SLOVENSKI STANDARD
SIST EN 16274:2012

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Analizne metode za alergene - Kvantitativno določevanje domnevnih dišavnih alergenov v izdelkih za potrošnike - Stopnja 1: Plinsko kromatografska analiza vzorcev, ki so pripravljeni za injeciranje

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

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

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

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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

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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of an identical text or by endorsement, at the latest by March 2013, and conflicting national standards shall be withdrawn at the latest by March 2013.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.