
**Oils of bergamot, lemon, citron and lime,
fully or partially reduced in bergapten —
Determination of bergapten content by
high-pressure 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 pression
(CLHP)*



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Printed in Switzerland

Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 3.

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

ISO 7358 was prepared by Technical Committee ISO/TC 54, *Essential oils*.

Annex A of this International Standard is for information only.

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Oils of bergamot, lemon, citron and lime, fully or partially reduced in bergapten — Determination of bergapten content by high-pressure liquid chromatography (HPLC)

1 Scope

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

For essential oils having a bergapten content greater than or equal to a mass fraction of 0,001 %, the internal standard method is applicable.

For essential oils having a bergapten content between a mass fraction of 0,000 1 % and 0,001 %, the external standard method is applicable.

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 sample*

ISO 8432:1987, *Essential oils — Analysis by high performance liquid chromatography — General method*

3 Principle

Liquid chromatography is based on the physico-chemical phenomena of adsorption, separation, ion exchange or exclusion. It enables a small quantity of essential oil to be analysed using a chromatographic column with an appropriate packing, under appropriate conditions, with identification of the different constituents and the quantitative determination of specific compounds.

4 Reagents

Use only reagents of recognized analytical grade.

DANGER — Attention is drawn to the hazard deriving from the specified use of chloroform, a toxic solvent.

4.1 Reference substance: bergapten, of known purity ≥ 95 %.