

Accurate Analysis of Low Levels of Mercury in Fish by Vapor Generation AA

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Key Words

Atomic Absorption, Fish, Hydride Generation, Mercury, Methyl Mercury, Microwave Digestion, Vapor Generation

Key Benefits

- The dedicated vapor generation accessory (VP100) offers fast, repeatable and robust analysis
- The sensitivity and precision of the method easily meets the detection limits required by all current international guidelines
- The SOLAAR software is easy to use and gives step-by-step instructions to allow quick set-up and optimization of the method

Summary

The Thermo Scientific iCE 3000 Series Atomic Absorption Spectrometers are the perfect tool for the measurement of low levels of mercury in fish. With the addition of a VP100 vapor generation accessory the iCE 3000 Series Atomic Absorption Spectrometers are capable of reaching detection limits of 0.07 ppb ($\mu\text{g/L}$) in solution. This is equivalent to 0.014 mg/kg in the initial fish sample (based on a 0.5 g in 100 mL preparation), which easily meets the standards demanded by food safety legislation throughout the world. This method is also very fast and allows analysis in around 90 seconds per sample.

Introduction

Mercury is a significant and toxic environmental pollutant that can be deadly to humans. It is found in three different forms: the metallic element, inorganic salts and organic compounds (e.g., methyl mercury, ethyl mercury and phenyl mercury). Elemental mercury can be released into the atmosphere by natural occurrences such as volcanic eruptions, but the majority is produced by human activities. 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 year¹.



Due to mercury's low boiling point it 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. The 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. The consumption of these fish and other marine organisms is the main route of human exposure to methyl mercury.

The toxicity of methyl mercury was first recognized 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. The reason for the acute toxicity of methyl mercury to humans is 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 recognition of the toxicity of methyl mercury and the realization that fish is the major source for humans has led to the development of legislation by governments and health organizations throughout the world. The majority of countries and global organizations now enforce maximum concentrations of mercury in fish of approximately 0.5 mg/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 too. For more detailed information see Table 1.

Table 1: The maximum or guideline levels for mercury in seafood adopted by various countries or international regulatory bodies

Organisation	Limit	Fish Type	Mercury Level	
			Total (mg/kg)	Organic (mg/kg)
EU Commission	Maximum Level ¹	Non-carnivorous fish & crustaceans ⁸		0.5
		Carnivorous fish ⁸		1
Codex Alimentarius	Guideline Level ²	Non-carnivorous fish & crustaceans ⁸		0.5
		Carnivorous fish ⁸		1
US FDA	Maximum Level ³	Fish, shellfish, crustaceans & other aquatic animals		1
China	Maximum Level ⁴	Fish (excluding carnivorous fish) & other aquatic products		0.5
		Carnivorous fish (e.g., shark, tuna, etc.)		1
Japan	Maximum Level ⁵	All fish, shellfish & aquatic products	0.4	0.3
Australia	Maximum Level ⁶	Crustaceans, molluscs & non-carnivorous fish	0.5	
		Carnivorous fish & fish samples with low sample numbers	1	
Canada	Maximum Level ⁷	Edible portion of all retail fish with six exceptions ⁸	0.5	
		Edible portion of six carnivorous fish ⁸	1	

- 1 Commission Regulation (EC) No 1881/2006 of 19 December 2006: Setting maximum levels for certain contaminants in foodstuffs
- 2 Codex General Standard for Contaminants and Toxins in Foods CODEX STAN 193-1995, Rev.3-2007
- 3 Action Levels for Poisonous or Deleterious Substances in Human Food and Animal Feed (2000)
- 4 USDA Foreign Agricultural Service, GAIN Report (CH6064), "China, Peoples Republic of; FAIRS Product Specific; Maximum Levels of Contaminants in Foods; 2006)
- 5 National Oceanic and Atmospheric Administration Technical Memorandum, "A survey on Japan's import regulations on fish and shellfish products" (1980)
- 6 Standard 1.4.1 – Contaminants and Natural Toxicants
- 7 Canadian Standards ("Maximum Limits") for Various Chemical Contaminants in Foods
- 8 See the specific standard for details of the exact fish species that fall under each limit

The iCE 3000 Series Atomic Absorption Spectrometers and accessories are perfect tools for the analysis of low levels of mercury in fish. For laboratories interested in total mercury measurements they provide

fast and accurate analysis of samples with detection limits below 0.07 ppb ($\mu\text{g/L}$) in solution. This equates to 0.014 mg/kg in the original fish sample, based on a 0.5g in 100 mL preparative method. For laboratories analyzing methyl mercury, the iCE 3000 Series Atomic Absorption Spectrometers provide an excellent screening tool. Their cost-effectiveness and ease-of-use makes them a perfect partner to more complex and expensive techniques, such as HPLC-ICP-MS or GC-ICP-MS.

This application note gives details of the reagents, sample preparation and instrument conditions needed to analyze low levels of mercury in fish. The method has been evaluated using both spiked fish samples and certified standard materials containing mercury levels relevant to current global legislation.

Instrumentation

The Thermo Scientific iCE 3500 Atomic Absorption Spectrometer was used during this analysis, although similar results could also be obtained on both the iCE 3300 and the 3400 models. The iCE 3000 Series Atomic Absorption Spectrometers combine high-precision optics, state-of-the-art design and user-friendly software to provide unrivalled analytical performance.

A VP100 vapor generation accessory is also necessary to perform this analysis. The unique VP100 uses a continuous flow system to produce a steady-state signal and provides excellent analytical precision. The continuous flow of reagents ensures that the system is self-cleaning, reducing memory effects and increasing sample throughput. The VP100 is entirely controlled by the SOLAAR software, meaning that setting up a method and running an analysis is extremely simple.

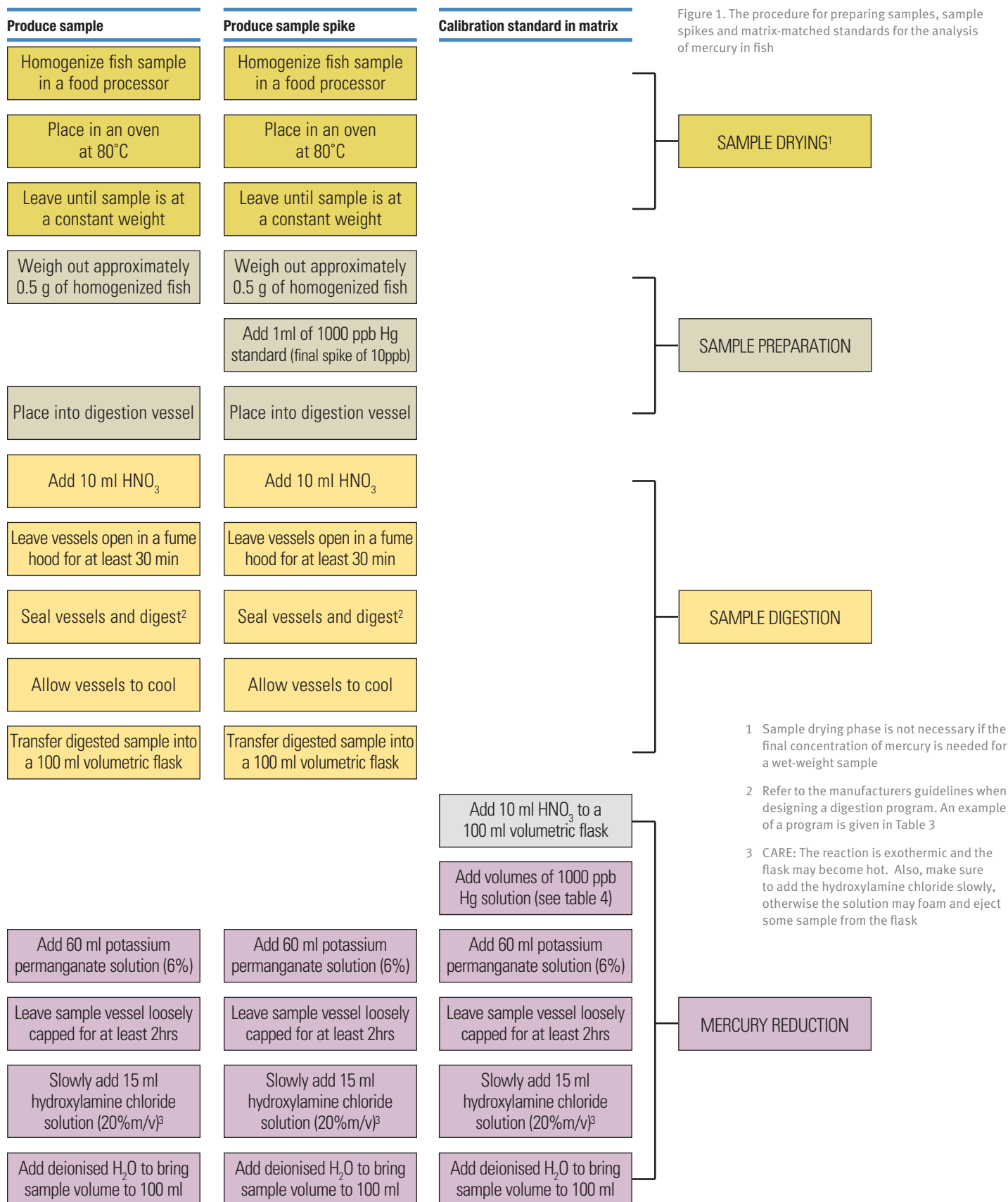
A mercury cell (provided as standard with the VP100) was also used. This accessory provides an increased path-length compared to a normal vapor cell and gives exceptionally low detection limits.



Sample and reagent summary

The sample preparation procedure is shown in Figure 1. There are four main sections: sample drying, sample preparation, sample digestion and mercury reduction. The drying section may not be applicable for all situations, as it is only necessary if the final mercury concentration is needed as a dry weight value, e.g., mg/kg dry weight.

Most countries and official regulatory bodies (e.g., Codex Alimentarius, US FDA, EU Commission) specify concentrations of mercury in a wet weight of sample.



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) stabilized in 10 % HCl. The acid solution was 50 % HCl. Refer to Table 5 for some guideline figures of how much reductant and acid might be needed.

Table 5. Estimate of reagent use during the analysis described in this application note

Pump Speed	Gas Flow	Reagents used per sample ¹			Signal Stabilization ²
		Acid (mL)	Reductant (mL)	Sample (mL)	(s)
40	200	1.40	3.20	15.00	70

- 1 Reagent use based on a total sample analysis time of 90 seconds. This is equivalent to the signal stabilization time and five resamples, each taking four seconds
- 2 This approximation is based on the time taken for the system to stabilize during the analysis of a 10 ppb standard solution

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. This was used 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. The parameters used for both the VP100 and spectrometer are shown in Table 6. For further details on how to optimize the VP100 parameters for your analysis, please refer to the iCE 3000 Series Operator Manual.

Table 6. Summary of the parameters used for the analysis of mercury in fish for this application note

Spectrometer Parameters	
Wavelength :	253.7 nm
Lamp Current :	75 %
Bandpass :	0.5 nm
Background :	D2 Quadline
Correction Resamples :	5
Measurement Time :	4.0s
VP100 Parameters	
Pump Speed :	40 rpm
Gas Flow :	200 ml/min
Acid Reagent :	50 % HCl
Reductant :	7.5 % stannous chloride in 10 % HCl
Measurement Delay :	70

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 0.5 g) with an R^2 value of 0.9989. This shows the superb performance of the iCE 3000 Series Atomic Absorption Spectrometers over a wide concentration range. This calibration is equivalent to concentrations of 0 – 20 mg/kg mercury in the original fish samples, assuming a sample mass of exactly 0.5 g. 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.

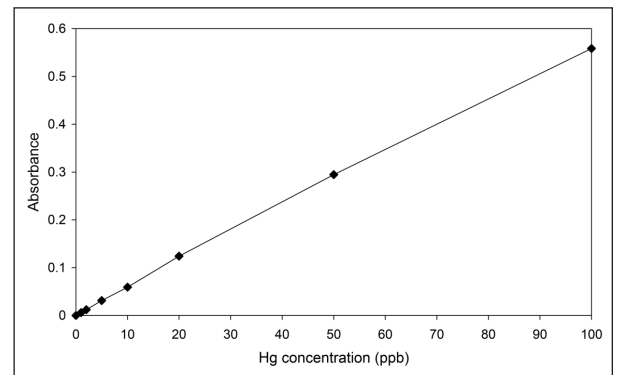


Figure 2. Calibration curve produced for the analysis of mercury in fish samples. Matrix matched standards were used

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 you through the steps necessary to quantify the performance of your 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 ($\mu\text{g/L}$) 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 you to confidently determine lower concentrations of mercury in your 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 0.5 g).

Table 7. Detection limit and characteristic concentration data

Pump Speed	Gas Flow	Detection Limit		Characteristic Concentration	
		Solution (ppb)	Sample (mg/kg) ¹	Solution (ppb)	Sample (mg/kg) ¹
40	200	0.07	0.01	0.7	0.1

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 0.5 g) and demonstrate the accuracy of the analysis at levels appropriate to current legislation. The spike recoveries are shown in Tables 8 and 9. The agreement with expected results is excellent, with the recovered values all falling within 6 % of the expected values. This demonstrates the repeatability and accuracy of both the sample digestion procedure and the vapor analysis using the Thermo Scientific iCE 3000 Series Atomic Absorption Spectrometers.

Table 8. 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 9. 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	94.7
Salmon 2	2	1.948	97.4
Salmon 3	2	1.99	99

To ensure the accuracy of the sample preparation, digestion and analysis, three separate samples of the DORM-2 standard reference material were also analyzed (Table 10). The recoveries from these samples were also excellent, with an accuracy of ± 2 % or better.

Table 10. 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 \pm 0.26	4.59	99
DORM-2 2	4.64 \pm 0.26	4.538	98
DORM-2 3	4.64 \pm 0.26	4.57	98

Conclusions

The results shown in this application note show that the iCE 3000 Series Atomic Absorption Spectrometers and VP100 vapor generation accessory offer excellent linear range, stability and accuracy during the analysis of trace levels of mercury in fish. Their superb sensitivity and excellent detection limits easily meet the levels required for all current worldwide legislation (Table 1). The speed and efficiency of the VP100 allows the analysis of a sample approximately every 90 seconds. The SOLAAR software controls every aspect of the spectrometer and VP100 and makes setting up the method quick and simple. These characteristics mean that the iCE 3000 Series Atomic Absorption Spectrometers provide 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, <http://www.chem.unep.ch/MERCURY/Report/GMA-report-TOC.htm>

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