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**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)**

*Huiles essentielles de bergamote, de citron, de bigarade et de  
limette complètement ou partiellement privées de bergaptène —  
Détermination de la teneur en bergaptène par chromatographie  
liquide à haute performance (CLHP)*

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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 second edition cancels and replaces the first edition (ISO 7358:2002), which has been technically revised.

The main change to the previous edition is as follows:

- the addition of an alternative method using a reversed phase column.

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

## 1 Scope

This document specifies a high-performance liquid chromatographic (HPLC) method, using either an internal standard or external standard, for the determination of the bergapten content in essential oil of bergamot [*Citrus aurantium* ssp. *bergamia* (Risso et Poit.) Wight et Arn. ex Engl.], in essential oil of lemon [*Citrus limon* (L.) Burm. f.], in essential oil of bitter orange (*Citrus bigaradia* Risso) and in essential oil of lime [*Citrus aurantiifolia* (Christm.) Swingle and *Citrus latifolia* Tanaka], all of them fully or partially reduced in bergapten.

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

ISO 8432, *Essential oils — Analysis by high performance liquid chromatography — General method*

## 3 Terms and definitions

No terms and definitions are listed in this document.

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

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

## 4 Principle

The bergapten contents of test samples to be measured are determined after dilution by reversed phase HPLC or by normal phase HPLC with gradient elution, using an internal standardization method or an external standardization method and diode-array UV spectrometric detection.

## 5 Reagents

Use only the following reagents of recognized analytical grade.

**5.1 Reference substance: bergapten** (5-Methoxypsoralen),  $C_{12}H_8O_4$ , MW = 216,19 g/mol of known purity  $\geq 95$  %.

**5.2 Internal standard: coumarin** (1-Benzopyran-2-one),  $C_9H_6O_2$ , MW = 146,14 g/mol of known purity  $\geq 98$  %.