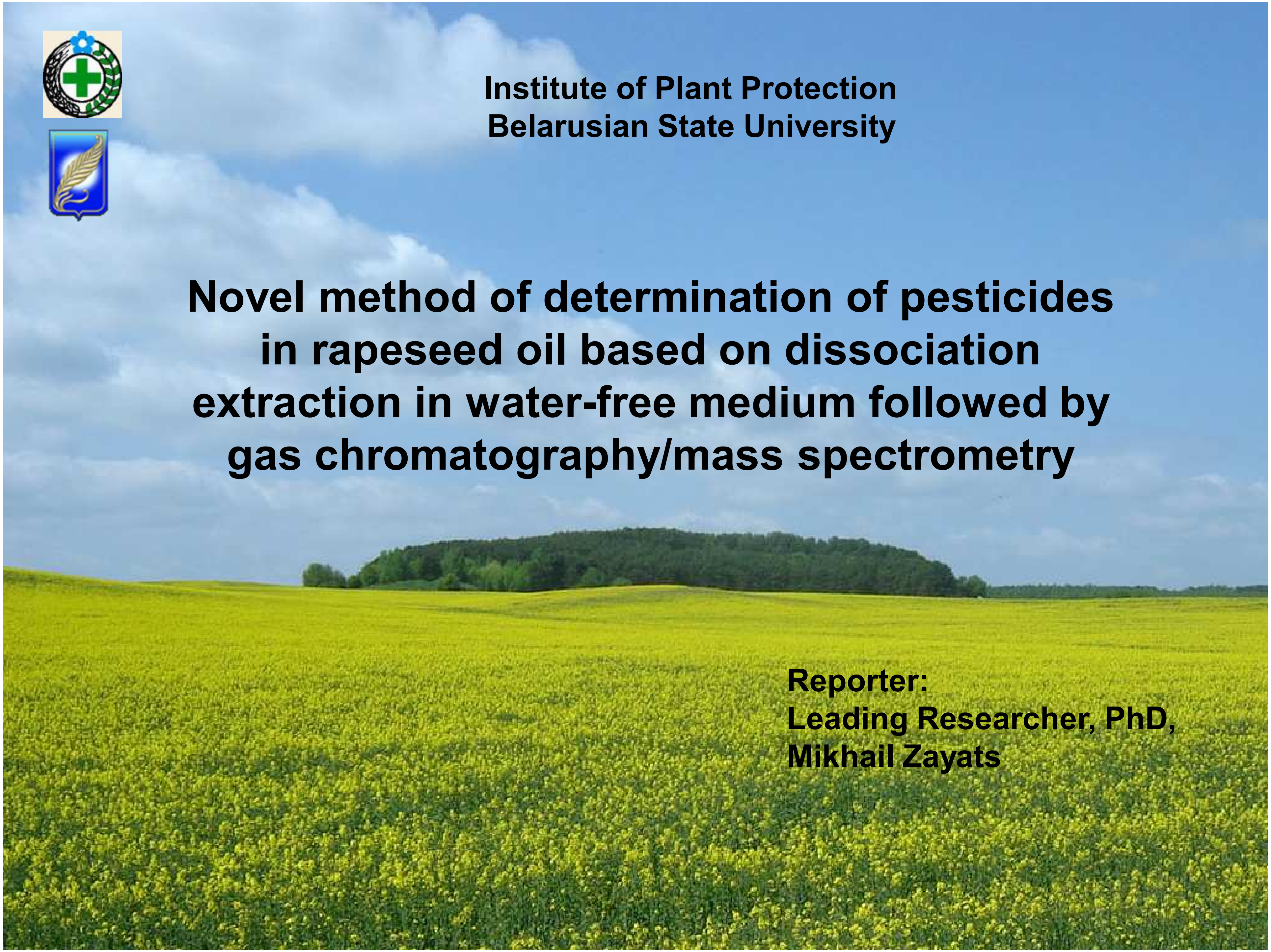
Institute of Plant Protection
Belarusian State University

Novel method of determination of pesticides in rapeseed oil based on dissociation extraction in water-free medium followed by gas chromatography/mass spectrometry



Reporter:
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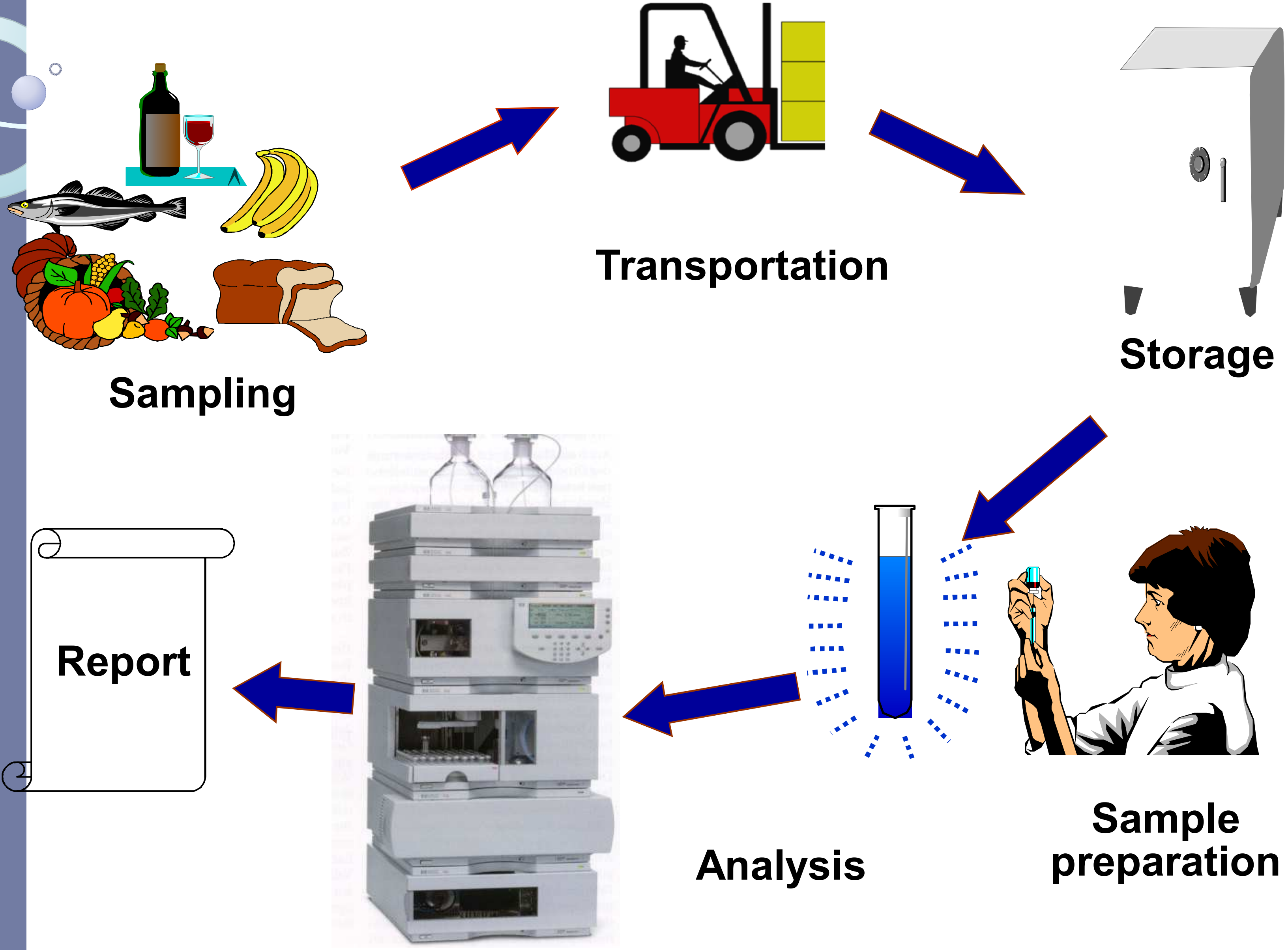
Summary

- ✓ The role of sample preparation in pesticide residues determination
- ✓ The relevance of the development of new methodology of sample preparation for the determination of pesticides in vegetable oils
- ✓ The determination of partition constants and partition coefficients of pesticides in different extraction systems
- ✓ The application of partition constants and partition coefficients for the development of sample preparation techniques

Stages of Pesticide Residues Determination



Sampling

Transportation

Storage

Sample preparation

Analysis

Report

The role of sample preparation in pesticide residues determination

Effectiveness of
Sample preparation

Choice of detection method

Lifetime of the instrument
(maintenance intervals)

Qualitative characteristics
(false positive/negative results)

Quantitative characteristics
(repeatability, reproducibility,
linearity, RSD, etc.)

Signal-to-noise ratio
(LOD, LOQ)

Etc.

Sample preparation techniques for pesticide residues determination in vegetable oils

Extraction with **acetonitrile** with following purification of extracts:

- Low-temperature lipid precipitation
- Gel permeation chromatography
- Solid-phase extraction (column/cartridge)
- QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe)
- Matrix solid-phase dispersion (MSPD)

Main drawbacks of existing sample preparation techniques

“Dirty extracts”	**“Clean extracts”**
Express methods	*Multistage*
	Time-consuming
	Solvent-consuming
	Expensive
	Additional equipment

The aim of the Study

Aim – the development of an improved (simple and effective) extraction sample preparation techniques of vegetable oils for further determination of pesticides of basic nature

Way of reaching the aim – study and application of partition coefficients of pesticides in different extraction systems (model systems and real objects)

Pesticides under study

100 pesticides of different nature:

- *Amides*
- *Anilinopyrimidines*
- *Carbamates*
- *Imidazoles*
- *Morpholines*
- *Neonicotinoids*
- *Organochlorines*
- *Organophosphorus pests*
- *Pyrazoles*
- *Pyrethroids*
- *Pyridazinones*
- *Strobilurins*
- *Thiazoles*
- *Triazines*
- *Triazoles*
- *Etc.*

Extraction systems under study

Model systems:

- Hexane – Acetonitrile (AcN)

- Hexane – 0.01 mol L^{-1} $HClO_4$ in AcN
- Hexane – 0.1 mol L^{-1} $HClO_4$ in AcN

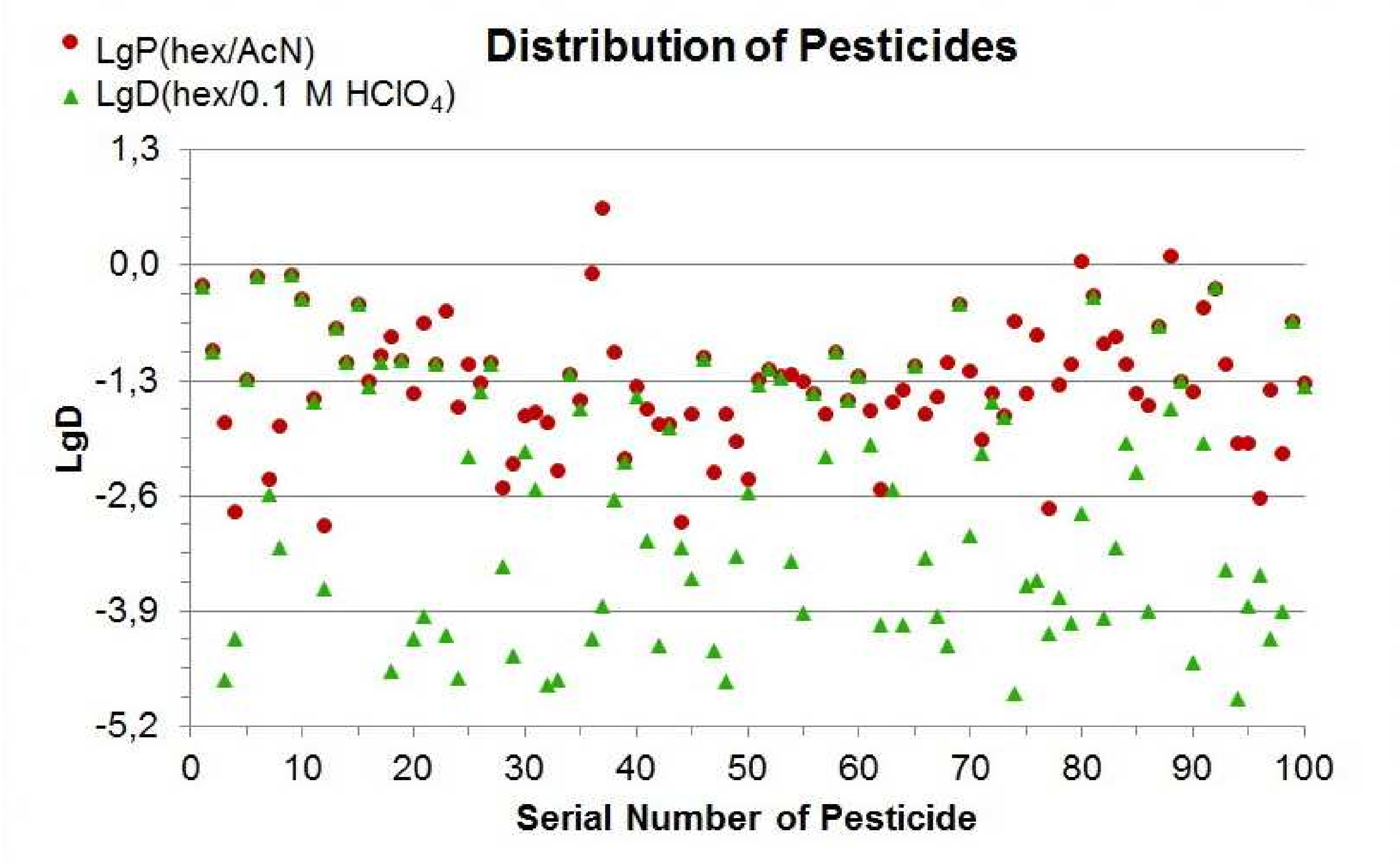
- Hexane – different mixtures of AcN and HCl (aq)

Real systems:

- Rapeseed Oil – Acetonitrile

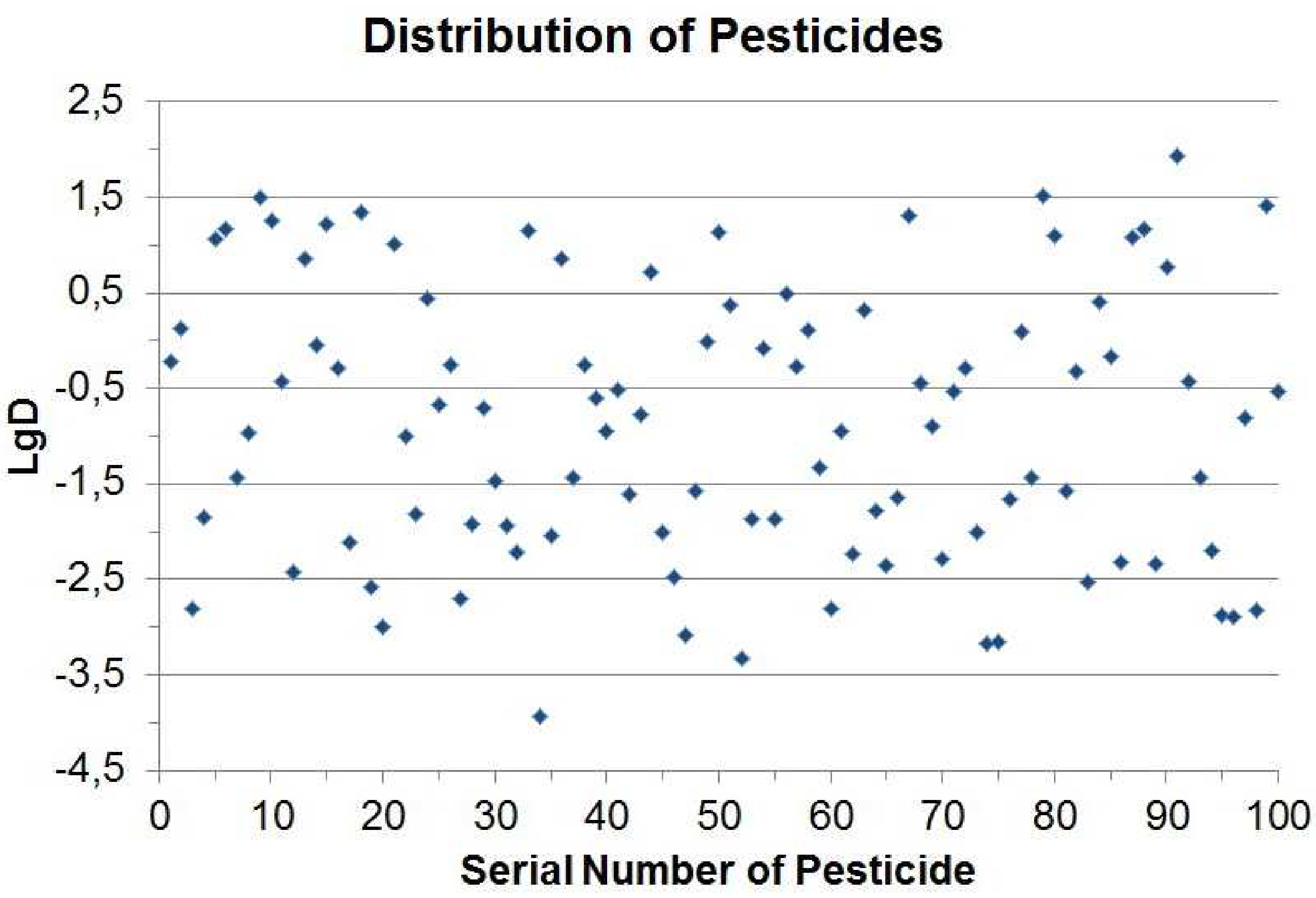
- Solution of Rapeseed Oil in Hexane (33 %) – 0.1 mol L^{-1} $HClO_4$ in AcN

Extraction systems Hexane – Acetonitrile and Hexane – 0.1 mol L^{-1} $HClO_4$ in Acetonitrile



$LgP_{hex/AcN}$(rapeseed oil)=1.80
$LgD_{hex/AcN}$(oleic acid)=0.01
$LgD_{hex/AcN}$(sum of rapeseed oil acids)=0.30

Extraction system Hexane – (Acetonitrile + 1 mol L^{-1} HCl (aq)=1+1)



Recovery and preconcentration of pesticides at a single-step extraction by acetonitrile:

- from hexane

$$R_{AcN/hex} = \frac{1}{D_{hex/AcN} \cdot V_{hex}/V_{AcN} + 1}$$

$$D_{hex/AcN} < 1/19$$

$R_{AcN/hex} \geq 0.95$ at or and $V_{hex}/V_{AcN} = 1$

$$LgD_{hex/AcN} < -1.28$$

- from oil

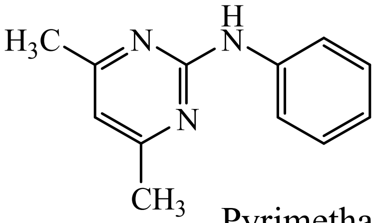
$$LgD_{oil/AcN} = LgD_{hex/AcN} + (0.7 - 1.5)$$

$$LgD_{hex/AcN} < -2.0 \ldots -2.8$$

Pesticides which are protonated in acetonitrile

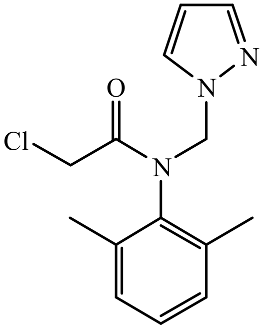
Substances of basic nature – N-containing pesticides
with sufficient electronic density on N atom

Anilinopyrimidines



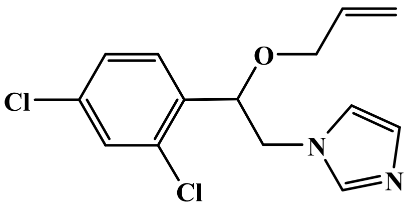
Pyrimethanil

Pyrazoles



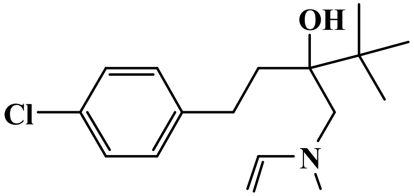
Metazachlor

Imidazoles



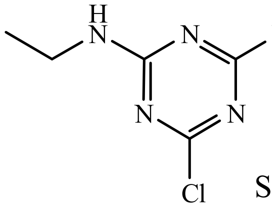
Imazalil

Triazoles



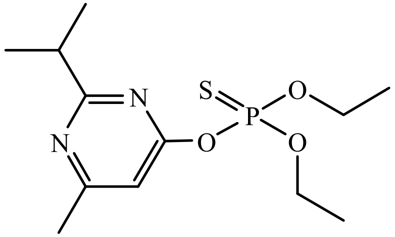
Tebuconazole

Triazines



Simazine

Pyrimidines

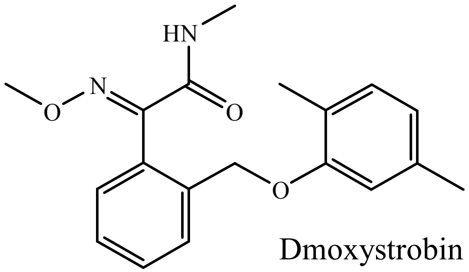


Diazinon

Total amount of protonatable pesticides is 50

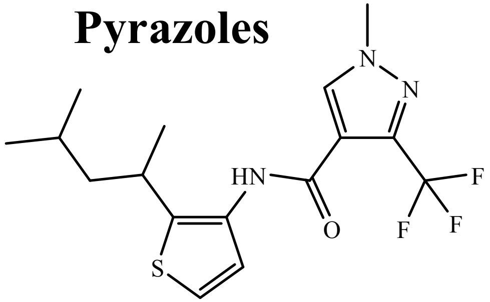
Pesticides which are poorly or not protonated in acetonitrile, but have low LgD

Strobilurins



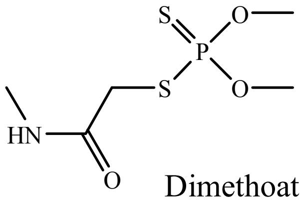
Dmoxystrobin

Pyrazoles



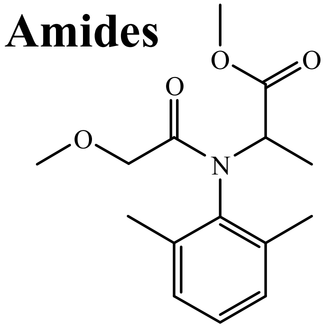
Penthiopyrad

Organodithiophosphates



Dimethoate

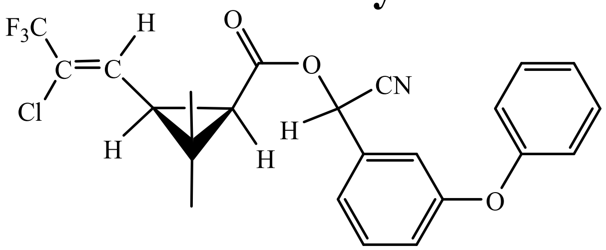
Amides



Metalaxyl

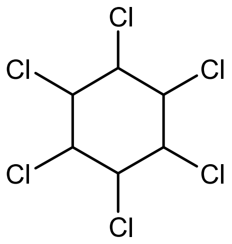
Pesticides which are not protonated in acetonitrile and have LgD>-1.9

Pyrethroids



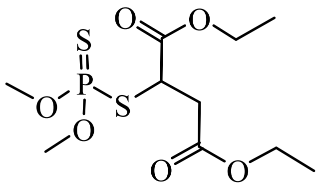
Gamma-cyhalothrin (isomers)

Organochlorines



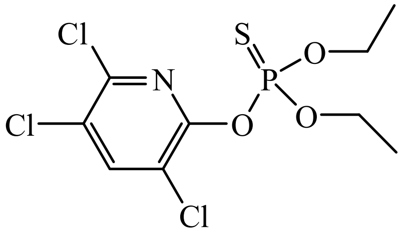
Lindane

Organodithiophosphates



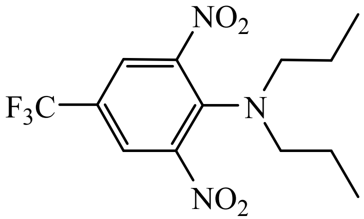
Malathion

Organothiophosphates



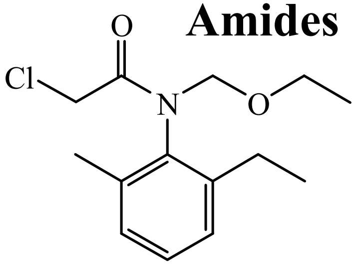
Chlorpyrifos

Dinitroanilines



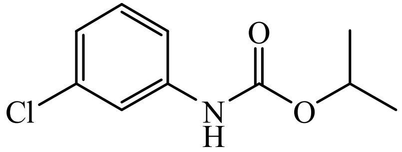
Trifluralin

Amides



Acetochlor

Carbamates



Chlorpropham

Extraction of pesticides from rapeseed oil by acidified acetonitrile in theory

The acidification of acetonitrile with perchloric acid (0.1 mol L^{-1} solution) allows theoretically :

- to use in some cases up to **16 000** times less volume of solvent for oil sample preparation (for fenpropidine, fenpropimorph, pirimiphos methyl) to achieve the same recoveries of basic pesticide residues;
- to carry out at a one-step extraction in some cases **100** times preconcentration of pesticides in acetonitrile from rapeseed oil (cyproconazole, flurtamon, flusilazole, etc.).

Extraction of pesticides from rapeseed oil by acidified acetonitrile in practice

Problems:

- Acetonitrile is partially soluble in oil.
- The separation of acetonitrile and oil phases is very slow.

Possible way to solve the problems:

- Larger volume of acetonitrile should be used for extraction (at least 2-3 mL per 5 mL of oil).
- Oil should be diluted at least 3-4 times by hexane (the density of oil solution in hexane should be less than the density of acetonitrile).

Extraction of pesticides from rapeseed oil by acidified acetonitrile in practice

For convenience of carrying out of the sample preparation the following amounts of sample and solvents can be used:

- 5 g of oil
- 15 mL of hexane
- 3 mL of 0.1 mol L^{-1} perchloric acid in acetonitrile

At these conditions a one-step extraction is enough for quantitative (>95 %) extraction of ~60 pesticides.

But the extract should be purified for further chromatographic analysis.

Purification of extracts

The acetonitrile extract can be purified from hydrophobic matrix components by washing with hexane.

To remove most pigments and residual matrix hydrophobic compounds, the extract can be diluted twice with 1 mol L^{-1} aqueous solution of hydrochloric acid and washed with small amount of hexane (1/3 of extract volume). In this system 55 from 100 pesticides have LgD<-0.5. It means that their loss on this stage is less then 10 %.

Then the extract can be neutralized to pH=7-8), using 10% dipotassium hydrogen orthophosphate (K_2HPO_4) solution and the pesticides can be extracted by dichloromethane (P>500). At this stage hydrophilic substances and remaining higher carboxylic acids are removed.

After that dichloromethane extract should be evaporated to dryness and dissolved in a suitable solvent for chromatographic analysis.

Sample preparation technique

1. Take 5 g of oil
2. Dissolve in 15 mL of hexane saturated with acetonitrile
3. Add 3 mL of freshly prepared 0.1 mol L^{-1} perchloric acid ($HClO_4$) in acetonitrile
4. Shake for 2 minutes
5. After full phase separation, discard the upper layer
6. Wash the lower layer 2 times with 10 mL of hexane saturated with acetonitrile and discard hexane.
7. Add 3 mL of 1 mol L^{-1} HCl (aq) to acetonitrile extract and wash the obtained solution one time with 2 mL of hexane and discard hexane.
8. Add 15 mL of 10 % (wt) dipotassium hydrogen orthophosphate (K_2HPO_4) to acetonitrile extract.
9. Extract basic pesticides twice with 5 mL of dichloromethane (CH_2Cl_2).
10. Combine the dichloromethane extracts and concentrate to about 0.5 mL on a rotary vacuum evaporator and blow out to dryness under a stream of air (or nitrogen).
11. Dissolve the dry residue in 1 mL of acetone and analyze by GC.

Sample preparation technique allow to

1. Obtain “very clean” samples for chromatographic analysis. Mass of residual matrix is less then 1 mg from 5 g of oil.

2. Conduct 5 time preconcentration of 54 pesticides from oil with recovery 85-110 %.

3. Analyze rapeseed oil for pesticide residues with LOD from 0.002 to 0.02 $mg \cdot kg^{-1}$ (below MRL, established in EU countries).

Comparison of developed method with published techniques

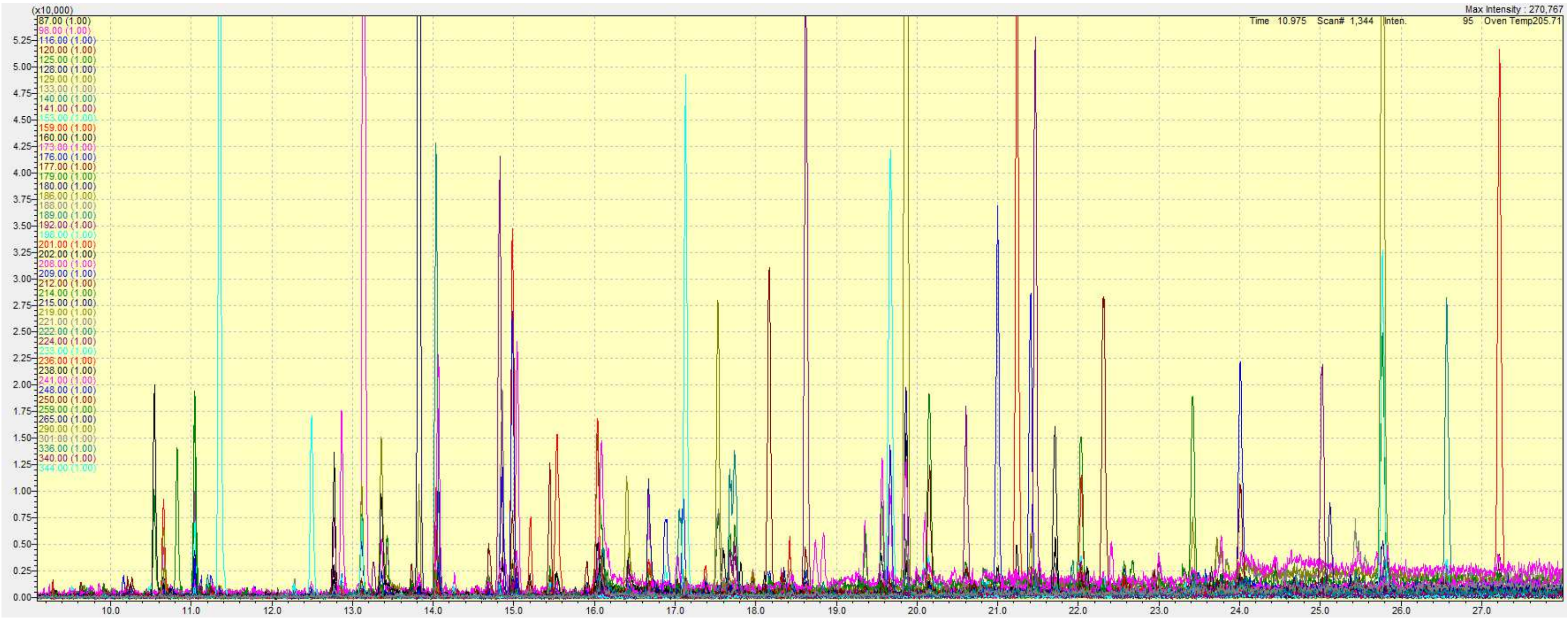
1. The method developed is very simple and consumes only cheap and rather common reagents that allow to perform such technique in any laboratory regardless its equipment. Only flasks, test-tubes and pipets are used for sample preparation.

2. The procedure of sample preparation lasts ~1.5 hour for 4 samples, that is rather quick especially while comparing with GC analysis of one sample (50 min).

3. The final extracts are much more pure in comparison with obtained by one-stage acetonitrile extraction and are clean enough to obtain good chromatograms without cleaning of injector liner at least at 200 injections.

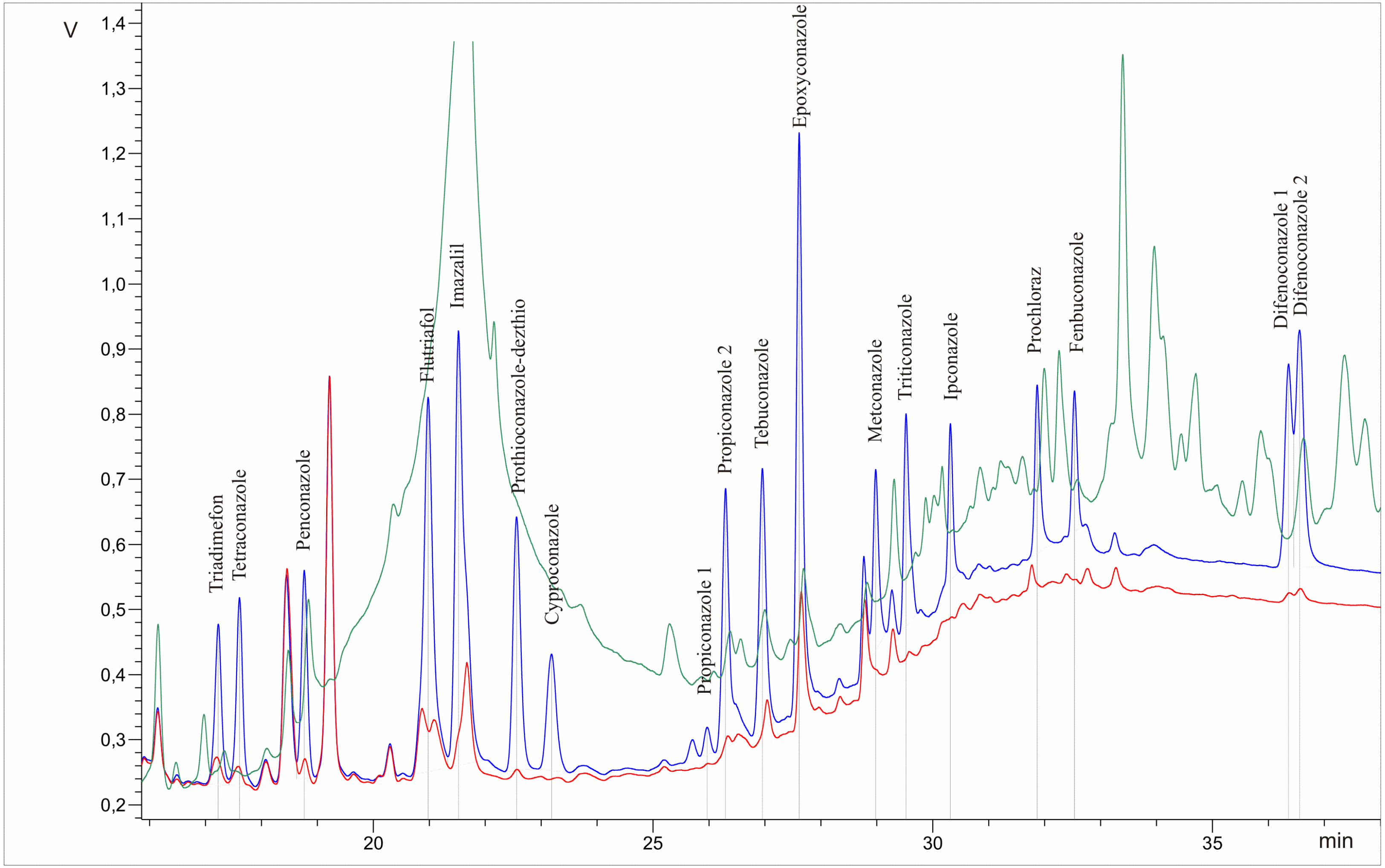
4. In most cases GC-ECD can be used for the pesticide residues determination instead of GC-MS.

GC-MS analysis of rapeseed oil



The overlaid SIM chromatograms of the acetonitrile extract of rapeseed oil with the addition of basic pesticides at a concentration of 0.1 mg·kg^{-1}, prepared by the proposed technique.

GC-ECD analysis of rapeseed oil



The overlaid chromatograms of the acetonitrile extract of rapeseed oil without addition of azole pesticides and without clean-up from co-extractants (green) and of the samples of rapeseed oil with the addition (blue) and without addition (red) of azole pesticides, prepared by the proposed technique.

Conclusion

In this study, we have applied, for the first time, dissociation extraction in a water-free medium as a sample treatment strategy for high-effective complete extraction of pesticide residues of basic nature from rapeseed oil.

The proposed methodology was combined with GC-MS (and GC-ECD) for quantitation of pesticide residues at limits of quantitation ranged from 0.002 to 0.02 μg kg^{-1}. The obtained detection limits are below the maximum residue levels (MRLs) set by the European Union for the majority of pesticides.

Dissociation extraction offers various attractive advantages compared with ordinary solvent extraction. It provides remarkably higher recoveries while using the same amount of extractant or allows consuming much less volume of solvent to achieve the same recoveries of basic pesticides.

The clean-up procedure by hexane from acidified water - acetonitrile mixture with following neutralization and purification step with aqueous solution of dipotassium hydrogen orthophosphate provides removing of acids and hydrophilic substances from extract. The application of this sample preparation strategy, undoubtedly, could be extended to other kinds of vegetable oils and other pesticides with basic properties.

THANK YOU FOR ATTENTION