

BS EN ISO 8534:2017



BSI Standards Publication

# Animal and vegetable fats and oils — Determination of water content — Karl Fischer method (pyridine free) (ISO 8534:2017)

**National foreword**

This British Standard is the UK implementation of EN ISO 8534:2017. It supersedes BS EN ISO 8534:2008 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee AW/307, Oilseeds, animal and vegetable fats and oils and their by-products.

A list of organizations represented on this committee can be obtained on request to its secretary.

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**Animal and vegetable fats and oils - Determination of  
water content - Karl Fischer method (pyridine free) (ISO  
8534:2017)**

Corps gras d'origines animale et végétale -  
Détermination de la teneur en eau - Méthode de Karl  
Fischer (sans pyridine) (ISO 8534:2017)

Tierische und pflanzliche Fette und Öle - Bestimmung  
des Wassergehalts - Karl-Fischer-Verfahren  
(pyridinfrei) (ISO 8534:2017)

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**CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels**

## **European foreword**

This document (EN ISO 8534:2017) has been prepared by Technical Committee ISO/TC 34 “Food products” in collaboration with Technical Committee CEN/TC 307 “Oilseeds, vegetable and animal fats and oils and their by-products - Methods of sampling and analysis” the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by August 2017, and conflicting national standards shall be withdrawn at the latest by August 2017.

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This document supersedes EN ISO 8534:2008.

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### **Endorsement notice**

The text of ISO 8534:2017 has been approved by CEN as EN ISO 8534:2017 without any modification.

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## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see [www.iso.org/directives](http://www.iso.org/directives)).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

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

This third edition cancels and replaces the second edition (ISO 8534:2008), of which it constitutes a minor revision to exclude the applicability for fat coming from milk and milk products.

## Introduction

The determination of the water content of fats and oils according to Karl Fischer is carried out by two different procedures. This document specifies the volumetric Karl Fischer method for the determination of higher milligram levels of water (high level moisture). It is used for samples having between 1 mg and 100 mg of water in the sample.

[Annex B](#) specifies a coulometric titration, which requires between 10 µg and 10 mg water in the sample. The coulometric method is more sensitive than the volumetric method and permits the determination of lower water contents.





# Animal and vegetable fats and oils — Determination of water content — Karl Fischer method (pyridine free)

## 1 Scope

This document specifies a method for the determination of the water content of animal and vegetable fats and oils (hereinafter referred to as fats) using Karl Fischer apparatus and a reagent which is free of pyridine.

Milk and milk products (or fat coming from milk and milk products) are excluded from the scope of this document.

## 2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 661, *Animal and vegetable fats and oils — Preparation of test sample*

## 3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at <http://www.electropedia.org/>
- ISO Online browsing platform: available at <http://www.iso.org/obp>

### 3.1

#### water content

mass, in grams per 100 g of sample, of water as determined in accordance with the method specified in this document

Note 1 to entry: The water content is expressed as a percentage mass fraction.

## 4 Principle

Dissolved fat is titrated against an iodine solution and sulfur dioxide (SO<sub>2</sub>) is oxidized by iodine in the presence of water. In principle, the chemical reaction in [Formula \(1\)](#) takes place:



The alcohol reacts with SO<sub>2</sub> and a nitrogenous base (RN) to form an intermediate alkylsulfite salt, which is then oxidized by iodine to an alkylsulfate salt. This oxidation reaction consumes water contained in the sample. The end point is monitored potentiometrically.

## 5 Reagents

**WARNING — Comply with any local regulations which specify the handling of hazardous substances. Technical, organizational and personal safety measures shall be followed.**

It is recommended that “ready for use” working solvents be used, either one-component reagents (5.1.1) or two-component reagents (5.1.2). Reagents with a titre of 1 mg and 2 mg water per millilitre are required for acceptable performance.

**5.1 Karl Fischer reagents**, consist of one-component reagents or two-component reagents for volumetric determination.

**5.1.1 One-component reagents**, contain all the reactant in the titrant solution: iodine, sulfur dioxide, and imidazole, dissolved in a suitable alcohol. Methanol is typically used as the working medium in the titration cell.

Absolute methanol is the solvent of choice. But for fats and oils, use a mixture of absolute methanol and absolute chloroform (the methanol content should be at least 25 % volume fraction, or optimally 50 % volume fraction).

**5.1.2 Two-component reagents**, consist of all necessary reactants for the titration, but in two different solutions. The titrating agent (usually known as the titrant) contains only iodine and methanol, while the solvent containing the other Karl Fischer reaction components is used as the working medium in the titration cell.

**5.2 Water standard**, commercially prepared standard with a certified concentration of 10 mg/g (1,0 % mass fraction).

## 6 Apparatus

Usual laboratory apparatus and, in particular the following.

**6.1 Karl Fischer apparatus**, set up according to the manufacturer’s recommendations for the determination of water in fats and oils. Set up and conduct protocols for routine maintenance as recommended by the manufacturer. Use an airtight vessel and do not place the instrument in high humidity areas. Do not place instruments or handle samples near water sources, such as taps, sinks, and dishwashers in the laboratory.

**6.2 Analytical balance**, readable to the nearest 0,1 mg.

**6.3 Syringes**, of capacity 1 ml, 2 ml, 5 ml, 10 ml, and 20 ml.

To ensure accurate and reproducible results from the water standard, use a glass gastight syringe. For water standard 10,0, use a 10 ml syringe and for either the water standard 1,00 or 0,10, use a 5 ml syringe. In addition to the appropriate size syringe, use a needle that is long enough to allow for a subsurface injection when injecting through the instrument’s septum.

## 7 Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

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

## 8 Preparation of the test sample

Prepare the test sample in accordance with ISO 661.

The determination of water is conducted by adjusting the sample size to have between 1 mg and 100 mg water for the volumetric titration (the main body of this document) and between 10 µg and 10 mg for the coulometric titration ([Annex B](#)) using Karl Fischer instruments and reagents which have been validated with standard water solutions over the necessary range. For the volumetric determination, a minimum amount of 0,5 ml Karl Fischer reagent shall be used for the titration.

## 9 Procedure

### 9.1 Titre

9.1.1 The titre shall be determined daily for each bottle of titrant.

9.1.2 Prepare the instrument according to the manufacturer's recommendations for calibration.

9.1.3 Add 20 ml to 40 ml of working solvent ([5.1](#)) to the titration vessel. The solvent should cover the platinum electrodes.

9.1.4 Titrate the vessel to a stable dry end point.

**CAUTION — Take care not to overtitrate.**

9.1.5 Determine the titre of the titrant using the water standard ([5.2](#)) and a syringe ([6.3](#)). Sample mass is determined by difference.

9.1.5.1 Weigh, to the nearest 0,1 mg, approximately 1 g of the water standard into a syringe, placed on the analytical balance ([6.2](#)).

Upon opening the ampoule, withdraw a small portion of the standard to rinse the syringe; 1 ml to 2 ml is sufficient. Rinse the entire interior of the syringe and discard the rinsings. Then, immediately transfer the remaining standard to the syringe and expel any air bubbles. Using mass by difference make at least three injections from the syringe.

9.1.5.2 When the mass displayed is stable, tare the balance.

9.1.5.3 Inject the water sample into the titration vessel and close the vessel.

9.1.5.4 Place the syringe back on the balance. Record the mass of the water injected to the nearest 0,1 mg. The mass will be displayed as a negative value.

9.1.5.5 Enter the sample mass in the instrument.

9.1.6 Start the titration and record the titre when a stable end point is reached. Some instruments may require calculation of titre from the displayed percentage of water.

9.1.7 Average a minimum of three titre determinations. Record the arithmetic average.

9.1.8 Update the instrument titre value with the new setting.

The titre,  $\rho_{\text{titrant}}$ , in milligrams per millilitre, can be calculated from [Formula \(2\)](#):

$$\rho_{\text{titrant}} = \frac{m_s w_{\text{H}_2\text{O},s}}{V_{\text{titrant}}} \quad (2)$$

where

$m_s$  is the mass, in grams, of the water standard;

$w_{\text{H}_2\text{O},s}$  is the water content of the certified water standard;

$V_{\text{titrant}}$  is the volume, in millilitres, of the titrant used.

**9.1.9** Titre should not change significantly from day to day with routine operation unless a fresh bottle of titrant has been opened. A problem may exist if more than a 10 % relative change in titre occurs between days for the same bottle of titrant. If the instrument has been set up more than 12 h before use, the burette should be flushed and the titrant vessel refilled prior to determination of titre. Change the desiccant on the reagent bottles as recommended by the manufacturer to minimize the drift in reagent titre.

## 9.2 Test portion

**9.2.1** By means of a syringe, weigh and introduce a portion of the sample into the instrument using the target masses shown in [Table 1](#). At least 0,5 ml Karl Fischer reagent shall be used for the titration.

**Table 1 — Test portion sizes for the volumetric titration**

Water content in the test portion % mass fraction	Min. mass of test portion <sup>a</sup> g	Water content in the test portion mg
0,001	20	0,2
0,01	20	2
0,1	5	5
1	1	10
5	0,2	10
10	0,1	10
20	0,05	10

<sup>a</sup> The exact test portion mass depends on the precision required and the burette used. However, the test portion mass shall not be greater than the solvent mass. The maximum mass ratio of test portion to solvent is 1 + 1.

**9.2.2** Test portion masses are determined by difference.

**9.2.2.1** Place the test portion, in an appropriately sized syringe, on the balance.

**9.2.2.2** When a stable weight has been achieved, tare balance.

**9.2.2.3** Immediately transfer the test portion from the syringe to the reaction vessel.

**9.2.2.4** Place the syringe back on the balance. When the weight is stable, record the mass to the nearest 0,1 mg.

### 9.3 Determination

**9.3.1** Add 20 ml to 40 ml working solvent (5.1) to the titration vessel. The solvent should cover the platinum electrodes.

**9.3.2** Titrate the vessel to a stable dry end point.

**CAUTION — Take care not to overtitrate.**

**9.3.3** Weigh and introduce the test portion into the instrument according to 9.2.

**9.3.4** Record the moisture content of the test portion when a stable dry end point is reached.

**9.3.5** Up to six test portions may be assayed before replacing and pre-titrating the working solvent.

**9.3.5.1** Replace the working solvent if a precipitate forms indicating that the test portions have not fully dissolved.

**9.3.5.2** Pre-titrate the working solvent if there is more than a 10 min delay between running test portions on the same vessel of working solution.

**9.3.5.3** The amount of solvent in a two-component solvent system added to the titration vessel determines the maximum amount of water that may be titrated. The volume of solvent or mixed solvent should provide 60 mg to 100 mg of water capacity.

**9.3.5.4** The number of test portions that may be titrated before replacing and redrying the working solution depends on sample solubility, reagent capacity of two-component reagent systems, and vessel capacity. The initial volume of working solvent added using the two-component reagents sets a maximum amount of water that can be titrated. The selection of six test portions serves as a guideline but may vary based on water content, reagent titre, and sample solubility.

## 10 Expression of results

Calculations completed automatically by the instrument.

The water content,  $w_{\text{H}_2\text{O},t}$ , in grams per 100 g of test portion, is calculated from Formula (3):

$$w_{\text{H}_2\text{O},t} = \frac{V_{\text{titrant}} \rho_{\text{H}_2\text{O}} \times 100}{m_t \times 1\,000} \quad (3)$$

where

$V_{\text{titrant}}$  is the volume, in millilitres, of titrant used;

$\rho_{\text{H}_2\text{O}}$  is the titre (water equivalent of the reagent, in milligrams of water per millilitre);

$m_t$  is the mass, in grams, of the test portion.

## 11 Precision of the method

### 11.1 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.

### 11.2 Repeatability

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 in not more than 5 % of cases exceed the values of  $r$  given in [Table A.1](#).

### 11.3 Reproducibility

The absolute difference between two single test results, obtained with the same method on identical test material in different laboratories by different operators using different equipment, will in not more than 5 % of cases exceed the values of  $R$  given in [Table A.1](#).

## 12 Test report

The test report shall include at least the following information:

- 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 document, i.e. ISO 8534;
- d) the result(s) obtained;
- e) if the repeatability has been checked, the final quoted result obtained;
- f) any operating details not specified in this document, or regarded as optional, together with details of any incidents which may have influenced the test result(s).

For further information on the test report, refer to ISO/IEC 17025:2005, 5.10.

## Annex A (informative)

### Results of interlaboratory tests

An international collaborative test involving 22 laboratories in five countries was carried out on the following samples:

- |                                                |                            |
|------------------------------------------------|----------------------------|
| A Medium chain triglyceride oil with 3 % water | E Castor oil/Vegetable oil |
| B Sample A/Vegetable oil (1 + 1)               | F Extra virgin olive oil   |
| C Sample A/Vegetable oil (1 + 3)               | G Vegetable oil            |
| D Castor oil                                   | H Vegetable oil            |

The test was organized by the German member body (DIN) in 2006 and the results obtained were subjected to statistical analysis in accordance with ISO 5725-1[2] and ISO 5725-2[3] to give the precision data in [Table A.1](#) for the volumetric method.

**Table A.1 — Statistical results for the volumetric method**

Sample	A	B	C	D	E	F	G	H
<b>No. participating laboratories, <math>n_p</math></b>	10	12	12	14	14	14	14	13
<b>No. laboratories retained after eliminating outliers, <math>n_p</math></b>	9	10	10	14	12	12	12	11
<b>No. test results in all remaining laboratories, <math>n_t</math></b>	18	20	20	28	24	24	24	22
<b>Mean water content, <math>\bar{w}_{H_2O,t}</math>, g/100 g</b>	2,939	1,439	0,728	0,295	0,181	0,075	0,044	0,010 4
<b>Repeatability standard deviation, <math>s_r</math>, g/100 g</b>	0,025	0,015	0,015	0,008	0,003	0,003	0,002	0,001 0
<b>Coefficient of variation of repeatability, <math>CV(r)</math>, %</b>	0,8	1,1	2,1	2,7	1,6	4,4	4,8	10,1
<b>Repeatability limit, <math>r</math>, g/100 g</b>	0,070	0,043	0,043	0,022	0,008	0,009	0,006	0,002 9
<b>Reproducibility standard deviation, <math>s_R</math>, g/100 g</b>	0,144	0,024	0,021	0,022	0,008	0,005	0,005	0,002 7
<b>Coefficient of variation of reproducibility, <math>CV(R)</math>, %</b>	4,9	1,7	2,9	7,5	4,2	6,9	10,4	26,4
<b>Reproducibility limit, <math>R</math>, g/100 g</b>	0,403	0,068	0,060	0,062	0,021	0,015	0,013	0,007 6

## Annex B (informative)

### Information and precision data on the use of the coulometric method

While the volumetric method specified in the main body of this document is used for the determination of higher milligram levels of water (high level moisture), the coulometric method is used for moisture levels in the range 10 µg to 10 mg of water in a sample (low level moisture). But, depending on the amount of test sample, both methods can be used for high and low level moisture determination. With the coulometric Karl Fischer titration, the amount of water is determined by measuring the quantity of electric charge, in coulombs, generated during the titration. The electric charge is calculated by multiplying the current, in amperes, by the titration time, in seconds. According to Faraday's law,  $2 \times 96\,485$  C are needed to generate 1 mole of iodine, and one molecule of this iodine subsequently reacts with one molecule of water during the Karl Fischer reaction.

In the coulometric variation of the Karl Fischer determination of water, iodine necessary for the reaction with water is produced by the anodic oxidation of iodide. Instead of dispensing Karl Fischer reagent as in volumetric Karl Fischer titration, the Karl Fischer instrumentation actually generates the reagent inside the reaction cell. A current flows through the reagent generating iodine at the anode electrode. The iodine produced is proportional to the quantity of electricity consumed. The commercial instruments which have been developed to make use of this principle are sophisticated, usually being fully automated and computerized. Such an instrument contains two cells, anodic and cathodic, separated by a membrane and containing electrolytes into which platinum electrodes dip. The reaction takes place in the anodic cell. The rest of the instrument facilitates the reaction, the measurement of the number of coulombs of charge consumed and its conversion into the water content. Based on the Faraday law relationship between electric charge and amount of substance, the exact amount of iodine generated is determined. Since one molecule of water reacts with one molecule of iodine, the amount of water can be calculated.

The coulometric method is more sensitive than the volumetric method and permits the determination of lower water contents (see [Table B.1](#)). It is also sensitive to atmospheric moisture and chemical side reactions.

**Table B.1 — Sample sizes**

Water content in the sample % mass fraction	Sample mass g	Water content in the sample mg
0,000 1	10	0,01
0,001	10	0,1
0,01	5	0,5
0,1	2	2
1	0,2	2



An international collaborative test involving 22 laboratories in five countries was carried out on the following samples:

- |   |                                              |   |                          |
|---|----------------------------------------------|---|--------------------------|
| A | Medium chain triglyceride oil with 3 % water | E | Castor oil/Vegetable oil |
| B | Sample A/Vegetable oil (1 + 1)               | F | Extra virgin olive oil   |
| C | Sample A/Vegetable oil (1 + 3)               | G | Vegetable oil            |
| D | Castor oil                                   | H | Vegetable oil            |

The test was organized by the German member body (DIN) in 2006 and the results obtained were subjected to statistical analysis in accordance with ISO 5725-1[2] and ISO 5725-2[3] to give the precision data in [Table B.2](#) for the coulometric method.

**Table B.2 — Statistical data for the coulometric method**

Sample	A	B	C	D	E	F	G	H
<b>No. participating laboratories, <math>n_p</math></b>	11	14	13	14	14	15	15	15
<b>No. laboratories retained after eliminating outliers, <math>n_p</math></b>	11	14	10	13	11	12	11	12
<b>No. test results in all remaining laboratories, <math>n_t</math></b>	22	28	20	26	22	24	22	24
<b>Mean water content, <math>\bar{w}_{H_2O,t}</math>, g/100 g</b>	2,994	1,436	0,708	0,284	0,178	0,075 3	0,043 8	0,007 1
<b>Repeatability standard deviation, <math>s_r</math>, g/100 g</b>	0,041	0,037	0,010	0,006	0,003	0,000 7	0,000 6	0,000 4
<b>Coefficient of variation of repeatability, <math>CV(r)</math>, %</b>	1,4	2,6	1,4	2,3	1,5	0,9	1,3	5,1
<b>Repeatability limit, <math>r</math>, g/100 g</b>	0,116	0,103	0,027	0,018	0,007	0,001 9	0,001 6	0,001 0
<b>Reproducibility standard deviation, <math>s_R</math>, g/100 g</b>	0,214	0,079	0,030	0,013	0,007	0,002 1	0,001 0	0,001 5
<b>Coefficient of variation of reproducibility, <math>CV(R)</math>, %</b>	7,1	5,5	4,2	4,6	3,8	2,8	2,3	21,1
<b>Reproducibility limit, <math>R</math>, g/100 g</b>	0,599	0,220	0,084	0,036	0,019	0,005 8	0,002 8	0,004 2

## Bibliography

- [1] ISO 5555, *Animal and vegetable fats and oils — Sampling*
- [2] ISO 5725-1, *Accuracy (trueness and precision) of measurement methods and results — Part 1: General principles and definitions*
- [3] ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results — Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*
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