Manual for Marine Monitoring in the
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Part B

General guidelines on quality assurance for monitoring in the Baltic Sea

Annex B-12

Technical note on the determination of heavy metals and persistent organic compounds in biota

Appendix 5

Technical note on the determination of total mercury in marine biota

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ANNEX B-12 TECHNICAL NOTE ON THE DETERMINATION OF HEAVY METALS AND PERSISTENT ORGANIC COMPOUNDS IN BIOTA

ANNEX B-12 APPENDIX 5. TECHNICAL NOTE ON THE DETERMINATION OF TOTAL MERCURY IN MARINE BIOTA

1. GENERAL REMARKS TO THE AVAILABLE ANALYTICAL METHODS

The most widely used method for the determination of total mercury in biological tissues and sediments is cold vapour atomic absorption spectrometry (CV-AAS), based on a technique elaborated in detail by Hatch and Ott (1968). In this method, (divalent) ionic mercury is reduced to its metallic form (Hg^o) in acidic solution using a powerful reducing agent. Subsequently, the elemental mercury is volatilized (purged) by a carrier gas and transported into an absorption cell, where the 253.65 nm wavelength absorbance of mercury atoms is measured.

More recent atomic fluorescence (AFS) was introduced as an alternative detection method. It is part of the US EPA Method 1631 Revision E (US EPA 2002), which describes the measurement of trace concentrations of mercury in water samples. Comparing both methods the CV-AAS is may be more appropriate for samples containing higher levels of mercury and the CV-AFS method more for lower level samples.

Analysis can be performed manually using batch operation or automatically using flow injection (FI) techniques. FI is a very efficient approach for processing and introducing liquid samples into the detector. The FI technique, combined with a built-in detector optimised for mercury determination, reduces sample and reagent consumption, has a higher tolerance of interference's, lower determination limits and improved precision compared with conventional cold vapour techniques.

The efficiency of various flow injection mercury systems has been reported by several groups (Tsalev *et al*., 1992a, 1992b; Welz *et al*., 1992; Guo and Baasner, 1993; Hanna and McIntosh, 1995; Kingston and McIntosh, 1995; Lippo *et al*., 1997).

Further improvement of sensitivity could be reached by the insertion of an amalgamation unit between reduction unit and detector. In this case the mercury vapour, released from the sample solution, will be pre concentrated on a gold adsorber (Welz and Melcher, 1984). This technique eliminates kinetic interferences due to a different vaporization rate or a different distribution function of the elemental mercury between the liquid and the gaseous phases. The amalgamation ability of the gold adsorber must be carefully and regularly checked, because volatile compounds, sulfur-containing in particular,evaporating together with the elemental mercury from the sample solution and may deactivate the adsorber surface. This means an increased risk of underestimation, as unknown quantities of mercury are not collected by the adsorber.

2. SAMPLE PRE-TREATMENT

It is generally agreed that oxidative conversion of all forms of mercury in the sample to ionic Hg (II) is necessary prior to reduction to elemental Hg and its subsequent measurement by CV-techniques. Therefore, the initial procedural step in mercury analysis is a sample pre-treatment, which is aimed at liberating the analyte element from its chemical bonding to the organic matrix and thus transforming all of the analyte species into a well-defined oxidation state. For this purpose, a wide variety of combinations of strong acids (HCl, H_2SO_4, and HNO_3) and combustion with oxidants (H_2O_2, $KMnO_4$, $K_2Cr_2O_7$, $K_2S_2O_8$) have been tested and recommended (Kaiser *et al*., 1978; Harms, 1988; Vermeir *et al*., 1989; Ping and Dasgupta, 1989; Baxter and Frech, 1990; Landi *et al*., 1990; Navarro *et al*., 1992; Lippo*et al*., 1997).

A suitable sample pre-treatment, which implies the complete transformation of all organomercury species into inorganic mercury ions, requires the following:

- oxidation mixtures with a high oxidation potential;
- rapid oxidation (usually promoted by high reaction temperatures), preferably in closed systems;
- compatibility with CV techniques;
- stability of sample solutions during storage (at least short term);
- no formation of solid reaction products

Several authors (Tsalev *et al*., 1992a 1992b; Welz *et. al*., 1992; Guo and Baasner, 1993) have demonstrated that microwave digestion can be successfully applied to the analysis of liquid samples.

3. CONTROL OF CONTAMINATION AND ANALYTE LOSSES

Major difficulties arise due to the mobility and reactivity of mercury and its compounds, respectively, during sample preparation, sample pre-treatment, and analysis. Therefore, the stability of samples and standard solutions is of prime importance, and it is advisable to test the stability of typical standard and sample solutions under typical laboratory conditions.

Mercury can disappear from solution due to several mechanisms, including volatilization of mercury compounds, reduction of such compounds followed by volatilization of elemental (metallic) mercury, adsorption on container walls, adsorption onto colloids or particles, incorporation into stable chemical complexes, or incorporation, upon reduction, into stable amalgams.

Thermodynamic considerations of Toribara *et al*. (1970) showed that loss of mercury from a solution containing the element in the monovalent form may occur readily through disproportion and subsequent loss of metallic mercury. Because of the high oxidation potential of the mercury(II)-mercury(I) system, almost any reducing substance could convert some divalent mercury ions into monovalent mercury ions, which then spontaneously disproportion into mercury(II) and mercury(o). The latter escapes as metallic vapour from the solution into the gas phase. Because of the almost impossibility of preventing the introduction of small amounts of reducing substances by reagents or solvents, the more dilute mercury(II) solutions would be less stable and lose mercury more readily. The only practical method for stabilizing such solutions is to add a small excess of an oxidizing substance (such as permanganate), which has a higher oxidation potential than the mercury(II)-mercury(I) system.

Similarly, Feldman (1974) concluded from his experiments that solutions with 0.1 µg divalent Hg dm^{-3} in distilled water could be stored in glass vials for as long as five months without deteriorating if the solutions contained 5 % (v/v) HNO_3 and 0.01 % $Cr_2O_7^{2-}$. Storage of such solutions was safe in polyethylene vials for at least 10 days if the solutions contained 5 % (v/v) HNO_3 and 0.05 % $Cr_2O_7^{2-}$. The efficiency of this mixture was probably due to its ability to prevent the hydrolysis of dissolved mercury and prevents its reduction to valances lower than +2.

4. REDUCING REAGENTS

Tin(II) chloride and sodium tetrahydroborate are predominantly used as reducing reagents for the determination of total mercury by CV-Methods. Sodium tetrahydroborate has been found advantageous for several applications owing to its higher reducing power and faster reaction (Toffaletti and Savory, 1975). In addition, this reductant has been successfully used even in the presence of interfering agents such as iodide and selenium (Kaiser *et al.*, 1978). However, potential interferences can occur from metal ions (e.g., Ag(I), Cu(II), Ni(II)), which are themselves reduced to the metallic state and so may occlude mercury through amalgamation.

Welz and Melcher (1984) showed that sodium tetrahydroborate could more readily attack those organic mercury compounds which were not reduced to metallic mercury by tin(II) chloride. However, they stated that sodium tetrahydroborate could not be recommended as the reducing reagent for the amalgamation technique. They found that, due to the rather violent reaction with sodium tetrahydroborate, fine droplets of the sample solution were carried by the gas stream and contaminated or deactivated the adsorber surface. Further, they considered even more important the fact that not only mercury but all gaseous hydride-forming elements (e.g., arsenic, antimony, and selenium) were volatilized when sodium tetrahydroborate was used as reductant. These hydrides reacted with the adsorber material and deactivated its surface, thus no longer permitting a sensitive and reproducible determination of mercury.

5. INTERFERENCES

Interferences by volatile nitrogen oxides in the determination of mercury by FI-CV-AAS were studied by Rokkjaer *et al*. (1993). The main symptom of the interference effects was suppression, broadening or even splitting of the mercury signal. The authors postulated that volatile nitrogen oxides formed as reaction products of nitric acid during sample decomposition scavenged the reducing agent and concomitantly inhibited the reduction of mercury(II). The rate of the reaction of nitrogen oxides with the reducing agent was considered to be so fast that it was consumed before the reduction of mercury was complete. Rokkjaer *et al*. (1993) demonstrated that the interference could easily be remedied by purging the sample solution with an inert gas prior to the introduction of the reducing agent.

Lippo *et al*. (1997) concluded from their experiments that nitrogen mono- and dioxide, having molecular absorption bands at 253.63 nm and 253.85 nm, respectively, might cause unspecific absorption at the specific mercury wavelength of 253.65 nm, leading to enhanced and broadened mercury signals if not properly compensated for by adequate instrumental background correction.

The presence of water vapour in the fluorescence detector may produce scattering effects, positive interferences and degradation of the analytical signal. The use of a dryer tube is required to remove any water vapour from the flow before reaching the detector.

6. INTERNAL (ROUTINE) QUALITY CONTROL

In order to demonstrate that the analytical method applied is fit for the purpose of the investigations to be carried out, control materials should be regularly analysed alongside the test materials (cf. Chapter 5, 6).

The control materials--preferably certified reference materials--should be typical of the test materials under investigation in terms of chemical composition, physical properties and analyte concentration.

Fitness for purpose is achieved if the results obtained from the analysis of the control materials are within the defined limits of permissible tolerances in analytical error (see Chapters 5).

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