Evaluation of Thin-Layer Chromatography—Laser Desorption Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometric Imaging for Visualization of Crude Oil Interactions

Ali Zahraei,‡ Peter W. F. Arisz,† Alexander P. van Bavel,‡ and Ron M. A. Heeren*‡

†Maastricht MultiModal Molecular Imaging (M4I) Institute, Division of Imaging Mass Spectrometry, Maastricht University, Maastricht, The Netherlands
‡Shell Global Solutions International BV, Amsterdam, The Netherlands

ABSTRACT: A light oil was separated into four chromatographic fractions that serve as proxy for SARA fractions. The fractions were (semi)quantified on a rod by TLC-flame ionization detection and characterized on a plate with laser desorption ionization—mass spectrometry imaging (TLC-LDI-MS). Comparisons of (semi)quantitative TLC-FID and qualitative TLC-LDI-MS results showed that LDI-MS was most sensitive for detection of molecules in the polar P1 fraction, and, to some extent, for the aromatics fraction, while no signal was observed for the most polar P2 and saturates fractions. Based on these results, limits of the compositional space, as observed by the laser ionization technique, were evaluated. The molecular speciation between and within the spots of the aromatics and the P1 fractions were analyzed and interpreted in terms of oil−SiO{sub}2 versus oil−solvent interactions, as a function of molecular characteristics such as DBE, aromaticity (H/C ratio), heteroatom content, degree of alkylation, and shielding of heteroatoms. In addition, the high oil loading resulted in an interesting bifurcation of the aromatics spot, which implies that oil−oil interactions can be enforced and studied in the TLC model system.

INTRODUCTION

The interface between oil and rock is believed to be mainly governed by polar components within the oil. These interactions alter the properties of the interface and can make it difficult to extract oil from reservoir rocks.¹,² Crude oil separation on silica-coated thin layer chromatography (TLC) plates with direct laser desorption ionization mass spectrometry (LDI-MS) readout³ may serve as a simple online model system to study oil interactions with silica.⁴ Ideally, fundamental knowledge about the physical—chemical behavior of oil at interfaces can be employed to improve oil recovery² and processing technologies, but we must make the reservation beforehand that subsurface conditions are far more complex and involve additional interactions with brines and other minerals at elevated temperature and pressure at geological time scales.

Crude oil is intrinsically very complex and contains a plethora of natural organic compounds.⁵⁻⁹ Structural complexity of hydrocarbon molecules includes their sizes and shapes; napthenic and aromatic ring systems, alkyl-substitution patterns and N, O, and S containing polar functional groups such as pyrrolic, pyridinic, quinolinic; hydroxyl, carbonyl, carboxyl, and thiophenic, sulfide, and sulfoxide units, respectively.¹⁰,¹¹ The number of elemental compositions increases exponentially as the molecular weight and heteroatom content in hydrocarbon compounds each increase.⁷,¹²,¹³ Tens of thousands of distinctive mass peaks with unique elemental compositions can be identified by high-resolution Fourier transform ion cyclotron resonance (FTICR) mass spectrometry (MS) measurements of crude oils.¹⁴

Each of the measured elemental compositions is composed of numerous isomeric structures, including different functional groups. Information about isomeric structures cannot be assessed by mass spectra of (pseudo)molecular ions alone, but, when combined with chromatographic separation, patterns are expected that are based on the type of functional group and their exposure or shielding by alkyl substituents, such as that convincingly demonstrated for carbazole model compounds by Li and Larter et al.,¹⁵ for example. Fractionation of crude oils often provides the opportunity to identify components that were not possible to detect directly in the parent crude oil.¹⁶

One of the approaches for petroleum characterization is by SARA analysis, which classifies oils in terms of their proportion of saturates, aromatics, resins, and asphaltenes fractions (SARA). SARA fractions are separated based on their solubility (as described in IP-143) in different solvents combined with their interaction with silica or alumina particles.¹⁷,¹⁸

Saturates fraction is composed of a mixture of linear, branched, and cyclic alkanes. The aromatics fraction contains molecules with one or more aromatic rings with attached saturated hydrocarbon side chains. The resins fraction consists of polar molecules that dissolve in alkane solvents such as heptane or pentane, while asphaltenes are polar molecules that, by definition, precipitate in alkane solvents such as pentane or heptane but dissolve in aromatic solvents such as benzene.
pyridine, or toluene.\textsuperscript{19} TLC is applied frequently as a rapid and convenient proxy for SARA fractions.\textsuperscript{20,21}

Iatroscan TLC-based analysis is typically coupled with flame ionization detection (FID) to quantify the fractions. TLC-FID can be employed without considerable sample preparation, but a drawback of TLC-FID is that it provides no information at the molecular level. TLC combined with LDI-FTICR-MS imaging is demonstrated as a direct chemical readout of fractionated petroleum samples\textsuperscript{3} but no quantitative response factors are known for this technique.

Here, we present the application and further validation of the TLC-LDI-FTICR-MS imaging method presented by Smith et al.\textsuperscript{3} to study molecular speciation resulting from oil–SiO\textsubscript{2}, oil–solvent, and oil–oil interactions that occur in a three-step chromatographic separation. A light crude oil sample was chosen with the expectation that light oil produces less complex data, compared to heavier oil types. The response of TLC-LDI-MS imaging was compared with TLC-FID data and optical observations of the developed TLC plate in order to (semi)quantify and evaluate the limits of the compositional space that is observed by LDI-MS. The TLC plate was imaged with a 16-fold-higher spatial resolution, namely, with a raster size of 250 μm, compared to 1 mm used in the previous study, that allowed chemical speciation both between and within chromatographic spots. Observed trends in the chemical speciation were tentatively correlated with chemical structures.

\section*{EXPERIMENTAL SECTION}

\textbf{Sample and Reagents.} Xylene, toluene, n-heptane, and dichloromethane (DCM), and methanol (MeOH) were purchased from Sigma–Aldrich (Zwijndrecht, The Netherlands). A hydrophobic-type petroleum sample (API, 41.6; density, 0.8179 g/cm\textsuperscript{3}; viscosity, 3.2769 cP at 20 °C; and %S, 0.231%) was provided by Shell Global Solutions International B.V. (The Netherlands). A hydrophobic-type petroleum sample (API, 41.6; density, 0.8179 g/cm\textsuperscript{3}; viscosity, 3.2769 cP at 20 °C; and %S, 0.231%) was provided by Shell Global Solutions International B.V. (The Netherlands). Xylene, toluene, and 95/5 DCM/MeOH mobile phase was eluted to line 2. The DCM/MeOH (95/5 vol/vol) was used with a mixture of toluene/−heptane (80/20 (vol/vol)) for 12 min, followed by a mixture of toluene/n-heptane (80/20 (vol/vol)) for 12 min, and finally with a mixture of (95/5) DCM/MeOH for 3 min. The developed rods were scanned with the Iatroscan at a speed of 30 s per scan. The data were collected with SES-i-Chromstar software.

\textbf{TLC Development for MS Imaging Analysis.} Silica gel 60 with a fluorescence indicator at 254 nm on TLC aluminum foil (Catalog No. S6524–25EA, Sigma–Aldrich, The Netherlands) was used with a pore volume of 0.75 mL/g. The sample was diluted in xylene (1/1) and 4 μL was aliquoted at line 1 in Figure 1 on the bottom of a TLC plate with a spot size ~2 mm in diameter. The first mobile phase consisting of n-heptane was eluted to line 4, followed by a mixture of toluene/n-heptane (80/20 (vol/vol)) to line 3. Subsequently, the DCM/MeOH (95/5 vol/vol) mobile phase was eluted to line 2. The plate was dried at room temperature for at least 5 min after every step. For reference MS measurement, a diluted oil sample was spotted at line 5 after the fluorescence picture was taken.

Photographs were taken of the fully developed plate exposed by room light and by fluorescent excitation with 254 nm UV light. LDI-FTICR-MS Imaging Analysis. Double-sided conductive tape (3M Science, USA) was used to attach the back of the developed TLC plate to a stainless-steel MALDI target. The LDI-FTICR-MS image was recorded with a raster size of 250 μm without a matrix in LDI-MS positive (+) ion mode on a SolariX XR FTICR mass spectrometer (Bruker Daltonics GmbH, Bremen, Germany) equipped with a 9.4 T magnet.

A frequency-tripled Nd:YAG laser (355 nm, 3.49 eV photons) was employed for laser desorption directly from the TLC foil with 250 laser shots per position at a repetition rate of 1 kHz. The laser spot size was ~30 μm. The laser power and number of laser shots were adjusted to obtain a signal with a sufficient signal-to-noise ratio. Only the ions in the mass range set between m/ε 250 and 600 were transferred with the quadrupole ion-guide from the ion source into the FTICR cell. Such selective ion accumulation in the FTICR cell made petroleum detection easier as the signal-to-noise ratio increased significantly during the acquisition.\textsuperscript{22} The selected mass window was taken similar to the mass ranges reported in recent LDI-MS publications of crude oils.\textsuperscript{23} The signal of light oils with API values larger than 40 hardly exceeds m/ε 600.\textsuperscript{24} LDI may form very abundant fullerenes above this mass that disturb the ICR signal.\textsuperscript{25}

Spectra were acquired with a 2 MW transient size and were zero-filled with an additional 2 MW. The transient length was 1.82 s and gave a resolving power of ~350,000 at m/ε 400. The mass spectra were externally calibrated using the red phosphorus in LDI-MS positive mode immediately prior to the experiment. After the experiment was completed, an internal recalibration was performed by using the crude oil N1 series spectrum in positive mode. Peak lists were obtained with the Compass Data Analysis software package (Bruker Daltonics) through the selection of peaks with a signal-to-noise ratio of >5. Spectral interpretation was performed with the PetroOrg (Corilo, Yuri. EnviroOrg, Florida State University, Tallahassee, FL, USA, 2013; http://software.petroorg.com).

\textbf{PetroOrg Spectral Analysis.} Peak lists of recalibrated spectra were created with Bruker DataAnalysis based on exact theoretical masses with a ±0.5 ppm error range. PetroOrg assignments were made with a maximum error of 2.0, 1.3, and 2.5 ppm for the whole oil, aromatics, and P1 spot, respectively. Normal boundary conditions for petroleum data (C\textsubscript{7}H\textsubscript{n}NO\textsubscript{S}, where \(c = 0–100, h = 0–200, n = 0–5, s = 0–5\) or \(c = 0–4\) were used for these assignments. The data were filtered for a minimum of two consecutive carbons per homologous series, which removed all noise from single scattered data points. Data of assigned mass peaks can be visualized by the software as relative abundance class plots and iso-abundance-contoured plots of double bond equivalents (DBE = (c − h)/2 + n/2 + 1\textsuperscript{7}) versus the carbon number (C).

\textbf{Principal Component Analysis.} Principal component analysis (PCA) was performed with an in-house written toolbox (Chemo-meTricks) for MATLAB (The MathWorks, Natick, MA, USA). 26 SPlite data were generated by the Bruker software and were peak-picked with 0.0001 Da bin size, n2 of 16, smoothing 2, and a threshold of 5. Background pixels were removed manually from the entire image (Figure 1C) until a clear separation was achieved between the aromatics and the P1 spots in PC1.

PCA of the selected region of the aromatics spot resulted in a separation of the bottom and top zone by PC2, as shown in Figure 6B (shown later in this work), and the top and bottom zones of the P1 spot were separated by PC1, as shown in Figure 6C (shown later in this work).

\section*{RESULTS AND DISCUSSION}

\textbf{TLC-FID Analysis.} Previous studies show that TLC development with a single mobile phase of 5% isopropanol in 95% heptane\textsuperscript{26,27} yields a continuous separation with poorly defined borders of the four proxy SARA fractions. In this study, four distinctly separated chromatographic fractions were obtained\textsuperscript{26,28} by a sequential rod or plate development with n-heptane, 80/20 toluene/n-heptane, and 95/5 DCM/MeOH as the mobile phase. The solvents subsequently eluted the fractions of saturates, aromatics, and the polar P1 fraction that was a proxy for the resins from the origin spot\textsuperscript{21,30,31} respectively. The most polar P2 fraction was a proxy for the asphaltene, which are nonmobile and remain at the origin of
Considering the high polarity and relatively high molecular saturates fraction, followed by the aromatics fraction.

The amount of volatiles is most abundant in the phases. The evaporation of volatiles probably mainly occurs during the

TLC-FID analysis. By fi

the plate.

TLC-FID analysis was used to estimate the sensitivity of LD-Ms for the chromatographic fractions. By first approxi-
mation, when the FID carbon-response factors for all fractions would be taken to be unity, the quanti-
fication, when the FID carbon-response factors for all fractions contains a relatively large amount of volatile compounds. Therefore, the measured amounts of saturates and aromatics were probably underestimated by the TLC-FID analysis.

Figure 1. (A) Image of developed TLC plate in visible light, (B) fluorescence with 254 nm UV, and (C) total ion count (heat map) from LDI FT-ICR-MS.

The separation was performed both on a flat TLC plate that is suitable for optical analysis and direct MS imaging readout, as well as on an Iatroscan rod for semiquantitative TLC-FID analysis.

Although the heteroatom content of oil is limited to a few mass percent, it increases steadily as the polarity of the fractions increases. Saturates are almost-pure hydrocarbons with a carbon-response factor of 1.0; the aromatics fraction contains some heteroatoms, while the P1 and P2 fractions contain substantial amounts; asphaltenes may contain even more than 10 wt %, respectively. Therefore, it would be expected that the carbon-response factor decreases in the order from saturates to P2.

However, the work by Bisht et al. revealed an opposite trend, where the response of saturates is significantly lower than that of asphaltenes. This reversed effect can be explained by the loss of volatile compounds with boiling points below 165 °C. The evaporation of volatiles probably mainly occurs during the drying of the rods after the development with the mobile phases. The amount of volatiles is most abundant in the saturates fraction, followed by the aromatics fraction. Considering the high polarity and relatively high molecular weight of P1 and P2, the amount of volatiles can be ignored for these fractions. Our light oil, which is rich in saturates, likely contains a relatively large amount of volatile compounds. Therefore, the data derived from TLC-FID were rounded to whole percentages in the following text. Nevertheless, it was clear from the TLC-FID measurement that saturates make up the bulk of the volume of this light crude oil. The large amount of saturates fits well with the small amount of asphaltenes, as indicated by P2. These two fractions are inherently incompatible with each other and asphaltenes would precipitate in situ, because of the high saturates content in such types of light oil. The TLC-FID results confirm the description that this was a type of hydrophobic oil with a polar fraction of ~14%.

TLC Plate Images. A flat TLC plate was sampled with a large amount of 1.6 mg (for comparison, Smith et al. applied 0.2 or 0.4 mg) of oil at the origin position “1” and developed subsequently with the three mobile phases. Sample overloading and multilayer formation was not a major concern, since enforcement of oil–SiO2 and oil–oil interactions was the primary interest in this study, rather than obtaining neat chromatographic separation of diluted oil compounds.

Figure 1 shows three images of the same plate as observed in visible light (Figure 1A), fluorescent emission with 254 nm excitation light (Figure 1B), and by the heat map of MS imaging, where the total ion count over the mass range m/z 250–600 was summed for each pixel (Figure 1C). The dotted lines indicate the original sample application position (1) and the three solvent fronts of DCM/MeOH (2), toluene/n-heptane (3), and n-heptane (4) development.

Isolated SARA-proxy spots with some tailing between the spots were observed for P2 at the dotted line labelled as “1”, P1 just below the dotted line labelled as “2”, and aromatics between the dotted lines labelled as “2” and “3”. None of the applied readout techniques detected the saturates fraction. The most striking feature in the images is the bifurcation of the aromatics spot, which was split into two equal branches. Data in Figure S1 in the Supporting Information showed that this phenomenon was completely absent for another oil type. Some forcing in the chromatographic process leads to the observed demixing of oil compounds from the saturates and the aromatics fractions. The capability to load a large amount of oil and study its development in two dimensions as partially driven by oil–oil interactions is a unique feature of the TLC plate model. The bifurcation phenomenon weakened some what at lower sample loadings, and therefore could be interpreted as an oil–oil interaction. Inspection of TLC separations in other publications showed that this phenomenon is frequent, but none of the papers discussed this effect. Overlay of the three images in Figure 1 allows the comparison of optical and mass spectrometric characteristics of the fractions, where enhanced examination in the bifurcation region is of special interest.

The colors of fractions in Figure 1A agree well with the observations described by Pantoja et al. colorless for saturates, yellow-orange for aromatics, and brown for P1 and P2. However, the darkness of the spots is a poor indicator for
the sample concentrations, because of large differences in light absorption properties of the oil fractions. First of all, the saturates, which comprise 60% of the oil sample, were not observed by either of the techniques (i.e., visible light, absorbance of the UV 254 nm excited fluorescence light, nor LDI-MS). The absence of any LDI-MS signal is explained by the absence of chromophores in saturates that can absorb the 355 nm laser light. Improvement of the LDI-MS signal by application of Ag-ion matrices\(^39\) might be possible but was beyond the scope of this study.

The aromatics fraction, which comprises 26% of the oil, showed only a weak yellow band in visible light (Figure 1A). Examples of non-alkyl-substituted core structures that absorb light of this wavelength are four-ring aromatic compounds (\(C_{18}H_{12}\), DBE of 12) as chrysene and tetracene, which substances are colored golden-yellow and pale orange, respectively. Fluorescence observed in Figure 1B after UV excitation revealed more details. There was a zone that emitted bright blue fluorescent light at the top and the inside of the bifurcated spot, and there was a dark band at the bottom and
outside of the spot. The dark band coincided both with the yellow band in Figure 1A and the LDI-MS signal in Figure 1C. The blue fluorescent light zone stretched significantly further inward in the bifurcation cavity in Figure 1B than indicated by the location of the yellow band in the visible light image and the band of the LDI-MS signal in Figures 1A and 1C. Apparently, inside the bifurcation gap, there is a family of molecules that does not absorb any visible light and does not ionize with 355 nm laser light, but that becomes fluorescent when excited with 254 nm UV light. Emission of bright blue fluorescent light after UV excitation is characteristic for molecules with small aromatic cores. It can be concluded from the optical properties that oil molecules with aromatic cores composed of less than four aromatic rings migrated toward the top of the aromatics spot.

The dark band at the bottom and outside of the bifurcated aromatics spot in Figure 1B was caused either by absorption of incoming UV excitation light or by absorption of emitted fluorescent visible light of chromophores in the aromatic molecules. The dark band coincided with the yellow-colored band in Figure 1A and the LDI-MS signal in Figure 1C. Apparently, the molecules in this band also interact strongly with 355 nm laser light, resulting in desorption and ionization of those molecules. These observations were indicative for the presence of stronger chromophores in the dark zone, but the exact mechanism for the dimmed light remains inconclusive. The light absorption might be caused by larger aromatic core structures, the absorption of which is known to scale with their size. However, it also might be the specific (conjugated) functional groups that are harboring lone pair electrons from heteroatoms in the oil molecules contributing to the light absorption.

The P1 fraction represented 13% of the oil, as quantified by TLC-FID. The P1 spot in Figures 1A and 1B gave the darkest bands in the visible and fluorescent light images, indicating that many P1 molecules had substantial light extinction coefficients. The bright background fluorescence from the silica plate made it impossible to infer conclusions about fluorescent properties of the P1 and P2 fractions. Although the P1 content (13%) in the oil is lower than the aromatics fraction (26%), Figure 1C shows that the strongest LDI-MS signal came from the P1 fraction. Apparently, the oil molecules in the P1 fraction were desorbed and ionized more efficiently by the LDI-MS process than the aromatics fraction. This result also implies that a large and probably a major portion of the aromatics fraction remained undetected by LDI-MS.

The small amount of only 1% P2 fraction still provided dark spots in Figures 1A and 1B. The lack of signal from the P2 fraction can be attributed to its low concentration, combined with the large binding energy of polar molecules with the silica surface, which could diminish desorption efficiency on LDI.

**LDI-MS Imaging.** The previous section concluded that the largest fraction of our light crude oil was not detected by LDI-MS, and some inferences were derived from optical properties. The next challenge is to establish the molecular space that was probed by LDI-MS and to link molecular characteristics with speciation on the TLC plate. Figure 2 shows the LDI-FTICR mass spectra of the entire crude oil and the spots from the TLC plate.
Next to the major mass peaks of the N1 DBE 12 ion series, the crude oil spectrum may contain thousands of other mass peaks. The mass spectra were processed by PetroOrg software, which assigns elemental compositions to mass peaks and also groups the assigned mass peaks into heteroatom classes. PetroOrg software has the option to summarize the signals of radical cations (M⁺) and protonated ions (M+H⁺) that both can be formed as follows by LDI of the same molecular species. The possibility of at least two ionization pathways required some further evaluation of LDI-MS spectra, which is done in the Supporting Information.

Figure 4 shows the class distribution of the sum of radical cations and protonated ions of the N1, hydrocarbon (HC), O1, N1O1, S1, N1S1, and N2 classes, as measured by LDI-MS imaging for the whole oil, the aromatics, and the P1 fractions. The class selection was based on two criteria. First, the relative abundance was at least 1% in either one of the three spectra. Second, only relative abundances of more than 0.5% were reported.

In all samples, the N1 class was most prevalent, followed by the hydrocarbon class. It was striking that the six major classes of the whole oil were also the major classes in both the P1 and the aromatics fractions. The minor N2 class was not detected above 0.5% abundance in the aromatics fraction. As observed through the selective LDI-MS ionization filter, the applied chromatography provided the resolution to separate heteroatom-free saturates from polar heteroatom-bearing molecules, but the partitioning of the latter over the aromatics and the P1 fractions was gradual and could not be specified on the heteroatom class.

Closer comparison of the relative abundances in Figure 4 shows that the N1, N1O1, and N2 classes were enriched in the P1 fraction, while the HC, O1, and S1 classes were enriched in the aromatics fraction. The relative abundance of the N1S1 class was approximately the same in all three samples. In terms of oil–SiO₂ interactions, these results can be interpreted that the group of N-containing molecules was more strongly bound by the silica and therefore ended up in the more-retained P1 fraction, while O1 and S1 molecules behaved more like hydrophobic entities that express less interaction with silica and dissolved better in the 80/20 toluene/n-heptane mobile phase.

Figure 5 shows the DBE versus carbon number plots for the summed intensities of protonated and radical cations of the seven selected heteroatom classes for the whole oil and the spots of the aromatics and the P1 fractions. The contour plots of the whole oil resemble the weighted sum of the plots for the P1 and aromatics fractions, where fraction weights are given in Figure 4. In the case of the HC, O1, and S1 classes,

### Table 1. Abundance Weighted Average Data of Molecular Weight, Carbon Number, DBE, and H/C, As Derived from PetroOrg for the Aromatics and the P1 Fractions

| class | Abundance Weighted Average Molecular Weight | Abundance Weighted Average #C | Abundance Weighted Average DBE | Abundance Weighted Average H/C |
|-------|---------------------------------------------|------------------------------|-------------------------------|--------------------------------|
|       | aromatic | P1 | Δ(Ar. – P1) | aromatic | P1 | Δ(Ar. – P1) | aromatic | P1 | Δ(Ar. – P1) | aromatic | P1 | Δ(Ar. – P1) |
| N1    | 421      | 382 | 39         | 31       | 28  | 3           | 13.9     | 14.9 | −0.9       | 1.21     | 1.06 | 0.15        |
| HC    | 388      | 423 | −35        | 30       | 32  | −3          | 15.4     | 16.9 | −1.5       | 1.06     | 1.06 | 0.00        |
| O1    | 411      | 440 | −28        | 30       | 32  | −2          | 15.9     | 15.4 | 0.5        | 1.05     | 1.15 | −0.10       |
| N1 O1 | 448      | 433 | 15         | 32       | 31  | 1           | 16.9     | 15.5 | 1.4        | 1.15     | 1.10 | 0.05        |
| S1    | 398      | 410 | −12        | 28       | 29  | −1          | 15.7     | 17.2 | −1.5       | 1.02     | 0.93 | 0.10        |
| N1 S1 | 437      | 400 | 37         | 30       | 28  | 3           | 16.8     | 17.2 | −0.4       | 1.15     | 0.89 | 0.26        |
| N2    | 489      |     |            | 35       |     |             | 19.5     |      |            |          |      | 1.07        |

### Figure 6. RGB plots of three PCAs. (A) PCA of the whole TLC LDI-MS image with PC1+ (green), PC1− (red), and PC2+ (blue); the P1 and aromatics spots are differentiated by PC1. (B) PCA of the aromatics spot with PC2+ (red), PC2− (green), and PC1+ (blue); PC2 differentiates the top and the bottom of the aromatics spot. (C) PCA of the P1 spot with PC1− (red), PC1+ (green), and PC3+ (blue); PC1 differentiates the top and the bottom of the P1 spot.

Aromatics and P1 fractions. All samples were measured under identical experimental conditions and the ratio of the total abundance of the aromatics to the P1 fraction was ~1:2, which also quantified the result in the heat map in Figure 1C.

The base peaks in spectra of the P1 and aromatics spots were identified as C₄H₄N₂ (m/z 273.1512) and C₅H₅N₂ (m/z 371.2607), respectively. Both peaks belonged to the same homologue series of the N1 class with DBE 12. Although no isomer information is obtained by LDI-MS, carbazoles derivatives are usually the most abundant N1 class compounds in crude oils and, therefore, these mass peaks can tentatively be assigned to benzo[c]carbazole core structures with 4 and 11 additional C atoms as methylene groups in alkyl substituents, respectively. This simple example clearly illustrates that, within one compound class and type (i.e., the same heteroatom content and DBE), the molecules with increasing alkyl substitution were more mobilized under normal phase chromatographic conditions. This trend fully agrees with the results of other studies and will be used for further interpretation.

The example in Figure 3 visualizes, in more detail, the capability of MS imaging to study how N1 DBE 12 compounds with C₄ through C₁₀ alkyl substitution were distributed over the P1 and the aromatics spot. Again, no isomer information was obtained by LDI-MS, but it can be expected that compounds with N atoms that were shielded by alkyl substituents at their vicinal positions move with the mobile phase to the aromatics spot, while isomers equal to the number of CH₂— groups but with exposed N atoms were more retained by the silica stationary phase through hydrogen bonding.

Next to the major mass peaks of the N1 DBE 12 ion series, the crude oil spectrum may contain thousands of other mass peaks. The mass spectra were processed by PetroOrg software, which assigns elemental compositions to mass peaks and also groups the assigned mass peaks into heteroatom classes.

PetroOrg software has the option to summarize the signals of radical cations (M⁺) and protonated ions (M+H⁺) that both can be formed as follows by LDI of the same molecular species. The possibility of at least two ionization pathways required some further evaluation of LDI-MS spectra, which is done in the Supporting Information.

Figure 4 shows the class distribution of the sum of radical cations and protonated ions of the N1, hydrocarbon (HC), O1, N1O1, S1, N1S1, and N2 classes, as measured by LDI-MS imaging for the whole oil, the aromatics, and the P1 fractions. The class selection was based on two criteria. First, the relative abundance was at least 1% in either one of the three spectra. Second, only relative abundances of more than 0.5% were reported.

In all samples, the N1 class was most prevalent, followed by the hydrocarbon class. It was striking that the six major classes of the whole oil were also the major classes in both the P1 and the aromatics fractions. The minor N2 class was not detected above 0.5% abundance in the aromatics fraction. As observed through the selective LDI-MS ionization filter, the applied chromatography provided the resolution to separate heteroatom-free saturates from polar heteroatom-bearing molecules, but the partitioning of the latter over the aromatics and the P1 fractions was gradual and could not be specified on the heteroatom class.

Closer comparison of the relative abundances in Figure 4 shows that the N1, N1O1, and N2 classes were enriched in the P1 fraction, while the HC, O1, and S1 classes were enriched in the aromatics fraction. The relative abundance of the N1S1 class was approximately the same in all three samples. In terms of oil–SiO₂ interactions, these results can be interpreted that the group of N-containing molecules was more strongly bound by the silica and therefore ended up in the more-retained P1 fraction, while O1 and S1 molecules behaved more like hydrophobic entities that express less interaction with silica and dissolved better in the 80/20 toluene/n-heptane mobile phase.

Figure 5 shows the DBE versus carbon number plots for the summed intensities of protonated and radical cations of the seven selected heteroatom classes for the whole oil and the spots of the aromatics and the P1 fractions. The contour plots of the whole oil resemble the weighted sum of the plots for the P1 and aromatics fractions, where fraction weights are given in Figure 4. In the case of the HC, O1, and S1 classes,
the aromatics fraction contributed more to the whole oil signal, whereas in the case of the N1, N1O1, N1S1, and N2 classes, the P1 fraction was more prevalent.

Comparison of the P1 versus the aromatics fraction shows that the contoured plots overlap to a great extent, which is in agreement with the rather similar relative class distributions in Figure 4. Table 1 lists abundance weighted parameters for the molecular weight, number of carbon atoms, DBE and H/C ratio per heteroatom classes in the aromatics, and the P1 fractions. Generally, most of the parameter values per class differ by <10% between both fractions, but several systematic trends can be observed that differentiate them. Except for the O1 class, all H/C values are smaller in the P1 fraction, which indicates that the polar behavior of P1 molecules is at least partially related to their aromaticity.

All the N-containing classes in the aromatics fraction are of higher molecular weight, combined with a lower degree of aromaticity (H/C). The higher molecular weights of 421, 448, and 437 Da versus 382, 433, and 400 Da for N1, N1O1, and N1S1, respectively, implied that higher degrees of alklylation of N-compounds reduced the silica interaction and improved the solubility in the mobile phases, as was illustrated by Figure 3. The H/C ratios for the corresponding classes were 1.21, 1.15, and 1.15 versus 1.06, 1.10, and 0.89, respectively. The effect of aromaticity is well-established by the normal-phase chromatographic retention of the N1 class alkylcarbazoles (DBE 9) and benzoazoles (DBE 12) model compounds on silica. Benzocarbazoles are much more retained, compared to tetramethyl carbazoles, i.e., both C16N1 compounds but different types with DBE 12 versus DBE 9, which emphasizes a significant increase in adsorption strength by the addition of more-aromatic C atoms in N-compounds. The adsorption strength of asphaltenes to silica was also reported to increase with increasing aromaticity of the molecules.

In contrast, lower molecular weight (388 Da versus 425 Da) combined with lower DBE (15.4 versus 16.9) were observed for the HC class in the aromatics fraction, while the H/C ratio of 1.06 was similar in both fractions. Apparently, the effect may also play a role.

The O1 class displayed a higher aromaticity, combined with a lower molecular weight for the HC class in the aromatics fraction, while the H/C ratio of 1.06 was similar in both fractions. Apparently, the partitioning of nonpolar aromatic hydrocarbon molecules between silica and the mobile phase is dependent on molecular size and absolute number of DBEs. A similar effect was observed for the S1 class, although the different H/C ratio of 1.02 versus 0.89 implied that an aromaticity effect may also play a role.

The O1 class displayed a higher aromaticity, combined with a lower molecular weight for the HC class in the aromatics fraction, while the H/C ratio of 1.06 was similar in both fractions. Apparently, the partitioning of nonpolar aromatic hydrocarbon molecules between silica and the mobile phase is dependent on molecular size and absolute number of DBEs. A similar effect was observed for the S1 class, although the different H/C ratio of 1.02 versus 0.89 implied that an aromaticity effect may also play a role.

Strong interactions of NO classes from asphaltenes with phenolic oxygen while the aromatics fraction may contain different functionality of the O atom in both fractions. For example, the P1 fraction may contain more-asaromatic C atoms in N-compounds, which emphasizes a significant increase in adsorption strength by the addition of more-aromatic C atoms in N-compounds. The adsorption strength of asphaltenes to silica was also reported to increase with increasing aromaticity of the molecules.
which is a common value for the most aromatic oil fraction, ratios for classes from the P1 fraction were smaller than 1.15, 1.21 were very low, especially for a light oil type. All H/C structures and that LDI-MS only detects the small subfraction bias of LDI-MS for compounds with condensed aromatic to elucidate and visualize individual related to the major di Figure 1C. Figure 6A shows an RGB-score plot image wherein supports the strong binding of N1O1 functionalities. highest molecular weight in the aromatics fraction, which to the N1 class with an average H/C of 0.98 ± 0.03 and a DBE of 14.0 ± 0.5, where the error is the standard error of the mean, ±(t_{n-1} × σ/√n) at p = 0.05. The top 30 PC1− loadings for the aromatics fraction consisted of 18 HC class mass peaks with an average H/C of 1.03 ± 0.04 and a DBE of 12.8 ± 0.5 and 12 N1 class masses with an average H/C of 1.21 ± 0.04 and a DBE of 10.9 ± 0.4. From these results, it can be concluded that the presence of an N atom, in combination with a relatively high degree of aromaticity, results in stronger retention.

The PCA interpretation can be extended with analyses of higher PCA functions and mass peaks of other heteroatom classes with lower loadings. However, processing the large amount of information generated by such analysis of oil data was outside the scope of our study. The PCA not only confirmed the results in Figures 5 and 6, namely, that the N1 class with high aromatic signature was specific for the P1 fraction and HC class elements were specific for the aromatics fraction, but the added value of PCA is the identification of those (top-30) features that contribute most to the differentiation between both fractions. The colored bands in the plots imply that PC loadings reveal information about groups of oil constituents that express similar SiO2 interactions. The PCA not only confirmed the results in Figures 5 and 6, namely, that the N1 class with high aromatic signature was specific for the P1 fraction and HC class elements were specific for the aromatics fraction, but the added value of PCA is the identification of those (top-30) features that contribute most to the differentiation between both fractions. The colored bands in the plots imply that PC loadings reveal information about groups of oil constituents that express similar SiO2 interactions.

Principal component analysis (PCA) is frequently employed to elucidate and visualize individual m/z features that are related to the major differences in an MS image plot such as Figure 1C. Figure 6A shows an RGB-score plot image wherein the P1 and aromatics fractions are separated along the first principal component (PC1) in the PC1+ and PC1− directions, respectively. This report mainly explored the visualization of score plot images, and therefore the PCA interpretation was limited to a high-level analysis. The top-30 mass peaks with the highest weighted scaled loadings are listed with their structural details in Table 2. All reported DBE values were integer values indicating that the observed masses were radical cations.

All of the top 30 PC1+ loadings for the P1 fraction belonged to the N1 class with an average H/C of 0.98 ± 0.03 and a DBE of 14.0 ± 0.5, where the error is the standard error of the mean, ±(t_{n-1} × σ/√n) at p = 0.05. The top 30 PC1− loadings for the aromatics fraction consisted of 18 HC class mass peaks with an average H/C of 1.03 ± 0.04 and a DBE of 12.8 ± 0.5 and 12 N1 class masses with an average H/C of 1.21 ± 0.04 and a DBE of 10.9 ± 0.4. From these results, it can be concluded that the presence of an N atom, in combination with a relatively high degree of aromaticity, results in stronger retention.

The PCA interpretation can be extended with analyses of higher PCA functions and mass peaks of other heteroatom classes with lower loadings. However, processing the large amount of information generated by such analysis of oil data was outside the scope of our study. The PCA not only confirmed the results in Figures 5 and 6, namely, that the N1 class with high aromatic signature was specific for the P1 fraction and HC class elements were specific for the aromatics fraction, but the added value of PCA is the identification of those (top-30) features that contribute most to the differentiation between both fractions. The colored bands in the plots imply that PC loadings reveal information about groups of oil constituents that express similar SiO2 interactions. The PCA not only confirmed the results in Figures 5 and 6, namely, that the N1 class with high aromatic signature was specific for the P1 fraction and HC class elements were specific for the aromatics fraction, but the added value of PCA is the identification of those (top-30) features that contribute most to the differentiation between both fractions. The colored bands in the plots imply that PC loadings reveal information about groups of oil constituents that express similar SiO2 interactions. The PCA not only confirmed the results in Figures 5 and 6, namely, that the N1 class with high aromatic signature was specific for the P1 fraction and HC class elements were specific for the aromatics fraction, but the added value of PCA is the identification of those (top-30) features that contribute most to the differentiation between both fractions. The colored bands in the plots imply that PC loadings reveal information about groups of oil constituents that express similar SiO2 interactions.
The PC1 highest scaled loadings, which, again, are all radical cations.
di were characterized by a higher degree of aromaticity that was retention on silica. The remaining top-30 PC1 aromaticity is an important parameter that determines the ±0.08. Also, this result again con-

dated in a rather narrow band at the bottom of the spot.

Figure 6C shows that the bottom and top of the P1 spot are differentiated by PC1. Table 4 lists the corresponding 30 highest scaled loadings, which, again, are all radical cations. The PC1− loadings for the top zone were all N1 class compounds with an average H/C of 0.99 ± 0.06 and a DBE of 13.3 ± 0.4. The highest PC1+ loadings corresponding to the bottom-zone encompass also 13 N1-class compounds, which were characterized by a higher degree of aromaticity that was reflected by an average H/C of 1.07 ± 0.03 and a DBE of 16.2 ± 0.08. Also, this result again confirms that the degree of aromaticity is an important parameter that determines the retention on silica. The remaining top-30 PC1− loadings were 16 highly aromatic hydrocarbons with an average H/C of 0.99 ± 0.05 and a DBE of 14.8 ± 0.7 and one N1 O1-class compound.

PCA of LDI-MS images confirmed that the retention of N-class compounds was favored by a low degree of alkylation, large aromatic core structures, and nitrogen-containing heteroatom classes. Figures 6B and 6C show that the heteroatom content was not distributed uniformly over the proxy SARA spots but that heteroatom classes were concentrated in chromatographic bands. The identification of this phenomenon implies that accurate TLC-FID quantifica-
tion should take chromatographic banding of heteroatom classes into account. The lack of such correction may explain some of the variance observed when TLC-FID results are correlated with other techniques.

Finally, we want to speculate about the origin of the bifurcation of the aromatics spot in Figures 1, 3, and 6. Obviously, the high sample loading enforced some sort of oil−oil interactions. The effect might result from enhanced viscosity caused by locally high concentrations of dissolved high-molecular-weight saturates as paraffins or waxes, for example. Dissolution of such compounds would increase the viscosity of the otherwise low viscous n- heptane mobile phase and, consequently, locally reduced the mobility of the liquid phase. Experiments with lower sample loadings indeed showed higher mobility of the bifurcation spot. When the solvent front of the second elution with 80/20 toluene/n-heptane hits the tail of the saturates fraction, the viscosity would locally increase and slowed down or even blocked further solvent flow. However, this simple description does not explain the presence of the blue fluorescent molecules inside the bifurcation gap. Apparently, more-complex molecular interactions occur in concentrated systems. Such interactions can be studied by MS imaging, whereas in regular liquid chromatography, they would give rise to undesired viscous finger effects.

### CONCLUSIONS

Comparison of (semi)quantitative TLC-FID and qualitative TLC-LDI mass spectroscopy imaging results demonstrated

---

### Table 4. Top-30 Scaled Loadings of PC1 of the P1 Spot in Figure 6C

| rank | m/z (exp) | scaled loading | formula | class | #C | H/C | DBE |
|------|-----------|----------------|---------|-------|----|-----|-----|
| 1    | 309.1511  | 5.2 C29H34N1   | N1      | 23    | 0.83| 15  |
| 2    | 323.1668  | 4.1 C28H34N1   | N1      | 24    | 0.88| 15  |
| 3    | 295.1354  | 3.2 C29H34N1   | N1      | 22    | 0.77| 18  |
| 4    | 359.1664  | 2.1 C29H34N1   | N1      | 27    | 0.78| 18  |
| 5    | 373.1819  | 1.8 C29H34N1   | N1      | 28    | 0.82| 18  |
| 6    | 347.1665  | 1.6 C29H34N1   | N1      | 26    | 0.81| 17  |
| 7    | 345.1509  | 1.5 C29H34N1   | N1      | 26    | 0.73| 18  |
| 8    | 364.2177  | 1.5 C29H34N1   | HC      | 28    | 1.00| 15  |
| 9    | 336.1868  | 1.4 C29H34N1   | HC      | 26    | 0.92| 15  |
| 10   | 361.1821  | 1.4 C29H34N1   | N1      | 27    | 0.85| 17  |
| 11   | 350.2023  | 1.3 C29H34N1   | HC      | 27    | 0.96| 15  |
| 12   | 378.2332  | 1.2 C29H34N1   | HC      | 29    | 1.03| 15  |
| 13   | 374.2021  | 1.2 C29H34N1   | HC      | 29    | 0.90| 17  |
| 14   | 337.1824  | 1.2 C29H34N1   | HC      | 29    | 0.92| 15  |
| 15   | 366.2333  | 1.2 C29H34N1   | HC      | 28    | 1.07| 14  |
| 16   | 324.1868  | 1.2 C29H34N1   | HC      | 25    | 0.96| 14  |
| 17   | 352.2177  | 1.1 C29H34N1   | HC      | 27    | 1.04| 14  |
| 18   | 338.2023  | 1.1 C29H34N1   | HC      | 26    | 1.00| 14  |
| 19   | 310.1545  | 1.1 C29H34N1   | N1      | 23    | 0.83| 15  |
| 20   | 333.1509  | 1.1 C29H34N1   | N1      | 25    | 0.76| 17  |
| 21   | 360.1867  | 1.1 C29H34N1   | HC      | 28    | 0.86| 17  |
| 22   | 322.1712  | 1.1 C29H34N1   | HC      | 25    | 0.88| 15  |
| 23   | 376.2177  | 1.0 C29H34N1   | HC      | 29    | 0.97| 16  |
| 24   | 310.1712  | 1.0 C29H34N1   | HC      | 24    | 0.92| 14  |
| 25   | 380.2487  | 1.0 C29H34N1   | HC      | 29    | 1.10| 14  |
| 26   | 371.2235  | 0.9 C28H34N1O1 | N1O1    | 26    | 1.12| 13  |
| 27   | 324.1700  | 0.9 C28H34N1   | N1      | 24    | 0.88| 15  |
| 28   | 447.2902  | 0.9 C28H34N1   | N1      | 33    | 1.12| 16  |
| 29   | 392.2486  | 0.9 C28H34N1   | HC      | 30    | 1.07| 15  |
| 30   | 370.2644  | 0.9 C28H34N1   | HC      | 28    | 1.21| 12  |
that, for light oil, only a minor fraction of the compositional space of all oil components is observed by LDI-MS. The two-photon ionization mechanism at 355 nm only provides information about the oil components with ionization energies of <7.0 eV, which are compounds with alkyl-substituted condensed aromatic rings. LDI-MS with a 355 nm laser source efficiently ionized a fraction of the P1 and aromatics, which was emphasized by the low H/C values of the observed classes in the aromatics (average 1.11) and especially the P1 (1.04) fractions. In our experiment, no saturates or P2 were detected.

Despite the fact that the molecular space that was probed by LDI-MS is limited to polar aromatic molecules, our results demonstrated that TLC-LDI-FTICR-MS imaging was a suitable model to study oil–SiO2 and oil–solvent interactions of those compounds that were detected. With high spatial resolution chromatographic trends in retention behavior could be monitored and were shown to be dependent on molecular features as the degree of alkylation and the degree of aromaticity. Although the online readout provides no direct information about isomers, based on examples such as the differentiation of one type of compounds over both the P1 and the aromatics spots, tentative conclusions can be inferred about shielded versus exposed functional groups. In addition, high sample loading was possible on the silica plate to enforce oil–oil interactions between different oil groups, as manifested by the bifurcation phenomenon. It was demonstrated that MS imaging data of oil could be analyzed much deeper by principal component analysis, which could reveal a wealth of information.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.energy-fuels.8b00639.

Comparison of TLC traces light oil with a heavy oil; evaluation of LDI-MS spectra; developed TLC plate flanked by a heavy oil trace (Figure S1); and DBE versus #C plots of radical cations and protonated ions of the N1 and the HC class (Figure S2) (PDF)

■ AUTHOR INFORMATION

Corresponding Author
*Tel.: +31-433881499. Fax: +31-43388415. E-mail: r.heeren@maastrichtuniversity.nl.

ORCID

Peter W. F. Arisz: 0000-0002-0283-2893
Ron M. A. Heeren: 0000-0002-6533-7179

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The work is part of the LINK program, which is financially supported by the Dutch Province of Limburg. We thank Ton van Loef and Irina Cotiuga (Latefakt BV, Koudekerk aan den Rijn, The Netherlands) for the Iatroscan measurements, and Gert Eijkel for assistance with the principal component analyses. We thank Erik Tegelaar, Pim Mul, and Frans Kordorffer (Shell Global Solutions International BV) for providing feedback to our work, and we acknowledge the funding support from Shell Global Solutions International BV.

■ REFERENCES

(1) Adams, J. J. Asphaltene Adsorption, a Literature Review. Energy Fuels 2014, 28 (5), 2831−2856.
(2) Strand, S.; Puntervold, T.; Austad, T. Water based EOR from clastic oil reservoirs by wettability alteration: A review of chemical aspects. J. Pet. Sci. Eng. 2016, 146, 1079−1091.
(3) Smith, D. F.; McKenna, A. M.; Corilo, Y. E.; Rodgers, R. P.; Marshall, A. G.; Heeren, R. M. A. Direct Analysis of Thin-Layer Chromatography Separations of Petroleum Samples by Laser Desorption Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry Imaging. Energy Fuels 2014, 28 (10), 6284−6288.
(4) Parida, S. K.; Dash, S.; Patel, S.; Mishra, B. K. Adsorption of organic molecules on silica surface. Adv. Colloid Interface Sci. 2006, 121 (1−3), 77−110.
(5) Terra, L. A.; Filgueiras, P. R.; Tose, L. V.; Romao, W.; de Castro, E. V. R.; de Oliveira, L. M. S. L.; Dias, J. C. M.; Vaz, B. G.; Popping, R. J. Laser desorption ionization FT-ICR mass spectrometry and CARSPLS for predicting basic nitrogen and aromatics contents in crude oils. Fuel 2015, 160, 274−281.
(6) Tissot, B. P.; Welte, D. H. Petroleum Formation and Occurrence; Springer−Verlag: Berlin, Heidelberg, 1984.
(7) Mullins, O. C.; Sheu, E. Y.; Hamami, A.; Marshall, A. G. Asphaltenes, Heavy Oils, and Petroleomics; Springer Science & Business Media: New York, 2007.
(8) Rodgers, R. P.; McKenna, A. M. Petroleum Analysis. Anal. Chem. 2011, 83 (12), 4665−4679.
(9) Marshall, A. G.; Rodgers, R. P. Petroleomics: Chemistry of the underworld. Proc. Natl. Acad. Sci. U. S. A. 2008, 105 (47), 18090−18095.
(10) Liu, P.; Shi, Q.; Chung, K. H.; Zhang, Y.; Pan, N.; Zhao, S.; Xu, C. Molecular characterization of sulfur compounds in Venezuela crude oil and its SARA fractions by electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. Energy Fuels 2010, 24 (9), 5089−5096.
(11) Acevedo, S. C.; Guzmán, K.; Labrador, H.; Carrier, H.; Bouyssiere, B.; Lobinski, R. Trapping of metallic porphyrins by asphaltene aggregates: a size exclusion microchromatography with high-resolution inductively coupled plasma mass spectrometric detection study. Energy Fuels 2012, 26 (8), 4968−4977.
(12) Blomberg, J.; Mes, E. P. C.; Schoenmakers, P. J.; van der Does, J. J. B. Characterization of complex hydrocarbon mixtures using online coupling of size-exclusion chromatography and normal-phase liquid chromatography to high-resolution gas chromatography. J. High Resolut. Chromatogr. 1997, 20 (3), 125−130.
(13) Lobodin, V. V.; Rodgers, R. P.; Marshall, A. G. Petroleomics and the analysis of complex organic mixtures with Fourier transform ion cyclotron resonance mass spectrometry. In Comprehensive Environmental Mass Spectrometry, Vol. 2; International Labmate Limited, Hertfordshire, U.K., 2012; Chapter 18, pp 415−442.
(14) Rodgers, R. P.; Marshall, A. G. Petroleomics: Advanced Characterization of Petroleum-Derived Materials by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS). In Asphaltenes, Heavy Oils, and Petroleomics; Mullins, O. C., Ed.; Springer: New York, 2007; pp 63−93.
(15) Li, M. W.; Larter, S. R.; Frolov, Y. B.; Bjoroy, M. Adsorptive Interaction between Nitrogen-Compounds and Organic and/or Mineral Phases in Subsurface Rocks—Models for Compositional Fractionation of Pyrrhotic Nitrogen-Compounds in Petroleum during Petroleum Migration. J. High Resolut. Chromatogr. 1994, 17 (4), 230−236.
(16) Marshall, A. G.; Rodgers, R. P. Petroleomics: The next grand challenge for chemical analysis. Acc. Chem. Res. 2007, 40 (1), 53−59.
(17) Terra, L. A.; Filgueiras, P. R.; Tose, L. V.; Romao, W.; de Souza, D. D.; de Castro, E. V. R.; de Oliveira, M. S. L.; Dias, J. C. M.; Popping, R. J. Petroleomics by electrospray ionization FT-ICR mass spectrometry coupled to partial least squares with variable selection methods: prediction of the total acid number of crude oils. Analyst 2014, 139 (19), 4908−4916.
(18) Panda, S. K.; Andersson, J. T.; Schrader, W. Mass-spectrometric analysis of complex volatile and nonvolatile crude oil components: A challenge. *Anal. Bioanal. Chem.* 2007, 389 (5), 1329–1339.

(19) Mullins, O. C. The Asphaltenes. *Annu. Rev. Anal. Chem.* 2011, 4, 393–418.

(20) Bisht, H.; Reddy, M.; Malvanker, M.; Patil, R. C.; Gupta, A.; Hazarika, B.; Das, A. K. Efficient and Quick Method for Saturates, Aromatics, Resins, and Asphaltenes Analysis of Whole Crude Oil by Thin-Layer Chromatography-Flame Ionization Detector. *Energy Fuels* 2013, 27 (6), 3006–3013.

(21) Khan, S. A.; Sarfraz, S.; Price, D. TLC-FID Calibration and Accurate Weight Determination of SARA Fractions in Heavy Crude Oil. *Pet. Sci. Technol.* 2012, 30 (23), 2401–2406.

(22) Anderson, D. M.; Mills, D.; Spraggins, J.; Lambert, W. S.; Calkins, D. J.; Schey, K. L. High-resolution matrix-assisted laser desorption ionization–imaging mass spectrometry of lipids in rodent optic nerve tissue. *Mol. Vision* 2013, 19, 581–592.

(23) Cho, Y.; Witt, M.; Kim, Y. H.; Kim, S. Characterization of Crude Oils at the Molecular Level by Use of Laser Desorption Ionization Fourier-Transform Ion Cyclotron Resonance Mass Spectrometry. *Anal. Chem.* 2012, 84 (20), 8587–8594.

(24) Smaniotti, A.; Montanari, L.; Flego, C.; Rizzi, A.; Ragazzi, E.; Seraglia, R.; Traldi, P. Can crude oils be distinguished by different component distribution obtained by laser desorption ionization mass spectrometry and evaluated by chemometrics? *Rapid Commun. Mass Spectrom.* 2008, 22 (10), 1597–1606.

(25) Santos, V. G.; Fasciotti, M.; Pudenzi, M. A.; Klitzke, C. F.; Nascimento, H. L.; Pereira, R. C. L.; Bastos, W. L.; Eberlin, M. N. Fullerenes in asphaltenes and other carbonaceous materials: natural constituents or laser artifacts. *Analyst* 2016, 141 (9), 2767–2773.

(26) Eijkel, G. B.; Kukker Kaletas, B.; van der Wiel, I. M.; Kros, J. M.; Luider, T. M.; Heeren, R. M. A. Correlating MALDI and SIMS imaging mass spectrometric datasets of biological tissue surfaces. *Surf. Interface Anal.* 2009, 41 (8), 675–685.

(27) Chirinos, J.; Oropesa, D.; Gonzalez, J.; Ranaudo, M.; Russo, R. E. Determination of Vanadium/Nickel Proportionality in the Asphaltene Fraction of Crude Oil Using Thin-Layer Chromatography with Femtosecond Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry. *Energy Fuels* 2013, 27 (5), 2431–2436.

(28) Chacon-Patino, M. L.; Blanco-Tirado, C.; Orrego-Ruiz, J. A.; Gomez-Escudero, A.; Combariza, M. Y. High Resolution Mass Spectrometric View of Asphaltene-SiO2 Interactions. *Energy Fuels* 2015, 29 (3), 1323–1331.

(29) Kim, E.; Cho, E.; Moon, S.; Park, J. I.; Kim, S. Characterization of Petroleum Heavy Oil Fractions Prepared by Preparatory Liquid Chromatography with Thin-Layer Chromatography, High-Resolution Mass Spectrometry, and Gas Chromatography with an Atomic Emission Detector. *Energy Fuels* 2016, 30 (4), 2932–2940.

(30) Fuhr, B. J.; Hawrelechko, C.; Holloway, L. R.; Huang, H. B. Comparison of bitumen fractionation methods. *Energy Fuels* 2005, 19 (4), 1327–1329.

(31) Carbognani, L.; Gonzalez, M. F.; Pereira-Almao, P. Characterization of athabasca vacuum residue and its visbroken products. Stability and fast hydrocarbon group-type distributions. *Energy Fuels* 2007, 21 (3), 1631–1639.

(32) Bisht, H.; Reddy, M.; Malvanker, M.; Patil, R. C.; Gupta, A.; Hazarika, B.; Das, A. K. Efficient and Quick Method for Saturates, Aromatics, Resins, and Asphaltenes Analysis of Whole Crude Oil by Thin-Layer Chromatography—Flame Ionization Detector. *Energy Fuels* 2013, 27 (6), 3006–3013.

(33) Bissada, K. K.; Tan, J.; Szymczyk, E.; Darnell, M.; Mei, M. Group-type characterization of crude oil and bitumen. Part I: Enhanced separation and quantification of saturates, aromatics, resins and asphaltenes (SARA). *Org. Geochem.* 2016, 95, 21–28.

(34) Karsen, D. A.; Larter, S. R. Analysis of Petroleum Fractions by Tlc-Fid - Applications to Petroleum Reservoir Description. *Org. Geochem.* 1991, 17 (5), 603–617.

(35) Holm, T. Aspects of the mechanism of the flame ionization detector. *J. Chromatogr A* 1999, 842 (1–2), 221–227.