



NSAI
Standards

Irish Standard
I.S. EN 16274:2021

Method for analysis of allergens -
Quantification of an extended list of 57
suspected allergens in ready to inject
fragrance materials by gas
chromatography mass spectrometry

I.S. EN 16274:2021

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National Foreword

I.S. EN 16274:2021 is the adopted Irish version of the European Document EN 16274:2021, Method for analysis of allergens - Quantification of an extended list of 57 suspected allergens in ready to inject fragrance materials by gas chromatography mass spectrometry

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EUROPEAN STANDARD

EN 16274

NORME EUROPÉENNE

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Supersedes EN 16274:2012

English Version

**Method for analysis of allergens - Quantification of an
extended list of 57 suspected allergens in ready to inject
fragrance materials by gas chromatography mass
spectrometry**

Méthode d'analyse des allergènes - Quantification de la
liste étendue des 57 allergènes suspectés dans les
matières premières de parfumerie et les compositions
parfumantes prêtes à être injectées, par
chromatographie en phase gazeuse/spectrométrie de
masse

Analyseverfahren für Allergene - Quantifizierung einer
erweiterten Liste von 57 zu vermutenden Allergenen
in einspritzfertigen Duftstoffen mittels
Gaschromatographie/Massenspektrometrie

This European Standard was approved by CEN on 8 February 2021.

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Contents	Page
European foreword	4
Introduction	5
1 Scope.....	6
2 Normative references.....	6
3 Terms and definitions	6
4 Principle	6
5 Reagents.....	7
5.1 Solvents	7
5.1.1 Methyl pivalate	7
5.1.2 Tert-Butyl Methyl Ether (MTBE)	7
5.2 Reference samples (suspected allergens)	7
5.2.1 General.....	7
6 Apparatus	11
6.1 Gas chromatograph equipped with flame ionization detector (GC-FID)	11
6.2 Gas chromatograph coupled to a mass spectrometer (GC-MS).....	11
6.2.1 General.....	11
6.2.2 GC-MS system.....	11
6.3 Capillary columns for GC	11
6.4 Analytical balance.....	12
7 Stock and sample solutions - preparation and storage.....	12
7.1 General information	12
7.1.1 Choice of the solvent.....	12
7.1.2 Miscellaneous.....	13
7.2 Preparation of stock solutions from reference samples	13
7.3 Preparation of internal standard solutions and calibration solutions	13
7.3.1 General.....	13
7.3.2 Stock Solutions of Internal Standards (1g/kg equivalent)	13
7.3.3 Calibration Solutions Concentrations	13
7.3.4 Preparation of Calibration solutions	14
8 Mass spectrometer acquisition conditions.....	16
8.1 Establishment of retention times and SIM chromatograph windows	16
8.2 SIM Window – Set up and criteria	17
8.3 GC-MS scan mode verification	17
9 Sample analysis	17
9.1 General.....	17
9.2 Sample preparation for analysis	17
9.3 Sequence	18
9.3.1 Blank.....	18
9.3.2 Calibration solutions	18
9.3.3 Check solution.....	18
9.3.4 Samples.....	19
10 Data validation and treatment.....	19
10.1 General.....	19
10.2 Examination of Q values	19
10.3 Relative intensity of characteristic ions in SIM.....	20

10.4	Relative ion intensity using scan data.....	20
10.5	Data verification scheme and reporting of final concentration	21
10.6	GC-MS scan mode verification.....	21
10.7	Mass spectrum evaluation in SCAN mode.....	21
11	Test report	21
	Annex A (informative) Determination of calibrant and reference sample purity	22
	Annex B (informative) GC capillary column parameters	29
	Annex C (informative) SIM Ions for SIM or SIM-SCAN Use.....	30
	Annex D (informative) Example of SIM windows.....	37
	Annex E (informative) Example of chromatograms.....	46
	Annex F (normative) Decisional tree for quantification of suspected allergens.....	48
	Annex G (informative) Preparation of stock solutions from reference samples.....	49
	Annex H (informative) Calibration methods and approach.....	54
	Annex I (informative) Quantification of allergens.....	56
	Annex J (informative) Suspected allergens selected as analytes targets – Rationale.....	59
	Annex K (informative) Other general information.....	69
	Bibliography	70

EN 16274:2021 (E)

European foreword

This document (EN 16274:2021) has been prepared by Technical Committee CEN/TC 347 “Methods for analysis of allergens”, the secretariat of which is held by SNV.

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 November 2021, and conflicting national standards shall be withdrawn at the latest by November 2021.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN 16274:2012.

The most significant technical changes made in comparison to the previous version are as follows:

- Number of allergens has been extended from 26 to 57 chemically defined molecules.
- Solvents: moves from methyl pivalate and ortho fluorotoluene and acetone to methyl pivalate and MTBE and other solvents provided they are tested prior being used.
- Sample preparation: allows to use weight/volume approach rather than only weight/weight.
- Data processing: provides explanations for a calibration curve using a forced through zero approach.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association, and supports essential requirements of EU Directive(s).

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Introduction

Directive 2003/15/EC amending Council Directive 76/768/EEC, relating to cosmetic products, regulates the obligation to inform consumers of the presence of 24 chemically-defined fragrance substances identified as potential allergens in cosmetic products. Following the publication of the Scientific Committee on Consumer Safety's document (SCCS/1459/11), it was proposed to extend that to 57 fragrance substances, some of them existing under several isomeric forms or as mixtures. This required the development of a new quantification method in response to the evolution of regulatory requirements.

The new analytical method has been developed using gas chromatography and mass spectrometry (GC-MS), to detect and to quantify the 57 fragrance substances and their relevant isomers in ready to inject fragrance compounds and fragrance raw materials.

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

The present document describes a working analytical method based on IFRA Analytical Working Group developments. The analytical method was validated based on a ring test performed by the CEN working group using the accuracy profile approach.

EN 16274:2021 (E)**1 Scope**

The present method permits the identification and quantification of the volatile compounds suspected as allergens, which are present in the fragrance compounds and fragrance raw materials used in cosmetic products. The analysis is performed by gas chromatography and mass spectrometry (GC-MS) on matrix samples which are “ready to be injected” and which are compatible with gas chromatography.

The analytes covered by this procedure are based on the contents of Tables 13.1 and 13.2 in the SCCS 1459/11 opinion document (1) and as listed in the legislation proposed by the European Commission. The rationale behind the final choice of procedure analytes is given in the table found in Annex J.

The method was validated at IFRA and CEN level.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at <http://www.electropedia.org/>
- ISO Online browsing platform: available at <https://www.iso.org/obp>

4 Principle

This procedure has a calibration range between 2 ppm and 240 ppm, this permits the quantification of suspected allergens in diluted matrices in the range 20 ppm to 2 400 ppm per analyte. Beyond the upper concentration level the recommendation is that the sample should be diluted further, or that GC-FID (GC with Flame Ionization Detection) is used preferably in combination with internal standard and response factors.

The matrix samples are analysed for suspected allergens by GC-MS in a total of 4 runs, using 2 analyte sets, both injected on two separate columns of differing polarity. Where necessary the matrix samples should first be diluted in an appropriate solvent.

Their identification and quantification are achieved by selected ion monitoring (SIM; SIM-SCAN) mode via the relative abundance of 3 characteristic fragment ions. The calculation and use of the corresponding Q value or similar data evaluation factor can be applied and a ‘Decisional Tree’ (see Annex F, Figure F.1) for the final inspection and validation of the data by a trained and experienced analyst is described. An additional full-SCAN analysis is recommended to confirm the presence of the allergen in matrix samples if only SIM methodology was used.

Their quantification is achieved in all modes by calibration using standard solutions and the internal standards 1,4-dibromobenzene and 4,4'-dibromobiphenyl. The ‘Decisional Tree’ is employed to determine the final concentration taking into account the different concentration values obtained from analysis on both columns.

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