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**THE DETERMINATION OF CADMIUM IN OILS AND
FATS BY DIRECT GRAPHITE FURNACE ATOMIC
ABSORPTION SPECTROMETRY**

(Technical Report)

Prepared for publication by

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The determination of cadmium in oils and fats by direct graphite furnace atomic absorption spectrometry (Technical Report)

Abstract: A description is given of the development by collaborative study of a standardised method for the determination of cadmium in crude and refined oils and fats by graphite furnace atomic absorption spectrometry. The procedure is rapid and sensitive allowing determination at levels of 1–10 µg/kg

INTRODUCTION

Cadmium in oils and fats is considered as an inorganic contaminant and its determination is important due to its high toxicity.

Graphite furnace atomic absorption spectrometry (GFAAS) has been accepted by IUPAC for the determination of copper, iron and nickel [1], and also lead [2] in oils and fats. A similar method for the determination of cadmium has been developed and tested by means of two ring tests.

METHODS OF ANALYSIS

Two different methods have been tested in this collaborative study (Table 1).

The procedure tested during the first ring test required a graphite tube furnace a L'vov platform both coated with niobium and an additional preashing step under oxygen-rich atmosphere in order to prevent losses of cadmium during thermal treatment [3]. But shortened furnace life-times have been observed by several participants, mainly due to the use of oxidising conditions during the pre-ashing step.

Therefore, a second procedure [4] using a palladium matrix modifier and an untreated pyrolytic platform was chosen and tested during the second ring test. This matrix modification method used to decrease the volatility of the analyte, allows to work under inert atmosphere and then avoids the above mentioned drawbacks.

Collaborative study

First ring test—realised in 1994 with the first analytical method

Materials provided for the study were edible oils (sunflower oil) containing cadmium at three concentration levels (high, medium, low). Each concentration level was provided in duplicate (blind coded) so that participants received in total six samples.

Second ring test—realised in 1994 with the second analytical method

Materials provided for the study were edible oils (sunflower oil) and fats (lard) spiked with cadmium at concentration levels (high, low). Each concentration level was provided in duplicate (blind coded) so that participants received in total eight samples.

Both methods have been subjected to an international collaborative study by laboratories world-wide. Participants were asked to analyse each sample in duplicate and to report both values obtained. A statistical evaluation of the data was made for each level and for type of sample separately according to ISO 5725.

Results of the first ring test (1992)

Five laboratories' data have been collected for evaluation. As the six samples were in fact three pairs of identical samples the mean of the two values obtained for each sample has been calculated. Then, for each

Table 1 Comparison of the two analytical procedures tested

	Method 1	Method 2
Wavelength	228.8 nm	228.8 nm
Slit	0.7	0.7
Furnace tube	graphite coated with niobium	graphite
Platform	pyrolytic coated with niobium	pyrolytic
Matrix modifier	–	Palladium
Pre-ashing step	600 °C/air	–
Ashing step	900 °C/argon	650 °C argon
Atomisation step	2400 °C	1600 °C
Limit of detection	0.25 µg/kg	0.2 µg/kg

concentration level, the differences between the blind duplicates have been tested for outliers according to Cochran & Dixon.

All the data have been in the evaluation due to the reduced number of participants, and the final calculations of repeatability and reproducibility have been achieved with five laboratories.

Concentration levels, average recoveries (µg/kg) and precision parameters are listed in Table 2. The Commission concluded that for a first test, the precision results were satisfying. It was decided to organise another interlaboratory study with more participants. Due to some comments of laboratory participants, the analytical procedure was modified as described above.

Table 2 Concentration levels, average recoveries (µg/kg) and precision parameters for the first ring test (1992)

Concentration level	Actual values	Averages (µg/kg)		Repeatability (<i>r</i>)			Reproducibility (<i>R</i>)		
		Sample	Level	<i>S_r</i>	<i>r</i>	<i>CV_r</i> (%)	<i>S_R</i>	<i>R</i>	<i>CV_R</i> (%)
High level samples	15.0	15.4	15.5	0.75	2.11	4.8	3.06	8.67	19.8
	15.0	14.2							
Medium level samples	8.1	7.5	7.6	0.55	1.56	7.2	0.89	2.52	11.6
	8.1	7.6							
Low level samples	2.0	1.8	1.7	0.28	0.78	16.0	0.31	0.89	18.2
	2.0	1.7							

S_r: repeatability standard deviation; *r* = repeatability limit ($2.83 \times S_r$); *CV_r*: repeatability coefficient of variation (as percentage of the mean values).

S_R: reproducibility standard deviation; *R* = reproducibility limit ($2.83 \times S_R$); *CV_R*: reproducibility coefficient of variation (as percentage of the mean values).

Results of the second ring test (1994)

Data screening. Eight laboratories data have been received for evaluation. It has been decided to omit the data from one laboratory because of deviation from the method, samples being prepared by microwave digestion instead of being directly injected in the AAS furnace.

The data from the remaining seven laboratories have been subjected tests for outliers according to Cochran & Dixon.

Cochran test. As the eight samples were in fact four pairs of identical samples, the mean of the values obtained for each sample has been calculated and then, for each concentration level, the differences between the blind duplicates have been tested for outliers according to Cochran's procedure.

One laboratory has been eliminated on the basis of the Cochran's test for the two 'high' concentrations levels (edible oil and fat).

Dixon test. After elimination of the significant outliers (i.e. significant at the 1% level) according to Cochran's test, the different concentration levels have been tested for outliers according to Dixon's procedure.

None of the laboratories have been eliminated. Hence, for the final calculations of precision parameters, there remained seven laboratories for the 'low' concentration levels (oil and fat) and six laboratories for the 'high' concentration levels (oil and fat).

Precision. In Tables 3 and 4, sample averages, concentration level averages and precision parameters in oil or in fat have been listed.

Table 3 Concentration levels, average recoveries ($\mu\text{g}/\text{kg}$) and precision parameters for the second ring test (1994)—edible oil (sunflower)

Concentration level	Actual values	Averages ($\mu\text{g}/\text{kg}$)		Repeatability (r)			Reproducibility (R)		
		Sample	Level	S_r	r	CV_r (%)	S_R	R	CV_R (%)
Medium level samples	8.0	7.87	7.87	0.31	0.87	3.9	0.99	2.94	13.2
	8.0	7.54							
Low level samples	2.0	1.54	1.58	0.39	1.11	24.9	0.55	1.55	34.8
	2.0	1.56							

S_r : repeatability standard deviation; r = repeatability limit ($2.83 \times S_r$); CV_r repeatability coefficient of variation (as percentage of the mean values).

S_R : reproducibility standard deviation; R = reproducibility limit ($2.83 \times S_R$); CV_R reproducibility coefficient of variation (as percentage of the mean values).

No simple relationship between repeatability limit, or reproducibility limit, and the cadmium concentration has been established.

Discussion (second ring test)

The relative repeatability values (CV_r) are included between 4 and 25% depending on the concentration level. These values are comparable to those obtained during the first ring test.

The relative reproducibility values (CV_R) are included between 13 and 35% depending on the concentration level. According to Horwitz [5], the relative reproducibility should be about 32% at the 10 $\mu\text{g}/\text{kg}$ level and 45% at the 1 $\mu\text{g}/\text{kg}$ level, for an analytical method to be acceptable.

The precision results obtained for both oil and fat during the second ring test are in agreement with this criterion.

Table 4 Concentration levels, average recoveries ($\mu\text{g}/\text{kg}$) and precision parameters for the second ring test (1994)—fat (lard)

Concentration level	Actual values	Averages ($\mu\text{g}/\text{kg}$)		Repeatability (r)			Reproducibility (R)		
		Sample	Level	S_r	r	CV_r (%)	S_R	R	CV_R (%)
Medium level samples	7.0	7.50	7.17	0.61	1.74	8.6	0.92	2.61	12.9
	7.0	6.96							
Low level samples	3.0	2.89	2.70	0.23	0.65	8.5	0.59	1.68	22.0
	3.0	2.71							

S_r : repeatability standard deviation; r = repeatability limit ($2.83 \times S_r$); CV_r repeatability coefficient of variation (as percentage of the mean values).

S_R : reproducibility standard deviation; R = reproducibility limit ($2.83 \times S_R$); CV_R reproducibility coefficient of variation (as percentage of the mean values).

CONCLUSION

Direct graphite furnace atomic absorption spectrometry is a rapid and very sensitive method which allows reliable determination of the concentration of cadmium in edible oils and fats.

After an extensive collaborative study where two different analytical procedures have been evaluated, the working group IUPAC WG 3/93 found the second procedure acceptable as an analytical method for the determination of traces of cadmium.

On the basis of the repeatability and reproducibility of the results obtained during the second ring test, the Commission has decided to adopt the method.

The text of the standardised procedure is given in the Appendix.

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APPENDIX: DETERMINATION OF CADMIUM BY DIRECT GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETRY

1. Scope and field of application

This standard describes a method for the determination of trace amounts ($\mu\text{g}/\text{kg}$) of cadmium in all types of crude or refined edible oils and fats. The limit of detection is defined as $0.2 \mu\text{g}/\text{kg}$.

2. Principle

Incineration and atomisation of the oil or fat in a suitable graphite tube furnace with platform connected to an atomic absorption spectrometer, previously calibrated using standard solutions of organo-compound of cadmium. Measurement of the metal content from the observed absorption at a wavelength of 228.8 nm. Addition of palladium as a matrix modifier in order to prevent loss of cadmium during the thermal treatment.

3. Apparatus

- 3.1 Polyethylene or polypropylene capped bottles—20 and 50 mL, metal free (see Note 1).
- 3.2 Micropipettor—10 and 20 μL .
- 3.3 Pipettor tips.
- 3.4 Volumetric flask—100 mL
- 3.5 Electric oven regulated at $60 \pm 5^\circ\text{C}$.
- 3.6 Atomic absorption spectrometer—Equipped with ‘peak area’ mode and ‘autocalibrate’ mode together with the appropriate electrode-less discharge lamp (or hollow cathode lamp) and deuterium background corrector or Zeeman atomic absorption spectrometer).
- 3.7 Graphite furnace atomizer—Placed in the atomic absorption spectrometer (3.6), equipped with a control unit for temperature programming.
- 3.8 Graphite tube uncoated or pyrolytically coated.
- 3.9 Platform—pyrolytic.
- 3.10 Autosampler for graphite furnace atomizer (optional)—Polyethylene sample cups (see Note 2).

4. Reagents

- 4.1 Cyclohexane—Analytical grade (see Note 3).
- 4.2 Hydrochloric acid—Analytical grade.
- 4.3 Water of grade 1 according to ISO 3696
- 4.4 Palladium chloride—Analytical grade
- 4.5 Matrix modifier—0.1% (m/v) palladium solution—Prepared by dissolving 0.167 g palladium chloride (4.4) in 50 mL water (4.3) in a 100-mL volumetric flask (3.4), adding 1 mL hydrochloric acid (4.2) and making up to volume with water.
- 4.6 Blank oil—Refined. Any edible oil is suitable—To be stored in a metal free polyethylene bottle. Cadmium content of oil not greater than 0.2 $\mu\text{g}/\text{kg}$.
- 4.7 Organometallic cadmium standard—(Conostan, 5000 mg/kg, available from Continental Oil Company, Ponca City, Oklahoma, USA).
- 4.8 Standard stock solution—Stock solution of 10 mg/kg Cd, prepared once every 3 months—Prepared by appropriate dilution of organometallic standard (4.7) with the blank oil (4.6).
- 4.9 Standard working solution—Prepare daily the following working solutions by diluting the stock solution with blank oil (4.6).
Cadmium 2.5–5–10 $\mu\text{g}/\text{kg}$
- 4.10 Argon—Purity 99.99% minimum.

5. Procedure

- 5.1 Treatment of samples, blank and standards
- 5.1.1 Place all samples, standard working solutions (4.9) and blank oil (4.6) in the oven (3.5) regulated at $60 \pm 5^\circ\text{C}$.
- 5.1.2 Shake samples vigorously. If the metal content of a sample is known to be outside the given range (4.9) dilute (m/m) with blank oil (4.6).
- 5.2 Preparation of apparatus
- 5.2.1 Switch on the atomic absorption spectrometer and the background correction (D_2 or Zeeman).
- 5.2.2 In accordance with the manufacturer's instructions supplied with the spectrometer, adjust: lamp current, slit (wide?), wavelength and amplification. The required wavelength is 228.8 nm.
- 5.2.3 Optimise the position of the graphite furnace atomizer (3.7) in the atomic absorption spectrometer (3.6) and set the required programme on the control unit of the furnace. Place platform in graphite tube.
- 5.2.4 Pretreat before each injection, the pipettor tip (3.3) by pipetting and then discarding 10 μL cyclohexane (4.1).

Programme for the graphite furnace atomiser is:

Step	Temp. ($^\circ\text{C}$)	Ramp time (s)	Hold time (s)	in. Gas Flow (mL/min)
1*	200	30	30	300
2	650	60	40	300
3	1600	0	5	0
4	2700	1	3	50

* Modifier: step 1. Analysis: step 1 to step 4

5.3 Determination

5.3.1 Graphite tube blank

Record the absorption, if any, of the graphite tube (3.8) and autozero this absorption.

5.3.2 Blank oil

By means of a micropipettor (3.2) inject 20 μL of the matrix modifier (4.5) into the graphite furnace (3.7) and initiate the modifier temperature programme (step 1). Inject 10 μL of the blank solution (4.6) into the graphite furnace (3.7), initiate the temperature programme (steps 1–4) and record the absorption (for the autosampler, see Note 2).

5.3.3 Calibration of the apparatus

By means of a micropipettor (3.2), inject 20 μL of the matrix modifier (4.5) into the graphite furnace (3.7) and initiate the modifier temperature programme (step 1). Inject 10 μL of the three standard working solutions prepared according to 4.9 into the graphite furnace (3.7), initiate the temperature programme (steps 1–4) and calibrate the spectrometer according to the operating procedure of the apparatus used (for the autosampler see Note 2). Plot the analytical curve.

5.3.4 Oil (liquid) samples

By means of a micropipettor (3.2), inject 20 μL of the matrix modifier (4.5) into the graphite furnace (3.7) and initiate the modifier temperature programme (step 1). Inject 10 μL of the oil sample into the graphite furnace (3.7), initiate the temperature programme (steps 1–4) and record the concentrations according to the operating procedure of the apparatus used (for the autosampler see Note 2).

5.3.5 Fat samples (m.p. 40 °C and higher)

Introduce an extra temperature programming step: temperature 60 °C—hold time 20 s—internal gas flow 0 mL/min. By means of a micropipettor (3.2), inject 20 μL of the matrix modifier (4.5) into the graphite furnace (3.7) and initiate the modifier temperature programme. Initiate the modified temperature programme. Inject 10 μL of the melted fat into the graphite furnace (3.7), allow the tip to remain in the injection opening to liquefy the fat and then inject. Record the concentration according to the operating procedure of the apparatus used (for the autosampler, see Note 2).

5.3.6 Number of determinations

Carry out two determinations in rapid succession.

6. Expression of results

The measured concentration is expressed in $\mu\text{g}/\text{kg}$.

Report as final result the mean of the results of two determinations.

7. Precision

7.1 Repeatability limit

When the mean value of two results, obtained from duplicate analysis of an oil sample (fat sample) carried out in rapid succession by the same operator, using the same apparatus under the same analysis conditions, lies between 2 and 8 $\mu\text{g}/\text{kg}$ (3 and 7 $\mu\text{g}/\text{kg}$), the difference between these two results should not be greater than 1.1 $\mu\text{g}/\text{kg}$ (1.7 $\mu\text{g}/\text{kg}$).

7.2 Reproducibility limit

When the values for the final result, obtained by operators in different laboratories using different apparatus for the analysis of the same oil sample (fat sample), lie between 2 and 8 $\mu\text{g}/\text{kg}$ (3 and 7 $\mu\text{g}/\text{kg}$), the difference between the values for the final result obtained by those laboratories should not be greater than 22.9 $\mu\text{g}/\text{kg}$ (2.6 $\mu\text{g}/\text{kg}$).

7.3 Statistical and other data derived from the results of the interlaboratory test

The interlaboratory test carried out at the international level by the IUPAC Commission on Oils, Fats and Derivatives, in which eight laboratories participated, gave the statistical results summarised in the Tables.

8. Notes

8.1 The polyethylene or polypropylene capped bottles are made metal-free in the following way: Clean the bottles thoroughly with warm nitric acid 2 M, rinse with distilled water and dry the bottles in a drying oven at about 80 °C.

8.2 Using an autosampler

—Autosampler equipped with a heating device regulated at 60 °C.

Fill sample cups with blank oil (4.6), standard working solutions (4.9) and oil samples.

Fat samples with a melting point of 40 °C and higher have to be diluted 1:1 (m/m) with blank oil.

Volume of sample injected: 15 µL.

—Autosampler without heating device

Dilute 1:1 (m/v) blank oil (4.6), standard working solutions (4.9) and oil or sample with an organic solvent, e.g. heptane and work at ambient temperature.

Volume of sample injected: 20 µL.

8.3 Instead of cyclohexane, isooctane can also be used.