

Allergenic Fragrance Testing - New Certified Reference Materials and GC-FID/GC-MS Application

Calibration mixes for the new IFRA method now available

Markus Obkircher, Head of Reference Materials R&D, markus.obkircher@sial.com

Hanspeter Sprecher, R&D Senior Scientist, hanspeter.sprecher@sial.com

Leonard Sidisky, Gas Separations R&D Manager, len.sidisky@sial.com

Kathleen H. Kiefer, Principal Scientist, kathy.kiefer@sial.com

Matthias Nold, Global Product Manager Reference Materials, matthias.nold@sial.com



Last year, the International Fragrance Association (IFRA) published a new method for the testing of 57 potentially allergenic fragrance compounds in response to an opinion by the European Scientific Committee on Consumer Safety (SCCS), which also proposed to correspondingly amend EC regulation No. 1223/2009.

This new analytical method has been developed by the IFRA Analytical Working Group to identify and to quantify the 57 fragrance substances and their relevant isomers at a concentration higher than 0.002% (20 mg/kg) in ready to inject fragrance materials and oils using Gas Chromatography with FID (GC-FID) or with Mass Spectrometry (GC-MS) detection. This new method will allow the screening of complex fragrance mixtures for the presence of these chemically defined allergens.

In this article we will discuss the introduction of new certified reference materials and their analysis by GC using an ionic liquid column as an alternative with unique selectivity and stability as compared to traditional wax/PEG phases.

New Certified Reference Material (CRM) Mixtures

We are proud to introduce two ready-to-use certified reference material mixtures suitable for use as calibrants for the above mentioned new IFRA method. The individual substances of these two mixes consist of a range of compound classes (predominantly of alcohol and carbonyl components), which were all individually thoroughly characterized and their content determined by quantitative nuclear magnetic resonance (qNMR) spectroscopy. qNMR is a relative primary method, as the signal intensity is proportional to the number of protons

contributing to the resonance. It is therefore possible to directly compare the signal intensities of an analyte via an internal reference standard to a NIST SRM being a different compound. The individual qNMR certifications were all performed following the well-established qualification workflow developed in Buchs, Switzerland, according to the ISO/IEC 17025 accreditation.

This workflow typically starts with a series of preliminary investigations such as hygroscopy and volatility of the substances. Further determinations ensure the compatibility of each analyte with the internal standard used for the qNMR measurements.

Since reliable weighing values are mandatory, having a direct influence on the result, they are performed in a metrological way. Using a micro-balance in a specific, dedicated setup is the key to success because less sensitive equipment will lead to higher uncertainty contributions deriving from the weighing procedure. This is of the utmost importance for these components as they often have relatively low boiling points. In the qNMR measurements, a value for the mass fractions (g/g) was obtained and direct traceability to the SI unit was achieved through the use of acknowledged NIST primary reference materials. In addition, early insight into the homogeneity and stability of the components could be gained. If signals of interest of different isomers could not be completely separated in NMR, the ratio was determined by Gas Chromatography (GC) measurement under ISO/IEC 17025 accreditation.

The raw materials were then gravimetrically dissolved and diluted in methyl tert-butyl ether following the ISO 17034 accreditation workflow. Amber glass ampoules were then filled with the resulting bulk solutions and the process subsequently controlled by GC-MS homogeneity testing.

The final ampoules were thoroughly investigated to guarantee stability throughout the entire shelf life of the product at the storage temperature, as well as during transport to the customer at a potentially higher temperature. In order to be fully compliant with ISO 17034, Long-Term Stability studies (LTS) as well as Accelerated Stability Tests (AST) were performed by GC-MS.

The results from stability testing, from the conducted homogeneity experiments and from the qNMR measurements themselves all contribute to the overall uncertainty budget that is included individually for each component in the certificate. In addition to the certified

(continued on next page)

values of the concentrations and their respective overall expanded uncertainties in g/kg and g/L (through a certified density measurement at 20 °C), the certificate contains the lot number, an expiry date, intended use, accreditation stamps and signatures of the producing and releasing chemists.

To guarantee a suitable solution for the customer, the mixtures were intensively tested in a series of interlaboratory comparison studies before the launch.

Table 1 shows product number and composition of the two mixes. These products and example certificates can be found on our website at [SigmaAldrich.com/fragrancestandards](https://www.sigmaaldrich.com/fragrancestandards).

Table 1. Fragrance Allergen Mixes A1 and A2 with Composition. The nominal concentration for most components is 2000 mg/kg (exceptions are marked with *). The lot specific certified values are given in the certificate provided with the products. For both mixes, 2 mL and 5 mL packages are available (amber glass ampoules).

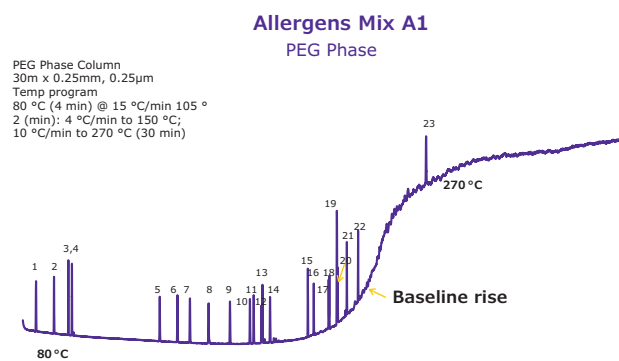
Fragrance Allergen Mix A1	Fragrance Allergen Mix A2
Cat. No.: 89131	Cat. No.: 16558
Qty.: 1 mL, 5 mL	Qty.: 1 mL, 5 mL
Solvent: Methyl-tert-butyl ether (MTBE)	Solvent: Methyl-tert-butyl ether (MTBE)
α -Amylcinnamyl alcohol	3-Propylidene phthalide
trans-Anethole	α -Acetyl Cedrene
Anise alcohol	Isoeugenyl Acetate
Benzyl alcohol	α -Amylcinnamaldehyde
β -Caryophyllene	Amyl salicylate
Cinnamyl alcohol	Benzaldehyde
Citronellol	Benzyl benzoate
Ebanol 1	Benzyl cinnamate
Ebanol 2	Benzyl salicylate
Eugenol	Butylphenyl methylpropional
trans,trans-Farnesol	Camphor
Geraniol	Carvone
Isoeugenol (E+Z)	Cinnamaldehyde
R(+)-Limonene	Neral
Linalool	Geranial
Menthol	Coumarin
α -Pinene	β -Damasconone (Rose Ketone-4)
β -Pinene	α -Damascone
α -Santalol	β -Damascone "E"
β -Santalol	δ -Damascone (Rose Ketone-3)
Sclareol	Dimethylbenzylcarbonyl acetate (DMBCA)
α -Terpinene	Eugenyl acetate
Terpineol, mainly α	Galaxolide 1
Trimethyl-benzenepropanol	Galaxolide 2
	Geranyl acetate
	Hexadecanolactone / Dihydroambrettolide
	α -Hexylcinnamaldehyde
	Hydroxycitronellal
	Hydroxyisohexyl 3-cyclohexene carboxaldehyde - major
	Hydroxyisohexyl 3-cyclohexene carboxaldehyde - minor*
	α -Isomethylionone
	Linalyl acetate
	Methyl salicylate
	Methyl-2-octynoate
	Salicylaldehyde
	Terpinolene
	α -Tetramethylacetyloctahydronaphthalene (ISO E® α)*
	β -Tetramethylacetyloctahydronaphthalene (ISO E® β)
	γ -Tetramethylacetyloctahydronaphthalene (ISO E® γ)*
	Vanillin

* Compounds deviate from the nominal concentration of 2000 mg/kg

GC-FID and GC-MS Application

In the following, the separation of the allergens of both mixes using an ionic liquid phase column is demonstrated. The chromatograms shown below have been generated using a polar phase SLB®-IL60i, which provides wax/PEG like polarity with unique selectivity. Unlike the wax/PEG column (**Figure 1**) there is minimal baseline rise when this polar column is used (**Figures 2 & 3**). The new IFRA methodology¹ discusses FID (higher concentrated samples) and MS use; therefore, the figures below show the bleed behavior of the SLB-IL60i for GC-FID (**Figure 2**) and GC-MS (**Figure 3**).

Figure 1. GC-MS Chromatogram of Mix A1 on a typical PEG phase column



Experimental

Two fragrance allergen samples Mix A1 (**Figure 2**) & Mix A2 (**Figure 3**) were dissolved in MTBE (100 μ L/mL) and analyzed by GC-FID or GC-MS using SLB®-IL60i ionic liquid column applying the conditions in **Tables 2 and 3**.

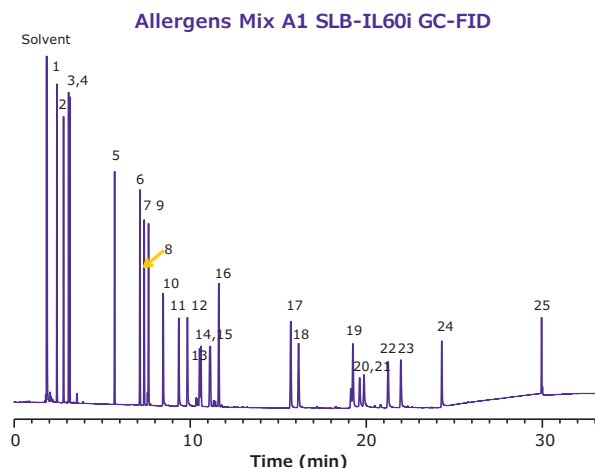
Table 2. Conditions GC-FID method Mix A1 (peak identification was done via separate GC-MS run)

Column:	SLB®-IL60i 30 m x 0.25 mm x 0.2 μ m (29832-U)
Inlet:	250 °C
Oven:	70 °C @ 10 °C/min. to 135 °C (2 min.); 3 °C/min. to 170 °C (1 min.); 10 °C/min. to 270 °C (3 min.)
Flow:	31.457 cm/s Helium
Detector:	FID: 310 °C
Liner:	Cup liner
Split:	100:1
Sample:	1 μ L (100 μ L Mix A1 cat. no. 89131 in 1 mL of MTBE)

Table 3. Conditions GC-MS method Mix A2

Column:	SLB®-IL60i, 30 m x 0.25 mm x 0.2 μ m (29832-U)
Inlet:	250 °C
Oven:	70 °C @ 10 °C/min. to 135 °C (2 min.); 3 °C/min. to 170 °C (1 min.); 10 °C/min. to 270 (30 min.)
Flow:	1.06 mL/min. (38 cm/s) Helium
Detector:	GC-MS, Ion Source: 250 °C, Interface Temp.: 200 °C
Liner:	Splitless with wool (Shimadzu 221-48876-03)
Split:	100:1
Sample:	1 μ L (100 μ L Mix A2 cat.no 16558 in 1 mL MTBE)

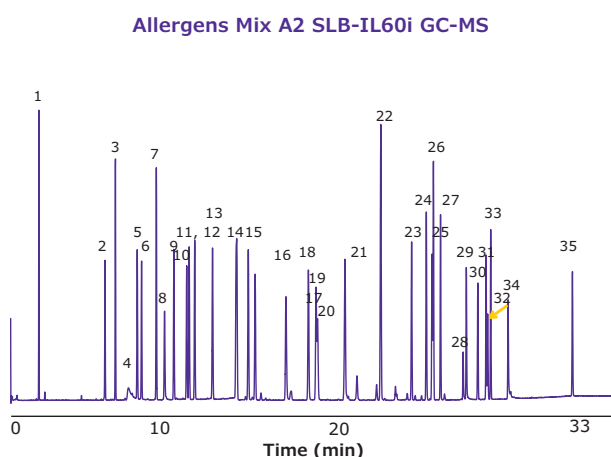
Figure 2. GC-FID Separation of Mix A1 on a SLB®-IL60i Column



Peak IDs

- | | |
|---------------------------|------------------------------------|
| 1. α -Pinene | 14. Ebanol 2 |
| 2. β -Pinene | 15. Anise alcohol |
| 3. Limonene | 16. trans-Anethole |
| 4. α -Terpinene | 17. Trimethylbenzenepropanol |
| 5. Linalool | 18. Eugenol |
| 6. β -Caryophyllene | 19. Santalol isomer |
| 7. Menthol | 20. Cinnamyl alcohol |
| 8. γ -Terpineol | 21. trans,trans-Farnesol |
| 9. α -Terpineol | 22. Santalol isomer |
| 10. Citronellol | 23. Isoeugenol (E+Z) |
| 11. Geraniol | 24. α -Amylcinnamyl alcohol |
| 12. Benzyl alcohol | 25. Sclareol |
| 13. Ebanol 1 | |

Figure 3. GC-MS of Fragrance Allergen Mix A2 on a SLB®-IL60i Column



Peak IDs

- | | |
|---|--|
| 1. Terpinolene | 20. Cinnamaldehyde |
| 2. Benzaldehyde | 21. Tetramethyl acetyl octahydronaphthalene (ISO E® Super) - all Isomers |
| 3. Linalyl acetate | 22. Galaxolide 1 & 2 |
| 4. Salicylaldehyde | 23. α -Amylcinnamaldehyde |
| 5. Camphor | 24. α -Acetyl cedrene |
| 6. Methyl-2-octynoate | 25. Eugenyl acetate |
| 7. Geranyl acetate | 26. Hexadecanolactone / Dihydroambrettolide |
| 8. Methyl salicylate | 27. α -Hexylcinnamaldehyde |
| 9. Neral | 28. Vanillin |
| 10. Carvone | 29. Hydroxyisohexyl 3-cyclohexene carboxaldehyde - major & minor |
| 11. Geranial | 30. 3-Propylidene phthalide |
| 12. Dimethylbenzylcarbonyl acetate (DMBCA) | 31. Isoeugenol acetate |
| 13. Damascone Isomer | 32. Coumarin |
| 14. β -Damasconone (Rose Ketone-4)/Damascone Isomer | 33. Benzyl benzoate |
| 15. α -Isomethylionone | 34. Benzyl salicylate |
| 16. Damascone Isomer | 35. Benzyl cinnamate |
| 17. Hydroxycitronellal | |
| 18. Butylphenyl methylpropional | |
| 19. Amyl Salicylate | |

Results and Discussion

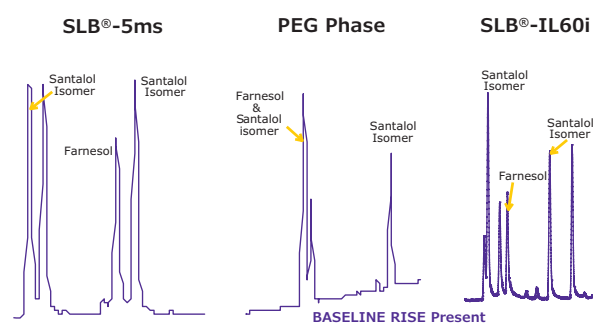
Figures 2 & 3 show the unique separation of the two Fragrance Allergen Mixes on a SLB®-IL60i. Both chromatograms show minimal baseline rise and unique selectivity. Figure 4 shows the separation of farnesol and santalol isomers in Fragrance Allergen Mix A1 on the SLB®-IL60i compared to other commonly used columns (methods not shown here). The ionic liquid phase provides the best selectivity to separate these compounds.

Summary

With the two new ready-to-use Certified Reference Materials, fragrance manufacturers and testing labs are being provided with reliable, high quality standard calibration solutions suitable for the new IFRA method. This is a major advancement in improving the safety of personal care products and allowing manufacturers to retain their customer's trust.

For the GC analysis the SLB®-IL60i ionic liquid phase column is an alternative to the traditional capillary columns used for fragrance analysis, providing unique separation patterns and often fewer co-elutions (will be demonstrated in a later article). It shows a significantly reduced column bleed compared to traditional polar columns like PEG/wax phases.

Figure 4. Comparison of Santalol & Farnesol Separation on 3 column phases



Reference

1. THE INTERNATIONAL FRAGRANCE ASSOCIATION (IFRA) Analytical Method to Quantify 57 Suspected Allergens (and Isomers) in Ready to Inject Fragrance Materials by Gas Chromatography and Mass Spectroscopy, November 15th, 2016

Featured Products

Description	Qty.	Cat. No.
Fragrance Allergen Standard A1	2 mL/5 mL	89131
Fragrance Allergen Standard A2	2 mL/5 mL	16558
SLB®-IL60i, 30 m x 0.25 mm I.D., 0.20 μ m	1 ea	29832-U