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SHORT COMMUNICATION

DECOMPOSITION OF FISH SAMPLES FOR DETERMINATION OF MERCURY

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The aim of the study was to compare the efficiency of acid and alkaline decomposition of biological materials using an open and a closed system for total mercury determination. Acid digestion was performed with concentrated HNO_3 in tubes at 80 °C and lasted five hours. Alkaline digestion was performed with a 45% NaOH and a 1% cysteine, heated at 120 °C for 20 minutes. Total mercury was measured by atomic absorption spectrometry using the cold vapour technique (CVAAS). The average recovery obtained for analysis of certified reference material in closed tubes for acid digested sample was superior to the alkaline one, 103±4% vs. 70±3%, respectively. In addition, the recoveries through the open system acid digestion (90±8%) and the open system alkaline digestion (57±2%) were lower than through the respective closed system digestions. Reproducibility of the acid decomposition method was superior to the alkaline one.

Key words:
acid digestion, alkaline digestion, biological material, closed system, cold vapour atomic absorption spectrometry, mercury recovery, open system

The analysis of total mercury in biological samples requires two separate processes: (i) the conversion of all forms of mercury to ionic mercury (Hg^{2+}) by various digestion/oxidation techniques and (ii) the reduction of Hg^{2+} to its atomic form (Hg^0) for analysis (1). Different methods for digestion of biological materials for quantitative determination of mercury are described in the literature. Some of these methods used alkaline (2, 3) and others acid digestion technique (4–6). So far, the results obtained

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using the two techniques on the same sample have not been compared. The aim of this study was to compare the efficiency of alkaline and acid decomposition of biological material (fish), carried out in an open and a closed digestion system.

MATERIAL AND METHODS

Reagents

All reagents (Kemika, Zagreb) were analytical grade and were selected for their low mercury content. Water was deionised to the conductivity of 0.06 μS/cm.

Stock solution of mercury (500 μg Hg^{2+}/ml) was prepared by dissolving 0.06767 g of mercuric chloride in 100 ml of 5% H_2SO_4. From this stock solution, 0.2 μg Hg^{2+}/ml solution was prepared for making working solutions of 2–20 ng Hg^{2+}/ml. Working solutions were prepared daily. Reduction agent was made by mixing $SnCl_2x2H_2O$ (2.4%) and $CdCl_2$ (0.5%), and by adding L-cysteine (0.2%) and NaCl (1%) to H_2SO_4 (2.25 M). Sodium hydroxide (35%) was used to reduce mercury in the alkaline medium.

Acid digestion

About 1 g of fish homogenate was first digested with 2 ml of concentrated HNO_3 in borosilicate glass tubes overnight at room temperature and then the next day at 80 °C for five hours in a programmed digestion system (DS-40, Tecator, Sweden). Digestion was performed in open or in closed tubes. After digestion samples were cooled to room temperature and the volume adjusted to 10 ml with deionised water, an aliquot of 0.5 ml was taken for mercury determination.

Alkaline digestion

About 1 g of fish homogenate was added 2 ml NaOH (45%) and 1 ml cysteine (1%), and heated in a glass tube at 120–150 °C for 20 minutes. Digestion was performed in open or in closed tubes. Samples were cooled to room temperature and the volume adjusted to 10 ml with deionised water. An aliquot of 0.5 ml was taken for mercury determination.

Sample preparation and method verification

Before digestion, a sample of fish muscle (hake,*Merlucius merlucius*) was prepared as a 50% homogenate in a 1% saline solution with Kinematica homogeniser (Switzerland). The accuracy of both digestion techniques was verified using the reference material (horse kidney H-8, IAEA, Austria) and expressed as percentage of recovery. Five replicates of 0.04 g lyophilised reference material were digested either with 0.5 ml of HNO_3 or with 0.5 ml of 45% NaOH and 0.25 ml of 1% L-cysteine. Samples were digested in open and in closed tubes as described in the fish digestion. To determine the reproducibility of the method, the whole procedure of digestion and mercury measurement was carried out with 9 replicates of the fish homogenate. Relative stan-

dard deviation (RSD) was calculated as percentage of standard deviation from arithmetic mean.

Mercury determination

Total mercury was analysed by cold vapour atomic absorption spectrometry (CVAAS) on Mercury Monitor LDC (Milton Roy, Riviera Beach, Fl) after reduction with stannous and cadmium chloride in an alkaline medium. This method was described as sensitive and reliable for determination of total mercury in aqueous solutions (2, 3, 7, 8). The detection limit of the method was calculated from 10 repeated measurements of the lowest standard solution (2 ng/ml).

RESULTS AND DISCUSSION

Table 1 shows the reproducibility of acid and alkaline digestion methods. Mercury was analysed in 9 replicates of the same fish homogenate. Calculated as relative standard deviation, the precision of the acid digestion method performed in a closed or an open system was 10% and 13%, respectively. The precision of the alkaline digestion performed in an open system was 14%.

Table 1 *Reproducibility of the method for mercury determination in a 50% fish homogenate digested using acid or alkaline technique (in a closed or an open system)*

Digestion	Acid (ng Hg/g)	RSD (%)	Alkaline (ng Hg/g)	RSD (%)
Closed	319 ± 31	10	–	–
Open	112 ± 14	13	150 ± 21	14

Results are presented as arithmetic mean±standard deviation of 9 replicates. RSD is relative standard deviation.

The accuracy of the methods after each digestion procedure was verified by measuring the certified reference material (Table 2). The average recovery for the sample digested with nitric acid in a closed and an open system was 103±4% and 90±8%, respectively. Alkaline digestion yielded lower recovery of only 70±3% and 57±2% for the respective closed and open systems. The detection limit of CVAAS method was 6 ng Hg/g of fresh tissue.

The results show that digestion of biological material in an open system results in considerable loss of mercury. Our study corroborates the results of *Jones and coworkers* (1) who recently found considerable losses of organic mercury in an open-vessel digestion. The above findings render questionable many earlier reports on mercury determination in undigested or open digested system of biological material (2, 3, 7).

Table 2 *The effect of acid or alkaline digestion methods (carried out in closed or in open system) on the accuracy of mercury analyses using standard reference material (horse kidney H-8, IAEA) containing 910±70 ng Hg/g.*

Digestion	ng Hg/g	Recovery (%)
Acid		
closed	934 ± 38	103 ± 4
open	815 ± 6	90 ± 8
Alkaline		
closed	641 ± 28	70 ± 3
open	519 ± 13	57 ± 2

Results are presented as arithmetic mean±standard deviation of 5 replicates.

Furthermore, our results show that alkaline digestion method yields lower recovery of mercury (Table 1 and 2), even if carried out in a closed system. The reason either lies in its loss during digestion or in its incomplete reduction in the reaction vessel. Alkaline digestion is probably unable to decompose methylmercury and, consequently, all mercury present in solution cannot be reduced in the reduction vessel (2). In general, acidic digestion yields much higher recovery than the alkaline.

There are many methods for the digestion of biological samples. Most of them involve an open-vessel digestion system and use a variety of acids (HCl, HNO_3, and H_2SO_4) (9–12). Strong oxidants such as $KMnO_4$, $K_2Cr_2O_7$ in acid mixture of HNO_3 and H_2SO_4 were used by some investigators (11, 13). These methods appear to result in complete digestion of biological material, but they are lengthy, may contaminate samples, exhibit high blank values, and give poor precision. Closed ampule digestion/oxidation technique prevents loss of organic-mercury compounds by volatilisation. A closed vessel also allows heat and pressure to break down the carbon-mercury bond in one step. Our experience confirms that once the samples are prepared in a closed vessel, they are stable with respect to the loss of mercury (1).

In the last few years, a simple method was developed using only one acid (concentrated nitric acid) in closed test tubes (1, 6, 12). In this study, we also applied a single (nitric) acid in digestion of samples. Our results show that acid digestion method using only nitric acid gives very good accuracy and precision (Table 1 and 2). Acid digestion performed in a closed system yields higher recovery than in the open one.

To conclude, our results indicate that acid digestion is superior to the alkaline in terms of accuracy and precision. A closed digestion system is also mandatory for quantitative mercury determination.

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Sažetak

RAZARANJE UZORAKA RIBE ZA ANALIZU ŽIVE

Svrha ovog rada bila je ispitati učinkovitost dviju metoda razaranja biološkog materijala pri analizi žive. Razaranje u kiselom mediju s koncentriranom HNO_3 primijenjeno je pri 80 °C tijekom 5 sati. Razaranje uzorka u alkalnom mediju primijenjeno je s 45% NaOH i 1% cisteinom pri 120 °C tijekom 20 minuta. Obje metode razaranja provedene su u otvorenim ili zatvorenim staklenim epruvetama. Ukupna živa određena je metodom atomske apsorpcijske spektrometrije tehnikom razvijanja hladnih para (CVAAS). Iskorištenje razaranja uzorka ispitano je na referentnom uzorku (konjski bubreg H-8, IAEA, Austrija). Rezultati su pokazali bolje iskorištenje određivanja žive u zatvorenim epruvetama u kiselom mediju (103±4%) negoli u alkalnom mediju (70±3%). Iskorištenje u otvorenim epruvetama je manje negoli u zatvorenima te iznosi za razaranje uzorka u kiselom mediju 90±8% i samo 57±2% u alkalnom mediju. Ponovljivost metode (koeficijent varijabilnosti izražen kao postotak standardne devijacije od aritmetičke sredine) ispitana je na uzorcima oslića (*Merlucius merlucius*). Metoda razaranja biološkog materijala u kiselom mediju preciznija je od metode razaranja u alkalnom mediju.

Ključne riječi:
atomska apsorpcijska spektrometrija razvijanjem hladnih para, biološki materijal, oslobađanje žive, otvoreni proces, razgradnja uzoraka kiselinom, razgradnja uzoraka lužinom, zatvoreni proces

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