



Erasmus+



NETCHEM



NETCHEM Remote Access Laboratory Guide

GC-MS analysis of pesticide pendimethalin in apples



In this exercise, you will:

- ✓ Get familiar with the basic steps performed in pesticide analysis in real samples
- ✓ Learn about principles of gas chromatography/mass spectrometry as analytical technique
- ✓ Perform GC-MS analysis of selected pesticide in selected fruit
- ✓ Gain experience in GC-MS usage: interpret obtained chromatogram and mass spectra.



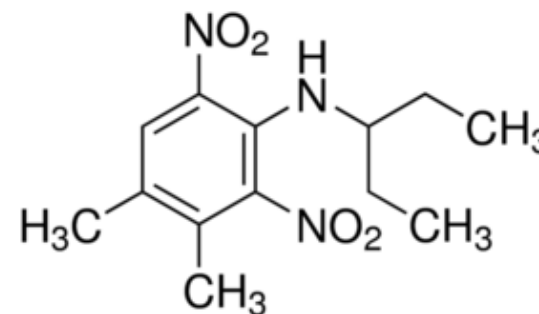


Background

To control weeds and pests, nowadays, more than 800 types of pesticides are used in agriculture. The most of applied pesticides become dangerous to human health through entering the food chain.

This exercise will demonstrate, on the example of **pendimethalin** in **apple**, the use of gas-chromatography coupled to mass spectrometry in analysis of pesticides in fruit samples.

Pendimethalin is a synthetic chemical compound belonging to the chemical family of *dinitroanilines* which acts as a selective pre-emergence and post-emergence herbicide. It is approved for a variety of crops in a large number of countries, including apple, blackberry, blackcurrant, cabbage, carrot, cherry, pear, plum, potato, raspberry. MRL (*maximum residue level*) for pendimethalin in apple is **0.5 mg/kg**.



Molar mass 281.31 g/mol

Pendimethalin





Sample preparation

The aim of sample preparation step is isolation of traces of analyte from complex matrices and elimination of the interfering compounds as much as possible. The most common steps performed within sample preparation for analysis are sampling, sample homogenization, extraction and extract purification. Having in mind reliable analyte assessment, within these steps the most critical are analyte extraction and extract purification.

Sample purification

One of the most commonly applied form of purification is **solid phase extraction (SPE)**. Adsorbent (solid phase) which is supposed to bond the analyte can be applied in two forms: packed into a column or in a dispersed form (*d*-SPE).

The principle of purification is the quantitative binding of the analyte to the adsorbent, while co-extractives pass through the adsorbent without any interaction or bind to a limited extent. The steps in SPE are: application of the analyte to adsorbent, adsorbent washing-out, elution of the analyte.

For satisfactory interaction of the analyte and adsorbent, both need to be similar in polarities i.e. to possess similar functional groups, therefore non-polar analytes will bind to non-polar adsorbents and vice versa.

Sorption of pesticides, which are usually polar and possess a range of functional groups within a molecule are usually performed with polar adsorbents (silica, alumina, Florisil). **Florisil®** is a synthetic magnesium silicate, with moderate polarity and formula $MgO \times nSiO_2$, where *n* represents the average molar ratio between SiO_2 and MgO .





Gas chromatography/Mass spectrometry technique

Gas chromatography with mass spectrometry (GC-MS) is an instrumental technique by which certain compounds from complex matrices can be separated, identified, and quantitatively determined. In order for a compound to be analyzed by this technique, it must be relatively volatile (medium and low molecular weight) and thermostable.

The instrument consists of two parts: a **gas chromatograph**, which serves to separate the components (analytes and other compounds present) and the **mass spectrometer** which allows the identification and quantification of the analyte. The entire system can be divided into several segments:

1. carrier gas;
2. chromatograph (system for introduction of analytes to a column, column);
3. mass spectrometer (ion source, mass analyzer);
4. detector.

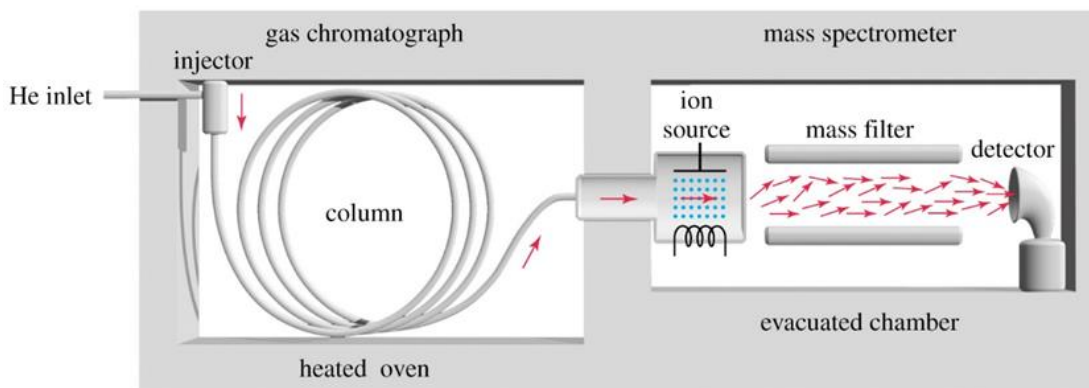


Figure 1. Schematic representation of instrumental technique





Gas chromatography/Mass spectrometry technique

Working principle

- a. *Sample prepared for analysis (usually in liquid state) is introduced with a syringe into instrument injector, heated to a high temperature, where the evaporation of the sample (analyte and solvent) occurs.*
- b. *Sample vapors are then transported with carrier gas (helium, nitrogen) to the separation column which is also heated to a high temperature. The principle of analyte separation is their different distribution between the liquid stationary phase (column) and the carrier gas (mobile phase).*
- c. *Passed the column, the analyte enters the ion source (the segment where the ions are formed, which are later analyzed by mass analyzer). Methods of ionization are different (electronic, chemical ionization etc.).*
- d. *The resulting ions are directed to a mass analyzer, which separates the ions according to the charge to mass ratio of the ions (m/z). As well as the ionization methods, there are many analyzers: single/triple quadrupole, time of flight analyzer, sector analyzer, etc.*
- e. *After separation, the ions are directed to the detector, producing a different signal depending on the abundance, which is then recorded and processed by the software.*












Material

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

Assessories

- blender
- measuring cylinder of 50 mL
- glass funnel
- volumetric flask of 100 mL
- glass pipette or automatic pipette (5 or 10 mL)
- Eppendorf
- Centrifuge for Eppendorf
- Vials (1.5 mL)

Reagents

- Florisil® (adsorbent)
- Hexane   
- Ethyl-acetate   
- Pendimethalin  : Stock solution of 100 µg/mL of pendimethalin pesticide in ethanol





Procedure:

- a) Measure 10 g of sample on a scale. Cut into small pieces and add to the blender together with 15 mL of water. After blending (with no large pieces of the sample remained), transfer complete volume of the mixture with a funnel into a volumetric flask of 100 mL of volume.
- b) Fortify the sample with the known amount of pesticide (e.g. 150 μL giving the concentration of 15 $\mu\text{g/g}$ of apple). Homogenize. After 3-5 minutes, add 5 mL of hexane and extract by shaking. Pour the water into the volumetric flask so that the hexane layer is lifted into the throat of the volumetric flask. Pipette out as much of a pure layer of hexane as possible and transfer it to the eppendorf. Centrifuge 3000 rpm 5 min. Record the amount of pesticide input and the amount of hexane extract taken for further steps.

SPE extract purification:

- a. Applying the sample to the adsorbent: Mix the certain volume of the extract (1.0 mL) with the adsorbent. Shake from time to time within 5 min. Then centrifuge 3000 rpm for 5 min. The liquid phase – the supernatant above the Florisil discard.
- b. Washing-out the impurities from the adsorbent: Add 0.5 ml of hexane to the solid phase and shake. Centrifuge. Discard the liquid phase. Repeat.
- c. Eluting the analyte from the adsorbent: Add 2×0.5 mL eluent (ethyl acetate) to the solid phase and shake. Centrifuge. Combine liquid phases (record volume) in a properly labeled vial of 1.5 mL.
- d. Keep the resulting extract in the refrigerator until the analysis.





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))	
host side (NOTE: Host side of NETCHEM Communication is defined as PC who invites other users to join the session)	participant's PC in classroom
guest side (NOTE: Guest side of NETCHEM Communication is defined as PC who joins the invitation to session)	participant's PC in laboratory
2. COMMUNICATION SOFTWARE	
Team Viewer	Meeting: No
	Remote control: Yes
	Meeting and Remote control simultaneously: No
Skype	Call 1:1: No
	Conference Call: Yes
3. COMMUNICATION HARDWARE	
on host side	1 PC for each participant
on guest side	1 PC, 1 headsets with microphone, camera
4. INFORMATION EXCHANGE TYPE	
Educational (one side is dominantly receptive)	Yes
	Place of Educator participant: guest side
	Number of educator(s): 1
	Place of student participant: host side
Consultative (two sides are equal in giving-receiving information)	Number of student participant(s): 15
	Number of host side participant(s): No
	Number of guest side participant(s): No





Erasmus+



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 choose 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 software packages, that will allow us to communicate instructions and answer questions during the session.

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

<https://www.teamviewer.com/en/index.aspx>

- **Skype**





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)**

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.





Erasmus+



Author, Editor and Referee References

This remote access laboratory was created thanks to work done primarily at University of Niš.

Contributors to this material were: Milica Brankovic

Date of creation: May, 2018.

Refereeing of this material was done by: ___A Warnet_____

Editing into NETCHEM Format and onto NETCHEM platform was completed by: _____





Erasmus+



References and Supplemental Material

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.

