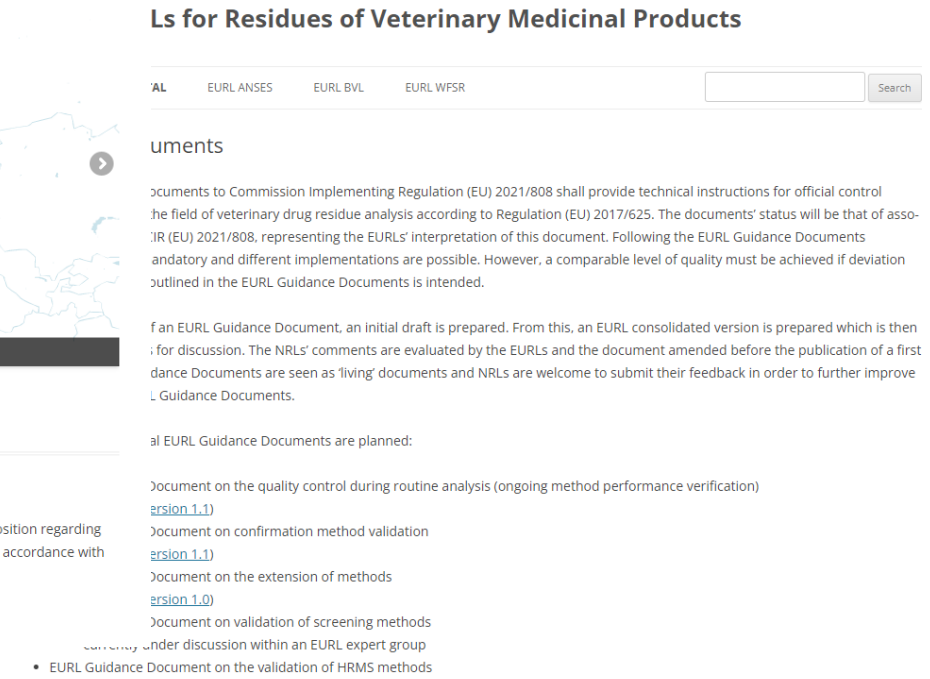
*EURL Cluster for VMP residues*

# CIR (EU) 2021/808

## Theoretical Principles of Validation and Identification

# …on behalf of the 3 EURLs

**EURL cluster website**
**https://eurl-residues.eu/**

# Validation and Identification

## Validation Definitions

**ISO 17025 : 2005**

**Validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled**

**ISO 17025 : 2017**

**verification (3.8), where the specified requirements are adequate for an intended use**

(ISO/IEC Guide 99:2007)

# Validation and Identification

**Verification**

**provision of objective evidence that a given item fulfils specified requirements**

**Example 2 : Confirmation that performance properties or legal requirements of a measuring system are achieved.**

**Identification ??**

# ....fit for purpose ??

**test items**
**“specified requirement”**
**measurement device**

**adequate for an intended use ?**

….**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 …. CDR (EU) 2022/1644

**provision of objective evidence that a given item fulfils specified requirements**

…**performance criteria for methods** to control for residues of pharmacologically active substances used in food-producing animals … CIR (EU) 2021/808

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

**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**

**(Text with EEA relevance)**

**"Legal part"**

**definitions and interpretation of results**

**"Technical part"**

**ANNEX I** ***details on methods and validation***

**ANNEX II** *sampling*

**enters into force 10 June 2021**

## Article 2 – Definitions

**level of interest means the concentration of a substance in a sample that is significant to determine its compliance with the legislation as regards:**

- **MRLs and MLs (CR 124/2009 + CR 37/2010 + …)**
- **RPAs (Regulation (EU) 2019/1871)**
- **a concentration as low as analytically achievable for prohibited or unauthorised substance (no RPA present)**

**=> new MMPR guidance V 2.0**

(**M**inimum **m**ethod **p**erformance **R**equirements)

# Article 5 - Interpretation of results

**The result of an analysis shall be considered non-compliant where it is equal to or above the decision limit for confirmation (CCα).**

# Article 2 – Definitions

**MRL or ML substances:**

the CCα shall be the concentration at and above which it can be decided with a statistical certainty of 1 – α that the permitted limit has been exceeded.

**(the α error shall be 5 % or lower)**

**Unauthorised or prohibited substances:**

CCα shall be the lowest concentration level at which it can be decided with a statistical certainty of 1 – α that the particular analyte is present.

**(the α error shall be 1 % or lower)**

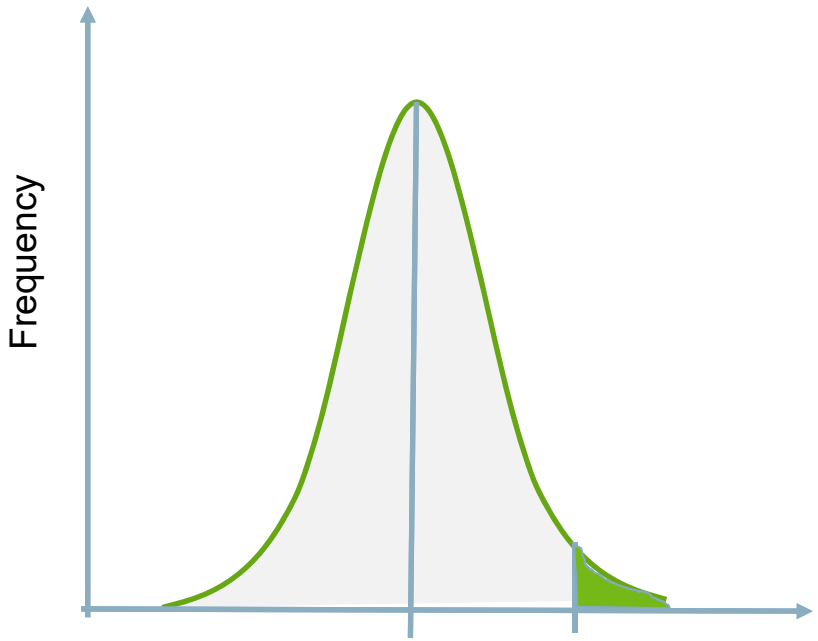
# Basics

**Decision limit CCα**

For confirmatory methods

Evaluation of **a sample !**

**$CC_\alpha$ > MRL / ML**
**≤ RPA, < MMPR**




False non-compliant decision, i.e even though the concentration is truly compliant, the result is non compliant ("=> risk")

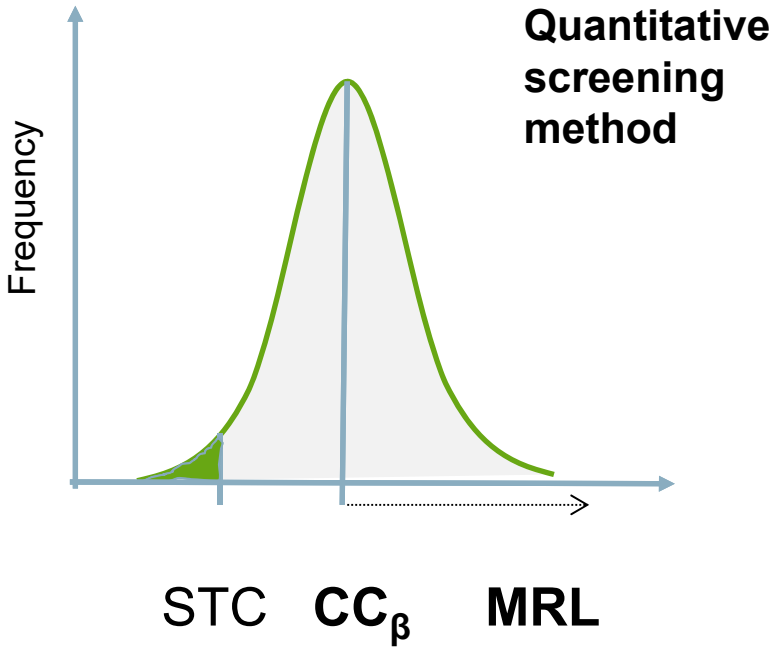
# Basics

## Detection capability

For screening methods

Evalution of **a method !**

**STC ≤ $CC_\beta$ < MRL, ML**
**or RPA / MMPR**




Risk of making a false compliant decision, i.e. the sample is **not send to confirmation**, even though the concentration is truly at the $CC_\beta$

# CIR (EU) 2021/808

## Annexes

### Annex 1 : Methods Requirements

Chapter 1: **Performance criteria and other requirements for analytical methods**

Chapter 2: **Validation**

Chapter 3: QC during routine analysis – ongoing method performance verification

Chapter 4: Extension of the validated scope of a previously validated method via quality control samples during routing analysis

### Annex 2 : Sampling procedures and official sample treatment

## 1.1 Requirements of screening methods

### 1.1.1 Categories of suitable screening methods

### 1.1.2 Requirements for biological, biochemical or physico-chemical screening methods

CCβ screening lower than MRL/ML/RPA/MMPR
**false compliant rate is lower than or equal to 5% (ß-error)**

## 1.2 Requirements of confirmatory methods

### 1.2.1 General requirements of confirmatory methods

**prohibited or unauthorised substances:**
the CCα shall be as low as analytically achievable
CCα shall be lower than or equal to the RPA / lower than MMPR
**For MRL substances** the CCα shall be higher than but as close as possible to the MRL or ML.

# Requirements for precision

## Requirements for identification

Group A – Prohibited or unauthorised pharmacologically active substances

Group B – Pharmacologically active substances authorised for use in food-producing animals

(CDR (EU) 2022/1644)

## 2.1 Performance characteristics for analytical methods (CIR (EU) 2021/808)

| 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 |

Quantitative methods can be "downgraded" and defined as e.g. qualitative or semiquantitative

## 1.2 Requirements of *(quantitative)* confirmatory methods

minor changes : 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 % |

| 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 is a **guideline** and should be as low as reasonably possible

repeatability conditions shall be equal or below two thirds of the table values

## 2.2 Trueness, repeatability and within-laboratory reproducibility

**Spike level / concentration range**

| Legal limit | CD 2002/657 | CIR 2021/808 |
|---|---|---|
| MRL/ML | 0.5, 1.0, 1.5 MRL/ML | 0.1 (0.5), 1.0, 1.5 MRL/ML |
| MRPL | 1.0, 1.5, 2.0 MRPL | MRPL concept has been revoked |
| RPA | - | 0.5 (1.0), 1.0, 1.5 RPA |
| MMPR | Concept first introduced in connection with CIR 2021/808 | Analytical methods need to be validated below MMPR, fortification levels can be similar to those for RPA compounds |
| Unauthorised compound | | 1.0, 2.0, 3.0 LCL |

**6 repetitions each – done at three different occasions (repeatability / within- lab reproducibility)**

**Remarks :**

**1) If reasonably achievable !!! - otherwise:**

- the lowest concentration between 0,5 times and 1,0 times the RPA / MRL

**2) If techniques allow to analyse substances below 0.5 times the RPA do so –**

**= > Anyhow the RPA itself should be one concentration level**

# Method validation using the conventional validation approach

- Validation in 3 separate series
- 4th series required in case absolute recovery and relative matrix effect need to be determined
- Minimum 21 different batches of matrix material required
- Spiking on three different levels

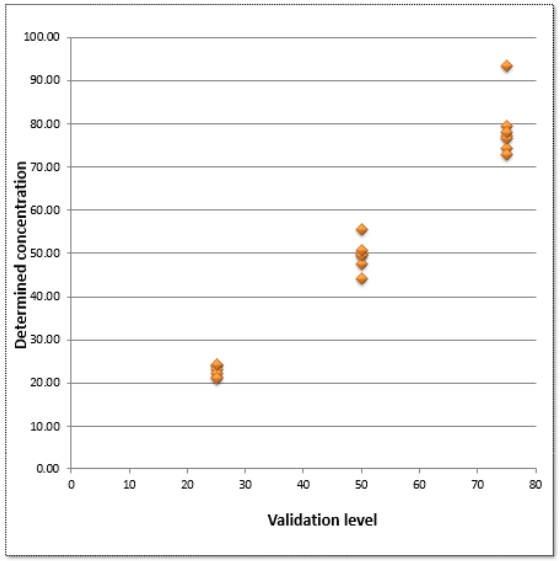
| | |
|---|---|
| Validation series 1: | 7 batches for specificity and fortification + 1 batch for the calibration curve[9] |
| Validation series 2: | 7 batches for specificity and fortification + 1 batch for the calibration curve[9,10] |
| Validation series 3: | 7 batches for specificity and fortification + 1 batch for the calibration curve[9,10] and ruggedness[11] |

+ validation series 4 for absolute recovery, relative matrix effect

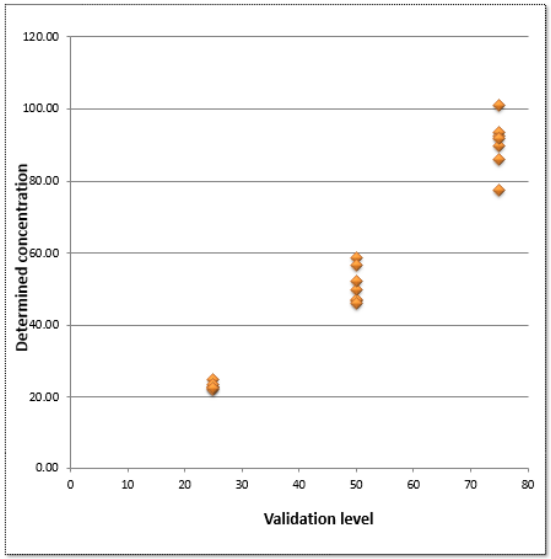
**Please refer to guidance document for details!**

# Data visualisation

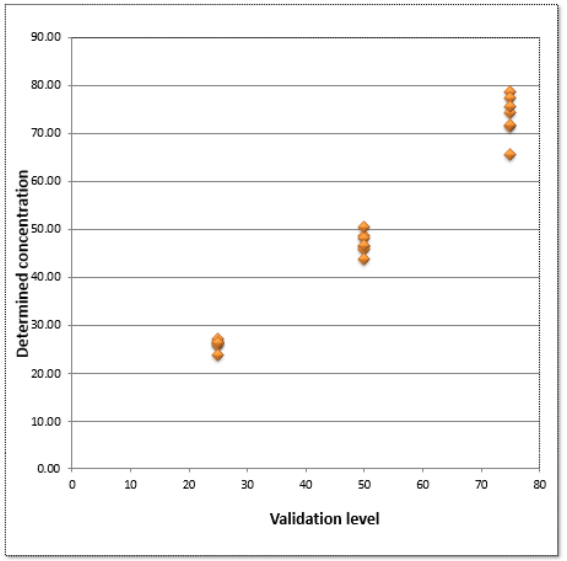
**Experiment 1**



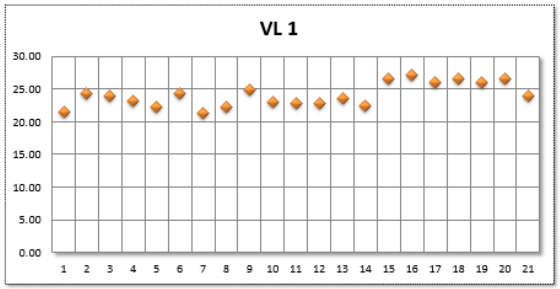

**Experiment 2**

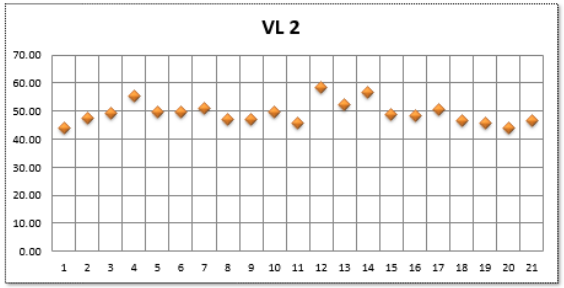


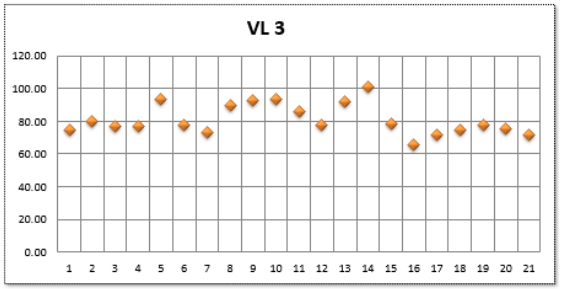

**Experiment 3**




**Overview of variation data exp 1-3**



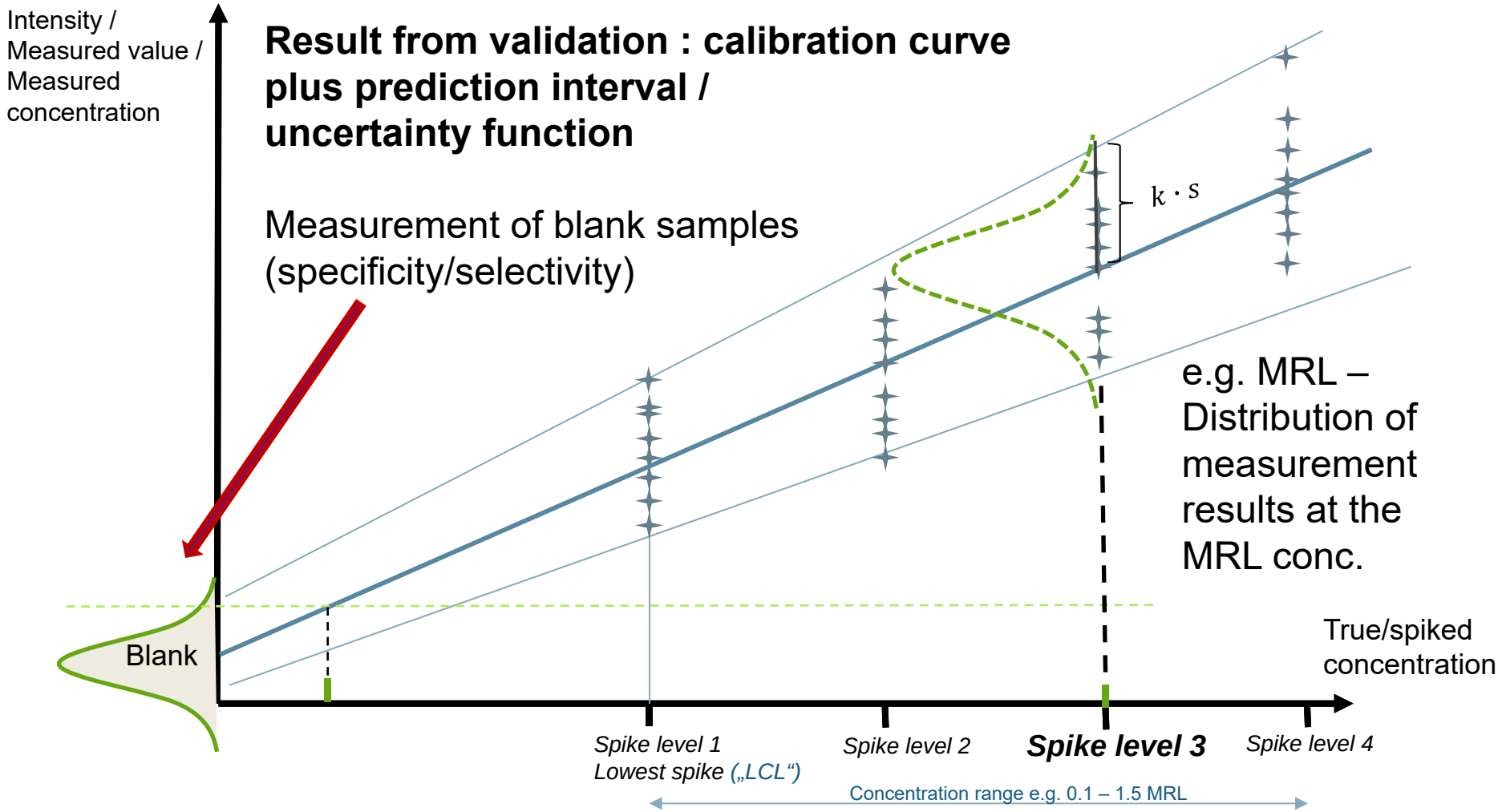



# Method validation according to alternative models (experimental design plans)

- Defining analytes and factors / factor levels
- Defining concentration **ranges** (spike levels)
- Preparation of the experimental plan
- Standard design : 8 runs at four to five concentration levels

| Block *j* | Breeding | Operator | HPLC | Extract storage | CL 01 (0.2) [µg/kg] | CL 02 (0.5) [µg/kg] | CL 03 (1) [µg/kg] | CL 04 (2) [µg/kg] | CL 05 (4) [µg/kg] | CL 06 (6) [µg/kg] |
|---|---|---|---|---|---|---|---|---|---|---|
| 01 | Conventional | Routine | Batch 1 (Old) | With | 0.22 | 0.49 | 0.82 | 2.11 | 4.66 | 6.45 |
| 02 | Conventional | Routine | Batch 2 (New) | With | 0.24 | 0.47 | 1.20 | 2.18 | 4.98 | 7.32 |
| 03 | Conventional | Occasional | Batch 1 (Old) | Without | 0.28 | 0.57 | 1.07 | 2.62 | 3.67 | 6.78 |
| 04 | Conventional | Occasional | Batch 2 (New) | Without | 0.22 | 0.55 | 1.06 | 1.74 | 3.72 | 5.56 |
| 05 | Organic | Routine | Batch 1 (Old) | Without | 0.16 | 0.42 | 0.91 | 2.00 | 4.42 | 5.53 |
| 06 | Organic | Routine | Batch 2 (New) | Without | 0.23 | 0.55 | 1.24 | 2.11 | 4.52 | 5.91 |
| 07 | Organic | Occasional | Batch 1 (Old) | With | 0.42 | 0.73 | 1.19 | 2.26 | 4.44 | 4.99 |
| 08 | Organic | Occasional | Batch 2 (New) | With | 0.32 | 0.56 | 1.08 | 1.82 | 3.25 | 5.01 |


Intensity / Measured value / Measured concentration
Result from validation : calibration curve plus prediction interval / uncertainty function
Measurement of blank samples (specificity/selectivity)
k · s
e.g. MRL – Distribution of measurement results at the MRL conc.
Blank
True/spiked concentration
Spike level 1
Lowest spike („LCL“)
Spike level 2
Spike level 3
Spike level 4
Concentration range e.g. 0.1 – 1.5 MRL

Requirements for precision

# Requirements for identification

Group A – Prohibited or unauthorised pharmacologically active substances

Group B – Pharmacologically active substances authorised for use in food-producing animals

(CDR (EU) 2022/1644)

# CIR (EU) 2021/808 - Annex 1

## Chapter 1:

### 1.2 Requirements of confirmatory methods

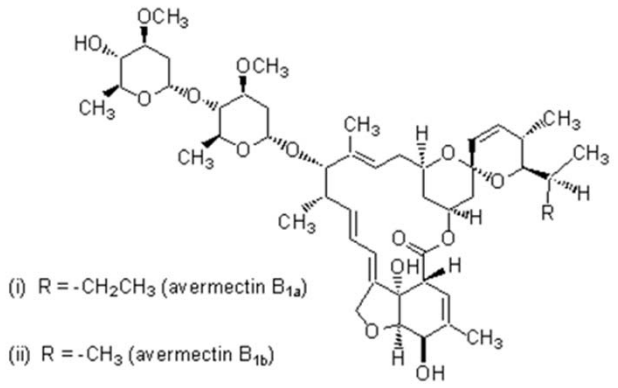
#### 1.2.1 General requirements of confirmatory methods

**usually : MS-based methods**

**In the case of mass spectrometry not being suitable for registered veterinary substances, also other methods like e.g. HPLC-DAD-FLD**

**e.g.**

Avermectin B1a bzw.
8,9-Z-Avermectin B1a
und
Avermectin B1b



## 1.2.3. Requirements for chromatographic separation

minimum acceptable retention time : twice the void volume retention time

The retention time of the analyte in the extract shall correspond to that of the ... standard (matrix, matrix matches, solution) ...

- with a tolerance **of ± 0.1 minute** or
- a deviation of less than 5% of the retention time, in case the retention time is below 2 minutes.

In case an internal standard is used, the relative retention time of the analyte, shall correspond to that of the ... standard ...(matrix, matrix matches, solution)

- with a maximum deviation 0.5 % for gas chromatography and
- **1 % for liquid chromatography** for methods.

## 1.2.4 Specific performance criteria for mass spectrometry

For all mass spectrometric analyses **at least one ion ratio** shall be determined.

The selected fragment or product ions shall **be diagnostic**

**The ion ratio** of the analyte to be confirmed shall correspond to those of the matrix-matched standards, matrix-fortified standards or standard solutions at comparable concentrations, measured under the same conditions, within **± 40 % relative deviation.**

**Definition HRMS**

the **mass deviation** of all diagnostic ions shall be **below 5 ppm** (or in case of m/z < 200 below 1 mDa). Resolution shall typically be greater than 10,000 for the entire mass range at 10 % valley or **20,000 at full width at half maximum** (FHWM).

## 1.2.3.3 Identification (identification points)

Identification points per technique

| Technique | Identification Points |
|---|---|
| Separation (mode GC, LC, SFC, CE) | 1 |
| LR-MS ion | **1** |
| Precursor ion selection at <±0,5 Da mass range | 1 (indirect) |
| LR-MSn product ion | **1,5** |
| HR-MS ion | 1,5 |
| HR-MSn product ion | 2,5 |

MRL substances : a minimum of **4 identification points**

non-authorized or prohibited substances:

a minimum of **5 identification points**

One point can originate from the **chromatographic separation**

# Decision limit and measurement uncertainty

## How has measurement uncertainty be taken into account in the compliant/non compliant decision and the calculation of the decision limit?

**ISO/IEC 17025:2017 - 3.7 Decision Rule**
rule that describes how measurement uncertainty is accounted for when stating conformity with a specified requirement.

**CIR (EU) 2021 / 808 -** § 5 Interpretation of Results
(1) The result of an analysis shall be considered **non-compliant** where it is **equal to or above** the decision limit for confirmation (**CCα**).

Annex I, 2.6
For the control of the **compliance** of samples, the **combined standard measurement uncertainty** has already been taken into account **in the CCα value** (decision limit for confirmation).

The **within-laboratory reproducibility and the trueness** are suitable to define the (combined) standard measurement uncertainty, if determined by taking into account all relevant influencing factors.

**ISO 17025:2017, 7.8.3.1**
...test reports shall, where necessary for the interpretation of the test results, include (...) the measurement uncertainty (...) when
the measurement uncertainty affects conformity to a specification limit.

# Decision rules and CCs

Point 2.6.1. An example on how calculate CCα value according Method 3 [CCα = **LCL** + k(one-sided, 99 %) × (combined) standard measurement uncertainty at **LCL**] are welcome“

Example Chloramphenicol :

Validation levels : e.g. **0.05** / 0.1 / 0.15 / 0.30 ug/kg
(validation range **0.05** – 0.3 ug/kg)

Within-laboratory reproducibility : 20 – 30 % (at the LCL 30 %)

(...combined standard MU if taken into account all relevant influencing factors)

k = 2.33 (Gaussian distribution; one-sided, n=∞) 99%

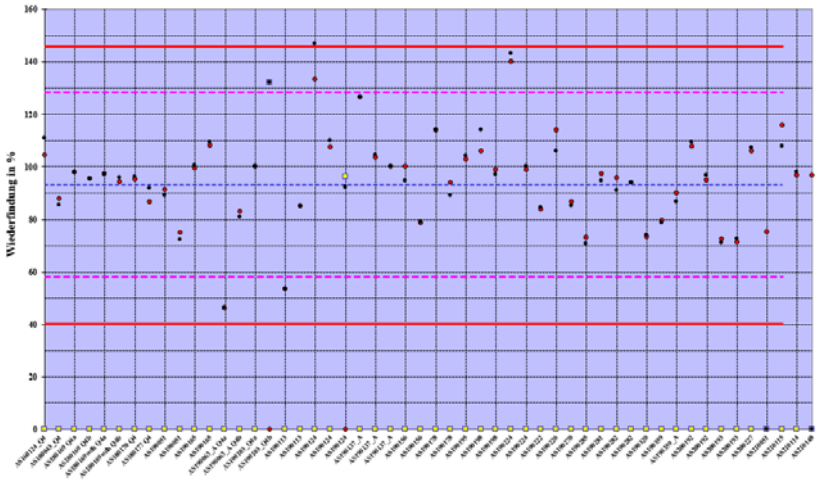
**CCα = 0.05 ug/kg + 2.33 x (0.3 x 0.05 ug/kg) = 0.085 ug/kg**

Fitness for purpose ?

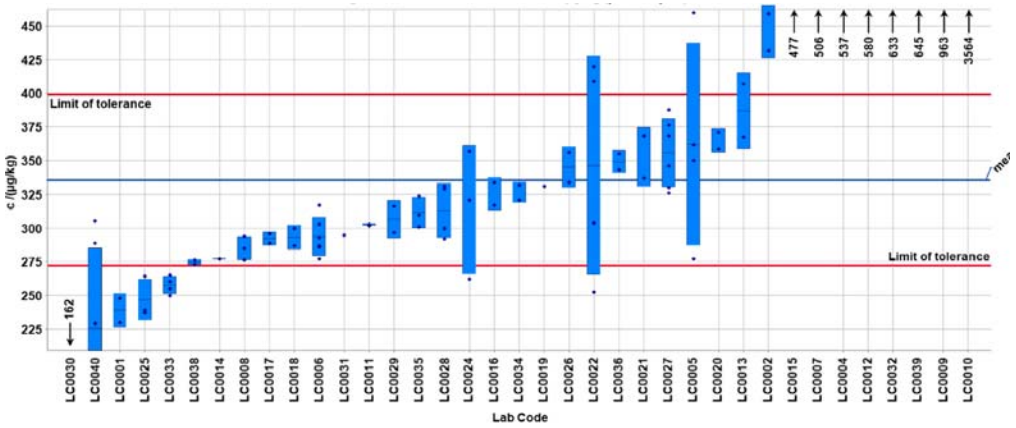
| Performance characteristic | Acceptance criteria |
|---|---|
| Identification | Sufficient amount of identification points as derived from the measurement technique, see 1.2.3.3, Annex of Commission Implementing Regulation (EU) No 11188/2018 |
| CCα | No numerical criteria<br>-authorised substances: higher than but as close to the MRL / ML as analytically achievable<br>-prohibited / unauthorised substances with RPA: lower than or equal to the RPA<br>-prohibited / unauthorised substances without RPA: as low as analytically achievable |
| CCβ | No numerical criteria<br>-authorised substances: lower than or equal to the MRL / ML<br>-prohibited / unauthorised substances with RPA: lower than or equal to the RPA<br>-prohibited / unauthorised substances without RPA: as low as analytically achievable |
| Precision | Concentration dependant, see 1.2.2.2, Annex of Commission Implementing Regulation (EU) No 11188/2018 |
| Trueness | Concentration dependant, see 1.2.2.1, Annex of Commission Implementing Regulation (EU) No 11188/2018 |
| Stability | See 2.5 |
| Relative matrix effect | See 2.10 |
| Absolute recovery | No fixed criteria for absolute recovery, specificity/selectivity and ruggedness. The results for these parameters shall be evaluated using expert knowledge. The responsible scientist shall identify critical aspects which may require method improvements. |
| Specificity / selectivity | |
| Ruggedness | |

# Ongoing method performance control

## Internal QC




## External QC



# Thanks for your attention !

**Contact:**

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