# INTERNATIONAL STANDARD

# ISO 12193

Second edition
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# Animal and vegetable fats and oils — Determination of lead by direct graphite furnace atomic absorption spectroscopy

*Corps gras d'origines animale et végétale — Détermination de la teneur en plomb par spectrométrie d'absorption atomique directe avec four en graphite*

Reference number
ISO 12193:2004(E)

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# Foreword

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The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 12193 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

This second edition cancels and replaces the first edition (ISO 12193:1994), which has been technically revised.

**INTERNATIONAL STANDARD** **ISO 12193:2004(E)**

# Animal and vegetable fats and oils — Determination of lead by direct graphite furnace atomic absorption spectroscopy

## 1 Scope

This International Standard specifies a method for the determination of trace amounts ($> 0{,}001$ mg/kg) of lead in all types of crude or refined edible oils and fats.

## 2 Principle

The oil or fat is atomized in a graphite furnace, with or without a platform, connected to an atomic absorption spectrometer which has been previously calibrated using standard solutions of an organo compound of lead. The metal content is measured from the observed absorbance at a wavelength of 283,3 nm.

## 3 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified, and distilled or demineralized water or water of equivalent purity.

**3.1 Cyclohexane***,* analytical grade.

**3.2 Lecithin**, containing 2 % phosphorus.

**3.3 Matrix modifier**: 2 % (by mass/volume) lecithin solution prepared by dissolving 2 g of lecithin (3.2) in 100 ml of cyclohexane (3.1).

**3.4 Blank oil**, refined.

Any edible oil is suitable, stored in a metal-free polyethylene bottle. The lead content of the oil shall not be greater than 0,001 mg/kg.

**3.5 Lead standard stock solution,** $c(\text{Pb}) = 10$ mg/kg.

This is prepared by appropriate dilution of an organometallic standard with the blank oil (3.4).

NOTE A suitable standard is available from the following companies:[1]

- Continental Oil Company, Ponca City, Oklahoma, USA (Conostan, 5 000 mg/kg);
- Merck KGaA, Darmstadt, Germany [Certipur 115051, lead standard dissolved in oil, $c(\text{Pb}) = 1$ g/kg] in standard oil;
- VWR International [Certipur 1.15051, lead standard dissolved in oil, $c(\text{Pb}) = 1$ g/kg] in standard oil;
- VWR International [Specpure ALFA019527.AD, lead, Organic AAS standard solution, Specpure, $c(\text{Pb}) = 1\,000$ mg/g].

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[1] These are examples of suitable products available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.

**3.6 Lead standard working solutions**: $c$(Pb) = 0,02 mg/kg, 0,05 mg/kg and 0,100 mg/kg, prepared daily by diluting the 10 mg/kg stock solution (3.5) with blank oil (3.4).

**3.7 Argon**, of minimum purity 99,99 %.

# 4 Apparatus

**4.1 Polyethylene or polypropylene capped bottles**, 20 ml, metal free.

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.

**4.2 Micropipettor**, 20 µl.

**4.3 Pipettor tips**.

**4.4 Electric oven**, regulated at 60 °C ± 2 °C.

**4.5 Atomic absorption spectrometer**, equipped with either “peak height” mode and printer, or “area” mode and pen-recorder (full-scale response in 0,2 s), together with the appropriate electrode-less discharge lamp (or hollow cathode lamp) and deuterium or Zeeman background corrector.

**4.6 Graphite furnace atomizer**, placed in the atomic absorption spectrometer (4.5), equipped with a control unit for temperature programming.

**4.7 Graphite tube**, normal (uncoated) or coated (pyrolytic).

**4.8 Pyrolytic platform**, in combination with uncoated or pyrolytically coated graphite tube.

Both atomization with and without the platform may be used. The accuracy and the sensitivity with the platform is twice as good as without the platform.

# 5 Sampling

Laboratory samples of at least 1,5 g shall be stored in metal-free bottles (4.1).

It is important that the laboratory receive a sample that is truly representative and has not been damaged or changed during transport or storage.

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 5555.

# 6 Procedure

## 6.1 Treatment of samples, blanks and standards

**6.1.1** Place all samples and standard working solutions (3.6) in the oven (4.4) set at 60 °C.

**6.1.2** Shake samples vigorously.

**6.1.3** Weigh 5,0 g of sample into a 20 ml bottle (4.1), together with 5,0 g of matrix modifier (3.3), and mix thoroughly.

**6.1.4** Carry out steps 6.1.2 and 6.1.3 also for the three standard working solutions (3.6) and the blank oil (3.4).

## 6.2 Preparation of apparatus

**6.2.1** Switch on the atomic absorption spectrometer (4.5) and the background correction ($D_2$ or Zeeman).

**6.2.2** In accordance with the manufacturer's instructions supplied with the spectrometer, adjust the lamp current, slit, wavelength and amplification. The required wavelength is 283,3 nm.

**6.2.3** Optimize the position of the graphite furnace atomizer (4.6) in the atomic absorption spectrometer and set the required programme on the control unit of the furnace. Use the measuring parameters given in Tables 1 and 2. If available, place the platform (4.8) in the graphite tube.

**6.2.4** Before each injection, pretreat the pipettor tip (4.3) by pipetting and then discarding 20 µl of cyclohexane.

Programmes for the graphite furnace atomizer are given in Tables 1 and 2.

**Table 1 — Uncoated tube without platform, with maximum power heating and gas-stop**

| Step No. | Temperature °C | Ramp-time s | Hold-time s | Internal gas flow ml/min |
|---|---|---|---|---|
| 1 | 100 | 10 | 20 | 300 |
| 2 | 650 | 60 | 40 | 300 |
| 3 | 1 900 | 0 | 5 | 0 |
| 4 | 2 700 | 1 | 3 | 50 |

**Table 2 — Pyro-coated tube with platform, with maximum power heating and gas-stop**

| Step No. | Temperature °C | Ramp-time s | Hold-time s | Internal gas flow ml/min |
|---|---|---|---|---|
| 1 | 200 | 10 | 20 | 300 |
| 2 | 650 | 60 | 40 | 300 |
| 3 | 1 700 | 0 | 5 | 0 |
| 4 | 2 700 | 1 | 3 | 50 |

## 6.3 Determination

### 6.3.1 Measurement of blank solution

Inject 20 µl of the blank solution, prepared according to 6.1.4, into the graphite furnace. Intiate the temperature programme and record the absorption.

### 6.3.2 Measurement of standard solutions

Inject 20 µl of the three standard solutions, prepared according to 6.1.4, into the graphite furnace and record the absorptions.

### 6.3.3 Measurement of sample solutions

Inject 20 µl of the sample solution, prepared according to 6.1.3, into the graphite furnace. Initiate the temperature programme and record the absorption.

# 7 Calculation and expression of results

## 7.1 Calculation

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

**7.1.2** Draw a calibration curve by plotting the absorption of the three standard solutions (6.3.2), corrected for the blank (6.3.1), against their respective metal content.

NOTE With the use of sophisticated equipment, autocalibration can be applied.

**7.1.3** Read the metal content of the sample from the calibration curve.

## 7.2 Expression of results

Express the results as milligrams per kilogram (to two significant figures).

# 8 Precision

## 8.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method are summarized in Annex A. The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.

The method has been tested with liquid oil (soyabean oil) and with solid fat (cocoa butter).

## 8.2 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 a short interval of time, will not in more than 5 % of cases be greater than the repeatability limit ($r$) as calculated from the formulae in Table 3.

**Table 3 — Repeatability ($r$) and reproducibility limits ($R$)**

| | $r$ | $R$ |
|---|---|---|
| Pb in edible oil | 0,19 $M$ | 0,30 $M$ |
| Pb in cocoa butter | 0,15 $M$ | 0,68 $M$ |
| NOTE $M$ is the corresponding mean concentration value. | | |

## 8.3 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, will in not more than 5 % of cases be greater than the reproducibility limit ($R$) as calculated from the formulae in Table 3.

## 8.4 Trueness (bias)

The bias of the method was demonstrated in the collaborative study of the method (see Table A.1) to be negligible when used for the determination of concentration levels of lead in the range 0,01 mg/kg to 0,10 mg/kg.

### 8.5 Sensitivity

The sensitivity of the method is demonstrated by the low values for $\rho$ and $P$ at the low concentration levels studied (see Table A.1). The limit of detection is 0,001 mg/kg and the limit of determination is 0,01 mg/kg.

Interference by other elements is not to be expected, provided that the measurements are carried out at the wavelength specific for lead (283,3 nm).

## 9 Test report

The test report shall specify:

a) all information necessary for the complete identification of the sample;

b) the sampling method used, if known;

c) the test method used, with reference to this International Standard;

d) all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

e) the test result(s) obtained, or, if the repeatability has been checked, the final quoted result obtained.

# Annex A
(informative)

## Results of interlaboratory test

An interlaboratory test was 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. The statistical results obtained (evaluated in accordance with ISO 5725:1986)[2)] are summarized in Table A.1.

**Table A.1 — Statistical results**

| | **Sample** | | | | | |
|---|---|---|---|---|---|---|
| | **Soyabean oil** | | | **Cocoa butter** | | |
| | **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, $s_r$ (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 ($r$) (2,8 × $s_r$) | 0,017 | 0,0095 | 0,0062 | 0,011 | 0,0087 | 0,004 |
| Reproducibility standard deviation, $s_R$ (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 ($R$) (2,8 × $s_R$) | 0,025 | 0,016 | 0,009 | 0,050 | 0,033 | 0,020 |

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2) ISO 5275:1986, *Precision of test methods — Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests* (now withdrawn).

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