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Original Research

Determination of Total Mercury in Fish and Cormorant Using Cold Vapour Atomic Absorption Spectrometry

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Abstract

One of the most frequently used methods for determining total mercury content in biological samples is cold vapour atomic absorption spectrometry (CV-AAS), which is extensively used in the biomonitoring of environmental pollution. Seabirds are often used as bioindicators of metal contamination because of their specific feeding habits, wide geographical ranges and long lifespan.

This paper describes the validation of CV-AAS for determining the total mercury content in biological samples (whole fish, cormorant tissues). The development and optimization of the procedure is outlined, and the main objective of this study was to calculate its validation parameters. The selectivity of the method was documented; linearity ($r>0.993$) ranged from 0.29 to 100 ng of total mercury per sample mass. For a total Hg content of 80-1,000 ng, a polynomial calibration curve derived directly the Lambert-Beer law was used. The method showed good recoveries (average 98.0%) and a relative standard deviation for repeatability of < 10%. The limit of detection was calculated at 0.096 ng of total Hg per sample mass. The uncertainty budget was evaluated according to the 'Guide to the Expression of Uncertainty in Measurement' (GUM) [1]; the relative expanded uncertainty was estimated at < 13%.

Keywords: mercury, cormorant, fish, cold vapour atomic absorption spectrometry, validation parameters

Introduction

Mercury is a dangerous xenobiotic, particularly its vapours and some of its water-soluble salts; one of its properties is the ability to accumulate in the internal organs of living organisms [2-4]. Anthropogenic inputs of mercury into the environment have significantly increased in the past century.

Seabirds are effective bioindicators of coastal and open-sea pollution [5-7]. Spending a significant proportion of their lives in a marine environment, these birds are exposed to a wide range of chemicals. Because most of them occupy higher trophic levels, they are susceptible to the bioaccumulation of certain pollutants [8, 9].

Monitoring and analytics are powerful tools providing the necessary data for reliably evaluating the state of the environment and the changes taking place within it, as well as for informing decisions regarding the implementation of conservation measures [10-15].

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One of the most frequently used methods for determining Hg concentrations in biological samples is cold vapour atomic absorption spectrometry (CV-AAS). Widely used in the biomonitoring of environmental pollution, this method gives the total amount of mercury in a sample.

A basic feature of any measurement is its reliability: the numerical value representing a given physicochemical property can only be called a measurement when it is reliable. Measurements are obtained in the course of procedures during which appropriate 'analytical tools' (i.e. analytical procedures and measuring equipment) are used [16]. These tools must be relevant if results are to be reliable. Assurance of the appropriate quality of analytical measurements involves checking the reliability of measuring equipment and establishing the range of applicability of analytical procedures. Every such procedure should be characterized as precisely as possible (i.e. validated) in order that highly reliable measurements can be obtained [17, 18]. Validation of any method is important to ensure the accuracy of results and the utility of the method [19-23].

The literature gives little information on the validation of procedures for determining total mercury levels in samples with a complex matrix composition.

The whole validation process leading to the determination of all metrological parameters characterizing procedures using CV-AAS, has been described in this paper.

Experimental

Materials and Methods

The cormorants were shot on the Vistula Lagoon, after which the birds were dissected. The fish were caught in the same area. The carefully separated tissues of the birds and the whole fish specimens were immediately deep-frozen, freeze-dried (lyophilised) and homogenized.

Reagents

- Mercury standard – MSHG – 100 μg g^{-1}, concentration 100.48±0.22 μg g^{-1} in 3.3% HCl, Inorganic Ventures, Inc., USA
- L-Cysteine, 98%, Nacalai Tesque, Inc., Kyoto, Japan
- Additive B, Wako Pure Chemical Industries, Ltd., Japan
- Additive M, POCh, Poland
- Buffer solution pH 7.00±0.05, POCh, Poland
- CRM: BCR-463 tuna fish (total and methyl mercury): 2.85±0.16 μg g^{-1}, IRMM, Geel, Belgium, ERM-CE278 mussel tissue (total and methyl mercury): 0.196±0.009 μg g^{-1}, IRMM, Geel, Belgium, DORM-2 dogfish muscle (total mercury): 4.64±0.26 μg g^{-1}, National Research Council, Canada
- Deionized water

Preparation of Standard Solutions

Various methods are available for preparing standard solutions. Nippon Instrument Corporation obtained good results using L-cysteine [24]. However, as the stability of such standard solutions deteriorates with age or as a result of prolonged storage in a warm place, they should be kept in a cool and dark place.

It is important that any mercury contained in standard solutions is in the form of $HgCl_2$. Some products contain $Hg(NO_3)_2$ as a mercury component. Since $Hg(NO_3)_2$ can react with L-cysteine, thereby losing its function as a fixing agent, such an undiluted $Hg(NO_3)_2$ standard solution should not be used.

The appropriate standard solutions (0.01, 0.1, 1.0 and 10 μg g^{-1}) were prepared by diluting a 100 μg g^{-1} standard Hg solution with an aqueous solution of 0.001% L-cysteine.

Apparatus

An automatic mercury analyzer (MA-2000; NIC, Japan) was used to determine total mercury content in the samples. It is a mercury analysis system that can measure mercury in liquid, solid, and gas (optional parts required) samples.

Analytical Procedure

The homogenized samples were directly weighed (10-50±0.1 mg) into pre-cleaned combustion boats and automatically inserted in the Mercury/MA-2000 system (NIC – Japan). They were then thermally decomposed at 800ºC under a clean airflow. To remove any interfering substances (generated during the thermal decomposition of a sample), gas washing was performed The total mercury content was determined by CV-AAS at 253.7 nm, three independent determinations being done for each sample.

The validation parameters of the method (linearity, repeatability, LOD, LOQ, range, trueness, accuracy and uncertainty) were determined prior to analysis.

Fig. 1 shows a schematic representation of the steps of the analytical procedure.

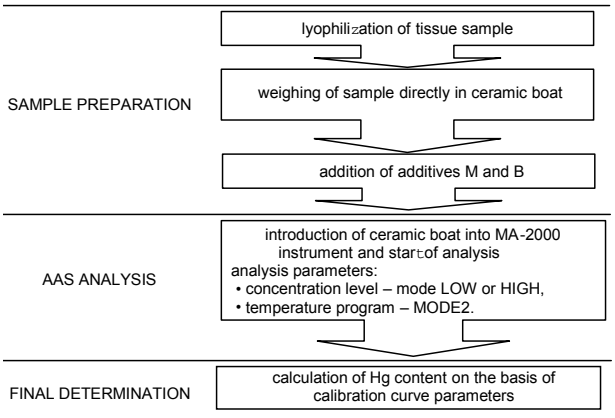

Fig. 1. Schematic presentation of the analytical procedure for determination of total mercury content in tissue samples.
additive B – activated alumina,
additive M – sodium carbonate + calcium hydroxide

Table 1. Validation parameters for developed method.

CALIBRATION				
instrument signal	peak height		peak area	
range of Hg content in standard solutions, ng	0.2÷2.0	2.0÷20	10÷100	80÷1000
regression coefficients	y=b*x*+a b=0.203 a=0.0373	y=b*x*+a b=0.0919 a=0.0933	y=b*x*+a b=2.17 a=6.11	$y=b_1x^3+b_2x^2+b_3x+a$ $b_1=1.10\cdot10^{-6}$ $b_2=0.00281$ $b_3=2.82$ a=24.0
LINEARITY				
coefficient of regression, *r*	0.993	0.998	0.999	1.000
LIMIT OF DETECTION				
LOD, ng	0.096			
LIMIT OF QUANTIFICATION				
LOQ, ng LOQ=3·LOD	0.29			
REPEATABILITY				
CV for standard solutions, %	5.4	4.0	4.3	1.4
CV for real samples, %	9.4	9.9	7.6	5.9
TRUENESS				
reference material		ERM-CE278	BCR-463	DORM-2
certified value, μg g^{-1}		0.196±0.009	2.85±0.16	4.64±0.26
mass of CRM sample used, mg		~ 30	~ 30	~ 50
R±U, %		98.9±5.2	97.1±8.2	101.8±5.8
UNCERTAINTY				
calibration, %	3.1	2.3	2.1	2.1
repeatability, %	5.4	5.7	4.4	3.5
trueness, %	2.6	2.6	4.2	2.8
expanded (k=2), %	13	13	13	10

R – recovery calculated as a ratio of determined value to certified one in %,
U – expanded uncertainty of R value for coverage factor k =2

Results and Discussion

The values of the following parameters were determined during the analytical validation procedure: selectivity, linearity, *LOD*, *LOQ*, range, repeatability, trueness, and uncertainty.

Selectivity

In the cold vapour technique, mercury is released from the sample, and then, after reduction to atomic mercury, trapped on the gold bed as an amalgam. The amalgam is subsequently heated to 600ºC and the atomic mercury released is carried in a stream of air to the absorption cell, where the absorption of the radiation (λ=253.7 nm) emitted by a hollow mercury cathode lamp, is measured [24]. This measurement method guarantees high selectivity of mercury determination for two reasons:

1. amalgamation is a selective reaction for mercury;
2. absorption is measured using a wavelength characteristic of mercury.

Linearity

The calibration was carried out as a function of instrument signal and mercury content. For total Hg content of <20 ng the peak height was used as the instrument signal; for total Hg content in the range 20-1,000 ng, the peak sur-

face area was used. Three independent measurements were made for each mercury mass. The linear function was used for calibration in the range 0.3-100 ng of total Hg per sample mass. For a range of 80-1,000 ng Hg, a polynomial calibration curve derived from the Lambert-Beer law was used.

The minimum mass of lyophilized tissue samples undergoing analysis is limited by the accuracy of the weight measurement as well as by its homogeneity. Taking this into account, this mass should not be less than 20 mg. However, the maximum sample mass is restricted by the maximum mass of substance that can be placed in the ceramic boat and then inserted into the furnace. This value should not exceed 200 mg.

A series of standard solutions was prepared with a mercury content of 0.2 to 1,000 ng. Three independent measurements were conducted for each of the solutions and, on the basis of the results, regression parameters were found and the calibration curve determined. Table 1 sets out the values obtained.

A high regression coefficient *r*, after fulfilling conditions for a 'uniform' concentration distribution in terms of the calibration curve, demonstrates a high linear procedure.

Limit of Detection (*LOD*) and Quantification (*LOQ*)

LOD is determined from a series of measurements of standard solution samples with the three lowest mercury levels (0.2, 0.4 and 0.6 ng). A calibration curve was plotted from the measurements, the parameters determining *LOD*, and the relationship [25-27]:

$$LOD = \frac{3.3 \cdot SD}{b} \quad (1)$$

LOD was taken to be 0.096 ng. If we assume that the mass of a sample for determination was 20 mg, this corresponds to an Hg concentration in tissue samples of 4.8 ng g^{-1}. However, LOQ was determined to be *LOQ*=3·*LOD*, i.e. 0.29 ng (14 ng g^{-1}).

Table 1 lists the relevant values.

Range

The measurement range is a concentration range from the *LOQ* section to the maximum standard solution concentration used for calibration; it is therefore equal to 0.29-1,000 ng. Assuming the mass of the sample determined to be 20 mg, this corresponds to a mercury concentration range of 14 ng g^{-1}-50 µg g^{-1}.

Repeatability

Repeatability was determined from a series of three independent measurements of standard solutions used for calibration and selected real samples with different mercury levels. It was determined as the *CV* value for the series. Table 2 lists the measurements for individual samples; Table 1 sets out the values obtained – the maximum values for each mercury content range have been listed.

Table 2. Measurement results for standard solutions and real samples, used for repeatability determination.

Sample	Hg content, ng	CV, %
Standard solution	0.2	5.45
Standard solution	0.6	4.15
Standard solution	2.0	4.03
Standard solution	10	3.55
Standard solution	20	1.28
Standard solution	30	4.31
Standard solution	50	2.35
Standard solution	100	1.43
Standard solution	500	0.53
Standard solution	1000	0.28
Tench	0.82	9.24
Prussian carp	5.02	8.92
Rufie	5.11	9.89
European smelt	5.21	6.33
Prussian carp	8.22	6.43
Roach	20.1	4.63
Herring	30.4	4.94
Roach	70.6	4.32
Great Cormorant – trachea	10.2	5.81
Great Cormorant – trachea	30.6	5.98
Great Cormorant – muscle	40.3	7.62
Great Cormorant – cardiac muscle	40.7	4.03
Great Cormorant – kidney	151	2.81
Great Cormorant – liver	204	2.49
Great Cormorant – liver	503	2.06
Great Cormorant – kidney	613	2.74
Great Cormorant – liver	809	5.86

Trueness

Trueness is based on the results for three samples of certified reference materials (BCR-463, ERM-CE278 and DORM-2) and is presented in the form of recovery values. A series of 5 independent determinations was conducted. The determined trueness values are listed in Table 1.

Uncertainty

The main components of the uncertainty budget were the uncertainty resulting from the determination of the calibration curve, the uncertainty related to the unrepeatability of measurements, as well as the uncertainty in the determination of trueness. The combined uncertainty value is estimated using the equation:

$$u_{smpl} = \sqrt{u_{cal}^2 + u_{rep}^2 + u_{true}^2} \quad (2)$$

...where:

u_{smpl} – represents the combined standard uncertainty in the determinations of real samples,

u_{cal} – represents the standard uncertainty in calibration-related determinations of real samples,

u_{rep} – represents the standard uncertainty in determinations of real samples in relation to measurement repeatability,

u_{true} – represents the standard uncertainty of results related to the determination of trueness.

The standard uncertainty related to the calibration step (the preparation of a series of standard solutions, measurements performed for a series of standard solutions, the approximation of measurement points on the calibration line using linear regression) is conducted on the basis of calibration parameters. Calculations are conducted for minimal masses for each of the real samples analyzed. Table 1 presents the results for all samples.

Conclusions

1. This analytical procedure fulfils the requirements for determining the total mercury content in lyophilized tissue samples from cormorants and fish.
2. The procedure is characterized by high selectivity, precision and repeatability (CV < 10%), trueness (recovery in the range 97.1%±8.2%-101.8%±5.8%) and, therefore, high accuracy [28].
3. The results obtained using this method have a low expanded uncertainty (< 15%), which is fully compliant with the requirements for this type of measurement [28].
4. The estimated limit of detection LOD=0.096 ng of total mercury per sample mass, assuming a minimal mass of 20 mg, corresponds to a concentration of 4.8 ng g^{-1} and allows for the detection of trace amounts of mercury in the analyzed samples [28].

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