INTERNATIONAL STANDARD

ISO 4728

Second edition
2003-09-01

Oil of Spanish wild marjoram (*Thymus mastichina* L.)

*Huile essentielle de marjolaine sauvage d'Espagne (*Thymus mastichina *L.)*

Reference number
ISO 4728:2003(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

Published in Switzerland

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 2.

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

ISO 4728 was prepared by Technical Committee ISO/TC 54, *Essential oils*.

This second edition cancels and replaces the first edition (ISO 4728:1992), which has been technically revised.

Oil of Spanish wild marjoram (*Thymus mastichina* L.)

1 Scope

This International Standard specifies certain characteristics of the oil of Spanish wild marjoram (*Thymus mastichina* L.), in order to facilitate assessment of its quality.

2 Normative references

The following referenced documents are indispensable for the application 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/TR 210, *Essential oils — General rules for packaging, conditioning and storage*

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

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

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

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

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 Terms and definitions

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

3.1
oil of Spanish wild marjoram
essential oil obtained by steam distillation of the flowering tops of *Thymus mastichina* L., of the Lamiaceae family, growing in different parts of Spain

NOTE For information on the CAS number, see ISO/TR 21092.

4 Requirements

4.1 Appearance

Liquid.

4.2 Colour

Almost colourless to yellow.

4.3 Odour

Characteristic, cineolic and spicy.

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

Minimum: 0,890

Maximum: 0,920

4.5 Refractive index at 20 °C

Minimum: 1,460

Maximum: 1,470

4.6 Optical rotation at 20 °C

Between –4° and +6°.

4.7 Miscibility in ethanol, 70 % (volume fraction), at 20 °C

It shall not be necessary to use more than 3 volumes of ethanol, 70 % (volume fraction), to obtain a clear solution with 1 volume of essential oil.

NOTE Sometimes opalescence is observed on dilution.

4.8 Acid value

Maximum: 2

4.9 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 %
α-Pinene	1	4,5
β-Pinene	2	5
Limonene	1	6
1,8-Cineole	30	68
Linalol	3	48
Camphor	0,1	2
δ-Terpineol	0,2	2
Borneol	0,1	1,8
Terpinen-4-ol	0,2	1,2
Linalyl acetate	0,2	4
β-Caryophyllene	0,5	1,5
NOTE The chromatographic profile is normative, contrary to typical chromatograms given for information in Annex A.		

4.10 Flashpoint

Information on the flashpoint is given in Annex B.

5 Sampling

See ISO 212.

Minimum volume of test sample: 25 ml.

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

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 Miscibility in ethanol, 70 % (volume fraction), at 20 °C

See ISO 875.

7.5 Acid value

See ISO 1242.

7.6 Chromatographic profile

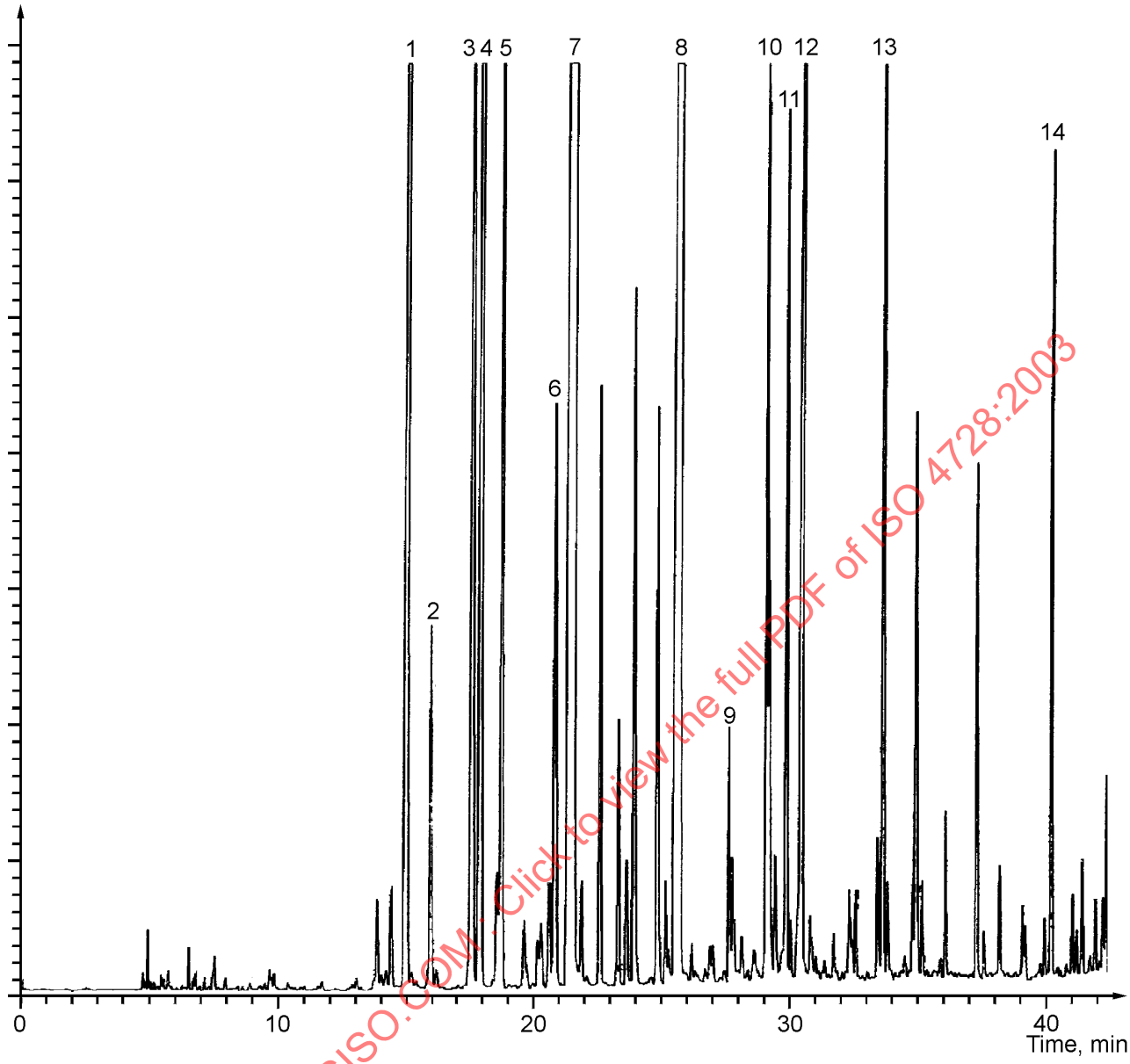
See ISO 11024-1 and ISO 11024-2.

8 Packaging, labelling, marking and storage

See ISO/TR 210 and ISO/TR 211.

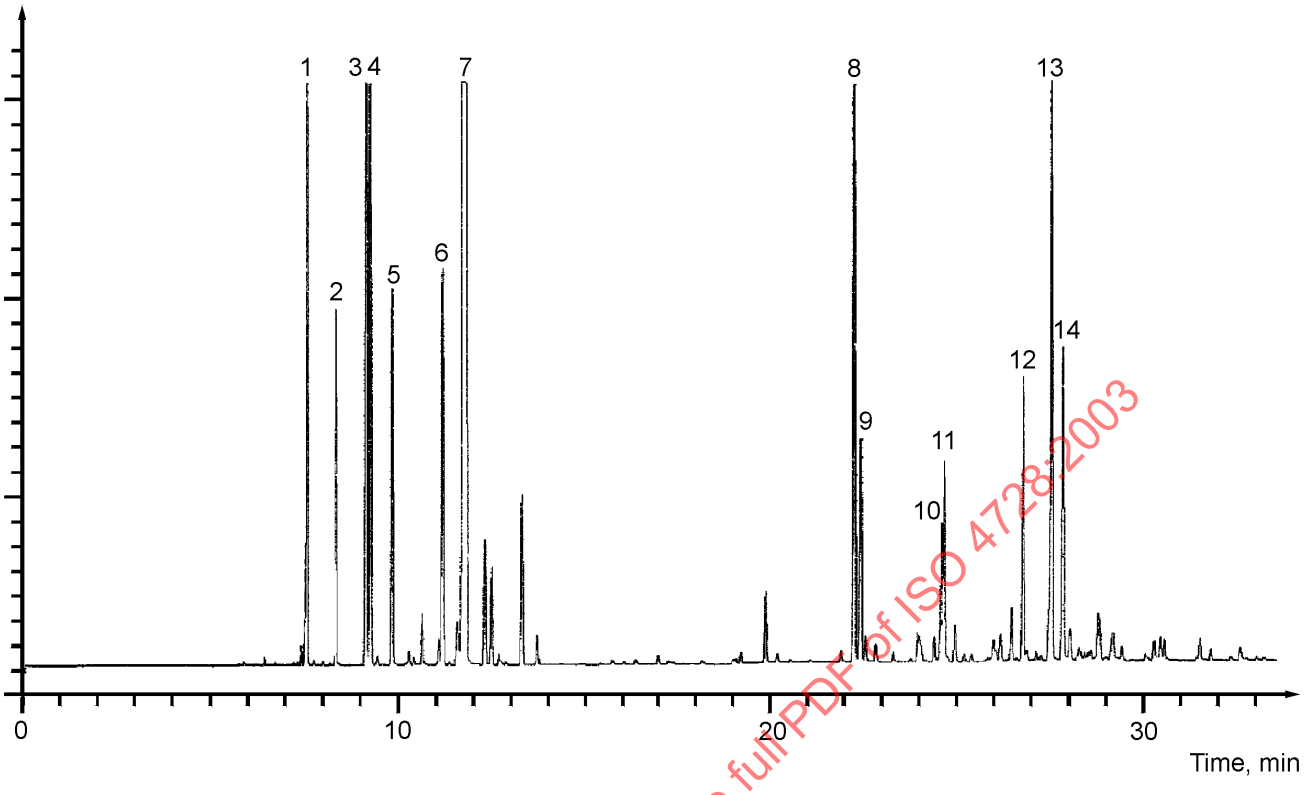
Annex A
(informative)

Typical chromatograms of the analysis by gas chromatography of the essential oil of Spanish wild marjoram (*Thymus mastichina* L.)



Peak identification		Operating conditions
1	α-Pinene	Column: fused silica capillary; length 60 m; internal diameter 0,25 mm
2	Camphene	Stationary phase: poly(5 % diphenyl – 95 % dimethylsiloxane)
3	Sabinene	Film thickness: 0,25 µm
4	β-Pinene	Oven temperature: temperature programming from 75 °C to 190 °C at a rate of 4 °C/min
5	Myrcene	Injector temperature: 250 °C
6	*p*-Cymene	Detector temperature: 270 °C
7	Limonene + 1,8-cineole	Detector: flame ionization type
8	Linalol	Carrier gas: nitrogen
9	Camphor	Injection volume: 0,6 µl
10	Borneol + δ-terpineol	Carrier gas flow rate: 1 ml/min
11	Terpinen-4-ol	Split ratio: 1/80
12	α-Terpineol	
13	Linalyl acetate	
14	β-Caryophyllene	

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



Peak identification		Operating conditions
1	α-Pinene	Column: fused silica capillary; length 60 m; internal diameter 0,25 mm
2	Camphene	Stationary phase: poly(ethylene glycol)-modified TPA (SP-1 000®)
3	β-Pinene	Film thickness: 0,25 µm
4	Sabinene	Oven temperature: temperature programming from 95 °C to 190 °C at a rate of 4 °C/min
5	Myrcene	Injector temperature: 250 °C
6	Limonene	Detector temperature: 250 °C
7	1,8-Cineole	Detector: flame ionization type
8	Linalol	Carrier gas: nitrogen
9	Linalyl acetate + camphor	Injection volume: 0,1 µl
10	β-Caryophyllene	Carrier gas flow rate: 1 ml/min
11	Terpinen-4-ol	Split ratio: 1/100
12	δ-Terpineol	
13	α-Terpineol	
14	Borneol	

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