BS EN 16274:2012

BSI Standards Publication

Methods for analysis of allergens — Quantification of suspected fragrance allergens in consumer products — Step 1: GC analysis of ready-to-inject sample

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National foreword

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The UK participation in its preparation was entrusted to Technical Committee CW/217, Cosmetics.

A list of organizations represented on this committee can be obtained on request to its secretary.

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ISBN 978 0 580 74898 1

ICS 71.100.60

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This British Standard was published under the authority of the Standards Policy and Strategy Committee on 31 October 2012.

Amendments issued since publication

Date	Text affected

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 16274

September 2012

ICS 71.100.60

English Version

Methods for analysis of allergens - Quantification of suspected fragrance allergens in consumer products - Step 1: GC analysis of ready-to-inject sample

Méthodes d'analyse des allergènes - Quantification des fragrances allergènes suspectées dans les produits de consommation - Étape 1 : Analyse par GC d'échantillons prêts à être injectés

Analyseverfahren für Allergene - Quantifizierung von mutmaßlichen Allergie auslösenden Duftstoffen in Verbrauchsgütern - Stufe 1: GC-Analyse von einspritzfertigen Proben

This European Standard was approved by CEN on 4 August 2012.

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EUROPÄISCHES KOMITEE FÜR NORMUNG

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Ref. No. EN 16274:2012: E

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Foreword

This document (EN 16274:2012) has been prepared by Technical Committee CEN/TC 347 “Methods for analysis of allergens”, the secretariat of which is held by DS.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by March 2013, and conflicting national standards shall be withdrawn at the latest by March 2013.

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Introduction

Human skin exposure to suspected allergenic fragrances can occur through diverse sources such as detergents and cosmetics intended to be rinsed or not. As a result of their possible effect, 26 fragrance substances have been restricted under Council Directives with labelling requirements in order to insure a high level of protection of consumers, particularly for sensitive population.

In this context, several analytical methods have been developed to detect and determine their presence in cosmetics such as Gas Chromatography/Flame Ionisation Detector (GC-FID), Gas Chromatography/Mass Spectrometry (GC-MS), comprehensive GC or MS-MS in raw materials and finished products.

The present analytical method uses GC-MS by combination of two GC columns of different polarity with a dedicated methodology for quantification [1]. This allows separation and quantification of the 24 volatile suspected allergens above 0,001 % (10 mg/kg) of each, in ready-to-inject sample from a cosmetic ingredient or product matrix. The present protocol has been validated thanks to a ring test [2].

1 Scope

This European Standard describes a method for the identification and determination of 24 volatile suspected allergens from ready-to-inject cosmetics and raw materials used in cosmetic products and are compatible with GC analysis. This analysis uses GC-MS after sample preparation. The 24 suspected allergens are restricted under Council Directives (7th amendment to the Cosmetic Directive 2003/15/EC).

The method described in this European Standard does not include requirements for the preparation of samples in matrices for which direct injection in GC is not feasible.

2 Principle

The method described in this European Standard is a comprehensive analysis of 24 volatile suspected allergens by Gas Chromatography coupled with Mass Spectrometry after dilution of the sample in an inert solvent.

Two assays are performed for the chromatographic separation of the 24 suspected allergens using two GC capillary columns of different polarities. Suspected allergen identification is achieved when possible using GC-MS in scan mode. Quantification is performed by single ion monitoring (SIM) using 1,4-dibromobenzene and 4,4'-dibromobiphenyl as internal standards.

The final result depends on the agreement of the different ion ratios obtained for both injections according to specific requirements.

3 Reagents

Unless otherwise stated, use only reagents of recognised analytical grade. The solvent shall be of quality for GC-MS analysis.

3.1 Solvents

3.1.1 **Methyl pivalate**, CAS no: [598-98-1], analytical grade or higher.

3.1.2 **Ortho-fluorotoluene**, CAS no: [95-52-3], analytical grade or higher.

3.1.3 **Acetone**, CAS no: [67-64-1], analytical grade or higher.

IMPORTANT — if other solvents are used, their inertness with the analytes shall be demonstrated. In any case, the same solvent shall be used both for calibration and determination.

3.2 Fragrance (suspected allergen) standards

3.2.1 **Amylcinnamic alcohol**, CAS no: [101-85-9], with known purity.

NOTE Possibly two isomers.

3.2.2 **Amylcinnamic aldehyde (flosal®),** CAS no: [122-40-7], with known purity.

NOTE Possibly two isomers.

3.2.3 **Anisyl alcohol**, CAS no: [105-13-5], with known purity.

3.2.4 **Benzyl alcohol**, CAS no: [100-51-6], with known purity.

3.2.5 **Benzyl benzoate**, CAS no: [120-51-4], with known purity.

3.2.6 Benzyl cinnamate, CAS no: [103-41-3], with known purity.

NOTE Possibly two isomers.

3.2.7 Benzyl salicylate, CAS no: [118-58-1], with known purity.

3.2.8 Butylphenyl methylpropional (lilial®), CAS no: [80-54-6], with known purity.

3.2.9 Cinnamic alcohol, CAS no: [104-54-1], with known purity.

NOTE Possibly two isomers.

3.2.10 Cinnamic aldehyde, CAS no: [104-55-2], with known purity.

NOTE Possibly two isomers.

3.2.11 Citral, CAS no: [5392-40-5], with known purity.

NOTE Two isomers, neral and geranial, which are determined separately.

3.2.12 Citronellol, CAS no: [106-22-9], with known purity.

3.2.13 Coumarine, CAS no: [91-64-5], with known purity.

3.2.14 Eugenol, CAS no: [97-53-0], with known purity.

3.2.15 Farnesol, CAS no: [4602-84-0], with known purity.

NOTE Possibly four isomers. The two major isomers are (E,E)-farnesol (CAS no [106-28-5]) and (Z,E)-farnesol (CAS no [3790-71-4]).

3.2.16 Geraniol, CAS no: [106-24-1], with known purity.

3.2.17 Hexylcinnamic aldehyde (jasmonal®), CAS no: [101-86-0] with known purity.

NOTE At least two isomers.

3.2.18 Hydroxycitronellal, CAS no: [107-75-5], with known purity.

3.2.19 Hydroxyisohexyl-3-cyclohexene carboxaldehyde (lyral®), CAS no: [31906-04-4], with known purity.

WARNING — This fragrance standard also contains hydroxyisohexyl-4-cyclohexene carboxaldehyde which shall not be quantified.

3.2.20 Isoeugenol, CAS no: [97-54-1], with known purity.

NOTE Possibly two isomers (cis, trans).

3.2.21 α-Isomethyl ionone, CAS no: [127-51-5], with known purity.

3.2.22 Limonene, CAS no: [5989-27-5], with known purity.

3.2.23 Linalool, CAS no: [78-70-6], with known purity.

3.2.24 Methyl-2-octynoate (folione®), CAS no [111-12-6], with known purity.

3.3 Internal standards (ISTD)

3.3.1 1,4-dibromobenzene, analytical grade or higher.

3.3.2 4,4'-dibromobiphenyl, analytical grade or higher.

4 Apparatus

Use standard laboratory glassware and equipment.

4.1 Analytical Balance

Capable of weighing to the nearest 0,000 1 g.

4.2 GC-FID (for solvent or standard purity only)

GC-FID equipped with a split/splitless injector with a glass insert maintained at 250 °C. The glass insert shall have an inner volume compatible with the expansion volume of the analytical solvent. A GC capillary column shall be connected to the FID.

4.3 GC-MS

GC-MS equipped with a split/splitless injector with a glass insert maintained at 250 °C. The glass insert shall have an inner volume compatible with the expansion volume of the analytical solvent. A GC capillary column shall be connected to the mass spectrometer, with the interface heated at least 10 °C above the final oven temperature.

CAUTION — Use of an ion trap mass spectrometer (ITD-MS) or time of flight mass spectrometer (ToF-MS) is possible if the conditions are adapted to such instruments, particularly for peak recognition and quantification. At least, the linearity of such instruments shall be checked in the calibration range used.

4.4 GC capillary columns

The two columns shall significantly differ in polarity and should be chosen according to Annex A.

When a new column is used (not listed in Annex A), it's characteristics shall be evaluated in terms of peak resolution during the column life. This shall be checked by either measuring the resolution between all analytes or by calculation of the mean resolution. The resolution between all peaks shall be > 1. The mean resolution $\overline{R}$ shall be > 5.

$$\overline{R} = 1{,}18\left[\prod_{i=1}^{i=n-1}\left(\frac{t_{R,i+1} - t_{R,i}}{w_{h,i} + w_{h,i+1}}\right)\right]^{\frac{1}{n-1}}$$

where

$t_{R,i}$ total retention time of the i^{th} peak;

$w_{h,i}$ width at half height of the i^{th} peak;

n number of peaks in the chromatogram.

If $\overline{R} = 0$,the column can only be used if there is no ion listed in Table 2 in common between 2 co-eluted allergens.

5 Standard preparation and preservation

5.1 General

Solvents used for analysis and storage shall comply with the following requirements:

— chemical inertia towards the allergen analytes,

— low volatility to ensure solution stability and concentration, and

— expansion volume compatible with the inner volume of injector insert.

The volume of injection shall be compatible with the volume of the insert and with the capacity of the column. Overflowing of the expanded volume of vaporised solvent shall be avoided.

Methyl pivalate (3.1.1) and ortho-fluorotoluene (3.1.2) are suitable for the preparation of all standard and sample solutions. Acetone (3.1.3) may be used for the preparation of calibration solutions or final sample dilutions. In any case, the stock solution shall not be diluted in a volatile solvent such as acetone.

NOTE Ethanol is normally not suitable. If present in high concentration in the sample, ethanol can be used provided that immediate injection is performed. Iso-octane is not suitable for polar fragrances.

The solvent purity shall be checked to ensure that no impurities interfere with any of the 24 fragrance suspected allergens analysed under the GC conditions used in this European Standard.

For ready-to-inject samples, the same solvent shall be used to prepare calibration solutions and sample dilution, except where a volatile solvent is used.

During analysis by GC-MS, a vial containing either the calibration solution or sample dilution shall be injected only once for analysis.

At least, half of the sample dilution solvent should be the same as the solvent used for calibration.

5.2 Standard preparation

5.2.1 General

The purity of each standard and each internal standard and the respective percentages for geometrical isomers (amylcinnamic alcohol (3.2.1), flosal® (3.2.2), benzyl cinnamate (3.2.6), cinnamic alcohol (3.2.9), cinnamic aldehyde (3.2.10), citral (3.2.11), farnesol (3.2.15), jasmonal® (3.2.17), lyral® (3.2.19), isoeugenol (3.2.20)) shall be determined by GC-FID, for further calculations of purity under the conditions used in this European Standard.

Preparation shall be undertaken according to either 5.2.2 or 5.2.3.

For citral and farnesol, the concentrations described hereafter (5.2.2 and 5.2.3) should be doubled in order to be in compliance with the final calibration range.

Standard solutions shall be prepared following 5.2.2 or 5.2.3 depending on storage facilities.

5.2.2 Stock solution of all allergens (5 g/l)

Prepare **stock solution (A)** of standard compounds (3.2) containing 5 g of each in 1 l of appropriate inert and non-volatile solvent (concentration 5 g/l).

NOTE 5 g/l level can be obtained by weighing 50 mg of each compound in 10 ml of solvent in order to use little quantity of fragrances and solvents.

Store this stock solution (A) in the absence of light in a freezer below -18 ºC. This solution can be used for one month.

5.2.3 Separate stock solutions (carbonyl / non carbonyl compounds) (10 g/l)

Prepare **stock solution (A-1)** of aldehydes and ketones standard compounds (3.2.2, 3.2.8, 3.2.10, 3.2.11, 3.2.17, 3.2.18, 3.2.19, 3.2.21) containing 10 g of each in 1 l of appropriate inert and non-volatile solvent (concentration 10 g/l).

Prepare **stock solution (A-2)** of other standard compounds (3.2.1, 3.2.3, 3.2.4, 3.2.5, 3.2.6, 3.2.7, 3.2.9, 3.2.12, 3.2.13, 3.2.14, 3.2.15, 3.2.16, 3.2.20, 3.2.22, 3.2.23, 3.2.24) containing 10 g of each in 1 l of appropriate inert solvent (concentration 10 g/l).

NOTE 10 g/l level can be obtained by weighing 100 mg of each compound in 10 ml of solvent in order to use little quantity of fragrances.

Store these separate stock solutions (A-1, A-2) in the absence of light in a refrigerator at approximately 4 °C. These solutions can be used for two months.

5.2.4 Internal standard solution

Prepare **internal standards solution (S-ISTD)** containing 1 g of 1,4-dibromobenzene (3.3.1) and 1 g of 4,4'-dibromobiphenyl (3.3.2) in 1 l of inert and non-volatile solvent.

Store this solution in the absence of light in a refrigerator at approximately 4 °C. This solution can be used for two months.

NOTE Such a solution can be prepared by weighing 100 mg of 1,4-dibromobenzene (3.3.1) and 100 mg of 4,4'-dibromobiphenyl (3.3.2) in 10 ml of solvent and then by diluting 1 ml of this solution in solvent to 10 ml. This procedure is adapted to expensive solvents.

5.2.5 Working solutions

Prepare **working solution (B)** by dilution of solution A containing 0,5 g of each in 1 l of same solvent than used in 5.2.2. This is a final concentration of 0,5 g/l.

If solutions A-1 and A-2 are used, prepare a **solution A'** by equally mixing both A-1 and A-2. This intermediate solution should be prepared daily.

Prepare **working solution (B')** by dilution of solution A' containing 0,5 g of each in 1 l of same solvent than used in 5.2.2. This is a final concentration of 0,5 g/l.

Solution B (or B') should be prepared with 1 ml of A (or A') in a total volume of 10 ml.

5.2.6 Calibration solution

Prepare **calibration solutions (C1, C2, C3, C4, C5, and C6)** by dilutions of solution B (or B') after addition of the internal standards (S-ISTD) at 10 mg/l. All solutions shall be stored in the absence of light in a freezer below -18 ºC. These solutions can be used for one week if a non-volatile solvent is used (e.g. ortho fluorotoluene or methyl pivalate) or one day if a volatile solvent is used (e.g. acetone).

Table 1 shows an example of a suitable calibration curve; if necessary the calibration range and the final concentration of ISTD may be adapted. Inject each of the calibration solutions to construct a standard calibration curve.

Table 1 — Calibration solutions

Calibration solutions	Stock solution volume (µl)	Total volume (ml)	Final concentration of allergens Level (mg/l)	Final concentration of ISTD Level (mg/l)
Calibration solution **C1**	800 µl of **B (or B') + 100 µl of S-ISTD**	10 ml	40	10
Calibration solution **C2**	600 µl of **B (or B') + 100 µl of S-ISTD**	10 ml	30	10
Calibration solution **C3**	400 µl of **B (or B') + 100 µl of S-ISTD**	10 ml	20	10
Calibration solution **C4**	200 µl of **B (or B') + 100 µl of S-ISTD**	10 ml	10	10
Calibration solution **C5**	100 µl of **B (or B') + 100 µl of S-ISTD**	10 ml	5	10
Calibration solution **C6**	20 µl of **B (or B') + 100 µl of S-ISTD**	10 ml	1	10

6 Procedure

6.1 General

Determination of allergen content is undertaken by acquiring in SIM mode using characteristic fragments I1, I2, I3 (see Table 2) on two GC capillary columns with different polarity.

Confirmation of the presence of allergens is undertaken by examining chromatograms obtained in SCAN and SIM modes on both GC capillary columns.

If an abundant fragrance ingredient is eluted from the GC capillary column just before an allergen analyte, the elution of the analyte may be delayed. The corresponding SIM acquisition window may be adjusted to acquire the characteristic fragment ions.

6.2 Chromatographic conditions

Two GC capillary columns of different polarity shall be used to separate the 24 suspected allergens for identification and quantification in the presence of other interfering fragrance ingredients.

Annex A describes suitable GC capillary columns by increasing polarity order and examples of temperature programmes.

6.3 MS conditions

6.3.1 General

A single quadrupole MS instrument is recommended with ionisation by electronic impact at 70eV.

Q-GC-MS-MS may be used in simple MS mode to comply with this European Standard.

Heat the MS in accordance with the instructions of the suppliers and the good practices of mass spectrometry.

Calibration shall be performed after each tune of the instrument and at least once a week.

6.3.2 SCAN mode

In SCAN mode, choose a mass range compatible with the lowest and highest fragments. Typically a mass range from 35 a.m.u to 350 a.m.u allows a structural confirmation of all allergen analytes.

6.3.3 SIM mode

In SIM mode, the fragment ions listed in Table 2 allow quantification by using one of the three ions as the quantification ion and the other two as qualifiers. Each of these three ions successively becomes the quantifier ions and the remaining two the qualifier ions.

NOTE 1 In Table 2, I1 is usually considered as the main ion for quantification.

The time spent on each ion (dwell time) shall be the same for all ions within a given window.

NOTE 2 A dwell time at 50 msec per ion for a SIM window including three fragments is correct. If 6 ions are present, a 20 msec per ion dwell time can be applied. At least, a minimum of 10 points of acquisition for one peak allows an acceptable quantification.

Depending on the instrument generation, an increase of the detector voltage, compared to that proposed by the instrument tune, may enhance the sensitivity, e.g. from 50 kV for recent instruments to 200 KV for old ones, for a 2 KV tune value.

The m/z values of Table 2 are rounded. Exact values should be used as SIM parameters, as obtained from the scan analysis.

Table 2 — List of ions for quantification

Name	I1 (a.m.u)	I2 (a.m.u)	I3 (a.m.u)
Amylcinnamic alcohol	133	115	204
Amylcinnamic aldehyde	202	201	129
Anisyl alcohol	138	137	109
Benzyl alcohol	108	79	107
Benzyl benzoate	105	212	194
Benzyl cinnamate	131	192	193
Benzyl salicylate	91	228	65
Butylphenyl methylpropional (lilial)	189	147	204
Cinnamic alcohol	92	134	115
Cinnamic aldehyde	131	132	103
Citral : Neral	69	94	109
Citral : Geranial	69	84	94
Citronellol	95	69	81
Coumarine	146	118	89
Eugenol	164	103	149
Farnesol	69	93	81
Geraniol	69	123	93
Hexylcinnamic aldehyde	216	215	129
Hydroxycitronellal	59	71	43
Hydroxyisohexyl-3-cyclohexene carboxaldehyde (lyral)	192	136	149
Isoeugenol	164	149	131
α-Isomethylionone	206	135	150
Limonene	68	93	67
Linalool	93	71	121
Methyl 2-octynoate	95	123	79
1,4-dibromobenzene	236	234	238
4,4'-dibromobiphenyl	312	310	314

Use the data obtained in GC-MS SCAN mode from a standard solution (e.g. the higher calibration level, 40 mg/l) to construct the SIM windows with no more than 6 ions per SIM window. For a given suspected allergen, the window may be chosen just before its integration start-time. In contrast, a delay is recommended where possible for the end of the SIM window after the integration end-time, as co-elution may delay the target peak.

Annex B presents an example of a programme in SIM mode according to the described chromatographic conditions.

6.4 Calibration

Inject each calibration solution (5.2.6) on both columns, and construct a calibration curve for each allergen analyte. One of the three ions is successively taken as the quantification ion, and the other two as qualifier ions. As a result 2 columns x 3 ions will give 6 different calibration curves for each allergen.

When an analyte is a mixture of isomers, the calibration curve is constructed for the major isomer, except for citral where calibration curves shall be constructed for neral and geranial and for farnesol where calibration curves shall be constructed for ((E,E)-farnesol and (Z,E)-farnesol). Use the purity of the major isomer for quantification of the other analytes.

Plot the calibration curves as follows:

$$\frac{\text{allergen area}}{\text{ISTD area}} = f\left(\frac{\text{allergen concentration}}{\text{ISTD concentration}}\right)$$

where

allergen area is the area of the ion of quantification of each analyte (3.2);

ISTD area is the area of ion m/z = 236 for 1,4-dibromobenzene (3.3.1) or the area of ion m/z = 312 for 4,4'-dibromophenyl (3.3.2).

Divide the retention time axis into two portions containing an ISTD, and quantify the analyte of each portion relatively to the ISTD of the same window.

The coefficients of determination of the calibration shall be greater than 0,995. Check that residues are randomly distributed on both sides of the calibration curve. If not, it may indicate a contamination of the injector insert and sometimes of the MS source or chromatography column.

For each sequence, a verification of the calibration and its linearity is required by injecting an independent check standard solution prepared at a concentration equivalent to the middle of the calibration range (for example 20 mg/l for a method range of 0 mg/l - 40 mg/l) (5.2.6). Calculate the analyte concentrations using the calibration curves. The concentration shall not differ more than ± 20 % from the expected value.

7 Sample analysis

Weigh a sample amount m (to the nearest mg) into a 10 ml volumetric flask. Add 100 µl of S-ISTD (5.2.4). The volume of ISTD added should be adapted if the ISTD concentration used in the calibration curve is not always fixed at 10 mg/ml (i.e. if a different calibration range is used, other than 0 mg/l - 40 mg/l). Make up to the mark in the 10 ml volumetric flask with the same solvent (5.2.6) used to prepare the calibration solutions, and mix. Transfer an aliquot of the prepared sample into a GC autosampler vial for GC-MS analysis.

Inject the calibration standards and samples under the same GC-MS condition in SCAN and SIM mode on both GC capillary columns.

Samples should be analysed by GC-MS within 24 h after preparation.

If the concentration of the allergen analyte in the sample is outside the calibration range following GC-MS determination, a further dilution is prepared from the 10 ml volumetric flask with the addition of S-ISTD. The samples are then analysed by GC-MS to obtain a concentration within the linear range of the calibration curve.

NOTE 1 The sample dilution could be performed at 1/10, weighing 1,0 g of sample and diluting to 10 ml in a volumetric flask with the same solvent used to prepare the calibration standards, after addition of internal standard.

NOTE 2 Typically, 1 µl in split mode (1:100) is suitable for usual expected values (> 10 mg/kg) for GC columns with internal diameter above 0,2 mm.

8 Data treatment and calculation of results

8.1 Identification of allergens

Following analyses of the sample on the 2 GC capillary columns, the presence and identity of all target allergen analytes are checked on the scan analysis.

Due to the lower sensitivity of the SCAN mode, a given analyte may not be detected by SCAN but should be detected in SIM mode. In such case, the analyte presence is confirmed if the abundance of its three ions fulfils the criteria in 8.3.2 or 8.3.3 using one of the two GC capillary columns.

An ion will be considered as missing if the signal/noise ratio is less than 10 for the considered mass.

8.2 Quantification of allergens

For all analytes on both GC capillary columns and each of the 3 ions, determine from the SIM analyses the concentrations by the ISTD method from the calibration curves. The concentrations are determined in mg/kg and correspond to the mass fractions in the sample which are then corrected for dilution.

When an analyte is a mixture of isomers, the final amount is performed by quantification of the major isomer except for:

— citral which is the sum of the amount of neral and the amount of geranial;

— farnesol which is the sum of the amount of four isomers (frequently, only three of them are detectable);

— lyral: only its isomer 3- is regulated (hydroxyisohexyl-3-cyclohexene carboxaldehyde).

Concentration C_{Ix} (mg/kg) of an analyte in the sample is:

$$C_{Ix} = Co\ 10/m$$

where

Co is the concentration (mg/l) of the compound in the vial by calculation from ion m/z = Ix, x = 1, 2, 3 on both columns (see Table 2);

m is the mass sample (mg);

10 is the volume of the flask in ml.

NOTE Co is currently obtained by plotting the ratio of the area of ion Ix on the area of m/z = base peak of ISTD against the concentration of calibrations vials as:

$$Co = (y - b)/a$$

where

y is the ratio of the area of ion Ix on the area of m/z = base peak of ISTD;

a is the slope of the calibration curve;

b is the intercept of the calibration curve.

8.3 Assessment of the analytical measurement

8.3.1 General

The validity of analytical measurement is checked for each sample with an allergen analyte concentration above 10 mg/kg.

Due to the usual high complexity of samples to be analysed by this European Standard, the relevance of the calculated concentration has to be carefully investigated. Different strategies to check the validity of the calculations are possible according to the knowledge of the operators and/or the functionalities of the software provided with the GC-MS.

The choice of the final concentration shall be undertaken according to the requirements of the decisional tree of Annex D.

The confirmation of the presence of an allergen analyses in GC-MS SCAN mode shall be firstly preferred whenever possible. If the SCAN mode fails to confirm the analyte identity, the presence of the analyte shall be confirmed at least by one of the 6 Q values ($Q \geq 90$, see 8.3.2), or by one of the 6 couples of ion ratios within the permitted tolerances (8.3.3).

Examination of chromatograms acquired in GC-MS SCAN mode can also identify if there is any co-elutions with the suspected allergen listed in 3.2. These co-elutions may or may not delay the chromatographic elution of some allergens analytes during the GC-MS scan and SIM mode acquisitions. Consequently, it may be necessary to specifically adjust the corresponding SIM acquisition window.

If the calculated concentration in SIM mode for an analyte is below the sensitivity in SCAN mode, one of the two approaches described below (8.3.2, 8.3.3) shall be taken into account.

8.3.2 Examination of the Q-values

The Q-value can be calculated from the raw peak area corresponding to the different ions according to the formula:

$$Q = 100 - \frac{\sum_{i=1}^{i=n}\left(100 * \left|r_i - r_i^{'}\right|\right)(\ln[100 r_i + 1])^2}{21.3 * \sum_{i=1}^{i=n} r_i}$$

where

n is the number of ions per compound;

r_i is the reference peak area ratio;

$r_i^{'}$ is the observed peak area ratio.

If the value of Q is 0, the corresponding amount shall not be considered.

The reference peak area ratio should be determined using a calibration level at the middle of the curve.

8.3.3 Maximum permitted tolerances

The relative intensities of the ion for quantification against respectively the two qualifiers, expressed as a percentage of the intensity of the most intense ion, shall correspond to those of the calibration standard solutions, at comparable concentrations, measured under the same conditions, within the tolerances described Table 3.

The calibration standard used as reference should be at the middle of the calibration curve.

Table 3 — Maximum permitted tolerances for relative ion intensities [3]

Relative intensity (% of base ion intensity)	**Relative range of the response**
> 50 %	± 10 %
> 20 % - 50 %	± 15 %
> 10 % - 20 %	± 20 %
≤ 10 %	± 50 %

9 Test report

The test report shall contain at least the following information:

— all information necessary for the identification of the sample,

— a reference to this European Standard,

— date and type of sampling (if known),

— date of receipt of the laboratory sample,

— date of test,

— the results and the units in which the results have been expressed,

— any particular points observed in the course of the test,

— any operations not specified in the method or regarded as optional which might have affected the results.

Annex A
(informative)

Column performances

NOTE Phases are listed in the increasing polarity order.

Table A.1 — Column performances *(1 of 2)*

Phase	Size Length x inner diameter x thickness	Recommended oven program	$\overline{R}$ a	Reported coelutions
100 % polydimethylsiloxane	60 m × 0,25 mm × 0,25 µm	100 °C for 2 min then 10 °C/min to 280 °C	0 (5,17)	Amylcinnamic aldehyde with first peak of hydroxyisohexyl-3-cyclohexene carboxaldehyde
100 % polydimethylsiloxane	20 m × 0,18 mm × 0,18 µm	100 °C for 2 min then 10 °C/min to 280 °C	0	Anisyl alcohol with geranial Cinnamic alcohol with methyl-2-octynoate
100 % polydimethylsiloxane	15 m × 0,1 mm × 0,1 µm	60 °C for 2 min then 15 °C/min to 135 °C for 5 min then 15 °C/min to 250 °C for 1 min	9,18	
5 % phenylmethylpolysiloxane 95 % polydimethylsiloxane	30 m × 0,25 mm × 0,25 µm	80 °C to 280 °C at 10 °C/min	0 (7,02)	Anisyl alcohol with hydroxycitronellal
5 % phenylmethylpolysiloxane 95 % polydimethylsiloxane	60 m × 0,25 mm × 0,25 µm	60 °C for 1 min then 3 °C/min to 150 °C then 6 °C/min to 280 °C	5,90	
50 % phenylmethylpolysiloxane 50 % polydimethylsiloxane	20 m × 0,18 mm × 0,18 µm	100 °C for 2 min then 10 °C/min to 280 °C	8,80	
75 % Methyl biphenyl - 25 % polysiloxane	30 m × 0,25 mm × 0,25 µm	60 °C for 1 min then 3 °C/min to 280 °C	0	Cinnamaldehyde with anisyl alcohol
33 % cyanopropylmethyl - 67 % dimethylpolysiloxane	30 m × 0,25 mm × 0,25 µm	90 °C for 1 min then 10 °C/min to 250 °C	0	Hydroxycitronellal with cinnamic alcohol Anisyl alcohol with lilial Amylcinnamic aldehyde with farnesol
50 % cyanopropylphenyl 50 % dimethylpolysiloxane	30 m × 0,25 mm × 0,25 µm	90 °C for 1 min then 8 °C/min to 250 °C	0	Methyl eugenol with cinnamic aldehyde

Table A.1 *(2 of 2)*

Phase	Size Length x inner diameter x thickness	Recommended oven program	$\overline{R}$ a	Reported coelutions
50 % dimethylpolysiloxane 50 % polyethylenglycol	30 m × 0,25 mm × 0,25 µm	65 °C for 1 min then 3 °C/min to 230 °C/min then 10 °C/min to 260 °C	9,78	
100 % polyethylenglycol	15 m × 0,1 mm × 0,1 µm	60 °C for 2 min then 20 °C/min to 75 °C then 15 °C/min to 135 °C then 10 °C/min to 260 °C for 2 min	11,17	
100 % polyethylenglycol	20 m × 0,18 mm × 0,3 µm	70 °C to 250 °C at 8 °C/min	8,64	
100 % polyethylenglycol	30 m × 0,25 mm × 0,25 µm	70 °C to 250 °C at 3 °C/min	12,2	

a $\overline{R}$ is calculated according to the formula given in 4.4. Values in parenthesis indicate the mean resolution without the reported co-elution.

Not recommended columns:

— High cyanopropyl modified phase due to the absorption of all phenols.

— 50 % trifluoropropyl- methylpolysiloxane due to the high bleeding.

IMPORTANT — For stability reasons, daily calibration is necessary for 100 % PEG-phase.

Annex B
(informative)

SIM windows

This table shows an example of SIM windows used with a DB17-MS-like column (30 mxID = 0,25 mm x df = 0,25 µm) programmed at 65 °C for 1 min then 3 °C/min to 230 °C then 10 °C/min to 260 °C at with a constant flow of 1,3 ml/min.

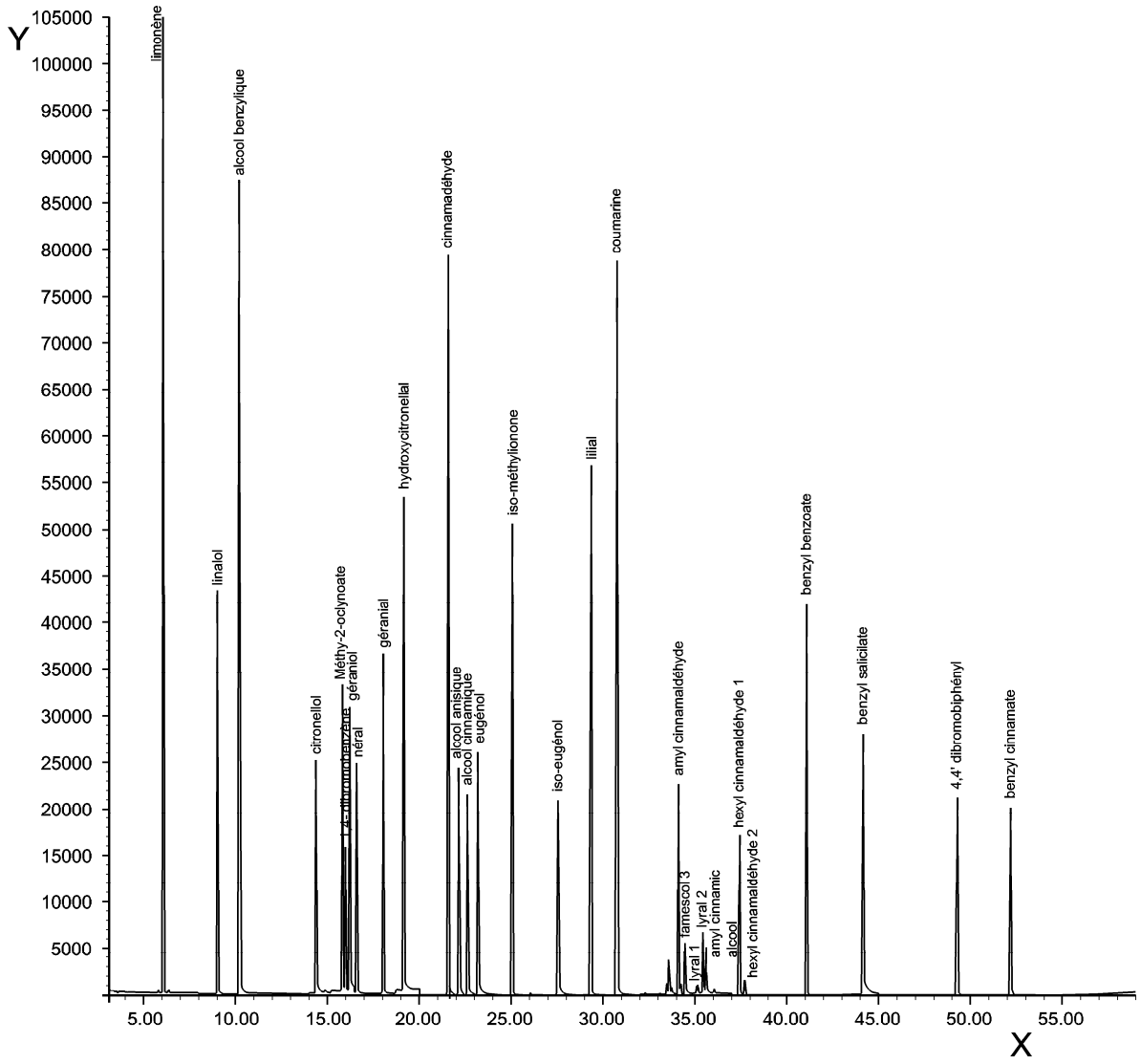
Allergen analytes above and below the double line refer to the first (3.3.1) and the second internal standard (3.3.2), respectively. The ions written in bold represent the best compromise between the sensitivity and the selectivity.

Table B.1 — SIM windows

Window	Name	Ions	Time window (min)	Dwell time (msec)
1	Limonene	**68**, 93, 67	3,00 (solvent delay) to 8,00	50
2	Linalool	**93**, 71, 121	8,00 to 9,50	50
3	Benzyl alcohol	**108**, 79, 107	9,50 to 14,00	50
4	Citronellol	**95**, 69, 81	14,00 to 15,20	50
5	Methyl 2-octynoate **1,4-Dibromobenzene** Geraniol	**95**, 123, 79 **236** **69**, 123, 93	15,20 to 16,45	20
6	Citral (neral)	**69**, 94, 109	16,45 to 17,20	50
7	Citral (geranial)	**69**, 84, 94	17,20 to 18,70	50
8	Hydroxycitronellal	**59**, 71, 43	18,70 to 20,00	50
9	Cinnamic aldehyde	**131**, 132, 103	20,00 to 21,90	50
10	Anisyl alcohol	**138**, 137, 109	21,90 to 22,40	50
11	Cinnamic alcohol	**92**, 134, 115	22,40 to 23,00	50
12	Eugenol	**164**, 103, 149	23,00 to 24,50	50
13	α-Isomethylionone	**135**, 206, 150	24,50 to 26,50	50
14	Isoeugenol	**164**, 149, 131	26,50 to 28,50	50
15	Lilial	**189**, 147, 204	28,50 to 30,00	50
16	Coumarine	**146**, 118, 89	30,00 to 32,00	50
17	(Z,E)-Farnesol	**69**, 93, 81	32,00 to 34,02	50
18	Amylcinnamic aldehyde	**202**, 201, 129	34,02 to 34,45	50
19	(E,E)-Farnesol	**69**, 93, 81	34,45 to 35,00	50
20	Lyral Amylcinnamic alcohol	**192**, 136, 149 **133**, 115, 204	35,00 to 37,00	20
21	Hexylcinnamic aldehyde	**216**, 215, 129	37,00 to 40,00	50
22	Benzyl benzoate	**105**, 212, 194	40,00 to 43,00	50
23	Benzyl salicylate	**91**, 228, 65	43,00 to 45,00	50
24	4,4'-Dibromobiphenyl	**312**, 310, 314	45,00 to 51,00	50
25	Benzyl cinnamate	**131**, 192, 193	51,00 to the end	50

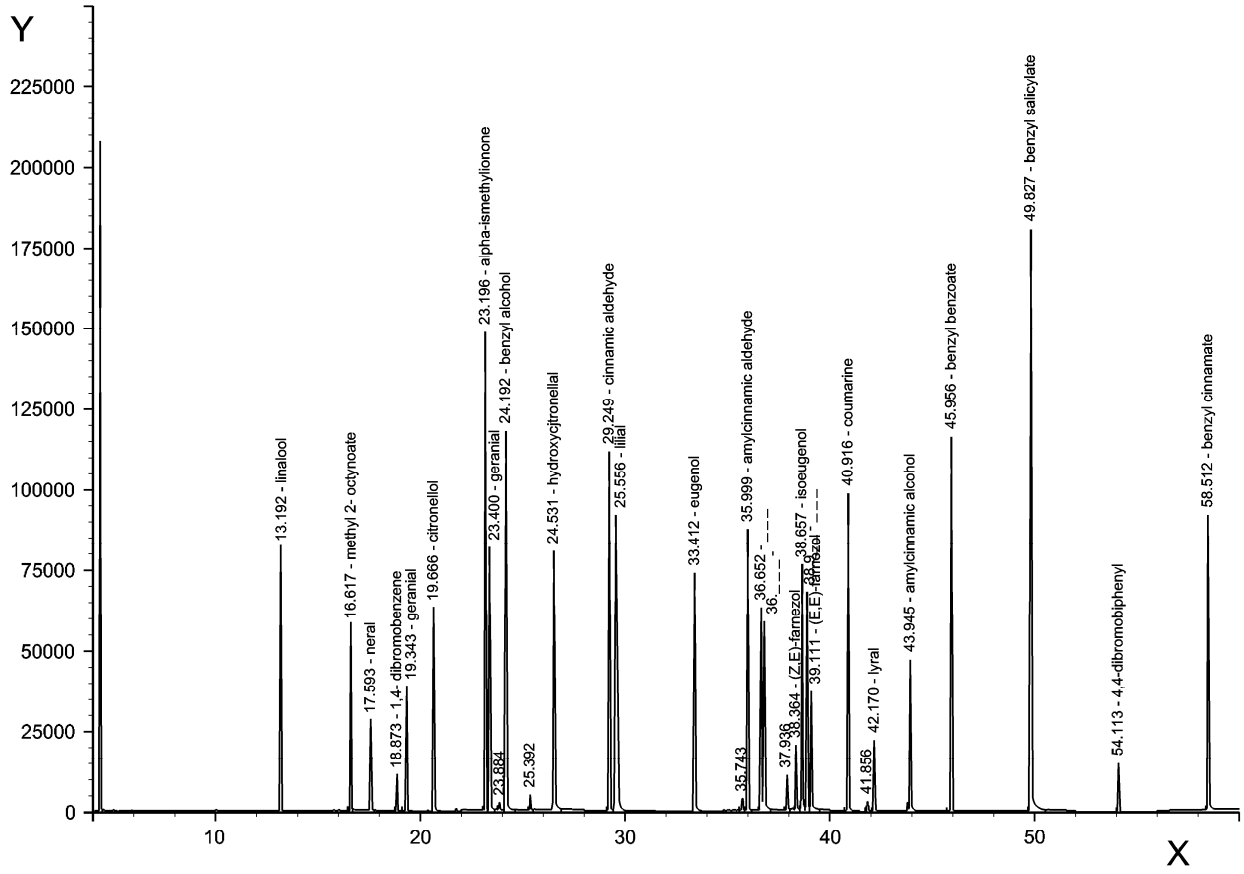
Annex C
(informative)

Example of chromatographic separation



Experimental conditions: Standard solution (100 mg/l) in SIM mode:
Column: length: 30 m; inner diameter: 0,25 mm
Stationary phase: (50 % phenyl)-methylpolysiloxane (DB 17-MS like)
Thickness: 0,25 µm
Temperature program: 65 °C for 1 min, then 3 °C/min to 230 °C, then 10 °C/min to 260 °C
Carrier gas: He
Flow: constant at 1,3 ml/min
Injection: split mode 1/100 for 1 µl injected
Injector temperature: 250 °C
Acquisition parameters: see Annex B

Figure C.1 — Example of chromatographic separation on mid-polar phase



Experimental conditions: Standard solution (100 mg/l) in SIM mode:
Column: length: 30 m ; inner diameter : 0,25 mm
Stationary phase: 100 % Polyethylenglycol (VF-Waxms)
Thickness: 0,25 µm
Temperature program: 70 °C then 3 °C/min to 250 °C
Carrier gas: He
Flow: constant at 1 ml/min
Injection: split mode 1/100 for 1µl injected
Injector temperature: 250 °C

Figure C.2 — Example of chromatographic separation on polar phase

Annex D
(normative)

Decisional tree

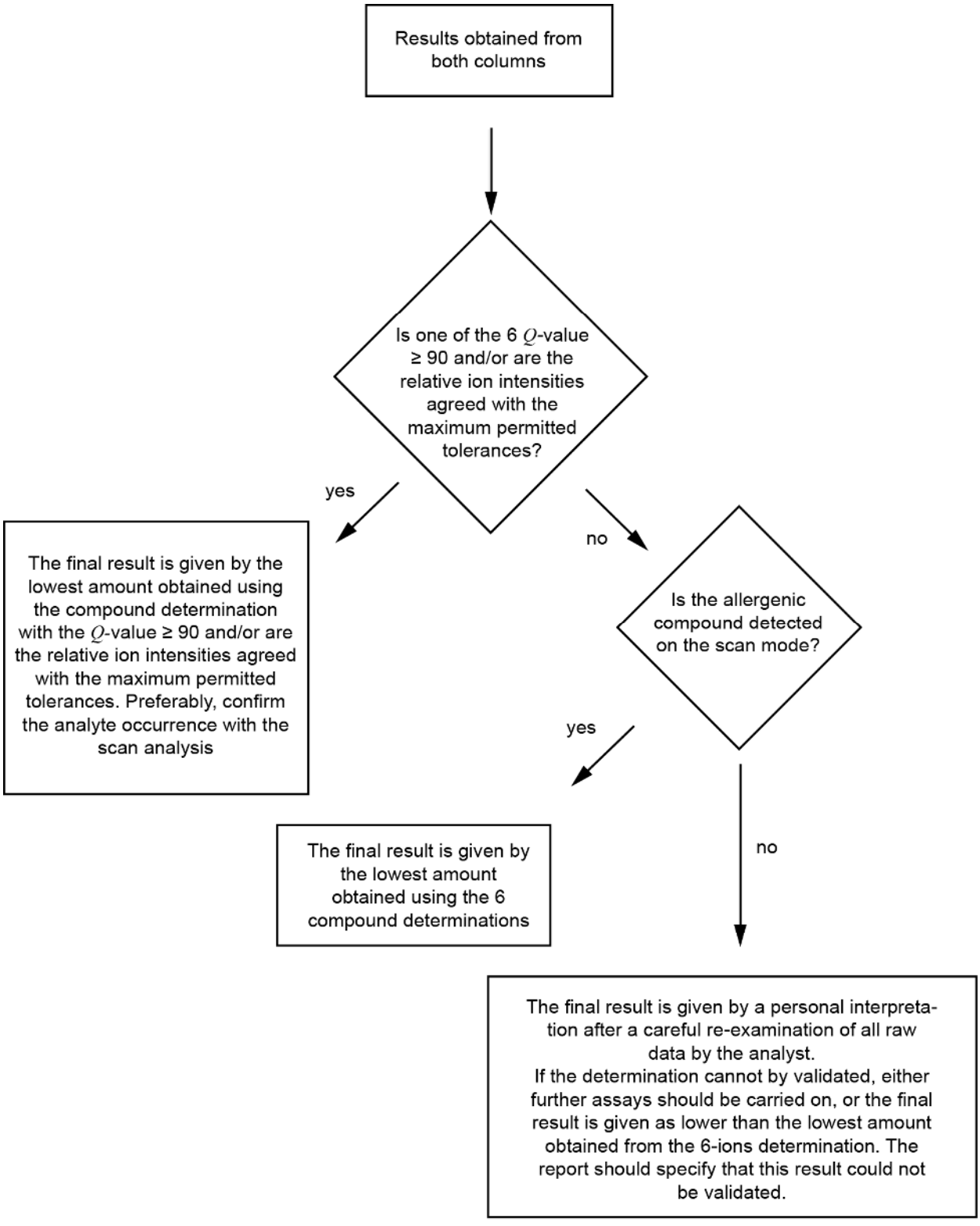


Figure D.1

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