Handbook of Analytical Methods for Chemical Elements as Adopted by National Reference Laboratories for Residues of the European Union

(Directive 96 / 23 / EC of April 29, 1996)

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Last update
December 2005

2005

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Preface

In conformity with a project of mutual interaction between CRLs and NRLs, the Community Reference Laboratory for Residues at the Istituto Superiore di Sanità prepares and up-dates the *Handbook of Analytical Methods for Trace Elements as Adopted by National Reference Laboratories for Residues of the European Union*. The aim of the book is to create a tool able to harmonise the different experimental strategies by collecting analytical methods performed in the laboratories of the various Member States. Each NRL is responsible for the content of their own analytical methods.

Arsenic

AUSTRIA
BELGIUM
CZECH REPUBLIC
DENMARK
ESTONIA
FINLAND
FRANCE
GERMANY
GREECE
HUNGARY
IRELAND
ITALY
LATVIA (AOAC 986.15)*
MALTA
NORTHERN IRELAND
POLAND
PORTUGAL
SPAIN
SWEDEN
THE NETHERLANDS
UNITED KINGDOM

**Official analytical methods are not reported*

Austrian Agency for Health and Food Safety

Wien, Austria

Arsenic

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Hydride Generation Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

There are no inquiries for determination of arsenic.

2. Samples throughput per year

No routine samples were analyzed during the last three years.

3. Procedure

Arsenic is assayed by HG-AAS.

3.1. Hydride Generation Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Reduction of As (V) to As (III).

2 ± 0.1 g of homogenized tissue is weighed in 40 ml Teflon vessels. 3 ml HNO_3 (65 %, suprapure) and 1.5 ml H_2O_2 (30 %) are added. The samples are digested in the microwave oven (MLS-Ethos 900) according to the following programme: 1. 100 W, 10 min; 2. 0 W, 1 min; 3. 250 W, 5 min; 4. 0 W, 1 min; 5. 400 W, 5 min; 6. 600 W, 10 min; 7. 500 W, 6 min. After cooling the digested sample solutions are transferred into 10 ml measuring flasks and filled up to 10 ml with deionized water.

Prior to analysis, As (V) is reduced to As (III) in a 10 ml measuring flask 1 ml sample solution is mixed with 1 ml of HCl conc., 5 % KI-solution, and 5 % ascorbic acid, each. The mixture is allowed to react 40-50 min at room temperature and then filled up to 10 ml with deionized water.

3.1.b. Analytical instrumentation

A SIMAA 6000 with FIMS/AS91 (Perkin-Elmer) is used.

3.1.c. Working conditions

Table 1. Equipment characteristics.

HG-AAS
Wavelength, 193.7 nm
Slit width, 0.7 nm
Lamp, As EDL
Injection volume, 500 µl sample/standard
Quartz cell temperature, 900 °C
Argon flow, 70 ml/min
Measurement mode, peak height
Smoothing points, 19
Read time, 15 s
Carrier solution, 3 % HCl
Reducing agent, 0.2 % $NaBH_4$ in 0.05 % NaOH

Table 2. Flow injection programme.

Step	**Time (s)**	**Pump 1 Speed**	**Pump 2 Speed**	**Valve Position**	**Read Step**
Pre-fill	15	100	120	Fill	-
1	5	100	80	Fill	-
2	20	100	80	Inject	X
3	10	100	0	Inject	-

The calibration curve is established by manual dilution of a stock solution (As_2O_5) to the following concentrations: 2, 4, 6 and 8 µg/l. Blank and standard solutions are preriduced as described under point 3.2.a. The blank is subtracted automatically from standards and samples.

4. Quality control

Calibration curves are accepted when the coefficient of correlation is > 0.995. The performance of the instrument is checked by a multielement standard (Perkin-Elmer). Recovery is calculated by spiked samples. CRM 186 (pig kidney) are analyzed in order to check the accuracy and reproducibility of the whole method.

5. Difficulties and limitations

Too high concentrations of HNO_3 prevent a complete reduction of As (V) to As (III) by HCl/KI/ascorbic acid. Dilution of the sample is the simplest way to avoid this problem.

6. Comments and remarks

A method for the more sensitive FIA-AAS (furnace) technique was not established yet. The equipment is available and it is planned to introduce this technique in our laboratory until the end of 1999.

Scientific Institute of Public Health – Louis Pasteur

Brussels, Belgium

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Analytical technique
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples frequently analyzed are fresh seafish, sometimes canned seafood depending on the programme.

2. Samples throughput per year

About. 50 samples per year.

3. Procedure

3.1. Analytical technique

Arsenic is assayed by HG-ICP-AES

3.1.a. Sample pretreatment

About 1 g is weighed on an analytical balance in a polyethylene cup and dropped in a quartz digestion tube. Wet digestion is carried out with the aid of 5 mL of HNO_3 conc. and 3 mL of H2SO4 conc. The tube is placed in mineralizator at 350 °C. After 5 ours, about 3mL of H_2O_2 is added. The tube is placed into mineralizator at 400 °C +/- 2 hour. After complete oxydation the tubes are cooled to ambient temperature. The digest is diluted into the tube to a volume of 5 mL with Milli-Q-water. If necessary, furthers dilutions are realised.

2.5 mL of pre-reduction solution (KI 2.5 % + Ascorbic Acid 2.5 %) and 2.5 mL of HCl Conc are added to the 5 mL of mineralizated solution.

3.1.b. Analytical instrumentation

A Perkin Elmer Optima 4300 DV instrument + FIAS 400.

3.1.c. Working conditions

HG–ICP-AES
Wavelength, 188.979 nm and 193.696 nm
Hydride generation with Natrium Borohydride and NaOH
Plasma: 15L/min
Aux: 0.2 l/min
Power 1400 w
Integration time: 1 to 10 s
Read delay: 70
Mode peak Area
Calibration mode, concentration
Normal resolution

Calibration is carried out with laboratory standard solution, freshly prepared with 2 % v/v conc. HNO_3. The stock is P. Elmer PE Pure. Linear working range is 1-20 ng/ml.

4. Quality control

An analytical run includes blanks and standards. Recovery test are realised on each samples. All glassware and polyethylene flasks and pipets are soaked in a 10 % HNO_3 solution and rinsed with Milli-Q-water just before use. All volumetric flasks, glass pipets are calibrated, plastic pipets are checked regularly. Intercalibration tests are regularly realised.

5. Difficulties and limitations

Matrix effects are often encountered

6. Comments and remarks

Not reported.

State Veterinary Institute Olomouc
Laboratory in Kromeriz

Kromeriz, Czech Republic

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Analytical technique
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are meat, fish, milk, babyfood, feedstuffs.

2. Sample throughput per year

About 500 samples per year.

3. Procedure

Arsenic is assayed by HG-AAS.

3.1. Hydride Generation Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

1 ± 0.1 g of homogenized sample with 5 ml 10 % w/v $Mg(NO_3)_2$ is evaporated to dryness. Sample is ashed at 450 ^oC in a muffle furnace overnight. Ashed sample is cooled down to room temperature, deionized water and 1 ml conc. HNO_3 are added. The sample is re-evaporated and heated up to 450 ^oC in the muffle furnace until ashes become white. The residue is dissolved in 2 ml of deionized water and 5 ml of conc. HCl. 3 ml of a solution containing 10 % KI and 5% ascorbic acid is added in order to reduce As (V) into As (III). The mixture is allowed to react three hours at room temperature and then filled up to 25 ml with deionized water.

3.1.b. Analytical instrumentation

A PerkinElmer AAnalyst-300 spectrometer equipped with a FIAS 400 and an AS 90 autosampler is used.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 1. and 2.

Table 1. Equipment characteristics

HG-AAS
Wavelength: 193.7 nm
Lamp: As EDL
Slit: 0.7 nm
Quarz cell temperature: 900 ^{o}C
Injection volume: 500 µl
Reducing agent: 0.25 % $NaBH_4$ in 0.05 % NaOH

Table 2. Flow injection programme

Step	Time (s)	Pump 1 Speed	Pump 2 Speed	Valve Position	Read Step
Prefill	12	100	0	Fill	
1	6	100	0	Fill	
2	6	100	120	Fill	
3	4	0	120	Inject	
4	15	0	120	Inject	x
5	4	70	0	Fill	
6	1	0	0	Fill	

The calibration curve is established by using As solutions of 5, 10 and 15 µg/l.

4. Quality control

The routine use of a method is preceded by analytical validation according to the IUPAC recommendations and the metod is accreditated. CRMs (BCR 185 R, GBW 8551, TORT 2, …) are use to check the accuracy and analyzed periodically. An analytical run includes two blanks, duplicates and in-house RM. The results of duplicate CRM, RM analysis samples are monitored on control charts.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Danish Institute for Food and Veterinary Research

Søborg, Denmark

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Inductively Coupled Plasma Mass Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are fish and meat.

2. Samples throughput per year

About 10 for ICP-MS.

3. Procedure

The samples are analyzed by ICP-MS.

3.1. Inductively Coupled Plasma Mass Spectrometry

3.1.a. Sample pretreatment

The sample (0.2-0.5 g) is weighed into the quartz bombs of a microwave assisted digestion The Multiwave high pressure microwave digestion system is used for sample decomposition. The maximum pressure is 70 bar and the digestion is finalised within 60 minutes including cooling. Following digestion the residue is taken to 20 ml by weighing using pure water.

3.1.b. Analytical instrumentation

An Elan 6100 DRC Perkin-Elmer/Sciex quadrupole mass spectrometer is used.

3.1.c. Working conditions

A volume of 5 ml of the sample solution is pipetted into a polyethylene autosampler tube and 5 ml of 1 % aqueous HNO_3 are added. Arsenic is measured at *m/z* = 75. Calibration is carried out by the standard addition method. Two additions are used for each sample. A mixture of usually Ga, In and Bi are used for internal standardization in the multielement method in which this element is an analyte.

4. Quality control

The method is accredited by the Danish Accreditation Organisation, DANAK. A matching CRM and blanks are analyzed in parallel with the samples in series containing up to 15 unknown samples. The results of CRMs are monitored on control charts (x- and R-charts). The risk of contamination of samples must always be considered. The critical

utensils are washed/rinsed with acid before use and ultrapure acids are used for the ashing process.

5. Difficulties and limitations

Care must be taken to prevent different ICPMS response from different As-species. If occurring, this is done by adding 3 % methanol to the sample solutions in combination with and increased RF power at 1350 W.

6. Comments and remarks

None reported.

Food Safety Veterinary and Food Laboratory

Tartu, Estonia

Arsenic

1. The equipment in use

1) Atomic Absorption Spectrophotometer-Thermo Elemental. It has equipped both with GFA and flame compartments. Zeemann and Deuterium correction are available. At the moment the Hydride Vapor System –for the determination of Arsenic is missing.
2) Atomic Absorption Spectrophotometer-Shimadzu - It has equipped both with GFA and flame compartments. Only deuterium correction is available.
3) Microwave owen AntonPaar- closed vessels for wet digestion
4) Muffle furnace Nabertherm

2. Analytical Methods

Element	**As**
Accreditation	**yes**
Reference for method	1) AA Spectrometers Methods Manual, 2003, Thermo Electron Corp., UK 2) EVS-EN 13804:2002 –Foodstuffs-Determination of Trace elements- Performance Criteria and general conciderations 3) EVS-EN 13805:2002 Foodstuffs-Determination of trace elements. Pressure digestion
Sample weight (g)	5 g -dry ashing > 1g- mw digestion
Sample preparation	Dry ashing Microwave digestion
Reagents used	HNO_3, H_2O_2, HCl
Modifier	Pd+ $Mg(NO_3)_2$ *$6H_2O$
Quantification (technique)	AAS Graphite Furnace
Wavelenght(nm)	193.7
LOQ (mg/kg)	0.01
U %	30

3. Reference materials

Element	**Matrix**	**Ref. value mg/kg**
As	CRM 422 cod mussel	21.1
	SRM 2976	13.3
	BCR 186	0.063

National Veterinary and Food Research Institute

Helsinki, Finland

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Hydride Generation Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are milk and animal tissues (meat, liver and kidney).

2. Samples throughput per year

About 50.

3. Procedure

The samples are analyzed by HG-AAS.

3.1. Hydride Generation Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

About 1 g of homogenized sample and 2 g of Mg $(NO_3)_2 \cdot 6H_2O$ are weighed into quartz dish, 5-10 ml of deionized water is added and evaporated to dryness on water bath. Sample is ashed at 450 °C in a muffle furnace overnight. Ashed sample is cooled down to room temperature, deionized water and 1 ml conc. HNO_3 are added. The sample is re-evaporated and heated up to 450 °C overnight in the muffle furnace. The residue is dissolved in 4 ml of conc. HCl and adjusted to appropriate volume with deionized water.

3.1.b. Analytical instrumentation

Samples are analyzed by HG-AAS (Perkin-Elmer Model 4100, FIAS 200, electrodeless discharge lamp and EDL System 2 power supply).

3.1.c. Working conditions

Instrumental parameters are reported in Table 1. Use is made of a 20 % KI solution to reduce As (V) to As (III). A solution of $NaBH_4$ is used as the reducing agent. The measurement is carried out with direct comparison with standard solution (Merck, Titrisol) in 10 % HCl.

Table 1. Equipment characteristics.

HG-AAS
Wavelength, 193.7 nm
Lamp, As EDL

More frequently encountered concentration ranges: <0.005-0.025 mg/kg.

4. Quality control

The method is accreditated. CRMs and blanks are analyzed together with each sample series. The results of CRMs samples are monitored on control charts. Analysis of spiked samples are performed. The risk of contamination of samples must always be considered. All glass- and plasticware are washed with acid before use.

5. Difficulties and limitations

Samples and standards must be given sufficient time to reduce any As (V) to As (III) while in contact with the KI solution. This is achieved by standing samples and standards three hours in the reducing KI solution at room temperature.

6. Comments and remarks

Not reported.

Agence Française de Sécurité Sanitaire des Aliments

Maisons-Alfort Cedex, France

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Hydride Generation Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are seafood.

2. Samples throughput per year

Not foreseeable.

3. Procedure

Arsenic is assayed by HG-AAS.

3.1. Hydride Generation Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Mineralization of animal tissues is carried out by dry ashing at 700 °C with $Mg(NO_3)_2$ and MgO as ashing aid for at least 5 h. Ashes are dissolved in 6 *N* HCl and made up to 25 ml. A solution containing 5 % KI and 5 % ascorbic acid is added in order to reduce As (V) into As (III). After 45 min the solution is made up to 50 ml with distilled water. A solution containing 0.2 % $NaBH_4$ in 0.05 % NaOH is used as the reducing agent.

3.1.b. Analytical instrumentation

A Varian SpectrAAA 220 FS instrument equipped with a SP5 is used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1. Use is made of $Mg(NO_3)_2$ and MgO as matrix modifiers.

Table 1. Equipment characteristics.

HG-AAS Wavelength, 193.7 nm As ULTRA AA Lamp Quartz cell temperature, 925 °C

4. Quality control

Blanks determined out with each batch of samples and CRMs (CRM 278R, mussel tissue; CRM 279, Sea lettuce, fish tissue, copepod homogenate) are periodically analyzed.

5. Difficulties and limitations

The critical step is the prereduction of As.

6. Comments and remarks

Not reported.

Federal Office for Consumer Protection and Food Safety

Berlin, Germany

Section I

1. Matrices

2. Samples throughput per year

3. Procedure
3.2. Hydride Generation Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

Section II

Sample ashing procedure

1. Matrices

2. Procedure

SECTION I

1. Matrices

Samples analyzed are meat, meat products, fish, fish products; plant materials, food.

2. Sample throughput per year

About 50.

3. Procedure

Arsenic is assayed by Hydride Generation – AAS.

3.1. Hydride Generation Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Mineralization is described in section II (Sample ashing procedure).

The mineralized sample solution is prepared at special conditions. After opening the mineralization vessels remove the NO_x gases by a gentle stream of nitrogen at the surface of the solution. Let stand the uncovered vessels over a period of 24 hours. Treat the solution in a ultrasonic bath for a minimum of 10 minutes at maximum power. Dilute to 20 ml solution with water. Repeat the ultrasonic treatment for 5 minutes. This solution is now ready for pre-reduction step. If a dilution step is necessary dilute the sample solution with 2.87 mol/l HNO_3 (blank solution) before pre-reduction is performed.

Pipette 4.0 ml of the blank-, standard- and diluted sample solutions in separate 50 ml polyethylene tubes. Add 4.0 ml HCl, 30 % (208 ml 36 % HCl + 42 ml water). and mix well. Add 2.0 ml KI-ascorbic acid solution (3 g KI + 5 g ascorbic acid / 100 ml water) and mix well. Let stand the open tubes at a minimum of 45 minutes at room temperature to complete the reduction to As^{III}. Add 10 ml water. A minimum concentration of 30 % HCl must be guaranteed in every case.

3.1.b. Analytical instrumentation

SpectrAA 300 - AAS with VGA 76 Hydride Generation System.

3.1.c. Working conditions

The calibration curve is plotted by using Arsenic solutions of 1.25, 2.5, 5.0 and 10.0 µg/l in 2.87 mol/l HNO_3 The analytical blank is a 2.87 mol/l HNO_3 (100 ml 65 % HNO_3 with 400 ml water). The calibration solutions and the blank solution pass through the same

procedure as the sample solutions. A $NaBH_4$ solution, (3 g $NaBH_4$ + 2.5 g NaOH / 500 ml) together with concentrated HCl is used as reducing agent.

The limit of detection is about 0.75 ng As/ml in sample solution.

4. Quality control

Analysis of certified reference materials (BCR, NIST, CBNM) is performed along with each analytical series. Analysis of blank and/or spiked solutions are carried out when CRM's are not available.

5. Difficulties and limitations

The most critical step of Arsenic analysis with HG-AAS is the complete prereduction of As^{V} to As^{III} with potassium iodide and ascorbic acid. This step is disturbed by HNO_3 and other strong oxidants like NO_x. Therefore it's necessary to remove all NO_x residues carefully before pre reduction step is performed. Incomplete reduction is indicated by a yellow colour of the mixture after addition of KI-ascorbic acid solution.

In some food products Arsenic is bounded in different chemical forms. For analysis of total Arsenic by HG-AAS it is absolutely necessary to mineralize the samples at a minimum temperature of 320 °C. These temperature must affect the sample for 90 minutes or longer.

6. Comments

These method is adapted to the draft of the official German Method for food analysis. The use of pressure ashing is recommended.

Sample preparation with a dry ashing procedure show identical results for total Arsenic.

Section II

Sample ashing procedure

1. Matrices

All materials will be mineralized following the same procedure.

2. Procedure

A high pressure ashing system HPA-S equipped with five 90 ml quartz vessels is used. Concentrated HNO_3, 65 % (FLUKA, pA plus) is used.

Between 300 mg and 1500 mg of homogenized sample material are weighed into the quartz vessels. Sample amounts are illustrated in Table 1.

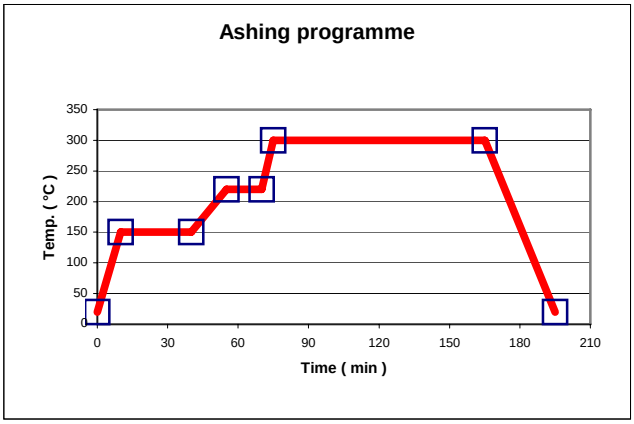
Table1. Sample amounts for use in the HPA-S

Sample	**Weight (mg)**	**Tolerance (mg)**	**Volume of HNO_3 (ml)**
dry samples (< 20 % water)	500	± 50	3.0
fresh samples	1000	± 100	3.0
fresh samples (> 50 % water)	1500	± 200	3.0

Dry samples should be wetted with some drops water before HNO_3 is added; it's to recommend to let stand the vessels over night before heating. The mineralization start by using a ashing temperature of 300 °C over a hold time of 90 minutes (Fig. 1).

For Arsenic determination by HG-AAS it's absolutely necessary to use a higher temperature at 320 °C for all sample materials. When ashing procedure is finished, let cool down to room temperature. Check the volume of HNO_3 in the vessels to recognize leaky vessels. Remove dissolved NO_x gases by a gentle stream of nitrogen at the surface of the sample solution. The solution must be treated in an ultrasonic bath. Add water up to 20 ml or 25 ml and store solutions in polyethylene tubes with screw cap for the AAS analysis.

Fig. 1: Ashing programme of the HPA-S



Institute of Food Hygiene of Athens

Athens, Greece

Arsenic

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Hydride Generation Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are biological materials, such as meat and fish.

2. Samples throughput per year

Not foreseeable.

3. Procedure

Arsenic is assayed by HG-AAS.

3.1. Hydride Generation Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

The test portion (0.5 g) is digested with HNO_3 (10 ml) in HP500 vessels. These vessels follow the next digestion programme.

Table 1. Digestion programme.

Step	Pressure (W)	Ramp Time (s)	Pressure (psi)	Temperature (°C)	Hold Time (s)
1	600 (Maximum)	12	0300	170	10

Depending on the number of vessels being used.

3.1.b. Analytical instrumentation

A Perkin-Elmer AAS instrument model 4110 with Zeeman background correction is used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1 and 2. Use is made of $NH_4H_2PO_4$ and $Mg(NO_3)_2 \cdot 6H_2O$ as matrix modifiers.

Table 2. Equipment characteristics.

HG-AAS
Wavelength, 193.7 nm
Slit width, 0.7 nm
Lamp, As EDL
Zeeman background correction
Flow Injection Mercury/Hydride Analyses

Table 2. Flow injection programme.

Step	Time (s)	Pump 1 Speed	Pump 2 Speed	Valve Position	Read Step
Pre-fill	20	120	-	Fill	
1	11	120	-	Fill	
2	20	120	-	Inject	X
3	1	120	-	Fill	

The calibration curve is plotted by using As solutions of ng/g, as summarized in Table 3.

Table 3. Sample parameters.

Volume (ml)			
Solution (ng/g)		**Reducing Agent**	**Carrier Solution**
Blank	**5**	**0.2 % $NaBH_4$ in 0.05 % NaOH**	**10 % (v/v) HCl**
Standard 1	3.2	"	"
Standard 2	6.25	"	"
Standard 3	11.8	"	"
Standard 4	16.7	"	"
Standard 5	21	"	"
Standard 6	25	"	"
Standard 7	33	"	"

4. Quality control

For the quality control, BCR standards, such as Bovine Muscle (BCR 184), Bovine Liver (BCR 185) and Pig Kidney (BCR 186), were used.

5. Difficulties and limitations

There were difficulties in the use of AAS and in the use of digestion system CEM MARS 5. The digestion system could not be stabilized at high pressures, as the membranes were not in a good case. Another point was that the temperature sensor was not termostatable, as one would prefer.

6. Comments and remarks

Not reported.

National Food Investigation Institute

Budapest, Hungary

Arsenic

Arsenic (As) determination via continuous-flow borohydride generation by AAS-technique as Standard Operation Procedure (SOP)

1. SOP-identification and-dating.
2. List of the amendments.
3. Nemes of the assembler and approver and their signatures subsequently.
4. Aim of the analytical method.
5. Technical background informations.
6. Principle of the method ($OH^- + GBH_4^- + As^{3+} + 4H^+ \rightarrow \underline{AsH_3\uparrow} + 3B_2H_6 + 3H_2 \uparrow + H_2O$).
7. Labour safety.

Matrices (specimen).
Chemicals, reagents, standards.
Solvents.
Materials.

Chemicals, reagents: certified elemental standard ($AsCl_3$/ its stock-and working-dilutions/, HCl, NaOH, dist.H_2O, $NaBH_4$, KI).

Instruments: AAS and As-hollow-cathode lamp, apparatus for continuous-flow hydride generation, test-flasks, certified digital pipettes, analytical balance, instr. for producing deionized water, electronic block-heater, temp. controlled furnace, platinum crucibles.

Description of the decontamination.
List of the technical documentations incl. the sample escorting note.

8. Method's description.

Arrival, recording and taking-over of the samples.
Storage of the samples.
Sample-preparation' steps.
Analytical weighing.

Carbonization and dry-ashing ($105^oC \rightarrow 450^oC$ in 50^oC increments for approx, 2 hours in Pt-crucibles, adding Mg-nitrate solution).

Cooling-off, rinsing and dilution with HCl solution.

$As^{5+} \rightarrow$ KI-red. $\rightarrow As^{3+}$.

9.4.1. Warming-up the AAS-Hollow-cathode-lamp (As) and slotted quartz-tube optimization/positioning for the selected resonance-line (193.7 nm).
9.4.2. Selection of the measurement-programme (slot, stabilization, integration-time, etc. managed by the computer).
9.4.3. AAS-interfacing with the instrument for continuous-flow borohydride-generation ($NaBH_4$-reagens, acidic-blank, $NaBH_4$-reagens, certified standard solutions, sample-solutions).
9.4.4. Determinaton of As-concentration of the sample solution.
9.4.5. Calculation of the As-concentration of the original sample as a functions of sample-solution's As-content/µg/cm^3/, final volume of the sample/cm^3/, the mass of the measured sample/g).
9.4.6. Determination of LOD and LOQ.
9.4.7. Electronic <u>data archiving</u>.

8.3. Main influencing parameters to be considered:
- interference-equations,
- MS-setup parameters,
- Nebulizer-parameters,
- Programme of the peristatic pump,
- Programme of autosampler.

8.4. Calibration procedure:
- Mass Calibration with tuning-solutions per month.
- Detector Plateau' determination with tuning-solutions, morthly.
- Cross Calibration with tuning-sulution, weekly.

8.5. Execution of the measurements.
8.6. Correction of the measurements (elimination of matrix-disturbance) with proper ISTD.
8.7. Calculations according to the equations executed by the computer-programmes.
8.8. Calculation of the measurement-uncertainty.
see: 2002/657 EC-Reg; ISO/EC 17025 std.
8.8.1. Uncertainty.
8.8.2. Recover.
8.8.3. Repeatability.
8.8.4. Within-laboratory reproducibility.
8.8.5. Limit of detection (LOD).
8.8.6. Limit of quantitation (LOQ).
8.8.7. Decision limit (CCalpha).
8.8.8. Detection capability (CCbeta).
8.8.9. Intercomparison with reference materials (e.g: MILK POWDER BCR # 150) (Cd, Cu, Hg, Pb).
8.8.10. Results of the participation in international proficiency testing (PT) programmes.

8.8.11. Evaluation of the possible interferences.

9. Calculations.
9.1. Determination of the elemental-concentration/s of the sample solution.
9.2. Calculation of the elemental concentration=/s of the original smaple.
9.3. Electronic data archiving.

Central Meat Control Laboratory

Celbridge, County Kildare, Ireland

Arsenic

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Hydride Generation Atomic Absorption Spectrometry (Hot Cell)
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are muscle, kidney and liver tissue.

2. Samples throughput per year

Not reported.

3. Procedure

Arsenic is assayed by HG-AAS (Hot Cell).

3.1. Hydride Generation Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

1 g of animal tissue is weighed into a digestion vessel. 4 ml of $HNO_3$are added and the solution is allowed to stand in fume hood for at least one hour. A spike control is prepared with 100 ppb of As and 1 ml of H_2O (Analytical grade water from Millipore Q system) is added to all samples subsequently. The samples are heated in a Microwave digestion system, MDS 2000 (CEM), following the digestion programme outlined in Table 1.

Table 1. Standard digestion programme.

Stage	Power	Pressure	Run Time	Run Time at press	Fan Speed
1	90 %	50	30.00	5.00	100 %
2	90 %	100	20.00	5.00	100 %
3	90 %	150	20.00	5.00	100 %
4	0 %	0	20.00	5.00	100 %
5	90 %	150	20.00	5.00	100 %

On completion the digest sequence, the vessels are allowed to cool and the pressure to dropped below 40 psi before removing from the oven to the fume hood. Using a capillary pipette the sample is purged with a steam of nitrogen for one minute to evolve any dissolved gases.

3.1.b. Analytical instrumentation

SpectrAA 600-AAS with the autosampler SPS-5. The vapour generation accessory VGA 76 is used to react the samples.

3.1.c. Working conditions

The vapour generation accessory is used to react the sample (flow rate 6 ml/min) with a 7 M HCl solution (flow rate 1 ml/min) and a Sodium Borohydride solution (0.3 % NaOH + 0.6% $NaBH_4$, flow rate 1 ml/min) and the gas generated feed on a nitrogen carrier gas through a hot cell for analysis.
1.5% KI w/v is added to all standards and samples as a pre-reduction step.

Instrumental parameters are reported in Table 2.

Table 2. Equipment characteristics.

HG-AAS
Wavelength, 193.7 nm
Slit width, 0.5 nm
Lamp, As HCL
Calibration Mode, concentration
Measurement Mode, integration
Sampling Mode, autonormal
Replicates All, 3
Air-acetylene flame

Premixed standards (1.0; 2.0; 4.0; 8.0; 12.0 ng/g) are used to generate a standard curve. Standards made up with 5 % HNO_3 + 1.5% KI w/v.
Samples are diluted to 1:50 with H_2O + 1.5% KI w/v.

4. Quality control

Reagent blanks, check and spiked samples are incorporated into each assay.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Final results are calculated, only if an acceptable standard curve has been achieved and takes into account sample weights and dilution factors.

National Institute of Health
Istituto Superiore di Sanità

Rome, Italy

1. Matrices

2. Samples throughput per year

3. Procedures
3.1. Hydride Generation Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions
3.2. Inductively Coupled Plasma Atomic Emission Spectrometry
3.2.a. Sample pretreatment
3.2.b. Analytical instrumentation
3.2.c. Working conditions
3.3. Inductively Coupled Plasma Mass Spectrometry
3.3.a. Sample pretreatment
3.3.b. Analytical instrumentation
3.3.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are meat, fresh and canned fish, milk, clams, mussels.

2. Samples throughput per year

Not foreseable.

3. Procedures

Three different techniques are used for the determination of As in the samples.

3.1. Hydride Generation Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

The sample is homogenized, weighed and digested at high pressure in a microwave oven. Usually an HNO_3-H_2O_2 (5:2) solution is used. Prior to analysis, samples are preriduced by KI and ascorbic acid. A 0.2 % $NaBH_4$ solution in 0.05 % NaOH is used as the reducing agent.

3.1.b. Analytical instrumentation

A Perkin-Elmer Model 5100 AAS equipped with a quartz cell (7 cm length) and a heat generator is used. The volatile hydride is generated and transported to the quartz cell by a Perkin-Elmer Flow Injection Analysis System.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1.

Table 1. Equipment characteristics.

HG-AAS
Wavelength, 193.7 nm
Slit width, 0.7 nm
Lamp, As EDL and HCL
Injection volume, 500 µl
Quartz cell temperature, 900 °C
Argon flow, 50 ml/min
Carrier solution, 10 % (v/v) HCl

The calibration procedure follows the standard addition method.

3.2. Inductively Coupled Plasma Atomic Emission Spectrometry

3.2.a. Sample pretreatment

The sample is homogenized, weighed and digested in HNO_3-H_2O_2 solution at high pressure in a microwave oven.

3.2.b. Analytical instrumentation

A Jobin-Yvon 38 VHR spectrometer equipped with a U-5000 AT CETAC ultrasonic nebulizer is used.

3.2.c. Working conditions

Instrumental parameters are reported in Table 2.

Table 2. Equipment characteristics.

ICP-AES
Wavelength, As (I) 193.7 nm
Torch, INSA, demountable
RF generator, Durr-JY 3848, frequency 56 MHz, maximum power output 2.2 kW
Nebulizer, ultrasonic, U-5000 AT, CETAC
Argon flows, plasma 16 l/min, auxiliary 0.3 l/min, aerosol 0.5 l/min Monochromator, HR 1000-M, focal length 1 m, Czerny-Turner mounting,
equipped with a 3600 grooves/mm holographic planegrating,
linear dispersion in the first order 0.27 nm/mm, spectral range 170-450 nm, entrance and exit slit widths, 40 µm

Calibration is carried out by using various As solutions depending on the As content in the sample.

3.3. Inductively Coupled Plasma Mass Spectrometry

3.3.a. Sample pretreatment

The sample is homogenized, weighed and digested in HNO_3-H_2O_2 solution at high pressure in a microwave oven.

3.3.b. Analytical instrumentation

A Perkin-Elmer/Sciex Elan 6000 quadrupole mass spectrometer is used.

3.3.c. Working conditions

Instrumental parameters are reported in Table 3.

Table 3. Equipment characteristics.

ICP-MS
Analytical mass, ^{75}As
Reference mass ^{103}Rh
Nebulizer, cross flow, Ryton spray chamber
Argon flows, plasma 16 l/min, auxiliary 0.9 l/min, aerosol 1 l/min
RF generator, maximum power output 1.0 kW
Scanning conditions: sweeps per reading, 5; readings per replicate, 4; number of replicates, 5; peak hopping; dwell time, 100 ms; replicate time, 2000 ms; normal resolution, 0.9-0.6 amu

Calibration is carried out by using various As solutions depending on the As content in the sample.

4. Quality control

An analytical run includes 1 blank, 1 CRM and unknown samples of similar composition. The accuracy of the method is estimated by means of CRMs (CRM 184, bovine muscle; CRM 185, bovine liver; CRM 186, pig kidney; CRM 150, skim milk). The calibration curves are accepted when the coefficient of correlation (r) is > 0.999.

5. Difficulties and limitations

If the sample matrix contains high levels of chlorine (> 1000 mg/l), the polyatomic ion $^{40}Ar^{35}Cl^{+}$ interferes with the As determination at *m/z* = 75. Chlorine should be removed

from the sample prior to each analysis, e.g. passing the solution through an $AgNO_3$ cartridge. Since $^{40}Ar^{35}Cl^{+}$ overlaps ^{75}As, a correction factor at $m/z = 77$ is applied.

6. Comments and remarks

Not reported.

Food and Veterinary Regulation Division

Albertown, Marsa, Malta

Arsenic

THE DETERMINATION OF TOTAL ARSENIC IN BOVINE / PORCINE LIVER AND FISH MUSCLE TISSUES

1.0 INTRODUCTION

This method is suitable for the determination of total arsenic (As) in food materials, freeze dried and fresh. The matrices covered by this SOP are: bovine and swine liver, and fish muscle.

2.0 EQUIPMENT

Atomic absorption spectrometer with D2 background correction, GBC932AA
Hydride generation system, GBC HG3000
Electric heating accessory, GBC EHG3000
Sample delivery system, GBC SDS-270
Arsenic super lamp
Electrically heated quartz absorption cell, open-ended
Membrane filters, 47 mm diameter, 0.45 µm pore size
Precision balance with a range of 300 g and an accuracy of 0.001 g
Analytical balance with a range of 300 g and an accuracy of 0.0001 g
Variable micropipettes (Finpipettes)
Kitchen homogeniser
Spatulas, fibre-based or plastic. ***(Note: No metal spatulas to be used)***.
Bottle brushes
Plastic bags
Carbolite ELF 11/14 muffle furnace equipped with Carbolite 201 Controller.
Sand bath
Hotplate

3.0 REAGENTS

3.1 Chemicals

Water, de-ionised.
Nitric acid, about 69%, s.g. 1.42 (High purity, such as BDH ARISTAR)
Hydrochloric acid, s.g. 1.18, about 12.4 M (BDH SpectrosoL or equivalent)
Magnesium nitrate hexahydrate (BDH AnalaR or equivalent)
Magnesium oxide (BDH AnalaR or equivalent).
Potassium iodide (Analytical grade)
L(+)-Ascorbic acid (Analytical grade)
Sodium borohydride (Aldrich 45,288-2 or equivalent)
Sodium hydroxide pellets (Analytical grade)
Decon 90
Arsenic standard solution, 1000 ppm (BDH or equivalent)

3.2 Solutions

3.2.1 Decon 90, 5%

Mix 20 mL Decon 90 with 380 mL of deionised water in a 500 mL measuring cylinder. Transfer to a wash bottle.

3.2.2 Nitric acid, 50% v/v
In a 100 mL measuring cylinder, add 50 mL of concentrated nitric acid to 50 mL of deionised water.

3.2.3 Ashing aid suspension (20% m/v $Mg(NO_3)_2$ and 2% m/v MgO)
In a 250 mL beaker, stir 20 g of $Mg(NO_3)_2.6H_2O$ and 2 g of MgO in 100 mL of deionised water until a homogeneous mixture is obtained.

3.2.4 3 M hydrochloric acid
In a 500 mL measuring cylinder, add 121 mL concentrated HCl (12.4 M) to 379 mL of deionised water.

3.2.5 Reducing solution (10% m/v KI and 5% m/v ascorbic acid)
Weigh 10 g of ascorbic acid in a 250 mL beaker and dissolve in about 170 mL of deionised water. Add 20 g of KI and stir until dissolved, adding more deionised water if necessary. Transfer to a 200 mL volumetric flask and make up to the mark with deionised water. Store in an amber bottle in a refrigerator. Use within 2 days.

3.2.6 Sodium borohydride reductant (0.6% $NaBH_4$ in 0.6% NaOH)
Weigh 3 g of sodium hydroxide pellets in a 250 mL beaker and dissolve in about 50 mL of deionised water. Then add 3 g of $NaBH_4$ to this solution, and stir well until a homogeneous mixture is obtained. Transfer this solution to a 500 mL volumetric flask and make up to the mark with deionised water. Shake well and filter under suction in order to remove any suspended particles. Discard any remaining solution after 3-4 days.

3.3 **Reference materials**
Certified Reference Materials, if available, e.g.:
BCR 185: Lyophilised Bovine Liver
DORM2: Lyophilised Dogfish muscle
Choose a reference material which closely resembles the test sample.

4.0 ARSENIC SPIKING SOLUTIONS

4.1 Arsenic intermediate solution (5 mg/L As)
Pipette 500 µL of arsenic stock standard (1000 mg/L) into a 100 mL volumetric flask and dilute to the mark with deionised water. Prepare on same day of use.

4.2 Arsenic spiking solution (500 µg/L As)
Pipette 5 mL of arsenic intermediate solution (4.1) into a 50 mL volumetric flask. Add 0.5 mL of concentrated nitric acid and dilute to the mark with deionised water. Prepare on same day of use.

5.0 ARSENIC WORKING STANDARDS (0.0, 2.5, 5.0, 7.5, 10.0, 12.5 µg/L arsenic)

Prepare these standard solutions on day before use and leave solutions to stand at room temperature in the dark overnight.

5.1 0.0 µg/L arsenic**: Transfer 150 mL of 3M HCl (3.2.4) and 20 mL of reducing solution (3.2.5) to a 200 mL volumetric flask. Dilute to volume with 3M HCl.**

5.2 **2.5, 5.0, 7.5, 10.0 µg/L arsenic**: Pipette 50, 100, 150, 200 µL of arsenic intermediate standard (5 mg/L, 4.1) into separate 100 mL volumetric flasks. To each, add 75 mL of 3M

HCl (3.2.4) and 10 mL of reducing solution (3.2.5). Dilute to volume with 3M HCl. The concentration of arsenic in these solutions is 2.5, 5.0, 7.5 and 10.0 µg/L respectively.

5.3 12.5 µg/L arsenic**: Pipette 500 µL of arsenic intermediate standard (5 mg/L, 4.1) into a 200 mL volumetric flask. Add 150 mL of 3M HCl (3.2.4) and 20 mL of reducing solution (3.2.5). Dilute to volume with 3M HCl.**

6.0 PLANNING THE DIGESTION

6.1 Each batch must contain a certified reference material in duplicate, duplicate samples, at least 2 blanks and 1 spiked sample in duplicate.

6.2 A maximum of 20 samples/blanks can be digested at one time in the muffle furnace. The digestion can be planned to incorporate two batches, which are then analysed together.

7.0 WEIGHING OF SAMPLES

7.1 Using the precision balance, weigh out 1.00 ± 0.10 g of homogenised sample in duplicate into clean and separate 50 mL beakers. Record the sample weights accurately up to 3 decimal places.

7.2 Weigh out aliquots (as in 7.1) of the samples to be spiked and transfer to separate clean 50 mL beakers. A minimum of one spiked sample should be included per species analysed in the batch. Record the sample weights accurately up to 3 decimal places.

7.3 Choose appropriate reference materials (3.3) and weigh out 0.20-0.50 g (according to Appendix I) of the chosen materials into separate clean 50 mL beakers, using the analytical balance. Record the sample weights accurately up to 4 decimal places. Add 1 mL deionised water to each beaker containing the reference materials.

8.0 SPIKING PROCEDURE

8.1 To the appropriate samples (7.2) pipette the appropriate volume of arsenic spiking solution (500 µg/L, 4.2) according to the following table:

Tissue	Concentration of spiking solution used	Volume of spiking solution added (µL)	Amount of spike added (ng)	Spike conc. in tissue (ng/g)
Bovine/swine liver (1 g)	500 µg/L As (4.2)	140	70	70
Fish muscle (1 g)	"	400	200	200

8.2 Allow the spiked samples to stand for 10 minutes before digesting.

9.0 DIGESTION PROCEDURE

9.1 Add 5 mL of 50% v/v HNO_3 (5.2.2) and 1 mL of ashing aid suspension (5.2.3) to each beaker and mix the solution well.

9.2 Evaporate to total dryness on a sand-bath.

9.3 Cover the beakers with watch-glasses and place in a muffle furnace. Subject the dry residue to a careful mineralization process (Appendix II, Programme I) at a temperature lower than 450˚C until a white ash is obtained. Allow the dry ashing programme to proceed overnight.

9.4 In general, it is necessary to wet the ash with 50% v/v HNO_3, evaporate in a sand-bath and perform a second short mineralization process (Appendix II, Programme II), once or twice, until the ash is completely white.

9.5 Treat the ash obtained according to the procedures outlined in 9.6-9.10.

9.6 Bovine/swine liver tissues:

9.6.1 Dissolve the ash with 5 mL of 3 M HCl (3.2.4) and transfer to a 20 mL volumetric flask. Add 2 mL of reducing solution (3.2.5) and dilute to volume with 3 M HCl. Transfer to specimen tubes and cap tightly.

9.7 BCR185 Bovine liver certified reference material:

9.7.1 Dissolve the ash with 5 mL of 3 M HCl (3.2.4) and transfer to a 10 mL volumetric flask. Add 1 mL of reducing solution (3.2.5) and dilute to volume with 3 M HCl. Transfer to specimen tubes and cap tightly.

9.8 Fish tissues:

9.8.1 Dissolve the ash with 5 mL of 3 M HCl (3.2.4) and transfer to a 20 mL volumetric flask. Dilute to the mark with 3M HCl.

9.8.2 Pipette 3 mL of the solution obtained in 9.8.1 to a 20 mL volumetric flask. Add 2 mL of reducing solution (3.2.5) and dilute to volume with 3 M HCl. Transfer to specimen tubes and cap tightly.

9.8.3 If the arsenic concentration of the solution obtained in 9.8.2 falls outside the calibration curve, dilute further as follows:- Pipette 1-2 mL (according to expected arsenic concentration) of the solution obtained in 9.8.1 to a 20 mL volumetric flask, add 2 mL of reducing solution (3.2.5) and dilute to volume with 3M HCl.

9.9 DORM2 dogfish muscle certified reference material:

9.9.1 Dissolve the ash with 5 mL of 3 M HCl (3.2.4) and transfer to a 20 mL volumetric flask. Dilute to the mark with 3M HCl.

9.9.2 Pipette 1 mL of the solution obtained in 9.9.1 to a 20 mL volumetric flask. Add 2 mL of reducing solution (3.2.5) and dilute to volume with 3 M HCl. Transfer to specimen tubes and cap tightly.

9.10 Blanks:

9.10.1 Prepare the blanks according to the procedures outlined for the bovine/swine and fish tissues in 9.6 and 9.8 respectively.

9.11 Following treatment with the reducing solution, leave the digest extracts (in stoppered specimen tubes) to stand in the dark at room temperature overnight in order to ensure complete reduction of As(V) to As(III). Analyze the reduced digests by HG-AAS (10.0) on the following day.

10.0 ANALYSIS BY HYDRIDE GENERATION AAS

10.1 Set the instrumental parameters for the analysis of total arsenic in the digest extracts (9.11) as in Appendix III.

10.2 Condition the HG3000 hydride system by alternately pumping the blank arsenic solution (5.1) and the most concentrated standard (5.3) for several minutes until a reproducible response is achieved.

10.3 Transfer the standards and samples to autosampler test-tubes and place in appropriate positions on the SDS-270 sample delivery system. Start the run. Re-calibration should be performed every 8-12 samples.

10.4 After analysis, flush the HG3000 hydride system with deionised water for at least 7 minutes to clean out all the tubing, mixing manifold, etc.

11.0 CALCULATION OF RESULTS

11.1 A Microsoft Excel spreadsheet, stored as 'Arsenic worksheet', is used to calculate results.

11.2 The concentration of total arsenic in the sample (ng/g), [As], is calculated directly by reference to the standard curve:-

$$[\text{ As }] = (C - C_{bl}) \times \frac{V_{dil}}{m}$$

where:

C = concentration of arsenic in the sample (µg/L), obtained by comparison with calibration curve.

C_{bl} = average concentration of arsenic in the blanks (µg/L).

V_{dil} = dilution volume of sample (see sections 13.6 and 13.8)

m = weight of sample, in g

11.3 The concentration of total arsenic in the certified reference materials is calculated using the following equation:-

$$[\text{ As }] = (C - C_{bl}) \times \frac{V_{dil}}{m} \times F$$

where: C = concentration of arsenic in the certified reference material (µg/L), obtained by comparison with calibration curve.

C_{bl} = average concentration of arsenic in the blanks (µg/L).

V_{dil} = dilution volume of reference material (see sections 13.7 and 13.9)

m = weight of reference material, in g

F = moisture correction factor

11.4 Recovery of analyte, R %, is calculated as follows:

$$R = \frac{[S] - [N]}{[A]} \times 100$$

where: [S] = the concentration of arsenic found in the spiked sample

[N] = the concentration of arsenic found in the respective unspiked sample

[A] = the concentration of arsenic added

The mean recovery figure for each batch is then calculated.

APPENDIX I

CERTIFIED REFERENCE MATERIALS USED FOR QUALITY CONTROL PURPOSES IN THE DRY ASHING OF ANIMAL TISSUES FOR DETERMINATION OF TOTAL ARSENIC

Certified Reference Material (CRM)	Indicative weight of CRM (g)
BCR185 Bovine liver	0.5
DORM2 Dogfish muscle	0.2

APPENDIX II

MUFFLE FURNACE PROGRAMME FOR ASHING OF SAMPLES

Programme	Step	Temperature (ºC)	Heating rate (ºC/min)	Time (hr) *
I	1	150	0.8	4
	2	200	0.8	1.5
	3	250	0.8	2
	4	300	0.8	4
	5	350	0.8	1.5
	6	450	0.8	12
II	1	150	0.8	4
	2	300	0.8	3.5
	3	450	0.8	12

* includes time taken to reach set temperature

APPENDIX III

Instrumental parameters for the determination of total As in animal tissues by HG-AAS

Instrument mode	Absorbance
Calibration mode	Conc. Least Squares
Measurement mode	Integration
Lamp current (mA)	20.0
Slit width (nm)	2.0
Slit height	Normal
Wavelength (nm)	193.7
Background Correction	On
Sample Introduction	Automatic
Read time (sec)	3.0
Time Constant	0.0
Replicates	3
Delay time (sec)	60.0
Rinse time (sec)	3.0
Final Method Rinse (sec)	10.0
Acid channel of HG3000	3M HCl (3.2.4)
Reductant	0.6% w/v $NaBH_4$ in 0.6% w/v NaOH (3.2.6)
Cell temperature (EHG3000)	900ºC
Separator gas	Argon (99.99% purity)
Separator gas flow rate	35 mL/min

Agriculture and Food Science Centre

Belfast, Northern Ireland

Arsenic

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Hydride Generation Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples most frequently analyzed are animal products such as kidney and liver, or of marine origin, such as fish, oysters, mussels and sediments.

2. Samples throughput per year

About 500 - 600.

3. Procedure

Arsenic is analyzed by HG-AAS.

3.1. Hydride Generation Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Samples are dry-ashed with addition of an ashing aid. 2 ml of a solution of 50 % w/v $Mg(NO_3)_2$ in water are added to the sample. Samples are then placed in a muffle furnace and temperature programmed from room temperature to 450 ^{o}C. The ash is dissolved in 10 ml of 50 % concentrated HCl containing 10 % w/v KI and 1 % w/v ascorbic acid. Standards are treated in the same manner. The solutions are left to stand at room temperature overnight. Samples are analyzed using a solution of 0.6 % $NaBH_4$ in 0.5 % NaOH as the reducing agent.

3.1.b. Analytical instrumentation

A Varian SpectrAA-400 Atomic Absorption Spectrometer equipped with a VGA-76 hydride generator system is used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1. Use is made of $Mg(NO_3)_2$ as matrix modifier.

Table 1. Equipment characteristics.

HG-AAS
Wavelength, 193.7 nm
Slit width, 0.5 nm
Lamp, As HCL
Flame,air-acetylene
Measurement time, 9s

A calibration curve is established by using As standard solutions as shown in Table 2.

Table 2. Sample parameters.

5 % HCl (ml)	**100 mg/ml As standard (µl)**	**10 % w/v KI and 1 % w/v ascorbic acid in 5 % HCl (ml)**	**As (ng)**
9.0	0	1	0
8.8	200	1	20
8.6	400	1	40
8.4	600	1	60
8.2	800	1	80
8.0	1000	1	100

4. Quality control

The laboratory quality system is based on GLP and UKAS principles.

Method validation is performed by using CRM's or spiked samples as appropriate. CRM's are analyzed periodically to check accuracy. During routine analysis, triplicate spiked samples or CRM's are included with each batch of samples analyzed, along with three reagent blanks to control potential contamination. The precision and accuracy is monitored on quality control charts using recovery results.

Instruments are optimized before each batch of analyses, and details recorded of lamp photomultiplier voltage and absorbance for a given standard. Plot linearity is also checked. The laboratory participates in proficiency testing exercises, such as the UK Food Analysis Performance Assessment Scheme (FAPAS).

5. Difficulties and limitations

Samples and standards must be given sufficient time to reduce any As (V) to As (III) while in contact with the KI solution. This is best achieved by allowing samples and standards to stand overnight in the reducing KI solution at room temperature.

6. Comments and remarks

Not reported.

National Veterinary Research Institute

Pulawy, Poland

Arsenic

Determination of arsenic in biological material by hydride generation atomic absorption spectrometry

Method description

Arsenic is determined by hydride generation MS after ashing samples. The samples are mixed together with 50% solution of magnesium nitrate hexahydrate in quartz crucible and dried. After them the samples is placed into cool muffle furnace and raise the temperature of the oven to 550°C. The ash is dissolved in hydrochloric acid solution (1 g sample in 1 ml acid solution).

The level of arsenic is determined in Perkin Elmer 4110 ZL equipped with FIAS 100 System.

Analytical parameters

Lamp: EDL
Stit: 0.7 nm
Wavelength: 193.7 nm
Cell temperature: 900°C
Argon: pure gas

Reagents

Carrier solution: 10% (v/v) HCL
Reducing agent: 0.3% $NABH_4$ in 0.1% NaOH
Sample solution: in 10% (v/v) HCL
Stock standard solution 1000 µg/ml from Merck

Instrument calibration:

The calibration curve far the determination of arsenic is prepared using a blank and working standard solution (3 - 20 µg/l).

Quality control

The method was validated in terms of the linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, recovery and uncertainty. Validation report is presented in Table.
The method was tested by studying the certified reference materials and regularly evaluated by participation in proficiency programmes organized by Food Analysis Performance Assessment Scheme (FAPAS) and European Community Reference Laboratories (Rome Italy, Geel Belgium).

Validation report – As determination by HG AAS

Parameters		Results					
Linearity (working range), mg/kg		0.003 – 0.020					
Limit of detection (LOD), mg/kg		0.0012					
Limit of quantitation (LOQ), mg/kg		0.0016					
Matrix		Muscle			Liver		
Levels of spiked samples, mg/kg		0,100	0,200	0,400	0,100	0,300	0,500
Repeatability	x mg/kg	0,085	0,203	0,362	0,112	0,350	0,590
	S mg/kg	0,016	0,010	0,029	0,003	0,003	0,004
	CV %	19,25	4,80	8,13	2,57	0,76	0,59
Intralaboratory Reproducibility	x mg/kg	0,085	0,206	0,392	0,100	0,326	0,531
	S mg/kg	0,013	0,011	0,064	0,002	0,014	0,010
	CV %	14,67	5,30	14,90	2,34	4,36	1,84
Recovery	%	85,40	103,10	98,00	101,30	105,60	105,40
Uncertainty							
combined (uc)		0,013			0,058		
expanded (U)		0,200 ± 0,027 mg/kg			0,500 ± 0,116 mg/kg		
coverage factor (k)		2			2		

Istituto de Investigação das Pescas e do Mar

Lisboa, Portugal

Arsenic

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Hydride Generation Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissue (liver).

2. Samples throughput per year

Not foreseeable.

3. Procedure

Arsenic is assayed by HG-AAS.

3.1. Hydride Generation Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Approximately 100 g of samples are homogenized, 1 g is weighed in quartz crucibles and 1 ml of 25 % $Mg(NO_3)_2$ are added. The sample is dry-ashed, followed by ashing in a muffle furnace at 450 °C during 1 h. The samples are then removed and wet with concentrated HNO_3 and put back to the furnace. This procedure is repeated every hour until ashes become white. Ashes are then quantitatively transferred to 10 ml volumetric flasks with 6 *N* HCl.

Samples are quantified transferring 1 ml of the digested sample, added with 0.5 ml of a 5 % NaI solution. The solutions were allowed to stand for 45 minutes at room temperature in order to reduce As (V) into As (III). At the end of this time the volumes were adjusted to 10 ml with deionized water and the determinations were performed. A solution containing 1 % $NaBH_4$ solution in 0.1 % NaOH is used as the reducing agent.

3.1.b. Analytical instrumentation

An FAAS Unicam 969 instrument attached to a Unicam VP 90 vapour system is used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1.

Table 1. Equipment characteristics.

HG-AAS
Wavelength, 193.7 nm
Slit width, 0.7 nm
Lamp, As HCL
Air-acetylene flame
Argon flow, 200 ml/min
Carrier solution, 5% HCl
Reducing agent 1% $NaBH_4$ in 0.1% NaOH

Calibration curve is plotted by using As solutions of 1.0, 2.0 5.0 and 10.0 µg/l for Unicam 969.

4. Quality control

Each analytical run includes blanks, commercial standard solutions and CRMs (BCR 186, pig kidney) and Quality Control Standards for Trace Metals – AA by RTC.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Ministerio de Agricultura, Pesca y Alimentación

Madrid, Spain

Arsenic

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Hydride Generation Inductively Coupled Plasma Atomic Emission Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are, in the order, wine, spirits, vinegar, beer, orange juice, water, cookies, various kinds of pasta, canned vegetables, liver and kidneys.

2. Samples throughput per year

About 800.

3. Procedure

Arsenic is assayed by HG-ICP-AES.

3.1. Hydride Generation Inductively Coupled Plasma Atomic Emission Spectrometry

3.1.a. Sample pretreatment

Sampling is not made by the Laboratory Staff, but by official inspectors. The techniques for storage and subsampling are different according to the product: milk and milk products, shake; meat, cut in small pieces; eggs, cook; fish, clean and cut; crustaceans, peal and cook and afterwards homogenizing in kitchen grinder.

Dry ashing of samples with 4 ml of 50 % w/w $Mg(NO_3)_2$ as ashing aid, is achieved in an muffle furnace with overnight digestion at 450 °C. Ashes are dissolved and made up to volume with 6 *N* HCl. An aliquot is taken and As (V) is reduced to As (III) with 30 % KI solution in 1 hr. Particularly if chloride is present, in order to prevent volatilization of As as the Trichloride, the sample must be heated first in presence of excess of HNO_3.

3.1.b Analytical instrumentation

A Varian Liberty 220 Inductively Coupled Plasma Atomic Emission Spectrometerer equipped with a Varian VGA-77 hydride generator system and a modular system with continuous flow is used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1.

Table 1. Equipment characteristics.

HG-ICP-AES Wavelength, 188.979 nm Nebulizer, 180 Kpa Argon flow, plasma 12 l/min, auxiliary 1.5 l/min Power output, 1.2 Kw High observation, 6 mm Integration time, 3 s

Calibration curve is made by using As solutions of 0, 5, 10 and 20 ng/ml.

4. Quality control

The routine use of each method is preceded by analytical validation according to the IUPAC recommendations in order to ascertain sensitivity, detection power, precision and accuracy. Certified Reference Materials of bovine liver (NIST) and mussel tissue (SMT) are used to check the accuracy and analyzed periodically. During routine assays, each analytical batch includes at least three reagent blanks to evaluate the detection limits and to control possible contaminations. Recoveries are calculated using two spiked reagent blanks and one spiked sample for each non-spiked sample assayed.

5. Difficulties and limitations

The critical point is the reduction with KI. It is important to remove the chloride in order to prevent volatilization of As as the Trichloride.

6. Comments and remarks

Not reported.

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Inductively Coupled Plasma Mass Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analysed are, , in the order, wine, spirits, vinegar, beer, orange juice, cookies, various kinds of pasta, canned vegetables, liver and Kidneys.

2. Samples throughput per year

About 1200 per year.

3. Procedure

As is assayed by ICP-MS

3.1. Inductively Coupled Plasma Mass Spectrometry

3.1.a. Sample pretreatment

Sampling is not made by the Laboratory Staff, but by official inspectors. The techniques for storage and subsampling are different according to the product; i.e., milk and milk products, shake; meat, cut in small pieces; eggs, cook; fish, clean and cut; crustaceans, peal and cook. Afterwards homogenising in Kitchen grinder is performed. Samples are digested in a microwave oven For 0.5 to1g homogeneized samples 2 ml concentrated HNO_3 and 8 ml H_2O are used. Microwave parameters are reported in the following table.

Stage	Power		Ramp Time	P(PSI)	ºC	Time
1	MAX	%	20 min	300	200	10 min
	600w	100				

After the digestion the samples are dissolved in deionized water in order to achieve a final concentration of 3% of nitric acid

3.1.b. Analytical instrumentation

The multielemental analysis is performed in a Perkin Elmer Elan 9000 Inductively Coupled Plasma Mass Spectrometer, with cross flow nebulizer, nickel cones and a autosampler placed inside a laminar flow cab**.**

3.1.c. Working conditions

The instrumental parameters adopted are reported in Tables 1 and 2.

Table 1: ICP conditions

Plasma power (W)	1100
Argon flow nebulizer (L/min)	0.78 - 0.96
Sample flow (ml/min)	1 ml / min

Table 2:Mass spectrometer conditions

	Quantitative mode	Semiquantitative mode
Vapour pressure(Torr)	7×10^{-7}	7×10^{-7}
Dwell Time (ms)	50	50
sweeps / readings	25	6
Readings / Replicates	1	1
Number of replicates	3	1
Reading mode	Peak Hopping	Peak Hopping

Calibration curve is plotted by using As solutions of 0.08, 0.8, 5, 10, 20 and 100 ug/l.

All the samples as well as the standards are read adding on-line a solution of 2% of isopropanol in order to counterbalance the effects of the remaining carbon in the samples.

Analytical mass:75

4. Quality control

The routine use of each method is precede by analytical validation according to the IUPAC recommendations in order to ascertain sensitivity, detection power, precision and accuracy. CMRs of bovine liver (SRM 1577b NIST), DORM-2 (Dogfish muscle certified material for trace metals), Spinach Leaves (SRM 1570a) and Corn Bran (8433 NIST) are used to check the accuracy and analysed periodically. During routine assays, each analytical batch includes at least three reagents blanks to evaluate the detection limits and to control possible contamination. Recoveries are calculated using two spiked reagent blanks and one spiked sample for each non spiked sample assayed. At de beginning and at the end of each assay and every five samples two quality controls are analysed. One with a concentration of Arsenic similar to the fifth standard of the calibration curve and the other with a concentration of Arsenic similar to the second standard.

5. Difficulties and limitations

In samples with high concentration of Cl is necessary to use a correction equation (-3.127*Se77+2.54*Se82)to eliminate any contribution to the mass 75 signal from ArCl in the plasma.

6. Comments and remarks

No reported.

National Food Administration

Uppsala, Sweden

Arsenic

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Inductively Coupled Plasma-Mass Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissues, cereals and vegetables.

2. Samples throughput per year

No analytical activities at the present time.

3. Procedure

Arsenic is determined by ICP-MS after microwave digestion.

3.1. Inductively Coupled Plasma-Mass Spectrometry

3.1.a. Sample pretreatment

A maximum of 0.5 g dried, homogenized sample is weighed into a Teflon vessel, followed by the addition of 5 ml conc. HNO_3 and 2 ml 30% H_2O_2. The digestion is carried out in a CEM (MDS 2000) microwave oven according to the detailed programme in Table 1.

Table 1. Digestion programme.

Step	Power (W)	Time (min)
1	250	3
2	630	5
3	550	22
4	0	15

3.1.b. Analytical instrumentation

Platform ICP, Micromass.

3.1.c. Working conditions

Instrumental parameters are reported in Table 2

Table 2. Equipment characteristics.

ICP-MS
Measured isotope, 75 amu Dwell time, 200 ms Number of scans, 16 Total measurement time, 3.2 s

External calibration using Rh as an internal standard is normally used. In certain cases the standard addition method may be necessary. Correction for ArCl is usually not necessary.

4. Quality control

An analytical run includes one-two blanks and one or more CRM or in-house RM. Samples are measured as singles or duplicates, depending on the requirements. Accuracy and precision are monitored by the use of control charts on which the results of duplicate CRM and RM analysis are plotted. The detection limit is based on three times the standard deviation of large number of blanks and is currently 30 ng/g., based on sample weight of 0.4 g.

5. Difficulties and limitations

The microwave digestion method is not suitable for fats, oils or extremely fatty products.

6. Comments and remarks

Not reported.

National Institute for Public Health and Environment
Rijksinstituut voor Volksgezondheid en Milieu

Bilthoven, The Netherlands

Arsenic

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Inductively Coupled Plasma Mass Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are meat, rain water, ground water, urine, animal feed and aerosol particles.

2. Samples throughput per year

About 1000.

3. Procedure

Arsenic is assayed by ICP-MS.

3.1. Inductively Coupled Plasma Mass Spectrometry

3.1.a. Sample pretreatment

Solid matrices are digested in a microwave oven. For 0.5 g of homogenised biological sample 1 ml of concentrated HNO_3 and 9 ml H_2O is used.
For soil samples (particle size < 250µm) an aqua regia digestion is performed.

3.1.b. Analytical instrumentation

An HP4500+ quadrupole ICP-MS is used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1.

Table 1. **ICP-MS HP4500+** Equipment characteristics.

Analytical mass, ^{75}As
Reference mass, ^{72}Ge (added on-line (dilution sample/IS: 1: 5.5))
Nebulizer, V-groove, Scott-type double pass spraychamber
Argon flow, nebulizer 1.17 l/min, R.f. power 1250W
Measuring conditions: 3 points / mass; integration time 1.5 s / mass, 3 replicates.
Two-point calibration, standards concentration, 0 and 20 µg/l
Matrix matching for calibration samples
Equation ^{75}As (-0.000116*Cl35), equation is updated in every measurement sequence using (a) suitable Interference Check Solution(s)

4. Quality control

Each analytical run includes 1 blank, 1 CRM and 1 RM. The instrumental conditions are controlled by the analysis of an in-house water reference material. The accuracy of the analytical, including sample digestion, is estimated by the use of CRMs. A number of matrix samples are analyzed and recovery experiments are carried out for As.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Central Science Laboratory

York, United Kingdom

Arsenic

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Inductively Coupled Plasma Mass Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions
3.2. Hydride Generation Inductively Coupled Plasma Mass Spectrometry
3.2.a. Sample pretreatment
3.2.b. Analytical instrumentation
3.2.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Sample most frequently analyzed are foods and beverages.

2. Samples throughput per year

Up to 1500.

3. Procedure

Arsenic is assayed by ICP-MS and confirmed by HG-ICP-MS.

3.1. Inductively Coupled Plasma Mass Spectrometry

3.1.a. Sample pretreatment

Aliquots of each sample are homogenized and portions (equivalent to 0.5 g dry weight) are weighed out into microwave digestion vessels. Concentrated HNO_3 (5 ml) is added and the samples are heated in a proprietary high pressure microwave digestion oven. The acid is transferred into a polycarbonate test tube with 18 MΩ cm^{-1} water and made up to 10 ml in volume. Arsenic levels are measured also by HG-ICP-MS.

3.1.b. Analytical instrumentation

A VG Instruments PlasmaQuad PQII+ Turbo quadrupole ICP-MS or Perkin Elmer ELAN 6000 ICP-MS quadrupole are used.

3.1.c. Working conditions

At least 7 calibration standards are used to cover the calibration range 0.1 µg/l to 100 µg/l. ^{75}As is monitored with In or Rh being used as an internal standard. Element concentrations in test solutions are determined by manufacturer software and processed further using an in-house written SuperCalc 5 macro. Automatic spike recovery and blank corrections are made during these calculations.

3.2. Hydride Generation Inductively Coupled Plasma Mass Spectrometry

3.2.a. Sample pretreatment

Aliquots of each sample are homogenized and portions (equivalent to 0.5 g dry weight) weighed out into boiling tubes. The sample is charred with H_2SO_4 and concentrated HNO_3 added. Small aliquots of HNO_3 are added and the mixture heated at increasing temperatures to 240 °C. H_2O_2 is then added after cooling slightly and the mixture reheated. At this stage $KMnO_4$ is added and the solution boiled to dryness.

The residue is taken up in H_2SO_4 and made up to 25 ml with water containing Sb internal standard. $NH_4OH{\cdot}HCl$ is added as prereductant beforeHG-ICP-MS.

3.2.b. Analytical instrumentation

A Perkin Elmer FIAS 200 automated hydride generation system is used with a VG Instruments PlasmaQuad PQII+ Turbo ICP-MS or Perkin Elmer FIAS 400 system connected to an ELAN 6000 ICP-MS.

3.2.c. Working conditions

Seven calibration standards are used to cover the calibration range 0.1 µg/l to 100 µg/l. ^{75}As is monitored using time resolved mode and Ge or Sb is used as an internal standard. Peak areas are determined by MassLynx software and processed further using an in-house written SuperCalc 5 macro. Automatic spike recovery and blank corrections are made during these calculations.

4. Quality control

All test batches contain reagent blanks, spiked blanks and CRMs and are performed according to UKAS accredited standards.

5. Difficulties and limitations

Hydride generation techniques should not be used for fish containing samples when microwave digestion pretreatment has been used, as this method does not completely destroy organo-arsenic compounds. This results in poor hydride generation efficiency from such digests.

Although adequate measurements are often possible by ICP-MS on such digests, a suitable alternative digestion method, which for use with HG-ICP-MS is given.

The latter approach eliminates any contribution to the mass 75 signal from ArCl in the plasma and is suitable for low-level measurements.

6. Comments and remarks

Hydride generation methods normally have lower limits of detection for As than direct nebulization.

Cadmium

AUSTRIA
BELGIUM
CZECH REPUBLIC
CYPRUS (AOAC 999.10)*
DENMARK
ESTONIA
FINLAND
FRANCE
GERMANY
GREECE
HUNGARY
IRELAND
ITALY
LATVIA (AOAC 986.15 / 999.11)*
MALTA
NORTHERN IRELAND
POLAND
PORTUGAL
SPAIN
SWEDEN
THE NETHERLANDS
UNITED KINGDOM

**Official analytical methods are not reported*

Austrian Agency for Health and Food Safety

Wien, Austria

Cadmium

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples most frequently analyzed are: liver and kidney from cattle, pig, sheep and poultry; muscle from horses and game.

2. Samples throughput per year

About 300-400.

3. Procedure

Cadmium is assayed by Z-ETA-AAS.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

a) 2 ± 0.1 g of homogenized tissue is weighed in 20 ml glass bottles. 3 ml HNO_3 (65 %, suprapure) are added. The samples are digested for at least 1h at 90 °C in a water bath. The solutions are filtered through paper filters into 10 ml measuring flasks and filled up to 10 ml with deionized water.

b) 2 ± 0.1 g of homogenized tissue is weighed in 40 ml Teflon vessels. 3 ml HNO_3 (65 %, suprapure) and 1.5ml H_2O_2 (30 %) are added. The samples are digested in the microwave oven (MLS-Ethos 900) according to the following programme: 1. 100 W, 10 min; 2. 0 W, 1 min; 3. 250 W, 5 min; 4. 0 W, 1 min; 5. 400 W, 5 min; 6. 600 W, 10 min; 7. 500 W, 6 min. After cooling the digested sample solutions are transferred into 10 ml measuring flasks and filled up to 10 ml with deionized water.

3.1.b. Analytical instrumentation

A SIMAA 6000 with Autosampler AS-72 (Perkin-Elmer) is used.

3.1.c. Working conditions

Equipment characteristics are reported in Table 1. Use is made of0.5 % $NH_4H_2PO_4$ + 0.03 % $Mg(NO_3)_2$ as matrix modifiers.

Table 1. Equipment characteristics.

Z-ETA-AAS
Wavelength: 228.8 nm
Lamp, Cd HCL
Zeeman background correction, longitudinal AC magnetic field with transversal heated graphite furnace
Injection volume, 20 µl sample/standard + 10 µl modifier
Measurement mode, peak area

The furnace conditions are summarized in Table 2.

Table 2. Graphite furnace programme.

Step	**Temperature (°C)**	**Ramp Time (s)**	**Hold Time (s)**	**Gas Flow (ml/min)**
Drying	110	1	30	250
Drying	130	15	40	250
Ashing	500	10	30	250
Atomization	1700	0	5	0
Clean-up	2450	1	3	250

The calibration curve is established by automatically dilution of one stock solution (5 µg/l Cd and 50 µg/l Pb) by the autosampler to following concentrations: 1, 2, 3 and 5 µg/l Cd and 10, 20, 30 and 50 µg/l Pb, respectively. The blank is subtracted automatically from standards and samples.

4. Quality control

Calibration curves are accepted when the coefficient of correlation is > 0.995. The performance of the instrument is checked by a multielement standard (Perkin-Elmer). Recovery is calculated by spiked samples. CRM 184 (bovine muscle), CRM 185 (bovine liver) or CRM 186 (pig kidney) are analyzed in order to check the accuracy and reproducibility of the whole method. Usually 10 samples are pretreated and measured together as one batch. One control sample is analyzed for every batch in order to check the repeatability.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Usually Cd and Pb are measured simultaneously.

Scientific Institute of Public Health – Louis Pasteur

Brussels, Belgium

Cadmium

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples frequently analyzed are meat,vegetables, sometimes babyfood

2. Samples throughput per year

About,*100* samples per year.

3. Procedure

Cadmium is assayed by Z-ETA-AAS.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

About 1 g is weighed on an analytical balance in a polyethylene cup and dropped in a PFA digestion tube. Wet digestion is carried out with the aid of 5 ml conc. HNO_3 and 0.2 ml conc. $HClO_4$. The tube is placed in an oven at 180 °C. After reducing of the volume to +/- 0.5 mL, 1 mL of H_2O_2 is added. The tube is placed into an oven at 80 °C +/- 1 hour. After complete oxydation the tubes are cooled to ambient temperature. The digest is diluted into the tube to a volume of 10 mL with Milli-Q-water. If necessary, furthers dilutions are realised.

3.1.b. Analytical instrumentation

Perkin Elmer SimAA 6000

3.1.c. Working conditions

Z-ETA-AAS

Wavelength, 228.8 nm Slit width, 0.5 nm Lamp, Cd EDL Zeeman background correction L'vov platform Injection volume, 20 µl sample + 10 µl modifier THGA graphite Furnace with End Cap Measurement mode, peak area Calibration mode, concentration Replicates, 2 Sample introduction, sampler automixing (5 points)

Step	Temperature (°C)
Injection	Ambient
Drying	110 -130
Pyrolysis	700
Atomization	1700
Clean-up	2600

Use is made of $NH_4H_2PO_4$ and $Mg(NO_3)_2$ as matrix modifiers. Calibration is carried out with standard solution, freshly prepared with 2 % v/v conc. HNO_3. The stock solution is Perlin Elmer PE Pure. Linear working range is 0-40 ng/ml.

4. Quality control

An analytical run includes blanks, standards, spike samples for recovery measurements on all samples and RMs (BCR 184, 185, 186, 422, 278) are regularly analysed. All glassware and polyethylene flasks and pipets are soaked in a 10 % HNO_3 solution and rinsed with Milli-Q-water just before use. All volumetric flasks, glass pipets are calibrated, plastic pipets are checked regularly. Control charts are used.

5. Difficulties and limitations

Little concentration range with ETAAS. Sometimes dilutions are necessary (Fish and some vegetables...)

6. Comments and remarks

Not reported.

State Veterinary Institute Olomouc
Laboratory in Kromeriz

Kromeriz, Czech Republic

Cadmium

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Analytical technique
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissues (meat, liver and kidney), meat products, fish, fish products, milk, milk products, babyfood, honey, feedstuffs.

2. Sample throughput per year

About 1400 samples per year.

3. Procedure

Cadmium is assayed by Z-ETA-AAS and F-AAS.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

a) 1-10 (± 0.1) g of homogenized sample is weighed and dry ashed at a temperature of 450±25 ^{o}C in a programmable furnace overnight. Use is made of 6 % w/w $Mg(NO_3)_2$ as an ashing aid. Ashed sample is cooled down to room temperature, deionized water and 1 ml conc. HNO3 are added. The sample is re-evaporated and heated up to 450 ^{o}C in the furnace. This procedure is repeated until ashes become white. Ashes are dissolved in 1 M HNO_3.

b) 1 ± 0.1 g of homogenized sample is weighed in 100 ml Teflon vessels. 5 ml HNO_3 (65 %, suprapure) and 1.5 ml H_2O_2 (30 %) are added. The samples are digested into the microwave oven (MLS – Ethos Plus) according to the following programme: 1. 500W, 10 min, 180 ^{o}C; 2. 500 W, 10 min, 200 ^{o}C. After cooling the digested sample solutions are transferred into 10 ml measuring flasks and filled up to 10 ml deionized water.

3.1.b. Analytical instrumentation

A PerkinElmer AAnalyst 800 spectrometer with THGA Graphite Furnace including AS-800 Autosampler is used.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 1. and 2. Use is made of Pd / $Mg(NO_3)_2$ as matrix modifiers.

Table 1. Equipment characteristics

Z-ETA-AAS
Wavelength: 228.8 nm
Lamp: Cd HCL
Zeeman background correction
Standard THGA Graphite Tubes with Integrated Platform
Injection volume: 20 µl samples + 10 µl modifier

Table 2. Graphite furnace programme

Step	Temperature (^{o}C)	Ramp Time (s)	Hold Time (s)	Internal Flow
1	110	1	30	250
2	130	15	30	250
3	500	10	20	250
4	1500	0	5	0
5	2450	1	3	250

The calibration curve is established by using Cd solutions of 10, 20, 50, 80, 100 µg/l.

3.2. Flame Atomic Absorption Spectrometry

3.2.a. Sample pretreatment

a) 1-10 (± 0.1) g of homogenized sample is weighed and dry ashed at a temperature of 450±25 ^{o}C in a programmable furnace overnight. Use is made of 6 % w/w $Mg(NO_3)_2$ as an ashing aid. Ashed sample is cooled down to room temperature, deionized water and 1 ml conc. HNO_3 are added. The sample is re-evaporated and heated up to 450 ^{o}C in the furnace. This procedure is repeated until ashes become white. Ashes are dissolved in 1 M HNO_3.

b) 1 ± 0.1 g of homogenized sample is weighed in 100 ml Teflon vessels. 5 ml HNO_3 (65 %, suprapure) and 1.5 ml H_2O_2 (30 %) are added. The samples are digested into the microwave oven (MLS – Ethos Plus) according to the following programme: 1. 500W, 10 min, 180 ^{o}C; 2. 500 W, 10 min, 200 ^{o}C. After cooling the digested sample solutions are transferred into 10 ml measuring flasks and filled up to 10 ml deionized water.

3.2.b. Analytical Instrumentation

A Perkin-Elmer 2100 Atomic Absorption Spectrometer is used.

3.2.c. Working conditions

Instrumental parameters are reported in Table 3.

Table 3. Equipment characteristics

F-AAS
Wavelength: 228.8 nm
Lamp: Cd HCL
Deuterium background correction
Air-acetylene flame

The calibration curve is established by using Cd solutions of 10, 20, 50, 80, 100 µg/l.

4. Quality control

The routine use of each method is preceded by analytical validation according to the IUPAC recommendations and the metods are accreditated.
CRMs (BCR 184, BCR 185 R, BCR 151, Tort-2, …) are use to check the accuracy and analyzed periodically. An analytical run includes two blanks, duplicates and in-house RM. The results of duplicate CRM, RM analysis samples are monitored on control charts.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Danish Institute for Food and Veterinary Research

Søborg, Denmark

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions
3.2. Inductively Coupled Plasma Mass Spectrometry
3.2.a. Sample pretreatment
3.2.b. Analytical instrumentation
3.2.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are fish, meat, flour, fruit and vegetables.

2. Samples throughput per year

About 10 for ETA-AAS and 50 for ICP-MS.

3. Procedure

Two different techniques are used for the determination of Cd in food although the ICP-MS based method of analysis will take over fully in the near future.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

The sample is homogenized in a blender equipped with titanium blades. A known amount of the homogenized sample (2-3 g wet weight) is weighed in a Berghof high pressure bomb and 4 ml of concentrated HNO_3 are added. The sample is digested in an oven at 160 °C for 4 h. This is typically done overnight. After the digestion distilled water is added up to a known weight of approximately 20 g.

Alternatively, the Multiwave high pressure microwave digestion system is used for sample decomposition for ICP-MS determination. The maximum pressure possible is 70 bar and the digestion is finalised within 60 minutes including cooling. The same sample intake and acid is used with his technique.

3.1.b. Analytical instrumentation

Perkin-Elmer Zeeman 3030 ET-AAS instrumentation is used.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 1 and 2. Use is made of $NH_4H_2PO_4$ and $Mg(NO_3)_2$ as matrix modifiers.

Table 1. Equipment characteristics.

ETA-AAS

Wavelength, 228.8 nm Slit width, 0.7 nm Lamp, Cd EDL Zeeman background correction L'vov platform

Table 2. Graphite furnace programme.

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)	Ar Flow (ml/min)
Drying	100 150	5 10	20 50	300 300
Charring	900	30	30	300
Atomization	1600	0	5	0
Clean-up	2300	1	4	300

Calibration is carried out by the standard addition method. Two additions are used for each sample.

3.2. Inductively Coupled Plasma Mass Spectrometry

3.2.a. Sample pretreatment

The sample is homogenized in a blender equipped with titanium blades. A known amount of the homogenized sample (2-3 g wet weight) is weighed intothe Multiwave high pressure microwave digestion system. The Multiwave high pressure microwave digestion system is used for sample decomposition. The maximum pressure is 70 bar and the digestion is finalised within 60 minutes including cooling. Following digestion the residue is taken to 20 ml by weighing using pure water.

3.2.b. Analytical instrumentation

An Elan 6100 DRC Perkin-Elmer/Sciex quadrupole mass spectrometer is used.

3.2.c. Working conditions

A volume of 5 ml of the diluted digest solution is pipetted into a polyethylene autosampler tube and 5 ml of 1 % aqueous HNO_3 are added. Cadmium is measured at *m/z* = 114. Calibration is carried out by the standard addition method. Two additions are used for

each sample. A mixture of usually Ga, In and Bi are used for internal standardization in the multielement method in which this element is an analyte.

4. Quality control

The method is accredited by the Danish Accreditation Organisation, DANAK. A matching CRM and blanks are analyzed in parallel with the samples in series containing up to 15 unknown samples. The results of CRMs are monitored on control charts (x- and R-charts). The risk of contamination of samples must always be considered. The critical utensils are washed/rinsed with acid before use and ultrapure acids are used for the ashing process.

5. Difficulties and limitations

Since both Cd and Sn have isotopes at m/z = 114, the cadmium detection is additionally carried out for ^{111}Cd. In case of a high Sn content in the sample, the latter isotope is used for quantification.

6. Comments and remarks

None reported.

Food Safety Veterinary and Food Laboratory

Tartu, Estonia

1. The equipment in use

1) Atomic Absorption Spectrophotometer-Thermo Elemental. It has equipped both with GFA and flame compartments. Zeemann and Deuterium correction are available. At the moment the Hydride Vapor System –for the determination of Arsenic is missing.
2) Atomic Absorption Spectrophotometer-Shimadzu - It has equipped both with GFA and flame compartments. Only deuterium correction is available.
3) Microwave owen AntonPaar- closed vessels for wet digestion
4) Muffle furnace Nabertherm

2. Analytical Methods

Element	**Cd**
Accreditation	**yes**
Reference for method	1) AA Spectrometers Methods Manual, 2003, Thermo Electron Corp., UK 2) EVS-EN 13804:2002 –Foodstuffs-Determination of Trace elements-Performance Criteria and general conciderations 3) EVS-EN 13805:2002 Foodstuffs-Determination of trace elements. Pressure digestion 4) AOAC method 999.11 Determination of Lead, Cadmium, Iron and Zinc in Foods. AAS after dry ashing First action 1999 5) Nordic Commitee of Food Analyses no 161, 1998. Metals. Determination by AAS after wet digestion in microwave owen.
Sample weight (g)	5g - dry ashing > 1g- mw digestion
Sample preparation	Dry ashing Microwave digestion
Reagents used	HNO_3, H_2O_2, HCl
Modifier	Pd GFA
Quantification (technique)	AAS Graphite Furnace AAS flame
Wavelenght(nm)	228.8
LOQ (mg/kg)	0.002 GFA 0.2 flame
U %	23 GFA 15 flame

3. Reference materials

Element	**Matrics**	**Ref. value mg/kg**
Cd	BCR 186	2.71
	SRM 2976	0.82
	CRM 185 R	0.544
	BCR 151	0.101

National Veterinary and Food Research Institute

Helsinki, Finland

Cadmium

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissues (meat, liver and kidney), milk and honey.

2. Samples throughput per year

About 300-500.

3. Procedure

The samples are analyzed by Z-ETA-AAS.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Sample (5-10 g) is ashed at 450 °C in a muffle furnace overnight. Ashed sample is cooled down to room temperature, deionized water and 1 ml conc. HNO_3 are added. The sample is re-evaporated and heated up to 450 °C overnight in the muffle furnace. The residue is dissolved in 0.5 ml of conc. HNO_3 and adjusted to appropriate volume with deionized water.

3.1.b. Analytical instrumentation

A Perkin-Elmer 5100 PC Atomic Absorption Spectrometer with Zeeman background corrector and hollow cathode lamp is used.

3.1.c. Working conditions

The instrumental parameters adopted are reported in Table 1. Measurement are carried out by direct comparison with standard solution (Reagecon) in 0.1 M HNO_3.

Table 1. Equipment characteristics.

ETA-AAS
Wavelength, 228.8 nm
Lamp, Cd HCL
Zeeman background correction

Most frequently encountered concentration ranges are:

< 0.001-0.002 mg/kg (meat, milk)
0.005-0.500 mg/kg (liver, kidney

4. Quality control

The method is accreditated. CRMs and blanks are analyzed together with each sample series. The results of CRMs samples are monitored on control charts. Analysis of spiked samples are performed. The risk of contamination of samples must always be considered. All glass- and plasticware are washed with acid before use.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Agence Française de Sécurité Sanitaire des Aliments

Maisons-Alfort Cedex, France

Cadmium

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions
3.2. Flame Atomic Absorption Spectrometry
3.2.a. Sample pretreatment
3.2.b. Analytical instrumentation
3.2.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissues (muscle, liver, kidneys).

2. Samples throughput per year

Not foreseeable.

3. Procedure

Cadmium is assayed by ETA-AAS and F-AAS.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Mineralization of animal tissues is carried out by dry ashing at 700 °C (50 °C/h) for at least 5 h. H_2SO_4 is added as an ashing aid. Ashes are dissolved in 0.4 % aqueous HNO_3 in 50 ml.

3.1.b. Analytical instrumentation

A Perkin-Elmer 4100 ZL or a VARIAN 220Z spectrometer is used.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 1, 2 and 3. Use is made of 0.05% $Mg(NO_3)_2$ and 1% $NH_4H_2PO_4$ as matrix modifiers.

Table 1. Equipment characteristics.

ETA-AAS
Wavelength, 228.8 nm
Slit width, 0.5 nm
Lamp, Cd HCL
Zeeman background correction
Pyrolitic coated graphite tubes with platform
Signal quantification, peak area

Table 2. Graphite furnace programme for Perkin Elmer.

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)	Ar Flow (ml/min)
1	130	30	10	250
2	250	20	5	250
3	725	30	5	250
4	1400	0	2	50
5	2400	2	2	250

Table 3. Graphite furnace programme for Varian.

Step	Temperature (°C)	Time (s)	Flow (l/min)
1	85	10.0	3.0
2	120	30.0	3.0
3	120	10.0	3.0
4	150	5.0	3.0
5	150	1.0	3.0
6	500	10.0	3.0
7	500	3.0	3.0
8	800	3.0	0.2
9	1900	0.7	0.2
10	1900	2.0	0.2
11	2300	3.0	3.0

3.2. Flame Atomic Absorption Spectrometry

3.2.a. Sample pretreatment

Mineralization of animal tissues is carried out by dry ashing at 700 °C (50 °C/h) for at least 5 h. H_2SO_4 is added as an ashing aid. Ashes are dissolved in 4 % aqueous HNO_3 in 50 mL.

3.2.b. Analytical instrumentation

A Varian SpectrAA 220 FS spectrometer equipped with an air-acetylene flame burner and a deuterium background corrector is used.

3.2.c. Working conditions

Instrumental parameters are reported in Table 3.

Table 3. Equipment characteristics.

F-AAS
Wavelength, 228.8 nm
Lamp, Cd HCL
Deuterium background correction
Air-acetylene flame

4. Quality control

Blanks determined out with each batch of samples and CRMs (CRM 278R, mussel tissue; CRM 184, bovine muscle; CRM 185, bovine liver; CRM 186, pig kidney; MA-B-3/TM, fish tissue and MA-A-1/TM, copepod homogenate and spike) are periodically analyzed.

5. Difficulties and limitations

Possibility of contaminations.

6. Comments and remarks

Not reported.

Federal Office for Consumer Protection and Food Safety

Berlin, Germany

Cadmium

Section I

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Zeeman Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

Section II

Sample ashing procedure

1. Matrices

2. Procedure

Section I

1. Matrices

Samples analyzed are meat, meat products, fish, fish products; plant materials, food.

2. Sample throughput per year

About 500.

3. Procedures

Cadmium is assayed by Z-ETA-AAS.

3.1. Zeeman Electrothermal Atomization Atomic Absorption Spectrometry.

3.1.a. Sample pretreatment

Mineralization is described in section II (Sample ashing procedure).

3.1.b. Analytical instrumentation

A SpectrAA 400 Zeeman - AAS with Autosampler GTA 96 (VARIAN) is used.

3.1.c.Working conditions

Instrumental parameters are reported in Table 1 and Table 2.

Table 1. Equipment characteristics.

ETA-AAS
Zeeman background correction
Pyrolytic coated tubes without platform
Injection volume: 20 µl + 5 µl modifier

The modifier used is a freshly prepared (1+1)-mixture of two solutions: (I) 0.5 ml of Pd-modifier + 9.5 ml HCl, 4 mol/l; (II) ascorbic acid, 10 g/l in water.

Table 2. Graphite furnace programme.

Step	Temperature (°C)	Time (s)	Gas Flow (L/min)
1	95	3	3
2	95	20	3
3	120	15	3
4	600	2	3
5	600	10	3
6	600	2	0
7	2000	1	0
8	2000	2	0
9	2400	2	3

The calibration curve is plotted by using Cadmium solutions of 0.2, 0.5, 1 and 2 ng/ml in 8 % HNO_3. The analytical blank is a solution of 8 % HNO3. Limit of detection is 0.1 ng/ml in sample solution.

4. Quality control

Analysis of certified reference materials (BCR, NIST, CBNM) is performed along with each analytical series.

5. Difficulties and limitations

Not reported

6. Comments

These method corresponds to the official German Method for food analysis.

Section II

Sample ashing procedure

1. Matrices

All materials will be mineralized following the same procedure.

2. Procedure

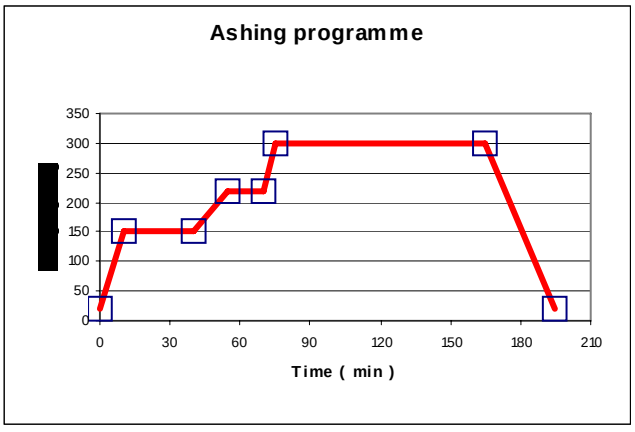
A high pressure ashing system HPA-S equipped with five 90 ml quartz vessels is used. Concentrated HNO_3, 65 % (FLUKA, pA plus) is used. Between 300 mg and 1500 mg of homogenized sample material are weighed into the quartz vessels. Sample amounts are illustrated in Table.

Table 1. Sample amounts for use in the HPA-S

Sample	Weight (mg)	Tolerance (mg)	Volume of HNO_3 (ml)
dry samples (< 20 % water)	500	± 50	3.0
fresh samples	1000	± 100	3.0
fresh samples (> 50 % water)	1500	± 200	3.0

Dry samples should be wetted with some drops water before HNO_3 is added; it's to recommend to let stand the vessels over night before heating. The mineralization start by using a ashing temperature of 300 °C over a hold time of 90 minutes. (see Fig. 1). When ashing procedure is finished, let cool down to room temperature. Check the volume of HNO_3 in the vessels to recognize leaky vessels. Remove dissolved NO_x gases by a gentle stream of nitrogen at the surface of the sample solution. Add water up to 20 ml or 25 ml and store solutions in polyethylene tubes with screw cap for the AAS analysis.

Fig. 1: Ashing programme of the HPA-S



Institute of Food Hygiene of Athens

Athens, Greece

Cadmium

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are foods of animal origin (fish meat and fishery products, meat, liver, kidney, milk and honey).

2. Samples throughput per year

Not foreseeable

3. Procedure

Cadmium is assayed by Z-ETA-AAS.

3.1.a. Sample pretreatment

Approximately 100g of samples (fish, meat, liver or kidney) are homogenized and 0.5g from the homogenized sample is digested with HNO_3 (5ml for meat, liver and kidney and 10 ml for fish and fishery products) in HP500 vessels. The digestion program is presented in Table 1.

Table 1. Digestion program for fish and fishery products

Step	Power(W)	Ramp Time(s)	Pressure (psi)	Temperature (^oC)	Hold Time (s)
1	600	25:00	190	200	10:00

Approximately 2g of milk is digested with HNO_3:H_2O_2 4:1. the digestion program is presented in Table 2.

Table 2. Digestion program for milk

Step	Power(W)	Ramp Time(s)	Pressure (psi)	Temperature (^oC)	Hold Time (s)
1	600	30:00	190	200	5:00

3.1.b. Analytical instrumentation

A Perkin-Elmer AAS instrument model 4110 with Zeeman background correction is used.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 3 and 4. A modifier of $NH_4H_2PO_4$ and $Mg(NO_3)_2$ is used.

Table 3. Equipment characteristics

Wavelength, 228.8 nm Slit width, 0.7nm Lamp, Cd EDL Zeeman background correction Graphite Tubes, End caped-THGA

Table 4. Graphite furnace program

Step	Temperature(°C)	Ramp Time (s)	Hold time (s)	Internal Flow	Read
1	110	1	30	250	
2	130	15	30	250	
3	500	10	20	250	
4	1500	0	5	0	x
5	2500	1	3	250	

Calibration curve is plotted by using aqueous solution 0.2, 0.5, 1.0, 1.5, and 2.0 ng/ml.

4. Quality control

An analytical run includes 1 CRM or in house RM and unknown samples of similar composition. For quality control, BCR 184, BCR 186, BCR 278R, BCR 422 and BCR 151 are used, depending on method.

The method for the determination of cadmium in fish and fishery products and the method for the determination of cadmium in meat, liver and kidney are accredited according to ISO 17025.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

National Food Investigation Institute

Budapest, Hungary

Cadmium

Determination of cadmium (Cd), copper (Cu) and lead (Pb) via AAS-technique as Standard Operation Procedure (SOP)

1. SOP-identification and-dating.
2. List of the amendments.
3. Names of the assambler and approver and their signatures subsequetly.
4. Aim of the analytical method.
5. Technical background informations.
6. Principle of the method/s atomic absorption spectrometry (AAS) at the underlisted resonance-lines: Cd – 228.8 nm; Cu – 324.8 nm and Pb – 217.0 nm respectively.
7. Labour safety.

Matrices (specimen).

Chemicals, reagents, standards.

Solvents.

8. Materials.

Chemical reagents: nitric acid-solutions, $Mg(NO_3)_2 \cdot 6H_2O$-solution, HCl-solution, deionized-water.

Instrument: AAS, hollow-cathode lamps, electrothermal atomizer, analytical balance, lab. water deionizer, certified automatic pipettes, blender, electronic block-heater, temp. controlled furnafe, platinum crucibles.

Description of the decontamination.

List of the technical documentations incl. the sample escorting note.

9. Method's description.

Arrival, recording and taking-over the samples.

Storage of the samples.

Sample preparation steps.

Analytical weighting.

Carbonization and dry-ashing ($105^oC \rightarrow 450^oC$ in 50^oC increments for approx. 2 hours in Pt-crucibles, adding Mg-nitrate solution.

Cooling-off, rinsing and dilution with HCl-solution.

See at N^o: 6.

9.4.1. Warming-up the hollow-cathode lamps and slotted quartz-tube optimization/positioning for the selected resonance lines; see at N^o: 6.

9.4.2. Selection of the measurement programme (slot, stabilization, integration-time, etc. managed by the computer.

9.4.3. Determination of the elemental (Cd, Cu, Pb)-concentrations of the sample solutions respectively.

9.4.5. Calculation of the individual elemental concentrations of the original sample as a function of the sample-solution's elemental-concents /$\mu g/cm^3$/, final volumes of the sample /cm^3/, the mass of the measured sample/g/.

9.4.6. Determination of LODs and LOQs.

9.4.7. Electronic data archiving.

Appendix-I

Microwave digestion of milk and milk-powder samples for multi-elemental (cadmium /Cd/, copper /Cu/, lead /Pb/, mercury /Hg/, stibium/antimory /Sb/, etc.) determination via inductively coupled plasma-mass spectrometric (ICP-MS) method

1. SOP-identification and dating.
2. Amendments.
3. Names of the assembler and approver and their signatures.
4. Aim and principle of the analytical method – The samples –
   - for designated multi-elemental analysis (Cd, Cu, Hg, Pb, Sb, etc.)
   - were digested by the mixture of HNO_3+H_2O_2+Au-solution,+Ge-, In-,

   Tl-ISTDs in TFE-bombs at temperature- and pressure-pogrammed, microwave-assisted apparatus. It had been supplemented with Y-standard solution and diluted into PFA/PP-tubes, in 1:33.3-rate dilution with high purity water for ICP-MS analysis.
   The sample solution was sprayed into – the high temperature, radiofrequency-assisted argon-plasma for elemental ionization – horizontal torch/vaporizer/. The emerging ions, via Ni-cone interface, will get into the mass spectrometer (MS). Within MS the positively charged ions were focused by ion-lens system and accelerated by negatively charged voltage. The accelerated and focused positively charged ions will get into the quadrupol-analyzer for radiofrequency assisted m/z-related sorting. The detection of defined isotopes was done by photomultiplier with sec-frequency and evaluated by the specific computer- programme.
5. Technical background informations.
6. Laborur safety.

6.1. Matrices (specimen).

6.2. Chemicals, reagents, standards.

6.3. Solvents.

6.4. Microwave digestion.

7. Materials.

7.1. Chemicals, reagents, standards, appliances for digestion: deionized water (0.05 μS), HNO_3, H_2O_2, Au, In, Ge, Ta, PFA-, PF-, PTX-, PTFE-beakers, measuring-tubes, centrif.-tubes, eprouvettes, digital pipettes and tips, dispensers.

7.2. Instruments: digital analytical balance, for digestion: Milestone Ethos Plus (with 100 ml TFM-teflon tubes, HPR-1000/10S type high-pressure segmented rotor); for deioized water: TKA-LAB (typ HP6UV/UF) water-purifier; for detection: Thermo Elemental X-series ICP-MS apparatus with CETAC ASX-510 robotic sample applier; computer.

7.3. List of technical documentations incl. smaple escorting note.

8. Method description.

8.1. Calibration-Measure of Au-, ISTD1/Ge-), ISTD2/In-), ISTD3/Tl-) ISTD4/Y-/solution.
Standard solutions: blanks, Au197-, Cu65-, Ge72-, In115-, Hg202-, Pb208-Sb121-, Tl205-, Zn66-, Y89-dilutions.

8.2. Set-up characteristics for ICP-MS apparatus:
   - Plasm-performance: 1300 W
   - Depth of sample immersion: 55 steps
   - Argon-gas-flow (outer): 0.7 $l.min^{-1}$
   - Argon-gas-flow (intermediary): 13 $l \cdot min^{-1}$
   - Argon-gas-flow (aerosol carrier): 0.87 $l \cdot min^{-1}$

- Sample volume (introduction): 1.0 ml·min^{-1}
- Nebulizer: concentric
- Nickel „sampler-cone”: ø1 mm
- Nickel „skimmer-cone”: ø 1.7 mm

Setup the integration-time in „peak-jump”

Mass/charge (m/z)	Element regarded to setted m/z	Integration time (m sec)
65	Cu	20
66	Zn	20
72	Ge	10
82	Se	20
89	Y	10
111	Cd	20
115	In	10
121	Sb	20
202	Hg	20
205	l	10
289	Pb	20

Programme of the peristaltic pump and the autosampler

Before data-collection	
Volume of the sampling-flow	1 ml/min
Sampling time	60 sec
Revolution per minute	34 rpm
Stabilization time	30 sec
Subsequent to data-collection	
Velocity of the flushing	3 ml/min
Duration of the flushing	45 sec
Revolution per minute	100 rpm

8.3. Main influencing parameters to be considered:
- interference-equations,
- MS-setup parameters,
- Nebulizer-parameters,
- Programme of the peristatic pump,
- Programme of autosampler.

8.9. Calibration procedure:
- Mass Calibration with tuning-solutions per month.
- Detector Plateau' determination with tuning-solutions, morthly.
- Cross Calibration with tuning-sulution, weekly.

8.10. Execution of the measurements.
8.11. Correction of the measurements (elimination of matrix-disturbance) with proper ISTD.
8.12. Calculations according to the equations executed by the computer-programmes.
8.13. Calculation of the measurement-uncertainty.
see: 2002/657 EC-Reg; ISO/EC 17025 std.
8.8.1. Uncertainty.
8.8.2. Recover.
8.8.12. Repeatability.
8.8.13. Within-laboratory reproducibility.
8.8.14. Limit of detection (LOD).
8.8.15. Limit of quantitation (LOQ).
8.8.16. Decision limit (CCalpha).
8.8.17. Detection capability (CCbeta).
8.8.18. Intercomparison with reference materials (e.g: MILK POWDER BCR # 150) (Cd, Cu, Hg, Pb).
8.8.19. Results of the participation in international proficiency testing (PT) programmes.

8.8.20. Evaluation of the possible interferences.

9. Calculations.
9.1. Determination of the elemental-concentration/s of the sample solution.
9.4. Calculation of the elemental concentration=/s of the original smaple.
9.5. Electronic data archiving.

Central Meat Control Laboratory

Celbridge, County Kildare, Ireland

Cadmium

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are muscle, kidney and liver tissue.

2. Samples throughput per year

Not repoted.

3. Procedure

Cadmium is assayed by Z-ETA-AAS.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

1 g of animal tissue is weighed into a digestion vessel. 4 ml of $HNO_3$are added and the solution is allowed to stand in fume hood for at least one hour. A spike control is prepared with 100 ppb of Cd and 1 ml of H_2O (Analytical grade water from Millipore Q system) is added to all samples subsequently. The samples are heated in a Microwave digestion system, MDS 2000 (CEM), following the digestion programme outlined in Table 1.

Table 1. Standard digestion programme.

Stage	Power	Pressure	Run Time	Run Time at press	Fan Speed
1	90 %	50	30.00	5.00	100 %
2	90 %	100	20.00	5.00	100 %
3	90 %	150	20.00	5.00	100 %
4	0 %	0	20.00	5.00	100 %
5	90 %	150	20.00	5.00	100 %

On completion the digest sequence, the vessels are allowed to cool and the pressure to drop below 40 psi before removing from the oven to the fume hood. Using a capillary pipette the sample is purged with a steam of nitrogen for one minute to evolve any dissolved gases

3.1.b. Analytical instrumentation

Cadmium analysis is carried out on Spectra AA-220Z Atomic Absorption Spectrophotometer with the Varian GTA 110Z Graphite Tube Furnace with autosampler.

3.1.c. Working conditions

The modifier used is a Palladium (0.1 %) with Magnesium nitrate (0.1 %) made up in a 25% Citric acid solution. Instrumental parameters are reported in Table 2 and Table 3.

Table 2. Equipment characteristics.

ETA-AAS
Wavelength, 228.8 nm
Slit width, 0.5 nm
Slit Height, normal
Lamp, Cd HCL
Calibration Mode, concentration
Measurement Mode, peak height
Sampling Mode, automix
Replicates All, 3

Standards made up with 5 % HNO_3. The samples are diluted to 1:50 with H_2O.

Table 3. Furnace programme.

Step	**Temperature (°C)**	**Time (s)**	**Gas Flow (L/min)**
1	85	5	3
2	95	40	3
3	120	10	3
4	250	5	3
5	250	1	3
6	250	2	0
7	1800	0.8	0
8	1800	2	0
9	2300	1	3
10	2300	3	3

4. Quality control

Reagent blanks, check and spiked samples are incorporated into each assay.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Final results are calculated, only if an acceptable standard curve has been achieved and takes into account sample weights and dilution factors.

National Institute of Health
Istituto Superiore di Sanità

Rome, Italy

Cadmium

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions
3.2. Inductively Coupled Plasma Atomic Emission Spectrometry
3.2.a. Sample pretreatment
3.2.b. Analytical instrumentation
3.2.c. Working conditions
3.3. Inductively Coupled Plasma Mass Spectrometry
3.3.a. Sample pretreatment
3.3.b. Analytical instrumentation
3.3.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are liver, kidneys, milk, canned vegetables and fruit juices.

2. Samples throughput per year

Not foreseeable.

3. Procedure

Three different techniques are used for the determination of Cd in the samples.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

The sample is homogenized, weighed and digested at high pressure in a microwave oven. Usually an HNO_3-H_2O_2 (5:2) solution is used. Use is made of KH_2PO_4 and $Mg(NO_3)_2 \cdot 6H_2O$ as matrix modifiers.

3.1.b. Analytical instrumentation

A Perkin-Elmer Model 5100 AAS with Zeeman background correction, equipped with an HGA-600 graphite furnace and an AS-60 autosampler is used.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 1 and 2.

Table 1. Equipment characteristics.

ETA-AAS
Wavelength, 228.8 nm
Slit width, 0.7 nm
Lamp, Cd EDL and HCL
Zeeman background correction
L'vov platform
Injection volume, 20 µl

Table 2. Graphite furnace programme.

Step	**Temperature (°C)**	**Ramp Time (s)**	**Hold Time (s)**	**Ar Flow (ml/min)**
Drying	130	10	20	300
Charring	300	15	20	300
Atomization	1800	0	3	0
Clean-up	2500	2	2	300

Calibration curve is performed by the standard addition method.

3.2. Inductively Coupled Plasma Atomic Emission Spectrometry

3.2.a. Sample pretreatment

The sample is homogenized, weighed and digested at high pressure in a microwave oven. Usually an HNO_3-H_2O_2 solution is used.

3.2.b. Analytical instrumentation

A Jobin-Yvon 38 VHR spectrometer equipped with a U-5000 AT CETAC ultrasonic nebulizer is used.

3.2.c. Working conditions

Instrumental parameters are reported in Table 3.

Table 3. Equipment characteristics.

ICP-AES
Wavelength, 228.8 nm Torch, INSA, demountable RF generator, Durr-JY3848, frequency 56 MHz, maximum power output 2.2kW Nebulizer, ultrasonic, U-5000 AT, CETAC Argon flows, plasma 16 l/min, auxiliary 0.3 l/min, aerosol 0.5 l/min Monochromator, HR 1000-M, focal length 1 m, Czerny-Turner mounting, equipped with a 3600 grooves/mm holographic plane grating, linear dispersion in the first order of 0.27 nm/mm, spectral range of 170-450 nm, entrance and exit slit widths of 40 µm

Calibration is carried out by using various Cd solutions depending on the Cd content in the sample.

3.3. Inductively Coupled Plasma Mass Spectrometry

3.3.a. Sample pretreatment

The sample is homogenized, weighed and digested at high pressure in a microwave oven. Usually an HNO_3-H_2O_2 solution is used.

3.3.b. Analytical instrumentation

A Perkin-Elmer/Sciex Elan 6000 quadrupole mass spectrometer is used.

3.3.c. Working conditions

Instrumental parameters are reported in Table 4.

Table 4. Equipment characteristics.

ICP-MS
Analytical mass, ^{114}Cd
Reference mass ^{103}Rh
Nebulizer, cross flow, Ryton spray chamber
Argon flows, plasma 16 l/min, auxiliary 0.9 l/min, aerosol 1 l/min
RF generator, maximum power output 1.0 kW
Scanning conditions: sweeps per reading, 5; readings per replicate, 4; number of replicates, 5; peak hopping; dwell time, 100 ms; replicate time, 2000 ms; normal resolution, 0.9-0.6 amu

Calibration is carried out by using various Cd solutions depending on the Cd content in the sample.

4. Quality control

An analytical run includes 1 blank, 1 CRM and unknown samples of similar composition. The accuracy of the method is estimated by means of CRMs (CRM 184, bovine muscle; CRM 185, bovine liver; CRM 186, pig kidney and CRM 150, skim milk). The calibration curves are accepted when the coefficient of correlation (r) is > 0.999.

5. Difficulties and limitations

Since both Cd and Sn have an isotope at *m/z* = 114, a correction factor at *m/z* = 118 is applied to estimate the actual Sn counts.

6. Comments and remarks

Not reported.

Food and Veterinary Regulation Division

Albertown, Marsa, Malta

Cadmium

THE DETERMINATION OF LEAD AND CADMIUM IN MILK

1.0 INTRODUCTION

This method is suitable for the determination of lead (Pb) and cadmium (Cd) in fresh and powdered milk. Lead and cadmium are non-essential elements, causing several adverse health effects in humans after accumulation in multiple organs in the body. The current maximum level of Pb in cows' milk set by the EU is 0.02 mg/kg wet weight.

2.0 EQUIPMENT

2.1 Precision balance with a range of 300 g and an accuracy of 0.001 g
2.2 Analytical balance with a range of 300 g and an accuracy of 0.0001 g
2.3 Variable micropipettes (Finpipettes)
2.4 Spatulas, fibre-based or plastic. ***(Note: No metal spatulas to be used)***.
2.5 Microwave digestion system (CEM MARS5)
2.6 Oven (Memmert ULE500)
2.7 Atomic absorption spectrometer with graphite furnace and D2 background correction (GBC932AA) and PAL3000 autosampler
2.8 Lead super lamp
2.9 Cadmium super lamp
2.10 Pyrolytic coated graphite tube with pyrolytic-coated platform

3.0 REAGENTS

3.1 Chemicals

3.1.1 Water, de-ionised.
3.1.2 Nitric acid, about 69%, s.g. 1.42 (High purity, such as BDH ARISTAR)
3.1.3 Hydrogen peroxide, 30% (SpectrosoL, BDH)
3.1.4 Ammonium dihydrogen orthophosphate (BDH AnalaR or equivalent)
3.1.5 Magnesium nitrate hexahydrate (BDH AnalaR or equivalent)
3.1.6 Lead standard solution, 1000 ppm (BDH or equivalent)
3.1.7 Cadmium standard solution, 1000 ppm (BDH or equivalent)

3.2 Solutions

3.2.1 Decon 90, 5%
Mix 20 mL Decon 90 (5.1.4) with 380 mL of deionised water in a 500 mL measuring cylinder. Transfer to a wash bottle.

3.2.2 Modifier (1% $NH_4H_2PO_4$, 0.2% $Mg(NO_3)_2$)
Weigh out 1 g of ammonium dihydrogen orthophosphate (5.1.4) in a 250 mL beaker and add about 50 mL of deionised water to dissolve. Add 0.2 g of magnesium nitrate hexahydrate (5.1.5) to this solution and stir to dissolve, adding more deionised water if necessary. Transfer the solution quantitatively to a 100 mL volumetric flask and make up to the mark with deionised water.

3.2.3 Nitric acid solution, 1%
Transfer about 80 mL of deionised water to a 100 mL volumetric flask, add 1 mL of concentrated nitric acid (3.1.2) and dilute to the mark with deionised water.

3.3 **Reference materials**

Certified Reference Materials, if available, e.g.:
BCR-150: Skim milk powder (spiked)
BCR-151: Skim milk powder (spiked)
Choose a reference material which closely resembles the test sample.

4.0 STANDARD SOLUTIONS

4.1 Combined lead and cadmium spiking solution (2 mg/L Pb + 0.5 mg/L Cd)

Pipette 200 µL of lead stock standard (1000 mg/L, 5.1.5) and 50 µL of cadmium stock standard (1000 mg/L, 5.1.6) into a 100 mL volumetric flask. Add 1 mL of concentrated nitric acid and dilute to the mark with deionised water. Store this solution at 0-5ºC. Prepare on same day of use.

4.2 Lead and cadmium intermediate standard solutions (10 mg/L Pb, 10 mg/L Cd)

Pipette 1000 µL of lead stock standard (1000 mg/L, 3.1.6) and 1000 µL of cadmium stock standard (1000 mg/L, 3.1.7) into separate 100 mL volumetric flasks. Add 1 mL of concentrated nitric acid to each and dilute to the mark with deionised water. Prepare on same day of use.

4.3 Lead working standard solution (40 µg/L)

Pipette 400 µL of lead intermediate standard (10 mg/L, 4.2) into a 100 mL volumetric flask. Add 1 mL of concentrated nitric acid and dilute to the mark with deionised water. Prepare on same day of use.

4.4 Cadmium working standard solution (10 µg/L)

Pipette 100 µL of cadmium intermediate standard (10 mg/L, 4.2) into a 100 mL volumetric flask. Add 1 mL of concentrated nitric acid and dilute to the mark with deionised water. Prepare on same day of use.

5.0 PLANNING THE DIGESTION

5.1 Each batch must contain a certified reference material, duplicate samples, at least 1 blank and 1 spiked sample.

5.2 A maximum of 14 samples/blanks can be digested at one time.

6.0 WEIGHING OF SAMPLES

6.1 Using the precision balance, weigh out 10 g ± 0.30 g of liquid milk sample in duplicate into separate clean vessel liners. Record the sample weights accurately up to 3 decimal places.

6.2 For powdered milk, weigh out 0.50 g ± 0.03 g of sample in duplicate into separate clean vessel liners, using the analytical balance. Record the sample weights

accurately up to 4 decimal places. Add 1 mL deionised water to each vessel liner containing powdered milk.

6.3 Weigh out aliquots (as in 6.1) of the samples to be spiked and transfer to separate clean vessel liners. A minimum of one spiked sample should be included in the batch. Record the sample weights accurately up to 3 decimal places.

6.4 Choose appropriate reference materials (3.3) and weigh out 0.50 g ± 0.05 g of the chosen material into separate clean vessel liners, using the analytical balance. Record the sample weights accurately up to 4 decimal places. Add 1 mL deionised water to each vessel liner containing the reference materials.

6.5 For the blank, weigh out 10 g of deionised water in a clean vessel liner. A minimum of one blank should be included in each batch.

7.0 SPIKING PROCEDURE

7.1 To the appropriate samples (6.3), add 200 µL of lead/cadmium spiking solution (2 mg/L Pb + 0.5 mg/L Cd, 4.1). This is equivalent to 40 ng Pb/g sample and 10 ng Cd/g sample.

7.2 Allow the spiked samples to stand for 10 minutes before digesting.

8.0 DIGESTION PROCEDURE

8.1 Place vessel liners containing the weighed out samples, spikes, certified reference materials and blanks in an oven. Heat overnight at 100ºC.

8.2 The next day, add 5 mL of concentrated HNO_3 and 1 mL of 30% m/v H_2O_2 to each vessel liner.

8.3 Allow the samples to pre-digest for 10-15 minutes. During this period, monitor the reaction in the vessel liners and place the most reactive sample (determined from the degree of frothing observed) in the control vessel.

8.4 Tightly cap the vessels and place in the microwave oven. Heat the contents of the vessels to 1200 W (100% power for 14 vessels) over a 15-minute ramp period and hold at this power for an additional 12 minutes (Sample digestion programme in Appendix I). Control the pressure and temperature at 350 psi and 190ºC respectively.

8.5 After the heating cycle, cool the contents of the vessels for 15 minutes.

8.6 When cool, loosen the vessel caps and expel the interior gas into a fume hood.

8.7 Then remove the caps and quantitatively transfer the contents into 10 mL volumetric flasks and dilute to volume with ddH_2O.

8.8 Analyse the diluted digests obtained in 8.7 with graphite furnace AAS for the determination of Pb and Cd.

9.0 ANALYSIS BY GRAPHITE FURNACE AAS

9.1 Set up the AAS instrument for analysis using graphite furnace. The instrument parameters are shown in Appendix II (Pb analysis) and Appendix III (Cd analysis).

9.2 The blank solution (diluent) used in 1% nitric acid (3.2.3).

9.3 The modifier used is 1% $NH_4H_2PO_4$, 0.2% $Mg(NO_3)_2$ (3.2.2).

9.4 Standard is 40 µg lead / L (4.3) and 10 µg cadmium / L (4.4) for Pb and Cd analysis respectively.

9.5 The instrument is calibrated by performing standard additions on the spiked milk digest. Recalibration should be carried out after every 8-10 samples.

10.0 CALCULATION OF RESULTS

10.1 The print out from the GBC AAS gives the concentration of lead or cadmium in the digest in µg/L, also % RSD.

10.2 A Microsoft Excel spreadsheet, stored as 'Milk HM worksheet', is used to calculate results.

10.3 The concentration of lead or cadmium in the sample (ng/g), [X], is calculated as follows:-

$$[X] = (C - C_{bl}) \times V_{sa} \times \frac{V_{dil}}{m}$$

where:

C	=	concentration of lead or cadmium in the digest (µg/L).
C_{bl}	=	average concentration of lead or cadmium in the blanks (µg/L).
V_{sa}	=	dilution of digest for standard additions, i.e. 4 for Pb; 2.667 for Cd
V_{dil}	=	dilution volume of sample, i.e. 10 mL (see section 8.7)
m	=	weight of sample, in g

Note: For certified reference material multiply also by the moisture correction factor to get lead or cadmium concentration (ng/g) on a dry weight basis.

10.4 From the duplicate assays, calculate the mean result for each sample.

10.5 Recovery of analyte, R %, is calculated as follows:

$$R = \frac{[S] - [N]}{[A]} \times 100$$

where

[S] =	the concentration of lead or cadmium found in the spiked sample
[N] =	the concentration of lead or cadmium found in the respective unspiked sample
[A] =	the concentration of lead or cadmium added

The mean recovery figure for each batch is then calculated.

APPENDIX I

MICROWAVE DIGESTION PROGRAMMES FOR CLEAN-UP OF THE VESSELS AND DIGESTION OF THE SAMPLES

Vessels: 14 HP-500 vessels

	Clean up	Sample digestion
Reagents	10 mL HNO_3	5 mL HNO_3 + 1 mL H_2O_2
Maximum power	1200 W	1200 W
% power	100	100
Ramp time (min)	10	15
Hold time (min)	10	12
Control pressure (psi)	350	350
Control temperature (ºC)	170	190
Cooling time (min)	15	15

APPENDIX II

INSTRUMENTAL PARAMETERS FOR THE ANALYSIS OF LEAD

System Type	Furnace
Graphite tube	Pyrolytic-coated with platform
Element	Pb
Lamp Current	8.0 mA
Wavelength	283.3 nm
Slit Width	1.0 nm
Slit Height	Reduced
Instrument Mode	Abs. BC On
Measurement Mode	Peak Area
Sample Introduction	Automatic
Time Constant	0.0
Replicates	3
Calibration Mode	Standard Additions

Graphite Furnace Operating Conditions

Step No.	Final Temperature (°C)	Ramp Time (sec)	Hold Time (sec)	Gas Type (*)	Read
1	Inject Sample				
2	150	5.0	1.0	Inert	Off
3	190	20.0	20.0	Inert	Off
4	450	10.0	1.0	Inert	Off
5	1200	15.0	15.0	Aux.	Off
6	1200	0.0	2.0	None	Off
7	1900	0.4	2.0	None	On
8	2600	0.5	2.0	Inert	Off
9	30	13.0	20.0	Inert	Off

(*) Gas Type:-
Inert (Nitrogen): Flow rate = 1.3 L/min (approx.); 2.5 units
Auxiliary (Argon): Flow rate = 1.0 L/min (approx.); 2.5 units

Sampler parameters for standard additions

	Volume (µL)				Added concentration of Pb (ppb)
	Diluent (1% HNO_3, 5.2.3)	Standard (40 µg/L Pb, 7.3)	Sample	Modifier (5.2.2)	
Sample	8	0	4	4	-
Blank	12	0	0	4	-
Addition 1	6	2	4	4	5.0
Addition 2	4	4	4	4	10.0
Addition 3	2	6	4	4	15.0

APPENDIX III

INSTRUMENTAL PARAMETERS FOR THE ANALYSIS OF CADMIUM

System Type	Furnace
Graphite tube	Pyrolytic-coated with platform
Element	Cd
Lamp Current	3.0 mA
Wavelength	228.8 nm
Slit Width	1.0 nm
Slit Height	Reduced
Instrument Mode	Abs. BC On
Measurement Mode	Peak Area
Sample Introduction	Automatic
Time Constant	0.0
Replicates	3
Calibration Mode	Standard Additions

Graphite Furnace Operating Conditions

Step No.	Final Temperature (°C)	Ramp Time (sec)	Hold Time (sec)	Gas Type (*)	Read
1	Inject Sample				
2	150	5.0	7.0	Inert	Off
3	180	40.0	20.0	Inert	Off
4	280	10.0	1.0	Inert	Off
5	800	10.0	10.0	Aux.	Off
6	800	0.0	2.0	None	Off
7	1600	0.4	3.0	None	On
8	2300	0.5	2.0	Inert	Off
9	30	13.0	20.0	Inert	Off

(*) Gas Type:-
Inert (Nitrogen): Flow rate = 1.3 L/min (approx.); 2.5 units
Auxiliary (Argon): Flow rate = 1.0 L/min (approx.); 2.5 units

Sampler parameters for standard additions

	Volume (µL)				Added concentration of Cd (ppb)
	Diluent (1% HNO_3, 5.2.3)	Standard (10 µg/L Cd, 7.4)	Sample	Modifier (5.2.2)	
Sample	6	0	6	4	-
Blank	12	0	0	4	-
Addition 1	4	2	6	4	1.25
Addition 2	2	4	6	4	2.50
Addition 3	0	6	6	4	3.75

Food and Veterinary Regulation Division

Albertown, Marsa, Malta

THE DETERMINATION OF LEAD AND CADMIUM IN ANIMAL TISSUES, USING MICROWAVE HEATING AS A DIGESTION PROCEDURE

1.0 INTRODUCTION

The method described in this SOP is suitable for the determination of lead (Pb) and cadmium (Cd) in animal tissues, freeze dried and fresh. The matrices covered by this SOP are: bovine and swine liver, poultry and rabbit muscle, and fish muscle. The current maximum levels set by the EU are the following:-

Product	Maximum level (mg/kg wet weight)	
	Lead	Cadmium
Bovine and swine liver	0.5	0.5
Poultry muscle	0.1	0.05
Muscle meat of fish	0.2 (a)	0.05 (b)
Muscle meat of fish	0.4 (c)	0.1 (d)

(a) Muscle meat of fish, excluding fish species listed in (c)
(b) Muscle meat of fish, excluding fish species listed in (d)
(c) Muscle meat of bonito, common two-banded seabream, eel grey mullet, grunt, horse mackerel or scad, sardine, sardinops, spotted seabass, tuna, wedge sole.
(d) Muscle meat of bonito, common two-banded seabream, eel, European anchovy, grey mullet, horse mackerel or scad, louver or luvar, sardine, sardinops, tuna, wedge sole.

2.0 EQUIPMENT

2.1 Precision balance with a range of 300 g and an accuracy of 0.001 g
2.2 Analytical balance with a range of 300 g and an accuracy of 0.0001 g
2.3 Variable micropipettes (Finpipettes)
2.4 Spatulas, fibre-based or plastic. ***(Note: No metal spatulas to be used)***.
2.5 Microwave digestion system (CEM MARS5)
2.6 Atomic absorption spectrometer with graphite furnace and D2 background correction (GBC932AA) and PAL3000 autosampler
2.7 Lead super lamp
2.8 Cadmium super lamp
2.9 Pyrolytic coated graphite tube with pyrolytic-coated platform

3.0 REAGENTS

3.1 Chemicals

3.1.1 Water, de-ionised.
3.1.2 Nitric acid, about 69%, s.g. 1.42 (High purity, such as BDH ARISTAR)
3.1.3 Hydrogen peroxide, 30% (SpectrosoL, BDH)
3.1.4 Ammonium dihydrogen orthophosphate (BDH AnalaR or equivalent)
3.1.5 Magnesium nitrate hexahydrate (BDH AnalaR or equivalent)
3.1.6 Lead standard solution, 1000 ppm (BDH or equivalent)
3.1.7 Cadmium standard solution, 1000 ppm (BDH or equivalent)

3.2 Solutions

3.2.1 Decon 90, 5%
Mix 20 mL Decon 90 with 380 mL of deionised water in a 500 mL measuring cylinder. Transfer to a wash bottle.

3.2.2 Modifier (1% $NH_4H_2PO_4$, 0.2% $Mg(NO_3)_2$)
Weigh out 1 g of ammonium dihydrogen orthophosphate (3.1.4) in a 250 mL beaker and add about 50 mL of deionised water to dissolve. Add 0.2 g of magnesium nitrate hexahydrate (3.1.5) to this solution and stir to dissolve, adding more deionised water if necessary. Transfer the solution quantitatively to a 100 mL volumetric flask and make up to the mark with deionised water.

3.2.3 Nitric acid solution, 1%
Transfer about 80 mL of deionised water to a 100 mL volumetric flask, add 1 mL of concentrated nitric acid (3.1.2) and dilute to the mark with deionised water.

3.3 **Reference materials**
Certified Reference Materials, if available, e.g.:
BCR 185: Lyophilised Bovine Liver
DORM2: Lyophilised Dogfish muscle
CRM 422: Lyophilised Cod muscle
Choose a reference material which closely resembles the test sample.

4.0 STANDARD SOLUTIONS

4.1 Combined lead and cadmium solutions (for spiking)

4.1.1 Combined lead and cadmium intermediate solution (5 mg/L Pb, 1.5 mg/L Cd)
Pipette 500 µL of lead stock standard (1000 mg/L) and 150 µL of cadmium stock standard (1000 mg/L) into a 100 mL volumetric flask. Add 1 mL of concentrated nitric acid and dilute to the mark with deionised water. Store this solution at 0-5ºC. Prepare every week.

4.1.2 Combined lead and cadmium spiking solution (250 µg/L Pb, 75 µg/L Cd)
Pipette 2.5 mL of combined lead and cadmium intermediate solution (4.1.1) into a 50 mL volumetric flask. Add 0.5 mL of concentrated nitric acid and dilute to the mark with deionised water. Prepare on same day of use. Use this solution for spiking (Section 7.0).

4.2 Separate lead and cadmium standard solutions (for calibration)

4.2.1 Lead and cadmium intermediate standard solutions (10 mg/L Pb, 10 mg/L Cd)
Pipette 1000 µL of lead stock standard (1000 mg/L, 3.1.6) and 1000 µL of cadmium stock standard (1000 mg/L, 3.1.7) into separate 100 mL volumetric flasks. Add 1 mL of concentrated nitric acid to each and dilute to the mark with deionised water. Store this solution at 0-5ºC. Prepare every week.

4.2.2 Lead working standard solution (40 µg/L)
Pipette 400 µL of lead intermediate standard (10 mg/L, 4.2.1) into a 100 mL volumetric flask. Add 1 mL of concentrated nitric acid and dilute to the mark with deionised water. Prepare on same day of use.

4.2.3 Cadmium working standard solution (20 µg/L)
Pipette 200 µL of cadmium intermediate standard (10 mg/L, 4.2.1) into a 100 mL volumetric flask. Add 1 mL of concentrated nitric acid and dilute to the mark with deionised water. Prepare on same day of use.

5.0 PLANNING THE DIGESTION

5.1 Each batch must contain a certified reference material, duplicate samples, at least 1 blank and 1 spiked sample for each species/matrix combination.

5.2 A maximum of 14 samples/blanks can be digested at one time.

5.3 Tissues which closely resemble each other (e.g. bovine liver and swine liver) may be digested in the same batch. Different tissues or animal products should be digested in separate batches.

6.0 WEIGHING OF SAMPLES

6.1 Using the precision balance, weigh out 1.00 ± 0.10 g of homogenised sample in duplicate into separate clean vessel liners. Record the sample weights accurately up to 3 decimal places.

6.2 Weigh out aliquots (as in 6.1) of the samples to be spiked and transfer to separate clean vessel liners. A minimum of one spiked sample should be included per species / matrix combination analysed in the batch. Record the sample weights accurately up to 3 decimal places.

6.3 Choose appropriate reference materials (3.3) and weigh out 0.40-0.50 g (according to Appendix II) of the chosen materials into separate clean vessel liners, using the analytical balance. Record the sample weights accurately up to 4 decimal places. Add 1 mL deionised water to each vessel liner containing the reference materials.

7.0 SPIKING PROCEDURE

7.1 To the appropriate samples (6.2), add 200 µL of lead/cadmium spiking solution (250 µg/L Pb, 75 µg/L Cd, 4.1.2). This is equivalent to 50 ng Pb/g sample and 15 ng Cd/g sample.

7.2 Allow the spiked samples to stand for 10 minutes before digesting.

8.0 DIGESTION PROCEDURE

8.1 Add 7 mL of concentrated HNO_3 and 1.5 mL of 30% m/v H_2O_2 to each vessel liner containing the samples, spiked samples and certified reference materials.

8.2 For the blank, add 7 mL of concentrated HNO_3 and 1.5 mL of 30% m/v H_2O_2 to a clean vessel liner. A minimum of one blank should be included in each batch.

8.3 Allow the samples to pre-digest for 10-15 minutes. During this period, monitor the reaction in the vessel liners and place the most reactive sample (determined from the degree of frothing observed) in the control vessel.

8.4 Tightly cap the vessels and place in the microwave oven. Heat the contents of the vessels to 1200 W (100% power for 14 vessels) over a 15-minute ramp period and hold at this power for an additional 12 minutes (Sample digestion programme in Appendix I). Control the pressure and temperature at 350 psi and 190ºC respectively.

8.5 After the heating cycle, cool the contents of the vessels for 15 minutes.

8.6 When cool, loosen the vessel caps and expel the interior gas into a fume hood.

8.7 Then remove the caps and quantitatively transfer the contents into 20 mL volumetric flasks and dilute to volume with ddH_2O.

8.8 Analyse the diluted digests obtained in 8.7 with graphite furnace AAS for the determination of Pb and Cd.

9.0 ANALYSIS BY GRAPHITE FURNACE AAS

9.1 Set up the AAS instrument for analysis using graphite furnace. The instrument parameters are shown in Appendix III (Pb analysis) and Appendix IV (Cd analysis).

9.2 The blank solution (diluent) used in 1% nitric acid (5.2.3).

9.3 The modifier used is 1% $NH_4H_2PO_4$, 0.2% $Mg(NO_3)_2$ (5.2.2).

9.4 Standard is 40 µg lead / L (7.2.2) and 20 µg cadmium / L (7.2.3) for Pb and Cd analysis respectively.

9.5 The instrument is calibrated by performing standard additions on a spiked tissue digest. Recalibration should be carried out after every 8-10 samples.

10.0 CALCULATION OF RESULTS

10.1 The print out from the GBC AAS gives the concentration of lead or cadmium in the digest in µg/L, also % RSD.

10.2 A Microsoft Excel spreadsheet, stored as 'Tissue HM worksheet', is used to calculate results.

10.3 The concentration of lead or cadmium in the sample (ng/g), [X], is calculated as follows:-

$$[X] = \frac{(C - C_{bl}) \times V_{sa} \times V_{dil}}{m}$$

where:

C = concentration of lead or cadmium in the digest (µg/L).
C_{bl} = average concentration of lead or cadmium in the blanks (µg/L).
V_{sa} = dilution of digest for standard additions, i.e. 2.667.
V_{dil} = dilution volume of sample, i.e. 20 mL (see section 8.7)
m = weight of sample, in g

Note: For certified reference material multiply also by the moisture correction factor to get lead or cadmium concentration (ng/g) on a dry weight basis.

10.4 From the duplicate assays, calculate the mean result for each sample.

10.5 Recovery of analyte, R %, is calculated as follows:

$$R = \frac{[S] - [N]}{[A]} \times 100$$

where

[S] = the concentration of lead or cadmium found in the spiked sample
[N] = the concentration of lead or cadmium found in the respective unspiked sample
[A] = the concentration of lead or cadmium added

The mean recovery figure for each batch is then calculated.

APPENDIX I

MICROWAVE DIGESTION PROGRAMMES FOR CLEAN-UP OF THE VESSELS AND DIGESTION OF THE SAMPLES

Vessels: 14 HP-500 vessels

	Clean up	Sample digestion
Reagents	10 mL HNO_3	7 mL HNO_3 + 1.5 mL H_2O_2
Maximum power	1200 W	1200 W
% power	100	100
Ramp time (min)	10	15
Hold time (min)	10	12
Control pressure (psi)	350	350
Control temperature (ºC)	170	190
Cooling time (min)	15	15

APPENDIX II

CERTIFIED REFERENCE MATERIALS USED FOR QUALITY CONTROL PURPOSES IN THE MICROWAVE DIGESTION OF ANIMAL TISSUES

Certified Reference Material (CRM)	Indicative weight of CRM (g)	Elements analysed
CRM422 Cod muscle	0.5	Pb, Cd
DORM2 Dogfish muscle	0.5	Pb, Cd
BCR185 Bovine liver	0.4	Pb, Cd

APPENDIX III

INSTRUMENTAL PARAMETERS FOR THE ANALYSIS OF LEAD

System Type	Furnace
Graphite tube	Pyrolytic-coated with platform
Element	Pb
Lamp Current	8.0 mA
Wavelength	283.3 nm
Slit Width	1.0 nm
Slit Height	Reduced
Instrument Mode	Abs. BC On
Measurement Mode	Peak Area
Sample Introduction	Automatic
Time Constant	0.0
Replicates	3
Calibration Mode	Standard Additions

Graphite Furnace Operating Conditions

Step No.	Final Temperature (°C)	Ramp Time (sec)	Hold Time (sec)	Gas Type (*)	Read
1	Inject Sample				
2	150	5.0	1.0	Inert	Off
3	190	30.0	25.0	Inert	Off
4	450	10.0	1.0	Inert	Off
5	1200	15.0	15.0	Aux.	Off
6	1200	0.0	2.0	None	Off
7	1900	0.4	2.0	None	On
8	2600	0.5	2.0	Inert	Off
9	30	13.0	20.0	Inert	Off

(*) Gas Type:-
Inert (Nitrogen): Flow rate = 1.3 L/min (approx.); 2.5 units
Auxiliary (Argon): Flow rate = 1.0 L/min (approx.); 2.5 units

Sampler parameters for standard additions

	Volume (µL)				Added concentration of Pb (ppb)
	Diluent (1% HNO_3, 3.2.3)	Standard (40 µg/L Pb, 4.2.2)	Sample	Modifier (3.2.2)	
Sample	6	0	6	4	-
Blank	12	0	0	4	-
Addition 1	4	2	6	4	5.0
Addition 2	2	4	6	4	10.0
Addition 3	0	6	6	4	15.0

APPENDIX IV

INSTRUMENTAL PARAMETERS FOR THE ANALYSIS OF CADMIUM

System Type	Furnace
Graphite tube	Pyrolytic-coated with platform
Element	Cd
Lamp Current	3.0 mA
Wavelength	228.8 nm
Slit Width	1.0 nm
Slit Height	Reduced
Instrument Mode	Abs. BC On
Measurement Mode	Peak Area
Sample Introduction	Automatic
Time Constant	0.0
Replicates	3
Calibration Mode	Standard Additions

Graphite Furnace Operating Conditions

Step No.	Final Temperature (°C)	Ramp Time (sec)	Hold Time (sec)	Gas Type (*)	Read
1	Inject Sample				
2	150	5.0	7.0	Inert	Off
3	180	40.0	20.0	Inert	Off
4	280	10.0	1.0	Inert	Off
5	800	10.0	10.0	Aux.	Off
6	800	0.0	2.0	None	Off
7	1600	0.4	3.0	None	On
8	2300	0.5	2.0	Inert	Off
9	30	13.0	20.0	Inert	Off

(*) Gas Type:-
Inert (Nitrogen): Flow rate = 1.3 L/min (approx.); 2.5 units
Auxiliary (Argon): Flow rate = 1.0 L/min (approx.); 2.5 units

Sampler parameters for standard additions

	Volume (µL)				Added concentration of Cd (ppb)
	Diluent (1% HNO_3, 3.2.3)	Standard (20 µg/L Cd, 4.2.3)	Sample	Modifier (3.2.2)	
Sample	6	0	6	4	-
Blank	12	0	0	4	-
Addition 1	4	2	6	4	2.5
Addition 2	2	4	6	4	5.0
Addition 3	0	6	6	4	7.5

Agriculture and Food Science Centre

Belfast, Northern Ireland

Cadmium

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Flame Atomic Absorption Spectrometry
3.1.a Sample pretreatment
3.1.b Analytical instrumentation
3.1.c Working conditions
3.2. Electrothermal Atomization Atomic Absorption Spectrometry
3.2.a. Sample pretreatment
3.2.b. Analytical instrumentation
3.2.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples most frequently analyzed are animal products such as kidney and liver, milk and milk products, or samples of marine origin, such as fish, oysters, mussels and sediments.

2. Samples throughput per year

About 500 - 600 samples per year.

3. Procedures

Cadmium is analyzed by two different instrumental techniques depending upon the concentration of the element in the sample. Flame atomic absorption spectrometry is the general method of analysis with electrothermal atomization atomic absorption spectrometry being used to a lesser extent for lower concentrations of elements.

3.1 Flame Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Samples are dry-ashed in a muffle furnace which is temperature programmed from room temperature to 450 °C. The ash is dissolved in 0.1 *N* HNO_3.

3.1.b. Analytical instrumentation

A Varian SpectrAA-400 Atomic Absorption Spectrometer with deuterium lamp background correction is used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1.

Table 1. Equipment characteristics.

F-AAS
Flame,air-acetylene
Wavelength, 228.8nm
Slit width, 0.5 nm
Deuterium background correction

A calibration curve is established by using Cd standard solutions of 0, 0.2, 0.4, 0.6, 0.8 and 1.0 µg/g in 0.1 *N* HNO_3.

3.2. Electrothermal Atomization Atomic Absorption Spectrometry

3.2.a. Sample pretreatment

Samples are dry-ashed in a muffle furnace which is temperature programmed from room temperature to 450 ^{o}C. The ash is dissolved in 0.1 *N* HNO_3.

3.2.b. Analytical instrumentation

A Varian SpectrAA-300 Atomic Absorption Spectrometer with Zeeman background correction, graphite furnace and autosampler is used.

3.2.c. Working conditions

Instrumental parameters are reported in Tables 2 and 3.

Table 2. Equipment characteristics.

ETA-AAS
Wavelength, 228.8 nm
Slit width, 0.5 nm
Zeeman background correction
Injection volume, 5 µl sample
Pyrotylitic coated graphite tube

Table 3. Graphite furnace programme

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)	Ar Flow (ml/min)
Drying	95 120	5 10	40 10	300 300
Charring	800	10	8	300
Atomization	1800	0	0.5	0
Clean-up	2400	1	2	300

A calibration curve is established using Cd standard solutions of 0.002, 0.004 and 0.006 µg/g in 0.1 *N* HNO_3.

4. Quality control

The laboratory quality system is based on GLP and UKAS principles. Method validation is performed by using CRM's or spiked samples as appropriate. CRM's are analyzed periodically to check accuracy. During routine analysis, triplicate spiked samples or CRM's are included with each batch of samples analyzed, along with three reagent blanks to control potential contamination. The precision and accuracy is monitored on quality control charts using recovery results.
Instruments are optimized before each batch of analyses, and details recorded of lamp photomultiplier voltage and absorbance for a given standard. Plot linearity is also checked.
The laboratory participates in proficiency testing exercises, such as the UK Food Analysis Performance Assessment Scheme (FAPAS)

5. Difficulties and limitations

The main problem is potential contamination and purity of chemicals. Dry-ashing requires careful control. The temperature should be kept as low as is possible because of the danger of loosing volatile inorganic compounds, but at the same time high enough to ensure complete combustion of all the organic matter in a reasonably short time. Fatty samples may require additional treatment and precautions when dry-ashing.

6. Comments and remarks

Not reported.

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Bull Vet Inst Pulawy 49, 89-92, 2005

DETERMINATION OF LEAD AND CADMIUM IN BIOLOGICAL MATERIAL BY GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETRY METHOD

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Received for publication August 06, 2004.

Abstract

A graphite furnace atomic absorption spectrometry method for lead and cadmium determination in biological material was prepared. The samples were digested in muffle furnace at 450°C. The ash was dissolved in 1N hydrochloric acid and the final solution was diluted in 0.2% nitric acid. The heavy metals were determined using a Perkin-Elmer atomic absorption spectrometer equipped with electrodeless discharge lamps (EDL) at 283.3 nm for Pb and 228.8 nm for Cd. Ammonium dihydrogen phosphate and magnesium nitrate were used as matrix modifiers for both Pb and Cd analyses. The method was validated in terms of basic analytical parameters. The mean recoveries of lead and cadmium were 82.05 and 98.40% and their analytical detection limits were 0.001 and 0.0001 µg/g, respectively. Certified reference materials and participation in national and international proficiency studies were used for analytical quality assurance programme.

Key words: lead, cadmium, atomic absorption spectrometry, biological materials.

Lead and cadmium are industrial pollutants which have strong negative effect on human and animal health. These metals are accumulated in the organism, mainly in the liver and kidneys. The exposure to toxic elements could be minimalised by regular control of food and feed and setting maximum levels for heavy metals in these products. Commission Regulation (EC) No 466/2001 of 8 March 2001, and Polish Regulation of the Ministry of Health of 30 April 2004 established limits for lead and cadmium in foodstuffs. The limits for food of animal origin range from 0.01 to 1.00 mg/kg (3, 14, 19). A fully validated analytical method was prepared to support introduction of legislation to practice and necessity of lead and cadmium control in monitoring programme. The National Veterinary Residue Control Programme which is organized in Poland according to Council Directive 96/23/EC of 29 April, 1966 and Polish Regulation of the Ministry of Agriculture and Rural Development of April 19, 2004 requires controlling the levels of lead and cadmium in animal tissues and food of animal origin.

Material and Methods

Reagents. All chemicals (concentrated nitric acid, hydrochloric acid, matrix modifiers - magnesium nitrate hexahydrate and ammonium phosphate) were of analytical grade. Stock standard solutions 1 000 µg/ml were the reference solution from Beaker.

Working standard solutions were prepared by dilution of stock and intermediate standards. The working standards were as follows: Pb - 10, 20, 30, 40, 50, and 60 µg/l and Cd - 1, 2, 3, 4, 5, and 10 µg/l diluted in 0.2% nitric acid.

Samples. Lead and cadmium were determined in animal tissues, milk, eggs and feedstuffs. Bovine muscle BCR No 184, Pig kidney BCR No 186 and Milk powder CRM 151 were used as reference materials.

Apparatus. An atomic absorption spectrometer (Perkin-Elmer 4110 ZL) equipped with graphite furnace and As-72 autosampler was used for the determination of Pb and Cd. Argon was used as the pure gas. Mechanical oven and muffle furnace was equipped with temperature controller. All containers (quartz crucibles, plastic tubes) were cleaned with detergent and treated successively by the hydrochloric acid and rinsed with de-ionized water.

Ashing procedure. Weigh 2-10 g of homogenized sample into 50 ml quartz crucible. Dry in an oven (120 ± 20°C) overnight until the sample is thoroughly dry. Place the sample into cool muffle furnace and raise the temperature of the oven to 450 ± 20°C (50°C/h). Next day, remove the samples from the oven and cool to room temperature. Add 1 ml concentrated nitric acid and put ash on a hot plate to get dry. Return the sample to the muffle furnace and raise the temperature to 450°C. Keep the sample at this

temperature about 1 h. Repeat that step if needed. The ash must be carbon free. Remove the sample from the muffle furnace and cool to room temperature. Dissolve the ash sample in 5-10 ml of 1N HCl (1 g sample in 1ml of HCl). Transfer the solution from the crucible to a clean tube. The final solutions of the samples are diluted in 0.2% nitric acid. Each batch should include reagent blank and control sample containing all the reagents in the same volumes.

Instrument conditions. Lead - wavelength 283.3 nm, slit 0.7 nm, atomization 2000°C, read time 3 s, sample volume 10 µl, modifiers volume 20 µl. Cadmium - wavelength 228.8 nm, slit 0.7 nm, atomization 1 550°C, read time 3 s, sample volume 10 µl, modifiers volume 20 µl.

Instrument calibration. The calibration curve for the determination of lead was prepared using a blank and working standards solution (10 – 60 µg/l). The calibration curve for the determination of cadmium was prepared using a blank and working standards solution (1 – 10 µg/l).

Metals determination by Graphite Furnace Atomic Absorption Spectrofotometer (GF AAS). Detailed instructions on the operation of the Perkin-Elmer model 4110 ZL are described in the operator's manual.

The sample (calibration blank, standards, reagent blank, control sample) and matrix modifiers were introduced to the furnace by an autosampler. After the atomisation steps, concentrations of the lead and cadmium were reported in the computer in µg of metals/g, wet weight of sample.

The calibration was periodically verified by analysing the standard at the frequency of 20 readings. If the recovery was outside the limits, the analysis was stopped. The problem was corrected and the system was recalibrated.

Statistical analysis. The data obtained from the analysis were evaluated in based statistical parameters using computer program Excel.

Results

A series of experiments was conducted to establish optimum of analytical parameters. Optimalization of digestion condition for different biological matrices and selection of instrumental programmes for the determination of lead and cadmium were done. Dry ashing procedure at 450°C with additional treatment of concentrated nitric acid produced satisfactory digestion of all biological samples (animal tissues, milk, eggs, feedstuffs).

The method was tested by studying the certified reference materials (Bovine muscle BCR No 184, Pig kidney BCR No 186, Milk powder CRM 151) with the certified values and was regularly evaluated by participation in proficiency programmes organised by Food Analysis Performance Assessment Scheme (FAPAS) and European Community Reference Laboratories. The results of proficiency tests were within 2 Z-scores for both metals. The results of the determination of lead and cadmium in certified reference materials are presented in Table 1.

Discussion

Different methodologies based on atomic absorption spectrometry have been reported for the determination of trace elements in various matrices (1, 2, 7, 8, 10-13, 15).

Sample digestion and temperature of atomisation are the most important stages in lead and cadmium analysis (4, 9, 16-18, 20, 21). Described dry ashing sample digestion procedure is very useful for lead and cadmium determination in biological materials. No significant loses of the metals (volatility) were observed during ashing procedure at 450°C. All recovery results above 80% confirmed this observation.

There are two major advantages of dry ashing procedure: possibility of ashing more than 30 samples in one set and lack of samples contaminations from reagents.

The method was validated in terms of the linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, recovery and uncertainty (5, 6). Validation reports are presented in Tables 2 and 3.

Atomisation at 2 000°C for lead and at 1 550°C for cadmium was satisfactory to run all kinds of biological samples (animals tissues, milk, egg, feedstuffs). Magnesium nitrate hexahydrate and ammonium phosphate were necessary to use as matrix modifiers when lead and cadmium were determined in biological material.

Table 1

Comparison of the found and certified values of lead and cadmium in the certified reference materials (accuracy), n = 6

Certified materials	Lead			Cadmium		
	Certified value µg/g	Found µg/g x ± S	Recovery (%)	Certified value µg/g	Found µg/g x ± S	Recovery (%)
Bovine muscle BCR No 184	0.239	0.285 ± 0.051	119.25	0.013	0.014 ± 0.001	107.69
Pig kidney BCR No 186	0.306	0.316 ± 0.042	103.27	2.71	2.59 ± 0.198	95.57
Milk powder CRM 151	2.002	1.894 ± 0.301	94.61	0.101	0.099 ± 0.006	98.02

Table 2
Validation report – Cd determination by GF AAS

Parameters	Results					
Linearity (working range), mg/kg	0.001 – 0.010					
Limit of detection (LOD), mg/kg	0.0001					
Limit of quantitation (LOQ), mg/kg	0.0002					
Matrix		Liver			Eggs	
Levels of spiked samples, mg/kg	0.200	0.300	0.400	0.010	0.040	0.070
Repeatability						
x, mg/kg	0.203	0.284	0.362	0.011	0.039	0.075
SD, mg/kg	0.011	0.031	0.019	0.001	0.002	0.002
RSD, %	5.41	10.91	5.24	9.09	5.12	2.66
Intralaboratory reproducibility						
x, mg/kg	0.212	0.273	0.394	0.012	0.036	0.072
SD, mg/kg	0.012	0.027	0.018	0.001	0.001	0.002
RSD, %	5.66	9.89	4.56	8.33	2.77	2.77
Recovery, %	105.80	90.80	98.60	107.50	91.10	102.50
Uncertainty						
combined (uc)		0.043			0.004	
expanded (U)		0.400 ± 0.086 mg/kg			0.040 ± 0.008 mg/kg	
coverage factor (k)		2			2	

Table 3
Validation report – Pb determination by GF AAS

Parameters	Results					
Linearity (working range), mg/kg	0.010 – 0.060					
Limit of detection (LOD), mg/kg	0.001					
Limit of quantitation (LOQ), mg/kg	0.002					
Matrix		Liver			Eggs	
Levels of spiked samples, mg/kg	0.200	0.300	0.450	0.050	0.200	0.400
Repeatability						
x, mg/kg	0.172	0.243	0.395	0.049	0.175	0.374
SD, mg/kg	0.022	0.041	0.054	0.005	0.011	0.017
RSD, %	12.79	16.87	13.67	10.20	6.28	4.54
Intralaboratory reproducibility						
x, mg/kg	0.161	0.247	0.376	0.044	0.170	0.346
SD, mg/kg	0.016	0.027	0.035	0.003	0.007	0.018
RSD, %	9.93	10.93	9.30	6.81	4.11	2.20
Recovery, %	80.30	82.20	83.60	87.70	84.90	86.50
Uncertainty						
combined (uc)		0.058			0.0074	
expanded (U)		0.450 ± 0.116 mg/kg			0.050 ± 0.015 mg/kg	
coverage factor (k)		2			2	

Value and suitability of the developed method was fully supported by validation results. The validation procedure is in agreement with the Commission Decision of 12 August 2002 (2002/657/EC) implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. All validation data confirm that the method could be used as routine procedure for the determination of lead and cadmium levels in food and feed in official monitoring control programme. LOD and LOQ values are much below the maximum levels which were settled for lead and cadmium in foodstuffs. Generally, good results of precision (RSD below 10%), recoveries (above 80%) and reasonable value of

uncertainty additionally support the described method as a routine procedure for lead and cadmium determination in biological materials.

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Cadmium

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissue (liver), milk and honey

2. Samples throughput per year

About 200 samples per year.

3. Procedure

Cadmium is assayed by Z- ETA-AAS.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Liver/Meat

Approximately 100 g of samples are homogenized, 1 g is weighed in quartz crucibles and 0.5 ml of 50 % $Mg(NO_3)_2$ are added. The sample is pretreated in pre-ashing apparatus until completely charred, followed by ashing in a muffle furnace at 450 °C during 1 h. The samples are then removed and wet with concentrated HNO_3 and put back to the furnace. This procedure is repeated every hour until ashes become white. The ashes are then quantitatively transferred to 10 ml volumetric flasks with 5 % HNO_3.

Or

Subsamples of 0.500 g were weighted into the MW teflon vessel and digested with 4ml of 65% nitric acid and 1ml of 30% hydrogen peroxide. acid using the microwave program shown in the table 1.

Table 1: Operative conditions for the microwave oven digestion

Step	Temperature (ºC)	Power (W)	Time (min.)
1	85	700	2.00
2	145	500	5.00
3	210	1000	3.00
4	210	1000	10.00

After digestion samples were cooled at room temperature and made up to 10ml with high purity deionized water in volumetric glass flasks.

Milk

2 ml of homogenised milk was measured into the MW Teflon vessels. Each sample was digested in 2 ml of concentrated nitric acid using the microwave program shown in the table 2.

Table 2: Operative conditions for the microwave oven digestion

Step	Temperature (ºC)	Power (W)	Time (min.)
1	85	500	3.00
2	85	0	1.00
3	145	500	5.00
4	145	1000	8.00

After digestion samples were cooled at room temperature and made up to 10 ml with high purity deionized water in volumetric glass flasks.

Honey

S. Caroli et al. /Talanta 1999.

3.1.b. Analytical instrumentation

A Perkin Elmer Atomic Absorption Spectrometer Aanalyst 600 with THGA Graphite Furnace including AS- 800 Autosampler.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 2 and 3. Use is made of $NH_4H_2PO_4$ and $Mg(NO_3)_2$ (v/v) as the matrix modifiers.

Table 3. Equipment characteristics.

Z-ETA-AAS
Wavelength, 228.8 nm
Lamp, Cd HCL
Zeeman background correction
L'vov platform
Injection volume, 10 µl samples +5 µl modifier
Pyrolytic coated graphite tubes

Table 4. Graphite furnace programme.

Step	Temperature (ºC)	Ramp Time (s)	Hold Time (s)	Internal Flow
1	100	5	30	250
2	130	15	30	250
3	900	10	20	250
4	1500	0	5	0
5	2450	1	3	250

Calibration plots are set up by using Cd solutions of 1; 2; 4 and 8 ng/g.

4. Quality control

Each analytical run includes blanks, commercial standard solutions, CRMs (BCR 185-R, bovine liver and BCR 184, bovine muscle) and Quality Control Standards for Trace Metals - AA by RTC.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Ministerio de Agricultura, Pesca y Alimentación

Madrid, Spain

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are, in order, wine, spirits, vinegar, beer, orange juice, water, cookies, various kinds of pasta, canned vegetables, liver and kidneys.

2. Samples throughput per year

About 1200.

3. Procedure

Cadmium is assayed by ETA-AAS.

3.1 Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Sampling is not made by the Laboratory Staff, but by official inspectors. The techniques for storage and subsampling are different according to the product; *i.e.*,: milk and milk products, shake; meat, cut in small pieces; eggs, cook; fish, clean and cut; crustaceans, peal and cook. Afterwards homogenizing in kitchen grinder is performed. Dry ashing of samples is achieved in a muffle furnace with overnight digestion at 450 °C. Use is made of 6 % w/w $Mg(NO_3)_2$ as ashing aid. Ashes are dissolved in HNO_3.

3.1.b. Analytical instrumentation

A Perkin-Elmer Model 3030 AAS with Zeeman background correction, equipped with the HGA-600 graphite furnace, AS-60 autosampler and PR-100 recorder, is used.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 1 and 2.

Table 1. Equipment characteristics.

ETA-AAS
Wavelength 228.8 nm
Slit width, 0.7 nm
Lamp, Cd HCL
Zeeman background correction
L'vov platform
Injection volume, 10 µl

Table 2. Graphite furnace programme.

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)	Ar Flow (ml/min)
Drying	110	20	10	300
Charring	400	20	10	300
Atomization	2150	1	5	0
Clean-up	2500	1	3	300

The calibration curve is plotted by using Cd solutions of 1, 2 and 4 ng/ml.

4. Quality control

The routine use of each method is preceded by analytical validation according to the IUPAC recommendations in order to ascertain sensitivity, detection power, precision and accuracy. CRMs of bovine liver (NIST) and mussel tissue (SMT) are used to check the accuracy and analyzed periodically. During routine assays, each analytical batch includes at least three reagent blanks to evaluate the detection limits and to control possible contaminations. Recoveries are calculated using two spiked reagent blanks and one spiked sample for each non-spiked sample assayed.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Inductively Coupled Plasma Mass Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analysed are, in the order, wine, spirits, vinegar, beer, orange juice, cookies, various kinds of pasta, canned vegetables, liver and Kidneys.

2. Samples throughput per year

About 1200 per year.

3. Procedure

Cd is assayed by ICP-MS.

3.1. Inductively Coupled Plasma Mass Spectrometry

3.1.a. Sample pretreatment

Sampling is not made by the Laboratory Staff, but by official inspectors. The techniques for storage and subsampling are different according to the product; i.e.,: milk and milk products, shake; meat, cut in small pieces; eggs, cook; fish, clean and cut; crustaceans, peal and cook. Afterwards homogenising in Kitchen grinder is performed. Samples are digested in a microwave oven. For 0.5 to1g homogeneized samples 2 ml concentrated HNO_3 and 8 ml H_2O are used. Microwave parameters are reported in the following table.

Stage	Power		Ramp Time	P(PSI)	ºC	Time
1	MAX	%	20 min	300	200	10 min
	600w	100				

After the digestion the samples are dissolved in deionized water in order to achieve a final concentration of 3% of nitric acid.

3.1.b. Analytical instrumentation

The multielemental analysis is performed in a Perkin Elmer Elan 9000 Inductively Coupled Plasma Mass Spectrometer, with cross flow nebulizer, nickel cones and a autosampler placed inside a laminar flow cab**.**

3.1.c. Working conditions

The instrumental parameters adopted are reported in Tables 1 and 2.

Table1: ICP Conditions

Plasma power (W)	1100
Argon flow nebulizer (L/min)	0.78 - 0.96
Sample flow (ml/min)	1 ml / min

Table 2: Mass spectrometer conditions

	Quantitative mode	Semiquantitative mode
Vapour pressure (Torr)	7×10^{-7}	7×10^{-7}
Dwell Time (ms)	50	50
sweeps / readings	25	6
Readings / Replicates	1	1
Number of replicates	3	1
Reading mode	Peak Hopping	Peak Hopping

Calibration curve is plotted by using Cd solutions of 0.04, 0.4, 2.5, 5, 10 and 50 ug/l.
Analytical mass: 114

4. Quality control

The routine use of each method is precede by analytical validation according to the IUPAC recommendations in order to ascertain sensitivity, detection power, precision and accuracy. CMRs of bovine liver (SRM 1577b NIST), DORM-2 (Dogfish muscle certified material for trace metals), Spinach Leaves (SRM 1570a) and Corn Bran (8433 NIST) are used to check the accuracy and analysed periodically. During routine assays, each analytical batch includes at least three reagents blanks to evaluate the detection limits and to control possible contamination. Recoveries are calculated using two spiked reagent blanks and one spiked sample for each non spiked sample assayed. At de beginning and at the end of each assay and every five samples two quality controls are analysed. One with a concentration of cadmium similar to the fifth standard of the calibration curve and the other with a concentration of cadmium similar to the second standard.

5. Difficulties and limitations

No reported.

6. Comments and remarks

No reported.

National Food Administration

Uppsala, Sweden

Cadmium

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissues, cereals and vegetables.

2. Samples throughput per year

About 400-600 samples per year.

3. Procedure

Cadmium is determined by ETA-AAS after either dry ashing or microwave digestion.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

a) About 1-10 g of sample is weighed into a platinum crucible and dry ashed at a temperature of 450±25 °C in a programmable furnace. The carbon-free ash is dissolved in 20-30 ml of 0.1 M HNO_3 and transferred to a plastic container.

b) A maximum of 0.5 g dried, homogenized sample is weighed into a Teflon vessel, followed by the addition of 5 ml conc HNO_3 and 2 ml 30% H_2O_2. The digestion is carried out in a CEM (MDS 2000) microwave oven according to the detailed programme in Table 1.

Table 1. Digestion programme.

Step	Power (W)	Time (min)
1	250	3
2	630	5
3	550	22
4	0	15

3.1.b. Analytical instrumentation

Perkin Elmer Z-5100 with HGA-600 autosampler AS-60.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 2 and 3 for the PE Z-5100 instrument. Matrix modifiers are generally not used.

Table 2. Equipment characteristics.

ETA-AAS
Wavelength, 228.8 nm
Slit width, 0.7 low
Lamp, Cd HCL
Zeeman background correction
L'vov platform
Injection volume, 10 µl sample
Pyrolytic coated graphite tubes

Table 3. Graphite furnace programme.

Step	**Temperature (°C)**	**Ramp Time (s)**	**Hold Time (s)**
Injection	Ambient	-	-
Drying	130	10	30
Pyrolysis	350	5	10
Atomization*	1300	0	2
Clean out	2500	2	2

*Gas stop

Calibrations are generally carried out by standard addition method.

4. Quality control

An analytical run includes one-two blanks and one or more CRM or in-house RM. Samples are measured as singles or duplicates, depending on the requirements. Accuracy and precision are monitored by the use of control charts on which the results of duplicate CRM and RM analysis are plotted. The detection limit is based on three times the standard deviation of large number of blanks and is currently 0.5 ng/ml in sample solution.

5. Difficulties and limitations

Extremely fatty or sugar rich samples require extra attention when dry ashing is used. The microwave digestion method is not suitable for fats, oils or extremely fatty products.

6. Comments and remarks

The dry ashing method has been collaboratively tried and was approved by the Nordic Committee and Food Analysis (NMKL) in 1990. The report was published in *JAOAC* Int. 76 (1993), no. 4. (Lars Jorhem, Determination of metals in foodstuffs by AA Spectrophotometry after dry ashing: An NMKL collaborative study of Cd, Cr, Cu, Fe, Ni, Pb and Zn). This method was validated on January 1st 1996. The microwave digestion method has been collaboratively tried and was approved by NMKL in 1997. The result of both collaboratively trials has been submitted to the AOAC Methods Committee and was accepted in December 1999.

National Institute for Public Health and Environment
Rijksinstituut voor Volksgezondheid en Milieu

Bilthoven, The Netherlands

Cadmium

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Inductively Coupled Plasma Mass Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are meat, rain water, ground water, urine, animal feed and aerosol particles.

2. Samples throughput per year

About 1000.

3. Procedure

Cadmium is assayed by ICP-MS.

3.1 Inductively Coupled Plasma Mass Spectrometry

3.1.a. Sample pretreatment

Solid matrices are digested in a microwave oven. For 0.5 g of homogenised biological sample 1 ml of concentrated HNO_3 and 9 ml H_2O is used.
For soil samples (particle size < 250µm) an aqua regia digestion is performed.

3.1.b. Analytical instrumentation

An HP4500equipped with the quadrupole ICP-MS is used.

3.1.c Working conditions

Instrumental parameters are reported in Table 1.

Table 1. **ICP-MS HP4500+** Equipment characteristics.

Analytical mass, ^{114}Cd
Reference mass, ^{103}Rh (added on-line (dilution sample/IS: 1: 5.5)
Nebulizer, V-groove, Scott-type double pass spraychamber
Argon flow, nebulizer 1.17 l/min, R.f. power 1250W
Measuring conditions: 3 points / mass; integration time 1.5 s / mass, 3 replicates
Two-point calibration. Standards concentration, 0 and 20 µg/l
Matrix matching for calibration samples
Equation ^{114}Cd (-0.00027*Mo95-0.0264*Sn118), equation is updated in every measurement sequence using (a) suitable Interference Check Solution(s)

4. Quality control

Each analytical run includes 1 blank, 1 CRM and 1 RM. The instrumental conditions are controlled by the analysis of an in-house water reference material. The accuracy of the analytical procedure, including sample digestion, is estimated by the use of CRMs. A number of matrix samples are analyzed and recovery experiments are carried out for Cd.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Central Science Laboratory

York, United Kingdom

Cadmium

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Inductively Coupled Plasma Mass Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are foods and beverages.

2. Samples throughput per year

Up to 1500.

3. Procedure

Cadmium is assayed by ICP-MS.

3.1. Inductively Coupled Plasma Mass Spectrometry

3.1.a. Sample pretreatment

Aliquots of each sample are homogenized and portions (equivalent to 0.5 g dry weight) are weighed out into microwave digestion vessels. Concentrated HNO_3 (5 ml) is added and the samples are heated in a proprietary high pressure microwave digestion oven. The acid is transferred into a polycarbonate test tube with 18 MΩ cm^{-1} water and made up to 10 ml in volume.

3.1.b Analytical instrumentation

A VG Instruments PlasmaQuad PQII+ Turbo quadrupole ICP-MS or Perkin Elmer ELAN 6000 quadrupole ICP-MS are used.

3.1.c. Working conditions

At least 7 calibration standards are used to cover the calibration range 0.1 µg/l to 100 µg/l. ^{111}Cd is monitored with In or Rh being used as an internal standard. Element concentrations in test solutions are determined by manufacturers software and processed further using an in-house written SuperCalc 5 macro. Automatic spike recovery and blank corrections are made during these calculations.

4. Quality control

All test batches contain reagent blanks and spiked blanks, CRMs and are performed according to UKAS accredited standards.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Chromium

AUSTRIA
BELGIUM
DENMARK
FINLAND
NORTHERN IRELAND
PORTUGAL
SPAIN
SWEDEN
THE NETHERLANDS

Austrian Agency for Health and Food Safety

Wien, Austria

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Animal tissue (muscle, liver, kidney)

2. Samples throughput per year

0-5 samples/year. No routine samples were analyzed during the last four years.

3. Procedure

Chromium is assayed by Z-ETA-AAS.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

a) 2 ± 0.1 g of homogenized tissue is weighed in 20 ml glass bottles. 3 ml HNO_3 (65 %, suprapure) are added. The samples are digested for at least 1 h at 90 °C in a water bath. The solutions are filtered through paper filters into 25 ml measuring flasks and filled up to 25 ml with deionized water.

b) 2 ± 0.1 g of homogenized tissue is weighed in 40 ml Teflon vessels. 3 ml HNO_3 (65 %, suprapure) and 1.5 ml H_2O_2 (30 %) are added. The samples are digested in a microwave oven (MLS-Ethos 900) according to the following programme: 1. 200 W, 30 s; 2. 0 W, 30 s; 3. 100 W, 30 s; 4. 0 W, 30 s; 5. 200 W, 30 s; 6. 0 W, 30 s; 7. 200 W, 5 min; 8. 250 W, 5 min; 9. 450 W, 5 min; 10. 600 W, 10 min. After cooling, the digested sample solutions are transferred into 25 ml measuring flasks and filled up to 25 ml with deionized water.

3.1.b. Analytical instrumentation

A SIMAA 6000 with Autosampler AS-72 (Perkin-Elmer) is used. Use is made of 0.15 % $Mg(NO_3)_2$ as matrix modifier.

3.1.c. Working conditions

Table 1. Equipment characteristics.

Z-ETA-AAS Wavelength: 357.9 nm Lamp: HCL **Zeeman background correction, longitudinal AC magnetic field with transversal heated graphite furnace** Injection volume: 20 µl sample/standard + 10 µl modifier Measurement mode: peak area

Table 2. Graphite furnace programme.

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)	Gas Flow (ml/min)
Drying	110	1	30	250
Drying	130	15	35	250
Ashing	1500	10	20	250
Atomization	2300	0	5	0
Clean-up	2490	1	4	250

The calibration curve is established by automatical dilution of one stock solution (5 µg/l Cr) by the autosampler to following concentrations: 1.25, 2.50, 3.75 and 5.00 µg/l Cr. The blank is subtracted automatically from standards and samples.

4. Quality control

Calibration curves are accepted when the coefficient of correlation is >0.995. The performance of the instrument is checked by a multielement standard (Perkin-Elmer). Recovery is calculated by spiked samples.

5. Difficulties and limitations

No CRM available in order to check the accuracy and reproducibility of the method.

6. Comments and remarks

Since Cd and Pb are measured at lower temperatures, Cr remains in the graphite tube and results in higher values for the blank when Cr analysis is started.

Scientific Institute of Public Health – Louis Pasteur

Brussels, Belgium

Chromium

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

3.2. Inductively Coupled Plasma - Atomic Emission Spectrometry
3.2.a. Sample pretreatment
3.2.b. Analytical instrumentation
3.2.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Paint of wood toys, migration solutions of ceramics and glasses, paper, plastics, fish, meat

2. Samples throughput per year

+/- 50 samples

3. Procedure

Chromium is assayed by Z-ETA-AAS of ICP-AES

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

- For food samples

About 1 g is weighed on an analytical balance in a polyethylene cup and dropped in a PFA digestion tube. Wet digestion is carried out with the aid of 5 ml conc. HNO_3 and 0.2 ml conc. $HClO_4$. The tube is placed in an oven at 180 °C. After reducing of the volume to +/- 0.5 mL, 1 mL of H_2O_2 is added. The tube is placed into an oven at 80 °C +/- 1 hour. After complete oxydation the tubes are cooled to ambient temperature. The digest is diluted into the tube to a volume of 10 mL with Milli-Q-water. If necessary, furthers dilutions are realised.

- For other samples

Microwave digestion with adapted program and acid(s)

3.1.b. Analytical instrumentation

A Perkin Elmer SimAA 6000 Zeeman AAS instrument.

3.1.c. Working conditions

Z-ETA-AAS

Wavelength, 357.9 nm; slit with 0.5 nm; lamp Cr HCL Zeema Background correction. Injection volume 20 µL + 10 µL Matrix Modifiers: $Mg(NO_3)_2$, $Pd(NO_3)_2$ Measurment Mode: Peak Area THGA Graphite Furnace Without End Cap Calibration mode, concentration Replicates, 2 Sample introduction, sampler automixing (5 points)

Step	Temperature (°C)
Injection	Ambient
Drying	110-130
Pyrolysis	1200
Atomization	2300
Clean-up	2600

Use is made of $Pd(NO_3)_2$ and $Mg(NO_3)_2$ as matrix modifiers. Calibration is carried out with standard solution, freshly prepared with 2 % v/v conc. HNO_3. The stock solution is Perkin Elmer PE Pure. Linear working range is 0-10.0 ng/ml.

3.2. Inductively Coupled Plasma - Atomic Emission Spectrometry

3.2.a. Sample pretreatment

- For food samples

About 1 g is weighed on an analytical balance in a polyethylene cup and dropped in a PFA digestion tube. Wet digestion is carried out with the aid of 5 ml conc. HNO_3 and 0.2 ml conc. $HClO_4$. The tube is placed in an oven at 180 °C. After reducing of the volume to +/- 0.5 mL, 1 mL of H_2O_2 is added. The tube is placed into an oven at 80 °C +/- 1 hour. After complete oxydation the tubes are cooled to ambient temperature. The digest is diluted into the tube to a volume of 10 mL with Milli-Q-water. If necessary, furthers dilutions are realised.

- For other samples

Microwave digestion with adapted program and acid(s)

3.1.b. Analytical instrumentation

A Perkin Elmer Optima 4300 DV, instrument.

3.1.c. Working conditions

Axially viewed ang Gemcone nebulizers Wavelength: 276.716 nm; 205.560; and 283.563 nm. Injection flow rate: 2.2 mL /min. Integration time: 5-20 sec Measurment Mode: Peak Area Normal resolution

4. Quality control

Three wavelength are used. The best wavelength is conservated for the expression of results based on correlation coefficient and recovery test at level of +/- 50 % of the concentration range of samples. An analytical run includes blanks and standards. All glassware and polyethylene flasks and pipets are soaked in a 10 % HNO_3 solution and rinsed with Milli-Q-water just before use. All volumetric flasks, glass pipets are calibrated, plastic pipets are checked regularly. Intercalibration tests are regularly realised.

5. Difficulties and limitations

More Matrix effects that Z-ETA-AAS.

6. Comments and remarks

Not reported.

Danish Institute for Food and Veterinary Research

Søborg, Denmark

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Inductively Coupled Plasma Mass Dynamic Reaction Cell Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Food samples in general and food-related samples for proficiency tests.

2. Samples throughput per year

10.

3. Procedure

The samples are analyzed by ICP-MS.

3.1. Inductively Coupled Plasma Mass Spectrometry

3.1.a. Sample pretreatment

The sample is homogenized in a blender equipped with titanium blades. A known amount of the homogenized sample (2-3 g wet weight) is weighed intothe Multiwave high pressure microwave digestion system. The Multiwave high pressure microwave digestion system is used for sample decomposition. The maximum pressure is 70 bar and the digestion is finalised within 60 minutes including cooling. Following digestion the residue is taken to 20 ml by weighing using pure water.

3.1.b. Analytical instrumentation

An Elan 6100 DRC Perkin-Elmer/Sciex quadrupole mass spectrometer is used.

3.1.c. Working conditions

A volume of 5 ml of the sample solution is pipetted into a polyethylene autosampler tube and 5 ml of 1 % aqueous HNO_3 are added. Chromium is measured at *m/z* =52 or 53. Calibration is carried out by the standard addition method. Two additions are used for each sample. The DRC is used for reduction of the ArC interference on ^{52}Cr with ammonia as the cell gas. No corrections for polyatomic interference is used at at *m/z* = 53 for ^{53}Cr.. A mixture of usually Ga, In and Bi are used for internal standardization in the multielement method in which this element is an analyte.

4. Quality control

The method is accredited by the Danish Accreditation Organisation, DANAK. A matching CRM and blanks are analyzed in parallel with the samples in series containing up to 15 unknown samples. The results of CRMs are monitored on control charts (x- and R-charts). The risk of contamination of samples must always be considered. The critical utensils are washed/rinsed with acid before use and ultrapure acids are used for the ashing process.

5. Difficulties and limitations

None reported.

6. Comments and remarks

None reported.

National Veterinary and Food Research Institute

Helsinki, Finland

1. Matrices

Samples more frequently analyzed are animal tissues (meat, liver and kidney).

2.1 Samples throughput per year

About 10.

3. Procedure

The samples are analyzed by Z-ETA-AAS.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Sample (5-10g) is ashed at 450^{o}C in a muffle furnace overnight. Ashed sample is cooled down to room temperature, deionized water and 1 ml conc. HNO_3 are added. The sample is re-evaporated and heated up to 450^{o} C overnight in the muffle furnace. The residue is dissolved in 0.5 ml of conc. HNO_3 and adjusted to appropriate volume with deionized water.

3.1.b. Analytical instrumentation

A Perkin-Elmer 5100 PC Atomic Absorption Spectrometry with Zeeman background corrector and hollow cathode lamp is used.

3.1.c. Working conditions

The instrumental parametets adopted are reported in Table 1. Measurement are carried out by direct comparison with standard solution (Reagecon) in 0.1 M HNO_3.

Table 1. Equipment characteristics.

ETA-AAS
Wavelenght, 357.9 nm
Lamp, Cr HCL
Zeeman background correction

4. Quality control

The method is accredited. Blanks are analyzed together with each sample series. Analysis of spiked samples are performed. The risk of contamination of samples must always be considered. All glass- and plasticware are washed with acid before use.

5. Difficulties and limitations

Lack of certified reference material. Consumption of graphite tubes is high because of the high atomization temperature.

6. Comments and remarks

Not reported.

Agriculture and Food Science Centre

Belfast, Northern Ireland

Chromium

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Flame Atomic Absorption Spectrometry
3.1.a Sample pretreatment
3.1.b Analytical instrumentation
3.1.c Working conditions
3.3. Electrothermal Atomization Atomic Absorption Spectrometry
3.2.a. Sample pretreatment
3.2.b. Analytical instrumentation
3.2.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples most frequently analyzed are of marine origin, such as fish, oysters, mussels and sediments.

2. Samples throughput per year

About 200 samples per year.

3. Procedures

Chromium is analyzed by two different instrumental techniques depending upon the concentration of the element in the sample. Flame atomic absorption spectrometry is the general method of analysis with electrothermal atomization atomic absorption spectrometry being used to a lesser extent for lower concentrations of elements.

3.1 Flame Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Samples are dry-ashed in a muffle furnace temperature programmed from room temperature to 450 °C. The ash is dissolved in 0.1 *N* HNO_3.

3.1.b. Analytical instrumentation

A Varian SpectrAA-400 Atomic Absorption Spectrometer with deuterium lamp background correction is used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1.

Table 1. Equipment characteristics.

F-AAS	Flame, air-acetylene
Wavelength, 357.9nm	
Slit width, 0.2 nm	
Deuterium background correction	
Lamp current 7.0mA	

A calibration curve is established by using Cr standard solutions of 0, 0.2, 0.4, 0.6, 0.8 and 1.0 µg/g in 0.1 *N* HNO_3.

3.2. Electrothermal Atomization Atomic Absorption Spectrometry

3.2.a. Sample pretreatment

Samples are dry-ashed in a muffle furnace which is temperature programmed from room temperature to 450 ^{o}C. The ash is dissolved in 0.1 *N* HNO_3.

3.2.b. Analytical instrumentation

A Varian SpectrAA-300 Atomic Absorption Spectrometer with Zeeman background correction, graphite furnace and autosampler is used.

3.2.c. Working conditions

Instrumental parameters are reported in Tables 2 and 3.

Table 2. Equipment characteristics.

ETA-AAS
Pyrotylitic coated graphite tube
Wavelength, 357.9 nm
Slit width, 0.5 nm
Zeeman background correction
Injection volume, 5 µl sample

Table 3. Graphite furnace programme

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)	Ar Flow (ml/min)
Drying	85 95 120	5 40 10	0 0 0	300 300
Ashing	400	5	12	300
Atomization	2400	1	2	0
Clean-up	2400	0	3	300

A calibration curve is established using Cr standard solutions of 0.002, 0.004 and 0.006 µg/g in 0.1 *N* HNO_3.

4. Quality control

The laboratory quality system is based on GLP and UKAS principles. Method validation is performed by using CRM's or spiked samples as appropriate. CRM's are analyzed periodically to check accuracy. During routine analysis, triplicate spiked samples or CRM's are included with each batch of samples analyzed, along with three reagent blanks to control potential contamination. The precision and accuracy is monitored on quality control charts using recovery results.
Instruments are optimized before each batch of analyses, and details recorded of lamp photomultiplier voltage and absorbance for a given standard. Plot linearity is also checked.
The laboratory participates in proficiency testing exercises, such as the UK Food Analysis Performance Assessment Scheme (FAPAS)

5. Difficulties and limitations

The main problem is potential contamination and purity of chemicals. Dry-ashing requires careful control. The temperature should be kept as low as is possible because of the danger of loosing volatile inorganic compounds, but at the same time high enough to ensure complete combustion of all the organic matter in a reasonably short time. Fatty samples may require additional treatment and precautions when dry-ashing.

6. Comments and remarks

Not reported.

Istituto de Investigação das Pescas e do Mar

Lisboa, Portugal

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissue (liver).

2. Samples throughput per year

Not foreseeable.

3. Procedure

Chromium is assayed by Z-ETA-AAS.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Approximately 100 g of samples are homogenized, 1 g is weighed in quartz crucibles and 0.5 ml of 50 % $Mg(NO_3)_2$ are added. The sample is pretreated in pre-ashing apparatus until completely charred, followed by ashing in a muffle furnace at 450 °C during 1 h. The samples are then removed and wet with concentrated HNO_3 and put back to the furnace. This procedure is repeated every hour until ashes become white. The ashes are then quantitatively transferred to 10 ml volumetric flasks with 5 % HNO_3.

3.1.b. Analytical instrumentation

A Perkin Elmer Atomic Absorption Spectrometer Aanalyst 600 with THGA Graphite Furnace including AS- 800 Autosampler.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 1 and 2. Use is made of $Mg(NO_3)_2$ (v/v) as the matrix modifier.

Table 1. Equipment characteristics.

Z-ETA-AAS
Wavelength, 357.9 nm
Cr hollow cathode lamp,
Zeeman background correction
L'vov platform
Injection volume, 10 µl samples + 5 µl modifier
Pyrolytic coated graphite tubes

Table 2. Graphite furnace programme.

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)	Internal Flow
1	100	4	30	250
2	130	15	30	250
3	1400	10	20	250
4	2300	0	5	0
5	2500	1	5	250

Extra furnace clean-out.

The calibration curve is plotted by using Cr solutions of 5; 10; 20 and 40 ng/g.

4. Quality control

Each analytical run includes blanks, commercial standard solutions, and Quality Control Standards for Trace Metals - AA by RTC.

5. Difficulties and limitations

Possibility of contamination.

6. Comments and remarks

Not reported.

Ministerio de Agricultura, Pesca y Alimentación

Madrid, Spain

1. Matrices

2. Samples throughput per year

3. Procedure
3.1 Electrothermal Atomization Atomic Absorption
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are, in order, wine, spirits, vinegar, beer, orange juice, water, cookies, various kinds of pasta, canned vegetables, liver and kidneys.

2. Samples throughput per year

About 400.

3. Procedure

Chromium is assayed by ETA-AAS.

3.1 Electrothermal Atomization Atomic Absorption

3.1.a. Sample pretreatment

Sampling is not made by the Laboratory Staff, but by official inspectors. The techniques for storage and subsampling are different according to the product; *i.e.*,: milk and milk products, shake; meat, cut in small pieces; eggs, cook; fish, clean and cut; crustaceans, peal and cook. Afterwards homogenizing in kitchen grinder is performed. Digestion of the samples is carried out in a microwave oven. The acids used for digestion are different depending on the nature of the sample processed. In the case of the samples included in the National Residue Investigation Plan, namely liver and kidneys, the reagents used for sample digestion are the following: HNO_3, $HclO_4$ and H_2O_2.

3.1.b. Analytical instrumentation

A CEM Model NDS-81D microwave oven and a Perkin Elmer Model 3030 Atomic Absorption Spectrometer with Zeeman background correction, equipped with the HGA-600 graphite furnace, AS-60 autosampler and PR-100 recorder, is used.

3.1.c. Working conditions

The instrumental parameters adopted are reported in Table 1 and 2.

Table 1. Equipment characteristics.

ETA-AAS
Wavelength, 357.9 nm
Slit width, 0.7 nm
Lamp, Cr HCL
Zeeman background correction
Injection volume, 20 µl

Table 2. Graphite furnace programme.

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)	Ar Flow (ml/min)
Drying	130	1	10	300
Charring	1200	10	10	300
Atomization	2300	0	2	0
Clean-up	2700	2	3	300

4. Quality control

The routine use of each method is preceded by analytical validation according to the IUPAC recommendations in order to ascertain sensitivity, detection power, precision and accuracy. CRMs of bovine liver (NIST) and mussel tissue (SMT) are used to check the accuracy and analyzed periodically. During routine assays, each analytical batch includes at least three reagent blanks to evaluate the detection limits and to control possible contaminations. Recoveries are calculated using two spiked reagent blanks and one spiked sample for each non-spiked sample assayed.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

National Food Administration

Uppsala, Sweden

Chromium

1. Matrices

2. Samples throughput per year

3. Procedure

3.2. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissues, cereals and vegetables.

2. Samples throughput per year

About 50 - 150 samples per year.

3. Procedure

Chromium is determined by ETA-AAS after dry ashing.

3.3. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

About 1-10 g of sample is weighed into a platinum crucible and dry ashed at a temperature of 450±25 °C in a programmable furnace. The carbon-free ash is dissolved in 20-30 ml of 0.1 M HNO_3 and transferred to a plastic container.

3.1.b. Analytical instrumentation

Perkin Elmer Z-5100 with HGA-600 autosampler AS-60.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 1 and 2 for the PE Z-5100 instrument. Matrix modifiers are generally not used.

Table 1. Equipment characteristics for PE Z-5100.

ETA-AAS
Wavelength, 357.9 nm
Slit width, 0.7 low
Lamp, Cr HCL
Zeeman background correction
Injection volume, 10 µl sample
Pyrolytic coated graphite tubes

Table 2. Graphite furnace programme for PE Z-5100.

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)
Injection	Ambient	-	-
Drying	130	10	30
Pyrolysis	1000	5	10
Atomization*	2300	0	2
Clean out	2700	2	2

*Gas stop

Calibrations are generally carried out by standard addition method.

4. Quality control

An analytical run includes one-two blanks and one or more 1 CRM or in-house RM. Samples are measured as singles or duplicates, depending on the requirements. Accuracy and precision are monitored by the use of control charts on which the results of duplicate CRM and RM analysis are plotted. The detection limit is based on three times the standard deviation of large number of blanks and is currently 1.3 ng/ml in sample solution.

5. Difficulties and limitations

Extremely fatty or sugar rich samples require extra attention when dry ashing is used.

6. Comments and remarks

The dry ashing method has been collaboratively tried and was approved by the Nordic Committee for Food Analysis (NMKL) in 1990. The report was published in *JAOAC* Int. 76 (1993), no. 4. (Lars Jorhem, Determination of metals in foodstuffs by AA Spectrophotometry after dry ashing: An NMKL collaborative study of Cd, Cr, Cu, Fe, Ni, Pb and Zn). This method was validated on January 1st 1996.

The result of the collaboratively trial has been submitted to the AOAC Methods Committee and was accepted in December 1999.

National Institute for Public Health and Environment
Rijksinstituut voor Volksgezondheid en Milieu

Bilthoven, The Netherlands

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Inductively Coupled Plasma Mass Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are meat, rain water, ground water, urine, animal feed and aerosol particles.

2. Samples throughput per year

About 1000.

3. Procedure

Chromium is assayed by ICP-MS.

3.1 Inductively Coupled Plasma Mass Spectrometry

3.1.a. Sample pretreatment

Solid matrices are digested in a microwave oven. For 0.5 g of homogenised biological sample 1 ml of concentrated HNO_3 and 9 ml H_2O is used.
For soil samples (particle size < 250µm) an aqua regia digestion is performed.

3.1.b. Analytical instrumentation

An HP4500+ quadrupole ICP-MS is used.

3.1.c Working conditions

Instrumental parameters are reported in Table 1.

Table 1. **ICP-MS HP4500+** Equipment characteristics

Analytical mass, ^{52}Cr or ^{53}Cr
Reference mass, ^{72}Ge (added on-line (dilution sample/IS: 1: 5.5))
Nebulizer, V-groove, Scott-type double pass spraychamber
Argon flow, nebulizer 1.17 l/min, R.f. power 1250W
Measuring conditions: 3 points / mass; integration time 1.5 s / mass, 3 replicates.
Two-point calibration, standards concentration, 0 and 20 µ/g
Matrix matching for calibration samples
Equation ^{53}Cr (-0.000141*Cl35-0.0094*C13), equation is updated in every measurement sequence using (a) suitable Interference Check Solution(s)

4. Quality control

Each analytical run includes 1 blank, 1 CRM and 1 RM. The instrumental conditions are controlled by the analysis of an in-house water reference material. The accuracy of the analytical procedure, including sample digestion, is estimated by the use of CRMs. A number of matrix samples are analyzed and recovery experiments are carried out for Cr.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Copper

AUSTRIA
BELGIUM
CZECH REPUBLIC
DENMARK
ESTONIA
FINLAND
GERMANY
HUNGARY (see Cd)
ITALY
LATVIA (AOAC 999.11)*
NORTHERN IRELAND
SPAIN
SWEDEN
THE NETHERLANDS
UNITED KINGDOM

**Official analytical methods are not reported*

Austrian Agency for Health and Food Safety

Wien, Austria

Copper

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Muscle, liver and kidney from animals, especially sheep (they are prone to poisoning by copper) and calves (copper is sometimes added to their feed in order to get light veal).

2. Samples throughput per year

About 0 - 10 samples per year.

3. Procedure

Copper is assayed by Z-ETA-AAS.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

a) 2 ± 0.1 g of homogenized tissue is weighed in 20 ml glass bottles. 3 ml HNO_3 (65 %, suprapure) are added. The samples are digested for at least 1 h at 90 °C in a water bath. The solutions are filtered through paper filters into 25 ml measuring flasks and filled up to 25 ml with deionized water.

b) 2 ± 0.1 g of homogenized tissue is weighed in 40 ml Teflon vessels. 3 ml HNO_3 (65 %, suprapure) and 1.5 ml H_2O_2 (30 %) are added. The samples are digested in a microwave oven (MLS-Ethos 900) according to the following programme: 1. 100 W, 10 min 2. 0 W, 1 min 3. 250 W, 5 min 4. 0 W, 1 min 5. 400 W, 5 min 6. 600 W, 10 min 7. 500 W, 6 min. After cooling, the digested sample solutions are transferred into 25 ml measuring flasks and filled up to 25 ml with deionized water.

3.1.b. Analytical instrumentation

A SIMAA 6000 with Autosampler AS-72 (Perkin-Elmer) is used. Use is made of 0.05 % Pd + 0.03 % $Mg(NO_3)_2$ as matrix modifier.

3.1.c. Working conditions

Table 1. Equipment characteristics.

Z-ETA-AAS
Wavelength 324.8nm
Lamp, Cu HCL
Zeeman background correction, longitudinal AC magnetic field with transversal heated graphite furnace
Injection volume, 20 µl sample/standard + 10 µl modifier
Measurement mode, peak area

Table 2. Graphite furnace programme.

Step	**Temperature (°C)**	**Ramp Time (s)**	**Hold Time (s)**	**Gas Flow (ml/min)**
Drying	110	1	30	250
Drying	130	15	30	250
Ashing	1200	10	20	250
Atomization	2200	0	5	0
Clean-up	2450	1	3	250

The calibration curve is established by automatical dilution of one stock solution (100 µg/l Cu) by the autosampler to following concentrations: 25, 50, 75 and 100 µg/l Cu. The blank is subtracted automatically from standards and samples.

4. Quality control

Calibration curves are accepted when the coefficient of correlation is >0.995. The performance of the instrument is checked by a multielement standard (Perkin-Elmer). Recovery is calculated by spiked samples. CRM 184 (bovine muscle), CRM 185 (bovine liver) or CRM 186 (pig kidney) are analyzed in order to check the accuracy and reproducibility of the whole method.

5. Difficulties and limitations

Not reported

6. Comments and remarks

Due to the high concentrations of Cu in animal tissue you have to be aware of likely memory effects in the graphite tube. Since Cd and Pb are measured at lower temperatures, Cu remains in the graphite tube and results in higher values for the blank when Cu analyses are started.

Scientific Institute of Public Health-Louis Pasteur

Brussels, Belgium

Copper

1. Matrices

2. Samples throughput per year

3. Procedure
3.1.Inductively Coupled Plasma Atomic Emission Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Meat, pruduct of bakkery and frequently calf livers.

2. Samples throughput per year

About 200 samples per year.

3. Procedure

Copper is assayed by ICP-AES.

3.1. Inductively Coupled Plasma - Atomic Emission Spectrometry

3.1.a. Sample pretreatment

About 1 g is weighed on an analytical balance in a polyethylene cup and dropped in a PFA digestion tube. Wet digestion is carried out with the aid of 5 ml conc. HNO_3 and 0.2 ml conc. $HClO_4$. The tube is placed in an oven at 180 °C. After reducing of the volume to +/- 0.5 mL, 1 mL of H_2O_2 is added. The tube is placed into an oven at 80 °C +/- 1 hour. After complete oxydation the tubes are cooled to ambient temperature. The digest is diluted into the tube to a volume of 10 mL with Milli-Q-water. If necessary, furthers dilutions are realised.

3.1.b. Analytical instrumentation

A Perkin Elmer Optima 4300 DV

3.1.c. Working conditions

Axially viewed ang Gemcone nebulizers Wavelength: 327.4 nm; 324.8; and 224.7 nm Injection flow rate: 2.2 mL /min Integration time: 5-20 sec Measurment Mode: Peak Area Normal resolution

Calibration is carried out with standard solution, freshly prepared with 2 % v/v conc. HNO_3. The stock solution is Perkin Elmer PE Pure. Working range is from 10 ppb to 10 ppm.

4. Quality controle

Three wavelength are used. The best wavelength is conservated for the expression of results based on correlation coefficient and recovery test at level of +/- 50 % of the concentration range of samples. An analytical run includes blanks and standards. All glassware and polyethylene flasks and pipets are soaked in a 10 % HNO_3 solution and rinsed with Milli-Q-water just before use. All volumetric flasks, glass pipets are calibrated, plastic pipets are checked regularly. Proficiency test are frequently realised.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

State Veterinary Institute Olomouc
Laboratory in Kromeriz

Kromeriz, Czech Republic

Copper

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Analytical technique
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are food, animal feed and water.

2. Sample throughput per year

About 150 samples per year.

3. Procedure

Cooper is assayed by F-AAS.

3.1. Flame Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

a) 1-10 (± 0.1) g of homogenized sample is weighed and dry ashed at a temperature of 450±25 ^{o}C in a programmable furnace overnight. Use is made of 6 % w/w $Mg(NO_3)_2$ as an ashing aid. Ashed sample is cooled down to room temperature, deionized water and 1 ml conc. HNO_3 are added. The sample is re-evaporated and heated up to 450 ^{o}C in the furnace. This procedure is repeated until ashes become white. Ashes are dissolved in 1 M HNO_3.

b) 1 ± 0.1 g of homogenized sample is weighed in 100 ml Teflon vessels. 5 ml HNO_3 (65 %, suprapure) and 1.5 ml H_2O_2 (30 %) are added. The samples are digested into the microwave oven (MLS – Ethos Plus) according to the following programme: 1. 500W, 10 min, 180 ^{o}C; 2. 500 W, 10 min, 200 ^{o}C. After cooling the digested sample solutions are transferred into 10 ml measuring flasks and filled up to 10 ml deionized water.

3.1.b. Analytical Instrumentation

A Perkin-Elmer 2100 Atomic Absorption Spectrometer is used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1.

Table 1. Equipment characteristics

F-AAS
Wavelength: 324.8 nm
Lamp: Cu HCL
Air-acetylene flame

The calibration curve is established by using Cu solutions of 0.50, 1.00, 2.00, 4.00 mg/l.

4. Quality control

The routine use of each method is preceded by analytical validation according to the IUPAC recommendations and the metods are accreditated.
CRMs (BCR 184, BCR 185 R, BCR 151, Tort-2, APS -1075…) are use to check the accuracy and analyzed periodically. An analytical run includes two blanks, duplicates and in-house RM. The results of duplicate CRM, RM analysis samples are monitored on control charts.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Danish Institute for Food and Veterinary Resaearch

Søborg, Denmark

Copper

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Inductively Coupled Plasma Optical Emission Spectrometry

3.1.a. Sample pretreatment

3.1.b. Analytical instrumentation

3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are food supplements, fish, meat, flour, fruit and vegetables.

2. Samples throughput per year

About 10.

3. Procedure

Copper is analyzed by ICP-OES.

3.1. Inductively Coupled Plasma Optical Emission Spectrometry

3.1.a. Sample pretreatment

The biological sample is homogenized in a blender equipped with titanium blades. A known amount (up to 0.5 g dry weight) is wet ashed in a Teflon-lined high-pressure steel bomb by 4 ml of suboiled HNO_3 at 160 °C for 4 h. After cooling of the bombs, the residue is taken to 20 g by water.

Alternatively, the Multiwave high pressure microwave digestion system is used for sample decomposition. The maximum pressure possible is 70 bar and the digestion is finalised within 60 minutes including cooling. The same sample intake and acid is used with his technique.

3.1.b. Analytical instrumentation

An Optima 300 DV Perkin-Elmer/Sciex ICP-OES instrument is used.

3.1.c. Working conditions

The residue is further diluted by 1 % HNO_3 in order to obtain a signal intensity of the instrumentation detector within the linear range. The signal intensity for Cu at 324,752 nm emission wavelength (axial viewing) is measured. The calibration is carried out by external standardization using a certified standard.

4. Quality control

The method is accredited by the Danish Accreditation Organisation, DANAK. A matching CRM and blanks are analyzed in parallel with the samples in series containing up to 15 unknown samples. The results of CRMs are monitored on control charts (x- and R-charts). The risk of contamination of samples must always be considered. The critical utensils are washed/rinsed with acid before use and ultrapure acids are used for the ashing process.

5. Difficulties and limitations

The external standard curve must be forced through zero (the origin) in order to minimize the risk of erroneous calibration of Cu emission signals of low intensity.

6. Comments and remarks

None reported.

Food Safety Veterinary and Food Laboratory

Tartu, Estonia

Copper

1. The equipment in use

1) Atomic Absorption Spectrophotometer-Thermo Elemental. It has equipped both with GFA and flame compartments. Zeemann and Deuterium correction are available. At the moment the Hydride Vapor System –for the determination of Arsenic is missing.
2) Atomic Absorption Spectrophotometer-Shimadzu - It has equipped both with GFA and flame compartments. Only deuterium correction is available.
3) Microwave owen AntonPaar- closed vessels for wet digestion
4) Muffle furnace Nabertherm

2. Analytical Methods

Element	**Cu**
Accreditation	**yes**
Reference for method	1) AOAC method 999.11 Determination of Lead, Cadmium, Iron and Zinc in Foods. AAS after dry ashing First action 1999. 2) AA Spectrometers Methods. Manual, 2003, Thermo Electron Corp., UK 3) EVS-EN 13804:2002 –Foodstuffs-Determination of Trace elements-Performance Criteria and general conciderations. 4) EVS-EN 13805:2002 Foodstuffs-Determination of trace elements. Pressure digestion. 5) Nordic Commitee of Food Analyses no 161, 1998. Metals. Determination by AAS after wet digestion in microwave owen.
Sample weight	5 dry ashing >1 mw dig.
Sample preparation	Dry ashing Microwave digestion
Reagents used	HNO_3, H_2O_2, HCl
Quantification (technique)	AAS Graphite Furnace AAS flame
Wavelenght(nm)	324.8
LOQ	0.01 GFA 0.2 flame
U %	20 GFA 15 flame

3. Reference materials

Element	**Matrics**	**Ref. value mg/kg**
Cu fl	CRM 185 R bovine liver	277
Cu gr	BCR 186	31.9
	BCR 151	5.23
	SRM 2976	4.02

National Veterinary and Food Research Institute

Helsinki, Finland

Copper

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Flame Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissues (meat, liver and kidney).

2. Samples throughput per year

About 20.

3. Procedure

The samples are analyzed by F-AAS.

3.1. Flame Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Sample (5-10 g) is ashed at 450 °C in a muffle furnace overnight. Ashed sample is cooled down to room temperature, deionized water and 1 ml conc. HNO_3 are added. The sample is re-evaporated and heated up to 450 °C overnight in the muffle furnace. The residue is dissolved in 0.5 ml of conc. HNO_3 and adjusted to appropriate volume with deionized water.

3.1.b. Analytical instrumentation

Samples are analyzed by air-acetylene AAS (Perkin-Elmer 5100 PC and hollow cathode lamp).

3.1.c. Working conditions

Copper is measured at 324.8 nm. Measurement are carried out with direct comparison by standard solution (Reagecon) in 0.1 M HNO_3.

4. Quality control

The method is accreditated. CRMs and blanks are analyzed together with each sample series. The results of CRMs samples are monitored on control charts. Analysis of spiked samples are performed. The risk of contamination of samples must always be considered. All glass- and plasticware are washed with acid before use.

5. Difficulties and limitations

Not reported

6. Comments and remarks

Not reported.

Federal Office for Consumer Protection and Food Safety

Berlin, Germany

Copper

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Flame Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

3.2. Electrothermal Atomization Atomic Absorption Spectrometry
3.2.a. Sample pretreatment
3.2.b. Analytical instrumentation
3.2.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

<u>Section II</u>

Sample ashing procedure

1. Matrices

2. Procedure

Section I

1. Matrices

Samples analyzed are meat, meat products, plant materials, food

2. Sample throughput per year

About 250.

3. Procedures

Copper is assayed by F-AAS or ETA-AAS

3.1. Flame Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Mineralization is described in section II (Sample ashing procedure)

3.1.b. Analytical instrumentation

A SpectrAA 300-AAS with acetylene-air-flame and D_2 background correction is used.

3.1.c. Working conditions

The calibration curve is plotted by using Copper solutions of 0.5, 1.0, 2.0 and 4.0 µg/ml in 3 % HNO_3.The analytical blank is a solution of 3 % HNO_3. Use of D_2 background correction and an oxidizing flame is recommended. Limit of detection is 0.04 µg/ml in sample solution.

3.2. Electrothermal Atomization Atomic Absorption Spectrometry

3.2.a. Sample pretreatment:

Mineralization is described in section II (Sample ashing procedure)

3.2.b. Analytical instrumentation

A SpectrAA 300 P- AAS (Deuterium BC) with Autosampler GTA 96 (VARIAN) or a SpectrAA 400 Zeeman - AAS with Autosampler GTA 96 (VARIAN) is used

3.2.c. Working conditions

Instrumental parameters are reported in Table 1 and 2. The modifier used is a freshly prepared (1+1)-mixture of two solutions: (I) 0.5 ml of Pd-modifier + 9.5 ml HCl, 4 mol/l; (II) ascorbic acid, 10 g/l in water

Table 1. Equipment characteristics

ETA-AAS
Either Zeeman or D_2 - background correction
Pyrolytic coated tubes without platform
Injection volume: 20 µl + 5 µl modifier

Table 2. Graphite furnace programme

Step	Temperature (°C)	Time (s)	Gas Flow (l/min)
1	95	3	3
2	95	20	3
3	120	15	3
4	700	2	3
5	700	10	3
6	700	2	0
7	2400	1	0
8	2400	2	0
9	2400	2	3

The calibration curve is plotted by using Copper solutions of 4, 8, 12, 16 ng/ml in 8 % HNO_3. The analytical blank is a solution of 8 % HNO_3. Limit of detection is 2.0 ng/ml in sample solution.

4. Quality control

Analysis of certified reference materials (BCR, NIST, CBNM) is performed along with each analytical series.

5. Difficulties and limitations

Not reported

6. Comments

The flame AAS method corresponds to the official German Method for food analysis.

Section II

Sample ashing procedure

1. Matrices

All materials will be mineralized following the same procedure.

2. Procedure

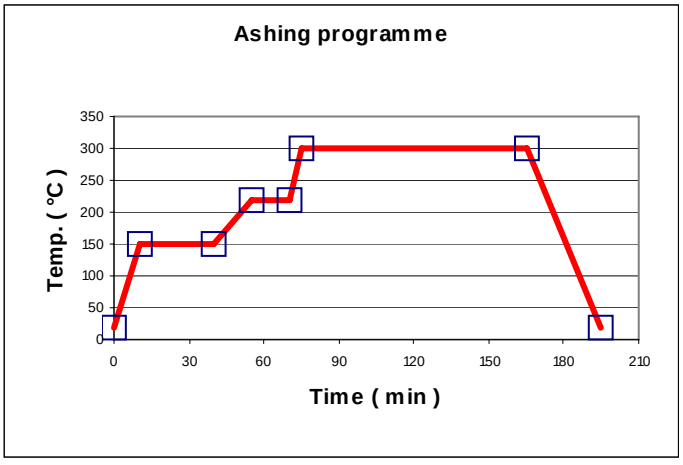
A high pressure ashing system HPA-S equipped with five 90 ml quartz vessels is used. Concentrated HNO_3, 65 % (FLUKA, pA plus) is used. Between 300 mg and 1500 mg of homogenized sample material are weighed into the quartz vessels. Sample amounts are illustrated in Table 1.

Table 1. Sample amounts for use in the HPA-S

Sample	Weight (mg)	Tolerance (mg)	Volume of HNO_3 (ml)
dry samples (< 20 % water)	500	± 50	3.0
fresh samples	1000	± 100	3.0
fresh samples (> 50 % water)	1500	± 200	3.0

Dry samples should be wetted with some drops water before $HNO_3$is added; it's to recommend to let stand the vessels over night before heating. The mineralization start by using a ashing temperature of 300 °C over a hold time of 90 minutes (Fig. 1). When ashing procedure is finished, let cool down to room temperature. Check the volume of HNO_3 in the vessels to recognize leaky vessels. Remove dissolved NO_x gases by a gentle stream of nitrogen at the surface of the sample solution. Add water up to 20 ml or 25 ml and store solutions in polyethylene tubes with screw cap for the AAS analysis.

Fig. 1: Ashing programme of the HPA-S



National Institute of Health
Istituto Superiore di Sanità

Rome, Italy

Copper

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Inductively Coupled Plasma Atomic Emission Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions
3.2. Inductively Coupled Plasma Mass Spectrometry
3.2.a. Sample pretreatment
3.2.b. Analytical instrumentation
3.2.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are liver, kidneys, milk, canned vegetables and fruit juices.

2. Samples throughput per year

Not foreseeable.

3. Procedure

Three different techniques are used for the determination of Cd in the samples.

3.1. Inductively Coupled Plasma Atomic Emission Spectrometry

3.1.a. Sample pretreatment

The sample is homogenized, weighed and digested at high pressure in a microwave oven. Usually a 5:2 HNO_3-H_2O_2 solution is employed.

3.1.b. Analytical instrumentation

A Jobin-Yvon 38 VHR spectrometer equipped with a U-5000 AT CETAC ultrasonic nebulizer is used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1.

Table 1. Equipment characteristics.

ICP-AES
Wavelength, 324.7 nm
Torch, INSA, demountable
Argon flows, plasma 16 l/min, auxiliary 0.3 l/min, aerosol 0.5 l/min
RF generator, Durr-JY 3848, frequency 56 MHz, maximum power output 2.2 kW
Nebulizer, ultrasonic, U-5000 AT, CETAC
Monochromator, HR 1000-M, focal length 1 m, Czerny-Turner mounting, equipped with a 3600 grooves/mm holographic plane grating, linear dispersion in the first order of 0.27 nm/mm, spectral range of 170-450 nm, entrance and exit slit widths of 40 μm

Calibration is carried out by using various Cu solutions depending on the Cu content in the sample.

3.2. Inductively Coupled Plasma Mass Spectrometry

3.2.a. Sample pretreatment

The sample is homogenized, weighed and digested at high pressure in a microwave oven. Usually an HNO_3-H_2O_2 solution is used.

3.2.b. Analytical instrumentation

A Perkin-Elmer/Sciex Elan 6000 quadrupole mass spectrometer is used.

3.2.c. Working conditions

Instrumental parameters are reported in Table 2.

Table 2. Equipment characteristics.

ICP-MS
Analytical mass, ^{65}Cu
Reference mass ^{103}Rh
Nebulizer, cross flow, Ryton spray chamber
Argon flows, plasma 16 l/min, auxiliary 0.9 l/min, aerosol 1 l/min
RF generator, maximum power output 1.0 kW
Scanning conditions: sweeps per reading, 5; readings per replicate, 4; number of replicates, 5; peak hopping; dwell time, 100 ms; replicate time, 2000 ms; normal resolution 0.9-0.6 amu

Calibration is carried out by using various Cu solutions depending on the Cd content in the sample.

4. Quality control

An analytical run includes 1 blank, 1 CRM and unknown samples of similar composition. The accuracy of the method is estimated by means of CRMs (CRM 184, bovine muscle; CRM 185, bovine liver; CRM 186, pig kidney; CRM 150, skim milk). The calibration curves are accepted when the coefficient of correlation (r) is > 0.999.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Agriculture and Food Science Centre

Belfast, Northern Ireland

Copper

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Flame Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are of marine origin such as fish, mussels, oysters and sediments.

2. Samples throughput per year

Not foreseeable.

3. Procedure

Copper is assayed by F-AAS.

3.1. Flame Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Samples are dry-ashed in a muffle furnace which is temperature programmed from room temperature to 450 °C. The ash is dissolved in 0.1 *N* HNO_3.

3.1.b. Analytical instrumentation

A Varian SpectrAA-400 AAS with deuterium lamp background correction is used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1.

Table 1. Equipment characteristics.

F-AAS
Flame, air-acetylene
Wavelength, 324.7 nm
Slit width, 0.5 nm
Deuterium background correction

A calibration curve is established by using Cu standard solutions of 0, 2.0, 4.0, 6.0, 8.0 and 10.0 µg/g.

4. Quality control

The laboratory quality system is based on GLP and UKAS principles. Method validation is performed by using CRM's or spiked samples as appropriate. CRM's are analyzed periodically to check accuracy. During routine analysis, triplicate spiked samples or CRM's are included with each batch of samples analyzed, along with three reagent blanks to control potential contamination. The precision and accuracy is monitored on quality control charts using recovery results.
Instruments are optimized before each batch of analyses, and details recorded of lamp photomultiplier voltage and absorbance for a given standard. Plot linearity is also checked.
The laboratory participates in proficiency testing exercises, such as the UK Food Analysis Performance Assessment Scheme (FAPAS).

5. Difficulties and limitations

The main problem is potential contamination, and purity of chemicals. Dry-ashing requires careful control. The temperature should be kept as low as is possible because of the danger of loosing volatile inorganic compounds, but at the same time high enough to ensure complete combustion of all the organic matter in a reasonably short time. Fatty samples may require additional treatment and precautions when dry-ashing.

6. Comments and remarks

Not reported.

Ministerio de Agricultura, Pesca y Alimentación

Madrid, Spain

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Inductively Coupled Plasma Atomic Emission Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are, in order, wine, spirits, vinegar, beer, orange juice, water, cookies, various kinds of pasta, canned vegetables, liver and kidneys.

2. Samples throughput per year

About 1200.

3. Procedure

Copper is assayed by ICP-AES.

3.1. Inductively Coupled Plasma Atomic Emission Spectrometry

3.1.a. Sample pretreatment

Sampling is not made by the Laboratory Staff, but by official inspectors. The techniques for storage and subsampling are different according to the product; *i.e.*,: milk and milk products, shake; meat, cut in small pieces; eggs, cook; fish, clean and cut; crustaceans, peal and cook. Afterwards homogenizing in kitchen grinder is performed. Digestion of the samples is carried out in a microwave oven. The acids used for digestion are different depending on the nature of the sample processed. In the case of the samples included in the National Residue Investigation Plan, namely liver and kidneys, the reagents used for sample digestion are the following: HNO_3, $HClO_4$ and H_2O_2.

3.1.b. Analytical instrumentation

A CEM Model NDS-81D microwave oven and a Varian Liberty 220 Inductively Coupled Plasma Atomic Emission Spectrometer is used.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 1.

Table 1. Equipment characteristics.

ICP-AES
Wavelength, 324.7 nm
Nebulizer, 180 kPa
Argon flows, plasma 10.5 l/min, auxiliary 1.5 l/min
Power output, 1.0 kw
High observation, 17 mm
Integration time, 3 s

The calibration curve is plotted by using Cu solutions of 0.1, 1, 5 and 10 ppm.

4. Quality control

The routine use of each method is preceded by analytical validation according to the IUPAC recommendations in order to ascertain sensitivity, detection power, precision and accuracy. CRMs of bovine liver (NIST) and mussel tissue (SMT) are used to check the accuracy and analyzed periodically. During routine assays, each analytical batch includes at least three reagent blanks to evaluate the detection limits and to control possible contaminations. Recoveries are calculated using two spiked reagent blanks and one spiked sample for each non-spiked sample assayed.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

National Food Administration

Uppsala, Sweden

Copper

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions
3.2. Flame Atomic Absorption Spectrometry
3.2.a. Sample pretreatment
3.2.b Analytical instrumentation
3.2.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissues, cereals and vegetables.

2. Samples throughput per year

About 100-200 samples per year.

3. Procedure

Copper is determined by ETA-AAS and F-AAS after either dry ashing or microwave digestion.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

a) About 1-10 g of sample is weighed into a platinum crucible and dry ashed at a temperature of 450 ± 25 °C in a programmable furnace. The carbon-free ash is dissolved in 20-30 ml of 0.1 M HNO_3 and transferred to a plastic container.

b) A maximum of 0.5 g dried, homogenized sample is weighed into a Teflon vessel, followed by the addition of 5 ml conc HNO_3 and 2 ml 30% H_2O_2. The digestion is carried out in a CEM (MDS 2000) microwave oven according to the programme detailed in Table 1.

Table 1. Digestion programme.

Step	Power (W)	Time (min)
1	250	3
2	630	5
3	500	22
4	0	15

3.1.b. Analytical instrumentation

Perkin Elmer Z-5100 with HGA-600 autosampler AS-60.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 2 and 3 for the PE Z-5100 instrument.

Table 2. Equipment characteristics.

Z-ETA-AAS
Wavelength, 324.8 nm
Slit width, 0.7 low
Lamp, Cu HCL
Zeeman background correction
L'vov platform
Injection volume, 10 µl sample
Pyrolytic coated graphite tubes

Table 3. Graphite furnace programme.

Step	Ramp Time (s)	Hold Time (s)	Temperature (°C)
Injection	-	-	Ambient
Drying	20	10	90/130
Pyrolysis	15	10	500
Atomization*	0	3	2100
Cleaning out	1	5	2600

* Gas stop

Calibrations are generally carried out by standard addition method.

3.2. Flame Atomic Absorption Spectrometry

3.2.a. Sample pretreatment

See section 3.1.a.

3.2.b. Analytical instrumentation

A Varian SpectrAA 220FS with D2-BC is used.

3.2.c. Working conditions

The instrument is equipped with a single slot burner and utilises an oxidizing air acetylene flame. Wavelength, 324.7 nm. Results are calculated from a standard curve.

4. Quality control

An analytical run includes one-two blanks and one or more CRM or in-house RM. Samples are measured as singles or duplicates, depending on the requirements. Accuracy and precision are monitored by the use of control charts on which the results of duplicate CRM and RM analysis are plotted. The detection limit is based on three times the standard deviation of large number of blanks and is currently 23 ng/ml in sample solution.

5. Difficulties and limitations

Extremely fatty or sugar rich samples require extra attention when dry ashing is used. The microwave digestion method is not suitable for fats, oils or extremely fatty products.

6. Comments and remarks

The dry ashing method has been collaboratively tried and was approved by the Nordic Committee and Food Analysis (NMKL) in 1990. The report as published in *JAOAC* Int. 76 (1993), no. 4. (Lars Jorhem, Determination of metals in foodstuffs by AA Spectrophotometry after dry ashing: An NMKL collaborative study of Cd, Cr, Cu, Fe, Ni, Pb and Zn.). This method was validated on January 1st 1996.

The microwave digestion method has been collaboratively tried and was approved by NMKL in 1997.

The result of both collaboratively trials has been submitted to the AOAC Methods Committee and was accepted in December 1999.

National Institute for Public Health and Environment
Rijksinstituut voor Volksgezondheid en Milieu

Bilthoven, The Netherlands

Copper

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Inductively Coupled Plasma Mass Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analysed are meat, rain water, ground water, urine, animal feed, soil and aerosol particles.

2. Samples throughput per year

About 1000.

3. Procedure

Copper is assayed by ICP-MS.

3.1 Inductively Coupled Plasma Mass Spectrometry

3.1.a. Sample pretreatment

Solid matrices are digested in a microwave oven. For 0.5 g of homogenised biological sample 1 ml of concentrated HNO_3 and 9 ml H_2O is used.
For soil samples (particle size < 250µm) an aqua regia digestion is performed.

3.1.b. Analytical instrumentation

An HP4500 equipped with the quadrupole ICP-MS is used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1.
Table 1. **ICP-MS HP4500+** Equipment characteristics.

Analytical mass, ^{65}Cu
Reference mass, ^{72}Ge (added on-line (dilution sample/IS: 1: 5.5))
Nebulizer, V-groove, Scott-type double pass spraychamber
Argon flow, nebulizer 1.17 l/min, R.f. power 1250W
Measuring conditions: 3 points / mass; integration time 0.3 s / mass, 3 replicates. Two-point calibration, standards concentration, 0 and 100 µg/l
Matrix matching for calibration samples
Equation, $^{65}Cu(-0.000041*Mg26-0.000069*S34-0.000076*Ca43-0.001195*Ba^{++}69)$, equation is updated in every measurement sequence using (a) suitable Interference Check Solution(s)

4. Quality control

Each analytical run includes 1 blank, 1 CRM and 1 RM. The instrumental conditions are controlled by the analysis of an in-house water reference material. The accuracy of the analytical procedure, including sample digestion, is estimated by the use of CRMs. A number of matrix samples are analyzed and recovery experiments are carried out for Cu.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Central Science Laboratory

York, United Kingdom

Copper

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Inductively Coupled Plasma Mass Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are foods and beverages.

2. Samples throughput per year

Up to 1500.

3. Procedure

Copper is assayed by ICP-MS.

3.1. Inductively Coupled Plasma Mass Spectrometry

3.1.a. Sample pretreatment

Aliquots of each sample are homogenized and portions (equivalent to 0.5 g dry weight) are weighed out into microwave digestion vessels. Concentrated HNO_3 (5 ml) is added and the samples are heated in a proprietary high pressure microwave digestion oven. The acid is transferred into a polycarbonate test tube with 18 MΩ cm^{-1} water and made up to 10 ml in volume.

3.1.b. Analytical instrumentation

A VG Instruments PlasmaQuad PQII+ Turbo quadrupole ICP-MS or Perkin Elmer ELAN 6000 ICP-MS quadrupole are used.

3.1.c. Working conditions

At least 7 calibration standards are used to cover the calibration range 0.1 µg/l to 100 µg/l. ^{63}Cu and ^{65}Cu are monitored with In or Rh being used as an internal standard. Element concentrations in test solutions are determined by manufacturers software and processed further using an in-house written SuperCalc 5 macro. Automatic spike recovery and blank corrections are made during these calculations.

4. Quality control

All test batches contain reagent blanks, spiked blanks and CRMs and are performed according to UKAS accredited standards.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Iron

BELGIUM
GERMANY
NORTHERN IRELAND
PORTUGAL
SPAIN
SWEDEN
THE NETHERLANDS

Scientific Institute of Public Health – Louis Pasteur

Brussels, Belgium

Iron

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Inductively Coupled Plasma - Atomic Emission Spectrometry 3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Fish, meat, product of bakkery

2. Samples throughput per year

About 200 samples per year.

3. Procedure

Iron is assayed by ICP-AES

3.2. Inductively Coupled Plasma - Atomic Emission Spectrometry

3.2.a. Sample pretreatment

About 1 g is weighed on an analytical balance in a polyethylene cup and dropped in a PFA digestion tube. Wet digestion is carried out with the aid of 5 ml conc. HNO_3 and 0.2 ml conc. $HClO_4$. The tube is placed in an oven at 180 °C. After reducing of the volume to +/- 0.5 mL, 1 mL of H_2O_2 is added. The tube is placed into an oven at 80 °C +/- 1 hour. After complete oxydation the tubes are cooled to ambient temperature. The digest is diluted into the tube to a volume of 10 mL with Milli-Q-water. If necessary, furthers dilutions are realised.

3.1.b. Analytical instrumentation

A Perkin Elmer Optima 4300 DV

3.1.c. Working conditions

Axially viewed ang Gemcone nebulizers Wavelength: 238.2 nm; 239.6 nm; and 259.9 nm. Injection flow rate: 2.2 mL /min. Integration time: 5-20 sec Measurment Mode: Peak Area Normal resolution

Calibration is carried out with standard solution, freshly prepared with 2 % v/v conc. HNO_3. The stock solution is Perkin Elmer PE Pure. Working range is from 10 ppb to 10 ppm

4. Quality controle

Three wavelength are used. The best wavelength is conservated for the expression of results based on correlation coefficient and recovery test at level of +/- 50 % of the concentration range of samples. An analytical run includes blanks and standards. All glassware and polyethylene flasks and pipets are soaked in a 10 % HNO_3 solution and rinsed with Milli-Q-water just before use. All volumetric flasks, glass pipets are calibrated, plastic pipets are checked regularly. Proficiency test are frequently realised.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Federal Office for Consumer Protection and Food Safety

Berlin, Germany

Iron

Section I

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Flame Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

Section II

Sample ashing procedure

1. Matrices

2. Procedure

1. Matrices

Samples analyzed are meat, meat products, plant materials, food

2. Sample throughput per year

About 100.

3. Procedures

Iron is assayed by F-AAS

3.1. Flame Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Mineralization is described in section II (Sample ashing procedure).

3.1.b. Analytical instrumentation

A SpectrAA 300 - AAS with acetylene-air-flame is used.

3.1.c. Working conditions

The calibration curve is plotted by using Iron solutions of 0.5, 1.0, 2.0 and 4.0 µg/ml in 3 % HNO_3. The analytical blank is a solution of 3 % HNO_3. Limit of detection is 0.1 µg/ml in sample solution.

4. Quality control

Analysis of certified reference materials (BCR, NIST, CBNM) is performed along with each analytical series.

5. Difficulties and limitations

Work with care to prevent contaminations from laboratory air!

6. Comments

These method corresponds to the official German Method for food analysis.

Section II

Sample ashing procedure

1. Matrices

All materials will be mineralized following the same procedure.

2. Procedure

A high pressure ashing system HPA-S equipped with five 90 ml quartz vessels is used. Concentrated HNO_3, 65 % (FLUKA, pA plus) is used. Between 300 mg and 1500 mg of homogenized sample material are weighed into the quartz vessels. Samples amounts are illustrated in Table 1.

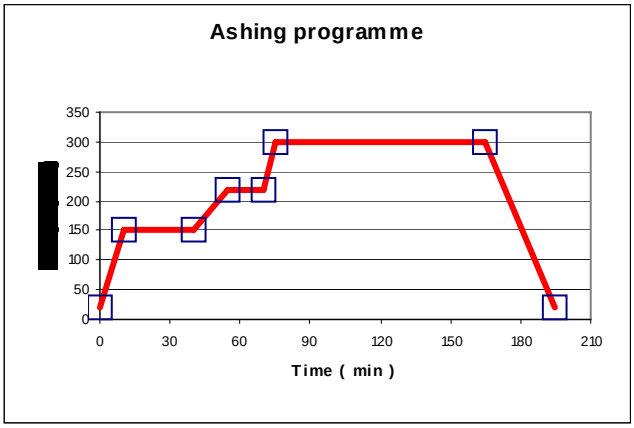
Table 1. Sample amounts for use in the HPA-S

Sample	**Weight (mg)**	Tolerance **(mg)**	**Volume of HNO_3 (ml)**
dry samples (< 20 % water)	500	± 50	3.0
fresh samples	1000	± 100	3.0
fresh samples (> 50 % water)	1500	± 200	3.0

Dry samples should be wetted with some drops water before HNO_3 is added; it's to recommend to let stand the vessels over night before heating.

Start the mineralization: use a ashing temperature of 300 °C over a hold time of 90 minutes (Fig. 1). When ashing procedure is finished, let cool down to room temperature. Check the volume of HNO_3 in the vessels to recognize leaky vessels. Remove dissolved NO_x gases by a gentle stream of nitrogen at the surface of the sample solution. Add water up to 20 ml or 25 ml and store solutions in polyethylene tubes with screw cap for the AAS analysis.

Fig. 1: Ashing programme of the HPA-S



Agriculture and Food Science Centre

Belfast, Northern Ireland

Iron

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Flame Atomic Absorption Spectrometry
3.1.a Sample pretreatment
3.1.b Analytical instrumentation
3.1.c Working conditions
3.4. Electrothermal Atomization Atomic Absorption Spectrometry
3.2.a. Sample pretreatment
3.2.b. Analytical instrumentation
3.2.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples most frequently analyzed are of marine origin, such as fish, oysters, mussels and sediments.

2. Samples throughput per year

About 200 samples per year.

3. Procedures

Iron is analyzed by two different instrumental techniques depending upon the concentration of the element in the sample. Flame atomic absorption spectrometry is the general method of analysis with electrothermal atomization atomic absorption spectrometry being used to a lesser extent for lower concentrations of elements.

3.1 Flame Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Samples are dry-ashed in a muffle furnace temperature programmed from room temperature to 450 °C. The ash is dissolved in 0.1 *N* HNO_3.

3.1.b. Analytical instrumentation

A Varian SpectrAA-400 Atomic Absorption Spectrometer with deuterium lamp background correction is used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1.

Table 1. Equipment characteristics.

F-AAS	Flame, air-acetylene
Wavelength, 248.3nm	
Slit width, 0.2 nm	
Deuterium background correction	
Lamp current 5.0mA	

A calibration curve is established by using Fe standard solutions of 0, 0.2, 0.4, 0.6, 0.8 and 1.0 µg/g in 0.1 *N* HNO_3.

3.2. Electrothermal Atomization Atomic Absorption Spectrometry

3.2.a. Sample pretreatment

Samples are dry-ashed in a muffle furnace which is temperature programmed from room temperature to 450 ^{o}C. The ash is dissolved in 0.1 *N* HNO_3.

3.2.b. Analytical instrumentation

A Varian SpectrAA-300 Atomic Absorption Spectrometer with Zeeman background correction, graphite furnace and autosampler is used.

3.2.c. Working conditions

Instrumental parameters are reported in Tables 2 and 3.

Table 2. Equipment characteristics.

ETA-AAS	Pyrotylitic coated graphite tube
Wavelength, 248.3 nm	
Slit width, 0.2 nm	
Zeeman background correction	
Injection volume, 5 µl sample	

Table 3. Graphite furnace programme

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)	Ar Flow (ml/min)
Drying	85 95 120	5 45 10	0 0 0	300 300
Ashing	1100	5	3	300
Atomization	2100	0.5	2	0
Clean-up	2100	1	2	300

A calibration curve is established using Fe standard solutions of 0.002, 0.004 and 0.006 µg/g in 0.1 *N* HNO_3.

4. Quality control

The laboratory quality system is based on GLP and UKAS principles. Method validation is performed by using CRM's or spiked samples as appropriate. CRM's are analyzed periodically to check accuracy. During routine analysis, triplicate spiked samples or CRM's are included with each batch of samples analyzed, along with three reagent blanks to control potential contamination. The precision and accuracy is monitored on quality control charts using recovery results.
Instruments are optimized before each batch of analyses, and details recorded of lamp photomultiplier voltage and absorbance for a given standard. Plot linearity is also checked.
The laboratory participates in proficiency testing exercises, such as the UK Food Analysis Performance Assessment Scheme (FAPAS)

5. Difficulties and limitations

The main problem is potential contamination and purity of chemicals. Dry-ashing requires careful control. The temperature should be kept as low as is possible because of the danger of loosing volatile inorganic compounds, but at the same time high enough to ensure complete combustion of all the organic matter in a reasonably short time. Fatty samples may require additional treatment and precautions when dry-ashing.

6. Comments and remarks

Not reported.

Istituto de Investigação das Pescas e do Mar

Lisboa, Portugal

Iron

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

3.2. Flame Atomic Absorption Spectrometry
3.2.a. Sample pretreatment
3.2.b. Analytical instrumentation
3.2.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissue (liver).

2. Samples throughput per year

Not foreseeable.

3. Procedure

Iron is assayed by Z-ETA-AAS and by F-AAS.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Approximately 100 g of samples are homogenized, 1 g is weighed in quartz crucibles and 0.5 ml of 50 % $Mg(NO_3)_2$ are added. The sample is pretreated in pre-ashing apparatus until completely charred, followed by ashing in a muffle furnace at 450 °C during 1 h. The samples are then removed and wet with concentrated HNO_3 and put back to the furnace. This procedure is repeated every hour until ashes become white. The ashes are then quantitatively transferred to 10 ml volumetric flasks with 5 % HNO_3.

3.1.b. Analytical instrumentation

A Perkin Elmer Atomic Absorption Spectrometer Aanalyst 600 with THGA Graphite Furnace including AS- 800 Autosampler.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 1 and 2. Use is made of $Mg(NO_3)_2$ (v/v) as the matrix modifier.

Table 1. Equipment characteristics.

Z-ETA-AAS
Wavelength, 248.3 nm
Fe hollow cathode lamp.
Zeeman background correction
L'vov platform
Injection volume, 10 µl samples + 5 µl modifier
Pyrolytic coated graphite tubes

Table 2. Graphite furnace programme.

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)	Internal Flow
1	100	4	30	250
2	130	15	30	250
3	1400	10	20	250
4	2100	0	5	0
5	2450	1	3	250

Extra furnace clean-out.

The calibration curve is plotted by using Fe solutions of 10; 20; 40 and 80 ng/g.

3.2. Flame Atomic Absorption Spectrometry

3.2.a. Sample pretreatment

Approximately 100 g of samples are homogenized, 1 g is weighed in quartz crucibles. The sample is pretreated in pre-ashing apparatus until completely charred, followed by ashing in a muffle furnace at 450 °C overnight. The samples are then removed and wet with concentrated HNO_3 and put back to the furnace. This procedure is repeated every hour until ashes become white. The ashes are then quantitatively transferred to 100 ml volumetric flasks with 5% HNO_3.

3.2.b. Analytical instrumentation

An F-AAS Unicam Model 969 instrument is used.

3.2.c. Working conditions

Instrumental parameters are reported in Tables 1.

Table 1. Equipment characteristics.

F-AAS
Wavelength, 213.9 nm
Deuterium background correction
Injection volume, 1000 µl samples
Fuel flame concentration, 1.0 mg/L

Calibration plots are set up by using Fe solutions of 0.5, 1.0, 2.0 and 3.0 µg/ml.

4. Quality control

Each analytical run includes blanks, commercial standard solutions, CRMs (BCR 186, pig kidney), from IRMM, and Quality Control Standards for Trace Metals - AA by RTC.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Ministerio de Agricultura, Pesca y Alimentación

Madrid, Spain

Iron

1. Matrices

2. Samples throughput per year

3. Procedure
3.2. Inductively Coupled Plasma Atomic Emission Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are, in order, wine, spirits, vinegar, beer, orange juice, water, cookies, various kinds of pasta, canned vegetables, liver and kidneys.

2. Samples throughput per year

About 1200.

3. Procedure

Iron is assayed by ICP-AES.

3.1 Inductively Coupled Plasma Atomic Emission Spectrometry

3.1.a. Sample pretreatment

Sampling is not made by the Laboratory Staff, but by official inspectors. The techniques for storage and subsampling are different according to the product; *i.e.*,: milk and milk products, shake; meat, cut in small pieces; eggs, cook; fish, clean and cut; crustaceans, peal and cook. Afterwards homogenizing in kitchen grinder is performed. Digestion of the samples is carried out in a microwave oven. The acids used for digestion are different depending on the nature of the sample processed. In the case of the samples included in the National Residue Investigation Plan, namely liver and kidneys, the reagents used for sample digestion are the following: HNO_3, $HclO_4$ and H_2O_2.

3.1.b. Analytical instrumentation

A CEM Model NDS-81 D microwave oven and a Varian Liberty 220 Iinductively Coupled Plasma Atomic Emission Spectrometer is used.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 1.

Table 1. Equipment characteristics.

ICP-AES
Wavelength, 259.940 nm
Nebulizer, 150 kPa
Argon flows, plasma 15.0 l/min, auxiliary 1.5 l/min
Power output, 1.50 kw
High observation, 15 mm
Integration time, 3 s

The calibration curve is plotted by using Fe solutions of 0.1, 1, 5 and 10 mg/l.

4. Quality control

The routine use of each method is preceded by analytical validation according to the IUPAC recommendations in order to ascertain sensitivity, detection power, precision and accuracy. CRMs of bovine liver (NIST) and mussel tissue (SMT) are used to check the accuracy and analyzed periodically. During routine assays, each analytical batch includes at least three reagent blanks to evaluate the detection limits and to control possible contaminations. Recoveries are calculated using two spiked reagent blanks and one spiked sample for each non-spiked sample assayed.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

National Food Administration

Uppsala, Sweden

Iron

1. Matrices

2. Samples throughput per year

3. Procedure

3.2. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissues, cereals and vegetables.

2. Samples throughput per year

About 100-200 samples per year.

3. Procedure

Iron is determined by ETA-AAS and F-AAS after either dry ashing or microwave digestion.

3.3. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

a) About 1-10 g of sample is weighed into a platinum crucible and dry ashed at a temperature of 450 ± 25 °C in a programmable furnace. The carbon-free ash is dissolved in 20-30 ml of 0.1 M HNO_3 and transferred to a plastic container.

b) A maximum of 0.5 g dried, homogenized sample is weighed into a Teflon vessel, followed by the addition of 5 ml conc HNO_3 and 2 ml 30% H_2O_2. The digestion is carried out in a CEM (MDS 2000) microwave oven according to the programme detailed in Table 1.

Table 1. Digestion programme.

Step	Power (W)	Time (min)
1	250	3
2	630	5
3	500	22
4	0	15

3.1.b. Analytical instrumentation

Perkin Elmer Z-5100 with HGA-600 autosampler AS-60.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 2 and 3 for the PE Z-5100 instrument.

Table 2. Equipment characteristics for the PE Z-5100.

Z-ETA-AAS
Wavelength, 248.3 nm
Slit width, 0.7 low
Lamp, Fe HCL
Zeeman background correction
L'vov platform
Injection volume, 10 µl sample
Pyrolytic coated graphite tubes

Table 3. Graphite furnace programme.

Step	**Ramp Time (s)**	**Hold Time (s)**	**Temperature (°C)**
Injection	-	-	Ambient
Drying	20	10	90/130
Pyrolysis	15	10	500
Atomization*	0	3	2000
Cleaning out	1	5	2600

* Gas stop

Calibrations are generally carried out by standard addition method.

3.2. Flame Atomic Absorption Spectrometry

3.2.a. Sample pretreatment

See section 3.1.a.

3.2.b. Analytical instrumentation

A Varian SpectrAA 220FS with D2-BC is used.

3.2.c. Working conditions

The instrument is equipped with a single slot burner and utilises an oxidizing air acetylene flame or a reducing nitrous oxide acetylene flame. Wavelength 248.3 nm. Results are calculated from a standard curve.

4. Quality control

An analytical run includes one-two blanks and one or more CRM or in-house RM. Samples are measured as singles or duplicates, depending on the requirements. Accuracy and precision are monitored by the use of control charts on which the results of duplicate CRM and RM analysis are plotted. The detection limit is based on three times the standard deviation of large number of blanks and is currently 37 ng/ml in sample solution.

5. Difficulties and limitations

Extremely fatty or sugar rich samples require extra attention when dry ashing is used. The microwave digestion method is not suitable for fats, oils or extremely fatty products.

6. Comments and remarks

The dry ashing method has been collaboratively tried and was approved by the Nordic Committee for Food Analysis (NMKL) in 1990. The report was published in *JAOAC* Int. 76 (1993), no. 4. (Lars Jorhem, Determination of metals in foodstuffs by AA Spectrophotometry after dry ashing: An NMKL collaborative study of Cd, Cr, Cu, Fe, Ni, Pb and Zn.). This method was validated on January 1st 1996.

The microwave digestion method has been collaboratively tried and was approved by NMKL in 1997.

The result of both collaboratively trials have been submitted to the AOAC Methods Committee and were accepted in December 1999.

National Institute for Public Health and Environment
Rijksinstituut voor Volksgezondheid en Milieu

Bilthoven, The Netherlands

Iron

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Inductively Coupled Plasma Mass Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are meat, rain water, ground water, urine, animal feed and aerosol particles.

2. Samples throughput per year

About 1000.

3. Procedure

Iron is assayed by ICP-MS.

3.1 Inductively Coupled Plasma Mass Spectrometry

3.1.a. Sample pretreatment

Solid matrices are digested in a microwave oven. For 0.5 g of homogenised biological sample 1 ml of concentrated HNO_3 and 9 ml H_2O is used.
For soil samples (particle size < 250µm) an aqua regia digestion is performed.

3.1.b. Analytical instrumentation

An HP4500equipped with the quadrupole ICP-MS is used.

3.1.c Working conditions

Instrumental parameters are reported in Table 1.

Table 1. **ICP-MS HP4500+** Equipment characteristics.

Analytical mass, ^{57}Fe
Reference mass, ^{72}Ge (added on-line (dilution sample/IS: 1: 5.5))
Nebulizer, V-groove, Scott-type double pass spraychamber
Argon flow, nebulizer 1.17 l/min, R.f. power 1250W
Measuring conditions: 3 points / mass; integration time 1.5 s / mass, 3 replicates
Two-point calibration, standards concentration, 0 and 2000 µg/l
Matrix matching for calibration samples
Equation ^{57}Fe (-0.00045*Mg26-0.023*Ca43), equation is updated in every measurement sequence using (a) suitable Interference Check Solution(s)

4. Quality control

Each analytical run includes 1 blank, 1 CRM and 1 RM. The instrumental conditions are controlled by the analysis of an in-house water reference material. The accuracy of the analytical procedure, including sample digestion, is estimated by the use of CRMs. A number of matrix samples are analyzed and recovery experiments are carried out for Fe.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Lead

AUSTRIA
BELGIUM
CZECH REPUBLIC
CYPRUS (AOAC 999.10)*
DENMARK
ESTONIA
FINLAND
FRANCE
GERMANY
GREECE
HUNGARY (see Cd)
IRELAND
ITALY
LATVIA (AOAC 986.15 / 999.11)*
MALTA (see Cd)
NORTHERN IRELAND
POLAND (see Cd)
PORTUGAL
SPAIN
SWEDEN
THE NETHERLANDS
UNITED KINGDOM

**Official analytical methods are not reported*

Austrian Agency for Health and Food Safety

Wien, Austria

Lead

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples most frequently analyzed are: liver and kidney from cattle, pig, sheep, poultry, muscle from horses and game.

2. Samples throughput per year

About 300-400.

3. Procedure

Lead is assayed by Z-ETA-AAS.

3.1.Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

a) 2 ± 0.1 g of homogenized tissue is weighed in 20 ml glass bottles. 3 ml HNO_3 (65 %, suprapure) are added. The samples are digested for at least 1 h at 90 °C in a water bath. The solutions are filtered through paper filters into 25 ml measuring flasks and filled up to 25 ml with deionized water.

b) 2 ± 0.1 g of homogenized tissue is weighed in 40 ml Teflon vessels. 3 ml HNO_3 (65 %, suprapure) and 1.5 ml H_2O_2 (30 %) are added. The samples are digested in the microwave oven (MLS-Ethos 900) according to the following programme: 1. 100 W, 10 min; 2. 0 W, 1 min; 3. 250 W, 5 min; 4. 0 W, 1 min; 5. 400 W, 5 min; 6. 600 W, 10 min; 7. 500 W, 6 min. After cooling the digested sample solutions are transferred into 25 ml measuring flasks and filled up to 25 ml with deionized water.

3.1.b. Analytical instrumentation

A SIMAA 6000 with Autosampler AS-72 (Perkin-Elmer) is used.

3.1.c. Working conditions

Equipment characteristics are reported in Table 1. Use is made of 0.5 % $NH_4H_2PO_4$ + 0.03 % $Mg(NO_3)_2$ as matrix modifier.

Table 1. Equipment characteristics.

Z-ETA-AAS
Wavelength, 283.3 nm
Lamp, Pb HCL
Zeeman background correction, longitudinal AC magnetic field with transversal heated graphite furnace
Injection volume, 20 µl sample/standard + 10 µl modifier
Measurement mode, peak area

The furnace conditions are summarized in Table 2.

Table 2. Graphite furnace programme.

Step	**Temperature (°C)**	**Ramp Time (s)**	**Hold Time (s)**	**Gas Flow (ml/min)**
Drying	110	1	30	250
Drying	130	15	40	250
Ashing	500	10	30	250
Atomization	1700	0	5	0
Clean-up	2450	1	3	250

The calibration curve is established by automatical dilution of one stock solution (5 µg/l Cd and 50 µg/l Pb) by the autosampler to following concentrations: 1, 2, 3 and 5 µg/l Cd and 10, 20, 30 and 50 µg/l Pb, respectively. The blank is subtracted automatically from standards and samples.

4. Quality control

Calibration curves are accepted when the coefficient of correlation is > 0.995. The performance of the instrument is checked by a multielement standard (Perkin-Elmer). Recovery is calculated by spiked samples. CRM 184 (bovine muscle), CRM 185 (bovine liver) or CRM 186 (pig kidney) are analyzed in order to check the accuracy and reproducibility of the whole method. Usually 10 samples are pretreated and measured together as one batch. One control sample is analyzed for every batch in order to check the repeatability.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Usually Cd and Pb are measured simultaneously.

Scientific Institute of Public Health-Louis Pasteur

Brussels, Belgium

Lead

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples frequently analyzed are meat,vegetables, sometimes babyfood

2. Samples throughput per year

About,*100* samples per year.

3. Procedure

Cadmium is assayed by Z-ETA-AAS.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

About 1 g is weighed on an analytical balance in a polyethylene cup and dropped in a PFA digestion tube. Wet digestion is carried out with the aid of 5 ml conc. HNO_3 and 0.2 ml conc. $HClO_4$. The tube is placed in an oven at 180 °C. After reducing of the volume to +/- 0.5 mL, 1 mL of H_2O_2 is added. The tube is placed into an oven at 80 °C +/- 1 hour. After complete oxydation the tubes are cooled to ambient temperature. The digest is diluted into the tube to a volume of 10 mL with Milli-Q-water. If necessary, furthers dilutions are realised.

3.1.b. Analytical instrumentation

Perkin Elmer SimAA 6000

3.1.c. Working conditions

Z-ETA-AAS

Wavelength, 283.3 nm Slit width, 0.5 nm Lamp, Pb HCL Zeeman background correction L'vov platform Injection volume, 20 µl sample + 10 µl modifier THGA graphite Furnace with End Cap Measurement mode, peak area Calibration mode, concentration Replicates, 2 Sample introduction, sampler automixing (5 points)

Step	Temperature (°C)
Injection	Ambient
Drying	110 -130
Pyrolysis	700
Atomization	1700
Clean-up	2600

Use is made of $NH_4H_2PO_4$ and $Mg(NO_3)_2$ as matrix modifiers. Calibration is carried out with standard solution, freshly prepared with 2 % v/v conc. HNO_3. The stock solution is Perlin Elmer PE Pure. Linear working range is 0-2.5 ng/ml.

4. Quality control

An analytical run includes blanks, standards, spike samples for recovery measurements o all samples and RMs (BCR CRM 185, bovine liver) are regularly analysed. All glassware and polyethylene flasks and pipets are soaked in a 10 % HNO_3 solution and rinsed with Milli-Q-water just before use. All volumetric flasks, glass pipets are calibrated, plastic pipets are checked regularly. Control charts are used.

5. Difficulties and limitations

Little concentration range with ETAAS. Sometimes dilutions are necessary (Fish and some vegetables...)

6. Comments and remarks

Not reported.

State Veterinary Institute Olomouc
Laboratory in Kromeriz

Kromeriz, Czech Republic

Lead

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Analytical technique
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissues (meat, liver and kidney), meat products, fish, fish products, milk, milk products, babyfood, honey, feedstuffs.

2. Sample throughput per year

About 1300 samples per year.

3. Procedure

Lead is assayed by Z-ETA-AAS and F-AAS.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

a) 1-10 (± 0.1) g of homogenized sample is weighed and dry ashed at a temperature of 450±25 ^{o}C in a programmable furnace overnight. Use is made of 6 % w/w $Mg(NO_3)_2$ as an ashing aid. Ashed sample is cooled down to room temperature, deionized water and 1 ml conc. HNO_3 are added. The sample is re-evaporated and heated up to 450 ^{o}C in the furnace. This procedure is repeated until ashes become white. Ashes are dissolved in 1 M HNO_3.

b) 1 ± 0.1 g of homogenized sample is weighed in 100 ml Teflon vessels. 5 ml HNO_3 (65 %, suprapure) and 1.5 ml H_2O_2 (30 %) are added. The samples are digested into the microwave oven (MLS – Ethos Plus) according to the following programme: 1. 500W, 10 min, 180 ^{o}C; 2. 500 W, 10 min, 200 ^{o}C. After cooling the digested sample solutions are transferred into 10 ml measuring flasks and filled up to 10 ml deionized water.

3.1.b. Analytical instrumentation

A PerkinElmer AAnalyst 800 spectrometer with THGA Graphite Furnace including AS-800 Autosampler is used.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 1. and 2. Use is made of Pd / $Mg(NO_3)_2$ as matrix modifiers.

Table 1. Equipment characteristics

Z-ETA-AAS
Wavelength: 283.3 nm
Lamp: Pb EDL
Zeeman background correction
Standard THGA Graphite Tubes with Integrated Platform
Injection volume: 20 µl samples + 10 µl modifier

Table 2. Graphite furnace programme

Step	**Temperature (°C)**	**Ramp Time (s)**	**Hold Time (s)**	**Internal Flow**
1	110	1	30	250
2	130	15	30	250
3	900	10	20	250
4	1800	0	5	0
5	2450	1	3	250

The calibration curve is established by using Pb solutions of 10, 20, 50, 80, 100 µg/l.

3.2. Flame Atomic Absorption Spectrometry

3.2.a. Sample pretreatment

a) 1-10 (± 0.1) g of homogenized sample is weighed and dry ashed at a temperature of 450±25 °C in a programmable furnace overnight. Use is made of 6 % w/w $Mg(NO_3)_2$ as an ashing aid. Ashed sample is cooled down to room temperature, deionized water and 1 ml conc. HNO_3 are added. The sample is re-evaporated and heated up to 450 °C in the furnace. This procedure is repeated until ashes become white. Ashes are dissolved in 1 M HNO_3.

b) 1 ± 0.1 g of homogenized sample is weighed in 100 ml Teflon vessels. 5 ml HNO_3 (65 %, suprapure) and 1.5 ml H_2O_2 (30 %) are added. The samples are digested into the microwave oven (MLS – Ethos Plus) according to the following programme: 1. 500W, 10 min, 180 °C; 2. 500 W, 10 min, 200 °C. After cooling the digested sample solutions are transferred into 10 ml measuring flasks and filled up to 10 ml deionized water.

3.2.b. Analytical Instrumentation

A Perkin-Elmer 2100 Atomic Absorption Spectrometer is use.

3.2.c. Working conditions

Instrumental parameters are reported in Table 3.

Table 3. Equipment characteristics

F-AAS
Wavelength: 217 nm
Lamp: Pb EDL
Deuterium background correction
Air-acetylene flame

The calibration curve is established by using Pb solutions of 0.20, 0.50, 1.00, 3.00 mg/l.

4. Quality control

The routine use of each method is preceded by analytical validation according to the IUPAC recommendations and the metods are accreditated.
CRMs (BCR 184, BCR 185 R, BCR 151, Tort-2, …) are use to check the accuracy and analyzed periodically. An analytical run includes two blanks, duplicates and in-house RM. The results of duplicate CRM, RM analysis samples are monitored on control charts.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Danish Institute for Food and Veterinary Research

Søborg, Denmark

Lead

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions
3.2. Inductively Coupled Plasma Mass Spectrometry
3.2.a. Sample pretreatment
3.2.b. Analytical instrumentation
3.2.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are fish, meat, flour, fruit and vegetables.

2. Samples throughput per year

About 20. for ICP-MS.

3. Procedure

Two different techniques are used for the determination of Pb in food although the ICP-MS based method of analysis will take over fully in the near future.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

The sample is homogenized in a blender equipped with titanium blades. A known amount of the homogenized sample (2-3 g wet weight) is weighed in a Berghof high pressure bomb and 4 ml of concentrated HNO_3 are added. The sample is digested in an oven at 160 °C for 4 h. This is typically done overnight. After the digestion distilled water is added up to a known weight of approximately 20 g.

Alternatively, the Multiwave high pressure microwave digestion system is used for sample decomposition for ICP-MS determination. The maximum pressure possible is 70 bar and the digestion is finalised within 60 minutes including cooling. The same sample intake and acid is used with his technique.

3.1.b. Analytical instrumentation

A Perkin-Elmer Zeeman 3030 ET-AAS instrumentat is used.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 1 and 2. Use is made of $NH_4H_2PO_4/Mg(NO_3)_2$ as matrix modifier.

Table 1. Equipment characteristics.

ETA-AAS
Wavelength, 283.3 nm
Slit width, 0.7 nm
Lamp, Pb EDL
Zeeman background correction
L'vov platform

Table 2. Graphite furnace programme.

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)	Ar Flow (ml/min)
Drying	100 150	5 10	20 50	300 300
Charring	850	20	30	300
Atomization	1600	0	5	0
Clean-up	2700	1	4	300

Calibration is carried out by the standard addition method. Two additions are used for each sample.

3.2. Inductively Coupled Plasma Mass Spectrometry

3.2.a. Sample pretreatment

The sample is homogenized in a blender equipped with titanium blades. A known amount of the homogenized sample (2-3 g wet weight) is weighed intothe Multiwave high pressure microwave digestion system. The Multiwave high pressure microwave digestion system is used for sample decomposition. The maximum pressure is 70 bar and the digestion is finalised within 60 minutes including cooling. Following digestion the residue is taken to 20 ml by weighing using pure water.

3.2.b. Analytical instrumentation

An Elan 6100 DRC Perkin-Elmer/Sciex quadrupole mass spectrometer is used.

3.2.c. Working conditions

A volume of 5 ml of the sample solution is pipetted into a polyethylene autosampler tube and 5 ml of 1 % aqueous HNO_3 are added. Lead is measured at m/z = 208. Calibration is carried out by the standard addition method. Two additions are used for each

sample. A mixture of usually Ga, In and Bi are used for internal standardization in the multielement method in which this element is an analyte.

4. Quality control

The method is accredited by the Danish Accreditation Organisation, DANAK. A matching CRM and blanks are analyzed in parallel with the samples in series containing up to 15 unknown samples. The results of CRMs are monitored on control charts (x- and R-charts). The risk of contamination of samples must always be considered. The critical utensils are washed/rinsed with acid before use and ultrapure acids are used for the ashing process. In this laboratory we continuously detect procedural blank signals for 208Pb of typically 1000-2000 cps. This reflects that Pb, in spite of its absence in modern gasoline, remains a problem as an environmental contaminant. This calls for extra care in reduction of the sources of this contaminant.

5. Difficulties and limitations

If there are differences in the isotopic composition between standards and samples, Pb should be measured using the sum of the isotopes, *i.e.*, $^{204+206+207+208}$Pb.

6. Comments and remarks

None reported.

Food Safety Veterinary and Food Laboratory

Tartu, Estonia

Lead

1. The equipment in use

1) Atomic Absorption Spectrophotometer-Thermo Elemental. It has equipped both with GFA and flame compartments. Zeemann and Deuterium correction are available. At the moment the Hydride Vapor System –for the determination of Arsenic is missing.
2) Atomic Absorption Spectrophotometer-Shimadzu - It has equipped both with GFA and flame compartments. Only deuterium correction is available.
3) Microwave owen AntonPaar- closed vessels for wet digestion
4) Muffle furnace Nabertherm

2. Analytical Methods

Element	**Pb**
Accreditation	**yes**
Reference for method	1) AOAC method 999.11 Determination of Lead, Cadmium, Iron and Zinc in Foods. AAS after dry ashing First action 1999. 2) AA Spectrometers Methods. Manual, 2003, Thermo Electron Corp., UK. 3) EVS-EN 13804:2002 –Foodstuffs-Determination of Trace elements-Performance Criteria and general conciderations. 4) EVS-EN 13805: 2002 Foodstuffs-Determination of trace elements. Pressure digestion.
Sample weight (g)	5 g -dry ashing > 1g- mw digestion
Sample preparation	Dry ashing Microwave digestion
Reagents use	HNO_3, H_2O_2, HCl
Modifier	Pd/Mg
Quantification (technique)	AAS Graphite Furnace
Wavelenght(nm)	283.3
LOQ (mg/kg)	0.01
U %	30

3. Reference materials

Element	**Matrics**	**Ref. value mg/kg**
Pb	BCR 151	2.002
	SRM 2976	1.19
	BCR 150 milk powder	1.00
	BCR 186	0.306

National Veterinary and Food Research Institute

Helsinki, Finland

Lead

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissues (meat, liver and kidney), milk and honey.

2. Samples throughput per year

About 300-500.

3. Procedure

The samples are analyzed by Z-ETA-AAS.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Sample (5-10 g) is ashed at 450 °C in a muffle furnace overnight. Ashed sample is cooled down to room temperature, deionized water and 1 ml conc. HNO_3 are added. The sample is re-evaporated and heated up to 450 °C overnight in the muffle furnace. The residue is dissolved in 0.5 ml of conc. HNO_3 and adjusted to appropriate volume with deionized water.

3.1.b. Analytical instrumentation

A Perkin-Elmer 5100 PC Atomic Absorption Spectrometer with Zeeman background corrector and hollow cathode lamp is used.

3.1.c. Working conditions

The instrumental parameters adopted are reported in Table 1. Measurements are carried out by direct comparison with standard solution (Reagecon) in 0.1 M HNO_3.

Table 1. Equipment characteristics.

ETA-AAS
Wavelength, 283.3 nm
Lamp, Pb HCL
Zeeman background correction

More frequently encountered concentration ranges are: < 0.01-0.02 mg/kg (meat, milk) and 0.01-0.10 mg/kg (liver, kidney).

4. Quality control

The method is accreditated. CRMs and blanks are analyzed together with each sample series. The results of CRMs samples are monitored on control charts. Analysis of spiked samples are performed. The risk of contamination of samples must always be considered. All glass- and plasticware are washed with acid before use.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Agence Française de Sécurité Sanitaire des Aliments

Maisons-Alfort Cedex, France

Lead

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions
3.2. Flame Atomic Absorption Spectrometry
3.2.a. Sample pretreatment
3.2.b. Analytical instrumentation
3.2.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissues (muscle, liver, kidneys).

2. Samples throughput per year

Not foreseeable.

3. Procedure

Lead is assayed by ETA-AAS and F-AAS.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Mineralization of animal tissues is carried out by dry ashing at 700 °C (50 °C/h) for at least 5 h. H_2SO_4 is added as an ashing aid. Ashes are dissolved in 0.4 % aqueous HNO_3 in 50 ml of distilled water.

3.1.b. Analytical instrumentation

A Perkin-Elmer 4100 ZL spectrometer is used.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 1 and 2. Use is made of 0.05 % $Mg(NO_3)_2$ and 1 % $NH_4H_2PO_4$ as matrix modifiers.

Table 1. Equipment characteristics.

ETA-AAS
Wavelength, 283.3 nm
Slit wisth, 0.5 nm
Lamp, Pb HCL
Zeeman background correction
Pyrolytic coated platform graphite tubes
Measurement mode, peak area

Table 2. Graphite furnace programme.

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)	Ar Flow (ml/min)
1	130	30	10	250
2	250	20	5	250
3	600	30	5	250
4	1400	0	2	0
5	2400	2	2	250

3.2. Flame Atomic Absorption Spectrometry

3.2.a. Sample pretreatment

Mineralization of animal tissues is carried out by dry ashing at 700 °C (50 °C/h) for at least 5 h. H_2SO_4 is added as an ashing aid. Ashes are dissolved in 0.4 % aqueous HNO_3 in 50 ml of distilled water.

3.2.b. Analytical instrumentation

A Varian/SpectrAA220 FS spectrometer equipped with an air-acetylene flame burner and a deuterium background corrector is used.

3.2.c. Working conditions

Instrumental parameters are reported in Table 3.

Table 3. Equipment characteristics.

F-AAS
Wavelength, 283.3 nm
Lamp, Pb HCL
Deuterium background correction
Air-acetylene flame

4. Quality control

Blanks determined out with each batch of samples and CRMs (CRM 278R, mussel tissue; CRM 184, bovine muscle; CRM 185, bovine liver; CRM 186, pig kidney; MA-B-3/TM, fish tissue and MA-A-1/TM, copepod homogenate and spike) are periodically analyzed.

5. Difficulties and limitations

Possibility of contamination phenomena.

6. Comments and remarks

Not reported.

Federal Office for Consumer Protection and Food Safety

Berlin, Germany

Lead

Section I

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Zeeman Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

3.1.b. Analytical instrumentation

3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

Section II

Sample ashing procedure

1. Matrices

2. Procedure

1. Matrices

Samples analyzed are meat, meat products, fish, fish products; plant materials, food

2. Sample throughput per year

About 500.

3. Procedures

Lead is assayed by Zeeman - ETA - AAS

3.1. Zeeman Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

See separate section II: (Sample ashing procedure)

3.1.b. Analytical instrumentation

A SpectrAA 400 Zeeman - AAS with Autosampler GTA 96 (VARIAN) is used

3.1.c. Working conditions

Instrumental parameters are reported in Table 1 and 2. The modifier used is a freshly prepared (1+1)-mixture of two solutions: (I) 0.5 ml of Pd-modifier + 9.5 ml HCl, 4 mol/l; (II) ascorbic acid, 10 g/l in water

Table 1. Equipment characteristics:

ETA-AAS
Zeeman background correction
Pyrolytic coated tubes without platform
Injection volume: 20 µl + 5 µl modifier

Table 2. Graphite furnace programme

Step	Temperature (°C)	Time (s)	Gas Flow (L/min)
1	95	3	3
2	95	20	3
3	120	15	3
4	700	2	3
5	700	10	3
6	700	2	0
7	2200	1	0
8	2200	2	0
9	2400	2	3

The calibration curve is plotted by using Lead solutions of 2, 4, 8 and 16 ng/ml in 8 % HNO_3. The analytical blank is a solution of 8 % HNO3. Limit of detection is 1.0 ng/ml in sample solution.

4. Quality control

Analysis of certified reference materials (BCR, NIST, CBNM) is performed along with each analytical series.

5. Difficulties and limitations

Not reported.

6. Comments

These method corresponds to the official German Method for food analysis.

Section II

Sample ashing procedure

1. Matrices

All materials will be mineralized following the same procedure.

2. Procedure

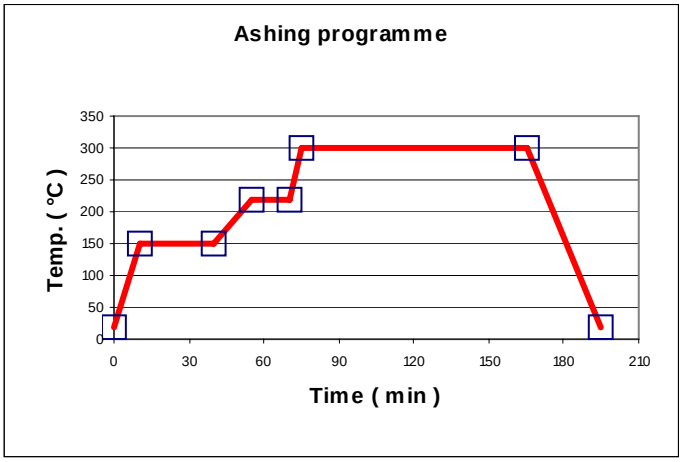
A high pressure ashing system HPA-S equipped with five 90 ml quartz vessels is used. Concentrated HNO_3, 65 % (FLUKA, pA plus) is used. Between 300 mg and 1500 mg of homogenized sample material are weighed into the quartz vessels. Sample amounts are illustrated in Table 3.

Table 3. Sample amounts for use in the HPA-S

Sample	Weight (mg)	Tolerance (mg)	Volume of HNO_3 (ml)
dry samples (< 20 % water)	500	± 50	3.0
fresh samples	1000	± 100	3.0
fresh samples (> 50 % water)	1500	± 200	3.0

Dry samples should be wetted with some drops water before HNO_3 is added; it's to recommend to let stand the vessels over night before heating. The mineralization start using a ashing temperature of 300 °C over a hold time of 90 minutes (Fig. 1). When ashing procedure is finished, let cool down to room temperature. Check the volume of HNO_3 in the vessels to recognize leaky vessels. Remove dissolved NO_x gases by a gentle stream of nitrogen at the surface of the sample solution. Add water up to 20 ml or 25 ml and store solutions in polyethylene tubes with screw cap for the AAS analysis.

Fig. 1: Ashing programme of the HPA-S



Institute of Food Hygiene of Athens

Athens, Greece

Lead

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are foods of animal origin (fish meat and fishery products, meat, liver, kidney, milk and honey).

2. Samples throughput per year

Not foreseeable.

3. Procedure

Lead is assayed by Z-ETA-AAS.

3.1.a. Sample pretreatment

Approximately 100 g of samples (fish, meat, liver or kidney) are homogenized and 0.5 g from the homogenized sample is digested with HNO_3 (10 ml for fish and 5 ml for meat, kidney, liver) in HP500 vessels. The digestion program is presented in Table 1.

Table 1. Digestion program for meat, liver and kidney

Step	Power(W)	Ramp Time(s)	Pressure (psi)	Temperature (^oC)	Hold Time (s)
1	600	20:00	170	200	5:00

Approximately 2g of milk is digested with HNO_3:H_2O_2 4:1. the digestion program is presented in Table 2.

Table 2. Digestion program for milk

Step	Power(W)	Ramp Time(s)	Pressure (psi)	Temperature (^oC)	Hold Time (s)
1	600	30:00	190	200	5:00

3.1.b. Analytical instrumentation

A Perkin-Elmer AAS instrument model 4110 with Zeeman background correction is used.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 2 and 3. A modifier of 0.005 mg Pd + 0.003 mg $Mg(NO_3)_2$ is used.

Table 3. Equipment characteristics

Wavelength, 283.3 nm Slit width,0.7nm Lamp, Pb EDL Zeeman background correction Graphite tubes: End caped-THGA

Table 4. Graphite furnace program

Step	Temperature(^{o}C)	Ramp Time (s)	Hold time (s)	Internal Flow	Read
1	110	5	20	250	
2	130	15	20	250	
3	950	10	20	250	
4	1900	0	4	0	x
5	2500	1	3	250	

Calibration curve is plotted by using Pb solution 5.0, 10.0, 20.0, 30.0, 40.0 and 50.0 ng/ml in 1% HNO_3.

4. Quality control

An analytical run includes 1 CRM or in house RM and unknown samples of similar composition. For quality control, BCR 184, BCR 186, BCR 278R, BCR 422 and BCR 151 are used, depending on method.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Central Meat Control Laboratory

Celbridge, County Kildare, Ireland

Lead

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

3.1.b. Analytical instrumentation

3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are muscle, kidney and liver tissue.

2. Samples throughput per year

Not repoted.

3. Procedure

Lead is assayed by Z-ETA-AAS.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

1 g of animal tissue is weighed into a digestion vessel. 4 ml of $HNO_3$are added and the solution is allowed to stand in fume hood for at least one hour. A spike control is prepared with 100 ppb of Pb and 1 ml of H_2O (Analytical grade water from Millipore Q system) is added to all samples subsequently. The samples are heated in a Microwave digestion system, MDS 2000 (CEM), following the digestion programme outlined in Table 1.

Table 1. Standard digestion programme.

Stage	Power	Pressure	Run Time	Run Time at press	Fan Speed
1	90 %	50	30.00	5.00	100 %
2	90 %	100	20.00	5.00	100 %
3	90 %	150	20.00	5.00	100 %
4	0 %	0	20.00	5.00	100 %
5	90 %	150	20.00	5.00	100 %

On completion the digest sequence, the vessels are allowed to cool and the pressure to drop below 40 psi before removing from the oven to the fume hood. Using a capillary pipette the sample is purged with a steam of nitrogen for one minute to evolve any dissolved gases.

3.1.b. Analytical instrumentation

Lead analysis is carried out on Spectra AA-220Z Atomic Absorption Spectrophotometer with the Varian GTA 110Z Graphite Tube Furnace with
autosampler.

3.1.c. Working conditions

The modifier used is a Ammonium dihydrogen orthophosphate (0.2 %) with Magnesium nitrate (0.2 %). Instrumental parameters are reported in Table 2 and Table 3.

Table 2. Equipment characteristics.

ETA-AAS
Wavelength, 283.3 nm
Slit width, 0.5 nm
Slit Height, normal
Lamp, Pb HCL
Calibration Mode, concentration
Measurement Mode, peak height
Sampling Mode, automix
Replicates All, 3

Standards made up with 5 % HNO_3. The samples are diluted to 1:50 with H_2O.

Table 3. Furnace programme.

Step	**Temperature (°C)**	**Time (s)**	**Gas Flow (L/min)**
1	85	5	3
2	95	40	3
3	120	10	3
4	400	5	3
5	400	1	3
6	400	2	0
7	2100	0.8	0
8	2100	2	0
9	2300	1	3
10	2300	3	3
11	2300	3	3

4. Quality control

Reagent blanks, check and spiked samples are incorporated into each assay.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Final results are calculated, only if an acceptable standard curve has been achieved and takes into account sample weights and dilution factors.

National Institute of Health
Istituto Superiore di Sanità

Rome, Italy

Lead

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions
3.2. Inductively Coupled Plasma Atomic Emission Spectrometry
3.2.a. Sample pretreatment
3.2.b. Analytical instrumentation
3.2.c. Working conditions
3.3. Inductively Coupled Plasma Mass Spectrometry
3.3.a. Sample pretreatment
3.3.b. Analytical instrumentation
3.3.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are milk, canned vegetables, fruit juices, wine and spirits.

2. Samples throughput per year

Not foreseeable.

3. Procedure

Three different techniques are used for the determination of Pb in the samples.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

The sample is homogenized, weighed and digested at high pressure in a microwave oven. Usually a 5:2 HNO_3-H_2O_2 solution is employed. Use is made of KH_2PO_4 and $Mg(NO_3)_2 \cdot 6H_2O$ as matrix modifiers.

3.1.b. Analytical instrumentation

A Perkin-Elmer Model 5100 AAS with Zeeman background correction, equipped with a HGA-600 graphite furnace and a AS-60 autosampler, is used.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 1 and 2.

Table 1. Equipment characteristics.

ETA-AAS
Wavelength, 283.3 nm
Slit width, 0.7 nm
Lamp, Pb EDL and HCL
Zeeman background correction
L'vov platform
Injection volume, 20 µl

Table 2. Graphite furnace programme.

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)	Ar Flow (ml/min)
Drying	130	10	20	300
Charring	450	15	20	300
Atomization	1800	0	3	0
Clean-up	2500	2	2	300

Calibration curve is performed by the standard addition method.

3.2. Inductively Coupled Plasma Atomic Emission Spectrometry

3.2.a. Sample pretreatment

The sample is homogenized, weighed and digested at high pressure in a microwave oven. Usually an HNO_3-H_2O_2 solution is used.

3.2.b. Analytical instrumentation

A Jobin-Yvon 38 VHR spectrometer equipped with a U-5000 AT CETAC ultrasonic nebulizer is used.

3.2.c. Working conditions

Instrumental parameters are reported in Table 3.

Table 3. Equipment characteristics.

ICP-AES
Wavelength, 220.4 nm
Torch, INSA, demountable
Argon flows, plasma 16 l/min, auxiliary 0.3 l/min, aerosol 0.5 l/min
RF generator, Durr-JY 3848, frequency 56 MHz,
Maximum power output 2.2 kW
Nebulizer, ultrasonic, U-5000 AT, CETAC
Monochromator, HR 1000-M, focal length 1 m, Czerny-Turner mounting, equipped with a 3600 grooves/mm holographic plane grating, linear dispersion in the first order of 0.27 nm/mm, spectral range of 170-450 nm
Entrance and exit slit widths of 40 µm

Calibration is carried out by using various Pb solutions depending on the Pb content in the sample.

3.3. Inductively Coupled Plasma Mass Spectrometry

3.3.a. Sample pretreatment

The sample is homogenized, weighed and digested at high pressure in a microwave oven. Usually an HNO_3-H_2O_2 solution is used.

3.3.b. Analytical instrumentation

A Perkin-Elmer/Sciex Elan 6000 quadrupole mass spectrometer is used.

3.3.c. Working conditions

Instrumental parameters are reported in Table 4.

Table 4. Equipment characteristics.

ICP-MS
Analytical mass, ^{208}Pb
Reference mass ^{103}Rh
Nebulizer, cross flow, Ryton spray chamber
Argon flows, plasma 16 l/min, auxiliary 0.9 l/min, aerosol 1 l/min
RF generator, maximum power output 1.0 kW
Scanning conditions: sweeps per reading, 5, readings per replicate, 4; number of replicates, 5; peak hopping, dwell time, 100 ms, replicate time, 2000 ms, normal resolution, 0.9-0.6 amu

Calibration is carried out by using various Pb solutions depending on the Pb content in the sample.

4. Quality control

An analytical run includes 1 blank, 1 CRM and unknown samples of similar composition. The accuracy of the method is estimated by means of CRMs (CRM 184, bovine muscle; CRM 185, bovine liver; CRM 186, pig kidney; CRM 150, skim milk). The calibration curves are accepted when the coefficient of correlation (r) is > 0.999.

5. Difficulties and limitations

For a correct measurement of Pb content in the samples, the sum of the four isotopes (*m/z* = 204, 208, 207 and 206) has to be used.

6. Comments and remarks

Not reported.

Agriculture and Food Science Centre

Belfast, Northern Ireland

Lead

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Flame Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions
3.2. Electrothermal Atomization Atomic Absorption Spectrometry
3.2.a. Sample pretreatment
3.2.b. Analytical instrumentation
3.2.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples most frequently analyzed are animal products such as kidney and liver, milk and milk products or samples of marine origin, such as fish, oysters, mussels and sediments.

2. Samples throughput per year

About 500 - 600 samples per year.

3. Procedure

Lead is analyzed by two different instrumental techniques depending upon the concentration of the element in the sample. Flame atomic absorption spectrometry is the general method of analysis with electrothermal atomization absorption spectrometry being used to a lesser extent for lower concentrations of elements.

3.1. Flame Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Samples are dry-ashed in a muffle furnace which is temperature programmed from room temperature to 450 °C. The ash is dissolved in 0.1 *N* HNO_3.

3.1.b. Analytical instrumentation

A Varian SpectrAA-400 Atomic Absorption Spectrometer with deuterium lamp background correction is used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1.

Table 1. Equipment characteristics.

F-AAS
Flame, air-acetylene
Wavelength, 217.0 nm
Slit width, 1 nm
Deuterium background correction

A calibration curve is established out by using Pb standard solutions of 0, 0.2, 0.4, 0.6, 0.8 and 1.0 µg/g in 0.1 *N* HNO_3.

3.2. Electrothermal Atomization Atomic Absorption Spectrometry

3.2.a. Sample pretreatment

Samples are dry-ashed in a muffle furnace which is temperature programmed from room temperature to 450 ^{o}C. The ash is dissolved in 0.1 *N* HNO_3.

3.2.b. Analytical instrumentation

A Varian SpectrAA-300 Atomic Absorption Spectrometer with Zeeman background correction, graphite furnace and autosampler is used.

3.2.c. Working conditions

Instrumental parameters are reported in Tables 2 and 3.

Table 2. Equipment characteristics.

ETA-AAS
Wavelength, 283.3 nm
Slit width, 0.5 nm
Zeeman background correction
Injection volume, 10 µl sample

Table 3. Graphite furnace programme.

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)	Ar Flow (ml/mim)
Drying	95 120	5 10	40 10	300 300
Charring	1000	10	8	300
Atomization	2200	0	0.6	0
Clean-up	2400	1	2	300

A calibration curve is established using Pb standard solutions of 0.02, 0.04 and 0.06, 0.08 and 0.10 µg/g in 0.1 *N* HNO_3.

4. Quality control

The laboratory quality system is based on GLP and UKAS principles. Method validation is performed by using CRM's or spiked samples as appropriate. CRM's are analyzed periodically to check accuracy. During routine analysis, triplicate spiked samples or CRM's are included with each batch of samples analyzed, along with three reagent blanks to control potential contamination. The precision and accuracy is monitored on quality control charts using recovery results.

Instruments are optimized before each batch of analyses, and details recorded of lamp photomultiplier voltage and absorbance for a given standard. Plot linearity is also checked. The laboratory also participates in proficiency testing exercises, such as the UK Food Analysis Performance Assessment Scheme (FAPAS).

5. Difficulties and limitations

The main problem is potential contamination, and purity of chemicals. Dry-ashing requires careful control. The temperature should be kept as low as is possible because of the danger of loosing volatile inorganic compounds, but at the same time high enough to ensure complete combustion of all the organic matter in a reasonably short time. Fatty samples may require additional treatment and precautions when dry-ashing.

6. Comments and remarks

Not reported.

Istituto de Investigação das Pescas e do Mar

Lisboa, Portugal

Lead

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissue (liver), milk and honey

2. Samples throughput per year

About 200 samples per year.

3. Procedure

Lead is assayed by Z-ETA-AAS.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Liver

Approximately 100 g of samples are homogenized, 1 g is weighed in quartz crucibles and 0.5 ml of 50 % $Mg(NO_3)_2$ are added. The sample is pretreated in pre-ashing apparatus until completely charred, followed by ashing in a muffle furnace at 450 °C during 1 h. The samples are than removed and wet with concentrated HNO_3 and put back to the furnace. This procedure is repeated every hour until ashes become white. The ashes are then quantitatively transferred to 10 ml volumetric flasks with 5 % HNO_3.

Or

Subsamples of 0.500 g were weighted into the MW teflon vessel and digested with 4ml of 65% nitric acid and 1ml of 30% hydrogen peroxide. acid using the microwave program shown in the table 1.

Table 1: Operative conditions for the microwave oven digestion

Step	Temperature (ºC)	Power (W)	Time (min.)
1	85	700	2.00
2	145	500	5.00
3	210	1000	3.00
4	210	1000	10.00

After digestion samples were cooled at room temperature and made up to 10ml with high purity deionized water in volumetric glass flasks.

Milk

2 ml of homogenised milk was measured into the MW Teflon vessels. Each sample was digested in 2 ml of concentrated nitric acid using the microwave program shown in the table 2.

Table 2: Operative conditions for the microwave oven digestion

Step	Temperature (ºC)	Power (W)	Time (min.)
1	85	500	3.00
2	85	0	1.00
3	145	500	5.00
4	145	1000	8.00

After digestion samples were cooled at room temperature and made up to 10 ml with high purity deionized water in volumetric glass flasks.

Honey

Ref.: S. Caroli, G Forte, A. Iamiceli, *Talanta* (1999) 50:627-336.

3.1.b. Analytical instrumentation

A Perkin Elmer Atomic Absorption Spectrometer Aanalyst 600 with THGA Graphite Furnace including AS- 800 Autosampler.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 3 and 4. Use is made of 0.2 mg $(NH_4)H_2PO_4$ as matrix modifier.

Table 3. Equipment characteristics.

Z-ETA-AAS
Wavelength, 283.3 nm
Lamp, Pb HCL
Zeeman background correction
L'vov platform
Injection volume, 10 µl samples + 5 µl modifier
Pyrolytic coated graphite tubes

Table 4. Graphite furnace programme.

Step	**Temperature (°C)**	**Ramp Time (s)**	**Hold Time (s)**	**Internal Flow**
1	100	4	30	250
2	130	15	30	250
3	950	10	20	250
4	1400	0	5	0
5	2450	1	3	250

Calibration plots one set up by using Pb solutions of 12.5; 25; 50 and 100 ng/g.

4. Quality control

Each analytical run includes blanks, commercial standard solutions, CRMs (BCR 185-R, bovine liver and BCR 184, bovine muscle) and Quality Control Standards for Trace Metals – AA by RTC.

5. Difficulties and limitations

Possibilities of contamination.

6. Comments and remarks

Not reported.

Ministerio de Agricultura, Pesca y Alimentación

Madrid, Spain

Lead

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomization Atomic Absorption
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are, in the order, wine, spirits, vinegar, beer, orange juice, water, cookies, various kinds of pasta, canned vegetables, liver and kidneys.

2. Samples throughput per year

About 1200.

3. Procedure

Lead is assayed by ETA-AAS.

3.1. Electrothermal Atomization Atomic Absoption

3.1.a. Sample pretreatment

Sampling is not made by the Laboratory Staff, but by official inspectors. The techniques for storage and subsampling are different according to the product; *i.e.*,: milk and milk products, shake; meat, cut in small pieces; eggs, cook; fish, clean and cut; crustaceans, peal and cook. Afterwards homogenizing in kitchen grinder is performed. Dry ashing of samples is achieved in a muffle furnace with overnight digestion at 450 °C. Use is made of 6 % w/w $Mg(NO_3)_2$ as ashing aid. Ashes are dissolved in HNO_3.

3.1.b. Analytical instrumentation

A Perkin-Elmer Model 3030 Atomic Absorption Spectrometer with Zeeman background correction, equipped with the HGA-600 graphite furnace, AS-60 autosampler and PR-100 recorder, is used.

3.1.c. Working conditions

The instrumental parameters adopted are reported in Tables 1 and 2.

Table 1. Equipment characteristics.

ETA-AAS
Wavelength, 283.3 nm
Slit width, 0.7 nm
Lamp, Pb HCL
Zeeman background correction
L'vov platform
Injection volume, 10 µl

Table 2. Graphite furnace programme.

Step	**Temperature (°C)**	**Ramp Time (s)**	**Hold Time (s)**	**Ar Flow (ml/min)**
Drying	110	20	10	300
Charring	450	20	10	300
Atomization	2300	1	5	0
Clean-up	2500	1	3	300

Calibration curve is plotted by using Pb solutions of 5, 25, 50 and 80 ng/ml.

4. Quality control

The routine use of each method is preceded by analytical validation according to the IUPAC recommendations in order to ascertain sensitivity, detection power, precision and accuracy. CRMs of bovine liver (NIST) and mussel tissue (SMT) are used to check the accuracy and analyzed periodically. During routine assays, each analytical batch includes at least three reagent blanks to evaluate the detection limits and to control possible contaminations. Recoveries are calculated using two spiked reagent blanks and one spiked sample for each non-spiked sample assayed.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Non reported.

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Inductively Coupled Plasma Mass Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analysed are, in the order, wine, spirits, vinegar, beer, orange juice, cookies, various kinds of pasta, canned vegetables, liver and Kidneys.

2. Samples throughput per year

About 1200 per year.

3. Procedure

Pb is assayed by ICP-MS.

3.1. Inductively Coupled Plasma Mass Spectrometry

3.1.a. Sample pretreatment

Sampling is not made by the Laboratory Staff, but by official inspectors. The techniques for storage and subsampling are different according to the product; i.e.,: milk and milk products, shake; meat, cut in small pieces; eggs, cook; fish, clean and cut; crustaceans, peal and cook. Afterwards homogenising in Kitchen grinder is performed. Samples are digested in a microwave oven. For 0.5 to1g homogeneized samples 2 ml concentrated HNO_3 and 8 ml H_2O are used. Microwave parameters are reported in the following table.

Stage	Power		Ramp Time	P(PSI)	ºC	Time
1	MAX	%	20 min	300	200	10 min
	600w	100				

After the digestion the samples are dissolved in deionized water in order to achieve a final concentration of 3% of nitric acid.

3.1.b. Analytical instrumentation

The multielemental analysis is performed in a Perkin Elmer Elan 9000 Inductively Coupled Plasma Mass Spectrometer, with cross flow nebulizer, nickel cones and a autosampler placed inside laminar flow cab.

3.1.c. Working conditions

The instrumental parameters adopted are reported in Tables 1 and 2.

Table 1: ICP Conditions

Plasma power (W)	1100
Argon flow nebulizer (L/min)	0.78 - 0.96
Sample flow (ml/min)	1 ml / min

Table 2:Mass spectrometer conditions

	Quantitative mode	Semiquantitative mode
Vapour pressure(Torr)	7×10^{-7}	7×10^{-7}
Dwell Time (ms)	50	50
sweeps / readings	25	6
Readings / Replicates	1	1
Number of replicates	3	1
Reading mode	Peak Hopping	Peak Hopping

Calibration curve is plotted by using Pb solutions of 0.024, 0.24, 1.5, 3, 6 and 30 ug/l.
Analytical mass: Pb 206+ Pb 207 + Pb208.

4. Quality control

The routine use of each method is precede by analytical validation according to the IUPAC recommendations in order to ascertain sensitivity, detection power, precision and accuracy. CMRs of bovine liver (SRM 1577b NIST), DORM-2 (Dogfish muscle certified material for trace metals), Spinach Leaves (SRM 1570a) and Corn Bran (8433 NIST) are used to check the accuracy and analysed periodically. During routine assays, each analytical batch includes at least three reagents blanks to evaluate the detection limits and to control possible contamination. Recoveries are calculated using two spiked reagent blanks and one spiked sample for each non spiked sample assayed. At the beginning and at the end of each assay and every five samples two quality controls are analysed. One with a concentration of lead similar to the fifth standard of the calibration curve and the other with a concentration of lead similar to the second standard.

5. Difficulties and limitations

No reported.

6. Comments and remarks

No reported.

National Food Administration

Uppsala, Sweden

Lead

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissues, cereals and vegetables.

2. Samples throughput per year

About 400-600 samples per year.

3. Procedure

Lead is determined by ETA-AAS after either dry ashing or microwave digestion.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

a) About 1-10 g of sample is weighed into a platinum crucible and dry ashed at a temperature of 450±25 °C in a programmable furnace. The carbon-free ash is dissolved in 20-30 ml of 0.1 M HNO_3 and transferred to a plastic container.

b) A maximum of 0.5 g dried, homogenized sample is weighed into a Teflon vessel, followed by the addition of 5 ml conc HNO_3 and 2 ml 30% H_2O_2. The digestion is carried out in a CEM (MDS 2000) microwave oven according to the programme detailed in Table 1.

Table1. Digestion programme.

Step	Power (W)	Time (min)
1	250	3
2	630	5
3	500	22
4	0	15

3.1.b. Analytical instrumentation

Perkin Elmer Z-5100 with HGA-600 autosampler AS-60.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 2 and 3 for the PE Z-5100 instrument. Matrix modifiers are generally not used.

Table 2. Equipment characteristics.

ETA-AAS
Wavelength, 283.3 nm
Slit width, 0.7 low
Lamp, Pb HCL
Zeeman background correction
L'vov platform
Injection volume, 10 µl sample
Pyrolytic coated graphite tubes

Table 3. Graphite furnace programme.

Step	**Ramp Time (s)**	**Hold Time (s)**	**Temperature (°C)**
Injection	-	-	Ambient
Drying	10	30	130
Pyrolysis	5	10	500
Atomization*	0	2	1900
Clean out	2	2	2500

* Gas stop

Calibrations are generally carried out by standard addition method.

4. Quality control

An analytical run includes one-two blanks and one or more CRM or in-house RM. Samples are measured as singles or duplicates, depending on the requirements. Accuracy and precision are monitored by the use of control charts on which the results of duplicate CRM and RM analysis are plotted. The detection limit is based on three times the standard deviation of large number of blanks and is currently 0.9 ng/ml in sample solution.

5. Difficulties and limitations

Extremely fatty or sugar rich samples require extra attention when dry ashing is used. The microwave digestion method is not suitable for fats, oils or extremely fatty products.

6. Comments and remarks

The dry ashing method has been collaboratively tried and was approved by the Nordic Committee and Food Analysis (NMKL) in 1990. The report was published in *JAOAC* Int. 76 (1993), no. 4. (Lars Jorhem, Determination of metals in foodstuffs by AA Spectrophotometry after dry ashing: An NMKL collaborative study of Cd, Cr, Cu, Fe, Ni, Pb and Zn.). This method was validated on January 1st 1996. The microwave digestion method has been collaboratively tried and was approved by NMKL in 1997. The result of both collaboratively trials has been submitted to the AOAC Methods Committee and was accepted in December 1999.

National Institute for Public Health and Environment
Rijksinstituut voor Volksgezondheid en Milieu

Bilthoven, The Netherlands

Lead

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Inductively Couples Plasma Mass Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analysed are meat, rain water, ground water, urine, animal feed, soil and aerosol particles.

2. Samples throughput per year

About 3000.

3. Procedure

Lead is analysed by ICP-MS.

3.1. Inductively Coupled Plasma Mass Spectrometry

3.1.a. Sample pretreatment

Solid matrices are digested in a microwave oven. For 0.5 g of homogenised biological sample 1 ml of concentrated HNO_3 and 9 ml H_2O is used.
For soil samples (particle size < 250µm) an aqua regia digestion is performed.

3.1.b. Analytical instrumentation

An HP4500 equipped with the quadrupole ICP-MS is used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1.

Table 1. **ICP-MS HP4500+** Equipment characteristics.

Analytical mass, ^{206}Pb, ^{207}Pb, ^{208}Pb
Reference mass, ^{209}Bi (added on-line (dilution sample/IS: 1: 5.5))
Nebulizer, V-groove, Scott-type double pass spraychamber
Argon flow, nebulizer 1.17 l/min, R.f. power 1250W
Measuring conditions: 3 points / mass; integration time 0.09 s / mass, 3 replicates
Two-point calibration, standards concentration, 0 and 20 µg/l
Matrix matching for calibration standards
Equation, $^{206}Pb + ^{207}Pb + ^{208}Pb$

4. Quality control

Each analytical run includes 1 blank, 1 CRM and 1 RM. The instrumental conditions are controlled by the analysis of an in-house water reference material. The accuracy of the analytical procedure, including sample digestion, is estimated by the use of CRMs. A number of matrix samples are analyzed and recovery experiments are carried out for Pb.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Central Science Laboratory

York, United Kingdom

Lead

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Inductively Coupled Plasma Mass Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are foods and beverages.

2. Samples throughput per year

Up to 1500.

3. Procedure

Lead is assayed by ICP-MS.

3.1. Inductively Coupled Plasma Mass Spectrometry

3.1.a. Sample pretreatment

Aliquots of each sample are homogenized and portions (equivalent to 0.5 g dry weight) are weighed out into microwave digestion vessels. Concentrated HNO_3 (5 ml) is added and the samples are heated in a proprietary high pressure microwave digestion oven. The acid is transferred into a polycarbonate test tube with 18 MΩ cm^{-1} water and made up to
10 ml in volume.

3.1.b. Analytical instrumentation

A VG Instruments Plasma Quad PQII+ Turbo quadrupole ICP-MS or Perkin Elmer ELAN 6000 quadrupole ICP-MS are used.

3.1.c. Working conditions

At least 7 calibration standards are used to cover the calibration range 0.1 µg/l to 100 µg/l. ^{208}Pb is monitored with In or Rh being used as an internal standard. Element concentrations in test solutions are determined by manufacturers software and processed further using an in-house written SuperCalc 5 macro. Automatic spike recovery and blank corrections are made during these calculations.

4. Quality control

All test batches contain reagent blanks, spiked blanks and CRMs and are performed according to UKAS accredited standards.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Manganese

ESTONIA

Food Safety Veterinary and Food Laboratory

Tartu, Estonia

Manganese

1. The equipment in use

1) Atomic Absorption Spectrophotometer-Thermo Elemental. It has equipped both with GFA and flame compartments. Zeemann and Deuterium correction are available. Atomic Absorption Spectrophotometer-Shimadzu - It has equipped both with GFA and flame compartments. Only deuterium correction is available.
2) Microwave owen AntonPaar- closed vessels for wet digestion
3) Muffle furnace Nabertherm

2. Analytical Method

Element	**Mn**
Accreditation	**yes**
Reference for method	1) AOAC method 999.11 Determination of Lead, Cadmium, Iron and Zinc in Foods. AAS after dry ashing First action 1999 2) AA Spectrometers Methods Manual, 2003, Thermo Electron Corp., UK 3) EVS-EN 13804:2002 –Foodstuffs-Determination of Trace elements-Performance Criteria and general conciderations 4) EVS-EN 13805:2002 Foodstuffs-Determination of trace elements. Pressure digestion 5) Nordic Commitee of Food Analyses no 161, 1998. Metals. Determination by AAS after wet digestion in microwave owen.
Sample weight	5 dry ashing >1 mw dig.
Sample preparation	Dry ashing Microwave digestion
Reagents used	HNO_3, H_2O_2, HCl
Quantification (technique)	AAS Graphite Furnace AAS flame
Wavelenght(nm)	279.5
LOQ	0.005 GFA 0.2 flame
U %	15 GFA 15 flame

Mercury

AUSTRIA
BELGIUM
CZECH REPUBLIC
CYPRUS (AOAC 974.14 and EN 13806)*
DENMARK
ESTONIA
FINLAND
FRANCE
GERMANY
GREECE
HUNGARY
IRELAND
ITALY
LATVIA (AOAC 971.21)*
MALTA
NORTHERN IRELAND
POLAND
PORTUGAL
SPAIN
THE NETHERLANDS
UNITED KINGDOM

**Official analytical methods are not reported*

Austrian Agency for Health and Food Safety

Wien, Austria

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Cold Vapour Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples most frequently analyzed are kidneys from cattle.

2. Samples throughput per year

100-200.

3. Procedure

Mercury is assayed by CV-AAS.

3.1. Cold Vapour Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

2 ± 0.1 g of homogenized tissue is weighed in 40ml Teflon vessels. 3 ml HNO_3 (65 %, suprapur) and 1.5ml H_2O_2 (30 %) are added. The samples are digested in the microwave oven (MLS-Ethos 900) according to the following programme: 1. 100 W, 10 min; 2. 0 W, 1 min; 3. 250 W, 5 min; 4. 0 W, 1 min; 5. 400 W, 5 min; 6. 600 W, 10 min; 7. 500 W, 6 min. After cooling the digested sample solutions are transferred into 25 ml measuring flasks and filled up to 25 ml with deionized water.

3.1.b. Analytical instrumentation

A FIMS (Perkin-Elmer) is used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1.

Table 1. Equipment characteristics.

CV-AAS
Wavelength, 253.7 nm
Slit width, 0.7 nm
Injection volume, 500 µl sample/standard
Argon flow, 70 ml/min
Measurement mode, peak height
Smoothing points, 19
Read time, 15 s
Carrier solution, 0.37 % HCl
Reducing agent, 0.2 % $NaBH_4$ in 0.05 % NaOH

Table 2. Flow injection programme.

Step	Time (s)	Pump 1 Speed	Pump 2 Speed	Valve Position	Read Step
Prefill	10	100	120	Fill	-
1	5	100	80	Fill	-
2	20	100	80	Inject	X
3	10	100	0	Inject	-

The calibration curve is established by manual dilution of a stock solution (usually to following concentrations: 0.5; 1.0; 1.5 and 2.0 µg/l). The blank is subtracted automatically from standards and samples.

4. Quality control

Calibration curves are accepted when the coefficient of correlation is > 0.995. The performance of the instrument is checked by the known solutions of the interlaboratory trial. Recovery is calculated by spiked samples. CRM 186, pig kidney is analyzed in order to check the accuracy and reproducibility of the whole method.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Scientific Institute of Public Health-Louis Pasteur

Brussels, Belgium

Mercury

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Cold Vapour Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are seafood and seafish.

2. Samples throughput per year

About 50 samples per year.

3. Procedure

Automated Mercury Analyser

3.1. Cold Vapour Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

no pretreatment necessary

3.1.b. Analytical instrumentation

AMA 254

3.1.c. Working conditions

Light source: low pressure mercury lamp.
Wavelength: 253.65 nm; Interference filter 254 nm, half-width 9 nm. Sample injection: 10 µl.
Drying time: 10s; Decomposition time: 120s; stabilization of temperature within the amalgamator: 30s.
Mercury is released from the amalgamator by a short heating up and transferred by carrier gas through the measuring cells. The same quantity of mercury is measured twice, using different sensitivity, resulting in a dynamic range of 0.05 – 600 ng of Hg.

4. Quality control

An analytical run includes blanks, standards, spike samples for recovery measurements and CRMs (BCR 422 and 278, tuna fish).

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

State Veterinary Institute Olomouc
Laboratory in Kromeriz

Kromeriz, Czech Republic

Mercury

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Analytical technique
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are meat, meat products, fish, fish products, milk, babyfood, feedstuffs.

2. Sample throughput per year

About 1000 samples per year.

3. Procedure

Mercury is assayed by CV-AAS using an Advanced Mercury Analyser.

3.1. Cold Vapor Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

The instrument is suitable for the direct analysis of solid and liquid samples without the need for any sample pretreatment.

3.1.b. Analytical instrumentation

An Advanced Mercury Analyser AMA 254 (made by ALTEC, Czech Republic, distributed by LECO) is used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1.

Table 1. Equipment characteristics

CV-AAS: AMA 254
Light source: low pressure mercury lamp
Wavelength: 253.65 nm
Interference filter: 254 nm
Half-width 9 nm

100-500 µl of liquid sample is injected or the homogenised solid sample is directly weighed (100 mg ± 0.1mg) into pre-cleaned combustion boat and automatically inserted into the AMA 254 analyser. The sample is dried at 120 ^{o}C for 60 s and thermally decomposed at 550 ^{o}C for 130 s under an oxygen flow. Decomposition products are carried by the oxygen flow to an Au-amalgamator. Selectively trapped

mercury is released from the amalgamator by a brief heat-up and finally quantified (measuring cycle, 60 s) as Hg^0 by cold-vapour AAS technique. The same quantity of mercury is measured twice, using different sensitivity, resulting in dynamic range of 0.05- 600 ng of Hg.

The calibration curve is established by using Hg solutions of 0.05, 0.1, 0.2, 0.3 and 0.4 ppm.

4. Quality control

The routine use of a method is preceded by analytical validation according to the IUPAC recommendations and the metod is accreditated.
CRMs (BCR 151, CRM 463, Tort-2, DORM 2…) are use to check the accuracy and analyzed periodically. An analytical run includes two blanks, duplicates and in-house RM. The results of duplicate CRM, RM analysis samples are monitored on control charts.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Danish Institute for Food and Veterinary Research

Søborg, Denmark

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Inductively Coupled Plasma Mass Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are fish, meat, flour, fruit and vegetables.

2. Samples throughput per year

About 10.

3. Procedure

The samples are analyzed by ICP-MS.

3.1. Inductively Coupled Plasma Mass Spectrometry

3.1.a. Sample pretreatment

The sample is homogenized in a blender equipped with titanium blades. A known amount of the homogenized sample (2-3 g wet weight) is weighed intothe Multiwave high pressure microwave digestion system. The Multiwave high pressure microwave digestion system is used for sample decomposition. The maximum pressure is 70 bar and the digestion is finalised within 60 minutes including cooling. Following digestion the residue is taken to 20 ml by weighing using pure water.

3.1.b. Analytical instrumentation

An Elan 6100 DRC Perkin-Elmer/Sciex quadrupole mass spectrometer is used.

3.1.c. Working conditions

A volume of 5 ml of the sample solution is pipetted into a polyethylene autosampler tube and 5 ml of 1 % aqueous HNO_3 and 10 µl of a 200 mg/l Au solution is additionally added as a matrix modifier to minimize adhesion of Hg to surfaces in the sample introduction system. The Hg ions are detected at m/z = 200 or 202. Calibration is carried out by the standard addition method. Two additions are used per sample. A mixture of usually Ga, In and Bi are used for internal standardization in the multielement method in which this element is an analyte.

4. Quality control

The method is accredited by the Danish Accreditation Organisation, DANAK. A matching CRM and blanks are analyzed in parallel with the samples in series containing up to 15 unknown samples. The results of CRMs are monitored on control charts (x- and R-charts). The risk of contamination of samples must always be considered. The critical utensils are washed/rinsed with acid before use and ultrapure acids are used for the ashing process.The calibration curves are accepted if the coefficient of correlation (r) is > 0.999.

5. Difficulties and limitations

Detection of Hg using ICP-MS can be hampered by memory effects. The use of a cyclonic spray chamber, the addition of Au^{3+} to the sample solutions and a 2 min. wash time between samples can eliminate the memory effects. If this process, in contrast to what is expected, is not effective (memory effects still prevail) this will be revealed by deviation from linearity of the standard additions calibration curve. In this case the time-resolved Hg intensity signal is studied and if indeed tailing of the signal occurs, then the analysis, including recording of new calibration curves, is repeated.

6. Comments and remarks

None reported.

Food Safety Veterinary and Food Laboratory

Tartu, Estonia

Mercury

1. The equipment in use

1) Atomic Absorption Spectrophotometer-Thermo Elemental. It has equipped both with GFA and flame compartments. Zeemann and Deuterium correction are available. At the moment the Hydride Vapor System –for the determination of Arsenic is missing.
2) Atomic Absorption Spectrophotometer-Shimadzu - It has equipped both with GFA and flame compartments. Only deuterium correction is available.
3) Fluorescense Mercury Analyzer-Analytic Jena. This instrument has an enrichment system (gold amalgam)
4) Microwave owen AntonPaar- closed vessels for wet digestion
5) Muffle furnace Nabertherm

2. Analytical Methods

Element	**Hg**
Accreditation	**yes**
Reference for method	1) EVS-EN 13804:2002 –Foodstuffs-Determination of Trace elements-Performance Criteria and general conciderations. 2) EVS-EN 13806:2002 Foodstuffs-Determination of mercury by cold vapour AAS (CVAAS) after pressure digestion. 3) EVS-EN 13805:2002 Foodstuffs-Determination of trace elements. Pressure digestion. 4) Mercury fuorescense analysator: Operating instructions, Analytic Yena AG, 2003.
Sample weight (g)	> 1g
Sample preparation	Microwave digestion
Reagents use	HNO_3, H_2O_2, HCl, $K_2Cr_2O_7$ $SnCl_2$
Modifier	
Quantification (technique)	CV AFS
Wavelenght(nm)	253.7
LOQ (mg/kg)	0.005
U %	30

3. Reference materials

Element	**Matrics**	**Ref. value mg/kg**
Hg	BCR 186—kidney	1.97
	CRM 422- cod mussel tissue	0.559
	BCR 151-milk powder	0.101

National Veterinary and Food Research Institute

Helsinki, Finland

Mercury

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Cold Vapour Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are fish meat and animal tissues (meat, liver and kidney).

2. Samples throughput per year

About 50.

3. Procedure

The samples are analyzed by CV-AAS.

3.1. Cold Vapour Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

1 g of homogenized sample is weighed into an Erlenmeyer and 1 ml of 10 *N* HCl and 5 ml of 14 M HNO_3 is added and mixed carefully. Then 2 ml of 18 M H_2SO_4 is added. The mixture is let to stand on the waterbath for 1.5 hours. The mixture is cooled down to room temperature and 20 ml of water and 20 ml of hexane is added and let to stand at room temperature for two hours. The hexane is extracted away. The Erlenmeyer is rinsed with water, 25 µl of 5 % $KMnO_4$ is added and solution is adjusted to 50 ml with water.

3.1.b. Analytical instrumentation

A Perkin-Elmer 4100 AAS instrument equipped with FIAS 200 is used. Amalgam system is used for samples of low concentration.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1.

Table 1. Equipment characteristics.

CV-AAS
Wavelength, 253.7 nm
Electrodeless discharge lamp
EDL System 2 power supply

Sample and standard solutions are treated with 5 % $KMnO_4$ to oxidize all the Hg to Hg (II). $NaBH_4$ is used as the reducing agent. The measurement is carried out with direct comparison with standard solution (Reagecon) in 0.1 M HNO_3. More frequently encountered concentration ranges: 0.10-1.00 mg/kg (fish, meat) and <0.005-0.010 mg/kg (animal tissue).

4. Quality control

The method is accredited. CRMs and blanks are analyzed together with each sample series. The results of CRMs samples are monitored on control charts. Analysis of spiked samples is performed. The risk of contamination of samples must always be considered. All glass- and plasticware are washed with acid before use.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Agence Française de Sécurité Sanitaire des Aliments

Maisons-Alfort Cedex, France

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Cold Vapour Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are fishes tissues (seafood).

2. Samples throughput per year

Not foreseeable.

3. Procedure

Mercury is assayed by CV-AAS.

3.1. Cold Vapour Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Mineralization of fishes tissues is carried out by wet ashing using Digiprep in the presence of a HNO_3 (2 mL) and H_2SO_4 (1.5 mL) mixture, as shown in Table 1.

Table 1. Digestion programme

Température	80 °c
Montée en température	100 °C/h soit 48mn
Palier	120 minutes
Fin de programme	off

The digest is made up to volume with doubled distilled water. A solution containing 20 % SnCl2 in 20 % HCl is used as the reducing agent.

3.1.b. Analytical instrumentation

A Varian Speectra AA 220FS instrument equipped with an SP5 is used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 2.

Table 2. Equipment characteristics.

CV-AAS
Wavelength, 253.7 nm
Slit width, 0.5 nm
UltrAA Lamp Hg

4. Quality control

Blanks determined out with each batch of samples and CRMs (CRM 151, spiked milk powders; CRM 278R, mussel tissue, CRM 463, Tuna fish, fish tissue and copepod homogenate) are periodically analyzed.

5. Difficulties and limitations

Possibility loss of Hg during the digestion step.

6. Comments and remarks

Not reported.

Federal Office for Consumer Protection and Food Safety

Berlin, Germany

Section I

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Cold Vapour Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

3.2. Cold Vapour Atomic Absorption Spectrometry with Amalgam Enrichment
3.2.a. Sample pretreatment
3.2.b. Analytical instrumentation
3.2.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

Section II

Sample ashing procedure

1. Matrices

2. Procedure

Section I

1. Matrices

Samples analyzed are meat, meat products, plant materials, food

2. Sample throughput per year

About 500.

3. Procedures

Mercury is assayed by CV-AAS or CV-AAS with amalgam enrichment

3.1. Cold Vapour Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Mineralization is described in section II (Sample ashing procedure)

3.1.b. Analytical instrumentation

A SpectrAA 300 - AAS equipped with the VGA 76 cold vapour system (VARIAN) is used.

3.1.c. Working conditions

A solution of 25 g/l $SnCl_2$ 2 H_2O in 50 ml 18 % HCl (100 ml 36 % HCl + 100 ml water) is used as reducing agent. The length of the absorption cell is 17 cm. The calibration curve is plotted by using Mercury solutions of 1.25, 2.5, 5 and 10 ng/ml in 8 % HNO_3. The blank is a solution of 8 % HNO_3. Limit of detection is 0.4 ng/ml in sample solution.

3.2. Cold Vapour Atomic Absorption spectrometry with Amalgam Enrichment

3.2.a. Sample pretreatment

Mineralization is described in section II (Sample ashing procedure)

3.2.b. Analytical instrumentation

A SpectrAA 300 - AAS equipped with the amalgam system M II (VARIAN) is used.

3.1.c. Working conditions

A solution of 25 g/l $SnCl_2$ 2 H_2O in 50 ml 18 % HCl (100 ml 36 % HCl + 100 ml water) is used as reducing agent. The length of the absorption cell is 17 cm. The sampling time is 5 minutes, followed by electrical heating of the Au/Pt-grid. The calibration curve is plotted by using aliquots of a Mercury solution of 10 ng/ml in 8 % HNO_3 as illustrated in Table 1. The blank is a solution of 8 % HNO_3.

Table 1. Pipetting scheme

	Blank (ml)	**Standards (ml)**	**Sample (ml)**
Sample solution			1 - 10
Blank solution	15	15	add.to 15
Standard solution with 10 ng Hg/ml		0.1 0.4 0.8	
$SnCl_2$ solution	2	2	2

Limit of detection is 0.4 ng Hg in the pipetted sample volume.

4. Quality control

Analysis of certified reference materials (BCR, NIST, CBNM) is performed along with each analytical series.

5. Difficulties and limitations

Sample solutions with less than 0.5 ng Hg/ml should be analyzed with enrichment system

6. Comments

These method corresponds to the official German Method for food analysis.

Section II

Sample ashing procedure

1. Matrices

All materials will be mineralized following the same procedure.

2. Procedure

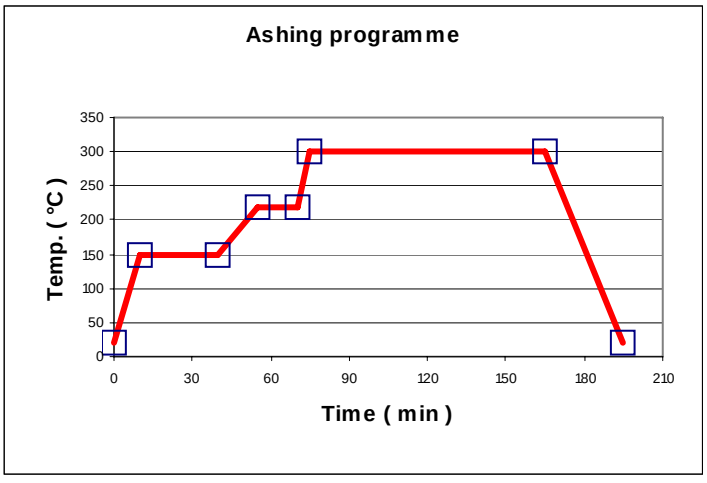
A high pressure ashing system HPA-S equipped with five 90 ml quartz vessels is used. Concentrated HNO_3, 65 % (FLUKA, pA plus) is used. Between 300 mg and 1500 mg of homogenized sample material are weighed into the quartz vessels. Sample amounts are illustrated in Table 2.

Table 2. Sample amounts for use in the HPA-S

Sample	Weight (mg)	Tolerance (mg)	Volume of HNO_3 (ml)
dry samples (< 20 % water)	500	± 50	3.0
fresh samples	1000	± 100	3.0
fresh samples (> 50 % water)	1500	± 200	3.0

Dry samples should be wetted with some drops water before HNO_3 is added; it's to recommend to let stand the vessels over night before heating. The mineralization start by using a ashing temperature of 300 °C over a hold time of 90 minutes (Fig. 1). When ashing procedure is finished, let cool down to room temperature. Check the volume of HNO_3 in the vessels to recognize leaky vessels. Remove dissolved NO_x gases by a gentle stream of nitrogen at the surface of the sample solution. Add water up to 20 ml or 25 ml and store solutions in polyethylene tubes with screw cap for the AAS analysis.

Fig. 1: Ashing programme of the HPA-S



Institute of Food Hygiene of Athens

Athens, Greece

Mercury

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

5. Matrices

Samples more frequently analyzed are foods of animal origin (fish and fishery products).

6. Samples throughput per year

Not foreseeable

7. Procedure

Mercury is assayed by CV-AAS.

3.1.a. Sample pretreatment

Approximately 100g of samples (fish and fishery products) are homogenized and 0.5g from the homogenized sample is digested with HNO_3 (10ml) in HP500 vessels. The digestion program is presented in Table 1.

Table 1. Digestion program for fish and fishery products

Step	Power(W)	Ramp Time(s)	Pressure (psi)	Temperature (^oC)	Hold Time (s)
1	600	25:00	190	200	10:00

3.1.b. Analytical instrumentation

A Perkin-Elmer AAS instrument model 4110 equipped with the FIAS-400 is used.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 2 and 3.

Table 2. Equipment characteristics

Wavelength, 253.7 nm Slit width, 0.7nm Lamp, Hg EDL Quartz cell temperature, 120^oC Sample loop volume, 500 µl

Table 3. Programme for FIAS 400

Step	Time(s)	Pump 1 speed	Pump 2 speed	Valve position	Read step
Prefill	20	100	120	Fill	
1	15	100	120	Fill	
2	30	0	120	Inject	x

Calibration curve is plotted by using aqueous solution 0.5, 2.5, 5.0, 10.0, 15.0 and 20.0 ng/ml in 10% HNO3. A solution containing 0.2 % NaBH4 and 0.05 % NaOH is used as reducing agent.

8. Quality control

An analytical run includes 1 CRM or most frequently in house RM and unknown samples of similar composition. For quality control, BCR 278R and BCR 422 are used periodically.

The method for the determination of mercury in fish and fishery products is accredited according to ISO 17025.

5.Difficulties and limitations

Not reported

6.Comments and remarks

Not reported

National Food Investigation Institute

Budapest, Hungary

Mercury

Determination of mercury (Hg) by AAS coldvapour technique as Standard Operation Procedure (SOP)

1. SOP-identification and-dating.
2. List of amendments.
3. Names of the assembler and approver and their signatures subsequently.
4. Aim of the analytical method.
5. Technical background informations.
6. Principle of the method/s – cold-vapour atomic absorption spectrometry (AAS) at the underlisted resonanceline: Hg — 253.7 nm.
7. Labour safety.

Matrices (specimen).

Chemicals, reagents, standards.

Solvents.

8. Materials.

Chemical reagents: H_2SO_4, HNO_3, H_2O_2, V_2O_5, $SnCl_2$.

Instrument: AAS, hollow-cathod lamp, analytical balance, lab. water deionizer, blender, certified automatic pipettes, shaker, liquid dispenser, gassware with polished stoppers (Erlenmeyer-type).

Description of the decontamination.

List of the technical documentations incl. sample escorting-note.

9. Method's description.

Arrival, recording and taking-over the sample.

Storage of the samples.

Sample preparation's steps.

Analytical weighting and addition of V_2O_5.

Pitting: Weighted sample placed into the Erlenmeyer-flash /reaction vessel/ with polished stopper, aa HNO_3+H_2SO_4, the stopper fixed, lab. shaker (100 cycle/min.) at ambient temperature and increased upto 50 ^oC for 20-24 hs.

The pitting after being completed the sample-falsk will be cooled-down and diluted with deionized water. During shaking added to it dropwise H_2O_2 until ceasing the formation of nitrous-gas. Finally the sample-solvent exhibits light yellow colour.

9.4.1. Warming-up the hollow-cathode lamp, position of the slotted quartz-tube.

9.4.2. Assembling the closed Hg-gas generation-system: air-pump → Dreschsel-setup → Erlenmayer-flask → doplet-separation ($Mg/ClO_4/_2$) → via the air-pump back to the reaction vessel.

9.4.3. Selection of measurement programme.

9.4.4. Air-flow started, via syringe added $SnCl_2$-solution into the reaction-vessel, the Dreschsel-setup will be closed soon. The formation of steady-state of the chemical reaction will be taken approx. 100 sec.

9.4.5. Sequence of absorption measurement (cold vapour at 253.7 nm: blank-solution, analitical standards (calibration), blank, serie of samples after 15 samples must be taken recalibration.

9.4.6. Determination of LOD and LOQ for Hg.

9.4.7. Determination of Hg-concentration/s of the sample solution/s.

9.4.8. Calculation of the Hg-concentration/s of the original sample/s.

9.4.9. Electric data archiving.

Appendix-I

Microwave digestion of milk and milk-powder samples for multi-elemental (cadmium /Cd/, copper /Cu/, lead /Pb/, mercury /Hg/, stibium/antimory /Sb/, etc.) determination via inductively coupled plasma-mass spectrometric (ICP-MS) method

1. SOP-identification and dating.
2. Amendments.
3. Names of the assembler and approver and their signatures.
4. Aim and principle of the analytical method – The samples –
   - for designated multi-elemental analysis (Cd, Cu, Hg, Pb, Sb, etc.)
   - were digested by the mixture of HNO_3+H_2O_2+Au-solution,+Ge-, In-,

   Tl-ISTDs in TFE-bombs at temperature- and pressure-pogrammed, microwave-assisted apparatus. It had been supplemented with Y-standard solution and diluted into PFA/PP-tubes, in 1:33.3-rate dilution with high purity water for ICP-MS analysis.
   The sample solution was sprayed into – the high temperature, radiofrequency-assisted argon-plasma for elemental ionization – horizontal torch/vaporizer/. The emerging ions, via Ni-cone interface, will get into the mass spectrometer (MS). Within MS the positively charged ions were focused by ion-lens system and accelerated by negatively charged voltage. The accelerated and focused positively charged ions will get into the quadrupol-analyzer for radiofrequency assisted m/z-related sorting. The detection of defined isotopes was done by photomultiplier with sec-frequency and evaluated by the specific computer- programme.
5. Technical background informations.
6. Laborur safety.

6.1. Matrices (specimen).

6.2. Chemicals, reagents, standards.

6.3. Solvents.

6.4. Microwave digestion.

7. Materials.

7.3. Chemicals, reagents, standards, appliances for digestion: deionized water (0.05 μS), HNO_3, H_2O_2, Au, In, Ge, Ta, PFA-, PF-, PTX-, PTFE-beakers, measuring-tubes, centrif.-tubes, eprouvettes, digital pipettes and tips, dispensers.

7.4. Instruments: digital analytical balance, for digestion: Milestone Ethos Plus (with 100 ml TFM-teflon tubes, HPR-1000/10S type high-pressure segmented rotor); for deioized water: TKA-LAB (typ HP6UV/UF) water-purifier; for detection: Thermo Elemental X-series ICP-MS apparatus with CETAC ASX-510 robotic sample applier; computer.

7.3. List of technical documentations incl. smaple escorting note.

8. Method description.

8.2. Calibration-Measure of Au-, ISTD1/Ge-), ISTD2/In-), ISTD3/Tl-) ISTD4/Y-/solution.
Standard solutions: blanks, Au197-, Cu65-, Ge72-, In115-, Hg202-, Pb208-Sb121-, Tl205-, Zn66-, Y89-dilutions.

8.2. Set-up characteristics for ICP-MS apparatus:

- Plasm-performance: 1300 W
- Depth of sample immersion: 55 steps
- Argon-gas-flow (outer): 0.7 l.min^{-1}
- Argon-gas-flow (intermediary): 13 l·min^{-1}
- Argon-gas-flow (aerosol carrier): 0.87 l·min^{-}1
- Sample volume (introduction): 1.0 ml·min^{-1}
- Nebulizer: concentric
- Nickel „sampler-cone”: ø1 mm
- Nickel „skimmer-cone”: ø 1.7 mm

Setup the integration-time in „peak-jump”

Mass/charge (m/z)	**Element regarded to setted m/z**	**Integration time (m sec)**
65	Cu	20
66	Zn	20
72	Ge	10
82	Se	20
89	Y	10
111	Cd	20
115	In	10
121	Sb	20
202	Hg	20
205	l	10
289	Pb	20

Programme of the peristaltic pump and the autosampler

Before data-collection	
Volume of the sampling-flow	1 ml/min
Sampling time	60 sec
Revolution per minute	34 rpm
Stabilization time	30 sec
Subsequent to data-collection	
Velocity of the flushing	3 ml/min
Duration of the flushing	45 sec
Revolution per minute	100 rpm

8.3. Main influencing parameters to be considered:
- interference-equations,
- MS-setup parameters,
- Nebulizer-parameters,
- Programme of the peristatic pump,
- Programme of autosampler.

8.14. Calibration procedure:
- Mass Calibration with tuning-solutions per month.
- Detector Plateau' determination with tuning-solutions, morthly.
- Cross Calibration with tuning-sulution, weekly.

8.15. Execution of the measurements.
8.16. Correction of the measurements (elimination of matrix-disturbance) with proper ISTD.
8.17. Calculations according to the equations executed by the computer-programmes.
8.18. Calculation of the measurement-uncertainty.
see: 2002/657 EC-Reg; ISO/EC 17025 std.
8.8.1. Uncertainty.
8.8.2. Recover.
8.8.21. Repeatability.
8.8.22. Within-laboratory reproducibility.
8.8.23. Limit of detection (LOD).
8.8.24. Limit of quantitation (LOQ).
8.8.25. Decision limit (CCalpha).
8.8.26. Detection capability (CCbeta).
8.8.27. Intercomparison with reference materials (e.g: MILK POWDER BCR # 150) (Cd, Cu, Hg, Pb).
8.8.28. Results of the participation in international proficiency testing (PT) programmes.

8.8.29. Evaluation of the possible interferences.

9. Calculations.
9.1. Determination of the elemental-concentration/s of the sample solution.
9.6. Calculation of the elemental concentration=/s of the original smaple.
9.7. Electronic data archiving.

Central Meat Control Laboratory

Celbridge, County Kildare, Ireland

Mercury

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Hydride Generation Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are muscle, kidney and liver tissue.

2. Samples throughput per year

Not reported.

3. Procedure

Mercury is assayed by CV-AAS.

3.1. Hydride Generation Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

1 g of animal tissue is weighed into a digestion vessel. 4 ml of $HNO_3$are added and the solution is allowed to stand in fume hood for at least one hour. A spike control is prepared with 100 ppb of Hg and 1 ml of H_2O (Analytical grade water from Millipore Q system) is added to all samples subsequently. The samples are heated in a Microwave digestion system, MDS 2000 (CEM), following the digestion programme outlined in Table 1.

Table 1. Standard digestion programme.

Stage	Power	Pressure	Run Time	Run Time at press	Fan Speed
1	90 %	50	30.00	5.00	100 %
2	90 %	100	20.00	5.00	100 %
3	90 %	150	20.00	5.00	100 %
4	0 %	0	20.00	5.00	100 %
5	90 %	150	20.00	5.00	100 %

On completion the digest sequence, the vessels are allowed to cool and the pressure to drop below 40 psi before removing from the oven to the fume hood. Using a capillary pipette the sample is purged with a steam of nitrogen for one minute to evolve any dissolved gases

3.1.b. Analytical instrumentation

SpectrAA 220FS-AAS with the autosampler SPS-5.
The vapor generation accessory VGA 77 is used to react the samples.

3.1.c. Working conditions

The vapour generation accessory is used to react the sample (flow rate 6 ml/min) with a 25 % solution of Stannous Chloride (flow rate 1 ml/min) to generate mercury vapour which is feed on a Nitrogen carrier gas through a cold cell for analysis.

Instrumental parameters are reported in Table 2.

Table 2. Equipment characteristics.

HG-AAS
Wavelength, 253.7 nm
Slit width, 0.5 nm
Lamp, Hg HCL
Calibration Mode, concentration
Measurement Mode, integration
Sampling Mode, autonormal
Replicates All, 3
Air flame

Premixed standards (0.5; 1.0; 2.0; 5.0; 10.0 ng/g) are used to generate a standard curve. Standards made up with 5% HNO_3 + 0.01% $K_2Cr_2O_7$ w/v.
The samples are diluted to 1:50 with HNO_3 + 0.01 % $K_2Cr_2O_7$ w/v.

4. Quality control

Reagent blanks, check and spiked samples are incorporated into each assay.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Final results are calculated, only if an acceptable standard curve has been achieved and takes into account sample weights and dilution factors.

National Institute of Health
Istituto Superiore di Sanità

Rome, Italy

Mercury

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Cold Vapour Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

3.2. Inductively Coupled Plasma Atomic Emission Spectrometry
3.2.a. Sample pretreatment
3.2.b. Analytical instrumentation
3.2.c. Working conditions

3.3. Inductively Coupled Plasma Mass Spectrometry
3.3.a. Sample pretreatment
3.3.b. Analytical instrumentation
3.3.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are meat, canned fish, milk, clams and mussels.

2. Samples throughput per year

Not foreseeable.

3. Procedure

Three different techniques are used for the determination of Hg in the samples.

3.1. Cold Vapour Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

The sample is homogenized, weighed and digested at room temperature in an HNO_3-H_2SO_4 (1:1) solution in a cooling apparatus. A solution of $SnCl_2$-$NH_2OH{\cdot}HCl$ or $NaBH_4$ in 0.05 % NaOH is used as the reducing agent.

3.1.b. Analytical instrumentation

A Perkin-Elmer 5100 AAS equipped with a quartz cell and peristaltic pump or a Perkin-Elmer Flow Injection Mercury System 400 is used.

3.2.c. Working conditions

Instrumental parameters for Perkin-Elmer 5100 AA spectrometer and for Perkin-Elmer FIMS 400 are reported in Tables 1 and 2, respectively.

Table 1. Equipment characteristics.

CV-AAS
Wavelength, 253.7 nm
Slit, 0.7 nm
Lamp, Hg EDL and HCL
Air flow, 50 ml/min

Table 2. Equipment characteristics.

CV-AAS
Wavelength, 253.7 nm
Slit, 0.7 nm
Lamp, Hg EDL and HCL
Argon flow, 70-100 ml/min
Carrier solution, 3 % (v/v) HCl

3.2. Inductively Coupled Plasma Atomic Emission Spectrometry

3.2.a. Sample pretreatment

The sample is homogenized, weighed and digested in HNO_3-H_2O_2 solution at high pressure in a microwave oven. A 1 % $NaBH_4$ solution in 0.1 *N* NaOH is used as the reducing agent.

3.2.b. Analytical instrumentation

A Jobin-Yvon 38 VHR spectrometer is used.

3.2.c. Working conditions

Instrumental parameters are reported in Table 3.

Table 3. Equipment characteristics.

ICP-AES
Wavelength, 253.7 nm
Torch, INSA, demountable
Argon flows, plasma 16 l/min, auxiliary 0.4 l/min, aerosol 0.5 l/min
RF generator, Durr-JY 3848, frequency 56 MHz, maximum power output 2.2 kW
Nebulizer, pneumatic concentric type, condensation chamber of the Scott type
Monochromator, HR 1000-M, focal length 1 m, Czerny-Turner mounting, equipped with a 3600 grooves/mm holographic planegrating, linear dispersion in the first order 0.27 nm/mm, spectral range 170-450 nm, entrance and exit slit widths 40 μm

Calibration is carried out by using different Hg solutions depending on the Hg content in the sample.

3.3. Inductively Coupled Plasma Mass Spectrometry

3.3.a. Sample pretreatment

The sample is homogenized, weighed and digested in HNO_3-H_2O_2 solution at high pressure in a microwave oven.

3.3.b. Analytical instrumentation

A Perkin-Elmer/Sciex Elan 6000 quadrupole mass spectrometer is used.

3.3.c. Working conditions

Instrumental parameters are reported in Table 4.

Table 4. Equipment characteristics.

ICP-MS
Analytical mass, ^{202}Hg
Reference mass ^{103}Rh
Nebulizer, cross flow, Ryton spray chamber
Argon flows, plasma 16 l/min, auxiliary 0.9 l/min, aerosol 1 l/min
RF generator, maximum power output 1.0 kW
Scanning conditions: sweeps per reading, 5; readings per replicate, 4; number of replicates, 5; peak hopping; dwell time, 100 ms; replicate time, 2000 ms; normal resolution, 0.9-0.6 amu

Calibration is carried out by using various Hg solutions depending on the Hg content in the sample.

4. Quality control

An analytical run includes 1 blank, 1 CRM and unknown samples of similar composition. The accuracy of the method is estimated by means of CRMs (CRM 184, bovine muscle; CRM 185, bovine liver; CRM 186, pig kidney; CRM 150, skim milk). The calibration curves are accepted when the coefficient of correlation (r) is > 0.999.

5. Difficulties and limitations

In order to eliminate the memory effects in ICP-AES and ICP-MS analyses a pre-established wash time between samples is mandatory. Stabilization of Hg solution is obtained by the addition of a 0.01 % w/w $K_2Cr_2O_7$.

6. Comments and remarks

Not reported.

Food and Veterinary Regulation Division

Albertown, Marsa, Malta

Mercury

THE DETERMINATION OF MERCURY IN BOVINE / PORCINE LIVER AND FISH MUSCLE TISSUES

1.0 INTRODUCTION

This method is suitable for the determination of mercury in food materials, freeze dried and fresh. The matrices covered by this SOP are: bovine and swine liver, and fish muscle.

2.0 EQUIPMENT

Digestion system (Gerhardt)
Vortex mixer
Atomic absorption spectrometer with D2 background correction, GBC932AA
Hydride generation system, GBC HG3000
Electric heating accessory, GBC EHG3000
Sample delivery system, GBC SDS-270
Mercury hollow cathode lamp
Quartz absorption cell, closed
Precision balance with a range of 300 g and an accuracy of 0.001 g
Analytical balance with a range of 300 g and an accuracy of 0.0001 g
Variable micropipettes (Finpipettes)
Kitchen homogeniser
Spatulas, fibre-based or plastic. ***(Note: No metal spatulas to be used)***.
Bottle brushes
Plastic bags
Hot plate

3.0 REAGENTS

3.1 Chemicals

Water, de-ionised.
Nitric acid, about 69%, s.g. 1.42 (High purity, such as BDH SpectrosoL or ARISTAR)
Hydrochloric acid, about 37%, s.g. 1.18 (BDH SpectrosoL or equivalent)
Tin(II) chloride dihydrate, $SnCl_2.2H_2O$ (BDH SpectrosoL or equivalent)
Decon 90
Mercury standard solution, 1000 ppm (BDH or equivalent)

3.2 Reference materials

Certified Reference Materials, if available, e.g.:
BCR 185: Lyophilised Bovine Liver
CRM 463: Tuna fish
DORM2: Dogfish muscle
Choose a reference material which closely resembles the test sample.

4.0 MERCURY SPIKING SOLUTIONS

4.1 Mercury intermediate solution (5 mg/L Hg)
Pipette 500 µL of mercury stock standard (1000 mg/L) into a 100 mL volumetric flask. Add 1 mL of concentrated nitric acid and dilute to the mark with deionised water. Prepare on same day of use.

4.2 Mercury spiking solution (500 µg/L Hg)
Pipette 5 mL of mercury intermediate solution (7.1) into a 50 mL volumetric flask. Add 0.5 mL of concentrated nitric acid and dilute to the mark with deionised water. Prepare on same day of use.

5.0 MERCURY WORKING STANDARDS

Prepare standard solutions in 20% v/v HNO_3 before use:

5.1 Calibration series 1 (0, 5, 10, 15, 20 µg/l): for the analysis of mercury in bovine/porcine liver and fish (excluding predatory fish) muscle tissues.

5.2 Calibration series 2 (0, 5, 10, 15, 20, 30, 50 µg/l): for the analysis of mercury in predatory fish (such as tuna and swordfish).

6.0 PLANNING THE DIGESTION

6.1 Each batch must contain a duplicate certified reference material, duplicate samples, at least 3 blanks, spiked samples and spiked blanks.

6.2 A maximum of 40 samples/blanks can be digested at one time.

6.3 The samples should be distributed in random positions along the digestor.

7.0 WEIGHING OF SAMPLES

7.1 Using the precision balance, weigh out 5.00 ± 0.25 g of homogenised sample in duplicate into clean and separate digestion tubes. Record the sample weights accurately up to 3 decimal places.

7.2 Weigh out aliquots (as in 7.1) of the samples to be spiked and transfer to separate clean digestion tubes. A minimum of one spiked sample should be included per species analysed in the batch. Record the sample weights accurately up to 3 decimal places.

7.3 Choose appropriate reference materials (3.2) and weigh out 0.25 g of the chosen materials into separate clean digestion tubes, using the analytical balance. Record the sample weights accurately up to 4 decimal places. Add 2 mL deionised water to each digestion tube containing a reference material.

8.0 SPIKING PROCEDURE

8.1 To the appropriate samples (7.2) pipette the appropriate volume of mercury spiking solution (500 µg/L, 4.2), according to the following table:

Tissue	Concentration of spiking solution used	Volume of spiking solution added (µL)	Amount of spike added (ng)	Spike conc. in tissue (ng/g)
Bovine/swine liver (5 g)	500 µg/L Hg (4.2)	500	250	50
Fish muscle (5 g)	"	1000	500	100

8.2 Allow the spiked samples to stand for 10 minutes before digesting.

9.0 DIGESTION PROCEDURE

9.1 Add 10 mL of concentrated HNO_3 to each digestion tube. Vortex mix each tube, so that any materials sticking to the sides of the tube will go down to the bottom.

9.2 Place the tubes in the digestion block and start the programme no. 12 on the digestor, as indicated in Appendix I.

9.3 After digestion, remove the tubes from the digestion block and allow to cool.

9.4 Transfer each digest to a 50 mL volumetric flask and dilute to the mark with deionised water. Dilute further if mercury concentration falls outside the calibration curve range.

9.5 For digests containing the DORM2 reference material, these should be diluted to 100 mL with deionised water in a volumetric flask.

10.0 ANALYSIS BY COLD VAPOUR AAS

10.1 Set the instrumental parameters for the analysis of mercury in the diluted digests (9.4) as in Appendix II.

10.2 Condition the HG3000 hydride system by alternately pumping the blank mercury solution and the 20 µg/L mercury working standard solution for several minutes until a reproducible response is achieved.

10.3 Transfer the standards and samples to autosampler test-tubes and place in appropriate positions on the SDS-270 sample delivery system. Start the run. Re-calibration should be performed every 8-12 samples.

10.4 After analysis, flush the HG3000 hydride system with deionised water for at least 7 minutes to clean out all the tubing, mixing manifold, etc.

11.0 CALCULATION OF RESULTS

11.1 A Microsoft Excel spreadsheet, stored as 'Mercury worksheet', is used to calculate results.

11.2 The concentration of mercury in the sample (ng/g), [Hg], is calculated directly by reference to the standard curve:

$$[\,Hg\,] = (C - C_{bl}) \times \frac{V_{dil}}{m}$$

where:

C = concentration of mercury in the sample (µg/L), obtained by comparison with calibration curve.

C_{bl} = average concentration of mercury in the blanks (µg/L).

V_{dil} = dilution volume of sample

m = weight of sample, in g

11.3 The concentration of mercury in the certified reference materials is calculated using the following equation:

$$[\,Hg\,] = (C - C_{bl}) \times \frac{V_{dil}}{m} \times F$$

where:

C = concentration of mercury in the certified reference material (µg/L), obtained by comparison with calibration curve.

C_{bl} = average concentration of mercury in the blanks (µg/L).

V_{dil} = dilution volume of reference material

m = weight of reference material, in g

F = moisture correction factor

11.4 Recovery of analyte, R %, is calculated as follows:

$$R = \frac{[S] - [N]}{[A]} \times 100$$

where

[S] = the concentration of mercury found in the spiked sample

[N] = the concentration of mercury found in the respective unspiked sample

[A] = the concentration of mercury added

The mean recovery figure for each batch is then calculated.

APPENDIX I

PROGRAMME FOR DIGESTION OF SAMPLES

Digestor Programme No. 12

Step No.	Temperature	Time (hrs:mins)
1	26°C	8:00
2	50°C	1:00
3	90°C	1:00
4	123°C	1:00
5	123°C	4:00

APPENDIX II

Instrumental parameters for the determination of mercury in animal tissues by CV-AAS

Instrument mode	Absorbance
Calibration mode	Conc. Least Squares
Measurement mode	Integration
Lamp current (mA)	3.0
Slit width (nm)	0.5
Slit height	Normal
Wavelength (nm)	253.7
Background Correction	On
Sample Introduction	Automatic
Read time (sec)	5.0
Time Constant	0.1
Replicates	3
Delay time (sec)	90.0
Rinse time (sec)	3.0
Final Method Rinse (sec)	10.0
Acid channel of HG3000	Distilled deionised H_2O
Reductant	10% w/v stannous chloride in 20% v/v HCl
Cell temperature (EHG3000)	35ºC
Mixer and Separator gas	Argon (99.99% purity)
Mixer gas flow rate	120 mL/min
Separator gas flow rate	35 mL/min

Agriculture and Food Science Centre

Belfast, Northern Ireland

Mercury

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Cold Vapour Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples most frequently analyzed are animal products such as kidney and liver, or of marine origin, such as fish, oysters, mussels and sediments.

2. Samples throughput per year

About 500 - 600.

3. Procedure

Mercury is analyzed by CV-AAS.

3.1 Cold Vapour Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Sample aliquots are placed in plastic 10 ml graduated screw-top test tubes. 1.5 ml of a 4:1 concentrated $HClO_4$-HNO_3 mixture are added to the samples. The samples are allowed to stand for at least 1 h at room temperature in the closed test tube to ensure they are completely wet by the acid. They are then placed in a waterbath at 65 ^{o}C overnight. Next day samples are cooled. 5 drops of a 50 % w/v $NH_2OH{\cdot}HCl$ in aqueous solution are added and the samples are made up to 10 ml in 1.5 % HNO_3. A solution of 3.0 % $NaBH_4$ in 1 % NaOH is used as the reducing agent.

3.1.b. Analytical instrumentation

A Varian SpectrAA-400 Atomic Absorption Spectrometer equipped with a Perkin-Elmer MHS-10 hydride generator system is used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1.

Table 1. Equipment characteristics.

CV-AAS
Wavelength, 253.7 nm
Slit width, 0.5 nm
Lamp, Hg HCL

A calibration curve is established by using standard solutions of Hg as shown in Table 2.

Table 2. Sample parameters.

1.5 % HNO_3 (ml)	100 mg/ml Hg standard (µl)	Drops of 50 % w/v NH_2OH HCl in H_2O	Hg (ng)
10	0	5	0
9.8	200	5	20
9.6	400	5	40
9.4	600	5	60
9.2	800	5	80
9.0	1000	5	100

4. Quality control

The laboratory quality system is based on GLP and UKAS principles. Method validation is performed by using CRM's or spiked samples as appropriate. CRM's are analyzed periodically to check accuracy. During routine analysis, triplicate spiked samples or CRM's are included with each batch of samples analyzed, along with three reagent blanks to control potential contamination. The precision and accuracy is monitored on quality control charts using recovery results.
Instruments are optimized before each batch of analyses, and details recorded of lamp photomultiplier voltage and absorbance for a given standard. Plot linearity is also checked.
The laboratory participates in proficiency testing exercises, such as the UK Food Analysis Performance Assessment Scheme (FAPAS).

5. Difficulties and limitations

Samples must be thoroughly wetted with the 4:1 $HClO_4$-HNO_3 digestion solution before they are placed in the waterbath at 65 ^{o}C. Dry powdered samples can explode if they are not completely wet when some of the dry sample comes in contact with the hot $HClO_4$-HNO_3 mixture.

6. Comments and remarks

Not reported.

National Veterinary Research Institute

Pulawy, Poland

Mercury

Determination of mercury in biological material by atomic absorption spectrometry

Method description

Total mercury concentration is determined in Advanced Mercury Analyser - AMA 254. This instrument is designed for the direct mercury determination in solid and liquid samples without the need of sample chemical pretreatment. In measuring liquid or solid samples the weight of sample is determined by the amount of mercury supposed to be contained on e sample (max. vol. 500 µl or 300 mg solid sample).

Analytical parameters

Detailed instructions on the determination of the mercury in the AMA 254 are described in the operator's manual.

Instrument calibration

The calibration curve for the determination of mercury is prepared using a basic solution 1000 mg/ml from Beaker. The calibration curve is prepared for the range 50 - 500 ng/ml.

Quality control

The method was validated in terms of the linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, recovery and uncertainty. Validation report is presented in Table.
The method was tested by studying the certified reference materials and regularly evaluated by participation in proficiency programmes organized by Food Analysis Performance Assessment Scheme (FAPAS) and European Community Reference Laboratories (Rome - Italy, Geel - Belgium).

Validation report – Hg determination by AAS - AMA

Parameters		Results					
Linearity (working range), mg/kg		0.002 – 0.500					
Limit of detection (LOD), mg/kg		0.0004					
Limit of quantitation (LOQ), mg/kg		0.0005					
Matrix		Muscle			Liver		
Levels of spiked samples, mg/kg		0,010	0,020	0,040	0,020	0,040	0,050
Repeatability	x mg/kg	0,010	0,020	0,037	0,021	0,039	0,047
	S mg/kg	0,0004	0,0022	0,023	0,0004	0,0011	0,0011
	CV %	7,51	12,94	6,94	1,79	2,72	2,34
Intralaboratory Reproducibility	x mg/kg	0,011	0,020	0,038	0,021	0,039	0,047
	S mg/kg	0,0005	0,0013	0,0020	0,0005	0,0008	0,0011
	CV %	4,97	6,59	5,21	2,22	2,03	2,34
Recovery	%	107,80	102,00	95,40	103,00	91,10	93,7
Uncertainty							
combined (uc)		0,0014			0,0013		
expanded (U)		0,020 ± 0,003 mg/kg			0,050 ± 0,003 mg/kg		
coverage factor (k)		2			2		

Istituto de Investigação das Pescas e do Mar

Lisboa, Portugal

Mercury

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Cold Vapour Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are livers of slaughter animals and fish.

2. Samples throughput per year

About 15 samples per day.

3. Procedure

The samples are analyzed by CV-AAS.

3.1. Cold Vapour Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

0.5 ± 0.05 g of homogenized tissue is weighed in Teflon vessels. 4 ml HNO_3 (65 %, suprapure) and 2ml H_2O_2 (30 %) are added. The samples are digested in the microwave oven (CEM MARS 5). The detailed program is described in Table 1.

Table 1. Digestion programme.

Step	Temp (ºC)	Time (min)
1	85	3
2	145	9
3	200	4
4	200	14

After cooling, the digested sample solutions are transferred into 25 ml measuring flasks and filled up to 25 ml with deionized water.

3.1.b. Analytical instrumentation

Perkin-Elmer Flow Injection Mercury System (FIMS - 100) is used.

3.1.c. Working conditions

Instrumental parameters for FIMS 100 are reported in Tables 2.

Table 2. Equipment characteristics.

CV-AAS
Wavelength, 253.7 nm Lamp, low pressure mercury Injection volume, 500µl samples/standard Argon flow, 70ml/min Measurement mode, peak height Read time, 15s Absorption cell length, 260 mm Sensitivity check, 10 µg/l mercury solution should provide a signal of about 0.07 A. Carrier solution, 3% HCl Reducing agent, 0.2% $NaBH_4$ in 0.05% NaOH

Table 3. Flow injection programme.

Step	Time (s)	Pump 1 Speed	Valve Position	Read Step
Prefill	15	100	Fill	-
1	10	100	Fill	-
2	15	100	Inject	X

The calibration curve is established by manual dilution of a stock solution (usually following concentrations: 0.5; 1.0; 2.0 and 5.0 µg/l). The blank is subtracted automatically from standards and samples.

4. Quality control

Ccalibration curves are accepted when the coefficient of correlation is > 0.995. The performance of the instrument is checked by the known solutions of the interlaboratory trial. CRM 186, pig kidney and CRM 422, cod muscle are analyzed in order to check the accuracy and reproducibility of the whole method.

5. Difficulties and limitations

Lectures performed by FIMS 100 have been giving BCR's values higher than the certified.

2. Comments and remarks

Not reported.

Ministerio de Agricultura, Pesca y Alimentación

Madrid, Spain

Mercury

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Cold Vapour Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are, in the order, wine, spirits, vinegar, beer, orange juice, water, cookies, various kinds of pasta, canned vegetables, liver and kidneys.

2. Samples throughput per year

About 800.

3. Procedure

Mercury is assayed by CV-AAS.

3.1. Cold Vapour Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Sampling is not made by the Laboratory Staff, but by official inspectors. The techniques for storage and subsampling are different according to the product; *i.e.*,: milk and milk products, shake; meat, cut in small pieces; eggs, cook; fish, clean and cut; crustaceans, peal and cook. Afterwards homogenizing in kitchen grinder is performed. Digestion of the samples is carried out in a microwave oven. The acids used for digestion are different depending on the nature of the sample processed. In the case of the samples included in the National Residue Investigation Plan, namely liver and kidneys, the reagents used for sample digestion are the following: HNO_3, $HClO_4$ and H_2O_2. Vanadium pentoxide is used as a catalyst. 5 ml of the digested sample are quantitative transferred into the cold vapour vessels. 5 ml of 1.5 % HCl and 1 ml of 5 % $KMnO_4$ are also added.

3.1.b. Analytical instrumentation

A CEM Model NDS-81D microwave oven and a Perkin-Elmer Model 2380 atomic absorption spectrometer equipped with a cold vapour generator system are used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1.

Table 1. Equipment characteristics.

CV-AAS
Wavelength, 253.6 nm
Slit width, 0.7 nm
Lamp, Hg HCL

4. Quality control

The routine use of each method is preceded by analytical validation according to the IUPAC recommendations in order to ascertain sensitivity, detection power, precision and accuracy. CRMs of bovine liver (NIST) and mussel tissue (SMT) are used to check the accuracy and analyzed periodically. During routine assays, each analytical batch includes at least three reagent blanks to evaluate the detection limits and to control possible contamination. Recoveries are calculated using two spiked reagent blanks and one spiked sample for each non-spiked sample assayed.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analysed are, , in the order, wine, spirits, vinegar, beer, orange juice, cookies, various kinds of pasta, canned vegetables, liver and Kidneys.

2. Samples throughput

About 1200 per year.

3. Procedure

3.1. Thermal decomposition, amalgamation, and atomic absorption spectrophotometry

3.1.a. Sample pretreatment

Sampling is not made by the Laboratory Staff, but by official inspectors. The techniques for storage and subsampling are different according to the product; i.e.,: milk and milk products, shake; meat, cut in small pieces; eggs, cook; fish, clean and cut; crustaceans, peal and cook. Afterwards homogenising in Kitchen grinder is performed. The sample is directly analysed in the atomic absorption spectrometer without previous digestion.

3.1.b. Analytical instrumentation

AAS-DMA. Atomic absorption spectrophotometry by direct mercury autoanalyser

3.1.c. Working conditions

Two calibration curves are used. One for the low response range of the instrument (0 to 35 ng) and another for the high response range of the instrument (35 to 500 ng)

The low calibration curve is plotted by using Hg solutions of 0.5, 1, 2.5, 5, 10, 20 and 30 ng.

The high calibration curve is plotted by using Hg solutions of 100, 200 and 500 ng.

4. Quality control

The routine use of each method is precede by analytical validation . Before the analysis of each batch of samples a 1 mg/ml standard of mercury and a reference material are analysed in order to check the accuracy of the equipment. The CMRs used is dogfish muscle (DOLT2, NIST).

5. Difficulties and limitations

No reported.

6. Comments and remarks

No reported.

National Institute for Public Health and Environment
Rijksinstituut voor Volksgezondheid en Milieu

Bilthoven, The Netherlands

Mercury

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Cold Vapour Atomic Fluorescence Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are meat, urine, animal feed and rain water.

2. Samples throughput per year

About 100.

3. Procedure

Mercury is assayed by CV-AFS.

3.1. Cold Vapour Atomic Fluorescence Spectrometry

3.1.a. Sample pre-treatment

Solid matrices are digested in a microwave oven. For 0.5 g homogenized meat samples or animal feed 1 ml of concentrated HNO_3 and 9 ml H_2O is used. An aliquot of the digested sample is acidified with HNO_3 and HCl (0.3 M HNO_3 and 0.7 M HCl) and pre-treated with bromine (0.001 M Br_2) in order to convert (organic-) mercury compounds into ionic mercury (II).

3.1.b. Analytical instrumentation

A Merlin Plus Atomic Fluorescence Spectrometer from PS Analytical Ltd. is used. The excess of bromine is removed by adding a solution of $NH_2OH{\cdot}HCl$. A solution of $SnCl_2$ is used as the reducing agent. Prior to detection a hygroscopic membrane from Perma Pure is used to dry the generated mercury containing vapour.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1.

Table 1. CV-AFS equipment characteristics.

Wavelength, 253.7 nm
Carrier-gas flow, Argon 0.3 l/min, Shield-gas flow, Argon 0.3 l/min and Dryer-gas flow, Argon or Nitrogen 2.5 l/min
Delay-time 15 s, Rise-time 20 s, Analysis-time 60 s, Memory-time 30 s
Sample injection flow 8.0 ± 2.0 ml/min, Blank injection flow 8.0 ± 2.0 ml/min, reductant injection flow 3.0 ± 1.0 ml/min
Calibration curve: 5, 50, 100 ng/l, linear calibration range: 0.6-100 ng/l

4. Quality control

Each analytical run includes 1 blank, 1 CRM and 1 RM. The instrumental conditions are controlled by the analysis of an in-house water RM. The accuracy of the analytical procedure, including sample digestion, is estimated by the use of CRM.

5. Difficulties and limitations

To prevent contamination it is essential that a clean laboratory work area and cleaned (with HNO_3 or Br_2 / HCL) labware is used. Cleaned labware and labware with measurement-solutions must be stored in double-bagged plastics. Water and chemicals used for sample-, blank- and standard preparation should contain mercury contents as low as possible. To remove traces of mercury in the reductant during 120 min an argon-, or nitrogen-flow of 1 l/min should bubble through the solution.

6. Comments and remarks

Samples of urine and rainwater are not digested with HNO3 in a microwave oven.
These samples are digested totally when pre-treated with bromine and hydrochloric acid. (Urine: 0.0025 M Br2 and 0.6 M HCl, rainwater: 0.001 M Br2 and 0.6 M HCL).

Central Science Laboratory

York, United Kingdom

Mercury

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Inductively Coupled Plasma Mass Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed to UKAS accredited are foods and beverages.

2. Samples throughput per year

Up to 1500.

3. Procedure

Mercury is assayed by ICP-MS.

3.1. Inductively Coupled Plasma Mass Spectrometry

3.1.a. Sample pretreatment

Aliquots of each sample are homogenized and portions (equivalent to 0.5 g dry weight) are weighed out into microwave digestion vessels. Concentrated HNO_3 (5 ml) is added and the samples are heated in a proprietary high pressure microwave digestion oven. The acid is transferred into a polycarbonate test tube with 18 MΩ cm^{-1} water and made up to 10 ml in volume.

3.1.b. Analytical instrumentation

A VG Instruments PlasmaQuad PQII+ Turbo ICP-MS or Perkin Elmer ELAN 6000 ICP-MS are used.

3.1.c. Working conditions

At least 7 calibration standards are used to cover the calibration range 0.1 µg/l to 100 µg/l. ^{202}Hg is monitored with In or Rh being used as an internal standard. Element concentrations in test solutions are determined by manufacturers software and processed further using an in-house written SuperCalc 5 macro. Automatic spike recovery and blank corrections are made during these calculations.

4. Quality control

All test batches contain reagent blanks, spiked blanks, RMs and are performed to UKAS accredited standards.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Nickel

BELGIUM
FINLAND
NORTHERN IRELAND
PORTUGAL
SPAIN
SWEDEN
THE NETHERLANDS

Scientific Institute of Public Health-Louis Pasteur

Brussels, Belgium

Nickel

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Zeeman Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Migration on skin of Nickel jewels, meat, livers

2. Samples throughput per year

Occasionnaly

3. Procedure

Nickel is assayed by ICP-AES

3.1. Zeeman Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

About 1 g is weighed on an analytical balance in a polyethylene cup and dropped in a PFA digestion tube. Wet digestion is carried out with the aid of 5 ml conc. HNO_3 and 0.2 ml conc. $HClO_4$. The tube is placed in an oven at 180 °C. After reducing of the volume to +/- 0.5 mL, 1 mL of H_2O_2 is added. The tube is placed into an oven at 80 °C +/- 1 hour. After complete oxydation the tubes are cooled to ambient temperature. The digest is diluted into the tube to a volume of 10 mL with Milli-Q-water. If necessary, furthers dilutions are realised.

3.1.b. Analytical instrumentation

Perkin Elmer SimAA 6000

3.1.c. Working conditions

Axially viewed ang Gemcone nebulizers Wavelength: 231.604 nm; and 221.648 nm Injection flow rate: 2.2 mL /min. Integration time: 5-20 sec Measurment Mode: Peak Area Normal resolution

Calibration is carried out with standard solution, freshly prepared with 2 % v/v conc. HNO_3. The stock solution is Perkin Elmer PE Pure. Working range is from 10 ppb to 10 ppm

4. Quality control

Twoe wavelength are used. The best wavelength is conservated for the expression of results based on correlation coefficient and recovery test at level of +/- 50 % of the concentration range of samples. An analytical run includes blanks and standards. All glassware and polyethylene flasks and pipettes are soaked in a 10 % HNO_3 solution and rinsed with Milli-Q-water just before use. All volumetric flasks, glass pipettes are calibrated, plastic pipettes are checked regularly.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported

National Veterinary and Food Research Institute

Helsinki, Finland

Nickel

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Zeeman Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissues (meat, liver and kidney)

2. Samples throughput per year

About 10.

3. Procedure

The samples are analyzed by Z-ETA-AAS

3.1. Zeeman Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Sample (5-10g) is ashed at 450^{o}C in a muffle furnace overnight. Ashed sample is cooled down to room temperature, deionized water and 1 ml conc. HNO_3 are added. The sample is re-evaporated and heated up to 450^{o} C overnight in the muffle furnace. The residue is dissolved in 0.5 ml of conc. HNO_3 and adjusted to appropriate volume with deionized water.

3.1.b. Analytical instrumentation

A Perkin-Elmer 5100 PC Atomic Absorption Spectrometry with Zeeman background corrector and hollow cathode lamp is used.

3.1.c. Working conditions

The instrumental parameters adopted are reported in Table 1. Measurement are carried out by direct comparison with standard solution (Reagecon) in 0.1 M HNO_3.

Table 1. Equipment characteristics.

Z-ETA-AAS
Wavelength, 232.0 nm
Lamp, Ni HCL Zeeman background correction

4. Quality control

The method is accredited. Blanks are analyzed together with each sample series. Analysis of spiked samples are performed. The risk of contamination of samples must always be considered. All glass- and plasticware are washed with acid before use.

5. Difficulties and limitations

Lack of certified reference material. Consumption of graphite tubes is high because of the high atomization temperature.

6. Comments and remarks

Not reported.

Agriculture and Food Science Centre

Belfast, Northern Ireland

Nickel

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Flame Atomic Absorption Spectrometry
3.1.a Sample pretreatment
3.1.b Analytical instrumentation
3.1.c Working conditions
3.2. Electrothermal Atomization Atomic Absorption Spectrometry
3.2.a. Sample pretreatment
3.2.b. Analytical instrumentation
3.2.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples most frequently analyzed are of marine origin, such as fish, oysters, mussels and sediments.

2. Samples throughput per year

About 200 samples per year.

3. Procedures

Nickel is analyzed by two different instrumental techniques depending upon the concentration of the element in the sample. Flame atomic absorption spectrometry is the general method of analysis with electrothermal atomization atomic absorption spectrometry being used to a lesser extent for lower concentrations of elements.

3.1 Flame Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Samples are dry-ashed in a muffle furnace temperature programmed from room temperature to 450 °C. The ash is dissolved in 0.1 *N* HNO_3.

3.1.b. Analytical instrumentation

A Varian SpectrAA-400 Atomic Absorption Spectrometer with deuterium lamp background correction is used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1.

Table 1. Equipment characteristics.

F-AAS
Flame, air-acetylene
Wavelength, 232.2nm
Slit width, 0.2 nm
Deuterium background correction
Lamp current 4.0mA

A calibration curve is established by using Ni standard solutions of 0, 0.2, 0.4, 0.6, 0.8 and 1.0 µg/g in 0.1 *N* HNO_3.

3.2. Electrothermal Atomization Atomic Absorption Spectrometry

3.2.a. Sample pretreatment

Samples are dry-ashed in a muffle furnace temperature programmed from room temperature to 450 ^{o}C. The ash is dissolved in 0.1 *N* HNO_3.

3.2.b. Analytical instrumentation

A Varian SpectrAA-300 Atomic Absorption Spectrometer with Zeeman background correction, graphite furnace and autosampler is used.

3.2.c. Working conditions

Instrumental parameters are reported in Tables 2 and 3.

Table 2. Equipment characteristics.

ETA-AAS Pyrotylitic coated graphite tube Wavelength, 232.0 nm Slit width, 0.2 nm Zeeman background correction Injection volume, 5 µl sample

Table 3. Graphite furnace programme

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)	Ar Flow (ml/min)
Drying	90 95 150	5 40 10	0 0 0	300 300
Ashing	400	5	7	300
Atomization	2500	1	2	0
Clean-up	2500	0	2	300

A calibration curve is established using Ni standard solutions of 0.002, 0.004 and 0.006 µg/g in 0.1 *N* HNO_3.

4. Quality control

The laboratory quality system is based on GLP and UKAS principles. Method validation is performed by using CRM's or spiked samples as appropriate. CRM's are analyzed periodically to check accuracy. During routine analysis, triplicate spiked samples or CRM's are included with each batch of samples analyzed, along with three reagent blanks to control potential contamination. The precision and accuracy is monitored on quality control charts using recovery results.
Instruments are optimized before each batch of analyses, and details recorded of lamp photomultiplier voltage and absorbance for a given standard. Plot linearity is also checked.
The laboratory participates in proficiency testing exercises, such as the UK Food Analysis Performance Assessment Scheme (FAPAS).

5. Difficulties and limitations

The main problem is potential contamination and purity of chemicals. Dry-ashing requires careful control. The temperature should be kept as low as is possible because of the danger of loosing volatile inorganic compounds, but at the same time high enough to ensure complete combustion of all the organic matter in a reasonably short time. Fatty samples may require additional treatment and precautions when dry-ashing.

6. Comments and remarks

Not reported.

Istituto de Investigação das Pescas e do Mar

Lisboa, Portugal

Nickel

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissue (liver).

2. Samples throughput per year

Not foreseeable.

3. Procedure

Nickel is assayed by Z-ETA-AAS.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Approximately 100 g of samples are homogenized, 1 g is weighed in quartz crucibles and 1 ml of 25 % $Mg(NO_3)_2$ are added. The sample is dry-ashed, followed by ashing in a muffle furnace and temperature programmed from 100ºC to 450 °C. After one hour at 450º C, the sample is then removed and wet with concentrated HNO_3 and put back to the furnace. This procedure is repeated every hour until ashes become white. The ashes are then quantitatively transferred to 10 ml volumetric flasks with 5 % HNO_3.

3.1.b. Analytical instrumentation

A Perkin Elmer Atomic Absorption Spectrometer Aanalyst 600 with THGA Graphite Furnace including AS- 800 Autosampler.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 1 and 2.

Table 1. Equipment characteristics.

Z-ETA-AAS
Wavelength, 232.0 nm
Ni HCL.
Zeeman background correction
Injection volume, 15 µl samples
Pyrolytic coated graphite tubes with L'vov platform

Table 2. Graphite furnace programme.

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)	Ar Flow (ml/min)
1	100	4	30	250
2	130	15	30	250
3	1300	10	20	250
4	2300	0	5	0
5	2500	1	5	250

The calibration curve is plotted by using Ni solutions of 12.5, 25, 50 and 100 ng/g.

4. Quality control

Each analytical run includes blanks, commercial standard solutions, and Quality Control Standards for Trace Metals - AA by RTC

5. Difficulties and limitations

Possibility of contamination.

6. Comments and remarks

Not reported.

Ministerio de Agricultura, Pesca y Alimentación

Madrid, Spain

Nickel

1. Matrices

2. Samples throughput per year

3. Procedure
3.1 Electrothermal Atomization Atomic Absorption
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analysed are, in order, wine, spirits, vinegar, beer, orange juice, water, cookies, various kinds of pasta, canned vegetables, liver and kidneys.

2. Samples throughput per year

About 400.

3. Procedure

Nickel is assayed by ETA-AAS.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Sampling is not made by the Laboratory Staff, but by official inspectors. The techniques for storage and subsampling are different according to the product; *i.e.*,: milk and milk products, shake; meat, cut in small pieces; eggs, cook; fish, clean and cut; crustaceans, peal and cook. Afterwards homogenizing in kitchen grinder is performed. Digestion of the samples is carried out in a microwave oven. The acids used for digestion are different depending on the nature of the sample processed. In the case of the samples included in the National Residue Investigation Plan, namely liver and kidneys, the reagents used for sample digestion are the following: HNO_3, $HclO_4$ and H_2O_2.

3.1.b. Analytical instrumentation

A CEM Model NDS-81D microwave oven and a Perkin Elmer Model 3030 Atomic Absorption Spectrometer with Zeeman background correction, equipped with the HGA-600 graphite furnace, AS-60 autosampler and PR-100 recorder, is used.

3.1.c. Working conditions

The instrumental parameters adopted are reported in Table 1 and 2.

Table 1. Equipment characteristics.

ETA-AAS
Wavelength, 232.0 nm
Slit width, 0.7 nm
Lamp, Ni HCL
Zeeman background correction
Injection volume, 20 µl

Table 2. Graphite furnace programme.

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)	Ar Flow (ml/min)
Drying	130	1	19	300
Charring	1200	5	20	300
Atomization	2500	0	2	0
Clean-up	2700	2	2	300

4. Quality control

The routine use of each method is preceded by analytical validation according to the IUPAC recommendations in order to ascertain sensitivity, detection power, precision and accuracy. CRMs of bovine liver (NIST) and mussel tissue (SMT) are used to check the accuracy and analyzed periodically. During routine assays, each analytical batch includes at least three reagent blanks to evaluate the detection limits and to control possible contaminations. Recoveries are calculated using two spiked reagent blanks and one spiked sample for each non-spiked sample assayed.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

National Food Administration

Uppsala, Sweden

Nickel

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Electrothermal Atomization Atomic Absorption Spectrometry 3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissues, cereals and vegetables.

2. Samples throughput per year

About 50-150 samples per year.

3. Procedure

Nickel is determined by ETA-AAS after either dry ashing or microwave digestion.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

a) About 1-10 g of sample is weighed into a platinum crucible and dry ashed at a temperature of 450±25 °C in a programmable furnace. The carbon-free ash is dissolved in 20-30 ml of 0.1 M HNO_3 and transferred to a plastic container.

b) A maximum of 0.5 g dried, homogenized sample is weighed into a Teflon vessel, followed by the addition of 5 ml conc HNO_3 and 2 ml 30% H_2O_2. The digestion is carried out in a CEM (MDS 2000) microwave oven according to the programme detailed in Table 1.

Table1. Digestion programme.

Step	Power (W)	Time (min)
1	250	3
2	630	5
3	500	22
4	0	15

3.1.b. Analytical instrumentation

Perkin Elmer Z-5100 with HGA-600 autosampler AS-60.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 2 and 3 for the PE Z-5100 instrument. Matrix modifiers are generally not used.

Table 2. Equipment characteristics for PE Z-5100.

ETA-AAS
Wavelength 232.0 nm
Slit width, 0.7 low
Lamp, Ni HCL
Zeeman background correction
Injection volume, 10 µl sample
Pyrolytic coated graphite tubes

Table 3. Graphite furnace programme for PE Z-5100.

Step	**Ramp Time (s)**	**Hold Time (s)**	**Temperature (°C)**
Injection	-	-	Ambient
Drying	10	30	130
Pyrolysis	5	10	1000
Atomization*	0	2	2500
Clean out	2	2	2700

*Gas stop

Calibrations are generally carried out by standard addition method.

4. Quality control

An analytical run includes one-two blanks and one or more CRM or in-house RM. Samples are measured as singles or duplicates, depending on the requirements. Accuracy and precision are monitored by the use of control charts on which the results of duplicate CRM and RM analysis are plotted. The detection limit is based on three times the standard deviation of large number of blanks and is currently 4 ng/ml in sample solution.

5. Difficulties and limitations

Extremely fatty or sugar rich samples require extra attention when dry ashing is used. The microwave digestion method is not suitable for fats, oils or extremely fatty products.

6. Comments and remarks

The dry ashing method has been collaboratively tried and was approved by the Nordic Committee for Food Analysis (NMKL) in 1990. The report was published in *JAOAC 76* (1993), 4. (Lars Jorhem, Determination of metals in foodstuffs by AA Spectrophotometry after dry ashing: An NMKL collaborative study of Cd, Cr, Cu, Fe, Ni, Pb and Zn.).

This method was validated on January 1st 1996. The result of the collaborative trial have been submitted to the AOAC Methods Committee and was accepted in December 1999.

National Institute for Public Health and Environment
Rijksinstituut voor Volksgezondheid en Milieu

Bilthoven, The Netherlands

Nickel

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Inductively Coupled Plasma Mass Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are meat, rain water, ground water, urine, animal feed and aerosol particles.

2. Samples throughput per year

About 1000.

3. Procedure

Nickel is assayed by ICP-MS.

3.1 Inductively Coupled Plasma Mass Spectrometry

3.1.a. Sample pretreatment

Solid matrices are digested in a microwave oven. For 0.5 g of homogenised biological sample 1 ml of concentrated HNO_3 and 9 ml H_2O is used.
For soil samples (particle size < 250µm) an aqua regia digestion is performed.

3.1.b. Analytical instrumentation

An HP4500+ quadrupole ICP-MS is used.

3.1.c Working conditions

Instrumental parameters are reported in Table 1.

Table 1. **ICP-MS HP4500+** Equipment characteristics.

Analytical mass, ^{60}Ni
Reference mass, ^{72}Ge (added on-line (dilution sample/IS: 1: 5.5))
Nebulizer, V-groove, Scott-type double pass spraychamber
Argon flow, nebulizer 1.17 l/min, R.f. power 1250W
Measuring conditions: 3 points / mass; integration time 1.5 s / mass, 3 replicates.
Two-point calibration, standards concentration, 0 and 20 µg/l
Matrix matching for calibration samples
Equation ^{60}Ni (-0.00022*Ca43) equation is updated in every measurement sequence using (a) suitable Interference Check Solution(s)

4. Quality control

Each analytical run includes 1 blank, 1 CRM and 1 RM. The instrumental conditions are controlled by the analysis of an in-house water reference material. The accuracy of the analytical procedure, including sample digestion, is estimated by the use of CRMs. A number of matrix samples are analyzed and recovery experiments are carried out for Ni.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Selenium

AUSTRIA
BELGIUM
DENMARK
GERMANY
NORTHERN IRELAND
SWEDEN
THE NETHERLANDS

Austrian Agency for Health and Food Safety

Wien, Austria

Selenium

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Hydride Generation Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Animal tissue (muscle, liver, kidney), blood, serum.

2. Samples throughput per year

About 0-10 samples per year. No routine samples were analyzed during the last four years.

3. Procedure

Selenium is assayed by HG-AAS (FIAS-Furnace Technique).

3.1. Hydride Generation Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Muscle, kidney, liver:

a) 2 ± 0.1 g of homogenized tissue is weighed in 20 ml glass bottles. 3 ml HNO_3 (65%, suprapure) are added. The samples are digested for at least 1 h at 90 °C in a water bath. The solutions are filtered through paper filters into 25 ml measuring flasks and filled up to 25 ml with deionized water.

b) 2 ± 0.1 g of homogenized tissue is weighed in 40 ml Teflon vessels. 3 ml HNO_3 (65%, suprapure) and 1.5 ml H_2O_2 (30%) are added. The samples are digested in a microwave oven (MLS-Ethos 900) according to the programme showing in following Table 1.

Table 1. Digestion programme

Step	Pressure (W)	Hold Time (s)
1	200	30
2	0	30
3	100	30
4	0	30
5	200	30
6	0	30
7	200	5
8	250	5
9	450	5
10	600	10

After cooling, the digested sample solutions are transferred into 25 ml measuring flasks and filled up to 25 ml with deionized water. 5mL of solution are prereduced by addition of 1mL conc. HCl and heated at 80°C for 20min. After cooling the solution is filled up to 10mL with deionized water.

Blood, serum:

0.5 mL blood or serum is transferred in 20 ml glass bottles. 1.5 mL HNO_3 (65 %, suprapure) are added. The samples are digested for at least 1 h at 80 °C in a water bath. The solutions are filtered through paper filters into 10 mL measuring flasks and filled up to 10 mL with deionized water. 5mL of solution are prereduced by addition of 1mL conc. HCl and heated at 80°C for 20min. After cooling the solution is filled up to 10mL with deionized water.

3.1.b. Analytical instrumentation

A SIMAA 6000 with FIAS/AS91 (Perkin-Elmer) is used (FIAS-Furnace Technique).

3.1.c. Working conditions

Instrumental parameters are reported in Table 2, Table 3 and Table 4.

Table 2. Equipment characteristics:

FIAS
Wavelength 196.0 nm Lamp, Se EDL Zeeman background correction, longitudinal AC magnetic field with transversal heated graphite furnace Measurement mode: peak area Injection volume: 500 µl sample/standards Carrier solution: 3 % HCl

A solution of 0.2 % $NaBH_4$ in 0.05 % NaOH is used as the reducing agent

Dry ashing in presence of magnesium nitrate:
- dissolve in 1+1 HCl for half an hour, then filter;
- hydride-AAS determination;
- interference from high Cu level can be overcome by adding KI to the $NaBH_4$ reagent.

Table 3. Flow injection program:

Step	**Time (s)**	**Pump 1 Speed**	**Pump 2 Speed**	**Valve Position**
Pre-fill	12	100	0	**Fill**
1	5	100	0	Fill
2	5	100	80	Fill
3	30	80	80	Inject

Table 4. Graphite furnace programme:

Step	**Temperature (°C)**	**Ramp Time (s)**	**Hold Time (s)**	**Gas Flow (ml/min)**
1	400	1	50	0
2	400	1	20	250
Atomization	2100	0	5	0
Clean-up	2300	1	3	250

The calibration curve is established by manual dilution of a stock solution to the following concentrations: 1, 3, 6 and 10 µg/l. Blank and standard solutions are prereduced as described under point 3.1a. The blank is subtracted automatically from standards and samples.

4. Quality control

Calibration curves are accepted when the coefficient of correlation is > 0.995. The performance of the instrument is checked by a multielement standard (Perkin-Elmer). Recovery is calculated by spiked samples. CRM 185 (bovine liver) or CRM 186 (pig kidney) are analyzed in order to check the accuracy and reproducibility of the whole method.

5. Difficulties and limitations

Not reported

6. Comments and remarks

The graphite tube must be treated with Iridiumchloride in order to guarantee a complete trapping of Se on the inner surface of the graphite tube.

Scientific Institute of Public Health-Louis Pasteur

Brussels, Belgium

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Electrothermal Atomization Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Complementary Food addtives, meat and products of bakkery.

2. Samples throughput per year

About 100 samples

3. Procedure

Selenium is assayed by Z-ETA-AAS.

3.1. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

About 1 g is weighed on an analytical balance in a polyethylene cup and dropped in a PFA digestion tube. Wet digestion is carried out with the aid of 5 ml conc. HNO_3 and 0.2 ml conc. $HClO_4$. The tube is placed in an oven at 180 °C. After reducing of the volume to +/- 0.5 mL, 1 mL of H_2O_2 is added. The tube is placed into an oven at 80 °C +/- 1 hour. After complete oxydation the tubes are cooled to ambient temperature. The digest is diluted into the tube to a volume of 10 mL with Milli-Q-water. If necessary, furthers dilutions are realised.

3.1.b. Analytical instrumentation

Perkin Elmer SimAA 6000

3.1.c. Working conditions

Z-ETA-AAS

Wavelength, 196.0 nm Slit width, 0.5 nm Lamp, Se EDL, Zeeman background correction Injection volume, 20 µl sample + 10 µl modifier THGA graphite Furnace without End Cap Measurement mode, peak area Calibration mode, concentration Replicates, 2 Sample introduction, sampler automixing (5 points)

Table 3. Graphite furnace programme.

Step	**Temperature (°C)**
Injection	Ambient
Drying	110 -130
Pyrolysis	1300
Atomization	1900
Clean-up	2600

Use is made of $Pd(NO_3)_2$ and $Mg(NO_3)_2$ as matrix modifiers. Calibration is carried out with standard solution, freshly prepared with 2 % v/v conc. HNO_3. The stock solution is Baker. Linear working range is 0-40.0 ng/ml.

4. Quality control

An analytical run includes blanks, standards, spike samples for recovery measurements on all samples and RMs are regularly analysed. All glassware and polyethylene flasks and pipets are soaked in a 10 % HNO_3 solution and rinsed with Milli-Q-water just before use. All volumetric flasks, glass pipets are calibrated, plastic pipets are checked regularly.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Danish Institute for Food and Veterinary Research

Søborg, Denmark

Selenium

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Inductively Coupled Plasma Mass Spectrometry 3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1.Matrices

Samples more frequently analyzed are animal tissues (meat and liver) and selenised yeast.

2. Samples throughput per year

About 50.

3. Procedure

The samples are analyzed by ICP-MS.

3.1. Inductively Coupled Plasma Mass Spectrometry

3.1.a. Sample pretreatment

The sample (0.2-0.5 g) is weighed into the quartz bombs of a microwave assisted digestion The Multiwave high pressure microwave digestion system is used for sample decomposition. The maximum pressure is 70 bar and the digestion is finalised within 60 minutes including cooling. Following digestion the residue is taken to 20 ml by weighing using pure water.

3.1.b. Analytical instrumentation

An Elan 6100 DRC Perkin-Elmer/Sciex quadrupole mass spectrometer is used.

3.1.c. Working conditions

A volume of 5 ml of the diluted digest solution is pipetted into a polyethylene autosampler tube and 5 ml of 1 % aqueous HNO_3 are added. Selenium is measured at *m/z* = 80 in the instrument's DRC mode, which minimizes the interfering argon dimer. Calibration is carried out by the standard addition method. Two additions are used for each sample. A mixture of usually Ga, In and Bi are used for internal standardization in the multielement method in which this element is an analyte.

4. Quality control

The method is accredited by the Danish Accreditation Organisation, DANAK. A matching CRM and blanks are analyzed in parallel with the samples in series containing up to 15 unknown samples. The results of CRMs are monitored on control charts (x- and R-charts). The risk of contamination of samples must always be considered. The critical

utensils are washed/rinsed with acid before use and ultrapure acids are used for the ashing process.

5. Difficulties and limitations

Care must be taken to prevent different ICPMS response from different Se-species. If occurring, this is done by adding 3 % methanol to the sample solutions in combination with and increased RF power at 1350 W.

6. Comments and remarks

None reported.

Federal Office for Consumer Protection and Food Safety

Berlin, Germany

Selenium

Section I

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Hydride Generation Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

Section II

Sample ashing procedure

1. Matrices

2. Procedure

1. Matrices

Samples analyzed are meat, meat products, fish, fish products; plant materials, food

2. Sample throughput per year

About 50.

3. Procedure

Selenium is assayed by HG-AAS

3.1. Hydride Generation Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

See separate section II (Sample ashing procedure)

For Selenium analysis it is necessary to prepare the mineralized sample solution at special conditions. After opening the mineralization vessels remove the NO_x gases by a gentle stream of nitrogen at the surface of the solution. Let stand the uncovered vessels over a period of 24 hours. Treat the solution in an ultrasonic bath for a minimum of 45 minutes at maximum power. Dilute to 20 ml solution with water. Repeat the ultrasonic treatment for 5 minutes. This solution is now ready for pre-reduction step.

Pipette 2.0 ml of the sample solutions in 20 ml reagent tubes. Add 8.0 ml HCl, 6 mol/l (100 ml 36 % HCl + 100 ml water). and mix well. Heat the open reagent tubes with the sample solutions in a water bath at 90 °C over a period of 30 minutes. let cool down at room temperature.

3.1.b. Analytical instrumentation

SpectrAA 300 - AAS with VGA 76 Hydride Generation System

3.1.c. Working conditions

The calibration curve is plotted by using Se^4 solutions of 2.0, 4.0, 6.0 and 8.0 µg/l in 6 mol/l HCl. The analytical blank is a 6 mol/l HCl (100 ml 36 % HCl + 100 ml water).

The calibration is performed without prereduction of standard solutions. Therefore it's necessary to check the procedure by analysing a standard solution that is treated at the same procedure as the sample solutions along with each analytical series.

A $NaBH_4$ solution, (3 g $NaBH_4$+2.5 g NaOH / 500 ml) together with 7 mol/L HCl is used as reducing agent.

The limit of detection is about 0.5 ng Se^4/ml in sample solution.

4. Quality control

Analysis of certified reference materials (BCR, NIST, CBNM) is performed along with each analytical series. Analysis of blank and/or spiked solutions are carried out when CRM's are not available.

5. Difficulties and limitations

The most critical step of Selenium analysis with HG-AAS is the complete prereduction of Se^6 to Se^4 in presence of HCl. This step is disturbed by HNO_3 and other strong oxidants like NO_x. Therefore it's necessary to remove all NO_x residues carefully before pre reduction step is performed.

In some food products Selenium is bounded in different chemical forms. For the analysis by HG-AAS it is necessary to mineralize the samples at a minimum temperature of 320°C. These temperature must affect the sample for 90 minutes or longer**.**

6. Comments

Sample preparation with a dry ashing procedure show identical results for Selenium.

Section II

Sample ashing procedure

1. Matrices

All materials will be mineralized following the same procedure.

2. Procedure

A high pressure ashing system HPA-S equipped with five 90 ml quartz vessels is used. Concentrated HNO_3, 65 % (FLUKA, pA plus) is used. Between 300 mg and 1500 mg of homogenized sample material are weighed into the quartz vessels. Sample amounts are illustrated in Table 1.

Table 1. Sample amounts for use in the HPA-S

Sample	Weight (mg)	Tolerance (mg)	Volume of HNO_3 (ml)
dry samples (< 20 % water)	500	± 50	3.0
fresh samples	1000	± 100	3.0
fresh samples (> 50 % water)	1500	± 200	3.0

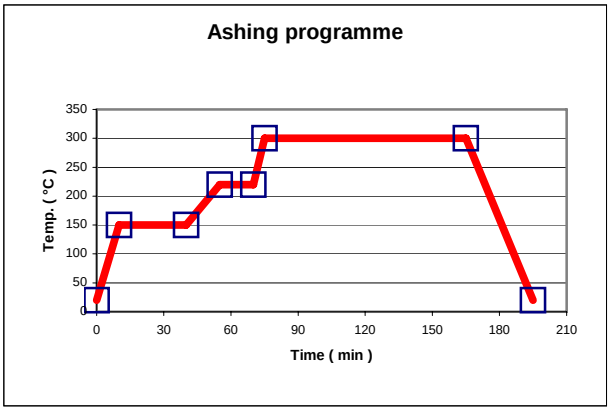
Dry samples should be wetted with some drops water before HNO_3 is added; it's to recommend to let stand the vessels over night before heating.

Start the mineralization: use a ashing temperature of 300 °C over a hold time of 90 minutes (Fig. 1).

For Selenium determination by HG-AAS it's absolutely necessary to use a higher temperature at 320 °C for all sample materials.

When ashing procedure is finished, let cool down to room temperature. Check the volume of HNO_3 in the vessels to recognize leaky vessels. Remove dissolved NO_x gases by a gentle stream of nitrogen at the surface of the sample solution. The solution must be treated in an ultrasonic bath. Add water up to 20 ml or 25 ml and store solutions in polyethylene tubes with screw cap for the AAS analysis.

Fig. 1: Ashing programme of the HPA-S



Agriculture and Food Science Centre

Belfast, Northern Ireland

Selenium

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Flame Atomic Absorption Spectrometry
3.1.a Sample pretreatment
3.1.b Analytical instrumentation
3.1.c Working conditions
3.5. Electrothermal Atomization Atomic Absorption Spectrometry
3.2.a. Sample pretreatment
3.2.b. Analytical instrumentation
3.2.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples most frequently analyzed are of marine origin, such as fish, oysters, mussels and sediments.

2. Samples throughput per year

About 200 samples per year.

3. Procedures

Selenium is analyzed by HG-AAS.

3.1 Hydride Generation Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Organic matter in the sample is destroyed by wet oxidation using a mixture of nitric/perchloric acids (4:1). Selenium present is converted into selenium (IV) by boiling gently with HCl (40%) at 90°C. The solutions are cooled and diluted with distilled water (10ml). Aliquots of these solutions are made up to a suitable volume with HCl (60%) for analysis by hydride generation.

3.1.b. Analytical instrumentation

A Varian SpectrAA-400 Atomic Absorption Spectrometer equipped with a VGA-76 hydride generator system is used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1. Use is made of $Mg(NO_3)_2$ as matrix modifier.

Table 1. Equipment characteristics.

F-AAS
Wavelength, 196.0nm
Lamp, Se HCL
Slit width, 1.0 nm
Flame, air-acetylene
Measurement time, 9s
Delay time, 30s

A calibration curve is established by using Se standards as shown in Table 2.

Table 2. Sample parameters

60% HCl (ml)	100mg/ml Se standard (µl)	Se (ng)
10	0	0
9.8	200	20
9.6	400	40
9.4	600	60
9.2	800	80
9.0	1000	100

4. Quality control

The laboratory quality system is based on GLP and UKAS principles. Method validation is performed by using CRM's or spiked samples as appropriate. CRM's are analyzed periodically to check accuracy. During routine analysis, triplicate spiked samples or CRM's are included with each batch of samples analyzed, along with three reagent blanks to control potential contamination. The precision and accuracy is monitored on quality control charts using recovery results.

Instruments are optimized before each batch of analyses, and details recorded of lamp photomultiplier voltage and absorbance for a given standard. Plot linearity is also checked.

The laboratory participates in proficiency testing exercises, such as the UK Food Analysis Performance Assessment Scheme (FAPAS).

5. Difficulties and limitations

The main problem is potential contamination and purity of chemicals. Dry-ashing requires careful control.

The temperature should be kept as low as is possible because of the danger of loosing volatile inorganic compounds, but at the same time high enough to ensure complete combustion of all the organic matter in a reasonably short time.

Fatty samples may require additional treatment and precautions when dry-ashing.

6. Comments and remarks

Not reported.

National Food Administration

Uppsala, Sweden

Selenium

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Inductively Coupled Plasma-Mass Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissues, cereals and vegetables.

2. Samples throughput per year

No analytical activities at the present time.

3. Procedure

Selenium is determined by ICP-MS after microwave digestion.

3.1. Inductively Coupled Plasma-Mass Spectrometry

3.1.a. Sample pretreatment

A maximum of 0.5 g dried, homogenized sample is weighed into a Teflon vessel, followed by the addition of 5 ml conc HNO_3 and 2 ml 30% H_2O_2. The digestion is carried out in a CEM (MDS 2000) microwave oven according to the detailed programme in Table 1.

Table 1. Digestion programme.

Step	Power (W)	Time (min)
1	250	3
2	630	5
3	550	22
4	0	15

3.1.b. Analytical instrumentation

Samples are analyzed by Platform ICP, Micromass.

3.1.c. Working conditions

Instrumental parameters are reported in Table 2

Table 2. Equipment characteristics.

ICP-MS
Measured isotopes: 77, 78, 80, 82
Dwell time, 200 ms
Number of scans, 16
Total measurement time, 3.2 s

External calibration using Rh as an internal standard is normally used. In certain cases the standard addition method may be necessary. Post run correction for interferences from K_2 and BrH may be needed

4. Quality control

An analytical run includes one-two blanks and one or more CRM or in-house RM. Samples are measured as singles or duplicates, depending on the requirements. Accuracy and precision are monitored by the use of control charts on which the results of duplicate CRM and RM analysis are plotted. The detection limit is based on three times the standard deviation of large number of blanks and is 100 ng/g on a sample weight of 0.4 g

5. Difficulties and limitations

The microwave digestion method is not suitable for fats, oils or extremely fatty products.

6. Comments and remarks

Not reported.

National Institute for Public Health and Environment
Rijksinstituut voor Volksgezondheid en Milieu

Bilthoven, The Netherlands

Selenium

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Inductively Coupled Plasma Mass Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analysed are meat, rain water, ground water, urine, animal feed, soil and aerosol particles.

2. Samples throughput per year

About 3000.

3. Procedure

Selenium is analysed by ICP-MS.

3.1 Inductively Coupled Plasma Mass Spectrometry

3.1.a. Sample pretreatment

Solid matrices are digested in a microwave oven. For 0.5 g of homogenised biological sample 1 ml of concentrated HNO_3 and 9 ml H_2O is used.
For soil samples (particle size < 250µm) an aqua regia digestion is performed.

3.1.b. Analytical instrumentation

An HP4500 equipped with quadrupole ICP-MS is used.

3.1.c Working conditions

Instrumental parameters are reported in Table 1.

Table 1. ICP-MS HP4500+ Equipment characteristics

Analytical mass, ^{82}Se
Reference mass, ^{72}Ge (added on-line (dilution sample/IS: 1: 5.5))
Nebulizer, V-groove, Scott-type double pass spraychamber
Argon flow, nebulizer 1.17 l/min, R.f. power 1250W
Measuring conditions: 3 points / mass; integration time 1.5 s / mass, 3 replicates.
Two-point calibration, standards concentration, 0 and 20 µg/l
Matrix matching for calibration standards
Equation ^{82}Se (-0.000001*S34-0.000042*Ca43-0.000364*Br81, equation is updated in every measurement sequence using (a) suitable Interference Check Solution(s)

4. Quality control

Each analytical run includes 1 blank, 1 CRM and 1 RM. The instrumental conditions are controlled by the analysis of an in-house water reference material. The accuracy of the analytical procedure, including sample digestion, is estimated by the use of CRMs. A number of matrix samples are analyzed and recovery experiments are carried out for Se.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Zinc

BELGIUM
CZECH REPUBLIC
ESTONIA
FINLAND
GERMANY
NORTHERN IRELAND
PORTUGAL
SPAIN
SWEDEN
THE NETHERLANDS

Scientific Institute of Public Health-Louis Pasteur

Brussels, Belgium

Zinc

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Inductively Coupled Plasma - Atomic Emission Spectrometry

3.1.a. Sample pretreatment

3.1.b. Analytical instrumentation

3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Meat and product of bakkery

2. Samples throughput per year

+/- 100 samples

3. Procedure

Zinc is assayed ICP -AES.

3.1. Inductively Coupled Plasma - Atomic Emission Spectrometry

3.1.a. Sample pretreatment

About 1 g is weighed on an analytical balance in a polyethylene cup and dropped in a PFA digestion tube. Wet digestion is carried out with the aid of 5 ml conc. HNO_3 and 0.2 ml conc. $HClO_4$. The tube is placed in an oven at 180 °C. After reducing of the volume to +/- 0.5 mL, 1 mL of H_2O_2 is added. The tube is placed into an oven at 80 °C +/- 1 hour. After complete oxydation the tubes are cooled to ambient temperature. The digest is diluted into the tube to a volume of 10 mL with Milli-Q-water. If necessary, furthers dilutions are realised.

3.1.b. Analytical instrumentation

A Perkin Elmer Optima 4300 DV.

3.1.c. Working conditions

Axially viewed ang Gemcone nebulizers Wavelength: 206.200 nm; 213.857; and 202.548 nm. Injection flow rate: 2.2 mL /min. Integration time: 5-20 sec Measurment Mode: Peak Area Normal resolution

Calibration is carried out with standard solution, freshly prepared with 2 % v/v conc. HNO_3. The stock solution is Perkin Elmer PE Pure. Working range is from 10 ppb to 10 ppm

4. Quality control

Three wavelength are used. The best wavelength is conservated for the expression of results based on correlation coefficient and recovery test at level of +/- 50 % of the concentration range of samples. An analytical run includes blanks and standards. All glassware and polyethylene flasks and pipets are soaked in a 10 % HNO_3 solution and rinsed with Milli-Q-water just before use. All volumetric flasks, glass pipets are calibrated, plastic pipets are checked regularly.

5. Difficulties and limitations

Analysis with Z-ETA-AAS is impossible, because of too high blanks and instability during calibration. At ppb concentrations, they are great risks of contamination. There fore, it is very necessary to limit the number of manipulations, such as described in 3.1.a.

6. Comments and remarks

Not reported.

State Veterinary Institute Olomouc
Laboratory in Kromeriz

Kromeriz, Czech Republic

Zinc

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Analytical technique
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are food, animal feed and water.

2. Sample throughput per year

About 150 samples per year.

3. Procedure

Zinc is assayed by F-AAS.

3.1. Flame Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

a) 1-10 (± 0.1) g of homogenized sample is weighed and dry ashed at a temperature of 450±25 ^{o}C in a programmable furnace overnight. Use is made of 6 % w/w $Mg(NO_3)_2$ as an ashing aid. Ashed sample is cooled down to room temperature, deionized water and 1 ml conc. HNO_3 are added. The sample is re-evaporated and heated up to 450 ^{o}C in the furnace. This procedure is repeated until ashes become white. Ashes are dissolved in 1 M HNO_3.

b) 1 ± 0.1 g of homogenized sample is weighed in 100 ml Teflon vessels. 5 ml HNO_3 (65 %, suprapure) and 1.5 ml H_2O_2 (30 %) are added. The samples are digested into the microwave oven (MLS – Ethos Plus) according to the following programme: 1. 500W, 10 min, 180 ^{o}C; 2. 500 W, 10 min, 200 ^{o}C. After cooling the digested sample solutions are transferred into 10 ml measuring flasks and filled up to 10 ml deionized water.

3.1.b. Analytical Instrumentation

A Perkin-Elmer 2100 Atomic Absorption Spectrometer is used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1.

Table 1. Equipment characteristics

F-AAS
Wavelength: 213.9 nm
Lamp: Zn HCL
Deuterium background correction
Air-acetylene flame

The calibration curve is established by using Zn solutions of 0.20, 0.50, 1.00, 2.00 mg/l.

4. Quality control

The routine use of each method is preceded by analytical validation according to the IUPAC recommendations and the metods are accreditated.
CRMs (BCR 184, BCR 185 R, P-ALFALFA, Tort-2, DORM 2, APS -1075…) are use to check the accuracy and analyzed periodically. An analytical run includes two blanks, duplicates and in-house RM. The results of duplicate CRM, RM analysis samples are monitored on control charts.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Food Safety Veterinary and Food Laboratory

Tartu, Estonia

Zinc

1. The equipment in use

1) Atomic Absorption Spectrophotometer-Thermo Elemental. It has equipped both with GFA and flame compartments. Zeemann and Deuterium correction are available. At the moment the Hydride Vapor System –for the determination of Arsenic is missing.
2) Atomic Absorption Spectrophotometer-Shimadzu - It has equipped both with GFA and flame compartments. Only deuterium correction is available.
3) Microwave owen AntonPaar- closed vessels for wet digestion
4) Muffle furnace Nabertherm

2. Analytical Methods

Element	**Zn**
Accreditation	**yes**
Reference for method	1) AOAC method 999.11 Determination of Lead, Cadmium, Iron and Zinc in Foods. AAS after dry ashing First action 1999. 2) AA Spectrometers Methods. Manual, 2003, Thermo Electron Corp., UK. 3) EVS-EN 13804:2002 –Foodstuffs-Determination of Trace elements-Performance Criteria and general conciderations. 4) EVS-EN 13805:2002 Foodstuffs-Determination of trace elements. Pressure digestion. 5) Nordic Commitee of Food Analyses no 161, 1998. Metals. Determination by AAS after wet digestion in microwave owen.
Sample weight	5 dry ashing >1 mw dig.
Sample preparation	Dry ashing Microwave digestion
Reagents used	HNO_3, H_2O_2, HCl
Quantification (technique)	AAS flame
Wavelenght(nm)	213.9
LOQ	0.1
U %	12

3. Reference materials

Element	**Matrics**	**Ref. value mg/kg**
Zn flame	BCR 184-bovine mussel	166
	SRM 2976	137
	BCR 186	128
	CRM 185R	138.6

National Veterinary and Food Research Institute

Helsinki, Finland

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Flame Atomic Absorption Spectrometry
3.1.a Sample pretreatment
3.1.b Analytical instrumentation
3.1.c Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissues (meat, liver and kidney), plasma and serum.

3. Samples throughput per year

About 20.

4. Procedure

The samples are analyzed by F-AAS

4.1. Flame Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Sample (5-10g) is ashed at 450^{o}C in a muffle furnace overnight. Ashed sample is cooled down to room temperature, deionized water and 1 ml conc. HNO_3 are added. The sample is re-evaporated and heated up to 450^{o} C overnight in the muffle furnace. The residue is dissolved in 0.5 ml of conc. HNO_3 and adjusted to appropriate volume with deionized water.

3.1.b. Analytical instrumentation

Samples are analyzed by air-acetylene AAS(Perkin-Elmer 5100 PC and hollow cathode lamp).

3.1.c. Working conditions

Zinc is measured at 213.9 nm. Measurement are carried out with direct comparison by standard solution (Reagecon) in 0.1M.

5. Quality control

The method is accredited. CRMs and blanks are analyzed together with each sample series. The results of CRMs are monitored on control charts. Analysis of spiked samples are performed. The risk of contamination of samples must always be considered. All glass- and plasticware are washed with acid before use.

6. Difficulties and limitations

Not reported.

7. Comments and remarks

Serum and plasma are analyzed without digestion.

Federal Office for Consumer Protection and Food Safety

Berlin, Germany

Section I

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Flame Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

Section II

Sample ashing procedure

1. Matrices

2. Procedure

Section I

1. Matrices

Samples analyzed are meat, meat products, plant materials, food

2. Sample throughput per year

About 150.

3. Procedures

Zinc is assayed by F-AAS

3.1. Flame Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

See separate section II: (Sample ashing procedure).

3.1.b. Analytical instrumentation

A SpectrAA 300 - AAS with acetylene-air-flame is used.

3.1.c. Working conditions

The calibration curve is plotted by using Zinc solutions of 0.1, 0.2, 0.4 and 0.8 µg/ml in 3 % HNO_3. The analytical blank is a solution of 3 % HNO_3. Limit of detection is 0.02 µg/ml in sample solution.

4. Quality control

Analysis of certified reference materials (BCR, NIST, CBNM) is performed along with each analytical series.

5. Difficulties and limitations

Not reported

6. Comments

These method corresponds to the official German Method for food analysis.

Section II

Sample ashing procedure

1. Matrices

All materials will be mineralized following the same procedure.

2. Procedure

A high pressure ashing system HPA-S equipped with five 90 ml quartz vessels is used. Concentrated HNO_3, 65 % (FLUKA, pA plus) is used. Between 300 mg and 1500 mg of homogenized sample material are weighed into the quartz vessels. Sample amounts are illustrated in Table 1.

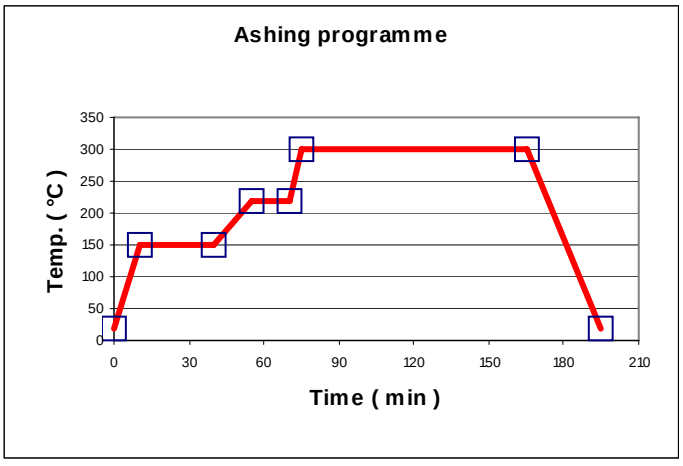
Table1. Sample amounts for use in the HPA-S

Sample	Weight **(mg)**	**Tolerance (mg)**	**Volume of HNO_3 (ml)**
dry samples (< 20 % water)	500	± 50	3.0
fresh samples	1000	± 100	3.0
fresh samples (> 50 % water)	1500	± 200	3.0

Dry samples should be wetted with some drops water before HNO_3 is added; it's to recommend to let stand the vessels over night before heating.

The mineralization start by using a ashing temperature of 300 °C over a hold time of 90 minutes (Fig. 1). When ashing procedure is finished, let cool down to room temperature. Check the volume of HNO_3 in the vessels to recognize leaky vessels. Remove dissolved NO_x gases by a gentle stream of nitrogen at the surface of the sample solution. Add water up to 20 ml or 25 ml and store solutions in polyethylene tubes with screw cap for the AAS analysis.

Fig. 1: Ashing programme of the HPA-S



Agriculture and Food Science Centre

Belfast, Northern Ireland

Zinc

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Flame Atomic Absorption Spectrometry
3.1.a Sample pretreatment
3.1.b Analytical instrumentation
3.1.c Working conditions
3.6. Electrothermal Atomization Atomic Absorption Spectrometry
3.2.a. Sample pretreatment
3.2.b. Analytical instrumentation
3.2.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples most frequently analyzed are of marine origin, such as fish, oysters, mussels and sediments.

2. Samples throughput per year

About 200 samples per year.

3. Procedures

Zinc is analyzed by two different instrumental techniques depending upon the concentration of the element in the sample. Flame atomic absorption spectrometry is the general method of analysis with electrothermal atomization atomic absorption spectrometry being used to a lesser extent for lower concentrations of elements.

3.1 Flame Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Samples are dry-ashed in a muffle furnace temperature programmed from room temperature to 450 °C. The ash is dissolved in 0.1 *N* HNO_3.

3.1.b. Analytical instrumentation

A Varian SpectrAA-400 Atomic Absorption Spectrometer with deuterium lamp background correction is used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1.

Table 1. Equipment characteristics.

F-AAS
Flame, air-acetylene
Wavelength, 213.9nm
Slit width, 1.0 nm
Deuterium background correction
Lamp current 5.0mA

A calibration curve is established by using Zn standard solutions of 0, 0.2, 0.4, 0.6, 0.8 and 1.0 µg/g in 0.1 *N* HNO_3.

3.2. Electrothermal Atomization Atomic Absorption Spectrometry

3.2.a. Sample pretreatment

Samples are dry-ashed in a muffle furnace temperature programmed from room temperature to 450 ^{o}C. The ash is dissolved in 0.1 *N* HNO_3.

3.2.b. Analytical instrumentation

A Varian SpectrAA-300 Atomic Absorption Spectrometer with Zeeman background correction, graphite furnace and autosampler is used.

3.2.c. Working conditions

Instrumental parameters are reported in Tables 2 and 3.

Table 2. Equipment characteristics.

ETA-AAS
Pyrotylitic coated graphite tube
Wavelength, 213.9 nm
Slit width, 1.0 nm
Zeeman background correction
Injection volume, 5 µl sample

Table 3. Graphite furnace programme

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)	Ar Flow (ml/min)
Drying	85 95 120	5 40 10	0 0 0	300 300 300
Ashing	300	5	7	300
Atomization	2000	1	2	0
Clean-up	2600	2	2	300

A calibration curve is established using Zn standard solutions of 0.002, 0.004 and 0.006 µg/g in 0.1 *N* HNO_3.

4. Quality control

The laboratory quality system is based on GLP and UKAS principles. Method validation is performed by using CRM's or spiked samples as appropriate. CRM's are analyzed periodically to check accuracy. During routine analysis, triplicate spiked samples or CRM's are included with each batch of samples analyzed, along with three reagent blanks to control potential contamination. The precision and accuracy is monitored on quality control charts using recovery results.
Instruments are optimized before each batch of analyses, and details recorded of lamp photomultiplier voltage and absorbance for a given standard. Plot linearity is also checked.
The laboratory participates in proficiency testing exercises, such as the UK Food Analysis Performance Assessment Scheme (FAPAS).

5. Difficulties and limitations

The main problem is potential contamination and purity of chemicals. Dry-ashing requires careful control. The temperature should be kept as low as is possible because of the danger of loosing volatile inorganic compounds, but at the same time high enough to ensure complete combustion of all the organic matter in a reasonably short time. Fatty samples may require additional treatment and precautions when dry-ashing.

6. Comments and remarks

Not reported.

Istituto de Investigação das Pescas e do Mar

Lisboa, Portugal

Zinc

1. Matrices

2. Samples throughput per year

3. Procedure
3.1. Flame Atomic Absorption Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissue (liver), and premixes for animal feeds.

2. Samples throughput per year

About 10 samples per week

3. Procedure

Zinc is assayed by F-AAS.

3.1. Flame Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

Approximately 100 g of sample are homogenized, 1 g is weighed in quartz crucibles and 1 ml of $MgNO_3$ 50 % are added. The samples are pretreated in a hot place until the test portion is completely carbonized, followed by ashing in a muffle furnace at 550 °C ± 10 °C overnight.

The samples are then removed and wet with concentrated HNO_3 and then put back into the furnace. This procedure is repeated every hour until ashes become white. The ashes are then quantitatively transferred to 25 ml volumetric flasks with 5 % HNO_3.

3.1.b. Analytical instrumentation

An F-AAS Unicam Model 969 instrument is used.

3.1.c. Working conditions

Instrumental parameters are reported in Table 1.

Table 1. Equipment characteristics.

F-AAS
Wavelength, 213.9 nm
Deuterium background correction
Injection volume, 1000 µl samples
Fuel flame concentration, 1.0 mg/L

Calibration plots are set up by using Zn solutions of 0.2, 0.5, 1.0 and 2.0 µg/ml.

4. Quality control

Each analytical run includes blanks, commercial standard solutions, CRM (BCR 185-R, bovine liver and BCR 184, bovine muscle) and Quality Control Standards for Trace Metals – AA by RTC.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

Ministerio de Agricultura, Pesca y Alimentación

Madrid, Spain

1. Matrices

2. Samples throughput per year

3. Procedure
3.3. Inductively Coupled Plasma Atomic Emission Spectrometry
3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are, in order, wine, spirits, vinegar, beer, orange juice, water, cookies, various kinds of pasta, canned vegetables, liver and kidneys.

2. Samples throughput per year

About 1200.

3. Procedure

Zinc is assayed by ICP-AES.

3.2. Inductively Coupled Plasma Atomic Emission Spectrometry

3.1.a. Sample pretreatment

Sampling is not made by the Laboratory Staff, but by official inspectors. The techniques for storage and subsampling are different according to the product; *i.e.*,: milk and milk products, shake; meat, cut in small pieces; eggs, cook; fish, clean and cut; crustaceans, peal and cook. Afterwards homogenizing in kitchen grinder is performed. Digestion of the samples is carried out in a microwave oven. The acids used for digestion are different depending on the nature of the sample processed. In the case of the samples included in the National Residue Investigation Plan, namely liver and kidneys, the reagents used for sample digestion are the following: HNO_3, $HclO_4$ and H_2O_2.

3.1.b. Analytical instrumentation

A CEM Model NSD-81D microwave oven and a Varian Liberty 220 Inductively Coupled Plasma Atomic Emission Spectrometer is used.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 1.

Table 1. Equipment characteristics.

ICP-AES
Wavelength, 213.856 nm
Nebulizer, 150 kPa
Argon flows, plasma 10.5 l/min, auxiliary 1.5 l/min
Power output, 1.00 kw
High observation, 16 mm
Integration time, 3 s

The calibration curve is plotted by using Zn solutions of 0.1, 1, 5 and 10 mg/l.

4. Quality control

The routine use of each method is preceded by analytical validation according to the IUPAC recommendations in order to ascertain sensitivity, detection power, precision and accuracy. CRMs of bovine liver (NIST) and mussel tissue (SMT) are used to check the accuracy and analyzed periodically. During routine assays, each analytical batch includes at least three reagent blanks to evaluate the detection limits and to control possible contaminations. Recoveries are calculated using two spiked reagent blanks and one spiked sample for each non-spiked sample assayed.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.

National Food Administration

Uppsala, Sweden

1. Matrices

2. Samples throughput per year

3. Procedure

3.4. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analyzed are animal tissues, cereals and vegetables.

2. Samples throughput per year

About 300-500 samples per year.

3. Procedure

Copper is determined by ETA-AAS and F-AAS after either dry ashing or microwave digestion.

3.5. Electrothermal Atomization Atomic Absorption Spectrometry

3.1.a. Sample pretreatment

a) About 1-10 g of sample is weighed into a platinum crucible and dry ashed at a temperature of 450 ± 25 °C in a programmable furnace. The carbon-free ash is dissolved in 20-30 ml of 0.1 M HNO_3 and transferred to a plastic container.

b) A maximum of 0.5 g dried, homogenized sample is weighed into a Teflon vessel, followed by the addition of 5 ml conc HNO_3 and 2 ml 30% H_2O_2.

The digestion is carried out in a CEM (MDS 2000) microwave oven according to the programme detailed in Table 1.

Table 1. Digestion programme.

Step	Power (W)	Time (min)
1	250	3
2	630	5
3	500	22
4	0	15

3.1.b. Analytical instrumentation

Perkin Elmer Z-5100 with HGA-600 autosampler AS-60.

3.1.c. Working conditions

Instrumental parameters are reported in Tables 2 and 3 for the PE Z-5100 instrument.

Table 2. Equipment characteristics for PE Z-5100.

Z-ETA-AAS
Wavelength, 213.9 nm
Slit width, 0.7 low
Lamp, Zn HCL
Zeeman background correction
L'vov platform
Injection volume, 10 µl sample
Pyrolytic coated graphite tubes

Table 3. Graphite furnace programme.

Step	**Ramp Time (s)**	**Hold Time (s)**	**Temperature (°C)**
Injection	-	-	Ambient
Drying	20	10	90/130
Pyrolysis	15	10	400
Atomization*	0	3	1200
Cleaning out	1	5	2600

* Gas stop

Calibrations are generally carried out by standard addition method.

3.2. Flame Atomic Absorption Spectrometry

3.2.a. Sample pretreatment

See section 3.1.a.

3.2.b. Analytical instrumentation

A Varian SpectrAA 220FS with D2-BC is used.

3.2.c. Working conditions

The instrument is equipped with a single slot burner and utilises an oxidizing air acetylene flame. Wavelength 213.9 nm. Results are calculated from a standard curve.

4. Quality control

An analytical run includes one-two blanks and one or more CRM or in-house RM. Samples are measured as singles or duplicates, depending on the requirements. Accuracy and precision are monitored by the use of control charts on which the results of duplicate CRM and RM analysis are plotted. The detection limit is based on three times the standard deviation of large number of blanks and is currently 13 ng/ml in sample solution.

5. Difficulties and limitations

Extremely fatty or sugar rich samples require extra attention when dry ashing is used. The microwave digestion method is not suitable for fats, oils or extremely fatty products.

6. Comments and remarks

The dry ashing method has been collaboratively tried and was approved by the Nordic Committee for Food Analysis (NMKL) in 1990.
The report was published in *JAOAC* Int. 76 (1993), n. 4. (Lars Jorhem, Determination of metals in foodstuffs by AA Spectrophotometry after dry ashing: An NMKL collaborative study of Cd, Cr, Cu, Fe, Ni, Pb and Zn.). This method was validated on January 1st 1996.

The microwave digestion method has been collaboratively tried and was approved by NMKL in 1997.

The result of both collaboratively trials have been submitted to the AOAC Methods Committee and were accepted in December 1999.

National Institute for Public Health and Environment
Rijksinstituut voor Volksgezondheid en Milieu

Bilthoven, The Netherlands

Zinc

1. Matrices

2. Samples throughput per year

3. Procedure

3.1. Inductively Coupled Plasma Mass Spectrometry

3.1.a. Sample pretreatment
3.1.b. Analytical instrumentation
3.1.c. Working conditions

4. Quality control

5. Difficulties and limitations

6. Comments and remarks

1. Matrices

Samples more frequently analysed are meat, rain water, ground water, urine, animal feed, soil and aerosol particles.

2. Samples throughput per year

About 3000.

3. Procedure

Zinc is analysed by ICP-MS.

3.1 Inductively Coupled Plasma Mass Spectrometry

3.1.a. Sample pre-treatment

Solid matrices are digested in a microwave oven. For 0.5 g of homogenised biological sample 1 ml of concentrated HNO_3 and 9 ml H_2O is used.
For soil samples (particle size < 250µm) an aqua regia digestion is performed.

3.1.b. Analytical instrumentation

An HP4500+ quadrupole ICP-MS is used.

3.1.c Working conditions

Instrumental parameters are reported in Table 1.

Table 1. **ICP-MS HP4500+** Equipment characteristics.

Analytical mass, ^{66}Zn
Reference mass, ^{125}Te (added on-line (dilution sample/IS: 1: 5.5))
Nebulizer, V-groove, Scott-type double pass spraychamber
Argon flow, nebulizer 1.17 l/min, R.f. power 1250W
Measuring conditions: 3 points / mass; integration time 1.5 s / mass, 3 replicates.
Two-point calibration, standards concentration, 0 and 2000 µg/l
Matrix matching for calibration standards
Equation ^{66}Zn (-0.000041*Mg26-0.000088*S34-0.000389*Ba^{2+}69), equation is updated in every measurement sequence using (a) suitable Interference Check Solution(s)

4. Quality control

Each analytical run includes 1 blank, 1 CRM and 1 RM. The instrumental conditions are controlled by the analysis of an in-house water reference material. The accuracy of the analytical procedure, including sample digestion, is estimated by the use of CRMs. A number of matrix samples are analysed and recovery experiments are carried out for Zn.

5. Difficulties and limitations

Not reported.

6. Comments and remarks

Not reported.