COLD VAPOUR ATOMIC ABSORPTION SPECTROSCOPIC
DETERMINATION OF MERCURY IN BRINE PRESERVED FISH

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Abstract: Five varieties of locally produced and imported brine preserved canned fish samples were
digested with a mixture of nitric and sulphuric acids, and then analysed for total mercury concentrations
by cold vapour atomic absorption spectrophotometry (CV-AAS). Samples of tuna, salmon, sardine,
kipper and mackerel were evaluated. The accuracy of the methods was tested by analyzing fish
samples spiked with 0.1 and 1.0 \(\mu g/g\) of inorganic and organic mercury. Recovery of mercury from
canned fish and brine solutions ranged from 85 to 106\% and 83 to 110\%, respectively, for inorganic
and organic mercury spikes. The mean total (wet) concentrations of mercury found in the canned fish
samples were 0.095 \(\mu g/g\) for tuna, 0.054 \(\mu g/g\) for salmon, 0.029 \(\mu g/g\) for sardine, 0.095 \(\mu g/g\) for kipper
and 0.056 \(\mu g/g\) for mackerel.

Keywords: Cold vapour atomic absorption spectrophotometry, mercury, wet digestion methods, fish.

Introduction

A major dietary source of mercury to humans is from various fish types, which are capable to
accumulating relatively large concentrations of this element in their tissues. The concern about the
concentrations of mercury in fish resulted mainly from an earlier incidence of mercury poisoning
through the consumption of mercury contaminated seafood obtained from Minamata Bay in Japan
(Kurando, Faro & Seidler, 1960). In this particular incidence the concentration of mercury found in
the contaminated seafood ranged from 5 to 20 \(\mu g/g\). However, since then regulatory limits have been
introduced to avoid similar incidences in other countries. At present, the WHO recommended level for
total mercury (wet) concentration in fish is 0.5 \(\mu g/g\) (WHO, 1972).

Several developments in analytical instrumentation over the past four decades have made the
reliable determination of mercury at the trace and ultra-trace concentrations possible. In particular, cold
vapour atomic absorption spectrophotometry (CV-AAS) has gained most use for the determination of
mercury in various biological and environmental materials (Chapman, & Dale, 1978). However, recent
studies have indicated that in spite of the relatively high sensitivity of this technique, its reliability
for the quantification of mercury in samples can be affected by the choice of decomposition methods
and presence of certain substances (Ade\(\text{le}^{ou}\), Dhindsa & Tandon, 1994; Ade\(\text{le}^{ou}\) & Mann, 1987;
Analytical Methods committee, 1960 & 1965; Gorsuch, 1959 & 1970; Louie, 1983). In a study in our
laboratories (Ade\(\text{le}^{ou}\), Dhindsa & Tandon, 1994), we found that the use of \(\text{HNO}_3\cdot\text{H}_2\text{SO}_4\) mixture was
most effective for the decomposition of biological and environmental materials and for the reliable
CV-AAS determination of mercury in these samples.

The objective of the present study was to establish the adequacy of this decomposition
method for the rapid and reliable determination of mercury in a variety of canned fish samples by CV-
AAS. The reliability of the wet decomposition of fish samples with the \(\text{HNO}_3\cdot\text{H}_2\text{SO}_4\) mixture and of
the CV-AAS measurement was investigated with spiked samples.
Experimental Materials and Methods

Reagents and Standards
All reagents used in this study were of analytical grade. Mercury stock solution (1 g/l) was purchased from PROLABO, Paris (France). A mercury standard (0.1 mg/l) was prepared daily by diluting the stock in 2% v/v hydrochloric acid. Stock solution of organic mercury (1 g/l) was prepared by dissolving appropriate amount of methymercury chloride in ethanol (12 ml of 95% v/v) and diluting to volume (100ml) with water. Stamnos Chloride solution (30% w/v) was prepared daily by dissolving appropriate amount in 20% v/v hydrochloric acid and stabilized by the addition of a piece of tin. All solutions were prepared with Milli-Q water.

Instrumentation and Glassware
CV-AAS measurements were performed on a Varian Spectra 20 atomic absorption spectrophotometer operated in a double beam mode. The conditions employed for the measurements were: wavelengths, 253.7 nm; slit width, 0.5 nm; lamp current, 0.3 mA.
All glassware and plastic containers were soaked in nitric acid (2M) for at least 24 hours and rinsed 4-5 times with Milli-Q water prior to use.

Procedure
Preparation of Fish Samples. After decanting the preservative brine solution into a clean plastic container, the fish content in each can was weighed and subsequently homogenized in a steel blade blender. Three portions (5g each) of the homogenized fish were freeze-dried and subsequently ground to powder before being used for the CV-AAS measurement. The preservative brine solution was filtered through an acid-washed Whatman filter paper 42 and the filtrate was used to determine the total mercury concentration leached form the fish tissue. The acid washing of the filter paper involves soaking in 2M HNO₃ for 24 hours, washing 4-5 times with Milli-Q water and soaking in water until required.

Wet Digestion Methods. A 0.3 g of the freeze-dried fish sample (or 4g of brine preservative) was weighed into a pre-cleaned 100ml Erlenmeyer flask, followed by the addition of 2.5ml (or 5.0ml for brine preservative) of the 2:25: 1 HNO₃-H₂SO₄ mixture. The loosely stoppered flask was then heated at 90°C for 60 minutes. The flask was then cooled to room temperature and the stopper as well as the inner sides of the flask was rinsed with Milli-Q water. The final volume was made up to 20ml and this was used for the mercury measurement, as described below.

CV-AAS Measurement. A 100ml Erlenmeyer flask containing 20ml sample or standard solution was connected to the mercury vapour generation system, 1ml of stamnos chloride solution was then added and the contents of the flask were stirred with a magnetic stirrer at maximum speed for 3 minutes. The resulting mercury vapour was displaced from the flask to the mercury cell by the water displacement method (Chapman, & Dale, 1978) at a rate of 10ml/sec. After recording the mercury response, the cell was flushed out with instrumental grade air.

Results and Discussion
Optimization of the Double Beam System for CV-AAS Measurement of Mercury
In a previous study (Adeloja, Dhindsa & Tandon, 1994), we used a single beam atomic absorption spectrophotometer for the CV-AAS measurement of mercury and accomplished a detection limit and sensitivity of 4.1 ng and 2.7 ng/0.0044 Abs, respectively, in the HNO₃-H₂SO₄ mixture. The double beam spectrophotometer used in the present study enabled substantial lowering of these parameters to 1.8 ng and 2.0 ng/0.0044 Abs, respectively. The observed improvement may also be associated with the replacement of the heated close ended T-Cell with the non-heated open ended T-Cell for the mercury measurement. In any case, the observed improvement will further increase the ability to obtain reliable mercury concentrations in the canned fish samples.
Table 1: Recovery of Spiked Organic and Inorganic Mercury from Different Canned Fish Samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hg Added (µg/g)</th>
<th>% HgCl₂ recovered</th>
<th>% CH₂HgCl recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuna</td>
<td>0.1</td>
<td>105.8</td>
<td>95.2</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>90.0</td>
<td>83.8</td>
</tr>
<tr>
<td>Salmon</td>
<td>0.1</td>
<td>94.7</td>
<td>110.0</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>85.5</td>
<td>101.0</td>
</tr>
<tr>
<td>Sardine</td>
<td>0.1</td>
<td>95.9</td>
<td>110.0</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>88.8</td>
<td>87.7</td>
</tr>
<tr>
<td>Brine</td>
<td>0.1</td>
<td>93.7</td>
<td>88.7</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>95.8</td>
<td>105.2</td>
</tr>
</tbody>
</table>

* Added as HgCl₂ or CH₂HgCl.

Recovery of Inorganic and Organic Mercury Spikes

It was demonstrated in our previous study (Adeloju, Dhindsa & Tandon, 1994) for other sample materials that the recovery of inorganic and organic mercury spikes was influenced considerably by the sample matrix. Similarly, in the present study, the recovery of the spiked mercury was influenced by the nature of the sample matrix and the amount of mercury added. The data in Table 1 shows that lower recoveries (83.8 – 101.0%) were generally obtained for the different fish samples when higher mercury concentration (1 µg/g) was added. This may be due to the occurrence of the absorbance reading for the higher mercury concentration in the upper-end of the linear concentration range of the CV-AAS measurement. This view is well supported by the much higher recoveries (94.7 – 110.0%) obtained when lower mercury spike (0.1 µg/g) was employed. Overall, the average percentage recovery was much better for the organic mercury (97.7%) than for the inorganic mercury (93.8%). Previous application of the HNO₃-H₂SO₄ digestion to the CV-AAS determination of mercury in a standard reference fish homogenate (Adeloju, Dhindsa & Tandon, 1994) gave a good agreement between the certified value (0.47 ± 0.03 µg/g) and the experimental results (0.472 ± 0.004 µg/g).

Table 2: Concentrations of Mercury found in Different Canned Fish Samples by CV-AAS

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of Samples</th>
<th>Mean±(µg/g)</th>
<th>Range (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuna</td>
<td>8</td>
<td>0.095 ± 0.053</td>
<td>0.037 – 0.178</td>
</tr>
<tr>
<td>Salmon</td>
<td>18</td>
<td>0.054 ± 0.047</td>
<td>0.015 – 0.204</td>
</tr>
<tr>
<td>Sardine</td>
<td>6</td>
<td>0.029 ± 0.016</td>
<td>0.009 – 0.057</td>
</tr>
<tr>
<td>Kipper</td>
<td>3</td>
<td>0.095 ± 0.027</td>
<td>0.064 – 0.111</td>
</tr>
<tr>
<td>Mackerel</td>
<td>2</td>
<td>0.056 ± 0.037</td>
<td>0.019 – 0.093</td>
</tr>
</tbody>
</table>

* Based on three measurements on each canned fish sample.
Mercury in Canned Fish Samples

Table 2 gives the mean and range of total mercury concentrations found in the samples of tuna, salmon, sardine, kipper and mackerel digested with the HNO₃-H₂SO₄ mixture and analysed by CV-AAS. The mean mercury concentrations decreased in the following order: tuna > salmon > sardine which is consistent with trends reported in previous studies (Acra, Namaan & Raffoul, 1981; Kyle, & Chami, 1983) and is related to their position in the food chain, as well as their size (Acra, Namaan & Raffoul, 1981; Ghoshdastidar & Chakrabarti, 1991).

![Graph 1](image1)

**Figure 1**: Variation of total mercury concentrations in brine preserved canned tuna from Australia and Thailand.

![Graph 2](image2)

**Figure 2**: Variation of total mercury concentrations in brine preserved canned salmon from various countries.

The examination of the mercury concentrations in the canned fish samples on the basis of their origin revealed some interesting results. Figures 1 and 2 revealed that the mean mercury (wet) concentrations in Australian produced canned tuna (0.137 ± 0.005 μg/g) and salmon (0.141 ± 0.005 μg/g) were among the highest concentrations obtained for the samples. In contrast, the mean mercury in canned tuna imported from Thailand (0.094 ± 0.004 μg/g) was significantly lower (p<0.005). Similarly, the mean concentration of mercury in imported canned salmon from developed countries (USA and Canada grouped together as G1; 0.033 ± 0.002 μg/g) and developing countries (Russia, Chile, Thailand, Malaysia and Korea grouped together as G2; 0.041 ± 0.002 μg/g) were significantly lower (p<0.0005 in both G1 and G2) than those found in Australian produced canned salmon. In
general, the mercury concentrations found in Australian produced salmon (Figure 2) were about 2-4 times higher than the imported produce. The observed differences may be indicative of the differences in the quality of locally consumed and exported products.

![Graph showing mercury concentrations](image)

**Figure 3:** Variation of total mercury concentrations in brine preserved canned sardine from Canada, Norway and Thailand.

The mean (wt) concentration of mercury in canned sardine samples in this study (0.029 ± 0.016 µg/g) was lower than 0.08 ± 0.04 µg/g reported for sardines imported to Papua New Guinea (Kyle, & Ghani, 1983) and the range (0.009 to 0.057 µg/g) was higher than those (0.004 to 0.030 µg/g) reported for the same country, but lower than 0.05 to 0.14 reported for another study (NHMRC, 1985). Figure 3 shows that the highest mercury concentration (0.057 µg/g) was obtained in canned sardine imported from Norway. Also, the mean (0.056 ± 0.040 µg/g) and range (0.019 to 0.093 µg/g) of mercury concentration obtained for mackerel in this study were lower than 0.17 ± 0.09 µg/g and 0.05 to 0.51 µg/g reported for canned mackerel imported to Papua New Guinea (Kyle, & Ghani, 1983). Generally, the mercury concentrations found in the various sardine samples were much lower than the levels found in the tuna, salmon, kipper and mackerel samples.

**Table 3:** Variations of Mercury Concentrations found in Different Canned Fish Samples by CV-AAS

<table>
<thead>
<tr>
<th>Sample</th>
<th>Country of origin</th>
<th>Can 1 (µg/g)</th>
<th>Can 2 (µg/g)</th>
<th>Can 3 (µg/g)</th>
<th>Mean±σ (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuna</td>
<td>Thailand</td>
<td>0.068</td>
<td>0.075</td>
<td>0.089</td>
<td>0.077</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(± 0.003)</td>
<td>(± 0.003)</td>
<td>(± 0.002)</td>
<td></td>
</tr>
<tr>
<td>Salmon</td>
<td>Australia*</td>
<td>0.103</td>
<td>0.115</td>
<td>0.204</td>
<td>0.141</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(± 0.009)</td>
<td>(± 0.006)</td>
<td>(± 0.001)</td>
<td></td>
</tr>
<tr>
<td>Sardine</td>
<td>Canada</td>
<td>0.024</td>
<td>0.033</td>
<td>0.031</td>
<td>0.029</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(± 0.005)</td>
<td>(± 0.003)</td>
<td>(± 0.001)</td>
<td></td>
</tr>
</tbody>
</table>

*Produced for local consumption.

The random analysis of three cans of brine preserved fish samples form the same supplier and country of origin revealed, as shown in Table 3, that the mercury concentrations are reasonably consistent and are often within the specified limit (WHO, 1972). Also, no mercury was found in the brine preservative for all the canned fish samples tested in this study and, thus, indicate that mercury is not leached from the samples during storage in the cans.
Conclusions

The wet decomposition of the brine preserved canned fish with the HNO3-H2SO4 mixture provide a rapid and reliable approach for the accurate determination of mercury by cold vapour atomic absorption spectrophotometry. Variation of mercury concentrations based on fish type and origin were easily detected by this method. Also, the test of the accuracy of the method with spiked fish samples revealed that it is effective for complete recovery of inorganic and organic mercury. Australian produced brine preserved canned tuna and salmon for local consumption were found to contain significantly higher mercury concentrations that those found in similar imported fish. Nevertheless, the mercury concentrations found in all brine preserved canned fish samples tested in this study were lower than the maximum limit of 0.5 µg/g recommended for fish consumption (WHO, 1972).

References