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Quantitation of suspected allergens in fragrances Part II. Evaluation of comprehensive gas chromatography–conventional mass spectrometry

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Abstract

The European legislation requires that fragranced products are evaluated for their content in 24 compounds that are suspected to be skin sensitizers. Their quantitation in fragrance concentrates may not be achieved with GC–flame ionization detection (FID), due to the complexity of these mixtures and even comprehensive GC–FID does not provide sufficient resolution. This paper reports the first example of quantitation based on the hyphenation of comprehensive GC with a low-cost quadrupole MS. A detection frequency of 30.7 Hz can be obtained by monitoring a single ion. This allows a satisfactory evaluation of the area sum over the 2–3 modulations of a given compound and linear calibration curves are obtained. Analyses are completed within 35 min.

Keywords: Allergens; Gas chromatography, comprehensive two-dimensional; Perfumes; Fragrances

1. Introduction

Allergens are a much-debated question in the European Union. Among them, some compounds are suspected to participate in the skin irritation sometimes observed by perfume users according to the Scientific Committee on Cosmetic Products [1]. However, the scientific literature dealing with their quantitation remains very scarce. A first paper proposes their identification using GC–MS [2] and their subsequent quantitation with a GC–flame ionization detection, after peak attribution owing to their retention times. A second publication bases the quantitation on the GC–MS monitoring of specific ions [3].

A GC–MS method is currently under development for the routine evaluation of allergens in quality control laboratories [4]. However, difficult cases may be observed when co-elutions of allergens with isobaric ions occur. To overcome this drawback, the capabilities of comprehensive GC have recently been investigated [4]. In spite of the dramatic resolution of such a bidimensional technique, the detection still requires more specificity than FID to clearly distinguish allergen peaks from other fragrance constituents in the second dimension and to overcome two-dimensional peak overlappings. Therefore the hyphenation of GC × GC with mass spectrometry (GC × GC–MS) was considered.

As comprehensive GC implies a short, very narrow second column operated in fast mode, a high sampling rate is required (>50 Hz). The resulting scan rate can not be achieved with a conventional quadrupole MS. In contrast, the hyphenation is quite compatible with time-of-flight MS (TOF–MS) as shown by Shellie et al. [5] and optimized by Dallüge et al. [6].

However, TOF–MS systems are costly instruments that are not compatible with quality control laboratory's budgets. Therefore, the use of a conventional MS was investigated in this work. To overcome the instrument limit of 2.43 scan/s (Hewlett-Packard, Model 5972), Frysinger and Gaines [7] proposed to broaden chromatographic peaks. Shellie et al. [8] preferred to limit the scanning range to achieve a rate of 20 scans/s. In the present paper, as the aim was not the identification of unknown peaks, but the quantitation of known compounds, the analysis was performed in single-ion monitoring (SIM) mode.

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2. Experimental

2.1. Materials

Suppliers and purities of reference compounds have been reported elsewhere [4]. 1,4-Dibromobenzene and 4,4′-dibromobiphenyl (Aldrich, Steinheim, Germany) were used as internal standards (I.S.s). *o*-Fluorotoluene (Acros) was used as a solvent. Two concentrated fragrance models, free of target compounds, were used for the spiking experiments (Proton and SVB). They consisted of 32 and 168 constituents, respectively.

2.2. GC × GC

All analyses were performed using an Agilent 6890 model gas chromatograph (Agilent Technologies, Wilmington, DE, USA). The GC system was equipped with a longitudinally modulated cryogenic system (LMCS, Everest model, Chromatography Concepts, Doncaster, Australia). GC × GC experiments were performed using two capillary columns that were coupled in series with a zero dead-volume connector (Gerstel, Mülheim, Germany). The primary (first dimension) column was a DB1 (Agilent), 60 m (30 m in Fig. 2A) × 0.25 mm i.d., 0.25 μm phase thickness. The secondary (second dimension) column was either a SPWax-10 (Supelco, Bellefonte, PA, USA), or a DB 225 (Agilent) stationary phase column of dimensions 1.0 m × 0.10 mm (0.10 μm film thickness). The modulation period was 3 s in all analyses. The events control function of the GC system was used to instruct modulation to commence (9.0 min) and cease (39.5 min) at precise times. Between these times, the thermostatically controlled cryogenic trap was maintained at ca. 0 or −40 °C for analyses performed with SPWax and DB1 in the modulation tube, respectively. Temperature-programmed conditions were performed for all analyses, using an initial oven temperature of 80 °C (constant for 2 min), then temperature programmed at a rate of 5 °C/min to a final oven temperature of 230 °C (constant for 14 min). The carrier gas was helium and was supplied at 380 kPa head pressure (200 kPa without open-split). A 7683 series autosampler (Agilent Technologies) was used to deliver an injection volume of 2.0 μl. The split–splitless injector was operated at 250 °C in split mode, providing a split ratio of 20:1.

2.3. Mass detection

The second column outlet was connected, through a laboratory-made open-split to a quadrupole mass spectrometer (Model 5972, Agilent Technologies). Acquisitions were performed in the SIM mode, with under-resoluted mass peaks. The electron-multiplier voltage was increased of 700 V above the autotune adjustment. The dwell time was 10 ms, for a sampling rate of only 30.7 Hz, due to dead intervals between two samplings. The analysis duration was divided into time windows corresponding to the elution zone of each allergen in the first dimension. Target compounds were successively monitored by choosing a single and characteristic ion in each window (Table 1).

2.4. Quantitation

A stock solution of the 24 allergens, plus phenylacetaldehyde, estragole, methyl eugenol and methyl 2-nonynoate was prepared in isooctane (10 g/l of each compound). From this stock solution, calibration solutions were prepared in *o*-fluorotoluene at individual concentrations of 2, 5, 10, 20, 50, and 100 mg/l of each compounds and 100 mg/l of

Table 1
Ions used to monitor target compounds

Name/CAS Reg. no.	Ions	Name/CAS Reg. no.	Ions
Amyl cinnamic alcohol [101-85-9]	133	Farnesol[a] [106-28-5]	Isomer 1: 81; Isomer 2: 93
Amyl cinnamic aldehyde [122-40-7]	202	Geraniol [106-24-1]	69
Anisyl alcohol [105-13-5]	138	Hexyl cinnamic aldehyde [101-86-0]	216
Benzyl alcohol [100-51-6]	108	Hydroxycitronellal [107-75-75]	59
Benzyl benzoate [120-51-4]	105	Isoeugenol [97-54-1]	164
Benzyl cinnamate [103-41-3]	131	Butylphenyl methylpropional [80-54-6]	189
Benzyl salicylate [118-58-1]	91	Limonene [5989-27-5]	68
Cinnamic alcohol [104-54-1]	92	Linalool [78-70-6]	93
Cinnamic aldehyde [104-55-2]	131	Hydroxyisohexyl-3-cyclohexene carboxaldehyde [31906-04-4]	Isomer 1: 136; Isomer 2: 136
Citral [5392-40-5]	Neral: 69 Geranial: 69	Methyl 2-nonynoate[b] [111-80-8]	79
Citronellol [106-22-9]	69	Methyl 2-octynoate [111-12-6]	95
Coumarine [91-64-5]	146	Methyl eugenol[b] [93-15-2]	178
Estragole[b] [140-67-0]	148	Phenylacetaldehyde[b] [122-78-1]	91
Eugenol [97-53-0]	164	α-Isomethylionone [127-51-5]	135
1,4-Dibromobenzene	236	4,4′-Dibromobiphenyl	312

[a] Isomers 3 and 4 were not considered for the farnesol quantitation due to their low abundance.
[b] Other compounds, not in the list of 24 allergens [1].

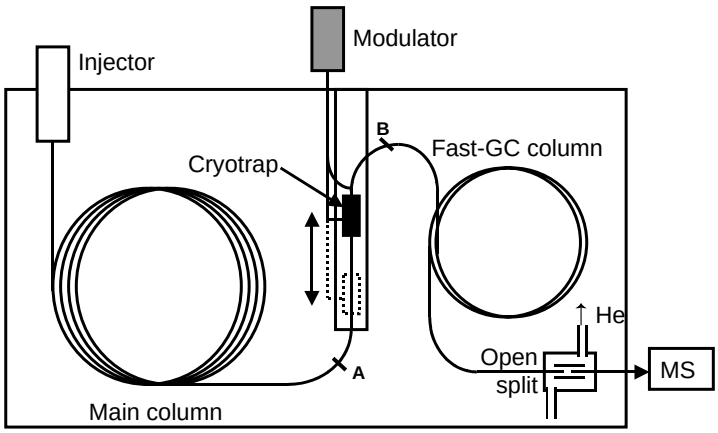

Fig. 1. Scheme of the GC × GC–MS system. The modulator tube is between A and B.

each internal standards. An aliquot (2 μl) of these calibration solutions was injected with the autosampler using the above-mentioned GC–MS conditions. Allergen areas were reported to the closest eluting internal standard, and calibration curves were drawn as:

$$\frac{\text{allergen area}}{\text{I.S. area}} = f\left(\frac{\text{allergen amount}}{\text{I.S. amount}}\right)$$

The fragrance to be evaluated was spiked with both internal standards (100 mg/l) and then diluted in *o*-fluorotoluene (50 g/l) depending on the sample polarity.

2.5. Software

Data acquisition and peak integration were performed using standard GC–MS software (Chemstation G1701 BA Version B.01.00, Agilent Technologies). Importation of the raw data matrix or integration results in spreadsheets (Excel 97 SR-25, Microsoft, Redmond, WA, USA) was done in ASCII format.

3. Discussion

In previous papers attempting to couple mass spectrometry to comprehensive GC, the second column seems to be directly connected to the MS source under vacuum [5–7,9]. This configuration implies that flow conditions are imposed by vacuum performances of the MS system. Therefore an open-split was inserted between the second column outlet and the mass spectrometer (Fig. 1). Using the DB1 × DB225 configuration, peak shapes were compared with and without open-split. For instance, the methyl eugenol peak widths were $^{2}\omega_h = 66$ and 90 ms, and skewness at 4.4% of the height were $\eta = 1.46$ and 1.6 with and without open-split, respectively. These values might suggest that the carrier gas velocity was slightly increased by the source vacuum. However, as the carrier gas flow had to be decreased when the second dimension was directly connected to the source, the contribution of the delayed elution to peak broadening and tailing was unclear. Because the open-split also provided a greater versatility (column change without stopping the MS, same flow conditions as with FID conditions), it was used throughout this study. The linearity of the MS response was unaffected as shown hereafter with the calibration curves.

3.1. Peak broadening

In a previous paper, the GC × GC–FID analysis of the same compounds that are strongly retained in the second dimension (e.g. anisyl alcohol) gave rise to large peakwidths and biased peak areas [10]. Using the same two columns coupled to MS obviously led to similar observations. Therefore the influence of the phase within the modulator tube and in the second dimension was investigated.

Carbowax-type columns are widely used in fragrance analysis due to their capability of resolving a great number of perfume ingredients. Therefore, this phase was first selected for the second dimension, in association with a polydimethylsiloxane-type for the first dimension. Although the resulting large polarity difference was expected to provide the largest orthogonality, the broadening of polar compounds in SPWax 10 (e.g. benzyl alcohol, $^{2}\omega_h = 762$ ms, Fig. 2A) gave rise to an incomplete resolution of these peaks from those of the perfume ingredients. Lowering ^{2}D polarity down to that of a cyano-based phase, yielded acceptable peak widths for all compounds (benzyl alcohol Fig. 2B, $^{2}\omega_h = 78$ ms).

The above-mentioned trials were achieved by using the beginning of the second column as a modulator tube where analytes eluting from the first dimension were cryotrapped (Fig. 1, column sections A and B: SPWax-10). In the literature, several variants of the modulator tube phase have been proposed. Some authors used a transfer line coated with a thicker phase film than both analytical columns, either with the same polarity as the first dimension [11], or with an intermediate polarity between those of both dimen-

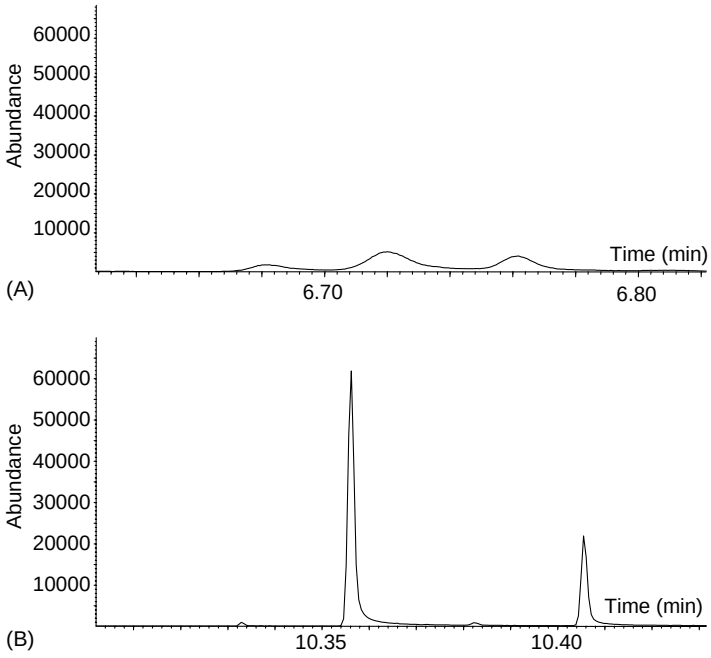

Fig. 2. Elution of benzyl alcohol using (A) DB1(30 m) × SPWax10 and (B) DB1(60 m) × DB225.

sions [12,13]. Alternatively, the end of the first column can be used to achieve the trapping [14].

As the phase of the modulator tube could delay the thermal desorption of analytes, a phase-free, deactivated capillary was tested, but led to an unsatisfactory cryotrapping of analytes (Fig. 3A) in spite of a low temperature in the trap (Fig. 3B). Coupling both columns after the modulator (Fig. 1, connection in B) and trapping analytes at −40 °C in the end of the first column (Fig. 3C) led to the same peakwidth ($^2\omega_h$ = 78 ms) as trapping in the beginning of the second column (Fig. 2B). Since trapping in the beginning of the second dimension or the end of the first dimension was equivalent, this later configuration was used in the continuation of this work.

3.2. Integration accuracy and calibration

As the sampling rate of a quadrupole MS detector was limited to 30.7 Hz, the number of datapoints could be questionable for an accurate measurement of peak areas. For instance, the software used 28 datapoints to evaluate the area of limonene, a very narrow peak ($^2\omega_h$ = 54 ms). However, 92% of the total area was represented by only six datapoints (Fig. 4). Using five replications of the allergen mixture, the repeatability of limonene peaks was evaluated (Table 2). Over three modulations where it was eluted, relative standard deviations (R.S.D.s) were 169, 16 and 59%, respectively. In contrast, the sum of these three peaks only exhibited a R.S.D. of 4.2%. This clearly indicates that the variability of individual peaks is mainly due to the distribution of the amount eluting from the first dimension into different modulations as a function of minor variations of the retention time in the first column.

The overall R.S.D., calculated from all peaks (25 mg/l each) of the calibration mixture and three injections was 6.3%, with a median of individual R.S.D.s of 4.1%. The difference between the median and the mean was notably explained by the first-eluting isomer of farnesol (R.S.D. = 24%), the least repeatable peak, in agreement with a previous observation from its GC–MS quantitation [4]. Therefore this isomer was not used to quantify farnesol. For other compounds, as total areas resulted from more than 10 representative datapoints corresponding to (at least) two modulations, they were considered repeatable enough for quantitation purposes.

The MS response was calibrated between 2 and 100 mg/l for the 28 compounds. All curves were linear with correlation coefficients in the 0.999–1.000 range. The satisfactory integration accuracy was indirectly confirmed by this excellent fit to straight lines. As an example, the limonene calibration is shown in Fig. 5, together with its 99% confidence interval.

3.3. Quantitation

GC × GC of complex perfumes usually gives rise to hundreds of peaks in chromatograms. Due to the absence of

Table 2
Repeatability (5 injections of the mix of standards) of the limonene peak in each modulation and of its total area

Modulation	Retention time (min)	Mean	R.S.D. (%)
1	10.839	44627	169
2	10.889	481623	16
3	10.939	21306	59
Sum (modulation 1–3)	–	547556	4.2

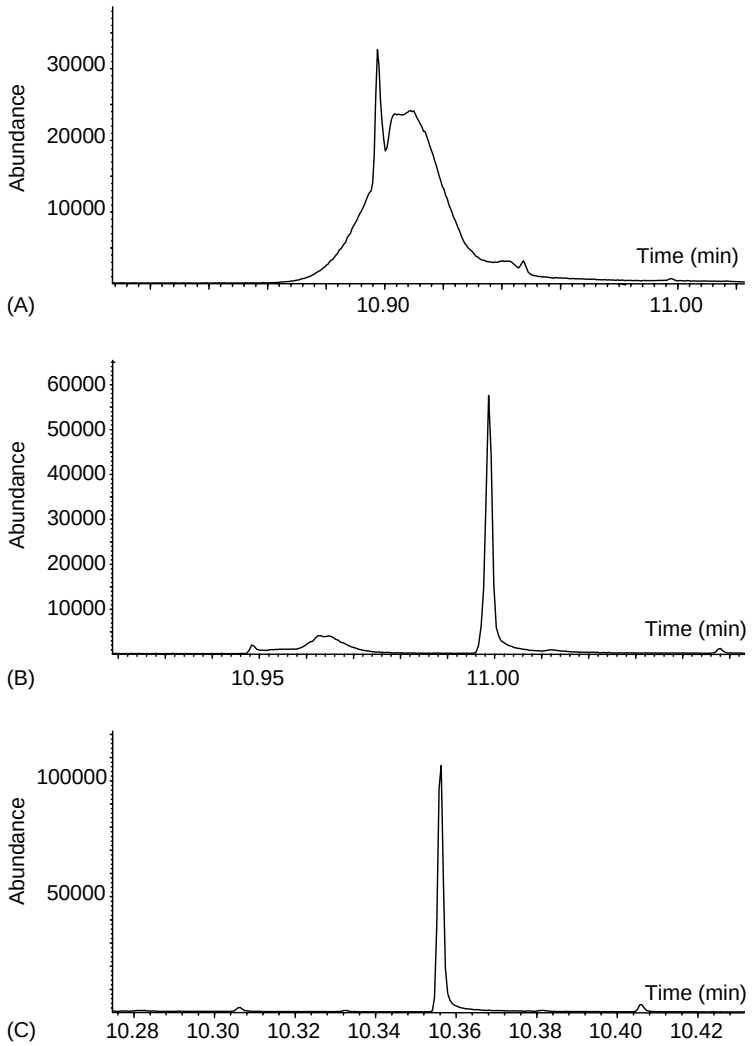

Fig. 3. Peak shape of benzyl alcohol using a modulator tube made of: (A) a deactivated capillary 0 °C, (B) a deactivated capillary, −40 °C; (C) the end of the first column (DB1), −40 °C.

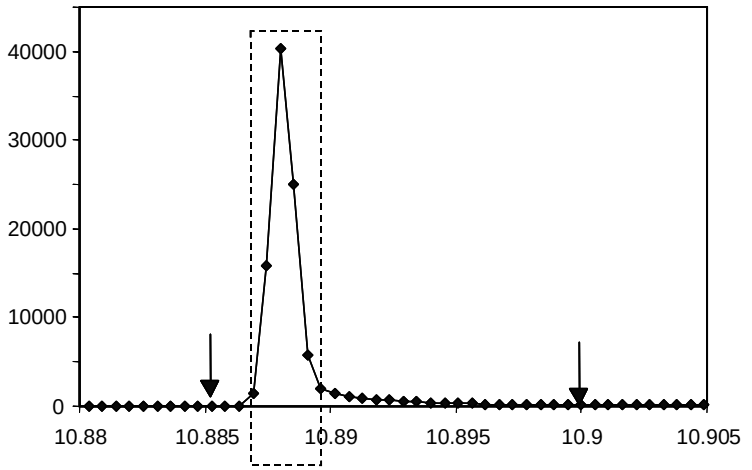

Fig. 4. Software integration of the limonene peak (between arrows). Six datapoints in the dotted-line rectangle represent 92% of the total area.

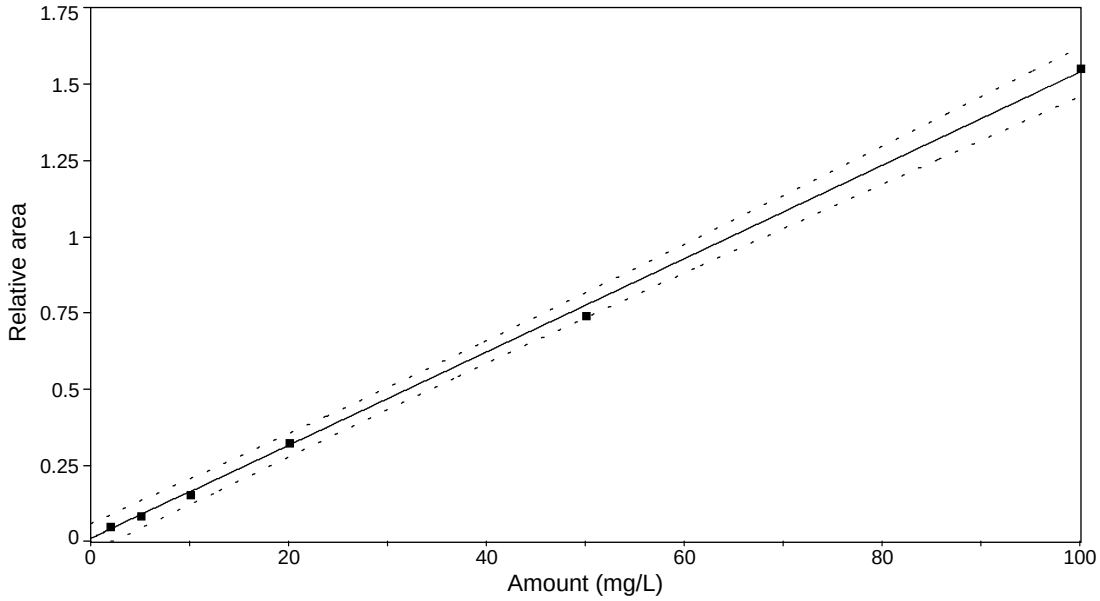

Fig. 5. Limonene calibration curve (straight line) and 99% confidence intervals: $y = 0.015306x + 0.009798$ with $R^2 = 0.9991$.

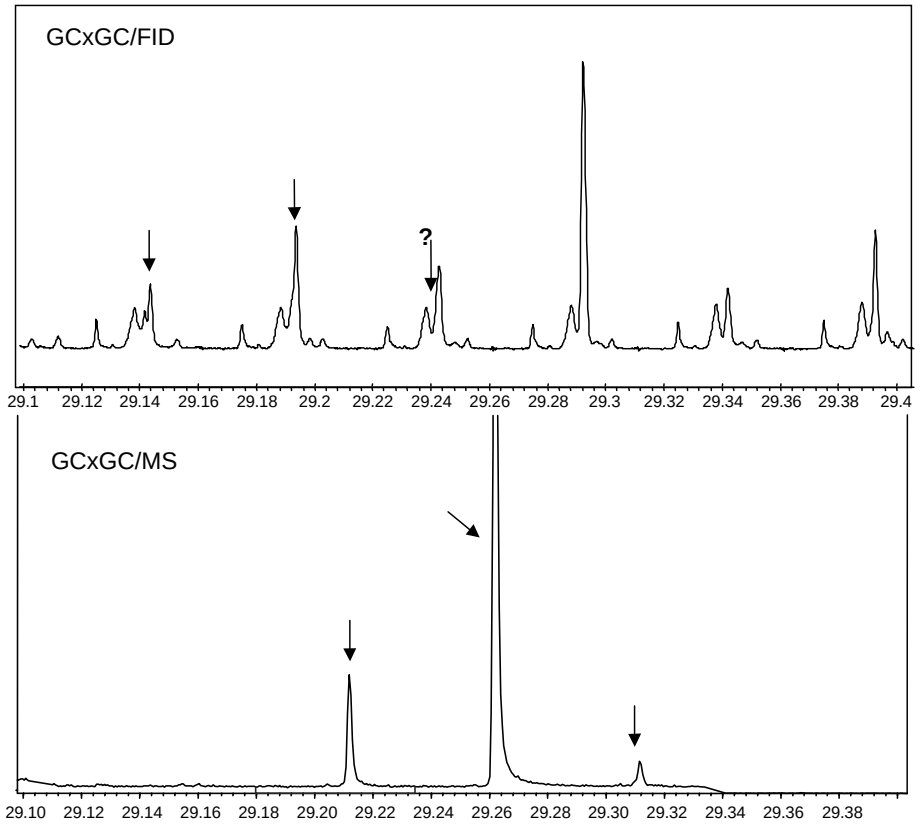

Fig. 6. Peaks of hexyl cinnamaldehyde (marked with arrows and a question mark) within a real fragrance using FID and MS in the SIM mode (both analyses with a DB1 × DB225 configuration).

commercial integration software dedicated to this technique, the target peak selection and integration over several modulations where it is eluted is a tedious task. An additional advantage of using MS detection in the SIM mode was the dramatic simplification of the chromatogram. In many instances, the target compound became the only detected peak within a modulation and its integration was made much easier and free of interference from overlapping peaks (Fig. 6).

To evaluate the quantitation capabilities of the GC × GC–quadrupole MS system, two fragrance concentrates

Table 3
GC–MS and GC × GC–MS quantitation of a fragrance concentrate ("proton") spiked with five compounds at a 50 ppm level (DB1 × SPWax configuration)

Compound	GC–MS	GC × GC–MS
Estragole	46.5	48
Geraniol	45.1	39
Hydroxycitronellol	44.5	55
Methyl nonynoate	42.7	58
Methyl eugenol	47.7	44
Mean	45.2	48.8
R.S.D. (%)	4.1	16.0

Table 4
GC–MS, GC × GC–FID and GC × GC–MS quantitation of a fragrance concentrate ("SVB") spiked with five compounds at a 50 ppm level (second line: phase of the single or second dimension column associated with a DB1)

Compound	GC–MS, DB1	GC × GC–FID, SPBWax	GC × GC–MS	
			SPBWax	DB225
Linalool	6296[a]	510[a]	66	57
Anisyl alcohol	782[a]	n.q.[a]	78	54
Eugenol	43	50	97	70
γ-Methyl ionone	64	52	33	53
Benzyl benzoate	54	54	29	61
Mean	n.a.	n.a.	61	59
R.S.D. (%)	n.a.	n.a.	48	12

[a] Co-elution; n.q.: not quantitable; n.a.: not applicable.

were spiked with five of the target compounds and analyzed in comparison with the GC–MS technique [4]. The simplest fragrance gave an evaluation close to the GC–MS, with a higher R.S.D., but a mean estimation closer to the expected value (Table 3).

The most complex fragrance (168 constituents) could not be quantitated by GC–MS in a single analysis. Linalool and anisyl alcohol were co-eluted with compounds exhibiting the same ion as the one used for the target compound, as shown by the calculation of the *Q*-value (Table 4, second column, see details in [4]). In a previous evaluation [10], GC × GC–FID also failed to evaluate the same two compounds because of unresolved peaks value (Table 4, third column). In the present study, the increased detection specificity allowed to evaluate the five spiked compounds with the same column configuration as the GC × GC–FID evaluation (DB1 × SPWax), but with deviations due, e.g. to the peak broadening inherent in the elution of alcohols with a SPWax, as previously investigated (Table 4, fourth column). In contrast, using a DB225 as the second dimension, values were less dispersed around the expected amounts (Table 4, fifth column).

4. Conclusion

From this first investigation of the quantitative capabilities of quadrupole MS coupled with comprehensive GC, this system shows good performances when applied to the evaluation of allergens in fragrance concentrates. Its improved selectivity, compared to GC–MS and GC × GC–FID, allows the determination of target compound concentrations which may not be achieved in a single run with these two other techniques.

The optimization of analytical parameters also indicates that: (1) the greatest phase orthogonality does not lead to a satisfactory peak shape; and (2) a good evaluation of the total area associated with a given compound is observed over its 2–3 modulations, in spite of the few representative data points within a single peak.

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