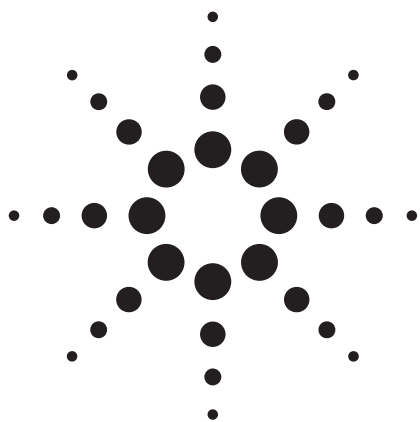




Low Level Measurement of Cadmium in Foods

Application Note

Atomic Absorption

Author

Bruce Shelley

Abstract

This method is applicable to a wide range of animal and plant tissues including dairy products, grains, fruit and vegetables and oil seeds such as poppy seeds. It is suitable for cadmium concentration in the range 0.01-0.3 $\mu\text{g/g}$. Sample is digested by wet ashing and cadmium is then complexed and extracted into an organic solvent. After removal of the spent aqueous phase the organo metallic complex is destroyed by the addition of dilute HNO_3 . This aqueous phase containing the stripped cadmium is then analyzed by Graphite Furnace Atomic Absorption Spectroscopy (GFAAS). The additional stripping step is employed to improve precision and facilitate the use of aqueous standards.

Introduction

Of the naturally occurring elements, only about 26 are known to be essential. A large number of elements occur in foodstuffs as environmental contaminants, sometimes at levels which are considered toxic. Cadmium is a toxic element whose levels in food needs to be carefully monitored.



Agilent Technologies

Reagents and Chemicals

- Ammonium acetate, BDH AnalaR grade
- Ammonium dihydrogen orthophosphate, UNIVAR analytical reagent grade
- Ammonium pyrrolidine dithiocarbamate (APDC), BDH spectrosol grade
- Di-isobutyl ketone (DIBK), UNILAB laboratory grade
- Milli Q Water, 18 mΩ - cm
- Nitric acid, MALLINCKRODT Transistar grade: CAUTION CORROSIVE
- Perchloric acid, MALLINCKRODT, AR Select grade (70%) : CAUTION CORROSIVE
- Titrisol cadmium standard solution, MERCK

50% w/v Ammonium Acetate

500 g of $\text{CH}_3\text{COONH}_4$ dissolved in Milli-Q water and diluted to 1 liter in a standard flask. Possible cadmium contamination is removed by the following procedure. Add approximately 0.5 g APDC, mix thoroughly and add 10 mL DIBK. After shaking for 60 seconds the mixture is allowed to settle before removal of the DIBK fraction. Successive 10 mL portions of DIBK are added, shaken and removed until the aqueous layer gives an absorbance ≤ 0.04 Abs when using the graphite furnace program listed. This buffer solution is stable for 1 month.

0.25% Ammonium Phosphate/0.75% HNO_3

5 g of $\text{NH}_4\text{H}_2\text{PO}_4$ is dissolved in Milli Q water. 15 mL concentrated HNO_3 is added and made up to 2 litres. Cadmium contamination is removed using the same procedure as for the 50% ammonium acetate. This solution is stable for 1 year.

2% Ammonium Pyrrolidine Dithiocarbamate

1 g of APDC is dissolved in 50 mL of Milli-Q water. The solution does not need to be filtered but must be made up fresh each time it is used.

Standard Cadmium Solution

One liter of a 1000 $\mu\text{g/mL}$ Cd solution is made up using Titrisol cadmium standard solution. This stock solution is diluted to 10 $\mu\text{g/mL}$, with subsequent dilutions to 0.10 $\mu\text{g/mL}$, 0.0125 $\mu\text{g/mL}$ and 0.0063 $\mu\text{g/mL}$. The latter three solutions should be made up weekly.

Calibration Standards: (See Appendix 1)

The calibrating standards are prepared according to the following dilution scheme.

Vol. of 0.1 mg/mL Cd	$\mu\text{g/mL}$	Typical Abs (mL)
0	0	0.000
1	0.001	0.140
2.5	0.0025	0.330
5.0	0.005	0.560
7.5	0.0075	0.750

Standards made up to 100 mL in 3% HNO_3 .

The calibration standards and 0.1 mg/mL Cd solution should be made up weekly.

Instrumentation

An Agilent GTA 95 graphite furnace atomizer with autosampler was used with an Agilent AA-875. Furnace parameters are shown.

Editor's Note - This program will be directly transferrable to a SpectrAA instrument with GTA 96.

Temp ($^{\circ}\text{C}$)	Time (secs)	Flow (L/min)	Gas	Read command
75	5.0	3.0	Ar	
90	60.0	3.0	Ar	
600	10.0	3.0	Ar	
600	2.0	0		
2100	0.9	0		*
2100	2.0	0		*
2400	1.0	3.0	Ar	

Sample injection volume 10 μL . Modifier injection volume 15 μL .

Sample Preparation

Rubber is a major potential source of contamination. Rubber gloves should not be used for sample preparation, digestion or subsequent manipulations including acid washing of vessels. Vinyl examination gloves may be satisfactory.

Fruit and Vegetables

Following the procedure laid out in Appendix 2, samples are dried at 80 °C for 3–5 days. The higher the sugar content the longer this takes. Once dry the sample is fine ground in a non-metallic ring mill for 30–90 seconds according to quantity and hardness of sample. Oily samples, for example, avocados, should be freeze dried prior to grinding.

Grains

Grains are analyzed as received but require fine grinding using a Makla mill.

Egg

A representative sample is taken from each packet of eggs and the contents blended together to form a homogeneous mixture using a kitchen blender or similar mixer. Homogenized egg samples must be kept refrigerated.

Dairy Products

Dairy products are analyzed as received. If possible the sample (for example milk, yoghurt, butter) should be blended with a hand mixer to ensure homogeneity. Where this may not be possible for example mature tasty cheese, the sample should be grated and mixed thoroughly before subsampling. Dairy products must be kept refrigerated.

Oil Seeds

Oil seeds are analyzed as received. Small seeds such as poppy seeds can be analyzed intact or may be freeze dried and ground as in fruits and vegetables.

Procedure

Before proceeding with the analysis, ensure adherence to the Quality Assurance procedure.

1. Weigh 0.5 g grain/egg/dairy product/oil seed or 0.25 g vegetable/fruit into clean, dry digestion tube.
2. Add 2 mL conc HClO_4 and 4 mL conc HNO_3 .

3. Leave overnight to cold digest.
4. Place in digestion block. Allow 1 1/2 hrs to reach 210 °C from room temperature.
5. After 1 hour check samples for fumes of HClO_4 , continue checking until fumes appear.

Warning - HClO_4 is a hazardous chemical and extreme care must be taken in handling this reagent. Perchloric acid digests – particularly of organic materials – are also hazardous. Their use can cause a fire or explosion resulting in injury or death. ALWAYS evaporate digests in a suitable fume cupboard. NEVER evaporate to dryness.

6. Remove to cool.
7. Add 18 mL of Milli Q water.
8. Add 3 mL of the 50% ammonium acetate solution.
9. Add 1 mL of the 2% APDC, swirl tube to mix.
10. Add 10 mL of DIBK into tube and shake for 1 minute.
11. After 15 minutes remove aqueous layer using a suitable pipette pump (See Appendix 3).
 - Precision deteriorates with increasing time at this step. It is best to have two operators at this point. As one removes the aqueous phase the other adds DIBK and shakes the solution. A new disposable glass pasteur pipette is used for each sample.
 - When using the modified pi pump raise the plunger a couple of millimeters before inserting the pasteur pipette. When the pipette tip has entered the aqueous phase push the plunger home to expel any organic phase that may have entered the pipette.
 - The aqueous phase is removed by withdrawing the plunger. Keep pipette tip at the lowest point of the tube and note the rate of reduction in the meniscus to avoid over-shooting.
 - Keep discarded aqueous phases (collective) to check the pH range is between 3.0–4.0. It has been found that precision deteriorates with decreasing pH and sensitivity deteriorates with increasing pH.
12. Add 10 mL of 3% HNO_3 (for grain, egg, dairy, and oilseed samples) or 20 mL of HNO_3 (for vegetable and fruit samples), cap tube and shake for 1 minute.

13. After 1 hour remove the sample of aqueous phase and analyze.

- Time is not critical after 1 hour.
- Extra back stripping step improves precision of the determination.
- Samples may be kept and analyzed up to three days later provided the spent organic phase is removed. Failure to do so will produce erratic results.

14. Set up GFAAS as per manufacturer's instructions.

- Use 0 µg/mL Cd standard in Blank position.
- Use 0 µg/mL to 0.0075 µg/mL Cd calibration standards.
- Calibrate instrument zero before the run.
- During run calibrate zero on 0 µg/mL Cd in the Blank position, thus the zero calibration can then be rechecked during the calibration.
- Operate in peak height mode with two replicate readings for each sample.
- Reslope after every 10 samples using 0.0025 µg/mL Cd standard.
- Reagent Blank is run as a sample and the result subtracted from the subsequent samples.

An Agilent DS15 computer is used in conjunction with the Agilent Rational Algorithm to calculate results. Due to the curve-linear response, linear, quadratic and higher polynomials are unsuitable.

Results must have dry matter corrections made before reporting.

Accuracy

The accuracy of the procedure was checked by analyzing an NIST standard reference material (wheat flour). The results are presented here for 18 replicate measurements.

SRM1567 wheat flour	specified 0.032 + 0.007 mg/g Cd
	obtained 0.029 + 0.006 mg/g Cd
	[± 1σ]

Detection Limit

Based on ten readings for both a blank solution and 0.0005 mg/mL cadmium standard, the detection limit is better than 0.0005 mg/mL Cd in solution (5s). For grains, this equates to better than 0.01 mg/mL Cd in the sample.

Reporting limit is therefore 0.02 mg Cd/g for grains (10σ)

The detection limit may be improved by using up to 20 µL injected volume into the graphite furnace and as little as 5 mL of 3% HNO₃ in the back stripping step.

Precision

3% RSD at 0.032 µg/g Cd level, and typical precision measurements have shown better than 13% at 0.015 µg/g Cd.

Quality Assurance

Every tenth sample is analyzed in duplicate. The thirtieth sample is analyzed in triplicate with 1 mL of 0.0065 µg/g Cd (fruit,vegetables) or 1 mL of 0.0125 µg/g Cd (grains, egg, dairy, oil seeds) added to the third replicate. A recovery calculation is made on those samples as follows:

$$\frac{\mu\text{g/g Cd in spiked sample} - \mu\text{g/g Cd in sample}}{\mu\text{g/g Cd in sample due to spike}} \times 100$$

The recovery should be between 80% and 120%. A correction may be made for recovery: Corrected result = Apparent result × 100/recovery. In addition a primary standard is analyzed in each batch (eg. NBS 1567, wheat flour). The certified value for NBS 1567 is 0.032 ± 0.007 µg/g Cd. The value for NBS 1567a is 0.026 ± 0.002 µg/g Cd.

Results

Maximum recommended levels set by the Australian National Health and Medical Research Council (NH & MRC) are as follows:

Cadmium in Wheat Germ = 0.2 µg/g

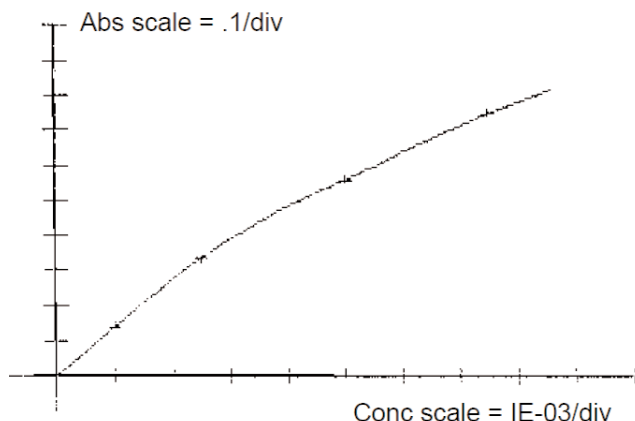
Cadmium in all other foods = 0.5 µg/g

Typical levels found from this study are shown below:

Grains	< 0.02 – 0.07 µg/g Cd
Vegetables	< 0.02 – 0.07 µg/g Cd
Fruit	< 0.02 µg/g Cd
Eggs	< 0.02 µg/g Cd
Milk	< 0.02 – 0.05 µg/g Cd
Cheese	< 0.02 – 0.05 µg/g Cd
Yoghurt	< 0.02 – 0.05 µg/g Cd
Butter	< 0.02 – 0.05 µg/g Cd
Oil seeds	< 0.05 – 0.2 µg/g Cd

Appendix 1

Typical calibration graph



Conclusion

The method has shown that cadmium can be measured at low levels in a variety of food stuffs by GFAAS. Samples are digested, cadmium is complexed and extracted into an organic solvent, then back extracted into an aqueous phase.

Appendix 2

Preparation Methods for Analysis of Chemical Residues

Samples for analysis should contain tissue from a number of items of the one commodity. If not already established, a moisture determination must be made for each commodity.

Vegetables

Beans	Wash prior to analysis.
Beans (broad)	Remove seed, thick stems and use sections of floral heads.
Bean Sprouts	Remove outer leaves and wash for analysis.
Cabbage	Remove outer leaves and wash radial sections of the heart for analysis.
Capsicums	Wash and use sections of flesh for analysis.
Carrot	Peel, top and tail, and wash root sections for analysis.
Cauliflower	Remove thicker stems and wash curd sections for analysis.
Celery	Wash stem sections for analysis.
Leek	Wash sections of leaves for analysis.
Lettuce	Remove outer leaves and wash radial sections of the heart for analysis.

Melon	Section radially, remove skin and seeds and analyze the flesh.
Onion	Remove outer skin and analyze the flesh.
Potatoes	Peel, wash and analyze flesh.
Silverbeet	Use section of washed stem for analysis.
Sweetcorn	Remove and wash the seeds for analysis.
Tomato	Wash and use sections of flesh with skin attached for analysis.
Zucchini	Wash and use mid-sections for analysis.

Fruit

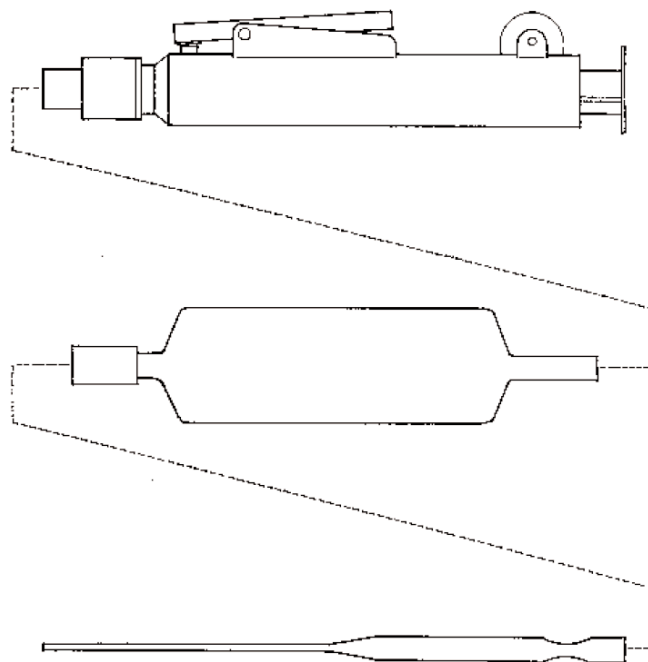
Apples	Wash remove core and analyze sections of flesh with skin attached.
Apricots	Wash, remove stone and analyze sections of flesh with skin attached.
Avocados	Remove skin and stone and analyze sections of flesh only.
Bananas	Remove skin and analyze flesh.
Berries	Wash, remove the small green leaves at the calyx and analyze the berries.
Cherries	Wash, remove stalk and stones and analyze the flesh with the skin attached.
Citrus	Peel and use segments for analysis.
Grapes	Remove berries from the stem and wash.
Kiwifruit	Remove peel and analyze sections of flesh.
Mango	Remove skin and stone and analyze sections of flesh.
Pawpaw	Remove skin and seeds and analyze sections of flesh.
Olive	Wash, remove stones and analyze the flesh with the skin attached.
Peach	Wash, remove stone and analyze the flesh with the skin attached.
Nectarine	Wash, remove stone and analyze the flesh with the skin attached.
Plum	Wash, remove stone and analyze the flesh with the skin attached.
Pear	Wash, remove core and analyze the flesh with the skin attached.
Persimmon	Wash, and analyze flesh with the skin attached.
Pineapple	Remove skin and analyze flesh.
Strawberries	Remove leaves and calyx and wash lightly.

Appendix 3

The tool for removing aqueous phase consists of a 25 mL pipette pump with a cut down 50 mL pipette.

A piece of tygon tubing is used to attach disposable glass pasteur pipettes. A new pasteur pipette is used for each sample.

If the pasteur pipette is found to contaminate the sample then it should be acid washed before use.



References

1. B. E. Limbek, and C. J. Rowe, (1986). Curve Correction in Atomic Absorption. "Varian Instruments at Work" No. AA-58, April 1989. Varian Techtron Pty Ltd., Mulgrave, Victoria, Australia.
2. K. M. Bone, and W. D. Hibbert, (1979). *Analytica Chimica Acta*, 107, 219-229.
3. S. R. Koirtiyohann, and J. W. Wen, (1979). *Anal. Chem.* 45, 1986-89.
4. M. P. C. De Vries, and K. G. Tiller, and R. S. Beckwith, (1975). *Comm. Soil Science & Plant Analysis* 6, 629-640.
5. I. Okuno, et al, (1978). *J. Assoc. Off. Anal. Chem.* 61, 664-667.
6. R. W. Dabeka, (1979). *Anal. Chem.*, 51, 902-907.
7. Vollard et al (1978). *Anal. Chem.* 61, 664.

For More Information

For more information on our products and services, visit our Web site at www.agilent.com/chem

www.agilent.com/chem

Agilent shall not be liable for errors contained herein or for incidental or consequential damages in connection with the furnishing, performance, or use of this material.

Information, descriptions, and specifications in this publication are subject to change without notice.

© Agilent Technologies, Inc., 1990
Printed in the USA
November 1, 2010
AA097



Agilent Technologies