

---

---

**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*

STANDARDSISO.COM : Click to view the full PDF of ISO 18054:2004



**PDF disclaimer**

This PDF file may contain embedded typefaces. In accordance with Adobe's licensing policy, this file may be printed or viewed but shall not be edited unless the typefaces which are embedded are licensed to and installed on the computer performing the editing. In downloading this file, parties accept therein the responsibility of not infringing Adobe's licensing policy. The ISO Central Secretariat accepts no liability in this area.

Adobe is a trademark of Adobe Systems Incorporated.

Details of the software products used to create this PDF file can be found in the General Info relative to the file; the PDF-creation parameters were optimized for printing. Every care has been taken to ensure that the file is suitable for use by ISO member bodies. In the unlikely event that a problem relating to it is found, please inform the Central Secretariat at the address given below.

STANDARDSISO.COM : Click to view the full PDF of ISO 18054:2004

© ISO 2004

All rights reserved. Unless otherwise specified, no part of this publication may be reproduced or utilized in any form or by any means, electronic or mechanical, including photocopying and microfilm, without permission in writing from either ISO at the address below or ISO's member body in the country of the requester.

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](mailto:copyright@iso.org)  
Web [www.iso.org](http://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 18054 was prepared by Technical Committee ISO/TC 54, *Essential oils*.

STANDARDSISO.COM : Click to view the full PDF of ISO 18054:2004

## 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.

STANDARDSISO.COM : Click to view the full PDF of ISO 18054:2004

# 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<sup>1)</sup> 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  $\alpha$ -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 <sup>®</sup> , Supelcowax <sup>®</sup> ), 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**

$\alpha$ -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**

<i>trans</i> -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**

$\alpha$ -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**

<i>trans</i> -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

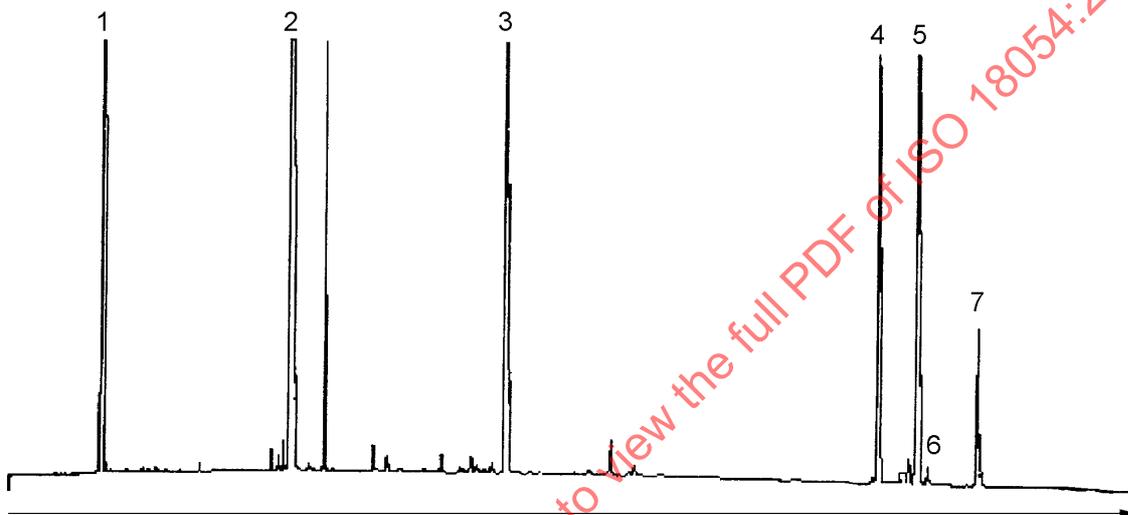
See ISO 7609.

STANDARDSISO.COM : Click to view the full PDF of ISO 18054:2004

**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**

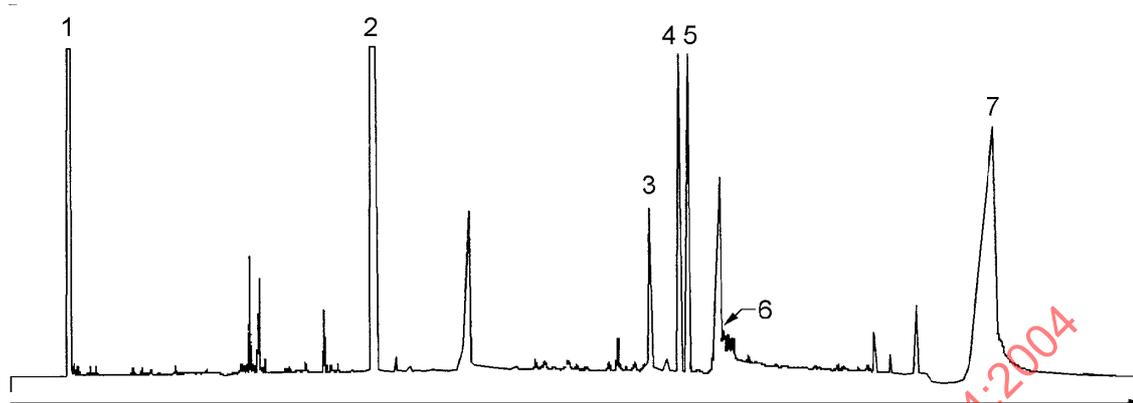
- 1 Dichloromethane
- 2 Linalool
- 3 *trans*-Anethole
- 4 *trans*- $\alpha$ -Irone
- 5 *cis*- $\alpha$ -Irone
- 6 *cis*- $\gamma$ -Irone
- 7  $\beta$ -Irone

**Operating conditions**

Column: quartz capillary; length 50 m; internal diameter 0,32 mm  
 Stationary phase: poly(dimethyl siloxane) (CPSil 5 CB®)  
 Film thickness: 0,4  $\mu$ m  
 Oven temperature: temperature programming from 100 °C to 270 °C at a rate of 2 °C/min  
 Injector temperature: 230 °C  
 Detector temperature: 250 °C  
 Detector: flame ionization type  
 Carrier gas: helium  
 Volume injected: 1  $\mu$ l  
 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

- 1 Dichloromethane
- 2 *trans*-Anethole
- 3 *trans*- $\alpha$ -Irone
- 4 *cis*- $\alpha$ -Irone
- 5 *cis*- $\gamma$ -Irone
- 6  $\beta$ -Irone
- 7 Myristic acid

### Operating conditions

Column: quartz capillary; length 50 m; internal diameter 0,32 mm  
 Stationary phase: poly(dimethyl siloxane) (CPSil 5 CB®)  
 Film thickness: 0,4  $\mu$ m  
 Oven temperature: temperature programming from 100 °C to 250 °C at a rate of 2 °C/min  
 Injector temperature: 230 °C  
 Detector temperature: 250 °C  
 Detector: flame ionization type  
 Carrier gas: helium  
 Volume injected: 1  $\mu$ l  
 Carrier gas flow rate: 1 ml/min

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