INTERNATIONAL STANDARD

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Essential oils — General guidance on chromatographic profiles —

Part 1:
Preparation of chromatographic profiles for presentation in standards

Huiles essentielles — Directives générales concernant les profils chromatographiques —

Partie 1: Élaboration des profils chromatographiques pour la présentation dans les normes

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Contents

International Organization for Standardization
Case postale 56 • CH-1211 Genève 20 • Switzerland
Internet iso@iso.ch

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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.

International Standard ISO 11024-1 was prepared by Technical Committee ISO/TC 54, *Essential oils*.

International Standard ISO 11024 consists of the following parts, under the general title *Essential oils — General guidance on chromatographic profiles*:

— *Part 1: Preparation of chromatographic profiles for presentation in standards*

— *Part 2: Utilization of chromatographic profiles of samples of essential oils*

Annexes A and B of this part of ISO 11024 are for information only.

Essential oils — General guidance on chromatographic profiles —

Part 1:
Preparation of chromatographic profiles for presentation in standards

1 Scope

This part of ISO 11024 describes general guidelines on the determination of the chromatographic profile of an essential oil by gas chromatography on a capillary column.

The chromatographic profile is one of the specifications which enables assessment of the quality of an essential oil in the same way as the physico-chemical characteristics. It is determined at the time of finalizing the standard on the essential oil.

It is not a determination of the true concentration of the components, it is only an evaluation of its relative proportions.

NOTE Refer also to ISO 11024-2[1)] for use of chromatographic profiles of samples of essential oils.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this part of ISO 11024. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this part of ISO 11024 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 356, *Essential oils — Preparation of test samples.*

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

3 Terms and definitions

For the purposes of this part of ISO 11024 the following terms and definitions apply.

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1) ISO 11024-2, *Essential oils — General guidance on chromatographic profiles — Part 2: Utilization of chromatographic profiles of samples of essential oils.*

3.1
representative components
components present in all of the samples of the essential oil involved, whether major or minor ones

EXAMPLE Geranyl formate, isomenthone, citronnellal, geraniol, etc. in the essential oil of geranium.

3.2
characteristic components
one or more representative components, the concentration of which is characteristic for a given essential oil

NOTE The concentration may be nil.

EXAMPLES

Guaia-6,9-diene is present in traces in the Africa geranium and present in higher concentrations in the Bourbon geranium.

10-Epi-gamma-eudesmol is absent in the Bourbon geranium and present in the Africa geranium.

Camphor is present in quantities of less than 0,5 % in lavender.

3.3
typical chromatogram
graphical representation obtained by injection into the chromatograph of a sample of an essential oil considered to be representative of production, together with the operating conditions under which it was obtained

NOTE The chromatogram is for information only.

3.4
chromatographic profile
list of components selected among the representative and characteristic components of an essential oil, accompanied, for each of them, by concentration limits and, possibly, by the ratios between these concentrations

4 Principle

A sample of the essential oil is analysed using gas chromatography on a capillary column in accordance with ISO 7609.

The representative and characteristic components of the essential oil are selected.

The concentration of the components is determined by the peak area normalization method (internal normalization method in accordance with ISO 7609). The minimum and maximum concentrations which are acceptable for each of them are decided and, possibly, also the concentration ratio limits, by applying data analysis statistical methods.

5 Samples of the essential oil to be examined

The chromatographic profile of an essential oil shall be determined after having examined a very large number of commercially produced samples of the essential oil which are considered to be pure and of good organoleptic quality. They shall have been sampled from several years of production and originate from plants or parts of plants of which the botanical and, possibly, geographical origin is well defined and for which the preparation method is known.

6 Apparatus

6.1 Gas chromatograph, provided with split injector, capillary column.

6.2 Flame ionization detector.

6.3 Data-processing system (integrator, calculator, etc.), complying with the indications given in ISO 7609.

Verify the performance of the apparatus using the test described in clause 7.

7 Performance of the apparatus

7.1 General

Set-up the assembly comprising the chromatograph and the data-processing system (6.3) in such a manner that permits the correct resolution and total elution of all volatile components of the essential oil, and the chromatographic profile obtained with the test mixture defined in Table 1 conforms to the chromatographic profile defined by the interlaboratory test (see Table 2).

7.2 Preparation and composition of the standard test mixture

Prepare a standard test mixture as indicated in Table 1. (An example of use of the test mixture is given in annex A.)

Table 1 — Composition of the standard test mixture

Chemical name	CAS [a]	EINECS [b]	Minimum purity	Mass fraction %
n-Hexanol	111-27-3	203-852-3	99 %	0,80
α-Pinene	7785-70-8	232-087-8	99 %	5,00
1,8-Cineole (eucalyptol)	470-82-6	207-431-5	99,5 %	50,00
Linalool	78-70-6	201-134-4	99 %	10,00
n-Decanal [c]	112-31-2	203-957-4	98 %	0,20
Linalyl acetate	115-95-7	204-116-4	99 %	25,00
Eugenol	97-53-0	202-589-1	99 %	3,00
β-Caryophyllene	87-44-5	201-746-1	99 %	5,00
Benzyl salicylate	118-58-1	204-262-9	99 %	1,00

[a] Chemical Abstract Service Registration No.

[b] Registration No. of the European Inventory of existing commercially available chemical substances.

[c] Freshly distilled and/or chemically stabilized.

Check the purity of each of the components by gas chromatography and by the usual physico-chemical methods.

Store the mixture in full sealed bottles, sheltered from the light, and at a temperature between −5° C and +5 °C. Under these conditions, the test mixture may be stored at least a year.

7.3 Procedure

Carry out the chromatographic analysis of the test mixture by injecting the latter under the usual operating conditions for essential oils in practice in the laboratory.

7.4 Results

The results are obtained directly from the data-processing system.

To be agree formally, the obtained data, expressed as area percent, shall be within the limits given in Table 2.

Table 2 — Chromatographic profile of the standard test mixture

Chemical name	Minimum %	Maximum %
n-Hexanol	0,65	0,75
α-Pinene	5,85	6,25
1,8-Cineole (eucalyptol)	49,0	50,5
Linalool	10,10	10,50
n-Decanal	0,15	0,20
Linalyl acetate	22,80	23,50
Eugenol	2,50	2,75
β-Caryophyllene	5,85	6,30
Benzyl salicylate	0,75	0,95
n-Hexanol/benzyl salicylate ratio	0,75	0,95

For the peak of the *n*-decanal, the signal-to-noise ratio shall be greater than 100. This ratio may be calculated as follows:

— signal is the height of the peak of decanal;

— noise is the half of the difference between the maximum and the minimum signal value in the absence of a peak for 30 s.

8 Preparation of test sample

Comply with the method specified in ISO 356.

9 Identification and selection of the representative and characteristic components of the essential oil

Conduct a chromatographic examination of all of the samples of the essential oil being studied by complying with the method given in ISO 7609, and by applying the previously defined appropriate procedural conditions.

Identify the principal chemical components using usual analysis methods.

Select a few components among the most representative and characteristic ones of the essential oil being studied (12 maximum).

10 Fixing the concentration limits

Set the integrator so as to eliminate the background noise.

Assess the approximate concentration of the representative and characteristic components for each of the samples of essential oil being studied using the peak area normalization method (internal normalization method) in accordance with ISO 7609. This method allows one to assess, for each of the components, the peak area percentage in relation to the sum of the areas of all of the peaks of the chromatogram of the essential oil taken into consideration by the integrator. This percentage, which can be assimilated to a concentration, is read directly from the data system.

For each of the constituents of all of the samples being studied, calculate the mean m_1 of the concentrations and the standard deviation σ_1.

Define the confidence interval at 95 %, using the equation:

$$m_1 \pm 1{,}96\ \sigma_1$$

All values which are outside this confidence interval shall be considered as outliers and shall be eliminated.

On the remaining values, calculate a new mean m_2 and a new standard deviation σ_2.

Define a new confidence interval using the equation:

$$m_2 \pm 1{,}96\ \sigma_2$$

All values which are outside this confidence interval shall in turn be considered as outliers and shall be eliminated.

Proceed in this manner conducting successive truncations until such times as there is no longer any value to be eliminated.

The values of this last confidence interval then form the upper and lower limits of the acceptable concentrations.

It is possible to indicate, if need be, the ratio limits between the components if it allows one to improve the efficiency of a quality evaluation.

Round off, if necessary, the limit values obtained to integers or half integers.

Annex B shows a practical example of the application of the method, for information only.

11 Expression of results

The chromatographic profile of the essential oil in question is expressed by a list of the representative and characteristic components accompanied by their minimum and maximum concentration limits and, possibly, by ratios between these said concentrations.

12 Test report

The test report shall specify the method used and the results obtained. It shall also mention all operating conditions or statistical methods used but not specified in this part of ISO 11024, or regarded as optiona, together with details of any incidents which may have influenced the test result.

The test report shall include, in particular, the number of samples of the essential oil studied and the number of years of production which have been considered.

Annex A
(informative)

Example of use of the test mixture to verify a gas chromatographic installation

When verifying the installation, use a new column, a new septum and a new insert. See Tables A.1 and A.2.

Table A.1 — Use of certain peaks for setting a gas phase chromatographic installation

Eucalyptol peak	Its height shall not exceed 30 % to 40 % of the full scale (e.g. 300 mV to 400 mV for a 1 V outlet). Its shape shall be symmetrical; in particular, an eucalyptol peak of which the ascending part is very irregular generally indicates an overload of the stationary phase.
Decanal peak	Use its height for determining the sensitivity of the integrator (elimination of background noise).

Table A.2 — Observations made on the basis of the test mixture

Causes	**Consequences**
Column in poor condition	Decanal less than the range, tailing peak Eugenol less than the range, tailing peak Benzyl salicylate less than the range, tailing peak
Dirty reactive injector	Linalyl acetate < 22 %, and appearance of a myrcene peak
Poor flame setting (insufficient oxygen)	Eucalyptol > 52 %, linalyl acetate < 22 %
Poor injection (insufficient volatilization)	Eucalyptol > 55 %, hexanol > 0,8 %, α-pinene > 7 % All others well below the lower limit.

Annex B
(informative)

Practical example of the elaboration of the chromatographic profile of the essential oil of sage (*Salvia officinalis* L.)

B.1 Application of clause 5

The profile has been worked out after having analysed over 80 samples of essential oil corresponding to 6 years of production and coming from 9 different countries.

B.2 Application of clause 7

Choose the appropriate operating conditions for the chromatographic analysis of the essential oil and process to the validation of the performance of the apparatus by using the standard test mixture as indicated.

B.3 Application of clause 9

B.3.1 Analytical study

According to the indications given in clause 9, an analytical study of 80 samples of the essential oil of sage (*Salvia officinalis* L.) enable identification of 25 components present in all of the samples:

1	α-pinene	14	α-thujone
2	camphene	15	β-thujone
3	β-pinene	16	camphor
4	sabinene	17	linalool
5	myrcene	18	linalyl acetate
6	α-terpinene	19	bornyl acetate
7	limonene	20	β-caryophyllene
8	1,8-cineole	21	terpinen-4-ol
9	*cis*-ocimene	22	α-humulene
10	γ-terpinene	23	α-terpineol + borneol
11	*trans*-ocimene	24	caryophyllene oxide
12	*para*-cymene	25	viridiflorol
13	terpinolene		

B.3.2 Selection of the samples

Among the 80 samples of essential oil being studied, 9 samples were eliminated by agreement among experts, their chemical composition having been considered as being very different from that of the essential oils of sage (*Salvia officinalis* L.*)*, the latter being considered as pure and of good organoleptic quality.

NOTE For these 9 eliminated samples, the α- and β-thujone content (less than 2 %) and 1,8-cineole content (more than 20 %) were very abnormal. The reason for this could be possible mixing or confusion between essential oils of sage of different botanical species.

B.3.3 Selection of the representative components

From the 71 samples of essential oil remaining after this elimination, the experts selected the following 12 components, which are considered **representative** of the sage (*Salvia officinalis* L.) essential oil, according to the definition given in 3.1:

α-pinene	terpinolene
camphene	β-thujone
α-terpinene	bornyl acetate
limonene	terpinen-4-ol
1,8-cineole	α-humulene
γ-terpinene	borneol + α-terpineol

B.3.4 Selection of the characteristic components

B.3.4.1 A very small concentration of linalool and of linalyl acetate allows differentiation of the essential oil of sage (*Salvia officinalis* L.) from that of clary sage (*Salvia sclarea* L.) because *Salvia officinalis* contains less than 1 % of it, whereas clary sage generally contains over 60 %.

Consequently, linalool plus linalyl acetate (compounds which sometimes are not very well separated) are selected.

B.3.4.2 The 1,8-cineole content enables avoidance of all confusion between the essential oil of Spanish sage (*Salvia lavandulifolia*) which contains over 20 % of it, and that of *Salvia officinalis* which contains between 5 % and 13 %.

1,8-Cineole is therefore selected.

B.3.4.3 The essential oil of *Salvia officinalis* presents a certain toxicity due to the presence of camphor and of the two isomers α- and β-thujone, and national and European Community regulations limit the concentration of these components in foodstuffs. It is therefore necessary to evaluate these products in the essential oils.

As β-thujone already figures in the list shown above, it has been considered necessary to add α-thujone and camphor to this list.

These components are therefore characteristic within the meaning of the definition given in 3.2.

B.3.5 Composition of the list

To the 12 representative components are added the characteristic components, i.e α-thujone and camphor for toxicity reasons, and linaool plus linalyl acetate in order to avoid any confusion. Therefore a list of 16 components is obtained (in the example chosen, the 1,8-cineole is both representative and characteristic).

As stated in clause 9, it is advisable not to go beyond 12 components, and the list of 16 is therefore too big.

After examination, it was decided to eliminate those components having too small a concentration, namely:

α-terpinene

γ-terpinene

terpinolene

terpinen-4-ol

as well as the pair "borneol + α-terpineol" which is badly separated under the procedural conditions adopted.

B.3.6 Conclusions

For the chromatographic profile of sage (*Salvia officinalis* L.), **10 representative and/or characteristic** components have been chosen:

α-pinene	β-thujone
camphene	camphor
limonene	linalool + linalyl acetate
1,8-cineole	bornyl acetate
α-thujone	α-humulene

B.4 Application of clause 10

B.4.1 Fixing the concentration limits

The assessment of the approximate concentration of the 10 representative and characteristic components in the 71 samples of sage (*Salvia officinalis* L.) essential oil was conducted using the peak area normalization method (internal normalization method) according to ISO 7609.

The mean concentration m_1 and the standard deviation σ_1 were calculated for each of the components.

Minimum and maximum concentrations were then calculated, using the equation:

$m_1 \pm 1{,}96\ \sigma_1$

Those essential oils having values situated outside these lower and upper limits were eliminated.

A new mean m_2 and a new standard deviation σ_2 were calculated. This allows calculation of new upper and lower limits and, consequently, elimination of those values situated outside these new limits.

By successive truncations, a stage is reached where there is no longer any value to be eliminated. From the last mean determined, the lower and upper limits are calculated which constitute the values to be taken into account for the component in question. These values are to be rounded off.

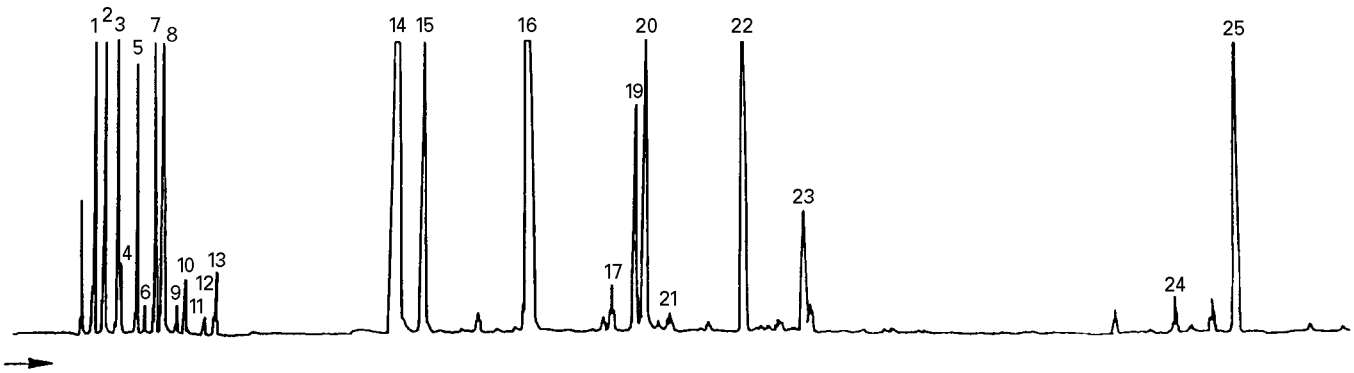
B.4.2 Example

See Table B.1.

Table B.1 — Examples of permissible concentration limits

Constituent	Mean	Standard deviation	Lower limit	Upper limit	Concentration limit %	
	%	σ	%	%	Min	Max.
α-Pinene	3,56	1,47	1,27	6,39	1	6,5
Camphene	3,74	1,55	1,69	6,54	1,5	7
Limonene	1,66	0,58	0,71	2,62	0,5	3
1,8-Cineole	8,52	2,80	5,65	12,72	5,5	13
α-Thujone	31,60	6,90	18,64	42,64	18	43
β-Thujone	5,16	1,73	3,04	8,22	3	8,5
Camphor	13,34	6,96	4,92	24,09	4,5	24,5
Linalool	0,43	0,14	0,26	0,64	0	1
Linalyl acetate	0	0	0	0		
Bornyl acetate	1,10	0,45	0,27	2,18	0	2,5
α-Humulene	5,56	3,32	0	11,93	0	12

Figures B.1 and B.2 show, for information only, some typical chromatograms obtained on conducting the chromatographic analysis of a sage (*Salvia officinalis* L.) essential oil by gas chromatography on a capillary column using two types of phases: polyethylene glycol 20 000 (polar phase) and OV 1701 (apolar phase).



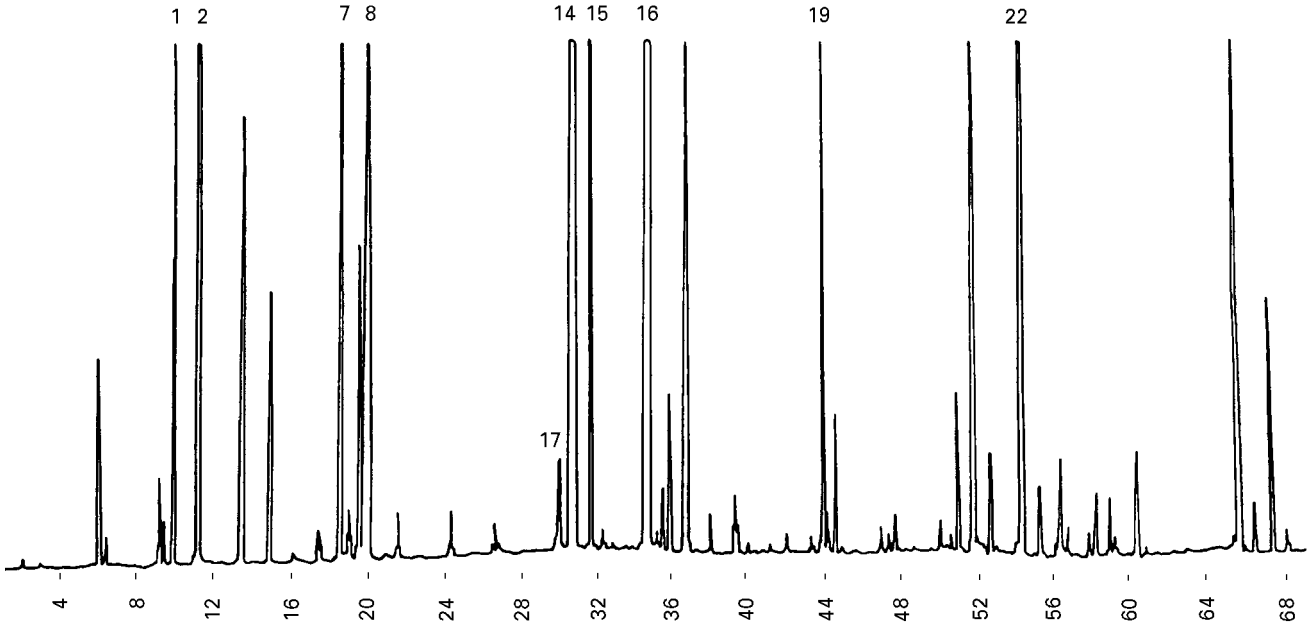
Peak identification

1	α-Pinene	14	α-Thujone
2	Camphene	15	β-Thujone
3	β-Pinene	16	Camphor
4	Sabinene	17	Linalool
5	Myrcene	18	Linalyl acetate
6	α-Terpinene	19	Bornyl acetate
7	Limonene	20	β-Caryophyllene
8	1,8-Cineole	21	Terpinen-4-ol
9	*cis*-Ocimene	22	α-Humulene
10	γ-Terpinene	23	α-Terpineol + borneol
11	*trans*-Ocimene	24	Caryophyllene oxide
12	*para*-Cymene	25	Viridiflorol
13	Terpinolene		

Operating conditions

Column: capillary column, fused silica, length 50 m, internal diameter 0,32 mm
Stationary phase: polyethylene glycol 20 000
Oven temperature: initial isotherm at 70 °C for 15 min, then from 70 °C to 180 °C at a rate of 2 °C/min, and final isotherm at 180 °C for 15 min
Injector temperature: 180 °C
Detector temperature: 200 °C
Detector: flame ionization detector
Carrier gas: hydrogen, 0,5 bar
Volume injected: 0,1 µl
Split ratio: 1/100

Figure B.1 — Typical chromatogram of *Salvia officinalis* L. on a polar phase



Peak identification

1	α-Pinene	14	α-Thujone
2	Camphene	15	β-Thujone
3	β-Pinene	16	Camphor
4	Sabinene	17	Linalool
5	Myrcene	18	Linalyl acetate
6	α-Terpinene	19	Bornyl acetate
7	Limonene	20	β-Caryophyllene
8	1,8-Cineole	21	Terpinen-4-ol
9	*cis*-Ocimene	22	α-Humulene
10	γ-Terpinene	23	α-Terpineol + borneol
11	*trans*-Ocimene	24	Caryophyllene oxide
12	*para*-Cymene	25	Viridiflorol
13	Terpinolene		

Operating conditions

Column: capillary column, fused silica, length 50 m, internal diameter 0,32 mm

Stationary phase: OV 1701

Oven temperature: initial isotherm at 70 °C for 15 min, then from 70 °C to 180 °C at a rate of 2 °C/min, and final isotherm at 180 °C for 15 min

Injector temperature: 180 °C

Detector temperature: 200 °C

Detector: flame ionization detector

Carrier gas: hydrogen, 0,5 bar

Volume injected: 0,1 µl

Split ratio: 1/100

Figure B.2 — Typical chromatogram of *Salvia officinalis* L. on an apolar phase

ICS 71.100.60

Descriptors: oils, essential oils, chemical analysis, chromatographic analysis, gas phase chromatography, general conditions.

Price based on 12 pages