



Version 1
September 25, 2003

INTERNATIONAL FRAGRANCE ASSOCIATION

ANALYTICAL PROCEDURE

GC/MS Quantitation of potential fragrance allergens* in fragrance compounds

*as defined by the SCCNFP in its opinion 0450/01, final and reflected in the 7th Amendment to the European Cosmetics Directive as published in the Official Journal N° L 66 of the European Union on March 11, 2003.

The above-cited regulation requires indicating the presence of 26 fragrance ingredients in finished cosmetic products if exceeding a threshold of 0.01% for rinse-off and 0.001% for leave on products.

From the 26 materials selected by the SCCNFP two are natural extracts (oak moss and tree moss) so the method as specified below restricts itself on the determination of the 24 volatile chemicals.

This method is intended to provide a reliable analytical method that can be used as reference in all quality control labs in the fragrance manufacturers industry, the customer industry but also serve the needs of public analysts.

The method has been developed by the EFFA / IFRA Analytical Working Group and has been accepted for publication by the Journal of Agricultural and Food Chemistry (A. Chaintreau, D. Joulain, C. Marin, C-O. Schmidt, M. Vey, GC-MS Quantitation of Fragrance Compounds Suspected to Cause Skin Reaction. Part 1, J. Agric. Food Chem., accepted for publication).

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1. Scope of the method

With view to the applicability of the method for routine analysis the following decisions regarding the scope of the method have been taken. The names describing the materials are the INCI (International Nomenclature of Cosmetic Ingredients) names that will be used for indicating the fragrance ingredients on the finished product packaging if present above the agreed thresholds.

1. Amyl Cinnamal

CAS: 122-40-7

CAS name: Heptanal, 2-(phenylmethylene)-

Both the (E) and (Z) isomers. .

2. Benzyl Alcohol

CAS: 100-51-6

CAS name: Benzenemethanol

Only one substance is described by this nomenclature.

3. Cinnamyl Alcohol

CAS: 104-54-1

CAS name: 2-Propen-1-ol, 3-phenyl-

Both (E)- and (Z)- isomers are included in our analysis.

4. Citral

CAS: 5392-40-5

CAS name: 2,6-Octadienal, 3,7-dimethyl-

Both (Z)- (Neral: CAS 106-26-3) and (E)- (Geranial: CAS 141-27-5) isomers are included.

5. Hydroxycitronellal

CAS: 107-75-5

CAS name: Octanal, 7-hydroxy-3,7-dimethyl-

Both (R)- and (S)- enantiomers are included.

6. Eugenol

CAS: 97-53-0

CAS name: Phenol, 2-methoxy-4-(2-propenyl)-

Only one substance described by this nomenclature.

7. Isoeugenol

CAS: 97-54-1

CAS name: Phenol, 2-methoxy-4-(1-propenyl)-

Both (E)- and (Z)- isomers are included.



8. Amylcinnamyl Alcohol

CAS: 101-85-9

CAS name: 1-Heptanol, 2-(phenylmethylene)-

Both the (E) and (Z) isomers.

9. Benzyl Salicylate

CAS: 118-58-1

CAS name: Benzoic acid, 2-hydroxy-, phenylmethyl ester

Only one substance described by this nomenclature.

10. Cinnamal

CAS: 104-55-2

CAS name: 2-Propenal, 3-phenyl-

Both (E)- (trans- CAS 14371-10-9) and (Z)- (cis- CAS 57194-69-1) isomers are included.

11. Coumarin

CAS: 91-64-5

CAS name: 2H-1-Benzopyran-2-one

Only one substance described by this nomenclature.

12. Geraniol

CAS: 106-24-1

CAS name: 2,6-Octadien-1-ol, 3,7-dimethyl-, (E)-

Only one substance described by this nomenclature. The (Z)- isomer (Nerol: CAS 106-25-2) is not included.

13. Hydroxyisohexyl- 3-cyclohexene carboxaldehyde

CAS: 31906-04-4

CAS name: 3-Cyclohexene-1-carboxaldehyde, 4-(4-hydroxy-4-methylpentyl)-

Only this isomer is included in our analysis although some commercial qualities of this substance usually contain lesser quantities of the 3-Cyclohexene-1-carboxaldehyde, 3-(4-hydroxy-4-methylpentyl)- isomer.

14. Anise Alcohol

CAS: 105-13-5

CAS name: Benzenemethanol, 4-methoxy-

Only one substance described by this nomenclature.

15. Benzyl Cinnamate

CAS: 103-41-3

CAS name: 2-Propenoic acid, 3-phenyl-, phenylmethyl ester

Both (E)- and (Z)- cinnamate isomers are included.



16. Farnesol

CAS: 4602-84-0

CAS name: 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-

Undefined mixture of four isomers (predominantly the (E,E) ("trans, trans-Farnesol":

CAS 106-28-5) and (Z,E) ("trans, cis-Farnesol": CAS 3790-71-4) .

17. Butylphenyl Methylpropional

CAS: 80-54-6

CAS name: Benzenepropanal, 4-(1,1-dimethylethyl)-.alpha.-methyl-

Both (R)- and (S)- enantiomers are included in our analysis. The isomeric 2-(3-tert-Butylbenzyl) propionaldehyde is not included.

18. Linalool

CAS: 78-70-6

CAS name: 1,6-Octadien-3-ol, 3,7-dimethyl-

Both (R)- (CAS # 126-91-0) and (S)- (CAS # 126-90-9) enantiomers are included.

19. Benzyl Benzoate

CAS: 120-51-4

CAS name: Benzoic acid, phenylmethyl ester

Only one substance described by this nomenclature.

20. Citronellol

CAS: 106-22-9

CAS name: 6-Octen-1-ol, 3,7-dimethyl-

Both (R)- ((-): CAS 1117-61-9)and (S)- ((+): CAS 7540-51-4) enantiomers are included.

21. Hexyl Cinnamal

CAS: 101-86-0

CAS name: Octanal, 2-(phenylmethylene)-

Both the (E) and (Z) isomers. .

22. Limonene

CAS: 5989-27-5

CAS name: Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (R)-

Both (R)- and (S)- (CAS # 5989- 54-8) enantiomers may have to be taken into account . (See special note on LIMONENE).

23. Methyl 2-Octynoate

CAS: 111-12-6

CAS name: 2-Octynoic acid, methyl ester

Only one substance described by this nomenclature.



24. Alpha-Isomethyl Ionone

CAS: 127-51-5

CAS name: 3-Buten-2-one, 3-methyl-4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-

Both (E)- and (Z)- isomers of the specified structural isomer are included in the analysis. Even though other structural isomers are often present in commercial quantities of this substance, they are not included.

SPECIAL NOTE ON d-LIMONENE

Strictly speaking, only the (R)- enantiomer would need be taken into account. Users should however be aware that because chiral differentiation techniques (e.g. Chromatography with chiral columns) are unlikely to be used by verifying laboratories, the (R)- and (S)- enantiomers will not be routinely distinguishable. . For that reason, it is recommended to provide information about the total levels of both enantiomers. If specific analysis has been performed which allows quantitative distinction of each enantiomer, then in addition to the total concentration of both enantiomers, more detailed information may be given. .

2. Procedure

Materials

Allergen standards must have purity higher than 95%, except α -isomethylionone (88%) due to the absence of supplier for a higher quality.

Two internal standards (ISTD) are added in each fragrance: 1,4 dibromobenzene and 4,4'-dibromobiphenyl.

Solvent: *o*-fluorotoluene for lipophilic and hydrophilic fragrances.

Suppliers for Standard materials

Attached is a list of suppliers that delivered the material the procedure is referring too. This certainly is just indicative as you might use material of comparable purity from other sources.

Name / CAS Reg. N°	Supplier
Amylcinnamic alcohol [101-85-9]	Mane Fils (F)
Amylcinnamic aldehyde [122-40-7]	Whyte Chemicals (UK)
Anisyl alcohol [105-13-5]	Symrise (D)
Benzyl alcohol [100-51-6]	Whyte Chemicals (UK)
Benzyl benzoate [120-51-4]	Symrise (D)
Benzyl cinnamate [103-41-3]	Symrise (D)
Benzyl salicylate [118-58-1]	Quest Int'l (UK)

Cinnamic alcohol [104-54-1]	Noveon (USA)
Cinnamic aldehyde [104-55-2]	Noveon (USA)
Citral [5392-40-5]	BASF AG (D)
Citronellol [106-22-9]	Takasago (NL)
Coumarine [91-64-5]	Buckton Page Ltd (UK)
Estragole [140-67-0]	Bordas SA (SP)
Eugenol [97-53-0]	Indonesian Essential Oils
Farnesol [106-28-5]	S. Black Ltd (UK)
Geraniol [106-24-1]	IFF (UK)
Hexylcinnamic aldehyde [101-86-0]	IFF (UK)
Hydroxycitronellal [107-75-75]	BASF AG (D)
Limonene [5989-27-5]	R.C. Treatt Co. Ltd (UK)
Linalool [78-70-6]	Millenium Products (USA)
Hydroxyisohexyl-3-cyclohexene carboxaldehyde (HMPCC) [31906-04-4]	IFF (UK)
Isoeugenol [97-54-1]	Indonesian Essential Oils
Butylphenyl methylpropional (BMHCA) [80-54-6]	S. Black Ltd (UK)
Methyl 2-nonynoate [111-80-8]	S. Black Ltd (UK)
Methyl 2-octynoate [111-12-6]	S. Black Ltd (UK)
Methyleugenol [93-15-2]	Symrise (D)
Phenylacetaldehyde [122-78-1]	Symrise (D)
α -Isomethylionone [127-51-5]	IFF (USA)
1,4-Dibromobenzene	Aldrich (USA)
4,4'-Dibromobiphenyl	Aldrich (USA)

GC conditions

Only two columns have been selected by IFRA/EFFA (Table 1), the final choice is not yet decided. For both columns, injector and transfer line temperatures are 250 and 280°C, respectively. The split ratio was 1/100. The carrier gas is delivered under constant pressure.

Table 1. GC conditions

Column	Oven program	Time min	Initial gas velocity cm/sec	Co-elutions
DB1, 60m × 0.25mm × 0.25 μ m	100°-2min; 10°/min; 280°C	25	50	Amylcinnamic ald./1 st peak of HMPCC*
DB17, 20m × 0.18mm × 0.18 μ m	100°-2 min; 10°/min; 280°C	17	60	No co-elution

* The first peak of HMPCC (CAS 31906-04-4) is not the most abundant (# 28 %) and it is not used for its quantitation. Its ions do not interfere with ions of amylicinnamic

aldehyde.

MS conditions

When injection conditions have been modified (e.g. installation of a new column, long period without injection, etc.), first inject the mix of allergens plus the ISTD in scan mode to determine the SIM windows. After integration of the file, determine the start- and end-time of each allergen peak (example in Table 2). The SIM window of a given allergen may be chosen just before its integration start-time. In contrast, a delay is recommended for the end of the SIM window after the integration end-time, as co-elutions may delay the target peak.

The electron-multiplier voltage is operated 200 V above the autotune adjustment. Three ions must be used for each allergen: 1 for the quantitation, 2 for the identification (the "qualifiers"). Their values are given in Table 2.

The dwell time is normally 50 msec for each ion. In case of coelution or overlap of 2 peaks of interest (e.g. estragole and the first ISTD in Table 2), 6 ions must be monitored simultaneously and the dwell time is lowered to 20 msec.

Table 2. Example of a SIM table using a DB1 column (conditions in Table 1).

Compounds above and below the double line refer to 1,4-dibromobenzene and 4,4'-dibromobiphenyl, respectively.

Window	Name / CAS Reg.Number	Ions	Time window (min)	Dwell time (msec)	Comment
1	Benzyl alcohol [100-51-6]	108, 79, 107	3.5	50	
2	Phenylacetaldehyde *	91, 120, 92	4.73	50	
3	Limonene [5989-27-5]	68, 93, 67	4.89	50	
4	Linalool [78-70-6]	93, 71, 121	5.6	50	
5	Methyl 2-octynoate [111-12-6]	95, 123, 79	6.75	50	
6	Estragole *	148, 147, 117	6.92	20	No co-elution but risk of peak overlap
6	1,4-dibromobenzene [106-37-6]	236, 234, 238	6.92	20	
7	Citronellol [106-22-9]	95, 69, 81	4.40	50	
8	Citral (neral) [5392-40-5]	69, 94, 109	7.54	20	
9	Geraniol [106-24-1]	69, 123, 93	7.77	20	No co-elution but risk of peak overlap
9	Cinnamic aldehyde [104-55-2]	131, 132, 103	7.77	20	
10	Citral (geranial) [5392-40-5]	69, 84, 94	7.94	20	No co-elution but risk of peak overlap
10	Anisyl alcohol [105-13-5]	138, 137, 109	7.94	20	

11	Hydroxycitronellal [107-75-5]	59, 71, 43	8.08	50	
12	Methyl 2-nonynoate * [111-80-8]	79 , 137, 100	8.26	50	
13	Cinnamic alcohol [104-54-1]	92 , 134, 115	8.37	50	
14	Eugenol [97-53-0]	164 , 103, 149	6.2	50	
15	Methyleugenol * [93-15-2]	178 , 163, 147	9.75	50	
16	Coumarine [91-64-5]	146 , 118, 89	10.13	50	
17	Isoeugenol [97-54-1]	164 , 149, 131	10.48	50	
18	α -Isomethylionone [127-51-5]	206 , 135, 150	11.16	50	
19	BMHCA [80-54-6]	189 , 147, 204	11.6	50	
20	Amylcinnamic aldehyde [122-40-7]	202 , 201, 129	13.16	50	
21	HMPCC [31906-04-4]	192 , 136, 149	13.27	50	
22	Amylcinnamic alcohol [101-85-9]	133 , 115, 204	13.6	50	
23	Farnesol [106-28-5]	69 , 93, 81	14.10	50	
24	Hexylcinnamic aldehyde [101-86-0]	216 , 215, 129	14.38	50	
25	Benzyl benzoate [120-51-4]	105 , 212, 194	14.49	50	
26	Benzyl salicylate [118-58-1]	91 , 228, 65	15.72	50	
27	4,4'-Dibromobiphenyl [92-86-4]	312 , 310, 314	17.2	50	
28	Benzyl cinnamate [103-41-3]	131 , 192, 193	17.96	50	

* Not in the SCCNFP list (24 volatile allergens).

Calibration

A stock solution is prepared using the 28 above-mentioned allergens (10 g/L of each) in *o*-fluorotoluene. This solution, stored in a hermetically closed bottle, is stable for 1 month in the freezer (-18°C) if the vial is not open. Alternatively, two separate solutions: 1) aldehydes + ketones, 2) non-carbonyl compounds, are stable for 2 months at 4°C (without being opened in the meantime).

Calibration solutions are prepared by diluting in *o*-fluorotoluene both internal standards, and the stock solution down to allergen concentrations of 2, 5, 10, and 25 mg/L and an ISTD concentration of 50 mg/L. An aliquot (1 μ L) of these calibration solutions is injected with a 5 μ L syringe and an autosampler using the above-mentioned GC/MS conditions. The chromatogram is divided into two areas: allergens eluting close to the



first eluting ISTD are reported to this one, and the last eluting ones to the second ISTD. After peak area calculation for each allergen, either areas and corresponding allergen amounts are recorded using the MS software, or curves are drawn using Excel as:

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

When re-injecting standards to re-calibrate the instrument, area ratios between the 3 ions have to be up-dated.

Quantitation

CAUTION: Agilent recommends to perform an autotune of the MS at least once a week which alters calibration curves of allergens. Therefore calibrations must be run after each autotune, and - at least - once a week. Cleaning the injector weekly prior to the calibration is a useful precaution as low-volatile fragrance ingredient may accumulate in the insert.

The MS resolution must be "low". The electron multiplier is operated 200 V above the voltage resulting from the autotune.

Spike the fragrance with both ISTDs (50 mg/L), then dilute (100 g/L) this mixture in o-fluorotoluene (ACROS, purity > 99 %).

The aliquot of the fragrance solution, at the same dilution level as for the calibration solution, is injected (1 µL). After peak integration, the amount of allergens is either directly calculated by the MS software or from the Excel calibration curves (as for the calibration, use area ratios of each allergen to the closest eluting ISTD).

Resulting concentrations are only valid if they fall within the calibration range (calibration between 2 and 25 mg/L, corresponding to an initial range of 20-250 mg/L in the fragrance before its 10% dilution).

For each allergen detected in the fragrance, check its identity in the following way:

1. Agilent GC/MS software

- 1.1. Choice of quantitation parameters in the Agilent software

- 1.1.1. Quant.type: Target compound

- 1.1.2. Measure response by: Area

- 1.1.3. Ident. by: best RT match

- 1.1.4. Subtraction method: Low first and last

- 1.2. Peaks exhibiting a Q-value between 90 and 100 are considered to be positively identified if the "Peak purity" option does not detect any co-elution.

1.3. Peaks exhibiting a Q-value below 90 are only tentatively identified: they may be absent, or co-eluted with another compound. Verify their purity using the "Peak purity" option. Re-inject the sample in scan mode with the same GC program. Check the presence of the allergen. If it is present, verify whether the mass used for its quantitation is altered by the co-eluting peak:

No → the previous result of the SIM quantitation is valid

Yes → choose one of the 2 qualifier ions that is free of co-elution to quantify the allergen. Draw the calibration curve based on this ion, and determine the amount.

In practice it may be time-saving to systematically draw the 3 calibration curves for each allergen, using successively 1 of the 3 ions as the target one, and the 2 others as qualifiers. Then, 3 quantitation reports are easily generated after a fragrance analysis.

2. Excel

The calculation of the Q-value used in Agilent software may be achieved according to the following formula:

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

With: n : Number of ions per compound

r_i : Reference peak area ratio

r_i' : Observed peak area ratio

A Q-value between 90 and 100 indicates a positive recognition of the target peak.

Note: if the amount of (an) allergen(s) is higher than the upper limit of the calibration, determine a rough concentration by extrapolation, dilute the sample down to the calibration range and re-determine the concentrations.

3. Closing remark

The EFFA / IFRA Analytical working group is continuously working to further improve the method.