence of several chemical species. Closer inspection of raw data showed that this chromatographic peak consists mainly of a single mass spectral peak (which may represent a mixture of isomeric species) with m/z 206.1907 and an assigned elemental composition of $C_{14}H_{24}N$. This may be the quasi-molecular ion of an alkylated pyridine or aniline,²⁸ and based on retention time it is suspected that an alkylated aniline (with a primary amine group) is more likely as previously discussed (Figure 3 a). It is possible that the elution profile of CD3 seems different from the other samples simply due to lower intensities of peaks eluting between 0.75 and 1.25 minutes (mostly CHN_1 species according to Figure 3 a).

For the negative ion classes, the CHO_1 class is relatively more prominent when chromatographic pre-separation is employed (Figure 1 c and Figure 7 b). This illustrates the effect of a matrix on ionization efficiency. Previous results have shown that the CHO_1 and CHO_2 classes are completely separated. It is suspected that the CHO_2 class consists of quasi-molecular ions of carboxylic acids, whilst the CHO_1 class consists mostly of quasi-molecular ions of alkylated phenols. In the absence of CHO_2 , the CHO_1 class ionizes more readily and therefore is relatively more abundant (Figure 7 b).

The results also show that the CHO_1 class is the most prominent class in the California sample (Figure 7 b). This is reflected in the negative ion chromatogram (Figure 6 b), where an intense chromatographic peak is observed between 0.80 and 1.00 minutes (representing ions from the

CHO₁ class) and a very small chromatographic peak is observed at 1.50 minutes where the CHO₂ ions predominantly elute.

Conclusions

Hydrophilic interaction liquid chromatography was used to separate methanol extracts of diesel fuel in terms of heteroatom content (i.e. group-type separation) for the first time. The solvents used, i.e. methanol, acetonitrile and water, makes the proposed HILIC method greener and more compatible with electrospray ionization (ESI-MS) compared to traditional normal phase HPLC.

The effect of chromatographic pre-separation on observable (ionizable) species was investigated by first considering results obtained without chromatographic pre-separation, i.e. direct infusion into the ion source. Different ambient ionization methods (ESI and APCI) were employed, although similar results were obtained: compounds containing one N-atom and compounds containing two O-atoms were the most prominent for positive and negative ion modes, respectively. All tested diesels gave unique heteroatom and DBE profiles, potentially useful in identification of different diesels according to their origins, blends and additives utilized.³⁸

In comparison, results obtained with chromatographic pre-separation was investigated by considering a mixture of diesel fuel extracts. Due to limited chromatographic resolution, individual chromatographic peaks were not considered, but retention time resolved data analysis was achieved by considering chromatographic fractions (retention time windows). Analysis of these chromatographic fractions showed that class separation (group-type separation) was achieved to a certain extent and that the retention mechanism cannot simply be described by the polarity and/or polarizability of the analytes. Compound class separation was further confirmed by investigating

extracted ion chromatograms of species representing ion classes. One of the limitations of the proposed method was the low ion signal intensities that were observed and ways to overcome this should be investigated in future.

Trends with regards to retention characteristics of specific ion classes were identified based on results obtained for the mixture of fuel extracts and applied to the interpretation of chromatograms (in conjunction with mass spectral data) of individual fuel extracts. As could be expected, chromatographic separation of compounds gives access to chemical class information not obtainable by elemental composition of ions only.¹¹ Chromatographic pre-separation had a significant effect on the relative intensities of selected ion classes, illustrating how this technique can be used to minimize matrix effects.

Despite some limitations, including low ion signal intensities and limited chromatographic resolution, the proposed HILIC method presents unique advantages compared to traditional normal phase HPLC and has a significant effect on the observable (ionizable) species when combined with ESI-MS.

Acknowledgements. The financial assistance of Sasol (Sasol Energy, Energy Technology), the Deutscher Akademischer Austauschdienst (DAAD) and the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the authors and are not necessarily to be attributed to the DAAD-NRF.

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