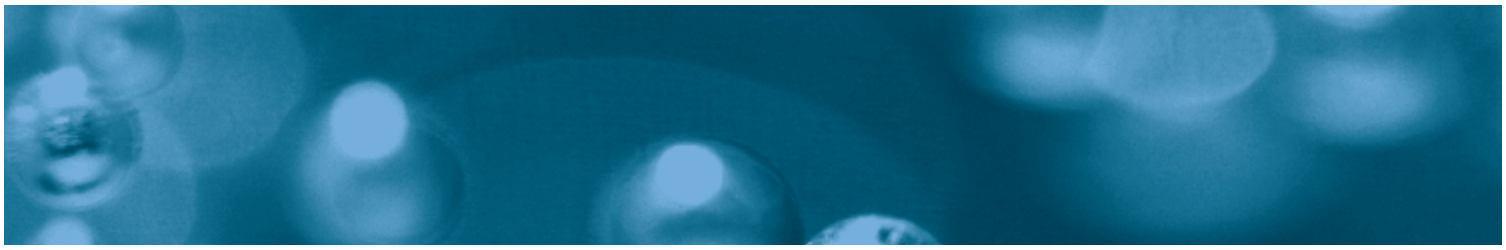
COLIPA GUIDELINES

TECHNICAL GUIDANCE DOCUMENT FOR THE DETERMINATION OF FRAGRANCE MATERIALS IN COSMETIC PRODUCTS

December, 2006



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23 December 2006

1. INTRODUCTION

The 7th Amendment to the EU Cosmetics Directive (Directive 2003/15/EC of 27.02.03) introduced a new legal requirement related to the labelling of 26 specific ingredients (listed in Appendix 2 to this guidance document) if present in a cosmetic product above the following concentration thresholds: 0.001% (10 mg/kg) for leave-on products and 0.01% (100 mg/kg) for rinse-off products.

The labelling requirement is linked to the presence of the substance in concentrations higher than the above-mentioned thresholds, irrespective of the substanceôs function and irrespective of its source (i.e. whether added as such or as component of a complex cosmetic ingredient such as botanical extracts, essential oils, fragrance compositions, aroma composition etc.).

For cosmetic products consisting of different components that are mixed immediately prior to application, these thresholds refer to the concentration of the substances in the final mix, as applied to the body.

Compliance with the labelling requirement needs to be based on the information received from the suppliers of the ingredients concerned. For further details, please see the Colipa/EFFA guidelines on the exchange of information between fragrance suppliers and cosmetic manufacturers, 2003.

2. OBJECTIVES

This guidance document is addressed to the experienced chemical analyst. Its objectives are to:

- specify the different physico-chemical and technical factors that allow for a correct identification and an accurate quantification of target fragrance compounds, in various types of cosmetic products,

- provide guidance to cosmetic manufacturers and control authorities regarding the analysis of these target fragrance compounds in cosmetic products.

3. SCOPE

The fragrance compounds that are subject to this guidance document are those listed in the 7th Amendment to the Cosmetics Directive (see Appendix 2). The two natural extracts (tree-moss and oak-moss) are not yet covered by this document, in consistency with the learning of Lepoittevin et al. (J. Sep. Sci. 2004, 27, 537-540).

Given the huge variety of cosmetic finished products ï in terms of composition and physico-chemical properties - available on the market, it is not possible to provide one common analytical approach for all cosmetic products.

4. GUIDANCE REGARDING THE ANALYTICAL APPROACH

Generally, and for fragranced products in particular, several factors need to be taken into account, such as the appropriate storage and shipment conditions (e.g. temperatures, exposure to light, exposure to irradiation, storage time), in order to avoid evaporation, degradation, segregation, and thus to ensure samplesôintegrity and representativeness.

Independent of the analytical approach applied ï as recommended in this document or developed in-house - the validation criteria described under 4.4.3 should be met and should be provided along with the analysis results.

4.1 ï Sample Preparation

4.1.1 Recommended approaches

- For perfumes, solvent dilution followed by direct injection is suitable. In case of atomizers (e.g. spray-bottles with or without gas propellant), the product must be sprayed prior to dilution.
- For most other cosmetic products (skin care, oral care, shower & bath, hair care, make-up), an extraction procedure, such as solvent extraction, sonication, centrifugation, is required. This procedure may consist of a first step (aqueous dilution, pH trimming, salting out) before the actual solvent extraction (e.g. with methoxylates, ethoxylates, ethers, dichloromethane, etc.).

4.1.2 Other possible approaches

- Specific instrumentation is available for automated fragrance isolation, for instance, Accelerated Solvent Extraction (ASE) equipment, Automatic Liner Exchange, micro-waves, prior to Gas Chromatography (GC) analysis. In any case, these approaches must meet validation criteria described under 4.4.3 which need to be provided along with the reported data.

4.2 ï Analysis

4.2.1 Recommended techniques

The target fragrance compounds in the sample extracts, as obtained according to 4.1.1, are separated by Capillary Gas Chromatography (CGC), and detected, identified and quantified preferably by quadrupole Mass Spectrometry (MS). For this purpose, **MS acquisition in full scan mode is strongly recommended**. Full scan MS in EI+ mode at 70eV is industry standard and is compatible with mass spectral databases such as the NIST/EPA/NIH mass spectral library.
Acceptable mass analyzers could also include single quadrupole, magnetic sector (HRMS) and triple quadrupole (for precursor - product ion confirmation of peak identity).

Selection of capillary columns must take into account a balance between analysis speed and efficiency in order to enable reliable quantification.
For checking chromatographic performance, a minimal resolution (R) of Ó1 and optimally Ó1.5 must be achieved with the chromatographic configuration (cfr. below equation ratio of retention time (tR) difference and actual peak widths (W) at 50% height for neighboring peaks A and B).

$$R = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B}$$

As a general guideline, typical columns should be:

- at least 20 m long
- with an internal diameter between 0.18 and 0.25 mm
- a film thickness between 0.10 and 0.30 µm
- with an apolar to medium-polar stationary phase (very polar phase not recommended due to over-time instability and bleeding phenomena).
- with at least 3000 ï 4000 plates / m for column efficiency.

For direct analysis (perfumes) or after solvent extraction, pulsed/splitless mode of injection is recommended in order to improve the sensitivity, especially for cases where very low amounts have to be detected.

Electron Impact (EI at 70 eV) Mass Spectrometry with a full scan range of 30-350 amu (atomic mass units) is recommended.

4.2.2 Other possible techniques

In line with the alternative sample preparation methods (under 4.1.2), e.g. direct thermal desorption of finished products is also a possibility, however, any alternative approaches must be validated (under 4.4.3.).

4.3 - Positive Identification Criteria in MS

For each of the target fragrance compounds, a selection of minimum 3 specific ions (at least one marker and two qualifier ions) with typically known response ratio on MS, is required, this in addition to forward and reverse match of obtained mass spectrum from sample with a reference library spectrum (e.g. NIST 05).

INGREDIENTS*	ISOMERS	IONS (proposals)
Limonene		67-**68**-**93**-136
Linalool		**71**-**93**-121-136
Benzyl alcohol		**79**-107-**108**
Citronellol		**69**-81-**95**-123-156
Methyl 2-octynoate		67-79-**95**-123
Geraniol		**69**-93-123-154
Citral		**69**-94-109-119
Citral		**69**-84-94-137
Hydroxycitronellal		43-**59**-71-96-139
Cinnamal		51-77-103-**131**-132
Anisic alcohol		109-137-**138**
Cinnamyl alcohol		**92**-105-115-**134**
Eugenol		103-131-149-**164**
Alpha-isomethyl ionone		107-**135**-**150**-**206**
Isoeugenol		131-149-**164**
Butylphenyl methylpropional		147-**189**-204
Coumarin		63-89-90-**118**-**146**
Amyl cinnamal		**129**-145-201-**202**
Farnesol	isomers	**69**-81-93-136
Hydroxyisohexyl-3-cyclohexene carboxaldehyde	Isomer 2	**105**-107-118-**192**
Hydroxyisohexyl-3-cyclohexene carboxaldehyde	Isomer 1	93-**136**-149-**192**
Amylcinnamyc alcohol		91-115-**133**-204
Hexyl cinnamal		**129**-145-215-**216**
benzyl benzoate		91-**105**-194-212
benzyl salicylate		65-**91**-228
benzyl cinnamate		**91**-**131**-192-193

* See the Appendix for the CAS numbers, EINECS numbers and INCI names.

Only main isomers are listed in this table. In case different isomers exist or occur, their contribution need to be taken into account for quantification (both standard and sample analysis).

Ions underlined in bold-face are typically selected (and recommended) for quantification. Other specific ions can be used for quantification and in peak purity test (e.g. in case of chromatographic co-elution).

The Council Directive 96/23/EC is recommended in order to ensure high quality of obtained mass spectral quality, taking into account acceptable tolerances on specific ions abundances as listed in table 4 below (ex. CD 96/23/EC):

Table 4

Maximum permitted tolerances for relative ion intensities using a range of mass spectrometric techniques

Relative intensity (% of base peak)	EI-GC-MS (relative)	CI-GC-MS, GC-MS^n LC-MS, LC-MS^n (relative)
> 50 %	± 10 %	± 20 %
> 20 % to 50 %	± 15 %	± 25 %
> 10 % to 20 %	± 20 %	± 30 %
≤ 10 %	± 50 %	± 50 %

4.4 - Quantification

4.4.1 Principle

Both ISTD and ESTD (provided a verified linear response for split injection) are possible standard calibrations. Typically, calibration curves with concentrations of target fragrance compounds are ranging from 0-200 mg/kg. In case of linearity problems, concentration ranges may be tailored towards actually measured concentrations or chromatographic conditions may be altered accordingly (e.g. change polarity of stationary phase). The fragrance calibration mixtures must be prepared in a solvent system similar to the one used for the fragrance extraction from the product.

4.4.2 Basic requirements

Since extraction efficiency is always matrix-dependent, recovery checks should be carried out for all tested matrices. Our recommended approach is standard addition of all fragrance compounds in the product matrix in order to verify recovery ratios for the individual fragrances and thus allow for accurate quantification.

In addition, the extraction procedure must also be checked by addition of a check standard (for example 1,4-dibromobenzene and 4,4ôdibromobiphenyl) and by determination of its recovery, in order to correct for the extraction (and/or final) volume and to account for possible organic solventsô interaction from matrix. This correction should be taken into account for the calculations of the target fragrances levels.
When applying internal standard calibration method, both 1,4-dibromobenzene and 4,4ô dibromobiphenyl may be considered as suitable internal standards, which would subsequently require addition of another standard for checking the extraction procedure (as mentioned earlier).

4.4.3 Validation criteria

- Provide correlation coefficient (typically minimum 0.99 for the 0-200 mg/kg range) or demonstrate acceptable linearity in the reported concentration range

- Checks with independent fragrance compound standards at known concentrations (preferably at 10 and 100 mg/kg level) should be run in order to ensure the validity of the applied calibration curve

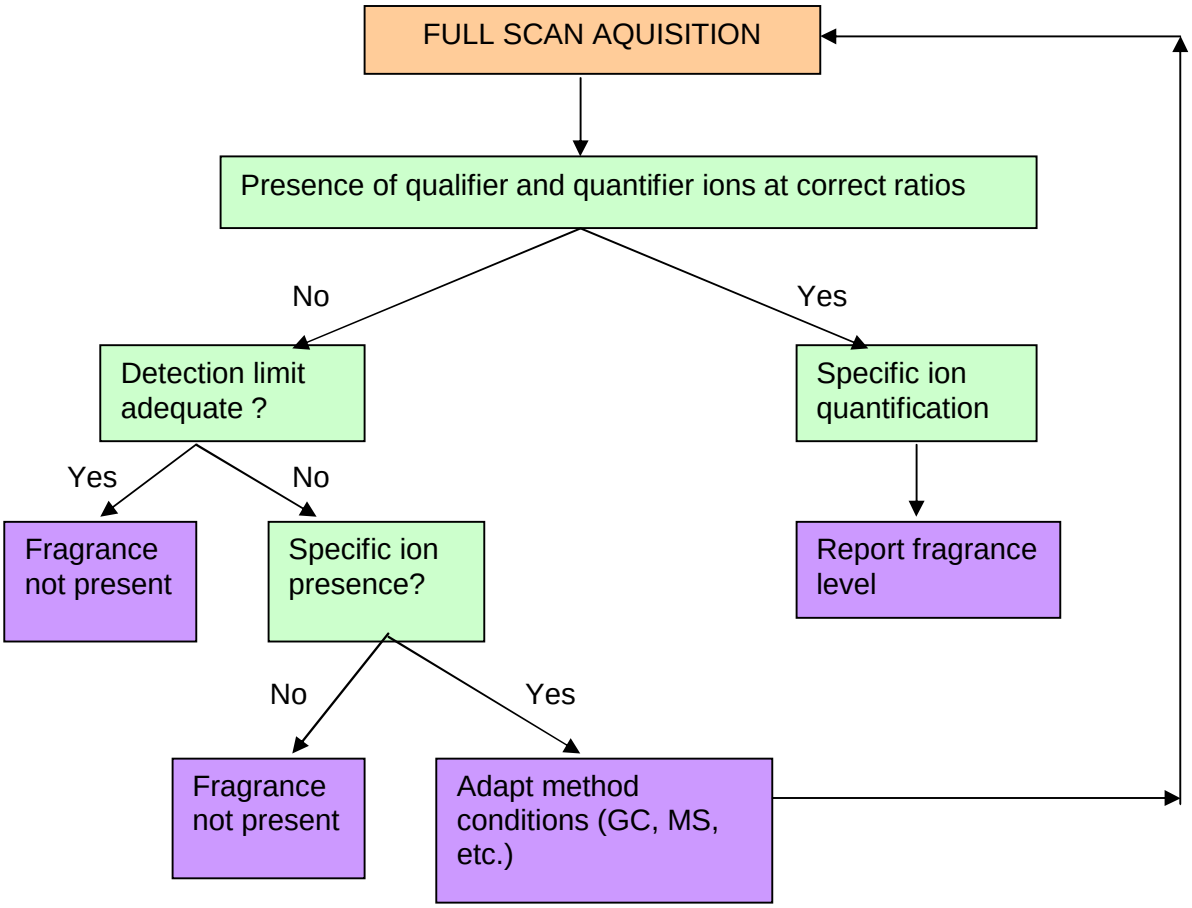
- The accuracy must be determined by standard addition of the target fragrance compounds in a known product matrix.

- Relative standard deviation: typically $\leq$ 10%. E.g., for replicate analyses of the target fragrance compound spiked into the product matrix at 10 mg/kg, the repeatability should not exceed 10% (cfr. also ISO 17025)

- Calculations of concentrations are made by using the verified external or internal calibration curve, followed by a correction for extraction efficiency (for example, final extraction volume, matrix adsorption, etc.).

- Regardless of the applied method, all target fragrance compounds should be quantifiable down to 1-5 mg/kg level, by this meeting the requirements of the Cosmetics Directive (76/768/EEC). This would typically require a signal/noise ratio of >10 for the specific masses of the target fragrance compound, when spiked at 5 mg/kg level in the product matrix.

4.4.4 General approach for interpreting data (with full scan data as a basis)



4.4.5 Specific considerations (ñwatch-outsò)

- Ion selection for quantification: refer to table under chapter 4.3 (the underlined ions are proposed for quantification).

- In cases where concentrations of target fragrance compounds exceed the typical calibration range by at least 20%, it is recommended to dilute the extracts to an appropriate level

4.4.6 Standards / solvents

ï Standards for target fragrance compounds:
Quality, purity (verified by GC or GC-MS), origin, stability data (storage conditions and shelf-life) should be provided for each of the used fragrance standards.

ï Check standards (for ISTD and/or ESTD calibration purpose):
Same as IFRA procedure, for example at 50 ppm (1,4-dibromobenzene and 4,4ô-dibromobiphenyl)
Others (based on own experience). Purity to be verified by GC or GC-MS)

ï Solvents for dilutions
Same as IFRA procedure (o-fluorotoluene or iso-octane)
Others (based on own experience).

ï Solvents for extractions
Water, tert-butylmethyl ether, ethanol, methanol, dichloromethane, etc. (based on own experience)
Quality, purity, origin should be provided for each of the used solvents.

4.5 ï Reporting

The information in the report is preferably consistent with ISO 17025 but should, as a minimum, contain:

- validation data (according to the criteria set)
- names and CAS numbers of the target fragrance compounds
- concentrations of the target fragrance compounds
- product matrix and production date
- summary of the applied method
- unique and unambiguous identification of the test product
- product receipt date
- analysis date
- name/address of the laboratory that conducted the tests

5. GENERAL CONSIDERATIONS

Given the high complexity of the matrices analysed, even with optimized methods and suitable instrumentation, some false positives and negatives for target fragrance compounds cannot be fully excluded. This phenomenon can be solved to a certain extent by either:

- changing the quantifier ion(s)
- using enhanced deconvolution software
- optimizing/changing the chromatographic conditions such as column selection or oven programming
- considering different sample preparation steps (change extraction and pre-treatment parameters ï see chapter 4.1.1)

On the condition that

- GC-MS analysis has proven that target fragrances show perfect peak purity for extracts from preset matrices, and
- samples contain high concentrations of target fragrances

GC-FID may be considered as an alternative approach given a preset composition of the sample matrix.

Finally, more selective chromatographic configurations (e.g. dual columns, coupled columns, GC x GC) and/or more selective or combined detection techniques (e.g. HR-MS, MS/MS and simultaneous SIM/Full-Scan MS) may be considered as alternative approaches.

It is the intention of the authors to closely follow new developments / trends in this field, and to update this guidance document as and when needed.

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This list has been compiled to the best of the authorsô knowledge; it may be not exhaustive and, therefore, it is subject to regular update.

APPENDIX 1

List of Abbreviations

ALE: Automatic Liner Exchange

AMU: Atomic mass units

ASE: Accelerated Solvent Extraction

CAS: Chemical Abstracts Service

CGC: Capillary Gas Chromatography

EI: Electron Impact

eV: electron Volt

EINECS: European inventory of existing commercial chemical substances

ESTD: External Standard Calibration

GC: Gas Chromatography

GC-MS: Gas Chromatography ï Mass Spectrometry

GC-FID: Gas Chromatography ï Flame Ionisation Detection

INCI: International Nomenclature Cosmetic Ingredients

ppm: parts per million

HR-MS: High Resolution Mass Spectrometry

APPENDIX 2

INCI NAMES FOR 26 SUBSTANCES ADDED TO ANNEX III OF THE COSMETICS DIRECTIVE

Annex III ref.	Directive Description	INCI Name	CAS N°	EINECS N°
67	Amyl Cinnamal	Amyl Cinnamal	122-40-7	204-541-5
68	Benzyl Alcohol	Benzyl Alcohol[1]	100-51-6	202-859-9
69	Cinnamyl Alcohol	Cinnamyl Alcohol[1]	104-54-1	203-212-3
70	Citral	Citral[1]	5392-40-5	226-394-6
71	Eugenol	Eugenol[1]	97-53-0	202-589-1
72	Hydroxy-citronellal	Hydroxycitronellal	107-75-5	203-518-7
73	Isoeugenol	Isoeugenol[1]	97-54-1	202-590-7
74	Amylcin-namyl Alcohol	Amylcinnamyl Alcohol	101-85-9	202-982-8
75	Benzyl Salicylate	Benzyl Salicylate[1]	118-58-1	204-262-9
76	Cinnamal	Cinnamal[1]	104-55-2	203-213-9
77	Coumarin	Coumarin[1]	91-64-5	202-086-7
78	Geraniol	Geraniol[1]	106-24-1	203-377-1
79	Hydroxy-methylpentylcyclohexenecarboxaldehyde	Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde	31906-04-4	250-863-4
80	Anisyl Alcohol	Anise Alcohol[1]	105-13-5	203-273-6
81	Benzyl Cinnamate	Benzyl Cinnamate[1]	103-41-3	203-109-3
82	Farnesol	Farnesol[1]	4602-84-0	225-004-1
83	2-(4-tert-butylbenzyl) Propionaldehyde	Butylphenyl Methylpropional	80-54-6	201-289-8
84	Linalool	Linalool[1]	78-70-6	201-134-4
85	Benzyl Benzoate	Benzyl Benzoate[1]	120-51-4	204-402-9
86	Citronellol	Citronellol[1]	106-22-9	203-375-0
87	Hexyl cinnam-aldehyde	Hexyl Cinnamal	101-86-0	202-983-3
88	d-Limonene[1, 2]	Limonene[1, 2]	5989-27-5	227-813-5
89	Methyl heptin carbonate	Methyl 2-Octynoate	111-12-6	203-836-6
90	3-Methyl-4-(2,6,6-tri-methyl-2-cyclohexen-1-yl)-3-buten-2-one	Alpha-Isomethyl Ionone [5]	127-51-5	204-846-3
91	Oak Moss extract	Evernia Prunastri [4]	90028-68-5	289-861-3
92	Treemoss extract	Evernia Furfuracea [4]	90028-67-4	289-860-8

Notes

1. *These ingredients are also found in some natural essential oils and extracts.*
2. *DL-Limonene is a mixture of the D and L isomers. If used in the cosmetic product, strictly speaking the relative proportions of the isomers would have to be worked out to determine whether the concentration requires d-*

Limonene to be labelled under its new INCI name ñLimoneneò In practice, because of the technical difficulty of the analysis, the total level of both isomers will be used to establish whether the threshold is exceeded and labelling is required.

3. *The Directive specifies the restrictions for each ingredient as:*

   The presence of the substance must be indicated in the list of ingredients referred to in Article 6(1)(g) when its concentration exceeds:

   - 0.001 % in leave-on products
   - 0.01 % in rinse-off products

   This applies if these ingredients are present in the product for any reason ï not just as constituents of fragrances.

4. *Evernia Prunastri: as listed in 1996 inventory, we expect this to change to Evernia Prunastri extract in a future update.*
   Evernia Furfuracea: for consistency we have used the format that would have appeared in the 1996 inventory. We expect this to change to Evernia Furfuracea extract in a future update.

5. *Alpha-Isomethyl Ionone is the name which appears in the current CTFA On-line listing of the INCI name for 3-Methyl-4-(2,6,6-tri-methyl-2-cyclohexen-1-yl)-3-buten-2-one. Previous hard copy listings omitted ñisoò from the name.*

COLIPA IS THE EUROPEAN TRADE ASSOCIATION REPRESENTING
THE INTERESTS OF THE COSMETIC, TOILETRY AND PERFUMERY INDUSTRY.

OUR VISION

The cosmetics, perfumery and personal care industry and its products significantly contribute to individual and social well-being in our everyday lives.

OUR MISSION

To help maintain and develop a sustainable, competitive and respected industry in Europe

- ➔ by demonstrating the inherent value of our industry
- ➔ by striving to create the most favourable economic and regulatory environment in which to operate
- ➔ and by advocating best practices, thereby ensuring that consumers benefit from continuously innovative and safe products.

OUR GOALS

Colipa, as THE recognised voice of the European cosmetics, perfumery and personal care industry, must:

Earn public trust

by fostering transparent and reliable relationships with public authorities and stakeholders, to best communicate the social and economic relevance of our industry in terms of satisfying consumer needs.

Achieve effective public policy

by actively contributing to the shaping of workable and fair policy frameworks regulating the industry. To this end, proactive and effective networking and communication are of the essence. Opportunities for achieving alignment on an international scale should be created and optimised.

Enhance member value

by addressing members' needs in an efficient and transparent way, through timely information and decision making processes and focusing on the issues and activities which are important to them.

Best use should be made of members' expertise and dedication to optimise both efficiency and one-voice positions.

COLIPA - The European Cosmetic, Toiletry and Perfumery Association

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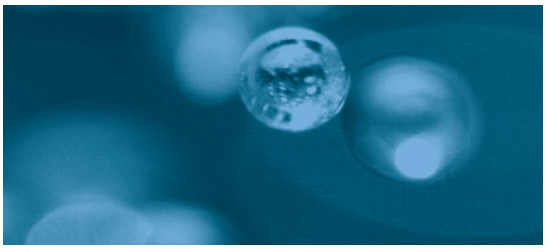

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