Bundesamt für
Verbraucherschutz und
Lebensmittelsicherheit

European
Commission

CIR (EU) 2021/808

Changes to Revision CD 2002/657 EC

Bundesamt für Verbraucherschutz und Lebensmittelsicherheit

CIR (EU) 2021/808

European Commission

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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

(Text with EEA relevance)

2021

2015

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General approach

European Commission

Keeping proven principles

(criteria approach, concept of identification points, concept of decision limit and detection capability)

Consideration of technical progress

(mass spectrometry, chromatography)

Consideration of guidelines in addition to 2002/657

("SANCO/2004/2726", "screening guideline")

Consideration of ISO 17025:2017 requirements

Reference to practical EURL guidance

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Contents

European Commission

CIR (EU) 2021/808 (Rev. Decision 2002/657 EC)

"Legal part"

Article 1 - Subject matter and scope

Article 2 - Definitions

Article 3 - Methods of analysis

Article 4 - Quality control — *reference to ISO 17025*

Article 5 - Interpretation of results

Article 6 - Methods for sampling — *former Decision 98/179/EC*

Article 7 - Repeals and transitional measures — ***old data valid until 2026 !!***

Article 8 - Entry into force — ***10 June 2021 !!***

"Technical part"

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

ANNEX II — *sampling*

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)

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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

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

Article 2 - Definitions

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 (the β error shall be 5 % or lower) :

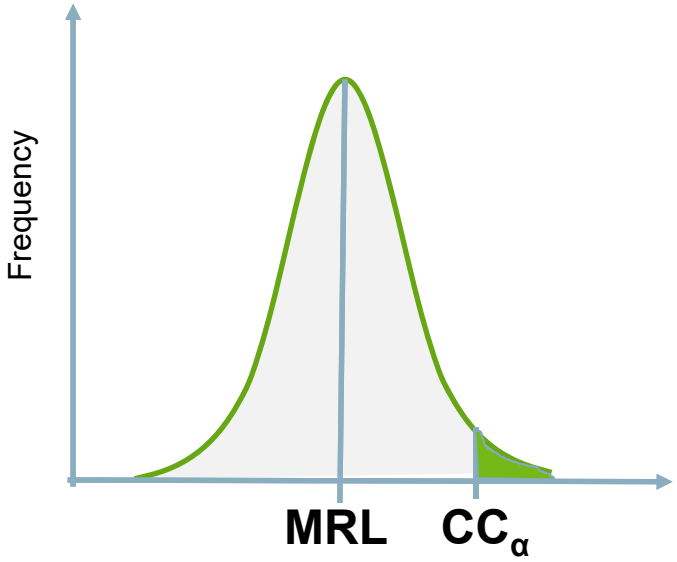
prohibited or unauthorised substances:
CCβ is the lowest concentration at which a method is able to detect or quantify with a statistical certainty of 1 – β, samples containing residues of prohibited or unauthorised substances;

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

Basics

Decision limit

For confirmatory methods



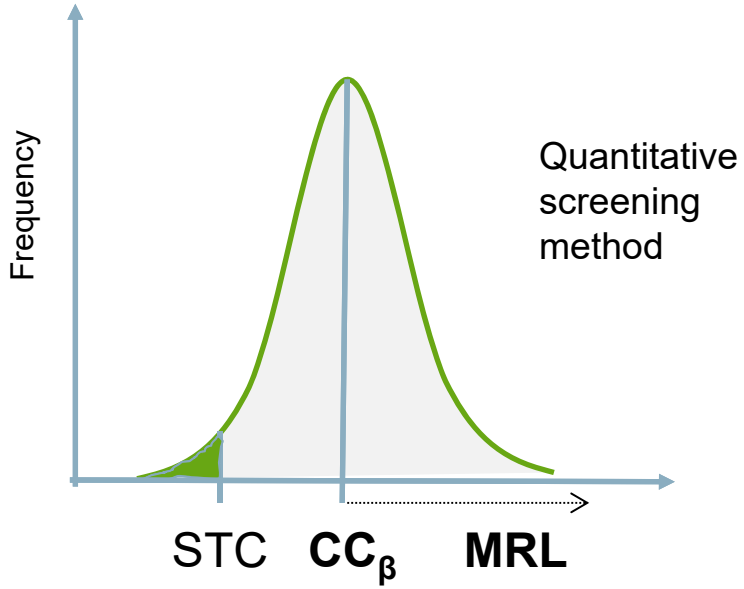
Evaluation of **a sample !**

CC_α **>** MRL / ML
≤ RPA, **<** MMPR

False non-compliant decision, i.e even though the concentration is truly compliant, the result is non compliant

Detection capability

For screening methods



Evalution of **a method !**

STC **≤** CC_β < MRL, ML or RPA /MMPR

False compliant result, i.e. the sample is **not send to confirmation**, even though the concentration is truly at the CC_β

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Article 2 - Definitions

“Rules” for results of screening methods with regard the interpretation of results:

screening target concentration (STC) means the concentration lower than or equal to the CCβ at which a screening measurement categorises the sample as potentially non-compliant ‘Screen Positive’ and triggers a confirmatory testing

lowest calibrated level (LCL) means the lowest concentration on which the measuring system has been calibrated

“lowest spike level which can be reliably detected”

(the concentration at which the analyte can be confirmed in at least 50 % of the cases)

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

CIR (EU) 2021/808 - Annex 1

Chapter 1: Performance criteria and other requirements for analytical methods

1.1 Requirements of screening methods

1.1.1 Categories of suitable screening methods

Qualitative, semi-quantitative or quantitative methods

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

CCβ screening shall be as low as analytically achievable
i.e. lower than MRL/ML/RPA/MMPR
false compliant rate is lower than or equal to 5% (ß-error)
(validated methods / demonstrated in a documented traceable manner)

= > Introduction of STC

CIR (EU) 2021/808 - Annex 1

Chapter 1:

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.
false non-compliant rate (α-error)
less or equal to 1% for prohibited or unauthorised substances
less or equal to 5% for MRL substances
(validated methods / demonstrated in a documented traceable manner)

CIR (EU) 2021/808 - Annex 1

Chapter 1:

1.2 Requirements of confirmatory methods

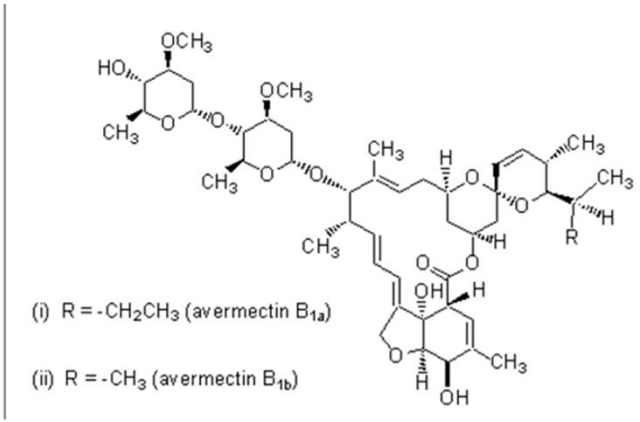
1.2.1 General requirements of confirmatory methods

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



CIR (EU) 2021/808 - Annex 1

1.2 Requirements of confirmatory methods

1.2.2 General performance criteria for 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 %

minor changes: Acceptable coefficient of variation (precision) :

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

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1.2.3. Requirements for chromatographic separation

the minimum acceptable retention time for the analyte(s) shall be 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.

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1.2.4 Specific performance criteria for mass spectrometry

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).

The selected fragment or product ions shall be diagnostic…. Non-selective transitions (e.g. the tropylium cation or loss of water) shall be omitted whenever possible.

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

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.**

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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**

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Summary of important Changes

European Commission

Performance characteristic Changes from CD 2002/657 to CIR 2021/808		
Identification	Change in the concept for the identification points	ion ratios ! (+/- 40 % in general) HRMS definition for mass accuracy One IP for chromatographic separation
Chromatography	General requirements for validation remain the same, requirements for identification (RT time) have been adjusted	New: absolute RT time criteria LC : permitted relative retention time deviation reduced to 1 %

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Annex 1 - Validation

European Commission

2.1 Performance characteristics to be determined for analytical methods

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

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Annex 1 – Chapter 2 : Validation

European Commission

2.2 Trueness, repeatability and within-laboratory reproducibility

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
- the lowest concentration between 0,1 times and 0,5 times the 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

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CIR (EU) 2021/808 - Annex 1 - Validation

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Chapter 2.6 / 2.7 : CC_α and CC_β

Measurement uncertainty

"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)"

= decision rule

"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."

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MU- ISO technical specification

European Commission

New working Item proposal

1 Scope
2 Normative references / 3 Terms and definitions 4 Symbols
5 General principles
5.1 General
5.2 Principles of conventional approach
5.3 Principles of factorial approach
6 Blocking approach
7 Factorial approach

Validation - Chapter 2.6 CCα

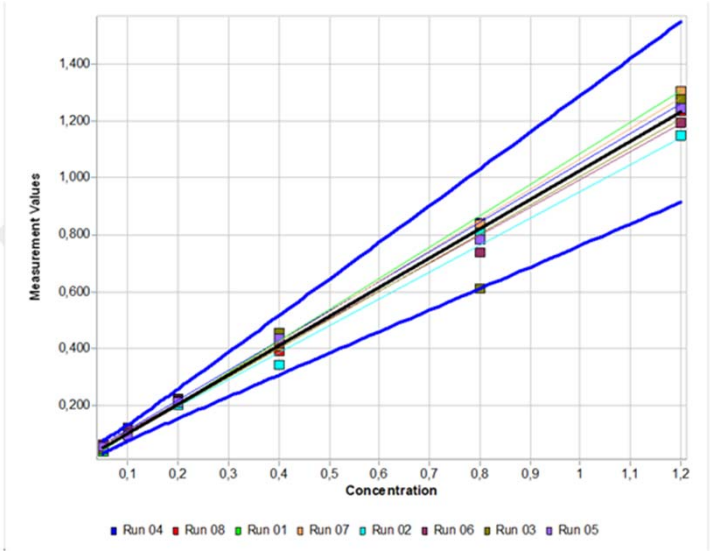
CCα (banned) : (three) possible calculation methods

Method 1: by the calibration curve procedure according to ISO 11843-1:1997

…blank material shall be used, which is fortified at and above the RPA or LCL in equidistant steps…

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

…calibration curves from 8 runs + confidence interval …



Validation - Chapter 2.6 CCα

CCα (banned) continued

Method 2: by analysing at least 20 representative blank materials per matrix to be able to calculate the signal to noise ratio

1+2 shall be verified by analysing blank matrix fortified at the calculated decision limit ; 2 : transition period for old validations until end 2025

CCα (MRL/ML)

comparable to banned substances, but use of MRL/ML levels for calculation

For authorised substances in matrix/species combinations for which no MRL has been set, **no residues** shall be present

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Validation - Chapter 2.6 CCα

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Sum-MRL : the CCα of the substance with the highest concentration in the sample shall be used as the CCα

Examples:

Thiabendazol: MRL : sum of thiabendazole and hydroxy-thiabendazol.
MRL in muscle is **100 µg/kg**.

Validation: thiabendazole (spike: 100 µg/kg, **CCα : 109 µg/kg**) ,
hydroxy-thiabendazole (spike: 100 µg/kg, **CCα : 116 µg/kg**)

Sample 1 : thiabendazole: 20 µg/kg + hydroxy-thiabendazole: 70 µg/kg
=> sum is **90 µg/kg** => **compliant**;

Sample 2 : containing thiabendazole: 40 µg/kg + hydroxy-thiabendazole: 80 µg/kg
=> sum is **120 µg/kg** => **non compliant**

= > take into account the CCalpha (at the MRL level !) for the substance with the highest concentration
i.e. here hydroxy-thiabendazole with a CCα: 116 µg/kg

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CIR (EU) 2021/808 - Annex 1 –Validation -Chapter 2.7 CCβ

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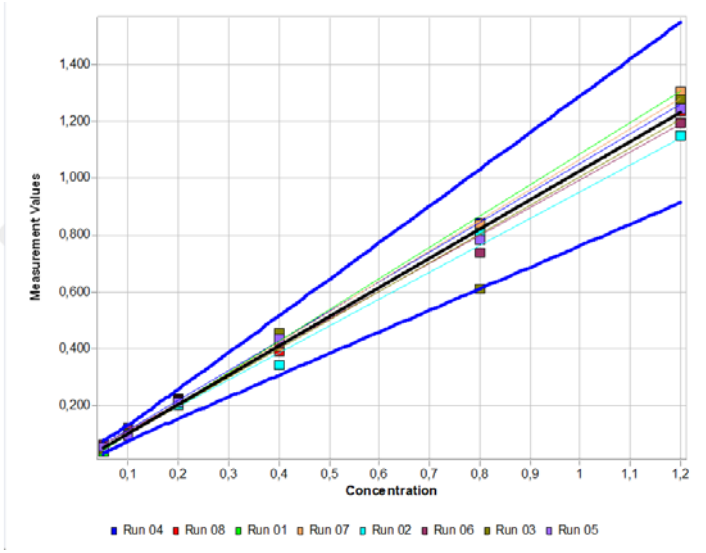
CCβ : three possible calculation methods

Method 1: by the calibration curve procedure according to ISO 11843-1:1997

…material fortified at and below the RPA (if no RPA around the STC) in equidistant steps…

Method 3: CCβ = STC + k(one-sided, 95 %) × (combined) standard measurement uncertainty above the STC

…calculate the maximum STC for control of a given level of interest …



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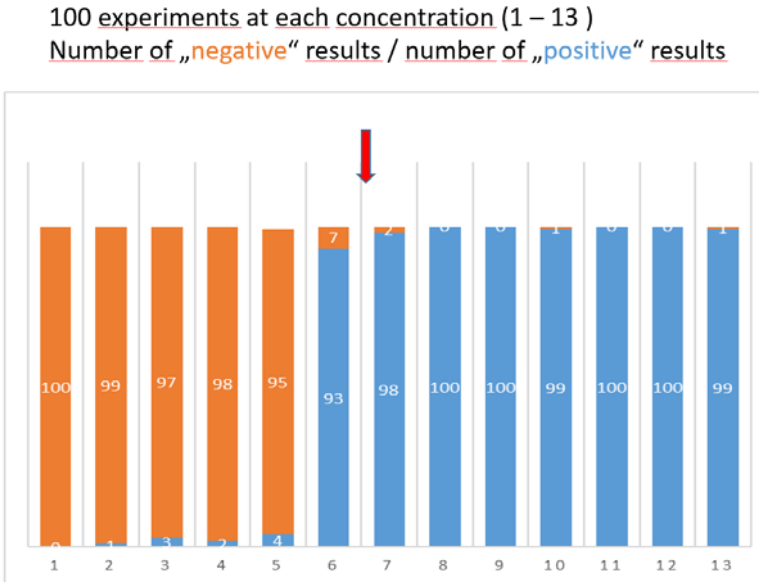
CCβ continued

Method 2: fortified blank material at concentration levels at and above the STC. The level, where only ≤ 5 % false compliant results remain, equals CCβ

Counting experiment / Theoretical example

i.e. at concentration levels > „6“
≤ 5 % false compliant results remain



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Summary of Changes

European Commission

Performance characteristic Changes from CD 2002/657 to CIR 2021/808		
Concentrations levels/ranges	Levels/ranges which should be validated have been revised	lower starting points (if reasonably achievable)
Precision	Acceptable coefficients of variation have been revised	more generously
Trueness	Acceptable ranges for analyte mass fractions >1 µg/kg have been revised	more generously
CCα	Additional calculation method	Inclusion of the MU
CCβ	Change of the concept of the CCβ	only of interest for screening !
Measurement uncertainty	Not explicitly mentioned in CD 2002/657	example of interpretation is provided
Relative matrix effect	Not explicitly mentioned in CD 2002/657	to be determined either separately or as part of the validation scheme
Absolute recovery	Previously referred to as “recovery”	t.b.d. if no internal standard is used
Calibration curve	No changes in the requirements	
Specificity / selectivity	No changes in the requirements	
Stability	No change in the requirements	
Ruggedness	No changes, but less detailed	

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European Commission

Thank you for your attention!

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