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Standardized protocols for the more common samples -Iteration#1

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Document Validation

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P1 CNRS – Christian Rolando	2021-07-26	christian.rolando@univ-lille.fr

Document Abstract

The following deliverable provided in the form of a public report document is the first iteration (#1) of the deliverable “Standardized protocols for the more common samples”. Motivation of this deliverable is to create a set of protocols with the same type of structure, parameters, and metadata for multiple types of ionization and coupling types in ultra-high resolution mass spectrometry, e.g., direct infusion or chromatographic approaches, as well as various types of sample materials. In this iteration, the formula type, i.e., parameters, requested metadata, general structure, was defined. Moreover, this standardized protocol was filled for the first six examples of experimental types and matrices covering the following issues:

- direct infusion positive and negative mode electrospray ionization (+/- ESI FT-ICR MS) for vacuum gas oils (a heavy fraction of fossil petroleum)
- direct infusion atmospheric pressure photo- and chemical ionization (APP/APCI FT-ICR MS) for crude oils
- gas chromatography coupled to atmospheric pressure chemical ionization (GC-APCI FT-ICR MS) for diesel fuels
- direct infusion positive and negative mode electrospray ionization (+/- ESI FT-ICR MS) for diesel fuels
- thermal analysis coupled to atmospheric pressure chemical ionization (GC-APCI FT-ICR MS) for heavy petroleum fractions, such as bitumen or asphaltenes

The overall content will be filled and completed based on the created structure in the following iterations #2 and #3.

Table of Contents

Abbreviations

APCI – atmospheric pressure chemical ionization
APPI – atmospheric pressure photoionization
APLI – atmospheric pressure laser ionization
DBE – double bond equivalent
DNA – deoxyribonucleic acid
ESI – electrospray ionization
GC – gas chromatography
HDX – hydrogen deuterium exchange
KMD – Kendrick mass defect
MALDI – matrix assisted laser desorption ionization
MS/MS – tandem mass spectrometry
RNA – ribonucleic acid
VGO – vacuum gas oil
Vpp – Volt peak to peak

1 INTRODUCTION OF THE CONSORTIUM EXPERTISE AND FUNDAMENTALS

1.1 EXPERTISE COVERED BY THE CONSORTIUM

The general trend for analytical applications of high magnetic field FT-ICR instruments is the analysis of complex samples, requiring either ultra-high mass accuracy or ultra-high resolution. The major applications range from small molecule analysis (Petroleomics, dissolved organic matter, metabolomics, tissue imaging with small molecules) to very large molecules or molecule assemblies (top-down proteins and protein complex analysis, glycomics). In the first case, the high resolving power of the instrument allows simultaneous analysis of a huge number of compounds, with mass accuracy allowing the assignment of elemental composition to each compound. In the area of larger molecules, 1 Da mass accuracy can be achieved up to 200 kDa, and the use of high resolution MS/MS allows unambiguous attribution of large fragments, providing for example enhanced sequence information on these large biomolecules.

	1	2	3	4	5	6	7
Partners	petroleomics, biofuels, environmental science, polymers	Top-down and middle down proteomics	physical chemistry, ion molecule reactions, ion spectroscopy	metabolomics, glycomics, lipidomics	cultural heritage, art, archaeology	mass spectrometry imaging	ultrahigh resolution, 2D FTICR MS
CNRS-LILL				x	x		x
CNRS-ORSA			x				
LIEG						x	
PRAG		x					
UHRO	x						
UEF	x	x		x			
ROMA			x				
LISBOA		x		x			
MOSC					x	x	x
WARW	x				x		x

1.1.1 Top-down Proteomics

Using FT-ICR mass spectrometry, proteins, can be analyzed intact (top-down) rather than in pieces (bottom-up). Top-down mass spectrometry is an emerging technology which

strives to preserve the post-translationally modified forms of proteins present in vivo by measuring them intact, rather than measuring peptides produced from them by proteolysis. The top-down technology is beginning to capture the interest of biologists and mass spectrometrists alike, with a main goal of deciphering interaction networks operative in cellular pathways.

1.1.2 Structural Proteomics

Genomes of many organisms including human have already been sequenced and decoded. Currently there is a strong demand to solve high order structures of proteins. Even if there are well established methods for structural biology, structural mass spectrometry becomes a very powerful technique in combination with molecular dynamics to build l ab initio protein model or explain protein-protein and/or protein-ligand interactions. These techniques are based on hydrogen-deuterium exchange (HDX), chemical cross-linking (CXMS) and ion-mobility.

1.1.3 Characterization of ribonucleic acids

Gene expression in living organisms is regulated at both the ribonucleic acid (RNA) and protein levels, and involves transcription of desoxyribonucleic acid (DNA) into RNA prior to translation into proteins. Large-scale, high-throughput sequencing studies have established that more than 60% of a mammalian genome is transcribed or predicted to be transcribed into RNA, but only a stunningly small fraction, ~2%, is actually translated into proteins. Currently, new mass spectrometry based approaches for the characterization of RNA are being developed that rely not only on the high mass accuracy and resolving power of FT-ICR instruments but also on their unique capability for ion-electron reactions that constitute the basis of new techniques such as electron detachment dissociation (EDD).

1.1.4 Metabolomics

Metabolic profiling is an emerging discipline under the umbrella concept of systems biology. The goal of metabolomics is understanding concentrations and fluxes of endogenous metabolites within a living biological system. Challenges in metabolomics include the presence of a wide range of molecular weights and large variations in concentration, but also the presence of polar and nonpolar as well as organic and inorganic molecules. In this regard, FT-ICR has unique advantages, as mass spectrometric method, to provide extensive mapping of the molecular diversity and attribution of unique elemental composition for the whole sample.

1.1.5 Imaging mass spectrometry

Imaging mass spectrometry can be roughly divided into two major contributions: mapping and identification. Quantification is still a challenging task. The 2D maps (or 3D on adjacent slices) allow defining regions of interest through an unsupervised analysis of spectral patterns. In histopathology, the regions of interest can be used for classification purposes of diseases. Once defined, the regions of interest may be analysed in depth either in situ or after separation from the rest of the section, e.g. to detect and quantify biomarkers. The growing developments in top down proteomics also allow to microextract soluble proteins

from the regions of interest and to perform identification by MS/MS of entire small to medium size proteins. Imaging mass spectrometry has also very important applications in the field of chemical communication. Inhibition zones between bacteria, between bacteria and pathogens, symbiotic activity, cell signalling through extracellular matrix are, among others, applications where bidimensional (2D) information leads to the detection of a biological activity.

1.1.6 Environmental science

Particulate matter and polluted effluents are highly complex mixtures comprising organic and inorganic components with completely different physical chemical properties and they are generally associated with a huge dynamic range. Non-targeted *in situ* approaches for the analysis of such mixtures have emerged a few years ago, relying on high resolution mass spectrometers which allow the identification of a large number of different compounds in a narrow mass range. Resolution and mass accuracy provided by FT-ICR instruments allow an accurate fingerprint of environmental samples for the identification of new pollutant families through the statistical analysis of a large number of samples.

1.1.7 Petroleomics

Ultra-high-resolution FT-ICR mass analysis offers the possibility to resolve species with very close elemental compositions (C3 vs SH_4, i.e. 0.0034 Da for example) and thus to contemplate the ultimate characterization of all of the chemical constituents of petroleum, along with their interactions and reactivity, a concept denoted as “petroleomics”. Such knowledge already showed its capability for distinguish petroleum from its distillates according to their geochemical origin, maturity, distillation cut, and extraction method, catalytic processing, etc. Such analysis can also be applied to analysis of humic and fulvic acids, coals, and other complex natural mixtures, often without prior or on-line chromatographic separation. In recent years, methodology used in petroleomics has also been successfully applied to the analysis of liquid biofuels such as wood derived bio-oils.

1.1.8 Organic and Organometallic chemistry

Characterization of unknown Organic and organometallic entities (natural or synthetic) is greatly facilitated by the high-resolution capabilities of the FT-ICR technique. The ability of performing MS/MS experiments and obtain accurate elemental composition of fragments greatly contributes to the structural elucidation of those new entities, especially when the MS/MS experiments can be performed with different fragmentation methods that can give insights into the involved mechanism.

1.1.9 Inorganic chemistry

One difficulty associated with the analysis of inorganic and organometallic material is the impossibility to dissolve a large part of the mineral compounds and the complexity, in term of isotopic distribution, associated with the presence of various metallic elements. Desorption techniques (LA, LD, SIMS) in combination with high field FT-ICR mass spectrometry is a unique tool to investigate insoluble compounds in complex matrix (composite) with the ultrafine resolution necessary for their unambiguous identification.

1.1.10 Polymer science

Most of the structural features of polymers, such as the nature of the repeat unit, average molecular weights or polymerization degree, can be obtained from a single-stage mass spectrum, as long as any peak overlap can be efficiently resolved, but microstructural details can only be obtained by MS/MS. In particular, mass data can be converted to elemental compositions but an increase in the polymer average molecular weight requires an appropriate increase in the resolving power of the mass analyzer to afford meaningful compositional analysis. Functional materials are prepared from macromolecules such as copolymers of various composition and microstructure as well as supramolecular assemblies, for which FT-ICR mass spectrometers provides the capacity to resolve overlapping peaks in highly complex mixtures and offers alternative fragmentation techniques elucidating the copolymer structures.

1.1.11 Physical Chemistry

The highly versatile ion storage and manipulating ability of FT-ICR mass spectrometry are an invaluable tool to ascertain fundamental physical chemical properties of isolated species, unaffected by the environmental effects that play a major role in condensed phases. Basic thermodynamic quantities (e.g. scales of electron and proton affinities, positive and negative ion affinities, sequential ligand binding and solvation energies) can be acquired studying equilibria of electron, proton, and atom or ligand transfer reactions in the presence of stationary concentrations of neutrals. An appropriate configuration involves multiple inlet systems to admit selected compounds in the FT-ICR cell. Ion-molecule reactivity is obtained by recording kinetics in both forward and reverse direction under the prevailing highly dilute pressure regime.

1.1.12 Ion spectroscopy

FT-ICR mass spectrometers provide an efficient method to confine ions in a small volume for periods of time that can reach hours. Although other mass spectrometry devices such as radiofrequency ion traps can also partly achieve this aim, FT-ICR mass spectrometers have the unique qualities to provide both ion trapping, high mass resolution and the possibility of electron activation. This leads to the investigation of a high number of issues in the characterization of gas phase structures with implications in multiple areas of mass spectrometry (peptide fragmentation, investigation of the chemistry of desolvation processes, gas phase chiral recognition, investigation of catalytic processes without solvent interference).

2 INTRODUCTION OF FUNDAMENTALS

2.1 FT-ICR MASS SPECTROMETRY

2.1.1 Ionisation techniques

- Electrospray Ionisation (ESI)
- Atmospheric Pressure Chemical Ionisation (APCI)
- Atmospheric Pressure Photo Ionisation (APPI)
- Atmospheric Pressure Laser Ionisation (APLI)
- Matrix-assisted Laser Desorption Ionisation (MALDI)

2.1.2 Measurement modes

- Direct Infusion
- Chromatographic/ion mobility separation
- Gas chromatography
- Liquid chromatography
- Ion Mobility
- Thermal separation/introduction
- Thermogravimetry
- Direct Inlet Probe
- Imaging

STANDARD MEASUREMENT PROCEDURES

3 GENERAL PROTOCOL STRUCTURE

For each protocol, the following main points (Introduction, Materials, etc.) need to be answered. The given categories under the main points are examples and can be extended or only partly chosen. They shall help to give the necessary information.

3.1 INTRODUCTION

3.1.1 Application field

Graphical illustration of the procedure and short description.

3.2 MATERIALS

3.2.1 Reagents

Needed chemicals (level of purity, vendor), also used gases

- ➔ In alphabetical order

3.2.2 Equipment

Used instruments, e.g. autosampler, chromatography, software, mass spectrometer, sampling equipment

- ➔ In alphabetical order

3.2.3 Reagent Setup

Dilution of standard mixtures, preparation of reagents before usage

3.2.4 Equipment Setup

Setup description (figure), Quality check

3.3 PROCEDURE

3.3.1 Sampling
3.3.2 Extraction
3.3.3 Derivatisation
3.3.4 Dilution
3.3.5 Cleaning
3.3.6 Measurement
3.3.7 ESI/APCI/APPI Injection Parameters
➔ as a table

3.3.8 MALDI Parameters
➔ as a table

3.3.9 Chromatographic Parameters
3.3.10 Ion Mobility Parameters
3.3.11 FT-ICR Mass spectrometry parameters

3.4 DATA HANDLING AND ANTICIPATED RESULTS

3.4.1 Calibration
3.4.2 Peak assignment
3.4.3 Deconvolution
3.4.4 Retention time Index
3.4.5 Drift Time
3.4.6 Calculated sum formulae
3.4.7 Identification of novel compounds and contaminants
3.4.8 Data visualization

STANDARD MEASUREMENT PROTOCOL,

4 DIRECT INFUSION (+)- AND (-)-ESI-FT-ICR MS FOR VACUUM GAS OILS (VGOS)

4.1 INTRODUCTION

4.1.1 Application field

Vacuum gas oils are high complex mixtures containing species with high molecular weight and a high content of heteroatoms, such as oxygen, sulphur and nitrogen or organometallic compounds. During catalytic hydrocracking, the activity of the conversion catalyst is decreased in the presence of heteroatoms and therefore, their removal is essential.

4.2 MATERIALS

4.2.1 Reagents

Chemicals

- Formic acid 98-100 % (Merck LiChropur for HPLC)
- Ammonia solution 25% (Merck LiChropur for HPLC)

Solvents

- Methanol (Romil-UpS Ultra Gradient for HPLC-MS,>99.9%)
- Toluene (Sigma−Aldrich Chromasolv Plus for HPLC,≥99.9%)

Gases

- Nitrogen 5.0 (for FT ICR MS)

4.2.2 Equipment

Instruments

- FT-ICR MS (12T Solarix XR with para cell, Bruker Daltonics GmbH)

Consumables

- Glass vials, with screw caps (1.5 ml, BGB analytics)
- Eppendorf pipettes, adjustable (1000 µl, 100 µl, 10 µl)
- Glass syringe for ESI (250 µl, blunt needle, Hamilton)

4.2.3 Equipment Setup

For the ESI measurements, a commercial ESI ion source (Bruker Daltonics GmbH) is used. The general set-up can be abstracted from Figure 1.

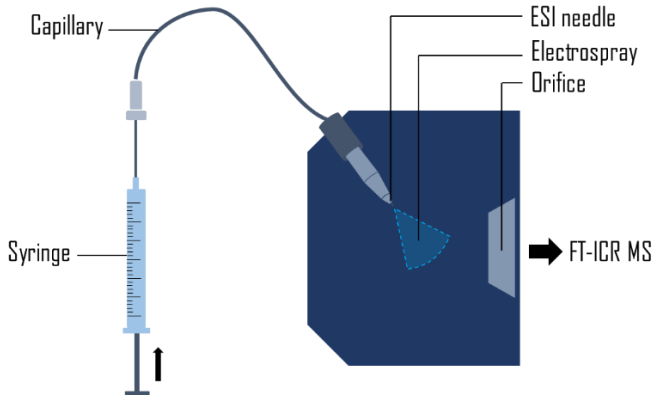

Figure 1: Schematic ESI setup for direct infusion measurements.

4.3 PROCEDURE

Besides the sample preparation, it is recommended to make the same preparation with a blank sample for blank value correction or for removing contaminants. Record a blank spectrum prior each sample introduction.

4.3.1 Dilution

1) Dissolve the vacuum gas oil in toluene.
2) Dilute the dissolved sample further with methanol/toluene (50:50 v/v) to a final concentration of 0.5 mg/ml
3) Add 1 volume-% of formic acid for acidification to the sample for positive ESI measurements and 3 volume-% of ammonia for negative ESI measurements.

4.3.2 Measurement

4) For direct infusion ESI, fill approximately 200 µl of the diluted extract into the syringe. Be careful, that there are no air bubbles left in the syringe. The measurement parameters are given below.

4.3.3 Cleaning

5) After each measurement, it is necessary to clean the ESI syringe and capillary. Therefore, flush the syringe with methanol.
6) Subsequent, flush the capillary several times with methanol with the help of the cleaned syringe. If there are sticky contaminants, use different solvents in the order of polarity to prevent a clocking of the ESI needle.

4.3.4 ESI Injection Parameters

Positive mode

capillary voltage	-4500 V
dry gas flow	4 l/min
flow rate (syringe)	400 µl/h
nebulizer gas flow	0.5 l/min
source temperature	146 °C

Negative mode

capillary voltage	+4500 V

dry gas flow	4 l/min
flow rate (syringe)	400 µl/h
nebulizer gas flow	0.5 l/min
source temperature	150 °C

4.3.5 FT-ICR Mass Spectrometry Parameters

Positive mode

accumulation time	0.025 s
mass-to-charge ratio (m/z) range	147-1300 Da
mode	Broadband
octopole energy	350 V_{pp}
quadrupole collision energy	1200 V_{pp}
quadrupole lower cut-off	200
resolution	500 000-900 000 @m/z400
spectra number	400
transient length	3.4 s *
Time-of-flight	0.8 ms

*For a 12 T FT-ICR MS with para cell; for other magnetic field strength or ICR cells, these parameters have to be adjusted.

Negative mode

accumulation time	0.035 s
mass-to-charge ratio (m/z) range	147-1300 Da
mode	Broadband
octopole energy	350 V_{pp}
quadrupole collision energy	1200 V_{pp}
quadrupole lower cut-off	200
resolution	500 000-900 000 @m/z400
spectra number	400
transient length	3.4 s *
Time-of-flight	0.8 ms

*For a 12 T FT-ICR MS with para cell; for other magnetic field strength or ICR cells, these parameters have to be adjusted.

4.4 DATA HANDLING AND ANTICIPATED RESULTS

4.4.1 Calibration

The mass spectra can be externally m/z calibrated by standards from quality measurements. For higher mass accuracy, the mass spectra can be calibrated on internal homologues rows of the measured samples. Therefore, more than one homologues row should be used and the whole mass range should be covered. The spectra should be calibrated with a mass accuracy of 1 ppm.

4.4.2 Peak assignment

High mass accuracy and ultra-high resolving power enable the possibility to calculate sum formulae from the measured m/z values due to mass defect, but also other chemical-based

validation rules (H/C ratio, homologues rows, etc.). For calculation, we recommend to limit the assignment boundaries as following: Signal-to-noise ratio above 6, even electron configuration, a double bond equivalent of -1.5 to 30, a mass accuracy of 0.2-1 ppm and sum formula parameters of $C_{6\text{-}50}H_{4\text{-}100}N_{0\text{-}2}O_{0\text{-}2}S_{0\text{-}2}$ (positive mode), and of $C_{6\text{-}50}H_{4\text{-}100}N_{0\text{-}2}O_{0\text{-}4}S_{0\text{-}1}$. For positive ion mode, 0-1 Na atom can be considered due to possible adduct formation.

4.4.3 Kendrick mass defect

The Kendrick mass defect is a retransformation of the mass scale, typical based on CH_2. It is applied in the fields of environmental science, proteomics, petroleomics, metabolomics, polymer science, etc. The transformation enables the alignment of homologues rows as a horizontal line. The Kendrick mass for CH_2 and the Kendrick mass defect (KMD) are calculated as follows:

$$Kedrick\ mass = IUPAC\ mass\ \frac{14.00000}{14.01565}$$

$$Kendrick\ mass\ defect = nominal\ mass - Kendrick\ mass$$

A Kendrick Plot (KMD vs. nominal mass) allows an estimation of the alkylation degree of the present species.

4.4.4 Calculated sum formulae

Direct infusion ESI measurements result in a large number of calculated sum formulae, which are in the field of Petroleomics most often too complex to be investigated manually. A variety of data visualisation techniques may help to investigate differences between different sample sets. Different compound classes can be grouped as bar plots and compared regarding the summed intensity.

4.4.5 H/C ratio and van Krevelen plots

Van Krevelen plots are plotted H/C vs. a heteroatom/C value. For Petroleomics, mostly O, S and N are interesting as heteroatom/C ratios. The plots give an evidence for aromaticity, alkylation and separates different heteroatom-containing classes. For example, the H/C value of the aromatic benzene is 1, whereas linear alkanes have a H/C value above 2.

4.4.6 Double bond equivalent

The double bond (DBE) equivalent gives the number of rings and double bonds present in a molecule and can be seen as a measure for the level of unsaturation and aromaticity. The DBE is calculated as follows:

$$DBE = C + 1 - \frac{H}{2} - \frac{X}{2} + \frac{N}{2}$$

C is the number of Carbon atoms present, *H* is the number of Hydrogen atoms, *X* is the number of halogen atoms (Cl, Br, I, F), N is the number of Nitrogen atoms present. For example, benzene has a DBE value of 4, consisting of three double bonds and one ring. DBE vs. #C plots can give evidence on the aromatic distribution and alkylation/size of the molecules present.

STANDARD MEASUREMENT PROTOCOL

5 DIRECT INFUSION APPI- AND APCI-FT-ICR MS FOR CRUDE OIL-DERIVED SAMPLES

5.1 INTRODUCTION

5.1.1 Application field

Atmospheric pressure chemical ionization (APCI) is a gas phase ionization technique, which is able to ionize polar to semi-polar species. In APCI, especially oxygen-containing molecules are pronounced. Atmospheric pressure photo ionisation (APPI) is a gas phase ionisation technique which is able to ionize semi-polar species and pronounces preferentially sulphur-containing and aromatic species.

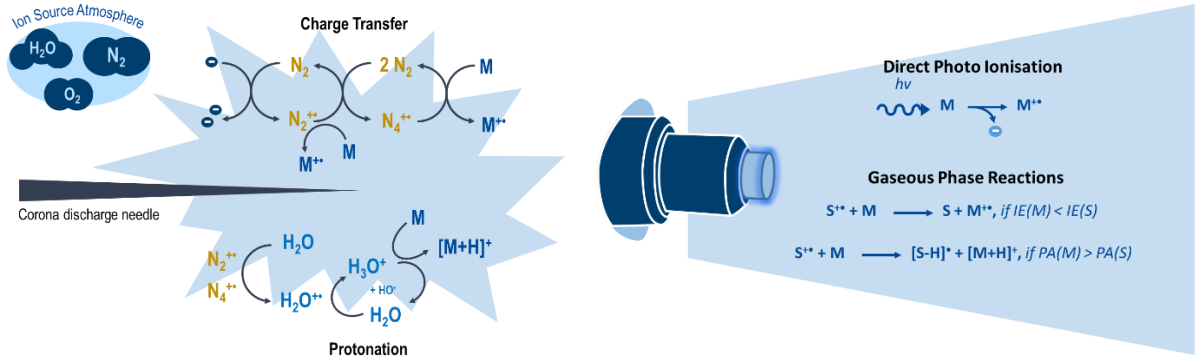

Figure 2: Schematic operating principle of the APCI and APPI ionisation mechanisms

5.2 MATERIALS

5.2.1 Reagents

Solvents

- Methanol (Romil-UpS Ultra Gradient for HPLC-MS,>99.9%)
- Toluene (Sigma−Aldrich Chromasolv Plus for HPLC,≥99.9%)

Gases

- Nitrogen 5.0 (for FT ICR MS)

5.2.2 Equipment

Instruments

- FT-ICR MS (12T Solarix XR with para cell, Bruker Daltonics GmbH)

Consumables

- Glass vials, with screw caps (1.5 ml, BGB analytics)

- Eppendorf pipettes, adjustable (1000 µl, 100 µl, 10 µl)
- Glass syringe for sample introduction (250 µl, blunt needle, Hamilton)

5.2.3 Equipment Setup

For the APCI measurements, a commercial APCI ion source (Bruker Daltonics GmbH) with vaporizer is used. The general set-up can be abstracted from Figure 1 a). For APPI measurements, an additional Krypton discharge lamp with 10.0 and 10.6 eV is needed. The general set-up is shown in Figure 2 b).

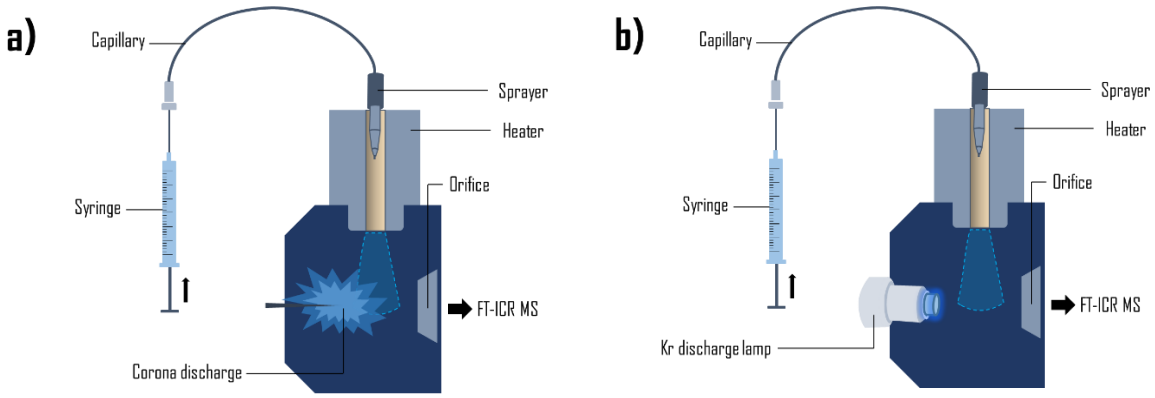

Figure 3: Schematic ESI setup for direct infusion measurements.

5.3 PROCEDURE

Besides the sample preparation, it is recommended to make the same preparation with a blank sample for blank value correction or for removing contaminants. Record a Blank spectrum prior each sample introduction.

5.3.1 Dilution

7) Dissolve the petroleum-derived sample in toluene.
8) Dilute the dissolved sample further with methanol/toluene (50:50 v/v) to a final concentration of 0.5 mg/ml

5.3.2 Measurement

9) For direct infusion APCI or APPI, fill approximately 200 µl of the diluted extract into the syringe. Be careful, that there are no air bubbles left in the syringe. The measurement parameters are given below.
10) Before starting the measurement, be sure that the vaporizer reached the desired temperature.

5.3.3 Cleaning

11) After each measurement, it is necessary to clean the syringe and capillary. Therefore, flush the syringe with methanol.

12) Subsequent, flush the capillary several times with methanol with the help of the cleaned syringe. If there are sticky contaminants, use different solvents in the order of polarity to prevent a clocking of the sprayer needle.

5.3.4 APCI Injection Parameters

capillary voltage	-4000 V
corona needle	9000 nA
dry gas flow	3 l/min
flow rate (syringe)	600 µl/h
nebulizer gas flow	2.5 l/min
source temperature	220 °C
vaporizer temperature	300 °C

5.3.5 APPI Injection Parameters

capillary voltage	-900 V
dry gas flow	3 l/min
flow rate (syringe)	600 µl/h
nebulizer gas flow	2.5 l/min
source temperature	220 °C
vaporizer temperature	300 °C

5.3.6 FT-ICR Mass Spectrometry Parameters

5.3.6.1Positive mode

accumulation time	0.025 s
mass-to-charge ratio (m/z) range	147-1300 Da
mode	broadband
octopole energy	350 V_{pp}
quadrupole collision energy	1200 V_{pp}
quadrupole lower cut-off	200
resolution	300 000 – 900 000 @ m/z400 (depending on sample)
spectra number	150
transient length	3.4 s * (depending on sample)
Time-of-flight	0.8 ms

*For a 12 T FT-ICR MS with para cell; for other magnetic field strength or ICR cells, these parameters have to be adjusted.

5.4 DATA HANDLING AND ANTICIPATED RESULTS

5.4.1 Calibration

The mass spectra can be externally m/z calibrated by standards from quality measurements. For higher mass accuracy, the mass spectra can be calibrated on internal homologues rows of the measured samples. Therefore, more than one homologues row should be used and

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The double bond (DBE) equivalent gives the number of rings and double bonds present in a molecule and can be seen as a measure for the level of unsaturation and aromaticity. The DBE is calculated as follows:

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C is the number of Carbon atoms present, *H* is the number of Hydrogen atoms, *X* is the number of halogen atoms (Cl, Br, I, F), N is the number of Nitrogen atoms present. For example, benzene has a DBE value of 4, consisting of three double bonds and one ring. DBE vs. #C plots can give evidence on the aromatic distribution and alkylation/size of the molecules present.

STANDARD MEASUREMENT PROTOCOL

6 GC-APCI-FT-ICR MS FOR POLAR AND SEMI-POLAR SPECIES IN DIESEL SAMPLES AND SIMILAR DISTILLATION CUTS

6.1 INTRODUCTION

6.1.1 Application field

Polar species in diesel contribute significantly to the fuels' physical properties, such as lubricity and stability and are therefore important to analyse.

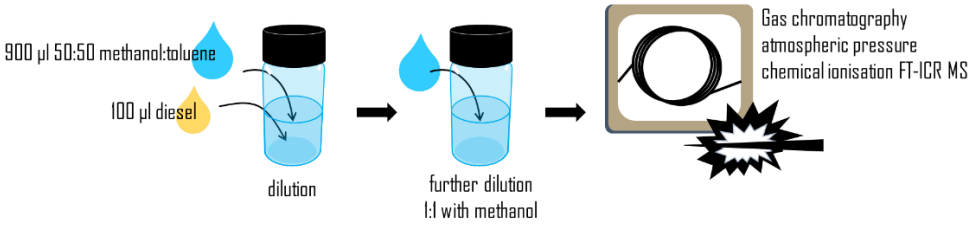

Figure 4: Graphical illustration of the preparation procedure. For GC experiments, only two dilution steps are necessary. 1) Dilution of the sample 1:10 in methanol:toluene mixture (50:50) 2)Dilution of the mixture 1:1 in methanol 3) GC-APCI-FT-ICR MS measurements.

6.2 MATERIALS

6.2.1 Reagents

Solvents

- Methanol (Romil-UpS Ultra Gradient for HPLC-MS,>99.9%)
- Toluene (Sigma−Aldrich Chromasolv Plus for HPLC,≥99.9%)

Gases

- Nitrogen 5.0 (for FT ICR MS)
- Helium 5.0 (carrier gas for GC)

6.2.2 Equipment

Instruments

- 7T FT-ICR MS and software (APEX Qe Series II with infinity cell, Bruker Daltonics GmbH and Bruker Compass Data Analysis 4.0 SP 5 software package, Bruker Daltonics GmbH)
- Gas chromatography (Model CP 3800, Varian Technologies) equipped with a programmed temperature vaporizing injector

Consumables

- Eppendorf pipettes, adjustable (1000 µl, 100 µl, 10 µl)
- GC column (BPX5, SGE Analytical Science, Australia)
- Glass microvials for ChromatoProbe (Agilent Technologies, 8010-0419)
- Glass syringe (10 µl, conical needle, Hamilton)
- Glass vials, with screw caps (1.5 ml, BGB analytics)

6.2.3 Equipment Setup

For the GC-APCI-FT-ICR MS measurements, a gas chromatograph (Model CP 3800, Varian) is connected via the commercial GC-APCI II source (Bruker Daltonics GmbH) to the 7 T FT-ICR MS (Bruker Daltonics GmbH). The chromatographically separated analytes are transferred into the ion source via a heated transferline (300 °C) and then ionised with atmospheric pressure chemical ionisation (APCI).

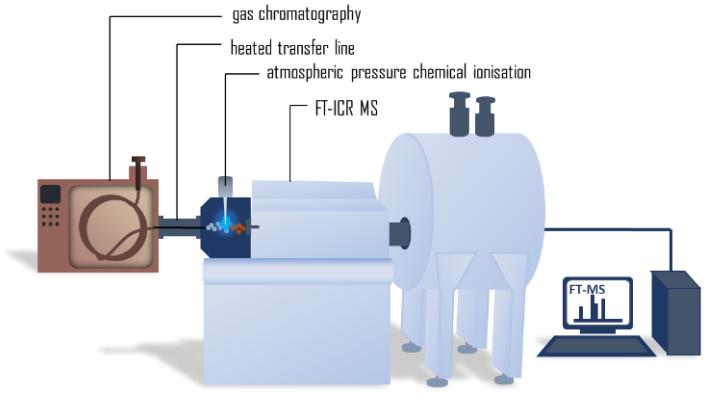

Figure 5: Schematic GC-APCI-FT-ICR MS setup.

6.3 PROCEDURE

Besides the sample preparation, it is recommended to make the same preparation with a blank sample for blank value correction or for removing contaminants.

6.3.1 Dilution

13) Dilute the neat fuel 1:10 (v/v) with a mixture of toluene and methanol (50:50).
14) Dilute the previous obtained mixture 1:1 with methanol.

6.3.2 Measurement

15) For GC-APCI-FT-ICR MS, fill 1 µl of the diluted diesel into the syringe and insert the sample into the microvial. Insert the vial with the ChromatoProbe in the injector. The measurement parameters are given below. The total measurement time is about 52 minutes.

6.3.3 GC Parameters

The method is optimized to also address higher boiling point species.

Carrier gas	Helium 5.0
Column	BPX5, 15 m, 250 µm inner diameter, 0.10 µm film
Flow rate	10 ml/min
GC oven program	50 °C (5min) → 5 K/min → 200 °C → 10 K/min → 250 °C → 20 K/min → 330 (10 min)
Injector oven program	50 °C (1 min) → 10 K/min → 80 °C → 60 K/min → 320 °C (20 min)
Transferline	300 °C
Split ratio	1:2 – 1:5

6.3.4 APCI Parameters

capillary voltage	+ 3 kV
Collision induced dissociation (in-source)	30 V
Corona needle	3000 nA
dry gas flow	2-3 l/min
dry gas temperature	230-250 °C
nebulizer gas flow	2-3.5 l/min

6.3.5 FT-ICR Mass Spectrometry Parameters

Chromatography mode	enable
mass-to-charge ratio (m/z) range	120-1000 Da
resolution	340 000 @m/z 200 *
spectra number	1
transient length	1.15 s *
Time-of-flight	0.6-0.7 ms

*For a 7 T FT-ICR MS with infinity cell; for other magnetic field strength or ICR cells, these parameters have to be adjusted.

6.4 DATA HANDLING AND ANTICIPATED RESULTS

6.4.1 Calibration

For high mass accuracy, the mass spectra can be calibrated on internal homologues rows of the measured samples. Therefore, more than one homologues row should be used and the whole mass range should be covered. The spectra should be calibrated with a mass accuracy of 1 ppm.

6.4.2 Peak assignment

High mass accuracy and ultra-high resolving power enable the possibility to calculate sum formulae from the measured m/z values due to mass defect, but also other chemical-based validation rules (H/C ratio, homologues rows, etc.). For calculation, we recommend to limit the assignment boundaries as following: Signal-to-noise ratio above 6, even and odd electron configuration, a double bond equivalent of -1.5 to 30, a mass accuracy of 1 ppm and sum formula parameters of $C_{6\text{-}50}H_{4\text{-}100}N_{0\text{-}2}O_{0\text{-}5}S_{0\text{-}3}$. The GC mode enables besides the analysis of the summed spectra over the analysis time also the investigation of the time resolved measurements. The peak annotation can be done in Bruker Data Analysis or with self-written scripting.

6.4.3 Retention index and structure assignment with data bases

The retention index (RI) and the exact mass are necessary to assign structures from data bases to the found compounds in the sample. Therefore, a retention index calibration is necessary for each spectrum. The retention index can be calibrated by an added internal standard or on sample internal species, for example, core structure poly cyclic aromatics which are normally present in petroleum-derived samples. For the calibration, assign a fixed retention times to the internal markers. Calculate the retention times for all other compounds

eluting between two standards. Be careful, because the described GC method changes the heating ramp in between which causes a non-linear shape for the retention index calibration.

6.4.4 Kendrick mass defect

The Kendrick mass defect is a retransformation of the mass scale, typical based on CH_2. It is applied in the fields of environmental science, proteomics, petroleomics, metabolomics, polymer science, etc. The transformation enables the alignment of homologues rows as a horizontal line. The Kendrick mass for CH_2 and the Kendrick mass defect (KMD) are calculated as follows:

$$Kedrick\ mass = IUPAC\ mass\ \frac{14.00000}{14.01565}$$

$$Kendrick\ mass\ defect = nominal\ mass - Kendrick\ mass$$

A Kendrick Plot (KMD vs. nominal mass) allows an estimation of the alkylation degree of the present species.

6.4.5 Calculated sum formulae

Direct infusion ESI measurements result in a large number of calculated sum formulae, which are in the field of Petroleomics most often too complex to be investigated manually. A variety of data visualisation techniques may help to investigate differences between different sample sets. Different compound classes can be grouped as bar plots and compared regarding the summed intensity.

6.4.6 H/C ratio and van Krevelen plots

Van Krevelen plots are plotted H/C vs. a heteroatom/C value. For Petroleomics, mostly O, S and N are interesting as heteroatom/C ratios. The plots give an evidence for aromaticity, alkylation and separates different heteroatom-containing classes. For example, the H/C value of the aromatic benzene is 1, whereas linear alkanes have a H/C value above 2.

6.4.7 Double bond equivalent

The double bond (DBE) equivalent gives the number of rings and double bonds present in a molecule and can be seen as a measure for the level of unsaturation and aromaticity. The DBE is calculated as follows:

$$DBE = C + 1 - \frac{H}{2} - \frac{X}{2} + \frac{N}{2}$$

C is the number of Carbon atoms present, *H* is the number of Hydrogen atoms, *X* is the number of halogen atoms (Cl, Br, I, F), N is the number of Nitrogen atoms present. For example, benzene has a DBE value of 4, consisting of three double bonds and one ring. DBE vs. #C plots can give evidence on the aromatic distribution and alkylation/size of the molecules present.

STANDARD MEASUREMENT PROTOCOL

7 DIRECT INFUSION ESI FT-ICR MS FOR TRACE POLAR SPECIES IN DIESEL SAMPLES

7.1 INTRODUCTION

7.1.1 Application field

Polar species in diesel contribute significantly to the fuels' physical properties, such as lubricity and stability and are therefore important to analyse.

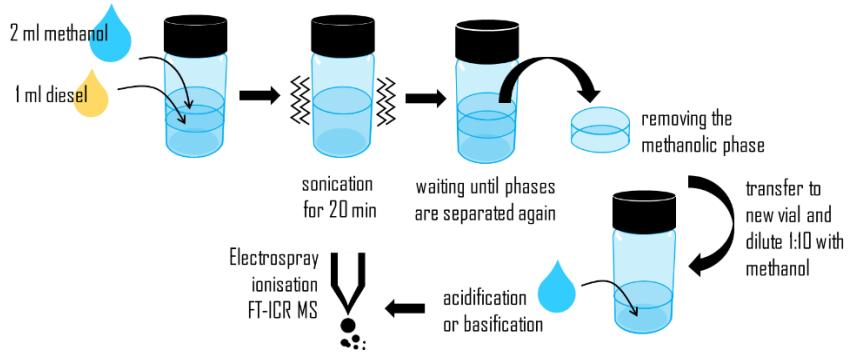

Figure 6: Graphical illustration of the preparation procedure. 1) Methanolic extraction of the fuel. 2) Sonication of the extraction mixture. 3) Remove of the methanolic phase. 4) Dilution of the extract 1:10 with methanol. 5) Adding of 1 volume-% formic acid/ammonia solution. 6) ESI positive/negative measurement.

7.2 MATERIALS

7.2.1 Reagents

Chemicals

- Arginine in methanolic solution (2 mg/ml)
- Ammonia solution 25% (Merck LiChropur for HPLC)
- Formic acid 98-100 % (Merck LiChropur for HPLC)

Solvents

- Methanol (Romil-UpS Ultra Gradient for HPLC-MS,>99.9%)

Gases

- Nitrogen 5.0 (for FT ICR MS)

7.2.2 Equipment

Instruments

- FT-ICR MS and software (7T Solarix with infinity cell, Bruker Daltonics GmbH and Bruker Compass Data Analysis 4.0 SP 5 software package, Bruker Daltonics GmbH)

Consumables

- Glass vials, with screw caps (8 ml, BGB analytics)
- Glass vials, with screw caps (1.5 ml, BGB analytics)

- Eppendorf pipettes, adjustable (1000 µl, 100 µl, 10 µl)
- Glass syringe for ESI (250 µl, blunt needle, Hamilton)

7.2.3 Reagent Setup

Dilute the arginine solution (2 mg/ml) 1:10 with methanol to obtain an arginine solution of 0.2 mg/ml for quality check and pre-calibration of the instrument.

7.2.4 Equipment Setup

For the ESI measurements, a commercial ESI ion source (Bruker Daltonics GmbH) is used. The general set-up can be abstracted from Figure 1.

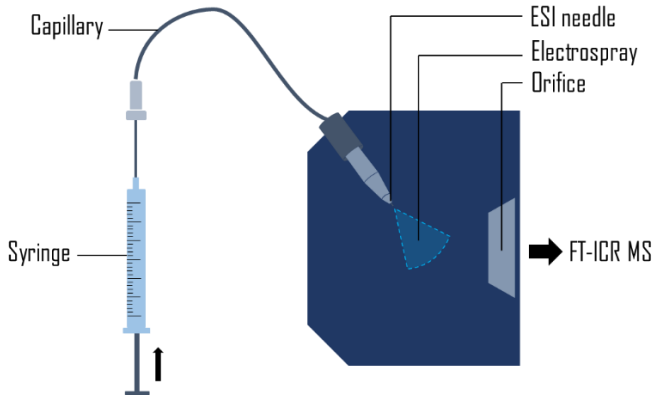

Figure 7: Schematic ESI setup for direct infusion measurements.

For pre-calibration of the instrument, take 200 µl of the diluted arginine mixture with the glass syringe. The parameters for pre-calibration should be the similar to the parameters used for the measurements and can be therefore taken from the section “procedure”. The pre-calibration should be repeated every day before the measurements.

7.3 PROCEDURE

Besides the sample preparation, it is recommended to make the same preparation with a blank sample for blank value correction or for removing contaminants.

7.3.1 Extraction

16) Extract the polar fraction by mixing 1 ml of diesel fuel with 2 ml of methanol in a 8 ml glass vial and seal the vial with a screw cap.
17) Sonicate the mixture for 20 min and wait afterwards until the phases are separated again.
18) Remove the methanol phase (top layer) for further dilution.

7.3.2 Dilution

19) Dilute the methanolic phase from the extraction 1:10 with methanol in smaller 1.5 ml vials. Set at least to dilutions, one for positive ESI and one for negative ESI.
20) Add 1 volume-% of formic acid for acidification to the extract for positive ESI measurements and 1 volume-% of ammonia for negative ESI measurements.

7.3.3 Measurement

21) For direct infusion ESI, fill approximately 200 µl of the diluted extract into the syringe. Be careful, that there are no air bubbles left in the syringe. The measurement parameters are given below. The total analysis time amounts to 2.5 min.

7.3.4 Cleaning

22) After each measurement, it is necessary to clean the ESI syringe and capillary. Therefore, flush the syringe with methanol.

23) Subsequent, flush the capillary several times with methanol with the help of the cleaned syringe. If there are sticky contaminants, use different solvents in the order of polarity to prevent a clocking of the ESI needle.

7.3.5 ESI Injection Parameters

Positive mode

collision induced dissociation	45 V
dry gas flow	adjust for stable electrospray
flow rate (syringe)	2.5 µl/min
nebulizer gas flow	adjust for stable electrospray
spray voltage	+4 kV

Negative mode

collision induced dissociation	45 V
dry gas flow	adjust for stable electrospray
flow rate (syringe)	5 µl/min
nebulizer gas flow	adjust for stable electrospray
spray voltage	-3 kV

7.3.6 FT-ICR Mass Spectrometry Parameters

mass-to-charge ratio (m/z) range	100-1000 Da
resolution	150 000 @m/z 200 *
spectra number	200
transient length	0.490 s *
Time-of-flight	0.6-0.7 ms

*For a 7 T FT-ICR MS with infinity cell; for other magnetic field strength or ICR cells, these parameters have to be adjusted.

7.4 DATA HANDLING AND ANTICIPATED RESULTS

7.4.1 Calibration

The mass spectra can be externally m/z calibrated by arginine and its oligomeric clusters from quality measurements. For higher mass accuracy, the mass spectra can be calibrated on internal homologues rows of the measured samples. Therefore, more than one homologues row should be used and the whole mass range should be covered. The spectra should be calibrated with a mass accuracy of 1 ppm.

7.4.2 Peak assignment

High mass accuracy and ultra-high resolving power enable the possibility to calculate sum formulae from the measured m/z values due to mass defect, but also other chemical-based validation rules (H/C ratio, homologues rows, etc.). For calculation, we recommend to limit the assignment boundaries as following: Signal-to-noise ratio above 6, even electron configuration, a double bond equivalent of -1.5 to 30, a mass accuracy of 1 ppm and sum formula parameters of $C_{6\text{-}50}H_{4\text{-}100}N_{0\text{-}2}O_{0\text{-}5}S_{0\text{-}3}$. For positive ion mode, 0-1 Na atom can be considered due to possible adduct formation.

7.4.3 Kendrick mass defect

The Kendrick mass defect is a retransformation of the mass scale, typical based on CH_2. It is applied in the fields of environmental science, proteomics, petroleomics, metabolomics, polymer science, etc. The transformation enables the alignment of homologues rows as a horizontal line. The Kendrick mass for CH_2 and the Kendrick mass defect (KMD) are calculated as follows:

$$Kedrick\ mass = IUPAC\ mass\ \frac{14.00000}{14.01565}$$

$$Kendrick\ mass\ defect = nominal\ mass - Kendrick\ mass$$

A Kendrick Plot (KMD vs. nominal mass) allows an estimation of the alkylation degree of the present species.

7.4.4 Calculated sum formulae

Direct infusion ESI measurements result in a large number of calculated sum formulae, which are in the field of Petroleomics most often too complex to be investigated manually. A variety of data visualisation techniques may help to investigate differences between different sample sets. Different compound classes can be grouped as bar plots and compared regarding the summed intensity.

7.4.5 H/C ratio and van Krevelen plots

Van Krevelen plots are plotted H/C vs. a heteroatom/C value. For Petroleomics, mostly O, S and N are interesting as heteroatom/C ratios. The plots give an evidence for aromaticity, alkylation and separates different heteroatom-containing classes. For example, the H/C value of the aromatic benzene is 1, whereas linear alkanes have a H/C value above 2.

7.4.6 Double bond equivalent

The double bond (DBE) equivalent gives the number of rings and double bonds present in a molecule and can be seen as a measure for the level of unsaturation and aromaticity. The DBE is calculated as follows:

$$DBE = C + 1 - \frac{H}{2} - \frac{X}{2} + \frac{N}{2}$$

C is the number of Carbon atoms present, *H* is the number of Hydrogen atoms, *X* is the number of halogen atoms (Cl, Br, I, F), N is the number of Nitrogen atoms present. For example, benzene has a DBE value of 4, consisting of three double bonds and one ring.

DBE vs. #C plots can give evidence on the aromatic distribution and alkylation/size of the molecules present.

STANDARD MEASUREMENT PROTOCOL

8 TG-APCI-FT-ICR MS FOR HEAVY PETROLEUM FRACTIONS SUCH AS ASPHALTENES AND BITUMEN

8.1 INTRODUCTION

8.1.1 Application field

Heavy petroleum fractions contains species with high molecular weight and a large number of heteroatoms such as nitrogen, oxygen or sulphur. Because of their enormous complexity, high boiling fractions are still an analytical challenge. Because of its high mass resolution and high mass accuracy, Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR MS) enables the identification of thousands of mass peaks in one spectrum. The coupling to thermogravimetry enables the temperature resolved analysis of the desorbable and pyrolysable material of the samples without prior sample treatment.

8.2 MATERIALS

8.2.1 Reagents

Standards

- Polystyrene (NIST standard with narrow molecular weight distribution, Merck)

Gases

- Nitrogen 5.0 (for FT ICR MS and for TG)

8.2.2 Equipment

Instruments

- 7T FT-ICR MS and software (APEX Qe Series II with infinity cell, Bruker Daltonics GmbH and Bruker Compass Data Analysis 4.0 SP 5 software package, Bruker Daltonics GmbH)
- Thermobalance (TG 209 cell, NETZSCH Gerätebau)

Consumables

- Aluminium crucibles (85 µl, up to 600 °C, Thepro GbR)
- Alumina crucibles (85 µl, up to 1000 °C, Thepro GbR)

8.2.3 Equipment Setup

For the TG-APCI-FT-ICR MS measurements, a thermobalance (Model TG 209 cell, NETZSCH Gerätebau) is connected via the commercial GC-APCI II source (Bruker Daltonics GmbH) and an additional heated transferline (300 °C) to the 7 T FT-ICR MS (Bruker Daltonics GmbH). The evaporated compounds are transferred into the ion source via a slight overpressure of 8 mbar over the heated transferline and then ionised with atmospheric pressure chemical ionisation (APCI). The TG oven atmosphere is flushed with

nitrogen during the measurements for avoiding oxidation and combustion of the material. Because of the comparatively slow evaporation of the material when using heating rates of 5 °C/min to 10 °C/min, alternating MSMS experiments can be conducted to get a reflection of the core structures of the previous spectrum.

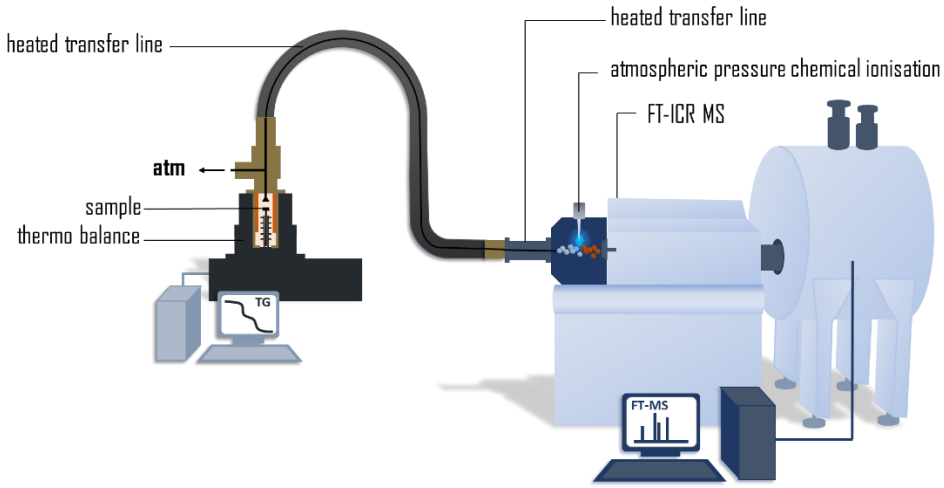

Figure 8: Schematic TG-APCI-FT-ICR MS setup.

8.3 PROCEDURE

In the beginning of each measurement day, it is recommended to measure a polystyrene standard with the same settings as for the sample measurement to test the sensitivity of the set-up. One measurement needs to be conducted with an empty crucible for uplift correction of the TG curve.

8.3.1 Measurement

24) Weigh the crucible before filling in the sample material for determine the residual mass after the measurement.
25) Fill in approximately 1 mg of sample for bitumen or 0.5 mg for asphaltenes. Weigh the crucible again to get the exact mass.
26) Place the crucible carefully inside the TG oven on the sample holder and close the TG oven. Shortly wait until the sample is in thermal equilibrium.
27) Start the measurement.
28) After the measurement, wait until the oven has cooled down. Weigh again the crucible for determine the residual mass.

8.3.2 TG Parameters

Carrier gas	Nitrogen 5.0
Overpressure	8 mbar
Temperature Program	for Bitumen: 20 °C (2 min) → 10 K/min → 600 °C (10 min) for Asphaltenes: 20 °C (2 min) → 5 K/min → 600 °C (10 min)
Transferline	deactivated fused silica capillary, 300 °C

8.3.3 APCI Parameters

capillary voltage	- 3.5 kV
Corona needle	3000 nA
dry gas flow	2 l/min
dry gas temperature	220 °C
nebulizer gas flow	3 l/min

8.3.4 FT-ICR Mass Spectrometry Parameters

Accumulation time	0.1 s (MS), 1 s (MSMS)
Chromatography mode	enabled
CID voltage (MSMS)	30 V
mass-to-charge ratio (m/z) range	100-1000 Da
resolution	290 000 @m/z 400 *
spectra number	5
transient length	2 s *
Time-of-flight	0.7 ms

*For a 7 T FT-ICR MS with infinity cell; for other magnetic field strength or ICR cells, these parameters have to be adjusted.

8.4 DATA HANDLING AND ANTICIPATED RESULTS

8.4.1 Calibration

For high mass accuracy, the mass spectra can be calibrated on internal homologues rows of the measured samples. Therefore, more than one homologues row should be used and the whole mass range should be covered. The spectra should be calibrated with a mass accuracy of 1 ppm. For first calibration in DataAnalysis, the spectrum can be summed over the time frame, in which sample signals occur. Calibrate the summed spectrum and recalculate each line spectrum. Each line spectrum can be further recalculated after export with self-written routines.

8.4.2 Peak assignment

High mass accuracy and ultra-high resolving power enable the possibility to calculate sum formulae from the measured m/z values due to mass defect, but also other chemical-based validation rules (H/C ratio, homologues rows, etc.). For calculation, we recommend to limit the assignment boundaries as following: Signal-to-noise ratio above 6, even and odd electron configuration, a double bond equivalent of 0 to 30, a mass accuracy of 1 ppm and sum formula parameters of $C_{4\text{-}100}H_{4\text{-}200}N_{0\text{-}2}O_{0\text{-}5}S_{0\text{-}3}$. The TG mode enables besides the analysis of the summed spectra over the analysis time also the investigation of the temperature resolved measurements. The peak annotation can be done in Bruker Data Analysis or with self-written scripting.

8.4.3 TG curves

The TG curves show the mass loss of the sample with increasing temperature. Different stages of evaporation or pyrolysis can be extracted from the data. An uplift correction is necessary due to the changing viscosity properties of the nitrogen carrier gas with increasing temperature.

8.4.4 Kendrick mass defect

The Kendrick mass defect is a retransformation of the mass scale, typical based on CH_2. It is applied in the fields of environmental science, proteomics, petroleomics, metabolomics, polymer science, etc. The transformation enables the alignment of homologues rows as a horizontal line. The Kendrick mass for CH_2 and the Kendrick mass defect (KMD) are calculated as follows:

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A Kendrick Plot (KMD vs. nominal mass) allows an estimation of the alkylation degree of the present species.

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Van Krevelen plots are plotted H/C vs. a heteroatom/C value. For Petroleomics, mostly O, S and N are interesting as heteroatom/C ratios. The plots give an evidence for aromaticity, alkylation and separates different heteroatom-containing classes. For example, the H/C value of the aromatic benzene is 1, whereas linear alkanes have a H/C value above 2.

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The double bond (DBE) equivalent gives the number of rings and double bonds present in a molecule and can be seen as a measure for the level of unsaturation and aromaticity. The DBE is calculated as follows:

$$DBE = C + 1 - \frac{H}{2} - \frac{X}{2} + \frac{N}{2}$$

C is the number of Carbon atoms present, *H* is the number of Hydrogen atoms, *X* is the number of halogen atoms (Cl, Br, I, F), N is the number of Nitrogen atoms present. For example, benzene has a DBE value of 4, consisting of three double bonds and one ring. DBE vs. #C plots can give evidence on the aromatic distribution and alkylation/size of the molecules present.