PRATICAL APPLICATIONS FROM TWO FRENCH NRLS OF THE NEW EUROPEAN REGULATION 2021/808 AND THEIR IMPLEMENTATION ON FORBIDDEN AND AUTHORIZED SUBSTANCES

SARAF, 8th of December

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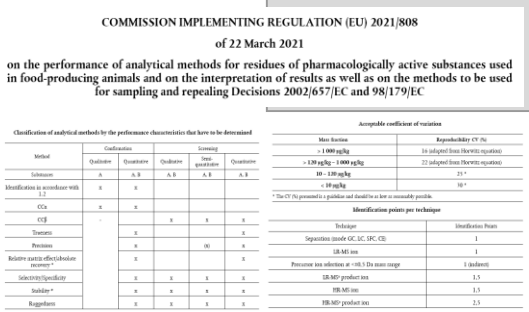


COMMISSION IMPLEMENTING REGULATION (EU) 2021/808

of 22 March 2021

on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results as well as on the methods to be used for sampling and repealing Decisions 2002/657/EC and 98/179/EC

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
Identification in accordance with 1.2	x	x			
CCα	x	x			
CCβ	-		x	x	x
Trueness		x			x
Precision		x		(x)	x
Relative matrix effect/absolute recovery *		x			x
Selectivity/Specificity		x	x	x	x
Stability *		x	x	x	x
Ruggedness		x	x	x	x

Acceptable coefficient of variation

Mass fraction	Reproducibility CV (%)
> 1 000 µg/kg	16 (adapted from Horwitz equation)
> 120 µg/kg – 1 000 µg/kg	22 (adapted from Horwitz equation)
10 – 120 µg/kg	25 *
< 10 µg/kg	50 *

* The CV (%) presented is a guideline and should be as low as reasonably possible.

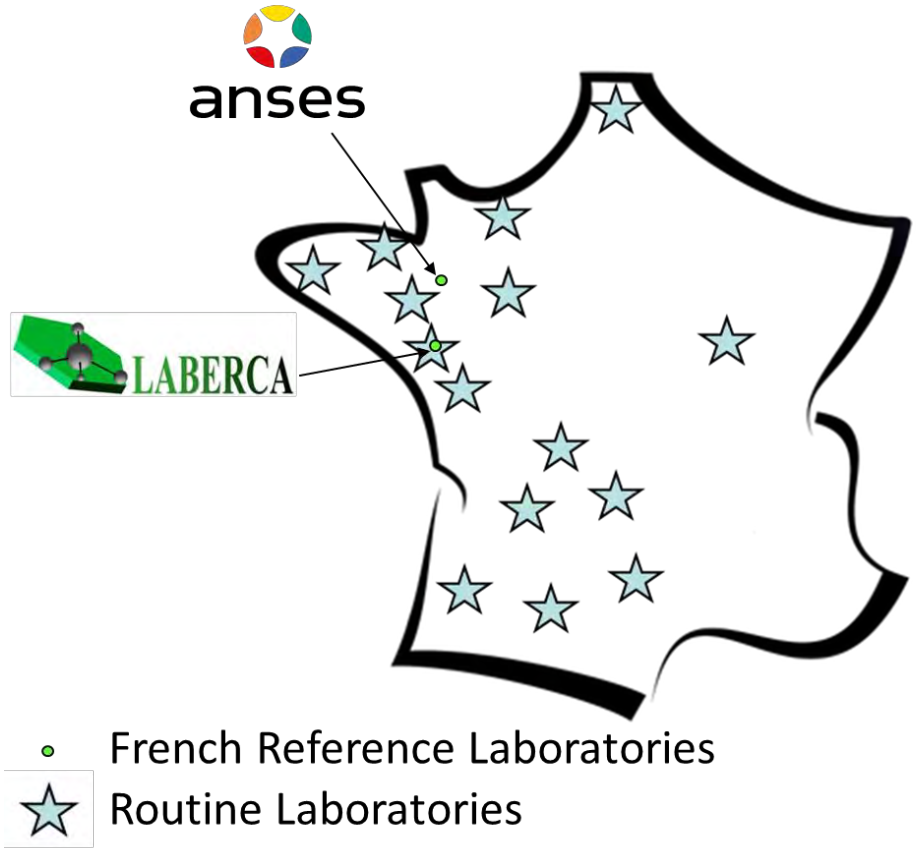
Identification points per technique

Technique	Identification Points
Separation (mode GC, LC, SFC, CE)	1
LR-MS ion	1
Precursor ion selection at >50,5 Da mass range	1 (indirect)
LR-MS² product ion	1,5
HR-MS ion	1,5
HR-MS² product ion	2,5

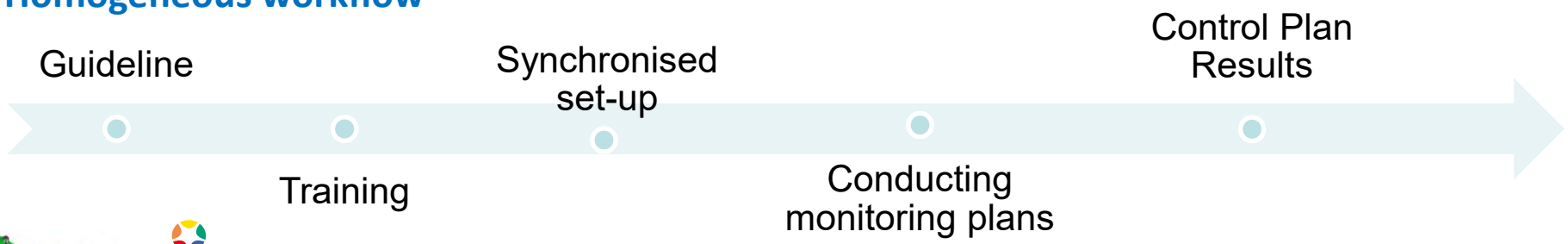
SARAF
School for Advanced Residue Analysis in Food

ORIGINS OF THE INITIATIVE

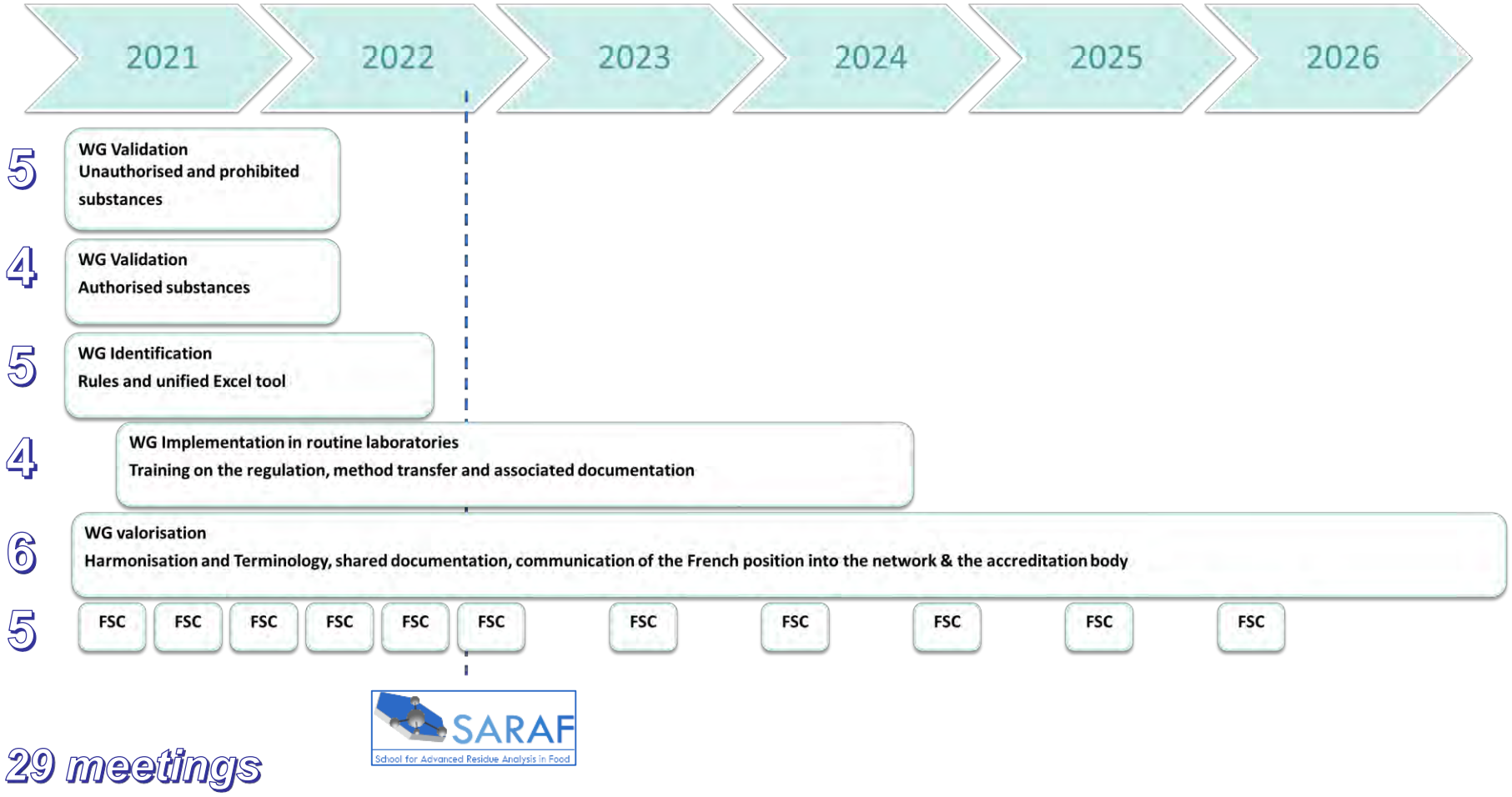
French laboratory network for the control of pharmacologically active substances



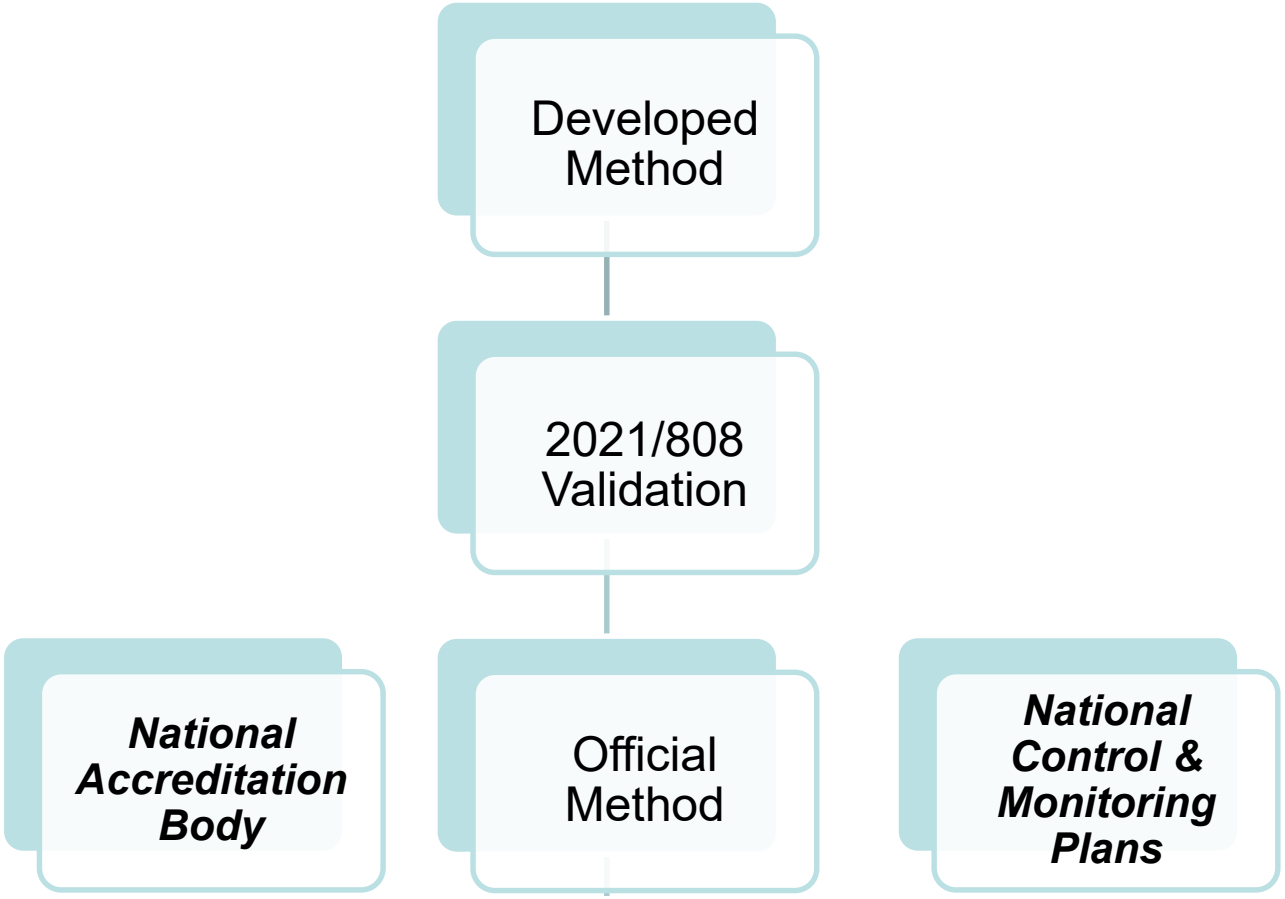
Homogeneous workflow

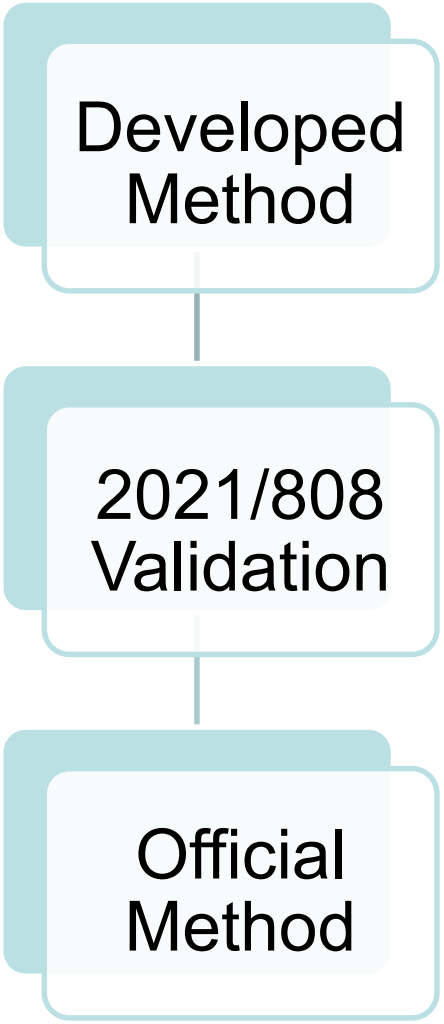


ESTABLISHMENT OF WORKING GROUPS AND TIMETABLE



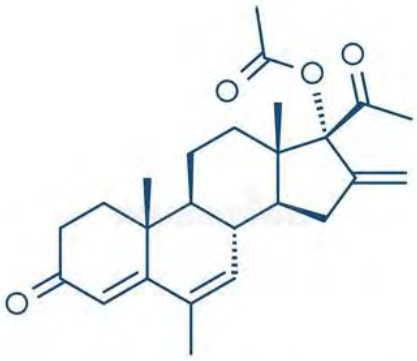
GENERAL IMPLEMENTATION WORKFLOW



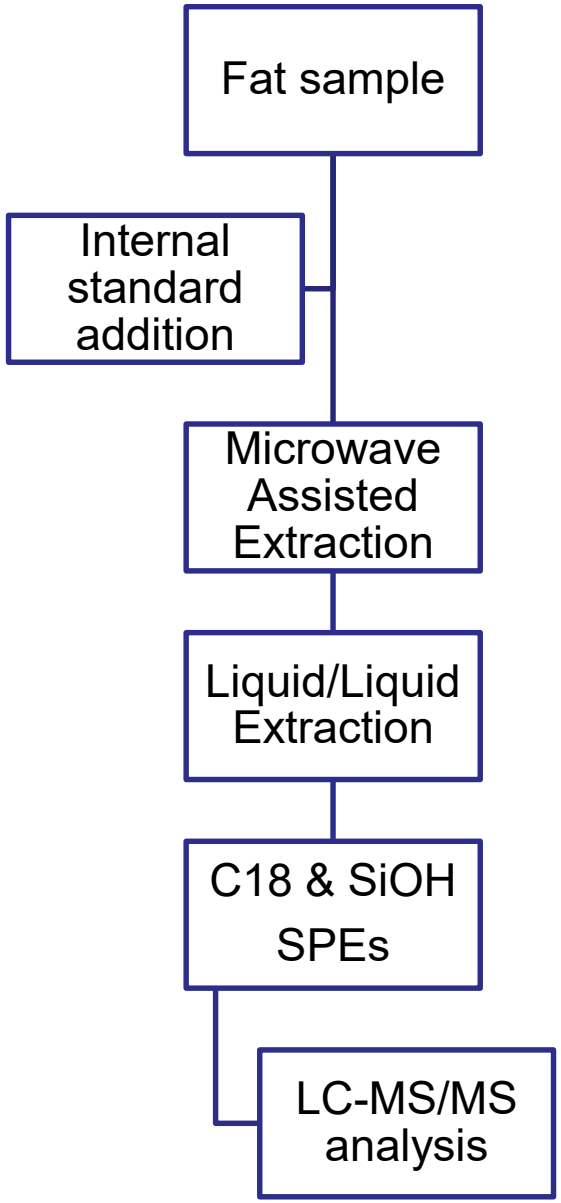

Developed Method
2021/808 Validation
Official Method

Example on progestagen esters in fat

Analytical methodology



melengestrol acetate



Example on progestagen esters in fat

Regulation

26.9.2022 EN Official Journal of the European Union L 248/3

COMMISSION DELEGATED REGULATION (EU) 2022/1644

of 7 July 2022

supplementing Regulation (EU) 2017/625 of the European Parliament and of the Council with specific requirements for the performance of official controls on the use of pharmacologically active substances authorised as veterinary medicinal products or as feed additives and of prohibited or unauthorised pharmacologically active substances and residues thereof

(Text with EEA relevance)

ANNEX I

Group A – Prohibited or unauthorised pharmacologically active substances in food-producing animals

1. Substances with hormonal and thyrostatic action and beta agonists the use of which is prohibited under Council Directive 96/22/EC ([1]):

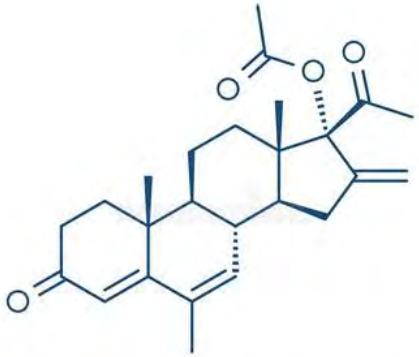
(a) Stilbenes;

(b) Antithyroid agents;

(c) Steroids;

Example on progestagen esters in fat

Level of interest



melengestrol acetate

Version 2.0
June 2022

European Union
Reference Laboratories
supported by the

European Commission

EURL GUIDANCE ON MINIMUM METHOD PERFORMANCE REQUIREMENTS (MMPRs) FOR SPECIFIC PHARMACOLOGICALLY ACTIVE SUBSTANCES IN SPECIFIC ANIMAL MATRICES

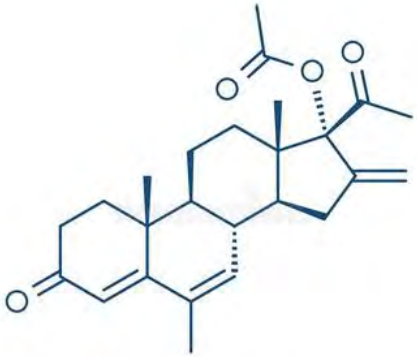
Substances	Marker residue-metabolite[$]	Matrix	MMPR*
Melengestrol	Melengestrol (acetate)	Kidney fat	5 ppb
		Muscle	1.0 ppb

**CCβ for screening methods or CCα for confirmatory methods should be lower than the value expressed in this column.*

Example on progestagen esters in fat

Analytical performances

L 180/84 EN Official Journal of the European Union 21.5.2021



melengestrol acetate

COMMISSION IMPLEMENTING REGULATION (EU) 2021/808

of 22 March 2021

on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals and on the interpretation of results as well as on the methods to be used for sampling and repealing Decisions 2002/657/EC and 98/179/EC

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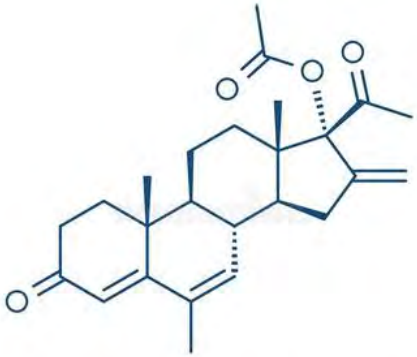
Identification in accordance with 1.2	x	x			
CCα	x	x			
CCβ	-		x	x	x
Trueness		x			x
Precision		x		(x)	x
Relative matrix effect/absolute recovery *		x			x
Selectivity/Specificity		x	x	x	x
Stability #		x	x	x	x
Ruggedness		x	x	x	x

Example on progestagen esters in fat

Analytical performances

L 180/84 EN Official Journal of the European Union 21.5.2021



melengestrol acetate

COMMISSION IMPLEMENTING REGULATION (EU) 2021/808

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Substances	A	A, B	A, B	A, B	A, B
Identification in accordance with 1.2	x	x			
CCα	x	x			
CCβ	-		x	x	x
Trueness		x ?			x ?
Precision		x ?		(x)	x ?
Relative matrix effect/absolute recovery *		x ?			x ?
Selectivity/Specificity		x	x	x	x
Stability #		x	x	x	x
Ruggedness		x	x	x	x

Example on progestagen esters in fat

Analytical performance

Version 1.1, 25 November 2021
EURL Guidance Document on
Confirmation Method Validation

European Union
Reference Laboratories
supported by the
European Commission

EURL Guidance Document on Confirmation Method Validation

French Touch

Level of interest	Level 1 : LCL	Level 2	Level 3	Level 4
MMPR	**≤ ½ MMPR**	**½ MMPR**	**MMPR**	**1.5xMMPR**

Choice of LCL :

- as low as possible
- Where validation of the concentration of 0.5 times the level of interest is not reasonably possible, this value may be replaced by the lowest concentration between 0.5 and 1 time the level of interest (see § 2.2.1.2, 2.2.1.3 and 2.2.1.4 of the Annex to the Regulation).

Melengestrol Acetate

Level of interest	Level 1 : LCL	Level 2	Level 3	Level 4
MMPR = 5 ng/g	**0.5 ng/g**	**2.5 ng/g**	**5 ng/g**	**7.5 ng/g**

Experimental design

Serie 1

Matrix effect

20 different batches of matrices representative to the method application field

Serie 2	**Serie 3**	**Serie 4**
Calibration curve (n≥5)	Calibration curve (n≥5)	Calibration curve (n≥5)
Blank samples (n≥6)	Blank samples (n≥6)	Blank samples (n≥6)
Level 1 (n≥6)	Level 1 (n≥6)	Level 1 (n≥6)
Level 2 (n≥6)	Level 2 (n≥6)	Level 2 (n≥6)
Level 3 (n≥6)	Level 3 (n≥6)	Level 3 (n≥6)
Level 4 (n≥6)	Level 4 (n≥6)	Level 4 (n≥6)

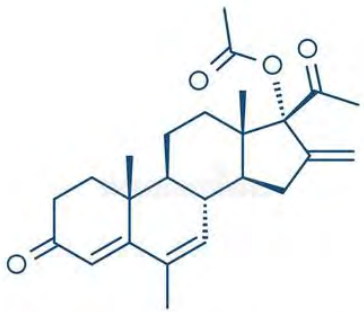
Melengestrol Acetate experimental design

Serie 1 : the 14th of june 2022

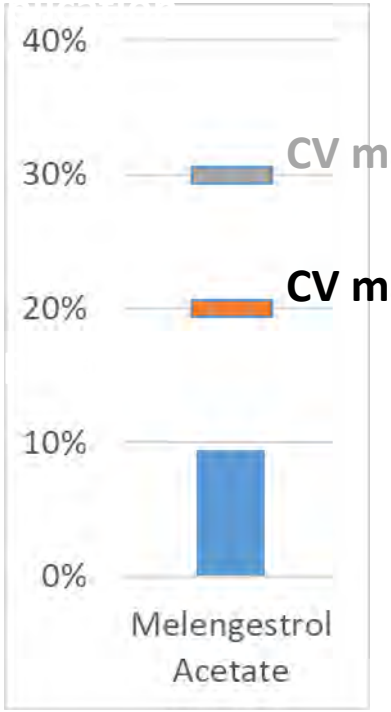
Matrix effect on the scope of the analytical method

21 fat samples from 8 Bovine, 2 Equine, 2 Ovine and 9 Porcine species spiked at the MMPR

Matrix effect corrected by the internal Standard
REG 2021/808 Criterion : CV < 20%



melengestrol acetate



No significant effect
=> validation on the complete scope

Melengestrol Acetate experimental design

Serie 1 : the 14th of June 2022

Matrix effect on the scope of the analytical method

21 fat samples from 8 Bovine, 2 Equine, 2 Ovine and 9 Porcine species spiked at the MMPR

Serie 2 : 1st of July 2022

Calibration curve on pool fat 1	7 different samples spiked at
0 ng/g	
0.25 ng/g	0 ng/g
0.5 ng/g	0.5 ng/g
1.25 ng/g	2.5 ng/g
2.5 ng/g	5 ng/g
5 ng/g	7.5 ng/g
10 ng/g	

Serie 3 : 8th of July 2022

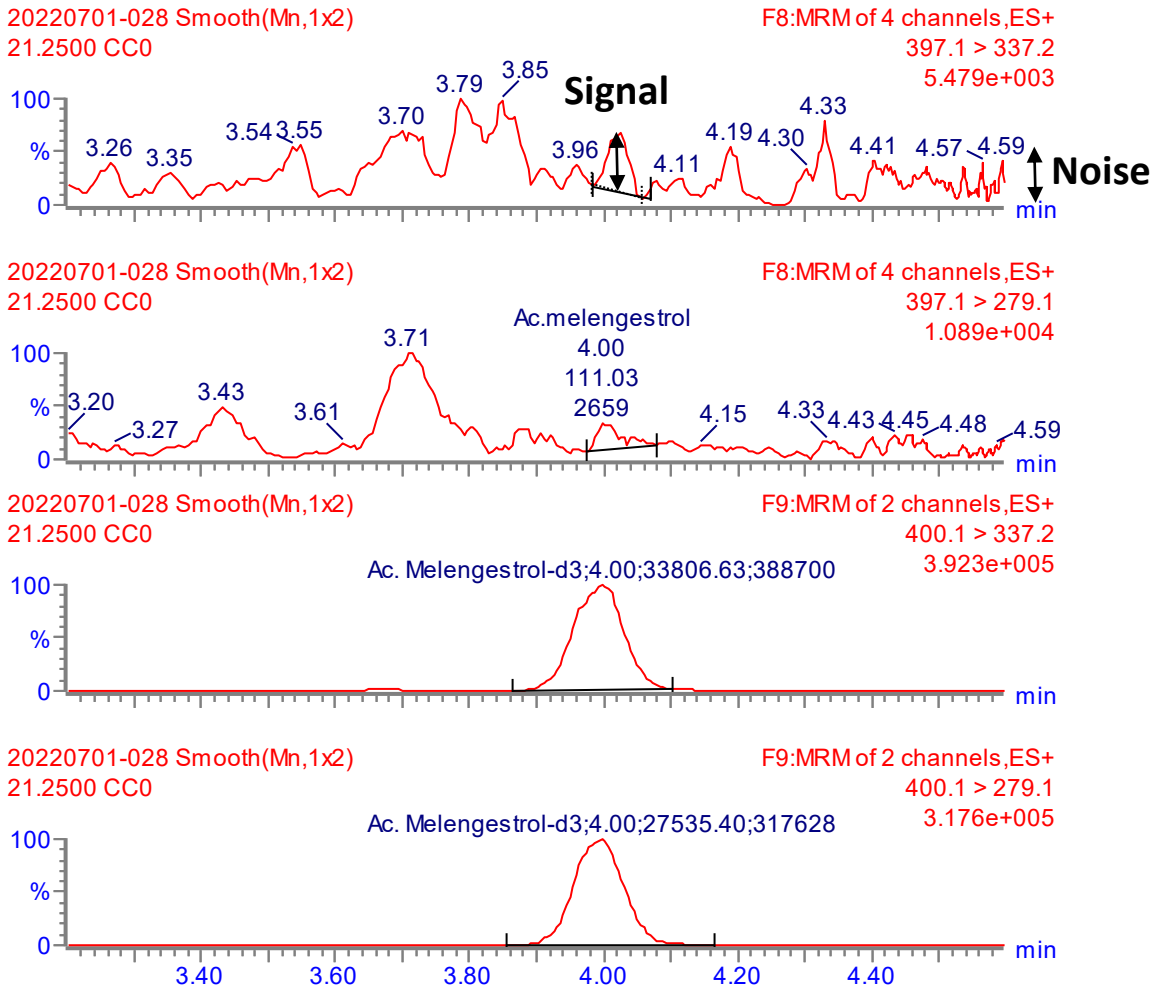
Calibration curve on pool fat 2	7 different samples spiked at
0 ng/g	
0.25 ng/g	0 ng/g
0.5 ng/g	0.5 ng/g
1.25 ng/g	2.5 ng/g
2.5 ng/g	5 ng/g
5 ng/g	7.5 ng/g
10 ng/g	

Serie 4 : 28th of July 2022

Calibration curve on pool fat 2	7 different samples spiked at
0 ng/g	
0.25 ng/g	0 ng/g
0.5 ng/g	0.5 ng/g
1.25 ng/g	2.5 ng/g
2.5 ng/g	5 ng/g
5 ng/g	7.5 ng/g
10 ng/g	

Specificity

No interference in the 21 blank samples



Melengestrol Acetate

Signal/Noise < 3

Melengestrol Acetate - d3

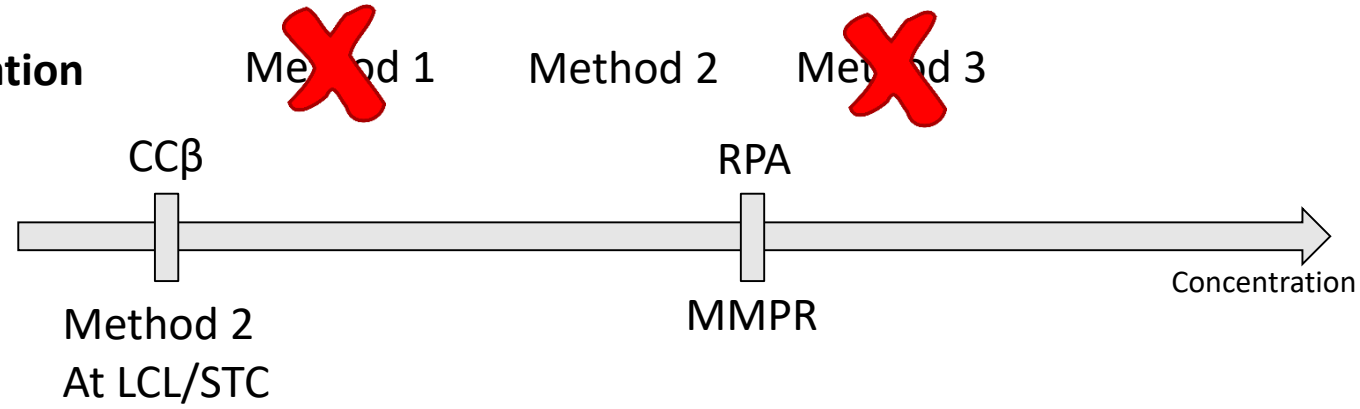
CCβ

Definition from the REG (EU) 2021/808

(15) 'detection capability for screening (CCβ)' means the smallest content of the analyte that may be detected or quantified in a sample with an error probability of β:

(a) in the case of prohibited or unauthorised pharmacologically active substances, the CCβ is the lowest concentration at which a method is able to detect or quantify, with a statistical certainty of 1 – β, samples containing residues of prohibited or unauthorised substances;

Determination



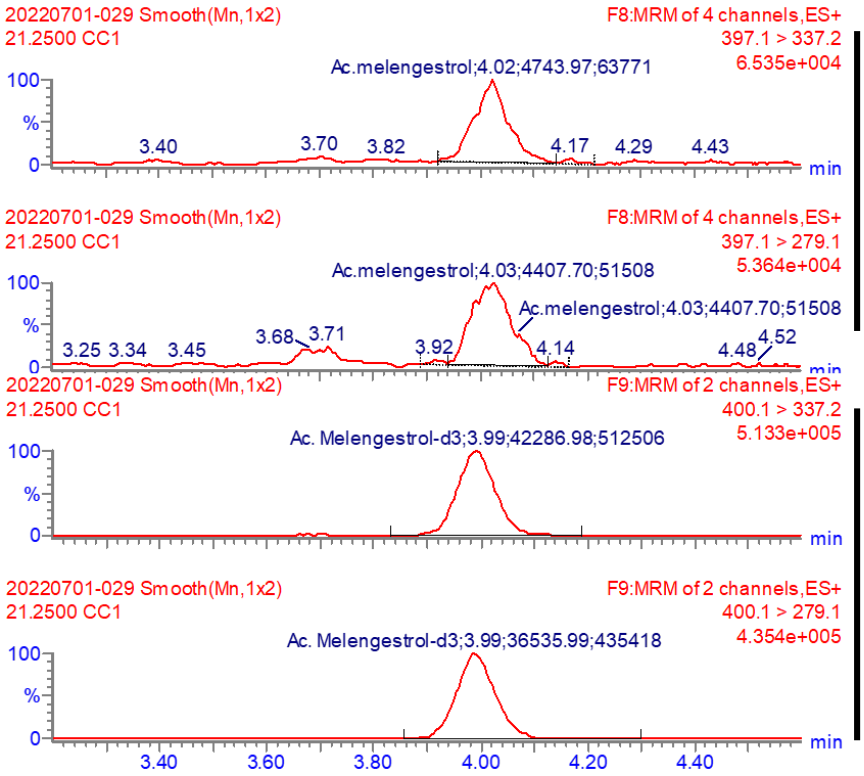
(b) Method 2: Investigation of fortified blank material at concentration levels at and above the STC. For each concentration level 20 fortified blanks shall be analysed in order to ensure a reliable basis for this determination. The concentration level, where only ≤ 5 % false compliant results remain, equals the detection capability of the method.

CCβ Melengestrol acetate in fat

Identification criteria in screening :

2 specific transitions detected above S/B = 3 @ the relative retention time (RRT) ± 1 %

@ Level 1 : LCL/STC = 0,1 x MMPR => 0.5 ng/g



Melengestrol Acetate

RRT < 1% for 21/21 spiked samples ✓

S/B > 3 for both transitions ✓

Melengestrol Acetate - d3

Melengestrol acetate

CCβ = 0,5 ng/g

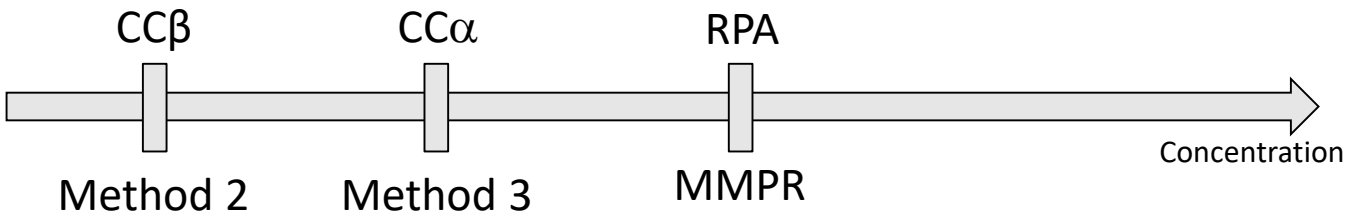
CCα

Definition from the REG (EU) 2021/808

(14) 'decision limit for confirmation (CCα)' means the limit at and above which it can be concluded with an error probability of α that a sample is non-compliant and the value 1 – α means statistical certainty in percentage that the permitted limit has been exceeded;

Determination ~~Method 1~~ ~~Method 2~~ Method 3

2021/808 requirement: CCa as low as possible and below RPA/MMPR



CCα

(c) Method 3: CCα = LCL + k(one-sided, 99 %) × (combined) standard measurement uncertainty at LCL

$CC\alpha = CC\beta + 2.33 \times u_c$ *with* u_c *= combined measurement uncertainty at CCβ*

u_c Determination $$u_c = \sqrt{u_{Precision}{}^2 + u_{Trueness}{}^2}$$

Precision

REG (EU) 2021/808 Requirements

Table 2

Acceptable coefficient of variation

Mass fraction	Reproducibility CV (%)
> 1 000 µg/kg	16 (adapted from Horwitz equation)
> 120 µg/kg – 1 000 µg/kg	22 (adapted from Horwitz equation)
10 – 120 µg/kg	25 *
< 10 µg/kg	30 *

* The CV (%) presented is a guideline and should be as low as reasonably possible.

Determination for melengestrol acetate

	level 1	level 2	level 3	level 4
Spike (ng/g)	0.5	2.5	5	7.5

u_c Determination $u_c = \sqrt{u_{Precision}{}^2 + u_{Trueness}{}^2}$

Trueness

REG (EU) 2021/808 Requirements

Table 1

Minimum trueness of quantitative methods

Mass Fraction	Range
≤ 1 µg/kg	−50 % to +20 %
> 1 µg/kg to 10 µg/kg	−30 % to +20 %
≥ 10 µg/kg	−20 % to +20 %

Determination for melengestrol acetate

	level 1	level 2	level 3	level 4
Spike (ng/g)	0.5	2.5	5	7.5

u_c Determination $u_c = \sqrt{u_{Precision}{}^2 + u_{Trueness}{}^2}$

At CCβ = Level 1

Melengestrol acetate : **u_c = 16 %**

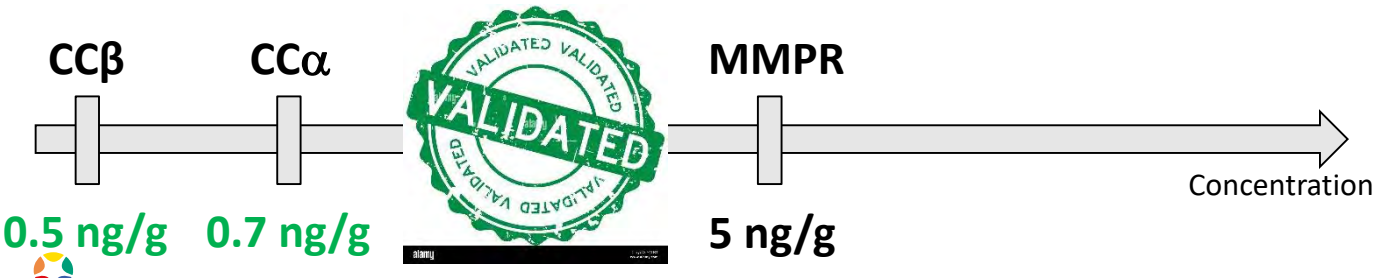
CCα

Determination

CCα=CCβ+2.33 x u_c *with u_c = combined measurement uncertainty at CCβ*

Melengestrol acetate

CCα = 0,7 ng/g



Identification criteria in confirmation

2021/808 Requirements

2 specific transitions detected above S/B = 3

Transition ratio < 40 % of deviation to the reference

@ the relative retention time (RRT) ± 1 %

Melengestrol Acetate: 84 spiked samples on the range [0.5 – 7.5] ng/g

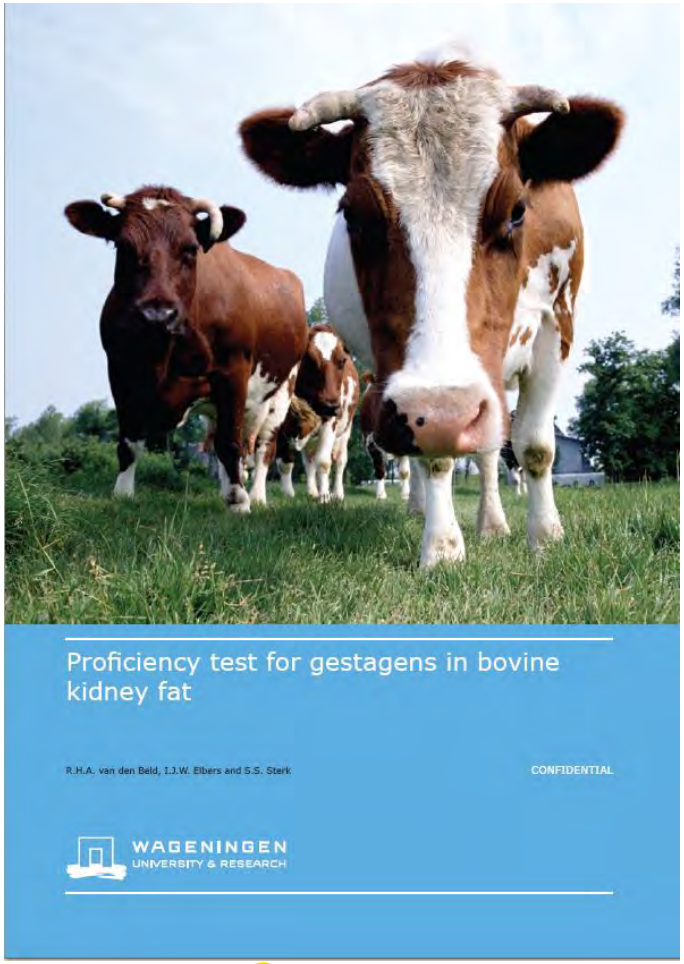
S/B > 3 for 84/84 spiked samples on both transitions ✓

RRT < 1% for 84/84 spiked samples ✓

Signal ratios below 40 % of deviation for 84/84 spiked samples ✓

Stability

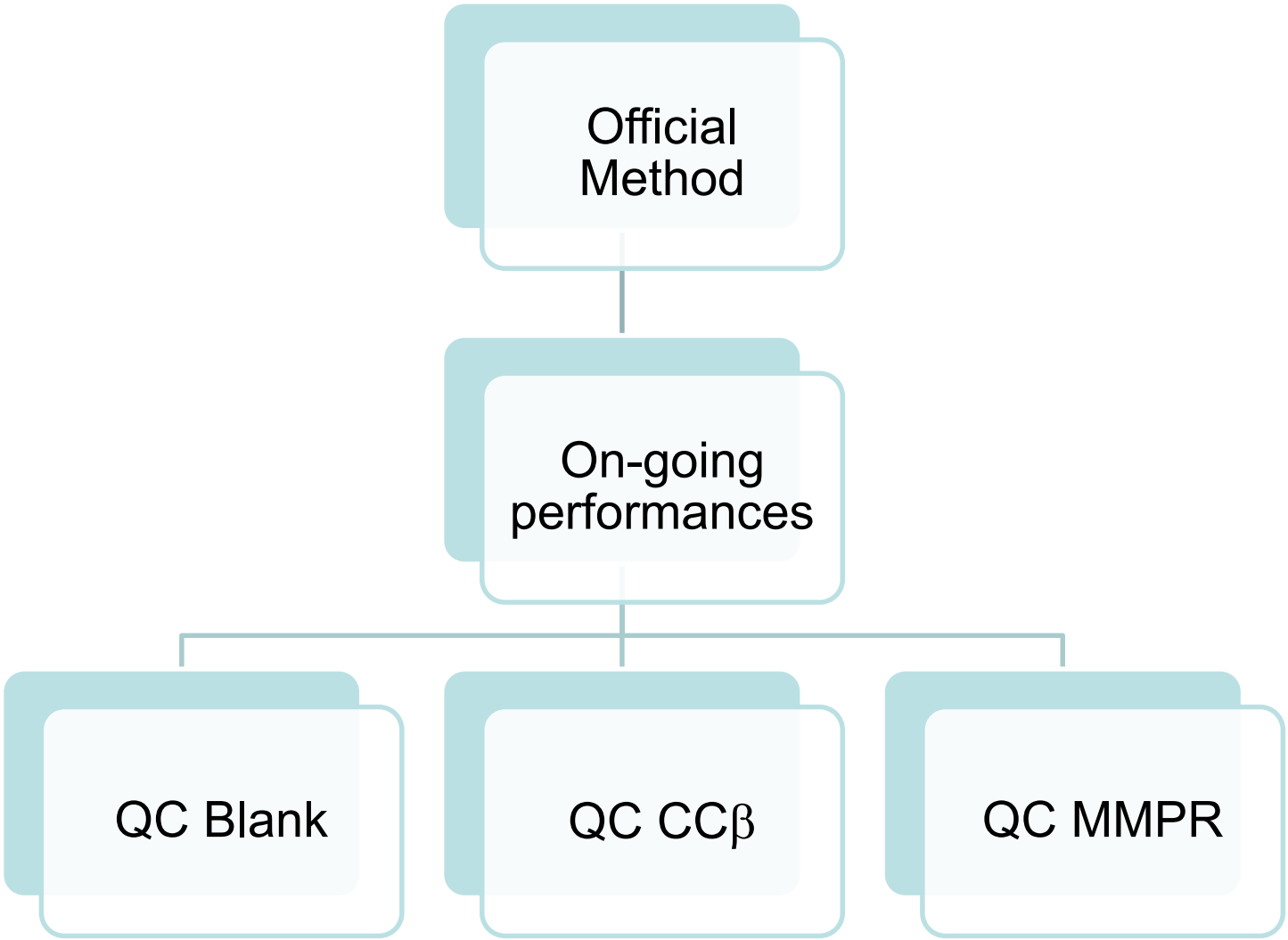
From EURL Data when available



Statistical evaluation for MLGA in material D			
Storage temp	-80 °C	<-20°C	7 days RT
Time in freezer (days)	0	70	70
Calculated amounts (µg/kg)	4.5	4.5	4.5
	4.7	4.5	4.6
	4.6	4.7	4.5
	4.4	4.6	4.5
	4.6	4.6	4.6
	4.5	4.6	4.7
Average amount (µg/kg)	4.5	4.6	4.6
n	6	6	6
st. dev (µg/kg)	0.10	0.07	0.07
Difference		-0.02	-0.03
$0.3\sigma_p$		0.30	0.30
Consequential difference? Diff < 0.3 σ_p		NO	NO

Performance criteria to be validated :

Criteria	Screening	Confirmation	
		Quantitative	Qualitative
Identification	ΔRRT < 1 % 2 signals with S/B > 3	ΔRRT < 1 % 2 signals with S/B > 3 and Δratio < 40 %	
CCβ	method 2 < MMPR		
CCα		method 3 < MMPR	
Trueness		2021/808 criteria	from bias at the CCβ level
Precision		2021/808 criteria	from CV at the CCβ level
Matrix effect	RSD < 20 % or $<RSD_{PRECISION}$		
Recovery	For information		
Stability	According to the EURL information		
Specificity	Assessment on at least 20 different blank samples		
Robustness	To be determine during the development step		


Official Method
On-going performances
QC Blank
QC CCβ
QC MMPR

QC Blank

QC CCβ

QC MMPR

<u>Matrices</u> representative of the scope of the method

Batch acceptation

MLGA:Level 0 ng/g
- ISTD detection
- No interference

MLGA:Level 0.5 ng/g
- ISTD detection
- Analyte detection

MLGA:Level 5 ng/g
- ISTD detection
- Analyte identification
- Control chart monitoring

Developed Method

2021/808 Validation

Official Method

On-going performances

QC Blank

QC CCβ

QC MMPR

15 official methods

≥ 147 samples/method for forbidden substances

Total : 2200 samples

+ Administrative work *validation reports*
method revision and edition
operator's training

Within the 3 next years

To be ready in **2026**

Let's start ASAP

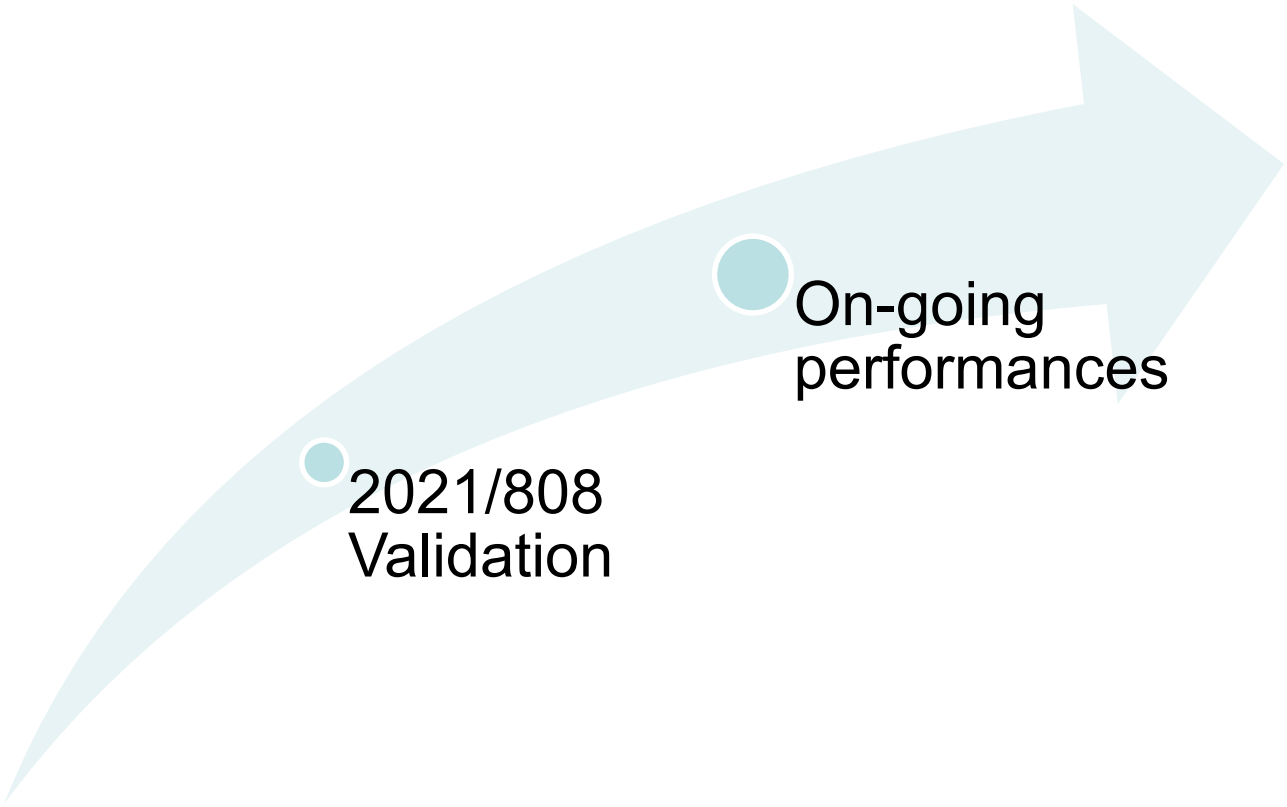
THANK YOU


SPACIBO
ARIGATO
TESEKKURLER
HVALA
MERCI
KHUBLEI
BLAGODARYA
MAHALO
GRAZIAS
ZIKOMO
TACK
THANK YE
DANKE
GRAZIE
MAULIATE
PALDIES
MERCI
VINAKA
MULTUMESC
SALAMAT
TENKIU
THANK YOU
DANKJE
KIITOS
BARKAL
D'AKUJU
FAKAAUE
MISAOTRA
SUWUN
OBRIGADU
GRACIES
TANGKIU
CHOKRAN
GRACIIS
AGUYJE
SHUKURIYAA
MURAKOZE
ARIGATO
GRAZAS
MITELA
GRACIAS
THANK YOU
GRASSIE
DANKON
QUJANAQ
TRUGAREZ
OBRIGADO
GRACIES
WILIWNI
VINAKA

INITIAL VALIDATION OF AN « AUTHORISED SUBSTANCES » METHOD:

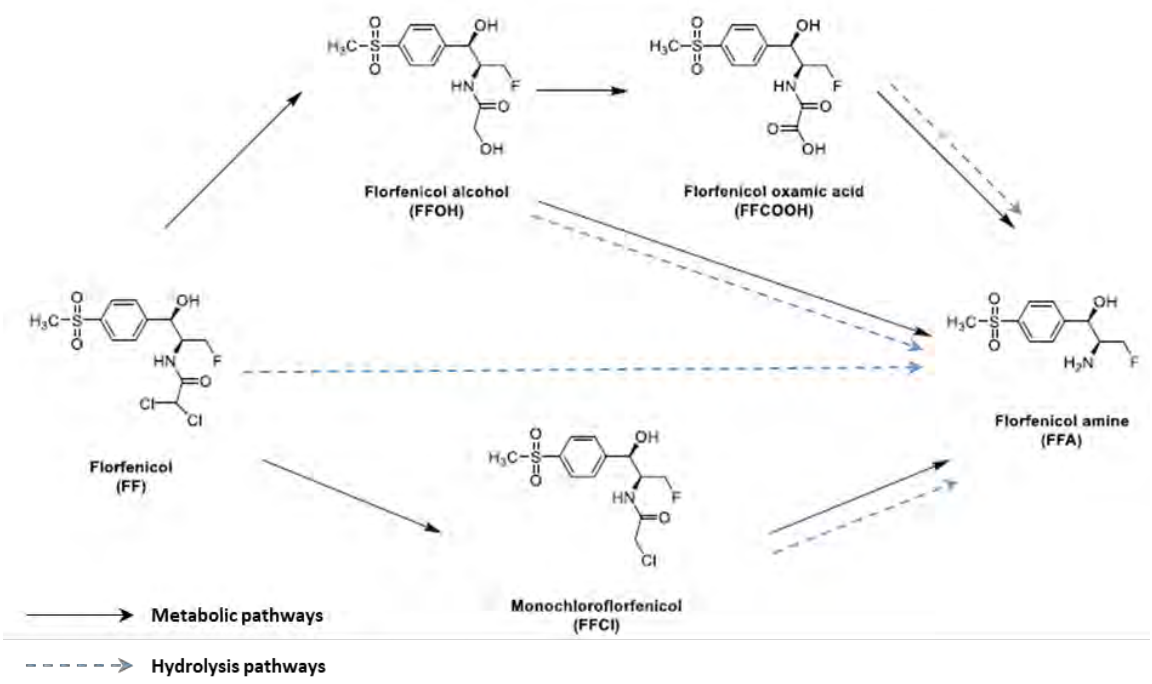
Method for the detection and confirmatory quantification of florfenicol residues in muscle and flesh matrices using LC-MS/MS

AUTHORISED SUBSTANCES

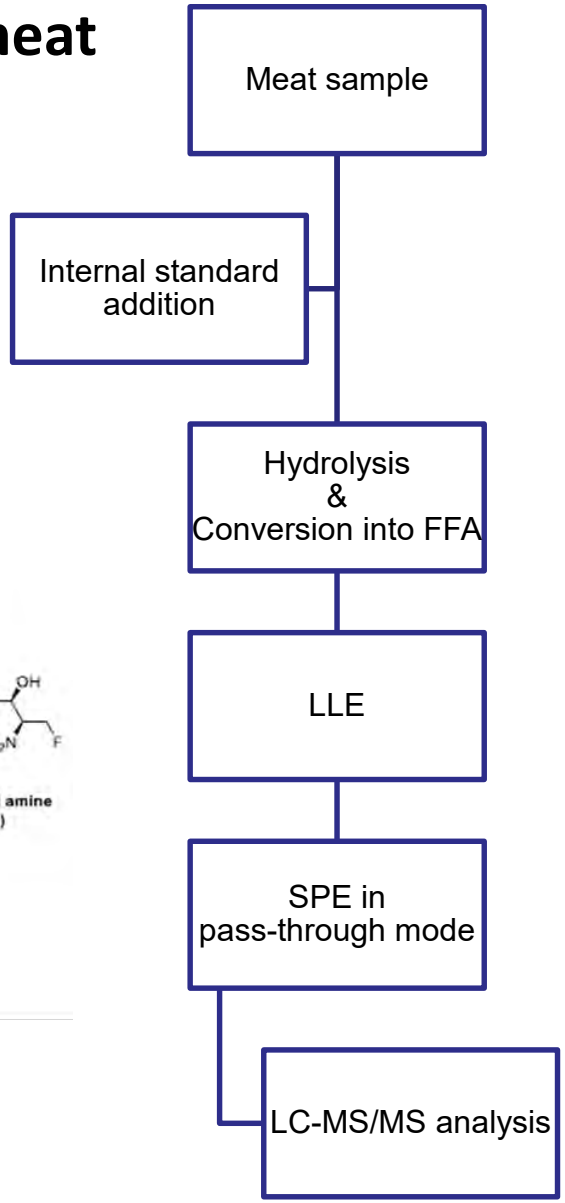


Example on total florfenicol residues in meat

Analytical methodology



(Saito-Shida et al. 2019)



Example on total florfenicol residues in meat

Analytical methodology

COMMISSION REGULATION (EU) No 37/2010

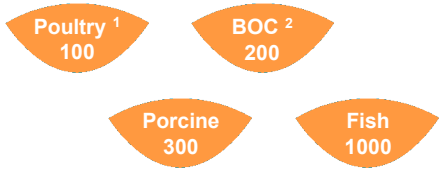
of 22 December 2009

on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin

(Text with EEA relevance)

Florfenicol	Sum of florfenicol and its metabolites measured as florfenicol-amine	Bovine, ovine, caprine	200 µg/kg 3 000 µg/kg 300 µg/kg	Muscle Liver Kidney	Not for animals from which milk is produced for human consumption. Not for animals from which eggs are produced for human consumption.	Anti-infectious agents/Antibiotics
		Porcine	300 µg/kg 500 µg/kg 2 000 µg/kg 500 µg/kg	Muscle Skin and fat Liver Kidney		
		Poultry	100 µg/kg 200 µg/kg 2 500 µg/kg 750 µg/kg	Muscle Skin and fat Liver Kidney		
		Fin fish	1 000 µg/kg	Muscle and skin in natural proportions.		
		All other food producing species	100 µg/kg 200 µg/kg 2 000 µg/kg 300 µg/kg	Muscle Fat Liver Kidney		

- Group B.1.a according to Regulation (EU) 2022/1644
- Authorised substance (antibiotic) present in Table 1 of Reg (EU) No 37/2010
- 4 different MRLs are set in muscle (µg/Kg):



[1] : Poultry and all other food producing species (OFPS)
[2] : Bovine. Ovine and Caprine species

Levels	Level of interest	Level 1: LCL	Level 2	Level 3
Authorised substances	MRL	**[0.1-0.5] x MRL**	**MRL**	**1.5 x MRL**

Choice of LCL :

- Equal to 0.1 x MRLs
- Where validation of the concentration of 0.1 times the level of interest is not reasonably possible, this value may be replaced by the lowest concentration between 0.1 and 0.5 time the level of interest (see § 2.2.1.2, 2.2.1.3 and 2.2.1.4 of the Annex to the Regulation).

Florfenicol:

Matrices	MRLs	Level 1: LCL	Level 2	Level 3	Level 4	Level 5
Poultry OFPS	**100 µg/kg**	10 µg/kg	20 µg/kg	50 µg/kg	100 µg/kg	150 µg/kg
Bovine, Ovine, Caprine	**200 µg/kg**	20 µg/kg	40 µg/kg	100 µg/kg	200 µg/kg	300 µg/kg
Porcine	**300 µg/kg**	30 µg/kg	60 µg/kg	150 µg/kg	300 µg/kg	450 µg/kg
Fish	**1000 µg/kg**	100 µg/kg	-	500 µg/kg	1000 µg/kg	1500 µg/kg

Day 1: Specificity

Evaluation requirements: n≥20 per meat categories

Day 2: Matrix effects

Evaluation requirements: n≥20 for the scope of the method

Days 3 to 5: Validation

D3	D4	D5
Calibration curve (n≥5)	**Calibration curve** (n≥5)	**Calibration curve** (n≥5)
Validation standards	**Validation standards**	**Validation standards**
Level 1 (n≥6)	Level 1 (n≥6)	Level 1 (n≥6)
Level 2 (n≥6)*	Level 2 (n≥6)*	Level 2 (n≥6)*
Level 3 (n≥6)	Level 3 (n≥6)	Level 3 (n≥6)
Level 4 (n≥6)	Level 4 (n≥6)	Level 4 (n≥6)
Level 5 (n≥6)	Level 5 (n≥6)	Level 5 (n≥6)

**** Not studied for aquaculture products***

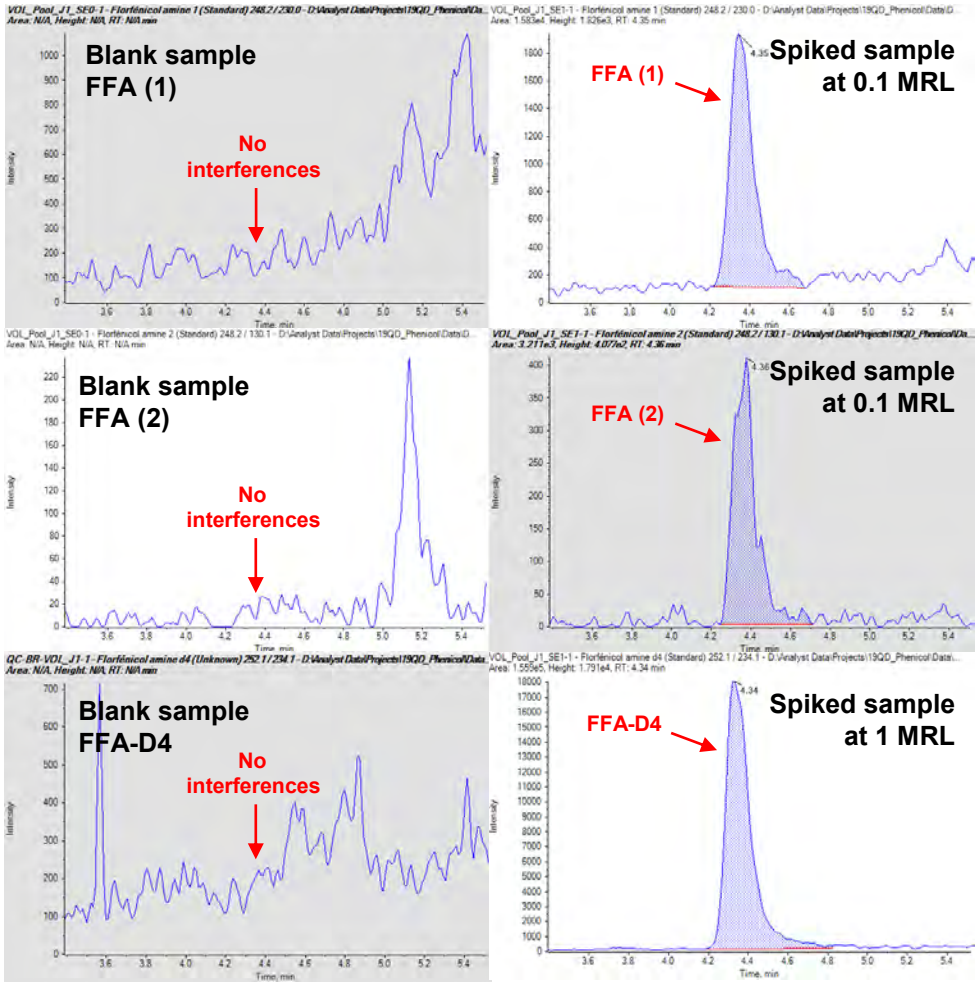
Day 1: Specificity

Evaluated on 84 different batches of matrices (n≥20 per meat categories)
21 poultry + OFPS ; 23 bovine + ovine + caprine ; 21 porcine ; 21 fish

- Absence of interference in matrix blanks at Tr of interest (on all batches)
- 1 positive sample (bovine) was found at Conc. < 0.1 MRL level
  => sample not included in validation design

Poultry sample (MRL = 100 µg/kg) →

- No interferences in blank samples
- No Veterinary Drug Residues from Florfenicol

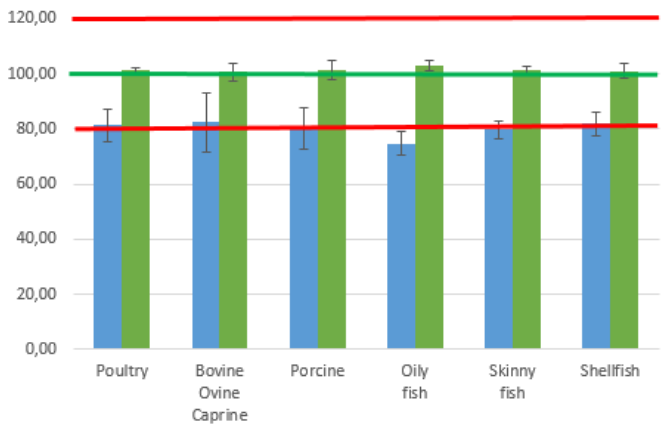


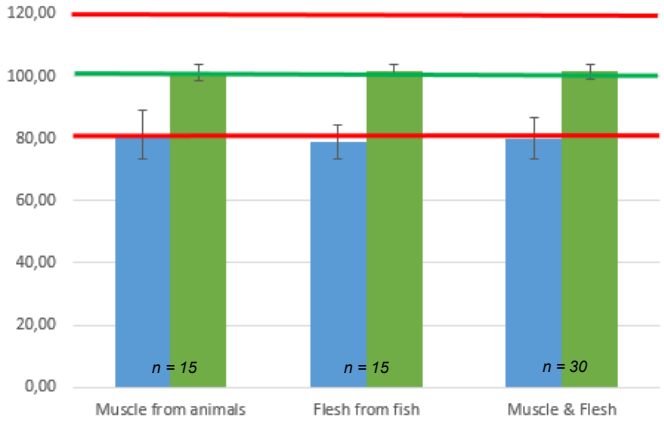
Day 2: Matrix effects

Evaluated on 30 differents batches of matrices representative of the scope of the method including at least 5 per meat category (n≥20 for the scope of the method)

Matrix Effets (ME) :

n = 5 per categories	*Poultry : 3 chicken + 2 turkey* *Bovine, Ovine, Caprine : (2+2+1)* *Porcine : 5 pork* *Oily fish : 3 salmon + 2 trout* *Skinny fish : 2 bass + 3 see bream* *Shellfish : 5 shrimp*





CV ≤ 20%
No matrix effects within or between species is observed when IS is used

Possibility to validate different matrices simultaneously within the same category or between categories.

Days 3 to 5: Validation (x4 meat categories)

	Animal species				Number of replicates	
	Poultry & OFPS	Bovine. Ovine. Caprine	Porcine	Fish		
CS0 : 0.0 MRL	0	0	0	0	1 (injected twice)	Calibration curve
CS1 : 0.1 MRL	10	20	30	100	1 (injected twice)	
CS2 : 0.2 MRL	20	40	60	-	1 (injected twice)	
CS3 : 0.5 MRL	50	100	150	500	1 (injected twice)	
CS4 : 1.0 MRL	100	200	300	1000	1 (injected twice)	
CS5 : 1.5 MRL	150	300	450	1500	1 (injected twice)	
VS1 : 0.1 MRL	10	20	30	100	7	Precision Trueness Uncertainty CCα & CCβ
VS2 : 0.2 MRL	20	40	60	-	7	
VS3 : 0.5 MRL	50	100	150	500	7	
VS4 : 1.0 MRL	100	200	300	1000	7	
VS5 : 1.5 MRL	150	300	450	1500	7	

CS : Calibration Standards
VS : Validation Standards

Validation against MRLs

Days 1 to 3 → Poultry & OFPS
Days 4 to 6 → Bovine, Ovine, Caprine
Days 7 to 9 → Porcine
Days 10 to 12 → Fish

Use of matrix pools

CS → composed of 7 ≠ matrices each days (pool)
VS → ≠ from those used in CS composition

➡ 21 matrices per meat category x 4

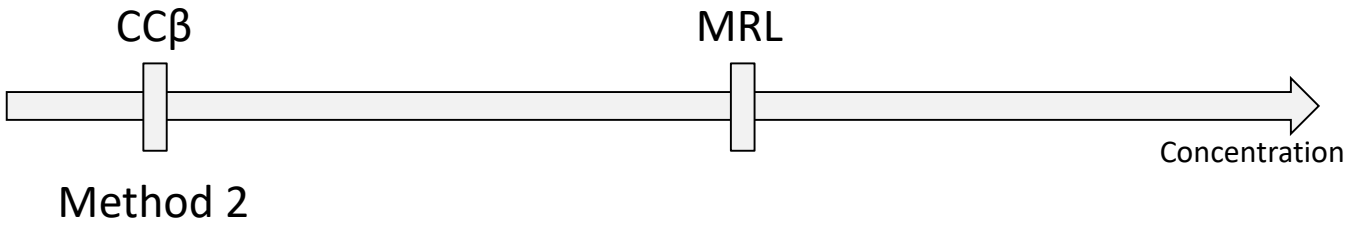
CCβ: Definition from the REG (EU) 2021/808

(15) 'detection capability for screening (CCβ)' means the smallest content of the analyte that may be detected or quantified in a sample with an error probability of β:

(b) in the case of authorised substances, the CCβ is the concentration at which the method is able to detect concentrations below the permitted limit with a statistical certainty of 1 – β;

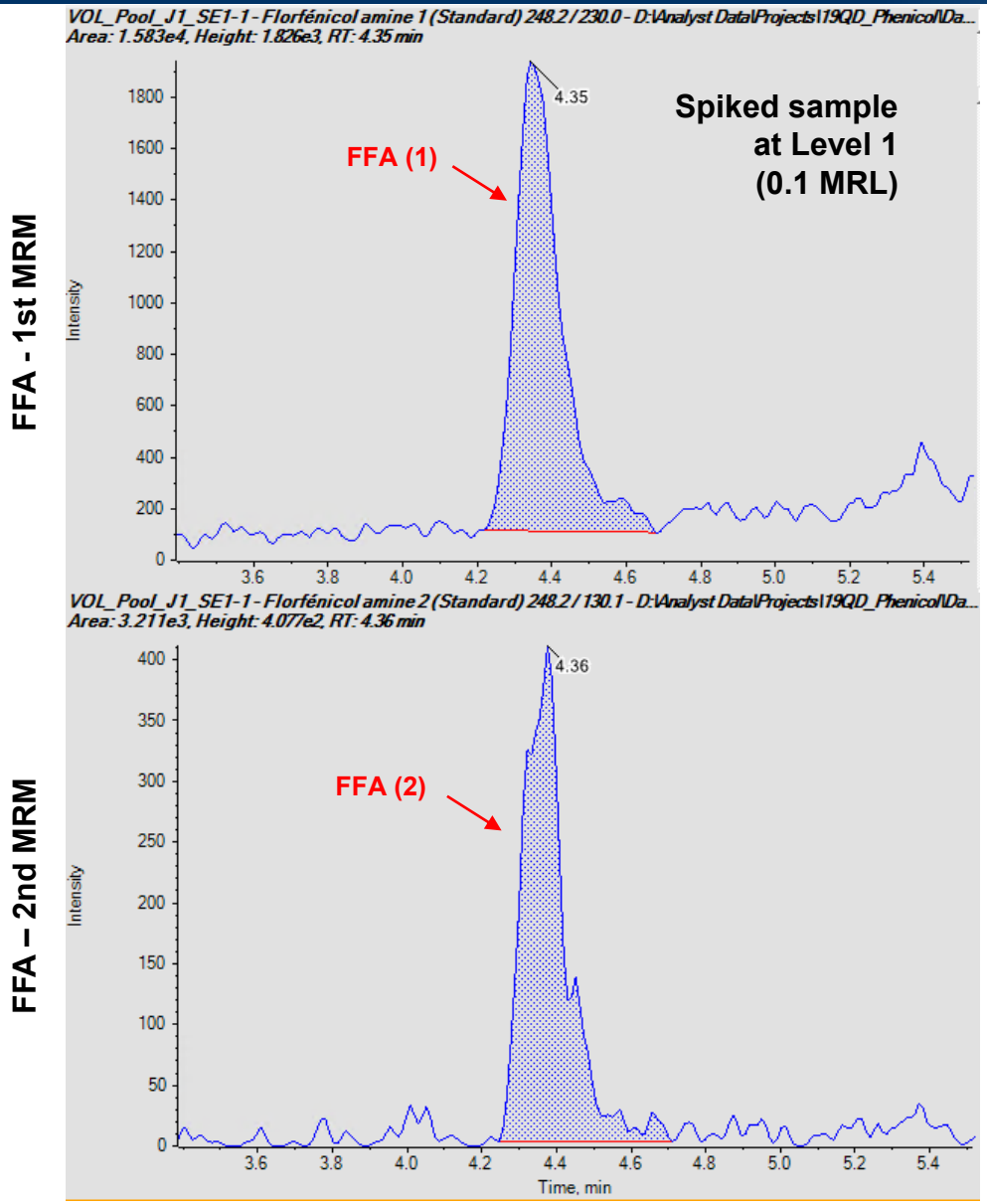
Determination acording to Method 2 (2.7.2.b):

2021/808 requirement: CCβ must be below the MRL



2. For authorised substances, a maximum β error of 5 % shall be ensured. The CCβ shall be calculated as follows:

(b) Method 2: by investigation of fortified blank material at concentration levels below the permitted limit. For each concentration level 20 fortified blanks shall be analysed in order to ensure a reliable basis for this determination. The concentration level, where only ≤ 5 % false compliant results remain, equals the detection capability of the method.



CCβ for FFA in Poultry & OFPS

Poultry sample
MRL = 100 µg/kg

$\beta = 5\% \rightarrow$ 1/21 false neg. tolerated

- S/N ≥ 3 for 21/21 spiked samples at 0.1 MRL for both transitions
- RRT < ±1% for 21/21 spiked samples for each levels (0.1 to 1.5 MRL)
- Relative ion ratio < ±40 % for 20/21 spiked samples at this level

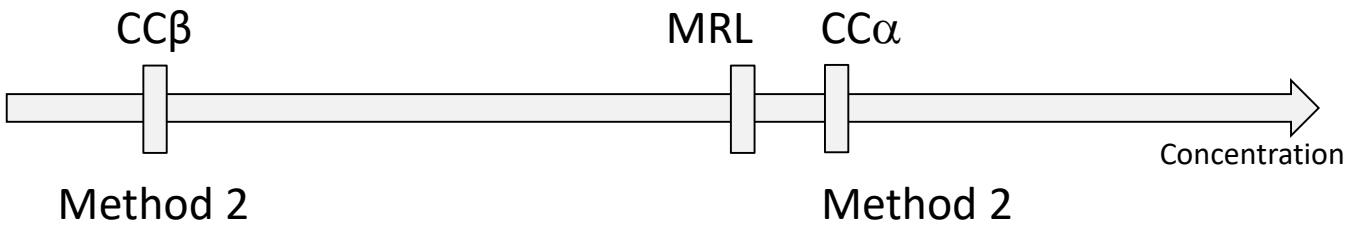
CCβ = Level 1 = 10 µg/kg

CCα: Definition from the REG (EU) 2021/808

(14) 'decision limit for confirmation (CCα)' means the limit at and above which it can be concluded with an error probability of α that a sample is non-compliant and the value 1 – α means statistical certainty in percentage that the permitted limit has been exceeded;

Determination acording to Method 2 (2.6.2.a.ii):

2021/808 requirement: CCα must be as close as possible to MRL and above MRL



2. For authorised substances, the CCα shall be calculated as follows:

(a) For authorised substances in matrix/species combinations for which an MRL or ML has been set:

(ii) Method 2: CCα = MRL (or ML) + k(one-sided, 95 %) × (combined) standard measurement uncertainty at the MRL or ML.

For authorised substances, depending on the validation experiment (and its respective degrees of freedom) the t-distribution might be reasonably applied, or – if the Gaussian distribution (one-sided, n=∞) is taken as a basis, a k-factor of 1,64 shall be used.

$$CC\alpha = MRL + 1.64 * u_c$$

with u_c : combined measurement uncertainty at MRL level

$$CC\alpha_{max} = MRL + 1,64 * u_{c_{max}}$$

$$u_{c_{max}} = \sqrt{(CV_{R_{max}})^2 + (u_{b_{max}})^2}$$

$u_{c_{max}}$: maximum uncertainty at the MRL level (µg/kg)

$CV_{R_{max}}$: maximum allowed intermediate fidelity CV at MRL level

$u_{b_{max}}$: maximum bias uncertainty allowed at the MRL level

$$u_{b_{max}} = \frac{maximum\ allowed\ bias\ at\ the\ MRL\ level}{\sqrt{3}} \quad *$$

MRL (µg/Kg)	Reproducibility $CV_{R_{max}}$ (%) (Reg 2021/808 chap. 1.2.2.2)	Trueness Bias max (%) (Reg 2021/808 chap. 1.2.2.1)	$u_{b_{max}}$(%)	$u_{c_{max}}$ (%)
10 ≤ MRL < 120	25**	20	12	28
120 ≤ MRL < 1000	22	20	12	25
MRL ≥ 1000	16	20	12	20

Exemple : at MRL = 100 µg/kg

$u_{c_{max}} = 28\% = 28\ \mu g/kg$

$$CC\alpha_{max} = 100 + 1.64 * 28 = 145.92\ \mu g/kg$$

* according to the rectangular distribution law defined in ISO 11352: 2012

** The CV (%) presented is a guideline and should be as low as reasonably possible (1.2.2.2. of Reg 2021/808)

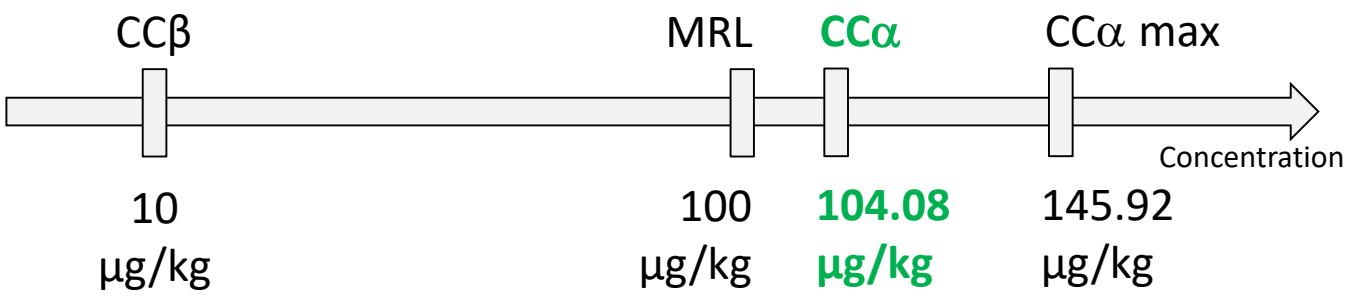
Meat categories	Concentration levels (µg/Kg)	n	Reproducibility			Trueness			u_c (µg/Kg)
			CV_R (%)	CV_R max (%)	CV_R (µg/kg)	u_b (%)	u_b max (%)	u_b (µg/Kg)	
Poultry & OFPS *MRL = 100 µg/kg*	10	21	27.9	25*	2.79	6.10	12	0.61	2.86
	20	21	15.7	25*	3.14	3.42	12	0.68	3.21
	50	21	2.84	25*	1.42	0.93	12	0.47	1.50
	100	21	2.43	25*	2.43	0.53	12	0.53	2.49
	150	21	3.78	22	5.67	1.23	12	1.84	5.96

* The CV (%) presented is a guideline and should be as low as reasonably possible (1.2.2.2. of Reg 2021/808)

$$CC\alpha = MRL + 1,64 * u_c$$

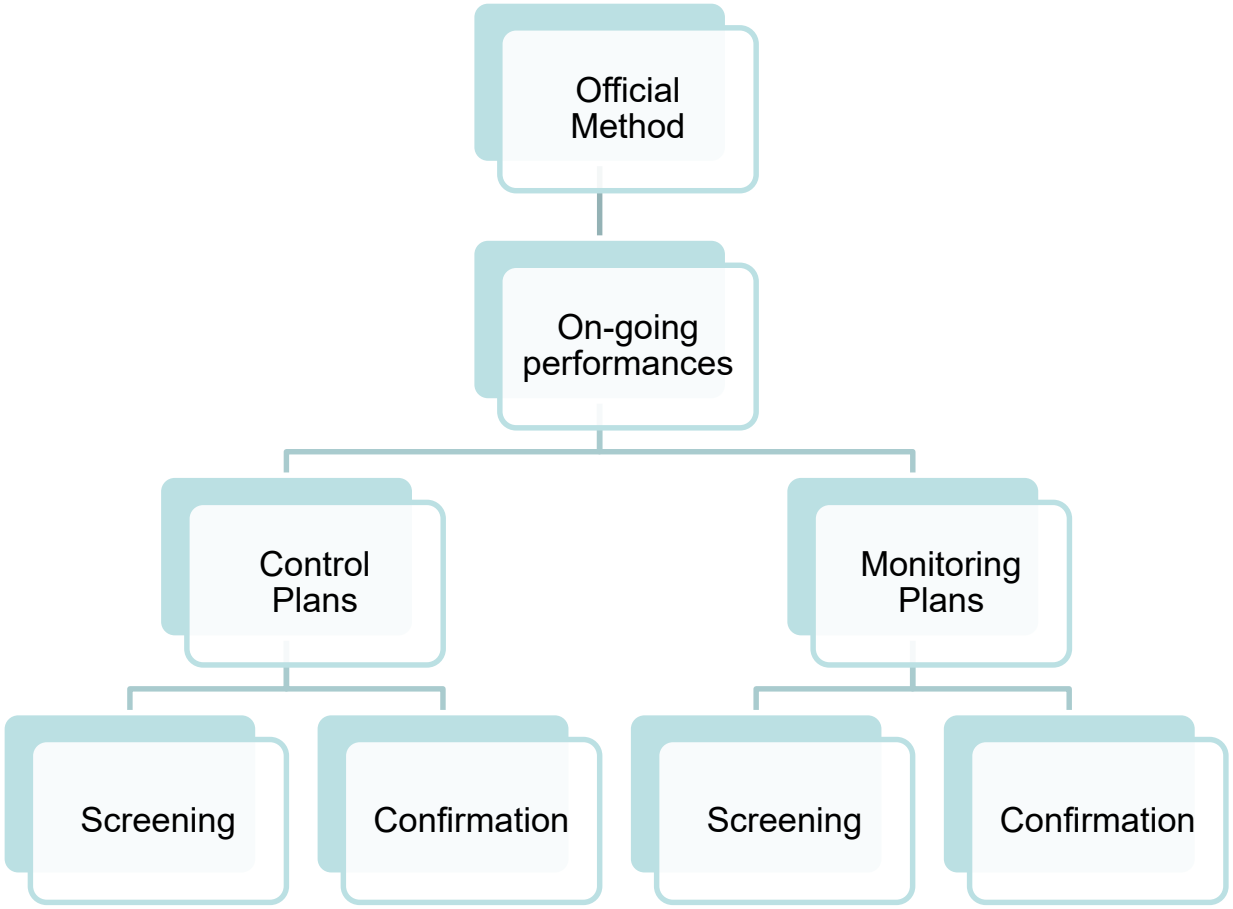
$$CC\alpha = 100 + 1,64 * 2.49$$

$$CC\alpha = 104.08\ \mu g/kg < CC\alpha\ max = 145.92\ \mu g/kg$$



Performance criteria to be validated :

Criteria	Screening		Confirmation
	Qualitative	Quantitative	Quantitative
Identification	ΔRRT < 1% 2 signals with S/N > 3		ΔRRT < 1% 2 signals with S/N > 3 Δratio < 40%
CCβ	method 2 CCb < MRL		
CCα			method 2 close to MRL & below CCα max
Trueness		2021/808 criteria	2021/808 criteria
Precision		2021/808 criteria	2021/808 criteria
Matrix effect	RSD < 20% or < $RSD_{PRECISION}$		
Recovery	For information		
Stability	According to the EURL information		
Specificity	Assessement on at least 20 different blank samples		
Robustness	To be determine during the development step		


Official Method
On-going performances
Control Plans
Monitoring Plans
Screening
Confirmation
Screening
Confirmation

QC for Control Plans:

	Screening	Confirmation	Batch acceptation
QC 1	Matrix blank	Matrix blank	Absence of the compound ISTD detection No interference
QC 2	0.5 MRL	/	ISTD & Analyte detection Qualitative with screening identification criteria
QC 3	/	1 MRL	ISTD & Analyte detection Projection on control chart

QC for Monitoring Plans:

	Screening	Confirmation	Batch acceptation
QC 1	Matrix blank	Matrix blank	Absence of the compound ISTD detection No interference
QC 2	CCβ	CCβ	ISTD & Analyte detection Qualitative with screening identification criteria
QC 3	/	1 MRL	ISTD & Analyte detection Projection on control chart

Specificity
- Studied on 84 different batches

Matrix Effects
- Studied on 30 different batches

Validation
- Cal. Standards: 6 levels
- Val. Standards: 5 levels x 7 replicates

J1 → J2 → J3

Poultry & OFPS | BOC | Porcine | Fish

Total samples

84

+

30

+

41 x 3 x 4 = 492

606

+/- consequent validation

Method validation: Reg (EU) 2021/808
Regulatory limits: Reg (EU) 37/2010 ; 2017/880 & 2018/470
Official control: Reg (EU) 2017/625 ; 2022/1644 & 1646

The validated method takes into account MRLs, cascade MRLs, Control & Monitoring Plans

THANK YOU


thank you
danke
謝謝
ngiyabonga
teşekkür ederim
gracias
спасибо
Баярлалаа
рахмат
dank je
tapadh leat
bedankt
dziękuje
obrigado
hvala
mauruuru
go raibh maith agat
mochchakkeram
sukriya
kop khun krap
terima kasih
grazie
arigatô
takk
dakujem
мерси
merci
ευχαριστώ
감사합니다
ありがとう
хвала
asante
manana
mersi
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welalin
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vinaka
faafetai lava
kiitos
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enkosi
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gracies
sulpay
taiku
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dhanyavadagalu
shukriya
trugarez
tanemirt
rahmet
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mahalo
mamnun
murakoze
tenki