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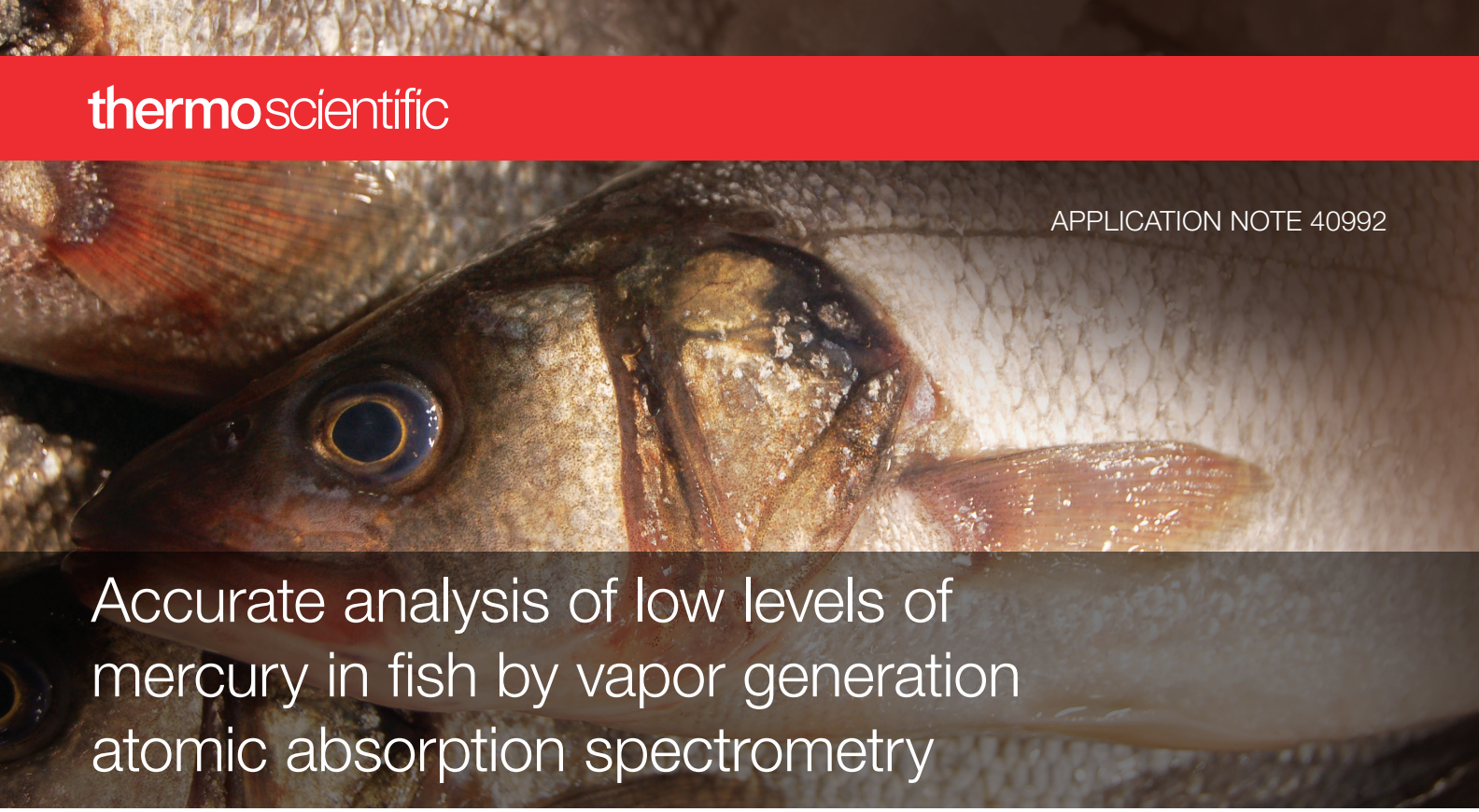

APPLICATION NOTE 40992

Accurate analysis of low levels of mercury in fish by vapor generation atomic absorption spectrometry

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Keywords

Atomic absorption, Fish, Hydride generation, Mercury, Methyl mercury, Microwave digestion, Vapor generation

Goal

The Thermo Scientific™ iCE™ 3000 Series Atomic Absorption Spectrometers coupled with the Thermo Scientific™ VP100 continuous flow vapor generation system offers fast, repeatable and robust analysis of mecury in fish according to a range of international guidelines.

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 year.

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. Mercury enters and accumulates 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 mercury.

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The majority of countries and global organizations now enforce maximum concentrations of mercury in fish of approximately 0.5 mg·kg^{-1} wet weight. There are differences in maximum mercury levels between countries and some variations depending on the type of fish (Table 1).

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

Organisation		Maximum mercury level mg·kg^{-1}
Japan	All fish, shellfish & aquatic products	0.4
Australia	Crustaceans, molluscs & non-carnivorous fish	0.5
	Carnivorous fish & fish samples with low sample numbers	1
Canada	Edible portion of all retail fish with six exceptions	0.5
	Edible portion of six carnivorous fish	1

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 µg·L^{-1} in solution. This equates to 0.014 mg·kg^{-1} in the original fish sample, based on a 0.5 g in 100 mL preparative method.

Sample preparation

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.

Produce sample	Produce sample spike	Calibration standard in matrix
Homogenize fish sample in a food processor	Homogenize fish sample in a food processor	
Place in an oven at 80 ˚C	Place in an oven at 80 ˚C	
Leave until sample is at a constant weight	Leave until sample is at a constant weight	
Weigh out approximately 0.5 g of homogenized fish	Weigh out approximately 0.5 g of homogenized fish	
	Add 1 ml of 1000 µg·L^{-1} Hg standard (final spike of 10 µg·L^{-1})	
Place into digestion vessel	Place into digestion vessel	
Add 10 ml HNO_3	Add 10 ml HNO_3	
Leave vessels open in a fume hood for at least 30 min	Leave vessels open in a fume hood for at least 30 min	
Seal vessels and digest[2]	Seal vessels and digest[2]	
Allow vessels to cool	Allow vessels to cool	
Transfer digested sample into a 100 ml volumetric flask	Transfer digested sample into a 100 ml volumetric flask	
		Add 10 ml HNO_3 to a 100 ml volumetric flask
		Add the required volumes of 1000 µg·L^{-1} Hg solution
Add 60 ml potassium permanganate solution (6%)	Add 60 ml potassium permanganate solution (6%)	Add 60 ml potassium permanganate solution (6%)
Leave sample vessel loosely capped for at least 2hrs	Leave sample vessel loosely capped for at least 2 hrs	Leave sample vessel loosely capped for at least 2 hrs
Slowly add 15 ml hydroxylamine chloride solution (20%m/v)[3]	Slowly add 15 ml hydroxylamine chloride solution (20%m/v)[3]	Slowly add 15 ml hydroxylamine chloride solution (20%m/v)[3]
Add deionised H_2O to bring sample volume to 100 ml	Add deionised H_2O to bring sample volume to 100 ml	Add deionised H_2O to bring sample volume to 100 ml

Sample drying[1]

Sample preparation

Sample digestion

Mercury reduction

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 program. An example of a program is given in Table 3.
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.

Figure 1. The procedure for preparing samples, sample spikes and matrix-matched standards for the analysis of mercury in fish.

Three different types of fish sample were used during the evaluation of this method: fresh fish (salmon) obtained from a local supermarket; canned fish (sardine), also obtained from a local supermarket; and DORM-2 certified reference material (National Research Council of Canada, Institute for National Measurement Standards, Ottawa, Canada).

If dry weight measurements are needed then the fish samples should be homogenized and dried in an oven at 80 ˚C until they reach a constant weight. Alternatively, the fish tissue can be freeze-dried and homogenized using a mortar and pestle. After drying, portions of approximately 0.5 g should be accurately weighed out for digestion. For wet weight measurements the fresh fish should be homogenized in a food processor and a portion of approximately 0.5 g should be accurately weighed and placed in a microwave digestion vessel. This provides a representative fish sample.

Following preparation in this manner, 1 mL of 1000 µg·L^{-1} Hg standard solution was added to half of the salmon and sardine samples. This spike gave a concentration of 10 µg·L^{-1} Hg in the final 100 mL sample. The other half of the samples did not have mercury added to them to allow the calculation of spike recoveries.

The microwave digestion vessels containing the samples were placed in a fume extraction hood before adding 10 mL concentrated HNO_3. The vessels were left for at least 30 minutes without their lids on to allow gases to escape. After this time the vessels were placed into a microwave digestion system and digested according to the manufactures guidance.

After digestion the samples were transferred to a 100 mL graduated flask and 60 mL 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, 15 mL 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 is essential to add the hydroxylamine chloride slowly during this 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 make the volume up to 100 mL.

VP100 continuous flow vapor generation system reagent preparation

The VP100 continuous flow vapor generation system 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.

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 continuous flow vapor generation system and spectrometer are shown in Table 2. For further details on how to optimize the VP100 continuous flow vapor generation system parameters for your analysis, please refer to the iCE 3000 Series Operator Manual.

Table 2. 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 continuous flow vapor generation system 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 µg·L^{-1} (Figure 2), which is equivalent to 20 mg·kg^{-1} 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^{-1} 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 continuous flow vapor generation system.

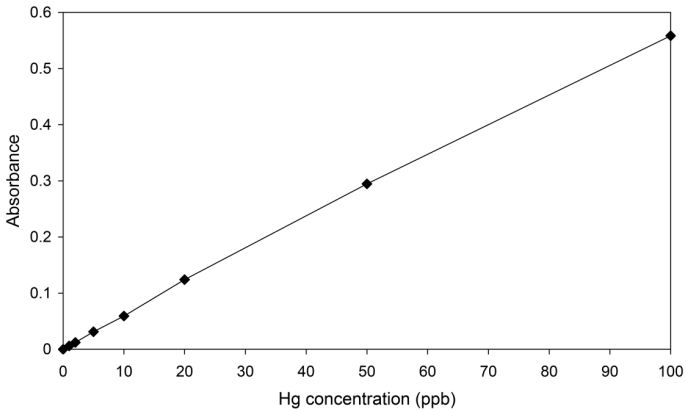

Figure 2. Calibration curve produced for the analysis of mercury in fish samples. Matrix matched standards were used.

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 µg·L^{-1} in solution. This equates to a MDL of 0.014 mg·kg^{-1} 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 µg·L^{-1} in solution. This would be the equivalent of 0.145 mg·kg^{-1} in the initial fish sample (assuming a sample weight of 0.5 g).

Table 3. Detection limit and characteristic concentration data.

Detection limit		Characteristic concentration	
Solution µg·L^{-1}	Sample mg·kg^{-1}	Solution µg·L^{-1}	Sample mg·kg^{-1}
0.07	0.01	0.7	0.1

Salmon and sardine samples were spiked with 10 µg·L^{-1} mercury prior to digestion and compared with unspiked samples to calculate recoveries. These 10 µg·L^{-1} spikes would correspond to a concentration of 2 mg·kg^{-1} 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 4 and 5. 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 4. Table of results showing the expected concentration, measured concentration and percentage spike recovery for three separate sardine samples.

Sample	Expected concentration mg·kg^{-1}	Measured concentration mg·kg^{-1}	Percentage recovery (%)
Sardine 1	2	1.93	97
Sardine 2	2	2.08	104
Sardine 3	2	1.91	95

Table 5. Table of results showing the expected concentration, measured concentration and percentage spike recovery for three separate salmon samples.

Sample	Expected concentration mg·kg^{-1}	Measured concentration mg·kg^{-1}	Percentage recovery (%)
Salmon 1	2	1.89	947
Salmon 2	2	1.948	974
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 6). The recoveries from these samples were also excellent, with an accuracy of ±2% or better.

Table 6. 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 $mg \cdot kg^{-1}$	Measured concentration $mg \cdot kg^{-1}$	Percentage recovery (%)
DORM-2 1	4.64 ± 0.26	4.59	99
DORM-2 2	4.64 ± 0.26	4.538	98
DORM-2 3	4.64 ± 0.26	4.57	98

Conclusions

The results shown in this application note show that the iCE 3000 Series Atomic Absorption Spectrometers and VP100 continuous flow vapor generation system 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 continuous flow vapor generation system allows the analysis of a sample approximately every 90 seconds. The SOLAAR software controls every aspect of the spectrometer and VP100 continuous flow vapor generation system 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.

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