NETCHEMA Remote Access Laboratory Guide

GC-MS analysis of petroleum pollutants
Gordana Gajica

In this exercise, you will:

✓ Become familiar with the analysis of petroleum and petroleum pollutants in real samples
✓ Learn about basic principles of gas chromatography mass spectrometry GG-MS
✓ Perform GC-MS analysis of petroleum and petroleum pollutants in real samples
✓ Gain experience in data analysis of obtained GC-MS chromatograms (identification of biomarkers, calculation of their parameters, determination type of petroleum pollutants based on distribution and abundance of biomarkers)
Background

Fuels, lubricants, polymers, asphalt and organic solvents are only a part of about 3000 products obtained by petroleum processing.

During the exploration of oil, transportation, storage, the production of petroleum products and their use pollution of oil and its derivatives can occur in the environment.

Petroleum and petroleum pollutants in the environment are subject to numerous physical, chemical and biological transformations.

Petroleum represents a migrated and accumulated product of the diagnostic and catagenetic transformations of the organic matter of sedimentary rocks. Since petroleum is one of the furthermost forms in the organic carbon cycle in the geosphere, petroleum can be defined as the most maturated form of organic matter of the geosphere.

The composition and characteristics of the petroleum depend on the origin of biomass, the conditions of depositional environment, the degree of thermal maturity, the length of the migration path, the lithological composition of the source and reservoir rocks, biodegradation, water rinsing and deasphalting.
Background

Petroleum is a mixture of a large number of hydrocarbons (90-95%), and nitrogen, sulfur and oxygen (NSO) compounds (5-10%).

According to the size of the molecules, the petroleum constituents can be classified into three fractions:

- fraction of easily volatile compounds,
- fraction of maltene (saturated, aromatic hydrocarbons and NSO-compounds),
- asphaltene fraction.

Due to different chemical properties of petroleum, four fractions of organic compounds can be isolated:

- saturated hydrocarbons (*n*-alkanes, isoprenoid alkanes, branched hydrocarbons, alicyclic alkanes including steranes and terpanes)
- aromatic hydrocarbons (mono-, di-, tri- and polycyclic hydrocarbons, naphthenoaromatic HC, small molecules with sulphur)
- NSO-compounds (porphyrins, fatty acids, alcohols, aliphatic and cyclic ketones, sulfuric aliphatic and aromatic compounds)
- asphaltene (condensed aromatic compounds with a large number of heteroatoms).
Background

Biomarkers are compounds that are identical in structure or close to compounds known to exist in the biosphere. Although the concentration of biomarkers in the sediment is relatively small, their different types and structures provide invaluable information on the origin, maturation and depositional environment of the organic matter.

These compounds have great application in organic-geochemical studies. Biomarkers are mostly used for assessing the origin and degree of maturation of bitumen. Furthermore, biomarkers can be reliably used to determine the source, nature and type of petroleum pollutants, as well as to distinguish petroleum pollutants from other already present hydrocarbons. Also, they can be used for monitoring the behavior of pollutants of the petroleum type in the environment over time.

For the analysis of biomarkers in petroleum it is necessary to do separate bitumen into saturated HC, aromatic HC and NSO-compounds.

For the analysis of petroleum pollutants firstly it is necessary to isolate soluble form of OM (bitumen) by some type of extraction and then separate into saturated HC, aromatic HC and NSO-compounds.

Gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS), gas hromatography-tandem mass spectrometry (GC-MS/MS) are used for the analysis of biomarkers.
Analytical procedure for the analysis of petroleum and petroleum pollutants in water and sediment samples

**Extraction of organic matter**

Water samples → Sediment samples

**Clean up of extracted organic matter and petroleum samples**

Column chromatography → Saturated fraction, Aromatic fraction, NSC fraction

**Instrumental analysis**

**Data analysis**

* petroleum samples don’t need extraction

This project has been funded with support from the European Commission. This publication reflects the views only of the authors, and the Commission cannot be held responsible for any use which may be made of the information contained therein.
Sample preparation

Liquid-liquid extraction of soluble organic matter from water samples

For the extraction of organic matter from the wastewater samples liquid-liquid extraction is used by chloroform.

Material

For this lab exercise, you will need the following material:

Accessories

- separatory funnel of 250 ml
- measuring cylinder of 50 mL
- measuring cylinder of 100 mL
- round-bottom flask of 250 ml
- pipette teats
- Pasteur glass pipette
- desiccator
- weighing bottles
- rotary evaporator

Reagents

- chloroform
- Na₂SO₄ anhydrous
**Procedure of liquid-liquid extraction**

1. Transfer the water samples into a separatory funnel.

2. For the extraction of 200 ml of water sample use 120 ml of chloroform.

Firstly, add 30 ml of the extraction solvent in a separatory funnel and shake. Then for a more efficient extraction use small volumes of extraction solvents (10 ml) several times. The extracts should be collected in the same round-bottom flask.

3. Remove the solvent from the extracts by rotary evaporator, and quantitatively transfer the residual in a weight bottle. The rest of the solvent remove in the nitrogen stream or at air stream at room temperature.

4. The difference in the weight of weighing bottles with the extract (m2) and the empty weighing bottles (m1) is the mass of the extracted soluble organic substance from the water sample (morg)
Soxhlet extraction of soluble organic matter from sediments samples

For the extraction of organic matter from the sediments samples is used Soxhlet extraction by azeotropic mixture of methylene-chloride and methanol (88:12, v/v).

**Material**

For this lab exercise, you will need the following material:

**Accessories**
- aperture for Soxhlet extraction
- measuring cylinder of 100 mL
- round-bottom flask of 250 ml
- pipette teats
- Pasteur glass pipette,
- desiccator
- weighing bottles
- cellulose extraction thimble
- rotary evaporator
- reagent spoon

**Reagents**
- methanol
- dichloromethane
Procedure of Soxhlet extraction

1. Measure 20 g of sediment samples on balance into a cellulose extraction thimble and put it in the Soxhlet extractor.

2. Add 125 ml of azeotropic mixture in a round-bottom flask.

3. Mount the Soxhlet extraction apparatus. Run the extraction for 36-42 hours.

4. After the completion of the extraction, remove the solvent from the extracts by rotary evaporator, and quantitatively transfer the residual in a weight bottle. The rest of the solvent remove in the nitrogen stream or at air stream at room temperature.

5. The difference in the weight of the weighing bottles with the extract (m2) and the empty weighing bottles (m1) is the mass of the extracted soluble organic substance from the water sample (morg).
Clean up of extracted organic matter and petroleum pollutants

The extracts of soluble organic matter (and petroleum samples) were separated into saturated, aromatic and NSO fractions using column chromatography over SiO2 and Al2O3.

**Material**

For this lab exercise, you will need the following material:

**Accessories**
- glass column with a teflon tap (about 1 cm in diameter and about 50 cm in length)
- measuring cylinder of 100 mL
- measuring cylinder of 50 mL
- measuring cylinder of 10 mL
- 3 x round-bottom flask of 250 ml
- cotton wool
- pipette teats
- Pasteur glass pipette,
- desiccator
- weighing bottles
- rotary evaporator

**Reagents**
- silica gel (Merck 60 Å, 70-230 mesh)
- aluminium oxide (Merck 90 Å, 70-230 mesh)
- petroleum ether
- benzene
- methanol
1. Fill the column by activated silica gel and aluminium oxide. For 10 mg of organic matter it is necessary to use 2.25 g of SiO2 and 1.65 g of Al2O3.

2. When column is filled add 30 mg of sample (petroleum or extract of soluble organic matter)

3. For the elution of fractions, the following solvents are used:

   - petroleum ether for the fraction of saturated hydrocarbons (for 10 mg of organic matter - 22.5 ml of petrol ether)

   - petroleum ether/benzene (2:1, v/v) for the fraction of aromatic hydrocarbons (for 10 mg of organic matter - 37.5 ml of petrol-ether/benzene)

   - chloroform/methanol (1:1, v/v) - for the fraction of the NSO-compound (for 10 mg of organic matter - 50 ml of mixture chloroform/methanol).

4. After the completion of the extraction, remove the solvent from the extracts by rotary evaporator, and quantitatively transfer the residual in a weight bottle. The rest of the solvent remove in the nitrogen stream or at air stream at room temperature.

5. The difference in the weight of weighing bottles with the extract (m2) and the empty weighing bottles (m1) is the mass of the fractions.
Instrumental of analysis of saturated fraction

Gas chromatography Mass spectrometry (GC-MS) technique

The saturated hydrocarbons of bitumen were analyzed by gas chromatography-mass spectrometry (GC-MS).

GC-MS was performed using an Agilent 7890A gas chromatograph coupled to an Agilent 5975C mass selective detector (70 eV).

The instrument that will be used in these exercises is shown in Figure below.
The numbers indicate the following parts of GC-MS instrument:

1. Gas (He)

**GC part** 2a. Injector
   
   2b. Autosampler

3. Column, which is placed in the oven

4. Transfer line – connects units of GC and MS

5. **MS part** – ion source (electron ionization, EI)
   
   - mass analyzer (quadrupole mass analyzer)
   
   - detector (electron multiplier)

6. Vacuum system - foreline pump (+ turbomolecular pump which is placed in the MS part)

7. Computer
1. In order to analyse the saturated fraction the first step is to set up the method for analysis. The parameters for GC and MS are listed below:

**GC parameters**

- Injection type – manual
- Injector temperature – 250 °C
- Mode – split, split ratio 5:1
- Column – HP5-MS column, 30m x 0.25 mm, 0.25 μm film thickness
- Temperature program – from 80 to 310 °C, at a rate of 2 °C/min, 310 °C was maintained for additional 25 min
- Carrier gas – He, flow 1.5 cm³/min

**MS parameters**

- Temperature of ion source – 250 °C
- Temperature of mass analyser – 250 °C
- EMV mode – Gain factor
- Gain factor – 1
- Solvent delay – 3.5 min
- Acquisition type – scan
- Mass range – 45-550
- Threshold – 150
2. Run the analysis on GC/MS.

Procedure explained in Lab manual that is on Moodle platform.

3. Data analysis - detailed analyses of the target compounds were conducted using the following ion chromatograms $m/z$ 71 (n-alkanes), $m/z$ 217 (steranes) and $m/z$ 191 (terpanes).

The individual peaks were identified in comparison with literature data and on the basis of the mass spectra (library: NIST5a). Biomarker parameters were calculated from GC-MS chromatogram peak areas (software GC-MS Data Analysis).

Procedure explained in Lab manual that is on Moodle platform.
## DESCRIPTION OF REMOTE ACCESS

### 1. NETCHEM COMMUNICATION SIDES
(NOTE: NETCHEM Communication is defined as event that involves all kinds of internet interactions (in real time and not in real time) between participants via devices (PCs, laptops, tablets and mobilephones))

<table>
<thead>
<tr>
<th>Side</th>
<th>Definition</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>host side</td>
<td>Host side of NETCHEM Communication is defined as PC who invites other users to join the session</td>
<td>participant’s PC in classroom</td>
</tr>
<tr>
<td>guest side</td>
<td>Guest side of NETCHEM Communication is defined as PC who joins the invitation to session</td>
<td>participant’s PC in laboratory</td>
</tr>
</tbody>
</table>

### 2. COMMUNICATION SOFTWARE

<table>
<thead>
<tr>
<th>Software</th>
<th>Meeting: No</th>
<th>Remote control: Yes</th>
<th>Meeting and Remote control simultaneously: No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Team Viewer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skype</td>
<td>Call 1:1: No</td>
<td>Conference Call: Yes</td>
<td></td>
</tr>
</tbody>
</table>

### 3. COMMUNICATION HARDWARE

<table>
<thead>
<tr>
<th>Side</th>
<th>Equipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>on host side</td>
<td>1 PC for each participant</td>
</tr>
<tr>
<td>on guest side</td>
<td>1 PC, 1 headsets with microphone, camera</td>
</tr>
</tbody>
</table>

### 4. INFORMATION EXCHANGE TYPE

<table>
<thead>
<tr>
<th>Type</th>
<th>Yes</th>
<th>Educational (one side is dominantly receptive)</th>
<th>Consultative (two sides are equal in giving-receiving information)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Place of Educator participant</td>
<td>guest side</td>
<td>Number of educator(s): 1</td>
<td></td>
</tr>
<tr>
<td>Place of student participant</td>
<td>host side</td>
<td>Number of student participant(s): 15</td>
<td></td>
</tr>
<tr>
<td>Number of host side participant(s)</td>
<td>No</td>
<td>Number of guest side participant(s): No</td>
<td></td>
</tr>
<tr>
<td>Number of guest side participant(s)</td>
<td>No</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Remote Access Connection Instructions

What makes these labs different and unique from other classroom experiments is that we have incorporated a section in each activity to remotely characterize your samples from your classroom. Request a remote lab session specifying information such as: the day, the time, and the instrument you are interested in using by visiting our web site:

http://netchem.ac.rs/remote-access

You will see the list of partners with the instruments provided to chose from. You will be contacted by a Remote Access staff member to set up a test run to ensure you are set up properly and have the required infrastructure.

Send samples or verify the in-house sample you would like us to prepare and load for characterization.

Send your samples to the Remote Access center that you chose on your request. There are two communications soft-ware packages, that will allow us to communicate instructions and answer questions during the session.

- **TeamViewer**: You can obtain a free download at:
- **Skype**

This project has been funded with support from the European Commission. This publication reflects the views only of the authors, and the Commission cannot be held responsible for any use which may be made of the information contained therein.
Remote Access Connection Instructions

You will need:

a) Computer with administrator access to install plug-ins and software
b) An internet connection
c) Speakers
d) Microphone
e) Projector connected to the same computer
f) Web browser (Firefox preferred)
g) Laptop

During the test run you can refer to this guide to perform the following steps, but it’s very important that you only proceed with these steps during your scheduled times. You may interfere with other remote sessions and potentially damage equipment if you log in at other times.

a) Open and logon to your Zoom/Team-viewer account. You will be given the access code to enter at the time of your test and then again during the remote session.
   - If you are using the Zoom software, Remote Access staff will give you the access code.
   - If you are using the Team-viewer software, Remote Access staff will give you the ID & password.

b) You should soon see the Remote Access desktop and at this point you can interact with the icons on the screen as if it were your desktop.

c) Switch to full screen mode by selecting the maximize screen option in the top right corner of the screen.

d) Upon completion of the session, move your mouse to the top right corner of the screen, and click on the X to disconnect the remote session. It will ask if you want to end the remote session. Click Yes.
This remote access laboratory was created thanks to work done primarily at University of Belgrade.

Contributors to this material were: Gordana Gajica

Date of creation: September, 2018.

Refereeing of this material was done by: ______________

Editing into NETCHEM Format and onto NETCHEM platform was completed by: ______________
The NETCHEM platform was established at the University of Nis in 2016-2019 through the Erasmus Programme.

Please contact a NETCHEM representatives at your institution or visit our website for an expanded contact list.

The work included had been led by the NETCHEM staff at your institution.