BRITISH STANDARD

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Oils of orris rhizome (*Iris pallida* Lam. or *Iris germanica* L.) — Determination of irone content — Method using gas chromatography on a capillary column

ICS 71.100.60

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Summary of pages

This document comprises a front cover, an inside front cover, the ISO title page, pages ii to iv, pages 1 to 8, an inside back cover and a back cover.

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INTERNATIONAL STANDARD

ISO 18054

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Oils of orris rhizome (*Iris pallida* Lam. or *Iris germanica* L.) — Determination of irone content — Method using gas chromatography on a capillary column

*Huile essentielle de rhizomes d'iris (*Iris pallida *Lam. ou* Iris germanica *L.) — Détermination de la teneur en irones — Méthode par chromatographie en phase gazeuse sur colonne capillaire*

Reference number
ISO 18054:2004(E)

Foreword

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ISO 18054 was prepared by Technical Committee ISO/TC 54, *Essential oils*.

Introduction

Since the description of methods of analysis by gas chromatography is very long, it is considered useful to establish general methods giving detailed information on all the recurrent parameters, apparatus, products, methods, formulae, etc. and then standards with short details on the determination of specific constituents in essential oils, giving only those operating conditions specific to the pertinent determination.

This is the case with the present International Standard, which refers to the general standard ISO 7609 for the general paragraphs.

Oils of orris rhizome (*Iris pallida* Lam. or *Iris germanica* L.) — Determination of irone content — Method using gas chromatography on a capillary column

1 Scope

This International Standard specifies a method for the determination of irone content in oils of orris[1)] rhizome (*Iris pallida* Lam. or *Iris germanica* L.) using gas chromatography on a capillary column.

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 356, *Essential oils — Preparation of test samples*

ISO 7609, *Essential oils — Analysis by gas chromatography on capillary columns — General method*

3 Principle

Gas phase chromatography on small quantities of orris essential oils is carried out on a capillary column, under the specified analytical conditions.

The irone content is determined by the internal standard method.

4 Reagents and products

4.1 Reference substance: synthetic irones of which the sum of the isomers is greater than 90 %, determined by chromatography under the test conditions.

4.2 Internal standard

Select either one of the following recently distilled substances, depending on whether a polar or apolar column is being used.

The internal standard selected shall elute as near as possible to the compound(s) to be determined, and shall not overlap any of the peaks corresponding to the constituents of the essential oil in question.

4.2.1 α-Ionone, having a minimum purity of 98 %, determined by gas chromatography under the test conditions, for determination on a polar column.

4.2.2 *trans*-Anethole, having a minimum purity of 98 %, determined by gas chromatography under the test conditions, for determination on an apolar column.

1) Commercially, this is usually called “orris butter”.

4.3 Dilution solvent: dichloromethane, having a minimum purity of 99 %, determined by gas chromatography under the test conditions, or **any other suitable solvent**, of identical analytical quality.

WARNING — Attention is drawn to the hazards of using dichloromethane, which is a toxic solvent.

4.4 Additive substance: linalool, having a minimum purity of 99 %, determined by chromatography under the test conditions.

5 Apparatus

5.1 Chromatograph, recorder and integrator.

See ISO 7609.

5.2 Column.

Capillary column having the characteristics given in 5.2.1 and 5.2.2.

5.2.1 Polar capillary column.

Length	10 m to 100 m
Internal diameter	0,2 mm to 0,5 mm
Stationary phase	poly(ethylene glycol) 20 000 (FFAP®, Supelcowax®), for example

5.2.2 Apolar capillary column.

Length	10 m to 100 m
Internal diameter	0,2 mm to 0,5 mm
Stationary phase	methyl silicone, for example

5.3 Flame ionization detector.

6 Preparation of test sample

See ISO 356.

7 Operating conditions

7.1 Temperatures

7.1.1 Oven temperature

The oven temperature shall be chosen so that the irone isomers are well separated from the other essential oil constituents. The following temperatures are given by way of indication.

a) Polar capillary column

Linear temperature programming from 100 °C to 220 °C, at a rate of 2 °C/min to 3 °C/min.

b) Apolar capillary column

Linear temperature programming from 100 °C to 250 °C, at a rate of 2 °C/min to 3 °C/min.

7.1.2 Injection temperature

About 230 °C.

7.1.3 Detection temperature

a) Polar capillary column

About 230 °C.

b) Apolar capillary column

About 260 °C.

7.2 Carrier gas and auxiliary gases flow rates

See ISO 7609.

8 Column performance

8.1 Chemical inertness test

Carry out the test as specified in ISO 7609.

8.2 Column efficiency

Determine the efficiency of the column as specified in ISO 7609.

The efficiency shall be at least 50 000 theoretical plates, determined on the linalol peak, under the test conditions.

The irone isomers shall be well separated.

9 Determination of retention indexes

See ISO 7609.

10 Identification of the irone isomers

See the typical chromatograms given in Annex A.

11 Determination by internal standard method

11.1 Determination of the response factor

Determine the response factor in accordance with ISO 7609, using the irone (4.1) as the reference substance and either a) or b) of the following standard mixtures, depending on whether the determination is conducted on a polar or an apolar capillary column.

Prepare the standard mixtures by weighing, taking into account the analytical purity of the standards in the calculation of the masses of the weighed products.

a) Standard mixture for polar capillary column

α-Ionone (4.2.1)	0,1 g
Synthetic irones	0,1 g
Linalool (4.4)	0,8 g
Dichloromethane (4.3)	4 g approximately

b) Standard mixture for apolar capillary column

trans-Anethole (4.2.2)	0,1 g
Synthetic irones	0,1 g
Linalool (4.4)	0,8 g
Dichloromethane (4.3)	4 g approximately

NOTE 1 The purpose of adding linalool to the standard mixtures is to obtain irone concentrations close to those of the synthetic irones.

NOTE 2 The weighing accuracy given in ISO 7609 is not applicable to the dilution solvent [dichloromethane (4.3)]. A relative weighing accuracy of 5 % suffices.

NOTE 3 For the calculation of the response factor, K, A_R is the sum of the areas of the peaks of the reference irone isomers. It is assumed that the response factors of the isomers are identical.

11.2 Determination of the irones in the sample

Carry out a determination of the irone content of the essential oil being analysed in accordance with the internal standard method specified in ISO 7609, using either a) or b) of the following test mixtures, depending on whether a polar or an apolar capillary column is used.

Prepare the mixtures by weighing.

a) Test mixture for polar capillary column

α-Ionone (4.2.1)	0,1 g
Synthetic irones	0,9 g
Dichloromethane (4.3)	4 g approximately

b) Test mixture for apolar capillary column

trans-Anethole (4.2.2)	0,1 g
Synthetic irones	0,9 g
Dichloromethane (4.3)	4 g approximately

NOTE 1 The weighing accuracy given in ISO 7609 is not applicable to the dilution solvent [dichloromethane (4.3)]. A relative weighing accuracy of 5 % suffices.

NOTE 2 For the determination of the irone content of the synthetic irones, A_x is the sum of the areas of the peaks of the irone isomers. It is assumed that the response factors of the isomers are identical.

12 Expression of results

See ISO 7609.

NOTE Typical chromatograms of the analysis are given in Annex A, for information.

13 Precision

13.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method are summarized in Annex B.

13.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time, should not be greater than 5 %.

13.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, should not be greater than 10 %.

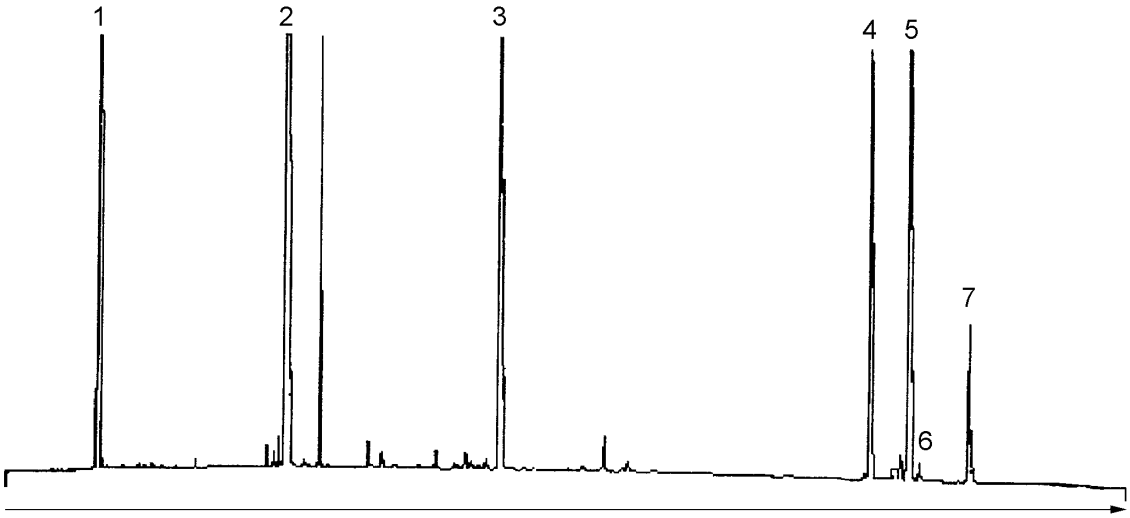
14 Test report

See ISO 7609.

Annex A
(informative)

Typical chromatograms of the determination by gas chromatography of the irone content in the oil of orris rhizome (*Iris pallida* Lam. or *Iris germanica* L.), of Moroccan origin

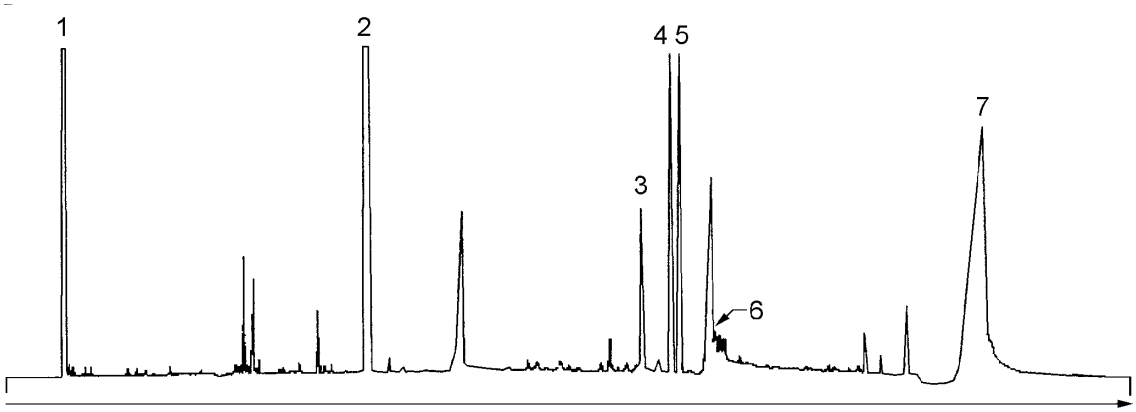
A.1 Identification of irone isomers and determination of the response factor on an apolar phase [standard mixture 11.1 b)]



Peak identification		Operating conditions
1	Dichloromethane	Column: quartz capillary; length 50 m; internal diameter 0,32 mm
2	Linalool	Stationary phase: poly(dimethyl siloxane) (CPSil 5 CB®)
3	*trans*-Anethole	Film thickness: 0,4 µm
4	*trans*-α-Irone	Oven temperature: temperature programming from 100 °C to 270 °C at a rate of 2 °C/min
5	*cis*-α-Irone	Injector temperature: 230 °C
6	*cis*-γ-Irone	Detector temperature: 250 °C
7	β-Irone	Detector: flame ionization type
		Carrier gas: helium
		Volume injected: 1 µl
		Carrier gas flow rate: 1 ml/min

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

A.2 Identification of irones on an apolar phase [test mixture 11.2 b)]



Peak identification		Operating conditions
1	Dichloromethane	Column: quartz capillary; length 50 m; internal diameter 0,32 mm
2	*trans*-Anethole	Stationary phase: poly(dimethyl siloxane) (CPSil 5 CB®)
3	*trans*-α-Irone	Film thickness: 0,4 µm
4	*cis*-α-Irone	Oven temperature: temperature programming from 100 °C to 250 °C at a rate of 2 °C/min
5	*cis*-γ-Irone	Injector temperature: 230 °C
6	β-Irone	Detector temperature: 250 °C
7	Myristic acid	Detector: flame ionization type
		Carrier gas: helium
		Volume injected: 1 µl
		Carrier gas flow rate: 1 ml/min

Figure A.2 — Typical chromatogram taken on an apolar column

Annex B
(informative)

Results of the interlaboratory tests

Interlaboratory tests were carried out in 1995, with the collaboration of nine laboratories.

Table B.1 — Determination of the irone content in oils of orris rhizome

	Laboratories							
	1	**3**	**4**	**5**	**6**	**7**	**8**	**9**
	7,89	8,42	8,15	7,75	8,89	7,42	7,82	8,17
	7,92	8,26	8,12	7,77	8,81	7,47	7,98	7,94
	7,67	8,54	8,12	8,07	8,82	7,58	7,92	7,90
	7,75	8,50	8,36	8,09	8,81	7,63		7,69
					8,83			7,63
					8,15			7,55
					8,07			
					8,08			
					8,10			
Minimum per laboratory	7,67	8,26	8,12	7,75	8,07	7,42	7,82	7,55
Maximum per laboratory	7,92	8,54	8,36	8,09	8,89	7,63	7,98	8,17
Mean per laboratory	7,81	8,43	8,19	7,92	8,51	7,53	7,91	7,81
Deviation, %	1,76	2,02	2,11	2,15	5,13	1,40	1,10	4,56
Repeatability limit (r)	5,13 %							
Variance	0,013 9	0,015 3	0,013 4	0,034 3	0,149 9	0,009 4	0,006 5	0,053 8
Standard deviation	0,117 9	0,123 8	0,115 9	0,185 1	0,387 1	0,096 8	0,080 8	0,231 9
General mean	8,07 (minimum 7,42/maximum 8,89)							
Maximum deviation from general mean	1,43 %							
Reproducibility limit (R)	10,16 %							

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