XVIII IMEKO WORLD CONGRESS
Metrology for a Sustainable Development
September, 17 – 22, 2006, Rio de Janeiro, Brazil

ESTIMATE OF UNCERTAINTY OF MEASUREMENT IN THE DETERMINATION OF METHYLMERCURY IN FISH SAMPLES BY FIA-CV-AAS

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Abstract: The uncertainty for methylmercury determination in fish samples by cold vapor atomic absorption spectrometry technique was estimated based in the guide for quantifying uncertainty in analytical measurement of EURACHEM and ISO to the expression of uncertainty in measurement. The expanded uncertainty (9,5%) was obtained with 95% confidence level (k = 2).

Keywords: uncertainty, methyl-mercury, FIA-CV-AAS

1. INTRODUCTION

In the Amazon region, fish constitutes the main protein source mainly for the indigenous and riversides populations. It is well known that methylmercury (MeHg), the most toxic form of Hg, represents 73-98% of the total mercury present in fish [1][2]. For this reason, mercury speciation plays an important role for the understanding on the impact of the mercury pollution in the environmental and health of the inhabitants of the region, since the environment toxicity and biological availability of Hg depend on their different chemical forms or oxidation states as well as their quantities [3].

Nowadays is widely recognized that quality of the analytical results is an essential part of quality control program. Uncertainty is defined by VIM [4] as “parameter, associated with the result of a measurement that characterizes the dispersion of the values that could reasonably be attributed to the measurand”.

The measurement of associated uncertainty, to the analytical laboratories, is the beginning to have a higher profile and also demonstrate their competence by seeking accreditation to the appropriate standards, such as NBR ISO/IEC 17025 [5].

Several approaches for estimating uncertainty in analytical measurements are proposed [6], [7]. Both texts involve the identification of all the possible sources of uncertainty for the method, the estimation of their magnitude and the combination of these individual uncertainties to give standard and expanded estimates.

This paper describes the application of a strategy to estimate the uncertainty of the MeHg determination method in fish samples by cold vapor atomic absorption spectrometry technique (FIA-CV-AAS).

2. MATERIALS AND METHODS

2.1 Instrumentation

All the determinations were carried out using a Varian AA-220 FS atomic absorption Spectrophotometer, at 253.7 nm. This spectrophotometer was coupled to a typical flow analysis injection (FIA) manifold. Before all analyses, the instrument was checked according to the instrument manufacturer´s instructions.

2.2. Sample Preparation

Inorganic and organic Hg were extracted from 0,1 g of the certified reference sample (DORM-2 “Dogfish Muscle Certified Reference Materials for Trace Metals") by leaching with 10 mL 6 M HCl and shaken in the darkness for at least 3 hours. The acidic extract and solid residue were separated by centrifugation. The separation of inorganic and organic Hg from liquid phase was carried out by ion exchange column (Dowex 1X8 resin, Cl form, 100-200 mesh) where inorganic Hg was adsorbed as a chloro-complex ($HgCl_4^{2-}$) and methylmercury was eluted as MeHgCl. The decomposition of methylmercury in Hg^{2+}, in the column effluents collected in quartz tubes, was obtained with irradiation UV (15W UV lamp) at least 6 hours after addition of 1 ml of BrCl solution [8].

2.3. Mercury determination

The mercury determination was made by FIA-CV-AAS with a manual injection valve that injects 500 µl of sample at a flow of deionized water (10 mL min^{-1}). The Hg^{2+} of UV-irradiation of sample is reduced on line by $SnCl_2$ 25% (m/v) in HCl 25% (v/v) at flow 1 mL min^{-1}. Argon was used as carrier gas at constant flow at 200 mL min^{-1} [9].

The mercury quantification was calculated against the mercury standard curves.

2.4. Identification of sources of uncertainty

The sources of uncertainty for the method were identified by constructing a cause-and-effect diagram (Fig. 1) widely cited by some authors [10], [11].

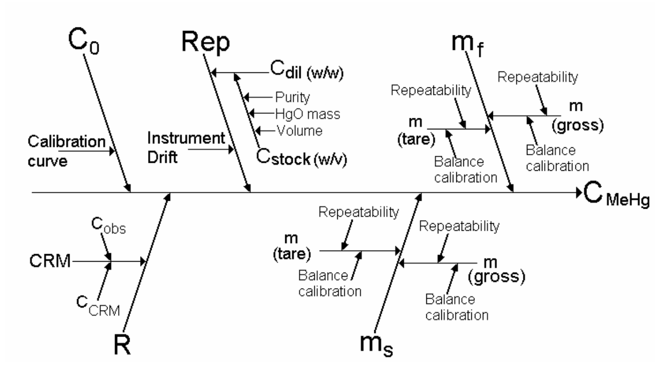

Fig. 1. Cause-and-effect diagram for the analysis of MeHg

The cause-and-effect diagram shows the main sources of uncertainty used in this work. The main cause branches represent the main parameters controlling the result. These are shown in Eq. (1).

$$C_{MeHg} = \frac{C_0 \times m_f \times 10^{-3}}{R \times m_s} \times rep \qquad (1)$$

Where: C_0 is the concentration of aliquot sample; m_f is the final mass of dilution; R is the recovery, m_s is the mass initial of the sample and rep is the repeatability.

The uncertainties associated with these parameters will contribute to the overall uncertainty in the final result.

2.4.1 *Concentration of aliquot sample, C_0*

As shown in Fig. 1 the uncertainty associated with the concentration of the MeHg in the sample aliquot is estimated from calibration curve.

The uncertainty of aliquot sample analyzed, represented by $u(c_0)$, is given by [7]

$$u(c_0) = \frac{S}{B_1}\sqrt{\frac{1}{p} + \frac{1}{n} + \frac{\left(c_o - \bar{c}\right)^2}{S_{xx}}} \qquad (2)$$

Where:
S = residual standard deviation,

$$S = \sqrt{\frac{\sum_{j=1}^{n}[A_j - (B_0 + B_1 \times c_j)]^2}{n-2}}$$

B_1 = slope of the calibration curve,
p = number of measurements to determine C_o,
n = number of measurements for the calibration,
$\bar{c}$ = mean value of the different calibration standards,
$S_{xx} = \sum_{j=1}^{n}\left(c_j - \bar{c}\right)^2$ Where j = index for the number of measurements to obtain the calibration curve.

2.4.2 *The final dilution mass, m_f*

In this experiment, the initial sample mass and final dilution mass were weighted on the same balance within a short period of time. Thus, the standards uncertainty calculated in the final dilution mass is equal the standard uncertainty in the initial mass (show in 3.2).

2.4.3 *Recovery, R*

The recovery is calculated as percentage recovery from comparable reference material. The recovery, R, has an uncertainty associated with the certified reference material value used and with the variability of the particular measurement of the solution analyzed [10].

2.4.4 *The sample initial mass, m_s*

In the AAS method employed, all samples are prepared and diluted on w/w basis. The contributions to the uncertainty associated with these mass, are the uncertainty about of standard deviation of balance control chart, uncertainty of calibration certificate and repeatability of the balance. All the mass were obtained by difference between the tare and gross weights taken on the same balance, and then balance "zero bias" is canceled.

2.4.5 *Repeatability, rep*

Fig.1 shows two major contributions to the uncertainty associated with the repeatability, instrument drift and precision associated with the dilution of calibration solutions

Instrument drift

The drift is monitored by periodically analyzing one of the calibration standards during a batch of analyses.

Precision associated with the dilution of calibration solutions

The calibration of instrument is realized by calibration solutions. These solutions are prepared from mercury solution (100 µg L^{-1}), obtained after the two dilution of 1/ 100 on w/w basis from mercury stock solution (1000.14 µg mL^{-1}).

3. RESULTS AND DISCUSSION

The sources of uncertainty identified in the cause-and-effect diagram were calculated and the values of the magnitude of uncertainty components were obtained. The Table 1 shows the uncertainty values calculated for each source.

3.1 Concentration of aliquot sample, C_0

The MeHg content of the aliquot sample was calculated against the mercury standard curves. For this purpose were prepared six mercury standards solutions with concentration of 2 µg kg^{-1}, 5 µg kg^{-1}, 10 µg kg^{-1}, 15 µg kg^{-1}, 20 µg kg^{-1} e 25 µg kg^{-1}. Those solutions were obtained of the dilution of a standard solution of 1000.14 µg mL^{-1} prepared from the mercury oxide.

The six calibration standards were measured three times each. The sample solution was measured and leading to a concentration C_0 of 13.412 μg kg^{-1}.

The uncertainty of aliquot sample analyzed, is

$$u(C_o) = \frac{0.00472}{0.0098}\sqrt{\frac{1}{2}+\frac{1}{18}+\frac{(13.412-12.8)^2}{1174.56}}$$

$$u(C_o) = 0.359$$

3.2 *The final dilution mass, m_f*

As mentioned above, the sample is weighted in the balance, and the mass used in this experiment is 0.100 g. The uncertainty associated with standard deviation of control chart is 0.0006 g, the associated with calibration certificate is 0.0005 g and uncertainty associated with repeatability of the balance is 0.0003 g. Combining these values, the uncertainty of mass final of the sample, $u(m_f)$, is:

$$u(m_f) = \sqrt{(0.0006)^2 + (0.0005)^2 + (0.0003)^2} = 0.0008366$$

3.3 *Recovery, R*

The estimate of R and u(R), method recovery and method recovery uncertainty, was obtained from standards prepared of a certified reference material DORM-2 dogfish muscle. The concentration of MeHg in this material is certified as 4.47 ± 0.32. The uncertainty is quoted at the 95% confidence level. The concentration of MeHg, in the aliquot sample, obtained by analysis was 13.412. The uncertainty, $u(C_{CRM})$, is:

$$u_{C_{CRM}} = 13.412\times\sqrt{\left(\frac{0.16}{4.47}\right)^2} = 0.480$$

R is calculated using Eq. (3) [10]:

$$R = \frac{C_{obs}}{C_{CRM}} \tag{3}$$

Where C_{obs} is the mean of the results obtained from the replicate analysis of the solution and C_{CRM} is the concentration of the solution in the certified reference material. For this analysis, R = 0.949.

The uncertainty associated with R, u(R), is obtained by combining the uncertainty in the reference value, $u(C_{CRM})$, obtained from solution of the certified reference material with the uncertainty in the mean of the observations [10]:

$$u(R) = 0.949\times\sqrt{\left(\frac{u(C_{CRM})}{C_{CRM}}\right)^2 + \frac{S_{obs}^2}{n\times C_{obs}^2}} \tag{4}$$

Where C_{CRM} is the concentration of aliquot sample measured, S_{obs} is the standard deviation obtained from the replicate analyses of the solution and n the number of replicates.

Then, the uncertainty u(R) is:

$$u(R\ ) = 0.949\times\sqrt{\left(\frac{0.480}{13.412}\right)^2 + \frac{(0.115)^2}{8\times(13.412)^2}}$$

$$u(R\ ) = 0.034$$

3.4 *The initial sample mass, m_s*

The calculation of the uncertainty associated of the initial sample mass of, $u(m_s)$, is equal the uncertainty associated of final dilution mass, as explicated in 2.4.2. Thus, the value of uncertainty associated of initial sample mass, $u(m_s)$, is 0.0008366.

3.5 *Repeatability, rep*

Uncertainty from instrument drift

To calculate the uncertainty in the instrument drift a calibration standard solution with concentration of 10μg kg^{-1} was analyzed. The instrument showed a repeatability of 1.5%. This value can be directly used for the calculation of the combined standard uncertainty [7].

Uncertainty from concentration of the stock solution

This uncertainty depends upon the purity of the mercury oxide, its mass and the volume of the liquid in which it is dissolved.

Purity

The purity of the mercury oxide is quoted in the supplier's certificate as 99.99 ± 0.01 %. P is therefore 0.9999 ± 0.0001. The standard uncertainty u(P) of the mercury oxide, using the assumption of a rectangular distribution, is obtained dividing 0.0001 by $\sqrt{3}$

$$u(P) = \frac{0.0001}{\sqrt{3}} = 0.000058$$

Mass

The uncertainty associated with the mass of the mercury oxide for this preparation is $u(m_s)$ = 0.0008366, using combination of the following components: standard deviation of control chart, uncertainty of calibration certificate and repeatability of the balance.

Volume

The uncertainty associated with the volume has three major influences: calibration, repeatability and temperature.

Calibration

The manufacturer quotes a volume for the flask of 250 mL ± 0.2 mL measured at a temperature of 20 °C. The standards uncertainty is calculated assuming a triangular distribution.

$$u(V_C) = \frac{0.2}{\sqrt{6}} = 0.082$$

Repeatability

This uncertainty was estimated by a series of ten fill and weigh experiments on a 250 mL volumetric flask. The standards deviation obtained in this experiment was 0.017 mL, this can be used directly as a standard uncertainty.

Temperature

The uncertainty from this effect can be calculated from the estimated of the temperature range and coefficient of the volume expansion. The laboratory temperature varies between the limits of ±4 °C and of volume expansion for water is $2.1\text{x}10^{-4}$, which leads to a volume variation of

$$\pm (250\text{x}4\text{x}2.1\text{x}10^{-4}) = \pm 0.21 \text{ mL}$$

The standards uncertainty for the temperature variation is calculated assuming a rectangular distribution.

$$u(V_T) = \frac{0.21mL}{\sqrt{3}} = 0.12mL$$

The three contributions are combined to give the standard uncertainty of the volume (u(V)).

$$u(V) = \sqrt{0.082^2 + 0.017^2 + 0.12^2} = 0.146 \ mL$$

Combining the intermediate standard uncertainties, above presented, the standard uncertainty of the mercury stock solution concentration can be calculated by:

$$u_{Cstock} = C_{stock} \times \sqrt{\left(\frac{\mu(P)}{P}\right)^2 + \left(\frac{\mu(m)}{m}\right)^2 + \left(\frac{\mu(V)}{V}\right)^2}$$

$$u_{Cstock} = 1000.14 \times \sqrt{\left(\frac{0.000058}{0.9999}\right)^2 + \left(\frac{0.0008366}{0.270}\right)^2 + \left(\frac{0.146}{250}\right)^2}$$

$$u_{Cstock} = 3.154$$

Then, the stock solution used in these experiments was quoted as having a concentration and uncertainty of 1000.14 ± 3.154.

As were prepared two solutions on a weight-by-weight basis, replicate weightings of 1 g and 100 g calibrated weights were measured and obtained standard deviations of 0.0050 and 0.0055 g, respectively. Combining these values with the uncertainty calculated for the concentration of the stock solution gives an uncertainty in the concentration of the dilute working standards of:

$$u_{C\ 10\mu g/g} = 10 \times \sqrt{\left(\frac{3.154}{1000.14}\right)^2 + \left(\frac{\frac{0.0050}{\sqrt{10}}}{1}\right)^2 + \left(\frac{\frac{0.0055}{\sqrt{10}}}{100}\right)^2}$$

$$u_{C10ug/g} = 0.0353 \ \mu\text{g g}^{-1}$$

$$u_{C\ 100\mu g/kg} = 100 \times \sqrt{\left(\frac{0.0353}{10}\right)^2 + \left(\frac{\frac{0.0050}{\sqrt{10}}}{1}\right)^2 + \left(\frac{\frac{0.0055}{\sqrt{10}}}{100}\right)^2}$$

$$u_{C100ug/kg} = 0.387 \ \mu\text{g kg}^{-1}$$

Combining the intermediate standard uncertainties, above presented, the standard uncertainty of the repeatability can be calculated by:

$$u_{rep} = rep \times \sqrt{\left(\frac{\mu(drift)}{drift}\right)^2 + \left(\frac{\mu(c_{100})}{c_{100}}\right)^2}$$

$$u_{rep} = 1 \times \sqrt{\left(\frac{0.015}{1}\right)^2 + \left(\frac{0.367}{100}\right)^2}$$

$$u_{rep} = 0.015$$

Table 1. Summary of contributions to the measurement uncertainty for the determination of MeHg by FIA-CV-AAS

Parameter	Value x	Standard uncertainty u(x)	Relative standard uncertainty u(x)/x
Concentration of aliquot sample (C_0) (µg kg^{-1})	13.412	0.359	0.0268
Recovery (R)	0.949	0.034	0.03583
Final mass (m_f) (g)	30.000	0.0008366	0.0000278
Sample mass (m_a) (g)	0.100	0.0008366	0.008366
Repeatability	1	0.015	0.015

3.6 Calculation of standard and expanded uncertainty

The measurement uncertainty was calculated from the data given in Table 1. The combined standard uncertainty is calculated from the root sum of squares of the individual components, according to the rules set out in the Eurachem Guide [7].

$$\frac{u(C_{MeHg})}{4.24} = \sqrt{(0.0268)^2 + (0.03583)^2 + (0.0000278)^2 + (0.008366)^2 + (0.015)^2}$$

$$u(C_{MeHg}) = 0.20 \mu g \ g^{-1}$$

For this experiment, the relative standard uncertainty was calculated as 0.20 µg g^{-1}. Expanded uncertainty was calculated using a coverage factor of two, which gives a level of confidence of approximate 95%, thus the expanded uncertainty for this experiment was 0.40 µg g^{-1}.The contributions of the different parameters and influence quantities to the measurements uncertainty are illustrated in Fig. 2.

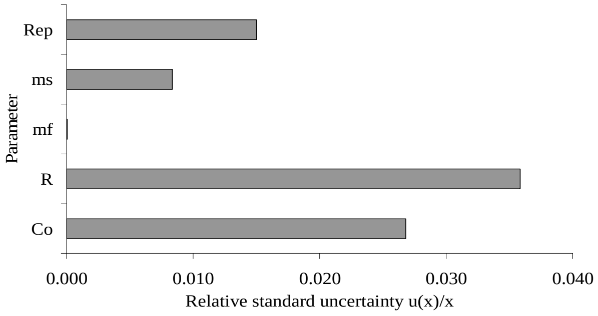

Fig. 2.-. Illustration of contributions to the uncertainty budget.

4. CONCLUSION

This study illustrates the application of cause-and-effect analysis to uncertainty estimation in atomic absorption technique.

When compared our result (4.24 ± 0.40 µg g^{-1}), obtained by application of equation 1, with the certified of reference material that presents a MeHg concentration as 4.47 ± 0.32 µg g^{-1}, we concluded that analytical method is appropriate, sensitive and provides a global estimation of the uncertainty.

The estimation of the uncertainty components showed to be a suitable tool for the experimental design in order to obtain a small uncertainty in the analytical result.

In this experiment, the major contributions to the uncertainty budget were found to be the recovery and concentration of aliquot sample (C_o) (see Fig. 2). While the contribution from the uncertainty associated with final mass was insignificant.

REFERENCES

[1] L. Maurice-Bourgoin, I. Quiroga, J. Chincheros and P. Courau, "Mercury distribution in waters and fishes of the upper Madeira rivers and mercury exposure in riparian Amazonian populations", The Science of the Total Environmental, 260, pp. 73-86, 2000.

[2] A.P.S. Lima, J.E.S. Sarkis, H.M. Shihomatsu and R.C.S. Muller, "Mercury and selenium concentrations in fish samples from Cachoeira do Piriá Municipality, Pará State, Brazil", Environmental Research, 97, pp. 236-244, 2005.

[3] J.R. Ikinguru and H. Akagi, "Methylmercury production and distribution in aquatic systems", The Science of the Total Environmental, 234, pp. 109-118, 1999.

[4] INMETRO. Vocabulário internacional de termos fundamentais e gerais de metrologia. 2. ed. 75p, Convênio SENAI/DN/INMETRO, Brasília, 2000.

[5] NBR ISO/IEC 17025 Requisitos gerais para competência de laboratórios de ensaio e calibração. ABNT, Rio de Janeiro, 2005.

[6] ISO Guide to the Expression of Uncertainty in Measurement. 1. Edition, Switzerland, 1995.

[7] EURACHEM/CITA Guide CG4. Quantifying Uncertainty in Analytical Measurement. 2. Edition, QUAM: 2000.1.

[8] K. May, M. Stoeppler and K. Reisinger, " Studies in the ratio total mercury/methylmercury in the aquatic food chain", Toxicological and Environmental Chemistry, 13, pp. 153-159, 1987.

[9] M.A. Hortellani, J. E. S. Sarkis; J. Bonetti; C. Bonetti; Evaluation of mercury contamination in sediments from Santos - São Vicente Estuarine System, São Paulo State, Brazil, *J.Braz. Chem. Soc.*, 16(6A), pp. 1140-1149, 2005.

[10] V. J. Barwick, S. L. R. Ellison, B. Fairman, "Estimation of uncertainties in ICP-MS analysis: a pratical methodology", Analytica Chimica Acta 394, pp. 281-291, 1999.

[11] J. H. Buchmann, J. E. S. Sarkis, "O conceito de incerteza aplicado aos processos de medição associados à preparação de uma solução de referência para calibração", Química Nova, vol. 25, No. 1, pp. 111-116, 2002