Pure & Appl. Chem., Vol. 63, No. 8, pp. 1183–1190, 1991.
Printed in Great Britain.

ADONIS 206922209100111B

INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY

APPLIED CHEMISTRY DIVISION
COMMISSION ON OILS, FATS AND DERIVATIVES*

DETERMINATION OF LEAD IN OILS AND FATS BY DIRECT GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETRY

Results of a collaborative study and the standardized method

Prepared for publication by

P. W. HENDRIKSE[1], F. J. SLIKKERVEER[1], A. FOLKERSMA[1] and A. DIEFFENBACHER[2]

[1]Unilever Research Laboratory, Vlaardingen, The Netherlands
[2]Nestec Ltd, Central Quality Assurance Laboratory, 55 Avenue Nestlé, CH-1800 Vevey, Switzerland

*Membership of the Commission during the period of the collaborative study (1987–89) was as follows:

Chairman: J. Beare-Rogers (Canada); *Vice-Chairman*: A. Dieffenbacher (Switzerland); *Secretary*: W. D. Pocklington (United Kingdom); *Titular Members*: P. W. Hendrikse (Netherlands); É. Kurucz (Hungary); G. Zwerenz (Austria); *Associate Members*: H. Brüschweiler (Switzerland); E. Fedeli (Italy); T. Hashimoto (Japan); G. Hølmer (Denmark); A. Karleskind (France); F. H. Koelman (Netherlands); Ö. Levin (Sweden); R. Manning (USA); R. Ohlson (Sweden); G. Ostermann (Germany); M. Pike (United Kingdom); A. Visapää (Finland); *National Representatives*: R. R. Brenner (Argentina); A. Johnson (Australia); P. Dysseler (Belgium); J. Pokorný (Czechoslovakia); J. V. Holm (Denmark); A. Gad (Egypt); R. Linko (Finland); J. P. Wolff (France); H. Wessels (Germany); D. Boscou (Greece); M. Jeránek (Hungary); A. Mathew (India); A. Sharkey (Ireland); A. Dolev (Israel); E. Tiscornia (Italy); S. Watanabe (Japan); S. Ong (Malaysia); A. Krab (Netherlands); L. Eyres (New Zealand); W. Zwierzykowski (Poland); R. Stoica (Romania); L. Du Plessis (South Africa); M. Gassiot Matas (Spain); T. Tuğlular (Turkey); A. E. Waltking (USA); V. L. Roubailo (USSR).

Determination of lead in oils and fats by direct graphite furnace atomic absorption spectrometry: results of a collaborative study and the standardized method

Abstract - A description is given of the development by collaborative study of a standardized method for the determination of lead in oils and fats by direct graphite furnace atomic absorption spectrometry. The procedure is both rapid and sensitive allowing determination at levels of 0.01 - 0.1 mg/kg.

INTRODUCTION

Lead in edible oils and fats is considered an inorganic contaminant. Its maximum allowable concentration is laid down by the FAO/WHO Codex Alimentarius.

In the oils and fat industry a rapid, accurate and standardized method for the determination of lead is very important for quality control. Graphite furnace atomic absorption spectrometry (GFAAS) has been accepted by IUPAC and AOCS† for the determination of copper, iron and nickel in oils and fats (1). A similar method for the determination of lead has been developed and tested by means of a collaborative study.

METHOD OF ANALYSIS

Due to the volatility of lead, the method (1) had to be modified by the introduction of a 'matrix modifier'. The matrix modification technique is generally used to decrease the volatility of the analyte element in order to prevent losses during thermal pretreatment. With reference to an earlier report (2) on the determination of phosphorus in oils, an organic matrix modifier was used. A solution of lecithin in cyclohexane (1%) proved to be adequate, both for atomization 'off the wall' and 'off the platform'.

Comparable results were found by atomization off the wall as well as by atomization off the platform, using both peak area integration and peak height integration. In the method under study it is preferred to use atomization off the platform and peak height integration, because better sensitivity and precision is obtained in that case.

COLLABORATIVE STUDY

In order to check the validity of the method as an international standard method for IUPAC and eventually for ISO‡‡, the method has been subjected to an international collaborative study by laboratories worldwide. The method studied took into account that various types of equipment of graphite furnaces can be used either with or without platform.

Materials provided for the study were edible oils (soyabean oil) and fats (cocoa butter) containing lead at three concentration levels (high, medium and low). Each concentration level was represented by two batches. Each sample was provided in duplicate (blind coded) so that participants received in total 24 samples. Participants were asked to analyze each sample in duplicate and to report both values obtained. A statistical evaluation of the data was made for each level and for each type of sample separately in accordance to ISO 5725 - 1986 using a method published earlier (3).

† American Oil Chemists' Society

‡‡ International Organisation for Standardization

RESULTS

Data screening From 20 laboratories data have been received for evaluation. At a first survey three laboratories have been excluded from the test because of deviations from the method. Moreover the data from one laboratory were judged unsuitable for inclusion in the test. The data from the remaining 16 laboratories have been subjected to tests for outliers according to Cochran and Dixon.

Cochran tests As the 24 samples analyzed were in fact 12 pairs of corresponding samples, the differences between these blind (hidden) duplicates have been tested for stragglers and outliers according to Cochran's procedure.

Dixon tests After elimination of the significant outliers (i.e. significant at the 1% level) according to Cochran's test, the 12 batch averages per laboratory have been tested for stragglers and outliers according to Dixon's procedure.

Most of the deviations concerned the 'oil data' in which two laboratories displayed so many deviations that it had to be decided to omit the data of these laboratories from further evaluation. All other data have been maintained in the evaluation after discarding the outliers. Hence, for the final calculations of repeatability and reproducibility, there remained a total of 14 and 16 laboratories for the soyabean oil and the cocoa butter respectively.

Precision

In tables 1 and 2 the batch averages at each concentration level in liquid oil or in solid fat have been listed. Averages per concentration level have been calculated because the results of two batches at one concentration level are close enough to each other to be representative of the same concentration.

When the values for $\underline{r}$ (repeatability) and $\underline{R}$ (reproducibility) as given in tables 1 and 2 are expressed as functions of their corresponding mean concentration values (m), the following equations are obtained:

Pb in edible oil	$\underline{r} = 0.19\ m$	$\underline{R} = 0.30\ m$	[Eq.1]
Pb in cocoa butter	$\underline{r} = 0.15\ m$	$\underline{R} = 0.68\ m$	[Eq.2]

Table 1 Concentration levels, average recoveries (mg/kg) and precision parameters in edible oil (soyabean oil) - [14 accepted laboratories]

Concentration level	Actual values	Averages		Repeatability			Reproducibility		
	Batch	Batch	Level	$\underline{s}_r$	$\underline{r}$	$\underline{CV}_r$(%)	$\underline{s}_R$	$\underline{R}$	$\underline{CV}_R$(%)
High	0.080 0.090	0.083 0.091	0.087	0.0059	0.017	6.8	0.0089	0.025	10.2
Medium	0.047 0.054	0.049 0.057	0.053	0.0034	0.009	6.4	0.0057	0.016	10.9
Low	0.018 0.023	0.020 0.024	0.022	0.0022	0.006	10.1	0.0034	0.009	15.4

[$\underline{s}_r$: repeatability standard deviation; $\underline{r}$: repeatability limit; $\underline{CV}_r$: repeatability coefficient of variation; $\underline{s}_R$: reproducibility standard deviation; $\underline{R}$: reproducibility limit; $\underline{CV}_R$: reproducibility coefficient of variation;]

Table 2 Concentration levels, average recoveries (mg/kg) and precision parameters in cocoa butter - [16 accepted laboratories]

Concentration level	Actual values	Averages		Repeatability			Reproducibility		
	Batch	Batch	Level	s_r	r	CV_r(%)	s_R	R	CV_R(%)
High	0.080 0.090	0.084 0.090	0.087	0.0039	0.011	4.5	0.0180	0.051	20.6
Medium	0.047 0.054	0.051 0.057	0.054	0.0031	0.009	5.7	0.0117	0.033	21.7
Low	0.018 0.023	0.024 0.029	0.026	0.0013	0.004	5.0	0.0073	0.021	27.7

I DISCUSSION

From the equations 1 and 2, describing the precision of the lead concentration it can be concluded that the straight lines corresponding with these equations pass through the origin.

The relative repeatability (the coefficient of variation) does not depend on the level, but the relative reproducibility decreases more or less linearly with the lead concentration. According to Horwitz (4) for an analytical method to be acceptable, the relative reproducibility (CV_R) should be about 23% at the 0.1 mg/kg level and 32% at the 0.01 mg/kg level. For both oil and fat this criterion is fully met.

From tables 1 and 2 it can be seen that the reproducibility of the 'fat data' is considerably larger than that of the 'oil data'. For this reason the results of the two laboratories which were omitted from the 'oil data' could be maintained in the 'fat data', resulting in 14 and 16 laboratories respectively.

CONCLUSION

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

After an extensive collaborative study it was concluded that the method meets the criterion for an analytical method to determine trace amounts of analyte as stated by Horwitz in 1982.

Based on the repeatability and reproducibility of the results obtained in the collaborative study the Commission has decided to adopt the method. The text of the standardized procedure is given on the following pages.

Acknowledgement

The Commission is thankful to the collaborators in Austria, Belgium, Denmark, France, Federal Republic of Germany, Hungary, the Netherlands, Republic of South Africa, Sweden, Turkey, United Kingdom and the United States of America for their participation and valuable cooperation.

REFERENCES

1. P.W. Hendrikse, F.J. Slikkerveer, J. Zaalberg and A. Hautfenne, Pure & Appl. Chem., 1988, 60 893.

2. F.J. Slikkerveer, A.A. Braad and P.W. Hendrikse, At. Spectr., 1980, 1 30.

3. J. Zaalberg, J. Assoc. Off. Anal. Chem., 1989, 72 34.

4. W. Horwitz, Anal. Chem., 1982, 54 67A.

2.632 DETERMINATION OF LEAD BY DIRECT GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETRY

1. SCOPE AND FIELD OF APPLICATION

This Standard describes a method for the determination of trace amounts (µg/kg) of lead in all types of crude or refined edible oils and fats.

2. PRINCIPLE

Vaporisation of the oil or fat in a suitable graphite furnace with or without a platform connected to an atomic absorption spectrometer, previously calibrated using standard solutions of organo-compound of lead and the measurement of the metal content from the observed absorption at a wavelength of 283.3 nm.

3. APPARATUS

3.1 Polyethylene or polypropylene capped bottles - 20 ml, metal free (see Note 1).
3.2 Micropipettor - 20 µl.
3.3 Pipettor tips.
3.4 Electric oven - Regulated at 60 ± 2 °C.
3.5 Atomic absorption spectrometer. Equipped with either "peak height" mode and printer, or "continuous" mode and pen recorder (full scale response in 0.2 sec.) together with the appropriate electrode-less discharge lamp (or hollow cathode lamp) and deuterium background corrector (or Zeeman atomic absorption spectrometer).
3.6 Graphite furnace atomizer - Placed in the atomic absorption spectrometer (3.5), equipped with a control unit for temperature programming.
3.7 Graphite tube - Normal (uncoated).
3.8 Platform - Pyrolytic, in combination with uncoated or pyrolytically coated graphite tube (see Note 2).

4. REAGENTS

4.1 Cyclohexane - Analytical grade.
4.2 Lecithin - A well defined lecithin containing 2% phosphorus.
4.3 Matrix modifier - 2 % (m/v) Lecithin solution is prepared by dissolving 2 g lecithin (4.2) in 100 ml cyclohexane (4.1).
4.4 Blank oil - Refined. Any edible oil is suitable - To be stored in a metal free polyethylene bottle. Lead content of oil not greater than 1 µg/kg.
4.5 Standard stock solution - A stock solution of 10 mg Pb/kg is prepared by appropriate dilution of an organometallic standard with the blank oil (4.4). A suitable standard (Conostan, 5000 mg/kg) is available from Continental Oil Company, Ponca City, Oklahoma, U.S.A.
4.6 Standard working solutions - 0.020 mg Pb/kg, 0.050 mg Pb/kg and 0.100 mg Pb/kg, to be prepared daily by diluting the 10 mg/kg stock solution (4.5) with blank oil (4.4).
4.7 Argon, purity 99.99% minimum.

5. PROCEDURE

5.1 Treatment of samples, blank and standards

5.1.1 Place all samples and standard working solutions in the oven (3.4), regulated at 60 ± 2 °C.
5.1.2 Shake samples vigorously.
5.1.3 Weigh 5.00 g sample in a 20 ml bottle (3.1) together with 5.00 g matrix modifier (3.3) and mix thoroughly.
5.1.4 Carry out instructions 5.1.2 - 5.1.3 also for the three standard working solutions (4.6) and the blank oil (4.4).

5.2 Preparation of apparatus

5.2.1 Switch on the atomic absorption spectrometer and the background correction (D_2 of Zeeman).

5.2.2 In accordance with the manufacturer's instructions supplied with the spectrometer, adjust: lamp current, slit, wavelength and amplification. The required wavelength is 283.3 nm.

5.2.3 Optimize the position of the graphite furnace atomizer (3.6) in the atomic absorption spectrometer (3.5) and set the required programme on the control unit of the furnace. If available, place platform in graphite tube (see Note 2).

5.2.4 Pretreat before each injection, the pipettor tip (3.3) by pipetting and then discarding 20 µl cyclohexane.

Programmes for the graphite furnace atomizer are: (for Varian Spectrometer, see note 4).

Uncoated tube (3.7) off the wall - max power heating - gasstop

Step	Temp. (°C)	Ramptime (s)	Holdtime (s)	Int. Gasflow (ml/min)
1	100	10	20	300
2	650	60	40	300
3	1900	0	5	0
4	2700	1	3	50

Pyro coated tube (3.8) with platform - max power heating - gasstop

Step	Temp. (°C)	Ramptime (s)	Holdtime (s)	Int. Gasflow (ml/min)
1	200	10	20	300
2	650	60	40	300
3	1700	0	5	0
4	2700	1	3	50

5.3 Determination

5.3.1 Measurement of the blank - Inject 20 µl of the blank solution prepared according to 5.1.4 into the graphite furnace, initiate the temperature programme and record the absorption.

5.3.2 Measurement of standards - Inject 20 µl of the three standard solutions prepared according to 5.1.4 into the graphite furnace and record the absorptions.

5.3.3 Measurement of sample solutions - Inject 20 µl of the sample solution prepared according to 5.1.3 into the graphite furnace, initiate the temperature programme and record the absorption.

6. CALCULATION AND EXPRESSION OF RESULTS

6.1 Calculation

6.1.1 Measure the peak height on the recorder-chart or take the reading of the display or printer.

6.1.2 Draw a calibration curve by plotting the absorption of the three standards (5.3.2), corrected for the blank (5.3.1), against their respective metal content (note 3).

6.1.3 Read the metal content of the sample from the calibration curve.

6.2 Expression of results

Express the results as mg/kg (to two significant figures)

7. QUALITY ASSURANCE

7.1 For general principles of analytical quality control see the section on Quality Assurance in the introductory part of the Compendium of the Standard Methods.

7.2 For specific applications of analytical quality control see the Annexe to this standard method.

8. NOTES

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

2. Both atomization off the wall and atomization of the platform can be used. The accuracy and the sensitivity off the platform is two times higher than off the wall.

3. With the use of sophisticated equipment auto-calibration can be applied.

4. **Modification for Varian spectrometers**

   For those having a Varian apparatus the following modifications should be made:

Clause 5.2.3
Varian programme for uncoated tube (3.7) - off the wall

Step No.	Temperature °C	Time (s)	Gasflow (l/min)	Gastype	Read command
1	100	20.0	3.0	Normal	No
2	100	40.0	3.0	Normal	No
3	550	60.0	3.0	Normal	No
4	550	40.0	3.0	Normal	No
5	550	1.0	0.0	Normal	No
6	2500	1.0	0.0	Normal	Yes
7	2500	5.0	0.0	Normal	Yes
8	2500	3.0	3.0	Normal	No

Varian programme for pyro coated tube with platform

Step No.	Temperature °C	Time (s)	Gasflow (l/min)	Gastype	Read command
1	200	10.0	3.0	Normal	No
2	200	20.0	3.0	Normal	No
3	650	60.0	3.0	Normal	No
4	650	40.0	3.0	Normal	No
5	650	1.0	0.0	Normal	No
6	2000	0.7	0.0	Normal	Yes
7	2000	5.0	0.0	Normal	Yes
8	2000	3.0	3.0	Normal	No

Clause 5.3: Use 10 µl for injection

APPENDIX

1. Repeatability limit

The absolute difference between two independent single test results, obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time, should not be greater than the repeatability limit (r) as calculated from the formulae in table 1.

Table 1 repeatability (r) and reproducibility limits (R)

Pb in edible oil	r = 0.19 m	R = 0.30 m
Pb in cocoabutter	r = 0.15 m	R = 0.68 m

Key: m = corresponding mean concentration value.

2. Reproducibility limit

The absolute difference between two single test results, obtained with the same method on identical test material in different laboratories with different operators using different equipment, should not be greater than the reproducibility limit ($\underline{R}$) as calculated from the formulae in table 1.

3. trueness (bias) - The bias of the method was demonstrated in the collaborative study of the method (see table of statistical data below) to be negligible when used for the determination of concentration levels of lead in the range 0.01 - 0.10 mg/kg.

4. The sensitivity of the method is demonstrated by the low values for $\underline{r}$ and $\underline{R}$ at the low concentration levels studies (see table of statistical data below), the limit of detection is 0.001 mg/kg, the limit of determination is 0.01 mg/kg.

Interference by other elements is not to be expected provided the measurements are carried out at the wave length specific for lead (283.3 nm).

5. Statistical and other data derived from the results of the interlaboratory test

The interlaboratory test carried out at the international level in 1988 by the IUPAC Commission on Oils, Fats and Derivatives, in which 20 laboratories participated, each obtaining two test results for each sample, gave the statistical results (evaluated in accordance with ISO 5725-1986) summarized in the following table:

Sample	Soyabean oil			Cocoa butter		
Batch	A	B	C	A	B	C
Number of laboratories retained after eliminating outliers	14	14	14	16	16	16
Number of outliers (laboratories)	6	6	6	4	4	4
Number of accepted results	112	112	112	128	128	128
Mean value (mg/kg sample)	0.087	0.053	0.022	0.087	0.054	0.026
True or accepted value (mg/kg)	0.085	0.050	0.020	0.085	0.050	0.020
Repeatability standard deviation ($\underline{S}_r$ in mg/kg)	0.0059	0.0034	0.0022	0.0039	0.0031	0.0013
Repeatability relative standard deviation (%)	6.8	6.4	10.1	4.5	5.7	5.0
Repeatability limit ($\underline{r}$) [2.8 x $\underline{S}r$]	0.017	0.0095	0.0062	0.011	0.0087	0.004
Reproducibility standard deviation ($\underline{S}_R$ in mg/kg)	0.0089	0.0057	0.0034	0.0180	0.0117	0.0073
Reproducibility relative standard deviation (%)	10.2	10.9	15.4	20.6	21.7	27.7
Reproducibility limit ($\underline{R}$) [2.8 x $\underline{S}R$]	0.025	0.016	0.009	0.050	0.033	0.020