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COMMISSION IMPLEMENTING REGULATION (EU) 2024/2052

of 30 July 2024

amending Implementing Regulation (EU) 2021/808 as regards its scope and certain performance criteria of analytical methods for residues of pharmacologically active substances used in food-producing animals

(Text with EEA relevance)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products, amending Regulations (EC) No 999/2001, (EC) No 396/2005, (EC) No 1069/2009, (EC) No 1107/2009, (EU) No 1151/2012, (EU) No 652/2014, (EU) 2016/429 and (EU) 2016/2031 of the European Parliament and of the Council, Council Regulations (EC) No 1/2005 and (EC) No 1099/2009 and Council Directives 98/58/EC, 1999/74/EC, 2007/43/EC, 2008/119/EC and 2008/120/EC, and repealing Regulations (EC) No 854/2004 and (EC) No 882/2004 of the European Parliament and of the Council, Council Directives 89/608/EEC, 89/662/EEC, 90/425/EEC, 91/496/EEC, 96/23/EC, 96/93/EC and 97/78/EC and Council Decision 92/438/EEC (Official Controls Regulation) ([1]), and in particular Article 34(6) thereof,

Whereas:

(1) Commission Implementing Regulation (EU) 2021/808 ([2]) lays down rules on the performance of analytical methods for residues of pharmacologically active substances used in food-producing animals, on the interpretation of results and on the methods to be used for sampling.

(2) Implementing Regulation (EU) 2021/808 concerns, among others, performance criteria for analytical methods in relation to residues of pharmacologically active substances in feed. It should, however, be clarified that Implementing Regulation concerns only the methods used to verify compliance with certain rules fixing regulatory levels in feed, covered by the multi-annual national control plans in the area of residues of pharmacologically active substances mentioned in Commission Implementing Regulation (EU) 2022/1646 ([3]), and does not concern the methods used to verify compliance with rules on cross-contamination of antimicrobial active substances in non-target feed, referred to in Commission Delegated Regulation (EU) 2024/1229 ([4]). The scope of Implementing Regulation (EU) 2021/808 should be amended accordingly.

(3) Since the adoption of Implementing Regulation (EU) 2021/808, several international standards have been updated. In order to ensure that the relevant references remain accurate, they should be updated accordingly.

([1]) OJ L 95, 7.4.2017, p. 1, ELI: http://data.europa.eu/eli/reg/2017/625/oj.

([2]) 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 (OJ L 180, 21.5.2021, p. 84, ELI: http://data.europa.eu/eli/reg_impl/2021/808/oj).

([3]) Commission Implementing Regulation (EU) 2022/1646 of 23 September 2022 on uniform practical arrangements for the performance of official controls as regards 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, on specific content of multi-annual national control plans and specific arrangements for their preparation (OJ L 248, 26.9.2022, p. 32, ELI: http://data.europa.eu/eli/reg_impl/2022/1646/oj).

([4]) Commission Delegated Regulation (EU) 2024/1229 of 20 February 2024 supplementing Regulation (EU) 2019/4 of the European Parliament and of the Council by establishing specific maximum levels of cross-contamination of antimicrobial active substances in non-target feed and methods of analysis for these substances in feed (OJ L, 2024/1229, 30.4.2024, ELI: http://data.europa.eu/eli/reg_del/2024/1229/oj).

(4) In order to ensure that performance criteria are adequately checked, it should be explicitly mentioned in Implementing Regulation (EU) 2021/808 that any deviations from the established technical criteria should be documented and analysed with traceable evidence kept. Therefore, this requirement should be added to the general requirements of the analytical methods.

(5) The transition period for certain provisions laid down in Article 7 of Implementing Regulation (EU) 2021/808 has ended. Consequently, it is appropriate to amend that Article accordingly.

(6) To improve the readability of general requirements for confirmatory methods, certain parts of the relevant provisions should be included in a specific subchapter referring to the specific use of co-chromatography.

(7) Based on the experience gained during the implementation of Implementing Regulation (EU) 2021/808, the coefficient of variation under repeatability conditions in certain cases cannot fulfil the requirements laid down as regards their precision and therefore this requirement should be amended to take into account reproducibility conditions.

(8) According to the performance characteristics, screening methods can be of three different types. Although qualitative and quantitative methods are defined in Implementing Regulation (EU) 2021/808, an explanation of the semi-quantitative screening method is missing. Therefore, an explanation of this type of method should be added to the classification of analytical methods.

(9) The requirements for performing several individual experiments for every major change currently refers to ruggedness. Since also the other performance characteristics are to be checked with the major change, a reference to all the necessary performance characteristics should be mentioned and therefore the relevant provisions should be amended accordingly.

(10) For a non-allowed pharmacologically active substance, validation of a concentration of 0,5 times the reference points per action (RPA) is requested. However, sometimes it is not reasonably achievable as the concentration is too low from the analytical point of view and, therefore, the concentration of 0,5 times the RPA can be replaced by the lowest concentration between 0,5 times and 1,0 times the RPA, which is reasonably achievable. For some cases, the lowest calibrated level can be lower than 0,5 times the RPA and therefore the possibility of validation at this concentration level should be added into the relevant footnotes.

(11) To clarify the total number of replicates required for the determination of repeatability and within-laboratory reproducibility, this number should be explicitly mentioned in the relevant subcategories.

(12) Validation of the analytical methods can be performed according to alternative models using an experimental plan. Currently, there is an international standard ISO/TS 23471:2022 available and, therefore, the reference to it should be added as another possibility for calculation of the method characteristics.

(13) When determining the stability of an analyte, an isochronous approach allows an improved determination of potential analyte instabilities as well as an estimation of appropriate storage periods. Therefore, this approach should be added to the options for determination of the stability of the analyte.

(14) During the implementation of Implementing Regulation (EU) 2021/808, the procedure describing the determination of the stability of analyte in matrix resulted in different interpretations. It is therefore appropriate to further clarify that procedure, in particular in the steps of fortification of the analyte and in the use of proper terms of aliquots and portions.

(15) Currently. the calculation of the detection capability for screening (CCβ) for Method 2 for unauthorised or prohibited pharmacologically active substances includes only the cases where the chosen screening target concentration provides less than or equal to 5 % false compliant results. Therefore, a provision for the case where the percentage of false compliant results is higher than 5 should be added.

(16) For the screening methods, only the CCβ for the individual substance is reported. Therefore, the additional provision for the sum of CCβ, included in the provisions for calculation of CCβ, is redundant and should be deleted.

(17) The need to determine the absolute recovery of the method depends on the unavailability of the internal standard or on whether a matrix-fortified calibration is used or not. The current wording that the absolute recovery of the method is to be determined when no internal standard or no matrix-fortified calibration is used can be confusing, as it could be understood that both cases occur together, when only one of two conditions is sufficient to determine the absolute recovery.

(18) Regarding relative matrix effects, currently, the value of the coefficient of variation refers to a maximum numerical percentage without differentiation of the mass fractions. Since Table 2 of Annex I to Implementing Regulation (EU) 2021/808 presents various acceptable coefficients of variations depending on the different mass fractions, the acceptable coefficient of variation should refer to the values listed in that table.

(19) Implementing Regulation (EU) 2021/808 should therefore be amended accordingly.

(20) The measures provided for in this Regulation are in accordance with the opinion of the Standing Committee on Plants, Animals, Food and Feed,

HAS ADOPTED THIS REGULATION:

Article 1

Implementing Regulation (EU) 2021/808 is amended as follows:

(1) Article 1 is replaced by the following:

'*Article 1*

Subject matter and scope

This Regulation lays down rules concerning the methods of analysis used for sampling and for laboratory analyses in relation to residues of pharmacologically active substances in the frame of national plans as defined in Article 3 of Commission Implementing Regulation (EU) 2022/1646 (*). It also lays down rules for the interpretation of analytical results of these laboratory analyses.

This Regulation applies to official controls aimed at verifying compliance with the requirements on the presence of residues of pharmacologically active substances.

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(*) Commission Implementing Regulation (EU) 2022/1646 of 23 September 2022 on uniform practical arrangements for the performance of official controls as regards 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, on specific content of multi-annual national control plans and specific arrangements for their preparation (OJ L 248, 26.9.2022, p. 32, ELI: http://data.europa.eu/eli/reg_impl/2022/1646/oj).';

(2) in Article 2, the second subparagraph is amended as follows:

(a) point (36) is replaced by the following:

'(36) "reproducibility" means precision under conditions, where test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment (*);

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(*) ISO 5725-1:2023 Accuracy (trueness and precision) of measurement methods and results – Part 1: General principles and definitions (Chapter 3).';

(b) point (47) is replaced by the following:

'(47) "units" means those units described in ISO 80000-1:2022 (*) and Council Directive 80/181/EEC (**);

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(*) ISO 80000-1:2022 Quantities and units – Part 1: General (Introduction).

(**) Council Directive 80/181/EEC of 20 December 1979 on the approximation of the laws of the Member States relating to units of measurement and on the repeal of Directive 71/354/EEC (OJ L 39, 15.2.1980, p. 40, ELI: http://data.europa.eu/eli/dir/1980/181/oj).';

(3) in Article 3, in point (4), after the first subparagraph, a second subparagraph is added, as follows:

'When deviations from the criteria established in Tables 1 and 2 of Annex I have been observed during validation, impact of those deviations on the outcome of the validation shall be analysed in a documented traceable manner.';

(4) in Article 7, the third paragraph is deleted;

(5) Annex I to Implementing Regulation (EU) 2021/808 is amended in accordance with the Annex to this Regulation.

Article 2

This Regulation shall enter into force on the twentieth day following that of its publication in the *Official Journal of the European Union*.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 30 July 2024.

For the Commission
The President
Ursula VON DER LEYEN

ANNEX

Annex I to Implementing Regulation (EU) 2021/808 is amended as follows:

(1) Point 1.2.1. is replaced by the following:

'1.2.1. *General requirements for confirmatory methods*

For prohibited or unauthorised substances, the CCα shall be as low as reasonably achievable. For prohibited or unauthorised substances, for which an RPA is established under Regulation (EU) 2019/1871 the CCα shall be lower than or equal to the reference point for action.

For authorised substances, the CCα shall be higher than, but as close as possible to, the MRL or ML.

For confirmation purposes, only analytical methods for which it can be demonstrated in a documented traceable manner that they are validated and have a false non-compliant rate (α error) which is less or equal to 1 % for prohibited or unauthorised substances, or which is less or equal to 5 % for authorised substances, shall be used.

Confirmatory methods shall provide information on the structural chemical composition of the analyte. Consequently, confirmatory methods based only on chromatographic analysis without the use of mass spectrometric detection are not suitable on their own for use as confirmatory methods for prohibited or unauthorised pharmacologically active substances. In the case of mass spectrometry not being suitable for authorised substances, other methods such as HPLC-DAD and HPLC-FLD, or a combination of them, can be used.

When required according to the confirmatory method, a suitable internal standard shall be added to the test portion at the beginning of the extraction procedure. Depending on availability, either stable isotope-labelled forms of the analyte, which are particularly suited for mass spectrometric detection, or analogue compounds that are structurally closely related to the analyte, shall be used.';

(2) Point 1.2.1a. is inserted between Points 1.2.1. and 1.2.2.:

'1.2.1a. *Specific use of Co-chromatography when no internal standard available*

When no suitable internal standard can be used, the identification of the analyte shall preferably be confirmed by co-chromatography (*). In this case, only one peak shall be obtained, the enhanced peak height (or area) being equivalent to the amount of added analyte. If this is not practicable, matrix-matched or matrix-fortified standards shall be used.

(*) Co-chromatography is a procedure in which the sample extract prior to the chromatographic step(s) is divided into two parts. Part one is chromatographed as such. Part two is mixed with the standard analyte that is to be measured. Then this mixture is also chromatographed. The amount of added standard analyte has to be similar to the estimated amount of the analyte in the extract. Co-chromatography is used to improve the identification of an analyte when chromatographic methods are used, especially when no suitable internal standard can be used.';

(3) in Point 1.2.2.2., the sentence after Table 2 is replaced by the following:

'For analyses carried out under repeatability conditions, the coefficient of variation under repeatability conditions is usually below two thirds of the values listed in Table 2 and shall be lower than or equal to the coefficient of variation under reproducibility conditions.';

(4) Point 1.2.3. is replaced by the following:

'1.2.3. *Requirements for chromatographic separation*

1.2.3.1. Minimum acceptable retention time

For liquid (LC) or gas chromatography (GC), the minimum acceptable retention time for the analyte(s) under examination shall be twice the retention time corresponding to the void volume of the column.

1.2.3.2. Retention time of the analyte in the extract

The retention time of the analyte in the extract shall correspond to that of the calibration standard, a matrix-matched standard or a matrix-fortified standard with a tolerance of ± 0,1 minute. For fast chromatography, where the retention time is below 2 minutes, a deviation of less than 5 % of the retention time is acceptable.

1.2.3.3. Retention time in case of using of internal standard

In case an internal standard is used, the ratio of the chromatographic retention time of the analyte to that of the internal standard, that means the relative retention time of the analyte, shall correspond to that of the calibration standard, matrix-matched standard or matrix-fortified standard with a maximum deviation 0,5 % for gas chromatography and 1 % for liquid chromatography for methods validated from the date of entry into force of this Regulation.';

(5) in Point 2.1., Table 5 is replaced by the following:

'Table 5

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

Method	Confirmation		Screening		
	Qualitative	Quantitative	Qualitative	Semi-quantitative (+)	Quantitative
Substances	A	A, B	A, B	A, B	A, B
Identification in accordance with 1.2.	x	x			
CCα	x	x			
CCβ	—		x	x	x
Trueness		x			x
Precision		x		(x)	x
Relative matrix effect/absolute recovery (*)		x			x
Selectivity/Specificity		x	x	x	x
Stability (#)		x	x	x	x
Ruggedness		x	x	x	x

x: It is required to prove by means of the validation that the requirements for the performance characteristic are met.
(x) The precision requirements of Chapter 1.2.2.2. do not need to be met for semi-quantitative screening methods. However, the precision shall be determined to prove the suitability of the method for avoiding false compliant analytical results.
A: prohibited or unauthorised substances
B: authorised substances
(+) A semi-quantitative screening method is a screening method which gives quantitative results but does not fulfil the precision requirements given in Table 2 of Annex I to this Regulation.
(*) Relevant for MS methods to prove by means of the validation that the requirements for the performance characteristics are met. The relative matrix effect of the method shall be determined when this effect was not assessed during the validation procedure. The absolute recovery of the method shall be determined when no internal standard or no matrix-fortified calibration is used.
(#) If stability data for analytes in a matrix are available from scientific literature or from another laboratory, these data do not need to be determined again by the concerned laboratory. However, a reference to available stability data of analytes in solution is only acceptable if identical conditions are applied.'

(6) Point 2.2.1. is replaced by the following:

'2.2.1. *Conventional validation*

The calculation of the parameters in accordance with conventional methods requires the performance of several individual experiments (see Table 5 in this Annex). For major changes the ongoing validity of the performance characteristics has to be verified. For multi-analyte methods, several analytes can be analysed simultaneously, as long as possibly relevant interferences have been ruled out. Several performance characteristics can be determined in a similar way. Therefore, in order to minimise the workload, it is advised to combine experiments as much as possible (e.g., repeatability and within-laboratory reproducibility with specificity, analysis of blank samples to determine the decision limit for confirmation and testing for specificity).';

(7) in Point 2.2.1.2., in point 1., subpoint (a) is replaced by the following:

'(a) 0,5 (**), 1,0 and 1,5 times the RPA; or

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(**) Where, for a non-allowed pharmacologically active substance validation of a concentration of 0,5 times the RPA is not reasonably achievable, the concentration of 0,5 times the RPA can be replaced by the lowest concentration between 0,5 times and 1,0 times the RPA, which is reasonably achievable or by the LCL in case that it is lower than 0,5 times the RPA.';

(8) Point 2.2.1.3. is amended as follows:

(a) in point 1, subpoint (a) is replaced by the following:

'(a) 0,5 (***), 1,0 and 1,5 times the RPA; or

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(***) Where, for a non-allowed pharmacologically active substance validation of a concentration of 0,5 times the RPA is not reasonably achievable, the concentration of 0,5 times the RPA can be replaced by the lowest concentration between 0,5 times and 1,0 times the RPA, which is reasonably achievable or by the LCL in case that it is lower than 0,5 times the RPA.';

(b) point 6 is replaced by the following:

'6. Repeat these steps on at least two other occasions for a total minimum of 18 replicates per level.';

(9) Point 2.2.1.4. is amended as follows:

(a) in point 1., subpoint (a) is replaced by the following:

‘(a) 0,5 (****), 1,0 and 1,5 times the RPA; or

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(****) Where, for a non-allowed pharmacologically active substance validation of a concentration of 0,5 times the RPA is not reasonably achievable, the concentration of 0,5 times the RPA can be replaced by the lowest concentration between 0,5 times and 1,0 times the RPA, which is reasonably achievable or by the LCL in case that it is lower than 0,5 times the RPA.’;

(b) point 5 is replaced by the following:

‘5. Repeat these steps on at least two other occasions (for a total minimum of 18 replicates per level) with different batches of blank material, different operators and as many different environmental conditions as possible, e.g. different batches of reagents, solvents, different room temperatures, different instruments or a variation of other parameters.’;

(10) in Point 2.2.2., the sentence after Table 6 is replaced by the following:

‘The calculation of the method characteristics shall be performed as described by Jülicher et al. (*****) or by ISO/TS 23471:2022 (******).

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(*****) 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.

(******) ISO/TS 23471:2022 Experimental designs for evaluation of uncertainty – Use of factorial designs for determining uncertainty functions.’;

(11) in Point 2.5., the third paragraph is replaced by the following:

‘In case the required stability data are not available, the following approaches should be used. In addition, also the application of an isochronous approach (*******) with a storage temperature scheme similar to the one in Table 7 of this Annex allows a determination of potential analyte instabilities and also an estimation of appropriate storage periods, and may also be used.

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(*******) Lamberty, A., Schimmel, H. and Pauwels, J. (1998) The study of the stability of reference materials by isochronous measurements. Fres. J. Anal. Chem. 360, 359.’;

(12) Point 2.5.2. is replaced by the following:

‘2.5.2. *Determination of the stability of analyte(s) in matrix*

1. Use, where possible, incurred samples. When no incurred matrix is available, a blank matrix fortified with the analyte shall be used.

2. When incurred matrix of interest is available, homogenise it, preferably while the matrix is still fresh. Divide the matrix into five portions and analyse one aliquot from each portion.

3. If no incurred matrix is available, take some blank matrix and homogenise it. Divide the matrix into five portions. Fortify each portion with the analyte around the level of interest, which should preferably be prepared in a small quantity of aqueous solution. Analyse one aliquot from each portion immediately.

4. Store the portions (subsamples) of the homogenised incurred matrix or the fortified blank matrix at a temperature reflecting the storage conditions adopted in the laboratory for a given analyte/matrix combination and determine the concentrations of the analyte after short-term storage, medium-term storage and long-term storage (at least as long as the sample is usually retained in the laboratory).

5. The mean value of five aliquots of one portion, which was stored, shall not differ by more than the within-laboratory reproducibility of the method from the mean value of the five freshly prepared aliquots. The mean value of the five freshly prepared aliquots shall be used as the basis for calculating the percentage difference.

6. Record the maximum acceptable storage time and the optimum storage conditions.';

(13) Point 2.7. is amended as follows:

(a) in point 1, point (b) is replaced by the following:

'(b) Method 2: Investigation of fortified blank material at the concentration level of the initially chosen STC. At this concentration level 20 fortified blanks shall be analysed in order to ensure a reliable basis for this determination. If at this concentration level ≤ 5 % false compliant results remain, the level equals the detection capability of the method. If > 5 % false compliant results are obtained, the selected STC shall be increased, and the investigation repeated to verify compliance with the ≤ 5 % false compliant results requirement.';

(b) in point 2, the second paragraph is deleted;

(14) in Point 2.9., the first paragraph is replaced by the following:

'The absolute recovery of the method does not need to be determined if an internal standard, a matrix-fortified calibration or both mentioned are available. In all other cases, the absolute recovery of the method shall be determined.';

(15) in Point 2.10., the last paragraph is replaced by the following:

'The coefficient of variation shall not be greater than the values listed in Table 2 of this Annex for the MF (standard normalised for IS).';

(16) in Chapter 3, the third paragraph is replaced by the following:

'During routine analysis, the analysis of certified reference materials (CRMs) is the preferable option to provide evidence of method performance. Since CRMs that contain the relevant analytes at the required concentration levels are seldom available, also reference materials provided and characterised by the EURLs or laboratories that hold an ISO/IEC 17043:2023 (********) accreditation may be used as an alternative. As another alternative in-house reference materials, which are controlled regularly, may be used.

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(********) ISO/IEC 17043:2023 Conformity assessment – General requirements for proficiency testing.'.