

INTERNATIONAL STANDARD

**ISO
4728**

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

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

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Reference number
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Foreword

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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.

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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 $+6^{\circ}$.

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.

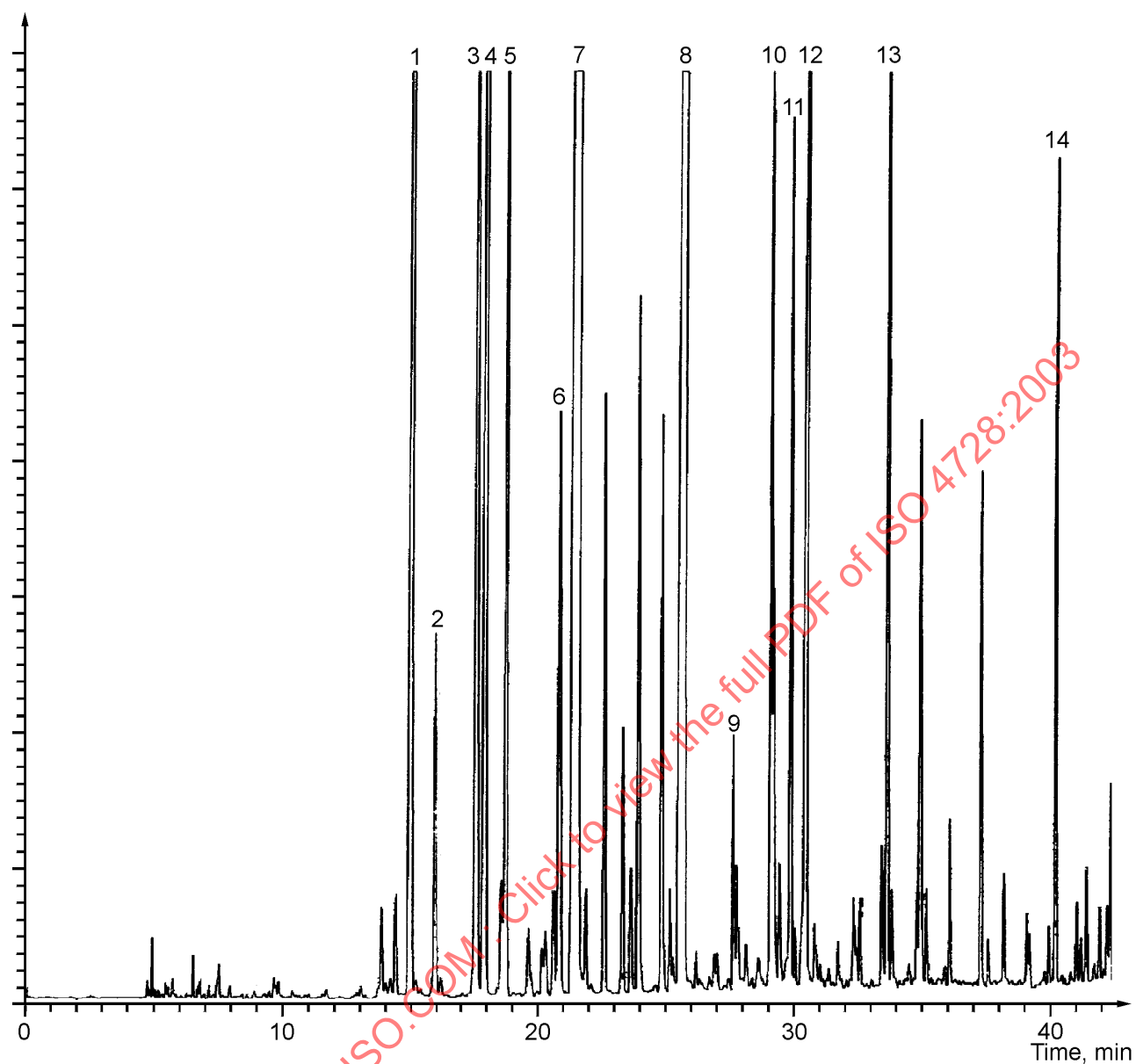
8 Packaging, labelling, marking and storage

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.)

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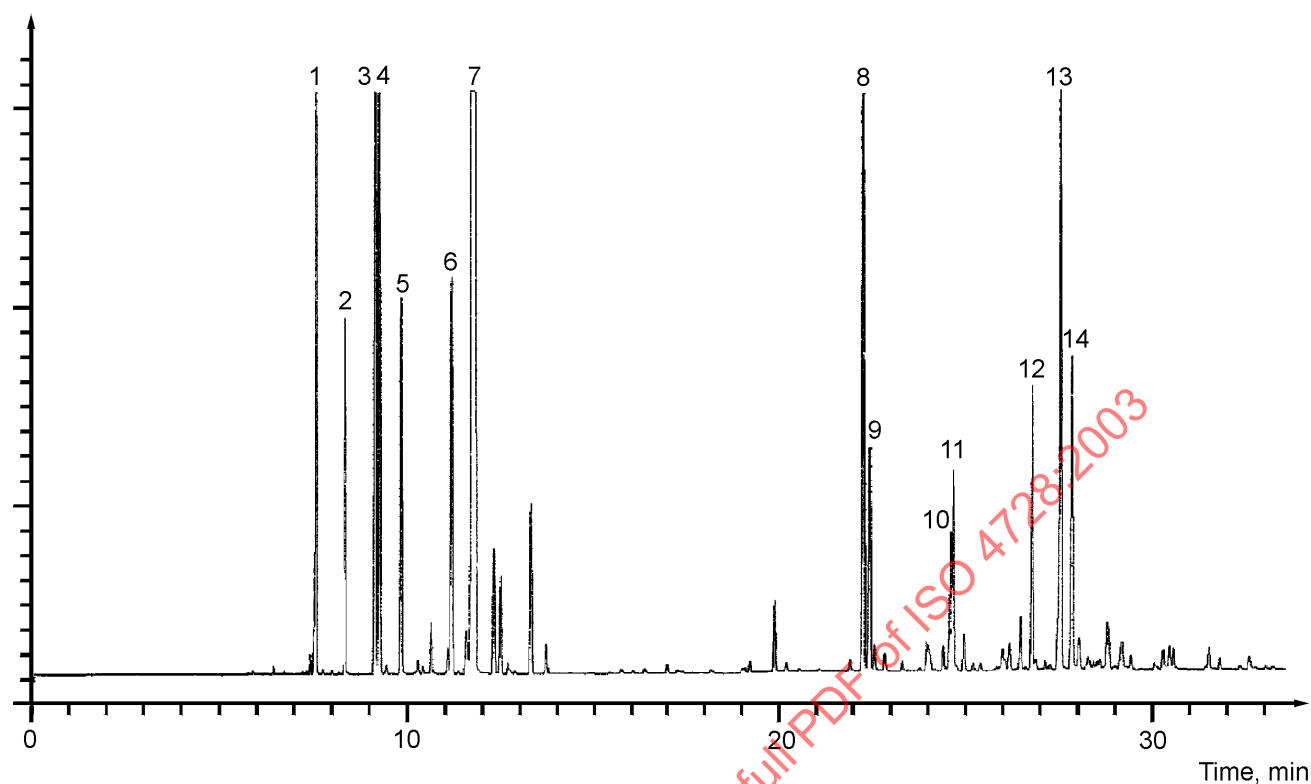
**Peak identification**

- 1 α -Pinene
- 2 Camphene
- 3 Sabinene
- 4 β -Pinene
- 5 Myrcene
- 6 *p*-Cymene
- 7 Limonene + 1,8-cineole
- 8 Linalol
- 9 Camphor
- 10 Borneol + δ -terpineol
- 11 Terpinen-4-ol
- 12 α -Terpineol
- 13 Linalyl acetate
- 14 β -Caryophyllene

Operating conditions

Column: fused silica capillary; length 60 m; internal diameter 0,25 mm
 Stationary phase: poly(5 % diphenyl – 95 % dimethylsiloxane)
 Film thickness: 0,25 μ m
 Oven temperature: temperature programming from 75 °C to 190 °C at a rate of 4 °C/min
 Injector temperature: 250 °C
 Detector temperature: 270 °C
 Detector: flame ionization type
 Carrier gas: nitrogen
 Injection volume: 0,6 μ l
 Carrier gas flow rate: 1 ml/min
 Split ratio: 1/80

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 | 1,8-Cineole |
| 8 | Linalol |
| 9 | Linalyl acetate + camphor |
| 10 | β -Caryophyllene |
| 11 | Terpinen-4-ol |
| 12 | δ -Terpineol |
| 13 | α -Terpineol |
| 14 | Borneol |

Operating conditions

Column: fused silica capillary; length 60 m; internal diameter 0,25 mm
Stationary phase: poly(ethylene glycol)-modified TPA (SP-1 000®)
Film thickness: 0,25 μ m
Oven temperature: temperature programming from 95 °C to 190 °C at a rate of 4 °C/min
Injector temperature: 250 °C
Detector temperature: 250 °C
Detector: flame ionization type
Carrier gas: nitrogen
Injection volume: 0,1 μ l
Carrier gas flow rate: 1 ml/min
Split ratio: 1/100

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