Spotlight

Food & Beverage Analysis

Accurate Analysis of Low Levels of Mercury in Fish Using Vapour Generation AA

Mercury is a toxic environmental pollutant that can be deadly to humans. It is found in three different forms: the metallic element, inorganic salts and organic compounds. Elemental mercury can be released into the atmosphere by natural occurrences such as volcanic eruptions, but the majority is produced by human activities. It has been estimated that coal fired power plants, waste incineration, metal processing and cement production produce approximately 75% of the 5,500 tons of mercury that are released into the atmosphere each year [1].

Due to its high volatility, mercury becomes airborne very easily. Once in the atmosphere, it can travel huge distances before eventually being deposited in rivers or oceans. In aquatic environments, mercury is transformed into methyl mercury by both microorganisms and abiotic reactions. Methyl mercury becomes increasingly concentrated in the marine food chain, in a process referred to as biomagnification, and can reach extremely high levels in predatory fish such as swordfish, tuna, king mackerel and shark. Methyl mercury can make up more than 90% of the total mercury in fish and seafood. The consumption of these fish and other marine organisms is the main route of human exposure to methyl mercury.

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The toxicity of methyl mercury was first recognised in Japan after a chemical company released large amounts of methyl mercury into Minamata Bay. This caused severe mercury poisoning in local people, with symptoms including damage to hearing and speech, muscle weakness and visual impairment. In severe cases, paralysis, coma and death followed within weeks of the onset of symptoms. Methyl mercury is so acutely toxic to humans because of its ability to pass through the meninges into the brain. Similarly, in pregnant women, methyl mercury can cross the placenta and damage the developing nervous system of the fetus.

The Zero Mercury Working Group, a coalition of different environmental organisations, claims that fish and seafood products should carry labelling informing consumers on the potential risks associated with their consumption. In its recent report [2], the group demands an effective response from governments and the United Nations in relation to the human health hazards caused by mercury in fish, especially for vulnerable groups of people such as pregnant women or breastfeeding women. According to the report, fish tested in different locations around the world show that internationally accepted exposure levels for methyl mercury are exceeded, often by wide margins.

The recognition of the toxicity of methyl mercury and the realisation that fish is the major source of human exposure has led to the development of legislation by governments and health organisations throughout the world.

REGULATIONS

The majority of countries and global organisations now enforce maximum concentrations of mercury in fish of approximately 0.5mg/kg wet weight. There are differences in maximum mercury levels between countries and some variations depending on the type of fish. Most countries also legislate specifically for methyl mercury, although there are some that provide guidelines for total mercury levels as well.

The European Food Safety Authority (EFSA) endorses the provisional tolerable weekly intake of 1.6mg/kg of methyl mercury. The European Commission has introduced the EC 1881/2006 [3] regulation, setting maximum levels for certain contaminants in foodstuffs. With regards to mercury in fishery products and muscle meat of fish, the rule mandates a maximum concentration of 0.5mg/kg wet weight. This maximum level applies to crustaceans, however excludes the brown meat of crab and the head and thorax meat of lobster and similar large crustaceans. For the muscle meat of carnivorous fish, including anglerfish, Atlantic catfish, redfish shark, snake mackerel, swordfish and tuna, the regulation specifies a maximum concentration of 1 mg/kg wet weight.

The Codex Alimentarius 193-1995 [4] general standard for contaminants and toxins in foods requires a maximum concentration of 0.5mg/kg wet weight of methyl mercury in fresh or processed fish and fish products moving in international trade, except for predatory fish. The guideline level intended for methyl mercury in predatory fish such as shark, swordfish, tuna and pike is 1 mg/kg wet weight.

In order to comply with these stringent regulations, companies need a reliable method for the analysis of mercury in fish. Atomic Absorption (AA) spectrometry is the perfect tool for the measurement of low levels of mercury in fish. For laboratories interested in total mercury measurements, the technique provides fast and accurate analysis of samples with detection limits below 0.07ppb (µg/L) in solution, when combined with a vapour generation accessory. This equates to 0.014mg/kg in the original fish sample, based on a 0.5g in 100 mL preparative method, which easily meets the standards demanded by food safety legislation. For laboratories analysing methyl mercury, AA spectrometry provides an excellent screening tool. Its cost-effectiveness and ease-of-use make it a perfect partner to more complex and expensive techniques, such as HPLC-ICP-MS or GC-ICP-MS. This method is also very fast and allows analysis in around 90 seconds per sample.

An experiment was developed to demonstrate the capability of AA spectrometry to achieve precise, dependable analysis of low levels of mercury in fish.

EXPERIMENTAL

A Thermo Scientific iCE 3500 AA spectrometer was used to perform this analysis, combining high-precision optics, state-of-the-art design and user-friendly software to provide unrivalled analytical performance. The spectrometer was coupled to a Thermo Scientific VP100 vapour generation accessory using a continuous flow system to produce a steady-state signal providing excellent analytical precision. The continuous flow of reagents ensured that the system was self-cleaning, reducing memory effects and increasing sample throughput. The VP100 was entirely controlled by the Thermo Scientific SOLAAR software, meaning that setting up a method and running an analysis was extremely simple. A mercury cell provided as standard with the VP100 was also used. This accessory provided an increased pathlength compared to a normal vapour cell and achieved exceptionally low detection limits.

SAMPLE PREPARATION

Three different types of fish sample were used during the evaluation of this method: fresh fish (salmon) obtained from a supermarket; canned fish (sardine), also obtained from a supermarket; and DORM-2 certified reference material (National Research Council of Canada, Institute for National Measurement Standards, Ottawa, Canada). Sample preparation involved a four-step procedure including sample drying, sample preparation, sample digestion and mercury reduction *(Figure 1)*.

Sample drying may not be applicable to all situations, as it is only necessary if the final mercury concentration is needed as a dry weight value, however, Codex Alimentarius and EU Commission specify concentrations of mercury in a wet weight of sample. If dry weight measurements are needed, then the fish samples should be homogenised and dried in an oven at 80°C until they reach a constant weight. Alternatively, the fish tissue can be freeze-dried and homogenised using a mortar and pestle. After drying, portions of approximately 0.5g should be accurately weighed out for digestion. For wet weight measurements, the fresh fish should be homogenised in a food processor and a portion of approximately 0.5g should be accurately weighed and placed in a microwave digestion vessel. This provides a representative fish sample.

Following preparation in the manner, 1mL of 1000ppb Hg standard solution was added to half of the salmon and sardine samples. This spike gave a concentration of 10ppb Hg in the final 100mL sample. The other half of the samples did not have mercury added to them to allow for the calculation of spike recoveries. The microwave digestion vessels containing the samples were placed in a fume extraction hood before adding 10mL concentrated HNO_3. The vessels were left for at least 30 minutes without their lids on to allow gases to escape and they were subsequently placed into a microwave digestion system and digested. Alternatively, a hot-block digestion could have been used to obtain suitable results.

Following digestion, the samples were transferred to a 100mL graduated flask and 60mL of 6% potassium permanganate solution was added. The sample vessels were left for at least 2 hours to ensure that all the mercury in the sample was reduced to Hg^{2+}. It is very important to check that the vessels are not sealed during this stage, as gases are produced that could cause pressure to build up. After the mercury was reduced, 15mL of 20% hydroxylamine chloride solution was added to remove the excess potassium permanganate. Care was taken during the addition of the hydroxylamine chloride, as this produces an exothermic reaction and the vessel may become hot.

It was essential to add the hydroxylamine chloride slowly during that stage and to gently mix the solution during the addition. Without these precautions, a violent reaction may occur that could eject some sample from the flask, leading to inaccurate results. After allowing the solution to cool, deionised water was added to increase the volume up to 100mL.

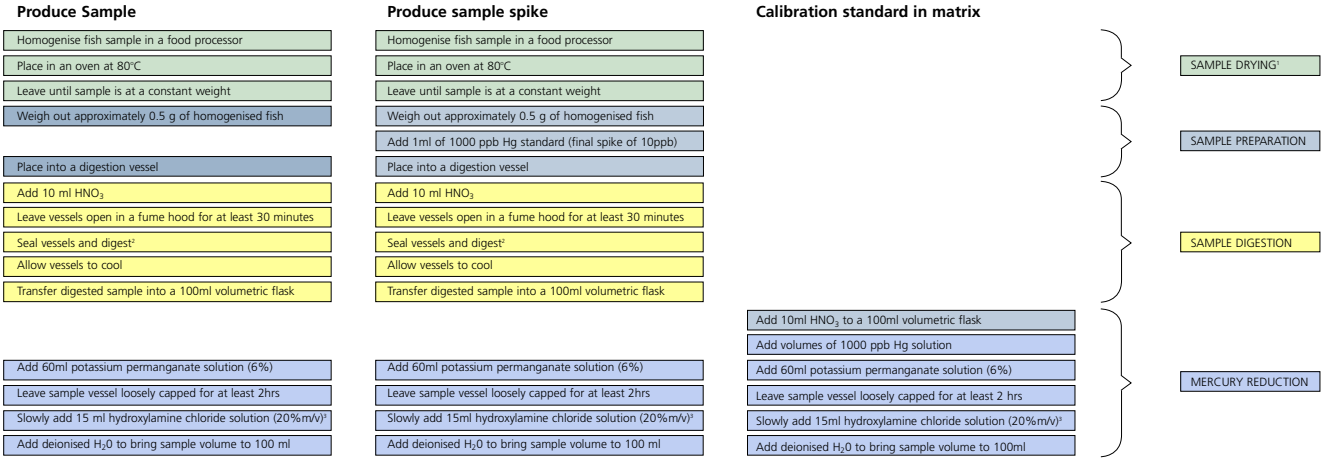

Figure 1. The procedure for preparing samples, sample spikes and matrix-matched standards for the analysis of mercury in fish.
1. Sample drying phase is not necessary if the final concentration of mercury is needed for a wet-weight sample
2. Refer to the manufacturers guidelines when designing a digestion programme.
3. CARE: The reaction is exothermic and the flask may become hot. Also, make sure to add the hydroxylamine chloride slowly, otherwise the solution may foam and eject some sample from the flask.

STANDARD PREPARATION

Standards were prepared from a 1000 ppm (mg/L) mercury standard solution. This standard was first diluted to produce a 1000 ppb (µg/L) stock solution to allow simple preparation of a range of standards. To demonstrate the linear range of AA spectrometry, a wide range of standards were used (1 – 100 ppb). The standards were matrix matched and prepared in the same order as the samples.

VP100 REAGENT PREPARATION

The VP100 requires both a reductant and an acid solution to perform the reactions that form the gaseous mercury. For this application, the reductant was a solution of 7.5% stannous chloride ($SnCl_2$) stabilised in 10 % HCl. The acid solution was 50% HCl.

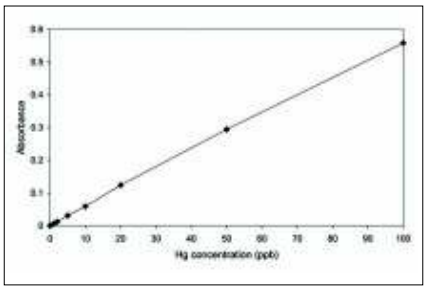

Figure 2. Calibration curve produced for the analysis of mercury in fish samples. Matrix matched standards were used.

INSTRUMENT CONDITIONS

The analysis was performed using the most sensitive absorption wavelength for mercury at 253.7 nm. Five resamples were used, with each resample taking four seconds, to thoroughly assess the short-term stability of the instrument during the development of this method. For normal use, three resamples would be adequate. Deuterium background correction was used throughout the analysis.

RESULTS

The calibration curve showed excellent linearity up to 100 ppb (*Figure 2*), which is equivalent to 20 mg/kg in a fish sample (assuming a sample weight of 0.5g) with an R^2 value of 0.9989. This demonstrates the superb performance of AA spectrometry over a wide concentration range. This calibration is equivalent to concentrations of 0 – 20mg/kg mercury in the original fish samples, assuming a sample mass of exactly 0.5g. The % relative standard deviations (%RSDs) for each of the standards were less than 2.5%. This demonstrates the excellent stability of both the spectrometer and the VP100 accessory. The method detection limit (MDL) and characteristic concentration were calculated using the automated 'Instrument Performance' Wizard in the SOLAAR software. This user-friendly feature guides users through the steps necessary to quantify the performance of the method. It also automates all of the data processing, making the entire procedure quick and easy. The method was found to have a detection limit of 0.068 ppb (µg/L) in solution. This equates to a MDL of 0.014 mg/kg in the original fish sample (assuming a sample mass of 0.5 g).

The MDL provides a measure of the noise and stability of the system. A lower detection limit allows allows for confident determination of lower concentrations of mercury in samples. The characteristic concentration is related to the sensitivity of the method. The characteristic concentration of this method was found to be 0.724 ppb in solution. This would be the equivalent of 0.145 mg/kg in the initial fish sample (assuming a sample weight of 0.5g).

Salmon and sardine samples were spiked with 10 ppb mercury prior to digestion and compared with unspiked samples to calculate recoveries. These 10 ppb spikes would correspond to a concentration of 2 mg/kg in normal fish samples (assuming a sample weight of 0.5g) and demonstrate the accuracy of the analysis at levels appropriate to current legislation. The spike recoveries are shown in *Tables 1 and 2*. The agreement with expected results was excellent, with the recovered values all falling within 6% of the expected values. This demonstrated the repeatability and accuracy of both the sample digestion procedure and the vapor analysis using AA spectrometry.

To ensure the accuracy of the sample preparation, digestion and analysis, three separate samples of the DORM-2 standard reference material were also analysed (*Table 3*). The recoveries from these samples were also excellent, with an accuracy of ±2% or better.

Table 1. Table of results showing the expected concentration, measured concentration and percentage spike recovery for three separate sardine samples.

Sample	Expected Concentration (mg/kg)	Measured Concentration (mg/kg)	Percentage Recovery (%)
Sardine 1	2	1.93	97
Sardine 2	2	2.08	104
Sardine 3	2	1.91	95

Table 2. Table of results showing the expected concentration, measured concentration and percentage spike recovery for three separate salmon samples.

Sample	Expected Concentration (mg/kg)	Measured Concentration (mg/kg)	Percentage Recovery (%)
Salmon 1	2	1.89	94
Salmon 2	2	1.94	97
Salmon 3	2	1.99	99

Table 3. Table of results showing the expected concentration, measured concentration and percentage spike recovery for three samples of the DORM-2 reference material.

Sample	Expected Concentration (mg/kg)	Measured Concentration (mg/kg)	Percentage Recovery (%)
DORM-2 1	4.64 ± 0.26	4.59	99
DORM-2 2	4.64 ± 0.26	4.53	98
DORM-2 3	4.64 ± 0.26	4.57	98

CONCLUSION

AA spectrometry combined with a vapor generation accessory offers excellent linear range, stability and accuracy for the analysis of trace levels of mercury in fish. The superb sensitivity, precision and excellent detection limits of this method easily meet the levels required by the Codex Alimentarius and EU Commission regulations. The speed and efficiency of the vapour generation accessory allows the analysis of a sample approximately every 90 seconds. The method is quick and simple to set up offering repeatable and robust analysis, providing an ideal solution for the screening and analysis of fish samples for potential mercury contamination.

REFERENCES

1. United Nations Environmental Programme (2002) Global Mercury Assessment.
2. Food Safety Information Website, http://www.foodhaccp.com.
3. Commission Regulation (EC) No 1881/2006 of 19 December 2006: Setting maximum levels for certain contaminants in foodstuffs.
4. Codex General Standard for Contaminants and Toxins in Foods CODEX STAN 193-1995, Rev.3-2007.