EURL Guidance Document on Confirmation Method Validation

Contents

1. Scope

This document serves as a guidance to support official control laboratories in the planning of method validation studies and shall contribute to the harmonisation of official control methods. Laboratories operating under Commission Implementing Regulation (EU) 2021/808 are not obliged to follow this guidance minutely; different approaches are acceptable if they provide the same level and quality of information. The guidance document supplements Commission Implementing Regulation (EU) 2021/808 regarding the validation of quantitative confirmation methods, but can also be used for the validation of (semi-)quantitative screening methods. The latter can be validated in the same manner outlined in this document for quantitative methods without the requirement to fulfil identification according to 1.2.3, 1.2.4 and 1.2.5 of Commission Implementing Regulation (EU) 2021/808.

2. Definitions

For the purpose of this guidance document the definitions given in Commission Implementing Regulation (EU) 2021/808 apply. The following definitions shall also apply:

2.1. Number of samples

Is a counter for all the extractions / tubes / individual samples in one analytical series.

2.2. Batch

Is a term that means one individual matrix material. For example, 7 batches of bovine muscle mean 7 muscle samples from 7 different bovines.

2.3. Aliquot

Is a term that defines one weighing portion of one and the same batch.

2.4. Experimental design

Is a term that here encompasses the planning of the validation study including the choice of factors and factor levels as well as the resulting (randomised) experimental plan.

2.5. Experimental plan

Is an overview of the (validation) experiments which need to be conducted.

2.6. Factor

Is a variable which may influence the analytical result. Up to seven factors can be evaluated using the orthogonal experimental design plan described in this document.

2.7. Noise factor

Is a variable which cannot be controlled in routine analysis but which may influence the result, e. g. sampling conditions, fat content, feeding, transport conditions, storage conditions, different lots of HPLC columns, different lots of SPE cartridges, different instruments (if applicable).

2.8. Design factor

Is a characteristic of the method to be developed which can be laid down e. g. in a method description and which may influence the result. Whether or not a factor is a design factor depends on the requirements specified in the standard operating procedure of the analytical method. If there are no regulations in the method description regarding the concerned characteristic, it is to be assumed that it varies by chance in routine analysis and thus has to be considered a noise factor.

2.9. Factor level

Is a variation of a factor. The number of possible factor levels is usually two, they are specified within the experimental design.

2.10. Factorial effect

Is the calculated influence on the analytical result which arises from the change from one factor level to another.

2.11. Experimental run

Is a single analysis using the factor levels specified in the randomised experimental plan.

2.12. Power function

Is a plot of the error probability 1-β against the concentration from which the critical concentrations CCα and CCβ can be graphically determined.

3. General remarks

Chapter 2 of Annex I to Commission Implementing Regulation (EU) 2021/808 describes the requirements for the validation of analytical methods for the determination of pharmacologically active substances[1]. According to this document, analytical methods can be classified into five different cases based on the type of analysis (screening, confirmation) and the mode of analyte determination (qualitative, semi-quantitative, quantitative). For each of the possible combinations, different performance characteristics are of importance as given in Table 5 of Commission Implementing Regulation (EU) 2021/808 (version adopted on 22 March 2021). All of the relevant parameters need to be validated and addressed in the validation report. In addition, calibration curve characteristics should be defined and the analytical scope of the respective method shall always be included in the validation report.

[1] The specific scope is laid down in CIR 2021/808.

Strictly qualitative confirmation methods are a very special case which is currently more of theoretical importance, as most detection methods suitable for confirmation allow semi-quantitative data evaluation to some extent. Therefore, a qualitative confirmation method can, for the time being, be understood as a quantitative confirmation method which does not fulfil all requirements for such methods laid down in Commission Implementing Regulation (EU) 2021/808. Validating a strictly qualitative confirmation method for unauthorised compounds would require very large sample numbers in order to fulfil the requirements regarding the β-error and is therefore usually not considered (economically) feasible in practice.

For the design of the validation study, the legal status of a substance (authorised, unauthorised) needs to be considered. In the case of authorised substances, the maximum residue level (MRL)[2] and the maximum level (ML)[3] are the reference for any validation. Regarding unauthorised substances, the reference point for action (RPA)[4] provides the benchmark for a validation endeavour. If a minimum method performance requirement (MMPR)[5] has been established for an unauthorised substance, a validation may be approached in the same fashion as for unauthorised substances. Since RPA are established only for prohibited substances, the confirmation of their presence at any concentration level triggers a follow-up investigation. Both RPA and MMPR are not to be understood as limit values and therefore analytical methods designed for the confirmation of substances for which an RPA or MMPR has been established shall be validated at concentration levels as low as reasonably achievable[6].

Unauthorised substances for which neither of the above mentioned values exists need to be validated based on the lowest calibrated level (LCL), meaning they should be validated for concentrations which are as low as reasonably achievable. With current multi-substance methods, it is likely that a combination of authorised and unauthorised substances is to be validated in one study. So for the general validation procedures described in this guidance document, no distinction is made between authorised and unauthorised substances or substances listed in either Group A or Group B of Annex I to Council Directive 96/23/EC[7]. But of course the requirements regarding the concentration levels to be validated need to be considered depending on the residues' legal status.

In order to guarantee adequate method performance, it is favourable to develop and validate methods using incurred reference materials. However, as reference materials containing all relevant residues at the required concentration levels are not readily available, the validation experiments are usually carried out on blank material fortified with the analytes of interest. With regard to an acceptable representation of naturally occurring matrix variations which might influence the method performance e. g. by matrix suppression or co-eluting matrix components, as many different (blank) matrix materials (batches) as

[2] Commission Regulation (EU) 37/2010
[3] A regularly updated list of references is available from the EURL Berlin.
[4] Commission Regulation (EU) 2019/1871
[5] EURL guidance on minimum method performance requirements (MMPRs) for specific pharmacologically active substances in specific animal matrices, September 2020
[6] Compare section 1.2.1 of Commission Implementing Regulation 2021/808
[7] Transitional period ends in 2022; changes are discussed in the annex to SANTE 11987:2017

feasible should be included in the validation study, irrespective of the selected approach to method validation. The proposed minimum of different matrix materials for each method validation approach is given in the respective sections. Nevertheless, it remains of significant importance to verify the suitability of methods developed and validated using fortified matrix material by analysing incurred matrix reference material. In addition, all analytical methods should be continuously assessed using matrix reference materials (if procurable) following method validation as a measure of ongoing quality control. For these purposes, the EURLs offer a variety of in-house reference materials characterised in proficiency tests to eligible laboratories.

Regardless of the selected approach to method validation, all relevant details of an analytical procedure should be investigated and defined in the method description before performing the actual validation experiments. Apart from the fundamental method requirements it is also necessary to check whether quantification needs to be carried out using a matrix-fortified or a matrix-matched calibration series, standard addition[8] or if quantification using standard solutions is sufficient. If preliminary experiments convey that certain matrix influences can only be compensated by specific matrix calibrations (e. g. porcine muscle samples cannot be quantified using a matrix calibration prepared from bovine muscle) these have to be employed throughout the entire validation study and naturally, this practice needs to be continued in routine application of the method.

Analytical methods previously validated in accordance with the requirements of Commission Decision 2002/657/EC need to be reviewed regarding the fulfilment of the requirements of Commission Implementing Regulation (EU) 2021/808. In many cases, only a limited number of experiments might be required in order to gather all the data necessary for an analytical method validated in accordance with CIR (EU) 2021/808. Considerations on this are given in the Annex of this EURL guidance document.

4. Practical implementation

4.1. Conventional validation approach

Section 2.2.1 of Annex I to Commission Implementing Regulation (EU) 2021/808 deals with the validation of analytical methods using the conventional validation approach, which demands individual experiments for every parameter to be validated, as well as for each major change in the methodology which will impact the performance characteristics. However, it is possible and recommended to reduce the overall number of required samples by combining certain validation experiments. The blueprint of a conventional validation study presented in this guidance document spans three analytical series ideally conducted over the course of several weeks and includes a ruggedness investigation. In case absolute

[8] An exemplary approach for confirmation method validation using quantification by standard addition will be discussed and included in future versions of this Guidance Document.

recovery and matrix effect have to be determined, a fourth validation series may be necessary. Information on the matrix effect can partly also be gathered from preliminary experiments.

A minimum of 21 different batches are needed for a full validation in accordance with the proposed exemplary validation approach (Table 1).

Table 1: Overview of batches required per validation series in the proposed conventional validation approach.

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]

The use of pooled samples, e.g. for the calibration curve, is possible. However, the minimum number of batches to be included in the validation needs to be respected. Exceptions may only be accepted for unusual matrices.

All of these batches, except the batch used for the calibration curve, need to be fortified with the analytes of interest to a minimum of the fortification levels given in Table 1; the exact concentrations depend on the legal status of the residue in question. Samples will also need to be fortified with internal standard solution, if applicable. See 4.1.1 for the required validation series.

Table 2: Required fortification levels in a conventional validation study as given in Commission Implementing Regulation 2021/808 (version adopted on 22 March 2021).

Residue	**Level 1**	**Level 2**	**Level 3**
Unauthorised with RPA[12]	0.5[13] RPA	1.0 RPA	1.5 RPA
Unauthorised[14]	1.0 LCL	2.0 LCL	3.0 LCL
Authorised	0.1[15] MRL/ML	1.0 MRL/ML	1.5 MRL/ML

[9] Calibration curve batch can be one of the 7 batches for specificity/fortification.
[10] For calibration curve: use a different batch for every validation series.
[11] For ruggedness the same batch as for the calibration curve can be used.
[12] The concentration levels given here for RPA substances are to be understood as exemplary concentration levels. Analytical methods for the confirmation of substances for which an RPA has been established shall be validated at concentrations as low as reasonably achievable.
[13] Where 0.5 RPA is not reasonably achievable, this level can be replaced by the lowest reasonably achievable concentration between 0.5 RPA and 1.0 RPA.
[14] The same approach can be applied for substances for which an MMPR has been established.
[15] Where 0.1 MRL/ML is not reasonably achievable, this level can be replaced by the lowest reasonably achievable concentration between 0.1 and 0.5 MRL/ML.

4.1.1. Validation experiments and calculation

Validation experiments

This paragraphs shows the required individual validation series for a full validation.

Validation series 1: 7 batches, 28 matrix samples (does not include a matrix-fortified calibration, quantification with standard calibration) or 33 matrix samples (includes a matrix-fortified calibration);

Validation series 2: 7 batches other than those used for validation series 1, 28 matrix samples (does not include a matrix-fortified calibration, quantification with standard calibration) or 33 matrix samples (includes a matrix-fortified calibration);

Validation series 3: 7 batches other than those used for validation series 1 and 2, 34 matrix samples (does not include a matrix-fortified calibration, quantification with standard calibration) or 39 matrix samples (includes a matrix-fortified calibration);

Validation series 4: 20 batches, 32 matrix samples, at least 33 analyses (only needed in case absolute recovery and relative matrix effect have to be determined).

Absolute recovery shall be determined when no internal standard or no matrix-fortified calibration is used. The relative matrix effects shall be determined when these effects have not been evaluated in preliminary experiments (paragraphs 2.9 and 2.10 in Annex I to Commission Implementing Regulation (EU) 2021/808).

Calculations

After analysis, concentrations, retention times, and ion ratios are used to calculate the performance characteristics using statistical software. The calculations can be based on an analysis of variances (ANOVA) approach to determine the trueness, repeatability and within-laboratory reproducibility and should respect the requirements of ISO 11843-1:1997 (critical concentrations). Alternatively, calculations may be based on ISO 5725-2:2019 and Codex CAC/GL 59-2006. Any software or spreadsheets required for these calculations need to fulfil the criteria of ISO 17025:2017. These tools can be developed in house, but are also available commercially and from the EURLs.

Total measurement uncertainty

According to Commission Implementing Regulation (EU) 2021/808 the within-laboratory reproducibility and the trueness need to be considered for the total measurement uncertainty. Although approaches for the inclusion of both aspects have been proposed[16], there is no generally accepted concept. Since method validation studies are often performed on fortified blank matrix material, the method's trueness contributes to the total measurement uncertainty by design. The within laboratory reproducibility can therefore be

[16] Magnusson and Elisson (2008), *Anal. Bioanal. Chem.* 390, 201-213.

taken as a good estimate of the total measurement uncertainty if all relevant factors were taken into account during the validation study.

Table 3: Experiments required for the validation series 1 of the exemplary approach to a complete, conventional validation.

Validation series 1	**Fortification level**	**# Extractions**	**Performance characteristic**
5 aliquots of 1 batch, fortified prior to extraction (if applicable)	five different levels including 0	5	calibration curve
1 aliquot of 7 different blank batches, no fortification	no fortification	7	specificity
1 aliquot of 7 different blank batches (same as used for specificity) fortified prior to extraction	level 1	7	CCα, trueness, repeatability*, within-lab reproducibility
1 aliquot of 7 different blank batches (same as used for specificity) fortified prior to extraction	level 2	7	
1 aliquot of 7 different blank batches (same as used for specificity) fortified prior to extraction	level 3	7	

Table 4: Experiments required for the validation series 2 of the exemplary approach to a complete, conventional validation.

Validation series 2	**Fortification level**	**# Extractions**	**Performance characteristic**
5 aliquots of 1 batch, fortified prior to extraction (if applicable)	five different levels including 0	5	calibration curve
1 aliquot of 7 different blank batches	no fortification	7	specificity
1 aliquot of 7 different blank batches (same as used for specificity) fortified prior to extraction	level 1	7	CCα, trueness, repeatability*, within-lab reproducibility
1 aliquot of 7 different blank batches (same as used for specificity) fortified prior to extraction	level 2	7	
1 aliquot of 7 different blank batches (same as used for specificity) fortified prior to extraction	level 3	7	

Table 5: Experiments required for the validation series 3 of the exemplary approach to a complete, conventional validation.

Validation series 3	Fortification level	# Extraction	Performance characteristic
5 aliquots of 1 batch, fortified prior to extraction (if applicable)	five different levels including 0	5	calibration curve
1 aliquot of 7 different blank batches	no fortification	7	specificity
1 aliquot of 7 different blank batches (same as used for specificity) fortified prior to extraction	level 1	7	CCα, trueness,
1 aliquot of 7 different blank batches (same as used for specificity) fortified prior to extraction	level 2	7	repeatability*,
1 aliquot of 7 different blank batches (same as used for specificity) fortified prior to extraction	level 3	7	within-lab reproducibility
6 aliquots of 1 batch (same as used for calibration curve) for 3 changes in method in duplicate**	level 2	6	ruggedness

* The significance of the repeatability for the characterisation of an analytical method, as well as its calculation is currently under discussion, also taking into account the definition for the repeatability condition of measurement as provided in the *International Vocabulary of Metrology*[17]. In this document repeatability conditions of measurement are defined as "a set of conditions that includes the same measurement procedure, same operators, same measuring system, same operating conditions and same location, and replicate measurements on the same or similar objects over a short period time". It is to be noted that opposed to the reproducibility, the repeatability is not the relevant parameter for the derivation of the critical concentrations.

** For ruggedness the Youden-approach (see section 2.4 of Annex I to Commission Implementing Regulation (EU) 2021/808) can be used, but also an alternative method. Choose for example 3 changes to the method and process the samples in duplicate.

Table 6: Experiments required for the validation series 4 of the exemplary approach to a complete, conventional validation.

Validation series 4	Fortification level	# Extraction	Performance characteristic
1 aliquot of 6 different blank batches, fortified prior to extraction	level 2	6	absolute recovery
1 aliquot of 6 different blank batches (same as used for line above), fortified after extraction	equal to level 2	6	
1 aliquot of 20 different blank batches, fortified after extraction	equal to level 2	20	relative matrix effect
1 standard solution	equal to level 2		

[17] International Vocabulary of Metrology – Basic and General Concepts and Associated Terms (VIM), 3rd edition, Internal Organization of Legal Metrology (OIML), 2007.

4.2. Alternative validation approach

The principle of an experimental design based approach to validation is described in section 2.2.2 of Annex I to Commission Implementing Regulation (EU) 2021/808. An example for an alternative approach using a factorial orthogonal experimental design plan is given in detail in this guidance document. The factorial approach aims at establishing reliable precision and measurement data by controlled variation of up to seven selected factors, which might have an influence on the analytical result. At the same time this approach also requires a smaller number of samples for the same quality of method performance data compared to non-experimental design approaches due to the stratification of the orthogonal design. Factorial validation studies are designed in such a way that precision, recovery (based on fortified samples), sensitivity, measurement uncertainty, and critical concentrations can be determined simultaneously. Additionally, this concept allows the evaluation of the combined impact of factorial effects as well as of random effects on the method performance. The experimental design also encompasses the investigation of the ruggedness of the analytical method and the determination of the in-house reproducibility standard deviation across different batches and matrices[18], which can be taken as a good estimate of the measurement uncertainty if influencing factors were properly selected. Stability experiments are not included in the model approach to a factorial validation presented in this guidance document and need to be conducted separately following the requirements outlined in section 2.5 of Annex I to Commission Implementing Regulation (EU) 2021/808.

In general, the procedure for a factorial method validation succeeding the method development is as follows:

1. Design of the study
    a. Selection of relevant analytes
    b. Selection of relevant concentration levels
    c. Selection of factors and factor levels
    d. Generation of the randomised experimental plan
2. Conduction of the experimental runs
3. Statistical evaluation and calculation of method parameters

4.2.1. Selection of analytes and concentration range

The analytes and the concentration range of the quantitative confirmation method to be validated are chosen according to the expected kind of samples and the purpose and requirements of the method. Additionally, the requirements of Commission Implementing Regulation (EU) 2021/808 need to be satisfied.

[18] If different matrices were included as a factor in the experimental design.

For confirmation purposes, the lowest calibrated level in the validation study must primarily be selected such that a reliable signal is guaranteed. The fulfilment of the identification criteria (required number of identification points for confirmation methods as given in Annex I to Commission Implementing Regulation (EU) 2021/808) has to be evaluated separately as described below.

The alternative validation approach may also be used for (semi-)quantitative screening methods. In this case the test method applied does not need to be able to confirm the identity of the analyte unequivocally. It is sufficient for the test method to produce signals referring to defined retention times and/or to the corresponding wave lengths/masses/frequencies, etc. Therefore, the lowest concentrations delivering a reliable signal (e. g. around the classical limit of quantification) can be used as a starting point.

Unlike in the conventional validation approach, with the alternative validation approach a concentration range is validated instead of discrete concentration levels. It is therefore not necessary to fortify the samples used for validation at the exact concentration levels given in Annex I of Commission Implementing Regulation (EU) 2021/808. However, the required concentration levels have to be covered adequately by the selected validated concentration range. At minimum the concentration ranges given in Table 7 need to be validated.

Table 7: Fortification levels in a factorial validation study as given in Commission Implementing Regulation 2021/808 (version adopted on 22 March 2021).

Residue	Concentration range
RPA[19]	0.5[20]·- 1.5 RPA
Unauthorised[21]	1.0·- 3.0 LCL
Authorised	0.1[22]·- 1.5 MRL/ML

It is preferable to define the concentration range to be validated before deciding on the factors to be included in the validation study, because extreme concentration ranges may enhance or diminish the importance of certain factors commonly included in factorial validation studies.

A minimum of five different concentration levels (including a blank level, if applicable) is considered appropriate to cover these concentration ranges. For multi-methods covering a large number of analytes and possibly also several matrices for which different legal limits have been established for the same substance, a validation for an extended concentration range may be necessary. This usually requires

[19] The concentration range given here for RPA substances are to be understood as an example. Analytical methods for the confirmation of substances for which an RPA has been established shall be validated at concentrations as low as reasonably achievable.
[20] Where 0.5 RPA is not reasonably achievable, this level can be replaced by the lowest reasonably achievable concentration between 0.5 RPA and 1.0 RPA.
[21] The same approach can be applied for substances for which an MMPR has been established.
[22] Where 0.1 MRL/ML is not reasonably achievable, this level can be replaced by the lowest reasonably achievable concentration between 0.1 and 0.5 MRL/ML.

the inclusion of additional fortification levels within the validation study and consequently, the linearity of the calibration needs to be assessed prior to the start of the method validation study. In some cases, it can be the better choice to validate the method separately for differing concentration ranges.

The calibration levels and the validation concentration levels do not need to be equidistant. Nevertheless, the fortified concentration levels to be assessed during validation need to be covered by the standard and/or matrix calibration range. In general, the standard/matrix calibration should span a larger range than the concentration levels to be validated. An example of the selected concentration levels for the validation of an analytical method for the determination of metronidazole (MNZ) in plasma and serum is given in Table 8.

Table 8: Example for fortification levels (CL) for metronidazole (MNZ) (MMPR=1.0 µg/kg) for a method validation in plasma and serum. Note that for compounds for which an MMPR has been established there are no fixed requirements as regards the validated concentration range. It is merely necessary that concentrations below the MMPR are validated.

Fortification level / Analyte	CL01	CL02	CL03	CL04	CL05	CL06
MNZ	0.05µg/kg	0.10 µg/kg	0.20 µg/kg	0.40 µg/kg	0.80 µg/kg	1.20 µg/kg

4.2.2. Design of experiment

Selection of factors and factor levels

The experimental design of the validation study is usually based on up to seven factors which are varied on two levels within the validation study. A factor is any parameter in the analytical procedure which might influence the analytical result. Which factors to assess during method validation depends on the method in question and is therefore always a case by case decision. Valuable information on factors with a large impact on the method performance can be gained during method development.

Factors can roughly be classified as “design factors” (mainly method-specific) and “noise factors” (mainly sample-specific). In general, design factors are parameters which can be defined in the method description. Only design factors which cannot be investigated during method development or which need a certain flexibility in routine analysis should be included in the validation study. All other design factors should be evaluated and fixed before method validation. For the validation study, all factor levels should be chosen based on the analyst’s experience or constraints specific to the laboratory. A non-exhaustive overview of possible factors and factor levels is given in Table 9.

If the analyst has gained sufficient experience with the method and tested different species or matrices during method development which yielded satisfactory results, those factors can potentially also be included as factor levels. However, it is not advisable to include too many matrices and/or species in the initial 8-run validation study.

Table 9: Non-exhaustive overview of possible factors and examples for factor levels for consideration in a factorial method validation study.

Factors	Explanation / examples for factor levels
Matrix	
species	bovine, porcine, ovine
animal class	mammal, poultry
matrix	kidney, muscle, liver
fat content	
sample condition	fresh, fresh-frozen, lyophilised
agricultural production	conventional, organic
Measurement	
quantification	standard calibration, matrix calibration
dilution	
injection volume	
instrument	
reconstitution volume	
Operator	
operator	familiar, unfamiliar with method
Sample preparation	
sample amount	weighed-in sample amount
SPE phase material	different lots or manufacturers
centrifugation	yes, no, centrifugation speed
defatting step	yes, no, different methods
derivatisation agent	
SPE elution speed	
enzymatic digestion	yes, no, different methods
extraction duration	
extraction volume	
extraction solvent	
filtration	
filter material	
hydrolysis	
pH	
reconstitution volume	
Sample storage	
storage conditions	-25 °C, +4 °C, +25 °C
storage of extract	none, overnight at +4 °C, 2-3 days at +4 °C
Technical factors	
cartridge manufacturer	
cartridge lot	
HPLC column	different lots, similar columns by different manufacturers
evaporation temperature	

Example

A method for the determination of nitroimidazoles is to be validated. During method development and from previous method validation studies there was sufficient evidence that the newly developed method is suitable for the species pig and turkey and the matrices plasma and serum. Therefore, the factors matrix (I) and species (II) were included as factors in the validation study (Table 10). In routine application it is to be expected that analyses following the procedure will have to be carried out by personnel both familiar and unfamiliar with the method. Therefore, a factor “operator” is also included in the validation study (III). The amount of matrix, i. e. the sample amount per analysis (IV) may also vary as it might be necessary to use a larger sample quantity for samples which contain only low concentrations of the analytes of interest. If a larger sample amount is used, it might be necessary to filter the final extract in order to remove suspended particles (VI) or to use a larger volume of solvent for

reconstitution of the sample (VII) in order to compensate for matrix effects. Often it cannot be guaranteed that a sample extract is analysed immediately after the sample preparation procedure, as the measuring instrument might not be available right away. It is therefore good practice to also include the noise factor "storage of final extract" (V) with the factor levels "no storage" and e. g. "storage 2-3 days at +4 °C" in the method validation study.

Table 10: Factors chosen for a validation study for a method for the determination of nitroimidazoles in pig and turkey plasma and serum.

Factor		Level A	Level B
I	matrix	plasma	serum
II	species	pig	turkey
III	operator	unfamiliar	familiar
IV	amount of matrix	2 g	1 g
V	storage of final extract	2-3 days of storage at +4 °C	immediate analysis
VI	filtration	none	100 kDa
VII	final volume	250 µL	150 µL

4.2.3. Validation experiments

After all the factors and factor levels have been chosen, the experimental plan can be generated by using either the EURL Excel template or a commercially available software (a general design plan is given in Table 11). Each line of the table represents one validation experiment ("run") which encompasses a specific combination of factor levels. Note that every factor level appears four times throughout all of the runs. This allows for the calculation of the change in recovery when switching from one factor level to the other. The order of these experimental runs is randomised to minimise the influence of possible systematic effects. An example of an experimental plan for a confirmation method for the determination of nitroimidazoles in pork and turkey plasma and serum is given in Table 12. Validation experiments will be performed in this randomised order, i. e. in the given example experiments will start with run 04 (validation series 1) which will be followed by run 08 (validation series 2), run 01 (validation series 3), and so forth. For the first analytical series of the validation study (run 04) the experiments will be carried out on 1 g of turkey plasma by an operator who is familiar with the method. Samples will not be filtered and will be reconstituted in 250 µL of solvent before being analysed immediately. To obtain a realistic data set that accounts for random deviations and gives a better representation of the intermediate precision of the laboratory, no more than two runs should be performed per week. A different batch of matrix shall be used for every run.

Table 11: Example of an orthogonal experimental design plan with 7 factors (I – VII) varied on two levels (A/B) in a validation study with eight runs (factor level combination).

Factor	I	II	III	IV	V	VI	VII
run 01	A	A	A	A	A	A	A
run 02	A	A	B	A	B	B	B
run 03	A	B	A	B	A	B	B
run 04	A	B	B	B	B	A	A
run 05	B	A	A	B	B	A	B
run 06	B	A	B	B	A	B	A
run 07	B	B	A	A	B	B	A
run 08	B	B	B	A	A	A	B

Table 12: Example of a randomised experimental plan for a method validation study for the determination of nitroimidazoles in plasma and serum.

Validation series	Run	Matrix	Species	Operator	Amount of matrix	Storage of extract	Filtration	Final volume
1	run 04	plasma	turkey	familiar	1 g	immediate analysis	no	250 µL
2	run 08	serum	turkey	familiar	2 g	2-3 days of storage at +4 °C	no	150 µL
3	run 01	plasma	pig	unfamiliar	2 g	2-3 days of storage at +4 °C	no	250 µL
4	run 07	serum	turkey	unfamiliar	2 g	immediate analysis	yes	250 µL
5	run 02	plasma	pig	familiar	2 g	immediate analysis	yes	150 µL
6	run 06	serum	pig	familiar	1 g	2-3 days of storage at +4 °C	yes	250 µL
7	run 03	plasma	turkey	unfamiliar	1 g	2-3 days of storage at +4 °C	yes	150 µL
8	run 05	serum	pig	unfamiliar	1 g	immediate analysis	no	150 µL

4.2.4. Validation study and samples

The validation study covers eight runs according to the experimental plan (see Table 12 for an example) and the factor level combinations given per run need to be followed strictly. In practice, a blank material is fortified to the desired concentration levels and analysed according to the conditions specified per run in the experimental plan. The quantification of the validation samples is carried out by using either a standard calibration curve, a (different) matrix or matrix-matched calibration curve, or a standard addition approach. It is also possible to employ multiple quantification approaches during the validation study and derive the most suitable approach for future routine measurements from the validation data. In addition to the quantitative evaluation, the fulfilment of the identification criteria (required number of identification points for confirmation methods as given in Annex I to Commission Implementing Regulation (EU) 2021/808) has to be evaluated for each fortification level in each run.

Different (blank) matrices should be used for every run. Also, the matrix used for matrix or matrix-matched calibration (if applicable) needs to differ from the matrix sample used for that day's run. If different batches of blank material are not easily procurable, it is possible to use a single batch for all

the required matrix calibrations in a validation study. This means that between 9 and 16 different batches are required for the validation study in total. The usual set of quality control samples per run is also recommended. These can be conveniently used to assess specificity and the (relative) matrix effect. Guidance on the QC samples can be found in the “Guidance on Ongoing Method Performance Verification”.

Table 13 illustrates the minimum amount of samples for one experimental run. For eight runs the total minimum amount of samples would be 104 (144). The repeatability is derived from the different concentration levels of each experimental run and no additional experiments are required. The experimental plan does however not include stability experiments. Therefore, stability has to be assessed in an additional study adhering to the requirements laid down in section 2.5, Annex I of Commission Implementing Regulation (EU) 2021/808.

Table 13: Experiments required for a single run of the exemplary approach to a complete validation in accordance with the alternative validation approach.

Minimum required samples for one run (one validation series)	# Samples	Performance characteristic
5 aliquots from 1 batch, fortified prior to extraction at 5 different levels[#]	5	matrix calibration curve
5 standard solutions[#]	(5)	standard calibration curve
5 aliquots from 1 batch, fortified prior to extraction at 5 different levels[#]	5	within-lab reproducibility, repeatability, trueness, CCα, (CCβ[‡]), absolute recovery*, ruggedness
1 matrix blank sample**	1	specificity / selectivity
1 matrix blank sample fortified with internal standard(s)	1	specificity / selectivity
1 matrix blank sample fortified with analyte(s) and internal standard(s) at a relevant level	1	relative matrix effect***
Total	13 (18)	

[#] including a blank level, if applicable

[‡] Only relevant for (semi-)quantitative screening methods.

* Only necessary when no internal standard is used or no matrix-fortified calibration curve is used.

** Only necessary if no blank level is included in the fortified levels (line above)

*** Additional experiments may be necessary in order to satisfy the requirements of Commission Implementing Regulation 2021/808 which foresees the investigation of the matrix effect on 20 blank samples.

4.2.5. Parameter calculation

Detailed information on the calculation of the parameters can be found in:

Jülicher, B., Gowik, P. and Uhlig, S. (1998) Assessment of detection methods in trace analysis by means of a statistically based in-house validation concept. Analyst, 123, 173.

Gowik, P., Jülicher, B. and Uhlig, S. (1998) Multi-residue method for non-steroidal anti-inflammatory drugs in plasma using high performance liquid chromatography-photodiode-array detection. Method description and comprehensive in-house validation. J. Chromatogr., 716, 221.

Jülicher, B., Gowik, P. and Uhlig, S. (1999) A top-down in-house validation based approach for the investigation of the measurement uncertainty using fractional factorial experiments. Analyst, 124, 537.

4.2.6. Interpretation of results

Graphical evaluation

The graphical display of the results obtained for the concentration levels during each run can be helpful in visually determining runs with significantly different performance. Furthermore, it is recommended to generate diagrams for all the different factors and to use different colours for runs with differing factor levels (Figure 1). This can assist in noticing trends in the results caused by individual factors. Another convenient graph for obtaining a first impression of the validation results is to display all experimental results together with the mean calibration curve and the prediction interval. Such a graph allows for direct identification of outliers. An example of a graphical evaluation including measurements, run calibration functions and overall calibration functions as well as the prediction interval is given in Figure 2. For this example, all calibration levels were included in the calculation, there was no removal of outliers.

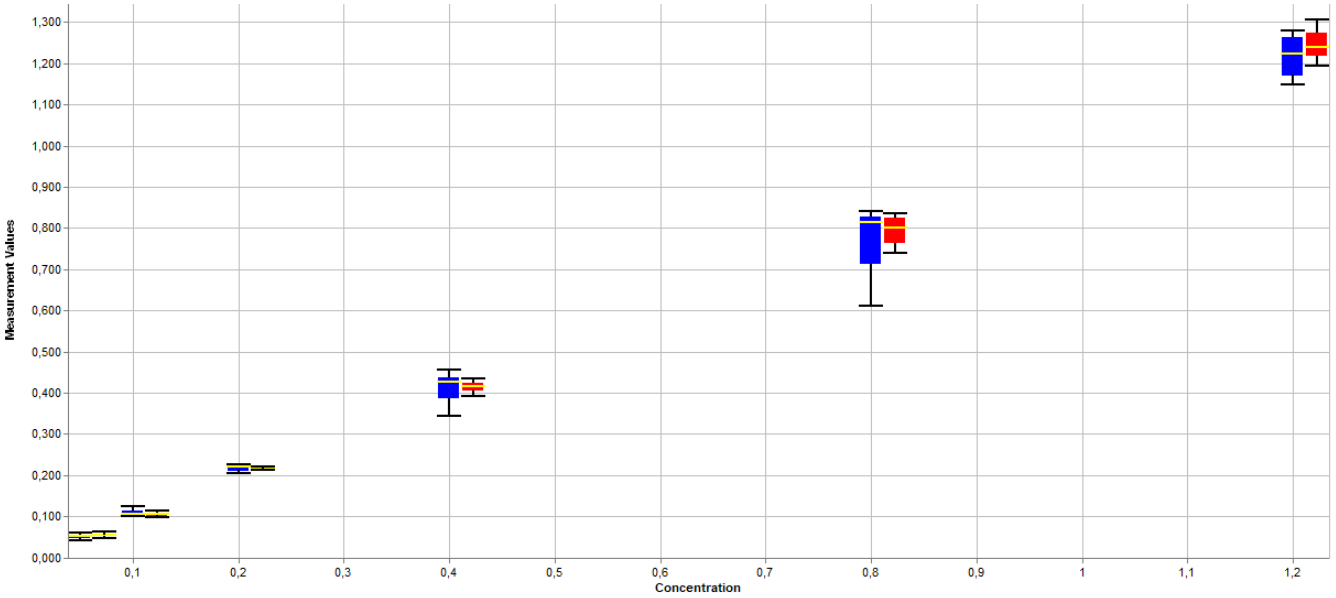

Figure 1: Box plot displaying the measurement results for metronidazole (MNZ) obtained for the runs using plasma samples (blue) and serum samples (red) versus the fortified concentration level (in µg/kg). The results between plasma and serum do not differ significantly.

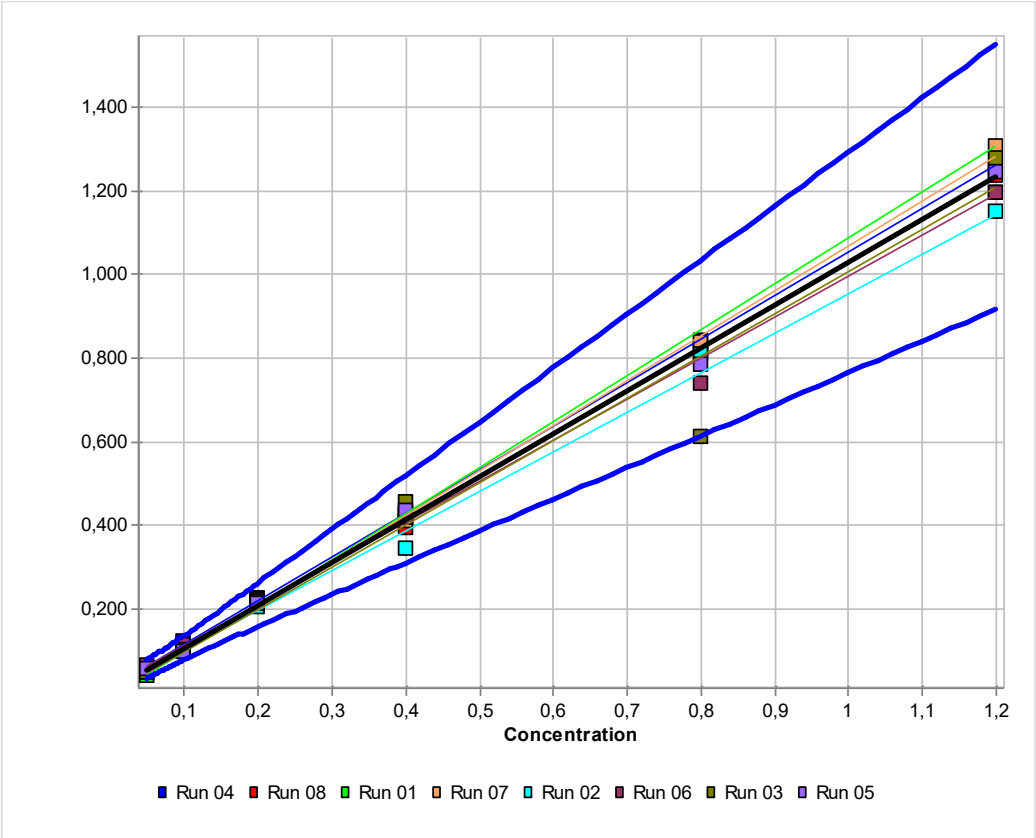

Figure 2: Calibration curves obtained for the different experimental runs for metronidazole (MNZ) fortified in plasma and serum in a factorial validation study given as µg/kg. The prediction interval of the curves is given in dark blue.

Recovery, repeatability and in-house reproducibility

The acceptance criteria for these data have to be evaluated separately for every analyte. For an overview of the criteria to apply refer to the respective articles in Commission Implementing Regulation (EU) 2021/808. Exemplary method performance data for the determination of metronidazole (MNZ) in plasma and serum are given in Table 14. All requirements regarding the performance parameters are fulfilled.

Table 14: Exemplary method performance data obtained for the validation of MNZ in plasma and serum.

Analyte	Calibration interval	Number of values	CCα	Recovery [%] at CCα	Rel sR [%] at CCα
MNZ	0.050 - 1.200	48	0.072	107.0	10.7

Power function and decision limit CCα

Statistical software packages may offer the calculation of a so-called power function which is generated by plotting the error probability 1-β against the concentration. The slope of the power function depends on the determined dispersion, the limits laid down in Commission Implementing Regulation (EU) 2021/808, and the kind of method (screening/confirmation) per chosen analyte. Assuming a normal distribution, CCα lies at about 50 % of the β-error probability. The steeper the slope of the power function, the better the method.

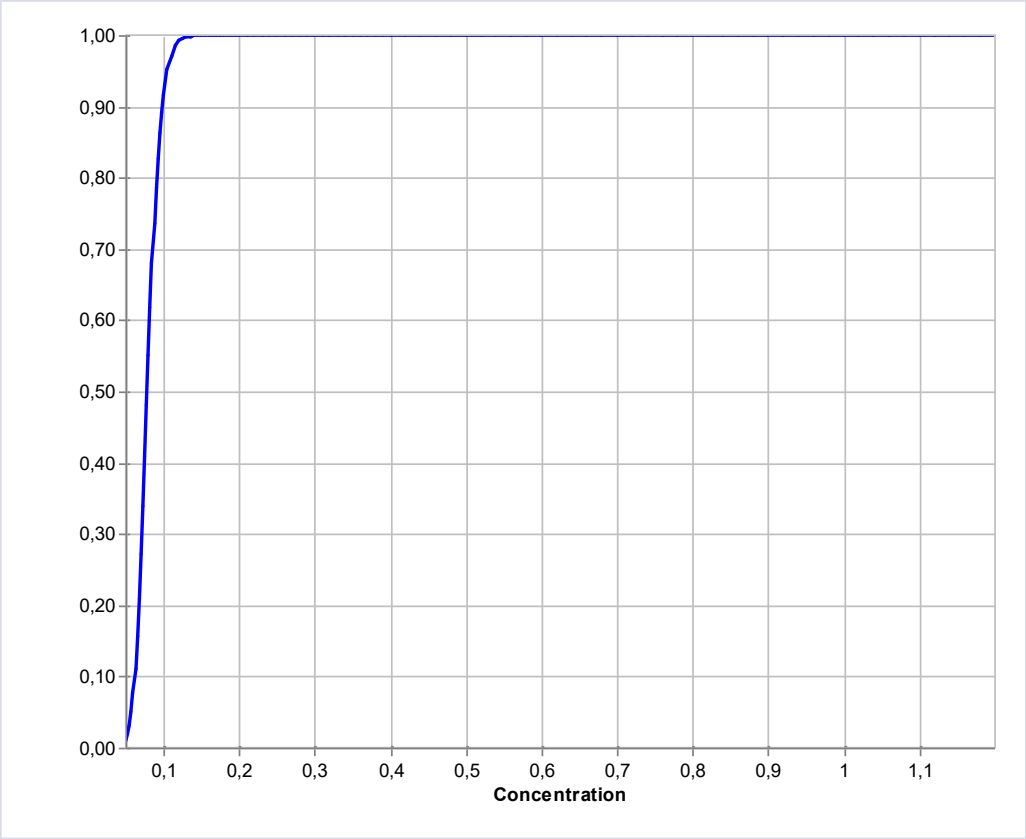

Figure 3: Power function for the determination of metronidazole (MNZ) in plasma and serum. The concentration is given in µg/kg.

Factorial effects and uncertainties

For every factor, the proportional deviation and the constant deviation between the factor levels can be calculated from the underlying statistical model. From these data, the contribution of each influencing factor to the total uncertainty can be estimated. The proportional deviation (i. e. the slope of the curve) denotes the average deviation of the slope of the respective calibration curve from the overall mean. The constant deviation (i. e. the position of the curve) denotes the average deviation of the level. Examples for deviations are given in the references cited under 4.2.5. A real example is also presented in Table 15. Here, for the factor level “unfamiliar operator” of the factor “operator” the proportional deviation is -2.56 %, which means that the average slope for this factor level is 2.56 % below the overall slope and the difference to the factor level “familiar operator” is 5.12 %.

Table 15: Factorial effects for the analyte MNZ calculated for a method for the determination of nitroimidazoles in plasma and serum.

Factor	Level	Proportional deviation	Constant deviation
matrix	plasma (+); serum(-)	0.13	0.28
species	turkey(+); pig(-)	1.13	0.98
operator	unfamiliar (+); familiar(-)	-2.56	-1.18
amount of matrix	2 g(+); 1 g(-)	0.98	0.13
storage of extract	direct analysis(+); 2-3 days of storage(-)	-0.23	-0.01
filtration	yes (+); no(-)	-2.25	-2.04
volume	200 uL final volume(+); 120 uL final volume(-)	2.33	2.24

These factorial effects contribute considerably to the total uncertainty. However, if the values of the calculated critical concentrations (CCs) are acceptable, it is not necessary to perform separate method validations for separate factors (e. g. matrices). Factorial effects of up to 10 % can usually be accepted. If the data evaluation leads to the conclusion that the factor levels differ significantly from one another, it should be reviewed whether this stems from a systematic or a random deviation. To counteract systematic deviations, additional specifications can be added in the method description (e. g. a factor can be fixed to one level). Random deviations however have to be accepted. If deviations over 20 % to 30 % are calculated, it may be necessary to exclude the factor from the validation, or to find arguments in favour of an inclusion. Alternatively, it is always possible to validate factor levels separately. In this case additional experiments are required.

In Figure 4 an overview of the uncertainties contributing to the total uncertainty of the determination of metronidazole (MNZ) in plasma and serum is given. Except for the relative standard solution uncertainty which is fixed at 3 %[23], all uncertainties are estimated from the experimental run data. In general, the repeatability uncertainty has the biggest influence on the total uncertainty whereas the uncertainty of recovery correction, standard solution and run uncertainty have a minor influence. At CCα level the matrix uncertainty and the repeatability uncertainty have the largest impact. The total uncertainty is calculated by combining all measurement uncertainty contributions using the law of propagation of uncertainties:

$$u(total) = \sqrt{u(matrix)^2 + u(run)^2 + u(repeatability)^2 + u(recovery)^2 + u(standard)^2}$$

[23] As determined from a bottom-up uncertainty evaluation for the preparation of dilutions as practiced by the EURL Berlin.

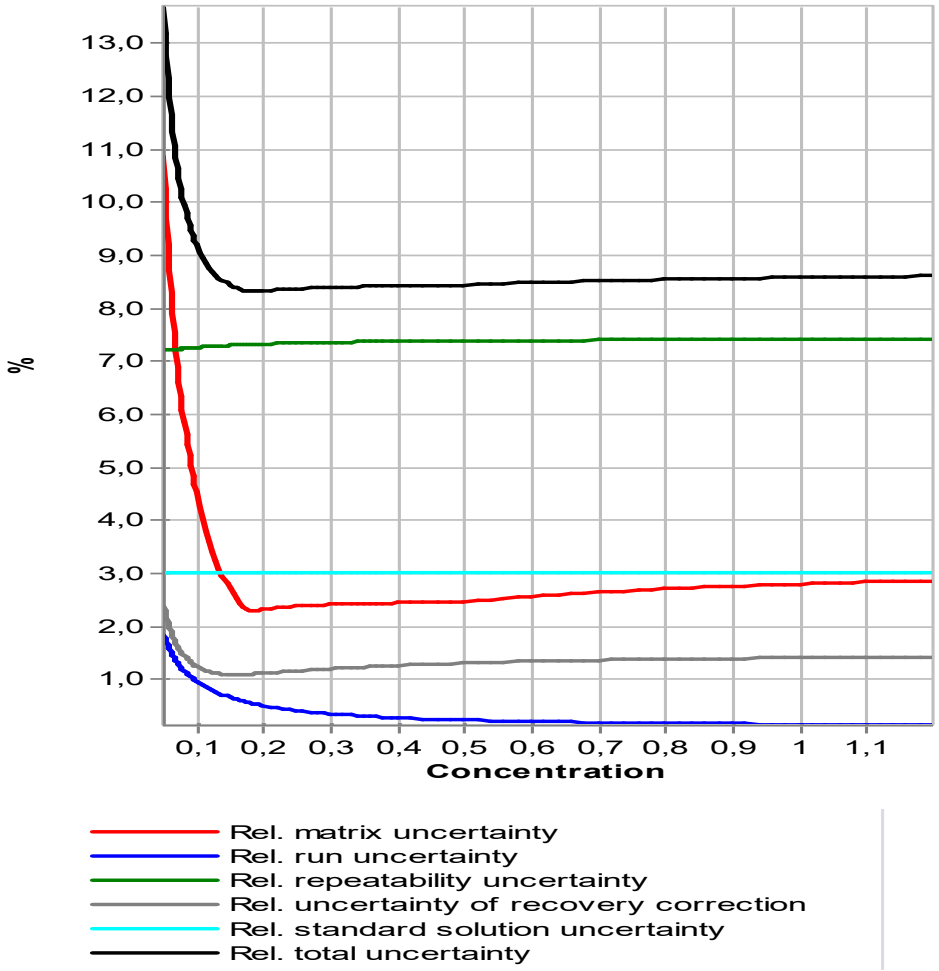

Figure 4: Contributions to the total uncertainty for the determination of metronidazole (MNZ) in plasma and serum.

Total measurement uncertainty

According to Commission Implementing Regulation (EU) 2021/808 the within-laboratory reproducibility and the trueness need to be considered for the total measurement uncertainty. Although approaches for the inclusion of both aspects have been proposed[24], there is no generally accepted concept. Since method validation studies are often performed on fortified blank matrix material, the method's trueness contributes to the total measurement uncertainty by design. The within laboratory reproducibility can therefore be taken as a good estimate of the total measurement uncertainty if all relevant factors were taken into account during the validation study.

4.2.7. EURL service

The use of commercially available software for the alternative validation approach might not be suitable for every laboratory, especially if the conventional validation approach has so far been used exclusively. Therefore, the EURL Berlin provides the NRLs with a tool for the planning of factorial validation studies.

[24] Magnusson and Elisson (2008), *Anal. Bioanal. Chem.* 390, 201-213.

The laboratories may choose the factors and factor levels for their validation study in consultation with the EURL, if required, and set up their experimental plan using a template. After completing the experiments, the NRLs may submit their analytical results to the EURL Berlin where the necessary calculations will be carried out using statistical software. Finally, the NRLs will be provided with a detailed validation report for each analyte included in the method. The use of this service is voluntary and offered free of charge.

5. Fitness for purpose

Regardless of which validation approach has been used it is necessary to assess the outcome of the validation study for the method's fitness for purpose and address all relevant aspects in the validation report. The acceptance criteria by which to judge whether or not a method can be considered adequately valid for a certain substance are given in Table 16.

If any of these criteria are not met for a substance, the method is not fit for the intended purpose. The consequences would be

- to define the applicability of the method accordingly (e. g. only applicable for the quantitative determination for 3 out of 4 initially intended substances)
- to define the method for a different purpose (e. g. only qualitative screening) if the required data is available
- to continue method development followed by another attempt at method validation.

In cases where the validation data implies that an analytical method does not fulfil all requirements for quantitative confirmation methods as laid down in Commission Implementing Regulation (EU) 2021/808, the method may still by applicable as a qualitative confirmation method (for A-substances).

Table 16: Performance characteristics and respective acceptance criteria.

Performance characteristic	Acceptance criteria
Identification	Sufficient amount of identification points for the applied measurement technique, requirements for chromatographic separation, mass spectrometric detection or other; see 1.2.3, 1.2.4 and 1.2.5., Annex of Commission Implementing Regulation (EU) 2021/808,
CCα	Qualitative criteria -authorised substances: higher than but as close to the MRL / ML as analytically achievable -prohibited / unauthorised substances with RPA: lower than or equal to the RPA -prohibited / unauthorised substances without RPA: as low as analytically achievable
CCβ‡	Qualitative criteria -authorised substances: lower than or equal to the MRL / ML -prohibited / unauthorised substances with RPA: lower than or equal to the RPA -prohibited / unauthorised substances without RPA: as low as analytically achievable

Trueness	Concentration dependant, see 1.2.2.1, Annex of Commission Implementing Regulation (EU) 2021/808
Precision	Concentration dependant, see 1.2.2.2, Annex of Commission Implementing Regulation (EU) 2021/808
Relative matrix effect	See 2.10, Annex of Commission Implementing Regulation (EU) 2021/808
Absolute recovery	No fixed criteria. The results for the parameter shall be evaluated using expert knowledge. The responsible scientist shall identify critical aspects which may require method improvements.
Specificity / selectivity	No fixed criteria. The results for the parameter shall be evaluated using expert knowledge. The responsible scientist shall identify critical aspects which may require method improvements.
Stability	See 2.5, Annex of Commission Implementing Regulation (EU) 2021/808, experiments for stability are not included in the exemplary validation plans given in this guidance
Ruggedness	No fixed criteria. The results for the parameter shall be evaluated using expert knowledge. The responsible scientist shall identify critical aspects which may require method improvements.

‡ Only relevant for (semi-)quantitative screening methods.

Annex

This annex provides an overview of the main differences between Commission Implementing Regulation (EU) 2021/808 (version of 22 March 2021) and Commission Decision (EU) 2002/657 with special regard to the (re-)validation of analytical methods. Due to the number of changes and the range and complexity of the topics covered by the legal documents, this annex cannot provide a complete list of revisions. For details, always refer to the legal documents. A summary of the most relevant changes is given in Table 17, the differences are discussed more specifically in the following paragraphs.

Table 17: Overview of changes in the requirements for method performance characteristics from CD 2002/657 to CIR 2021/808.

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

Identification criteria

In CIR 2021/808 a new concept for the identification points is introduced which also attributes one point to the applied separation technique (Table 18).

Table 18: Identification points in accordance with CD 2002/657 and CIR 2021/808.

Technique	Identification points 2002/657	Identification points 2021/808
Separation (GC, LC, SFC, CE)		1.0
LR-MS ion	1.0	1.0
Precursor ion	1.0	1.0 (indirect, selection at <±0.5 Da mass range)
LR-MS^n product ion	1.5	1.5
HR-MS ion	2.0	1.5
HR-MS^n product ion	2.5	2.5

Chromatography

The general requirements which need to be respected for the validation remain the same. However, specific requirements for the identification of analytes (e. g. maximum allowed relative retention time, maximum allowed deviation of ion ratio) have been adjusted and need to be evaluated for methods previously validated under CD 2002/657 (see below). Furthermore, under CIR 2021/808 HPLC-DAD and HPLC-FLD methods can only be employed as confirmation methods for authorised substances and are not considered suitable for use as methods for the confirmation of unauthorised or prohibited substances.

Retention time

Both the CD 2002/657 and the CIR 2021/808 require a minimum retention time for GC and LC of two times the void volume. Furthermore, in accordance with CIR 2021/808 a deviation of the analyte retention time in the extract of ±0.1 min compared to the retention time of the analyte in the calibration solution is allowed. For fast chromatography with a retention time of the analyte of <2.0 min, a deviation of <5 % is acceptable. These criteria have not been previously included in CD 2002/657.

Regarding the relative retention times (quotient of the retention time of the analyte and the internal standard), the allowed deviation for GC is given as ±5 % in CD 2002/657 and CIR 2021/808. The acceptable deviations of the relative retention time have been adjusted for LC from ±2.5 % (CD 2002/657) to ±1.0 % (CIR 2021/808).

Relative ion intensities

Commission Decision 2002/657 listed maximum permitted tolerances for relative ion intensities in samples versus the calibration for different mass spectrometric techniques. In Commission Implementing Regulation 2021/808 this has been replaced by a general requirement that the differences in relative intensities should not exceed ±40 %.

Concentration levels/ranges

The concentration levels which need to be investigated in a validation study have been revised for CIR 2021/808 (Table 19). This is partly due to the fact that the concept of the minimum required performance limit (MRPL) no longer applies. Also, a novel legal limit, the reference point for action

(RPA), and a new minimum technical requirement, the minimum method performance requirement (MMPR), have been established in the meantime. For unauthorised compounds for which no RPA has been established the concept of the lowest calibrated level (LCL) has been introduced. In case the validation is performed using a factorial approach, the concentration levels given in Table 19 are to be covered by the validated concentration range.

Table 19: Concentration levels to be validated for the different performance characteristics in accordance with CD 2002/657 and CIR 2021/808.

Legal limit	CD 2002/657	CIR 2021/808
MRL/ML	0.5, 1.0, 1.5 MRL/ML	0.1 (0.5)[25], 1.0, 1.5 MRL/ML
MRPL	1.0, 1.5, 2.0 MRPL	MRPL concept has been revoked
RPA[26]		0.5 (1.0)[27], 1.0, 1.5 RPA
MMPR[28]	Concept first introduced in 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

Precision

Reproducibility

In general, the reproducibility for the repeated analysis of reference or fortified material, should not exceed the HORWITZ standard deviation corresponding to the analyte mass fraction. As the equation may give very high values for low concentrations, the acceptable reproducibility has been adapted based on the HORWITZ function for both CD 2002/657 and CIR 2021/808, but is still dependent on the analyte mass fraction. The ranges of the mass fractions, as well as the acceptable reproducibilities were revised for CIR 2021/808 (Table 20) but the majority of methods validated in accordance with CD 2002/657 should fulfil the new criteria.

[25] Where 0.1 MRL/ML is not reasonably achievable, this level can be replaced by the lowest reasonably achievable concentration between 0.1 and 0.5 MRL/ML.
[26] The concentration levels given for RPA substances are to be understood as exemplary concentration levels. Analytical methods for the confirmation of substances for which an RPA has been established shall be validated at concentrations as low as reasonably achievable.
[27] Where 0.5 RPA is not reasonably achievable, this level can be replaced by the lowest reasonably achievable concentration between 0.5 and 1.0 RPA.
[28] Analytes for which an MMPR has been established can be validated analogously to unauthorised compounds as given in the table.

Table 20: Allowed coefficients of variation per analyte mass fraction in accordance with CD 2002/657 and CIR 2021/808.

CD 2002/657		CIR 2021/808	
Mass fraction	**Reproducibility CV (%)**	**Mass fraction**	**Reproducibility CV (%)**
1 µg/kg	As low as possible	<10 µg/kg	30 (guideline), as low as possible
10 µg/kg	As low as possible	10-120 µg/kg	25 (guideline), as low as possible
100 µg/kg	23	>120 µg/kg to 1000 µg/kg	22
1000 µg/kg	16	>1000 µg/kg	16

Repeatability

Regarding the repeatability, no strict requirements were given in CD 2002/657. It was only noted, that the repeatability should typically be as large as one third to two thirds of the values given for the reproducibility (Table 20). In CIR 2021/808 it is stated that the repeatability shall not be larger than two thirds of the coefficients of variation proposed for the reproducibility. Therefore, the majority of methods previously validated in accordance with CD 2002/657 should fulfil the new requirements for repeatability. Since the repeatability is not required for the derivation of the critical concentrations its significance as a method parameter is currently under discussion.

Within-laboratory reproducibility

In accordance with CD 2002/657 the within-laboratory reproducibility for substances for which a legal limit has been established should not exceed the values given for the reproducibility at 0.5x the legal limit. For methods validated in accordance with CIR 2021/808, the within-laboratory reproducibility shall not exceed the values given in Table 20.

Trueness

The minimum requirements for the trueness of quantitative methods have been adjusted in CIR 2021/808 (Table 21). Any method previously validated in accordance with the requirements of CD 2002/657 fulfils the updated requirements. The trueness should ideally be assessed by repeated analysis of certified reference material. If suitable CRM is not available, other materials, e. g. from inter-laboratory studies or a fortification of blank material can be used for recovery studies. For the latter, 18 aliquots are fortified on three concentration levels (6x3). Note that the required concentration levels have changed (Table 19).

Table 21: Minimum requirements for trueness of quantitative methods in accordance with CD 2002/657 and CIR 2021/808.

Mass fraction	Range CD 2002/657	Range CIR 2021/808
≤ 1 µg/kg	-50 % to +20 %	-50 % to +20 %
> 1 µg/kg to 10 µg/kg	-30 % to +10 %	-30 % to +20 %
≥ 10 µg/kg	-20 % to +10 %	-20 % to +20 %

Measurement uncertainty

An estimation of the measurement uncertainty was not a requirement of CD 202/657. Considerations on the derivation of an analytical method's associated total measurement uncertainty are given in sections 4.1.1 and 4.2.6 of this document.

Matrix effect

In accordance with CIR 2021/808 it is mandatory to assess the relative matrix effect of any quantitative confirmation or screening method if it has not been determined as part of the validation study or preliminary experiments. As this was not a direct requirement of CD 2002/657 the relative matrix effect has to be evaluated for revalidations. If sufficient data on the relative matrix effect can be gathered from previous analyses carried out using the method in question (e. g. data from quality control samples), these can be referred to. A new experimental study is necessary when the method itself has changed, though.

Absolute recovery

Under CD 2002/657 it was only required to assess an analytical method's recovery, the absolute recovery was not explicitly required. With CIR 2021/808, the absolute recovery is to be determined for all quantitative confirmation or screening methods which do not employ matrix-fortified calibrations or internal standards. If no such studies are available, then these experiments need to be included in a revalidation study.

Ruggedness

In general, the requirements remained identical. However, they were given in more detail in CD 2002/657. A ruggedness investigation is included in the alternative validation approach and an example for the conventional validation approach is given in the guidance document on confirmation method validation. Additionally, templates for the layout of ruggedness studies are available from the EURLs upon request.

Critical concentrations

Decision limit CCα for unauthorised and prohibited substances

Methods 1 and 2 for the calculation of CCα given in CIR 2021/808 are identical to the methods given in CD 2002/657. The newly added calculation method 3 provides a statistics based approach to the calculation of the decision limit. Note that the application of method 2 is only justified for analytical methods validated before the entry into force of CIR 2021/808 and may only be referred to until 01 January 2026. For all analytical methods validated after the entry into force of CIR 2021/808, method 2 may not be applied.

Decision limit CCα for authorised substances

Method 1 for the calculation of CCα given in CIR 2021/808 is identical to method 1 given in CD 2002/657. Method 2 has been replaced by a statistics-based approach to the calculation of the decision limit.

Detection capability CCβ

Regarding the detection capability, the concept has been revised. With the new CIR 2021/808 it is no longer required to determine the CCβ for confirmation methods as from now on this parameter is only used to assess the performance of screening methods. The calculation methods are in principle identical to those laid down in CIR 2021/808 for the calculation of the CCα but of course the reference concentration is different (e. g. screening target concentration STC). The most suitable calculation method depends on the type of analytical method and is i. a. dependent on the detection mode (e. g. immunochemical methods, mass spectrometric methods) and the quantification (e. g. qualitative method, (semi-)quantitative method with or without a calibration). A valid CCβ according to the requirements of CIR 2021/808 can for semi-quantitative methods usually also be calculated retrospectively for existing methods for example by setting a suitable fortification level as the STC. For more details on the validation of screening methods refer to CIR 2021/808 and the EURL Guidance document on screening method validation[29].

[29] In preparation