

Results of a Proficiency Testing for the Analysis of Dye Residues in Aquaculture Products Organised by the EU-RL



anses



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Overview

- Dyes are banned substances because of their toxicity for humans
- Dye residues have to be controlled in foodstuffs
- Participants performed a good analysis of dye residues at the RPA shown through the PT results.



Introduction

The organization of proficiency tests (PT) for European Union National Reference Laboratories (EU-NRLs) is one of the duties of the European Union Reference Laboratory (EURL). The EURL PTs are primarily targeted towards the National Reference Laboratories (NRLs), which in accordance with Article 101, 1(a), Regulation (EU) 2017/625 are obliged to participate. The participation in these PT allows NRLs to assess their competence and to prove the reliability of their results. The structure and statistical analysis of this PT was performed according to ISO/IEC 17043 and 13528 standards. The goal of this PT was to evaluate the proficiency of the participants to identify and quantify dye metabolite residues in aquaculture products and to take a decision regarding these samples according to the criteria of the European Decision 2002/657/EC.

Regulation and Dye metabolites:

According to the decision 2005/34/EC, an MRPL (Minimum Required Performance Limit) has been set at 2.0 µg/kg in European Union for the sum of malachite green (MG) and leuco malachite green (LMG) in tissues of aquaculture products. A reference point of action (RPA) is now set at 0.5 µg/kg according to the Commission regulation (EU) 2019/1871. Consequently, the methods of analysis used to monitor malachite green residues are supposed to detect and confirm the residues at least down to a level of 0.5 µg/kg. There is no MRPL/RPA set for crystal violet (CV) and leucocrystal violet (LCV) nor for brilliant green (BG) and leucobrilliant green (LBG) so analytical methods must be capable of detecting CV and/or LCV, BG and/or LBG at levels as low as reasonably achievable and presumably as low as the MG/LMG RPA according to the EU-RL recommendation. These molecules have limits set at 0.5 µg/kg in « EURL Guidance on minimum method performance requirements (MMPRs) for specific pharmacologically active substances in specific animal matrices » in version 2 of June 2022. Any findings of MG, LMG, CV, LCV, BG or LBG should be confirmed according to criteria of European Decision 2002/657/EC. A result at and above the CCalpha with all the matching identification criteria leads to the non-compliance of the sample.

Banned Dyes

Parent molecules

Malachite green(MG)
Cristal violet(CV)

Metabolites

Leuco malachite green (LMG)
Leuco cristal violet (LCV)

EU Regulation for Dyes

Decision 2005/34/EC

Commission Regulation (2019/1871/EC)

No but EURL guidance.

Limits

MRPL (Minimum Required Performance Limit) **2 µg/kg** before 2019

RPA for MG and LMG (Reference Point for Action) lower than the MRPL set at **0,5 µg/kg**

Method

PT samples:

- Provided by the EU-RL Anses-Fougères.
- The matrix was trout flesh
- Absence of dye residues in the "blank" material
- Two materials (2 and 3) spiked with dyes and their metabolites content in dyes
- Checked at the EU-RL premises by relevant validated and accredited LC-MS/MS procedure
- Homogeneity and stability of materials were checked. Stability was ok by using the expanded criterion. To consider this issue the evaluation of participants result was carried out by means of z'-scores.

Analysis: EU-NRL received three different samples to analyse with their routine method, for possible content in dyes

Proficiency evaluation: The assigned value (X_{pt}) was calculated as being the robust average of the confirmatory results of all the participants as proposed in the Standard ISO13528:2015 and calculated by means of the Algorithm A.

Individual z'-scores: performance characteristics of the participant for accuracy measurement Z'-scores are calculated as following:

$$z'_i = \frac{x_i - x_{pt}}{\sqrt{\sigma_{pt}^2 + u^2(x_{pt})}}$$

Where: - z' is the "z-score",

- x is the reported concentration,

- X_{pt} is the assigned value,

- σ_{pt} is the target value for standard deviation of the assigned value

- σ_{modified} is the target value for standard deviation

$$\hat{\sigma}_{modified} = \sqrt{\sigma_{pt}^2 + u^2(x_{pt})}$$

- u (x_{pt}) is the standard uncertainty of the assigned value

Interpretation of the z'-scores:

| z' | ≤ 2.0 ⇒ satisfactory

2.0 < | z' | < 3.0 ⇒ questionable

| z' | ≥ 3.0 ⇒ unsatisfactory

Table 1: description of the materials

	Material 1	Material 2	Material 3
Prohibited substance	none	MG and LMG	CV and LCV
Matrix	Trout flesh	Trout flesh	Trout flesh
Range of Targeted concentration (µg/kg)	0	0.7 for each	0.7 for each
EU RPA (µg/kg)	/	0.5 (new RPA)	0.5

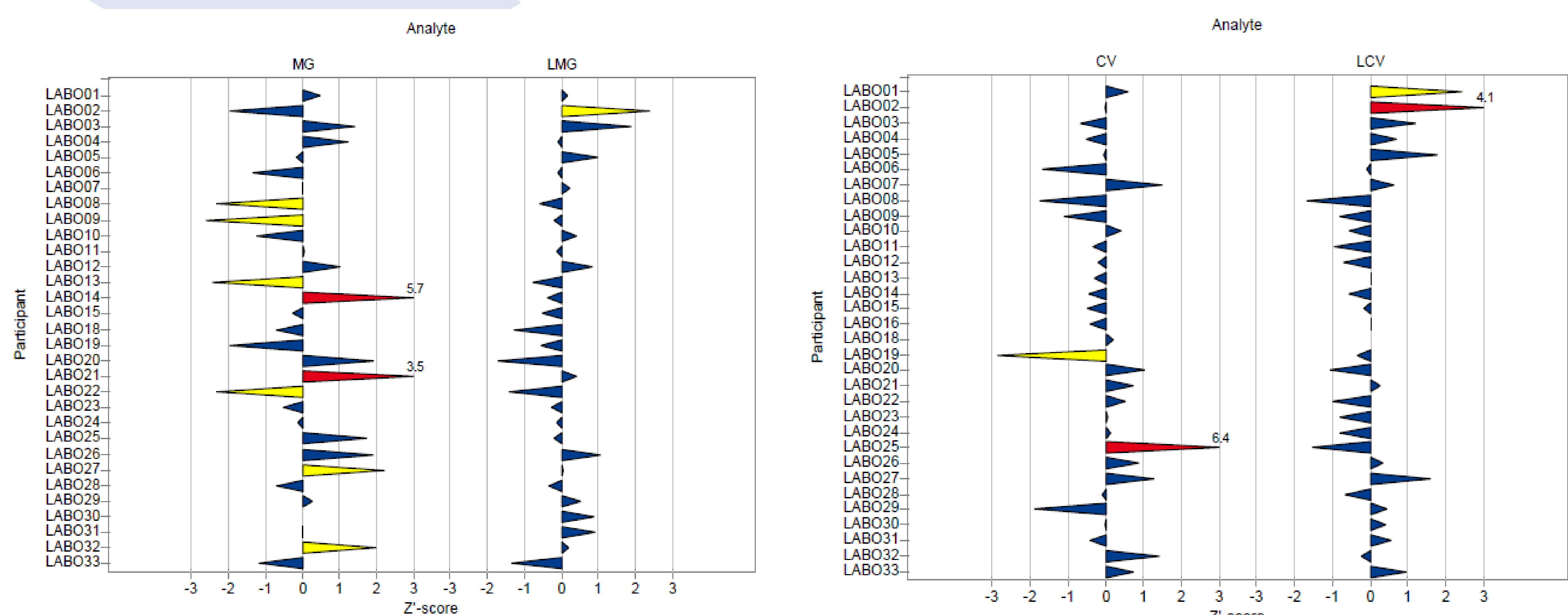
Table 2: confirmation step expected decision

	Material 1	Material 2	Material 3
Analyte	None	MG+LMG	CV+LCV
Expected decision Compliant (C), Non compliant (NC)	C	NC	NC

Results

Table 3: assigned values and their uncertainties for the analysed materials

Results	Material 1	Material 2	Material 3		
Analyte	none	MG	LMG	CV	LCV
Minimum value (µg/kg)	/	0.190	0.380	0.417	0.205
Maximum value (µg/kg)	/	1.240	0.945	1.300	1.420
Assigned value : x _{pt} (µg/kg)	/	0.518	0.615	0.583	0.677
Standard uncertainty of the assigned value u(x _{pt}) (µg/kg)	/	0.053	0.026	0.029	0.036
Target value for standard deviation : σ _{pt} (µg/kg)	/	0.114	0.135	0.149	0.128
u(x _{pt}) / σ _{pt}	/	0.19	0.47	0.22	0.24



- 33 participants included 22 NRL returned results for this PT
- Almost all participants used LC-MS/MS validated methods to analyse the samples, excepted one used GC-MS
- The method of analysis of dyes is updated to strictly follow the changing regulatory
- The ccalpha are already below the RPA of 0,5 µg/kg
- Proficiency expressed as Z'-score is satisfactory for dye leucobase for almost all participants

Keywords

Dye residues, veterinary drugs, LC-MS/MS, proficiency testing

