Effect of Sample Preparation on the Characterization of Crude Oil and its Complex Fractions by High Resolution Mass Spectrometery

Dissertation

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Sami Lababidi
aus
Damaskus

Fakultät für Chemie
der
Universität Duisburg-Essen

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Prof. Dr. Oliver Schmitz
Vorsitzender: Prof. Dr. Mathias Ulbricht

“Anybody who has been seriously engaged in scientific work of any kind realizes that over the entrance to the gates of the temple of science are written the words: Ye must have faith. It is a quality which the scientist cannot dispense with.”

Max Planck

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Abstract

The dependency of the modern society on affordable energy resources, which fuels the economy and social development, is growing. The rapid rise of prices on the world's oil markets in the last years clearly demonstrates that a better exploitation of the remaining supplies becomes distinctively more valuable. Despite the rather expensive chemical work-up procedures to yield clean and useful energy supplies, higher oil prices push the exploitation of unconvensional oils. Heavier crude oils and the higher boiling fractions of standard crude oils have thus become important resources to the expense of significantly elevated impurities levels, *e.g.*, sulfur-, nitrogen-, oxygen- or metal-compounds. Many of these compounds have adverse effects on processing as well as on performance of the final products. The optimization of catalytic refinement processes is thus heavily dependent on the quality of the chemical characterization of the raw material. In this regard, Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) has proven to provide analytical data of sufficient accuracy and resolution. Nevertheless, even with the ultimately high resolving power of FT-ICR MS, the accurate mass spectrometric analysis of crude oil components relies on appropriate ionization and separation methods. The role of sample preparation is immense in this regard. Without simplification of the complex crude oil sample it is practically impossible to analyze the sample even with high resolution mass spectrometry by one-shot-measurement approach. FT-MS instruments operate best when smaller number of ions is present during each transient. This cannot be accomplished in the analysis of crude oil samples without simplification of the sample. This study is focusing on the investigation of sample preparation methods which simplify the complexity of crude oil samples and at the same time show selectivity towards crude oil classes. Combining these analytical approaches with ultrahigh resolution mass spectrometry gives indispensable data that lead to the elemental composition of the sample components. Accordingly, statistical information that allows characterization of the compounds is gained. Extraction methods and chromatographic separations are applied in this study to analyze and characterize complex crude oil samples using high resolution FT-MS instruments.

Kurzfassung

Die Abhängigkeit der modernen Gesellschaft auf verfügbare Energie-Ressourcen wächst. Gleichzeitig, der rasante Anstieg der Preise auf den weltweiten Öl-Märkte in den letzten Jahren zeigt deutlich, dass eine bessere Ausnutzung der restlichen Quellen deutlich wichtiger wird. Trotz der eher teuren chemischen Aufarbeitungsmethoden, um saubere und nützliche Energieversorgung zu erzielen, wird die Aufarbeitung von unkonvetionellen Rohölen notwendig. Anderseites, haben höher siedenden Fraktionen von Standard Rohöle und schwere Rohöle erhöhten Konzentrationen von Heteroatomen, z. B. Schwefel-, Stickstoff-, Sauerstoff-oder Metall-Verbindungen. Viele dieser Verbindungen haben nachteilige Auswirkungen auf die Verarbeitung sowie auf die Leistung der Endprodukte. Die Optimierung der katalytischen Raffinierungsverfahren ist daher stark abhängig von der Qualität der chemischen Charakterisierung der Rohstoffe. In diesem Zusammenhang hat Fourier-Transform-Ionenzyklotronresonanz-Massenspektrometrie (FT-ICR MS) als analytische Methode bewiesen, dass die resultierenden Daten von ausreichender Genauigkeit und Auflösung sind. Dennoch, auch mit der letztlich hohen Auflösungsstärke des FT-ICR MS, beruht die genaue massenspektrometrische Analyse von Rohölkomponenten auf geeigneten Ionisationsmethoden und Trennverfahren. Die Rolle der Probenvorbereitung ist immens in dieser Hinsicht. Ohne Vereinfachung der komplexen Rohöl Probe ist es praktisch unmöglich, die Probe auch mit hochauflösender Massenspektrometrie einer einzelnen Messung zu analysieren. FT-MS Geräte arbeiten am besten, wenn eine kleinere Anzahl von Ionen bei jedem Transient verfügbar ist. Dies kann nicht in die Analyse von Rohöl Proben ohne Vereinfachung der Probe durchgeführt werden. Diese Studie basiert auf der Untersuchung der Probenvorbereitungsmethoden, die die Komplexität von Rohöl Proben vereinfacht und zur gleichen Zeit die Selektivität gegenüber Rohöl Klassen zeigt. Die Kombination dieser analytischen Ansätze mit höchster Auflösung der Massenspektrometrie gibt unverzichtbare Daten, die zur elementaren Zusammensetzung der Bestandteile der Probe führen. Dementsprechend werden statistische Informationen, die die Charakterisierung der Verbindungen ermöglicht, gewonnen. Vor allem, werden mit Hilfe hoch auflösende FT-MS Instrumente Extraktionsverfahren und Chromatographische Trennung in dieser Studie eingesetzt, damit komplexe Erdölproben analysiert und charakteriziert werden können.

Contents

Chapter 1 General Introduction

Crude oil, also known as petroleum, is the term used to describe a myriad of hydrocarbon-rich fluids that have accumulated in subterranean reservoirs [1]. The word *petroleum* derives from the Greek words *petra*, meaning rock, and *oleum*, denoting oil, which combined literally means *rock-oil*. The term petroleum was first used by the German mineralogist Georgius Agricola (1546) in the treatise *De Natura Fossilium*. The ancient Greek word *naphtha* was often used to describe any petroleum-like substance and in older texts was often used as a synonym for petroleum. Some languages, such as Arabic, still use variants of naphtha as the word for petroleum.

More than a century and a half after its discovery, crude oil continues to play an essential role in the global economy. Despite the change in the global energy map and the continuous rapid growth in the use of wind and solar technologies, fossil fuels remain the dominant source of energy [2]. In one of the expectations, oil demand reaches 99.7 millions of barrels per day (mb/d) in 2035, up from 87.4 mb/d in 2011 with China, India and the Middle East accounting for 60% of the increase [2]. On the other hand, the world oil reserves are being depleted at an annual rate of 2.1%, on the basis of 2005 world crude oil production and current recovery techniques [3].

Hence, a surge into unconventional oil supplies is anticipated [4]. Examples of unconventional oil resources include oil shale [5], oil sands [6, 7], and extra heavy oils [8] (see Figure 1-1). Other unconventional fuels are produced by chemical processes such as gas to liquids (GTLs), coal to liquids (CTLs), which liquefies coal using Fischer-Tropsch process for instance, and biofuels [9]. For the upgrading of unconventional oil resources into petroleum, development in chemical processes has to be achieved [10, 11]. However, a rapid expansion of non-conventional production is extremely challenging because of numerous technical, economic and environmental constraints. One of the major technical problems encountered in unconventional oils is the high viscosity of the feeds making them resistant to flow [12]. Additional important technical processing challenge is the higher content of heteroatoms in heavy crudes for instance in comparison with lighter crudes. Therefore, understanding the composition and chemical properties of these heavy oils is important to meet future energy needs.

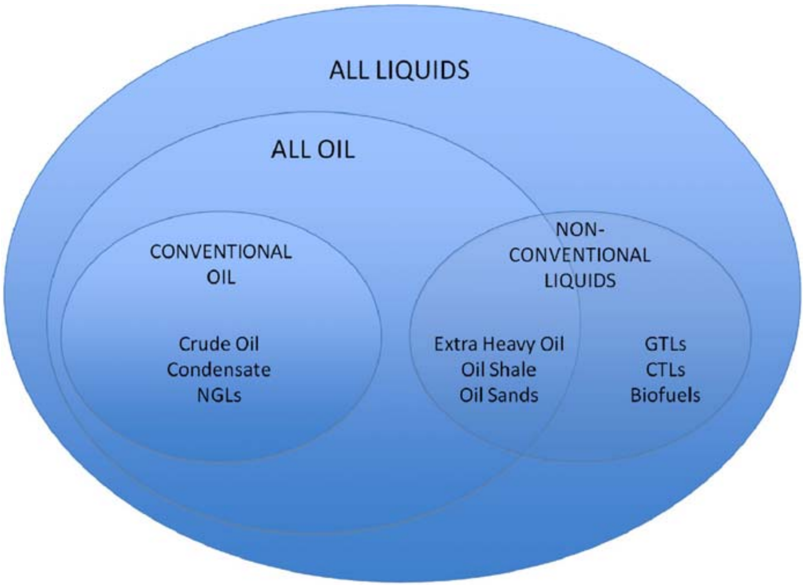

Figure 1-1: Classification of hydrocarbon liquids [9].

1.1 Crude Oil Composition

The composition of a crude oil correlates closely to its origin. The structures of hydrocarbons vary in the complex mixture of crude oil from alkanes and naphthenes to aromatic compounds. Alkanes (C_nH_{2n+2}) are saturated hydrocarbons composed entirely of straight or branched alkyl chains. Naphthenes are saturated hydrocarbon ring structures that may have varying degrees of alkyl substitution. Aromatic hydrocarbons contain one or more conjugated five- to six-carbon member rings, such as benzene or naphthalene and may be bonded to naphthenic rings and alkyl side chains [1].

Heteroatoms refer mainly to the organic compounds of nitrogen, oxygen, and sulfur beside trace metals such as nickel, vanadium and iron. Despite heteroatoms only account for less than 15% by weight of the heavy crude oil composition, but they are responsible for numerous problems in production and refining [13]. Examples of these problems include acid rain formation as result of the combustion of fuels with high sulfur content and the poisoning of catalyst in chemical processes in refineries as result of the presence of nitrogen containing compounds in feedstocks [14]. These compounds are the focus of this dissertation and will be discussed in more detail throughout subsequent chapters. Figure 1-2 illustrates examples of heteroatomic compounds found in crude oils, where **R** represents one or multiple alkyl chains of different lengths and attaching positions to the alicyclic or aromatic ring core.

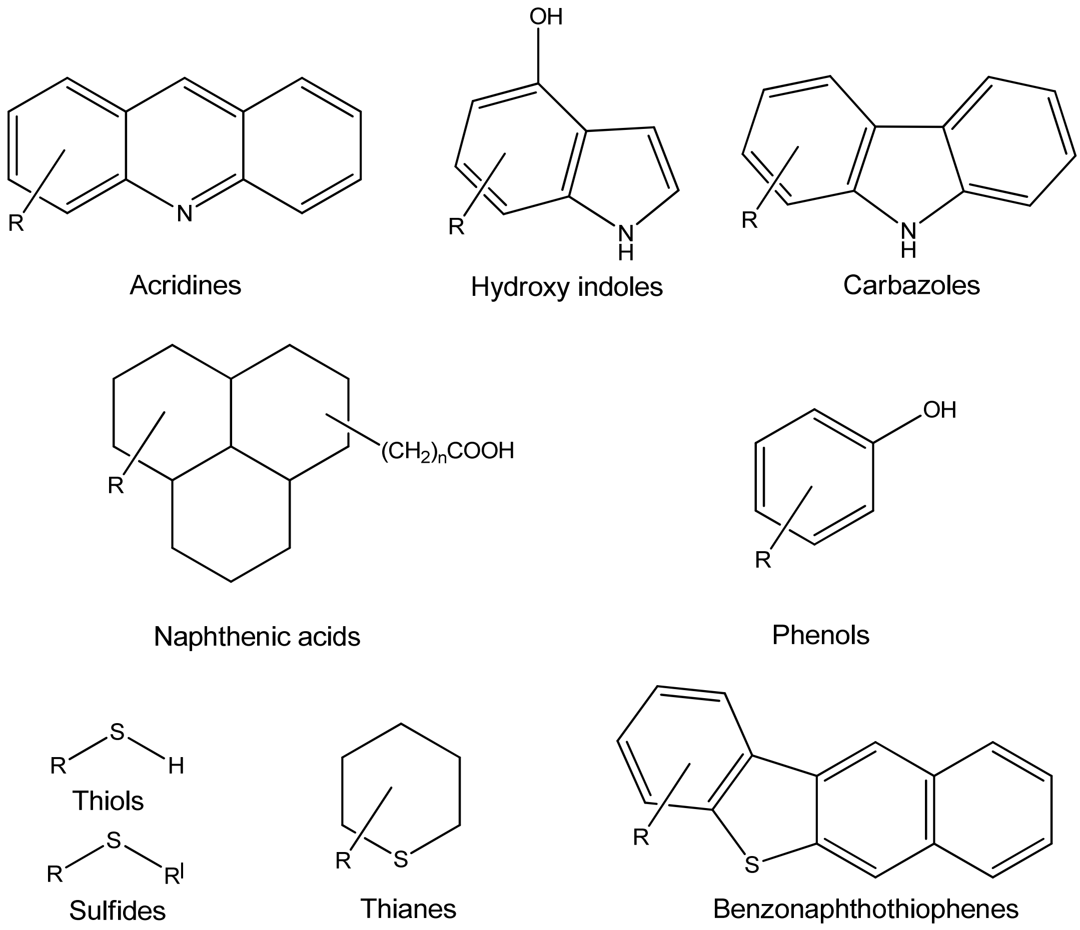

Figure 1-2: Examples of heteroatomic compounds present in crude oils.

Nitrogen-containing compounds in crude oils can be classified as basic and nonbasic including neutrals [1]. The nonbasic nitrogen compounds are usually pyrrole, indole, and carbazole types, whereas basic nitrogen compounds are composed mainly of pyridine derivatives. Nitrogen species occur throughout the boiling point ranges, but have a tendency to exist in higher abundance in the high boiling point fractions and residues [1]. The nitrogen content of crude oils tends to fall in the range of 0.1% - 0.9%.

Oxygen species in crude oil comprises less than 2% by weight, and they are found mostly in the form of phenols [15] and carboxylic acids [16]. The latter compounds, especially in the form of naphthenic acids have significance because of their use as markers in geochemistry and because of their corrosivity to refinery units [17].

Sulfur compounds are the most abundant of the heteroatomic species in crude oils and are broadly distributed across the range of crude oil fractions. Most of the sulfur compounds in crude oils belong to thiophene and sulfide derivatives [1]. The total sulfur in crude oil can vary from 0.05% up to about 14% by weight in the heaviest forms of crude oil [18]. However, oils containing more than 1% by weight are considered sulfur-rich oils (sour crudes), which are more difficult to process and turn into usable refined products than low-sulfur oils (sweet crudes). Sulfur is removed from crude oils in a process called hydrodesulfurization (HDS) [19].

Crude oil is considered as one of the most complex mixture in the nature. In order to examine crude oil composition properly, fractionation techniques have been applied, since prior simplification increases the amount of compositional information gained as a step towards thorough characterization of individual molecular types. The fractionation methods used in crude oil industry are aimed to separate the constituents without alteration of their molecular structure. Thus, for instance, fractionation techniques depending on intrinsic properties of the constituents, such as boiling points and the adsorption behavior on active surfaces, were designed to separate the constituents according to their molecular size and molecule type [13, 20, 21].

1.2 Crude Oil Fractionation

Distillation has been a common mean for the fractionation of crude oils since the beginning of crude oil analysis. Hence, terms such as *light* and *heavy* are often used to describe different types of crude oils. Light crude is usually rich in low boiling constituents, whereas heavy crude have components of higher boiling points. Generally, hydrocarbons in crude oil have boiling points ranging -160 °C to 600 °C [22]. In a process known as thermal cracking the carbon bonds of the heavier components begin to break at temperatures of approximately 350 °C, therefore, components that have higher boiling points are transferred from atmospheric distillation chambers to vacuum distillation towers [22]. The molecular size and complexity of crude oil components are directly related to the boiling point range. However, this method of classification is not directly related to composition.

Another typical classification of crude oils is based on the solubility of different types of compounds in various solvents. SARA-separation is an example of such classification, separating the crude oils in four main chemical classes based on differences in solubility and polarity [23-26]. The four SARA-fractions are Saturates, Aromatics, Resins, and the Asphaltenes. In this procedure the asphaltene fraction is collected as the insoluble fraction from n-alkane, typically n-pentane (C5-asphaltene) or n-heptane (C7-asphaltene). The remaining soluble fraction, which is called maltenes, is further separated by chromatography on a silica and/or an alumina packed column [27]. The non-polar nature of the saturate fraction makes them soluble in most alkanes. The aromatic and resin fractions, which are partially composed of polar NSO-containing species, are separated successively by increasing the polarity of the eluents. Asphaltenes is the most aromatic and heteroatom-rich fraction of crude oil and comprises of condensed aromatic and naphthenic molecules [28].

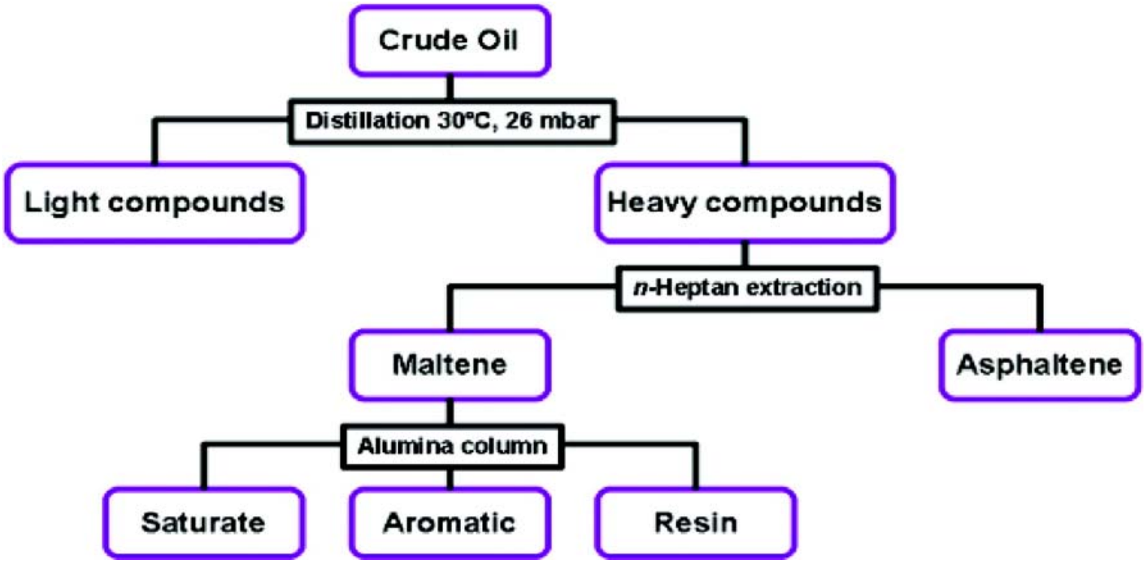

Figure 1-3: SARA fractionation according to the solubility of each fraction [29].

1.3 Crude Oil Analysis

Various methods have been introduced and developed for a complete characterization of the components in a crude oil and its fractions. The higher the boiling point of a fraction, the more difficult it is to analyze its composition due to the increased complexity. Higher molecular weight species found in higher boiling point fractions have more carbon atoms per structure and therefore the number of structural rearrangements increases rapidly [13]. A single analytical technique can be applied to determine molecular composition of low boiling fractions, which have reduced complexity [30, 31]. Conversely, the complexity of higher boiling fractions pushes the analytical techniques to their limits in order to elucidate compositional information. A variety of analytical techniques have been employed to examine the structure of crude oil constituents. Examples of these techniques include: gas chromatography (GC) [32-34], two-dimensional gas chromatography (GC X GC) [35], gas chromatography/mass spectrometry (GC/MS) [36], different liquid chromatographic (LC) techniques (such as high performance liquid chromatography (HPLC) with different stationary phases [37-39], gel permeation chromatography (GPC) [40]), near-infrared spectroscopy (NIR) [41], fluorescence spectroscopy [42], nuclear magnetic resonance (NMR) [43] and X-ray scattering techniques; such as X-ray absorption near edge structure (XANES) and X-ray photoelectron spectroscopy (XPS) [44-46]. Boduszynski et al published a series of papers with comprehensive characterization of heavy crude oils by a variety of analytical methods including HPLC, ^{13}C NMR, vapor pressure osmometry (VPO), and field ionization mass spectrometry (FIMS) [47-50].

Unlike the majority of analytical techniques, high resolution mass spectrometry nowadays is well suited for the analysis of complex mixture such as crude oil, because the number of mass spectral peaks per analyte is of order one, in other words; by using soft ionization methods that avoids fragmentation, each analyte has one signal [51]. Early mass spectrometers based on a magnetic sector had a mass resolving power of about 10,000, which was not sufficient for the characterization of crude oil species. Various mass analyzers have been developed throughout the last century such as quadrupole, ion trap, time-of-flight (TOF), Fourier transform ion cyclotron resonance (FT-ICR) [52], and Orbitrap [53]. Only FT-ICR and long-transient-Orbitrap mass spectrometers are capable to provide the peak capacity needed to resolve individual components of a complex data matrix in combination with the necessary accuracy [54, 55].

Another significant factor in analyzing heavy crude oil samples and fractions using mass spectrometry is the ionization efficiency of large molecules with different ionization sources. Therefore, several methods have been developed to identify whole ranges of compounds (polar and non polar). For the characterization of lighter fractions from crude oil, the electron ionization technique (EI) was employed as the method of choice at the early years of crude oil analysis. For the ionization energy a lower voltage was applied in order to avoid fragmentation of the analytes. Since the introduction of low-voltage electron ionization (LVEI) [56], it has been most frequently used in the crude oil industry. For instance, the speciation of neutral and basic nitrogen compounds from an off-shore Californian crude oil was achieved with LVEI as a source for ions [57]. However, the experiments were performed on a double focusing magnetic sector instrument with a resolution of 10,000. In another attempt to characterize nitrogen and sulfur compounds in gas oil and kerosene samples, an ammonia chemical ionization (CI) was implemented enabling selective speciation of these heterocycles [58]. Chemical formulas of nitrogen, oxygen and sulfur containing compounds found in crude oil distillates were identified by coupling the LVEI source to an FT-ICR mass spectrometer [59]. Field desorption/field ionization was used for the characterization of heavy crude oil fractions and residues [48, 60]. A vast number of nonpolar components from crude oil samples were determined by an FT-ICR mass spectrometer interfaced with a field desorption ion source [61]. Other ionization techniques such as thermospray [62] and matrix assisted laser desorption ionization (MALDI) [63, 64] were applied on a small scale in the field of crude oil analysis. However, samples in the previously mentioned ionization techniques are introduced to the mass spectrometer under vacuum.

On the other hand, atmospheric pressure ionization (API) methods nowadays are more frequently used in the field of crude oil analysis. The most common API sources used in the analysis of crude oil samples are electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), atmospheric pressure photo ionization (APPI) and atmospheric pressure laser ionization (APLI). The applicability of the mentioned ionization techniques depends on the polarity of the analyte.

1.4 Sample Simplification

The presence of tens of thousands of components makes crude oil a very complex mixture. The complexity of crude oil is high enough that even after initial fractionation complex fractions are obtained especially in the high-boiling range [27]. The number of isomers of the alkyl side chains grows enormously with an increase in the number of carbon atoms. For instance, decane ($C_{10}H_{22}$) has 75 isomers, but triacontane ($C_{30}H_{62}$) is estimated to possess over four billion [27]. There is no individual analytical instrument or technique capable to perform a complete analysis of crude oil. Mass spectrometry is a very powerful analytical tool for the characterization of crude oil samples indeed, however, even with the highest resolving mass spectrometer; spectral complexity can hinder correct identification of elemental compositions. Space charge effects, which arise from the influence of the electric field of ions in the trapped analyzer cell upon each other, limit mass accuracy of measurements in FT-ICR mass spectrometry [65]. It is unrealistic to expect individual compounds to be analyzable in such supercomplex mixtures with one single mass spectrometric measurement. FT-MS instruments operate best when smaller number of ions is present during each transient. This cannot be accomplished in the analysis of crude oil samples without simplification of the sample.

1.5 Scope of the Study

This study is focusing on the investigation of sample preparation methods which simplify the complexity of crude oil samples and at the same time show selectivity towards crude oil classes. Combining these analytical approaches with ultrahigh resolution mass spectrometry gives indispensable data that lead to the elemental composition of the sample components. Accordingly, statistical information that allows characterization of the compounds is gained.

In Chapter 3, the importance of a mass analyzer with a high resolving power is highlighted with the application of ultra-high resolution FT-MS instruments for the analysis of a heavy crude oil sample. A new research-grade Orbitrap instrument is introduced that is capable of reaching the necessary benchmarks to study such complex samples as crude oil mixtures. High mass accuracy and mass resolution values are obtained using a long–transient Orbitrap for the study of crude oil samples.

Chapter 4 addresses the role of solvent extraction on the mass spectrometric results of a crude oil deep well deposit. The solid deposit was dissolved in six different solvents covering a wide range of polarity. ESI FT-ICR MS measurements in positive and negative modes provide information about the class and type distribution of polar species in the sample. Population-based data representation is introduced as an alternative to intensity-based data calculation reducing thereby discrimination against low abundant species. The non-complete dissolution of the sample in toluene was indicative that the deep well deposit does not consist entirely of asphaltenes.

Chapter 5 introduces the first reported direct coupling of normal-phase HPLC using a polar aminocyano column to a Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS) for the characterization of a deasphalted crude oil sample using atmospheric pressure laser ionization (APLI) for ionization. The unique selectivity toward aromatic hydrocarbons and the high sensitivity of APLI accompanied with the high resolution of the mass spectrometer gives detailed compositional change in the chromatogram in offline and online measurements. Comparable UV- and ion chromatograms reflect the scan-to-scan sensitivity of the coupling system. Calculated *reconstructed class chromatograms (RCC)* are introduced, which provide along with the corresponding double bond equivalent (DBE) distribution effective and important tools for tracing differences in class composition along the retention time. A differentiation between basic and non-basic species in the same polar peak depending on radical and protonated nitrogen species generated by APLI is described, reflecting the significant role of selective matrix simplification of complex crude oil samples.

Chapter 6 investigates the effects of different ionization methods on the characterization of complex crude oil mixtures by online normal phase HPLC/FT-ICR MS coupling. Each one of the used atmospheric pressure ionization sources: electrospray ionization (ESI), atmospheric pressure photo ionization (APPI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure laser ionization (APLI) provides uniquely assigned components differing in polarity and aromaticity. Thereby, a detailed characterization of the complex sample is achieved.

Chapter 7 characterizes basic nitrogen compounds in crude oil and asphaltenes by combining extraction via metal-ion complexation with positive ESI-FT-MS measurements. Higher carbon number and double bond equivalent (DBE) values of nitrogen species obtained in the extracted samples is compared with a simple electrospray measurement of the sample, showing extra data deconvolution obtained by the extraction procedure.

Finally, the findings of this work are summarized in Chapter 8.

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Chapter 2 Instrumentation and Data Interpretation

2.1 Ionization Techniques

2.1.1 Electrospray

Electrospray ionization (ESI) is one of the most commonly used ionization method in the field of mass spectrometry. Depending on the polarity of the dispersing field, the ionization process in electrospray produces quasi-molecular ions, which are defined as protonated molecules $[M+H]^+$ or ions formed from deprotonated molecules $[M-H]^-$ [1]. The electrospray is created by applying a large potential between the metal inlet needle and the first skimmer in an API source. The electrical charge density at the surface of the droplets increases as solvent evaporates from the droplets until a critical point known as the Rayleigh stability limit. At this critical point, the droplets divide into smaller droplets (*Coulomb explosion*), because the electrostatic repulsion is greater than the surface tension and as a result, short bursts of charges through Taylor cone structures are ejected [1, 2]. Two different models try to explain the mechanism of gas-phase ion formation in electrospray. One of them is called *ion evaporation model*, in which desorption of solvent-ion clusters from the charged droplets happens in response to a rising electric field on the droplet surface [3]. The other one is the *charged residue model*, in which the formation of gas-phase ions is considered as the result of repeated droplet fission when continuous solvent evaporation leads to droplet shrinkage and Rayleigh instability [4].

2.1.2 Atmospheric Pressure Chemical Ionization

Atmospheric pressure chemical ionization (APCI) is a soft ionization technique, which uses gas-phase ion-molecule reactions at atmospheric pressure. In APCI, the analyte in solution is sprayed into fine droplets through the contact with a stream of nebulizing gas, typically nitrogen. The resulting droplets are vaporized in a high temperature tube. The analyte compounds and the hot gas are carried along a corona discharge electrode where ionization occurs. The ionization processes in APCI is equivalent to the processes that take place in chemical ionization (CI) but under atmospheric pressure. In the positive ion mode, either proton transfer or adduction of reactant gas ions can occur to produce the ions of molecular species, depending on the relative proton affinities of the reactant ions and the gaseous analyte molecules. In the negative mode, the ions of the molecular species are produced by either proton abstraction or adduct formation [5].

2.1.3 Atmospheric Pressure Photo Ionization

Atmospheric pressure photo ionization (APPI) involves the use of a vacuum gas discharge lamp; often krypton lamp is used, as a source of photons responsible for the ionization of analytes. Most organic molecules have ionization potentials in the range of 7-10 electron-volts (eV) [6], thus, molecular ions from most organic molecules can be formed by using discharge lamps of 10 eV or more. In APPI, the sample is mixed with a nebulizing gas, typically nitrogen, inside a heated sprayer. The resulting vapor exits the chamber and passes orthogonal to the UV lamp, where photo ionization occurs at atmospheric pressure. The absorption of a photon by a molecule causes the ejection of an electron forming a molecular radical cation. Direct photo-ionization of the analyte can happen when the photon energy is greater than the ionization potential (IP) of the molecule. However, the photons collide with gases and other molecules found in the atmosphere as well forming radical cations, which are subjected to subsequent charge exchanges with the analyte. The sensitivity in APPI is increased by adding a dopant, such as toluene, which enhances proton-transfer and charge-exchange reactions [6]. In dopant assisted APPI, photons first react with the dopant molecule which then undergoes charge exchange or proton transfer reactions with the analyte.

2.1.4 Atmospheric Pressure Laser Ionization

Atmospheric pressure laser ionization (APLI) is the newest developed technique among atmospheric pressure ionization methods. APLI is a two-photon resonance-enhanced multiphoton ionization (REMPI) method using fixed frequency laser radiation [7]. REMPI technique typically uses a stepwise resonant excitation of a molecule via stable intermediate energy levels. In the first step, a molecule is brought into an electronically excited state by the absorption of one photon. A second photon can be absorbed in the lifetime of the first reached electronic state forming a radical cation. The required power density has to be sufficiently high to ensure reaching the ionization potential. However, these requirements are ensured with an appropriate pulsed laser system. The source of photons in APLI is typically a KrF* excimer gas laser radiating at the wavelength of 248 nm corresponding to a photon energy of around 5 eV. Sensitive and selective analyses of polyaromatic compounds, with and without heteroatoms were reported in different APLI-MS measurements [7].

2.2 Mass Analyzers

2.2.1 Quadrupole Ion Trap

The quadrupole ion trap consists of a ring shaped electrode with curved caps on the top and bottom. Ions are injected from the source through one of the caps, and by applying a combination of voltages to the ring and capping electrodes, the ions can be trapped in a three-dimensional trajectory. The electric field is constructed in such a way that the force on an ion is proportional to its distance from the center of the trap. As a cooling gas, a constant low flow of helium is maintained in the cell to remove excess energy from the ions, which would otherwise repel each other to the extent that their trajectories became unstable, causing loss of ions from the trap. Once the ions are trapped, the electrode potentials can be manipulated so that the motion of the ions become unstable in order of their mass-to-charge ratios and they are ejected from the trap into an external detector, a process called mass-selective ejection. Mass analysis in this way takes less than one tenth of a second, and the resolution obtained is comparable to that of a quadrupole [8]. A prerequisite of this method of mass selective ion ejection is that ions be herded initially to the centre of the ion trap under the action of momentum-dissipating collisions; helium atoms are used for this purpose [9].

A linear quadrupole ion trap is similar to a quadrupole ion trap, but it traps ions in a two dimensional quadrupole field, instead of a three-dimensional quadrupole field as in a 3D quadrupole ion trap [10]. A set of quadrupole rods is used to confine ions radially and static electrical potential on-end electrodes to confine the ions axially [11]. Increased ion storage capacity and fast scan times are obtained by linear quadrupole ion traps. The introduction of a scanning linear quadrupole ion trap in front of an FT-ICR instrument has been lately incorporated in commercial mass spectrometer (like LTQ-FT mass spectrometers from Thermo Scientific). This configuration provides rapid and automated MS and MS/MS analyses with substantially improved internal scan dynamic range, mass measurement accuracy, mass resolution, and detection limits [12]. Combing fast scan rate with high resolution makes this hybrid system compatible for LC/MS measurements.

2.2.2 Fourier Transform Ion Cyclotron Resonance

Fourier-transform ion cyclotron resonance (FT-ICR) mass spectrometers offered higher resolution and mass accuracy more than any other mass analyzer [13]. The general principle of FT-ICR is described in detail in two reviews by Marshall et al [14, 15]. In FT-ICR mass spectrometers, a superconducting magnet is providing a strong magnetic field that allows trapping ions in a small sample cell. A cyclotron cell consists of three pairs of adjacent electrodes (plates) that are assembled like a cube or, in other cell designs, like a cylinder. The front and end plate work as trapping plates to trap the ions inside the cyclotron cell. The two excitation plates are connected with a radio-frequency (RF) transmitter to ‘‘excite’’ the ions and the detection plates register the induced mirror current of the ions for detection. In general, ICR spectrometry is based on the principle of cyclotron motion in a uniform magnetic field.

Specifically [1], an ion moving in the presence of a spatially uniform magnetic field is bent into a circle by a Lorentz force F_L that is perpendicular to the ion motion and the magnetic field as shown in the Equation:

$$F_L = z\,(\boldsymbol{v} \times \mathbf{B}) \tag{2-1}$$

where z is the charge of the ion, $\boldsymbol{v}$ is the velocity, and $\mathbf{B}$ is the magnetic field strength.
An ion of mass m and charge z travelling at a velocity $\boldsymbol{v}$ in a direction perpendicular to homogenous magnetic field $\mathbf{B}$ will follow a circular path of radius r that fulfils the condition of equilibrium of Lorentz force $F_\mathbf{L}$ and centripetal force $F_\mathbf{c}$:

$$z\,v\,B = m\,v^2 / r \tag{2-2}$$

$$r = m\,v / z\,B \tag{2-3}$$

Angular velocity is equal to

$$v = \omega\, r \tag{2-4}$$

where ω is the angular frequency.

Substitution of Eq. (2-3) in Eq. (2-4) and simplification produces Eq. (2-5):

$$\omega = z\,B / m \tag{2-5}$$

Regardless of velocity, all ions of a given mass-to-charge ratio *m/z* have the same frequency. This property makes ion cyclotron resonance (ICR) more useful for mass spectrometry, because translational energy "focusing" is not essential for the precise determination of *m/z*. Another form of the cyclotron equation is given n Eq. (2-6):

$$\nu_c = \omega / 2\pi = 1.535611 * 10^7\,B / (m/z) \tag{2-6}$$

where ν_c is the cyclotron frequency in Hertz, B is the magnetic field strength in Tesla, *m* is the ion mass in Dalton and *z* is multiples of elementary charge.

Ion cyclotron motion alone is not useful, as the cyclotron radius is too small to be detected. The ions are thus ''stored'' inside the cyclotron cell. If an RF pulse is sent to the cell the ions will gain energy when the RF field is equal to the cyclotron (resonance) frequency of the ion; they therefore move into a bigger orbit. The RF frequency is transmitted by the excitation plates of the cyclotron cell. Most instruments use an RF sweep that covers the range between several kilohertz and the lower megahertz range to accelerate the ions trapped inside the cell. This sweep pulse is called a chirp [14].

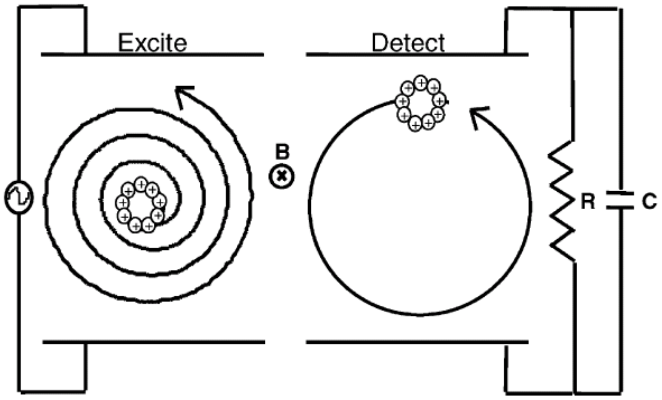

Figure 2-1: Scheme of the ICR excitation and detection [15].

After this energy uptake the ions circulate the cell in the higher orbit, thus getting closer to the detection plates and inducing a stronger electrical ‘‘mirror current’’; this signal is subsequently amplified by the electronics. The circular rotation produces a signal from all ions at approximately the same orbit, but at different frequencies for each ion which, with time, loses energy and drops back to a lower orbit. The transient signal from all the ions is then digitized and processed with a fast Fourier transform algorithm, converting the time domain data to frequency domain spectrum resulting in a ‘‘traditional’’ mass spectrum with ion abundance versus *mass-to-charge* ratio *(m/z).*

2.2.3 Orbitrap

A relatively new and already commercially available mass analyzer, invented by Makarov et al., is called Orbitrap [16]. It consists of an outer barrel-like electrode and a coaxial inner spindle-like electrode that form an electrostatic field with quadro-logarithmic potential distribution [17]. The ions are injected perpendicular to the z-axis and trapped in orbits, due to the balance between the electrostatic attraction to the inner electrode and the centrifugal forces. Since the ions are injected at a point displaced from z = 0, they move along the z-axis of the orbitrap. These oscillations can be detected and transformed into a mass spectrum via Fourier transfomation. Orbitrap mass analyzers enable measurements with high resolution and high mass accuracy near to what can be obtained by FT-ICR mass spectrometers, however, without needing liquid helium to cool the superconductive magnet.

A new research-grade high-field Orbitrap hybrid mass spectrometer, which allows 3-second-transient is used for characterization of complex mixtures. Its application on a heavy crude oil sample is explained in Chapter 3.

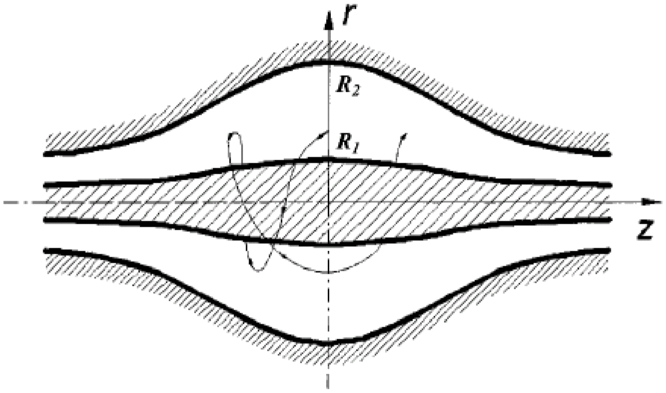

Figure 2-2: Example of a stable ion trajectory in the orbitrap [17].

2.3 Data Interpretation

2.3.1 Definitions

Mass Resolution

Generally peaks obtained from mass spectrometers are Gaussian in shape and the Resolution (R) definition is R = $m/\Delta m$, where m is the mass of an ion peak, but Δm is generally calculated in three different ways: (a) 10 % valley definition, (b) 5 % valley definition and (c) full width of the peak at half maximum intensity (FWHM) [1]. Resolution based on FWHM is commonly used for FT-MS instruments.

Mass Accuracy

The mass accuracy of a spectrometer is the difference observed between the calculated mass of an ion and its observed mass expressed relative to the observed mass. It is usually reported in parts per million (ppm). Generally, instruments capable of providing a mass accuracy of 2 ppm or better are chosen for accurate mass measurements. Moreover, the trend is shifting towards parts per billion (ppb) levels with the advent of modern mass spectrometers.

2.3.2 Introduction

With high resolving power and high mass accuracy of the recent Fourier transform mass spectrometers it is possible to obtain molecular formula assignments of the detected species. For instance, C_3/SH_4, $^{13}C/CH$, CH_2/N, and other mass doublets encountered in crude oil samples were baseline resolved leading into the speciation of heteroatomic species, which are unobservable by other mass analyzers [18]. Since the ion charge (z) can be determined in high resolution mass spectrometry by the spacing between isotopic peaks [19, 20], it has to be noticed that almost all species detected in crude oil analysis by mass spectrometry are singly charged. This is evident by the frequent 1 Da spacing between each monoisotopic species and its corresponding nuclide containing one ^{13}C-atom [21]. Thus, the peaks can be denoted by their mass in Daltons (Da) rather than as *mass-to-charge* ratio (m/z). Although high resolution mass spectrometers deliver the best accurate and resolved results in mass spectrometric measurements, it is necessary to have preliminary knowledge of constituent elements, which enables the assignment of exact compositions of species found in fossil fuels. Previous investigations using elemental analysis restricts the elements to C, H, N, O, and S as major constituents with the presence of V, Ni, and Fe. However, the latter elements are found only in heavy fractions and in very low concentration (less than 300 ppm) [22]. Therefore, data interpretation of the measured crude oil samples considers the first mentioned group of elements with their corresponding isotopes as composition matching constraints.

Tens of thousands of different compounds can be resolved and identified from a single crude oil sample by means of high resolution mass spectrometry. Due to the presence of an enormous number of isomers it is practically impossible to analyze each individual molecular species in heavy crude oil samples [23]. Therefore, the analysis of a complex mixture, such as a crude oil sample, present challenges in terms of data analysis and visualization [24].

2.3.3 Z number and Double Bond Equivalents

In order to obtain detailed compositional information about the complex crude oil samples, a strategy based on group-type analysis is quite helpful. One criterion that defines the grouping of the compound series is the hydrogen deficiency, which is based on the number of hydrogen atoms relative to the number of carbon atoms, i.e., in $C_nH_{2n+z}X$ as a general formula; where z is the "hydrogen deficiency" relative to mono-olefins or one ring naphthenes, n is the number of carbon atoms, X represents heteroatoms such as N, O, and S. For convenience, it can be explained by an example. If we consider a series of molecules, like C_6H_6 (benzene), C_7H_8 (toluene), and C_8H_{10} (xylenes/ethylbenzene), this type of compounds are designated as $z = -6$ series. Each addition of one ring or one double bond will make the z value more negative by 2. This concept was commonly utilized in data interpretation of crude oil samples [25].

Another term is used to describe the degree of hydrogen deficiency is the double bond equivalent (DBE), which is calculated by the sum of the number of rings and double bonds present in a molecule. This index can be determined from the elemental composition, $C_cH_hN_nO_oS_s$, obtained from high resolution mass spectrometric measurements [26, 27]:

$$\text{Double bond equivalents (DBE)} = c - h/2 + n/2 + 1 \tag{2-7}$$

An increase in DBE value reflects an increase in the number of rings and/or double bonds, which indicates to a possible aromatic structure of the system.

The determination of DBE values is quite important for the characterization of hydrocarbon systems in crude oil field [28]. For instance, the information from other techniques such as chromatography, in conjunction with the DBE value can give information on the size of the aromatic system [29].

2.3.4 Kendrick Mass and Kendrick Plots

An FT-ICR mass spectrum of a crude oil sample is one of the most crowded spectra in the field of mass spectrometry. However, a close inspection of the investigated mass spectrum reveals repeated patterns of mass differences throughout the entire mass range. Figure 2-3 depicts two segments of zoomed mass spectrum obtained by positive ion ESI FT-ICR MS measurement of a short residue; a residual of distillation of crude oil under vacuum. A series of repeated signals with 14.0157 Da difference is recognized, which corresponds to the addition of CH_2 units (Figure 2-3, bottom). The periodicity of multiple CH_2 units for the same class (number of heteroatoms N, O, and S) and type (DBE; number of rings and double bonds) of compounds comprises a series reflecting the alkylation state. This property of homologous series is observed in crude oil samples and reflects the degree of carbon number distribution. Another series with a difference of 2.0157 Da (Figure 2-3, top) is observed. The calculated difference is exactly the mass of two hydrogen atoms. Thus, a series consisting of compounds of the same class and carbon number differs only in their type, which can reflect the degree of aromaticity.

The IUPAC mass scale defines ^{12}C as having a mass of exactly 12 Da. Kendrick introduced a mass scale in the early 1960's [30] on the basis of considering CH_2 as having a mass of 14.00000 Da instead of the IUPAC value of 14.01565 Da. The IUPAC mass can be simply converted to Kendrick mass by the following equation:

$$\text{Kendrick mass} = \text{IUPAC mass} \times (14.00000/14.01565) \qquad (2\text{-}8)$$

Addition of CH_2 units will increase the mass by exactly 14. As a result, compounds containing the same heteroatoms (class) and the same number of rings and double bonds (type) will have the same Kendrick mass defect (KMD) in the homologous series.

$$\text{KMD} = (\text{Nominal mass} - \text{Kendrick mass}) \times 1000 \qquad (2\text{-}9)$$

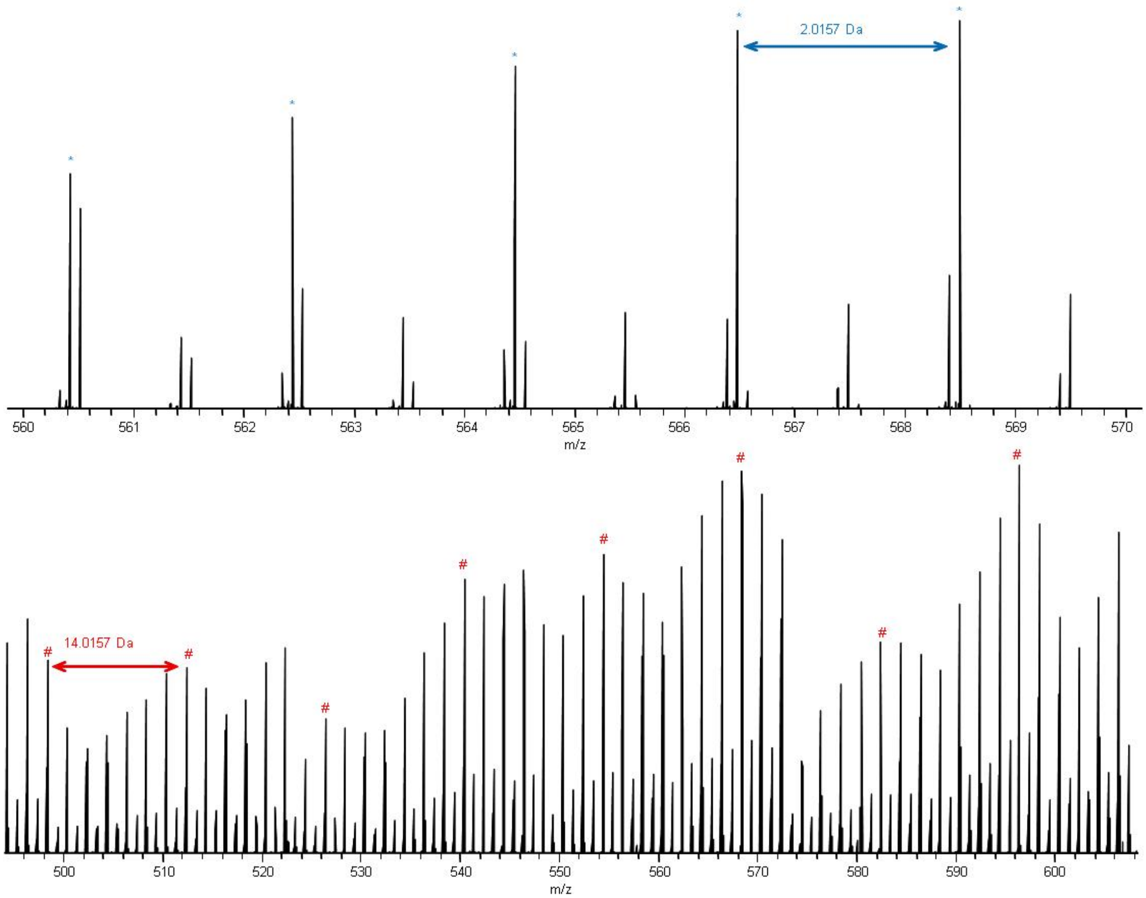

Figure 2-3: Zoom in mass scale of positive-ion ESI FT-ICR MS of a short residue sample (residual of vacuum distillation of crude oil). 14.01565 Da difference (bottom) represent members of a homologous series which differ only in alkylation (CH_2 units) and 2.0157 Da difference represent compounds differing only by two hydrogen atoms, indicative of different aromaticity (DBE values).

A series of alkylcarbazoles, namely, methylcarbazole, ethylcarbazole, and propylcarbazole can be taken as an example for explanation. These compounds share the same class (N_1) and type (DBE = 9; three rings and six double bonds) and differ only in the number of CH_2 units. Thus, all members of a pyridine series, which have the same Kendrick mass defect, can be represented by a single horizontal line in a plot of Kendrick nominal mass versus KMD (see Figure 2-4). If the identity of a data point in a series is known, then adjacent neighbors represent the species with the same hydrogen deficiency and heteroatom content, albeit with one fewer or additional CH_2 group [24].

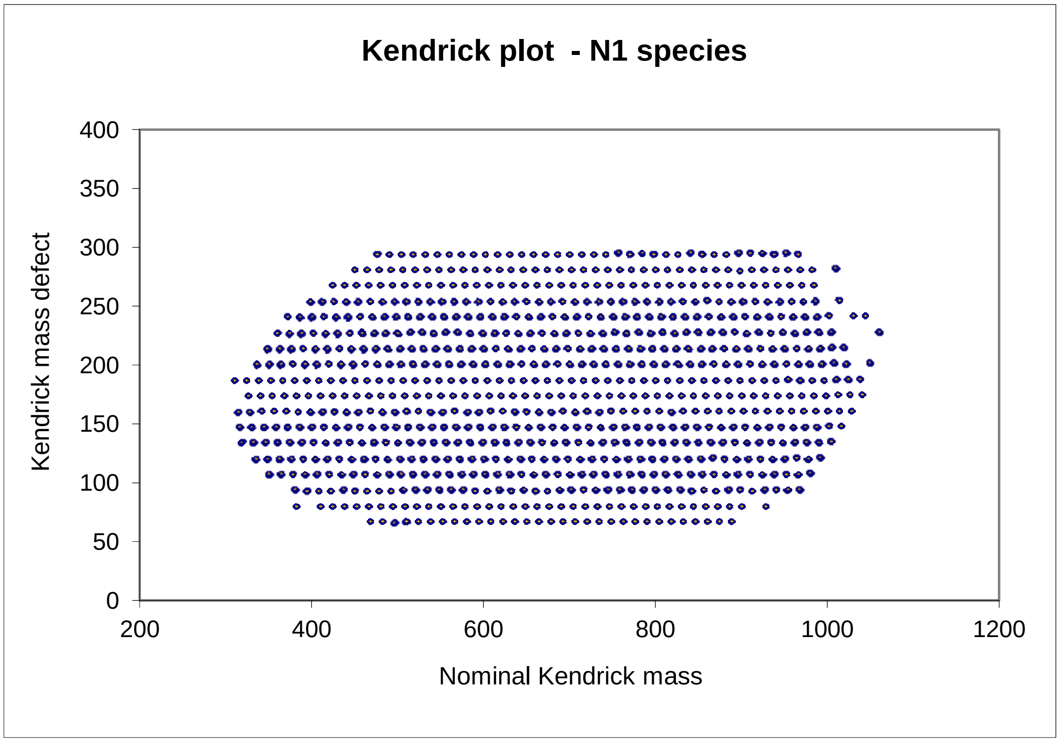

Figure 2-4: Kendrick plot of the N_1 class obtained from the data analysis of Positive ESI FT-ICR mass spectrum of a short residue sample, showing type and carbon number distribution at the same time. The vertical increase in Kendrick mass defect values reflects the increase in aromaticity, whereas the horizontal increase indicates to the addition of CH_2 unit within the same homologous series.

2.3.5 Data Representation

Elemental compositions data obtained from exact mass spectrometric measurements provide three levels of chemical information: class, type, and carbon number distribution as illustrated in Figure 2-5:

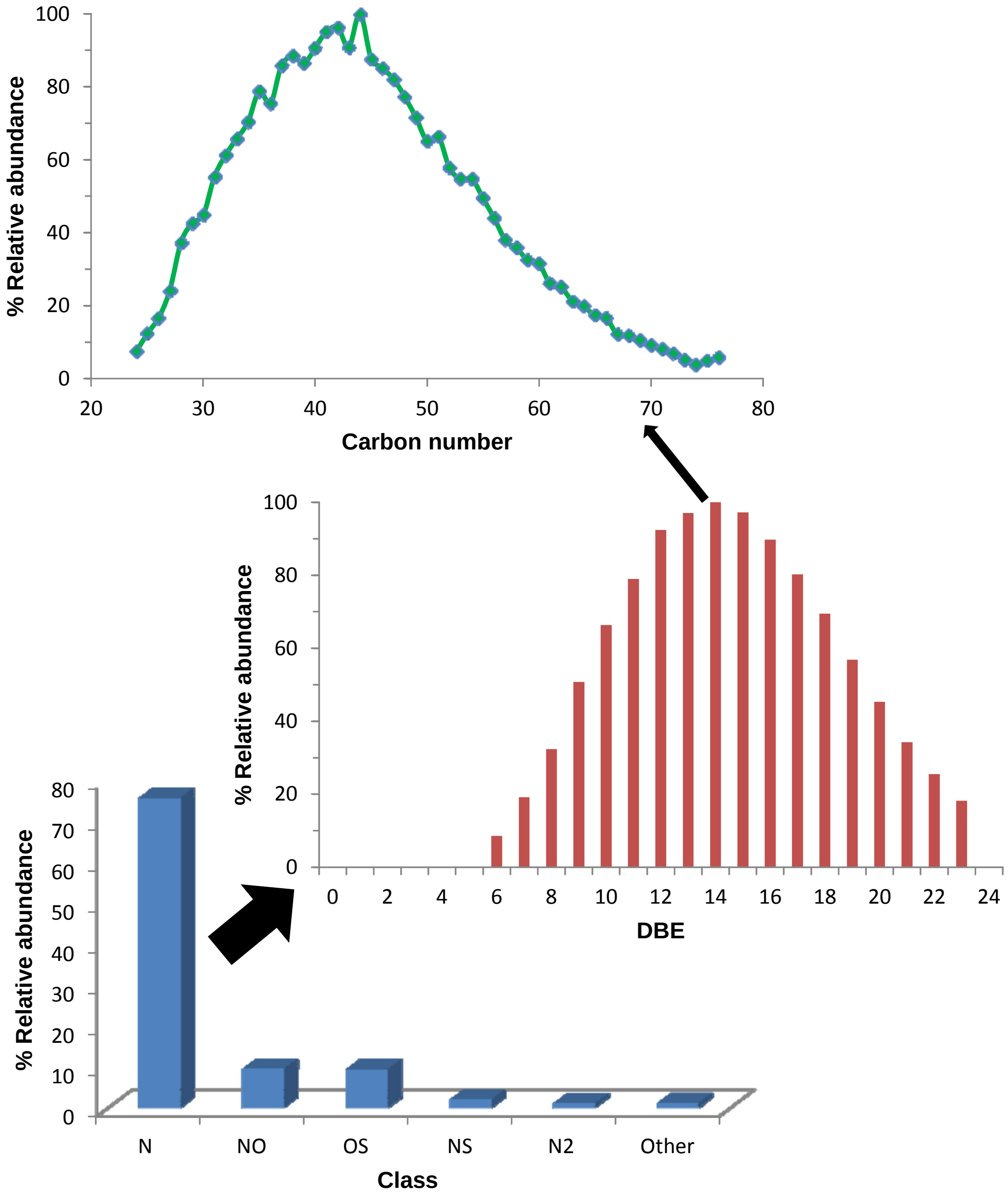

Figure 2-5: Deconvolution of the data obtained from ESI FT-ICR MS measurement of a short residue (residual of vacuum distillation of a crude oil). The compositional information is represented first as a distribution of the classes of compounds present in the sample (bottom left). Each class of compounds has its corresponding DBE distribution (middle). Finally, for every type of a given class, the carbon number distribution gives an indication of the extent of alkylation in the measured sample (top left).

As we see from Figure 2-5 above, the ordinate in the class distribution of the data is the percent relative abundance, which is the sum of the class abundance divided by the sum of every identified peak in the mass spectrum. For type and carbon number distribution, the abundance is scaled to the highest member for the facilitation of the comparison. It is not easy to relate relative abundance to the actual abundance in the crude oil, due to differences in ionization efficiency and matrix effects. As an alternative method for the presentation of the data, different (hetero) atom classes can be easily visualized as bar charts, in which abundances refer to the number of the assigned molecular formulas. This principle has been adopted in our group recently [31-33]. One of the major benefits of population-based distribution over the classic relative abundance distribution is the possibility to eliminate differences derived from signal intensities. Diverse molecules with variable ionization efficiencies are present in the analyte and may not represent the sample effectively.

2.3.6 Counter Plots and Heat Maps

The relative intensities of the species are not typically indicated in Kendrick plots. In recent years, a color-coded contour plot reflecting the abundance of identified species at different KMDs (DBEs) and at different masses (carbon numbers) was presented [34]. In this mode of presentation, the x-axis is carbon number, which indicates the extent of alkylation, the y-axis indicates the aromaticity and the third dimension is the percent relative abundance represented by color. The mass spectrum is thereby converted to an image. However, the visualization of the data with this method does not expose class based compositional differences [35]. Additionally, the information may be lost where different families of compounds overlap in terms of DBE [24]. In this regard, the visualization of the data using heat maps is helpful, where the DBE distribution of each individual class is compared in terms of relative intensity or population to each other. This way of representation is more convenient for highly complex samples, since it facilitates the comparison of DBE values of the species of different classes in the same plot rather than using multiple plots for different classes. The usefulness of this method is apparent when different type distributions (DBE distributions) are compared in regard to different ionization techniques [31], different crude oil fractions [32], or different retention times in the case of LC/MS coupling (see Chapter 5).

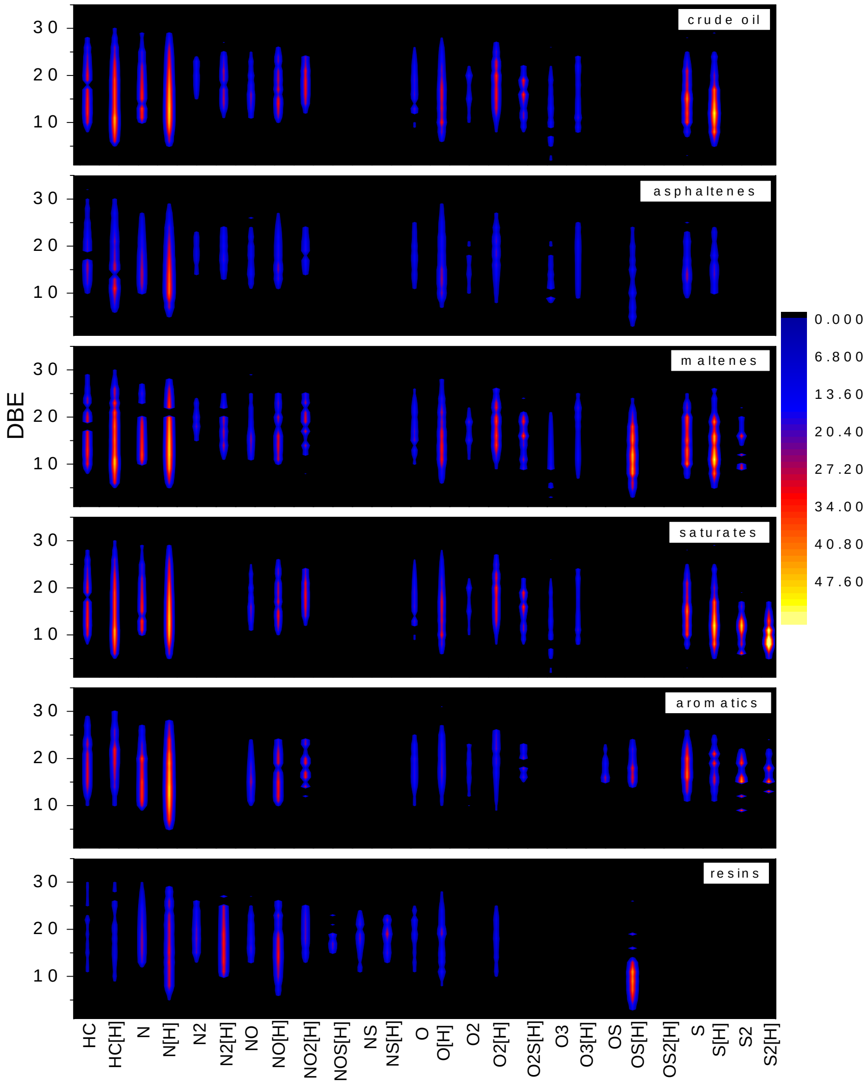

Figure 2-6: Heat map showing DBE distribution of the individual compound classes in each SARA fraction, scaled in the third axis according to the number of assigned molecules [32].

2.3.7 Visualization of Aromaticity Distribution

The application of statistical methods in the evaluation of the data is quite helpful, especially when the number of assigned peaks is used for visualization. One of these methods frequently used in descriptive statistics is using box plots as way for graphically depicting groups of numerical data. The use of such a method becomes an advantage when distributions between several groups or sets of data are compared. Although it takes less space, but it gives valuable statistical data about the smallest observation (sample minimum), lower quartile, median, upper quartile, largest observation (sample maximum), and the mean value. In order to illustrate the method, an example is depicted in Figure 2-7, where different ionization techniques were applied in the online HPLC/FT-ICR MS coupling set up for the investigation of a crude oil sample (see Chapter 6). Unambiguous and informative comparison is achieved by box plots visualization of the data in this example.

A normalization of the DBE values to the number of carbons within the given molecule (DBE/C) can provide further detail of the corresponding aromaticity. This method was successfully demonstrated to follow the *in situ* degradation of charcoal in soils and the description of black carbon content in the environment [36, 37]. Figure 2-7 shows the distribution of aromaticity enabling comparison of the diverse ionization methods. A second indicator of the aromaticity can be provided by the calculation of the H/C ratio from the assigned elemental compositions. An example of this distribution is depicted in Figure 7-8 in Chapter 7.

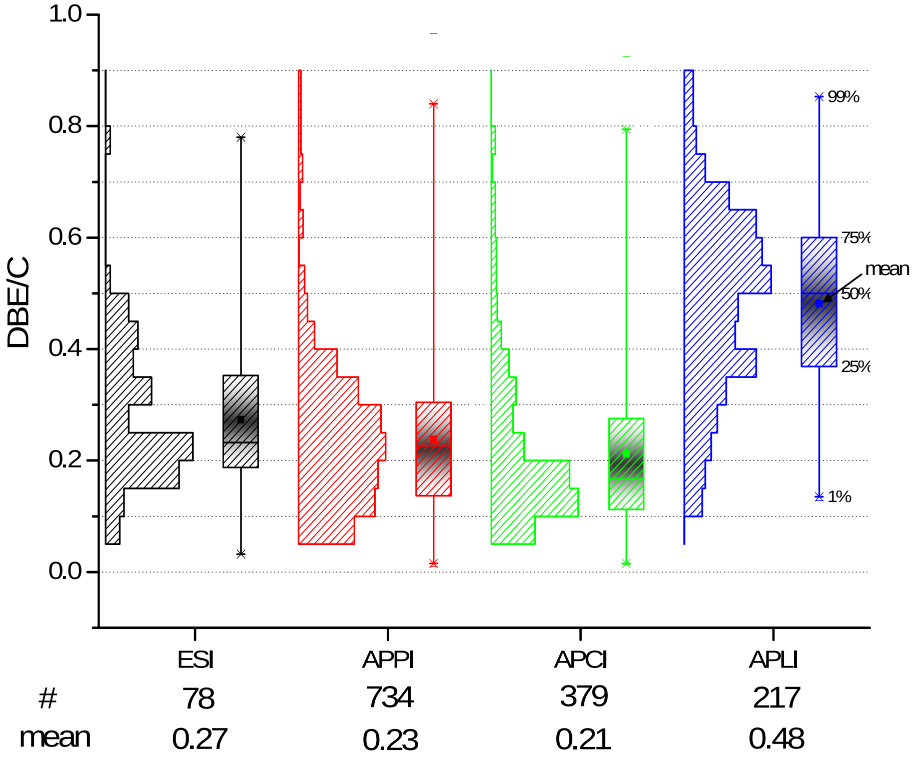

Figure 2-7: Box plots of frequency distributions of unique species found for each ionization techniques in the HPLC/FT-ICR MS coupling setup of a crude oil sample. Statistical information can be obtained easily in the same plot including smallest observation (sample minimum), lower quartile (25%), median (50%), upper quartile (75%), largest observation (sample maximum), and the mean value.

2.3.8 Isotopic Fingerprints

One of essential data reduction methods used in the analysis of crude oil samples using high resolution mass spectrometry is the validation of elemental compositions using isotopic fingerprints. ^{13}C isotopic fingerprint is commonly observed in crude oil analysis, since it composes mainly of hydrocarbons. The exact mass difference between ^{12}C and ^{13}C equals 1.0033 Da at an abundance of 1%; therefore, once a molecular formula is assigned it can be further validated from its ^{13}C isotope. On the other hand, the verification of elemental composition for sulfur containing species, which are concentrated in heavy oil fractions, can be achieved by ^{34}S isotope. The mass difference between ^{34}S and ^{32}S is 1.9958 Da. Thus, isotopic fingerprinting provides internal verification of elemental composition assignment.

2.4 References

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Appendix for Chapter 2

Abstract

The appendix for chapter 2 contains additional information about the process of data analysis throughout this work. The peak lists obtained from the calibrated high resolution mass spectrometric measurements were converted to molecular assignments by Composer™ (Sierra Analytics, Pasadena, CA, USA). The calculated molecular formulas were sorted into compound classes based on their denoted Kendrick mass defects and their DBE distribution. The obtained mass lists were transferred into Excel and Origin for data evaluation and visualization.

Contents

Appendix 2-1 Assignment of elemental composition by Composer

The algorithm used by Composer for the assignment of elemental composition is based on the following basic steps:[1]

- Conversion of the mass scale from IUPAC to Kendrick mass scale according to Eq. (2-8).
- Calculation of Kendrick mass defect for each peak according to Eq. (2-9).
- Sorting the peaks by their Kendrick mass defect into groups.
- Computing the elemental composition of the lowest *m/z* peak with a sufficiently S/N ratio from the highest abundance group.
- Expansion of the assignment to the rest of the group by adding or subtracting multiple of the repeat unit to the base chemical formula.
- Repetition of the process on the rest of unassigned groups after removal of the assigned from consideration
- Determination of chemical class, double bond equivalents (DBE), and carbon number

Chemical and technical constrains are applied to obtain reliable results. Typical parameters used for the analysis with Composer of the data in this work were the followings:

- *m/z* matching tolerance ~ 1 ppm
- minimum relative abundance ~ 1 %
- Number of heteroatoms N,O, and S is constrained to 2
- mass range between 200 – 1200 Da

As a result of the analysis, the elemental compositions are sorted hierarchically and displayed in a tree structure as it shown in Figure A2-1.

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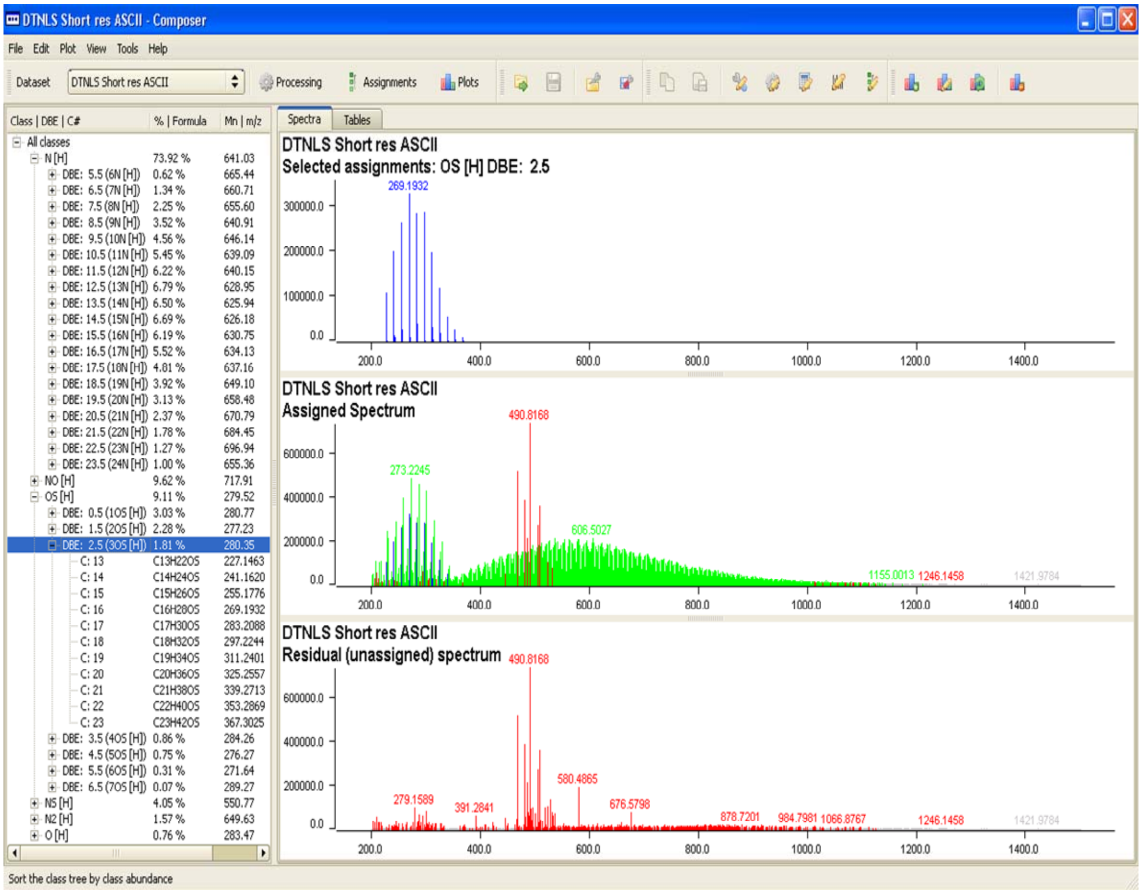
[1] Summerized from Composer software user manual version 1.0.2 – April 2009.



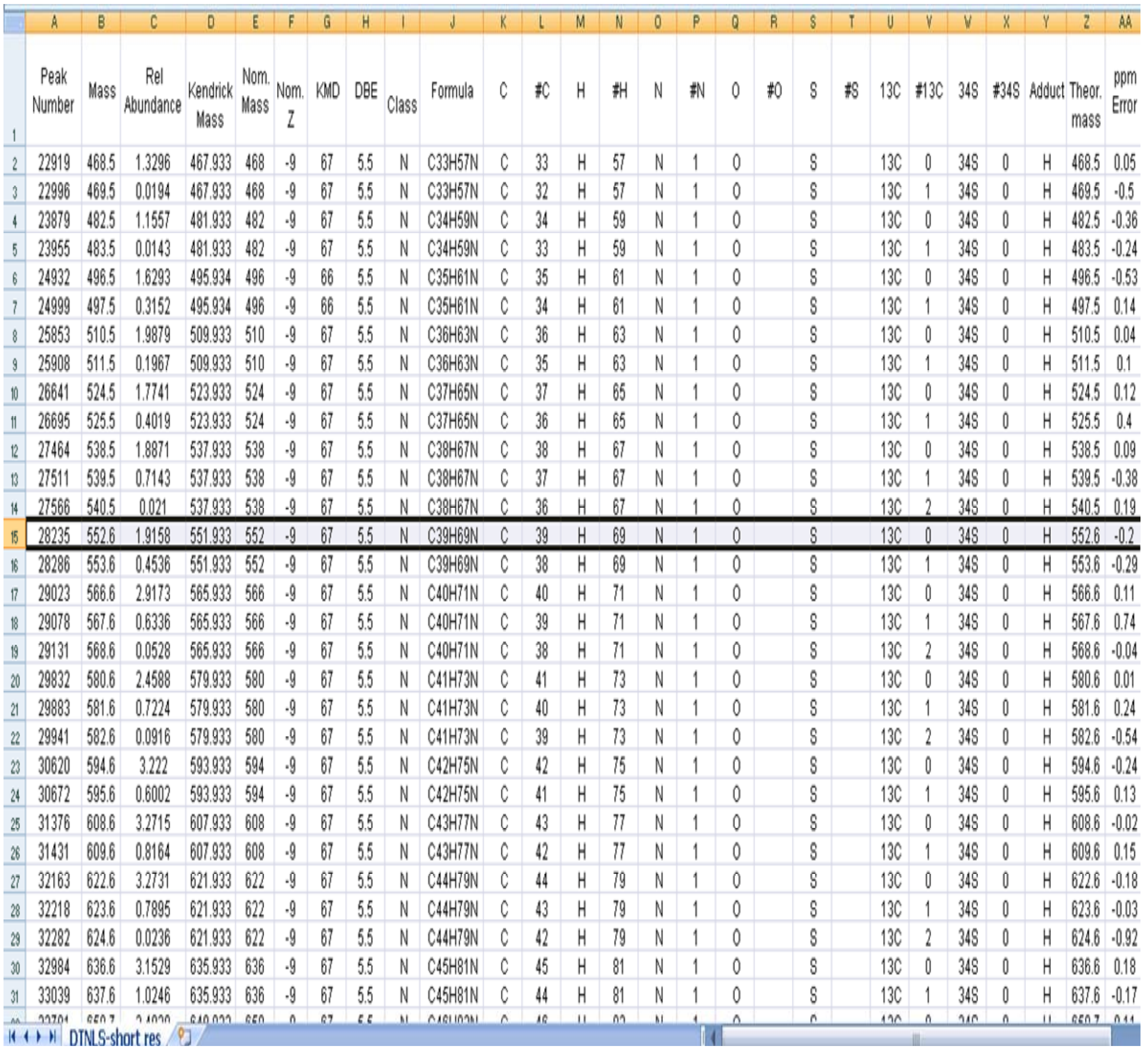
Class | DBE | C#	% | Formula	Mn | m/z
All classes		
N [H]	73.92 %	641.03
DBE: 5.5 (6N [H])	0.62 %	665.44
DBE: 6.5 (7N [H])	1.34 %	660.71
DBE: 7.5 (8N [H])	2.25 %	655.60
DBE: 8.5 (9N [H])	3.52 %	640.91
DBE: 9.5 (10N [H])	4.56 %	646.14
DBE: 10.5 (11N [H])	5.45 %	639.09
DBE: 11.5 (12N [H])	6.22 %	640.15
DBE: 12.5 (13N [H])	6.79 %	628.95
DBE: 13.5 (14N [H])	6.50 %	625.94
DBE: 14.5 (15N [H])	6.69 %	626.18
DBE: 15.5 (16N [H])	6.19 %	630.75
DBE: 16.5 (17N [H])	5.52 %	634.13
DBE: 17.5 (18N [H])	4.81 %	637.16
DBE: 18.5 (19N [H])	3.92 %	649.10
DBE: 19.5 (20N [H])	3.13 %	658.48
DBE: 20.5 (21N [H])	2.37 %	670.79
DBE: 21.5 (22N [H])	1.78 %	684.45
DBE: 22.5 (23N [H])	1.27 %	696.94
DBE: 23.5 (24N [H])	1.00 %	655.36
NO [H]	9.62 %	717.91
OS [H]	9.11 %	279.52
DBE: 0.5 (1OS [H])	3.03 %	280.77
DBE: 1.5 (2OS [H])	2.28 %	277.23
DBE: 2.5 (3OS [H])	1.81 %	280.35
C: 13	C13H22OS	227.1463
C: 14	C14H24OS	241.1620
C: 15	C15H26OS	255.1776
C: 16	C16H28OS	269.1932
C: 17	C17H30OS	283.2088
C: 18	C18H32OS	297.2244
C: 19	C19H34OS	311.2401
C: 20	C20H36OS	325.2557
C: 21	C21H38OS	339.2713
C: 22	C22H40OS	353.2869
C: 23	C23H42OS	367.3025
DBE: 3.5 (4OS [H])	0.86 %	284.26
DBE: 4.5 (5OS [H])	0.75 %	276.27
DBE: 5.5 (6OS [H])	0.31 %	271.64
DBE: 6.5 (7OS [H])	0.07 %	289.27
NS [H]	4.05 %	550.77
N2 [H]	1.57 %	649.63
O [H]	0.76 %	283.47

Figure A2-1: Screenshot of Composer software applied for the analysis of short residue sample.

Assignment of elemental composition to each of the thousands of peaks in a typical high resolution mass spectrum of a crude oil related sample relies on knowledge of the sample, ionization method, and behavior of the mass spectrometer.

Appendix 2-2 Exporting results from Composer

Composer saves the results in XML format, which is an acronym of eXtensible Markup Language. The results saved in XML format can be processed by XSLT (Extensible Stylesheet Language transform) scripts into scripting language XSL. One of these XSLT scripts provided by Composer is called Xalan, which is an open source software library. MIDASFilter is a script targets the results saved as XML format and transforms it into a CSV (Comma-separated values) file, which can be read by MS Excel (see Figure A2-2).



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA
1	Peak Number	Mass	Rel Abundance	Kendrick Mass	Nom. Mass	Nom. Z	KMD	DBE	Class	Formula	C	#C	H	#H	N	#N	O	#O	S	#S	13C	#13C	34S	#34S	Adduct	Theor. mass	ppm Error
2	22919	468.5	1.3296	467.933	468	-9	67	5.5	N	C33H57N	C	33	H	57	N	1	O		S		13C	0	34S	0	H	468.5	0.05
3	22996	469.5	0.0194	467.933	468	-9	67	5.5	N	C33H57N	C	32	H	57	N	1	O		S		13C	1	34S	0	H	469.5	-0.5
4	23879	482.5	1.1557	481.933	482	-9	67	5.5	N	C34H59N	C	34	H	59	N	1	O		S		13C	0	34S	0	H	482.5	-0.36
5	23955	483.5	0.0143	481.933	482	-9	67	5.5	N	C34H59N	C	33	H	59	N	1	O		S		13C	1	34S	0	H	483.5	-0.24
6	24932	496.5	1.6293	495.934	496	-9	66	5.5	N	C35H61N	C	35	H	61	N	1	O		S		13C	0	34S	0	H	496.5	-0.53
7	24999	497.5	0.3152	495.934	496	-9	66	5.5	N	C35H61N	C	34	H	61	N	1	O		S		13C	1	34S	0	H	497.5	0.14
8	25853	510.5	1.9879	509.933	510	-9	67	5.5	N	C36H63N	C	36	H	63	N	1	O		S		13C	0	34S	0	H	510.5	0.04
9	25908	511.5	0.1967	509.933	510	-9	67	5.5	N	C36H63N	C	35	H	63	N	1	O		S		13C	1	34S	0	H	511.5	0.1
10	26641	524.5	1.7741	523.933	524	-9	67	5.5	N	C37H65N	C	37	H	65	N	1	O		S		13C	0	34S	0	H	524.5	0.12
11	26695	525.5	0.4019	523.933	524	-9	67	5.5	N	C37H65N	C	36	H	65	N	1	O		S		13C	1	34S	0	H	525.5	0.4
12	27464	538.5	1.8871	537.933	538	-9	67	5.5	N	C38H67N	C	38	H	67	N	1	O		S		13C	0	34S	0	H	538.5	0.09
13	27511	539.5	0.7143	537.933	538	-9	67	5.5	N	C38H67N	C	37	H	67	N	1	O		S		13C	1	34S	0	H	539.5	-0.38
14	27566	540.5	0.021	537.933	538	-9	67	5.5	N	C38H67N	C	36	H	67	N	1	O		S		13C	2	34S	0	H	540.5	0.19
15	28235	552.6	1.9158	551.933	552	-9	67	5.5	N	C39H69N	C	39	H	69	N	1	O		S		13C	0	34S	0	H	552.6	-0.2
16	28286	553.6	0.4536	551.933	552	-9	67	5.5	N	C39H69N	C	38	H	69	N	1	O		S		13C	1	34S	0	H	553.6	-0.29
17	29023	566.6	2.9173	565.933	566	-9	67	5.5	N	C40H71N	C	40	H	71	N	1	O		S		13C	0	34S	0	H	566.6	0.11
18	29078	567.6	0.6336	565.933	566	-9	67	5.5	N	C40H71N	C	39	H	71	N	1	O		S		13C	1	34S	0	H	567.6	0.74
19	29131	568.6	0.0528	565.933	566	-9	67	5.5	N	C40H71N	C	38	H	71	N	1	O		S		13C	2	34S	0	H	568.6	-0.04
20	29832	580.6	2.4588	579.933	580	-9	67	5.5	N	C41H73N	C	41	H	73	N	1	O		S		13C	0	34S	0	H	580.6	0.01
21	29883	581.6	0.7224	579.933	580	-9	67	5.5	N	C41H73N	C	40	H	73	N	1	O		S		13C	1	34S	0	H	581.6	0.24
22	29941	582.6	0.0916	579.933	580	-9	67	5.5	N	C41H73N	C	39	H	73	N	1	O		S		13C	2	34S	0	H	582.6	-0.54
23	30620	594.6	3.222	593.933	594	-9	67	5.5	N	C42H75N	C	42	H	75	N	1	O		S		13C	0	34S	0	H	594.6	-0.24
24	30672	595.6	0.6002	593.933	594	-9	67	5.5	N	C42H75N	C	41	H	75	N	1	O		S		13C	1	34S	0	H	595.6	0.13
25	31376	608.6	3.2715	607.933	608	-9	67	5.5	N	C43H77N	C	43	H	77	N	1	O		S		13C	0	34S	0	H	608.6	-0.02
26	31431	609.6	0.8164	607.933	608	-9	67	5.5	N	C43H77N	C	42	H	77	N	1	O		S		13C	1	34S	0	H	609.6	0.15
27	32163	622.6	3.2731	621.933	622	-9	67	5.5	N	C44H79N	C	44	H	79	N	1	O		S		13C	0	34S	0	H	622.6	-0.18
28	32218	623.6	0.7895	621.933	622	-9	67	5.5	N	C44H79N	C	43	H	79	N	1	O		S		13C	1	34S	0	H	623.6	-0.03
29	32282	624.6	0.0236	621.933	622	-9	67	5.5	N	C44H79N	C	42	H	79	N	1	O		S		13C	2	34S	0	H	624.6	-0.92
30	32984	636.6	3.1529	635.933	636	-9	67	5.5	N	C45H81N	C	45	H	81	N	1	O		S		13C	0	34S	0	H	636.6	0.18
31	33039	637.6	1.0246	635.933	636	-9	67	5.5	N	C45H81N	C	44	H	81	N	1	O		S		13C	1	34S	0	H	637.6	-0.17

Figure A2-2: Data transformed by MIDASFilter XSL script into an Excel sheet displaying results of data analysis of the short residue sample.

After obtaining the results in CSV format it is possible to subject the data into further filtration to have reliable results. Depending on the conditions of the measurements, following filtration steps can be processed:

- Removal of assignments having high ppm error.
- Sorting the dataset according to KMD values to validate assigned homologous series.
- Exclusion of weird molecular assignments which do not follow chemical sense of structure.
- Calculation of DBE/C values to visualize the aromaticity of the species and to exclude weird molecular assignments.
- Controlling the isotopic fingerprints of the assigned species

Appendix 2-3 Calculation of the population of species

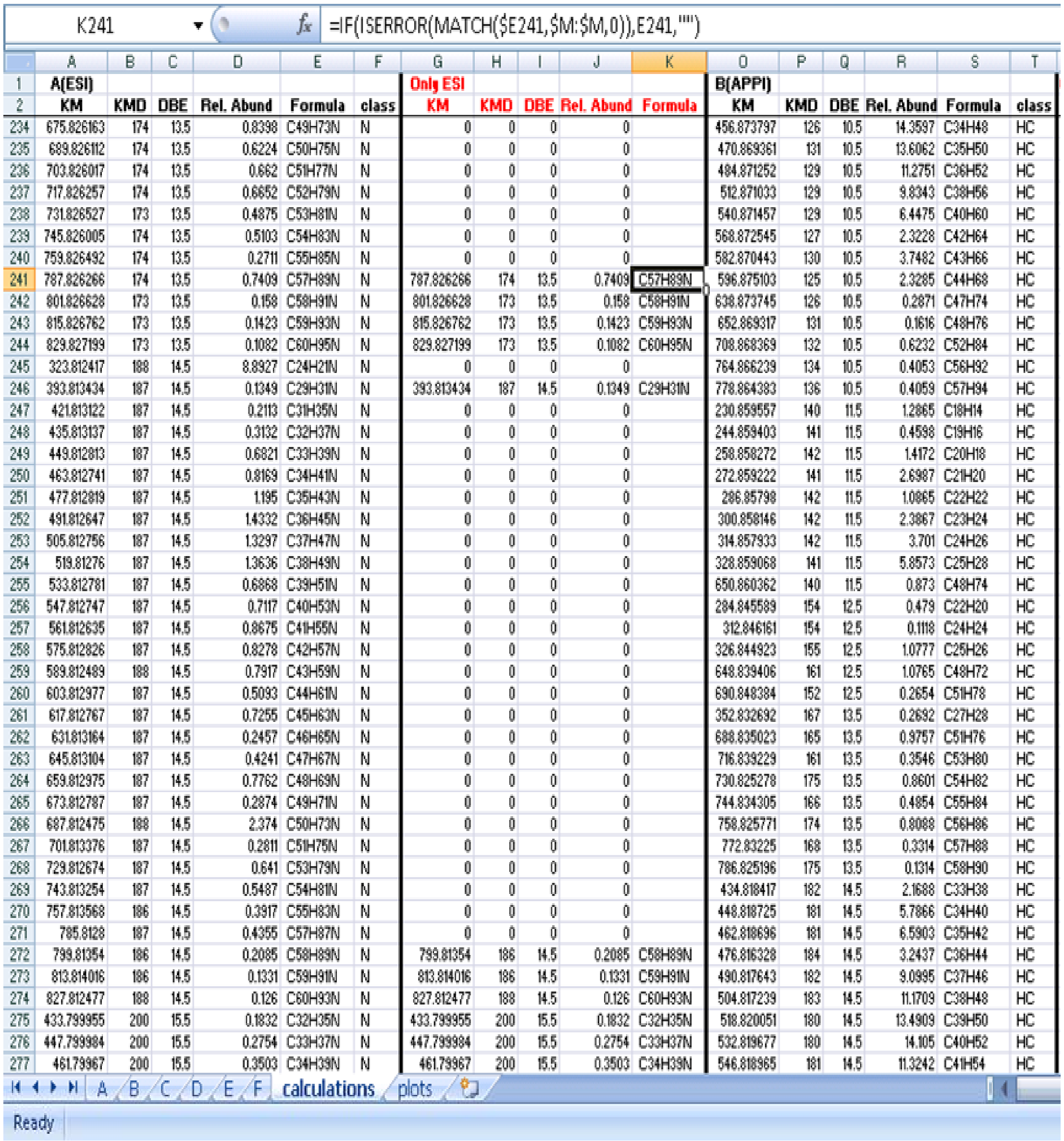
Population based distribution in this work were calculated by an Excel script written in our group based on the class and DBE distribution data in addition to MIDAS table. The calculations are based on the following points:

- A list of classes obtained from Composer class distribution is issued, taking into consideration that protonated ions were differentiated from radical ions by the addition of [H] symbol to the corresponding class. However, the brackets [ ] were removed for the formulas in the script functions.
- For each individual cell from MIDAS table, the values of class, adduct, and rounded DBE were merged. For example, in Figure A2-2, the values of the highlighted cells of the columns I, Y, and H are merged giving the result of NH6.
- A series of possible DBE values from 0 to 40 serves as basis for matching. If a merged value obtained from MIDAS table matches with this series it would be counted.

As a result, the number of assigned formulas for each type of classes is determined. Thereby, we were able throughout this work to depict class distribution histograms depending on the population of the assigned species.

Appendix 2-4 Determination of the common and unique constituents

Common and unique constituents were determined by comparing the MIDAS table obtained from Composer. An excel script written in our group enable us to calculate the molecular assignment found exclusively in each dataset. The resulting unique lists were exported into Origin to process descriptive statistical methods to enhance the visualization of comparison. Figure A 2-3 shows a screenshot of the Excel sheet used for the determination.



```
=IF(ISERROR(MATCH($E241,$M:$M,0)),E241,"")
```

	A	B	C	D	E	F	G	H	I	J	K	O	P	Q	R	S	T
1	A(ESI)						Only ESI					B(APPI)					
2	KM	KMD	DBE	Rel. Abund	Formula	class	KM	KMD	DBE	Rel. Abund	Formula	KM	KMD	DBE	Rel. Abund	Formula	class
234	675.826163	174	13.5	0.8398	C49H73N	N	0	0	0	0		456.873797	126	10.5	14.3597	C34H48	HC
235	689.826112	174	13.5	0.6224	C50H75N	N	0	0	0	0		470.869361	131	10.5	13.6062	C35H50	HC
236	703.826017	174	13.5	0.662	C51H77N	N	0	0	0	0		484.871252	129	10.5	11.2751	C36H52	HC
237	717.826257	174	13.5	0.6652	C52H79N	N	0	0	0	0		512.871033	129	10.5	9.8343	C38H56	HC
238	731.826527	173	13.5	0.4875	C53H81N	N	0	0	0	0		540.871457	129	10.5	6.4475	C40H60	HC
239	745.826005	174	13.5	0.5103	C54H83N	N	0	0	0	0		568.872545	127	10.5	2.3228	C42H64	HC
240	759.826492	174	13.5	0.2711	C55H85N	N	0	0	0	0		582.870443	130	10.5	3.7482	C43H66	HC
241	787.826266	174	13.5	0.7409	C57H89N	N	787.826266	174	13.5	0.7409	C57H89N	596.875103	125	10.5	2.3285	C44H68	HC
242	801.826628	173	13.5	0.158	C58H91N	N	801.826628	173	13.5	0.158	C58H91N	638.873745	126	10.5	0.2871	C47H74	HC
243	815.826762	173	13.5	0.1423	C59H93N	N	815.826762	173	13.5	0.1423	C59H93N	652.869317	131	10.5	0.1616	C48H76	HC
244	829.827199	173	13.5	0.1082	C60H95N	N	829.827199	173	13.5	0.1082	C60H95N	708.868369	132	10.5	0.6232	C52H84	HC
245	323.812417	188	14.5	8.8927	C24H21N	N	0	0	0	0		764.866239	134	10.5	0.4053	C56H92	HC
246	393.813434	187	14.5	0.1349	C29H31N	N	393.813434	187	14.5	0.1349	C29H31N	778.864383	136	10.5	0.4059	C57H94	HC
247	421.813122	187	14.5	0.2113	C31H35N	N	0	0	0	0		230.859557	140	11.5	1.2865	C18H14	HC
248	435.813137	187	14.5	0.3132	C32H37N	N	0	0	0	0		244.859403	141	11.5	0.4598	C19H16	HC
249	449.812813	187	14.5	0.6821	C33H39N	N	0	0	0	0		258.858272	142	11.5	1.4172	C20H18	HC
250	463.812741	187	14.5	0.8169	C34H41N	N	0	0	0	0		272.859222	141	11.5	2.6987	C21H20	HC
251	477.812819	187	14.5	1.195	C35H43N	N	0	0	0	0		286.85798	142	11.5	1.0865	C22H22	HC
252	491.812647	187	14.5	1.4332	C36H45N	N	0	0	0	0		300.858146	142	11.5	2.3867	C23H24	HC
253	505.812756	187	14.5	1.3297	C37H47N	N	0	0	0	0		314.857933	142	11.5	3.701	C24H26	HC
254	519.81276	187	14.5	1.3636	C38H49N	N	0	0	0	0		328.859068	141	11.5	5.8573	C25H28	HC
255	533.812781	187	14.5	0.6868	C39H51N	N	0	0	0	0		650.860362	140	11.5	0.873	C48H74	HC
256	547.812747	187	14.5	0.7117	C40H53N	N	0	0	0	0		284.845589	154	12.5	0.479	C22H20	HC
257	561.812635	187	14.5	0.8675	C41H55N	N	0	0	0	0		312.846161	154	12.5	0.1118	C24H24	HC
258	575.812826	187	14.5	0.8278	C42H57N	N	0	0	0	0		326.844923	155	12.5	1.0777	C25H26	HC
259	589.812489	188	14.5	0.7917	C43H59N	N	0	0	0	0		648.839406	161	12.5	1.0765	C48H72	HC
260	603.812977	187	14.5	0.5093	C44H61N	N	0	0	0	0		690.848384	152	12.5	0.2654	C51H78	HC
261	617.812767	187	14.5	0.7255	C45H63N	N	0	0	0	0		352.832692	167	13.5	0.2692	C27H28	HC
262	631.813164	187	14.5	0.2457	C46H65N	N	0	0	0	0		688.835023	165	13.5	0.9757	C51H76	HC
263	645.813104	187	14.5	0.4241	C47H67N	N	0	0	0	0		716.839229	161	13.5	0.3546	C53H80	HC
264	659.812975	187	14.5	0.7762	C48H69N	N	0	0	0	0		730.825278	175	13.5	0.8601	C54H82	HC
265	673.812787	187	14.5	0.2874	C49H71N	N	0	0	0	0		744.834305	166	13.5	0.4854	C55H84	HC
266	687.812475	188	14.5	2.374	C50H73N	N	0	0	0	0		758.825771	174	13.5	0.8088	C56H86	HC
267	701.813376	187	14.5	0.2811	C51H75N	N	0	0	0	0		772.83225	168	13.5	0.3314	C57H88	HC
268	729.812674	187	14.5	0.641	C53H79N	N	0	0	0	0		786.825196	175	13.5	0.1314	C58H90	HC
269	743.813254	187	14.5	0.5487	C54H81N	N	0	0	0	0		434.818417	182	14.5	2.1688	C33H38	HC
270	757.813568	186	14.5	0.3917	C55H83N	N	0	0	0	0		448.818725	181	14.5	5.7866	C34H40	HC
271	785.8128	187	14.5	0.4355	C57H87N	N	0	0	0	0		462.818696	181	14.5	6.5903	C35H42	HC
272	799.81354	186	14.5	0.2085	C58H89N	N	799.81354	186	14.5	0.2085	C58H89N	476.816328	184	14.5	3.2437	C36H44	HC
273	813.814016	186	14.5	0.1331	C59H91N	N	813.814016	186	14.5	0.1331	C59H91N	490.817643	182	14.5	9.0995	C37H46	HC
274	827.812477	188	14.5	0.126	C60H93N	N	827.812477	188	14.5	0.126	C60H93N	504.817239	183	14.5	11.1709	C38H48	HC
275	433.799955	200	15.5	0.1832	C32H35N	N	433.799955	200	15.5	0.1832	C32H35N	518.820051	180	14.5	13.4909	C39H50	HC
276	447.799984	200	15.5	0.2754	C33H37N	N	447.799984	200	15.5	0.2754	C33H37N	532.819677	180	14.5	14.105	C40H52	HC
277	461.79967	200	15.5	0.3503	C34H39N	N	461.79967	200	15.5	0.3503	C34H39N	546.818965	181	14.5	11.3242	C41H54	HC

Figure A2-3: Excel sheet displaying the determination of unique constituents.

Chapter 3 A new Research-grade High-Field Orbitrap Hybrid Mass Spectrometer Capable of Studying Complex Hydrocarbon Mixtures from Energy Resources at Ultra-high Mass Resolution*

**Redrafted from "Lababidi S., Denisov E., Makarov A., Schrader W.; A new Research-grade High-Field Orbitrap Hybrid Mass Spectrometer Capable of Studying Complex Hydrocarbon Mixtures from Energy Resources at Ultra-high Mass Resolution, submitted to Analytical Chemistry, in revision.*

3.1 Abstract

Crude oil is one of the most complex natural mixtures available and has shown to be a very difficult matrix to be analyzed. Due to the enormous complexity only ultra-high resolution methods that also have the ability to produce highly accurate data to characterize these ten-thousands of different compounds. Here, a new research-grade Orbitrap instrument is introduced that is capable of reaching the necessary benchmarks to study such complex samples as crude oil mixtures. This Orbitrap is capable to run longer transients up to 3 s, which makes it possible to practically reach resolutions above 800 000 at *m/z* 400 and even at higher mass ranges at m/z 1000 a resolution of 500 000 can be observed while the error is usually below 0.5 ppm.

3.2 Introduction

One of the major tasks in the near future will be to find suitable, environmental friendly, sustainable and economically affordable resources for the production of energy. Important studies have been carried out that devise different paths to a new energy supply and most of them conclude that a diversion of different resources will be key. Currently, our technology is strongly dependent on hydrocarbon based energy resources. Until new resources and ways of storing and transporting energy will be developed chemical processes especially for catalytical upgrading of these hydrocarbon based materials will play a major role.

Crude oil is still available in sufficient quantities to bridge the time gap until other means for the energy supply will be available. The difficulty will be that the light and sweet crude oils are diminishing and that the still available more heavy and sour resources are much dirtier and will need chemical upgrading before they can be used as a quality product. Especially highly aromatic polyaromatic hydrocarbons with and without different heteroatoms as well as metals like Vanadium, Iron or Nickel contaminate the crude oil [1]. Here catalytic step like hydrodesulfurization and other cleaning steps are necessary to remove these components. Additionally, the heavy components that are available are highly aromatic and need to be reduced to a molecular size suitable for use. Hydrocarbons from different sustainable resources like biomass or algae contain also different classes of components and are by definition not clean mixtures.

All this resources need catalytic chemical transformation. To study such immensely complex reactions sophisticated analytical equipment is needed [2]. The method of choice for this task is using Fourier Transform Ion Cyclotron Resonance Mass spectrometry (FT-ICR MS), which is known for its unsurpassed resolution and accuracy [3, 4] and has found application in different fields of complex analytical problems with natural organic matter [5, 6] or protein crosslinking [7] being some of them. Connected to different ion sources from ESI [8] to APPI [9, 10], APCI [11, 12] and APLI [13] it has shown that this method allows describing the complexity of such resources. These mixtures are so complex that more than 100 different chemical formulas can be calculated from the measurements of one nominal mass alone. In the end a high resolving power is needed to be able to observe the most important mass splits in hydrocarbon based materials. Here, the most important ones are 3.4 mDa, which represents the difference between SH_4 and C_3 or 1.1 mDa, which represents the difference between $SH_4{}^{13}C_1$ and C_4 [14]. Additionally, for the assignments of the thousands of signals in a hydrocarbon mixture high accuracy is needed that is below 1 ppm. The limiting task is that FT-ICR MS instruments need a large and expensive superconducting magnet; currently magnets up to 15 T are available while a limited number of 21 T instruments are under construction in multi-million dollar research programs [15]. Superconducting magnets are cost intensive from an investment side and also are cost intensive in operation due to the need for cryogens like liquid helium.

Several years ago a new mass analyzer has been introduced by Makarov and co-workers [16, 17]. Being based on orbital trapping of ions in electrostatic fields, it has been called Orbitrap analyzer and combines the capabilities of high resolving power with high mass accuracy but without the need for a superconducting magnet. Orbitrap mass spectrometers are fully established by now in different fields of analytical chemistry, especially in life science applications. They have not found much application in studies of hydrocarbon mixtures from energy related resources like biomass or crude oil components due to the limitation in resolving power, where the first generation allowed resolving powers up to 100,000 and the latest generation is capable of reaching 240,000 both at *m/z* 400. Here, a new research-grade Orbitrap that is capable of much higher resolutions is used to study real complex crude oil mixtures and the overall capabilities of the instrument are reported.

3.3 Experimental Section

3.3.1 SARA Fractionation

The heavy crude oil used for this study is from a North-American source. To avoid chemical transformation it is stored under argon. A SARA fractionation procedure that separates the crude oil into different subfractions was carried out to obtain the asphaltene fraction [18, 19]. The asphaltene fraction was obtained by precipitation from the heavy crude oil using n-heptane (HPLC grade, Merck, Germany). 40 mL of n-heptane per g of crude oil was added and the mixture was refluxed for 2 hours at 150 °C in a Soxhlet apparatus. The precipitate was filtered and dried under inert gas flow. The sample filter was extracted with 300 mL toluene (HPLC-grade, Overlack, Germany) in a Soxhlet apparatus until no color changes were observed.

3.3.2 Mass Spectrometric Analysis

The analysis was performed on a research-grade Orbitrap Elite (Thermo Fisher Scientific, Bremen, Germany) instrument that is capable to operate with 3 s transients. The instrument was manually tuned and mass-calibrated using standard TuneView software. The spectra of the toluene solution were collected in positive mode using an atmospheric pressure photo ionization source (APPI) at 10 and 10.6 eV for a one-photon ionization at flow rates of 20 µL/min. ESI measurements were carried out in positive mode operation at 4 kV ionization voltage at flow rates of 5 µL/min. Data were collected using full-scan mode and additionally the spectral stitching method, where smaller scan ranges of up to 100 Da were stitched together [20]. Additional measurements were carried out using a 12 T LTQ FT-ICR (Thermo Fisher Scientific, Bremen, Germany), MS equipped with both an ESI and an APPI source.

3.3.3 Data analysis

The data collection and processing was done with the LTQ FT Ultra 2.5.5 (Thermo Fisher Scientific, Bremen, Germany) data acquisition system. The mass spectra were externally calibrated and processed afterwards. For assignments of each signal the peak lists of the accurate data were converted to molecular formulae by Composer (Sierra Analytics, USA). The chemical constraints were set as follows: Number of H unlimited, $0<C<100$, $0<O<3$, $0<N<3$, $0<S<3$, $0<DBE<40$. A molecular formula was considered only if the corresponding ^{13}C peak was also detected. Radical cations and protonated molecules were distinguished and indicated separately (X, X[H], respectively). The calculated molecular formulae were sorted into compound classes based upon their Kendrick mass defects and their DBE distribution.[21] The mass lists were exported into Excel and Origin for data evaluation and preparation of the figures.

3.4 Results and Discussion

A new research-grade hybrid mass spectrometer combining a linear ion trap with a high-field Orbitrap analyzer is capable of reaching a much better performance due to the careful selection of the Orbitrap assembly (see Figure 3-1) from a batch of serial assemblies on the basis of machining accuracy and spectral quality. The construction of a high-field trap is a delicate endeavor [22]. The major point of the new assembly is that the instrument software was modified to allow transient durations up to 3.04 seconds, which need to be supported by the exactly manufactured hardware. The acquired transients were processed using enhanced Fourier Transform (eFT) method [23].

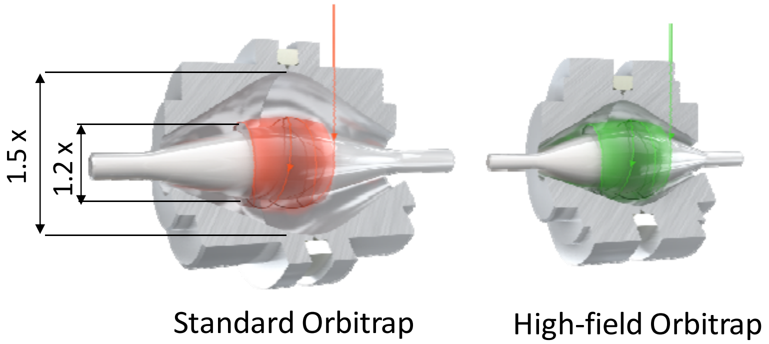


Figure 3-1: Comparison of a standard Orbitrap analyzer with a new high-field Orbitrap analyzer. (Reprinted with permission of Thermo Fisher Scientific (Bremen) GmbH)

This method takes advantage of the *excitation-by-injection* mechanism inherent to the Orbitrap analyzer [16, 17] and resulting in almost complete independence of initial phase of ion oscillations on mass-to-charge ratio. This synchronization allows conversion of spectra to so-called absorption spectra (corresponding to zero initial phase) that exhibit narrower peaks. The present implementation of eFT uses a combination of magnitude and absorption spectra along with Hanning apodization triple zero-filling and additional filtering to improve mass accuracy and peak shape. As the result, resolving power for the given duration of transient is doubled. Along with the use of high-field trap, it allows to provide nominal resolving power of 960,000 at *m/z* 400 for transients with duration of 3.04 second. However, experimental resolving powers may be lower due to noticeable signal decay over transient duration. This effect is investigated here to find out how good the resolving power of the Orbitrap with really complex mixtures can be.

Asphaltenes are of great interest in petroleum upgrading because until now they are mostly used to pave streets and have not really been converted into quality products. This philosophy is changing as research programs are carried out to investigate this heavy resources [18, 24-28]. By definition, asphaltenes are the components of a crude oil that is insoluble in normal paraffinic solvents (*e.g.* n-heptane) but are soluble in toluene. The first step in the characterization of a crude oil traditionally is the SARA fractionation [19], an often-used method that allows fractionation of ultra-complex crude oil mixtures into solubility based fraction, where the asphaltene residue in n-heptane is the first step. Recently, we have studied the analysis of asphaltenes in detail using a 12 T FT-ICR MS [18]. This instrument is capable of ultra-high mass resolutions of above 4 million when longer transients up to 24 s are used. Here, we are testing the new research type Orbitrap mass spectrometer using a 3 s transient and show how useful this cheaper instrument is for the analysis of extremely complex hydrocarbon mixtures.

In Figure 3-2 an example is shown combining a spectrum of the asphaltene sample and a spectrum showing the range of one mass unit. As can be seen here, up to 150 different signals can be observed, indicating the high resolving power in addition to the ability to store these large numbers of ions in the cell.

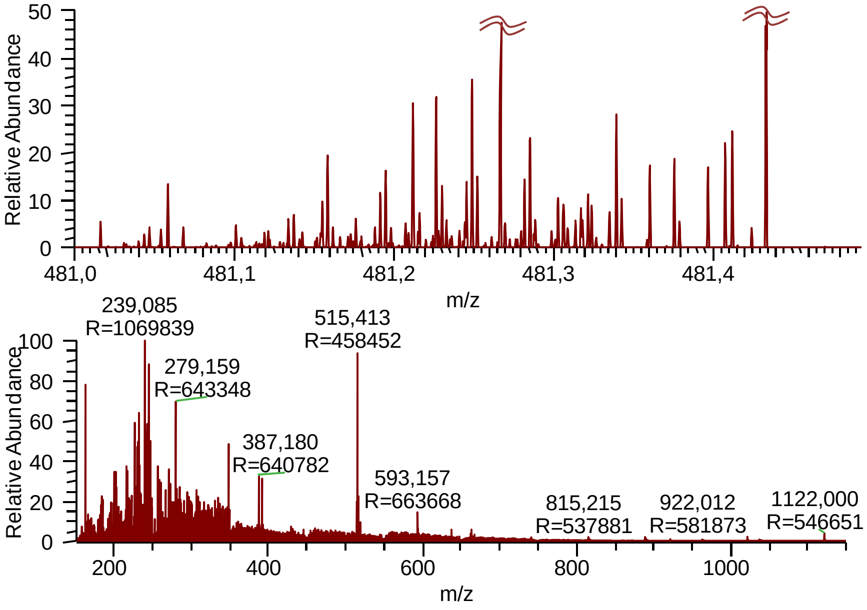


Figure 3-2: Ultra-high resolution spectra obtained from an asphaltene sample using APPI ionization. bottom: overall spectrum obtained by using selected ion monitoring (SIM) windows of 100 Da and combining them together by using spectral stitching as described elsewhere [20]. Top: zoomed-in spectrum displaying *m/z* 481.

Comparable data can be obtained from electrospray measurements of nitrogen rich crude oil mixtures as shown in Figure 3-3. Electrospray is the better choice as ionization method because the nitrogen components present are polar enough for this method. Here, data sets are compared that are obtained on one side from the standard method for complex mixture analysis, a 12 T FT-ICR MS, and on the other side from an Orbitrap mass spectrometer. For both instruments 3 s transients were used to keep the parameter in a comparable range. The data show that the results are very much comparable. A 3 s transient for a 12 T FT-ICR MS shows similar results than a 3 s transient for the Orbitrap.

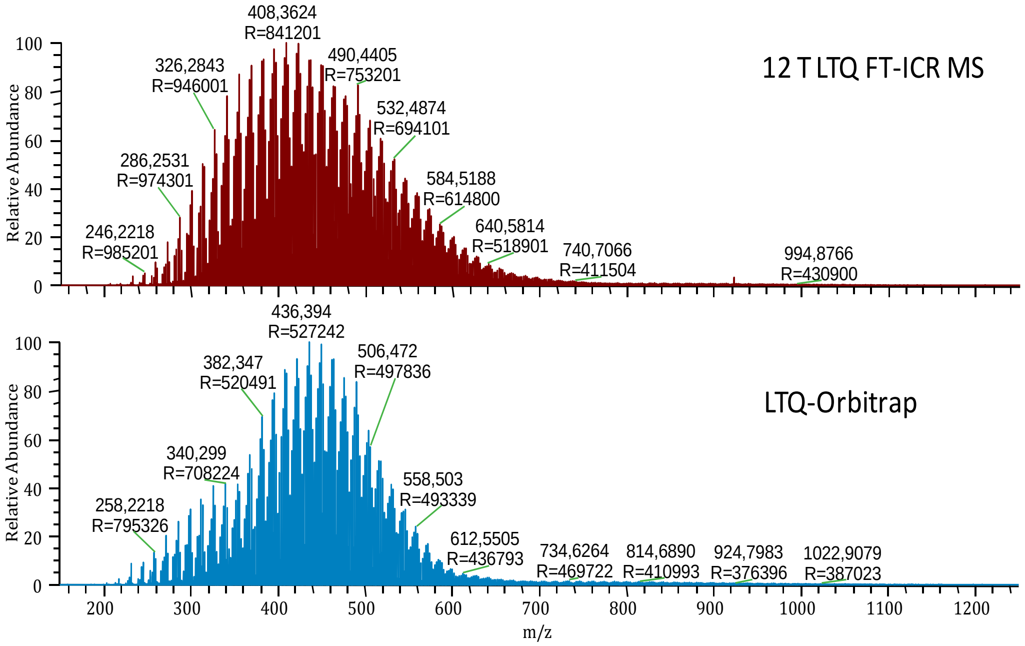


Figure 3-3: Comparison of ESI spectra of a nitrogen rich crude oil obtained from a. FT-ICR MS and b. high field Orbitrap MS.

One important indicator of performance is the resolution along the mass range that defines the capabilities of the instrument. An overview of the resolving power along a whole spectrum can be seen in Figure 3-2 (bottom), while in Figure 3-4 the resolving power from Orbitrap data are shown at different mass ranges for a 3 s transient. The results of this complex asphaltene sample show that the Orbitrap has its advantages at the higher mass scale, where even at *m/z* 1000 a resolution of 500,000 can be achieved.

For each signal in the spectrum now the elemental composition can be calculated based on the accurate mass data. This calculation allows assignments of each peak into classes depending on their elemental composition.

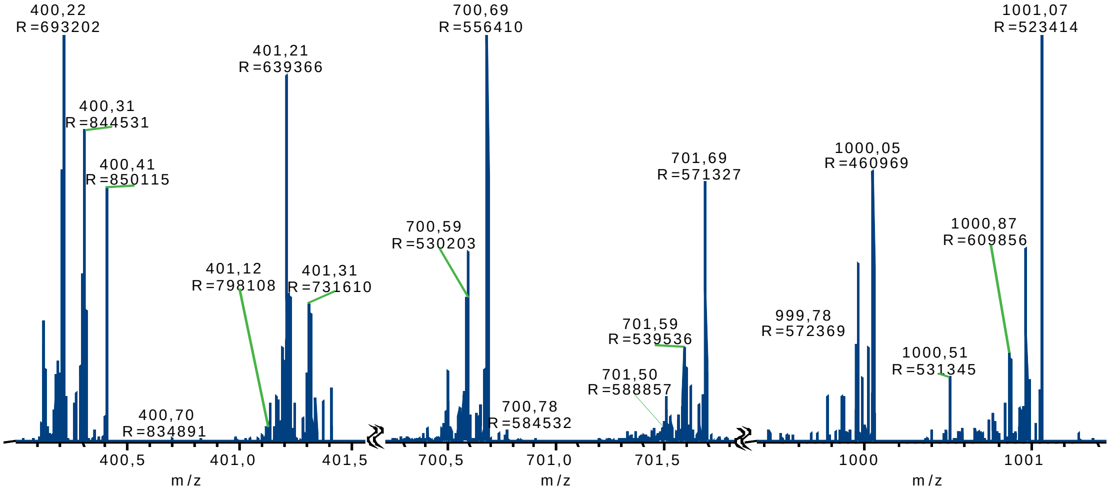


Figure 3-4: Mass spectrum of an asphaltene samples displaying the resolution R at different *m/z* values.

The best way to observe the capabilities of the instrument is by comparing the error of the assignments. This is done in Figure 3-5 where the calculated errors from the data of the ESI measurements are shown. The results indicate that the Orbitrap assignments are in the majority better that 0.5 ppm while especially in the lower mass region the error is still slightly higher for this research type instrument. Here, an adjusted calibration routine like the *walking calibration* that was already introduced for FT-ICR MS could be of help to improve the data [29].

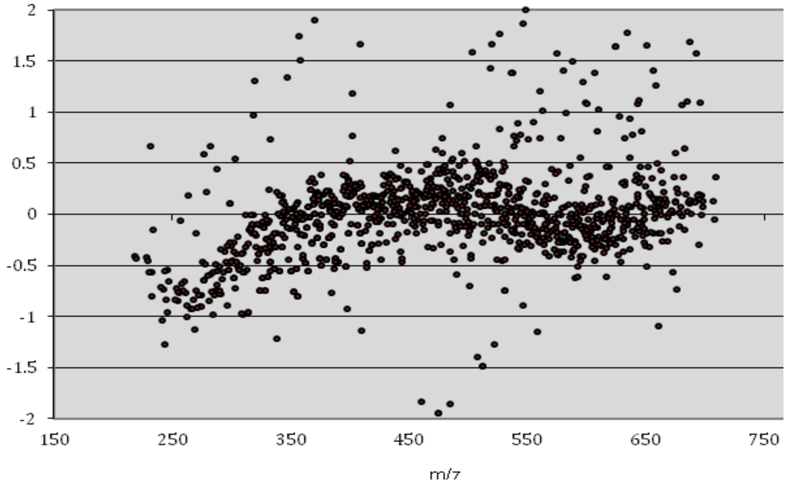


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Figure 3-5: Calculated error along the mass range obtained from the ESI-Orbitrap MS data shown in Figure 3-3.

3.5 Conclusion

These data show that this research type Orbitrap is a capable instrument to study very complex mixtures of hydrocarbons from energy related resources. The experimental data indicate that the resolving power starting with 800,000 at *m/z* 400 is a little lower than the highest possible theoretically value but still is a very reasonable value when considering that this ultra-high resolving power is obtained without a super conducting magnet that is used for FT-ICR mass spectrometers. Even at higher masses high resolutions can be obtained as at *m/z* 1000 still a resolving power of 500,000 can be achieved. Since all ion sources can be used, other applications with high complexity can be solved.

Acknowledgments

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Chapter 4 Deep Well Deposits: Effects of Extraction on Mass Spectrometric Results *

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4.1 Abstract

A crude oil deep well deposit was investigated using solvent extraction and electrospray ionization FT-ICR MS in both positive and negative mode. Six extraction solvents were used, covering a wide range of polarity, namely toluene, dichloromethane, chloroform, acetonitrile, dimethylformamide, and dimethyl sulfoxide. The highest extraction efficiency (about 56 %) was obtained with chloroform whereas dimethyl sulfoxide offered the least efficiency (about 21 %) among all the solvents subjected for extraction. With our analytical protocol using both solvent extractions and ultra-high resolution mass spectrometry with electrospray ionization (ESI-MS), we were able to detect the N_1 class, containing most likely pyridinic and carbazolic species, as dominant one in the deposit although each solvent was able to extract a wide variety of heterocyclic compounds of varying intensities. Although toluene - by definition - should dissolve the full asphaltene fraction here only a part of the sample was soluble including mostly the N-containing classes indicating that the deep well deposit does not consist entirely of asphaltenes. In comparison, DMSO showed the lowest mass balance but one of the broadest class distribution.

4.2 Introduction

Since the prices in the worlds' oil markets have skyrocketed, unconventional resources are gaining a lot of interest. Such resources often contain highly aromatic and low volatility compounds of an extremely complex nature, making the analytical task of identifying the exact molecular content extremely challenging, as can be seen in the most recent literature [1]. Analytical chemistry plays an important role in devising methodologies to investigate such supercomplex mixtures. The composition of crude oil is exceedingly complex and has been characterized as supercomplex [1]. This complexity is considered as one of the biggest challenges in analytical chemistry [2]. As a step for simplification, the organic components are traditionally analyzed after their separation into four fractions based on their solubility or polarity properties: Saturates, Aromatics, Resins and Asphaltenes (SARA), where the asphaltene fraction is defined as that part that is not soluble in an n-alkane solvent (typically n-pentane or n-heptane) [3, 4]. The unraveling of the chemical structure of asphaltene components has made progress in the last years [5, 6], but many points are still not understood or controversial as can be seen by the animated discussions about the molecular mass and structure of asphaltenes in the recent literature [7, 8].

There are several reasons why it is important to understand the composition of asphaltenes. Despite the current huge efforts to develop renewable energy sources, fossil fuels will continue to provide mankind with the largest part of its energy needs for several decades to come. The remaining fossil resources should therefore be used in a manner that is as efficient as possible. This includes making use of the heavier fractions of crude oils as well as the use of crudes that contain larger amounts of heteroatoms than traditional sources. Such materials can cause considerable problems, for instance through the formation of deposits in oil wells or pipelines [9]. Waxes and asphaltenes are the major components in organic deposits from petroleum fluids [10] , where the asphaltene fraction plays an important role during the crude oil wax crystallization [11, 12], although there is no synergistic interaction between these two components [10].

However, the asphaltene content alone does not predict the precipitation likelihood since even crudes with a high asphaltene content have been reported not to cause deposit problems in the field [13]. Experimental studies with model fluid systems suggest that it is the most polar fraction that has a tendency to precipitate [14]. This fraction may deposit ("*stick*") in the reservoir itself and/or on the walls of tubing and process equipment [15] and interrupt the flow of crude. Obviously, there is a major incentive to find ways to predict and avoid this problem. This task necessitates a thorough characterization of deep well deposits to gather information on their molecular constituents with a focus on polar compounds.

Mass spectrometry remains as the technique of choice for characterizing crude oil and related samples when traditional chromatographic methods, like gas chromatography fails due to non-volatility of very polar or high-molecular mass compounds. Here, ultra-high resolution mass spectrometry (Fourier Transform Ion Cyclotron Resonance MS, FT-ICR MS) has provided invaluable information [16]. Since only ions are analyzed in MS, the ionization exerts a decisive influence on the results. Electrospray ionization (ESI) is routinely used for the analysis of polar compounds believed to be the major cause of deep well deposits, although it is realized that discrimination and ion suppression have been observed during ionization [17]. Additionally, the separation or fractionation of crude oil into subfractions containing less components revealed that more signals were detected from the fractions than from the whole crude oil [18, 19]. Different ionization methods for the analysis of crude oil fractions have been compared and it has been shown that for aromatic compounds in asphaltene fractions every one of the five tested ionization techniques gave rise to a unique mass spectrum [1, 20]. All these studies show that suppression and discrimination effects are present during the analysis of really complex crude oil samples, so now it is important to understand how these effects affect the analysis.

Deep well deposits contain the elements carbon, hydrogen, nitrogen, oxygen, sulfur and a variety of metals like vanadium, nickel and iron in different proportions [21, 22]. The complexity is immense and it is probable that many compounds can contain several functional groups. For such deposits, a method based on FT-ICR MS has been described [23]. However, the analysis was limited to the acidic components of the toluene-soluble fraction.

The solubility parameters of petroleum residua were investigated in detail, and extractions were performed by vapor pressure osmometry resulting in the development of a molecular weight /polarity map of this material [24]. Still, the information is being kept at a bulk material level and no molecular information is available. One field where extraction of crude oil components are studied in much detail is the water extraction of bitumen from oil sands and especially the water soluble content that remains in the water solution. Here, mass spectrometry has shown to be a very valuable tool already [25].

We are interested in exploring the dependence of the mass spectra of deep well deposits on the sample extraction method to better understand both the chemical constituents of the deposits and the particularities of the extraction on a more molecular level. Since these samples can vary so much and contain so many compound classes, it can be expected that the extraction solvents may have an influence on the subsequent mass spectral picture obtained. For a fundamentally sound analysis it is imperative to comprehend the behavior of these materials. The focus of this work is to gain a better understanding of how the sample preparation of deep well deposits, with an emphasis on the extraction, influences the analytical results and how the deep well deposits correlate to the asphaltene fraction.

4.3 Experimental Section

4.3.1 Chemicals and Crude Oil Solid Deposit

All the HPLC grade solvents for extraction and mass spectrometry experiments were obtained from Sigma-Aldrich (Taufkirchen, Germany). A deep well deposit was provided by Shell Global Solutions, Amsterdam.

4.3.2 Solvent Extraction

About 5 mg of solid deposit was taken for each individual solvent extraction. The solvents used were toluene (TOL), dichloromethane (DCM), chloroform (CLF), acetonitrile (ACN), dimethylformamide (DMF), and dimethyl sulfoxide (DMSO). 1 mL of solvent was added to the deposit and sonicated for 15 minutes. After this the solution was filtered using Blue Ribbon filter paper with a pore size of 2 μm and the weight balance of the insoluble part was determined after drying. The filtrates obtained from the extractions were analyzed by FT-ICR MS.

4.3.3 Mass Spectrometry

ESI positive ion mass spectra were acquired using an APEX III FT-ICR MS (Bruker Daltonics, Bremen, Germany) equipped with a 7 T actively shielded super conducting magnet and an Agilent ESI source. The extracted samples (200 μL) was diluted with 800 μL methanol and spiked with 2 µL acetic acid or ammonium hydroxide for positive or negative ion analysis, respectively, and injected at a flow rate of 2 µL/min. The spray voltage was maintained at 4.5 kV. After ionization, the ions were accumulated for up to 3 s in an octapole before transfer to the cyclotron cell. For a better signal-to-noise ratio up to 500 scans in the mass range between *m/z* 200 and 2000 were accumulated. For ESI negative ion spectra, the extracted samples were also diluted with methanol and ammonia was added. The other conditions were as above, except that up to 700 scans were accumulated. Internal and external calibrations were performed using a mixture of the Agilent electrospray calibration solution with the masses 322.04812, 622.02896 and 922.00980 with the addition of indolacrylic acid with the masses 397.11589 $[M+Na]^+$ and 584.17923 $[2M+Na]^+$, thus covering the whole mass range in the samples.

4.3.4 Data analysis

The unprocessed mass data were imported into the Composer software (Sierra Analytics, Modesto, CA, USA) and calculated according to their elemental composition. The following chemical constraints were applied: Number of H unlimited, $0<C<100$, $0<O<2$, $0<N<2$, $0<S<2$, $0<DBE<40$ with a maximum mass error of 2 ppm and an S/N ration above 3. The IUPAC mass obtained from mass spectrometry can be easily converted to the Kendrick mass by using the formula:

Kendrick mass = IUPAC mass x (14.00000/14.01565)

This scale has been adopted as a standard protocol for the analysis of data obtained from ultra-high resolution mass spectrometry in complex mixtures.[26] After multiple sorting based on the Kendrick scale, different classes of compounds were grouped depending on the hetero-element present [27]. The masses from each class of compounds were further grouped based on double bond equivalent (sum of rings and double bonds). The assignments of the most abundant ions were further confirmed through their isotopic peaks. The obtained mass lists were transferred into Excel and Origin for data evaluation and preparation of the figures shown.

4.4 Results and Discussion

Although ultra-high resolution mass spectrometry is a powerful tool for complex mixtures, it cannot be expected that all compounds in the present sample can be completely and accurately analyzed by any single available analytical method. A minor change in extraction procedure or the addition of a different acid or a different base in place of acetic acid and ammonia may influence the representation of the sample composition that is obtained. Thus, this investigation can be a preliminary step towards the establishment of a suitable method for the successful characterization of asphaltenes and deep well deposits. A deep well deposit of Caribbean origin was investigated after extraction with six different solvents and the results from MS measurements will be discussed here. We used six solvents for comparison of the extraction of the deep well deposit. Each sample was subjected to ESI FT-ICR MS measurement in both the positive and the negative ionization mode. In an earlier investigation, negative electrospray ionization was preferred for the study of the asphaltenes since the acidic compounds were believed to be the sole cause of the deposit [23], but we extended the analysis to encompass all ionizable classes of polar components in deep well deposits. These deposits were taken from deep wells and represent a mixture of deposited material. The first observation about these extraction experiments was that - independently of the solvent - a part of the material could not be dissolved. Gravimetric calculation reveals that a maximum of ca. 60 % of the sample is dissolved in any of the solvents. In this case, the amount of solvent does not play a role. A mass balance obtained after the extractions is summarized in Table 4-1, showing that the more polar solvents performed fairly poorly compared to the less polar ones.

A first overview of the ESI-MS spectra can be gained from the spectra shown in Figure 4-1 that depicts the six extracts of the asphaltene deposit. The spectra show some differences as the various extraction solvents seem to accentuate different components. The high mass accuracy of the measurements makes a calculation of the elemental composition of the ions possible. Mass spectra of the toluene and acetonitrile extracts occupy similar mass range of *m/z* 250-550, whereas the rest covers the mass range up to *m/z* 650.

Table 4-1: Dissolved fraction of the deep well deposits for six solvents.

	TOL	**DCM**	**CLF**	**ACN**	**DMF**	**DMSO**
Amount dissolved	47.2 %	49.4 %	56.3 %	26.0 %	31.3 %	21.1 %

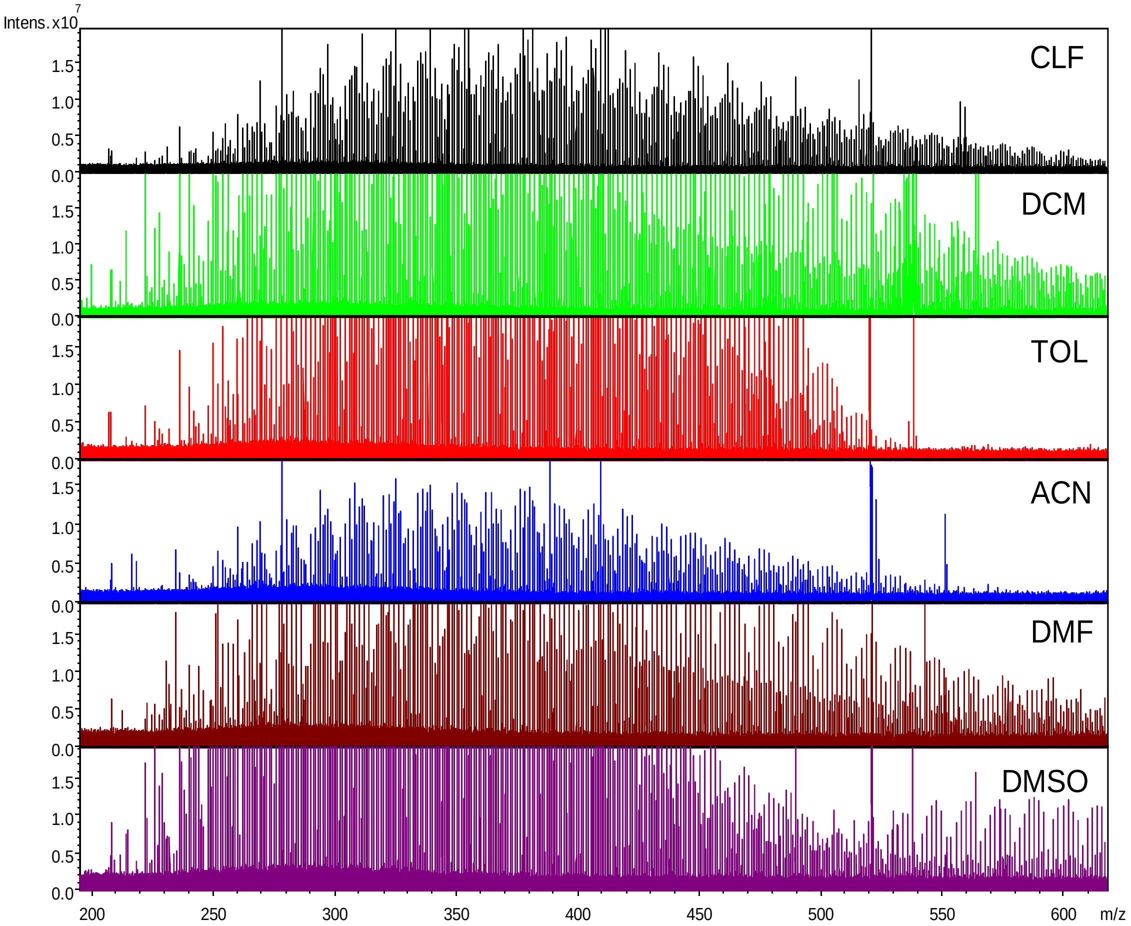

Figure 4-1: Comparison of different ESI (+) FT-ICR MS spectra of the solid asphaltene deposit.

These data clearly show that there is discrimination and, in general, it has to be assumed that each extraction method emphasizes a certain subset of the whole complex sample and makes this accessible for analysis. In particular the data reveal that not all components are present in all extracts. Therefore, each extraction solvent dissolves only a part of the initial sample.

Before going into details of these mass spectra, we want to discuss briefly how these calculations are being done and what kind of data are being compared. Some components in crude oils are discriminated during ionization [18, 20]. It has been shown that if a crude oil sample is fractionated more signals can be detected combined from the fractions than from the initial crude, meaning that the less components present, the more signals were detected overall [19]. Due to the enormous complexity in a crude oil sample there are several different functionalities present for even a single class. Different functional groups are ionized to very different degrees. The ease of ionizing a component in ESI strongly depends on its functional group - or more precisely - on the polarity of it where O_2 or N_1 classes are prime examples. Carboxylic acids form negative ions some ten times more easily than neutral nitrogen species [23], and therefore the O_2 class of compounds (fatty acids and naphthenic acids) are strongly enhanced when compared to the N_1 class of compounds if only the ion abundance is regarded. Comparing the ratio of intensity of oxygen species O_n along with nitrogen containing species NO_n can be a helpful fingerprint information as has been shown in Anthabasca oil sands polar organics [28]. This effect is more pronounced in electrospray ionization which is very effective for polar components where it shows a strong response and less effective for non-polar components where the response is less than ideal. But it is not limited to ESI because other ionization methods have other components that have a better response factor and because of the complexity of crude oil samples there are always components that get discriminated.

When summarizing the results for the different classes the question arises how to order these data into their respective class. There are two different approaches: the first is to add the intensities of all signals of the individual classes (intensity based), and the second one is to summarize the number of assignments to a certain class, here called the population based plot. None of the methods gives a 1:1 depiction of the situation in the sample, though, but each method has its merits which need to be known. A comparison of both calculations is displayed in Figure 4-2 where the data from the extraction with chloroform are presented both as a population plot and an intensity plot.

The differences can be best discussed when looking at the N_1O_1-class. In this case there is a relatively large number of signals in the spectrum but they are all of very low intensity. This does not necessarily mean that the total amount of the N_1O_1 species is low as indicated in the intensity based plot. The intensity cannot directly be related to a concentration since neither is any information available about the response factors of each component nor is their ionization efficiency known. The population plot shows that some 300 signals were assigned to the N_1O_1-class, a very significant number. How different the two ways of viewing the results are becomes obvious when comparing the N_1O_1 class with the N_1 class. The relative abundance class distribution shows a ratio of 1:6 between these two classes, while the ratio is almost 1:2 in the population based class distribution.

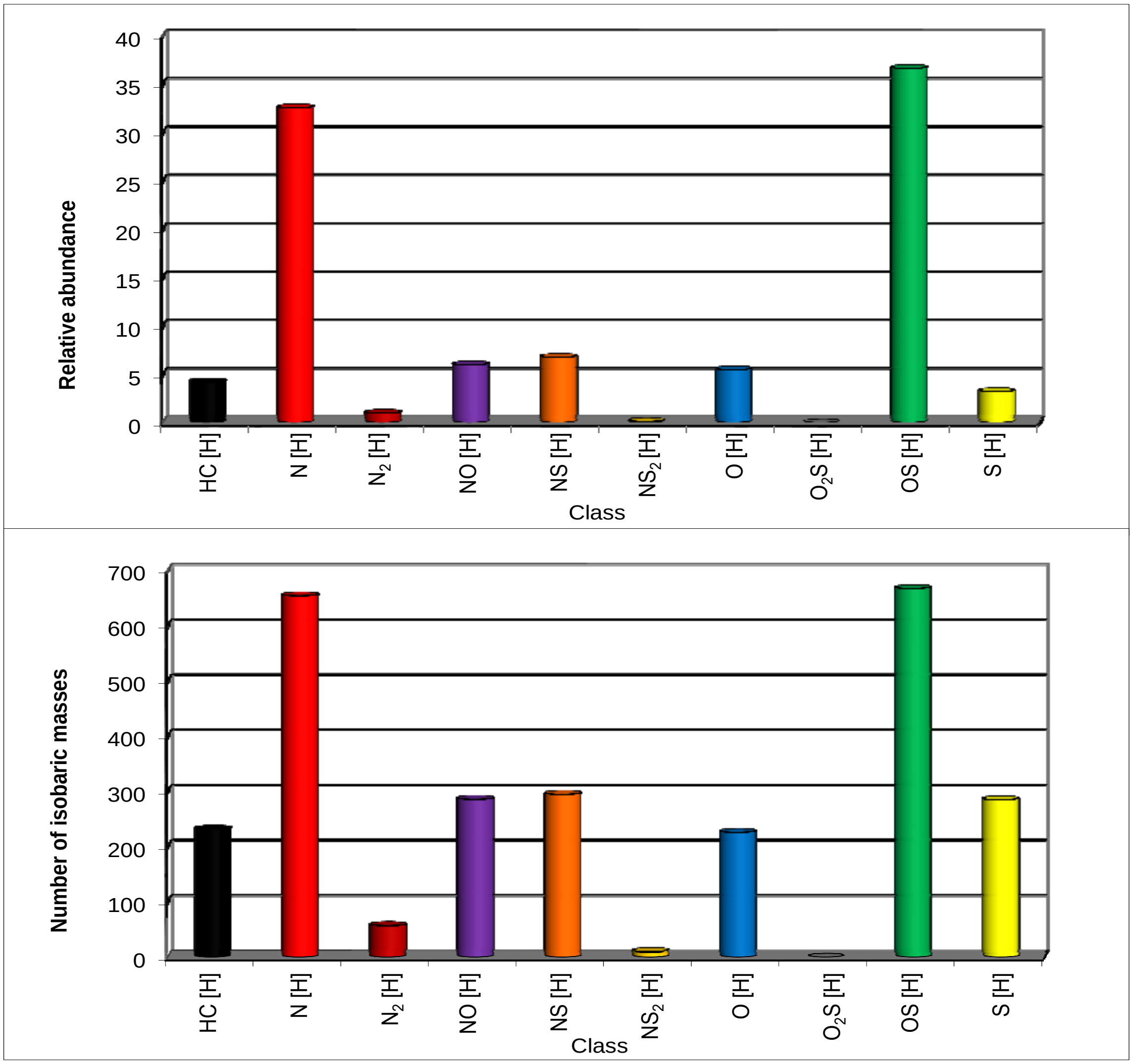

Figure 4-2: Comparison between intensity based (top) and number of isobaric masses (population based plot; bottom) assignments observed by positive ion ESI FT-ICR MS in the chloroform extracted sample. Protonated molecules are denoted as X[H].

This example shows that since no information is available for the response factors of the different classes of compounds, there cannot be even a semi precise way to correlate the intensity of a signal with its concentration. Therefore, we have to assume that each signal can in principle be derived from the same amount, irrespective of the signal intensity. For this reason the use of a relative intensity scale can lead to a significant distortion of the true distribution of the sample constituents if they contain different functional groups. We recognize that no absolute scale has been devised to provide a true quantitative representation of the constituents in a complex sample and prefer the population plot instead of the relative abundance for presentation of data in this work. This representation treats every assignment equally regardless of ionization efficiencies and response factors among others.

4.4.1 Chloroform extracted sample

The amount of data generated is very large and a detailed presentation for all the solvent results unnecessary. Therefore, only the extraction with chloroform will be documented in some detail for a better understanding of the kind of compounds extracted before the six solvents are compared. The class distribution in the chloroform extract measured in the positive mode is shown in Figure 4-2. The N_1 class dominates, followed by O_1S_1, N_1O_1, N_1S_1 and S_1. Surprisingly, in addition to the more polar groups that contain nitrogen or oxygen atoms, it was possible to detect compounds that contain only sulfur and hydrocarbons. Although sulfur compounds are thought not to be polar enough for ESI measurements, a large number of them – here more than 250 assignments - were detected. Presumably they are not of a thiophenic nature and most likely should contain a more polar functional group such as sulfide or thiol. The addition of a minute amount of acetic acid helps in the protonation of basic compounds, so the observed N_1 compounds are probably basic nitrogen heterocycles of the pyridine type. However, further detailed information on these species can only be achieved using multidimensional methods where a combination of chemical methods, chromatography or electrophoretic separation, selective isolation and spectroscopic detection is used.

Ten compound classes were observed in the negative polarity mode (Figure 4-3). In this case a large number of negative ions are detected which shows the significance of acidic components and neutral nitrogen heterocycles in solid deposits. The dominating class is N_1 followed by N_1O_1, N_1S_1 and N_2. The N_1 compounds observed here would be neutral nitrogen species because of their tendency to form deprotonated ions with ammonia in the negative ionization mode.

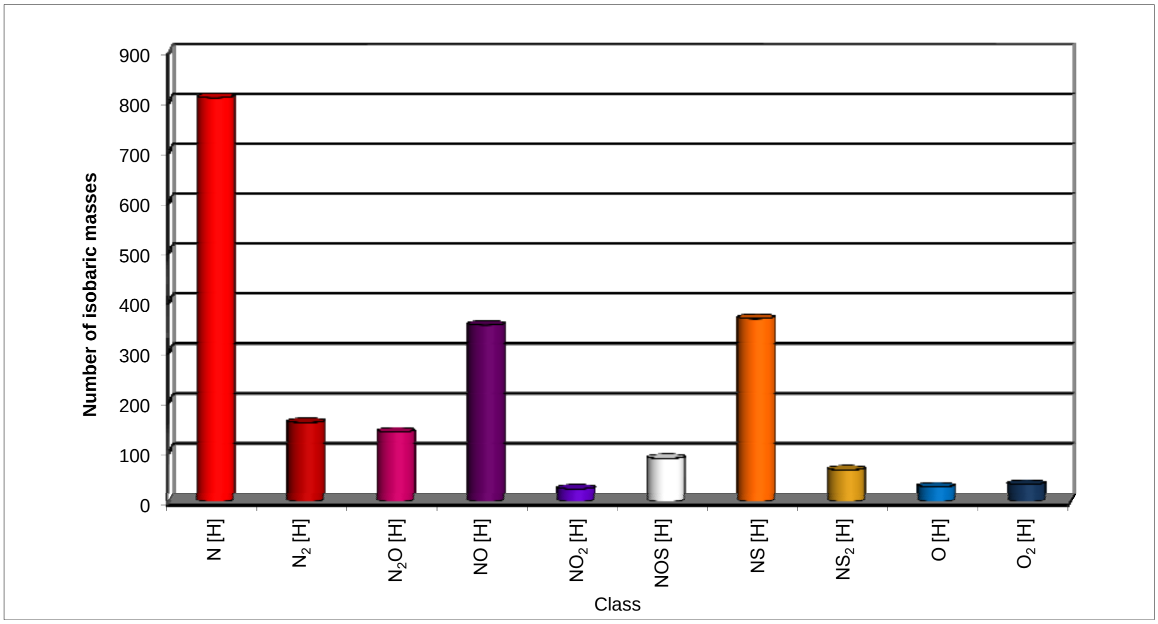

Figure 4-3: Number of assigned components for various hetero classes observed by negative ion ESI FT-ICR MS in the chloroform extracted sample.

The number of different isobaric masses present in a certain class gives a good overview of the data. In Figure 4-4 we compared three major classes of compounds, namely N_1, N_1O_1 and N_1S_1, observed both in the positive and the negative electrospray ionization, in a Kendrick plot that allows the correlation of the elemental composition with the mass defect or double bond equivalent (DBE). The DBE value can provide some information on the chemical structure since it is equivalent to the sum of the number of double bonds and rings in the compound.

The distribution pattern across DBE series of the N_1 class was quite different for the two polarity modes. In the positive mode the lowest DBE observed was 5, possibly pyridines with one naphthenic ring (DBE 4 represents pyridine as one of several possible structures), while the highest DBE was 22. The abundance of the signals reaches a maximum at DBE 10. The heaviest mass lies at about 700 Da. In the negative mode the trend was quite different where the lowest and highest DBEs were 9 and 21, respectively. The DBE 9, 12 and 15 series represent the most intense series.

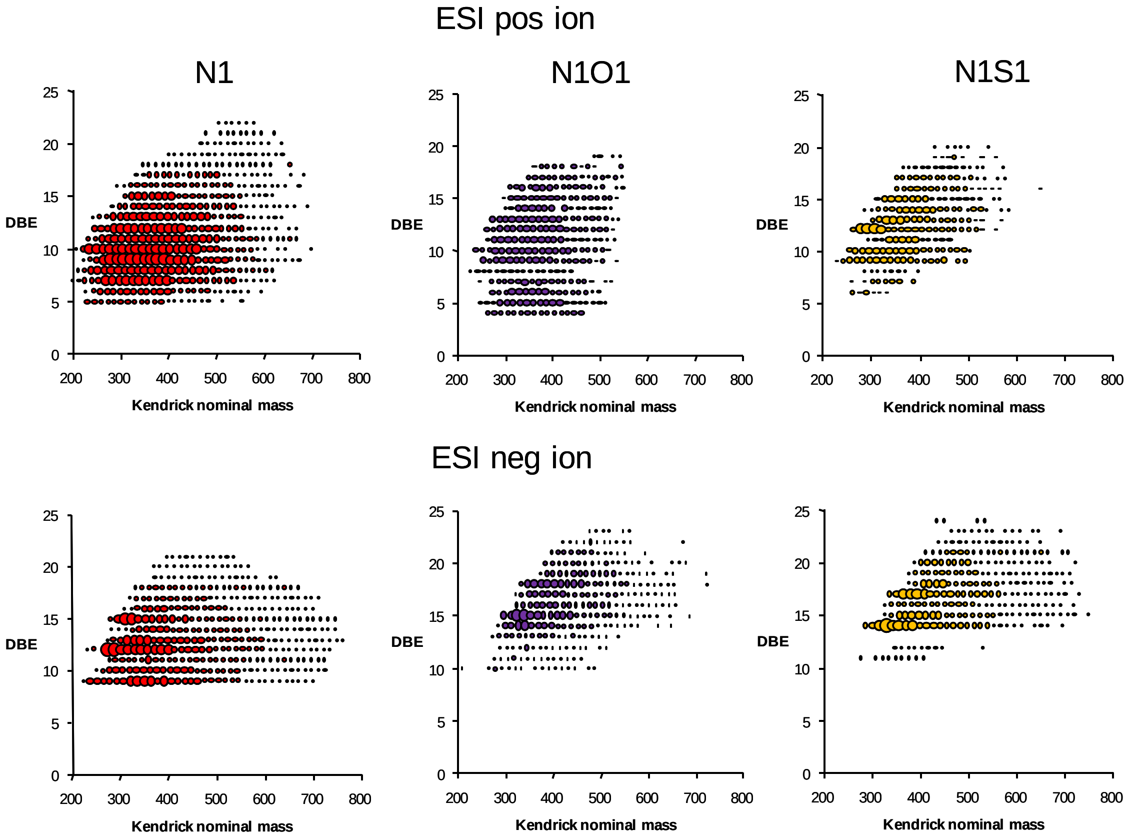

Figure 4-4: Kendrick plots of the N1, N1O1 and N1S1 classes observed both in positive and negative ESI FT-ICR MS in the chloroform extracted sample.

The lowest DBE was 4 and the highest DBE 19 for the N_1O_1 class in the positive mode. Among the negative ions, the lowest DBE was 10 and the highest 23 with DBE 15 and 18 as the most intense series as it illustrated in Figure 4-5. Thus it can be assumed that the N_1O_1 class contains a carbazole in addition to one oxygen atom (the first DBE observed was above the DBE of carbazole which is 9). In the positive mode, the DBE ranges from 6 to 20 and in the negative mode 11 to 24. In the positive mode, the highest mass observed for N_1, N_1O_1 and N_1S_1 was about 700 Da, 550 Da and 600 Da, respectively, whereas for the negative ions the highest mass was about 750 Da, 700 Da and 750 Da, respectively. The lowest DBE for the negative ions was in the region of 9-11 and for the positive ions in the range of 4-6, the same as for the N_1 compounds in both modes.

This typical distribution pattern was also seen for the thiophenes in a crude oil sample where the DBE 6, 9 and 12 are the most intense followed by 7 and 8, and 10 and 11, and 13 and 14 [29]. Using chromatography, UV spectroscopy and FT-ICR mass spectrometry, it was verified that DBE 6, DBE 9 and DBE 12 correspond to benzothiophenes, dibenzothiophenes and benzonaphthothiophenes, respectively [30]. Different DBE values have recently been connected to structural components for S containing components, where DBE 7-11 are benzothiophenes with 1-5 naphthenorings and DBE 10-14 are dibenzothiophenes with 1-5 naphthenorings [31]. From this it can be inferred that in the present deposit sample DBE 9, 12 and 15 correspond to carbazoles, benzocarbazoles and dibenzocarbazoles. The heaviest masses lie beyond 750 Da.

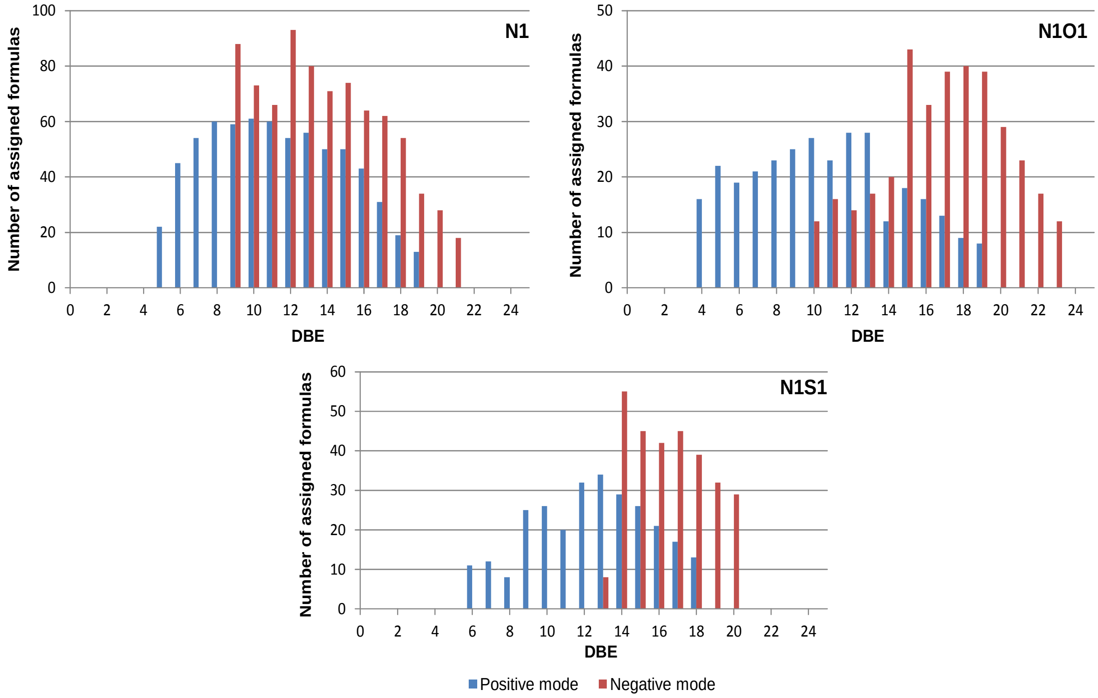

Figure 4-5: DBE distribution of the classes N_1, N_1O_1, and N_1S_1 of the chloroform extracted sample in both ionization modes.

4.4.2 Comparison of extractions

To investigate how dependent the result of an ultra-high-resolution MS analysis of a very complex mixture is on the workup of the sample, we show the data of different extraction solvents for a solid deposit from an oil-well in both the positive and negative ion mode. All the samples were measured under the same instrumental conditions in order to observe the effect of sample preparation on the representation of different classes. In the positive mode, ten major classes of compounds from six solvents are considered (see Figure 4-6). In all six extracts the three classes N_1, N_1S_1 and O_1S_1 were present. In four extracts, two further classes were found, namely N_1O_1, and O_1. The S_1 class was repeatedly observed only in the chloroform extracted sample whereas three solvents were able to extract at least some members of the N_2 class. Chloroform extracted the largest number of isobaric masses for all common classes, possibly due to its medium range of polarity, which helps in the extraction of both semi-polar and polar components. On the other hand, the extraction with acetonitrile delivered the least number of assignments among the six solvents. The N_1 class is by far the most widely detected class in this study but distinct differences between the extraction solvents emerge when the mass spectra are analyzed in detail.

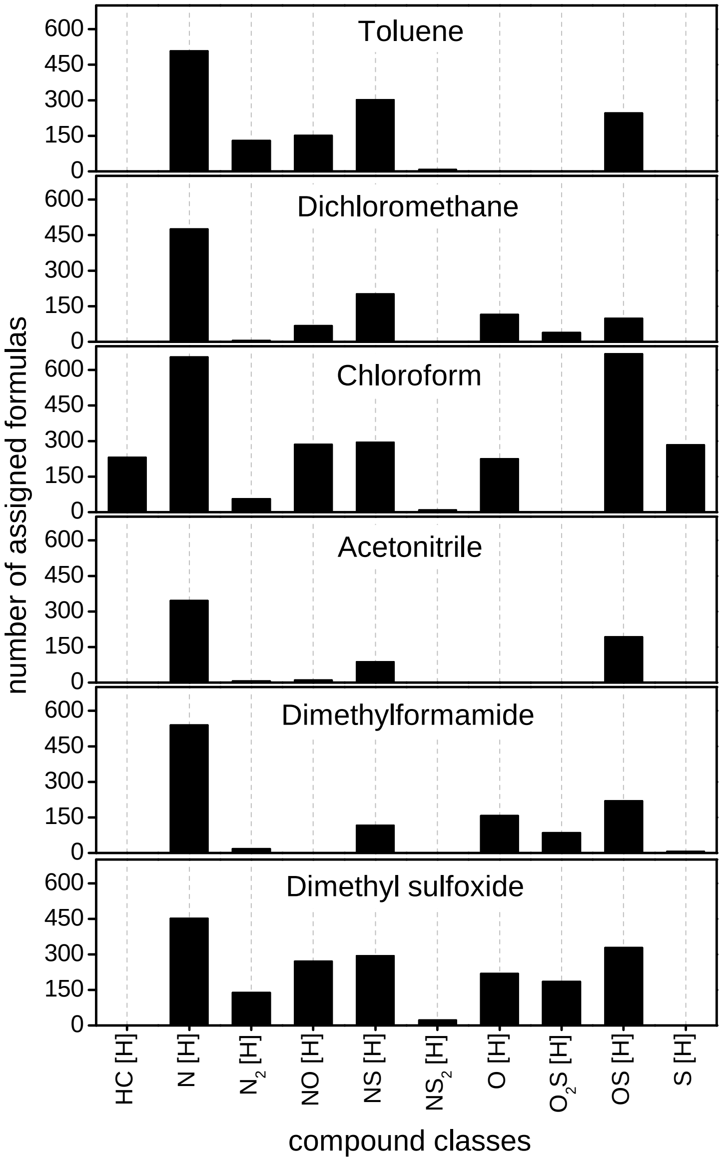

Figure 4-6: Major heteroatom classes observed in positive ion ESI FT-ICR MS by using six extraction solvents.

Figure 4-7 illustrates the contribution of the most dominant compound class types within each extraction solvent presented in heat map mode. The DBE distribution was calculated on the basis of the number of isobaric masses. N_1 is the dominating class in all cases, and the DBE distribution covering the range between DBE 6 – 21 was observed in toluene, chloroform and DMSO, whereas the other three solvents were more limited either in regard to the mass range or to the aromaticity.

While DMSO has shown to extract the least amount from the deposit it also shows a very broad spectrum of components. This could be due to the fact that DMSO should extract a higher amount of polar components, which are also easier to ionize by ESI. This shows that sample preparation and ionization play hand in hand in the analysis of such complex samples as deep well deposits. Similar observations were found in regard to the class O_1S_1, covering a lower DBE range though. The most abundant DBE distribution of the classes N_1O_1 and N_1S_1 was observed by DMSO extraction. Highly polar classes such as N_2 and O_1 have the most abundant DBE distribution in the most polar solvent used, namely dimethyl sulfoxide.

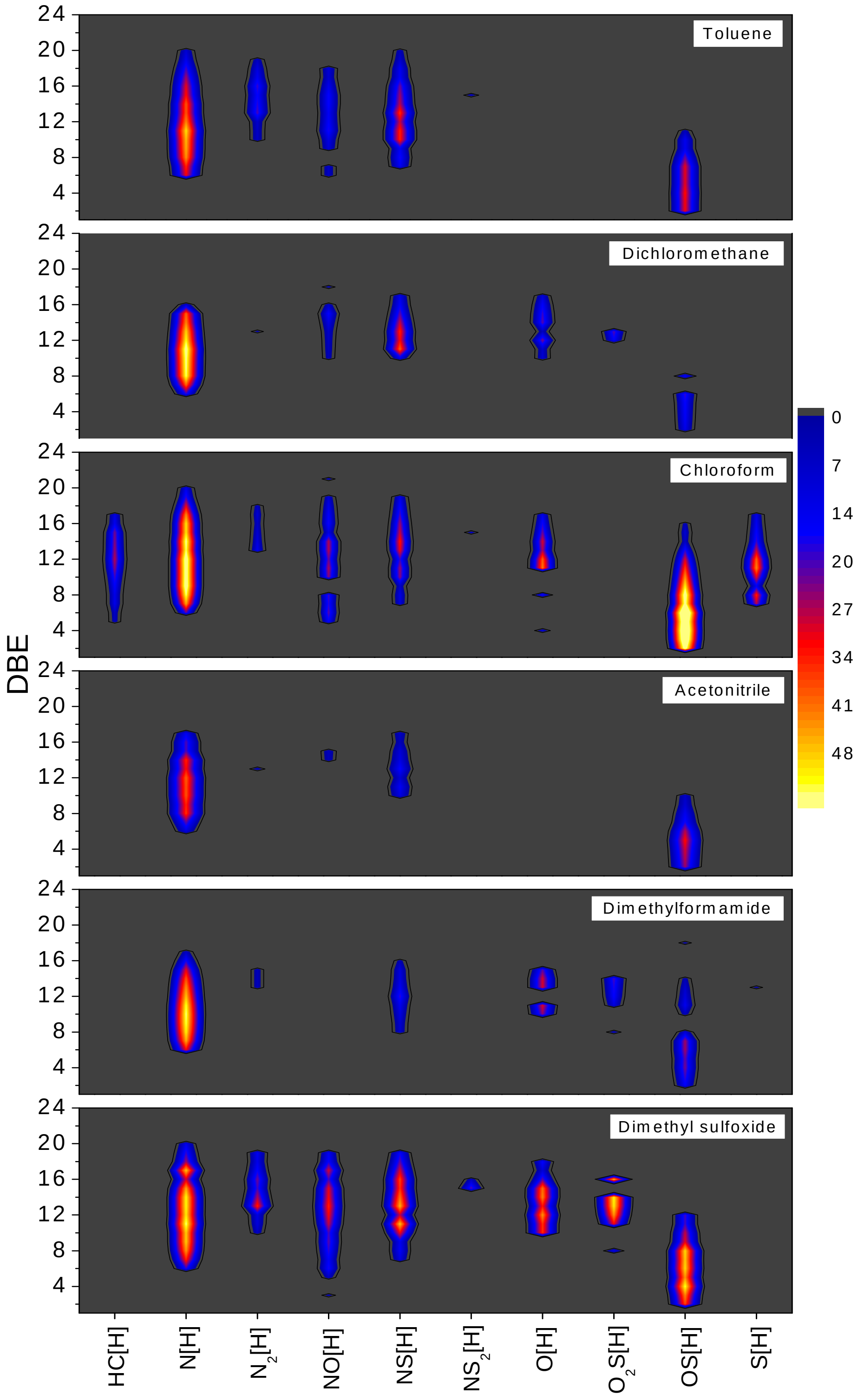

Figure 4-7: DBE distribution (heatmap) of the individual compound classes of a solid asphaltene deposit after extraction. (Data obtained in the positive mode).

In order to accentuate the role of each solvent and its characteristics on the extraction procedure, the unique compound assignments of each solvent is illustrated in Figure 4-8.

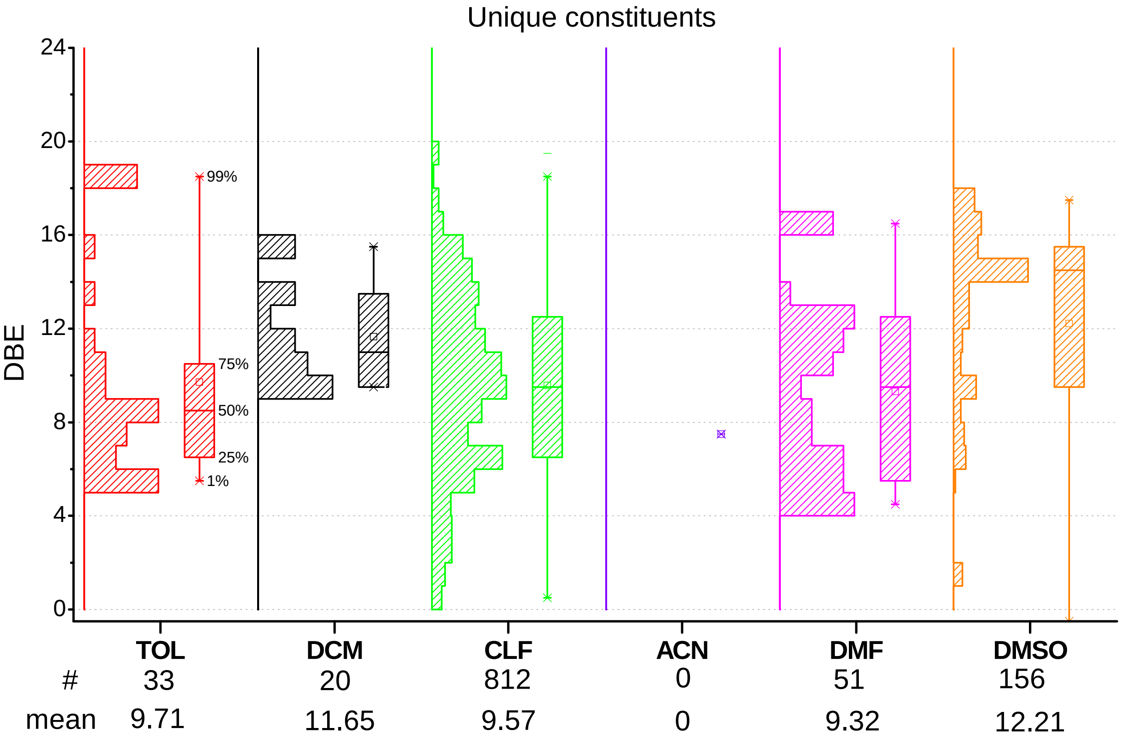

Figure 4-8: DBE distribution of the unique assigned molecules within the different extraction solvents in the positive mode.

The highest number of unique assignments was detected in the chloroform extraction covering almost the entire DBE range. On the other hand, all of the assigned peaks in the acetonitrile extraction were shared by at least one solvent of the other five, therefore no unique assignments were observed, which was expected according to the previously observed class and DBE distribution of acetonitrile. About half of the unique constituents found in the DMSO extraction were in the DBE range 12 - 14, which corresponds mainly to the class O_2S_1.

In a similar approach, mass spectrometric data in the negative mode obtained from four solvents were analyzed and class distribution is illustrated in Figure 4-9.

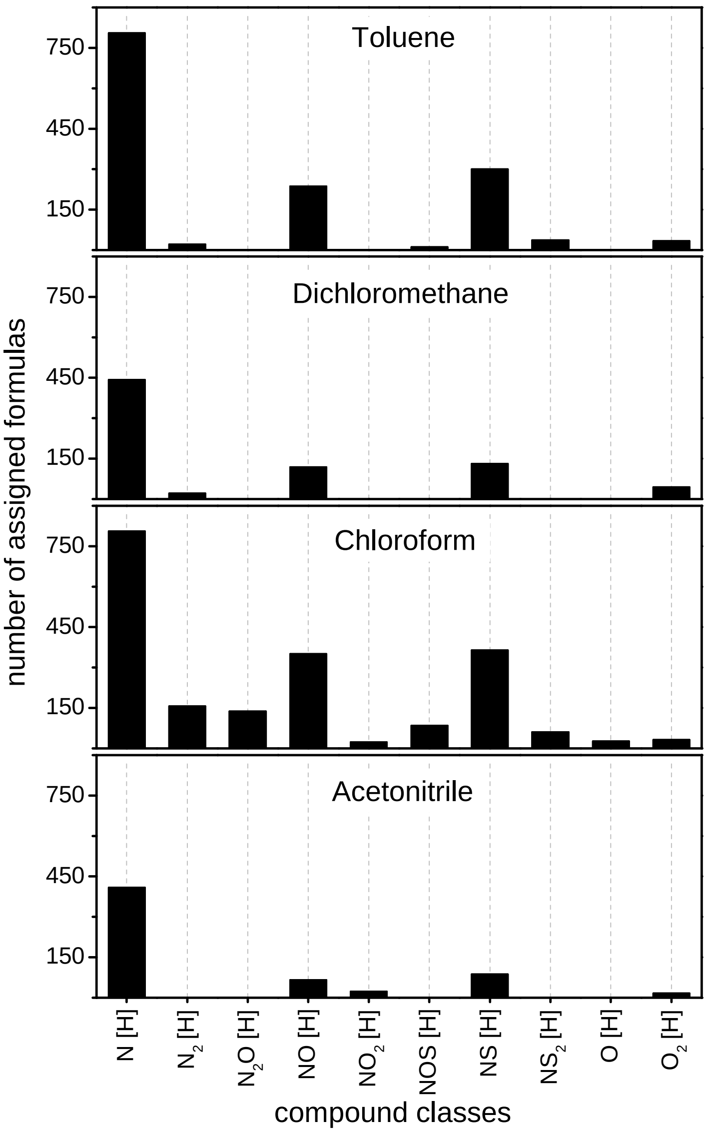

Figure 4-9: Major hetero classes observed by negative ion ESI FT-ICR MS in extracted samples.

N_1, N_1O_1, and N_1S_1 are the dominant common classes in all four extraction solvents, whereas classes such as N_2O_1 were only detected in chloroform for instance. Sulfur species were detected in the negative mode only accompanied with nitrogen, like $N_1O_1S_1$, N_1S_1, and N_1S_2, whereas a combination class of oxygen and sulfur was only detected in the positive mode. The dominance of the N_1 class is visualized in the DBE distribution (see Figure 4-10), especially in the toluene and chloroform extracts where the DBEs range between 11 and 24. The O_2 class, which can correspond to naphthenic acids, was present in all solvents; however, it was limited to DBE 3 in most of the cases.

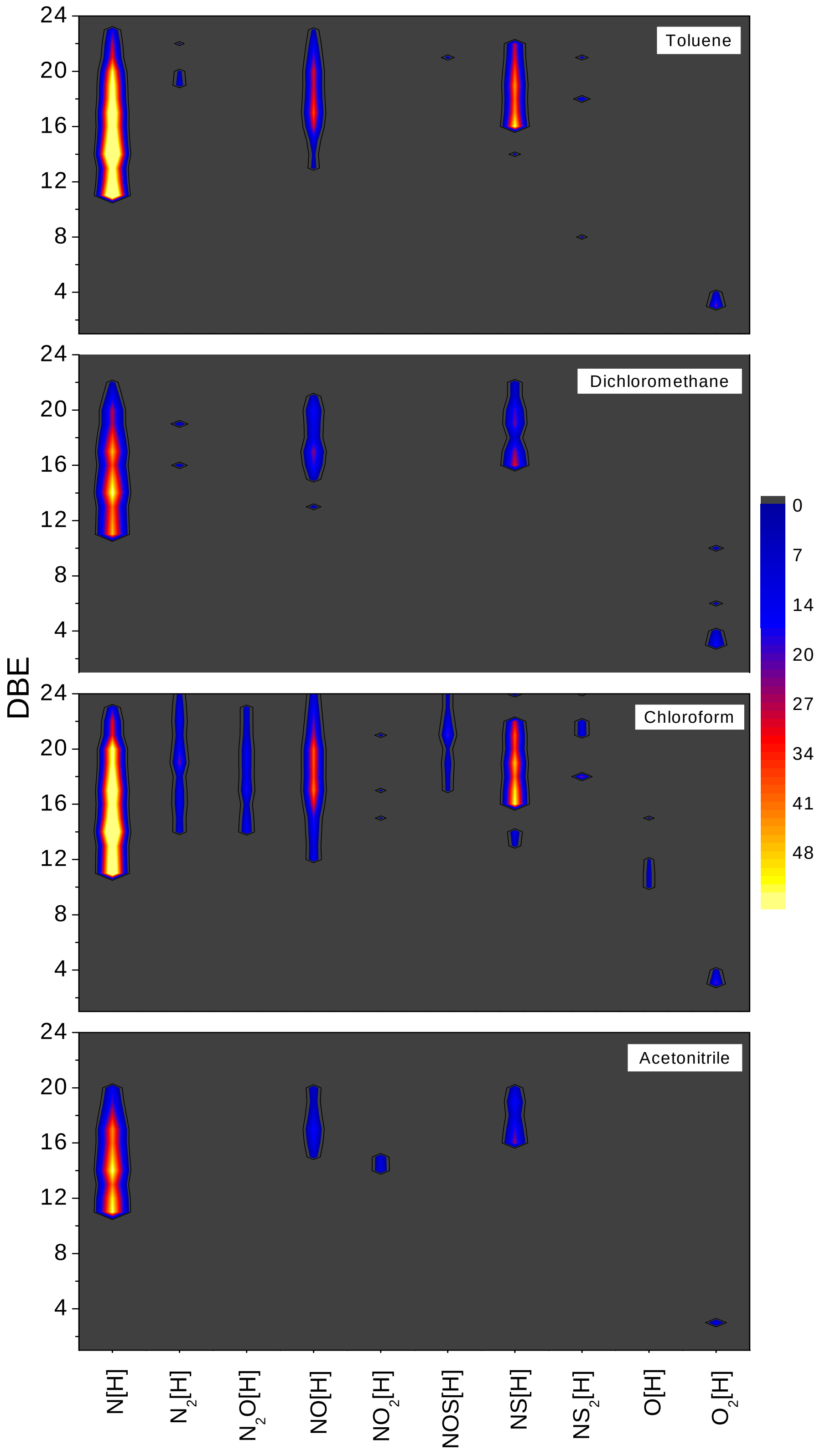

Figure 4-10: DBE distribution (heatmap) of individual classes of a solid asphaltene deposit after extraction (Data obtained in the negative mode).

As in the positive mode, the chloroform extraction delivers the largest number of assigned signals in the negative mode. A total of 660 unique formulas were assigned in the negative mode for the chloroform extract, almost 12 times more than for dichloromethane. The chloroform extracted species also show higher aromaticity than those of other solvents, with a mean value of DBE 17 as shown in Figure 4-11.

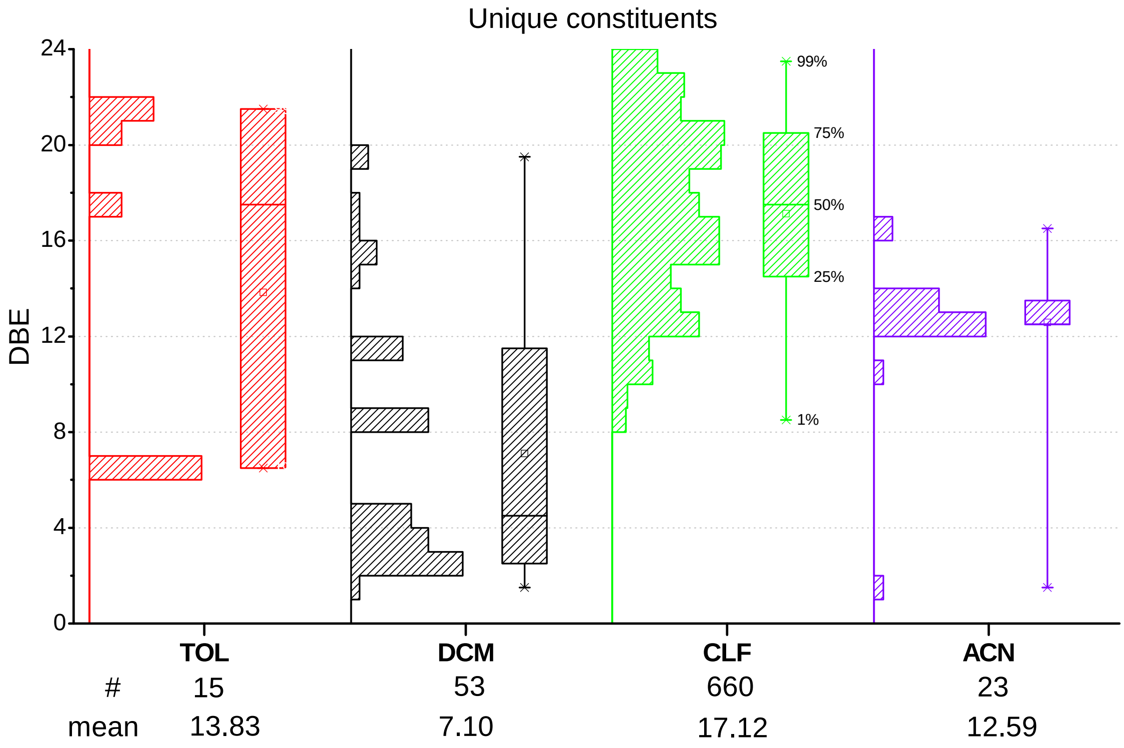

Figure 4-11: DBE distribution of the unique assigned molecules within the different extraction solvents in the negative mode.

The conclusion is that every extraction solvent has a certain affinity towards some classes of compounds. This selectivity makes it impossible to say what the true composition of the asphaltene deposit is. That only a partial dissolution of the sample is obtained, independent of the solvent, shows that even such a simple procedure as extraction plays a significant role in the analysis of such complex samples.

These data also point to the difficulty of comparing results obtained in different laboratories that besides instrumental parameters results can only be compared when detailed sample preparation data are provided since it cannot be excluded that small variations in the procedure can have a great influence on the MS result. Unfortunately, MS data cannot reveal the characteristics of the individual molecules but this study shows the tremendous differences that can occur after using extraction as a necessary tool of sample preparation for this particular crude oil fraction. Therefore, greater emphasis must be placed on understanding the compound classes in crude oils – both for analytical accuracy and for the optimization of upgrading processes for such heavy and unconventional materials as deep well deposits.

4.5 Conclusion

Ultra-high resolution mass spectrometry is a highly informative method for the analysis of incredibly complex samples such as deep well deposits. However, before the sample is introduced into the mass spectrometer, it must undergo some sample preparation, even though this may be as simple as dissolution. In this work the effect of different extraction solvents on the mass spectrometric information collected for such a complex material as a solid deposit is discussed. All six solvents of varying polarity were able to extract a wide variety of compound classes. Although similarities were seen in the extracts from some solvents, it was also obvious that there are major differences. Furthermore, from MS data alone it is impossible to learn about the nature and structure of heteroatom containing compounds, although the use of the positive and the negative ionization modes provides some insight into the functional groups that may be present. Although the deep well deposits are considered a fraction rich on polar components the results also show that ESI might not be the best method to analyze this fraction which results in lower intensities of the spectra.

These data clearly show that there is not one procedure or one analytical method that is able to characterize a crude oil fully, and that a comprehensive characterization of deep well deposits may be possible only through the combined use of various analytical methods, each of which must be carefully chosen for its selectivity. Each method has to be verified for the range of components it can cover and which kind of selectivity it provides for difficult problems such as the analysis of asphaltene and deep well deposits. Although mass spectrometry provides a very powerful analytical system that has no competition for such an endeavor, the need arises to combine this method with chemical, chromatographic or electrophoretic methods that allow for a better understanding of the chemical species through selective separation. The analysis of such a material emphasizes the need for unconventional approaches to gain a better understanding as recently suggested by Cooks *et al.* who emphasized the need to think outside the box and use other methods, such as implementation of chemical methods in analytical chemistry [32].

Acknowledgments

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Chapter 5 Direct Coupling of Normal-phase HPLC to Atmospheric Pressure Laser Ionization FT-ICR MS for the Characterization of Crude Oil Samples*

**Redrafted from "Lababidi S., Panda S.K., Andersson J.T., Schrader W.; Direct Coupling of Normal-phase HPLC to Atmospheric Pressure Laser Ionization FT-ICR MS for the Characterization of Crude Oil Samples, submitted to Analytical Chemistry, in revision.*

5.1 Abstract

The high complexity of crude oil makes the use of chromatographic separation an important tool especially for sample simplification. The coupling of normal-phase HPLC using a polar aminocyano column to a Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS) offers the best attributes of good separation prior to ultra-high resolution MS detection. Atmospheric pressure laser ionization (APLI) was used as an ionization technique to analyze the nitrogen-containing aromatic compounds in a deasphalted crude oil sample due to its unique selectivity toward aromatic hydrocarbons and also due to its sensitivity.

Two main chromatographic peaks were observed during this separation indicating a class-based separation. Mass spectra obtained from fractions were collected along the entire retention time and compared to each other to assign the unique constituents. By coupling the HPLC system directly to the FT-ICR mass spectrometer comparable ion and UV chromatograms were obtained, reflecting the scan-to-scan sensitivity of the coupling system. The results show that it is possible to calculate reconstructed class chromatograms (RCC) with the corresponding double bond equivalents (DBE) distribution, allowing differences in class composition to be traced along the retention time. As an example radical and protonated nitrogen species generated by APLI were detected along the retention time which enabled a differentiation between basic and non-basic species in the same polar peak, thus overcoming the limitation of chromatographic resolution. This report represents the first online LC/FT-ICR MS coupling in the field of crude oil analysis.

5.2 Introduction

Energy is the engine that keeps the world economy and societies running. Different concepts based on various sources are under continuous investigation to broaden the resources for energy, especially with respect to renewable resources. Although a number of different sustainable resources are currently studied in detail, fossil resources will still play a major role at least for the next one or two decades. It is important to enhance the supply of sustainable energy but equally important is to efficiently utilize the remaining fossil materials. The preferred crude oils are light and sweet and easy to refine, but such crudes are diminishing while heavy resources like the Canadian oil sands remain available in large amounts. Chemical upgrading technologies have to be developed and existing ones improved to convert these unconventional materials into usable fuel and energy sources. In this area analytical chemistry plays an important role, for instance in catalytic conversion processes that can only be observed in detail by a thorough molecular-level characterization of the fuel constituents. Polyaromatic heterocycles, which are concentrated in heavy crude oil fractions, are important in the characterization because of their negative role in many refining processes. Despite this, they can be quite difficult to characterize because of the high complexity of crude oil, and its overall characterization still represents a major challenge for researchers [1].

Ultra-high resolution mass spectrometry (Fourier transform ion cyclotron resonance mass spectrometry - FT-ICR MS) allows a detailed characterization of complex petroleum samples at the level of the elemental composition of individual constituents to be made [2, 3]. In addition to sample complexity, various effects during mass spectrometric measurements such as suppression effects and discrimination against different components interfere in a comprehensive characterization. It has been shown that when a crude oil is being separated into fractions according to the SARA fractionation scheme [3], a scheme that separates the crude oil components into solubility fractions, that the combined number of assigned constituents in the different fractions is much higher than the number of the assigned species in the crude oil itself [4, 5]. This poses a challenge to analytical chemists who obviously need to develop simplification methods accompanied by high resolution and high accuracy detection, to correctly analyse such complex mixtures as petroleum.

One of the major techniques for sample simplification is separation through chromatography. Different types of chromatography have been introduced as analytical tools for the investigation of crude oil. Gas chromatography has been utilized frequently for the identification of crude oil fractions [6-8], however many compounds in crude oil are not volatile enough for GC analysis [3]. Recently, two-dimensional gas chromatography (GC x GC) has been used to characterize and quantify lighter distillate fractions of crude oil [9]. Size exclusion chromatography in combination with matrix assisted laser desorption ionization-mass spectrometry (MALDI) has been used for structural characterization of heavy petroleum residues [10] in that two petroleum residues were fractionated using solvent (heptane) separation and characterized by size exclusion chromatography (SEC) and by UV-fluorescence spectroscopy (UV-F). Gel permeation chromatography was also used to separate crude oil fractions [11], however, the obtained fractions were too complex to show individual compound peaks in programmed-temperature gas chromatography [12]. On the other hand, ligand exchange chromatography has been proven an important tool for the investigation of heterocycles in crude oil and refined products [13]. A palladium-(II) based stationary phase has been efficiently used to separate polycyclic aromatic sulfur heterocycles (PASH) and polycyclic aromatic hydrocarbons (PAH) [14, 15]. The different fractions of a group separation from a Pd(II) column were analyzed using FT-ICR mass spectrometry and this enabled the characterization of the polycyclic aromatic sulfur heterocycles [16] and revealed additional structural features of the condensed and noncondensed thiophene compounds [13]. Sulfur polycycles have been further separated using a bonded β-cyclodextrin stationary phase, which is selective to the number of aromatic double bonds [17, 18].

The coupling of liquid chromatography to mass spectrometry is nowadays well established in routine analysis. Reversed phase liquid chromatography is frequently utilized as the method of choice for the coupling with mass spectrometry, especially for the detection of polar species [19]. Long-winded and time-consuming offline separations by high-performance liquid chromatography (HPLC) are usually required to enhance mass spectral interpretations. Normal phase liquid chromatography on the other hand is not often used. An example where it was coupled to ion trap MS is the characterization of site-specific protein glycosylation [20]. An application from the crude oil field is the characterization of high-boiling aromatic hydrocarbons by thermospray MS [21]. The coupling of reversed phase liquid chromatography and FT-ICR MS has been reported in some research applications, such as the characterization of complex peptide mixture [22]. Online coupling to a high resolution mass spectrometer should give detailed information about compounds in crude oil samples, especially with the ability of scan-to-scan analysis of the chromatographic peaks. The biggest difficulty of coupling LC to FT-ICR MS is the balance between the elution time window of a separated compound and the acquisition time required for a sensitive detection [23]. Additionally, the solvents used for normal phase chromatography are not necessarily compatible with the most often used ionization source, electrospray (ESI). Atmospheric pressure photo ionization (APPI) [24] and atmospheric pressure chemical ionization (APCI) [19] have been used as ionization methods for LC/MS. Recently, atmospheric pressure laser ionization (APLI) was found to be a sensitive and selective ionization method for nonpolar compounds, such as condensed polyaromatic compounds, which may also contain heteroatoms [25]. Additionally, the performance of APLI in the analysis of vacuum gas oil was also investigated, and a good response for nonpolar aromatic hydrocarbons was established [26]. Therefore, APLI presents an excellent tool for petroleum analysis of samples that contain reasonable amounts of aromatic compounds.

In this study the APLI method was chosen as the ionization source for the offline and online coupling of normal phase liquid chromatography with ultrahigh resolution FT-ICR MS to characterize crude oil sample with high nitrogen content. The selectivity of the ionization process ensures a very high selectivity for aromatic species. The online coupling of a liquid chromatographic system and an ultra-high resolution mass spectrometer LC/FT-ICR MS for crude oil analysis is reported here for the first time.

5.3 Experimental Section

A nitrogen-rich crude oil sample obtained from Shell was deasphalted by diluting 20 µL of the crude with 100 µL dichloromethane, followed by subsequent addition of 6 ml of n-hexane. The content was sonicated for 30 min, left standing overnight at room temperature, and filtered to give the maltenes fraction. The chromatographic separations were performed on a stationary phase of polar aminocyano-bonded silica column (5 µm, 250 X 2.0 mm) (Göhler Analysentechnik, Chemnitz, Germany). A stepwise gradient of n-hexane (HEX) with isopropyl alcohol (IPA) was set as follows: 100% HEX (0-5 min), 99.5% HEX + 0.5% IPA (5-10 min), 99% HEX + 1% IPA (10-15 min), 98% HEX + 2% IPA (15- 20 min), and 95% HEX + 5% IPA (20-25 min) and then returned to 100 % n-hexane until 30 min. All experiments were performed on an Agilent 1100 HPLC system (Agilent technologies, Waldbronn, Germany). Direct coupling was realized to a 12 T LTQ FT-ICR MS (Thermo Fisher, Bremen, Germany) equipped with a home-built APLI source [26]. More experimental details are described in the Appendix 5-3 at the end of Chapter 5.

5.4 Results and Discussion

5.4.1 Chromatography

The difficulty in analyzing crude oil mixtures is the complexity of the sample that has been called supercomplex [1]. More than 50 000 components are expected to be present. With a concentration used for direct infusion experiments between 100 and 500 ppm of sample, this means that each individual component is available only in minute amounts during the analysis. This affects all phases of the analysis. The mass spectrometric detection is usually carried out by adding between 100 and 500 transients to gain statistical depth. But when using LC/MS it is not possible to have the time required to co-add a large number of transients because otherwise the chromatographic resolution would be impaired. On the other hand, when recording the spectra from an LC/MS experiment the sensitivity needs to be high enough that all the components can be detected in each individual scan. Furthermore, the concentration of the sample has to be at an optimum level in order to avoid overloading effects when standard chromatography columns are used. This is one probable reason why online coupling of LC/MS for crude oil analysis is so difficult to achieve and until now all work on separation of crude oil has been done offline by collection of individual fractions.

The compounds in crude oil display a variety of physical attributes, especially in regard to polarity, which represents probably the most important parameter for chromatographic separation. Due to a low solubility of a large number of crude oil constituents in polar mobile phases, frequently used in reversed phase chromatography, and because the majority of the compounds of interest in this case are of lower polarity, normal-phase (NP) liquid chromatography was chosen as the separation method. One of the successful application of NP chromatography was reported for the separation of aromatic nitrogen heterocycles from aromatic hydrocarbons and non polar heterocycles on a polar aminocyano-bonded silica HPLC column (PAC) [27]. For our purposes this methodology was adopted and optimized for the separation of crude oil components.

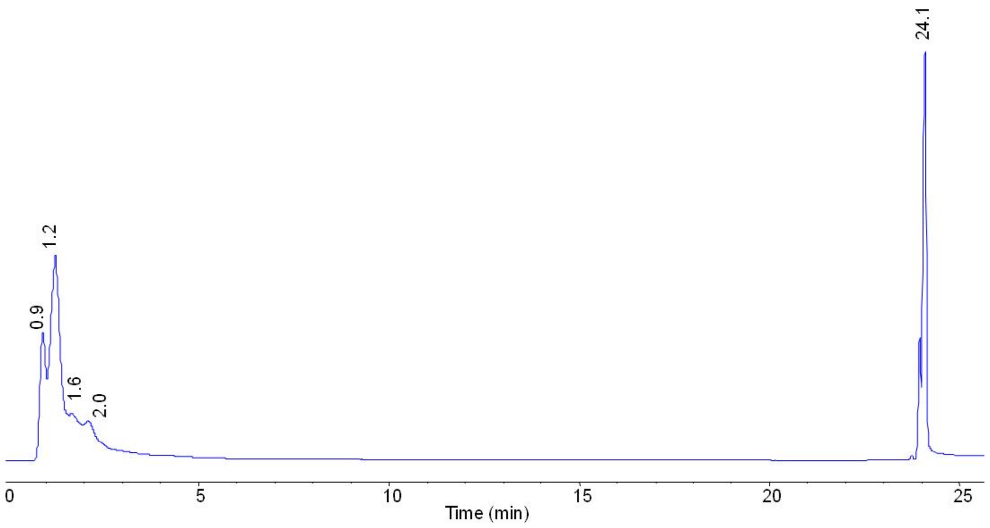

Figure 5-1: UV/Vis chromatogram obtained at λ=254 nm of deasphalted crude oil sample on the PAC column (5 µm, 250 X 2.0 mm).

A chromatographic method using an increasing concentration of isopropyl alcohol in n-hexane as the eluent was employed and optimized for the separation of a deasphalted nitrogen rich crude oil on a polar aminocyano column. The asphaltene fraction was removed from the crude oil sample due to its lack of solubility in the eluent. The remaining maltene fraction contains a wide spectrum of polar and nonpolar components. The separation of maltenes into two major fractions differing by polarity was achieved.

Figure 5-1 shows the resulting UV/Vis chromatogram with the corresponding retention times of the detected peaks. The first (non-polar) peak, composed of an array of four not fully resolved peaks, eluted between 0.6 and 3 minutes of retention time. At a retention time of 24 minutes, the second peak eluted after the last gradient change of isopropyl alcohol from 2 to 5 %. A comparison with the retention times of various standard compounds confirmed that the first peak consists of mainly nonpolar components, such as hydrocarbons and nonpolar heteroatomic constituents like sulfur compounds while the second peak represents the more polar nitrogen species. Although a group-type separation of polar nitrogen compounds into pyrrole and pyridine groups was reported for a North Sea crude oil by Larter et al [27]. However, it could not be repeated here.

5.4.2 High resolution mass spectrometry

Offline measurements

First results were obtained by using an off-line LC/MS approach. Fractions were collected once each minute between 3.0 and 26.0 min and afterwards concentrated for the mass spectrometric measurement (24 fractions of 60 second intervals). However, because of the multiple peaks found in the first nonpolar fraction, a higher time-resolution was desirable and thus fractions were collected every 0.3 min during retention times 0.6 and 3.0 min (8 fractions of 18 second intervals). Atmospheric pressure laser ionization (APLI) was used to obtain detailed information on the compounds in these fractions. The selectivity for aromatic compounds is due to a two photon ionization mechanism (1+1 REMPI – resonance enhanced multi photon ionization), achieved by using a standard excimer laser operating at 248 nm and the ability to ionize even at low concentrations [25] makes this ionization technique the method of choice for this study. No fragmentation of the analytes is expected since APLI is a soft ionization technique [28].

The high resolution mass spectra of fractions obtained at different retention times show a variable mass peak distribution in the mass range 150-700 Da (Figure A5-1, left column, see Appendix 5-1). All obtained spectra exhibited an average mass resolving power of 750,000 at *m/z* 400. The intensity of the detected signals was expected to reach their peak in those fractions that gave rise to the strongest UV absorption. The fractions between the two major peaks showed signals of lower intensity. To compare the detected signals in the spectra, a class distribution diagram was created from the intensity based calculations and is illustrated in Figure A5-1 (right column). It has to be noted that protonated compounds are described using [H] after the class, while the radical cation form is shown without an [H]. Radical hydrocarbons and nitrogen containing ions dominate the class distribution along the entire retention time scale as was expected for a nitrogen rich crude oil. Sulfur species were detected only in early fractions due to their nonpolar nature. On the other hand, oxygen species were present in early and late fractions and are not observed in the midsection. Early eluted oxygen species could correspond to nonpolar ethers, whereas late eluted species might correspond to polar phenolics.

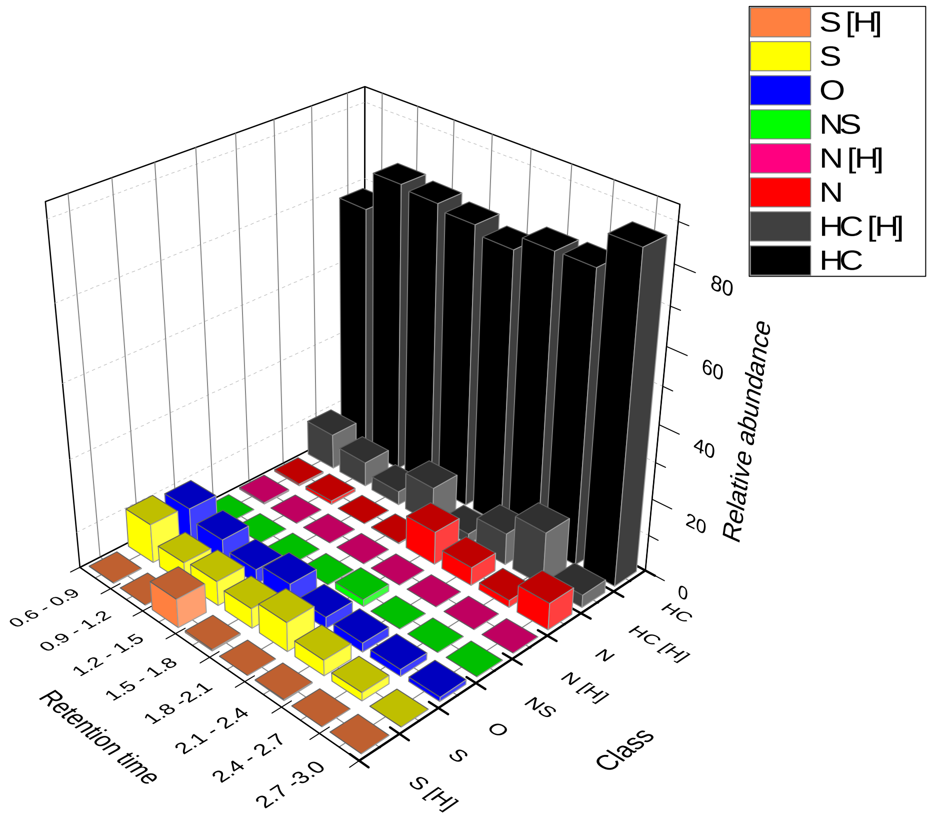

Figure 5-2: 3D compound class distribution plot of 20-second fractions collected in the retention range 0.6 – 3.0 minutes. (Protonated molecules are denoted using [H], while the cation radicals are shown without [H]).

The first chromatographic band shows four partially resolved peaks. To allow for a suitable time resolution in this range, fractions collected every 0.3 min (18 s) were collected. Their class distributions are compared with each other in Figure 5-2. The HC class dominates the distribution among the other abundant compound classes in these fractions. Sulfur and oxygen species did not exceed 10 % relative abundance and showed a continuous decrease along the retention time scale. The presence of the N class was observed in the later fractions collected between 1.8-3.0 minutes. The early retention time of these nitrogen species indicates their nonpolar nature. These could be less polar N compounds, such as N-alkylated nitrogen heterocycles and this could be confirmed by using N-substituted standards that were found to elute at this retention time.

The question whether each of the collected fractions contains original constituents can be easily answered in the case of the S[H] and NS classes, because they were detected exclusively in the fractions 1.2-1.5 and 1.8-2.1 minutes, respectively. The classes that are present in multiple fractions cannot be described adequately in the 3D class distribution especially when presented in a relative abundance scale. Therefore, in order to visualize the effectiveness of the separation, the unique constituents found exclusively in each single fraction were calculated based on the population (number of the assigned species). This population based distribution, which was adopted and reported [4, 29] by our group, eliminates differences caused by the signal intensities derived from differences in response factors.

Figure 5-3 depicts the double bond equivalent (DBE), which represents the number of double and ring closing bonds within a molecule, distribution of these unique assignments with the corresponding class distribution of these species. The first fraction between 0.6 and 0.9 min shows the highest number of selective assignments (465), comprising hydrocarbons (51%) as the main constituents with equal amounts of oxygen and sulfur compounds, and covering the DBE range from 3 to 20. However, hydrocarbons were assigned as unique compounds in the retention time between 1 and 2 minutes of retention time, referring to high number of similar compositions eluting at the same time. Unique hydrocarbons were observed again in the fraction 2.1-2.4 min, which has the highest DBE mean value among the fractions. The least number of the unique constituents was observed in the fraction 1.5-1.8 min, but at the same time included only sulfur with a small percentage of oxygen species. As was observed in Figure 5-3, the N and NS classes dominate the unique assignments in the fraction 1.8-2.1 min.

It is obvious that this offline fractionation is very useful for following the change in composition, especially in the case of samples of lower concentration. However, the analytes in the collected LC fractions are diluted with appreciable amounts of solvent that needs to be removed before the MS investigation. Since such operations can never be carried out without the risk of losses or contamination, and, in addition, is time consuming, the idea of applying an online coupling was realized to monitor the species in a scan-to-scan pattern simultaneously with their elution.

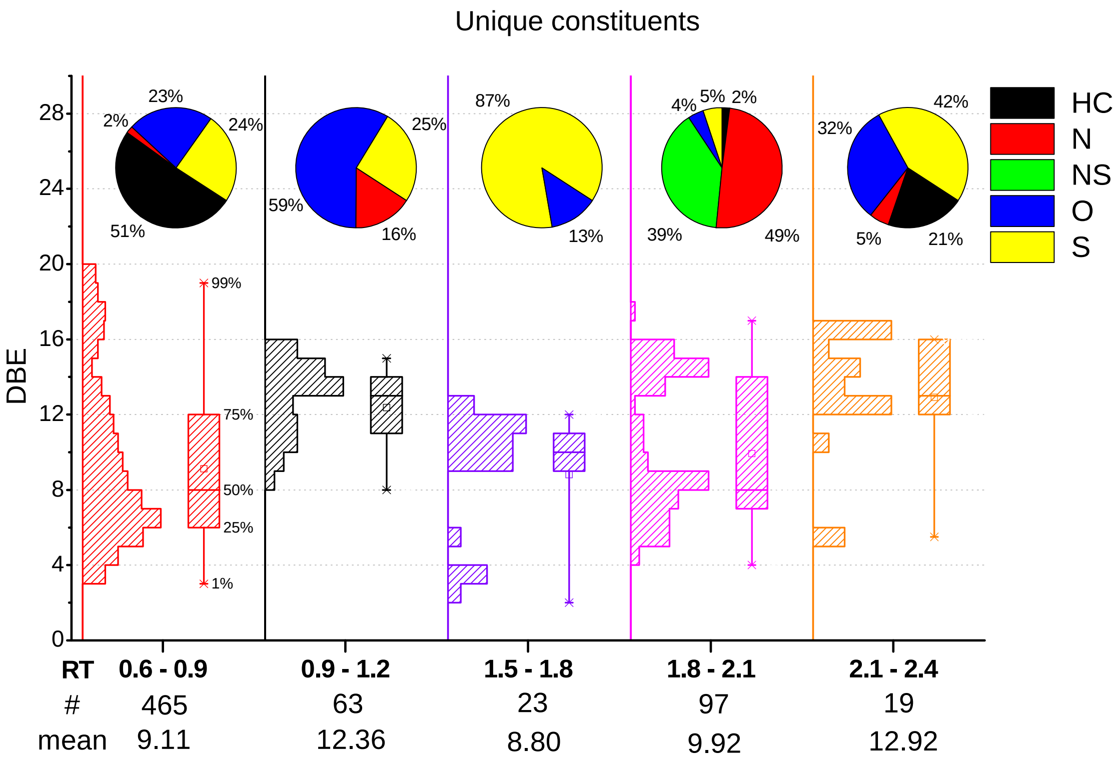

Figure 5-3: DBE distribution of the unique assigned elemental compositions in the fractions collected offline. The corresponding compound class distribution of the uniques (in percentage) for each fraction is shown in the pie charts, color-coded on the basis of classes (above). # denotes the number of unique elemental compositions found.

Online coupling

Usually the biggest difficulty of coupling LC to FT-ICR MS is the balance between the chromatographic resolution and the scanning time of each individual MS scan required for sensitive detection [23]. This problem was encountered when coupling capillary electrophoresis to the FT-ICR mass spectrometer [30]. The concentration needs to be balanced between good chromatographic resolution and enough mass spectrometric sensitivity. Therefore, short transients have to be selected to ensure a sufficiently large number of scans across the chromatographic peak. Performing the experiments using a hybrid linear quadrupole ion trap in front of the FT-ICR mass analyzer provides rapid MS analysis [31]. Additionally, instruments equipped with automatic gain control (AGC) provide mass measurements for LC/MS studies of proteins with an accuracy of 1-2 ppm for lower magnetic fields [31] and even better accuracies well below 1 ppm for higher magnetic field strengths[32] without internal standard.

As in the case of the offline measurements, atmospheric pressure laser ionization (APLI) was used for the ionization. Due to the different positions of the UV detector and the MS, a time delay of 3.6 seconds between the recordings of the two detectors was observed in the MS signal. The resulting total ion chromatogram (see Figure A5-2 in the Appendix 5-2) reveals great similarity with the UV/Vis chromatogram. Both chromatograms show two distinct areas. The first array of peaks elutes before 3.5 min while a second peak is present at 24 min. In the first peak the highest absorbance in the UV chromatogram was registered at about 1.2 min whereas in the TIC chromatogram the highest value was at around 2 min.

Here we want to introduce so called *reconstructed class chromatograms* (RCC), a chromatographic distribution similar to *reconstructed ion chromatograms* (RIC). While the RICs show the behavior of a single ion during the chromatographic separation, the *reconstructed class chromatograms* show the behavior of a class during the separation. To obtain these data, the class distribution is calculated for each MS scan. Based on this the distribution of a single class during the retention can be reconstructed. Similar calculations can also be arranged for *reconstructed DBE-chromatograms*, where the distribution of a single or multiple DBE value can be followed. It has to be noted that these calculations are extremely complex due to the large number of signals that can be present in each MS spectrum since a new spectrum is being recorded every 1-3 s, and each of those spectra needs to be calculated accordingly, resulting in a large amount of data that can only be handled with the aid of sophisticated software.

Details of the separation can be seen in Figure 5-4, where some *reconstructed class chromatograms* for the first three minutes are displayed. It is obvious that the radical and the protonated forms of each class do not run in parallel. While the S class RCC shows a higher number of assigned components than the S[H] class, it consists of three peaks at 1.3, 1.8 and 2.5 min. The S[H] class shows the same first two peaks albeit at lower intensities while the third peak is missing. The class chromatograms of the radical and protonated hydrocarbon classes are also differing. A big difference can be observed for the class chromatograms of the S and the HC classes; here a distinct chromatographic separation was achieved.

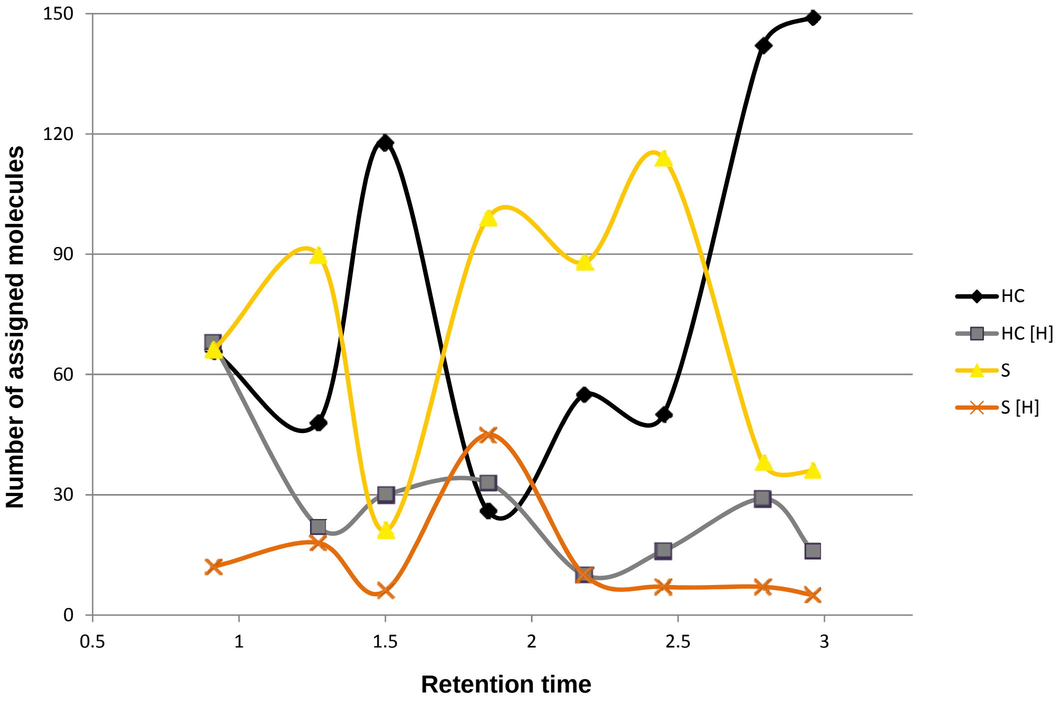

Figure 5-4: *Reconstructed class chromatograms* of the HC and S classes during the first 3.5 min, scaled according to the number of assigned elemental compositions (both radical and protonated hydrocarbon and sulfur ions are shown).

The class chromatograms reveal the changes in overall distribution as a function of the chromatographic separation but the full information about which components are separated can be better illustrated otherwise. This is achieved in Figure 5-5 that shows heat maps depicting the scan-to-scan DBE distribution calculated according to the number of assigned peaks at different retention times during the first three minutes. Here, the changes in the class chromatograms can be attributed to an increase in the DBE value with an increase in retention time. This means that even in the two not very well separated peaks (Figure 5-1); a fine-structure separation can be observed. With increased retention compounds with higher aromaticity elute. While the DBE value for the HC class at 0.91 min is between 9 and 13, it raises to a value of 11-19 at 2.9 min. The highest population of HC species occurred at DBE 12, which corresponds to polycyclic aromatic structures like that of pyrene. The increase of DBE values is accentuated for the HC[H] components. This phenomenon of increasing DBE with the retention time was reported for the separation of polycyclic aromatic sulfur heterocycles in crude oils on silver(I)-mercaptopropano stationary phase [18], and in nitrogen species found in the resin fraction of coker gasoil [33]; however in offline measurements.

Similar results were found for the S and S[H] classes, for which the DBE value grows during the first peak for both radical and the protonated ion class distributions. Until now, the broad separation into class peaks was the best that could be expected by collecting the separated fractions for further analysis. Now the tools are available that allow a more detailed description of the separation of this class separation even for such complex samples as crude oil.

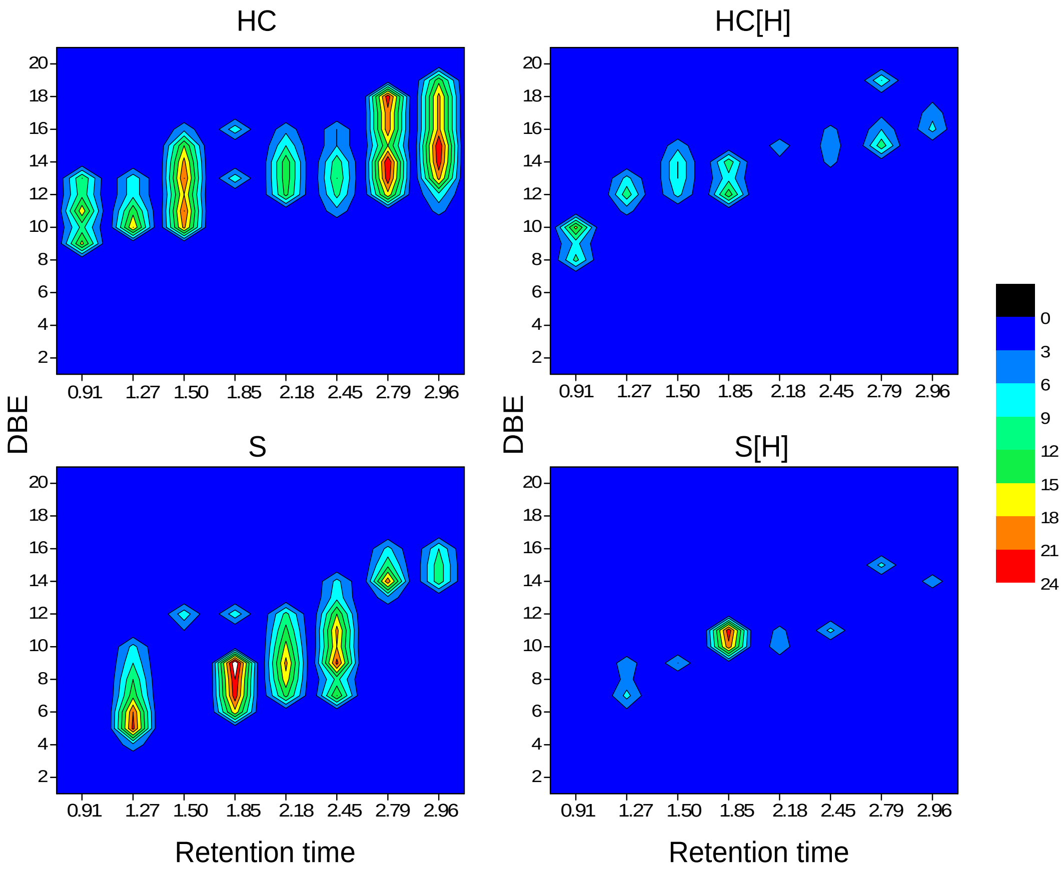

Figure 5-5: DBE distribution of the HC and S classes in the first chromatographic peak, scaled according to the number of assigned molecules. Hydrocarbons (top) and sulfur compounds (bottom) in both radical and protonated ion forms were detected in the online LC/APLI FT-ICR MS measurement. (Due to a better overview some scans are not shown because they overlap)

As was mentioned above, nonpolar nitrogen compounds were found at earlier retention times, while the second peak consisted mainly of more polar nitrogen compounds. The latter, according to injection of standards to HPLC system, could have pyrrole and/or pyridine nitrogen aromatic heterocyclic structures, which were not resolved due to the complexity of the samples and limited resolution of the stationary phase. On the other hand, the scan-to-scan DBE distribution of the N[H] class (Figure 5-6, bottom) reveals an interesting aspect of this investigation.

A significant increase of the assigned protonated ion N[H] was observed in the area between 24.39 and 24.48 min as indicated in Figure 5-6 (top). Here the ratio between N[H]/N is displayed with a strong increase at 24 min of retention This is a very significant point, especially when considering that APLI used for the ionization generates primarily radical cations, which are affected by subsequent ion molecules chemistry [34]. Since only analytes with rather high gas-phase basicities are converted to quasi-molecular ions in APLI [35], the protonated nitrogen species can be interpreted as polar and basic pyridine type compounds. The remaining nitrogen compounds which were mainly ionized as cation radicals belong probably to the neutral pyrrole type. This reveals that here a selective ionization of polar and basic compounds seems possible when APLI is being used after separation of the nitrogen species by normal phase chromatography.

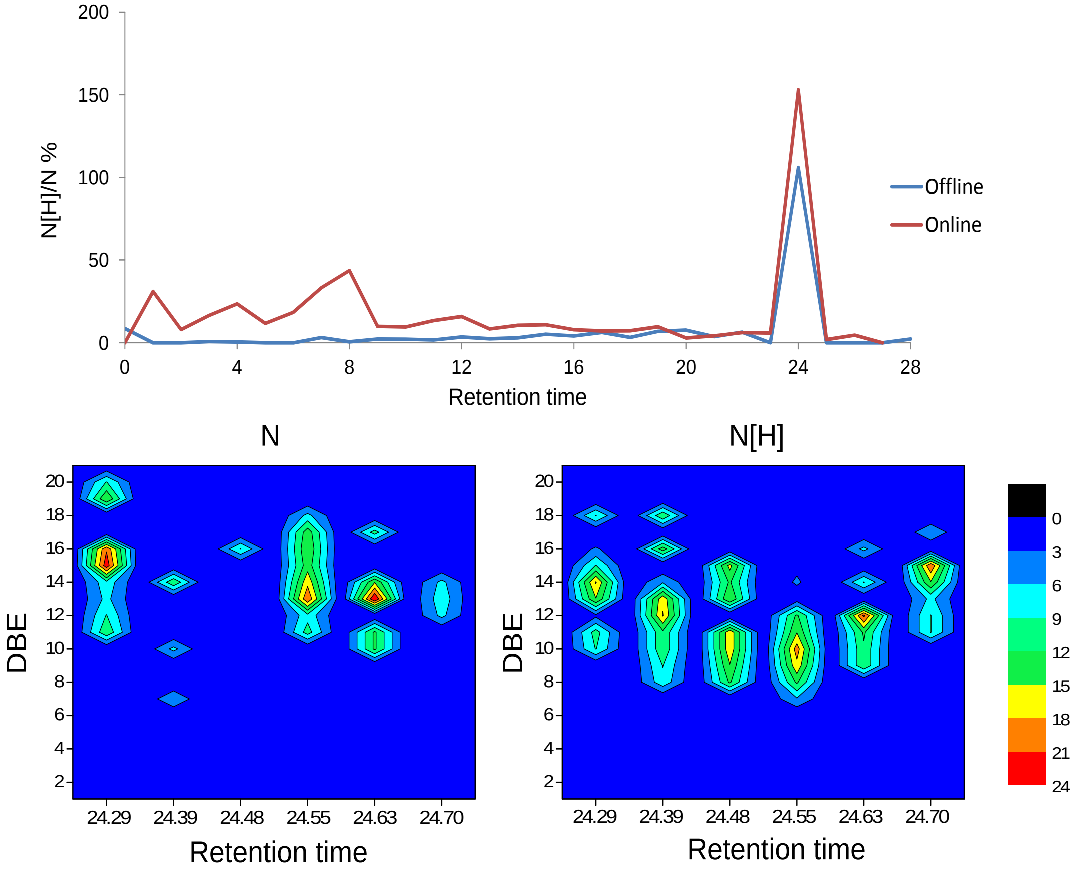

Figure 5-6: Ratio of assigned protonated and radical nitrogen species in both online and offline measurements detected at different retention times (top), and the heat maps displaying the DBE distribution along the retention time of the second peak (24.2 – 24.8 min).

5.5 Conclusion

In this work, we compared two methodologies (online and offline) of coupling normal-phase high performance liquid chromatography to an ultra-high resolution mass spectrometer for the investigation of a deasphalted crude oil sample using a REMPI based laser ionization method. APLI as the ionization technique made it possible to detect in detail the changes in compositions in the offline measurements of both nonpolar and polar fractions. It was also possible to trace polar nitrogen species and assign them selectively in the offline and online coupling schemes. In the online coupling, we were able to follow in a scan-to-scan manner the change of composition along the elapsed retention time. To the best of our knowledge, this is the first reported online HPLC/FT-ICR MS coupling for the investigation of a complex crude oil sample. Moreover, we introduced reconstructed class chromatograms as a method that allows tracking the changes of class compositions in great detail. These results show that both methodologies may receive great attention in the analysis of crude oils. While offline coupling allows longer transients and as a consequence a higher resolution of data, it is limited in that the fine structure in the separation is lost due to the rather coarse time-resolution. On the other hand, the fine structure analysis can only be achieved when faster transients are used, resulting in MS data of lower resolution.

Acknowledgment

The authors thank Dr. David Stranz (Sierra Analytics, Modesto, CA) for assistance in MS-data evaluation. The authors also gratefully acknowledge the generous funding and gift of samples from Royal Dutch Shell, Amsterdam and Houston, Tx, USA.

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Appendix for Chapter 5

Abstract

The appendix contains additional information about the detailed parameters of the experimental section including chromatographic and mass spectrometric setups beside data analysis. Two additional Figures are included; the first one about mass spectra of offline fraction and their corresponding class distribution diagram, and the second illustrates the total ion chromatogram of the online LC/FT-ICR MS coupling.

Contents

Appendix 5-1 Mass Spectra and the Corresponding Class Distribution of Offline Fractions

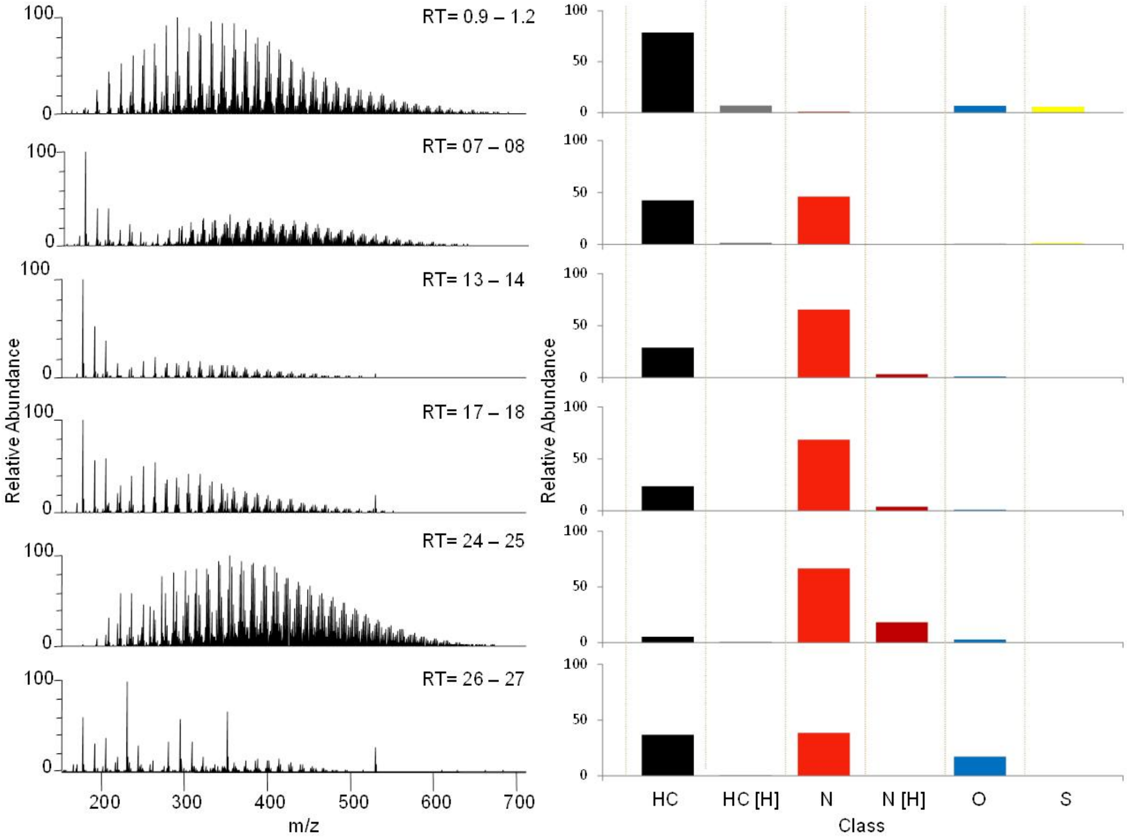


Figure A5-1: High resolution mass spectra of offline HPLC fractions of deasphalted crude oil obtained in positive APLI ionization mode at different retention times (left column) and the corresponding compound class distribution illustrated as relative abundance (right column). (Protonated molecules are denoted using [H], while the cation radicals are shown without [H]).

Appendix 5-2 Total Ion Chromatogram of Online HPLC/FT-ICR MS Setup

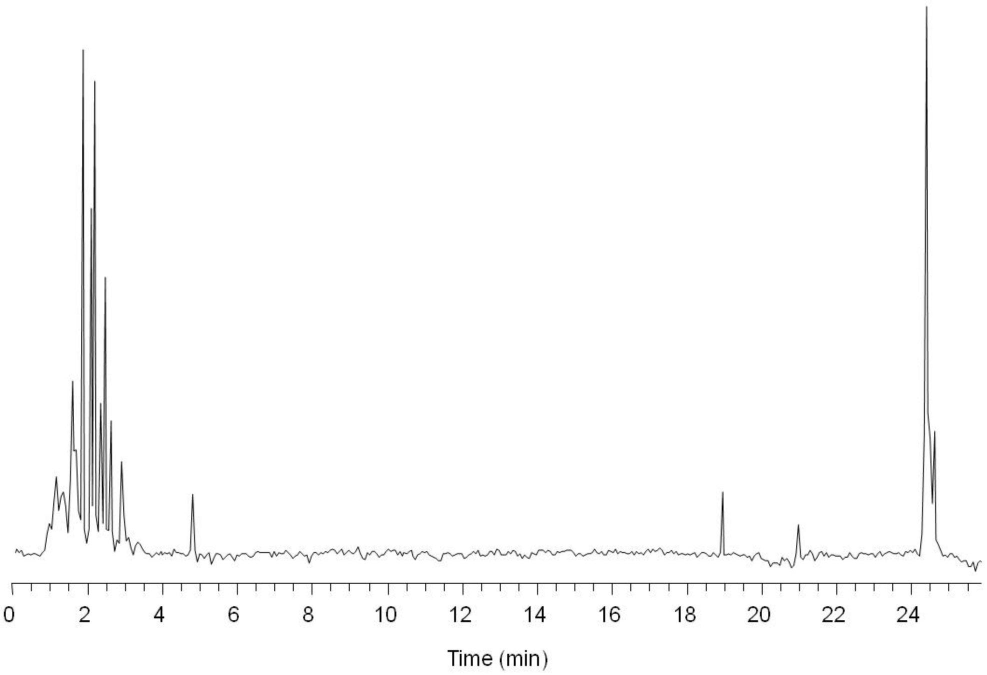


Figure A5-2: Total ion chromatogram of the online HPLC/APLI FT-ICR MS measurement.

Appendix 5-3 Experimental Section

Sample and chemicals

A nitrogen-rich crude oil sample obtained from Shell was deasphalted by diluting 20 µL of the crude with 100 µL dichloromethane, followed by subsequent addition of 6 ml of n-hexane. The content was sonicated for 30 min, left standing overnight at room temperature, and filtered to give the maltenes fraction. Chemicals used for sample preparation and solvents for the chromatographic separations are of HPLC grade and supplied by Sigma-Aldrich (Taufkirchen, Germany).

Liquid Chromatography

The chromatographic separations were performed on a stationary phase of polar aminocyano-bonded silica column (5 µm, 250 X 2.0 mm) (Göhler Analysentechnik, Chemnitz, Germany). A gradient of n-hexane (HEX) with isopropyl alcohol (IPA) was used as the mobile phase. An increase of the isopropyl alcohol concentration was achieved according to the following stepwise gradient: 100% HEX (0-5 min), 99.5% HEX+0.5% IPA (5-10 min), 99% HEX + 1% IPA (10-15 min), 98% HEX + 2% IPA (15- 20 min), and 95% HEX + 5% IPA (20-25 min) and then returned to 100 % n-hexane until 30 min. All experiments were performed on an Agilent 1100 HPLC system (Agilent technologies, Waldbronn, Germany). Eluents were degassed before and during the experiments and were pumped at a flow rate of 1 mL/min. All tubing and fittings were of stainless steel. 1 µL of the deasphalted crude oil was injected by an autosampler onto the chromatographic column, which was kept at room temperature and was connected to a diode array UV detector set at wavelength
λ = 254 nm. For offline measurements, fractions were collected from the outlet of the detector in 20 seconds segments from 0.6 – 3.0 minutes of retention time. From 3.0 minutes on, one-minute fractions were collected. The fractions that were collected every minute were concentrated to ca. 0.5 mL by removing the volatile n-hexane with a stream of argon. For online measurements, the outlet of the UV detector was connected to the mass spectrometer by using PEEK capillaries equipped with a micro-splitter to control the flow rate of the eluent, which was adjusted to 100 µL/min.

High-resolution mass spectrometry

Mass spectra for online measurements were obtained using a 12 T LTQ FT-ICR MS (Thermo Fisher, Bremen, Germany) equipped with a home-built APLI source [26]. Pulsed laser radiation (50 Hz, 10mJ) was obtained from a KrF* excimer laser (ATL Lasertechnik GmbH, Wermelskirchen, Germany), radiating at the wavelength of 248 nm. A commercial APCI sprayer (Thermo Fisher, Bremen, Germany) was used to introduce the sample to the mass spectrometer without the use of a corona needle. The sample was vaporized in the heated nebulizer at 300 °C with a stream of nitrogen as sheath gas. The generated cloud was ionized with the laser beam, positioned between the MS orifice and the exit of the ion source. Additional offline measurements were performed on a research type LTQ-Orbitrap capable of operating with 3 s transients. All obtained spectra exhibit an average mass resolving power of 750k (at *m/z* 400). The ionization conditions were kept the same as for the online mode. The sample was injected through the APCI nebulizer at a flow rate of 25 μL/min and was measured in the positive mode. The different fractions were diluted in toluene to a final concentration of 100 ppm. The measurements were carried out with a transfer capillary temperature of 275 °C, a skimmer voltage of 33 V, and a tube lens voltage of 125 V. The spectra were acquired with a mass range of *m/z* 150-1000 and processed with the LTQ FT Ultra 2.5.5 (Thermo Fisher) data acquisition system. For online measurements between 3 and 5 microscans were set resulting in a scan rate of one spectrum every 3 s.

Data analysis

Mass spectra were externally calibrated which resulted in a mass accuracy better than 1 ppm (0.77 and 0.65 ppm on average for offline and online measurements, respectively). The peak lists were converted to molecular formulae by Composer software package (Sierra Analytics, USA), and controlled using Xcalibur software (Thermo Fisher, Bremen, Germany). The following chemical constraints were applied: Number of H unlimited, $0<C<200$, $0<O<2$, $0<N<2$, $0<S<2$, $0<DBE<40$. The calculated molecular formulas were controlled by sorting them into compound classes based on Kendrick mass defects and their DBE distribution. The obtained mass lists were transferred into Excel and Origin for data evaluation and presentation of the figures shown. For selected ion monitoring (SIM) measurements, the peak list was exported directly from Xcalibur into Excel sheets, where multiple sorting based on the Kendrick scale was applied in order to group different classes of compounds according on the hetero-element present.

Chapter 6 Online Normal Phase HPLC/FT-ICR Mass Spectrometry: Effects of Different Ionization Methods on the Characterization of Highly Complex Crude Oil Mixtures*

**Redrafted from "Lababidi S., Schrader W.; Online Normal Phase HPLC/FT-ICR Mass Spectrometry: Effects of Different Ionization Methods on the Characterization of Highly Complex Crude Oil Mixtures, submitted to Rapid Communications in Mass Spectrometry, in revision.*

6.1 Abstract

Characterization of crude oil represents a challenge for researchers due to its complexity. While Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS) is the method of choice for such complex matrices the high number of ions present limits the efficiency of the analysis due to charge competition and space charge effects. One way to solve this problem is the direct coupling of FT-ICR MS with liquid chromatography.

Normal phase liquid chromatography was applied on a deasphalted crude oil sample by using a polar aminocyano bonded stationary phase with n-hexane and isopropyl alcohol as a mobile phase. The HPLC system was coupled online to a 12 T ultra-high resolution Fourier transform ion cyclotron resonance mass spectrometer (FT-ICR MS). Ion chromatograms were obtained with electrospray ionization (ESI), atmospheric pressure photo ionization (APPI), atmospheric pressure chemical ionization (APCI), and atmospheric pressure laser ionization (APLI).

The chromatographic separation yielded a group separation into two peaks according to the polarity of the components. Each ionization technique was able to uniquely assign components differing in polarity and aromaticity. This type separation was confirmed in a narrow selected ion monitoring (SIM) measurement. Additionally, an increase of aromaticity in the course of retention time for nonpolar species in the first peak was observed. Monitoring the ratio between protonated and radical mono-nitrogen species was achieved.

For the analysis of a crude oil sample, online coupling of a normal phase HPLC system to FT-ICR mass spectrometer was achieved. The results of the different ionization techniques were compared to each other, which enables a detailed characterization of the complex sample.

6.2 Introduction

Oil production from resources with light and sweet crudes is in decline [1]. This will result in an estimated increase of non-conventional oil from around 3 Million barrels per day (Mb/d) in 2012 to 8.8 Mb/d in 2030 [2]. The heavy resources contain more unwanted impurities like higher amount of heteroaromatic components and metals like V and Ni among others and need improved upgrading to reduce the level of heteroatomic containing species because most of the problems encountered in petroleum industry are related to the presence of heteroatoms [3]. A detailed evaluation of the properties of heavy crudes in terms of fouling and corrosion behavior could be very useful, as they can cause major problems to the refinery [4]. One of the heteroatoms of interest is nitrogen. The nitrogen content of crude oils ranges from 0.1% - 0.9% [5]. Although the nitrogen components comprise only a small percentage, their presence in crude oil is highly undesirable since they are responsible for catalyst poisoning [6], in addition to the formation of gums and solid precipitates during storage [7, 8]. Therefore, a thorough knowledge of types of nitrogen compounds in crude oil is essential for the optimization of denitrogenation processes, the speciation of safe handling methods of such materials, and for finding a relation between fouling properties of deposits in delayed coker furnaces and their nitrogen compound distribution. The difficulty is that such complex mixtures as crude oil are not easily analyzed. Ultra-high resolution mass spectrometry is a powerful method for the characterization of crude oil at a molecular level. Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS) enables determination on elemental scale in regard to its high-resolution and high mass accuracy [9, 10]. Recent studies however have shown that discrimination effects occur throughout the analytical scheme [11-14].

High performance liquid chromatography can be applied to reduce the complexity of the crude oil matrix. Boduszynski et al [15] described compound class separation of heavy crude oil fractions using HPLC, where the use of selective physical separations combined with high resolution mass spectrometry was required for the characterization of classes, which are defined by the number of heteroatoms N,O and S in a compound. Later et al [16] has developed a two-step open column liquid chromatography method for separation of nitrogen compounds. It consists of a separation of deasphalted crude oil or rock extract in a neutral alumina column followed by a silicic acid column. An application of this separation method was performed later by Zhang et al [17] for the characterization of neutral nitrogen compounds in crude oil using negative electrospray FTICR MS Li et al [18] described the use of a polar aminocyano-bonded silica HPLC column for the class separation of nitrogen compounds in crude oil. However, the experiments were performed on a low-resolution GC-MS instrument with its limitation on exact assignments [19]. Additionally, reproducibility problems associated with the separation of two distinct types of nitrogen compounds (pyrrole and pyridine) were encountered in our lab when applying this method on real crude oil sample. Therefore, the idea was to combine HPLC with FT-ICR mass spectrometry online to monitor the changes of composition in a class separation of crude oil components. Since it was shown that different ionization methods discriminate different components in a crude oil mixture, and there is no single ionization technique that allows the accurate characterization of all the components present in a whole crude oil sample, four different ionization techniques were used for this study [11, 20].

Electrospray ionization (ESI) is a technique used for detailed characterization of polar compounds in a petroleum system with leads to results that favors nitrogen components, the most polar class present in crude oil mixtures [21]. Atmospheric pressure photo ionization (APPI) on the other side, shows effective ionization of non-polar or low charge affinity aromatic compounds, reduced susceptibility to ion suppression, good sensitivity, and large linear dynamic range [22]. The major point with APPI is that for a broad range of compounds often a dopant needs to be added which results in both radical and protonated ions [23, 24]. Atmospheric pressure chemical ionization (APCI) is an ideal method for ionization of low- to medium-polar compounds, and it has been found that APCI is a more versatile LC/MS interface than ESI in many industrial applications for a wide range of compound mixtures [25]. Recently, atmospheric pressure laser ionization (APLI) was found to be a sensitive and selective ionization method for nonpolar aromatic compounds, such as condensed polyaromatic compounds, which may also contain heteroatoms [26]. The two-photon ionization at 248 nm is selective towards aromatic hydrocarbons, which are present in higher amounts in crude oil samples, and additionally allow easy coupling to chromatography [26].

The difficulty of coupling normal phase HPLC to a mass spectrometer for the analysis of crude oil components is that usually many thousands of different individual compounds are present in a crude oil sample. This can lead to sensitivity problems since usually only a minor amount is injected resulting in a very low concentration for each component. The amount of sample injected cannot really be increased because due to the complexity the separation of such a mixture is difficult enough even without overloading the column. Therefore, the online coupling of the normal-phase HPLC to a 12 T LTQ FT-ICR mass spectrometer with the different ionization techniques represents a unique and innovative step, which emphasizes the role of pre-sample treatment and preparation before MS analysis of crude oils.

6.3 Experimental Section

6.3.1 Crude oil and chemicals

A crude oil sample which has high nitrogen content was deasphalted by diluting 20 µL of the crude with 100 µL dichloromethane, followed by subsequent addition of 6 ml of n-hexane. The content was sonicated for 30 min, left standing overnight at room temperature and filtered to obtain a clear amber coloured fraction. All solvents used for sample preparation were of HPLC grade and supplied by Sigma-Aldrich (Taufkirchen, Germany).

6.3.2 Liquid Chromatography

The chromatographic separation was performed on a polar aminocyano-bonded silica column (5 µm, 250 X 2.0 mm) (Göhler Analysentechnik, Chemnitz, Germany). A gradient of n-hexane (HEX) with isopropyl alcohol (IPA) was used as the mobile phase. An increase of the isopropyl alcohol concentration was achieved according to the following: 100% HEX (0-5 min), 99.5% HEX+0.5% IPA (5-10 min), 99% HEX + 1% IPA (10-15 min), 98% HEX + 2% IPA (15- 20 min), and 95% HEX + 5% IPA (20-25 min) and then returned to 100 % n-hexane until 30 min.

All experiments were performed on an Agilent 1100 HPLC system (Agilent technologies, Waldbronn, Germany). Eluents were degassed before and during the experiments and were pumped with a flow rate of 1 mL/min. All tubing and fittings were of stainless steel. 1 µL of the deasphalted crude oil sample was injected by an autosampler onto the chromatographic column, which was kept at room temperature and was connected to a diode array UV detector set at wavelength $\lambda = 254$ nm.

6.3.3 LC/FT-ICR MS setup

Mass analysis was performed on a 12 T LTQ FT-ICR MS (Thermo Fisher, Bremen, Germany) equipped with commercially available ESI/APCI/APPI sources. The APLI source was laboratory-built as described elsewhere [23, 24, 26]. The data were collected and processed with the LTQ FT Ultra 2.5.5 (Thermo Fisher, Bremen, Germany) data acquisition system. The spectra were collected in positive mode using ESI, APCI, APPI and APLI. For online coupling measurements, the outlet of the UV detector was connected to the ionization chamber of the mass spectrometer by using PEEK capillaries and the flow rate of the eluent was controlled by a micro-splitter. Nitrogen was used as the drying gas for all ionization techniques. For the electrospray ionization (ESI) measurement a T-joint was installed allowing a mixture of methanol and formic acid (2 %) to be added to the eluent flow to stabilize the spray. The final flow rate was adjusted to 10 µL/min and the ions were generated from an electrospray source equipped with a metal-ESI needle. ESI(+) conditions were: needle voltage=4.0 kV, sheath gas=10 (arbitrary units). In case of the APPI, APCI and the APLI measurements the flow rate of the eluent was adjusted to 25 µL/min and the thermal nebulizer was set at 300°C with the continuous sheath gas flow of 20 (arbitrary units). For the APPI measurement a Syagen Krypton VUV lamp (Syagen, Tustin, CA, USA) was used, which emits photons at 10.0 and 10.6 eV. The analysis was performed without adding dopant to the system. In APCI measurement the current of the corona needle was set to 5 µA. For the APLI measurements pulsed laser radiation (50Hz, 10mJ) was obtained from a KrF* excimer laser (ATL Lasertechnik GmbH, Wermelskirchen, Germany), radiating at the wavelength of 248 nm. The sample was injected through the APCI nebulizer without corona needle and the generated cloud was ionized with the unfocused laser beam, positioned between the MS orifice and the exit of the ion source. The measurements were carried out with a transfer capillary temperature of 275 °C, a skimmer voltage of 33 V, and a tube lens voltage of 125 V. The spectra were acquired with the mass range of *m/z* 150-800. The number of microscans was set at 3 resulting in a scan rate of one spectrum every 3 seconds.

6.3.4 Data analysis

Mass spectra were internally calibrated resulting in a mass accuracy of less than 1 ppm with an average error of 0.07, 0.04, 0.93, and 0.57 ppm in ESI, APPI, APCI and APLI, respectively. The peak lists were converted to molecular formulas by the Composer software package (Sierra Analytics, USA), and the resulting assignments were checked by Xcalibur software (Thermo Fisher, Bremen, Germany). The following chemical constraints were applied: Number of H unlimited, $0<C<200$, $0<O<2$, $0<N<2$, $0<S<2$, $0<\mathrm{DBE}<40$. Radical cations and protonated molecules were distinguished and indicated separately (X, X[H], respectively). The calculated molecular formulas were processed by sorting them into compound classes based on Kendrick mass defects and their DBE distribution [27]. The obtained mass lists were imported into Excel (Microsoft) and Origin (OriginLab) packages for data evaluation and generation of the figures shown. For selected ion monitoring (SIM) measurements, the peak list was exported directly from Xcalibur into Excel sheets, where multiple sorting based on the Kendrick scale was applied in order to group different classes of compounds according to the hetero-element present [28].

6.4 Results and discussion

6.4.1 Comparison of UV/Vis and Total Ion Chromatograms (TIC)

The UV/Vis chromatogram of the crude oil shows two distinct peaks (Figure 6-1, top). The first peak was eluted in the range between 0.5 and 3 minutes of retention time. At a retention time of around 24 minutes, the second peak was detected after the last change of eluent composition in regard of isopropyl alcohol content from 2 to 5 %.

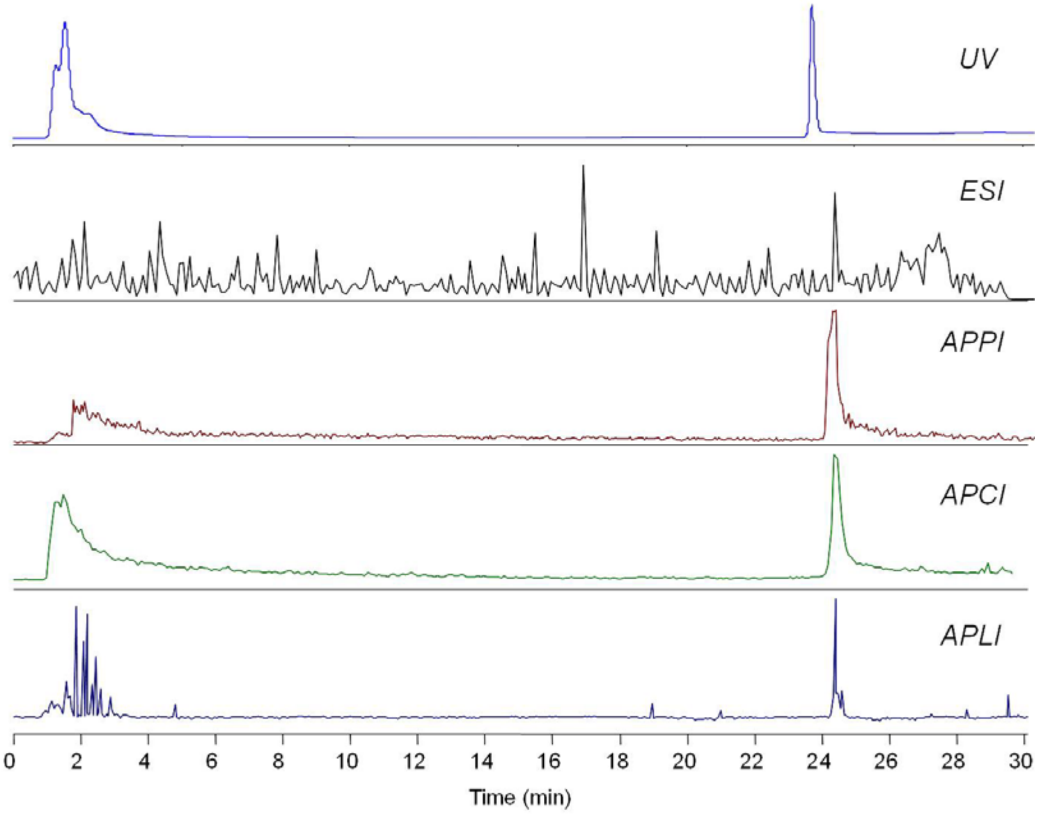


Figure 6-1: Total ion chromatograms for online LC/FTICR MS data collected by using different ionization techniques in comparison with UV/Vis chromatogram (top) of a deasphalted crude oil on PAC column (5 µm, 250 X 2.0 mm) obtained at λ = 254 nm.

The resulting total ion chromatogram of the online LC/FT-ICR MS coupling using ESI, APPI, APCI, and APLI is depicted in Figure 6-1. The similarity of the UV trace and the total ion chromatograms (TIC) confirms the synchronization between the HPLC system and high resolution mass analyzer, which allows following the changes in separation from scan-to-scan. However, the normalized ESI TIC showed a noisy baseline due to the presence of a number of high intense signals.

6.4.2 Class Distribution

The compound class distributions were calculated for each detected peak and were compared to the data from the other ionization techniques. The peaks were sorted into compound classes based on their denoted mass defects. Figure 6-2 shows the compound classes on the basis of the number of assigned formulas (population based assignments). Distributions based on the population of assigned species were favored over traditional intensity-based calculations in order to eliminate discrimination of classes caused by differences in ionization efficiencies of the species. This method of representing the data was adopted previously in our lab and published elsewhere [11, 12, 14]. A total of 17 different compound classes representing the majority of the assigned classes were plotted to visualize the differences. The compounds were observed in either radical or protonated (quasi-molecular) form. The protonated species are denoted as X[H], whereas radical species are denoted without the proton in brackets [H].

Electrospray was able to deliver protonated form of three nitrogen-containing classes (N[H], NS[H], and NO[H]) in first chromatographic peak, , whereas the higher abundant non polar species such as hydrocarbons and sulfur compounds were not ionized as expected. On the other hand, APPI was able to ionize the highest number of different classes among these ionization techniques. The number of assigned oxygen containing components for classes such as NO, NOS and OS in both radical and protonated form was larger than with other ionization methods. It has to be mentioned that APPI measurements in this study were performed without adding a standard dopant like toluene. Syage et al. described that the use of dopant was found unnecessary or non-critical in hexane-based mobile phases (eg. hexane/IPA) for normal phase LC/MS due to self-doping effects [29] since the ionization potentials of hexane and isopropyl alcohol (10.13 and 10.22 eV respectively) are lower than the Kr photon energies of 10.0 or 10.6 eV [22, 29]. APPI photon energies are high enough to ionize the mobile-phase solvents which react with target analytes forming analyte ions. The majority of the classes that were assigned in APPI have more protonated than radical species in the first peak. This was consistent with what was observed in the APCI measurement. However, in APCI fewer classes were observed and with a relative dominant presence of protonated hydrocarbons and sulfur species. In contrast to what is observed in APPI and APCI measurements, APLI was the only ionization technique where the number of radicals surpasses the number of protonated species of the same class.The class distribution of the second chromatographic peak (Figure 6-2, right column) is dominated by nitrogen containing compounds ESI delivered protonated species of the classes N[H], NS[H] and NS_2[H]. The presence of NS_2 class was only detectable when ESI is used, whereas NO[H] class was detected in APPI and APCI. The APLI distribution showed the prominent presence of radical form of N and NS classes in comparison to the other three ionization methods, where these classes were detected only in the protonated form. A more detailed investigation about this point will be discussed later.

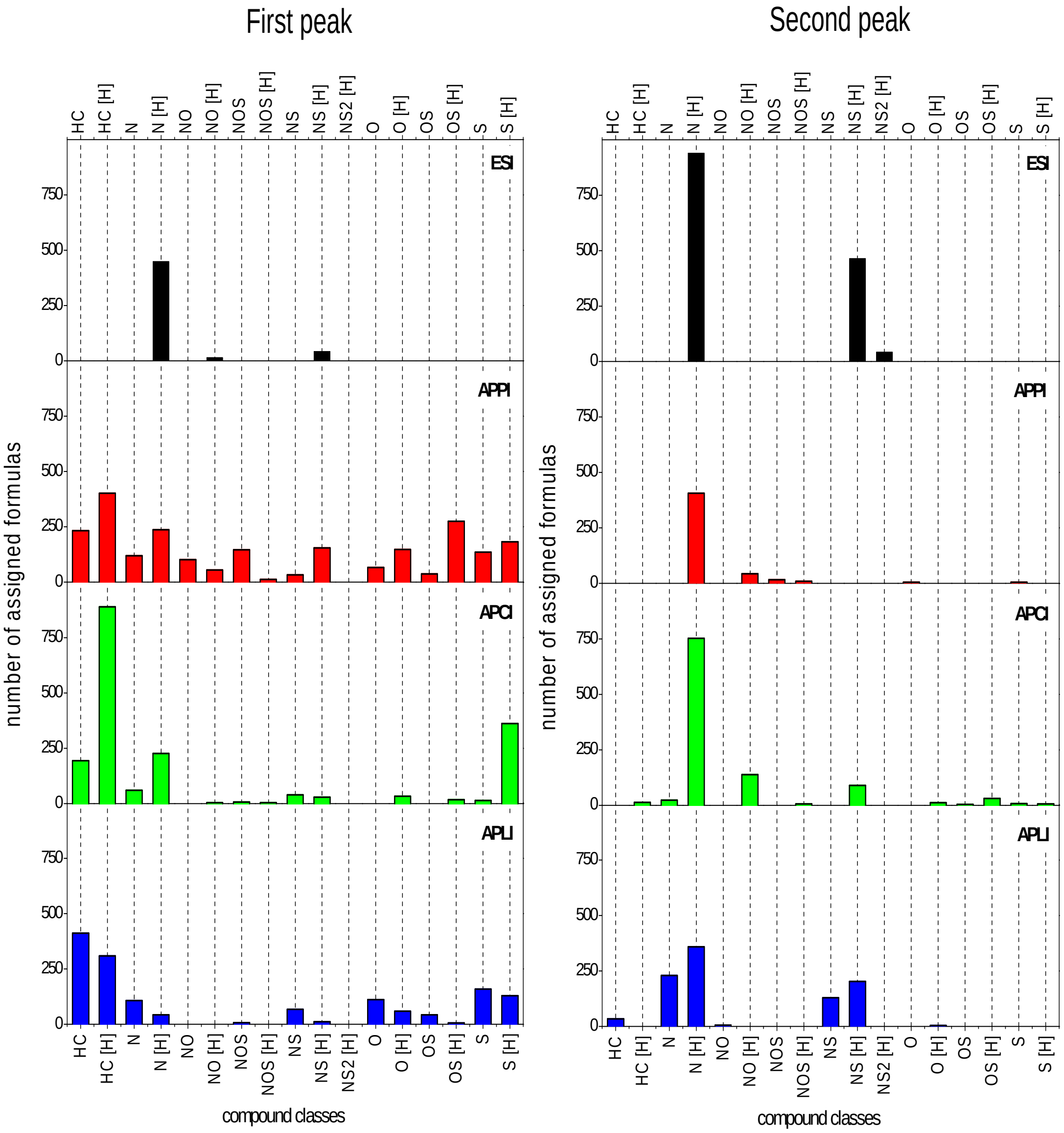


Figure 6-2: Class distribution based on the number of assigned formulas obtained by summing the scans in the total ion chromatograms (Figure 6-1) using ESI (top), APPI, APCI, and APLI (bottom) modes.

6.4.3 Unique Constituents

In order to visualize the differences between the examined ionization techniques in our study, the unique assignments of both peaks for each method was calculated with the corresponding class distribution presented in the inserted pie charts. Figure 6-3 depicts distributions of the unique assigned compounds in regard to their DBE/C values in each of the used ionization methods. Statistical data are illustrated in the accompanied box plots. It has to be mentioned that for the sake of simplification the class distributions of the classes inserted as pie charts refer to both protonated and radical ions. While ESI only shows 78 uniquely assigned signals in the first peak, APPI shows almost 10 folds (734) achieving the highest number of selective assignments among all ionization methods. This is probably due to the photo ionization at 124 nm which covers the broadest range of analytes. Oxygen containing classes (O, NO, OS, and NOS classes) comprise together 73% of all exclusively assigned peaks in APPI surpassing both APCI and APLI (8% and 40% respectively). This finding of elevated oxygen containing species was observed as well, when the crude oil was directly injected to the FT-ICR mass spectrometer without previous chromatographic separations [24]. Those oxygen species were the consequence of a gas-phase reaction of activated oxygen in the ion source which afterwards reacts with aromatic hydrocarbons. Since during these reactions highly active oxygen species such as OH-radicals can be formed, it was shown that this leads to compounds with multiple O-atoms in the elemental composition [24]. Here, only elemental assignments with one oxygen atom were found leading to the conclusion that they are really present in the crude oil sample.

The number of the unique assignments found exclusively in the second peak in all of the ionization methods used (Figure 6-3, bottom) is considerably lower than its counterpart in the first peak. This is due to the restricted number of classes contributing in the class distribution (Figure 6-2, right column). APCI delivered the highest number of the unique constituents in the second polar peak comprising with about 75% of N and NO classes. ESI and APLI show comparable dominance of NS class in distribution of their unique assignments, however, the DBE/C mean value in ESI was almost 4 times lower than in APLI, indicating that electrospray was able to ionize exclusively NS in the lower DBE/C range (0.06-0.35), while APLI covered the larger range (0.1-0.7). In general, APLI delivered data with a higher average aromaticity compared to the data obtained by other ionization techniques in both of chromatographic peaks.

The diversity of the class distributions in the unique assignments in the different ionization techniques confirms that there is no single ionization method that allows a crude oil sample to be truly described [20]. By this LC/MS approach, each ionization technique has its characteristic window where certain compounds are favored or discriminated. Although ESI delivered the least number of unique constituent in both chromatographic peaks, they were exclusively nitrogen containing class, reflecting their polar nature. APPI delivered the broadest view of assigned classes with dominant presence of oxygen containing classes in comparison with other ionization methods. On the other hand, APLI demonstrated that it can exclusively ionize highly aromatic species in both of chromatographic peaks, whereas in this study APCI delivered dominant species, HC[H] and S[H] in the first peak and N[H] and NS[H] in the second peak.

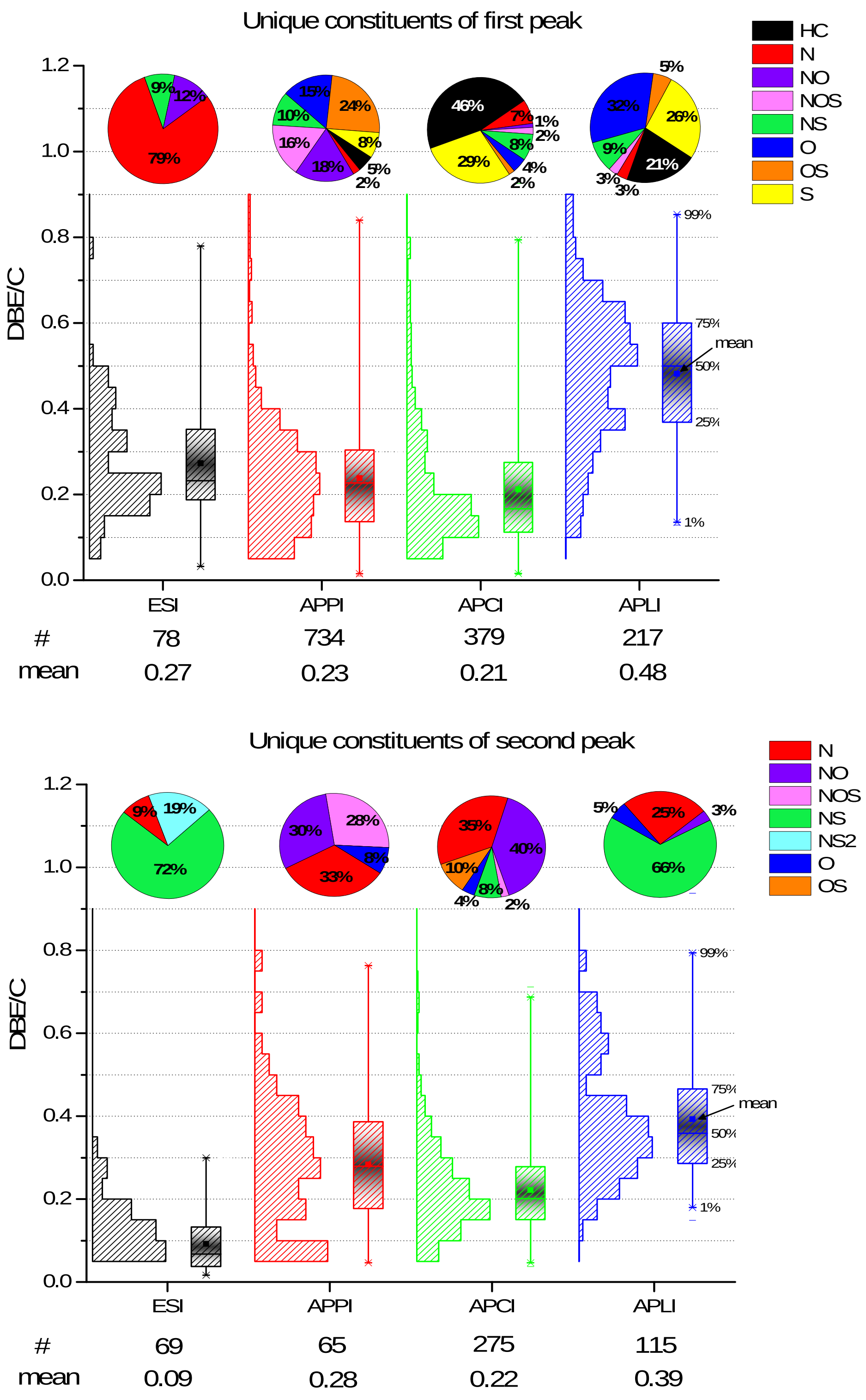


Figure 6-3: Unique assignments for each ionization method in both chromatographic peaks. The corresponding compound class distribution (in percentage) for each ionization technique is shown in the pie chart insert, color-coded based according to the classes.

6.4.4 Type separation

The ultra-high resolution capabilities of Fourier transform ion cyclotron resonance mass spectrometry enables the analysis of complex crude oil mixtures on a molecular level [30-32]. Figure 6-4 (top left) shows the mass spectrum of the direct infusion of the deasphalted crude oil sample (maltene fraction) using positive mode electrospray as an ionization method, with the molecular information designated above the main peaks in a zoomed 15 Da window (Figure 6-4, bottom).

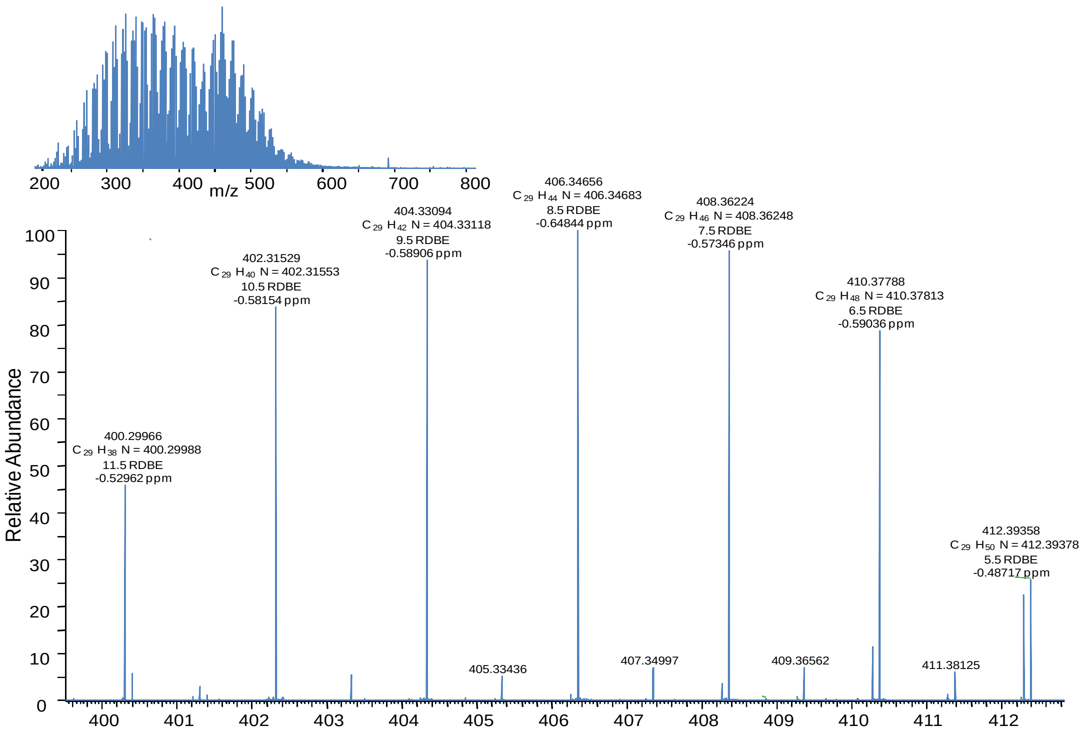


Figure 6-4: ESI(+) FT-ICR MS spectrum of direct infused crude oil sample (top left), and the corresponding 15 Da zoom showing the mass accuracy and the high resolution of components in addition to the molecular assignment and the corresponding DBE.

With the exact mass determination it is possible to assign the classes of the compounds in crude oil samples with their corresponding double bond equivalent value, which is the number of rings and double bonds serving as an indicator of the aromaticity of the detected species. However, the possibility of multiple isomers of one exactly assigned mass is quite high, especially in the case of complex materials such as crude oil samples. HPLC as a multi-dimensional analytical tool can provide a chemical dimension to the analysis when it is coupled to the FT-ICR MS achieving thereby a step towards the isolation and tracking of isomers.

In order to give depth into the online HPLC/FT-MS analysis approach of a crude oil sample, not only unique constituents in each of the different ionization techniques have to be considered, but also the common assignments. In the mass spectrometric study of a very complex matrix such as crude oil, the chance to have multiple isomers for one exact mass is quite high. The N_1 class is a good example for this demonstration, especially since type separation of nitrogen species is a significant issue in crude oil analysis. The species of N_1 class which are common in both chromatographic peaks (Table 6-1) have different chemical characteristics in regard to polarity, since the difference in retention times of the two peaks is large (1-3 and 24 min). As expected, electrospray delivered the highest number of assigned N_1 species in the second polar peak, and it has also the highest number of common assignments between the two peaks. Despite the considerable amount of N_1 species detected in both of chromatographic peaks, (150 and 589 in the first and second peak, respectively), APLI delivered the least number of commonly assigned species (80 assignments) among other ionization techniques. On the other side, none of these assignments possesses DBE lower than 9, in contrast to other ionization techniques, indicating the selectivity of APLI towards higher aromatic species.

Table 6-1: Number of N1 species assigned in both peaks individually and in common during the online LC/FT-ICR MS coupling for the analysis of deasphalted crude oil sample.

N_1 class	ESI	APPI	APCI	APLI
Number of assigned N_1 formulas in Peak 1	449	356	287	150
Number of assigned N_1 formulas in Peak 2	939	406	777	589
Number of common assigned formulas	206	187	186	80

6.4.5 SIM measurement

In order to investigate the behavior of N_1 species in crude oil along the retention time scale in the online HPLC/FT-MS setup, a selected ion monitoring experiment was performed. SIM was used for *"spectral stitching"* in order to expand the data depth for the analysis of complex crude oil samples when they are analyzed by FT-ICR MS [33]. By reducing the scan range each ion population is represented in a better proportion in the smaller mass range window, allowing for a better signal-to-noise ratio. APLI was selected as an ion source for this experiment. A window of 30 Da was chosen between *m/z* 385-415, which correspond to the maximum average intensity detected along the TIC. The signal at *m/z* 392.3312 which corresponds to the protonated ($[M+H]^+$) ion with a molecular assignment $C_{29}H_{43}N$ was chosen as an example for composition tracking. It belongs to Z=-15N series (Z is the hydrogen deficiency $C_nH_{2n-z}N$), which correspond to DBE 9. This exact molecular assignment was tracked along the retention time scale in a reconstructed ion chromatogram (Figure 6-5) with a mass tolerance set at 2 ppm.

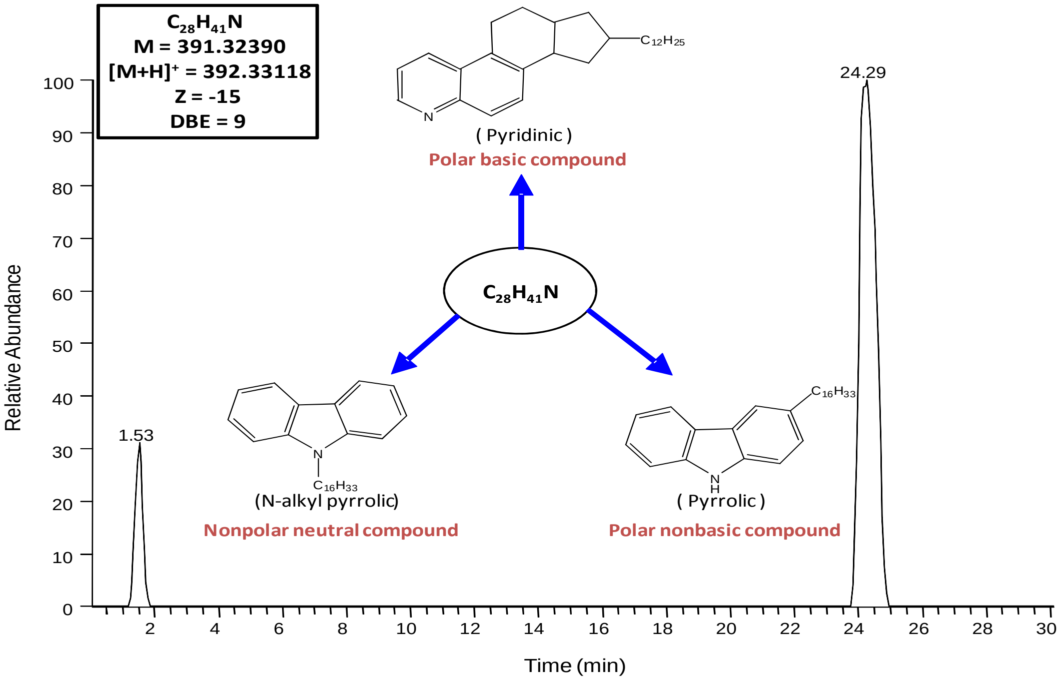
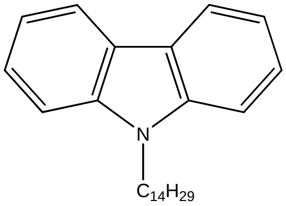
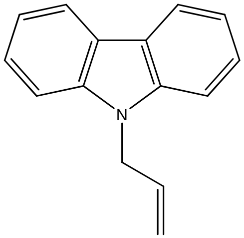
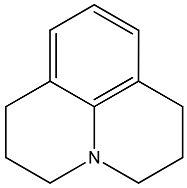
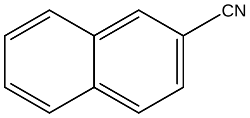
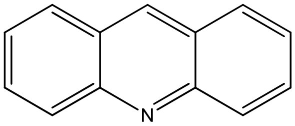
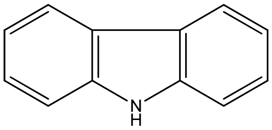
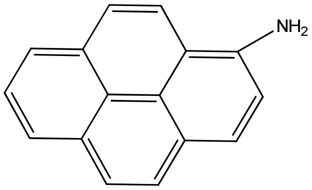


Figure 6-5: Reconstructed ion chromatogram (mass tolerance window of 2 ppm) for the ion *m/z* 392.3312 which corresponds to the molecular formula $C_{28}H_{42}N^+$ ($[M+H]^+$), and three postulated isomeric structures of the corresponding neutral compound.

The reconstructed ion chromatogram shows two peaks; a high intense signal at 24.29 min and a smaller peak at 1.53 min. Three possible isomer type's structures of N_1 class with DBE 9 were depicted in Figure 6-5. Carbazole derivatives (pyrrolic type) and quinoline derivatives (pyridinic type) represent nitrogen compound types commonly found in crude oil samples. Although that carbazole and quinoline has different basicity (pK_a = -6.8 for Carbazole [34] and pK_a = 4.9 for quinoline [35]), both of these compounds and their derivatives are polar and elute under these conditions at a retention time around 24 min. The nitrogen compounds eluting in the first peak could be N-alkylated carbazoles, because due to the alkylation there is no free electron pair resulting in less interaction with the stationary phase. These finding were confirmed by comparing retention times of standards as illustrated in Table 6-2.

Table 6-2: Retention times of standard compounds used in this work, injected onto a polar aminocyano column (PAC) (5µm, 250 X 2.0 mm) using n-hexane with isopropyl alcohol as a mobile phase with a gradient described above.

Name	Retention time (min)	Structure
9H-Carbazole, 9-tetradecyl	2.3	
9-Allyl-9H-carbazole	2.8	
Julolidine	4.4	
1-Cyanonaphthaline	6.1	
Acridine	23.8	
Carbazole	24.1	
1-Pyrenamine	24.6	

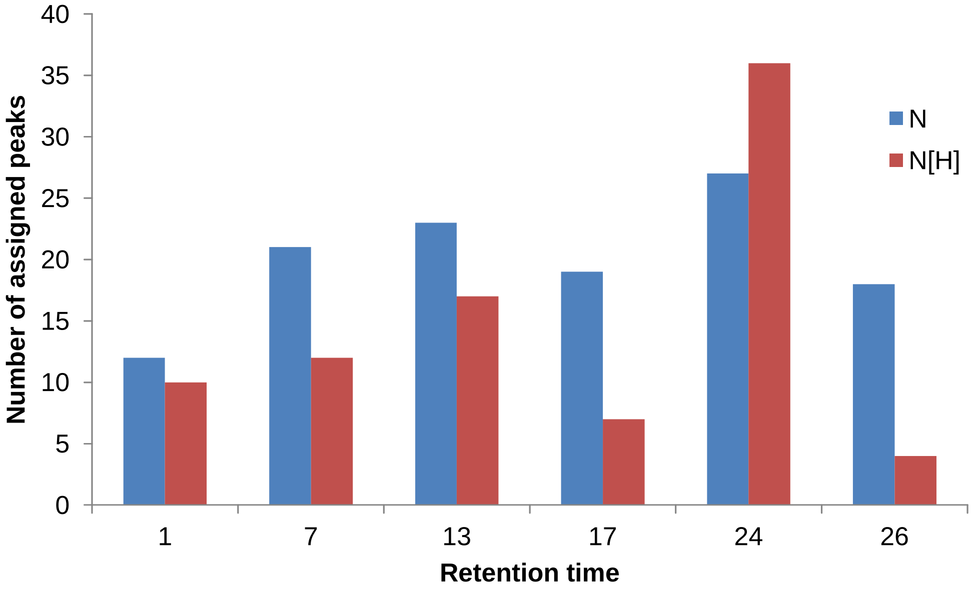


Figure 6-6: Comparison between the number of assigned protonated and radical nitrogen species in the online LC/APLI FT-ICR MS in the SIM mode for the mass range 385-415 Da observed at different retention times.

Since the composition of the eluent is changing after each 5 min time interval, the increasing amount of isopropanol is leading to the elution of higher polar components. From each interval one scan was selected and the number of assignments from both the protonated and the radical ion was plotted against each other. Here, in Figure 6-6 the changes in this ratio are shown from the online SIM measurement. The major result is that the scans at 24 min of retention time show an increase in the population of protonated nitrogen species and a decrease of the radical ion. This confirms also the results found in the full range in the case of nitrogen species that selectively the more polar components are protonated and the less polar components show a radical ion. Only analytes with rather high gas-phase basicities are converted to quasi-molecular ions in the APLI [36], hence, the protonated nitrogen species can be interpreted as polar and basic pyridine type compounds. The remaining nitrogen compounds which were favorably ionized as radicals belong probably to neutral pyrrole type compounds (see carbazole in Table 6-2).

The difference in the composition was also monitored in the nonpolar fraction by using the small SIM window. In Figure 6-7, the number of assigned molecular formulas for each class against the retention time of the nonpolar peak is plotted. Because a fixed 30 Da window contains at least two possible formulas belonging to the same homologous series, single assignments were not included in the counts. This compound class chromatogram shows nicely how the population of the different classes changes in a scan-to-scan detection manner with the retention time. An interesting example in these compound class chromatograms is the sulfur plot, where two peaks of radical sulfur are observed.

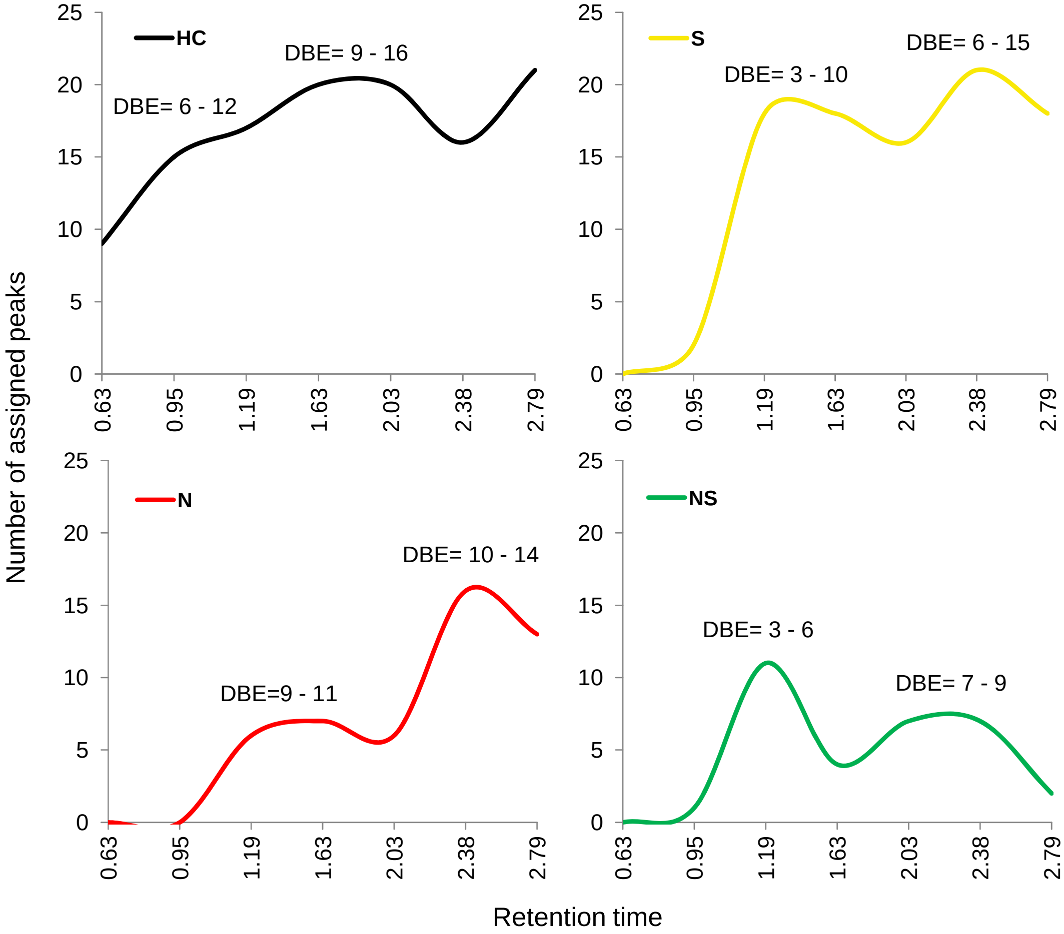


Figure 6-7: Population based reconstructed class chromatograms of assigned formulas of the HC, S_1, N_1, and NS classes with the corresponding DBE range. The online APLI HPLC/FT-ICR MS measurement was performed in the SIM mode within the mass range 385-415 Da of the first three minutes of retention time covering the first chromatographic peak.

The first one observed at 1.19 and the second one at 2.38 covering the DBE range from 3-10 and 6-15 respectively. That means the increase of DBE was also observed in lower intensity classes. The same observation was found for non-polar nitrogen and oxygen species (see Table 6-3).

Table 6-3: Double bond equivalent (DBE) range of the species observed at different retention times during the SIM mode within the mass range 385-415 Da.

Class / RT (min)	HC	HC[H]	S	S[H]	N	N[H]	NS	NS[H]	O	O[H]	OS	OS[H]
0.95	6-12	5-11	5-5	3-4	-	-	-	-	-	-	-	-
1.19	7-14	8-15	3-10	3-5	9-9	9-10	3-6	2-7	7-12	7-13	5-5	6-6
1.63	8-16	8-16	5-12	5-12	10-11	12-13	-	4-8	9-14	11-14	8-8	-
2.03	9-16	10-16	6-13	11-11	11-12	10-12	7-8	4-9	10-16	10-15	-	-
2.38	11-17	10-18	6-15	13-13	10-14	10-13	7-8	5-9	10-16	12-15	8-9	-
2.79	10-18	11-18	7-14	13-13	10-15	10-14	8-9	7-10	11-16	12-14	-	-

6.5 Conclusion

In this work, we compared four different ionization techniques: ESI, APPI, APCI, and APLI used in the online HPLC/FT-ICR MS coupling for the analysis of a deasphalted crude oil sample. Ion chromatograms using different ionization methods have been compared. Class distribution analysis on the detected peaks showed that the first peak comprises mainly of hydrocarbon assignments, whereas the second peak contains almost exclusively nitrogen containing species.

ESI as an ion source was only able to ionize nitrogen containing species in both peaks, whereas hydrocarbons and sulfur species were undetectable. On the contrary, APPI measurements delivered the widest spectrum of classes in the first nonpolar peaks, with emphasis on oxygen containing species. APCI was able to ionize the highest number of unique species in the second peak. Nevertheless, APCI delivered the fewest selective assignments among the different ionization techniques. On the other hand, APLI was selectively ionizing more highly aromatic species in the sample in comparison with other methods. Other selective assignments were observed in the second polar peak where APLI was the only ionization method which produces protonated as well as radical ions from nitrogen containing species enabling the distinction between pyrroles and pyridines. At the second polar chromatographic peak by online APLI-LC/MS measurement in the SIM mode, an increase in the population of protonated ions over the radical ions of the N_1 class species was observed. Additionally, an increase of aromaticity of different classes was shown in form of reconstructed class chromatograms, allowing tracing the change of composition in the course of retention time.

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Chapter 7 Extraction and Characterization of Basic Nitrogen Compounds from Crude Oils and Asphaltenes by Metal Ion Complexation and High Resolution Mass Spectrometry *

**Redrafted from "Lababidi S., Nachtigal F.M., Sripada K, Schrader W.; Extraction and Characterization of Basic Nitrogen Compounds from Crude Oils and Asphaltenes by Metal Ion Complexation and High Resolution Mass Spectrometry, will be submitted to Fuel.*

7.1 Abstract

Metal ion extraction was applied in this study to isolate nitrogen compounds by complexation in maltenes and asphaltenes samples using 95% ethanol solution containing Lewis acids ions.

The extracted basic nitrogen compounds were characterized using electrospray Fourier transform mass spectrometry enabling molecular assignments of the species with high resolution measurements. The selectivity towards nitrogen compounds in crude oil sample was verified by a methylation reaction of the extracted samples.

The metal ion-complexation principle on asphaltene samples was investigated. Higher carbon number and double bond equivalent (DBE) values were obtained in extracted samples compared with a simple electrospray measurement of the sample, showing extra data deconvolution obtained by the extraction procedure.

7.2 Introduction

The development of the price for crude oil on the world markets most recently has widened the focus about the remaining supplies of the crude oil. In the last 50 years mostly the lighter crudes have been of interest as an energy source. Since then, other less conventional resources, which have previously no economical interest, are gaining significance. Examples include oil sands or shale oils that are now becoming economically feasible to explore [1, 2]. The heavier crude oils and other higher boiling fractions of standard crude oils have thus become important resources to the expense of significantly elevated impurities levels, e.g., sulfur-, nitrogen-, oxygen- or metal-compounds. Many of these compounds have adverse effects on processing as well as on performance of the final products. In order to analyze such complex matrices, reliable analytical methods have to be utilized which enables elemental composition assignment of thousands of species. In this regard, Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS) has proven to provide analytical data of high accuracy and resolution [3] However, not all of the compounds present can be analyzed with the same ionization method for mass spectrometric analysis of crude oil samples [4]. Additionally, FT-ICR MS operates best when only a small amount of ions is present during each transient. This cannot be accomplished with crude oil samples without prior simplification of the complex matrix [5]. Therefore, to obtain high quality data it is imminent to simplify the approach and reduce the number of ions that are introduced into the mass spectrometer. Here, only a combination of separation techniques and the use of chemical knowledge by using derivatization and isolation procedures allow a thorough investigation of the complex matrix with FT-ICR MS instruments.

Nitrogen is one of the major heteroatoms found in fossil fuels [6]. Though present only in small quantities in most crude oil, typically between 0.1% and 2.0%, certain nitrogen species can probably decrease the efficiency of catalytic processes, contribute to the instability of the related products of crude oils, and promote tank and pipe corrosion and oil degradation during storage [7, 8]. For instance, it has been found that selective removal of nitrogen compounds from gas oil strongly enhanced the deep desulfurization [9]. Therefore, a detailed knowledge of the types and concentrations of the nitrogen compounds present in crude oils and its heavy fractions is clearly desirable in order to optimize methods for their removal and specify methods for the safe handling of such materials.

A wide range of methods have been reported over the years for the separation of organic nitrogen compounds from oil. Boduszynski et al [10] classified nitrogen compounds in crude oil into four major fractions, namely; basic, neutral, acidic, and pyrrolic compounds on the basis of HPLC separations of heavy crudes on alumina. Nitrogen content tends to exist in the higher-boiling fractions and residua such as asphaltene, and in general, the more asphaltic the oil, the higher its nitrogen content [11]. The majority of nitrogen compounds in asphaltenes are single-nitrogen-atom-containing molecules [12], in which the nitrogen atom is incorporated in the poly aromatic core [13]. Basic nitrogen was determined by acid extraction and liquid chromatography [14-16], by ion-exchange resin [17], and by liquid-liquid extraction as ammonium salts with an aqueous acid solution in continuous flow [18]. However, these procedures were complex and prohibitive for use in routine process monitoring, in addition to the low nitrogen recovery. The use of simple and efficient solvent extraction can be an alternative. It has been applied to extract nitrogen compounds efficiently from coal liquids with methanol and water [19].

On the other hand, chemical complexation based on coordinate bonds are generally stronger than van der Waals interactions, which controls conventional solvent extraction, yet they are weak enough to be reversible [20]. Therefore, extraction based on reversible chemical complexation such as Lewis acid coordinated complexes can achieve high selectivity. The formation of Lewis acid complexes is based on the interaction between an electron pair donor and an electron pair acceptor. According to hard/soft acid/base concept (HSAB) coined by Pearson [21], nitrogen bases are intermediate to strong Lewis bases, which preferentially interacts with a hard Lewis acid site such as Al^{3+} or Fe^{3+}. Hartung et al [22] utilized complex formation to isolate nitrogen compounds from crude oil products by using a mixture of ferric chloride and zinc chloride. The selectivity to nitrogen using the principle of complexation was recently applied to remove N-heterocyclic aromatic contaminants from fuel feeds by metal-organic-Frameworks (MOFs) [23]. Qi et al applied this method on catalytically cracked diesel oil using 95% ethanol solution containing a small amount of metal ions, resulting in an effective selective separation of basic nitrogen compounds [24]. The authors conclude that the separation principle involves the reversible complexation of nitrogen compounds (N) in crude oil phase with metal ions (M^{n+}) to form complexes $[N(M)_m]^{n+}$ which are soluble in ethanol-water phase [24].

The objective of this work is to study the validity of this method whether the selectivity of nitrogen compound in raw crude oil samples is possible, to investigate the role of Lewis acid site in the extraction process, and to answer the question regarding the extraction mechanism whether it is a metal-ion extraction or simply an acid-base extraction. In addition, a second objective was to expand this approach of the selective nitrogen extraction on an asphaltene fraction. Here, we utilize different extraction methodologies combined with derivatization to understand the selectivity towards a compound class of interest.

7.3 Experimental Section

7.3.1 Materials

All of the reagents and HPLC-grade solvents used, if not otherwise stated, were purchased from Sigma-Aldrich (high purity, Taufkirchen, Germany). A crude oil with high nitrogen content and low amount of asphaltene was used for the investigation of metal ion complexation. For asphaltene investigations, a heavy crude oil of North American origin was used to obtain the asphaltene fraction. The asphaltene fraction was precipitated from the corresponding heavy crude oil using n-heptane (HPLC grade, Merck, Germany). A slightly modified SARA fractionation procedure was used to obtain the asphaltene content [25]. A total of 30 mL of n-heptane/g of crude oil was added, and the mixture was refluxed for 2 h at 150 °C. The precipitated portion was filtered and dried under inert gas flow. The filter was extracted with 300 mL of toluene in a Soxhlet apparatus for 24 h. The redissolved asphaltene fraction was rotary-evaporated and, afterward, dried under a continuous stream of nitrogen. The asphaltene content of the heavy crude oil was 9.9 wt %.

7.3.2 Elemental Analysis

The C, H, N, and S contents of the asphaltene were determined in a Vario Elementar EL elemental analyzer using a TCD detector (Elementar Analysensysteme GmbH, Hanau, Germany). The oxygen content was estimated by the difference.

7.3.3 Metal ion extraction

Nitrogen compounds were extracted from crude oil sample by metal ion complexation according to the procedure described by Qi et al.[24] Extraction solutions containing Al^{3+}, Fe^{3+} ions were prepared by dissolving 1 wt% of $AlCl_3$ and $FeCl_3$, respectively, in 95% ethanol. An ethanol solution containing H^+ ions was prepared by adding hydrochloric acid (HCl) dropwise to the extraction solution till the pH value was adjusted at 2. The extraction solutions were mixed in a separation funnel with the crude oil at a volume ratio of solvent to crude oil of 0.2 for 5 minutes in a shaker at room temperature. After standing for 15 min, two separated phase were obtained; a residual oil phase at the bottom and ethanol-water phase at the top. The ethanol-water phase was evaporated in a rotary evaporator to have a final 5 mL concentrated solution for further mass spectrometric investigations.

For metal ion complexation of the asphaltene sample a modified procedure was adopted. To ensure maximized contact with the extraction solution, the precipitated clumps of asphaltene were ground with a mortar and pestle into a fine powder. About 30 mg of asphaltene powder were put in a round bottom flask with 100 mL of the extraction solution (1% wt. $AlCl_3$ in 95% EtOH) and stirred for 6 hours at 60°C. The resulting mixture, which has a pH value of about 3, was filtered using Blue Ribbon filter paper with a pore size of 2 µm to remove the asphaltene particles. The clear solution was evaporated in a rotary evaporator to have a final volume of 5 mL that was used for mass spectrometric investigations.

7.3.4 Methylation

The derivatization of crude oil components with methyl iodide is described in detail elsewhere [26-28]. Briefly, about 10 mg of the sample was treated with an excess of methyl iodide and silver tetrafluoroborate in 3 mL dichloroethane for 48 h. The yellow-brown precipitate of silver iodide was removed by filtration using Blue Ribbon filter paper with a pore size of 2 µm and washed with dichloroethane. The filtrate containing methylated products was subjected to the mass spectrometric analysis.

7.3.5 High resolution MS analysis

The concentrated extracts obtained from crude oil and asphaltene extraction procedures (200 µL) were individually diluted with 800 µL methanol for positive ESI measurements. Raw asphaltene sample (1.4 mg) was dissolved in 1 mL toluene (HPLC grade, Acros Organics); the stock solution (400 µL) was further diluted with acetonitrile and dichloromethane (HPLC grade, Sigma-Aldrich, Taufkirchen, Germany) to a concentration of 150 ppm. Mass analysis was performed on a research type LTQ Orbitrap (Thermo Fisher, Bremen, Germany). Additional measurements were carried out using a 12 T LTQ FT-ICR MS (Thermo Fisher, Bremen, Germany) equipped with a standard ESI source. The sample was infused at a flow rate of 2 µL/min and the ions were generated using the following conditions: needle voltage=3.8 kV, sheath gas=5 arbitrary units. Data were acquired in both instruments using a 3 s transient allowing a mass resolving power of 700,000 (at *m/z* 400). The data were collected and processed with the LTQ FT Ultra 2.5.5 (Thermo Fisher, Bremen, Germany) data acquisition system using full scan and spectral stitching [5, 29, 30] with a mass range 150-1150.

7.3.6 Data Interpretation

The peak lists were converted to molecular formulas by Composer (Sierra Analytics, U.S.A.). The following chemical constraints were applied: Number of H unlimited, $0 < C<100$, $0 < O<2$, $0 < N <2$, $0 < S<2$, $0 < DBE < 40$. A molecular formula was considered only if the corresponding ^{13}C peak was also found. The calculated molecular formulas were sorted into compound classes based on their denoted Kendrick mass defects and their DBE distribution.[31] The obtained mass lists were transferred into Excel and Origin for data evaluation and preparation of the figures shown.

7.4 Results and discussion

7.4.1 Metal-ion extraction of crude oil

Nitrogen compounds were extracted from the crude oil through the addition of pure ethanol solution and ethanol solution containing Al^{3+}, Fe^{3+} in the same manner that Qi et al. described [24]. To compare this procedure, additional studies were carried out using an acidified ethanol solution adjusted with HCl (pH = 2.0) to extract nitrogen species in a medium free of strong Lewis acid. The isolated N-species were characterized using Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS). Electrospray ionization (ESI) was utilized in this study as source of choice because of its suitability for polar constituents in crude oil samples [32]. The class distribution shown in Figure 7-1 (left) represents the summed relative abundances of each class (>1% relative abundance) that were detected in the spectra of the different extraction solutions. The majority of classes observed were nitrogen containing classes, in which N_1 class is the most abundant in all of the cases, since ESI efficiently ionizes the basic pyridinic moieties. Other polar nitrogen-containing classes such as N_2, N_1O_1, and N_1S_1 were detected, however, with about ten folds lower abundance than N_1.

In a further step, the extracted nitrogen compounds were submitted to a derivatization reaction using methyl iodide in the presence of silver tetrafluoroborate, and were subsequently analyzed by FT-ICR MS. Derivatization as a step following the extraction allowed to study the selectivity of the extraction method. The derivatization procedure allows following aromatic compounds containing sulfur and oxygen that are not polar enough to be ionized by ESI and which after derivatization would become ionizable. However, signals referent to S and O-species were found in the original crude oil and the ethanol extract while they were not detected in the other extracts (Figure 7-1, right). This shows that the extraction methods applied indeed showed a distinct selectivity towards nitrogen species.

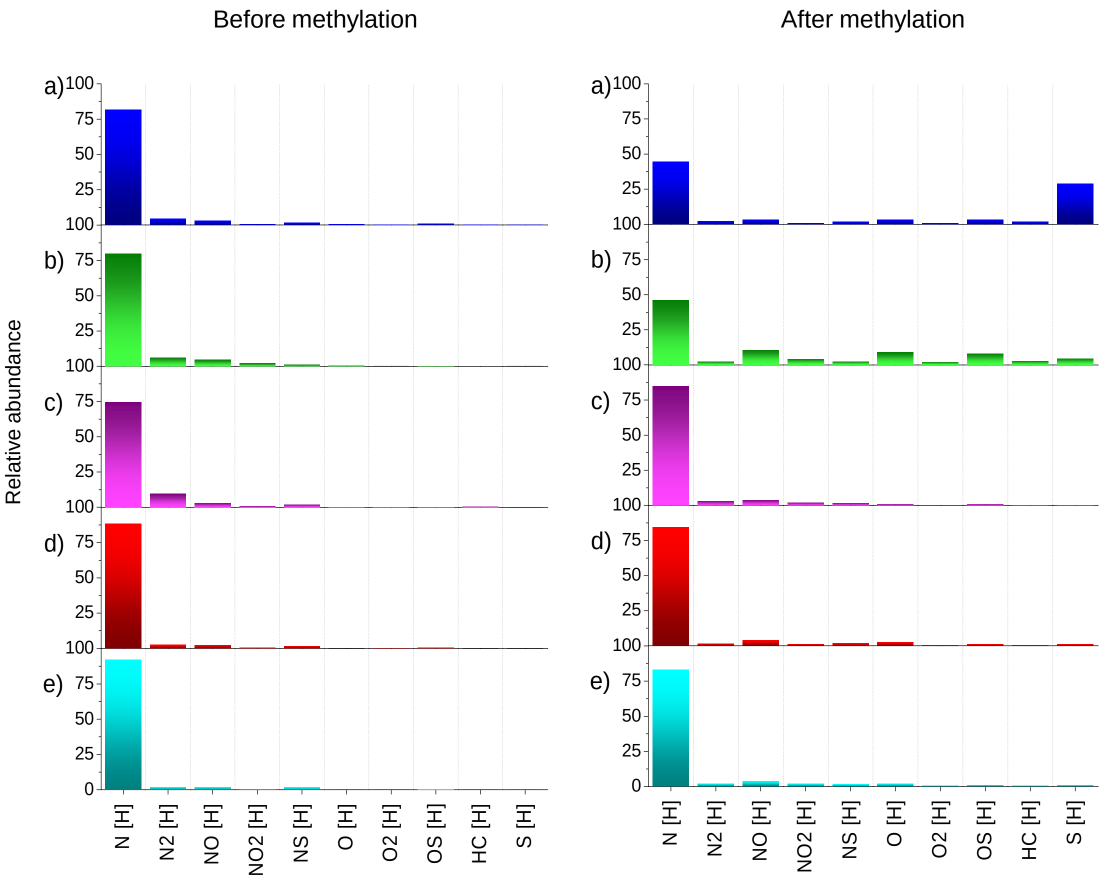

Figure 7-1: Comparisons between compound classes present in the crude oil (a) and extracted fractions (b) pure ethanol, (c) acid, (d) iron and (e) aluminium extractions before and after methylation.

In detail, extraction with pure ethanol (Figure 7-1, right row) presented a poor selectivity. For this extraction procedure a reasonable amount of O_1, O_1S_1 and S_1 compounds were observed. This was not the case for the extractions using metal ions or acidic conditions (pH = 2), where signals referent to S and O-species were almost not present. Additional information is derived from heat maps (Figure 7-2) illustrating the distribution of double bond equivalent (DBE; number of rings plus double bonds involving carbon) of the different classes before and after derivatization. The DBE distribution of N_1 class covered the range $4 < \text{DBE} < 20$ of all extraction solutions except of pure ethanol, which was restricted to a maximum DBE = 14. No change in the DBE distribution of N_1 was observed before and after methylation except for ethanol. Sulfur species were extracted by the ethanol-water phase only in very minute amounts and remain mostly in the residual oil phase. With the absence of sulfur species, which represent preferable targets for the methylation reaction, nitrogen species can also be derivatized.

No significant difference in terms of extracted nitrogen was observed whether Fe(III)- or Al(III)-ions were used in the extraction solution. Both of them delivered a very comparable DBE distribution of N_1 class covering the same range. On the other hand, comparable results were also obtained from the extraction solution of ethanol acidified with HCl, however, from acid extraction, nitrogen species with emphasis in lower DBE and carbon number were obtained in higher intensity. The selectivity of extraction of nitrogen compounds that was according to literature was due to Lewis acid interaction and reversible complexation in a complex matrix such as crude oil can also be achieved by an acid extraction. However, the Lewis acid aprotic complexation under mild conditions prevents possible modification and side reactions of the extracted samples when direct acid extraction is used, since it has been found that acid treatment of crude oil samples initiate polymerization of indoles [22].

Using the solvent extraction procedures, we were able to isolate and characterize basic nitrogen compounds present in the analyzed crude oil samples. As previously reported the extraction using pure ethanol showed a very low selectivity. The solvent extraction based on chemical complexation, as the procedure using ethanol solution containing metal ions, presented similar efficiencies for both Fe^{3+} and Al^{3+}. The results indicate that the extraction of nitrogen species under acidic conditions is preferred and an enrichment of such species can be achieved allowing a fairly selective sample preparation method for highly complex crude oil samples.

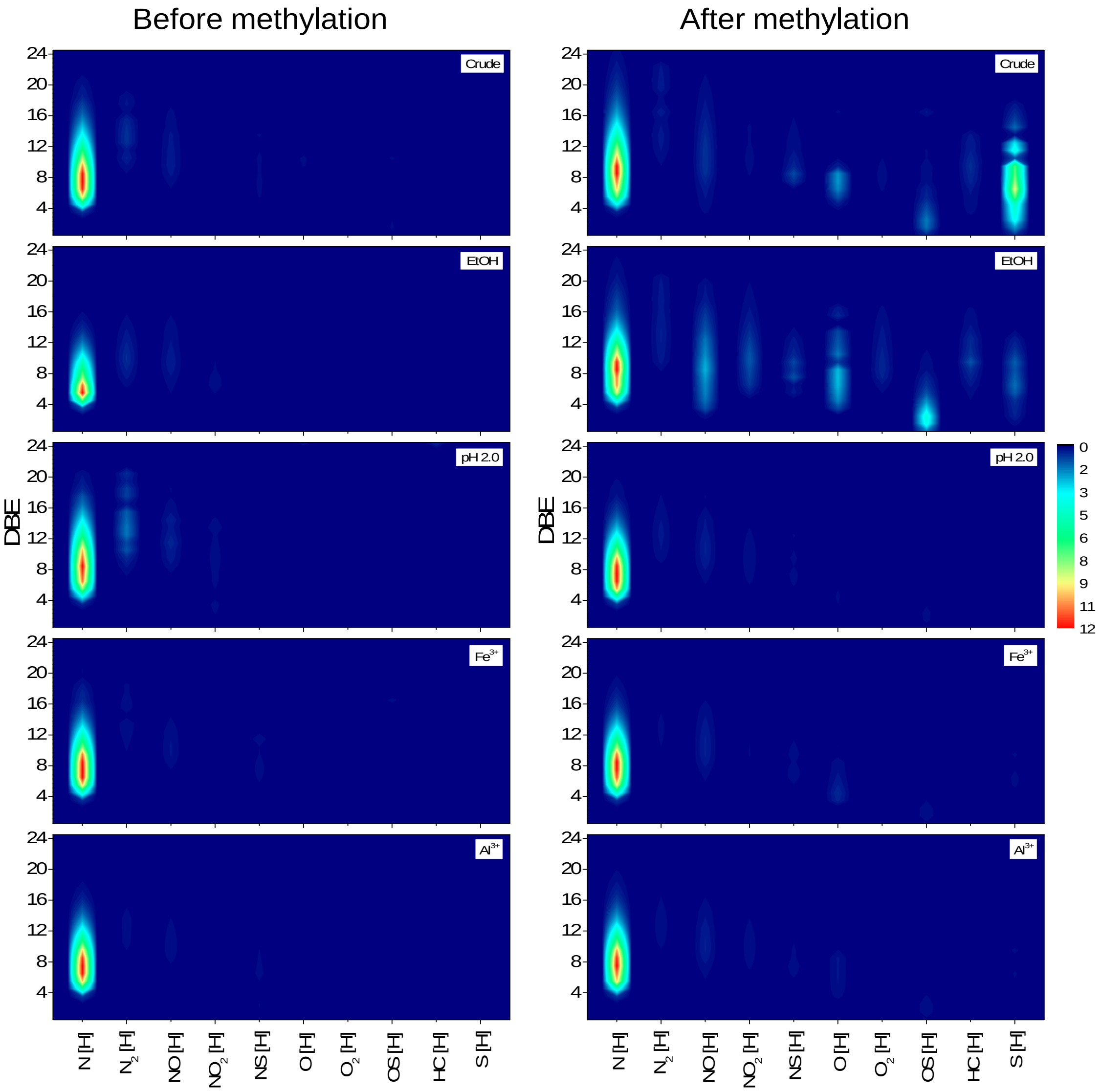

Figure 7-2: Comparison between compound classes present in the crude oil after different extractions, before and after methylation.

7.4.2 Metal-ion extraction of asphaltene sample

Asphaltenes are considered to contain higher nitrogen content in comparison with other lighter fractions. Basic polar nitrogen compounds in heavy fractions can be investigated by high resolution mass spectrometry using electrospray. However, asphaltenes have a very complex structure of polyaromatic hydrocarbon cores, which tend to stack and aggregate, making not all of the heteroatomic centres accessible. Since the results obtained here show the selectivity of the metal-ion extraction method towards basic nitrogen compounds, it was applied for the investigation of nitrogen species in asphaltene fraction.

The asphaltene fraction was obtained by SARA fractionation of a bitumen sample [33] (see experimental section) and was extensively analyzed by high resolution mass spectrometry using different ionization methods including electrospray [34]. However, the methodology of metal ion extraction could not be applied to asphaltene samples without modification, since a solvent mixture capable to dissolve the asphaltenes and form two immiscible layers with the ethanol solution was not practical. Hence, the method was changed in a way that a direct contact between fine powdered asphaltene and the extraction solution was achieved. The resulting extract was analyzed by high resolution mass spectrometry using the "*spectral stitching*" technique, which expands the data depth and allows a better signal distribution during the analysis of complex matrices such as asphaltenes [5]. Even if this technique is applied, ESI measurement of asphaltene is restricted because not many components from asphaltenes are being ionized [34].

The implementation of the metal ion extraction makes some of the components present in asphaltenes accessible for analysis by electrospray ionization because the more polar and nitrogen containing compounds are extracted. This is presented in Figure 7-3 where the number of assigned formulas from an electrospray measurement of an asphaltene and the asphaltene metal-ion extract are compared.

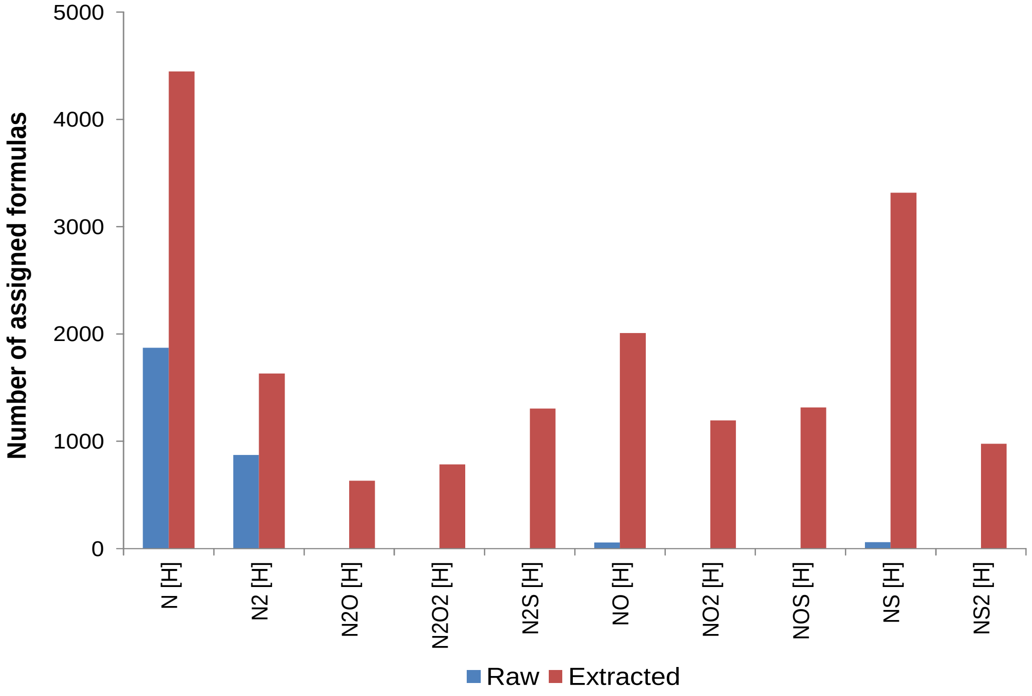

Figure 7-3: Comparison between the number of assigned nitrogen compounds present in the asphaltene obtained by positive electrospray FT-ICR MS measurements.

Various nitrogen containing compounds were ionized after extraction in comparison to simple dissolution procedure adopted for ESI measurement of asphaltene. Data presentation is based on the population of assignments (number of assigned formulas) which avoids discrimination resulting from different response factors of detected species [33-35].

The dominance of N_1 class was more than doubled in the extract compared to the original asphaltene. This extraction procedure enhances especially the ionization of N_1S_1 class compounds, where more than 3000 species were detected. A combination of sulfur and/or oxygen classes surpass the threshold of 500 assignments as observed in the data analysis of the extract. A visualization of the capability of enhancing the detection range of polar species is illustrated in Figure 7-4. A Kendrick plot [31] of N_1 class shows the DBE distribution against assigned masses along the Kendrick mass scale. Higher mass ranges and higher DBE values were observed in the extracted N_1 class. Almost all the signals assigned in ESI measurement of the original sample were found in the assignments calculated for the extract.

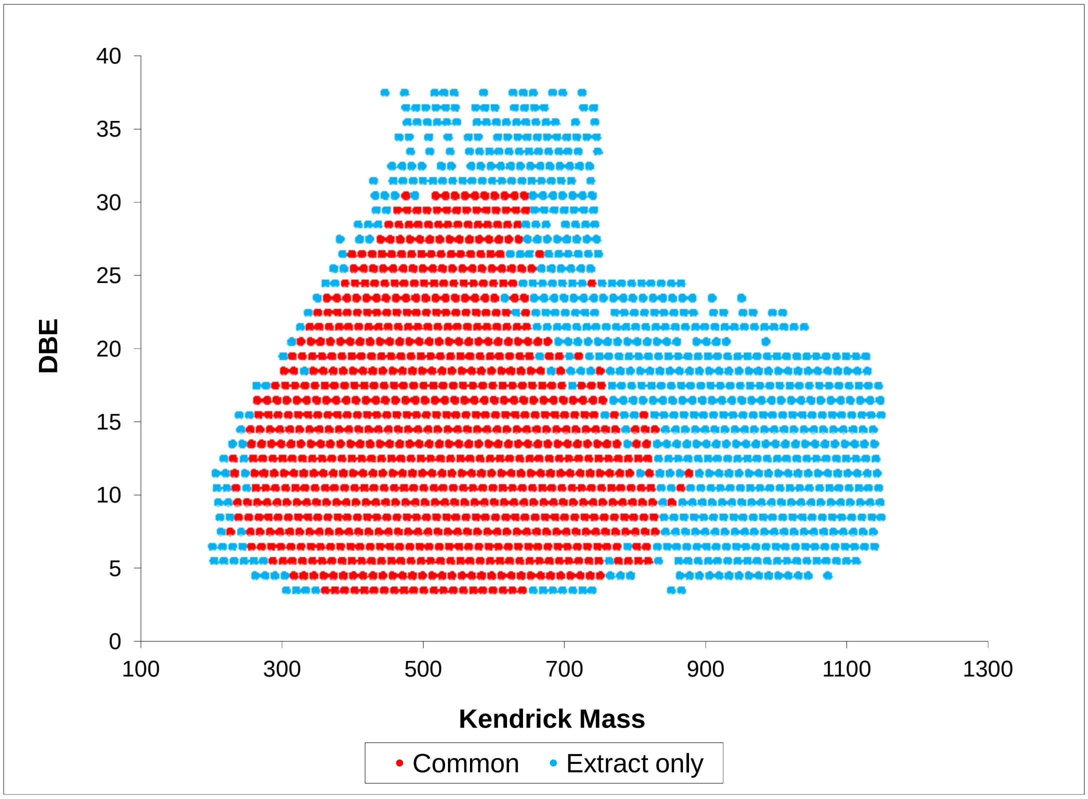

Figure 7-4: Kendrick plot of uniquely assigned formulas of N_1 class. This illustration is based on the assigned formulas regardless of the intensity. Common assignments detected in the ESI measurement of the original sample are marked with red, whereas unique assignments in the extract are marked with blue.

The expansion of detected range is probably due to reduced suppression effects because of less competing components in the simplified sample and could also be due to enrichment on nitrogen components. The extraction was performed under mild conditions; therefore no alteration in the asphaltene structure such as decomposition or polymerization was expected [22].

Mullins et al [12] postulated that asphaltenes in crude oil are composed mainly of seven fused polyaromatic hydrocarbon core with a mass about 750 Da, in contrast to coal asphaltenes [36], which have a smaller core and shorter alkyl-chains. Basic nitrogen constituents in asphaltene fractions are present in the form of pyridinic core incorporated in the polyaromatic core as it has been found using XANES spectroscopy [13].

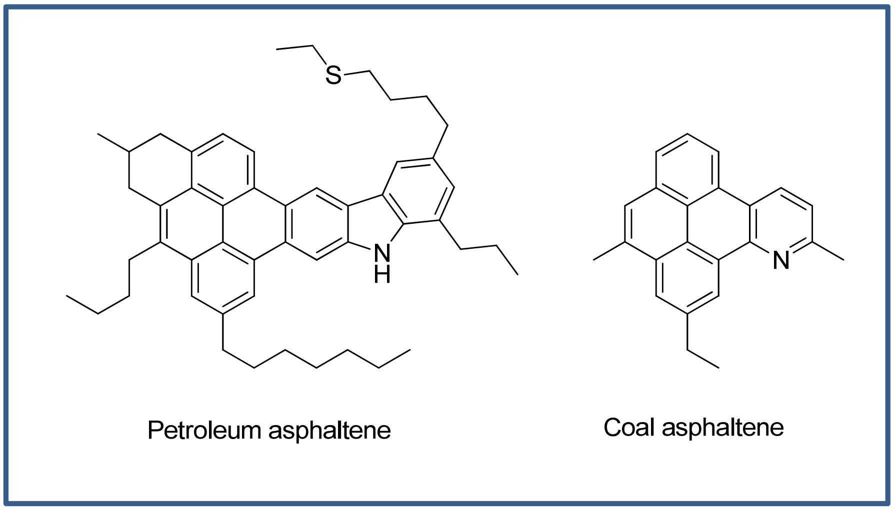

Figure 7-5: Proposed molecular structures for coal and petroleum asphaltenes by Groenzin and Mullins [37].

Closer examination of N_1 species, detected by positive ion electrospray measurement of the extract, reveals that the highest abundance of species is in the DBE range between 10 and 16 with a maximum intensity at DBE 12. This corresponds to 5-6 fused rings, a smaller structure than postulated for asphaltene. At the same time, elemental analysis of the asphaltene sample showed a composition: C 87.12, H 6.87, N 1.16, S 1.97, and an oxygen content of 2.88 calculated by difference. The C:H ratio gives a value of 1:0.938, which is relatively low for an asphaltene sample derived from petroleum and may relate closer to the coal asphaltenes [38]. On the other hand, coal asphaltenes have shorter alkylated chains, and the nitrogen species detected in the extract show carbon number over 70. Therefore, it can be suggested that the asphaltene sample measured in this study have a smaller aromatic core with 6 fused rings like coal asphaltene, but with longer alkyl chains or fused alicyclic structures. Solid state NMR measurements previously published [34] confirmed this suggestion.

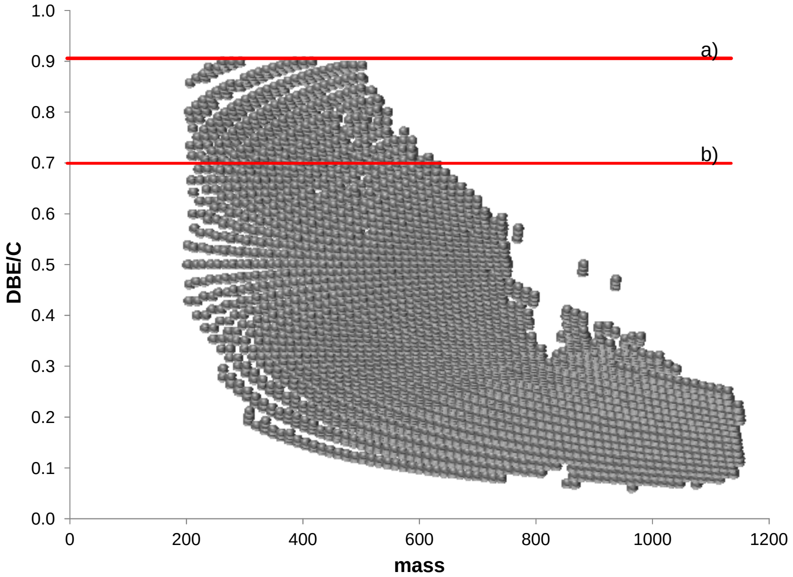

Figure 7-6: DBE/C versus the mass of all nitrogen-containing monoisotopic assignments obtained from positive ESI-FT-MS of the extract. The red lines represent (a) the “90%-rule”: theoretical upper boundary of DBE versus C and (b) the criterion for identifying species with condensed aromatic ring structures (CARS).

In order to obtain further details about the aromaticity of the assigned nitrogen-containing class compounds, a normalization of the DBE to the number of carbons within the given molecule (DBE/C) was calculated and depicted in Figure 7-6. The nitrogen containing species has a DBE/C range from 0.1 to 0.9 which covered the mass range 200-1200 Da. According to Hockaday et al [39] a threshold DBE/C value of 0.7 can serve as criterion for the identification of condensed aromatic ring structures (CARS). On the other hand, a theoretical barrier of chemically correct compositions is at the limit of DBE/C = 0.9 [40]. Hence, assignments following the DBE/C ≥ 0.7 are classified as polycyclic aromatic structure.

A total of 6732 unique assignments were calculated for the nitrogen containing species in the metal-ion extract of the asphaltene obtained by high resolution Fourier transform mass spectrometry. Only about 13% of these assignments fulfill the DBE/C ≥ 0.7 criterion. The major four dominant classes detected in the extract were N_1, N_1S_1, N_1O_1, and N_2, ordered by decreasing number of assignments (see Figure 7-3). With 18%, N_2 class is the second highest in unique assignments percentage within the boundaries of 0.9 > DBE/C ≥ 0.7 just below the N_1 class (22%). This indicates the possible highly aromatic structure of this dual nitrogen class. N_1S_1 and N_1O_1 registered merely 13% and 9.8% respectively. One assumption could be that the majority of N_1S_1 and N_1O_1 classes have a nitrogen-containing aromatic core with an alkyl chain which contains an additional sulfur or oxygen atom. It has to be mentioned, however, that the criterion of DBE/C ≥ 0.7 is restrictive and eliminates a significant amount of potential polycondensated structures [34].

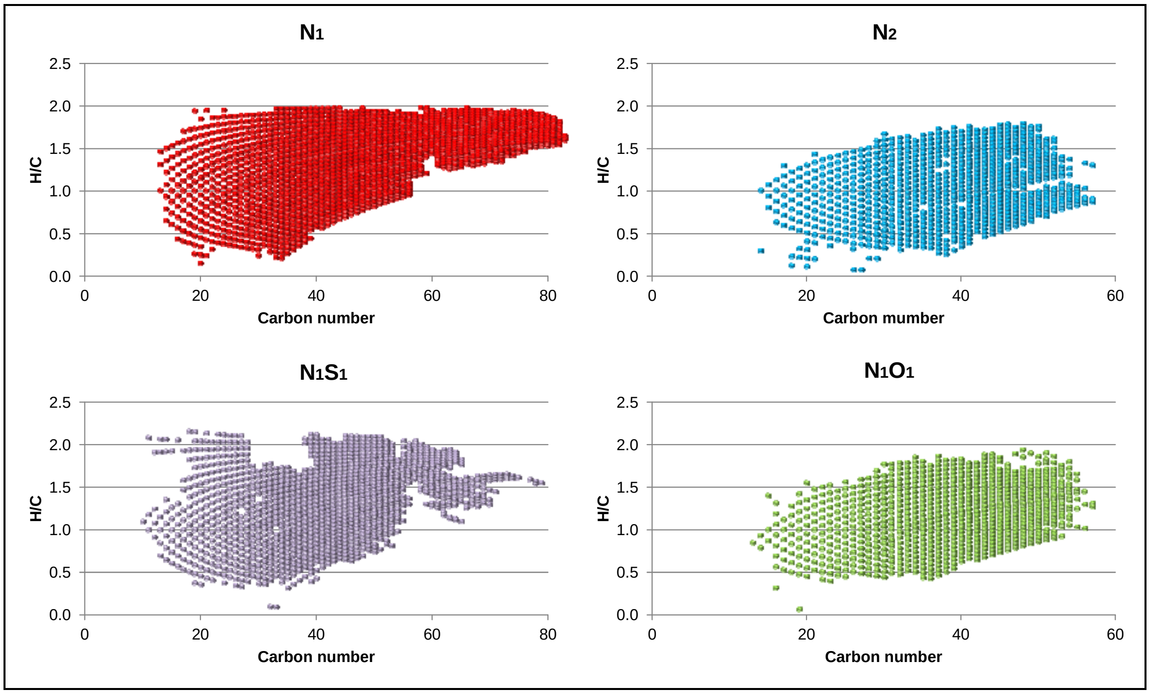

Figure 7-7: Bubble plot of H/C ratio versus carbon number for the species of the classes N_1, N_2, N_1S_1, and N_1O_1 assigned by the positive ESI-FT-MS measurement of the extract.

Another way of representation of the aromaticity of asphaltene constituents is by plotting H/C ratio against the carbon number [41], since asphaltene shows a bulk H/C ratio (~1) for asphaltenes and significantly higher ratio for maltenes [38, 42]. Statistically, 2430 uniquely assigned nitrogen containing species out of 6732 assignments (36%) have an H/C ratio less than 1. Figure 7-7 shows a bubble plot of H/C value of the unique constituents of the major four classes; N_1, N_2, N_1S_1, and N_1O_1 against their corresponding carbon number. N_2 class has the highest percentage of species having H/C < 1 (21%), whereas N_1 has about half the value of it (10%). From the N_1 and N_1S_1 plots it is obvious that these classes of compounds have more alkylated chains than their counterparts N_2 and N_1O_1. Both of N_1 and

N_1S_1 assignments have an average H/C value of 1.3, whereas N_2 and N_1O_1 have an average H/C value of 1.03 and 1.19, respectively.

This high hydrogen deficiency values indicates the ability of this procedure to extract highly aromatic nitrogen compounds. Additionally, ESI was able to efficiently ionize the extracted highly aromatic nitrogen-containing species present in the asphaltenes.

7.5 Conclusion

We have demonstrated that by using solvent extraction procedures, we were able to isolate and characterize basic nitrogen compounds present in the crude oil and bitumen asphaltene using high resolution Fourier transform mass spectrometry equipped with electrospray ionization. In the crude oil sample, the extraction with pure ethanol did not show selectivity towards a compound class, as expected. A comparison between extraction using both Fe(III) or Al(III) and acid showed similar distribution of carbon number x DBE however, from acid extraction, nitrogen species with emphasis in lower DBE and carbon number were obtained in higher intensity. This could mean that the extraction mechanism resembles more an acid directed extraction. However, the non complicated aprotic extraction with Lewis acid is more suitable for crude oil samples, because the complexation is reversible and no side reactions were observed. After methylation of the extracted fractions, no significant amount of other compound classes – S, O, CH – were found leading to the conclusion that the methodology is selective towards N-containing compounds and can be efficiently applied for complex mixtures.

A modified metal-ion extraction applied on an asphaltene sample showed selectivity towards nitrogen species. Once the basic nitrogen species were selectively extracted from the bulk asphaltene sample, a significant increase in the number of the assigned molecules was found when compared to the raw sample. The investigated asphaltene sample showed characteristic features of a polyaromatic core consisting of around 6 fused rings. The aromaticity of the extracted species was investigated by hand of DBE/C and H/C values in order to determine the highly abundant aromatic classes. N_2 class was the most aromatic class among other extracted species. This study revealed that by reducing the complexity of the sample an increased amount of analytical information can be achieved, allowing conclusions based on a better and deeper data set.

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Chapter 8 General Conclusion

The dependency of the modern society on affordable energy resources, which fuels the economy and social development, is growing. The rapid rise of prices on the world's oil markets in the last years clearly demonstrates that a better exploitation of the remaining supplies becomes distinctively more valuable. Despite the rather expensive chemical work-up procedures to yield clean and useful energy supplies, higher oil prices push the exploitation of heavier crude oils, Heavier crude oils and the higher boiling fractions of standard crude oils have thus become important resources to the expense of significantly elevated impurities levels, *e.g.*, sulfur-, nitrogen-, oxygen- or metal-compounds. Many of these compounds have adverse effects on processing as well as on performance of the final products. The optimization of catalytic refinement processes is thus heavily dependent on the quality of the chemical characterization of the raw material. In this regard, Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) has proven to provide analytical data of sufficient accuracy and resolution. Nevertheless, even with the ultimately high resolving power of FT-ICR MS, the accurate mass spectrometric analysis of crude oil components relies on appropriate ionization and separation methods. The role of sample preparation is immense in this regard. Without simplification of the complex crude oil sample it is practically impossible to analyze the sample even with high resolution mass spectrometry by one-shot-measurement approach. Prior sample preparations steps improve the detection capability by reducing matrix effects and ionization suppression. Thus, the combination of sample simplification and selective analytical approach towards crude oil classes achieves more data depth for the characterization of this complex mixture.

Even after initial simplification, the complexity of the obtained fractions is big, which assures the need of high resolving power mass spectrometers. In this regard FT-ICR MS play an important role. Additionally, a new research type Orbitrap instrument capable to study very complex crude oil sample was introduced. Reasonable resolving power values were achieved considering that they were obtained without a super conducting magnet that is used for FT-ICR mass spectrometers. Especially at higher mass ranges the obtained resolution is enough to resolve peaks in samples with high complexity.

The important role of sample preparation on the final results was investigated by dissolving the solid deep well deposit sample in different solvents and analyzing the extracts in positive and negative ESI FT-ICR MS. All six solvents of varying polarity were able to extract a wide variety of compound classes. Although similarities were seen in the extracts from some solvents, it was also obvious that there are major differences. Moreover, these data clearly show that there is not one procedure or one analytical method that is able to characterize a crude oil fully, and that a comprehensive characterization of deep well deposits may be possible only through the combined use of various analytical methods, each of which must be carefully chosen for its selectivity.

Selective separation can be achieved by applying chromatographic methods. In this work, two methodologies (online and offline) of coupling normal-phase high performance liquid chromatography to an ultra-high resolution mass spectrometer for the investigation of a deasphalted crude oil sample were compared. By using APLI as the ionization technique it was possible to detect in detail the changes in compositions in the offline measurements of both nonpolar and polar fractions. Furthermore, polar nitrogen species were traced in the offline and online coupling schemes. The change of composition was followed in a scan-to-scan manner along the elapsed retention time. Moreover, reconstructed class chromatogram was introduced as a method that allows tracking the changes of class compositions in great detail. Higher MS resolution was obtained by offline coupling because of longer transients; on the other hand, structure analysis can only be achieved when faster transients are used as in the case of online coupling.

Furthermore, by the online coupling HPLC/FT-MS setup for the analysis of a deasphalted crude oil sample, four different ionization techniques: ESI, APPI, APCI, and APLI were applied. Comparable UV/Vis and ion chromatograms were obtained and the class distribution analysis on the detected peaks showed that the first peak comprises mainly of hydrocarbon assignments, whereas the second peak contains almost exclusively nitrogen containing species. Unique assignments of each of the utilized ionization techniques showed selectivity towards classes of compounds in the crude oil sample. By monitoring the N_1 class species in the SIM mode of the APLI measurement it was found that the population of protonated species surpasses the radical species only in the second peak. This increase is an indicator of selective ionization of polar basic nitrogen compounds. Increase of aromaticity of different classes in course of retention time was observed as well.

Moreover, the isolation and characterization of basic nitrogen compounds present in the crude oil and bitumen asphaltene was achieved. An ethanol solution containing 1 % by weight Lewis acids such as Fe(III) or Al(III) showed simple and effective extraction behavior. ESI-FT MS measurements of the original and the methylated extracts showed selectivity towards nitrogen containing classes. It was found that the non complicated aprotic extraction with Lewis acid is more suitable for crude oil samples than acid extraction, because the complexation is reversible and no side reactions were observed. Additionally, a modified metal-ion extraction was applied on an asphaltene sample. A significant increase in the number of the assigned molecules was found in the extracted sample when compared to the raw sample. Furthermore, the investigated asphaltene sample showed characteristic structural features of the polyaromatic core.

Overall, this study revealed that by reducing the complexity of the crude oil sample an increased amount of analytical information can be achieved by high resolution mass spectrometric investigations.

List of Figures

List of Tables

List of Abbreviations

ACN	acetonitrile
AGC	automatic gain control
APCI	atmospheric pressure chemical ionization
APLI	atmospheric pressure laser ionization
APPI	atmospheric pressure photo ionization
CARS	condensed aromatic ring structure
CI	chemical ionization
CLF	chloroform
Da	Dalton
DBE	doule bond equivalent
DCM	dichloromethane
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
EI	electron ionization
ESI	electrospray
eV	electron volts
FT-ICR	Fourier-transform ion cyclotron resonance
FWHM	full width at half maximum
GC	gas chromatography
HDS	hydrodesulfurization
HEX	hexane
HPLC	high-performance liquid chromatography
IP	ionization potential
IPA	iso-propyl alcohol
IUPAC	international union of pure and applied chemistry
KMD	Kendrick mass defect
kV	kilovolts
m/z	mass-to-charge ratio
LC	liquid chromatography
LTQ	linear trap quadrupole
LVEI	low voltage electron ionization
MALDI	matrix-assisted laser desorption ionization
MS	mass spectrometry
NIR	near infrared
NMR	nuclear magnetic resonance
NP	normal phase
PAC	polar amino cyano
PAH	polycyclic aromatic hydrocarbons
PANH	polycyclic aromatic nitrogen heterocycles
PASH	polycyclic aromatic sulfur heterocycles
ppb	parts per billion
ppm	parts per million
R	resolution
REMPI	resonance enhanced multiphoton ionization
RF	radio frequency
RT	retention time
S/N	signal-to-noise ratio
SARA	saturates, aromatics, resins and asphaltenes

SIM	selected ion monitoring
T	tesla
TIC	total ion chromatogram
TOF	time-of-flight
TOL	toluene
UV/Vis	ultraviolet-visible
XANES	X-ray absorption near edge structure
XPS	X-ray photoelectron spectroscopy

List of Publications

Publications in peer-reviewed Journals

- Gaspar A., Zellermann E., Lababidi S., Reece J., Schrader W.; Impact of different ionization methods on the molecular assignments of asphaltenes by FT-ICR mass spectrometry, *Analytical Chemistry,* **2012**, 84, 5257.
- Gaspar A., Zellermann E., Lababidi S., Reece J., Schrader W.; Characterization of Saturates, Aromatics, Resins, and Asphaltenes heavy crude oil fractions by atmospheric pressure laser ionization Fourier transform ion cyclotron resonance mass spectrometry, *Energy & Fuels*, **2012**, 26, 3481.
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- Lababidi S., Schrader W., Denisov E., Markarov A.; A new research-grade high-field Orbitrap hybrid mass spectrometer capable of studying complex hydrocarbon mixtures from energy resources at ultra-high mass resolution (submitted to Analytical Chemistry).
- Lababidi S., Panda S., Andersson J.T., Schrader W.; Direct coupling of normal-phase HPLC to atmospheric pressure laser ionization FT-ICR MS for the characterization of crude oil samples (submitted to Analytical Chemistry).
- Lababidi S., Schrader W.; Online normal phase HPLC/FT-ICR mass spectrometry: Effects of different ionization methods on the characterization of highly complex crude oil mixtures (submitted to Rapid Communications in Mass Spectrometry).
- Lababidi S., Nachtigal F.M., Sripada K., Schrader W.; Extraction and characterization of basic nitrogen compounds from crude oils and asphaltenes by metal ion complexation and high resolution mass spectrometry (will be submitted to Fuel).

Poster and Oral Presentations

- Lababidi S., Schrader W.; HPLC/FT-ICR MS as an analytical approach for the characterization of crude oil samples, *60th ASMS conference on mass spectrometry and allied topics*, **20 – 24** May **2012** Vancouver, Canada (Poster).
- Lababidi S., Schrader W.; HPLC/FT-ICR MS as an approach for detailed analysis of crude oil samples, *Joint conference of polish mass spectrometry society and german mass spectrometry society, 45th DGMS annual meeting*, **04 – 07** March **2012**, Poznan, Poland (Oral presentation).
- Lababidi S., Nachtigal F., Schrader W.; Investigating the role of extraction methods on the analysis of polycyclic aromatic compounds in crude oil by FT-ICR mass spectrometry, *23rd International symposium on polycyclic aromatic compounds (ISPAC23),* **04 – 08** September **2011**, Münster, Germany (Poster).
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Curriculum Vitae

Der Lebenslauf ist in der Online-Version aus Gründen des Datenschutzes nicht enthalten.

Erklärung

Hiermit versichere ich, dass ich die vorliegende Arbeit mit dem Titel

„Effect of Sample Preparation on the Characterization of Crude Oil and its Complex Fractions by High Resolution Mass Spectrometery”

selbst verfasst und keine außer den angegebenen Hilfsmitteln und Quellen benutzt habe, und dass die Arbeit in dieser oder ähnlicher Form noch bei keiner anderen Universität eingereicht wurde.

Mülheim an der Ruhr, im Mai 2013

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