

Proficiency testing schemes for Cadmium in cocoa powder analysis for Colombian laboratories

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Abstract – This paper shows the experience in the operation of a proficiency test for the analysis of Cadmium in Cocoa. Due to the limited number of analytical laboratories participating, this proficiency test was developed with two reference materials (RMs), which were prepared from cocoa beans. The certified value for Cadmium in the two RMs (low and high concentration levels of Cadmium) was based on the agreement of results of at least two methods: ICP-MS and GF-AAS conducted at the National Metrology Institute of Colombia. The homogeneity and stability studies were performed according to guide ISO 35. In the first study, it was found an uncertainty associated to homogeneity between bottles of 0.06% for low concentration level and 0.89% for the high concentration level. Stability contribution was estimated by 0.24% in accelerated conditions and 0.47% in the long-term conditions. On the other hand, the assigned value was 0.202 ± 0.018 mg/kg by low concentration of the RM and 2.32 ± 0.15 mg/kg by the second one. The proficiency testing scheme counted with the participation of 10 laboratories with unsatisfactory results only for one participant. Following this scheme, some tools for improvement were identified such as the use of both internal and subrogate quality control standards for measurement, among others.

Keywords – Cadmium analysis, Cocoa powder, Proficiency testing schemes, Technical competence, Colombian laboratories.

I. INTRODUCTION

Cocoa beans are the most important commodity used for producers such as the European Union and the United States for the elaboration of products [1]. The main

producers of cocoa beans include Ghana, Ecuador, Nigeria, and Brazil, contributing with almost the 90% of the total production around the world [2]. In Latin America, the Andean region is the most important producer, but its production is only 8% of the of the total production. However, the cocoa from this region is particularly attractive in the global market due to the high quality in both flavour and aroma [3].

In addition to the low production, good quality Latin American cocoa in the international market has been significantly affected by the levels of Cadmium in cocoa beans, being a concern for the industry of high-cocoa content products [4] [5]. Likewise, the current diligence regulation for cocoa placed on the EU market turns the cocoa producers to address different challenges [6], which involve the verification of the conformity of cocoa beans, according to the regulation. For this, producers must have an offer of competent laboratories in the measurement of Cadmium in cocoa.

In order to maintain the competitiveness of the Latin American in the cocoa exports to the EU, the National Metrology Institute of Colombia designed and implemented a proficiency testing scheme for the measurement of Cd in cocoa. Thus, this work details of the design, planning and execution of this proficiency testing, as well as a presentation of the different steps during the characterization of the comparison item.

II. RELATED RESULTS IN THE LITERATURE

Proficiency tests are a type of interlaboratory comparison that allows to the laboratories to compare their performance against a valid benchmark that helps to take a preventive action and/or corrective procedures in order to meet a technical competence in a testing service. The evaluation of a proficiency testing performance to be faithfully representative of the service provided by a

testing laboratory, should include aspects as follows [7]:

- i) the similarity between the measurand and its concentration level inside the matrix of the test
- ii) accreditation in ISO/IEC 17043:2010 [8] by the supplier
- iii) comparability of measurement methods between the proficiency test and the analytical technique to be evaluated.

In the context of Cd analysis in cocoa beans, it has been found that some institutions offer this service. For example, FAPAS annually offers the scheme "Metallic contaminants in cocoa powder" with a cost of 200€, which is elevated in Latin American laboratories. In the proficiency testing of 2019 contaminants such as Arsenic, Cadmium, and Lead were evaluated. For a Cd analysis it was reported the use of ICP-MS and GF-AAS as analytical techniques, sample preparation by dry ashing and microwave digestion and hydrochloric acid, hydrogen peroxide, and nitric acid as reagents used in this step [9].

On the other hand, certified reference materials are a metrology tool that can be used for method validation, quality control and quality assurance purposes. Currently, only two reference materials related to the measurement of Cd in cocoa are available; however, these materials are chocolate derivatives, i.e. baking chocolate, and they have different physicochemical properties than cocoa grain.

III. DESCRIPTION OF THE METHOD

A. Reference material preparation

The proficiency test item consisted in two samples of cocoa in different concentration levels: RM1: low concentration level and RM2: high concentration level. The samples were prepared taking two batches of cocoa coming from local markets and transported to the laboratory for processing by the steps as follows: i) pulverized, ii) degreased, iii) sieved, iv) homogenized, v) bottled, vi) irradiated and vii) sampled for microbiological analysis. For the production of 70 bottles of each sample, 10 were taking to homogeneity test, 10 for stability analysis, 6 for assigned value, 10 for a distribution to the participants and the remaining 34 for monitoring.

Sample preparation: The extracts of the samples used in the characterization steps of comparison items were obtained by microwave-assisted extraction (MAE) using Anton Paar Multiwave Pro equipped with sixteen high-pressure PTFE vessels. The steps for digestion program were as follow: i) power ramp of 1400 W during 15 minutes, ii) power hold during 15 minutes, and iii) cooling up to 70°C. Each vessel contains at least 300 mg of cocoa powder from each bottle with 5 mL of sub boiled distilled HNO₃, 2 mL of H₂O₂ of 30% and 2 g of In and Rh solution of 12 µg/kg as surrogate standard. After, to the resultant extract, 10 g of Ge and U solution of 60 µg/kg were added as internal standards. Finally, it was diluted up to 50 g

using high purity water. This procedure was based on AOAC method 2015.01 [10].

B. Analytical methods

ICP-MS measurements on samples: A quadrupole-based NexION 300D ICP-MS instrument with an autosampler (Perkin-Elmer (PE), USA) was used to quantify Cd in cocoa extracts. Plasma was generated by a forward power of 1600 W and gas flow rates of coolant 15 L/min, auxiliary 1.2 L/min, and nebulizer 0.60 L/min. A cyclonic nebulizer, nickel sampler's and skimmer cones were used throughout the study. Torch position, ion lenses and gas output were optimized daily with the tuning solution (1 µg/kg of As, Cd, Ce, Hg, and Pb) to maximize ion signals, and to minimize interference effects from polyatomic ions and doubly charged ions.

GF-AAS measurements on cocoa extracts: Cd measurements by GFAAS were carried out using a PinAAcle 900T atomic absorption spectrometer (Perkin Elmer) with Zeeman background correction and an AS 900 autosampler. Electrodeless Discharge Lamp for Cd was operated to 228.8 nm. Aliquots of the 20 µL of the samples were injected with 5 µg of Pd as chemical modifier directly into the graphite tube. Pyrolysis and atomization temperatures were 500 °C and 1500 °C, respectively. Standard addition method was used by quantification method. SRM 3108 Cadmium Standard Solution from NIST was used as calibrant. ERM-BD 512 Dark Chocolate was used as analytical quality control.

Moisture determination on cocoa powder: Moisture determination was carried out using the oven drying method. A total of 2 g of cocoa was weighed for the analysis. The moisture of the cocoa samples was performed using oven drying with an oven temperature of 100 °C±2 °C for 2 hours. Moisture correction was applied to results to be reported on a dry basis.

C. Reference materials characterization

Homogeneity test: 7 sub-samples from each bottle were taken for a total of 70 observations. The uncertainty (u_{hom}) associated with the heterogeneity of the material was estimated by ANOVA analysis with replication using instrumental responses of ¹¹²Cd, ¹¹³Cd, ¹¹⁴Cd and ¹¹¹Cd relative to surrogate standards ¹¹⁵In, ¹⁰³Rh and internal standards ⁷²Ge, ⁷⁴Ge and ²³⁸U by ICP-MS.

Stability test: The short-term stability of the two reference materials was evaluated under three different conditions through an isochrone design. The reference temperature selected was -80°C. For this study, the bottles were randomly selected and stored at 40°C and 60% RH (ac 1), 60°C and 80% RH (ac 2) during 10 days. Finally, from the whole set of bottles, 5 sub-samples of cocoa powder were taken for each bottled. A total of 50 observations were digested by MAE and analysed by ICP-MS. The long-term stability study was conducted with measurements after 0, 3, 6 and 12 months under one

condition.

Reference value: Proficiency test item was characterized by two independent analytical procedures, using ICP-MS and GF-AAS. To achieve this, some set of bottles were taken by random sampling in different days and analysed by these techniques under repeatability conditions. From these results, the assigned value was obtained by the combination of values of each method, according to Levenson [11].

D. Proficiency testing design

This scheme was developed according to ISO/IEC 17043:2010 [8] with the participation of 10 laboratories. The evaluation for this exercise was conducted according to numeral 8.4 of ISO/IEC 13528:2015 [12] using the performance statistic z-score as indicated in, e.g. (1).

$$z_i = \frac{x_i - x_a}{\sigma_{pt}} \quad (1)$$

Where x_i is the measurement result for the proficiency testing scheme by laboratory i , x_a is the assigned value obtained from item characterization and σ_{pt} is the standard deviation for the proficiency test. Assessment of z-scores is based on the following criteria: (i) a $|z| \leq 2.0$ is considered as satisfactory, (ii) a result between $2.0 < |z| < 3.0$ is considered as questionable or warning, and (iii) $|z| > 3.0$ is deemed to be unacceptable (or action signal).

E. σ_{pt} estimation

The value of σ_{pt} , was estimated according to paragraph 8.4 of the ISO 13528:2015 [12] using the Horwitz curve according to the commission regulation (EU) No. 333/2007 [13], as indicated in e.g. (2):

$$\sigma_{pt} = \begin{cases} 0.22c & c < 1.2 \times 10^{-7} \\ 0.22c^{0.8495} & 1.2 \times 10^{-7} < c < 0.138 \\ 0.01c^{0.5} & c > 0.138 \end{cases} \quad (2)$$

Where c , is the Cd mass fraction in dry basis obtained from the value assignment and is in the range $0 < c < 1$.

Variance Components Model: Through the random effect model or variance components model, an alternative estimation of σ_{pt} was proposed using the model given in e.g. (3).

$$y_{ij} = \mu + u_i + \varepsilon_{ij} \quad (3)$$

Where y_{ij} proficiency test result from participant i in the measurement j , μ true value for the measurand, u_i , is the effect from the participant i distributed normal($0, \sigma_u$), ε_{ij} is the measurement error for participant i , distributed normal($0, \sigma_\varepsilon$) and u_i, ε_{ij} are independents. The variance components are estimated by Restricted Maximum Likelihood (REML) [14] and μ is estimated by Generalized Least Squares [15].

IV. RESULTS AND DISCUSSIONS

A. Homogeneity test

The uncertainty associated to the variation between bottles, u_{bb} and within bottles, u_{wb} , were estimated from SS_{bb} , SS_{wb} and SS_{Med} values, which are estimated by the ANOVA analysis as indicated in e.g. (4) and e.g. (5). Likewise, n_1 corresponds to the sub-samples from each bottle ($n_1=7$) and n_2 is the number of replicates in the ICP-MS instrument for each extract ($n_2=15$).

$$u_{bb} = \sqrt{\frac{SS_{bb} - SS_{wb}}{n_1}} \quad (4)$$

$$u_{wb} = \sqrt{\frac{SS_{wb} - SS_{Med}}{n_2}} \quad (5)$$

A major variation in SS_{bb} than in SS_{wb} was found when estimating the value of u_{hom} . Likewise, by two cocoa samples, u_{hom} was estimated as given in e.g. (6) according to the recommendation of the ISO 35: 2017 Guide [16]:

$$u_{hom} = \sqrt{\frac{SS_{wb}}{n_1}} * \sqrt[4]{\frac{2}{vSS_{wb}}} \quad (6)$$

Where, v correspond to degrees of freedom by these experiments ($v = N * (n_1 - 1)$). In this way, according to the relative response of Cd isotopes vs. surrogate and internal standards, it was found that u_{hom} is 0.06 % by RM1 and 0.89 % by RM2 vs. ^{115}In . This value for the two samples was adequate for the requirements of this material since the expected value was close to 1%. Likewise, it was found that the ^{238}U as an internal standard was not adequate for this study and the use of Ge isotopes increase u_{hom} close to three times.

B. Stability test

According to ISO 17034: 2016 [17], the evaluation of the stability study was carried out through linear regression models. On the other hand, through different transformations of the data, different kinetic models were evaluated: time (t) vs relative response (Rr) by order 0, vs Ln (Rr) by order 1, vs 1/Rr by order 2 and vs $1/(Rr)^2$ by order 3, see Fig.1. For these experiments, the ANOVA regression analysis shows that the degradation kinetic is not associated with the variation in the concentration of Cd in the bottles submitted to extremely (or environmental) conditions in function of time. Whereby, the uncertainty associated to stability in accelerated conditions (u_{sst}), thus, $u_{sst} = 0.24\%$

Likewise, the long-term stability contribution (u_{slt}) was estimated from the variation of the Cd determination in some cocoa bottles by ICP-MS under repeatability conditions. This study was carried out from t vs. Rr model by order 0 and u_{slt} was obtained for typical error of this model and estimating a variation during 90 days, see Table.1.

In both cases of the stability study, it was found that

is not a variation of the concentration of Cd as a function of time, whereby, u_{sst} and u_{slt} must be 0 according to ISO 17034: 2016 [17].

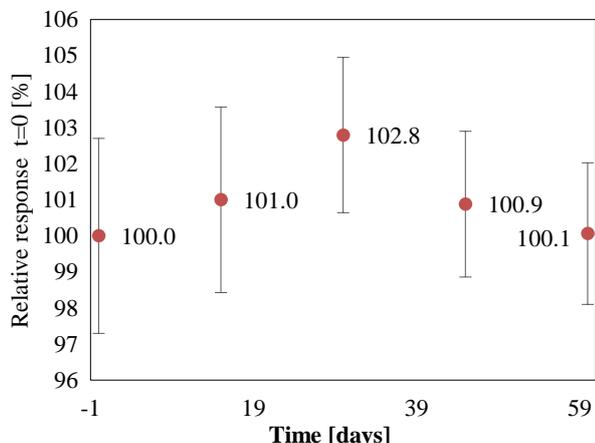


Fig. 1. Variation of the relative response as a function of time according to stability study in accelerated conditions.

However, it is necessary to consider the possible changes that may occur at the cocoa matrix. For this reason, the typical error of the t vs. R_r model was used by order 0, and the values obtained from stability contribution by the two samples were adequate for requirements of this material since the expected value was close to 1%.

Table 1. Results of long-term stability contribution

Sample	Typical error (%)	Time (days)	u_{slt} (%)
RM1	0.0170	90	0.47
RM2	0.0198		0.46

C. Reference values.

The value assignment of the two reference materials of the proficiency test was made from ICP-MS and GF-AAS measurements under intermediate precision conditions, see Table 2.

Table 2. Results of the Cd mass fraction in dry basis by ICP-MS and GF-AAS

Sample	Technique	[Cd] (mg/kg)	u_c (%)	u_s (%)
RM1	ICP-MS	0.186	4.98	4.95
	GF-AAS	0.210	3.54	
RM2	ICP-MS/AP	2.22	4.90	2.58
	ICP-MS/EQ	2.22	2.94	
	GF-AAS	2.42	2.51	

Where, u_c is the combined uncertainty of each analytic method used by quantification of comparison item samples, and u_s is the bias contribution obtained from a combination of ICP-MS and GF-AAS values of these samples. The assigned value was obtained from this set of values, according to Levenson [11], see Table.3.

Table 3. Assigned value of the proficiency test item

Sample	x_a (mg/kg)	u_{xa} (%)
RM1	0.202	4.53
RM2	2.32	3.02

The values of both samples were calculated as indicated in e.g. (7). By both samples, the estimation of the assigned value is considered as the uncertainty contribution by each analytic method through w_i value, which is inversely proportional. It means that, the GF-AAS technique has a major contribution on x_a in the both given samples and its measurement uncertainty u_c is smaller than the ICP-MS technique.

$$x_a = \sum w_i * x_i \quad (7)$$

On the other hand, the uncertainty value of x_a by both samples was estimated as indicated in e.g. (8).

$$u_{xa} = \sqrt{u_c^2 + u_s^2} \quad (8)$$

According to table 2, the u_c values from ICP-MS and GF-AAS and u_s obtained from the results of these techniques contribute similarly to u_{xa} . Also, it is possible to observe that u_{xa} by RM1 is major of u_{xa} by RM2, given that the challenge of measuring Cd in cocoa sample with a lower concentration level.

D. Final characterization of comparison item

Fulfilled the different steps of the characterization of the comparison item, the values and its uncertainties can be observed in table 4.

The uncertainty value u_{IPT} of each material was combined, as indicated in e.g. (9). This value contains contributions of all parameters studied for these materials. A major contribution by u_{xa} in both samples respect to others uncertainty sources can be observed.

$$u_{IPT} = \sqrt{u_{hom}^2 + u_{sst}^2 + u_{slt}^2 + u_{xa}^2} \quad (9)$$

These values obtained from uncertainty by the two samples were adequate for the requirements of this material since the expected value was close to 5%.

Table 4. assigned value of the proficiency test item

Contribution	RM1	RM2	Units	Observations
u_{hom}	0.06	0.89	%	^{111}Cd vs. ^{115}In
u_{sst}	0.24	0.24	%	By $t=10$ days
u_{slt}	0.47	0.46	%	By $t=90$ days
u_{xa}	4.53	3.02	%	Contain u_s

				contribution
u_{IPT}	4.56	3.19	%	According to ISO:GUM guide [18]
U_{IPT}	0.018	0.15	mg/kg	$k=1.97$, assuming normal distribution 95% confidence interval
Assigned value	0.202	2.32	mg/kg	In dry basis

F. Proficiency testing scheme implementation

Estimation of σ_{pt} : The estimated value of σ_{pt} according to Horwitz equation and Variance Components Model are listed in table 5.

Table 5. σ_{pt} estimation

Model	RM1	RM2
Horwitz equation	0.04	0.32
Variance Components Model	0.06	0.34

From these σ_{pt} estimation, the value obtained from the Horwitz equation is slightly lower than the estimate generated by the variance component model. Thus, by this proficiency testing scheme was implement σ_{pt} obtained by Horwitz equation model.

Proficiency test results: Results of the laboratory participants, see Fig.2, show only one unsatisfactory result (by participant 110). Likewise, for RM2 all participants are obtained a satisfactory result, see Fig.3.

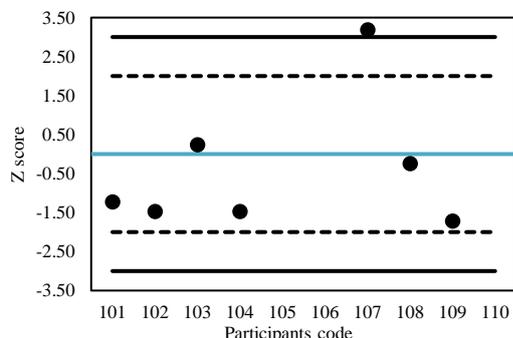


Fig. 2. Performance statistic (Z) results for RM1*

From the variance component model, the confidence intervals for μ at a 95% of confidence level correspond to 0.143 - 0.218 for RM1 and 1.96 – 2.40 for RM2. Thus, the assigned values for the two samples are contained in the

confidence intervals, meaning that the estimated values assigned from the comparison item characterization are consistent with the results reported by the participant's laboratories.

Observations found from measurement techniques by the participant's laboratories: From the results reported by the laboratories and its performance, the digestion process of the samples different among them. For seven laboratories, closed digestion was used, and three laboratories applied open digestion to the samples. Likewise, the proportion of digestion reagents were different. In general, HNO_3 and H_2O_2 were used for the laboratories as digestion reagents.

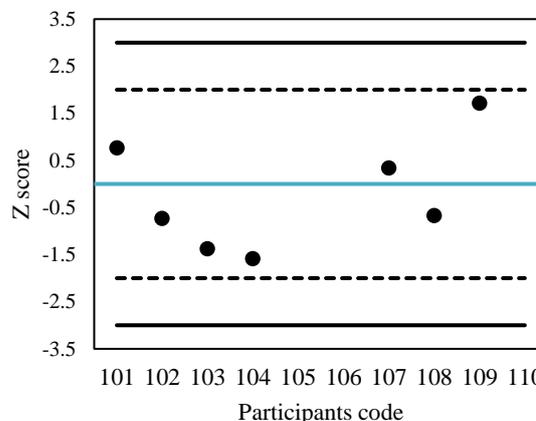


Fig. 3. Performance statistic (Z) results for RM2*. *do not show the results of laboratories 105, 106 and 110 because they did not authorize their use.

Regarding to the analytical techniques, of the total participants, three laboratories used graphite furnace, four used flame atomic absorption spectrometer, two used inductively coupled plasma with optical emission spectrometer (ICP-OES) and one laboratory used ICP-MS.

According to the results issued by participants, it was recommended to take into account the matrix effect in Cd on cocoa measurements. In the method validation for the characterization of the items, a rotational effect was observed. A rotational effect is met when the response signal from the analyte is affected by non-analyte constituents of the test material [19]. This effect causes a change in the slope of the calibration function, therefore, the Cd content in the sample may have a bias. Standard addition is a calibration method that can be applied to eliminates rotational effects.

For two samples, a negative bias was observed. This bias can be attributed to the calibration method that the laboratories used. For the sample analysis, participants used external calibration for analytical quantification.

None of the participants used standard addition, therefore, the negative bias of the results for two samples

might occur by the non-correction of the matrix effect. A last recommendation given to the laboratories was the use of certified reference materials, in matrix or calibrant, to provide traceability of the measurements and ensure comparability.

V. CONCLUSIONS AND OUTLOOK

Homogeneity evaluation and the stability study carried out for the proficiency test was adequate for this material since the values obtained by u_{hom} , u_{st} and u_{slt} were consistent with the planning activities for this reference material. Characterization of the comparison item showed that the uncertainty contribution by measurement of the mass fraction of Cd was the main uncertainty source, specifically from the difference between ICPMS and GF-AA.

It was found that σ_{pt} was adequately estimated from the Horwitz equation model and it was a suitable tool by the performance evaluation of this proficiency testing. It is important to highlight that the assigned value for both samples of the proficiency test item was adequate according to the confidence intervals estimated for μ from the variance component model.

From this scheme, some improvement tools by the participant laboratories were identified such as the use of internal and subrogate standards; quality control for measurement and also by the digestion process; use of MRC similar to cocoa for quality control such as the ERM-BD 512 dark chocolate and the measurement of blank reagents in different steps of the measurement process for evaluating the matrix effects.

VI. ACKNOWLEDGMENTS

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