

Proficiency Test and Method Comparison for Triphenylmethane Dyes in Aquaculture

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Introduction

The aim of the Proficiency Tests (PT) of the National Reference Laboratory (NRL) Berlin is to promote the residue analysis of veterinary drugs in different matrices in accordance with Article 101 (1c) of Council Regulation 625/2017/EC.

The main application of PT programmes is the assessment of the ability of Routine Field Laboratories (RFLs) in Germany to competently perform the required tests for entire substance groups (especially with multi-methods) in order to obtain comparable results.

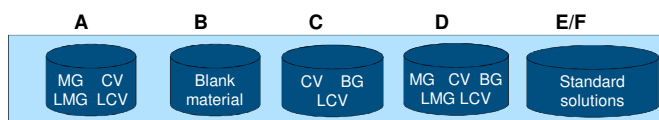
The dyes malachite green (MG), crystal violet (CV) and brilliant green (BG) are popular products for the treatment of aquaculture. However, due to the adverse health effects of these substances and their metabolites, their use in food-producing animals is no longer permitted in the EU. Nevertheless, positive findings have been reported for tests carried out in the framework of the national residue control plans of the EU Member States. In order to be able to detect cases of misuse, test methods covering the range of 1 µg/kg and below are required for these substances and their metabolites (leuco malachite green (LMG) and leuco crystal violet (LCV)).

Sample preparation

An important requirement for PTs is the use of incurred material in order to create conditions which are as similar as possible to real-life samples. For the production of positive samples for this PT, material from treated rainbow trouts as well as material from supermarkets was used.

Samples A, C and D were produced by mixing incurred material and blank material. For the production of sample B uncontaminated material was available. Samples E and F were standard solutions with unknown analyte concentrations.

Sample



Participants

The NRL carried out a PT for the German RFLs as well as for further national and international laboratories.

Each of the 19 participants (one lab with two data sets) analysed four trout fillet samples (test items) with, in total, twelve sample-analyte combinations, as well as two mixes of standard solutions with unknown concentrations. The analyte content in the test items was set to concentrations of approx. 0.7 to 2.6 µg/kg by mixing naturally incurred and blank materials.



Statistics

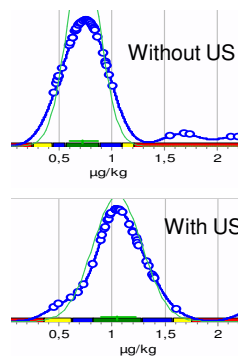
For the calculation of the z-score, two pivotal values must be determined: the assigned value and the target standard deviation.

- The assigned value is normally calculated as the mean of the outlier-free laboratory results.
- If the data of a PT are not normally distributed, it is recommended to carry out a so-called robust calculation of the mean, which was applied here. The model used was the calculation according to Huber (Q-Method-Median) [1].
- The target standard deviation (s) was calculated according to the Horwitz function [1]:

$$\sigma = 0.02 c^{0.8495}$$

c = mean value of the samples of this PT expressed as a power of ten (e.g. 1 µg/kg = 10⁻⁹)

Evaluation and Assessment of Results



In addition to the analyses for the PT, all 19 participants were asked to perform further analyses, which included an ultrasonic treatment (US) at a temperature of 70 °C in the sample preparation.

The evaluation are presented including the results of two different sample preparations for the multi-analysis of dyes and their metabolites.

Figure 1 and the table below provides a detailed picture of the results for the determination of MG and BG (robust statistics according to ISO 13528:2015, Annex C.5.2 and C.5.3, and consideration of different methods according to DIN EN ISO/IEC 17043:2010, point 4.7.2.2 c) [2].

Fig. 1: Kernel density estimation of the distribution of the laboratory mean values (Sample D – Malachite green)

Sample	Analyte	n	Ass. value [µg/kg]	S _R [µg/kg]	t-value	Diff. [%]
A	MG	15	0.819	32.5		
	MG*	16	1.288	26.2	4.144**	+57.2
D	MG	15	0.730	34.5		
	MG*	15	1.053	29.1	3.077**	+44.3
C	BG	18	1.078	34.1		
	BG*	17	1.566	48.2	2.343**	+45.2
D	BG	18	0.824	38.4		
	BG*	17	1.078	52.5	1.584***	+30.8

*with ultrasonic treatment **no equivalence ***no decision possible

Discussion

By means of a proficiency test and an equivalence assessment, it could be stated in a statistically ensured way that the impact of an ultrasonic treatment at a temperature of 70 °C in the sample preparation led to higher contents for MG and BG (31 to 57 %).

References

- [1] ISO 13528:2015: Statistical methods for use in proficiency testing by interlaboratory comparisons, Geneva, Switzerland, August 2015
- [2] ISO/IEC 17043:2010: Conformity assessment - General requirements for proficiency testing, Geneva, Switzerland, May 2010