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**Animal and vegetable fats and oils —  
Determination of ultraviolet absorbance  
expressed as specific UV extinction**

*Corps gras d'origines animale et végétale — Détermination de  
l'absorbance dans l'ultraviolet, exprimée sous la forme d'extinction  
spécifique en lumière ultraviolette*

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

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 3656 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

This fourth edition cancels and replaces the third edition (ISO 3656:2002), which has been technically revised.

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## Introduction

This International Standard describes a method for the determination of the absorbance of light in the ultraviolet (UV) spectrum by fats and oils. Changes in absorption in the UV region are used as quality, purity, and authenticity criteria for fats and oils. Refining causes the formation of conjugated dienes and trienes and increased values of  $K_{232}$  and  $K_{268}$ , which then indicate the presence of refined oils. The oxidation of linoleic and linolenic acids results in the formation of hydroperoxides in which the double bonds become conjugated. Furthermore, the formation of either carbon-carbon bonds or carbon-oxygen bonds ( $\alpha,\beta$ -unsaturated carbonyl compounds) as secondary autoxidation products are observed. These compounds all lead to an increase of the absorption in the region between 225 nm and 325 nm.

The third edition of this International Standard allowed the determination of the UV absorbance at 232 nm and 268 nm using three different solvents (isooctane, cyclohexane or *n*-hexane). However, it is known that the solvents themselves have an effect on the UV absorbance between 260 nm and 276 nm in vegetable oils. Recent investigations have shown that the measurement of  $K_{268}$  and  $K_{270}$  for the same oil in isooctane or in cyclohexane give significantly different results. In isooctane, the maxima appear at 267 nm to 268 nm whereas in cyclohexane, the maxima appear at 268 nm to 269 nm. In the IOC standards for the determination of the ultraviolet absorbance of (virgin) olive oils, the specified wavelengths are 232 nm and 270 nm.

Taking into account the above, the fourth edition of this International Standard specifies measurement at 268 nm when isooctane is used and measurement at 270 nm when cyclohexane is used. Moreover, the variation of the specific extinction,  $\Delta K$ , for olive oils has been introduced. Precision data from a new collaborative trial have also been taken into consideration for this revision.

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# Animal and vegetable fats and oils — Determination of ultraviolet absorbance expressed as specific UV extinction

## 1 Scope

This International Standard specifies a method for the determination of the absorbance at ultraviolet wavelengths of animal and vegetable fats and oils.

## 2 Normative references

The following referenced documents are indispensable for the application 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 (standards.iteh.ai)

**NOTE** The usual policy of ISO/TC 34/SC 11, of using symbols specified in International Standard 80000<sup>[5]</sup> is not followed in this International Standard. The symbols are those used, for example, in Commission Regulation (EEC) No 2568/91<sup>[6]</sup>.

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

### 3.1

**specific extinction** ( $K_{232} - K_{268} - K_{270}$ )

absorbance of 1 g sample, dissolved in 100 ml isooctane or cyclohexane, measured in a 10 mm cell at the specific wavelengths 232 nm, 268 nm, and 270 nm

## 4 Principle

A sample is dissolved in isooctane or cyclohexane and the absorbance is measured spectrophotometrically in a specified ultraviolet wavelength range. The specific absorbance at 232 nm and 268 nm in isooctane or 232 nm and 270 nm in cyclohexane for a concentration of 1 g per 100 ml in a 10 mm cell is calculated.

## 5 Reagents

**WARNING — Attention is drawn to the regulations which specify the handling of hazardous substances. Technical, organizational and personal safety measures shall be followed.**

During the analysis, unless otherwise stated, use only reagents of recognized analytical grade and distilled or demineralized water or water of equivalent purity.

**5.1 Solvent: isooctane** (2,2,4-trimethylpentane) for the measurement at 232 nm and 268 nm or **cyclohexane** for the measurement at 232 nm and 270 nm, having an absorbance less than 0,12 at 232 nm and less than 0,05 at 250 nm against water, measured in a 10 mm cell.

## 6 Apparatus

The glassware used for the determination shall be thoroughly cleaned and rinsed with the solvent (5.1) before use so that it is free from impurities having an absorbance within the wavelength range of 220 nm to 360 nm.

Usual laboratory apparatus and, in particular, the following.

**6.1 One-mark volumetric flasks**, capacity 25 ml, ISO 1042<sup>[1]</sup> class A.

**6.2 Analytical balance**, capable of being read to the nearest 0,000 1 g.

**6.3 Spectrometer**, with quartz cells of pathlength 10 mm, suitable for measurements at ultraviolet wavelengths (220 nm to 360 nm).

**6.3.1 General.** Before use it is recommended that the wavelength and absorbance scales of the spectrometer be checked as specified in 6.3.2 and 6.3.3.

**6.3.2 Wavelength scale.** Check this using a reference material consisting of an optical glass filter containing holmium oxide which has distinct absorption bands. The reference material is designed for the verification and calibration of the wavelength scales of visible and ultraviolet spectrophotometers having nominal spectral bandwidths of 5 nm or less. The holmium glass filter is measured in the absorbance mode against an air blank, over the wavelength range 640 nm to 240 nm. For each spectral bandwidth (0,10, 0,25, 0,50, 1,00, 1,50, 2,00, and 3,00), a baseline correction is performed with an empty cell holder. The wavelengths of the spectral bandwidth are listed in the certificate of the reference material<sup>1)</sup>.

**6.3.3 Absorbance scale.** This may be checked using a reference material consisting of four solutions of potassium dichromate in perchloric acid sealed in far UV quartz cells to measure the linearity and photometric accuracy reference in the UV. The potassium dichromate filled cells (40 mg/ml, 60 mg/ml, 80 mg/ml and 100 mg/ml) are measured against a perchloric acid blank (see Reference [7]). The net absorbance values are listed in the certificate of the reference material<sup>1)</sup>.

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## 7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 5555<sup>[2]</sup>.

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

## 8 Preparation of test sample

Prepare the test sample in accordance with ISO 661.

Ensure that the test sample is perfectly homogeneous and contains no suspended impurities. Filter oils which are liquid at ambient temperature through paper at a temperature of approximately 30 °C. Homogenize and filter hard fats at a temperature of not more than 10 °C above their melting point.

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1) Suitable holmium filters and potassium dichromate-sealed cells are available commercially, e.g. from Starna Scientific ([www.starnascientific.com](http://www.starnascientific.com)). This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this supplier.

## 9 Procedure

### 9.1 Test portion and preparation of the test solution

**9.1.1** Weigh, to the nearest 0,1 mg (6.2), an amount of the test sample, generally 0,05 g to 0,25 g, necessary to obtain absorbance values between 0,2 and 0,8, into a 25 ml volumetric flask (6.1).

**9.1.2**  $K_{232}/K_{268}$ . Dissolve the test portion at ambient temperature in a few millilitres of isooctane (5.1) for the determination of the specific absorbance at 232 nm and 268 nm and then make up to the mark with the same solvent. Mix thoroughly.

**9.1.3**  $K_{232}/K_{270}$ . Dissolve the test portion at ambient temperature in a few millilitres of cyclohexane (5.1) for the measurement of the specific absorbance at 232 nm and 270 nm and then make up to the mark with the same solvent. Mix thoroughly.

**9.1.4** Ensure that the solutions prepared in 9.1.2 and 9.1.3 are perfectly clear. If opalescence or turbidity is present, filter quickly through paper.

If the concentration of test sample in the test solution is greater than 1 g per 100 ml of solution, this shall be stated in the test report.

### 9.2 Determination

Rinse a quartz cell (6.3) three times with the test solution (9.1). Fill the cell with the test solution and measure the absorbance against the solvent used for dilution, by means of the spectrometer (6.3), over the wavelength range 220 nm to 360 nm, either continuously or at intervals of 1 nm or 2 nm, reducing the intervals to 0,5 nm in the regions of maximum and minimum absorbance.

NOTE It is possible that it is not necessary to measure the absorbance over the full wavelength range.

If the absorbance value obtained exceeds 0,8, dilute the test solution as appropriate and repeat the determination.

## 10 Expression of results

### 10.1 Specific extinction (extinction coefficients) at a specific wavelength

The specific extinction,  $K_{\lambda}$  (extinction coefficient) at a specific wavelength,  $\lambda$ , is calculated as follows:

$$K_{\lambda} = \frac{E_{\lambda}}{c s}$$

where

$E_{\lambda}$  is the extinction measured at wavelength  $\lambda$ ;

$c$  is the concentration, in grams per 100 ml, of the solution;

$s$  is the pathlength, in centimetres, of the cuvette.

Express the results to two decimal places.

## 10.2 Variation of the specific extinction, $\Delta K$

Spectrophotometric analysis of olive oil in accordance with Commission Regulation (EEC) No 2568/91<sup>[6]</sup> also involves the determination of the variation of the specific extinction,  $\Delta K$ , which is given by:

$$\Delta K = K_m - \frac{K_{m-4} + K_{m+4}}{2}$$

where  $K_m$  is the specific extinction at wavelength  $m$ , wavelength for maximum absorption which depends on the solvent used.

The results of this determination are expressed to two decimal places.

## 11 Precision

### 11.1 Interlaboratory test

Details of an interlaboratory test are summarized in Annex A. It is possible that the values derived from this interlaboratory test are not applicable to concentration ranges other than those given.

### 11.2 Repeatability

The absolute difference between two independent single test results, obtained using 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 be greater than the overall repeatability limit,  $r$ .

### 11.3 Reproducibility

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The absolute difference between two single test results, obtained using 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 overall reproducibility limit,  $R$ .

## 12 Test report

The test report shall contain 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 International Standard (ISO 3656:2011);
- 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;
- f) if the repeatability has been checked, the final quoted result obtained.



## Annex A (informative)

### Results of interlaboratory test

An interlaboratory test was carried out in 2009 in accordance with ISO 5725-1<sup>[3]</sup> and ISO 5725-2<sup>[4]</sup> by the International Olive Council. A total of 22 laboratories from seven countries participated. The results are summarized in Tables A.1 to A.6.

**Table A.1 — UV extinction at 232 nm in isooctane**

Parameter	Sample				
	A	B	C	D	E
No. participating laboratories, $n_p$	21	22	22	22	22
No. laboratories retained after eliminating outliers, $n_p$	20	18	18	21	17
No. test results in all laboratories, $n_t$	40	36	36	42	34
Mean specific extinction at 232 nm, $\bar{K}_{232}$	1,763	2,104	3,805	3,847	2,817
Repeatability standard deviation, $s_r$	0,026	0,013	0,016	0,036	0,019
Coefficient of variation of repeatability, $C_{V,r}$ , %	1,5	0,6	0,4	0,9	0,7
Repeatability limit, $r$	0,072	0,035	0,043	0,101	0,054
Reproducibility standard deviation, $s_R$	0,077	0,069	0,016	0,036	0,069
Coefficient of variation of reproducibility, $C_{V,R}$ , %	4,4	3,3	4,6	5,5	2,5
Reproducibility limit, $R$	0,216	0,194	0,488	0,582	0,194

**Table A.2 — UV extinction at 232 nm in cyclohexane**

Parameter	Sample				
	A	B	C	D	E
No. participating laboratories, $n_p$	21	21	21	21	21
No. laboratories retained after eliminating outliers, $n_p$	18	20	20	21	21
No. test results in all laboratories, $n_t$	36	40	40	42	42
Mean specific extinction at 232 nm, $\bar{K}_{232}$	1,758	2,122	3,827	3,863	2,789
Repeatability standard deviation, $s_r$	0,025	0,022	0,042	0,041	0,033
Coefficient of variation of repeatability, $C_{V,r}$ , %	1,4	1,0	1,1	1,1	1,2
Repeatability limit, $r$	0,070	0,060	0,119	0,113	0,093
Reproducibility standard deviation, $s_R$	0,049	0,073	0,151	0,138	0,100
Coefficient of variation of reproducibility, $C_{V,R}$ , %	2,8	3,4	4,0	3,6	3,6
Reproducibility limit, $R$	0,138	0,204	0,424	0,386	0,279