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Production of certified reference materials - homogeneity and stability study based on the determination of total mercury and methylmercury

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ABSTRACT

Reference materials (RMs) play an important role in all elements of the quality assurance system of measurements. In this work, ``package" 4 new CRMs (bottom sediment, herring tissue, cod tissue, cormorant tissue) were prepared and characterised to carry out the quality control in monitoring analysis of mercury and methylmercury in environmental samples. Materials - candidates were collected in Poland and south part of the Czech Republic. All materials were freeze-dried, milled, sieved, homogenised, sterilized and distributed in amber bottles. For the homogeneity and stability study of the samples of materials several statistical tests were applied. Materials - candidates for CRMs with certified mercury and methylmercury content meet the heterogeneity requirement and can be considered homogenous both between bottle and within bottle. Each material meets also the requirements for the stability condition of reference material with certified mercury content.

1. Introduction

Analytical laboratories are dealing with the need to provide objective proof of their technical competence and reliability of results. This need stems from the fact that many technical, economical and political decision based on results of analytical measurements [1]. It often happens that the measurements are directed to the detection, identification and quantitative determination of environmental poisons and other xenobiotics in the samples, which are typically characterized by a complex matrix composition [2]. If these results are the basis for drawing conclusions that might lead to a lot of negative consequences. For this reason, it is necessary to ensure an adequate systems of quality assurance and control (QA/QC) of the results of analytical measurement [3]. In order to obtain reliable data, it is necessary to:

- use appropriate certified analytical methods (method validation is needed),
- conduct analyzes in a professional manner, i.a. by accredited laboratories,
- use of systematic quality control of analytical work [4].

A key role in all elements of quality assurance and quality control system play reference materials (RM). RM certification, on the other hand, is an integrated process involving homogeneity and stability testing and characterisation. The relevant ISO guidelines [5-7] provide the best explanation of the requirements for RM's general quality.

Mercury pollution is considered as a global environmental issue. Mercury can be bioaccumulated at all levels of trophic chains [8]. Due to the specific properties of mercury (bioconcentration and biomagnification) [9], it became necessary to develop new types of reference materials, which will be a tool for obtaining reliable analytical information.

Commercially available reference materials (sediments, animal tissue) do not fully meet the expectations of analytical laboratories, due to other origins, different geochemical characteristics, other anthropogenic contaminants etc., compared to real samples. RMs should be similar to real samples, in terms of composition of the matrix, levels of analytes, potential interferences and physical state of the material [1]. Therefore, it is extremely important to continuously enrich the variety of available certified reference materials, so that it can ``mapping" the exact composition of the matrix of analyzed samples.

Considering the mercury cycle in nature and the transformation of this element in the environment, the aquatic ecosystems (oceans, seas, lakes, rivers and sediments) are most susceptible to contamination by mercury [10]. Herring, cod fish and cormorant tissues samples can be considered as representatives of particular groups of organisms from different levels of the aquatic food web, which could be widely use in biomonitoring of aquatic environments and e.g. quality control of food products.

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The hazards arising out of the presence of mercury in the environment, make it necessary to understand the transformation pathways of these metal and to monitor the content of mercury in the tissues of organisms at all levels of the trophic pyramid. The possibility that organic forms of mercury can get into the human body with food is a serious threat because these compounds are highly toxic [11]. That is why it is so crucial to monitor levels of organomercury compounds in samples of water, sediments and tissues from all levels of the trophic pyramid using the appropriate analytical methods and reference materials, which will be a tool for obtaining reliable analytical information.

The aim of the work was to produce a ``package" of 4 new type certified reference materials that could be considered as a response to the needs of analytical laboratories in the field of generally understood environmental analytics. In this study homogeneity and stability study of the candidates on reference materials (bottom sediment, herring tissue, cod tissue, cormorant tissue) based on the content of mercury and methylmercury was carried out. The advantage and innovation criterion for sampling candidates on CRMs is the fact that the whole package of the analyzed material is a kind of model of the food chain, consequence of which is the formation of new solutions, ideas and concepts relating to environmental research.

2. Materials and methods

2.1. Reagents, standards, and certified reference materials

Mercury standard-MSHG at a concentration 100.48 ± 0.22 μg mL-1 in 3.3% HCl was purchased from Inorganic Ventures, INC (USA). L-Cysteine (98%) and additive B (activated alumina) were obtained from Nacalai Tesque, Inc, Kyoto and Wako pure Chemical Industries, Ltd (Japan). Additive M (sodium carbonate + calcium hydroxide), sodium sulfate (99% purity) and buffer solution of pH 7.00 ± 0.05 were purchased from POCh (Poland). Nitric acid – Suprapur (65% purity), hydrobromic acid (45% purity) and toluene were obtained from Merck (Germany). Sodium acetate was purchased from Stanlab (Poland). Cerified reference materials BCR-463 and DOLT - 4 were supplied by IRMM (Belgium) and NRC (Canada) respectively.

2.2. Instrumentation

``Mercury/MA-2000″ supplied by Nippon Instruments Corporation (*NIC*, Japan) was used to analyse mercury by cold vapour technique and purified dry air was used as the carrier gas. Millipore – Milli-Q Water Purification System (USA), laboratory shaker Promax 2020 (*Heidolph*, Germany), centrifuge 5702 (*Eppendorf*, Germany) were used to methylmercury extraction.

2.3. Preparation and characteristic of the candidates on certified reference materials

2.3.1. Sampling location

In the first half of 2013 aprox.150 kg for each candidate on certified reference materials has been collected. Bottom sediment has been collected in three rounds from the Włocławek Reservoir. Then the sediment was sieved to obtain a suitable size fraction. Herring tissue used for the preparation of the CRM were imported from coastal waters Scotland and was supplied in the form of freeze-dried powder. Cod tissues used for the preparation of the CRM were collected in the southern part of the Baltic Sea near Władysławowo. They were transported to the laboratory in the form of frozen blocks of a weight of about 7.25 kg. Cormorant pectoral muscle was obtained from 663 individuals. The cormorants tissues used for the preparation of the CRM were collected in Poland and south part of the Czech Republic. All sampling points are shown in Fig. 1.

2.3.2. Sample handling and bottling

All materials were freeze-dried at low temperature (between −46 and −52 °C) and at low pressure (between ~0.17 and 0.22 mbar). The material was then homogenised before bottling to ensure constant concentrations throughout bottles [1]. Animal tissue materials were milled in mills that did not have metal parts and all materials were then sieved with a 100 μm mesh resulting in a fine powder. Then, the material was homogenized, mixed sterilized and distributed in amber bottles (50 g in each bottle).

2.4. Sample preparation for MeHg determination

Methylmercury in tissues was extracted following the method described by Maggi et al. [12] with few modifications. This method consists in a first step of isolating it from the sample matrix by hydrolysis with hydrobromic acid and sequentially toluene and L-cysteine extraction was carried out. A scheme of an analytical procedure for the extraction of methylmercury from environmental samples is shown in -Fig. 2.

2.5. Determination procedure

The total mercury and methylmercury content (pre-extracted analysis) in candidate for certified reference materials samples was determined using the MA-2000 Mercury Analyzer. The extracts and freeze-dried samples are thermally decomposed by controlled heating. Mercury is further atomized and free mercury vapour is collected by a mercury collection agent in the form of gold amalgam. The amalgam is heated to 600 °C and released mercury is detected using cold atomic absorption method at a wavelength of 253.7 nm in the detector's absorption cell. As a method of removing any substances that could interfere with measurement, Nippon Instruments Corporation uses two kinds of additives: additive B (activated alumina) and additive M (sodium carbonate + calcium hydroxide).

2.6. Method validation

The numerical values of the calibration curves regression parameters were the basis for estimating the value of the limit of detection and quantification of the analytical method. The limit of detection (LOD) was calculated using the equation [13]:

$$\mathrm{LOD} = \frac{3.3\, s_a}{b}$$

s_a – the standard deviation of the intercept of calibration curve$b$ – the slope of the calibration line

When calculating the numerical value of the limit of quantification (LOQ) were assumed dependence, described by the equation [13]:

$$\mathrm{LOQ} = 3 \cdot \mathrm{LOD}$$

Obtained numerical values LOD and LOQ were converted to the corresponding value of MDL and MQL - the limits of detection and quantification of the analytical method, assuming that the mass of the sample is 100 mg.

Repeatability was expressed as a coefficient of variation (CV) test samples results in a single analytical cycle. The intermediate precision was calculated as the coefficient of variation for all the results obtained in all the analyzed samples [10]. The trueness of the measurements for T-Hg and MeHg were accompanied by the analysis of two certified reference material BCR-463 and DOLT-4. Based on the results can be seen that the recovery of the analytical procedure is at a satisfactory level. Acceptable recovery for this type of analysis should be in the range of 80 to 120%. All of the validation parameters are presented in Supplementary materials in S5 (based on Konieczka et.al [14]).

Fig. 1. Sampling points for the candidates for certified reference materials (Google Maps/Snazzy Maps).

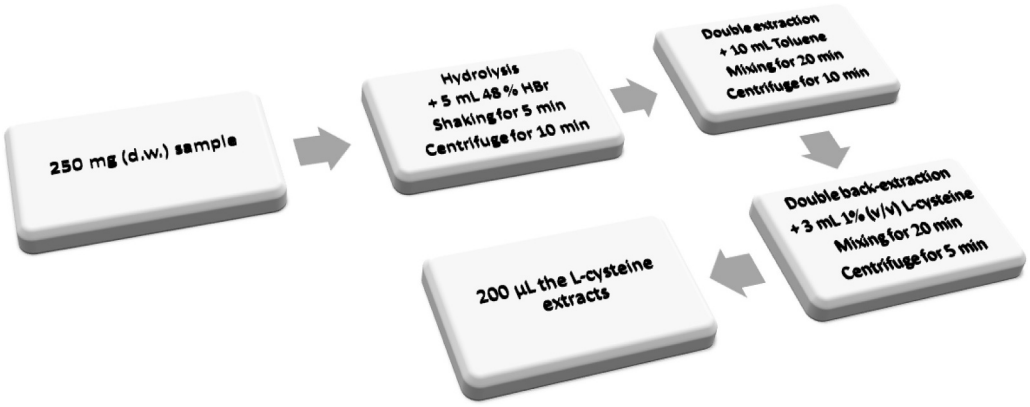

Fig. 2. Scheme of an analytical procedure for the extraction of methylmercury from environmental samples.

2.7. Homogeneity study

During the homogeneity study (within bottle homogeneity and between bottle homogeneity) of candidates for certification reference materials, the total mercury (soil, bottom sediment, herring tissue, cod tissue and cormorant tissue) and methylmercury (herring tissue, cod tissue, cormorant tissue) content were measured in randomized package. The samples were stored at room temperature (20°C) and in freezers (F_r) (−20 °C). In the case of the determination of the total mercury content, homogeneity was determined for the average mercury content of samples stored at room temperature and at reduced temperature. The homogeneity tested on the basis of the methylmercury content was determined separately for samples stored at room temperature and reduced temperature. Each of analyzed package was manually shaken before analysis to avoid possible segregation [15]. Seven independent sub-samples ($n = =7$) were analyzed for THg and six ($n = 6$) for MeHg. Several statistical tests were performed on the obtained data set, namely Grubbs, Q-Dixon, Cochran-Cox, *F*-Sendecor, *t* – Student tests – before between and within homogeneity is determined using the analysis of variance (ANOVA).

2.8. Stability study

The stability of the reference material is determined by analyzing the values of the certified parameters in the samples of materials stored at the temperatures recommended for the reference material, assuming that no change in composition of the reference material occurs at this temperature. In this work, the long-term stability was studied during 36 months at six or seven different times. The stability study was carried out on the basis of the total mercury content of the tested reference materials. Bottles of each storage temperature were randomly selected and analysed.

3. Results and discussion

3.1. Homogeneity study

The obtained results of analysis of variance of the total mercury and methylmercury content with the total extended uncertainty values in the individual samples are summarized in Table 1. The results analysis of variance of the total mercury content showed that for the samples of candidates for certified reference materials, the all *F* parameters values are less than the F_{crit} values ($F < F_{crit}$) in each measurement series, which means that the main component of heterogeneity of these materials is the within bottle inhomogeneity. The results of analysis of the variance of the methylmercury content showed that in most cases the *F* parameters values were greater than the F_{crit} values ($F > F_{crit}$). As a result, the CV_G parameter was additionally calculated. Summarizing, all the obtained coefficients CV_{BB}, CV_{WB} and CV_G values are low and do not exceed the 15% previously assumed, thus demonstrating that sample

Table 1
Results of analysis of analysis of variance of the total mercury and methylmercury content in candidate for reference materials samples.

Analyte	Sample type	Bottle No.	Average	$U(k = 2)$		Source of variance	F	F_{crit}	CV [%]	CV_G [%]
			[mg kg^{-1}]	[mg kg^{-1}]						
Mercury	Cormorant tissue	0101	2.319	0.013	Analysis of variance	Between bottle homogeneity	1.19	2.53	1.2	-
		0256	2.333	0.008		Within bottle homogeneity			1.1	
		0979	2.330	0.026						
		0172 F_r	2.341	0.022						
		0347 F_r	2.324	0.030						
		0780 F_r	2.309	0.017						
			[ng g^{-1}]	[ng g^{-1}]						
	Cod tissue	0122	315.3	3.6		Between bottle homogeneity	1.88	2.51	3.4	-
		0302	320.1	8.4		Within bottle homogeneity			2.5	
		0566	311.5	8.6						
		0200 F_r	311.0	5.4						
		0509 F_r	308.9	3.1						
		0899 F_r	310.4	3.0						
	Herring tissue	0221	202.3	6.9		Between bottle homogeneity	0.30	2.56	2.0	-
		0412	202.2	6.5		Within bottle homogeneity			3.7	
		0893	203.7	8.4						
		0104 F_r	201.9	4.2						
		0334 F_r	199.2	4.0						
		0739 F_r	204.2	5.5						
	Bottom sediment	0187	918	27		Between bottle homogeneity	1.06	2.56	3.5	-
		0277	911	19		Within bottle homogeneity			3.4	
		0689	933	18						
		0324 F_r	909	37						
		0799 F_r	894	24						
		0973 F_r	912	28						
Methylmercury	Herring Tissue	0120	121.4	4.1	Analysis of variance	Between bottle homogeneity	151.86	2.49	13	13
		0349	130.0	1.9		Within bottle homogeneity			1.1	
		0560	127.91	0.81						
		0724	139.76	0.65						
		0801	137.7	1.3						
		0992	135.17	0.70						
		0130 F_r	176.4	2.1		Between bottle homogeneity	26.76	2.48	8.2	8.3
		0206 F_r	165.1	1.7		Within bottle homogeneity			1.6	
		0347 F_r	161.6	2.0						
		0559 F_r	166.5	2.3						
		0680 F_r	168.7	1.2						
		0911 F_r	164.1	2.5						
	Cod tissue	0175	210.1	4.6		Between bottle homogeneity	50.32	2.51	9.0	9.1
		0239	209.2	1.2		Within bottle homogeneity			1.2	
		0412	226.5	1.0						
		0783	225.6	2.3						
		0814	225.84	0.49						
		0977	218.6	1.3						
		0021 F_r	210.3	2.9		Between bottle homogeneity	7.34	2.49	3.7	3.9
		0209 F_r	203.3	1.5		Within bottle homogeneity			1.4	
		0407 F_r	212.1	2.3						
		0671 F_r	207.6	1.7						
		0742 F_r	209.2	2.4						
		0975 F_r	209.0	2.2						
			[mg kg^{-1}]	[mg kg^{-1}]						
	Cormorant tissue	0007	1.629	0.022		Between bottle homogeneity	1.24	2.50	9.8	-
		0182	1.747	0.038		Within bottle homogeneity			8.8	
		0399	1.711	0.048						
		0509	1.694	0.022						
		0713	1.827	0.019						
		0900	1.604	0.017						
		0105 F_r	1.4988	0.0082		Between bottle homogeneity	39.15	2.49	11	12
		0368 F_r	1.528	0.011		Within bottle homogeneity			1.8	
		0557 F_r	1.351	0.038						
		0704 F_r	1.489	0.011						
		0879 F_r	1.477	0.027						
		0987 F_r	1.4271	0.0088						

candidates for reference materials with certified mercury and methylmercury content meet the heterogeneity requirement and can be considered homogenous both between bottle and within bottle. It was found that the samples (CRM candidates) were homogeneous for a minimum sample weight of 100 mg and could therefore be subjected to the next steps in the certification process.

Between bottle and within bottle homogeneity for the selected CRM candidate based on the results of the determination of the total content of mercury and methylmercury is presented graphically in figures S1 and S2 in Supplementary materials file. The individual results of determination of total mercury content in samples taken from a given package are within the range of the mean value for the package being analyzed, which confirms the conclusion about the within bottle homogeneity of the material.Whereas the calculated mercury content ranges in the individual bottle have a common part with a range corresponding to the mean for all analyzed bottles and it

confirms the conclusion about the between bottle homogeneity of the material.

3.2. Stability study

For each of the materials in the years 2013–2016 several series of measurements have been carried out. The results of the total mercury content with the total extended uncertainty values in the individual samples are summarized in the Table S6 in Supplementary materials.

A number of figures for all materials have been prepared for the confirmation of the proposals. Figures S3 and S4 in Supplementary materials show graphically the stability of each analyzed material. By analyzing the graphs, it can be seen that the average mercury content measured for each series of measurements is within the range of the mean of total mercury in the individual CRMsamples, which shows the stability of the produced materials.

The stability study of a package certified reference material was carried out using the Student's *t*-test based on the regression parameters of the stability graphs for all samples of reference materials. The results are summarized in Table S7 in Supplementary materials. The analysis of the obtained results proves that all slope of the regression are not statistically different from zero ($t < t_{crit}$) and also intercepts are not statistically different from the mean value of mercury ($t < t_{crit}$). In the case of total mercury content, the value is stable at both temperatures. Summarizing the results, it can be stated that each material meets the requirements for the stability condition of reference material with certified mercury content and these materials can be used for the quality control measurements.

4. Conclusions

Based on the results of the studies, each of the four analyzed samples (bottom sediment, herring tissue, cod tissue, cormorant tissue) is a suitable matrix for the reference material with certified mercury content. The confirmed homogeneity of the material determined on the basis of total mercury content was the basis for the stability studies. Based on the obtained results, it was found that each of the certified reference materials meets the stability requirements of the certified reference materials.

Material homogeneity was also determined based on the content of methylmercury in samples of three materials (herring tissue, cod tissue, cormorant tissue). However, in this case the instability of the analyte was observed.

New reference materials with certified mercury content, which are important complement to the current reference material offer available on the market and offered by reputable centers and institutions, have been produced (Table S8 Supplementary materials). In addition, the biomagnification effect of Hg and MeHg in the tissues of organisms in the following parts of the trophic chain is also observed. which is why, additional advantage and novelty of new CRMs is that the whole package of produced materials is a kind of food chain model, resulting in the creation of new solutions, ideas and concepts for environmental research. These materials are also a response to the ongoing need to produce new reference materials for specific analytical needs that will be representative in terms of the composition of the matrix and the content of analytes. Therefore applications of the new certified reference materials are very wide. They could be used for routine determinations of mercury in environmental or food laboratories or as a subject of study in interlaboratory comparision tests or research. These materials may be a reference in the study of bottom sediment, animal tissue or food samples collected from areas affected by environmental disasters or struggling with environmental contamination with mercury [16].

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.microc.2019.104338.

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