School for Advanced Residue Analysis in Food
TRAINING PROGRAMME

European Union Reference Laboratory

Commission Implementing Regulation (EU) 2021/808 of 22 March 2021 EURL Guidance Document on Confirmation Method Validation (conventional approach)

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Content

- Scope of guidance document
- Conventional approach method validation confirmatory methods

Scope

- Guidance represents the EURLs interpretation of CIR 2021/808 EU

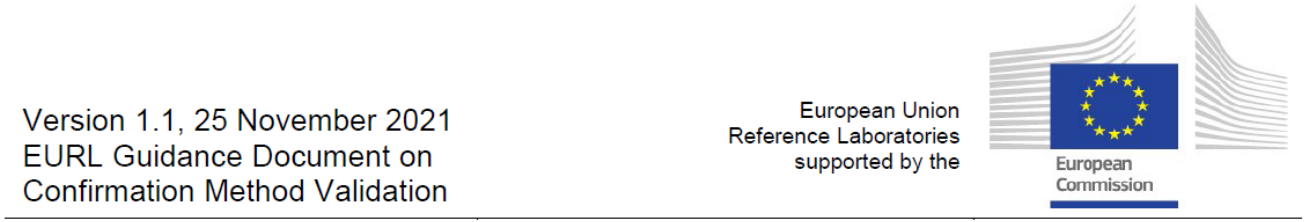
- Laboratories not obliged to follow approaches minutely
  - Other approaches need to provide same level and quality of information

- Guidance for (semi-)quantitative confirmatory methods
  - Presented approaches are also applicable to (semi-)quantitative screening methods

- Information on:
  - Conventional validation approach
  - Alternative validation approach (Joachim Polzer)

Guidance Document

EURL Webportal
www.eurl-residues.eu



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Classification of analytical methods by the performance characteristics that have to be determined

Method	Confirmation		Screening		
	Qualitative	Quantitative	Qualitative	Semi-quantitative	Quantitative
Substances	A	A, B	A, B	A, B	A, B

5 different analytical methods based on type (screening, confirmation) and group of analyte (A&B) determination (qualitative, semi-quantitative, quantitative)
Different performance characteristics are of importance depending on combination

General remarks (2)

Table 5

Classification of analytical methods by the performance characteristics that have to be determined

Method	Confirmation		Screening		
	Qualitative	Quantitative	Qualitative	Semi-quantitative	Quantitative
Selectivity/Specificity		x	x	x	x
Stability #		x	x	x	x
Ruggedness		x	x	x	x

Strictly qualitative methods are more of theoretical importance
Most detection methods for confirmation allow semi-quantitative data evaluation.

Therefore a qualitative confirmation method, for the time being can be understood as a quantitative confirmatory method which does not fulfill all requirements for such methods

General remarks (3)

- Authorised substances: MRL or ML used as reference for validation
- Unauthorised substances: RPA is benchmark for validation or MMPR, or LCL

- Note: RPA and MMPR are not to be used as limits!
- Methods shall be validated at concentration levels as low as reasonably achievable

Validation

- Guidance describes example how to perform validation
- To reduce overall number of samples combined validation experiments are described
- 3 analytical series ideally spread over some weeks
- In case absolute recovery and matrix effect have to be determined a fourth series may be needed
- Minimum 21 different batches* are needed for this approach

*batch meaning 1 individual matrix material coming from different animals

Validation levels

RPA - Reference point for action - nitrofurans, CAP, (L)MG in EU/2019/1871

MMPR – Minimum Method Performance Requirement Guidance of September 2020:

4 stilbenes	6 sedatives
6 thyrostats	7 NSAIDs
16 A3 steroids	crystal violet
4 resorcylic acid lactones	brilliant green
21 β-agonist	carbadox
8 nitroimidazoles	olaquindox
dapsone	several antibiotics in honey
chloorpromazin	

LCL – Lowest calibration level – for other unauthorized substances (CIR 2021/808/ EU)

MRL – Maximum residue level - residues in EU/2010/37

ML – Maximum level – for coccidiostats and histomonostats in EU/2009/124

Validation (2)

- Required fortification levels depending on the legal status of the residue

Residue	Level 1	Level 2	Level 3
Unauthorised with RPA	0.5 RPA	1.0 RPA	1.5 RPA
Unauthorised	1.0 LCL	2.0 LCL	3.0 LCL
Authorised	0.1 MRL/ML	1.0 MRL/ML	1.5 MRL/ML

Validation (3)

3 validation series with 7 different batches daily:

- Selectivity
- Trueness
- Repeatability
- Within-lab reproducibility
- CCα

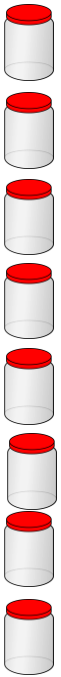
1 validation series with 20 different batches*:

- Absolute recovery
- Relative matrix effect

* To be determined when no internal standard or no matrix fortified calibration curve is used

Validation (4)

Fortify A at 5 relevant levels
Fortify A-G at 4 levels: 0, level 1, level 2, level 3



A matrix fortified calibration curve

A selectivity, trueness, repeatability, within labrep., CCalfa

B selectivity, trueness, repeatability, within labrep., CCalfa

C selectivity, trueness, repeatability, within labrep., CCalfa

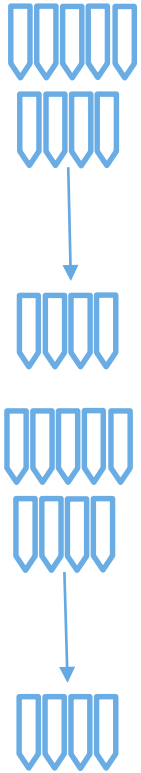
D selectivity, trueness, repeatability, within labrep., CCalfa

E selectivity, trueness, repeatability, within labrep., CCalfa

F selectivity, trueness, repeatability, within labrep., CCalfa

G selectivity, trueness, repeatability, within labrep., CCalfa

Validation (5)



Experiment 1

A calibration curve – 5 levels

A fortify at 0 ppb and levels 1-3

B
C
D
E
F

G fortify at 0 ppb and levels 1-3

33 matrix samples or 28 matrix samples without matrix calibration curve

Fortify each sample with IS

Experiment 2

H calibration curve – 5 levels

H fortify at 0 ppb and levels 1-3

I
J
K
L
M

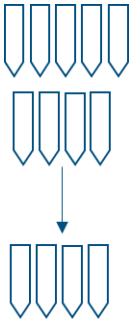
N fortify at 0 ppb and levels 1-3

33 matrix samples or 28 matrix samples without matrix calibration curve

Fortify each sample with IS

Validation (6)

Experiment 3



O calibration curve – 5 levels

O fortify at 0 ppb and levels 1-3

P
Q
R
S
T

U fortify at 0 ppb and levels 1-3

O for ruggedness – minor change 1

O for ruggedness – minor change 2

O for ruggedness – minor change 3

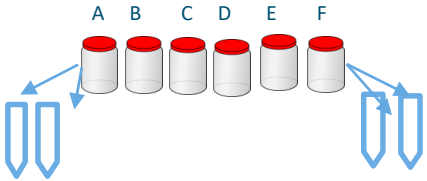
39 matrix samples or 34 matrix samples without matrix calibration curve

Fortify each sample with IS

Validation (7)

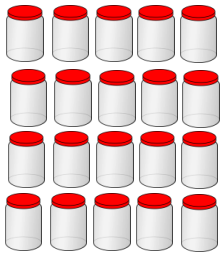
Experiment 4 (absolute recovery and matrix effect)

Absolute recovery: when no IS or matrix fortified calibration is used



A-F fortify at level 2 before and after extraction

Relative matrix effect: during validation or separate experiment



A-T (20 matrix samples), spike at level 2 after extraction

pure solution of analyte at level 2

Validation (8)

After 4 validation days:

- 21 results for selectivity (1 more than in CIR/2021/808)
- 21 results for repeatability/within labreproducibility/CCα (3 more)
- 6 results for ruggedness
- 6 results for absolute recovery
- 20 results for relative matrix effect

Calculations

- Trueness, repeatability, withinlab reproducibility -> Anova (for example)
- CCα -> 3 examples in paragraph 2.6 of CIR/2021/808
- Ruggedness -> no equation given in CIR/2021/808
- Absolute recovery and relative matrix effect -> equations in paragraphs 2.9 and 2.10

Validation (9)

Calculations: Resval (spreadsheet WFSR EURL)

$CC\alpha = LCL + 2.33$ (one-sided, 99%) x $s_{RL, LCL}$

$CC\alpha = M(R)L + 1.64$ (one-sided, 95%) x $s_{RL,M(R)L}$

$CC\beta$ (screening)= STC + 1,64 (eenzijdig, 95%) x $s_{RL, STC}$

For example EIA, AB-screening, HRMS

Validation (10)

Resval spreadsheet example:

Experiment 1

Instrument	WBA0321
Date	2-11-2021
Technician	
Project	Volledige validatie MVO152 (varkensnier)
Title method	MVO152
Compound	QCA
Internal standard	QCA-d4

Identifier (e.g. file names)	Sample name	Validation level	Concentration ug/kg	Confirmed X=Yes	Accuracy %
	202133446 (blanco E)	0	-0.04		-
	202133502 (blanco F)	0	-0.03		-
	202133596 (blanco G)	0	0.00		-
	202133629 (blanco H)	0	0.00		-
	202133630 (blanco I)	0	0.33		-
	202133649 (blanco J)	0	0.00		-
	202133742 (blanco K)	0	0.00		-
		0			-
		0			-
	202133446 (blanco E)	1	1.05	x	104.8
	202133502 (blanco F)	1	0.87	x	87.2
	202133596 (blanco G)	1	0.99	x	99.3
	202133629 (blanco H)	1	0.95	x	94.5
	202133630 (blanco I)	1	1.32	x	132.4
	202133649 (blanco J)	1	1.01	x	101.4
	202133742 (blanco K)	1	1.07	x	107.0
		1			
		1			
	202133446 (blanco E)	2	2.06	x	103.2
	202133502 (blanco F)	2	1.94	x	96.9
	202133596 (blanco G)	2	1.95	x	97.3
	202133629 (blanco H)	2	2.02	x	101.0
	202133630 (blanco I)	2	2.17	x	108.7
	202133649 (blanco J)	2	2.01	x	100.4
	202133742 (blanco K)	2	2.01	x	100.5
		2			
		2			
	202133446 (blanco E)	3	2.90	x	96.7
	202133502 (blanco F)	3	2.85	x	94.9
	202133596 (blanco G)	3	3.08	x	102.5
	202133629 (blanco H)	3	3.18	x	10
		3	3.43	x	11
		3	3.15	x	10
		3	3.18	x	10
		3			
		3			

Validation (11)

ResVal (v. 4.0) Validation Report

Validation conform EU/2021/808

Calculate

1. General Information

Instrument	WBA0321	WAA588	WBA0321
Date (exp 1, 2, 3)	2-11-2021	30-11-2021	11-11-2021
Technician			
Project	Volledige validatie MVO152 (varke	Volledige validatie MVO152 (varke	Volledige validatie MVO152 (varkensnier)
Title method	MVO152	MVO152	MVO152
Compound	QCA	QCA	QCA
Internal standard	QCA-d4	QCA-d4	QCA-d4

2. Validation Summary

Select the appropriate one and type 'X'

	?	Full validation (Exp 1, 2, 3)	
CCα Non-authorised compounds	x	1.27	
CCβ Screening non-authorised compounds			
CCα Authorised compounds			
CCβ Screening authorised compounds			

3. Performance characteristics full validation, Exp 1-3

Validation Level	1	2	3
Unit	ug/kg	ug/kg	ug/kg
Trueness	103%	101%	102%
Std. dev repeatability (s_r)	0.111	0.097	0.127
Relative st.dev. Repeat. (RSD_r)	11%	5%	4%
St. dev BL-reproducibility (s_R)	0.114	0.103	0.130
Relative st.dev. Reprod. (RSD_R)	11%	5%	4%
Expanded M.U.	22%	10%	9%
Confirmed	100%	100%	100%

Changes CD 2002/657 ⟶ CIR 2021/808 EU

- Annex highlighting differences between the documents
- Re-evaluation of validation data in terms of new CIR might be sufficient in some cases

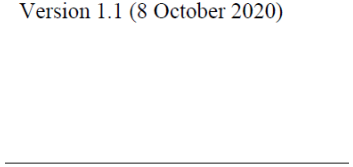
Performance characteristic	Changes from CD 2002/657 to CIR 2021/808
Identification	Change in the concept for the identification points
Chromatography	General requirements for validation remain the same, requirements for identification have been adjusted
Calibration curve	No changes in the requirements
Concentrations levels/ranges	Levels/ranges which should be validated have been revised
Precision	Acceptable coefficients of variation have been revised
Trueness	Acceptable ranges for analyte mass fractions >1 µg/kg have been revised
Measurement uncertainty	Not explicitly mentioned in CD 2002/657
Relative matrix effect	Not explicitly mentioned in CD 2002/657
Absolute recovery	Previously referred to as "recovery"
Specificity / selectivity	No changes in the requirements
Ruggedness	No changes in the requirements, but information is given in more detail in CD 2002/657
CCα	Additional calculation method
CCβ	Change of the concept of the CCβ
Stability	No change in the requirements

Take Home message

- Guidance document available on validation of confirmatory methods (conventional approach)
- Practical interpretation of CIR 2021/808 EU for laboratories
- Number of analyses for full validation decreased due to combinations of experiments
- Resval spreadsheet available from EURL WFSR for calculation if needed

Take Home message (2)

- More guidance documents available on:



Euroresidue IX conference 23-25 May 2022

- www.Euroresidue.nl for more information
- Sunday 22nd May Pre-conference workshop:

"Residue analysis for dummies - a pre-conference workshop to familiarize yourself with concepts and colleagues"

- Main topics:
  - Relevant EU legislation, current, and future trends
  - National Monitoring Plans
  - Registered and banned compounds (MRL/MMPR/RPA)
  - EURL-NRL-OL structure
  - Important topics of the EuroResidue IX conference

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Thank you for your attention

Thanks all colleagues from WFSR and NRLs who have participated in drawing up this guidance.

Questions?

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