Application Note: 40992

Food Safety Series – 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 range of atomic absorption (AA) spectrometers are a 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 instruments are capable of reaching detection limits of 0.07 ppb (µg/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 year[1].

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.

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Organisation	Limit Type	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	Maxiumum 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

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

The iCE 3000 Series AA 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 (µg/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 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

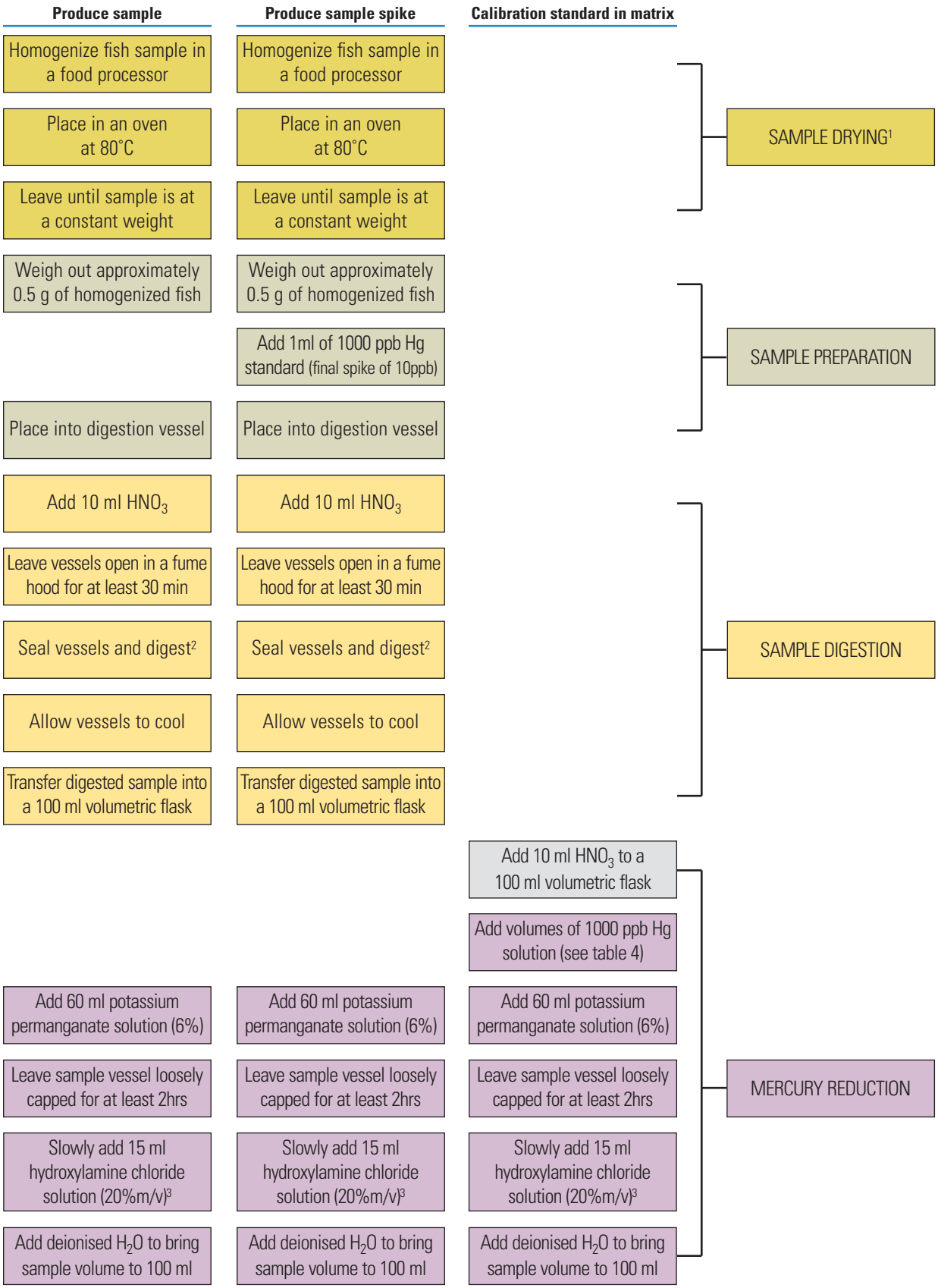
The Thermo Scientific iCE 3500 AA spectrometer was used during this analysis, although similar results could also be obtained on botth the iCE 3300 and the 3400 models. The iCE 3000 Series range of AA spectrometers combine high-precision optics, state-of-the-art design and user-friendly software to provide 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.



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).

Table 2 summarizes all the reagents necessary for the analysis.

Consumables
Nitric Acid
Potassium Permanganate
Hydroxylamine Chloride
Mercury Standard Solution (1000 ppm in 10 % HNO_3)
Stannous (Tin (II)) Chloride
Hydrochloric Acid

Table 2: Summary of all the reagents used during this analysis

If dry weight measurements are needed then the fish samples should 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 ppb Hg standard solution was added to half of the salmon and sardine samples. This spike gave a concentration of 10 ppb 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 using the program shown in Table 3. It is also possible to use a hot-block digestion to obtain suitable results.

Power Max (W)	%	Ramp (min)	Max. Pressure (psi)	Max Temperature (°C)	Hold (min)
800	100	30	180	190	15

Table 3. Microwave digestion program used

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.

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 the iCE 3000 Series AA spectrometers a wide range of standards were used (1 – 100 ppb). The standards were matrix matched and prepared in the same order as the samples. The procedure is summarized in Figure 1 and the exact volumes needed to prepare the standards are shown in Table 4.

		Standard							
		Blank	1	2	3	4	5	6	7
Final Concentration Hg	ppb	0	1	2	5	10	20	50	100
Volume 1000 ppb Hg Stock Solution	mL	0	0.1	0.2	0.5	1	2	5	10
Volume Conc. HNO_3	mL	10	10	10	10	10	10	10	10
Volume Potassium Permanganate Solution (6%)	mL	60	60	60	60	60	60	60	60
Volume Hydroxylamine Chloride (20%)	mL	15	15	15	15	15	15	15	15
Volume Deionized H_2O	mL	15	14.9	14.8	14.5	14	13	10	5
Total Volume	mL	100	100	100	100	100	100	100	100

Table 4: Volumes of stock standard and other reagents needed to prepare a range of standard solutions

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.

Pump Speed	Gas Flow	Reagents used per sample[1]			Signal Stabilization[2]
		Acid	Reductant	Sample	
(rpm)	(mL/min)	(mL)	(mL)	(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

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

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.

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

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

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 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.

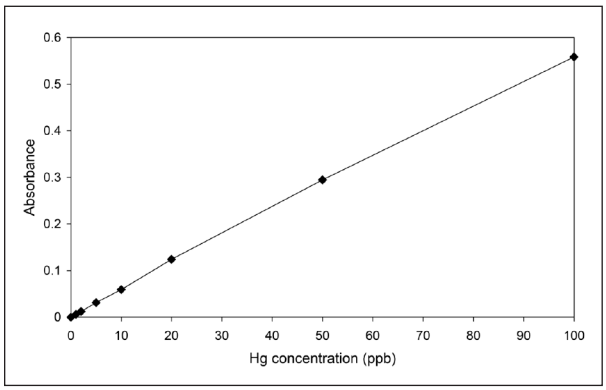


Figure 2: Calibration curve produced for the analysis of mercury in fish samples. Matrix matched standards were used.

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 (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 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).

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

1 The detection limit and characteristic concentration of the sample is based on a sample mass of 0.5 g

Table 7: Detection limit and characteristic concentration data

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 AA spectrometers.

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

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

Table 9: Table of results showing the expected concentration, measured concentration and percentage spike recovery for three separate salmon samples

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.

Sample	Expected Concentration (mg/kg)	Meaasured 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

Table 10: Table of results showing the expected concentration, measured concentration and percentage spike recovery for three samples of the DORM-2 reference material

Conclusions

The results shown in this application note show that the iCE 3000 Series AA 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 AA 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*

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

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AN40992_E 12/08C