INTERNATIONAL STANDARD

ISO 17494

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Aromatic extracts, flavouring and perfuming compounds — Determination of ethanol content — Gas chromatographic method on packed and capillary columns

Extraits aromatiques et compositions aromatisantes et parfumantes — Détermination de la teneur en éthanol — Méthode par chromatographie en phase gazeuse sur colonne remplie et sur colonne capillaire

Reference number
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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 3.

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.

International Standard ISO 17494 was prepared by Technical Committee ISO/TC 54, *Essential oils*.

Annexes A and B of this International Standard are for information only.

Introduction

Since the description of methods of analysis by gas chromatography is very long, it was considered useful to establish general methods giving detailed information on all the recurrent parameters, apparatus, products, methods, formulae, etc., and also to establish standards with brief details of 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 standards ISO 7359 and ISO 7609.

Aromatic extracts, flavouring and perfuming compounds — Determination of ethanol content — Gas chromatographic method on packed and capillary columns

1 Scope

This International Standard describes a method for determining the ethanol content in aromatic extracts and flavouring and perfuming compounds by gas chromatography, either on a packed column or a capillary column.

The method is applicable to those products having presumed ethanol contents over 0,5 %.

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 760, *Determination of water — Karl Fischer method (General method)*

ISO 7359, *Essential oils — Analysis by gas chromatography on packed columns — General method*

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

3 Term and definition

For the purposes of this International Standard, the following term and definition apply.

3.1
aromatic extracts, flavouring and perfuming compounds
liquid or diluted aromatic products, with the exclusion of dry aromatic substances (such as spices)

4 Principle

Aromatic extracts and flavouring and perfuming compounds are analysed by gas chromatography, either on a packed column or a capillary column, under specified conditions.

Their ethanol content is determined using the internal standard method.

5 Reagents

5.1 Reference substance: absolute ethanol, having a minimum purity of 99 %, verified in accordance with ISO 760 and by gas chromatography, under the test conditions.

5.2 Internal standard: **2-propanol** or ***n*-propanol**, free from ethanol, having a minimum purity of 99 %, determined by gas chromatography under the test conditions.

5.3 Solvent: methanol, or another suitable solvent for the product being analysed, free from impurities and which does not interfere with the results.

6 Apparatus

6.1 Chromatograph, recorder and integrator

See ISO 7359 or ISO 7609.

6.2 Column

Use a packed or capillary column. Examples of suitable columns are given in Tables 1 and 2.

Table 1 — Packed column

Length	3 m to 4 m
Internal diameter	2 mm to 4 mm
Stationary phase	vinyl benzene styrene, or equivalent, having a particle size from 100 mesh to 200 mesh

Table 2 — Capillary column

Length	10 m to 100 m
Internal diameter	0,2 mm to 0,5 mm
Stationary phase	dimethylpolysiloxane or polyethylene glycol 20 000, having a minimum film thickness of 0,2 μm

6.3 Flame ionization detector.

7 Operating conditions

7.1 Temperature

See Tables 3 and 4 for suitable temperatures.

Purge the column during the increase in temperature before proceeding to a new analysis.

Table 3 — Suitable temperatures with packed columns

Oven temperature	isothermal, about 150 °C
Injection temperature	about 180 °C
Detection temperature	about 180 °C

Table 4 — Suitable temperatures with capillary columns

Oven temperature	isothermal, about 50 °C
Injection temperature	about 230 °C
Detection temperature	about 230 °C

7.2 Flow rates of carrier gas and auxiliary gases

See ISO 7359 or ISO 7609.

8 Column performances

8.1 Chemical inertness test

Carry out the test as specified in ISO 7359 or ISO 7609.

8.2 Column efficiency

Determine the efficiency of the column as specified in ISO 7359 or ISO 7609.

9 Internal standard method

9.1 Determination of the response factor

Determine the response factor relative to the internal standard as specified in ISO 7359 or ISO 7609.

9.2 Determination of ethanol in the sample

Carry out the determination of the ethanol content in accordance with the method specified in ISO 7359 or ISO 7609.

In the case of viscous samples, first dilute in the solvent described in 5.3.

10 Expression of results

See ISO 7359 or ISO 7609.

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

11 Precision

11.1 Interlaboratory test

Details of an interlaboratory test on the precision of the method are summarized in annex B. The values derived from this interlaboratory test may not be applicable to concentration ranges and matrices other than those given.

11.2 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, will in not more than 5 % of cases be greater than 5 %.

11.3 Reproducibility

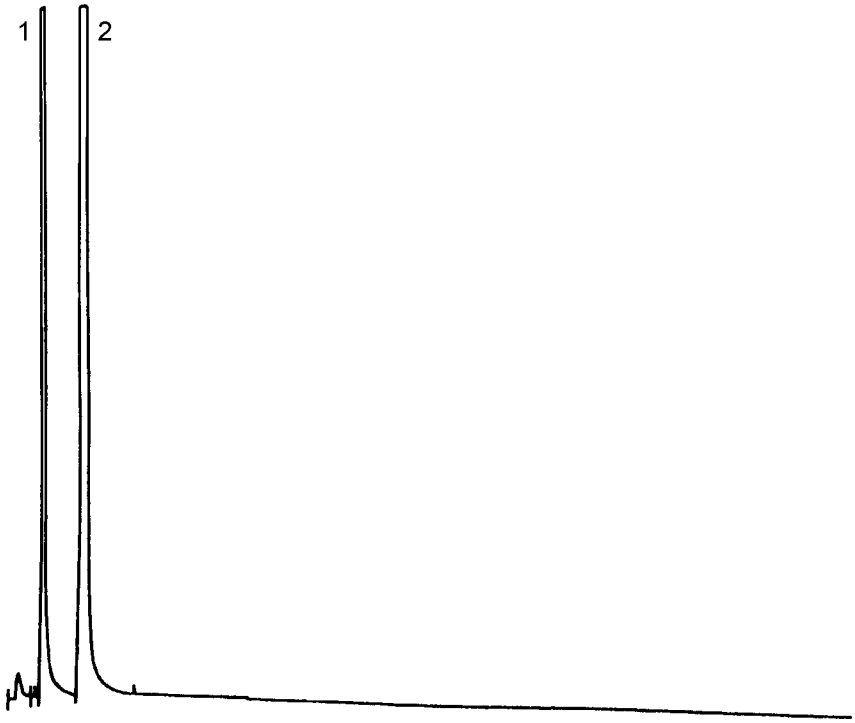
The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will in not more than 5 % of cases be greater than 10 %.

12 Test report

See ISO 7359 or ISO 7609.

Annex A
(informative)

Typical chromatograms



Peak identification

1 Ethanol

2 *n*-Propanol

Operating conditions

Column: packed; length 3 m; internal diameter 3 mm

Stationary phase: vinyl benzene styrene (Chromosorb 102)

Oven temperature: isotherm at 150 °C

Injection temperature: 180 °C

Detection temperature: 180 °C

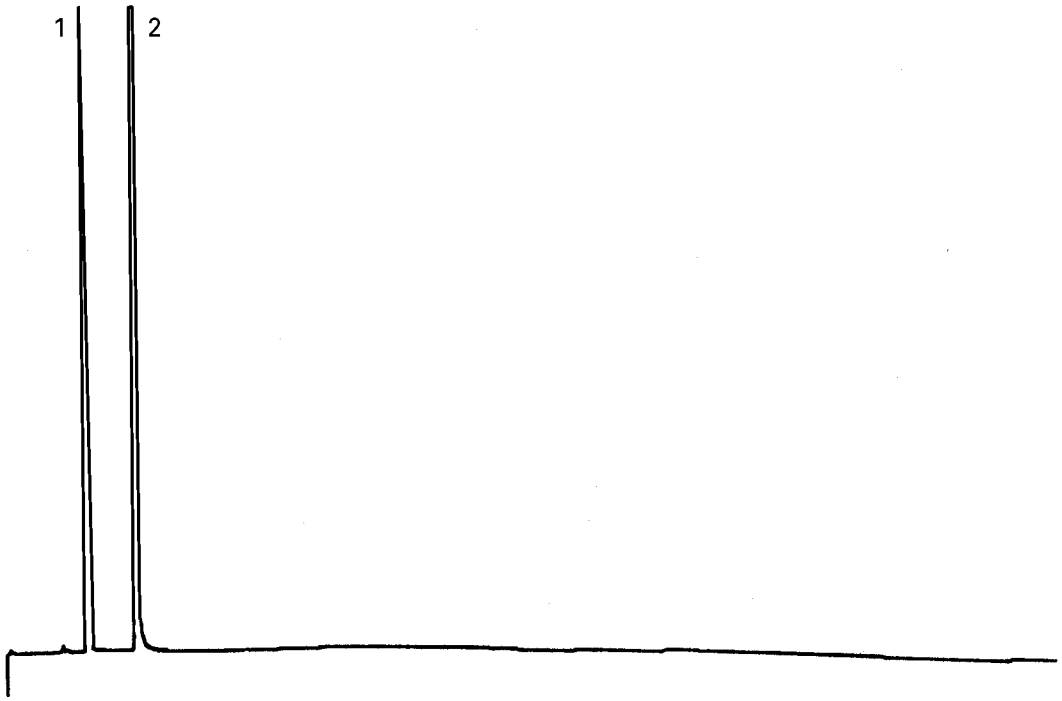
Detector: flame ionization type

Carrier gas: nitrogen

Volume injected: 0,1 µl

Carrier gas flow rate: 30 ml/min

Figure A.1 — Typical chromatogram taken on a packed column



Peak identification	Operating conditions
1 Ethanol	Column: capillary; length 30 m; internal diameter 0,32 mm
2 *n*-Propanol	Stationary phase: polyethylene glycol (Carbowax 20 M)
	Oven temperature: isotherm at 50 °C
	Injection temperature: 230 °C
	Detection temperature: 230 °C
	Detector: flame ionization type
	Carrier gas: nitrogen
	Volume injected: 0,1 µl
	Pressure at the head of the column: 50 kPa
	Split ratio: 1/100

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

Annex B
(informative)

Results of an interlaboratory test on apricot, banana and orange flavours and on a wine lees essential oil

An interlaboratory test on the precision of the method was carried out in 1995 with the participation of ten laboratories. The results are given in Tables B.1 to B.7.

NOTE Full data are available from the secretariat of ISO/TC 54 (AENOR).

Table B.1 — Determination of ethanol in an aroma of apricot

	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 7 bis	Lab 8	Lab 9	Lab 10
Number of measurements: 88											
Mean	13,36	13,20	13,32	14,94	13,49	13,05	13,75	13,80	13,03	13,44	13,89
Min. difference	0,41	0,20	0,24	0,13	0,20	0,05	0,06	0,06	0,03	0,52	0,12
Max. difference	0,49	0,32	0,53	0,21	0,33	0,05	0,20	0,08	0,07	0,63	0,10
Difference in %	3,67	2,45	3,96	1,38	2,42	0,38	1,46	0,59	0,51	4,71	0,84
Variance	0,07	0,02	0,06	0,03	0,03	0,00	0,00	0,00	0,00	0,12	0,00
Standard deviation	0,27	0,14	0,25	0,18	0,16	0,07	0,08	0,05	0,06	0,34	0,06
Repeatability limit, r (%)	4,71										
General mean	13,55 (min. 12,92; max. 15,15)										
Overall standard deviation	0,41										
Overall variance	0,17										
Max. difference in relation to the general mean, %	1,60										
Reproducibility limit, R (%)	11,81										

Table B.2 — Determination of ethanol in an aroma of apricot
(Limits taken into account: mean ± 2 × standard deviation)

	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 7 bis	Lab 8	Lab 9	Lab 10
Number of measurements: 85											
Mean	13,36	13,20	13,32		13,49	13,05	13,75	13,80	13,03	13,44	13,89
Min. difference	0,41	0,20	0,24	[a]	0,20	0,05	0,06	0,06	0,03	0,52	0,12
Max. difference	0,49	0,32	0,53		0,33	0,05	0,20	0,08	0,07	0,63	0,10
Difference in %	3,67	2,45	3,96		2,42	0,38	1,46	0,59	0,51	4,71	0,84
Variance	0,07	0,02	0,06		0,03	0,00	0,00	0,00	0,00	0,12	0,00
Standard deviation	0,27	0,14	0,25		0,16	0,07	0,08	0,05	0,06	0,34	0,06
Repeatability limit, r (%)	4,71										
General mean	13,50 (min. 12,92; max. 14,07)										
Overall standard deviation	0,31										
Overall variance	0,10										
Max. difference in relation to the general mean, %	0,58										
Reproducibility limit, R (%)	4,30										

[a] These values are not taken into account.

Table B.3 — Determination of ethanol in an aroma of banana

	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 7 bis	Lab 8	Lab 9	Lab 10
Number of measurements: 85											
Mean	47,19	47,69	47,16	46,82	44,85	46,70	47,79	47,96	46,05	48,20	46,87
Min. difference	0,35	1,58	0,09	0,73	0,65	0,10	0,75	0,49	0,35	0,70	0,17
Max. difference	0,20	1,01	0,15	0,50	1,09	0,10	0,87	0,39	0,60	0,47	0,17
Difference in %	0,73	3,32	0,31	0,71	2,43	0,21	1,82	1,01	1,30	1,45	0,37
Variance	0,03	0,85	0,00	0,42	0,34	0,02	0,23	0,10	0,27	0,15	0,01
Standard deviation	0,16	0,92	0,09	0,65	0,59	0,14	0,48	0,32	0,52	0,39	0,12
Repeatability limit, r (%)	3,32										
General mean	47,13 (min. 44,20; max. 48,70)										
Overall standard deviation	1,07										
Overall variance	1,14										
Max. difference in relation to the general mean, %	2,94										
Reproducibility limit, R (%)	6,24										

Table B.4 — Determination of ethanol in an aroma of banana
(Limits taken into account: mean $\pm$ 2 $\times$ standard deviation)

	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 7 bis	Lab 8	Lab 9	Lab 10
Number of measurements: 78											
Mean	47,19	47,69	47,16	46,82	45,59	46,70	47,79	47,96	46,05	48,20	46,87
Min. difference	0,35	1,58	0,09	0,73	0,47	0,10	0,75	0,49	0,35	0,70	0,17
Max. difference	0,20	1,01	0,15	0,50	0,35	0,10	0,87	0,39	0,60	0,47	0,17
Difference in %	0,73	3,32	0,31	0,71	1,02	0,21	1,82	1,01	1,30	1,45	0,37
Variance	0,03	0,85	0,00	0,42	0,18	0,02	0,23	0,10	0,27	0,15	0,01
Standard deviation	0,16	0,92	0,09	0,65	0,42	0,14	0,48	0,32	0,52	0,39	0,12
Repeatability limit, r (%)	3,32										
General mean	47,36 (min. 45,12; max. 48,70)										
Overall standard deviation	0,58										
Overall variance	0,76										
Max. difference in relation to the general mean, %	2,24										
Reproducibility limit, R (%)	4,73										

Table B.5 — Determination of ethanol in an aroma of orange

	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 7 bis	Lab 8	Lab 9	Lab 10
Number of measurements: 88											
Mean	55,41	55,65	55,53	56,67	54,57	55,40	55,68	55,51	53,90	56,54	56,46
Min. difference	0,24	0,55	0,34	0,76	1,15	0,20	0,25	0,13	0,20	0,70	0,23
Max. difference	0,36	0,66	0,40	0,55	1,66	0,20	0,47	0,48	0,20	0,53	0,17
Difference in %	0,66	1,18	0,55	1,35	3,04	0,36	0,85	0,86	0,37	1,24	0,41
Variance	0,03	0,11	0,04	0,46	0,56	0,08	0,04	0,03	0,04	0,14	0,02
Standard deviation	0,18	0,33	0,20	0,68	0,75	0,28	0,20	0,18	0,20	0,37	0,15
Repeatability limit, r (%)	3,04										
General mean	55,63 (min. 53,42; max. 57,12)										
Overall standard deviation	0,75										
Overall variance	0,56										
Max. difference in relation to the general mean, %	2,21										
Reproducibility limit, R (%)	3,97										

Table B.6 — Determination of ethanol in an aroma of orange
(Limits taken into account: mean ± 2 × standard deviation)

	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 7 bis	Lab 8	Lab 9	Lab 10
Number of measurements: 82											
Mean	55,41	55,65	55,53	56,67	54,89	55,40	55,68	55,51		56,54	56,46
Min. difference	0,24	0,55	0,34	0,76	0,58	0,20	0,25	0,13	[a]	0,70	0,23
Max. difference	0,36	0,66	0,40	0,55	1,34	0,20	0,47	0,48		0,53	0,17
Difference in %	0,66	1,18	0,55	1,35	1,35	0,36	0,85	0,86		1,24	0,41
Variance	0,03	0,11	0,04	0,46	0,40	0,08	0,04	0,03		0,14	0,02
Standard deviation	0,18	0,33	0,20	0,68	0,64	0,28	0,20	0,18		0,37	0,15
Repeatability limit, r (%)	1,35										
General mean	55,76 (min. 54,31; max. 57,12)										
Overall standard deviation	0,59										
Overall variance	0,35										
Max. difference in relation to the general mean, %	1,45										
Reproducibility limit, R (%)	2,60										

[a] These values are not taken into account.

Table B.7 — Determination of ethanol in an oil of wine lees

	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6[a]	Lab 7
Number of measurements: 23							
Mean	4,47	4,91	4,79	4,45	4,70	4,40	4.40
Min. difference	0,00	0,02	0,13		0,20		0,10
Max. difference	0,00	0,03	0,18		0,20		0,10
Difference in %	0,00	0,61	3,76		4,25		2,27
Variance	0,00	0,00	0,03		0,03		0,00
Standard deviation	0,00	0,03	0,16		0,18		0,06
Repeatability limit, r (%)	4,25						
General mean	4,59 (min. 4,30; max. 4,97)						
Overall standard deviation	0,22						
Overall variance	0,05						
Max. difference in relation to the general mean, %	0,38						
Reproducibility limit, R (%)	8,29						

[a] Mean of three results.

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