Voivodeship Sanitary-Epidemiological Station in Warsaw

Inside the Polish National Reference Laboratory - News on SRM

Iwona Bartosiewicz

A laboratory structure of control system for pesticide residues

National Reference Laboratory in Warsaw

5 Official Laboratories in

- Bydgoszcz
- Łódź
- Opole
- Wrocław
- Rzeszów

Types of analyzed samples

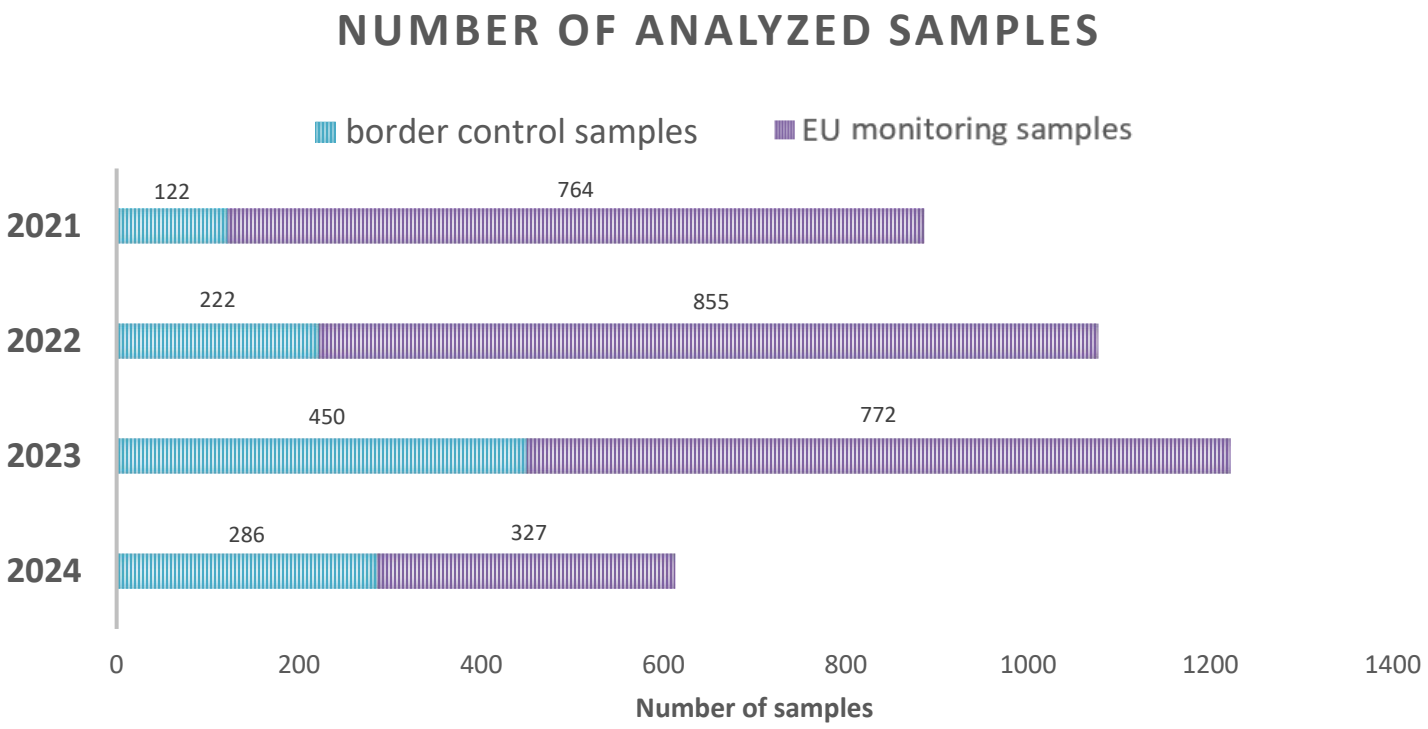
- **Food samples of plant origin**

  Commodity groups:

  - High water content
  - High acid and high water content
  - High oil and very low water content
  - High starch and protein content and low water and fat content
  - High oil content and intermediate water content
  - Difficult and unique

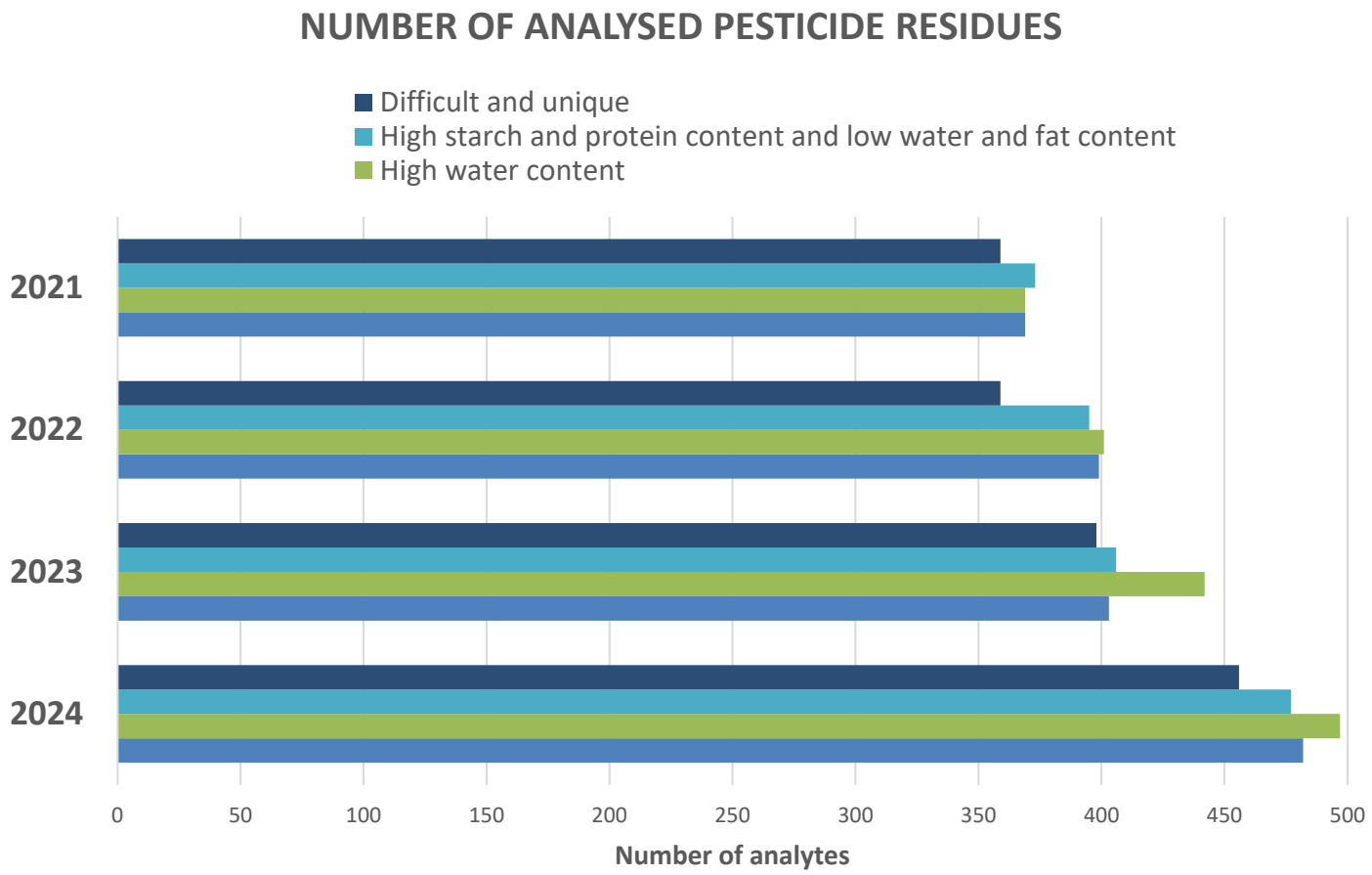
- **Food additives**
- **Water**

Types of analyzed samples (only food)



The total number of samples under official control and monitoring is consistently rising by approx. 15 % each year.

Laboratory progress in pesticide residue determinations



Expanding the scope of pesticide residue determinations in MRM and SRMs up to **nearly 500 compounds,** which covers all mandatory compounds required by EU Regulation 2023/731.

Implemented methods for pesticide residue determination

Technical equipment:

- LC/MS/MS (4) → SRM: Highly polar pesticides (base QuPPE method)
- LC/MS/MS (4) → SRM: Pesticides entailing conjugates or esters in their RD
- LC/MS/MS (4) → SRM/MRM method Multi-residue methods
- GC/MS/MS (3) → SRM/MRM method Multi-residue methods
- GC/MS/MS (3) → SRM: Ethylene oxide
- GC/MS (1) → SRM: Dithiocarbamates
- ICP-MS (1) → SRM: Copper compounds

Laboratory progress in pesticide residues determinations over last three years:

- Implementation of a new method for **ethylene oxide** determination (GC/MS/MS) in food samples, as well as in food additives (HS-GC/MS/MS)
- Implementation of a new method for **highly polar pesticide residues** (QuPPE)
- Implementation of a new method for residues of **pesticides entailing conjugates or esters** in their RD
- Implementation of a new method for **copper compound residues (ICP-MS) after initial microwave mineralization**

Alkaline hydrolysis
2-phenylphenol (sum of 2-phenylphenol and its conjugates, expressed as 2-phenylphenol)

Thermo Scientific Trace 1300 + TSQ 900 (GC-MS/MS) ≈OK

~~Agilent 7890A + 7000 Triple Quad~~
~~Shimadzu GC-2030 + TQ8040NX~~

Tea – GC/MS/MS

Weigh **2 g** of tea sample into a 50 mL centrifuge tube

↓

Add **10 ml of deionized water**, mix and put aside for 15 min.

↓

Add standard (spiked sample) mix and put aside for 30 min.

↓

Add **10,0 ml of ACN**, mix and add **2 ml of 5 M NaOH**.
close tube and shake thoroughly for 1 min by a mechanical shaker

↓

Heat for 1h at 60°C in ultrasound

↓

Cool down, add **2 ml of 5 M H_2SO_4** and **100 µl of HCOOH**
shake thoroughly for 1 min by a mechanical shaker

↓

Add **4 g of $MgSO_4$ + 1 g of NaCl**,
shake and cool the extract (tube) in ice water

↓

Shake thoroughly for 2 min by a mechanical shaker
Centrifuge 5 min at >3000 rpm

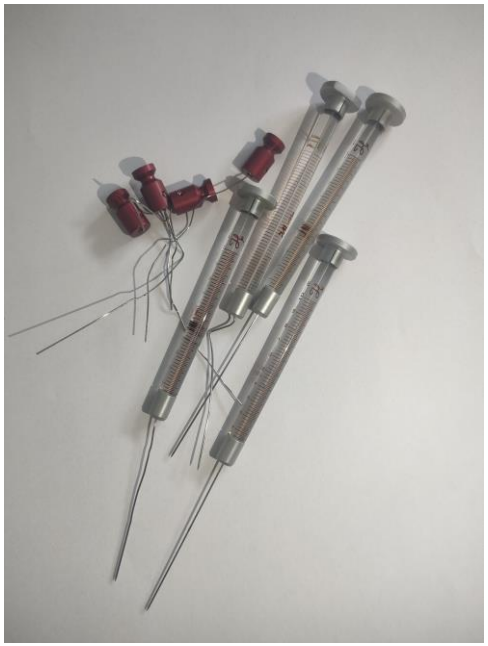
↓

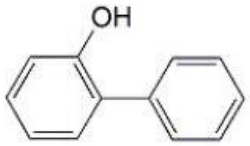
Take the extract and filter through a PTFE syringe filter

↓

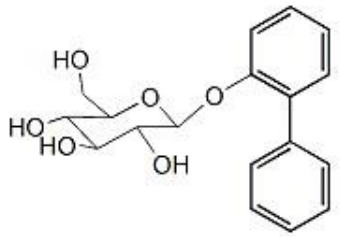
GC-MS/MS

Spiking level	Mean recovery	RSD %
0.05	96.7	11.5
0.25	96.9	6.1
2.0	99.7	4.9



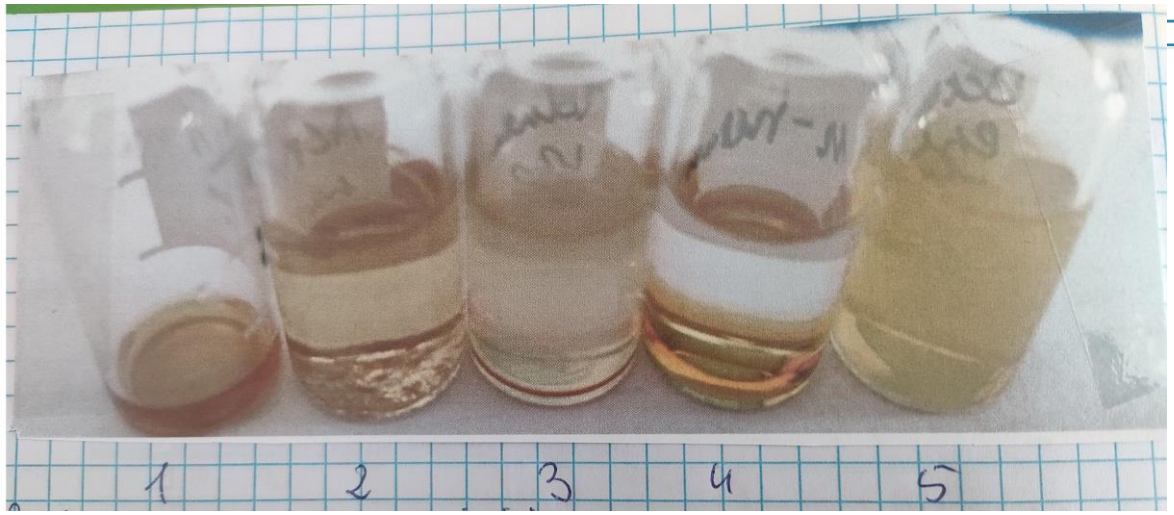


2-Phenylphenol



o-Phenylphenol-glucoside

Dilution / Filtration / Needle wash



1 – raw extract
2 – actonitrile – ”fluffy” precipitate
3 – toluene – a yellow precipitate has precipitated
4 – hexane – two phases
5 – ethyl acetate – turbidity of the extract (probably due to residual water – increase of MgSO4 in the procedure)

*methanol – total dissolution – caused very rapid degradation of the column

Plans – hydrolysis GC/MS/MS

Tea – GC/MS/MS

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↓

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↓

Add standard (spiked sample) mix and put aside for 30 min.

↓

Add **10,0 ml of ACN,** mix and add **2 ml of 5 M NaOH**.
close tube and shake thoroughly for 1 min by a mechanical shaker

↓

Heat for 1h at 60°C in ultrasound

STOP 1

↓

Cool down, add **2 ml of 5 M H_2SO_4** and **100 µl of HCOOH**
shake thoroughly for 1 min by a mechanical shaker

STOP 2

↓

Add **4 g of $MgSO_4$ + 1 g of NaCl**,
shake and cool the extract (tube) in ice water

↓

Shake thoroughly for 2 min by a mechanical shaker
Centrifuge 5 min at >3000 rpm

3)

↓

Take the extract and filter through a PTFE syringe filter

↓

GC-MS/MS

HCl, CH_3COOH

1)

Quantification of Phenolic Acids

CARTRIDGE	SiliaPrepX SAX 6 mL / 200 mg Part Number: SPE-P0010-06G
SAMPLE PRETREATMENT	pH of sample was adjusted to basic value with Sodium Hydroxide 1 N
CONDITIONNING STEP	6 mL of Methanol
EQUILIBRATION STEP	6 mL of water
LOADING STEP	Treated sample was slowly aspirated through the cartridge
WASHING STEP	3 mL of water, then 3 mL of Sodium Hydroxide 0.1 N and 3 mL of Methanol, dry the cartridge
ELUTION STEP	2 x 3 mL of Formic Acid 5 % in Methanol
FURTHER TREATMENT	Evaporation under Nitrogen, reconstitution with Methanol / water and quantification by LC/MS
RECOVERY	at 1 µg/mL

Syringic Acid	70 %
Vanillic Acid	86 %
p-Hydroxybenzoic Acid	97 %

2) Cartridge SPE C-18 (500 mg/6ml) Self-developed procedure for water testing in the laboratory.

Extraction of Bisphenol A, Triclosan & Ethynyl Estradiol from Water

CARTRIDGE	SiliaPrepX HLB 3 mL / 60 mg Part Number: SPE-P0002-03BB
SAMPLE PRETREATMENT	To 25 mL of sample water was added 250 µL of internal standard (*1 ppb of 17α-Ethynyl Estradiol d-6, 1 ppb of Bisphenol A d-16 and 0.4 ppb of Triclosan d-3 in Methanol*)
CONDITIONNING STEP	3 mL of Methanol
EQUILIBRATION STEP	3 mL of Water and 1 mL of Acetic Acid 100 mM
LOADING STEP	Treated sample was slowly aspirated through the cartridge
WASHING STEP	3 mL of Water, 1 mL of Acetic Acid 100 mM and 2 mL of 20 % Methanol in Water, dry the cartridge
ELUTION STEP	2 x 3 mL of Dichloromethane / Acetone (*50:50*)

https://aga-analytical.com.pl/wp-content/uploads/2024/03/silicycle-brosam-brochure-sample-preparation-web-1.pdf

Analysis of acidic pesticide residues in fruit and vegetables with methanol extraction using LC/MS/MS

Weigh a 5 g sample

↓

Add 5 ml of MeOH

↓

Adjust pH of the solution up to 5.5-7.5 using 5 M NaOH

↓

Shake intensively for 15 min
Centrifuge for 5 min at > 3000 rpm

↓

Take V ml of the extract and add V x 25 mg C18

↓

Shake intensively for 2 min
Centrifuge for 1 min at > 3000 rpm

↓

Filter the extracts through a syringe PTFE filter

↓

LC/MS/MS

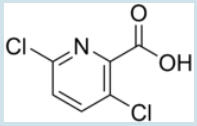
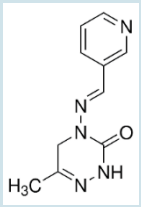
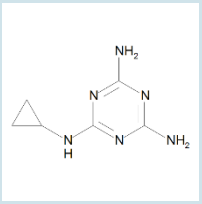
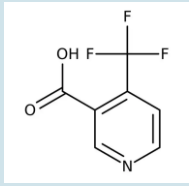
LC/MS/MS measurement parameters

Column:	Agilent ZORBAX Eclipse Plus C18, 2.1x150mm, 1.8 µm,
Eluent A:	H_2O + 0.01% formic acid + 5mM ammonium formate
Eluent B:	Methanol
Analysis time:	45 min

Time [min]	A [%]	B [%]	Flow rate [mL/min]	Max. pressure limit [bar]
0.00	95.0	5.0	0.2	600
0.10	95.0	5.0		
16.00	10.0	90.0		
24.00	10.00	90.0		
24.10	95.0	5.0		

Analysis of acidic pesticide residues in fruit and vegetables with methanol extraction using LC/MS/MS

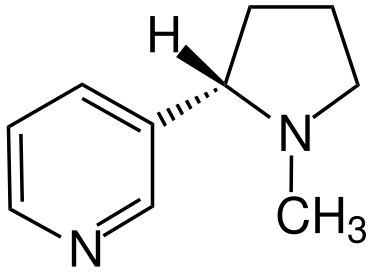
PB/PBP-14

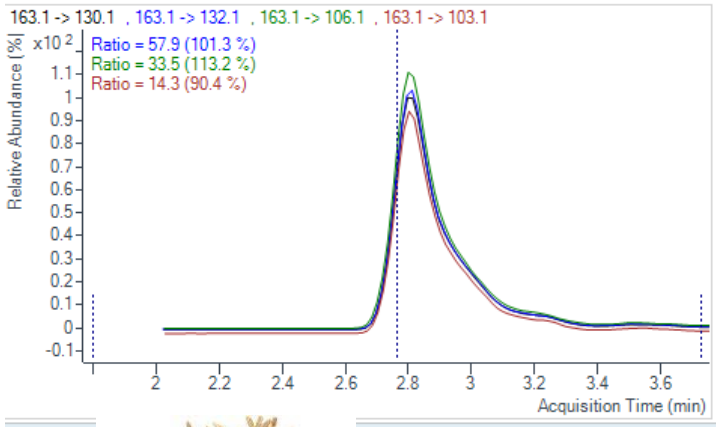
	CLOPYRALID	PYMETROZINE	CYROMAZINE	TFNA
				
pKa	2.32	4.40	5.22	2.62
Mean recoveries in Quechers method	1%	38%	33%	46%

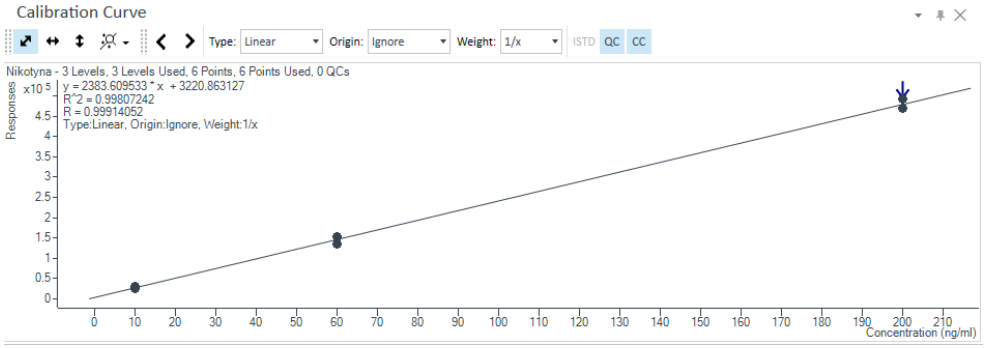
Group I COMPOUND	Mean recovery %	RSD %
Quizalofop-P	85,7	6,9
Clopyralid	96,0	11,7
Cymoxanil	103,5	8,8
Cyromazine	108,1	7,9
Dinotefuran	110,0	9,6
Flonicamid	108,4	10,3
Ioxynil	93,6	8,0
Propamocarb	105,4	9,3
Pymetrozine	102,4	7,6
TFNA	108,6	10,2
TFNG	101,4	13,5
Topramezone	95,0	13,8
Trinexapac	96,5	14,4

dicamba
flumioxazin
metalaxyl-M
MCPB
aldicarb
2,4-D
isoxaflutole
acefat
dinuron
spirotetramat
dichlophos
formetanat
dichlorprop
diethofencarb
135 compound
bentazon
linuron
nintenpyram
sulcotrion
metazachlor
triforin
malaoxon
cymoxanil
MCPA
dinoseb
aldicarb-suflon
paraoxon-methyl
piridafol

Nicotine







Sample					Nikotyna..	Nikotyna Results					Qual...	Qual...	Qual...
Name	Type	Level	Dil.	Acq. Date-Time	Exp. Conc.	RT	Resp.	Calc. Conc.	Final Conc.	Accuracy	Ratio	Ratio	Ratio
MeOH_oiw_MM	Sample		1.0	6/17/2024 10:05 AM		3.5	0	0.0000	0.0000		Accuracy (Percent Accu		
blank_banan_MM	Sample		1.0	6/17/2024 10:51 AM		3.0	0	0.0000	0.0000				
wz10_banan_MM	Cal	1	1.0	6/17/2024 12:37 PM	10.0000	2.8	25287	9.9711	9.9711	99.7	51.4	31.3	12.4
wz60_banan_MM	Cal	2	1.0	6/17/2024 1:23 PM	60.0000	2.8	129297	50.2465	50.2465	83.7	54.7	29.3	12.7
wz200_banan_MM	Cal	3	1.0	6/17/2024 2:16 PM	200.0000	2.8	504886	195.6834	195.6834	97.8	57.6	30.7	14.5
F548MM_1	Sample		1.0	6/17/2024 4:33 PM		2.8	29055	11.4303	11.4303		56.8	31.4	15.1
F548MM_2	Sample		1.0	6/17/2024 5:19 PM		2.8	29729	11.6911	11.6911		59.0	32.7	14.5
WZF548MM	Sample		1.0	6/17/2024 7:37 PM		2.8	29636	11.6552	11.6552		58.7	32.4	13.8
F560MM_1	Sample		1.0	6/18/2024 12:13 AM		2.8	46221	18.0775	18.0775		52.7	29.5	13.6
F560MM_2	Sample		1.0	6/18/2024 12:59 AM		2.8	46451	18.1665	18.1665		56.3	29.7	14.0
WZF560MM	Sample		1.0	6/18/2024 1:45 AM		2.8	56031	21.8761	21.8761		58.4	31.5	14.3
wz10_banan_MM_a	Cal	1	1.0	6/18/2024 4:03 AM	10.0000	2.8	28334	11.1512	11.1512	111.5	57.2	34.9	16.8
wz60_banan_MM_a	Cal	2	1.0	6/18/2024 4:49 AM	60.0000	2.8	156075	60.6153	60.6153	101.0	56.9	32.6	15.2
wz200_banan_MM_a	Cal	3	1.0	6/18/2024 5:35 AM	200.0000	2.8	547882	212.3326	212.3326	106.2	59.2	32.3	14.9

lp.	Compound	Spiking level	Spiking level x LOQ	Bias (systematic error) (%)					Mean$_{recovery}$	SD	RSD %
		mg/kg		test 1	test 2	test 4	test 4	test 5			
1	Nicotine	0.010	1.0	102.9	102.9	103.4	102.4	101.7	102.7	0.7	0.6

Preparation of sample for ethylene oxide (RD) analysis

sesame

Weigh 2 g of a homogenated sesame sample into 50 mL centrifuge tube + 1 ml saturated solution NaCl

Shake Vortex, leave for 15 min.

Add standards (spiked sample) mix and put aside fo 30 min.

Add 10 ml of 0.2 M HCl in ACN

Shake thoroughly for 30 min. by a mechanical shaker

Place the test tube in water ice and add 0.5g $MgSO_4$

Shake for 2 min.
Centrifuge 5 min. at > 3000 rpm

Take 8 ml of extract from the precipitate, add 400 mg C18 and 400 mg PSA (to 8 ml of extract)

Shake for 2 min
Centrifuge 5 min. at >3000 rpm

Take the maximum amount of the extract from above the precipitate.
The extract obtained is ready for analysis GC-MS/MS

spices, cereals, processed cereal-based products
Gum arabic, Xanthan gum, Cellulose gum, Konjac, Tara gum, Konjac, Guar gum, Locust bean gum

Weigh 4 g of a homogenated sample into 50 mL centrifuge tube

Add standards (spiked sample) mix and put aside for 30 min.

Add 10 ml of 0.2 M HCl w ACN

Shake Vortex

Add 1 ml of saturated NaCl solution

Shake thoroughly for 30 min. by a mechanical shaker

Place the test tube in water ice and add 0.5g $MgSO_4$

Shake for 2 min.
Centrifuge 5 min. at > 3000 rpm

Take 4 ml of extract from the precipitate, add 200 mg C18 and 200 mg PSA (to 4 ml of extract)

Shake for 2 min.
Centrifuge 5 min. at >3000 rpm

Take the maximum amount of the extract from above the precipitate.
The extract obtained is ready for analysis GC-MS/MS

Preparation of specific samples for ethylene oxide (RD) analysis

- calcium carbonate
- sodium hydroxymethyl cellulose

$$CaCO_3(s) + 2HCl(aq) \rightarrow CaCl_2(aq) + H_2O(l) + CO_2(g)$$

Weight 4 g of a homogenated sample into 50 mL centrifuge tube

Add 10 ml of cold hexane

Add standard (spiked sample) mix and put aside for 30 min. in a low temperature

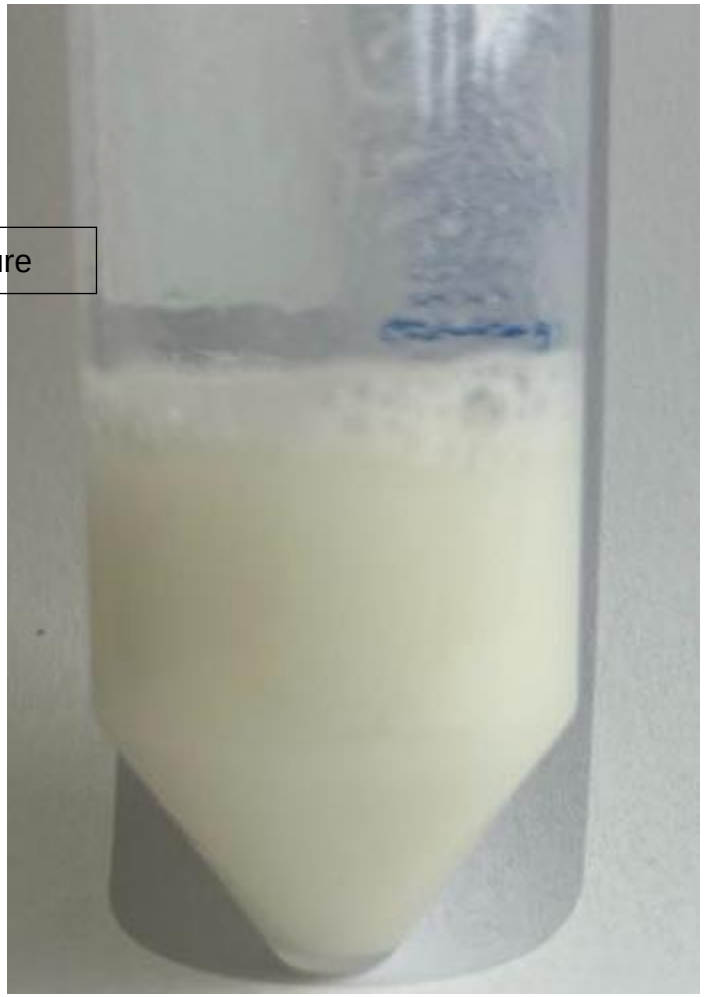
Shake for 2 min.
Centrifuge 5 min. at > 3000 rpm with cooling

Take 5 ml of the extract from the precipitate,
add 125 mg C18, 125 mg PSA and 750 mg $MgSO_4$ (to 5 ml of the extract)

Shake for 2 min.
Centrifuge 5 min. at > 3000 rpm

Take 4 ml of the extract from the precipitate

HS-GC-MS/MS



J&W HP-VOC GC Column, 30 m, 0.20 mm, 1.12

HS-GC-MS/MS

J&W HP-VOC GC Column, 30 m, 0.20 mm, 1.12

Analysis	
Syringe Tool	HS 1
Incubation Temperature	70 °C
Incubation Time	10 min
Syringe Temperature	70 °C
Agitator Speed	250 rpm
Pre Purge Time	0 s
Injector	Injector 2
Injection Flow Rate	25 mL/min
Post Purge Time	60 s
Analysis Time	18 min

Setup	
Gas Chromatograph	GC1
Sync Before Incubation ...	0 min
Agitator	Agitator 1
Do Agitation	False
Heat Agitator	True
Wait For Readiness Agit...	True
Sample Vial Depth	25 mm
Heat Syringe	True
Wait For Readiness Syrin...	True
Injection Signal Mode	PlungerUp
Injector Penetration Dep...	40 mm
Continuous Purge	False

Advanced	
Enable Pre Filling	True
Filling Strokes Count	5
Filling Strokes Volume	1.2 mL
Filling Strokes Aspirate F...	6 mL/min
Delay After Filling Strokes	30 s
Sample Aspirate Flow Ra...	6 mL/min
Sample Post Aspirate De...	0 s
Sample Vial Penetration ...	25 mm/s
Injector Penetration Spe...	25 mm/s
Pre Injection Dwell Time	3 s
Post Injection Dwell Time	10 s
Agitator On Time	5 s
Agitator Off Time	2 s

Temperature: 180.0 ℃
Injection Mode: Split
Sampling Time: 1.00 min

Carrier Gas: He
- Flow Control Mode: Linear Velocity
- Pressure: 151.3 kPa
- Total Flow: 8.0 mL/min
- Column Flow: 1.01 mL/min
- Linear Velocity: 34.5 cm/s
- Purge Flow: 3.0 mL/min
- Split Ratio: 4.0

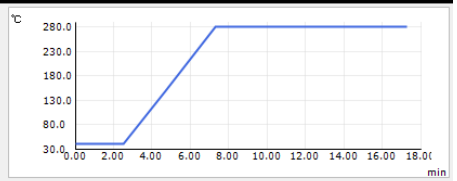
High Pressure Injection
- ◉ Off ○ Auto
- Pressure: 250.0 kPa Time: 2.00 min

Carrier Gas Saver
- ○ On ◉ Off
- Split Ratio: 20.0 Time: 1.00 min

Temperature: 40.0 ℃
Equilibration Time: 0.5 min

Column Information(HP-VOC)
- Column ID:
- Installation Date: 9/7/2021
- Column Max Temp: 280.0 ℃
- Length: 33.0 m
- Inner Diameter: 0.20 mm ID
- Film Thickness: 1.12 µm
- Comment:

Set...



Column Oven Temperature Program: Total Program Time: 17.30 min

	Rate	Temperature	Hold Time
	–	40.0	2.50
1	50.00	150.0	0.00
2	50.00	280.0	10.00
3	0.00	0.0	0.00
4	0.00	0.0	0.00
5	0.00	0.0	0.00
6	0.00	0.0	0.00
7	0.00	0.0	0.00
8	0.00	0.0	0.00

Oven Cooling Rate: Middle 200.0 ℃/min

GCMS-TQ Series

Ion Source Temp. : 225 ℃
Interface Temp. : 200 ℃
Solvent Cut Time : 0 min
☑ Use MS Program : Set...
☑ Acquire Data without Using CID Gas(Q3Scan)

Detector Voltage : ◉ Relative to the Tuning Result ○
0.9 kV
Threshold : 0
GC Program Time : 17.30 min Loop Time...

Ch1 m/z	Ch1 CE	Ch2 m/z	Ch2 CE	Ch3 m/z	Ch3 CE
44.00>14.00	18.00	44.00>29.00	6.00	44.00>28.00	6.00
80.00>31.00	6.00	80.00>44.00	5.00	82.00>31.00	6.00
84.00>33.00	6.00	86.00>33.00	6.00	0.00>0.00	0.00

	Compound Name	Start Time (min)	End Time (min)	Acq. Mode	Event Time(sec)	Scan Speed
1-1	Ethylene oxide	1.20	4.00	MRM	0.100	
2-1	2-chloroethanol	4.00	16.50	MRM	0.100	
2-2	2-chloroethanol-d4	4.00	16.50	MRM	0.100	

Product	Number of analyses in 2023	Number of detections	Content (range) [mg/kg]
Beta carotene	13		
Brown Rice	63		
Cellulose Gum	6		
Chili powder	2		
Coriander	1		
Cumin seeds	2		
Curry	1		
Fenugreek	2		
Ginger	3	1	0.024
Guar gum	43	1	0.039
Gum arabic	7		
Konjac	1		
Locust bean gum	9		
Nigella seeds	6		
Noodles & instant soups	103	6	(0.017-0.056)
Nutmeg	1		
Paprika powder	8		
Pepper	5	2	(0.015-0.066)
Sesame	89		
Turmeric powder	11		
Xanthan gum	1		
Xanthan gum	37	12	(0.011-0.055)
Total	414	22	

Ethylene oxide (RD)
LOQ = 0.011 mg/kg
MRL = 0.1 mg/kg

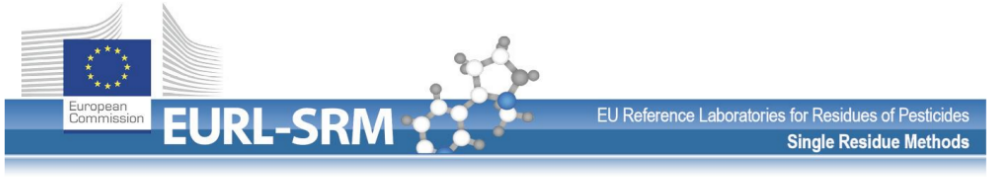
+ 8 dietary supplements

LGC – FC306 - 857
Ethylene oxide
in sesame paste
(z-score 0.0)

PROOF-ACS GmbH
Ring test P2201-RT
Ethylene oxide
in locust bean gum
(z-score 0.2)

LGC - FC316 - 868 -
Ethylene oxide
in spices
(z-score 0.0)

Own testing procedure, based on the QuPPe document



Quick Method for the Analysis of Highly Polar Pesticides in Food Involving Extraction with Acidified Methanol and LC- or IC-MS/MS Measurement

I. Food of Plant Origin (QuPPe-PO-Method)

Version 12 (22.07.2021, Document History, see page **98**)

Check for latest version of this Method under www.quppe.eu ; older versions: obsolete versions

Authors: M. Anastassiades; A.-K. Wachtler; D. I. Kolberg; E. Eichhorn; H. Marks; A. Benkenstein; S. Zechmann; D. Mack; C. Wildgrube; A. Barth; I. Sigalov; S. Görlich; D. Dörk; G. Cerchia

EU Reference Laboratory for pesticides requiring Single Residue Methods (EURL-SRM)

Contact: CVUA Stuttgart, Schaflandstr. 3/2, DE-70736 Fellbach, Germany, www.eurl-pesticides.eu; EURL@cvuas.bwl.de

Note: Changes from V11.1 to V12 are highlighted in yellow

- Eluent A 1% formic acid in water + 5% methanol
- Eluent B 1% formic acid in methanol
- 1:10 dilution
- Injection volume - 25uL

	Pesticide	[mg/kg]
1.	AMPA	0,050 – 2,5
2.	Chlorate	0,050 – 2,5
3.	Chlormequat	0,010 – 0,50
4.	Ethephon	0,050 – 35,6
5.	HEPA (ethephon-hydroxy)	0,020 – 15,0
6.	Fosetyl-Al	0,20 – 10,0
7.	Glyphosate	0,10 – 20,0
8.	Glufosinate ammonium	0,030 – 1,5
9.	Maleic hydrazide	0,10 – 40,0
10.	Bromide ion	1,0 – 40,0
11.	Trimethyl-sulfonium cation	0,10 – 1,0
12.	Cyanuric acid	0,040 – 2,0
13.	Phosphonic acid	0,10 – 60,0
14.	Matrine	0,020 – 0,40
15.	Mepiquat	0,010 – 0,50
16.	MPPA	0,010 – 0,50
17.	N-acetyl-glyphosate	0,030 – 1,5
18.	N-acetyl-glufosinate (NAG)	0,010 – 0,50

Residue findings of QuPPe-compounds

Compound	Number of samples		
Chlormequat	**<= MRL**	**> MRL**	**Compliant due to the uncertainty interval**
Pears	3		
Wheat flour	1		
Rye flour	2		
Brown rice	1	1	2
Rye grain	18		
Glyphosate	**<= MRL**	**> MRL**	**Compliant due to the uncertainty interval**
Onions	1		
Beans	8		
Pears	1		
Maleic hydrazide	**<= MRL**	**> MRL**	**Compliant due to the uncertainty interval**
Onions	28		
Potatoes	1		
Mepiquat	**<= MRL**	**> MRL**	**Compliant due to the uncertainty interval**
Pears		2	
Rye grain	1		
Chlorate	**<= MRL**	**> MRL**	**Compliant due to the uncertainty interval**
Beans	1		
Bromide ion	**<= MRL**	**> MRL**	**Compliant due to the uncertainty interval**
Oranges	1		

Cyanuric acid	Number of samples
Beans	1
Pears	3
Kiwi	2
Potatoes	11
Phosphonic acid	**Number of samples**
Pears	26
Kiwi	7
Carrots	4
Cucumbers	1
Oranges	21
Potatoes	4
Fosetyl	**Number of samples**
Pears	14
Carrots	2
Cucumbers	1
Oranges	7
Potatoes	2

Copper compounds – a high-pressure microwave system

Weigh 0.5 g of a sample

↓

Add 5 ml of HNO_3 + 2ml of H_2O_2

↓

Transfer the solution after digestion with deionized water into a 50 ml plastic tube

↓

Take the required volume into a 15 ml tube

↓

Perform ICP-MS analysis

Washing only with high purity nitric acid V, preferably distilled.

No. step	Time [min.]	MW [W]	Temperature [°C]
1	20	1800	210
2	15	1800	210
3	20	0	30

Copper compounds – ICP-MS

We determine Cu-63 in the KED mode.
We use an internal standard – indium or germanium.
The matrix effect is small.
Calibration standards in a solution of 1% nitric acid.

EUPT-SRM 19
Copper in grape
(z-score 0.2)

FAPAS FCCM45-FRU51
Copper in Grapefruit Purée
(z-score 0.5)

Purchases and plans to develop methods

- Amitrol
- Oxymatrine,
- Diketonitrile-metabolite

- Dinoterb (sum of dinoterb, its salts and esters, expressed as dinoterb)
- Fluroxypyr (sum of fluroxypyr, its salts, its esters, and its conjugates, expressed as fluroxypyr)
- Quizalofop (sum of quizalofop, its salts, its esters (including propaquizafop) and its conjugates, expressed as quizalofop (any ratio of constituent isomers)


Thank you for your attention