Hort4EUGreen

Strategic Partnership No. 2020-1-RO01-KA203-080398

SUSTAINABLE USE OF PESTICIDES AND THEIR RESIDUES MONITORING

Laboratory methods for pesticides residues analysis. Practical Training Module.

University of Agronomic Sciences and Veterinary Medicine of Bucharest
Лесотехнически университет
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"Enhancing practical skills of horticulture specialists to better address the demands of the European Green Deal"

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Laboratory methods for pesticides residues analysis.

Practical Training Module

INTRODUCTION

Monitoring studies in the field of pesticide residues are crucial for understanding the environmental and human health impact of these chemicals. These studies provide essential data on the levels of pollutants in various environments and matrixes and have a great contribution in the understanding of the environmental fate of pesticides, supporting initiatives like the European Green Deal, which aims for a more sustainable and less polluted environment. Monitoring pesticide residues through reliable analytical protocols is not just a scientific endeavor but also a critical component of environmental and human health and policy, emphasizing the need for skilled professionals and researchers in this field. The combination of theoretical knowledge and practical skills in this area is indispensable for the next generation of environmental scientists and policymakers.

The main key components of pesticide residue monitoring studies are:

Analytical Protocols

a. *Sampling*: ensure you follow the official regulation protocols for pesticide residue analysis. Insufficient sampling can lead to an unrealistic estimation of the pesticide residue concentration (overestimation or underestimation) due to the high heterogeneity of the sample.

b. *Sample Preparation*: the initial step in analyzing pesticide residues. It involves extracting pesticides from the sample matrix, which could be soil, water, or plant material, such as fruits or vegetables. This step is crucial for removing interferences and concentrating the pesticide residues.

c. *Instrumental Methods*: techniques like gas chromatography (GC), liquid chromatography (LC) and others, are commonly used. These methods separate, identify, and quantify pesticide residues in the samples.

d. *Detection Methods*: often coupled with GC or LC, methods like Mass Spectrometry (MS) provide highly sensitive and specific detection of pesticides.

Development and Optimization

a. *Tailoring the analytical methods* to specific needs is vital. It involves adjusting parameters such as solvent types, extraction conditions and chromatographic conditions to enhance efficiency and specificity.

Validation of the Method

a. *Recovery: Measures* the efficiency of extracting the analyte (pesticide residue, which is identified and measured) from the matrix. A high recovery rate indicates that the method is effective in extracting the pesticide from the sample.

b. *Limits of Detection (LOD) and Quantitation (LOQ)*: LOD refers to the lowest amount of substance that can be detected, whereas LOQ is the lowest amount that can be quantitatively measured with precision.

c. *Linearity Range*: Determines the concentration range over which the method provides a linear response.

d. *Precision*: Assesses the intra-day and inter-day repeatability and the reproducibility of the analytical method

Educational Implications

Teaching students about these analytical protocols, especially at the master's and doctoral levels, it's essential. It enhances their understanding not only of the methods themselves but also of their limitations and applicability. The practical skills in handling these techniques and interpreting the results are crucial for future researchers and professionals in environmental sciences.

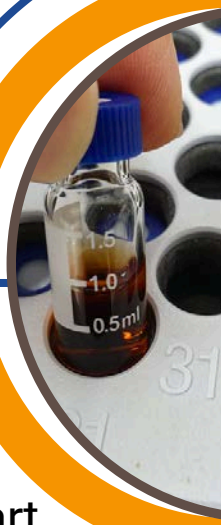
Relevance to the European Green Deal

The European Green Deal sets ambitious targets for reducing pollution and transitioning to a sustainable economy. Understanding and monitoring pesticide residues from food matrixes is a critical part of this initiative. Skilled professionals who can competently perform and interpret these analyses are essential for achieving these goals. The knowledge gained from these studies feeds into policy-making, helping to shape regulations and practices that align with the Green Deal's objectives.

Monitoring pesticide residues through reliable analytical protocols it is not just a scientific endeavor but also a critical component of environmental health and policy. It aligns closely with broader initiatives like the European Green Deal, emphasizing the need for skilled professionals and researchers in this field. The combination of theoretical knowledge and practical skills in this area is indispensable for the next generation of environmental scientists and policymakers.

Laboratory methods for pesticides residues analysis.

Practical Training Module



Summary

The Practical Training Module aims to impart foundational principles and hands-on experience in the instrumental analysis of environmental and food samples. This module includes hands-on laboratory work and interactive question-and-answer sessions focusing on key areas such as sample preparation and treatment, sample analysis, and data processing. The curriculum covers advanced analytical techniques, designed to enhance the practical skills and working knowledge of master's and doctoral students in this field.

Learning outcome descriptors

Upon completion of the Module, the trainee will be proficient in:

1. Comprehending the stages involved in the analytical protocol;
2. Grasping the process of working with an analytical method;
3. Recognizing the significance of preparing the analytical protocol.

General and transferable skills

1	Setting up a workstation for a particular analytical method.
2	Operating in the laboratory autonomously or with minimal supervision when necessary
3	Collaborating effectively in a team environment with limited guidance as needed
4	Exhibiting proficient laboratory skills
5	Displaying the ability to comprehend results and interpret data from diverse analytical methods

Knowledge, understanding and professional skills

1	Choosing the right analytical method for the detection of pesticide active ingredients
2	Acquiring practical laboratory skills through various characterization methods
3	Analyzing and making sense of results obtained using data collection

Unit 1.1 Determination of neonicotinoids using HPLC-UV

Mária Mörtl

1.1.1. Method principle

This method is suitable to determine the neonicotinoid content of a water sample using HPLC equipped with UV detection.

Exercise includes calibration, determination of recovery from water and optimization of the HPLC-UV method by changing the chromatographic conditions.

1.1.2. Apparatus

- HPLC equipped with UV detector and automatic injector;
- automatic pipets;
- vials of 2 mL;
- analytical balance, to the nearest ± 0.01 mg;
- 1 mL syringe;
- PTFE syringe filter 0.45 µm pore size;
- centrifuge tubes of 15 mL;
- vortex;
- evaporator (heating module).

1.1.3. Sample preparation

- *Calibration and determination of neonicotinoids*

Prepare stock solution containing different neonicotinoids (thiamethoxam, clothianidin, thiacloprid, acetamiprid, imidacloprid) at the level of 1 mg/mL in methanol;

- Prepare a solvent mixture, which has the same composition, as the initial composition of the eluent in the HPLC method (Table 1.1.);
- Prepare a common working solution (5 mL) containing 3 neonicotinoids at level of 10 µg/mL and then the calibration solutions containing each neonicotinoid at level of 50, 100, 200, 500 and 1000 ng/mL.
- Dilute the stock solution with the eluent solvent mixture (Table 1.1.) and do not forget to homogenize the samples;
- Inject 20 µL from each sample and from the unknown applying the chromatographic conditions shown in Table 1.1.

Table 1.1. Chromatographic conditions for isocratic determination of neonicotinoids

Column	**C18, 150mm × 4.6mm i.d., 5 µm**
Isocratic eluent composition	75% A (90% water:10% MeOH) + 25% B (Acetonitrile) OR 65% A + 35% Methanol
Wavelength	λ = 269 and 252 nm
Run time	10 min
Column temperature	40 °C
Injection	partial loop fill, 20 µL
Flow rate	1 mL/min

Recovery assessment of the neonicotinoids from water

- Add known amounts (200 ng) of neonicotinoids to 10 mL water sample (spiking) in four 15 mL centrifuge tube.
- Extract the samples with the two of the six different extraction solvents listed in Table 1.2.
- Shake them thoroughly manually and then for 30 seconds with a vortex.
- Do the extraction either once, or twice, when the two extracts are combined.
- Remove as much as possible from the organic solvent by a syringe.
- Evaporate the solvent to the dryness by a nitrogen stream in a laboratory fume hood and resolve the residue in 1 mL of solvent mixture, which has the same composition, as the initial composition of the eluent in the HPLC method.
- Filter the extract by using a PTFE syringe filter with a 0.45 µm pore size.
- As initial concentration in the water was 20 ng/mL, theoretically tenfold increase occurred during the sample preparation (100% recovery), thus the corresponding level of the extract is equal to 200 ng/mL.
- Prepare also this solution as a reference or use the measured value from the calibration.

Table 1.2. Composition of solvents used for the extraction of neonicotinoids

	Solvent	Volume (mL)	Co-solvent	Volume (mL)	Solvent
1	hexane	1.5	acetone	0.5	1
2	hexane	1.5	acetonitrile	0.5	2
3	hexane	2.0	-	0	3
4	dichloromethane	1.5	acetone	0.5	4
5	dichloromethane	1.5	acetonitrile	0.5	5
6	dichloromethane	2.0	-	0	6

Optimization of the method

One of the prepared solutions (e.g. 200 ng/mL) is used in this part of the laboratory exercise and initial parameters are indicated in Table 1.1. These are then modified as follows.

1.1.4. Sample preparation

Calibration and determination of neonicotinoids

Analysis is done by the method given in Table 1.1. Check the integration of the peaks and determine the peak areas for all calibration level and for the unknown. Summarize your results in a table and calculate the concentration of unknown.

Determination recovery of the neonicotinoids from water

Analysis is done by the HPLC method given in Table 1.1. Check the integration of the peaks and determine the areas of more intensive peaks (wavelength) for the 4 extracted (2 solvent mixture and 1 or two extraction) and the reference sample as well. Summarize your results in a table.

Optimization of the method

Initially use the parameters given in Table 1.1. and record the chromatogram.

• Modify the parameters, inject the same solution and record the chromatogram again.

•Change the ratio of organic modifier in the initial composition of the eluent by ±10%.

• Change the column temperature by ±5°C;

• Change the flow rate by ±0.1 mL/min;

• Change the wavelengths by ±20 nm;

• Summarize the retention times for each compound and conditions in a table.

Note: in some cases equilibration of the system is required, e.g. if the eluent composition is changed. Consider also that in the reversed phase liquid chromatography, decrease the ratio of organic modifier in the eluent results in increase of retention times for less polar components, which may increase the time of chromatographic run as well.

• Try to find a gradient method for the target neonicotinoids.

1.1.5. Evaluation

Calibration and determination of neonicotinoids

Plot the peak areas as a function of concentration for each component and based on this calibration curve, calculate the levels in the unknown. Calculate the peak ratios recorded at two different wavelengths as well. It should be constant, if there is no co-eluting component in the sample.

Determination recovery of the neonicotinoids from water

Compare the measured intensities (peak areas) with those of the reference solution and calculate the recoveries for 4 procedures (2 solvent mixture and 1 or two extraction) to each component. Recovery (rec%) is expressed as given by the equation:

$$rec\% = \frac{A_{extr,i}}{A_{ref,i}} \cdot 100\%$$

where:

rec% – recovery

$A_{extr,i}$ – peak area in the extracted sample for component i

$A_{ref,i}$ – peak area in the reference sample for component i

Optimization of the method

Compare the effect of different parameters to the retention times of neonicotinoids and consider also the peak widths and/or areas in the chromatogram. Summarize your observations and choose an optimal method.

Unit 6.2 Determination of pesticide residues from water using GC-MS

Mária Mörtl

1.2.1. Method principles

Pesticide active ingredients are present in the different environmental samples, for example in surface water. Multi-residue analysis of moderately volatile pesticides is performed by using GC-MS. Water samples are prepared by solid phase extraction (SPE), whereas soil samples are extracted by liquid extraction.

1.2.2. Apparatus

- SPE cartridges, Restek CarboPrep-90 (500 mg/6 mL) cartridge
- SPE extraction apparatus (See Fig. 1.1.);
- Erlenmeyer flasks;
- automatic pipets;
- 4 and 2 mL vials;
- tubes for collection of fractions;
- pesticide standards (e.g. dimethenamid, trifluralin, acetochlor, clomazone, tefluthrin, terbuthylazine, chlorpyrifos, metolachlor, propiconazole, penconazole, pendimethalin, etc.)
- BSTFA silylating agent for derivatization;
- GC-MS instrument;

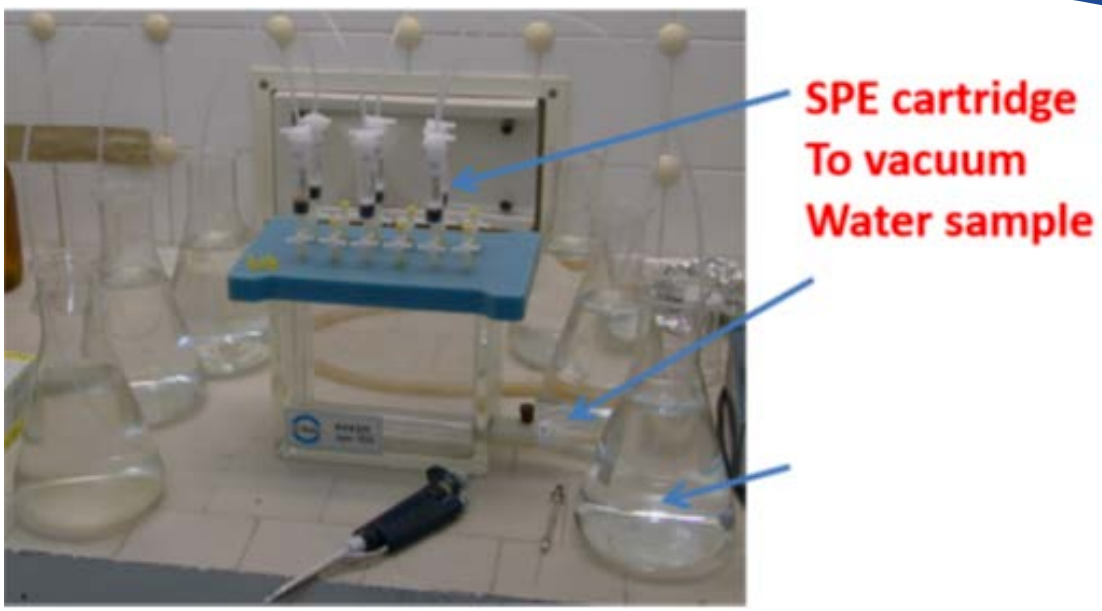


Figure 1.1. SPE extraction apparatus

1.2.3. Sample preparation

Multi-residue method for determination of pesticide active ingredients from water

Prepare 1 L (or 200 mL) of surface or ground water sample with solid phase extraction (SPE) according to the procedure shown in Figure 1.2. If the water contains a lot of suspended particles, then filter the sample through a paper. First, condition the cartridge prior to sample preparation, then load the sample and use vacuum to increase the flow rate. Removal of salts and other polar interferences are performed by washing, then dry the cartridge using vacuum and elute the components. Neutral and basic components are eluted slowly (without vacuum) in the first fraction by 1 mL of methanol, followed by mL of CH_2Cl_2/methanol (8:2). Acidic components are collected separately in a second fraction by a subsequent elution with a basic solution mixture containing

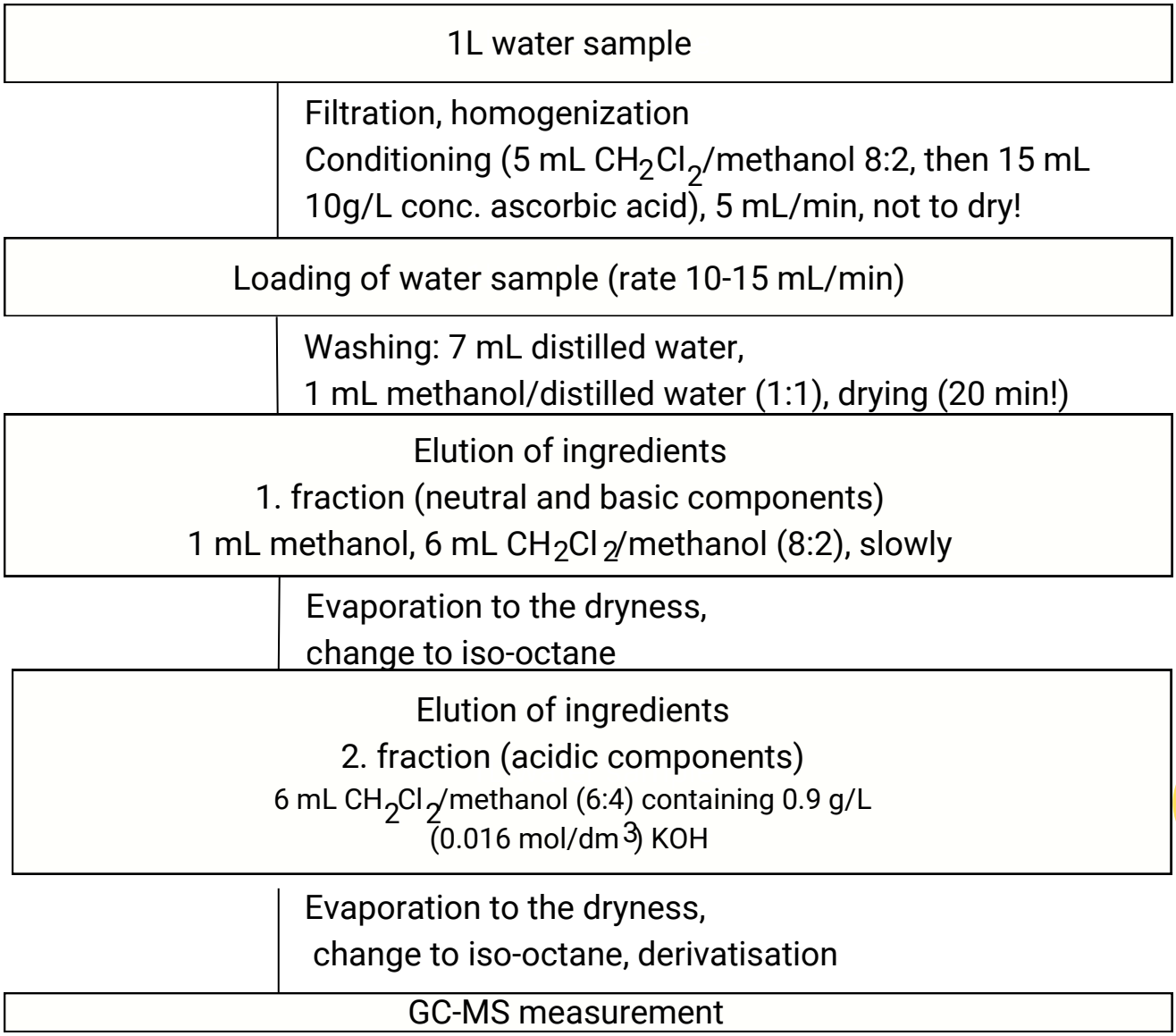


Figure 1.2. Sample preparation of water samples.

more methanol (CH_2Cl_2/methanol =6:4). These compounds (e.g. chlorophenoxy acid herbicides) are derivatized by using a silylating agent. Goal of the derivatization is to increase volatility, which is a prerequisite in GC. Remove the solvent by nitrogen stream and then resolve the dried residue in 1 mL of solvent suitable for GC measurements (e.g. iso-octane). If

you want to keep the 3 orders of magnitude in concentration and only 200 mL of water sample was prepared, then solve the dried residue only in 200 µL and use an insert to the vial. For the dried second fraction add the derivatizing reagent (e.g. 50 µL BSTFA + 10 µL trifluoroacetic acid) and heat at 60°C for half an hour. Note that the silylating agents as well as the silyl derivatives are moisture sensitive, thus apply the reagent in excess and close the vial to preserve stability toward the hydrolysis.

1.2.4. Sample analysis

Set the optimized GC-MS method for multi-residue analysis and inject 1 µL of the prepared sample to your GC-MS instrument. If the GC inlet allows larger injection volumes, then 5 µL or even more can be injected. Optimized GC-MS method may vary among the different laboratories. An example for parameters are given in Table 1.3.

Table 1.3. Chromatographic conditions of the multi-residue analysis by GC-MS

Column	Ultra-inert HP-5MS capillary column (30m x 0.25mm x 0.25 µm film thickness)
Oven temperature program	stationary at 70 °C for 0.5 min, heat from 70 °C to 100 °C at 60 °C / min, heat from 100 °C to 240 °C at 10 °C / min, stationary at 240 ° C for 11 min
Carrier gas	Helium (6.0) with a flow rate of 1.0 mL / min.
Injection	volume 5 µL, split from 1.5 min, split ratio 40
Injection temperature	stationary at 60 °C for 0.5 min, heat from 60 °C to 260 °C at 200 °C / min, held for 5 min, cool to 60 °C at 200 °C / min, held for 18 min
Mass spectrometer	Ionization energy 70 eV, on the mass range 60-350 m/z at 2 scan/s. Solvent delay 7.5 min. MS transfer line and ion source 280°C and 230°C, respectively

1.2.5. Evaluation

• Compare the mass spectra recorded to larger peaks of the chromatogram to the mass spectra in the database (e.g. NIST) by using the corresponding function of the software.

• Use evaluation function of the software, and try to find the target molecules, which are recorded in the optimized multi-residue method.

• If there are calibration curves for the target molecules, calculate the concentration as well. Worthy of note that recovery is probably not determined for each target compounds.

Unit 1.3 Determination of pesticide residues from soil using GC-MS

Mária Mörtl

1.3.1. Method principles

Pesticide active ingredients are present in the different environmental samples, for example in surface water. Multi-residue analysis of moderately volatile pesticides is performed by using GC-MS. Water samples are prepared by solid phase extraction (SPE), whereas soil samples are extracted by liquid extraction.

1.3.2. Apparatus

- SPE cartridges, Restek CarboPrep-90 (500 mg/6 mL) cartridge
- SPE extraction apparatus (See Fig. 1.1.);
- Erlenmeyer flasks;
- automatic pipets;
- 4 and 2 mL vials;
- tubes for collection of fractions;
- pesticide standards (e.g. dimethenamid, trifluralin, acetochlor, clomazone, tefluthrin, terbuthylazine, chlorpyrifos, metolachlor, propiconazole, penconazole, pendimethalin, etc.);
- BSTFA silylating agent for derivatization;
- GC-MS instrument.

1.3.3. Sample preparaton

Multi-residue method for determination of pesticide active ingredients from soil

Extract 10 g of air dried soil sample by using a mixture of hexane-acetone (1:1) according to the procedure presented in Figure 6.3. Extraction is carried out by manual shaking, followed by ultrasound agitation, then the solution is centrifuged in order to separate the phases. 10 mL of the supernatant is removed and the solvent is evaporated to the dryness by nitrogen stream in laboratory fume hood. Redisolve the dry residue in the solvent suitable for GC measurements (e.g. iso-octane).

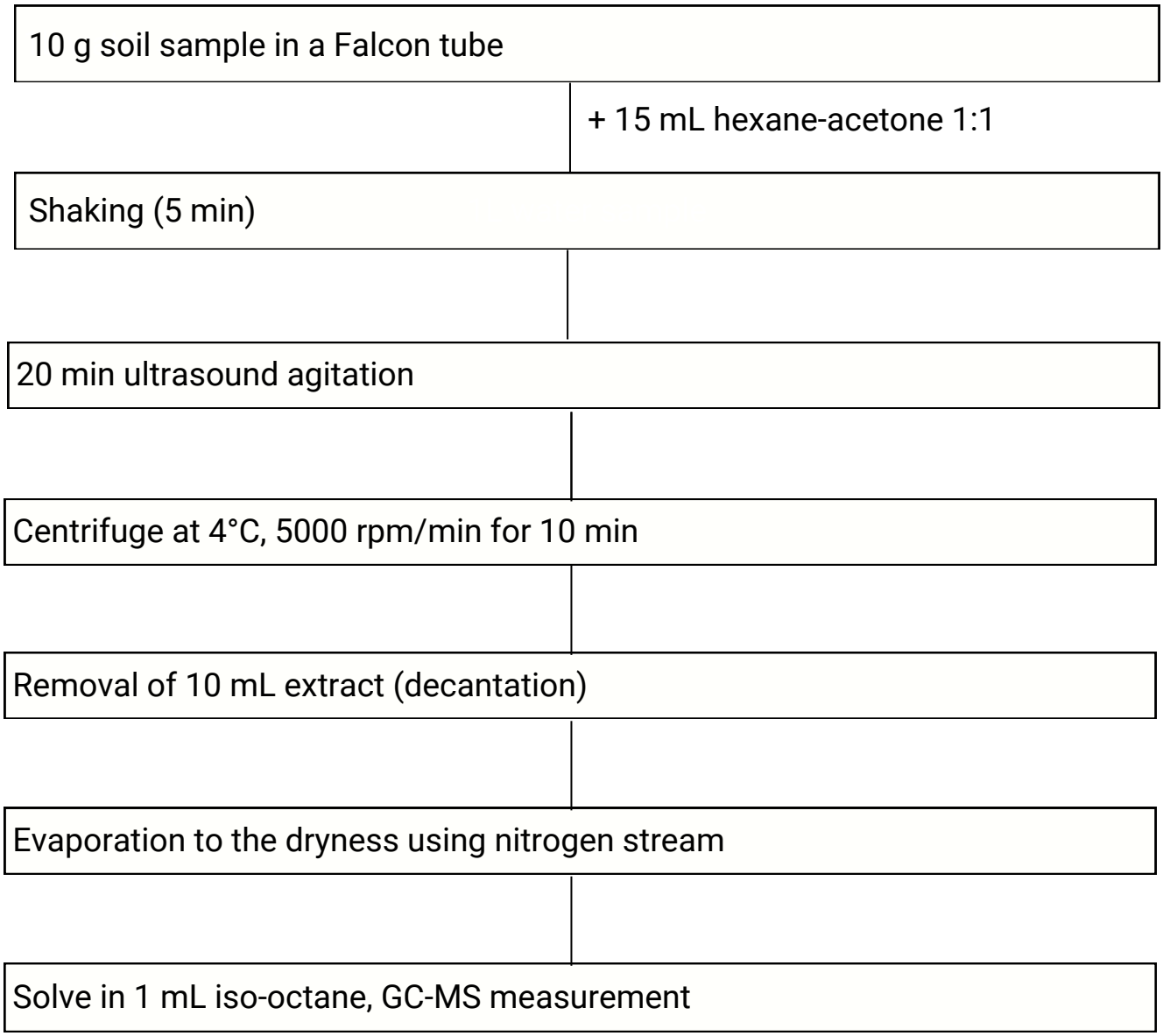


Figure 1.3. Sample preparation of soil samples.

1.3.4. Sample analysis

Apply the method developed for multi-residue analysis by GC-MS. Chromatographic conditions of the multi-residue analysis method are the same as in unit 1.2. Detailed parameters are given in Table 1.3.

Inject 1 µL (or more, e.g. 5 µL) of the sample. Inject also the solution of the normal alkanes using the same conditions. Identify the peaks on the bases of NIST database or by using the own library. The determination of the retention index is based on the retention times recorded for the n-alkane series. Retention indices (measured and/or calculated) are also given by NIST database (https://webbook.nist.gov/chemistry/).
Consider only those indices, which were determined on the same stationary phase.

1.3.5. Evaluation

Determination of residues in samples

Identify the pesticide active ingredients that polluted the soil sample. Identification of pesticide residues is based on the mass spectra and retention times have to be in accordance with that of measured earlier with analytical standard solutions and recorded in the method. Calculation of the levels in the sample for each component can be performed on the basis of the calibration curves, if available. Assuming 100% recovery, determine the levels in original sample if calibration for the pesticide active ingredient found in the sample are available.

Determination of retention indices

Plot the retention times of normal alkanes as a function of number of carbon atoms. Is it linear?

The indices of the normal alkanes are, by definition, a hundred times their carbon number. In the isothermal case, retention index (Kováts-index, RI) is expressed as given by the equation:

$$RI_i = 100\left(n + \frac{(\log(t_i - t_0) - \log(t_n - t_0)}{(\log(t_{n+1} - t_0) - \log(t_n - t_0)}\right) \text{ where}$$

- t_0 dead time
- t_i retention time of unknown
- t_n retention time of n-alkane eluting before the unknown
- n the number of carbon atoms in of n-alkane eluting before the unknown
- t_{n+1} retention time of n-alkanes eluting after the unknown, the number of carbon atoms is n+1

In temperature programmed case, if the temperature is increased linearly, then the index is calculated by the (van den Dool és Kratz) following equation:

$$RI_i = 100\left(n + \frac{t_i - t_n}{t_{n+1} - t_n}\right)$$

Calculate the retention index for the compounds identified by the mass spectral database and compare the calculated values with the literature data. Indices are valid to the

corresponding stationary phase and only slightly are influenced by the chromatographic conditions (oven temperature). Therefore, they are given in certain databases (e.g. NIST) and confirm identification of compound.

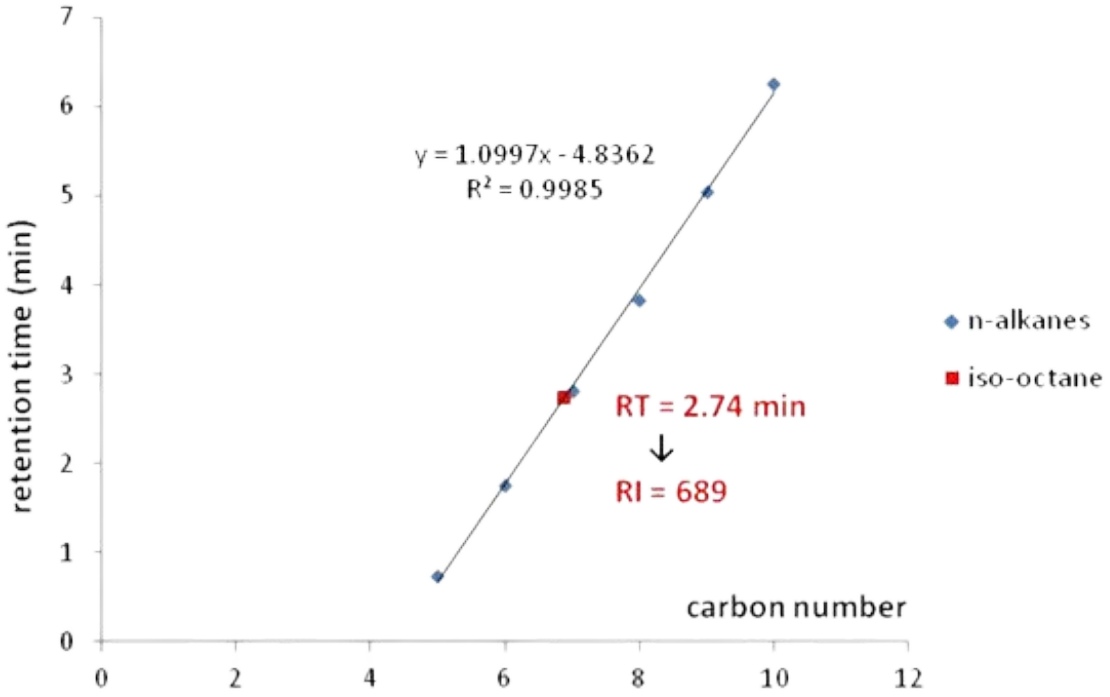


Figure 1.4. Determination of retention index (RI) for iso-octane. Equation was determined on the basis of retention times of normal alkanes. Note, that index is hundredfold of the carbon number.

Unit 1.4 Determination of pesticide residues from fruits and vegetables using GC-MS

Mária Mörtl

1.4.1. Method principles

The extraction of pesticide active ingredients from fruits and vegetables is performed by QuEChERS method and their determination is based on GC-MS measurement. QuEChERS is an acronym for Quick, Easy, Cheap, Effective, Rugged and Safe, and covers a variety of sample preparation and clean-up techniques for the analysis of multiple pesticide residues in agricultural matrices. Many varieties are published, which are optimized for preparation of environmental matrices (e.g. soil, plant leaves, etc.) and for other target compounds (e.g. mycotoxins) as well. Due to this variability, it is better to refer to these procedures as QuEChERS based methods. First in a salting out step pesticide residues are extracted by acetonitrile. Then clean-up of extract is performed. Three different sorbent types are applied in a dispersive way (dSPE) for elimination of matrix interferences. Primary secondary amine (PSA) is used in the removal of sugars and fatty acids, organic acids, lipids and some pigments. When PSA is used in combination with silica based C18 phase, additional lipids (long chain fatty compounds), sterols and other non-polar

interferences can be removed. The graphitized carbon black (GCB) is less commonly used as it is a strong sorbent for removing pigments, polyphenols, and other polar compounds, but some of the planar (polar aromatic) pesticides can be also removed.

1.4.2. Apparatus

- Falcon tubes 50 mL and 15 mL;
- dSPE phases (e.g. PSA, GCB and/or C18);
- NaCl and MgSO4;
- automatic pipets;
- 4 and 2 mL vials;
- syringe filter (0.45 or 0.22 µm pore size);
- pesticide standards (e.g. dimethenamid, trifluralin, acetochlor, clomazone, tefluthrin, terbuthylazine, chlorpyrifos, metolachlor, propiconazole, penconazole, pendimethalin, etc.)
- iso-octane or other organic solvent;
- GC-MS instrument;

1.4.3. Sample preparation

Weight 10 g of homogenized food sample into a 50 mL centrifuge tube and add 10 mL of acetonitrile as well. Apply one of the QuEChERS method (Fig. 1.5.). Note: if the sample has not a high water content (e.g. cereals), then lower amounts of the ground sample should be used and complete the weight to 10 g with water (hydrate).

Shake the sample thoroughly, then add the salts ($MgSO_4$ and NaCl) and shake again the sample. Let the sample for 10 minutes and repeat the shaking to achieve effective extraction pesticides in this salting out step. As some of the pesticide active ingredients are sensitive to pH, therefore buffer (acetate or citrate) may also be applied. Internal standard (ISTD) is also added to the solution in some cases to improve the precision and accuracy, correct method variability or eliminate the matrix effect occurring persive solid phase extraction (dSPE), recommended for the chosen food matrix. Follow the advice of your supervisor and apply one of the recommended procedures listed in Table 1.4. For example, for grains or cereals 150 mg $MgSO_4$ and 50 mg PSA per mL of extract is given in the guide. In contrast, for the highly pigmented samples (e.g. strawberry, tomato) 25 mg PSA and 2.5 mg GCB should be applied for the same amount of extract and salt. In addition to 150 mg $MgSO_4$ and 50 mg PSA, 50 mg of end capped C18 phases is applied for the clean-up of matrices with high lipid content (e.g. meat, fish, nut).

Centrifuge the sample in order to separate the phases and transfer an aliquot of the supernatant in a smaller tube. Add the magnesium salt and the phases to the extract. Instead of 1 mL more supernatant (e.g. 6 mL) can be also removed and use it in the dSPE clean-up process in order to increase the sample volume.

Shake, centrifuge and transfer an aliquot of the supernatant in a smaller tube. Prior to GC remove the solvent by nitrogen stream. Resolve the dried residue in the solvent suitable for GC measurements (e.g. iso-octane). Prior to LC measurements, the dried residue should be solved in the initial eluent, and filter through a syringe filter with 0.45 or 0.22 µm pore size.

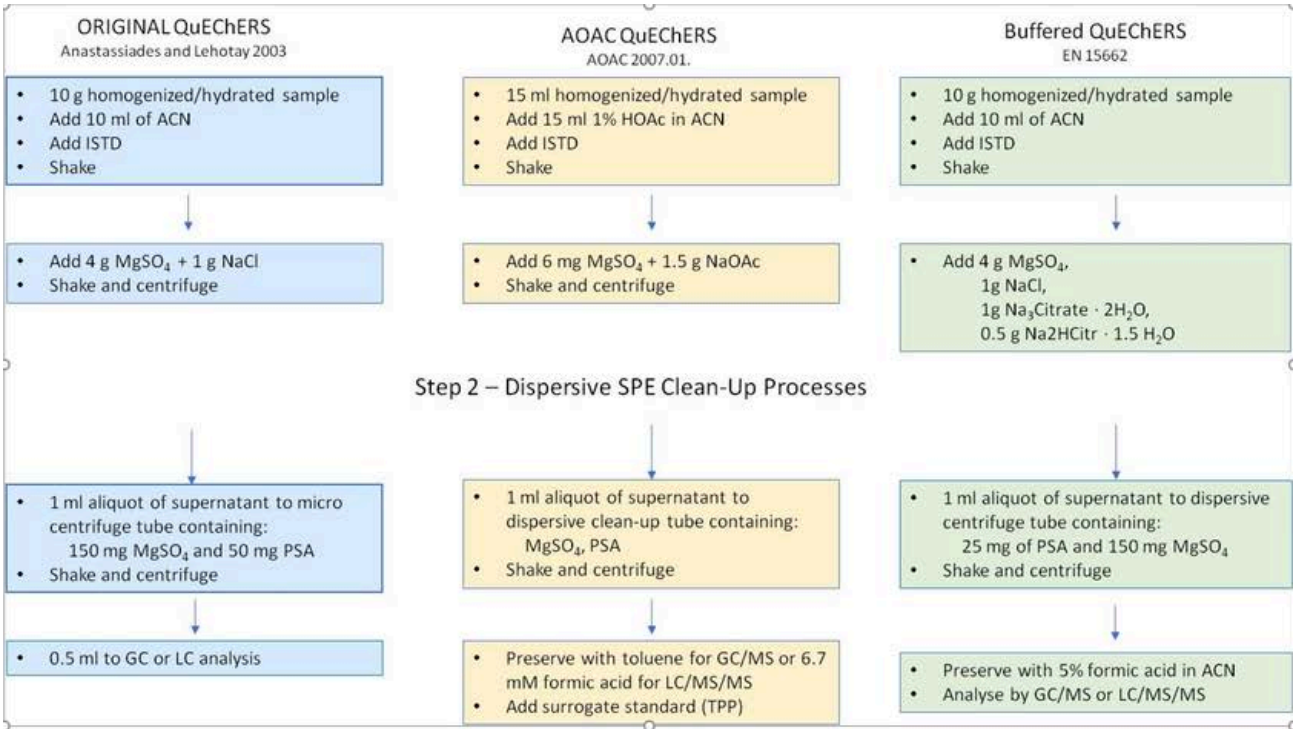


Figure 1.5. The original and the official QuEChERS procedures

Table 1.4. Dispersive SPE clean-up recommended for different matrices in the QuEChERS procedure

	Typical matrix	Example	dSPE clean-up
1	lightly pigmented fruit	potato	900 mg $MgSO_4$, 150 mg PSA
2	pigmented fruit	tomato, strawberry	900 mg $MgSO_4$, 150 mg PSA, 15 mg GCB
3	pigmented fruit with lipids	avocado	900 mg $MgSO_4$, 300 mg PSA, 45 mg GCB, 300 mg C18
4	Fatty products	fish, meat, nuts	900 mg $MgSO_4$, 300 mg PSA, 300 mg C18
5	Wine		900 mg $MgSO_4$, 300 mg PSA, 150 mg GCB
6	Olive oil		900 mg $MgSO_4$, 300 mg PSA
7	Cereals		900 mg $MgSO_4$, 300 mg PSA, (50 mg C18)
8	for acidic ingredients		900 mg $MgSO_4$, 300 mg C18

1.4.4. Sample analysis

Apply the method developed for multi-residue analysis by GC-MS. Chromatographic conditions of the multi-residue analysis method are the same as in unit 1.2. Detailed parameters are given in Table 1.3.

Inject 1 µL of sample and run the chromatographic method. Identify the peaks on the bases of NIST database or by using the own library. If the solution of the normal alkanes is also available, then inject it as well using the same conditions. Retention index (measured and/or calculated) is also given

by NIST database (*https://webbook.nist.gov/chemistry/*). Consider only those indices, which were determined on the same stationary phase.

1.4.5. Evaluation

Determination of residues in samples

Identify the pesticide active ingredients that polluted the sample. Identification of pesticide residues is based on the mass spectra and retention times have to be in accordance with that of measured earlier with analytical standard solutions and recorded in the method. Calculation of the levels in the sample for each component can be performed on the basis of the calibration curves, if available. Assuming 100% recovery, determine the levels in original sample if calibration for the pesticide active ingredient found in the sample are available.

Determination of retention indices

Check also the retention index and compare the calculated values with the literature data. Plot the retention times as a function of number of carbon atoms. Retention index (Kováts-index) (*RI*) is expressed as given by the equation in Unit 1.3.

Unit 1.5 Determination of glyphosate residues using ELISA method

Borbála Gémes,
Eszter Takács

1.5.1. Method principles

Enzyme linked immunosorbent assays (ELISAs) are using specific antibodies to recognize the target compound glyphosate in the sample. As competitive ELISA, the specific antibodies are able to bind glyphosate in the sample or the glyphosate-analogue conjugate coated to the microplate. Increasing concentration of the sample reduces the detectable signal. Quantification is possible by comparing the results to a 7-point calibration curve. Main steps of a competitive ELISA are described in the followings (based on the Abraxis Glyphosate Plate kit, Abraxis LLC, Warminster, PA).

Precautions:

⚠️ All reagents and plates are stored at 2-8°C. Let them to reach room temperature before testing!

⚠️ Avoid foam formation during vortexing.

1.5.2. Apparatus

The 96-well microplate format allows for the measurement of 25 samples in parallel with 7 points for the calibration curve.
Essential items for the performance of the test:

- Quantitative ELISA assay kit;
- single channel pipettes capable of delivering 50, 100, 150, 250 µl and 1 ml;
- 8-chennel pipettes capable of delivering 50 – 300 µl parafilm;
- test tubes for derivatization;
- glass vials for preparation of assay solutions;
- distilled or deionized water;
- vortex;
- microplate reader;
- for plant and animal samples you need also: Eppendorf tubes, mortars and pestles, analytical balance, centrifuge.

1.5.3. Sample preparation

According to manufacturer, ELISA is appropriate for determination of glyphosate in surface water samples without any dilution of samples. It can be also applied in plant/animal samples, however in this case the recommended dilution of sample extract is minimum 1:10. Apply matrix-based calibration in this case (Obtain the calibration curve in extract of control sample. Before preparing the calibration curve, dilute the control extract with the same dilution rate as for the samples).

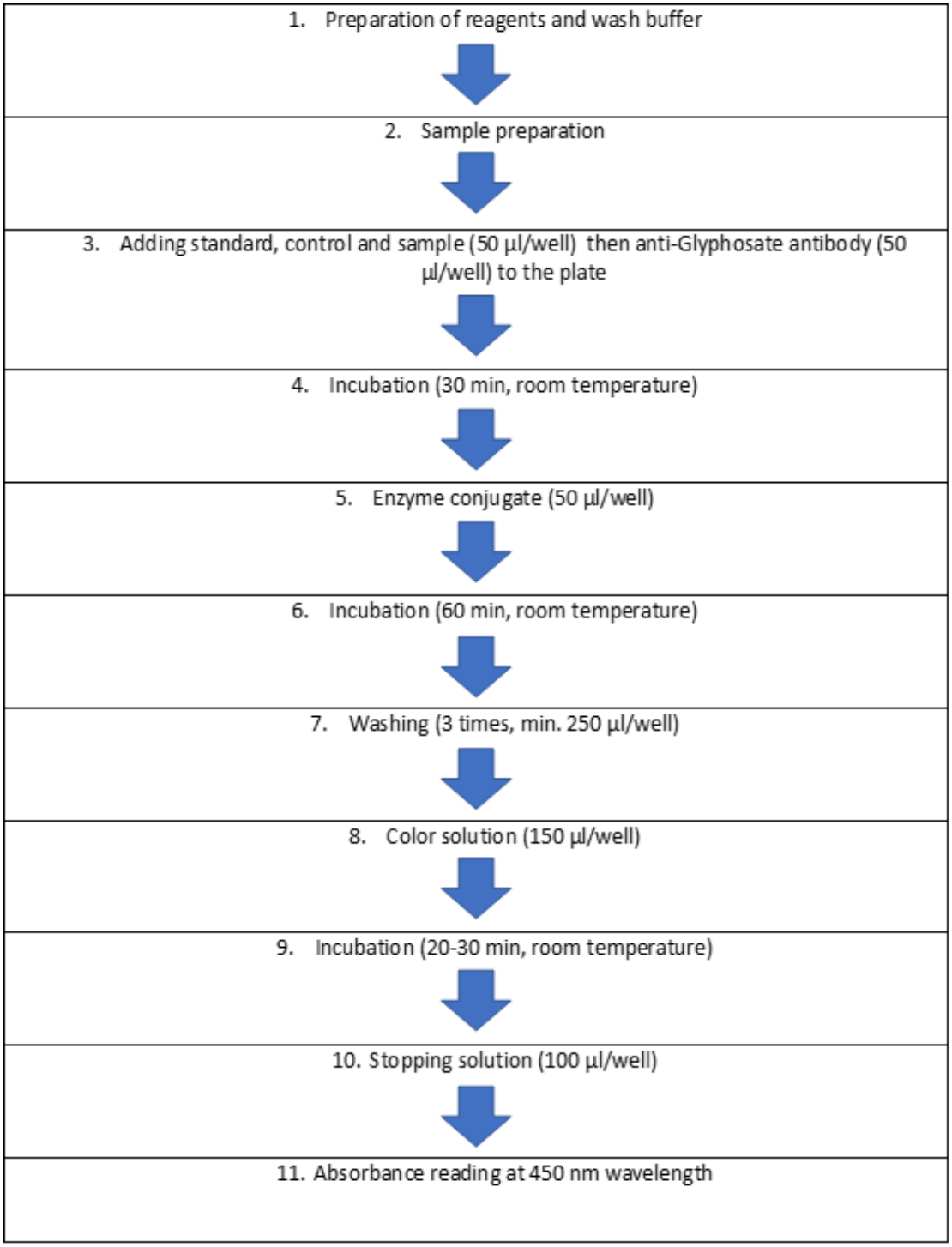
For plant/animal tissue add 40 mg of sample and 1 mL assay buffer into a mortar and homogenize them by pestle. Put the whole extract in an Eppendorf tube and centrifuge it for 10 mins, 12000 rpm and room temperature. The extract of control sample is the supernatant.

1. dilute the Derivatization Reagent with 3.5 ml of Derivatization Reagent Diluent
2. add 1 ml of Assay buffer to 250 µl standards, control and samples
3. vortex the mix
4. add 100 µl of the diluted derivatization reagent to each tubes and vortex until it gets homogenous,
5. incubate it at room temperature for 10 minutes.

1.5.4. Sample analysis/assay procedure

a. add 50 µl of derivatized standard, control or sample (using triplicates is recommended),
b. add 50 µl of the anti-Glyphosate antibody solution to each well,
c. cover the plate with parafilm, mix the content and incubate at room temperature for 30 minutes,
d. add 50 µl of the enzyme conjugate solution to each well,
e. cover the plate with parafilm, mix the content and incubate at room temperature for 60 minutes,
f. vigorously shake the whole content of the wells into a waste container, and wash the wells 3 times with 1X wash solution (volume at least 250 µl/well),

g. add 150 µl of color solution to each well, and incubate it for 20-30 minutes,
h. add 100 µl of Stopping solution to each well,
i. Read absorbance at 450 nm within 15 minutes after step h.



Other possibilities:

Beside this example there are other available ELISA kits on the market. All of them are based on the specific antibody-antigen reaction, but minor differences are possible e.g. in concentrations of the solutions or in the process. Always follow the manufacturer's guide!

1.5.5. Evaluation

Determine the glyphosate content of your samples based on the calibration curve.

Unit 1.6 Determination of organochlorine pesticides in organic fertilizers using GC-MS

Violeta Alexandra Ion,
Oana-Crina Bujor

1.6.1. Method principles

The purpose of this procedure is to quantitatively determine organochlorine pesticide (OCP) residues in inputs used in organic farming (fertilizers) by GC-MS analysis. The method is applied for the quantitative determination of organochlorine pesticide residues (α-HCH, β-HCH, γ-HCH, δ-HCH, Heptachlor, Aldrin, Heptachlor epoxide, Ensodulfan I, 4,4'-DDE, Dieldrin, Endrin, Endosulfan II, Endrin aldehydes, 4,4'-DDD, Endosulfan sulfate, 4,4'-DDT, Methoxychlor, PCB 18, PCB 105, PCB 118, PCB 126, PCB 169) from products used as inputs in organic agriculture. The limit of detection (LOD) for these organochlorine pesticides is DDT < 0.05 mg/kg dry matter, DDE < 0.05 mg/kg dry matter, DDD < 0.05 mg/kg dry matter, α-HCH < 0.002 mg/ kg dry matter, β-HCH < 0.001 mg/kg dry matter, γ-HCH < 0.001 mg/kg dry matter, δ-HCH < 0.001 mg/kg dry matter, total organochlorine pesticides: < 0.2 mg/kg dry matter, total polychlorinated biphenyls < 0.01 mg/kg dry matter (DM),

1.6.2. Apparatus and reagent

Reagents

- Ultrapure water, Grade 1 (MilliQ resistivity of 18 MΩ × cm at 25°C and TOC of 3 ppb);
- Hexane, ≥ 97%, GC grades;
- Acetone, ≥ 99.5 %, p.a.;
- Petroleum ether with a boiling point between 40 and 60 °C;
- Ethyl acetate;
- Anhydrous sodium sulfate ≥ 99%, p.a.;
- Sodium chloride ≥ 99.8 %, p.a..

Conditioning anhydrous sodium sulfate: dry for 6 h at 550 °C in the furnace, cool to approximately 200 °C and then to room temperature in a desiccator containing moisture absorbent material.

Pesticide stock solution (SS) of concentration 1000 µg/mL. Weigh individually to the nearest 0.1 mg an amount of the reference standard to give solutions of 1000 µg/mL in hexane. When weighing, the purity of the standard substances is taken into account. These solutions are stable for 6 months if stored at 4°C in the dark.

Pesticide intermediate solution (S1) concentration 20 µg/mL. Individually pipette 200 µL of the 1000 µg/mL stock solution (SS) into a 10 mL volumetric flask. Make up to volume with hexane. These solutions are stable for 6 months if stored at 4°C in the dark.

Pesticide working solution (SL) of concentration 200 ng/mL. Pipette into the mixture 10 µL of the intermediate solution (S1) with a concentration of 10 µg/mL in a 10 mL volumetric flask. Make up to volume with hexane. This solution is stable for one month if stored at 4°C in the dark.

Pipette each calibration standard on the domain 10-150 ng/mL.

Apparatus

- 5 mL, 10 mL volumetric flasks;
- Erlenmeyer beakers;
- Separating funnels with a capacity of 500 mL, 1000 mL;
- Graduated test tubes of 10 mL capacity and PTFE stoppers;
- Silica and aluminum oxide SPE cartridges;
- 100 mL round bottom flasks;
- 10 mL graduated cylinder;
- Medium porosity filter paper;
- Micropipette 10 - 100 µL;
- Micropipette 100 - 1000 µL;
- Syringe filters;
- Vortex;
- Rotary evaporator;
- Laboratory mill;
- Refrigerator combine;

- Analytical balance;
- GC-MS system;
- Chromatographic column HP - UI 5MS, length 30 m, internal diameter 0.250 mm, 0.25 µm film.

1.6.3. Sample preparation

Samples should be pretreated as soon as possible. Store the samples in a dark place at a temperature below 10 °C, if possible in a refrigerator. For OCP and PCB testing, sample storage time should not exceed 7 days. The samples are homogenized if they are non-homogeneous, in order to obtain a representative sample.

Before treating the samples, the determination of a control sample is carried out according to the work protocol using the same amounts of reagents as in the extraction, purification and analysis of a fertilizer sample. The values obtained from the analysis of the blank samples should be less than the detection limit for the analytes of interest.

a) Extraction of solid samples

Weigh 20 ± 0,1 g of a sample into an Erlenmeyer beaker. Add 50 mL of acetone to the test sample and shake vigorously for 15 min with the help of a stirrer at 200 rpm. Then add 50 mL of petroleum ether and stir for 15 min. Repeat the extraction with 50 mL of petroleum ether. Collect the extracts in a 500 mL separating funnel and remove the acetone by washing twice with 400 mL of water.

Dry the extract over sodium sulfate, wash three times with 10 mL of petroleum ether and add these washings to the extract.

b) Samples with high humidity

Weigh out 20 ± 0.1 g of the samples and place in an Erlenmeyer beaker. Add 50 mL of acetone to the test sample and shake vigorously for 15 minutes at 200 rpm, using a shaker. Then add 50 mL of petroleum ether and stir for 15 minutes. Repeat the extraction with 50 mL of petroleum ether. If the water content of the sample is greater than 25 %, increase the amount of acetone. The acetone:water ratio should be at least 9:1. The acetone:petroleum ether ratio should be kept constant at 1:2.

Collect the extracts in a 1 L separatory funnel and wash twice with 400 mL of water to remove the acetone. Dry the extract over anhydrous sodium sulfate and transfer the dried extract to the concentrator. Wash the sodium sulfate three times with 10 mL of petroleum ether and add these washings to the extract. Concentrate the extract to approximately 1 mL on a rotary evaporator.

NOTE - Too high temperatures and a high nitrogen flow can cause loss of highly volatile PCBs and OCPs.

Extract purification

With a pipette, transfer the extract to SPE with silica gel or florisil; wash the graduated test tube twice with 1 mL of petroleum ether and transfer the wash portions to the column with the same pipette as soon as the liquid level reaches the top of the column packing. Elute with approximately 20 mL of petroleum ether.

Divide the eluate into two equal parts and keep one part for eventual analysis of the diluted extract. The other part of the eluate is concentrated with a weak stream of nitrogen, without further heating, to a volume of about 1 mL.

The presence of sulfur in nonpolar PCB or OCP extracts can cause interferences in the chromatogram. If elemental sulfur is suspected to be present, it is removed as follows:

- Add 2 mL TBA sulfite reagent to 1 mL concentrated extract and shake for 1 minute.
- Then, add 10 mL of water and shake again for 1 minute.
- Separate the organic phase from the water using a Pasteur pipette and add a few crystals of anhydrous sodium sulfate to remove any remaining traces of water.

Caution: complete evaporation of the solvent is not allowed when using the rotoevaporator because dissolving the pesticides is very difficult.

1.6.4. Sample analysis

Inject 1 µL of the standard and sample solutions. If necessary, sample dilution is made. The peaks of each pesticide are identified based on retention times and mass spectra. If the results calculated on the basis of the working standard indicate levels of pesticide residues at or above 50 % of the corresponding MRL (maximum residue level), a calibration standard solution is used on the matrix specific to the sample type, prepared by adding to the blank extract amounts corresponding to the intermediate solutions of the pesticides identified in the sample solution so that the peak sizes of this standard reference solution are within ± 25 %. The concentration is automatically calculated by the data processing software (concentration reported in ng/mL).

The method parameters are:

- Injector: sample volume 1 µL, pulsed splitless, temp.225 °C.
- GC Column oven.

Ramp (°C/min.)	Temperature (°C)	Izotherm (min.)
0	90	1
12	160	5
3	250	1
10	280	3

- Transfer line temperature: 280 °C.
- Carrier gas: He with a flow rate of 1 mL/min.

MS parameters

- Ionization source: EI, 230 °C.
- Solvent delay 15 min.

Results

The concentration of an analyte **"x"** in the analyzed sample is calculated taking into account the concentration of the analyte **"x"** determined in the sample extract, by reference to the calibration curve of the respective analyte, which was drawn previously.

$$C_p = \frac{C_{ex} \times V_{ex}}{m \times S} \times R/1000$$

where:

C_p – concentration of analyte **"x"** in the analyzed sample, (mg/kg dry substance);

C_{ex} – the concentration of the same analyte **"x"** in the extract of the analyzed sample, (ng/mL);

V_{ex} – volume of extract obtained in the sample processing stage, (mL);

m_p – sample mass taken for extraction, (g);

S_u – dry substance content of the sample, (%);

R – recovery value.

Unit 1.7. Determination of triazine herbicides in organic fertilizers using HPLC-PDA

Violeta Alexandra Ion,
Oana-Crina Bujor

1.7.1. Method principles

The method is applied for the quantitative determination of residues of triazine herbicides (Atrazine, Ametryn, Hexazinon, Simazine, Simetryn, Propazine, Prometryn, Terbutylazine) from inputs used in organic agriculture (fertilizers) by UPLC-PDA. The minimum detection limit for these triazine herbicide residues is approximately 1 mg/kg dry matter, depending on the compound.

1.7.2. Apparatus and reagent

Reagents

- Ultrapure water Grade 1 (MilliQ with resistivity of 18 MΩ × cm at 25 °C and TOC of 3 ppb), CAS: 7732-18-5;
- Acetone ≥ 99.5%, p.a., CAS: 67-64-1;
- Sodium chloride ≥ 99.8 %, p.a., CAS: 7647-14-5;
- Petroleum ether with boiling point between 40 and 60°C, CAS: 64742-49-0;
- Ethyl acetate > 99%, CAS: 141-78-6;
- Anhydrous sodium sulfate ≥ 99%, p.a., CAS: 7757-82-6;
- Acetonitrile for HPLC, 99.9%, CAS: 75-05-8;

Conditioning anhydrous sodium sulfate: dry for 6 h at 550 °C in the furnace, cool to approximately 200 °C and then **kept** to room temperature in a desiccator containing moisture absorbent material.

Pesticide stock solution (SS) of concentration 1000 µg/mL. Weigh individually to the nearest 0.1 mg an amount of the reference standard to give solutions of 1000 µg/mL in methanol. When weighing, the purity of the standard substances is taken into account. These solutions are stable for 6 months if stored at 4°C in the dark.

⚠️ **Careful! Simazine is only soluble in acetone!**

Pesticide intermediate solution (S1) concentration 100 µg/mL. Pipette 2 mL of the 1,000 µg/mL Hexazinone (SS) solution and 1 mL of the 1,000 µg/mL (SS) solution of the other herbicides into a 10 mL beaker. Make up to volume with methanol. These solutions are stable for 3 months if stored at 4°C in the dark.

⚠️ **Careful! Each herbicide stock solution is ultrasonicated prior to preparation to ensure complete redissolution!**

Pesticide working solution (SL) of concentration 10 µg/mL. Pipette 1 mL of the intermediate solution (S1) of concentration 100 µg/mL into the 10 mL BC. Make up to volume with methanol. This solution is stable for 1 month if stored at 4°C in the dark.

Pipette each calibration standard on the calibration curve (0.2 - 20 µg/mL) for Hexazinone and (0.1 - 10 µg/mL) for the other herbicides.

Apparatus

- 5 mL, 10 mL volumetric flasks;
- Erlenmeyer beakers of 100 mL;
- Pear-shaped round-bottomed balloons;
- SPE silica cartridges;
- Graduated cylinders of 10 mL, 25 mL, 50 mL, 500 mL;
- Micropipette 10 - 100 µL;
- Micropipette 100 - 1,000 µL;
- 0.45 µm syringe filters;
- Vials for autosampler;
- Vortex;
- Refrigerator;
- Rotavapor system;
- SPE system;
- Orbital agitator;
- Analytical balance;
- UPLC system coupled with PDA detector;
- Chromatographic column C18, 4.6 x 100 mm, 5 µm.

1.7.3. Sample preparation

Determine the water and dry matter content of a test sample in accordance with standard moisture determination procedures. A specific amount of water calculated according to equation 1 is added to the sample mass (each prepared sample will have a water mass of 100 g).

$$m_w = 100 - \frac{m_s \times w_w}{100}$$

where:

m_w - *mass of water added, expressed in g;*

m_s – mass of the sample, expressed in g;

w_w – mass fraction of water, expressed in %.

Note: It is important that the addition of water has a fixed ratio (water:acetone:petroleum ether/ethyl acetate of 1:2:1.5). Under these conditions, the organic phase will represent the upper level.

Sample extraction and liquid/liquid distribution

Weigh a 5.0 g sample for analysis on a technical balance (to the nearest 0.1 g) in a 100 mL Erlenmeyer beaker. Add x grams of water and 20 mL of acetone. Close the Erlenmeyer beaker with the appropriate glass stopper and stir for 5 hours using a mechanical stirrer. Mixing of the two phases will be observed during stirring. Then add 3 g of sodium chloride and 15 mL of petroleum ether/ethyl acetate. Stir again for 15 minutes.

Concentration and dissolution of samples

Transfer 14 mL of the organic phase over anhydrous sodium sulfate, positioned in a filter funnel, with a Pasteur pipette into a 50 mL pear-shaped flask, then concentrate on a water bath at a maximum of 40 °C under vacuum using the rotavapor. Add 2 mL of acetonitrile before the sample reaches dryness. The petroleum ether is further evaporated under a stream of nitrogen, without bringing it to dryness, leaving only the

acetonitrile containing the extract of interest. Vortex the acetonitrile extract tube. Add another 2 mL of acetonitrile to the centrifuge tube, vortex, and transfer over the original extract in the glass tube.

Adjust the volume of the extract in acetonitrile to 1 mL and add an additional 1 mL of ultrapure water. Vortex, then filter the sample with a disposable syringe to which a 0.45 µm porosity filter is attached. The filtrate is collected in an autosampler vial. The extract is then injected and subjected to UPLC-PDA analysis.

Note: This method can also be used for the extraction of other compounds such as polychlorinated biphenyls (PCBs) or polycyclic aromatic hydrocarbons (PAHs) from fertilizers.

Note: Fertilizer samples with a high concentration of organic matter may show peak interference. If necessary, the extracts will be purified with the help of adsorbents (silica gel or florisil).

1.7.4. Sample analysis

For the chromatographic analysis of pesticides, the UHPLC chromatographic system with PDA detector will be used. Taking into account the specifications for the analytical chromatography column and the mobile phase in the SR ISO 15662:2009 standard, the following working conditions will be used:

- Injection volume: 10 µL

- Autosampler temperature: 10 °C;
- Column oven temperature: 30 °C;
- FMA1: Water;
- FMB1: Acetonitrile;
- Flow rate: 1 mL/min;
- Spectrum scanning in the range: 210 - 320 nm;
- Scanning wavelengths: 220 nm, 245 nm.
- Gradient:

Time	A1 %	B1 %
Initial	85	15
5	85	15
25	40	60
30	85	15
33	85	15

Results

The concentration of an analyte ”x” in the analyzed sample is calculated taking into account the concentration of the analyte ”x” determined in the sample extract, by reference to the calibration curve of the respective analyte, which was drawn previously.

$$C_p = \frac{C_{ex} \times V_{ex}}{m_p \times S_u} \times R$$

where:

C_p – concentration of analyte x in the analyzed sample, (mg/kg dry substance);

C_{ex} – the concentration of the same analyte x in the extract of the analyzed sample, (ng/mL);
V_{ex} – volume of extract obtained in the sample processing stage, (mL);
m_p – sample mass taken for extraction, (g);
S_u – dry substance content of the sample, (%);
R – recovery value.

Unit 1.8. Determination of dioxins and furans in organic fertilizers using GC-MS/MS

Violeta Alexandra Ion,
Oana-Crina Bujor

1.8.1. Method principles

The purpose of this procedure is to quantitatively determine dioxins (PCDDs) and furans (PCDFs) in inputs used in organic agriculture (fertilizers) by GC-MS/MS.

The method is applied for the quantitative determination of dioxin and furan residues (2,3,7,8-TeCDF; 2,3,7,8-TeCDD; 1,2,3,7,8-PeCDF; 2,3,4 ,7,8-PeCDF; 1,2,3,7,8-PeCDD; 1,2,3,4,7,8-HxCDF; 1,2,3,6,7,8-HxCDF; 2,3 ,4,6,7,8-HxCDF; 1,2,3,4,7,8-HxCDD; 1,2,3,6,7,8-HxCDD; 1,2,3,7,8,9 -HxCDD; 1,2,3,7,8,9-HxCDF; 1,2,3,4,6,7,8-HpCDF; 1,2,3,4,6,7,8-HpCDD; 1 ,2,3,4,7,8,9-HpCDF; OCDD and OCDF) from agricultural inputs using GC-MS QQQ. The minimum limit of determination of the amount of dioxins (PCDD) is 0.0001 mg/kg dry matter. The minimum determination limit for total furans (PCDF) is 0.0001 mg/kg dry matter.

1.8.2. Apparatus and reagent

Reagents

- Toluene, for GC, CAS: 108-88-3;
- n-Nonane, > 99 %, CAS: 111-84-2;

- Hexane ≥ 97%, HPLC, CAS: 60-29-7;
- Anhydrous sodium sulfate, ≥ 99%, p.a., CAS: 7757-82-6.

Conditioning anhydrous sodium sulfate: dry for 6 h at 550 °C in the furnace, cool to approximately 200 °C and then **kept** to room temperature in a desiccator containing moisture absorbent material.

Pesticide stock solution (SS) of concentration 1000 µg/mL. Weigh individually to the nearest 0.1 mg an amount of the reference standard to give solutions of 1000 µg/mL in methanol. When weighing, the purity of the standard substances is taken into account. These solutions are stable for 6 months if stored at 4°C in the dark.

Mix 25 analytes in n-nonane, different concentrations (eg: https://www.lgcstandards.com/IT/en/Modified-Method-8280-Calibration-Solutions-CC5-/p/CIL-EDF-4095-5#multianalytetable).

Standard solutions of dioxins and furans

Stock solution (SS) mix of 25 analytes in n-nonane, different concentrations. It is recommended to purchase ready-made solutions to minimize health risks (see analyte safety data sheet). Dilutions are made to the stock solution to reach 5 concentrations points, the highest being the stock solution.

Apparatus

- 5 mL, 10 mL volumetric flasks;
- Erlenmeyer beakers;
- Separating funnels with a capacity of 500 mL, 1,000 mL;
- Graduated test tubes of 10 mL capacity and PTFE

stoppers;
- Silica and aluminum oxide SPE cartridges;
- 100 mL round bottom flasks;
- 10 mL graduated cylinder;
- Medium porosity filter paper;
- Micropipette 10 - 100 µL;
- Micropipette 100 - 1,000 µL;
- Syringe filters;
- Vortex;
- Rotary evaporator;
- Laboratory mill;
- Refrigerator combine;
- Analytical balance;
- GC MS/MS system;
- Chromatographic column HP-UI 5MS, length 30 m, diameter in. 0.250 mm, 0.25 µm film.

1.8.3. Sample preparation

10 g of soil is spiked with internal standards (SI) containing 13C-labeled compounds (EPA 1613 LCS) as a surrogate. Extraction is performed using an ASE system with instrument settings as follows: 170°C, 1500 psi, 70% vol. washing, 2 static cycles of 6 min and toluene as extraction solvent. Aliquots are evaporated using a rotary evaporator, the solvent is replaced with n-hexane, and the samples are transferred through a multilayer silica gel column pre-equilibrated with 25 mL of n-hexane. After elution with 100 mL n-hexane, evaporate to dryness, then redissolve in 3 mL n-hexane and

transfer through an SPE column containing activated carbon to separate PCDD/Fs. The activated carbon SPE column was prewashed with 50 mL of DCM-hexane mixture (1:1, v/v) to separate the interfering material, and the extract was eluted with 100 mL of toluene. The eluted extract was evaporated to dryness with nitrogen, redissolved in 25 µL nonane, and internal standard with a final concentration of 100 ng/mL was added. The samples are then injected into the GC-MS/MS.

1.8.4. Sample analysis

Inject 3 µL of the standard and sample solutions. If necessary, sample dilution is made. The peaks of each analyte are identified based on the retention times and mass spectrum.

If the results calculated on the basis of the working standard indicate contaminant residue levels at or above 50% of the corresponding maximum residue level (MRL), a sample type-specific matrix calibration standard solution prepared by adding to the blank extract amounts corresponding intermediate solutions of the pesticides identified in the sample solution so that the peak sizes of this standard reference solution are within ± 25 %.

The concentration is automatically calculated by the data processing software (concentration reported in ng/mL).

The method parameters are:

- Injector: sample volume 3 µL, split 5:1, temp. 250°C;
- Transfer line temperature: 280°C;

- Carrier gas: He with a flow rate of 1 mL/min.;
- MS QQQ parameters;
- Ionization source: EI, 230°C;
- Solvent delay 8 min;
- GC Column oven:

Ramp (°C/min.)	Temperature (°C)	Izotherm (min.)
0	120	5
25	250	10
2	260	5
2	285	0
10	320	5

Results

The concentration of an analyte x in the analyzed sample is calculated taking into account the concentration of the analyte x determined in the sample extract, by reference to the calibration curve of the respective analyte, which was drawn previously.

$$C_p = \frac{C_{ex} \times V_{ex}}{m_p \times S_u} \times R/1000$$

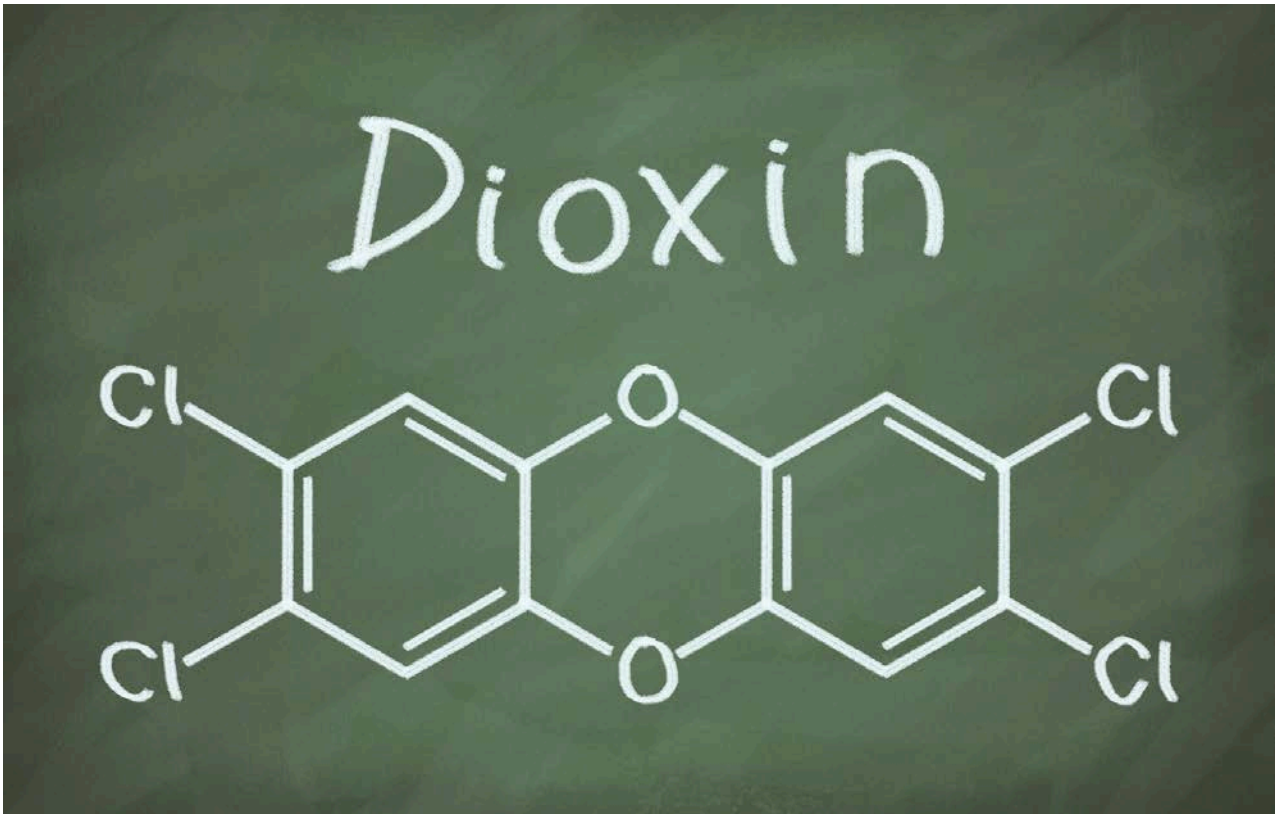
where:

C_p – concentration of analyte x in the analyzed sample, (mg/kg dry substance);

C_{ex} – the concentration of the same analyte x in the extract of the analyzed sample, (ng/mL);

V_{ex} – volume of extract obtained in the sample processing stage, (mL);

m_p – sample mass taken for extraction, (g);
S_u – dry substance content of the sample, (%);
R – recovery value.



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Food Safety Sample Preparation: QuEChERS Method for Pesticides
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- MORE $$$...
SIMPLE!
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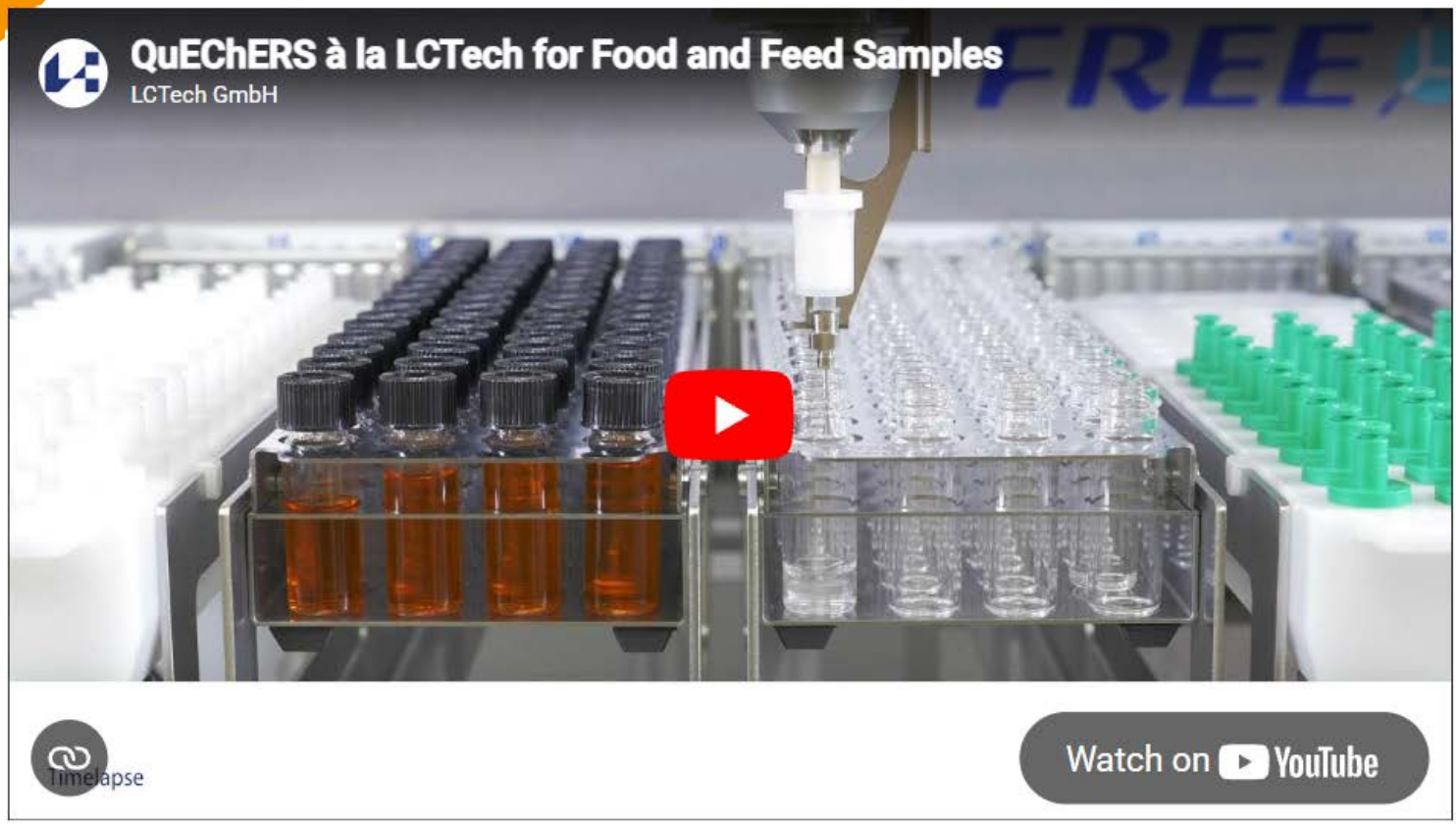
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