DETERMINATION OF TOTAL MERCURY
IN BIOLOGICAL MATERIAL
BY ATOMIC ABSORPTION SPECTROMETRY METHOD

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Abstract

A very simple procedure is proposed for the determination of total mercury in biological materials. The atomic absorption spectrometry method (AAS) for mercury determination was prepared. The Advanced Mercury Analyzer AMA 254 spectrometer was tested in order to determine the influence of the instrumentation on changes of the level of mercury in different biological samples (animal tissues, milk, feedstuffs). The method was validated in terms of basic analytical parameters. The mean recoveries of mercury was 101.70% for muscle and 98.30% for liver, and analytical detection limits was 0.001 µg/g. Certified reference materials and participation in national and international proficiency studies were used for analytical quality assurance programme.

Key words: mercury, atomic absorption spectrometry, biological materials.

Mercury occurs naturally in the environment and exists in several forms such as metallic mercury, inorganic mercury and organic mercury. Metallic mercury is still used in thermometers, barometers, batteries, and electrical switches. Some inorganic mercury compounds are used as fungicides and medicinal products. Mercuric chloride is a topical antiseptic or disinfectant agent. Other chemicals containing mercury are still used as antibacterials. Most of the mercury found in the environment is in the form of metallic and inorganic mercury compounds (1, 15, 18). Metallic mercury is a liquid at room temperature; it will evaporate into the air and can be carried for long distances. Microorganisms convert inorganic mercury to methylmercury. Inorganic or organic compounds of mercury can be released to water or soil. Mercury can enter and accumulate in the food chain. Some people can be exposed to higher levels of mercury in the form of methylmercury if they have a diet high in fish, shellfish, or marine mammals that come from mercury contaminated waters (7, 12, 34, 35). Inorganic mercury accumulates mostly in the kidneys but methylmercury is easily absorbed through the gastrointestinal tract and penetrates to the brain (1, 11, 17, 36). Mercury has been found in all foodstuffs (10, 12, 13, 21, 23, 28-32, 35, 37).

Commission Regulation (EC) No 466/2001 of 8 March 2001, and (EC) No 78/2005 of 19 January 2005 (4, 5), and Polish Regulation of Ministry of Health of 30 April, 2004 established the limits of Hg only for fish (25). Maximum levels for Hg in other foodstuffs existed in our country on legal basis until accession to the European Union (Polish Regulation of Ministry of Health of 13 January, 2003) (24). Actually, these values are established as the levels of action in the National Residue Control Plan in Poland which is realized by the Ministry of Agriculture and Rural Development (38). The limits for mercury are also established for feedstuffs (range 0.1 – 0.40 mg/kg) by the Polish Regulation of the Ministry of Agriculture and Rural Development of June 28, 2004 (26).

The exposure of mercury could be minimalized by regular control of food and feed and setting mercury maximum levels in these products. At the present time the National Veterinary Residue Control Programme which is organized in Poland according to Council Directive 96/23/EC of 29 April 1966 and Polish Regulation of the Ministry of Agriculture and Rural Development of April 19, 2004 requires controlling the levels of mercury in animal tissues, food of animal origin, and feedstuffs. A fully validated analytical method was developed to support introduction of legislation to practice and necessity of mercury control in monitoring programme.

Material and Methods

Reagents. Concentrated nitric acid was analytical grade. Stock standard solution containing 1 000 µg of Hg /ml was reference solution from Beaker.
Working standard solutions were prepared by dilution of stock and intermediate standards. The working standards were as follows:

- for the first range calibration – 0.05, 0.10, 0.20, 0.30 and 0.50 µg of Hg/ml prepared from the solution of 5.0 µg of Hg/ml concentration
- for the second range calibration – 1.0, 2.0, 3.0 and 5.0 µg of Hg/ml prepared from the solution of 100.0 µg of Hg/ml concentration

In the first range calibration, to every 100 ml flask 1, 2, 4, 6, and 10 ml of intermediate standard solution, containing 5 µg of Hg/ml, were measured and 1 ml of concentrated nitric acid was added and made up to 100 ml volume with deionized water. In the second range calibration, to every 100 ml flask 1, 2, 3, and 5 ml of intermediate standard solution, containing 100 µg of Hg/ml, were measured and 1 ml of concentrated nitric acid was added and made up to 100 ml volume with deionized water. At the same time a zero calibration solution was prepared in 100 ml flask, using 1 ml of concentrated nitric acid made up to 100 ml with deionized water. The stability of the standard solutions in the darkness is one week for the first range calibration and one month for the second range calibration.

Samples. Mercury was determined in animal tissues, milk, eggs, and feedstuffs. Cod muscle CRM 142, Pig kidney BCR 186, Milk powder BCR 151 and Milk powder BCR 150 were used as reference materials.

Apparatus. Advanced Mercury Analyzer AMA 254 spectrometer (Altec Ltd., Czech Republic). Oxygen of medical purity was used for sample combustion.

Instrument calibration. The calibration curve (first and second range) for the determination of mercury was prepared using a blank and working standard solutions.

Analytical method for mercury determination. Detailed instructions on the operation of the AMA 254 spectrometer are described in the operator’s manual.

The sample of known weight up to 300 mg or known volume up to 500 µl was placed to a sampling boat. The size of sample was adapted to expected concentration of mercury. The sample was introduced into a decomposition tube, dried for 70 s and thermally decomposed for 100 s at 900°C. The decomposition products of the sample are carried by oxygen flow to an amalgamator. The amalgamator and block of measuring cuvettes are thermostated to 120°C to prevent condensation of water. After completing sample decomposition and stabilization of temperature within the amalgamator the content of mercury trapped in amalgamator was measured in the optical cell system at 253.7 nm. After the atomization steps, concentrations of the mercury was reported in the computer in µg of Hg/g of wet weight of sample.

The calibration was periodically verified by analysing the standard and certified reference materials at the frequency of 20 readings. If the recovery was outside the limits, the analysis was stopped. The problem was corrected and the system was recalibrated.

Statistical analysis. The data obtained from the analysis were evaluated in based statistical parameters using computer program Excel.

Results

The Advanced Mercury Analyzer AMA 254 spectrometer was tested in order to determine the influence of the instrumentation on changes of the level of mercury in different biological samples (animal tissues, milk, feedstuffs). A series of experiments were conducted to establish optimum of analytical parameters. Optimization of analytical condition for different biological matrices and selection of instrumental programmes for this element were done.

The method was tested by studying of the certified reference materials (Cod muscle CRM 142, Pig kidney BCR 186 and Milk powder BCR 150 and 151) with the certified values and was regularly evaluated by participation in proficiency programmes organized by Food Analysis Performance Assessment Scheme (FAPAS) and European Community Reference Laboratories (CRL ISS). The result of Z-score of proficiency test organized by FAPAS in 2004 was -0.10, however, the result of Z-score of the 9th proficiency test in fish organized by CRL ISS in Rome in 2005 was 0.11. The results of the determination of mercury in certified reference material and validation reports are presented in Tables 1 and 2.

### Table 1

Comparison of the found and certified values of mercury in the certified reference materials (accuracy), n = 10

<table>
<thead>
<tr>
<th>Certified materials</th>
<th>Mercury Certified value µg/g</th>
<th>Found µg/g x ± SD</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cod muscle CRM 142</td>
<td>0.559</td>
<td>0.494 ± 0.019</td>
<td>88.37</td>
</tr>
<tr>
<td>Pig kidney BCR 186</td>
<td>1.97</td>
<td>1.76 ± 0.023</td>
<td>89.42</td>
</tr>
<tr>
<td>Milk powder BCR 151</td>
<td>0.101</td>
<td>0.092 ± 0.0007</td>
<td>90.59</td>
</tr>
<tr>
<td>Milk powder BCR 150</td>
<td>0.009</td>
<td>0.010 ± 0.0006</td>
<td>108.89</td>
</tr>
</tbody>
</table>
Table 2
Validation report – Hg determination by AAS (AMA-254)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity (working range), mg/kg</td>
<td>0.002 – 0.500</td>
</tr>
<tr>
<td>Limit of detection (LOD), mg/kg</td>
<td>0.0004</td>
</tr>
<tr>
<td>Limit of quantification (LOQ), mg/kg</td>
<td>0.0005</td>
</tr>
<tr>
<td>Matrix</td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td></td>
</tr>
<tr>
<td>Levels of spiked samples, mg/kg</td>
<td></td>
</tr>
<tr>
<td>Repeatability</td>
<td></td>
</tr>
<tr>
<td>x, mg/kg</td>
<td>0.010 0.020 0.037 0.021 0.039 0.047</td>
</tr>
<tr>
<td>SD, mg/kg</td>
<td>0.0004 0.00022 0.00023 0.004 0.0011 0.0011</td>
</tr>
<tr>
<td>RSD, %</td>
<td>7.51 12.94 6.94 1.79 2.72 3.25</td>
</tr>
<tr>
<td>Intralaboratory reproducibility</td>
<td></td>
</tr>
<tr>
<td>x, mg/kg</td>
<td>0.011 0.020 0.038 0.021 0.039 0.047</td>
</tr>
<tr>
<td>SD, mg/kg</td>
<td>0.0005 0.0013 0.0020 0.0005 0.0008 0.0011</td>
</tr>
<tr>
<td>RSD, %</td>
<td>4.97 6.59 5.21 2.22 2.03 2.34</td>
</tr>
<tr>
<td>Recovery, %</td>
<td>107.80 102.00 95.40 103.00 98.20 93.70</td>
</tr>
<tr>
<td>Uncertainty</td>
<td></td>
</tr>
<tr>
<td>combined (uc)</td>
<td>0.0014 0.0013</td>
</tr>
<tr>
<td>expanded (U)</td>
<td>0.020 ± 0.003 mg/kg 0.050 ± 0.0027 mg/kg</td>
</tr>
<tr>
<td>coverage factor (k)</td>
<td>2 2</td>
</tr>
</tbody>
</table>

Discussion

A very simple procedure is proposed for the determination of total and inorganic mercury in biological materials. Analytical and instrumental conditions are the most important stages in mercury analysis. Usually methods of atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), or neutron activation analysis (NAA) are used in the analysis (2, 3, 6, 12, 20, 22, 27, 30, 32). In addition, methods based on mass spectrometry (MS), spectrophotometry, and anodic stripping voltammetry (ASV) have also been used (14, 15). From the available methods, cold vapour-AAS is the most widely used (2, 14-16, 19, 30, 33). The wide range of biological materials (foods, feeds, plants, animal tissues and fluids) is characterized by a wide variety of chemical analytical measurements. In general, the classical method is composed of 3 steps: 1) sample pre-treatment, 2) analyte isolation/separation, and 3) quantitative measurement. These various materials were determined without preceding mineralization. Many of these materials contained large quantity of fat, protein, and carbohydrate which have influence on processing and determination steps in the investigated method.

In this study, the procedure of the determination of mercury in biological material by advanced mercury analyser – AMA 254 was tested. Selection of the operating parameters for AMA 254; oxygen flow, time of drying and time of decomposition in tube at 900°C were satisfactory to run all kinds of biological samples and give the satisfactory analytical parameters (limit of detection – LOD, and limit of quantification – LOQ below the levels of 0.001 mg/kg).

The method was validated in terms of the linearity, LOD, LOQ, precision, recovery, and uncertainty (8, 9). Results of the analysed certified reference materials and inter-laboratory comparison, and all validation data confirmed that the method could be used as a routine procedure for the determination of mercury levels in food and feed in official monitoring control programme. Generally good results of precision (RSD about 5% for muscles and 2% for liver), recoveries (about 100% for all analysed samples) and reasonable value of uncertainty additionally support the described method as a routine procedure for mercury determination in biological materials.

Value and simplicity of the developed method was fully supported by validation results. The validation procedure was in general agreement with the Commission Decision of 12 August 2002 (2002/657/EC) implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. The proposed analytical procedure is well suited for maximum level control of mercury in food and feedstuffs as a screening and confirmatory method in official food control laboratory.

References


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