Implementation of CIR (EU) 2021/808 Illustration on MRL compounds

Piotr Jedziniak

Department of Pharmacology and Toxicology, National Veterinary Research Institute in Puławy, Poland

National Reference Laboratory for chemical residues in food of animal origin

SARAF
School for Advanced Residue Analysis in Food
20 December 2023 - 9:00-12:00 CET
NANTES, FRANCE
WEBINAR ON EU REGULATION 2021/808 - PART III

National Veterinary Research Institute (NVRI, PIWet), Pulawy, Poland

Established in 1945 as a research and development institution, supervised by the Minister of Agriculture of Poland.

National Veterinary Research Institute (NVRI, PIWet), Pulawy, Poland

The main mission of the Institute -to do scientific research in the field of :

- Health protection of livestock with particular emphasis on infectious and invasive diseases
- Zoonotic diseases (zoonoses) and zoonotic agents
- Hygiene of food of animal origin and feed

PIWet

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Department of Pharmacology and Toxicology NVRI

- **National Reference Laboratory** for residues according to (old) 96/23 directive (vet drugs, homones, pesticides, elements, mycotoxins, feed additives) in food and feed.
- **Development of new analytical methods**, organization of proficiency tests, scientific research in the field of food safety
- **Certificate of accreditation AB 485** (since 2012 accreditation of tests in the flexible system)
- **45 accredited procedures (SOPs)** including 40 based on MS and MS/MS techniques

Department of Pharmacology and Toxicology NVRI

- 13 research scientists
- 38 laboratory technicians

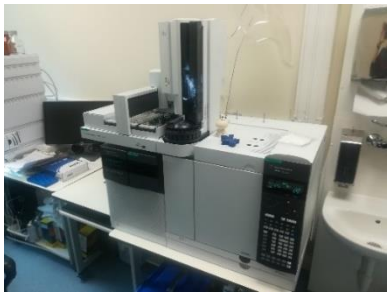
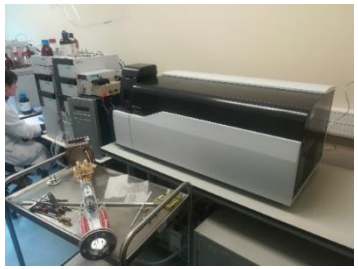
- 11 research teams:
  - **residues of nitroimidazoles and dyes**
  - **residues of antibacterial drugs**
  - **residues of prohibited drugs**
  - **residues of antiparasitic and anti-inflammatory drugs**
  - **residues of hormones and thyreostatics**
  - pesticides residues
  - mycotoxins content
  - content of elements
  - toxicological diagnostics
  - in vitro and in vivo toxicology

Department of Pharmacology and Toxicology NVRI

ICP-MS (2x) Agilent
GC-MS (2x) Agilent
GC-MS/MS (2x) Agilent
LC-MS/MS:
- **Sciex API 4000 (2x)**
- **Shimadzu LC8050**
- **Sciex Qtrap 4500**
- **Sciex Qtrap 5500 (3x)**
- **Sciex Qtrap 6500**
- **Sciex Qtrap 7500**

LC-HRMS (Thermo Orbitrap 120)





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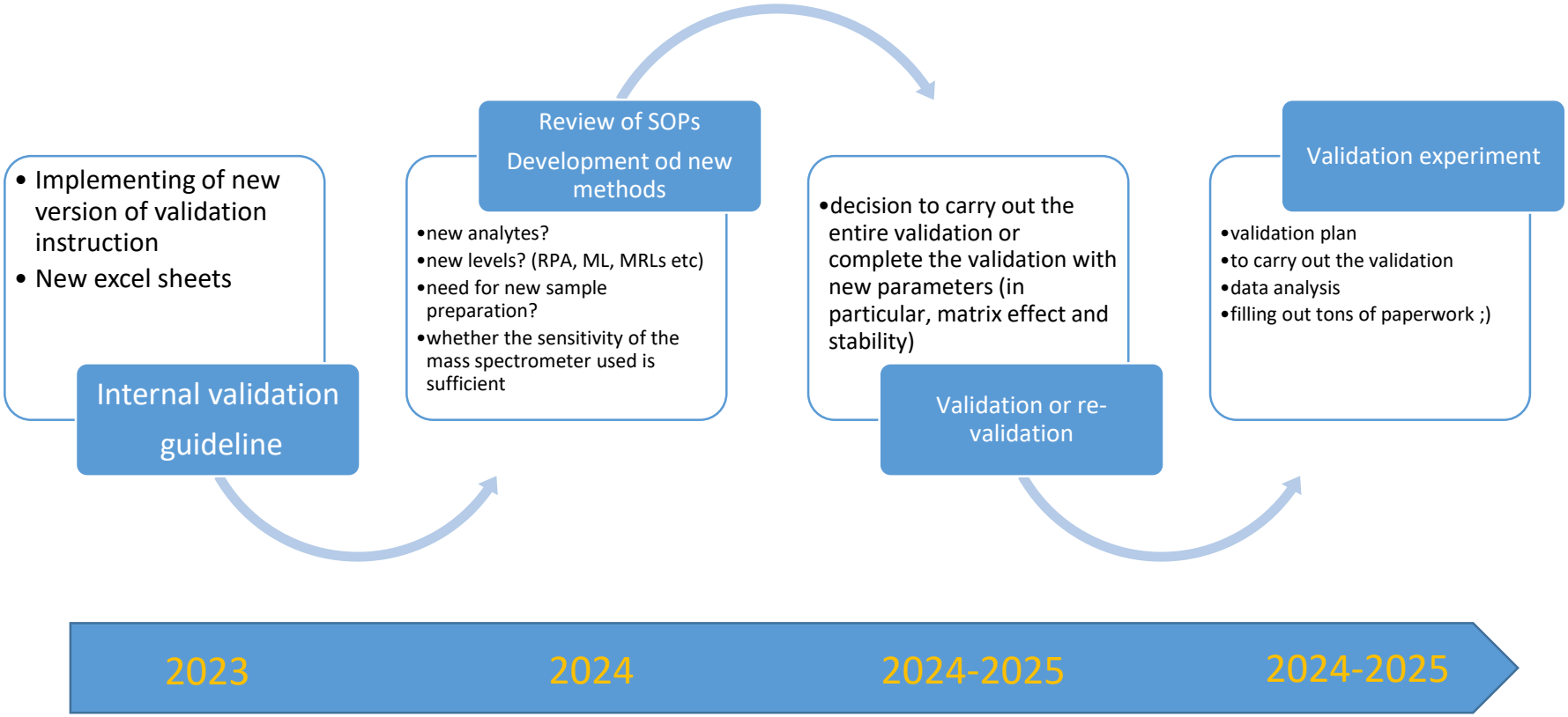
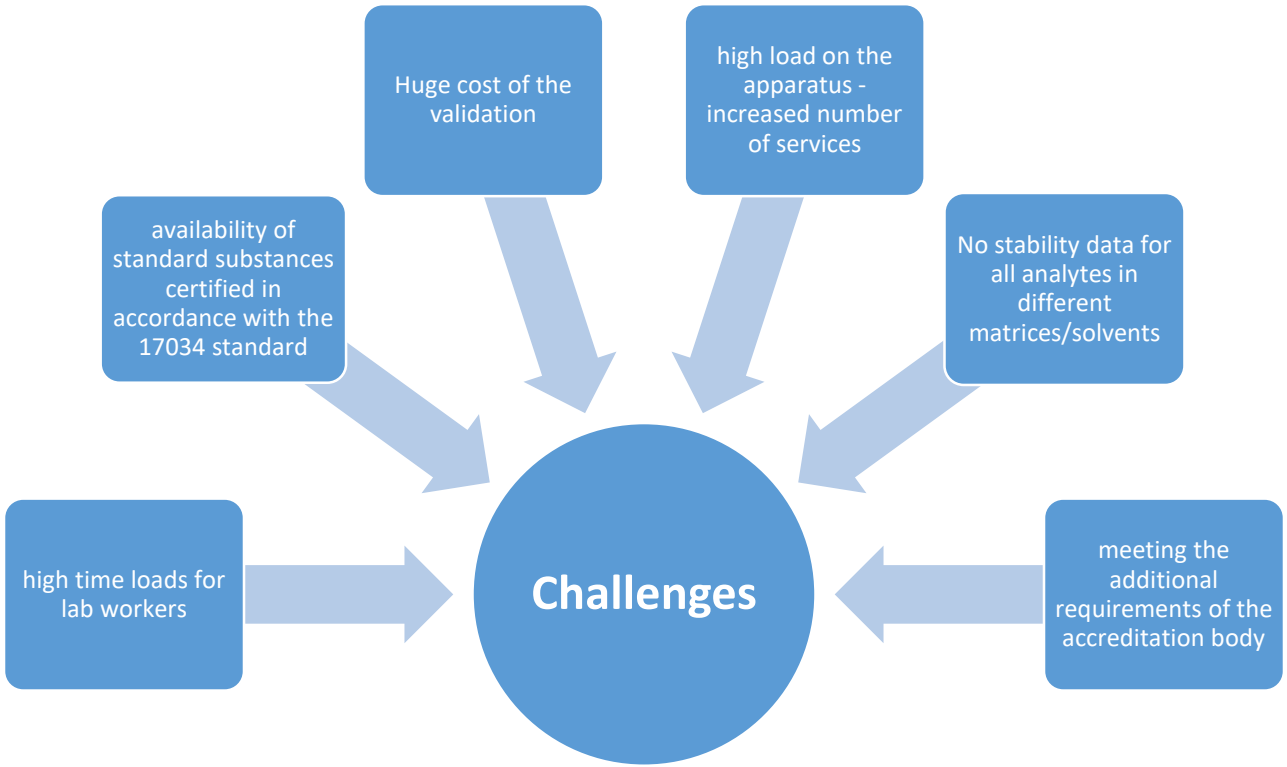
List of the SOPs +numer of analytes

Milestones of implementation of 2021/808



challenges

Submit to *Challenges*



Stability

Need for common base of stability data!

Meeting the requirements of the accreditation body

Uncertainty, LODs,

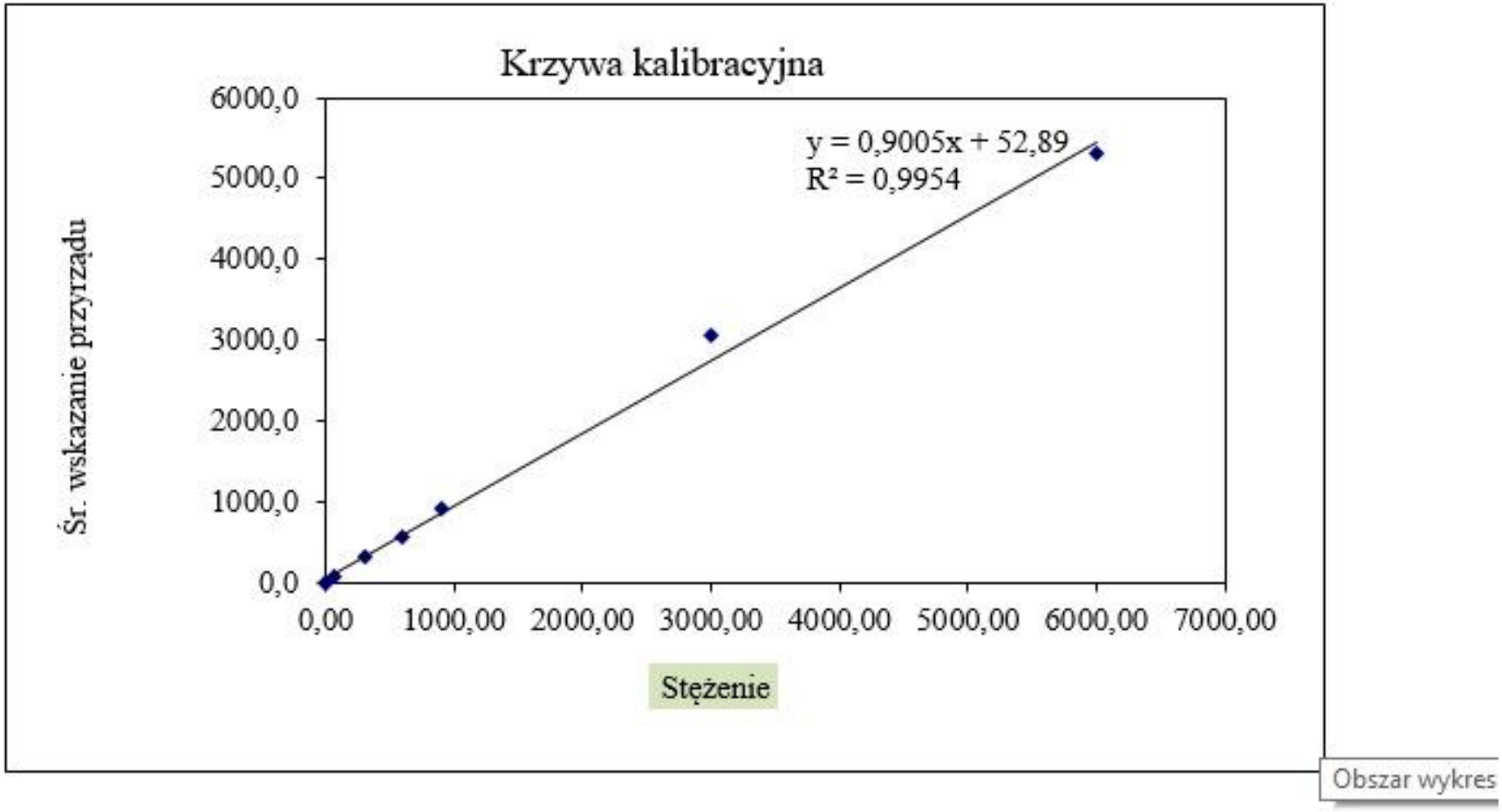
Practical example of validation MRL substance

Scheme of validation experiment

Practical example of validation MRL substance

Pharmacologically active Substance	Marker residue	Animal Species	MRL	Target Tissues	Other Provisions (*according to Article 14(7) of Regulation (EC) No 470/2009*)	Therapeutic Classification
Doxycycline	Doxycycline	Bovine	100 µg/kg 300 µg/kg 600 µg/kg	Muscle Liver Kidney	Not for use in animals from which milk is produced for human consumption.	Anti-infectious agents/Antibiotics
		Porcine, poultry	100 µg/kg 300 µg/kg 300 µg/kg 600 µg/kg	Muscle Skin and fat Liver Kidney	Not for use in animals from which eggs are produced for human consumption.	

Linearity


Krzywa kalibracyjna
y = 0,9005x + 52,89
R² = 0,9954
Śr. wskazanie przyrządu
6000,0
5000,0
4000,0
3000,0
2000,0
1000,0
0,0
0,00
1000,00
2000,00
3000,00
4000,00
5000,00
6000,00
7000,00
Stężenie
Obszar wykres

Matrix effect

Nr procedury badawczej:	ZFT/PB/02-20
Nr ewidencyjny przyrządu pomiarowego:	ZFT/PP/528/S
Technika analityczna	LC-MS/MS
Analit:	Doksycyklina
Matryca	mięso/ pr. mięsne (nerka)
MRL (MLP)	600 µg/kg

	MF (wzorzec)		MF (IS)		MF(wzorzec znormalizowany dla IS)
1	1,078212291	1	0,975687104	1	1,105079986
2	0,957894737	2	0,965714286	2	0,991902834
3	1,016304348	3	0,9810901	3	1,03589298
4	1,103030303	4	0,957422325	4	1,152083333
5	1,016574586	5	0,992018244	5	1,024753921
6	1,016393443	6	0,936681223	6	1,085100692
7	0,913265306	7	0,899774775	7	1,014993231
8	0,978142077	8	0,88952164	8	1,099627072
9	1,005494505	9	0,90326087	9	1,113182846
10	1,152941176	10	0,9213732	10	1,251329186
11	0,983870968	11	0,829718004	11	1,185789585
12	1,058139535	12	0,706243603	12	1,49826424
13	0,923913043	13	0,772391992	13	1,196171185
14	0,908163265	14	0,781350482	14	1,162299488
15	1,010869565	15	0,761609907	15	1,327279958
16	1,021505376	16	0,758996728	16	1,345862687
17	1,039106145	17	0,897377423	17	1,157936581
18	0,87755102	18	0,741100324	18	1,184119062
19	1,005464481	19	0,821164021	19	1,22443806
20	1,076923077	20	0,838187702	20	1,284823285

Kalkukacja	
x	1,17
sd	0,12
CV	10%

Conclusion
Matrix effect less than 20% - requirements mett

Repeatability

Spiked level[µg/kg]											
	60,000	µg/kg			600,00	µg/kg			900,000	µg/kg	
Oznaczone stężenia w kolejnych oznaczeniach[µg/kg]											
Powtórzenia	Dzień 1	Dzień 2	Dzień 3	Powtórzenia	Dzień 1	Dzień 2	Dzień 3	Powtórzenia	Dzień 1	Dzień 2	Dzień 3
1	58,40	62,90	51,40	1	596,00	648,00	600,00	1	828,00	910,00	836,00
2	57,50	63,10	59,00	2	549,00	517,00	576,00	2	931,00	963,00	808,00
3	60,70	62,70	63,60	3	614,00	617,00	605,00	3	868,00	934,00	898,00
4	56,50	64,80	60,60	4	592,00	636,00	564,00	4	943,00	887,00	917,00
5	56,60	60,60	62,70	5	581,00	623,00	547,00	5	818,00	1030,00	998,00
6	60,70	61,40	63,60	6	623,00	586,00	634,00	6	852,00	863,00	800,00
Powtarzalność											
x[µg/kg]	58,400	62,583	60,150	x[µg/kg]	592,50	604,50	587,67	x[µg/kg]	873,33	931,17	876,17
sr[µg/kg]	1,910	1,458	4,656	sr[µg/kg]	26,19	47,71	31,46	sr[µg/kg]	52,50	59,72	76,22
CV (%)	3,3	2,3	7,7	CV (%)	4,4	7,9	5,4	CV (%)	6,0	6,4	8,7
Recovery (%)	97,3	104,3	100,3	Recovery (%)	98,8	100,8	97,9	Recovery (%)	97,0	103,5	97,4

Within-laboratory reproducibility

Spiked level[µg/kg]											
	60,000	µg/kg			600,00	µg/kg			900,000	µg/kg	
Determined concentrations[µg/kg]											
Repetitions	Day 1	Day 2	Day 3	Repetitions	Day 1	Day 2	Day 3	Repetitions	Day 1	Day 2	Day 3
1	58,40	62,90	51,40	1	596,00	648,00	600,00	1	828,00	910,00	836,00
2	57,50	63,10	59,00	2	549,00	517,00	576,00	2	931,00	963,00	808,00
3	60,70	62,70	63,60	3	614,00	617,00	605,00	3	868,00	934,00	898,00
4	56,50	64,80	60,60	4	592,00	636,00	564,00	4	943,00	887,00	917,00
5	56,60	60,60	62,70	5	581,00	623,00	547,00	5	818,00	1030,00	998,00
6	60,70	61,40	63,60	6	623,00	586,00	634,00	6	852,00	863,00	800,00

x[µg/kg]	60,38		x[µg/kg]	594,89	x[µg/kg]	893,56
sR[µg/kg]	3,3452		sR[µg/kg]	34,86	sR[µg/kg]	65,72
CV (%)	5,5		CV (%)	5,9	CV (%)	7,4
Recovery (%)	100,63		Recovery (%)	99,1481	Recovery (%)	99,284

CCalfa

Repetitions	Sample spiked at MRL level	
1	596,0	µg/kg
2	549,0	µg/kg
3	614,0	µg/kg
4	592,0	µg/kg
5	581,0	µg/kg
6	623,0	µg/kg
7	553,0	µg/kg
8	618,0	µg/kg
9	569,0	µg/kg
10	620,0	µg/kg
11	625,0	µg/kg
12	612,0	µg/kg
13	538,0	µg/kg
14	587,0	µg/kg
15	578,0	µg/kg
16	620,0	µg/kg
17	536,0	µg/kg
18	560,0	µg/kg
19	598,0	µg/kg
20	531,0	µg/kg

Śr. stężenie	585,00	µg/kg
s	31,86	µg/kg
1,64 * s	52,25	µg/kg

Ccalfa	652	µg/kg

Matrix effect

Conclusions

- Implementation of regulation 2021/808 is a multi-year task for the laboratory
- must be well planned both in terms of cost but especially in terms of time burden on staff and instruments
- it is not clear how to proceed when the validation results of a given analyte in a multicomponent method do not meet the requirements (e.g., for the matrix effect)
- An important problem is the lack of data on the stability of analytes in the matrix -> perhaps it is worth developing a common database, and put there the information derived from different laboratories.

Thank you for your attention!

Have you any questions?