METROLOGICAL ASSESSMENT OF LABORATORY PERFORMANCE

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Abstract –The purpose of this communication is to present the contribution of EXHM/GCSL-EIM in two different approaches used to assess interlaboratory measurements at different metrological levels. In the first one, at the high metrological order - CCQM level, concerning the key comparison "CCQM K138 - determination of aflatoxins in dried figs", the method developed and the results reported by our institute are described. According to the second approach, participation in a Proficiency Testing scheme, the organization and evaluation of SCHEMA PTs reported results, are also introduced. Different methods for the determination of the assigned value, its uncertainty and the statistical evaluation of the SCHEMA 63 0X PTs series are demonstrated as well.

Keywords: *key comparison, proficiency testing, mycotoxins, uncertainty, metrology*

1. INTRODUCTION

Measurements underpinning food safety and food quality remain important issues in today's world. These measurements have to fulfill the criteria of the relevant legislation and therefore to be reliable and internationally equivalent. For these purposes, the OAWG (Organic Analysis Working Group) of the CCQM (Consultative Committee for the Amount of Substance) organizes key comparisons (KCs) between the NMIs/DIs (National Metrology Institutes/Designated Institutes) in order to evaluate their measurement capabilities for the execution of "high metrological order" measurements accompanied by the relevant uncertainty budget.

Among the topics of interest is the determination of mycotoxins in foodstuffs. Mycotoxins are highly toxic in mammals and could cause many adverse health effects. Commission Regulation 1881/2006 and its amendments set the maximum levels for various mycotoxins in different food categories (e.g. MPL of aflatoxins B1, B2, G1 and G2 in dried figs). In order to overcome the lack of Certified Reference Materials (CRM-a certified reference value and its uncertainty are given in the certificate) which could be used by routine laboratories, the Track C key comparison "CCQM-K138 Mass fractions of aflatoxins (AFB_1, AFB_2, AFG_1, AFG_2 and total AFs) in dried fig", was organized last year. For its participation in this key comparison, EXHM/GCSL-EIM developed an appropriate method based on liquid extraction coupled to an immunoaffinity clean-up (IAC) step and the determination was performed using an Isotope Dilution LC-MS/MS (ID-LC-MS/MS) method.

CRMs and reference methods are the best tools, which can be used by a routine laboratory in order to produce reliable measurements. In case these tools are not available, Proficiency Testing schemes (PTs) constitute an alternative way for an analytical laboratory to evaluate its ability to produce consistent and reliable results. EXHM/GCSL-EIM, as an accredited PTs provider (ISO 17043), offers PTs under the tradename SCHEMA. Taking into account the IUPAC harmonized protocol and ISO 13528:2015, different approaches could be used to determine the assigned value of a PT. In our contribution, three different approaches for the evaluation of laboratory performance in the SCHEMA PTs are presented: the formulation, the result of an expert laboratory and the consensus value of the participants' results, used.

2. EXPERIMENTAL

2.1 CCQM-K138-determination of mycotoxins

According to our method (based on AOAC 999.07), the sample was extracted with MeOH-H_2O and the extract was further purified by IAC clean-up step. The mycotoxins were separated in a Thermo Surveyor Plus HPLC system by means of a C18 chromatographic column (X-Terra, Waters) and a MeOH-H_2O gradient mobile phase. The analytes were identified by multiple reaction monitoring and quantified via an ID-MS/MS method using a Thermo TSQ Quantum Ultra AM mass spectrometer that operated in HESI mode.

IRMM CRMs solutions were used for the traceable calibration of the instrumentation, while in-house

calibration solutions were also prepared. The determination was performed using the exact matching and matrix matched techniques. $^{13}C_{17}$ labelled aflatoxin solutions were used (isotope dilution) in order to correct for recovery. The following transitions in the HESI(+) mode were monitored:

AfB1 (313.1 to 285Q, 241q), AflaB2 (315.1 to 243, 259Q, 287q), AflaG1 (329.1 to 200q, 215, 243Q), AflaG2 (331.1 to 201, 217, 245Q, 257q, 275, 313), ^{13}C-AfB1 (330.1 to 255, 301), ^{13}CAflaB2 (332 to 259, 303), ^{13}CAflaG1 (346 to 212, 317), ^{13}CAflaG2 (348 to 259, 313)

The trueness of the method is verified by the analysis of a surplus PTs sample. Finally, the uncertainty budget is presented.

2.2 SCHEMA PTs methods for laboratory performance evaluation

2.2.1. SCHEMA 63 03-determination of pesticide residues in foodstuffs

In line with market needs, orange juice was chosen as the testing substrate for this PTs. The test items were prepared gravimetrically from blank organic orange juice fortified with a known amount of eleven pesticide residues. This known amount was used as the assigned value of the PTs (by formulation). The uncertainty of this value was also calculated based on the contribution of the preparation, the homogeneity and the stability of the testing material.

2.2.2. SCHEMA 63 04-determination of pesticide residues in foodstuffs

The substrate of this intercomparison was organic tomato juice prepared from blank organic tomato juice spiked with a known amount of ten pesticide residues. The consensus value of the participant's results was used as the assigned value of the PT.

2.2.3 SCHEMA 63 02-determination of pesticide residues and acrylamide in foodstuffs

The testing material for this round was prepared by fortification of organic flour with a predefined amount of acrylamide. The assigned value was determined by an expert laboratory which used a primary ID-LC-MS/MS method and also performed a parallel analysis of a matrix CRM.

3. RESULTS AND DISCUSSION

3.1 CCQM K138-mycotoxins determination

The results (mass fraction of each measurand) were submitted to the organising NMI as well as their uncertainties. The mean of two samples with their expanded uncertainty were calculated and are presented in Table 1.

Table 1. EXHM/GCSL-EIM submitted results

Analytes	MassFraction (ng/g)	Mean Value (ng/g)	Combined Standard Uncertainty (ng/g)	ExpandedUncertainty (ng/g)
Aflatoxin B_1	6,121	5,994	0,123	0,249
	5,869			
Aflatoxin B_2	0,882	0,871	0,022	0,047
	0,861			
Aflatoxin G_1	2,032	2,093	0,061	0,125
	2,154			
Aflatoxin G_2	0,274	0,264	0,01	0,022
	0,254			
TotalAflatoxin	9,309	9,223	0,141	0,282
	9,138			

The preliminary evaluation of this intercomparison suggests that EXHM/GCSL-EIM has participated successfully.

3.2 SCHEMA PTs

3.2.1 SCHEMA 63 03-determination of pesticide residues in foodstuffs

The summary statistics and the evaluation of the PT are presented in Tables 2 and 3 respectively.

Table 2. SCHEMA 63 03 – Descriptive summary statistics

pesticides (mg/kg)	atrazine	chlorpyrifo	malathion	quinoxifen	dimethoat
Mean value	0.185	0.214	1.148	0.103	0.094

Standard Deviation	0.022	0.142	0.105	0.006	0.004
Target-value of standard deviation σ_p	0.050	0.050	0.250	0.025	0.025
Assigned value uncertainty (u)	0.01	0.01	0.09	0.01	0.01
Assigned Value $\bar{X}$	0.200	0.200	1.000	0.100	0.100

Table 3. SCHEMA 63 03 - Statistical evaluation of the submitted results

	Atrazine, assigned value 0.200 (mg/kg)		**Chlorpyrifos, assigned value 0.200 (mg/kg)**	
Lab code	result (mg/kg)	z-score	result (mg/kg)	z-score
PEST_01	0.149	-1.0	0.448	**5.0**
PEST_02	0.198	0.0	0.193	-0.1
PEST_03	0.180	-0.4	0.11	-1.8
PEST_04	0.202	0.0	0.225	0.5
PEST_05	0.195	-0.1	0.093	**-2.1**
PEST_06	-		-	
PEST_07	-		-	

The fortification level was used as the assigned value. The uncertainty of the assigned value was calculated and compared to the target-value of standard deviation for Proficiency Assessment $\sigma_p$as a measure of the reliability of the assigned value.

3.2.2 SCHEMA 63 04-determination of pesticides in foodstuffs

In this PT, the assigned value was determined from the participants' consensus value. The uncertainty of the assigned value was also calculated.

3.2.3 SCHEMA 63 02-determination of pesticide and acrylamide in foodstuffs

The summary statistics and the evaluation of the PT are presented in Table 4.

Table 4. SCHEMA 63 02: (A)-Descriptive Summary statistics, (B)-Statistical evaluation of the submitted results.

estimators	**acrylamide (mg/kg)**
Median	1.67
Mean value	1.62
Standard Deviation	0.18
Target-value of standard deviation σ_p	0.24
Assigned Value $\bar{X}$	1.56

(A)

Lab code	**result mg/kg)**	**z-score**
FHM 01	1.483	-0.3
FHM 02	1.67	0.5
FHM 03	-	
FHM 04	-	
FHM 05	1.700	0.6
FHM 06	-	
FHM 07	-	
FHM 08	-	
FHM 09	-	
FHM 10	-	
FHM 11	-	

(B)

Due to the limited number of participating laboratories, the assigned value was provided by an expert laboratory, which used a primary method for the determination of acrylamide in the SCHEMA 63 02 test sample and performed a within sequence matrix CRM analysis.

4. CONCLUSION

In an interlaboratory comparison, the assigned value and its uncertainty play a crucial role for the evaluation of the reported results. Even though the consensus value of the participant's results constitutes a common way for the determination of the assigned value, the majority of the PTs providers do not calculate the uncertainty of the consensus value. However, the determination of the assigned value by formulation (gravimetric preparation of the testing material) as well as by an expert laboratory seems to be advantageous:

1) PTs test items with small uncertainty of their assigned value could be used as a reference, secondary or in-house reference materials;

therefore, surplus samples could also be used for QC purposes, personnel training, etc.

2) metrological traceability of the certified value is assured,

3) provide an approved approach in order to evaluate the submitted results in PTs with a limited number of participants.

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