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APPLICATION NOTE 10691

Detection and quantification of potential fragrance allergens in complex matrices using GC Orbitrap MS technology

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Keywords

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Goal

Ascertain applicability of Orbitrap™-based GC-MS technology for routine analysis of potential fragrance allergens.

Introduction

Fragrance chemicals are organic substances of synthetic or natural origin widely used in the cosmetic industry around the globe to manufacture intermediate or final consumer goods such as personal care or cleaning products. Some of these chemicals may cause skin allergies and their use is regulated in the European Union.[1] As a result, some fragrance chemicals are forbidden to be used in cosmetic products in Europe (i.e., atranol, chloratranol), whereas others are allowed but are subjected to restrictions in regard to safe concentration limits and that the presence of these substances above the mentioned thresholds should be appropriately labeled.[2] Currently the EU lists 26 potential allergens that are regulated in cosmetic products and must be labeled when present at least at 0.001% in a leave-on product like a moisturizer, or at 0.01% in a rinse-off product like a shampoo. Moreover, the number of compounds in this list is likely to increase to 57 potential allergens in the future following the advice of the Scientific Committee on Consumer Safety (SCCS).[3] It is the responsibility of the manufacturer to ensure that the concentration limits of these potential allergens are respected and that the presence of these substances is appropriately labeled. The consumer must be informed about the content of the product to prevent a possible an allergic reaction. Furthermore this might serve as an aid for dermatologists to diagnose the cause of a patient's reaction.

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Accurate detection, identification, and quantification of potential fragrance allergenic chemicals are therefore important and require analytical instrumentation able to meet these requirements. Screening and quantifying of a large number of potential allergens in the presence of hundreds of other fragrance ingredients poses analytical challenges such as concentration range of potential allergens and complexity of matrices. The analytical method of choice is gas chromatography coupled to mass spectrometry detectors (GC-MS) with a quantification range of 2–100 mg/kg.[4] To address these challenges, laboratories use GC-MS couplings such as GC-QQQ (triple quadrupole), GC-ToF, or even a multi-instrument approach like the official IFRA GC-MS method. Limitations of such GC-MS platforms consist in a tedious configuration of SIM or MS/MS acquisition method, an important amount of data per compound (multiple calibration curves per compound and/or numerous dilutions per sample) but also limitations on the effective dynamic range and a lack in identification confidence. Additionally, SIM or MS/MS acquisition mode produces partial data and is more difficult to maintain in routine analysis than full scan acquisition due to the necessity to always align and check SIM or MS/MS windows to the actual retention time of the compound.

In this work the performance of an Orbitrap-based GC-MS was tested for the analysis of 57 potential fragrance allergens (60 analytes including isomers). Using the unparalleled high resolving power, linear dynamic range, and sensitivity, these potentially allergenic compounds were confidently detected, identified, and quantified at low to high concentration levels in a robust manner and from possibly complex samples.

Experimental

Samples

Solvent standards and a fragrance model were used to test the quantitative performance of the Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS system. Compound linearity, system sensitivity, peak area repeatability, and reproducibility of quantitation were tested using solvent standards prepared in methyl pivalate that contained all 60 potential allergens at 2, 10, 50, 100, 500, and 1000 mg/kg and two internal standards 1,4-dibromobenzene and 4,4'-dibromobiphenyl at 200 mg/kg. Quantification of potential allergens was made using a free of all potential allergens fragrance model, composed of 39 constituents and spiked with the potential allergens at two levels: "low" (spiked concentration varying from 0.4 to 4 mg/kg) and "high" (spiked concentration varying from 20 to 190 mg/kg).

Instrument and method setup

Data was obtained using a Q Exactive GC hybrid quadrupole-Orbitrap mass spectrometer coupled to a Thermo Scientific™ TRACE™ 1310 GC system. Sample injection was achieved using a Thermo Scientific™ TriPlus™ RSH autosampler, and the chromatographic separation was obtained on a Thermo Scientific™ TraceGOLD™ TG-1MS 30 m × 0.25 mm I.D. x 0.25 µm film capillary column (P/N 26099-1420).

The Q Exactive GC system was tuned and calibrated using perfluorotributylamine to achieve mass accuracy of <0.5 ppm. The system was operated in electron ionization mode (EI) using full scan and 60,000 mass resolution, full width at half maxima (FWHM), measured at *m/z* 200 (Table 2). These acquisition parameters ensured that chromatographic data was acquired with a minimum of 12 points/peak to ensure consistent peak integration.

Table 1. GC and injector conditions

TRACE 1310 GC System Parameters	
Injection Volume:	1.0 µL
Liner:	Precision split with wool (P/N 453A1315)
Inlet Temperature:	250 °C
Inlet Module and Mode:	Split/Splitless, hot split (200:1)
Carrier Gas:	He, 1.0 mL/min
Oven Temperature Program	
Temperature 1:	80 °C
Hold Time:	4 min
Temperature 2:	105 °C
Rate:	15 °C/min
Hold Time:	2 min
Temperature 3:	150 °C
Rate:	4 °C/min
Temperature 4:	270 °C
Rate:	10 °C/min
Hold Time:	3 min

Table 2. Mass spectrometer conditions

Q Exactive GC Mass Spectrometer Parameters	
Transfer Line Temperature:	250 °C
Ionization Type:	EI
Ion Source Temperature:	230 °C
Electron Energy:	70 eV
Acquisition Mode:	Full-scan
Mass Range:	50–400 *m/z*
Mass Resolution:	60,000 FWHM at *m/z* 200
Lock Masses:	207.03235 *m/z* 281.05114 *m/z*

Data processing

Data acquisition, processing, and reporting were performed with Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software. A database containing the names, expected retention times, and a minimum of three exact masses per compound was used to create a Chromeleon identification and quantification method for the target compounds.

Results and discussion

The objective of this study was to evaluate the performance of the Q Exactive GC system for the identification and quantification of potential fragrance allergens in perfume samples. Various analytical parameters such as compound chromatographic resolution, sensitivity and linearity over a large concentration range, mass accuracy, and reproducibility of quantification were assessed and the results of these experiments are described below.

Chromatography

The total GC run time per injection was ~37 min. An example of chromatography for a standard mixture at 100 mg/kg and a perfume sample spiked at 100 mg/kg is given in Figure 1. Using the GC conditions described in Table 1 excellent chromatographic separation was achieved even for isomeric compounds. The lowest resolution observed on the extract ion chromatogram is 0.94 for beta santalol / farnesol. Globally, only six pairs of compounds showed a resolution below 1.5 at a concentration of 1000 mg/kg.

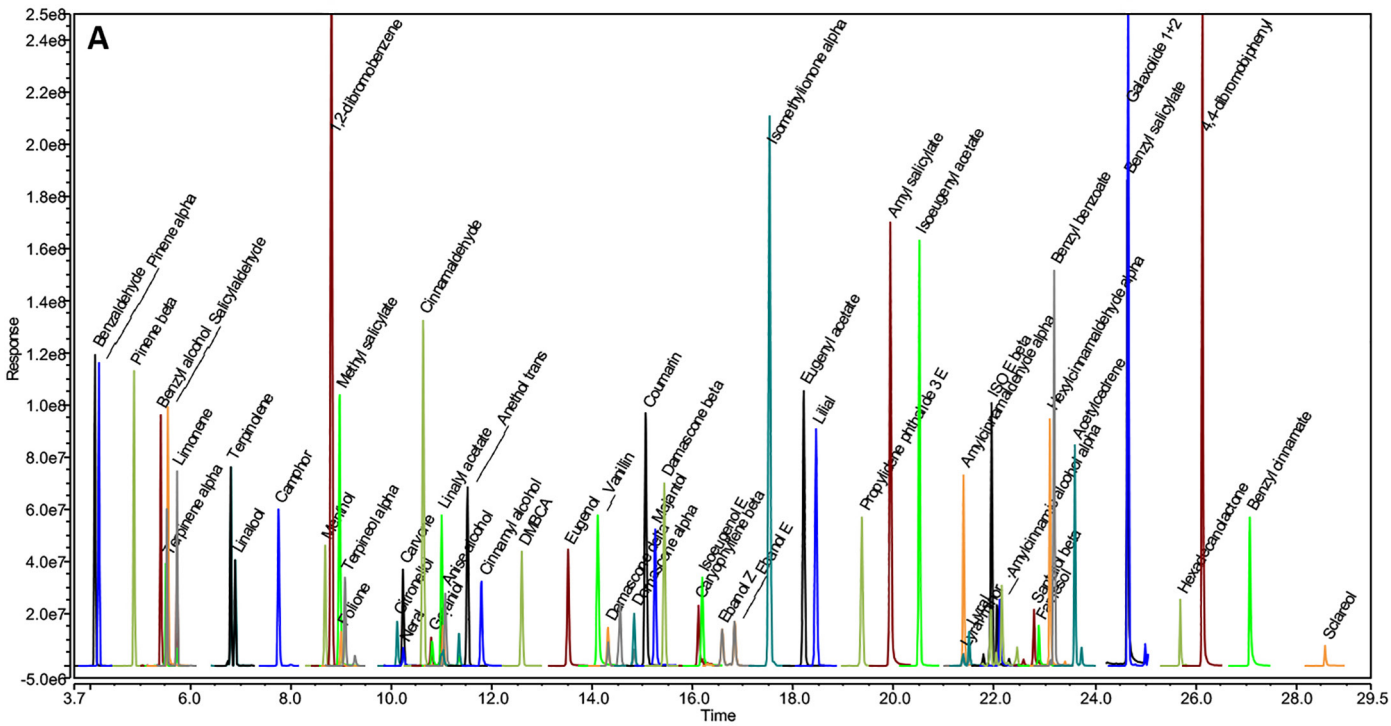


Figure 1A. TICs showing the chromatographic separation of 60 fragrance allergens in a solvent standard. The first (benzaldehyde) and the last (sclareol) eluting allergens are annotated. Data acquired in full scan (EI) at 60,000 resolving power (FWHM at *m/z* 200).

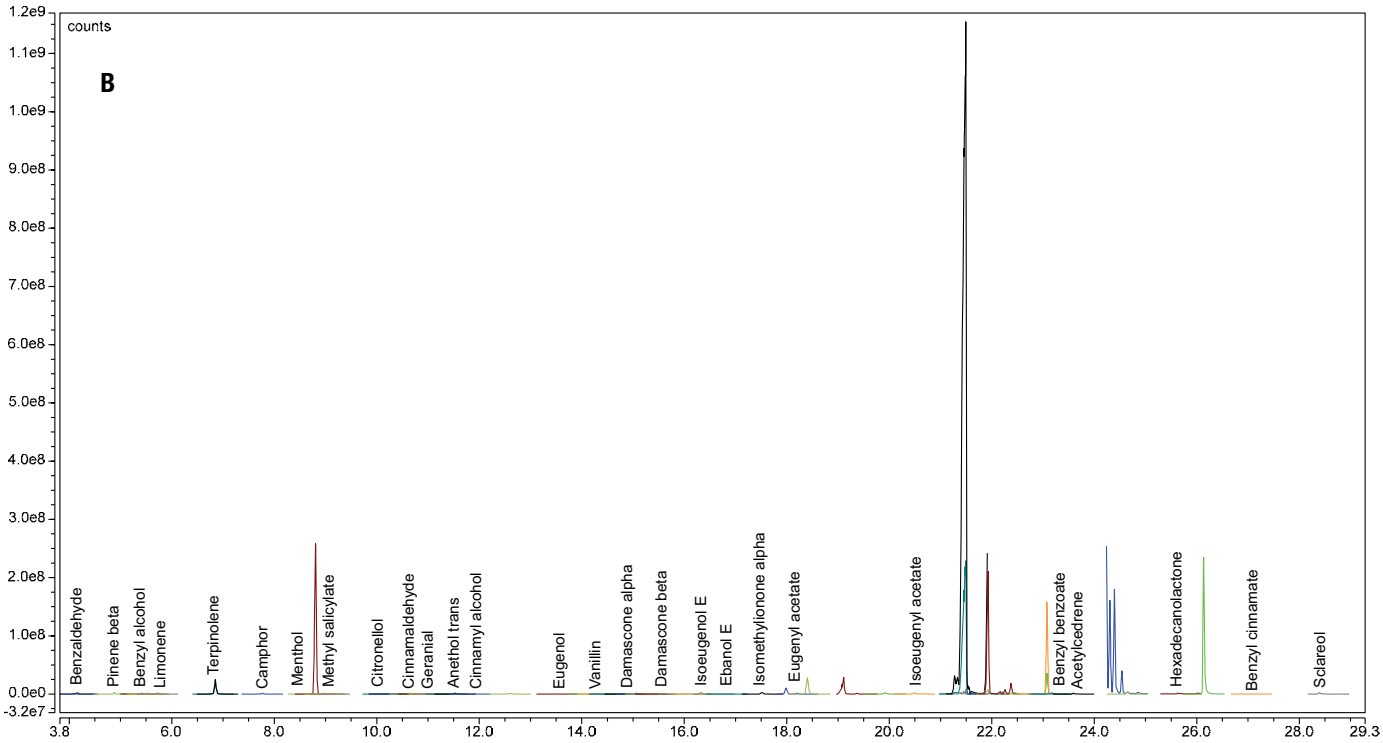


Figure 1B. TIC showing the chromatographic separation of 60 fragrance allergens with on column concentration 0.05 ng for both samples. The first (benzaldehyde) and the last (sclareol) eluting allergens are annotated. Data acquired in full scan (EI) at 60,000 resolving power (FWHM at *m/z* 200).

Sensitivity, selectivity, and linearity

Examples of chromatography, linearity of detector, and background subtracted spectra are shown in Figure 2 for carvone and coumarin.

Excellent sensitivity was achieved with all potential allergens detected in the lowest calibration standard of 2 mg/kg (0.01 ng on column). Moreover, outstanding selectivity was obtained using a resolution of 60,000

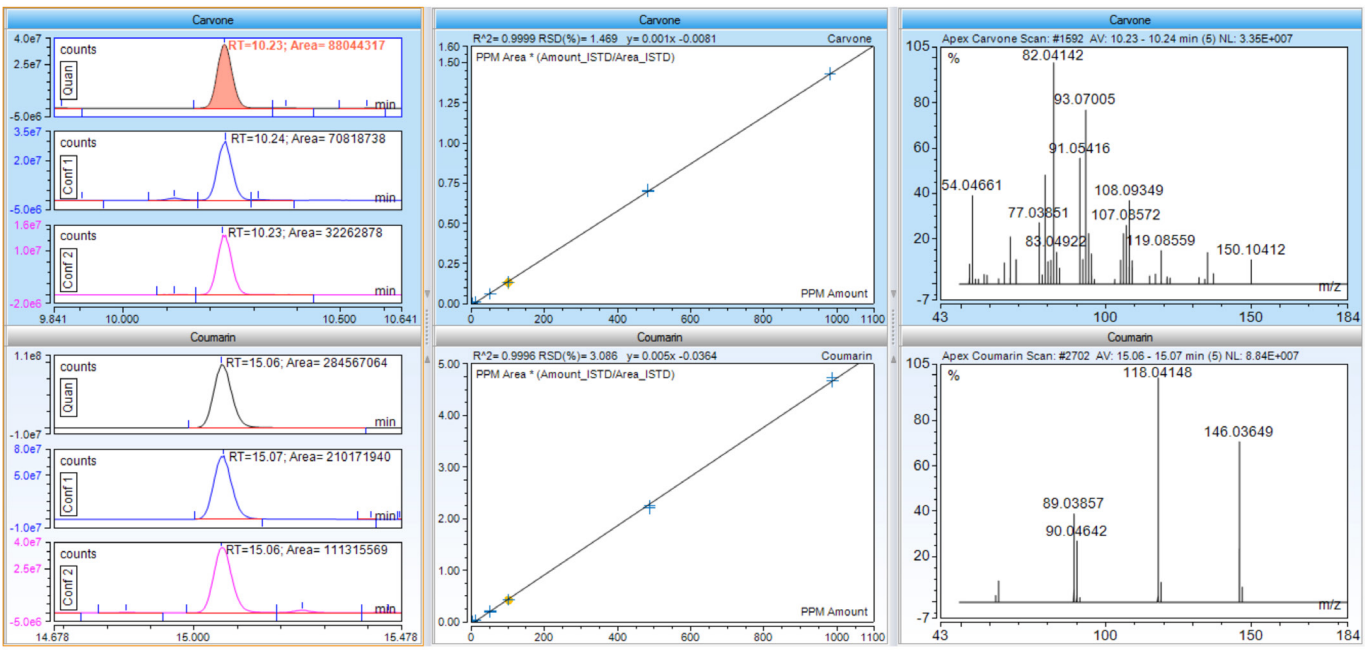


Figure 2. Chromatography of carvone (top) and coumarin (bottom) at 0.01 ng on column. Integrated peak area of the quantification ion, XIC stacked overlay of all the ions (quantitation and two confirmatory), linearity of response (R^2 and %RSD residuals) over 2 to 1000 ppm and background subtracted spectra are shown. Data acquired in full scan at 60,000 resolution (FWHM at *m/z* 200). Peak retention time (RT) as well as peak area counts (Area) are annotated.

as demonstrated in Figure 3 for compounds that are known to co-elute (Lyral™ and amylcinnamic aldehyde). In addition, an example of selectivity by the use of high resolution and accurate mass information is shown in Figure 4 where Lilial™ is easily resolved from the matrix (perfume) co-eluting component butylated hydroxytoluene (BHT).

An example of a co-elution at this concentration level is shown in Figure 4. At the 5 mg/kg level all the compounds detected have ion ratios within the 15% limit of the average ion ratio derived from the calibration curve across all concentrations, substantiating the identity of the compounds.

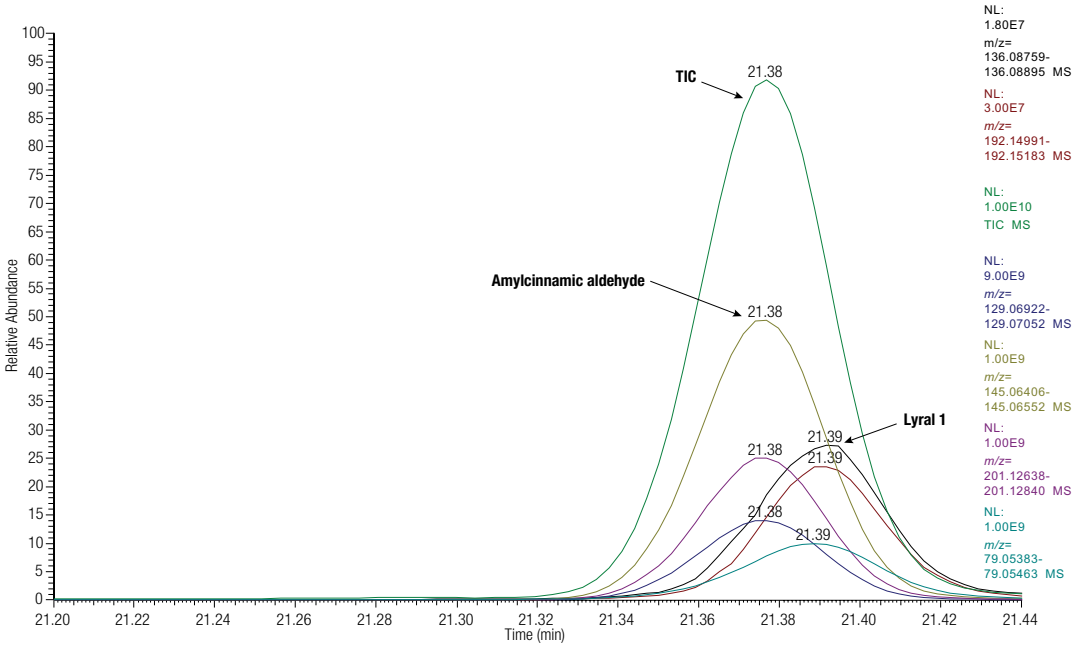


Figure 3. Deconvolution of lyral 1 (at 5 ng on column) and amylcinnamic aldehyde in a perfume sample. TIC trace as well as extracted ion chromatograms (XIC) of two masses for amylcinnamic aldehyde and for lyral 1 are shown. Data acquired in full scan at 60,000 resolution (FWHM at *m/z* 200).

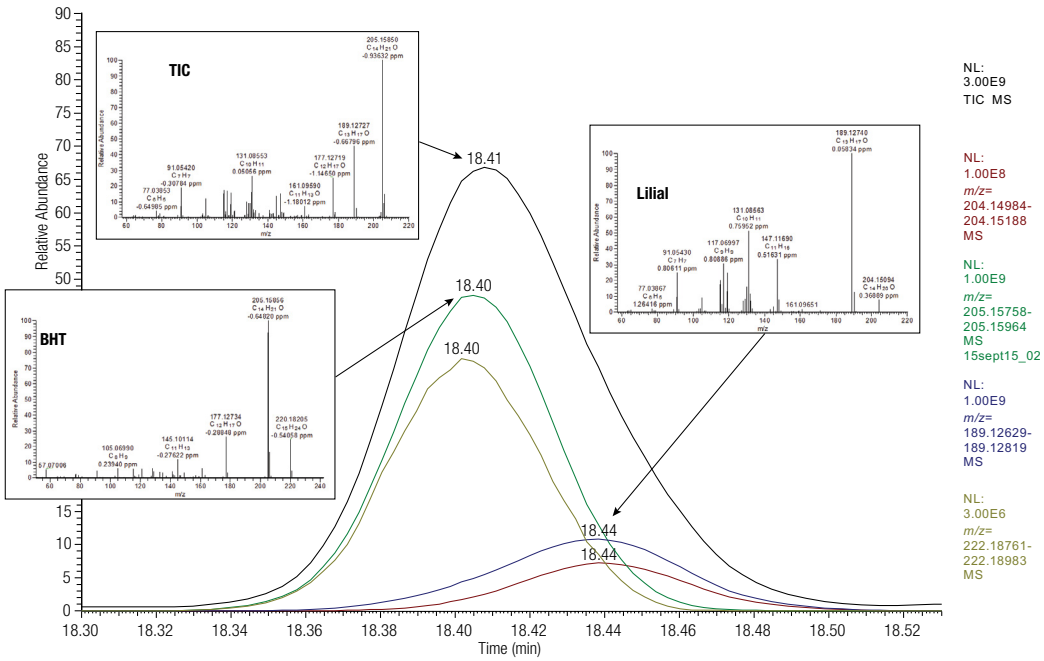


Figure 4. Co-elution of lilial (at 5 ng on column) and BHT resolved through spectral deconvolution in a perfume sample. TIC trace as well as extracted ion chromatograms (XIC) of two masses for BHT and for lilial are shown. Data acquired in full scan at 60,000 resolution (FWHM at *m/z* 200).

Repeatability and linearity of the Orbitrap detector

Detector linearity for the potential allergens was assessed over a concentration range of 2 to 1000 mg/kg (or 0.01 to 5 ng on column) using solvent calibration standards injected in duplicate at each level and taking into account the response of the two internal standard compounds (1,4-dibromobenzene and 4,4'-dibromobiphenyl). The same experiment was repeated after one week in order to test the robustness of the method. The results of these experiments are shown in Figure 5.

Obtaining consistent peak areas from injection to injection is very important for any analytical platform as this affects the accuracy of quantification. Excellent peak area repeatability was observed as demonstrated in Figure 6 for 1,4-dibromobenzene (internal standard), a compound that produced an RSD = 3.4% (number of replicate n=16).

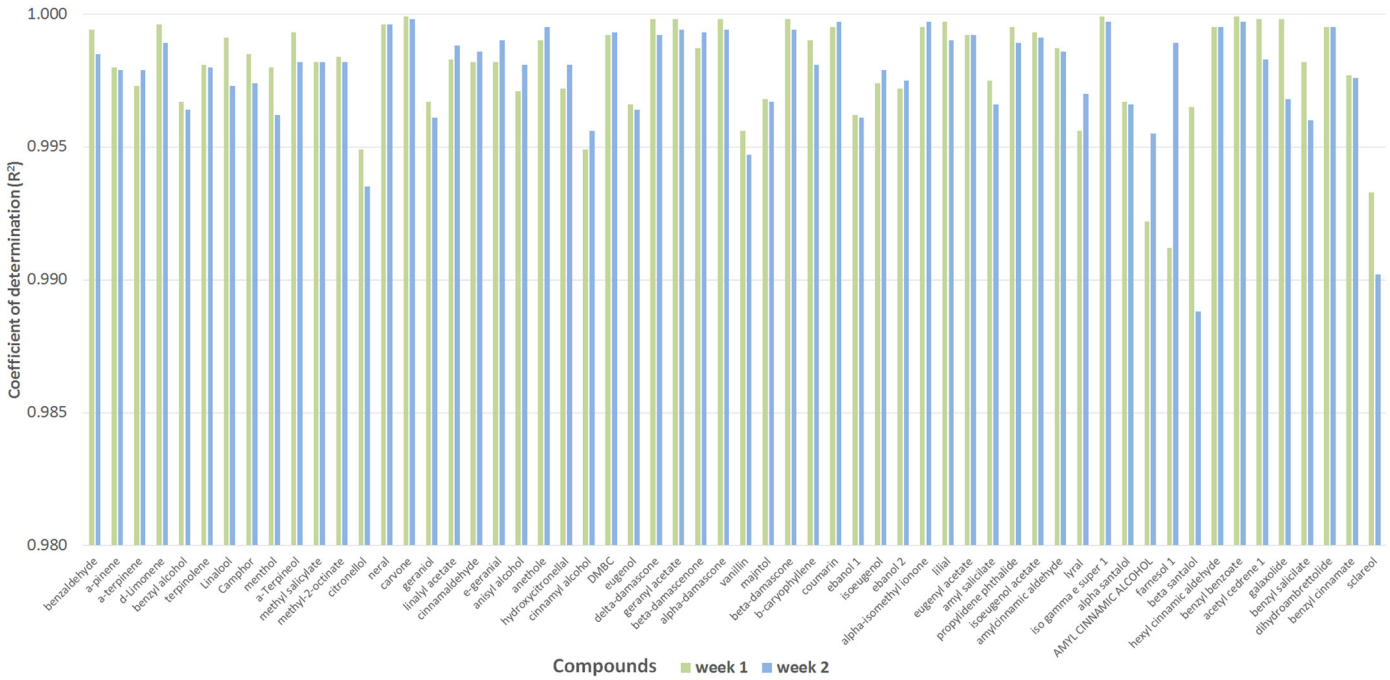
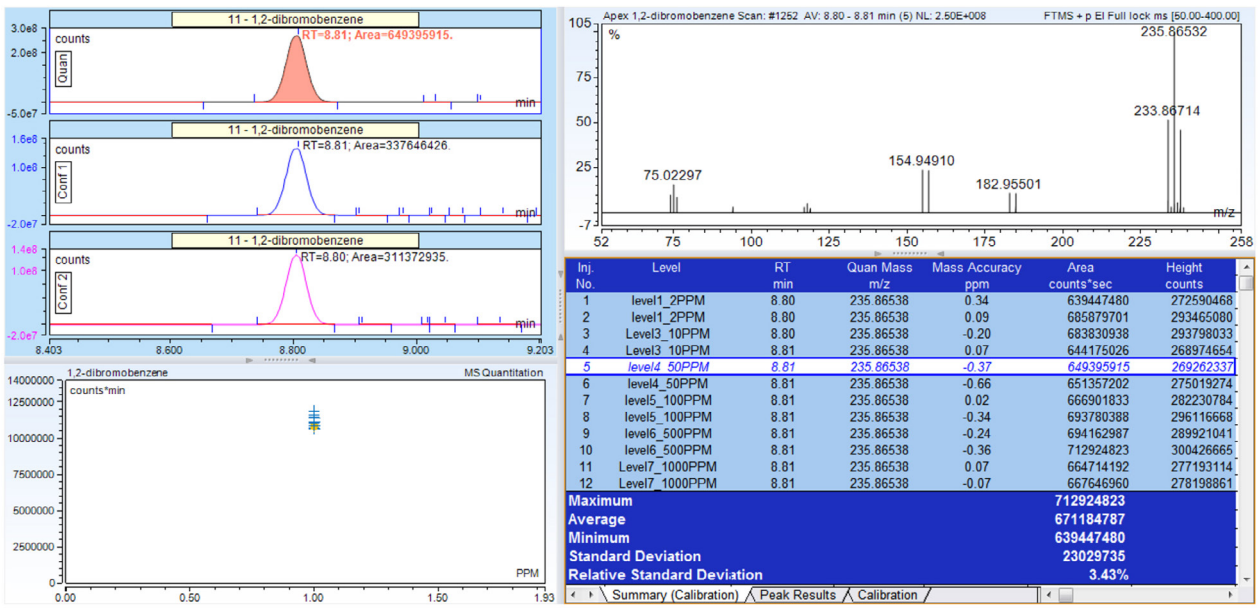


Figure 5. Coefficient of determination (R^2) derived from calibration curves of allergens over a concentration range of 2 to 1000 ppm (0.01 to 5 ng on column). Data are obtained from n=2 repeated injections of solvent standards at each calibration level for week 1 and week 2.



Inj. No.	Level	RT min	Quan Mass m/z	Mass Accuracy ppm	Area counts*sec	Height counts
1	level1_2PPM	8.80	235.86538	0.34	639447480	272590468
2	level1_2PPM	8.80	235.86538	0.09	685879701	293465080
3	Level3_10PPM	8.80	235.86538	-0.20	683830938	293798033
4	Level3_10PPM	8.81	235.86538	0.07	644175026	268974654
5	level4_50PPM	8.81	235.86538	-0.37	649395915	269262337
6	level4_50PPM	8.81	235.86538	-0.66	651357202	275019274
7	level5_100PPM	8.81	235.86538	0.02	666901833	282230784
8	level5_100PPM	8.81	235.86538	-0.34	693780388	296116668
9	level6_500PPM	8.81	235.86538	-0.24	694162987	289921041
10	level6_500PPM	8.81	235.86538	-0.36	712924823	300426665
11	Level7_1000PPM	8.81	235.86538	0.07	664714192	277193114
12	Level7_1000PPM	8.81	235.86538	-0.07	667646960	278198861
Maximum					712924823	
Average					671184787	
Minimum					639447480	
Standard Deviation					23029735	
Relative Standard Deviation					3.43%	

Figure 6. Peak area repeatability of 1,4-dibromebenzene across n=16 injections (including calibration standards and matrix perfume samples). Calculated %RSD <3.5%, quantification and confirmation extracted ion chromatograms as well as EI mass spectrum are shown.

Potential allergens quantification

The quantitative performance of the Q Exactive GC system was tested for all 60 targeted potential allergens. A calibration curve was calculated for a concentration range of 2 to 1000 mg/kg (or 0.01 to 5 ng on column). The potential allergens were quantified in the "low" and "high" perfume samples over two different weeks (Figure 7).

In the "low" sample, 57% of potential allergens were quantified with less than 20% error (calculated against the theoretical spikes amount). In the case of "high" sample, 95% of potential allergens were below this 20% limit and the mean error was 7%.

Consistent mass accuracy

In addition to the quantitative performance, the measured mass accuracy of the potential allergens was assessed across all the calibration concentrations and in the "low" and "high" perfume samples. Obtaining accurate mass information is critical in order to avoid misidentification of potential allergens, which can lead to either false positive or false negative results. For all targeted compounds, the mass accuracy was <1.5 ppm irrespective of concentration level. This is demonstrated in Figure 8 for 60 potential allergens over n=8 injections.

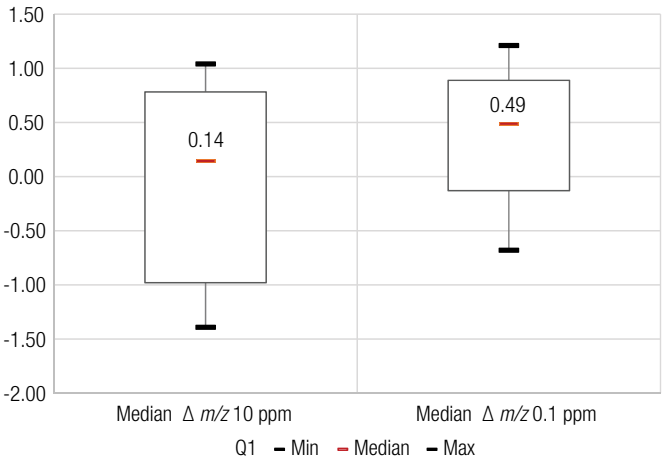


Figure 8. Mass accuracy (ppm) at 10 ppm (0.05 ng on column) and 0.1 ppm (0.0005 ng on column) concentration level assessed as an average value over n=8 consecutive injections for 54 compounds. Ninety percent of the allergens' population are included in the box plot.

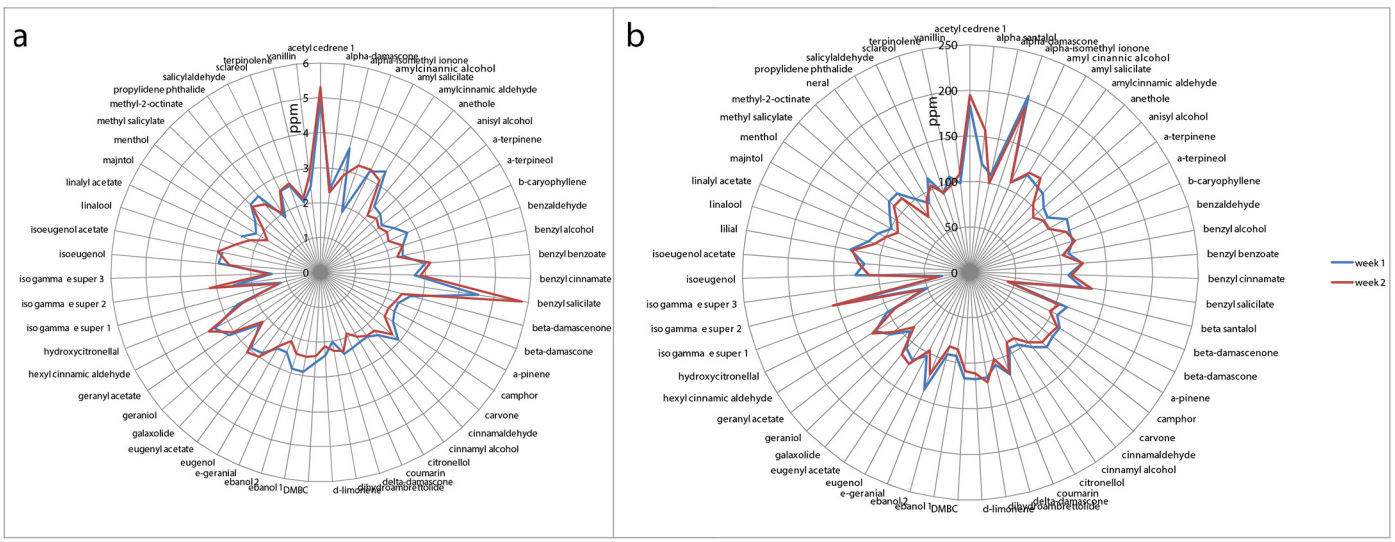


Figure 7. Quantification of 60 allergens in the "low" (A) and "high" (B) perfume samples showing reproducibility of the results. Blue and red lines represent the data obtained in two different weeks.

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Conclusions

- The results of this study demonstrate that the Q Exactive GC system could successfully be applied to quantify potential fragrance allergens.
- Full scan high-resolution accurate mass acquisition on the Q Exactive GC system allows for easy method setup and data interpretation compared to GC-MS-SIM or GC-QQQ but also facilitates the retrospective detection of new compounds in high resolution that might be added to the list of potential allergens in cosmetic products.
- Excellent sensitivity, consistent sub-ppm mass accuracy, and the large dynamic range of >5 orders of magnitude ensures that the target compounds are confidently detected, identified, and quantified, reducing the risk of false positives/negatives even in complex fragrance matrices with many co-eluting components, such as perfumes.
- Robust analytical performance of the Q Exactive GC system technology as demonstrated in this work saves laboratory time since fewer calibration curves per compound and dilution per sample are necessary compared to current GC-MS official IFRA method.

References

1. Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products of importance for the EEA (Official Journal of the European Union L 42/59, 22 December 2009).
2. EU: Annex III of EU Cosmetic Regulation 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products.
3. Scientific Committee on Consumers Safety, Opinion on Fragrance allergens in cosmetic products, SCCS/1459/11, 15th plenary meeting, 26-27 June 2012.
4. Chaintreau, A.; Joulain, D.; Marin, C.; Schmidt, C.O.; Vey, M. GC/MS quantitation of fragrance compounds suspected to cause skin reactions. *J Agric Food Chem.* **2003,** *51,* 6398–403.
5. The International Fragrance Association Analytical Working Group, Analytical method to quantify 57 suspected allergens (and isomers) in ready to inject fragrance materials by gas chromatography and mass spectrometry, IFRA Analytical guideline, November 2016.

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