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**Oils of *Citrus* — Determination of CD value  
by ultraviolet spectrometric analysis**

*Huiles essentielles de Citrus — Détermination de la valeur CD par analyse  
spectrométrique dans l'ultraviolet*

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Reference number  
ISO 4735:2002(E)

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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 3.

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 International Standard may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

International Standard ISO 4735 was prepared by Technical Committee ISO/TC 54, *Essential oils*.

This second edition cancels and replaces the first edition (ISO 4735:1981), which has been technically revised.



# Oils of *Citrus* — Determination of CD value by ultraviolet spectrometric analysis

## 1 Scope

This International Standard specifies a method for the determination of the CD value of the essential oils of *Citrus* by ultraviolet spectrometric analysis.

## 2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this International Standard. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this International Standard are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 356, *Essential oils — Preparation of test samples*

ISO 648, *Laboratory glassware — One-mark pipettes*

ISO 1042, *Laboratory glassware — One-mark volumetric flasks*

## 3 Term and definition

For the purposes of this International Standard, the following term and definition apply.

### 3.1

#### CD value

measure of segment CD, expressed in absorbance units, for the exact mass of test portion specified in the standard appropriate to the essential oil concerned

NOTE The segment CD is obtained as follows: draw the tangent common to the two parts of the curve (points A and B are obtained), and a line parallel to the absorbance axis which passes through the maximum absorption (point D). This parallel cuts the tangent at point C and the axis of the wavelength at point E (see Figure 1).

## 4 Principle

The absorbance curve of an ethanolic solution of the oil in the wavelength range from 220 nm to 440 nm is plotted and the CD value determined.

NOTE This method also permits determination of the ratio CD/DE (see Figure 1).



## 7 Sampling

Sampling is not part of the method specified in this International Standard. A recommended sampling method is given in ISO 212.

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

## 8 Preparation of test sample

Prepare the test sample according to ISO 356.

## 9 Procedure

### 9.1 Test portion

Weigh, to the nearest 0,001 g, the mass,  $m$ , of the test sample (see clause 8) specified in the standard appropriate to the essential oil concerned.

### 9.2 Preparation of test solution

Dissolve the test portion (9.1) in the ethanol (5.1) in a 100 ml one-mark volumetric flask (6.3). Dilute to the mark with ethanol and mix.

### 9.3 Determination

Introduce an aliquot portion of the test solution (9.2) into a quartz cell (6.2) of the spectrometer (6.1), and record the absorbance curve between 220 nm and 440 nm, using ethanol (5.1) as the reference liquid.

If a recorder is not available, determine the absorbance at intervals of 5 nm and, when approaching the expected minimum and maximum values, determine the absorbance at every 2 nm.

## 10 Expression of results

The CD value is expressed in absorbance units, to two decimal places, per gram of product, and is given by the formula:

$$CD = A_{CD} \times \frac{1}{m}$$

where

$A_{CD}$  is the segment CD directly obtained by measurement, expressed in absorbance units;

$m$  is the mass, in grams, of the test portion (9.1).

## 11 Test report

The test report shall specify:

- all information necessary for the complete identification of the sample;
- the sampling method used, if known;
- the test method used, with reference to this International Standard;

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- 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);
- the test result(s) obtained.



## Bibliography

- [1] ISO 212, *Essential oils — Sampling*

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