

INTERNATIONAL STANDARD

ISO
3520

Second edition
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Oil of bergamot [*Citrus aurantium* L. subsp. *bergamia* (Wight et Arnott) Engler], Italian type

*Huile essentielle de bergamote [Citrus aurantium L. subsp. bergamia
(Wight et Arnott) Engler], type Italie*

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Reference number
ISO 3520:1998(E)

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.

International Standard ISO 3520 was prepared by Technical Committee ISO/TC 54, *Essential oils*.

This second edition cancels and replaces the first edition (ISO 3520:1980), which has been technically revised.

Annexes A and B of this International Standard are for information only.

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Oil of bergamot [*Citrus aurantium* L. subsp. *bergamia* (Wight et Arnott) Engler], Italian type

1 Scope

This International Standard specifies certain characteristics of the oil of bergamot, Italian type, in order to facilitate assessment of its quality.

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 210, *Essential oils — General rules for packaging, conditioning and storage.*

ISO 211, *Essential oils — General rules for labelling and marking containers.*

ISO 212, *Essential oils — Sampling.*

ISO 279, *Essential oils — Determination of relative density at 20 °C (Reference method).*

ISO 280, *Essential oils — Determination of refractive index.*

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

ISO 592, *Essential oils — Determination of optical rotation.*

ISO 709, *Essential oils — Determination of ester value.*

ISO 875, *Essential oils — Evaluation of miscibility in ethanol.*

ISO 1242, *Essential oils — Determination of acid value.*

ISO 4715, *Essential oils — Quantitative evaluation of residue on evaporation.*

ISO 4735, *Oils of citrus — Determination of CD value by ultraviolet spectrophotometric analysis.*

ISO 7358, *Oils of bergamot, lemon, citron and lime, full or partially reduced in bergaptene — Determination of bergaptene content by high-performance liquid chromatography.*

ISO 11024-1, *Essential oils — General guidance on chromatographic profiles — Part 1: Preparation of chromatographic profiles for presentation in standards.*

ISO 11024-2, *Essential oils — General guidance on chromatographic profiles — Part 2: Utilization of chromatographic profiles of samples of essential oils.*

3 Term and definition

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

3.1 oil of bergamot, Italian type

essential oil extracted without heating, by a mechanical extraction process, from the fresh pericarp of the fruit of *Citrus aurantium* L. subsp. *bergamia* (Wight et Arnott) Engler, of the Rutaceae family, mainly picked in Italy

4 Requirements

4.1 Appearance

Clear, mobile liquid, sometimes with a solid deposit.

4.2 Colour

Green to yellow.

4.3 Odour

Characteristic, pleasant and cool, recalling that of the fresh pericarp of bergamot.

4.4 Relative density at 20 °C, d_{20}^{20}

Minimum: 0,876
Maximum: 0,883

4.5 Refractive index at 20 °C

Minimum: 1,465 0
Maximum: 1,470 0

4.6 Optical rotation at 20 °C

Between +15° and +32°.

4.7 Residue on evaporation

Minimum: 4,50 %
Maximum: 6,40 %

4.8 Miscibility in ethanol at 20 °C

1 volume of essential oil shall require a maximum of 1 volume of ethanol (85 % volume fraction) at 20 °C to obtain a clear solution.

4.9 Acid value

Maximum: 2

4.10 Ester value

Minimum: 86
Maximum: 129

4.11 Bergaptene content by high-performance liquid chromatography

Minimum: 0,18 %
Maximum: 0,38 %

4.12 CD value

Minimum: 0,760
Maximum: 1,180

Dilution of 0,1 g/100 ml of ethanol (95 % volume fraction).

4.13 Chromatographic profile

Analysis of the essential oil shall be carried out by gas chromatography. In the chromatogram obtained, the representative and characteristic components shown in Table 1 shall be identified. The proportions of these components, indicated by the integrator, shall be as shown in Table 1. This constitutes the chromatographic profile of the essential oil.

Table 1 — Chromatographic profile

Component	Minimum %	Maximum %
β-Pinene	5,5	9,5
Limonene	30	45
γ-Terpinene	6	10
Linalol	3	15
Linalyl acetate	22	36
Geranial	0,25	0,50
β-Bisabolene	0,30	0,55

NOTE The chromatographic profile is normative, contrary to typical chromatograms given for information in annex A.

4.14 Flashpoint

Information on the flashpoint is given in annex B.

5 Sampling

See ISO 212.

Minimum volume of final sample: 25 ml.

NOTE This volume allows each of the tests specified in this International Standard to be carried out at least once.

It is rather common with this oil for solid matter to settle. Special attention is therefore drawn to the need to sample only from well-homogenized material. The same applies to the preparation of the test sample.

6 Preparation of test sample

See ISO 356.

7 Test methods

7.1 Relative density at 20 °C, d_{20}^{20}

See ISO 279.

7.2 Refractive index at 20 °C

See ISO 280.

7.3 Optical rotation at 20 °C

See ISO 592.

7.4 Residue on evaporation

See ISO 4715.

Test portion: 5 g
Evaporation time: 6 h

7.5 Miscibility in ethanol at 20 °C

See ISO 875.

7.6 Acid value

See ISO 1242.

7.7 Ester value

See ISO 709.

Saponification time: 30 min

7.8 Bergaptene content by high-performance liquid chromatography

See ISO 7358.

7.9 CD value

See ISO 4735.

Point B: 278 nm approximately
Maximum value (point D): 312 nm approximately
Point A: 365 nm approximately

7.10 Chromatographic profile

See ISO 11024-1 and ISO 11024-2.

8 Packaging, labelling, marking and storage

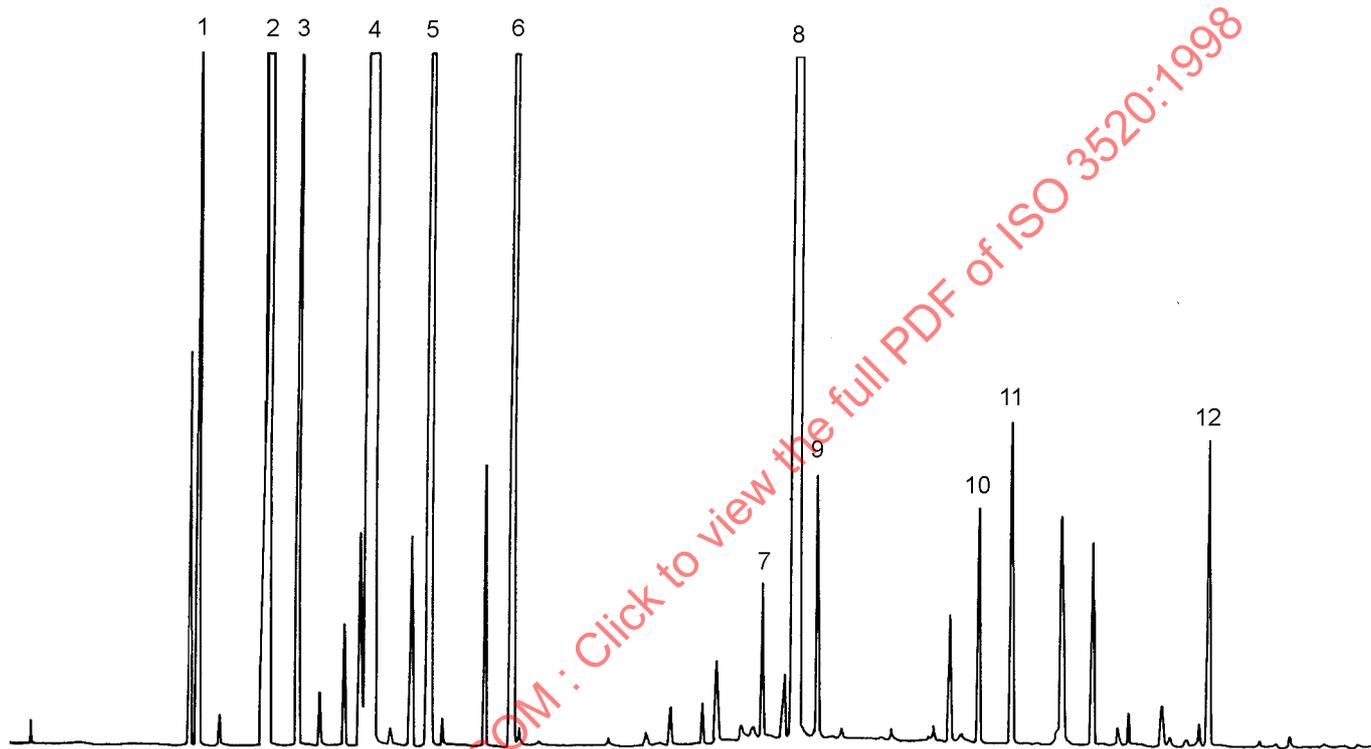
See ISO 210 and ISO 211.

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Annex A

(informative)

Typical chromatograms of the analysis by gas chromatography of the essential oil of bergamot, Italian type



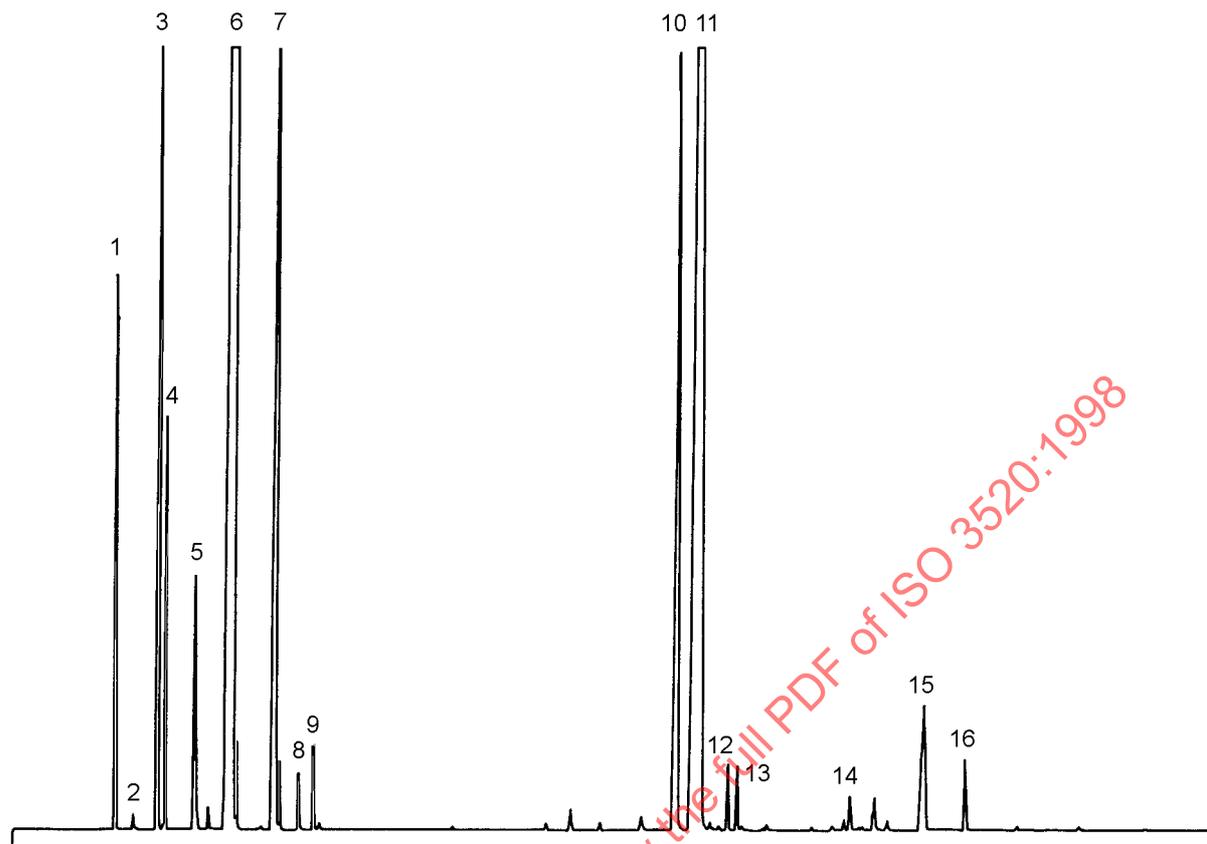
Peak identification

- 1 α -Pinene
- 2 Sabinene + β -pinene
- 3 Myrcene
- 4 Limonene
- 5 γ -Terpinene
- 6 Linalol
- 7 Neral
- 8 Linalyl acetate
- 9 Geranial
- 10 Neryl acetate
- 11 Geranyl acetate
- 12 β -Bisabolene

Operating conditions

Column: capillary in glass, 25 m long and 0,32 mm internal diameter
 Film thickness: 0,4 μ m to 0,45 μ m
 Stationary phase: SE 52
 Oven temperature: initial temperature of 60 $^{\circ}$ C for 8 min, then at a rate of 3 $^{\circ}$ C/min up to 100 $^{\circ}$ C, then 2,5 $^{\circ}$ C/min up to 130 $^{\circ}$ C, then 3 $^{\circ}$ C/min to a final temperature of 160 $^{\circ}$ C.
 Injector temperature: 280 $^{\circ}$ C
 Detector temperature: 280 $^{\circ}$ C
 Detector: flame ionization type
 Carrier gas: helium
 Volume injected: 1 μ l
 Split ratio: 1/150

Figure A.1 — Typical chromatogram taken on an apolar column

**Peak identification**

- 1 α -Pinene
- 2 Camphene
- 3 β -Pinene
- 4 Sabinene
- 5 Myrcene
- 6 Limonene
- 7 γ -Terpinene
- 8 *para*-Cymene
- 9 Terpinolene
- 10 Linalol
- 11 Linalyl acetate
- 12 Sesquiterpene
- 13 Sesquiterpene
- 14 Neral
- 15 Geranial + neryl acetate + β -bisabolene
- 16 Geranyl acetate

Operating conditions

Column: capillary in silica, 50 m long and 0,22 mm internal diameter
 Film thickness: about 0,2 μ m
 Stationary phase: polyethylene glycol 20 000
 Oven temperature: initial temperature of 65 °C for 5 min, then
 at a rate of 2 °C/min to 155 °C, then 3 °C/min up to 200 °C
 Injector temperature: 230 °C
 Detector temperature: 250 °C
 Detector: flame ionization type
 Carrier gas: hydrogen
 Volume injected: 0,2 μ l
 Carrier gas flow rate: about 0,40 m/s.

Figure A.2 — Typical chromatogram taken on a polar column

Annex B (informative)

Flashpoint

B.1 General information

For reasons of safety, transport companies, insurance companies, people in charge of safety services, etc. require information on the flashpoint of essential oils, which in most cases are flammable products.

A comparative study on the relevant methods of analysis (see ISO/TR 11018¹⁾) concluded that it was hard to find a single method for standardization purposes, given that:

- essential oils are varied and their chemical compositions differ to a large extent;
- the volume of the sample needed for certain test equipment is incompatible with the high price of essential oils;
- there are different types of equipment that satisfy the desired objective, but users cannot be obliged to use one type of equipment rather than another.

Consequently, it was decided to give a mean value for the flashpoint in an informative annex in each International Standard, for information purposes, in order to meet the requirements of the interested parties.

If possible, the method by which this value was obtained should be specified.

For further information see ISO/TR 11018¹⁾.

B.2 Flashpoint of oil of bergamot, Italian type

The mean value is +59 °C obtained with "Setaflash" equipment.

The mean value is +65 °C obtained with "Luchoire" equipment.

1) ISO/TR 11018:1997, *Essential oils — General guidance on the determination of flashpoint*.