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**Essential oil of bergamot [*Citrus bergamia* Risso et Poit], Calabrian type**

*Huile essentielle de bergamote [*Citrus bergamia* Risso et Poit], type calabrais*

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ISO copyright office  
CP 401 • Ch. de Blandonnet 8  
CH-1214 Vernier, Geneva  
Phone: +41 22 749 01 11  
Email: [copyright@iso.org](mailto:copyright@iso.org)  
Website: [www.iso.org](http://www.iso.org)

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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)).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, 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 [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

This document was prepared by Technical Committee ISO/TC 54, *Essential oils*.

This third edition cancels and replaces the second edition (ISO 3520:1998), which has been technically revised.

The main changes are as follows:

- the title has been changed;
- the structure has been revised;
- [3.1](#) has been reworded to update the botanical name to *Citrus bergamia* Risso et Poit and to add the Calabrian type;
- in [4.1](#), minor changes to a) relative density (formerly 0,883 max); b) refractive index (formerly 1,470 max); c) optical rotation (formerly +32° max); d) residue on evaporation (formerly 6,40 % max); and e) CD value (formerly 1,180 max) have been included;
- in [4.2](#),  $\beta$ -pinene % max has been reduced (formerly 9,5 %) and limonene % max has been increased (formerly 45 %);
- in [Annex A](#), the chromatograms in [Figures A.1](#) and [A.2](#) and the data have been updated.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at [www.iso.org/members.html](http://www.iso.org/members.html).

# Essential oil of bergamot [*Citrus bergamia* Risso et Poit], Calabrian type

## 1 Scope

This document specifies certain characteristics of the essential oil of bergamot (*Citrus bergamia* Risso et Poit), Calabrian type, with a view to facilitate the assessment of its quality.

## 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/TS 210, *Essential oils — General rules for packaging, conditioning and storage*

ISO/TS 211, *Essential oils — General rules for labelling and marking of 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 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 spectrometric analysis*

ISO 7358, *Essential oils of bergamot, lemon, bitter orange and lime, fully or partially reduced in bergapten — Determination of bergapten content by high-performance liquid chromatography (HPLC)*

ISO 11024 (all parts), *Essential oils — General guidance on chromatographic profiles*

## 3 Terms and definitions

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

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

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

**3.1 essential oil of bergamot, Calabrian type**  
essential oil extracted without heating, by mechanical extraction process, from the fresh pericarp of the fruit of *Citrus bergamia* (Risso et Poit), of the *Rutaceae* family, picked in the Calabria region of Italy

Note 1 to entry: This essential oil is cited as Protected Designation of Origin (PDO) in the COMMISSION REGULATION (EC) No 509/2001 of 15 March 2001 supplementing the Annex to Regulation (EC) No 2400/96 on the entry of certain names in the 'Register of protected designations of origin and protected geographical indications' provided for in Council Regulation (EEC) No 2081/92 on the protection of geographical indications and designations of origin for agricultural products and foodstuffs, available from: <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32001R0509&from=EN>.

Note 2 to entry: For information on the CAS number, see ISO/TR 21092.

## 4 Requirements

### 4.1 General requirements

Essential oil of bergamot, Calabrian type shall meet the requirements as given in [Table 1](#).

**Table 1 — Requirements for the Italian essential oil of bergamot, Calabrian type**

Characteristics	Requirements	ISO test method
Appearance	Clear, mobile liquid, sometimes with a solid deposit	—
Colour	Green to yellow	—
Odour	Characteristic, pleasant and cool, recalling that of fresh pericarp of bergamot	—
Relative density at 20 °C $d_{20}^{20}$	0,868 0 to 0,878 0	ISO 279
Refractive index at 20 °C	1,465 0 to 1,470 0	ISO 280
Optical rotation at 20 °C	Between +20° and +36°	ISO 592
Residue on evaporation	4,5 % to 6,0 %	ISO 4715
Miscibility in ethanol 85 % (volume fraction), at 20 °C	One volume of essential oil shall require a maximum of one volume of ethanol 85 % (volume fraction) at 20 °C to obtain a clear solution	ISO 875
Acid value	Maximum 2 mg KOH (potassium hydroxide) to neutralize 1 g of essential oil	ISO 1242
Ester value	86 mg to 129 mg KOH (potassium hydroxide) to neutralize acids liberated by hydrolysis of esters present in 1 g of essential oil	ISO 709
Bergaptene content by HPLC	0,18 % to 0,38 %	ISO 7358
CD value Dilution 1 g/l of ethanol 95 % (volume fraction)	0,65 to 1,15 absorbance unit	ISO 4735

### 4.2 Chromatographic profile

Carry out the analysis of the essential oil by gas chromatography. Determine the chromatographic profile in accordance with the ISO 11024 series. Identify in the chromatogram obtained the representative and characteristic components shown in [Table 2](#). The proportions of these components, indicated by the integrator, shall be as shown in [Table 2](#). This constitutes the chromatographic profile of the essential oil.

**Table 2 — Chromatographic profile**

<b>Component</b>	<b>Minimum %</b>	<b>Maximum %</b>
$\beta$ -Pinene	4,0	8,5
Limonene	32,0	47,0
$\gamma$ -Terpinene	6,0	10,0
Linalool	3,0	15,0
Linalyl acetate	22,0	36,0
Geranial	0,25	0,5
$\beta$ -Bisabolene	0,3	0,7
NOTE The chromatographic profile is normative, contrary to typical chromatograms given for information in <a href="#">Annex A</a> (see <a href="#">Figures A.1</a> and <a href="#">A.2</a> ).		

## 5 Flash point

Information on the flash point is given in [Annex B](#).

## 6 Sampling

Sampling shall be performed in accordance with ISO 212. The minimum volume of the test sample is 50 ml.

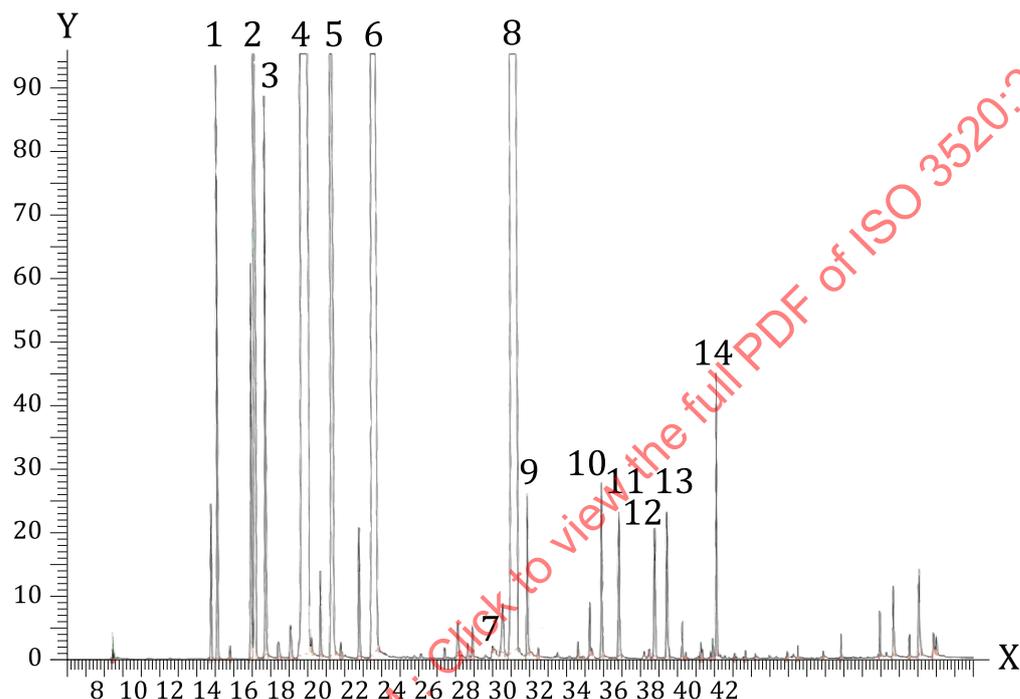
NOTE The volume allows each of the tests specified in this document to be carried out at least once.

## 7 Packaging labelling, marking and storage

These items shall be in accordance with ISO/TS 210 and ISO/TS 211.

## Annex A (informative)

### Typical chromatograms of the analysis by gas chromatography of essential oil of bergamot (*Citrus bergamia* Risso et Poit), Calabrian type



#### Key

X time (min)  
Y response (mV)

#### Peak identification

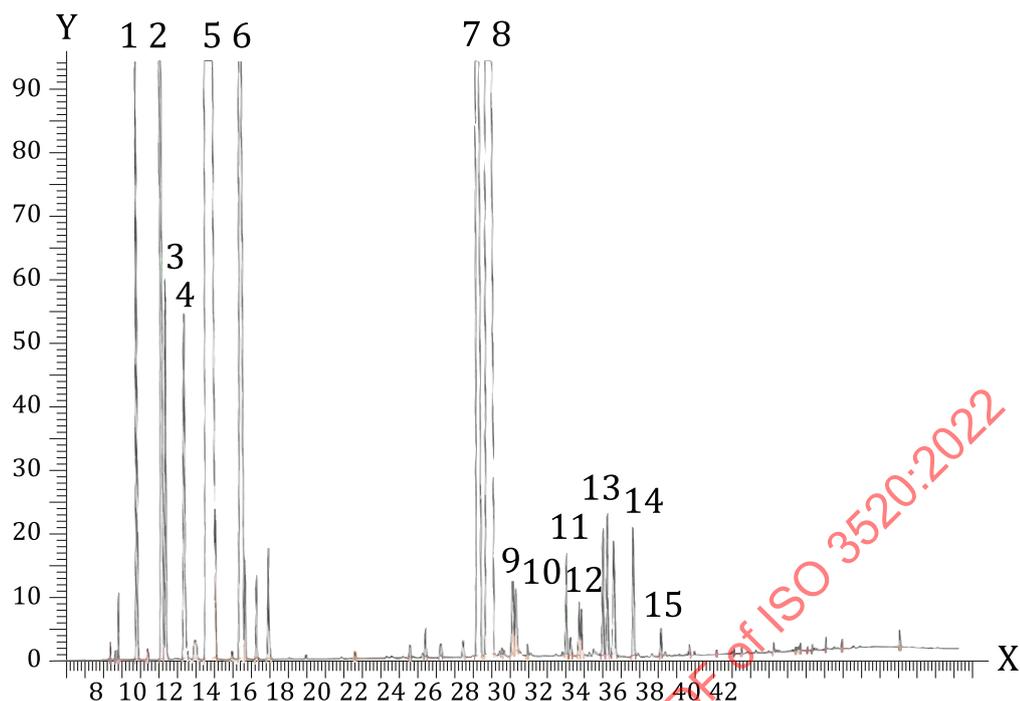
- |    |                              |
|----|------------------------------|
| 1  | $\alpha$ -pinene             |
| 2  | sabinene + $\beta$ -pinene   |
| 3  | myrcene                      |
| 4  | limonene                     |
| 5  | $\gamma$ -terpinene          |
| 6  | linalool                     |
| 7  | neral                        |
| 8  | linalyl acetate              |
| 9  | geranial                     |
| 10 | neryl acetate                |
| 11 | geranyl acetate              |
| 12 | $\beta$ -caryophyllene       |
| 13 | trans- $\alpha$ -bergamotene |
| 14 | $\beta$ -bisabolene          |

#### Operating conditions

column: capillary 30 m ID, 250  $\mu$ m  
film thickness: 0,25  $\mu$ m  
stationary phase: Perkin Elmer Elite 5 MS<sup>a</sup>  
oven temperature: initial temperature of 70 °C for 8 min, then at a rate of 3 °C/min up to 130 °C for 3 min, then 7 °C/min up to 220 °C for 5 min  
injection temperature: 250 °C  
detector temperature: 250 °C  
detector: flame ionization type  
carrier gas: helium  
volume injected: 0,2  $\mu$ l  
split ratio: 1/150

<sup>a</sup> Perkin Elmer Elite 5 MS is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

Figure A.1 — Typical chromatogram using an apolar column

**Key**

X time (min)  
Y response (mV)

**Peak identification**

- |    |                              |
|----|------------------------------|
| 1  | $\alpha$ -pinene             |
| 2  | $\beta$ -pinene              |
| 3  | sabinene                     |
| 4  | myrcene                      |
| 5  | limonene                     |
| 6  | $\gamma$ -terpinene          |
| 7  | linalool                     |
| 8  | linalyl acetate              |
| 9  | $\beta$ -caryophyllene       |
| 10 | trans- $\alpha$ -bergamotene |
| 11 | neral                        |
| 12 | geranial                     |
| 13 | neryl acetate                |
| 14 | $\beta$ -bisabolene          |
| 15 | geranyl acetate              |

**Operating conditions**

column: capillary 30 m, 250  $\mu$ m  
 film thickness: 0,25  $\mu$ m  
 stationary phase: Perkin Elmer Elite wax<sup>a</sup>  
 oven temperature: initial temperature of 70 °C for 8 min, then at a rate of 3 °C/min up to 130 °C for 3 min, then 7 °C/min up to 220 °C for 5 min  
 injection temperature: 250 °C  
 detector temperature: 250 °C  
 detector: flame ionization type  
 carrier gas: helium  
 volume injected: 0,2  $\mu$ l  
 split ratio: 1/150

<sup>a</sup> Perkin Elmer Elite wax is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

**Figure A.2 — Typical chromatogram using a polar column**