Detection and Quantification of Fragrance Allergens in Complex Matrices Using GC Orbitrap MS Technology

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ABSTRACT

Purpose: Quantitative analysis of fragrance allergens in complex matrices such as perfumes and other cosmetic product is challenging. It involves the accurate and reliable detection of an emerging list of compounds present at low and high levels. When using high-resolution mass spectrometry, the default acquisition mode is untargeted (full scan) meaning that all the ions are acquired at the same time across a specified mass range, making it simple to manage and giving the analyst the flexibility to decide post-acquisition which ions to measure. The objective of the experiments described below was to ascertain the applicability of Orbitrap-based GC-MS technology for routine analysis of fragrance allergens.

Methods: 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 allergens (2, 10, 50, 100, 500 and 1000 ppm) and two internal standards (200 ppm): 1,4-dibromobenzene and 4,4'-dibromobiphenyl. Quantification of allergens was made using the fragrance model, composed of 39 constituents, free of all allergens and spiked with the allergens at two levels: 'low' (spiked concentration varying from 0.4-4 ppm) and 'high' (spiked concentration varying from 20-190 ppm).

Results: Using a total GC run time of 37 min appropriate compound separation was achieved even for isomeric compounds. Excellent sensitivity was achieved with all allergens detected in the lowest calibration standard of 2 ppm (0.01 ng on column). Moreover, outstanding selectivity was obtained using 60k resolution for compounds that are known to coelute: lyral 1 and amylcinnamic aldehyde. Allergen linearity was assessed over a concentration range of 2-1000 ppm (or 0.01-5 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. Peak area repeatability experiments, as demonstrated for 1,4-dibromobenzene (internal standard), produced %RSDs < 3% (n=16 injections).

The quantitative performance of the Q Exactive GC was tested for all 60 target allergens. For this, a calibration curve was constructed over a concentration range of 2-1000 ppm (or 0.01-5 ng on column). The allergens were quantified in the 'low' and 'high' perfume samples in two different weeks. On the "low" sample, 57% of allergens were quantified with less than 20% error (calculated against the theoretical spike amount). In the case of the "high" sample, 95% of allergens were below this limit and the mean error was 7%.

INTRODUCTION

Fragrant chemicals are organic substances of synthetic or natural origin widely used in the cosmetic industry across the globe to manufacture intermediate or final consumer goods such personal care or cleaning products. Some of these chemicals may cause skin allergies and therefore their use is regulated in the European Union [1]. As a result, some of the fragrance chemicals are considered unsafe to be used in cosmetic products in Europe (ex: atranol, chloratranol), whereas others are allowed but subject to restrictions in regards to safe concentration limits [2]. Currently the EU lists 26 allergens that are regulated in cosmetic products and that must be clearly labelled when present at 0.001% in a leave-on product (ex: moisturiser) or 0.01% in a rinse-off product (ex: shampoo). Moreover, the number of compounds added to this list is likely to increase in the future following the advice from the Scientific Committee on Consumer Safety (SCCS) [3]. It is the responsibility of the manufacturer to ensure that the concentration limits of these allergens are met and that the presence of these substances are clearly labelled. The consumer has to be informed about the product chemical content, in the eventuality of an allergic reaction or to aid dermatologists trying to diagnose the cause of a patient's possible reaction.

Precise detection, identification and quantification of fragrance allergenic chemicals are especially important and require analytical instrumentation able to meet these requirements. Screening and quantifying of a large number of allergens in the presence of hundreds of other fragrance ingredients poses analytical challenges (such as variation in concentration levels of 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 ppm [4]. To address these challenges laboratories use GC-MS technologies such as GC triple quadrupole, GC-ToF or even a multi-instrument approach. With such GC-MS platforms limitations can include: dynamic range, sensitivity, selectivity in difficult matrices and sufficient resolution.

In this work the performance of an Orbitrap-based GC-MS was tested for the analysis of 56 (60 analytes including isomers) fragrance allergens. Using the unparalleled high resolving power, linear dynamic range and sensitivity, these allergenic compounds were confidently detected, identified and quantified at low to high levels in a robust manner and in potentially complex samples.

MATERIALS AND METHODS

Sample Preparation

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

Test Method(s)

Data was obtained using a Thermo Scientific™ Q Exactive™ GC Hybrid Quadrupole-Orbitrap™ mass spectrometer coupled to a Thermo Scientific™ TRACE™ 1310 GC. 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 x 0.25 mm I.D. x 0.25 µm film capillary column (P/N: 26099-1420). The Q Exactive GC was tuned and calibrated using FC43 to achieve routine 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.

Data Analysis

Data acquisition, processing and reporting was performed with Thermo Scientific™ Chromeleon™ 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..

Table 1. GC and MS experimental parameters.

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

Q Exactive GC Mass Spectrometer Parameters	
Transfer line (°C):	250
Ionization type:	EI
Ion source(°C):	230
Electron energy (eV):	70
Acquisition Mode:	Full scan
Mass range (m/z):	50-400
Mass resolution (FWHM at m/z 200):	60k
Lockmasses (m/z):	207.03235 281.05114

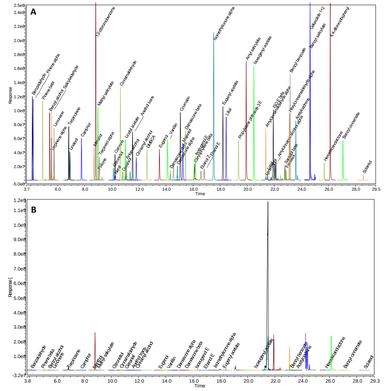
RESULTS

The objective of this study was to evaluate the performance of the Q Exactive GC for the quantification of fragrance allergens in perfume samples. Various analytical parameters such as compound chromatography, sensitivity, 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 solvent standard (100 ppm) and a perfume sample spiked at 100 ppm is given in Figure 1. Using the GC conditions described in Table 1 excellent compound separation was achieved even for isomeric compounds.

Figure 1: TIC showing the chromatographic separation of 60 fragrance allergens in a solvent standard (A) and in a spiked perfume sample (B), 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

All allergens were detected in the lowest calibration standard (0.01 ng on column amount) and examples of chromatography, linearity and background subtracted spectra are shown in Figure 2 for carvone and coumarin.

Excellent sensitivity with all allergens detected in the lowest calibration standard 2 ppm (0.01 ng on column). Moreover, outstanding selectivity was obtained using 60k resolution as demonstrated in Figure 3a for compounds that are known to coelute: lyral 1 and amylcinnamic aldehyde. In addition, an example of selectivity through the use of high resolution and accurate mass information is shown in Figure 3b where lilial is easily resolved from the matrix (perfume) co-eluting component butylated hydroxytoluene (BHT).

Figure 2: Chromatography of carvone (a) and coumarin (b) at 0.01 ng on column. Integrated peak area of the quantification ion, XIC overlay of all the ions (quantitation and two confirmatory), linearity of response (R2 and %RSD residuals) over 2 - 1000 ppm are shown. Data acquired in full scan at 60,000 resolution (FWHM at m/z 200).

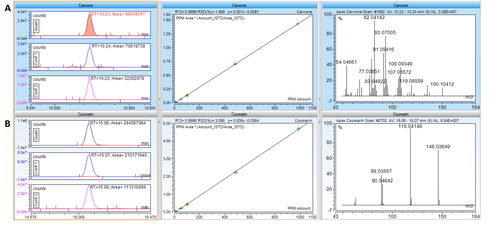
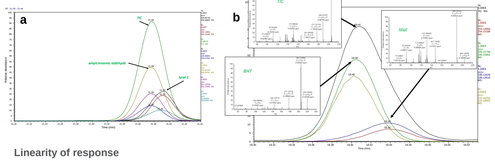


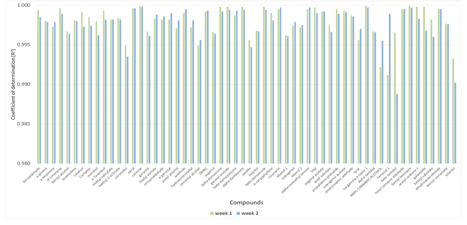
Figure 3a: Deconvolution of lyral 1 (at 5 ng on column) and amylcinnamic aldehyde in a perfume sample. TIC trace as well as extracted ion chromatograms (XIC) of two masses for amylcinnamic aldehyde and for lyral 1 are shown. 3b: co-elution of lilial (at 5 ng on column) and BHT resolved through spectral deconvolution in a perfume sample. TIC trace as well as extracted ion chromatograms (XIC) of two masses for BHT and for lilial are shown.



Linearity of response

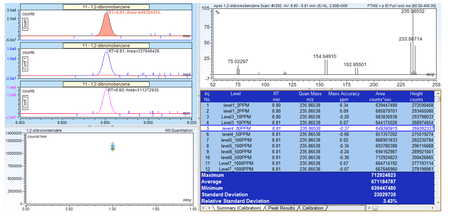
Allergen linearity was assessed over a concentration range of 2-1000 ppm (or 0.01-5 ng on column) using solvent calibration standards injected in duplicate at each level and taking into account the response of the two internal standard compounds (1,4-dibromobenzene and 4,4'-dibromobiphenyl). The same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

Figure 4: Coefficient of determination (R2) derived from allergens calibration curves over a concentration range of 2-1000 ppm (0.01 – 5 ng on column). Data obtained from n=2 consecutive injections of solvent standards at each calibration level for week 1 and week 2.



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 for 1,4-dibromobenzene (internal standard) in Figure 5, compound that produced an %RSD = 3.4% (n=16).

Figure 5. 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.

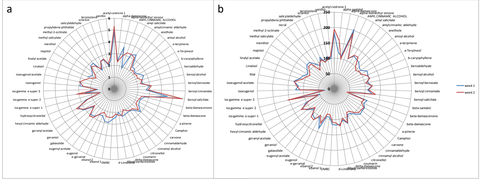


Allergens quantification

The quantitative performance of the Q Exactive GC was tested for all 60 targets allergens. For this, a calibration curve was constructed over a concentration range of 2-1000 ppm (or 0.01-5 ng on column). The allergens were quantified in the 'low' and 'high' perfume samples in two different weeks (Figure 6).

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

Figure 6. 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.



CONCLUSIONS

The results of this study demonstrate that the Q Exactive GC Orbitrap high resolution mass spectrometer, in combination with Chromeleon CDS software delivers sensitive quantitative performance for allergens in cosmetic products.

- The results of this study demonstrate that the Q Exactive GC could be successfully adopted for routine analysis of fragrance allergens.
- 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.
- The use of routine high resolving power allows for excellent selectivity, even in complex fragrance matrices with many co-eluting components, such as perfumes.

REFERENCES

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TRADEMARKS/LICENSING

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