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Original Paper

Fast gas chromatography-full scan quadrupole mass spectrometry for the determination of allergens in fragrances

The Scientific Committee for Cosmetics and NonFood Products in the 7th Amendment to the European Cosmetics Directive established 26 fragrance components, widely used in cosmetic products, as being responsible for a series of contact allergies (Directive 2003/15/EC, Official Journal of the European Union, L66/26, 11.3.2003). The regulation foresees that any allergen, present in excess of 100 mg/kg in rinse-off and of 10 mg/kg in leave-on formulations, must be reported on the label of the product. The present research reports a fast GC-full scan quadrupole mass spectrometric method (under 5 min) for the qualitative/quantitative analysis of allergens in perfumes. Reliable peak identification was achieved through a twin-filtered MS library matching procedure, considering a minimum degree of spectral similarity (90%) and retention data (a linear retention index window was applied). Peak quantification was carried out by using a specific extracted ion. In case a suspected allergen fell within its retention time window but presented a low degree of spectral purity (<90%), analyte determination was achieved by using three extracted ions (one quantifier and two qualifiers). The fast GC-MS method was validated in terms of intraday retention time and peak area precision, LODs and LOQs, and method linearity. Finally, peak skewing was also evaluated and was within more than acceptable limits.

Keywords: Allergens / Fast GC-MS / Fragrances / Microbore columns / Perfumes

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1 Introduction

In general, perfumes are complex mixtures characterized by fragrant essential oils and aroma compounds, fixatives, and solvents. The objective of their use is to generate pleasant and attracting smells in human bodies, living areas, and objects. The complete elucidation of perfume compositions is rather difficult, as the formulas are kept secret for obvious reasons [1]. Perfumes are also considered to be one of the causes of cosmetic-related allergic contact dermatitis [2, 3] and the European Commission Scientific Committee on Cosmetics and other Non-Food Products (SCCNFP) recently listed 24 volatile skin-sensitizing ingredients (therefore, amenable to GC analysis), commonly defined as allergens [4].

A sample (and related GC method) classification for the analysis of suspected allergens has been proposed. Perfumes can be classified in the class II, considering the high number of compounds (generally more than hundred) and their wide range of concentration [5]. In these cases, a single capillary column may be (or may not be) sufficient for satisfactory analyte separation. Whenever the complexity of a class II sample exceeds the potential of a monodimensional GC system, then a multidimensional approach must be considered [5, 6]. Mondello *et al.* [6] reported a comprehensive GC-MS experiment applied to a complex perfume: it was shown that a single peak in a monodimensional application was the summation of eight different components. In such a situation, the reliable identification and quantification of a fragrance constituent by using any of the most common GC-MS approaches (full scan, extracted ion, single ion monitoring (SIM), peak deconvolution) appears to be, at the least, problematic.

Nevertheless, single column GC-MS may be successfully applied to many “medium” complex class II samples. Hence, the development of both effective and rapid

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Abbreviation: EIC, extracted ion chromatogram

analytical procedures for the unveiling of perfume profiles is currently of great interest. The analysis of these sample-types by using direct injection and conventional GC-MS in the SIM mode [5, 7, 8], or in the full scan-extracted ion mode [9] has been reported. Chaintreau *et al.* [7] recognized that, although the SIM mode (three ions were monitored) provided satisfactory results, the identification of a given compound in the full scan mode was sometimes necessary. As a consequence, additional applications, recalibration, *etc.*, would be required. Leijts *et al.* [9] affirmed that, due to the specificity of the extracted ions, the chances of coelution with isobaric ions was minimized. Whatever method is applied, it seems that the possible coelution of ions must always be considered.

With respect to the GC velocity, the employment of reduced internal diameter capillaries is probably the most effective way of increasing sample throughput. It is well known, in fact, that band enlargement effects inside microbore columns are restricted and, thus, resolving power is enhanced. Consequently, column lengths may be shortened while separation power is not impaired if compared to conventional GC methods [10–12]. Additional advantages are related to higher optimum mobile phase linear velocities and to higher-than-ideal gas flows, which may be applied without significantly affecting column efficiency. Major drawbacks are the necessity of more drastic experimental conditions and the lower sample capacity. The former issue has been overcome by the introduction of adequate GC instrumentation while with regards to the other aspect, the generation of narrower analyte bands essentially counterbalances the injection of lower sample amounts. If the experimental parameters are properly tuned, conventional GC profiles may be entirely reproduced. Theoretical and practical discussions relative to microbore column fast GC have been widely reported in the literature [10–12].

In the present research, a fast full scan GC-MS method for the analysis of allergens in perfumes is proposed. The main novelty of the work, with respect to others, consists essentially in the very short run-time (the conventional GC-MS chromatographic profile was reproduced in less than 5 min) and in the use of a twin-filtered MS library search procedure for reliable peak identification, applied with satisfactory results in a previous comprehensive GC perfume application [6]. Quantification was carried out by using an extracted ion chromatogram (EIC). A suspected allergen overlapping with interfering compounds (an assumption derived from the low spectral quality) was determined by using multiple extracted ions.

2 Experimental

2.1 Samples and sample preparation

C₇–C₃₀ hydrocarbons in *n*-hexane solutions (0.1 µg/mL) were purchased from Supelco (Milan, Italy).

Amyl cinnamaldehyde, anisyl alcohol, benzyl alcohol, benzyl cinnamate, methyl 2-octynoate, citral, cinnamaldehyde, benzyl benzoate, benzyl salicylate, cinnamyl alcohol, amyl cinnamyl alcohol, coumarin, eugenol, isoeugenol, farnesol (four isomers with the *Z,E* and *E,E* isomers predominant), citronellol, geraniol, hydroxycitronellal, hexylcinnamaldehyde, limonene, α -isomethyl ionone, linal, linalool, lylal, and the two internal standards 1,4-dibromobenzene and of 4,4'-dibromobiphenyl were purchased from Sigma–Aldrich (Milan, Italy). The purity of each standard was verified and was accounted for prior to use, through GC-MS analysis: each was above 95% purity except for amyl cinnamyl alcohol (which was 50%; the other compound was dihydroamylcinnamic alcohol) and α -isomethyl ionone (85%). A special note should be made for *d*-limonene, as the EC directive refers to this specific enantiomer (CAS registry no. 5989-27-5). Although reference will be made to *d*-limonene throughout the paper, the method herein proposed does not enable the distinction between *d*- and *l*-limonene. All standard compounds were stored in hermetically close vials in a freezer (–25°C). A stock solution of approximately 10 g/L of each allergen was prepared in ethanol (Table 1). Calibration solutions were prepared by diluting the stock solution with ethanol to approximately the 1000, 100, 10, 5, 1, 0.5, and 0.1 mg/kg level. A 10 000 mg/kg internal standard solution was prepared by diluting 10 mg of 1,4-dibromobenzene (IS1) and 10 mg of 4,4'-dibromobiphenyl (IS2) in ethanol. Ten microliters of this was then added to each calibration solution. The allergen areas were reported to the closest eluting internal standard.

Four commercial perfumes (defined as samples I, II, III, and IV), purchased in a local store (Messina, Italy), were injected neat spiked with the internal standard solution (final concentration in the perfume: 100 mg/kg).

2.2 Experimental parameters

All analyses were carried out on a Shimadzu GCMS QP2010 gas chromatograph-quadrupole mass spectrometer, equipped with an AOC20i autoinjector and split/splitless injector (280°C) (Shimadzu, Milan, Italy). Fast GC-MS: column, SLB-5ms 10 m × 0.10 mm id, 0.10 µm film thickness (Supelco, Bellefonte, PA, USA); initial helium head pressure, 378 kPa; constant linear velocity, 55 cm/s; temperature program, from 40 to 250°C at 40°C/min; injection volume and mode, 0.2 µL, split (50:1).

All samples were analyzed in the full scan mode with a scan speed of 10 000 amu/s, a mass range of *m/z* = 40–350 and a sampling rate of 25 spectra/s; interface and ion source temperatures were 280 and 250°C, respectively. A 1 min solvent cut time was applied. All data were collected by the GCMS solution software (Shimadzu). The allergens were identified by using the FFNSC 1.2 mass spectral library database (Shimadzu).

Table 1. RSD values (RSD%) for retention times and peak areas (100 mg/kg solution); full scan LOD, extracted ion LOQ and LOD for a minimum 90% spectral similarity match (LOD 90%) for each allergen

No.	Allergen mix	RSD % t_R	RSD% areas	LOD (mg/kg)	LOQ (mg/kg)	LOD 90% (mg/kg)
1	Limonene	0.17	3.4	2.2	1.8	2.5
2	Benzyl alcohol	0.07	2.1	2.1	3.6	0.9
3	Linalool	0.07	0.1	1.4	3.1	0.9
4	Methyl 2-octynoate	0.02	2.2	3.5	4.3	2.5
5	Citronellol	0.02	3.2	0.9	5.7	0.9
6	Neral (citral)	0.02	3.4	2.4	2.7	2.2
7	Geraniol	0.07	0.1	0.9	2.4	0.9
8	Geranial (citral)	0.04	3.5	3.6	2.3	1.9
9	Cinnamaldehyde	0.05	3.1	4.4	3.4	4.8
10	Anysil alcohol	0.05	0.1	3.2	4.4	6.5
11	Hydroxycitronellal	0.32	4.4	3.3	3.1	2.4
12	Cinnamic alcohol	0.03	0.2	8.8	7.1	4.5
13	Eugenol	0.15	3.0	3.0	3.1	4.5
14	Coumarin	0.14	0.1	4.0	2.0	3.2
15	Isoeugenol	0.04	3.0	0.8	3.9	0.8
16	α -Isomethyl ionone	0.05	0.1	2.0	2.7	2.5
17	Butylphenyl methylpropional	0.04	5.0	2.9	2.2	2.5
18	Amyl cinnamic aldehyde	0.02	0.2	2.1	0.9	2.1
19	Hydroxyisohexyl-3-cyclohexene carboxaldehyde	0.01	0.2	1.1 (0.6 ^a)	2.0	1.1
20	Amyl cinnamic alcohol	0.02	0.1	1.7	3.2	3.1
21	Farnesol	0.04	1.3	5.4	5.1	4.7
22	Hexyl cinnamic aldehyde	0.03	0.1	2.1	6.0	2.9
23	Benzyl benzoate	0.01	0.1	2.9	1.1	2.9
24	Benzyl salicylate	0.12	2.5	3.8	3.0	4.8
25	Benzyl cinnamate	0.02	1.3	4.4	3.0	4.5

^a) Extracted ion value.

2.3 Method validation procedures

In all method validation experiments, mean values were derived from five consecutive applications.

All LODs were determined by measuring the mean noise value plus 3 SDs of the blank mean. All LOQs were determined by measuring the mean noise value plus 10 SDs of the blank mean.

3 Results and discussion

3.1 Fast GC-MS perfume analysis

Initially, four commercially available perfumes were subjected to conventional full scan GC-MS analysis (a 30 m $\times$ 0.25 mm id apolar column was employed) under what may be defined as ideal analytical conditions, using helium as a carrier gas for safety precautions (data not shown). The objective was to reproduce the conventional chromatographic profiles, in rapid GC-MS applications. Although conventional GC analyses times were between 65 and 90 min in the present study, it must be noted that these are slightly higher than those generally observed for such samples (30–50 min).

The positive effects of working under a vacuum column outlet conditions are well-known: high speed separations (the optimum linear velocity may near the 100 cm/s mark) on low-complexity samples may be attained by using a short, megabore column (*i.e.*, 0.53 mm id) with obviously few problems related with sample capacity. Moreover, peak widths are not as low as in microbore fast GC, therefore very high MS acquisition rates are not necessary [13]. Although the merits of this fast GC technique are undoubtful, it does not meet the column efficiency requirements for perfume analysis.

Although the positive effects of low pressure conditions are not exploited by using microbore columns, as high analyte–gas diffusion coefficients are present only in a short detector-end capillary segment, a 10 m $\times$ 0.10 mm id apolar column was tested: the helium linear velocity (55 cm/s) was near to ideal ($\sim$ 50 cm/s) while the temperature program rate (40°C/min) was slightly higher than the optimum, which is 10°C per void time ($\sim$ 30°C/min) [14]. The number of theoretical plates generated by the microbore column ($\sim$ 100 000) enabled the generation of similar chromatographic profiles with respect to the conventional applications, even though slight losses in resolution were observed. Most importantly, all target analytes remained sufficiently sepa-

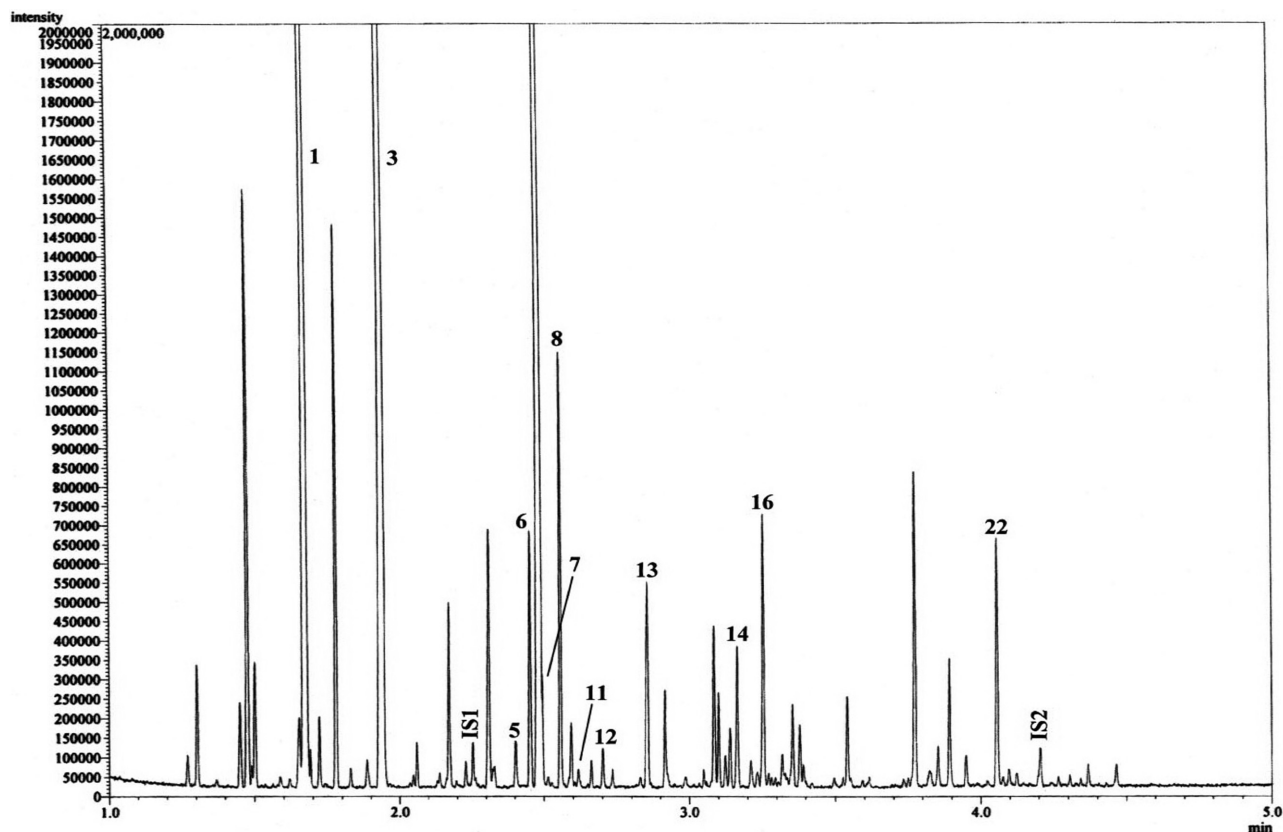


Figure 1. TIC fast GC-MS chromatogram (Sample I). For allergen identification, refer to Table 1.

rated. A total ion current (TIC) fast GC-MS chromatogram relative to sample I is reported in Fig. 1; the GC run-time was greatly reduced with respect to the conventional analysis (all applications were carried out in less than 5 min). Twelve allergens, numbered in the figure (for peak assignment refer to Table 1), were positively identified by using the twin-filtered MS library process: the library software automatically deleted from the list of matches, compounds with a lower than 90% spectral similarity and with an LRI value outside a rather wide range (for an apolar column) of ± 10 units. Prior to the fragrance analyses, a C_7 – C_{30} hydrocarbon solution was subjected to fast GC-MS. The LRI value, for any peak of interest, was directly calculated by the software. After peak assignment, quantitation was carried out by using an extracted ion. Reference/experimental LRI values, quantifier ions, spectral similarities and measured concentrations (in mg/kg) for all four samples are reported in Table 2. Altogether, 14 allergens were determined (compounds 6 and 8 correspond to citral); the spectral purity was generally very good: out of 39 matches, 33 were in the 94–99% range. In all applications, peak base widths ranged from approximately 900 to 1500 ms. The MS sampling frequency was 25 spectra/s, which guaranteed correct peak

reconstruction (at least 20 data points were attained for the fastest peaks). It must be noted that the quantitative results, which were attained by constructing calibration curves for each allergen and by using two internal standards (see Section 2.1), were not in contradiction with the allergens reported (or not reported) on the perfume labels (>10 mg/kg) and, therefore, with the current legislation. Obviously, no specific information on concentrations are reported and, hence, a direct comparison of data can not be made. Furthermore, it must be considered that fragrance manufacturers may label allergens which are very close but under the limit. The extracted ions used for the quantitation were the same as the SIM ions advised by the International Fragrance Association (IFRA) [7], except for geraniol: this allergen underwent partial coelution with considerable amounts of linalyl acetate in samples I (the large peak before peak 7 in Fig. 1), II, and III. A chromatogram was extracted for the quantifier ion $m/z = 69$, which is present in abundant amounts also in the spectrum of linalyl acetate. Geraniol was overestimated by approximately 20% in samples II (114 mg/kg) and III (92 mg/kg) but not in sample I (where linalyl acetate was contained in lower quantities). In order to overcome this source of inaccuracy, another

Table 2. Quantifier ions (EIC), library LRI values (LRI ref.), calculated LRI values (LRI calc.), % spectral similarity (Simil.%), and calculated concentrations (mg/kg) for allergens determined in the four perfumes by means of fast GC-MS

No.	EIC	LRI ref.	Sample I			Sample II			Sample III			Sample IV		
			LRI calc.	Simil.%	Conc. (mg/kg)	LRI calc.	Simil.%	Conc. (mg/kg)	LRI calc.	Simil.%	Conc. (mg/kg)	LRI calc.	Simil.%	Conc. (mg/kg)
1	68	1030	1037	97	2493	1034	97	3183	1035	97	1618	1035	93	1993
3	93	1101	1108	97	2185	1107	98	2559	1107	97	995	1106	98	886
5	95	1232	1225	94	43	1233	97	880	1235	97	233			
6	69	1238	1231	96	48	1245	95	7	1244	95	45	1245	91	5
7	123	1255	1259	96	36	1260	98	98	1260	97	80			
8	69	1268	1261	99	81	1275	95	13	1274	97	31	1275	94	7
9	131	1273										1280	95	127
11	59	1291	1298	92	11				1294	97	82	1295	97	254
12	92	1309	1313	94	37									
13	164	1357	1365	96	103							1365	94	26
14	146	1438	1441	92	43							1445	97	1460
16	206	1473	1480	95	80	1481	97	1030				1480	97	380
17	189	1535				1543	95	3222						
19	192	1671				1679	88	255	1679	92	302	1680	95	1357
22	216	1746	1750	98	90									

quantifier ion was chosen ($m/z = 123$), after consulting the mass tables. With regard to spectral similarity, after the twin-filtered GC-MS applications, each sample was subjected to a single filter analysis (only the LRI filter was used) in order to verify the possible presence of allergens with a low spectral purity: only lyral determined in sample II presented a less than 90% value, presumably due to the additional presence of one or more overlapping analytes. In this single case, two extracted ions (136, 149) were used for identification and a single one for quantification (192). The relative abundances of these ions were the same as those reported by IFRA [7]. As previously mentioned, a multidimensional GC method (either heart-cutting or comprehensive) would provide a more reliable result in this type of situation.

3.2 Method validation

In fast GC-quadrupole MS applications, rapid analyte bands are introduced into the ion source and the sampling rate should be high enough to guarantee that analyte concentration changes that occur therein should not generate substantial relative ion intensity differences across a peak. The possible generation of inconsistent mass spectra across a peak, an effect defined as “peak skewing”, is obviously an aspect that must be monitored. In fact, the direct consequence of peak skewing is that a single acquired spectrum relative to a specific analyte may present a different ion profile with respect to the mass spectrum of the same compound present in the MS library. The effect of mass spectral skewing was investigated for all allergens. In Fig. 2, mass ratios were plotted for two abundant ions in each acquired spectrum for standard linalool (19 data points; ions 121 and 41) and

amyl cinnamaldehyde (22 data points; ions 204 and 55). Mass ratio data was the mean of five consecutive injections. Mass spectral skewing appeared to be very restricted, as RSD% values of 2.7 and 4.0 were calculated for linalool and amyl cinnamaldehyde, respectively.

Fast GC-MS intra-assay retention time and peak area repeatability were determined; RSD% values relative to an application on an allergen 100 mg/kg standard solution are reported in Table 1. As it can be observed, RSD% values for retention times are quite satisfactory (0.32% was the maximum value), while those for peak areas are very good, with no component over 5%. Full scan fast GC-MS LODs, relative to all the allergens, were between 0.8 and 8.8 mg/kg. The extracted ion LOD value was determined only for lyral (0.6 mg/kg), as this was the only allergen that presented a spectral similarity of less than 90% (88% in sample II). The minimum analyte concentration necessary to generate a spectral similarity of at least 90% (reported as LOD 90% in Table 1) was also determined; the results attained were similar in most of the cases with the normal LOD values (0.8–6.5 mg/kg range). In some cases, the allergen concentration required to generate a spectral similarity of minimum 90% was higher than the normal LOD (*i.e.*, limonene and anisyl alcohol), while surprisingly it was slightly lower in others (*i.e.*, linalool and benzyl alcohol). Although it was expected that the LOD 90% values would always have been higher, in a series of cases, peaks below the “3 SDs of the noise mean” maintained a 90% (or higher) spectral similarity. It may rarely occur, therefore, that an allergen could be below its normal LOD value (and hence not be considered as an analyte) but could at the same time present a spectral similarity of $\geq 90\%$. With regards to the extracted ion GC-MS LOQs, these were between 0.9 and

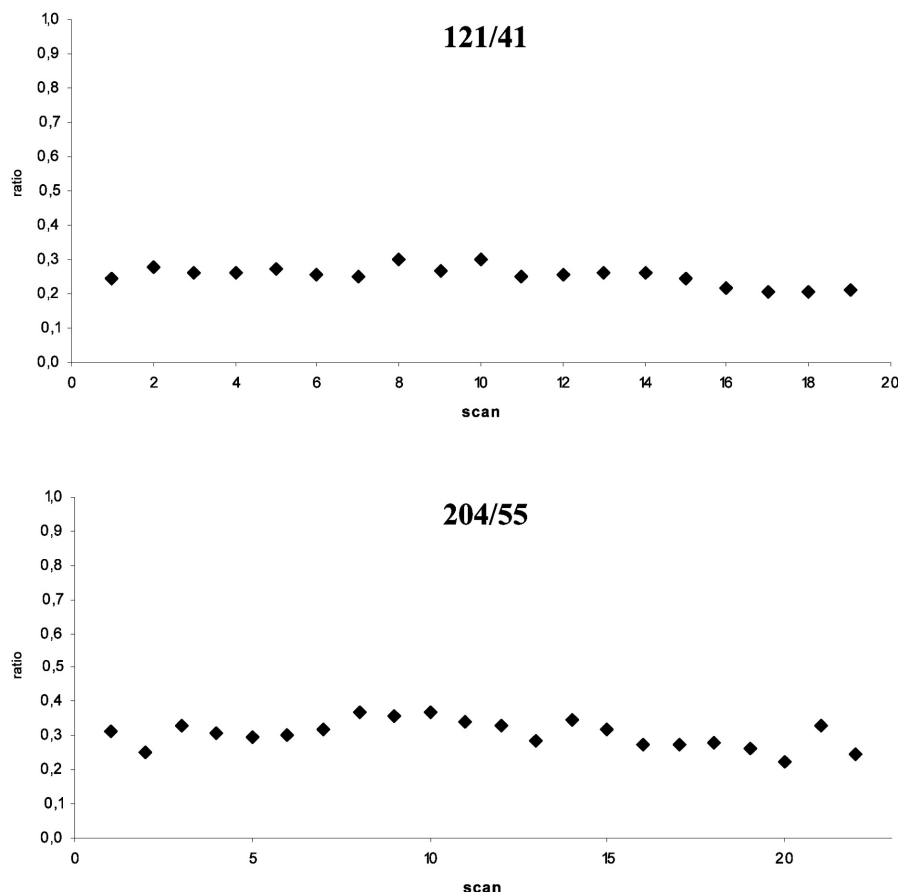


Figure 2. Graphs reporting mass ratios for ions 121 and 41 (linalool) and ions 204 and 55 (amyl cinnamaldehyde).

7.1 mg/kg. As it can be observed, all values were below the 10 mg/kg mark (all the aforementioned data are reported in Table 1). Finally, the method linear detection range was evaluated over approximately three orders of magnitude (LOD-1000 mg/kg), with regression coefficients (R^2) always over 0.9990.

4 Concluding remarks

The GC-MS method described in the present paper has proved to be a rapid and effective screening tool in the determination of allergens contained in mediumly complex perfumes. The option of a rapid response, for matrices of such importance (the method may also be extended to raw materials), may be considered a valuable one. In particular, the employment of a twin-filtered MS library search procedure was shown to be a powerful tool for reliable compound identification. The use of the full scan mode highlighted the presence of interferences co-eluting with a target component, whenever this occurred and, therefore, enabled the most appropriate ion chromatogram to be extracted (*i.e.*, geraniol in sample I) for effective quantitation. Moreover, the analytical benefits of full spectral information were provided for all sample

components. As for all monodimensional methods, it may fail when the number of sample volatiles greatly exceeds the peak capacity of a single column: the reliable qualitative/quantitative determination of 10–20 skin sensitizers amongst 500–1000 other volatiles would be a very difficult task. Obviously, the term “mediumly complex” is a general definition and does not provide a precise indication on the sample type that may be subjected to this kind of analysis: a good result may be attained for a 200-component fragrance and a bad one for a 150-compound perfume. The most appropriate approach to be used, if a multiple choice exists, is strictly dependent on the experience and judgement of the analyst.

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5 References

- [1] Pybus, D. H., Sell, C. S., *The Chemistry of Fragrances*, The Royal Society of Chemistry, Cambridge, UK 1999.
- [2] Rastogi, S. C., Menné, T., duus Johansen, J., *Contact Derm.* 2003, 48, 130.

- [3] Adam, R. M., Maihaeh, H. J., *J. Am. Acad. Dermatol.* 1985, 13, 1062–1069.
- [4] Fragrance Allergy in Consumers, SCCNFP/0017/98 Final, December 1999, EC: Brussels.
- [5] David, F., Devos, C., Sandra, P., *LC-GC Europe* 2006, 19, 602–616.
- [6] Mondello, L., Casilli, A., Tranchida, P. Q., Dugo, G., Dugo, P., *J. Chromatogr. A* 2005, 1067, 235–243.
- [7] Chaintreau, A., Joulain, D., Marin, C., Schmidt, C. O., Vey, M., *J. Agric. Food Chem.* 2003, 51, 6398–6403.
- [8] Analytical procedure for the GC/MS quantitation of potential fragrance allergens in fragrance compounds; version 1. *Analytical Procedures*, International fragrance Association, Brussels, Belgium 2003, pp. 1–10.
- [9] Leijts, H., Broekmans, J., van Pelt, L., Mussinan, C., *J. Agric. Food Chem.* 2005, 53, 5487–5491.
- [10] Korytár, P., Janssen, H.-G., Matisová, E., Brinkman, U. A. T., *Trends Analyt. Chem.* 2002, 21, 558–572.
- [11] Sandra, P., Proot, M., Diricks, G., David, F., in: Bicchì, C., Sandra, P. (Eds.), *Capillary Gas Chromatography in Essential Oil Analysis*, Hüthig, Heidelberg 1987.
- [12] Mondello, L., Tranchida, P. Q., Casilli, A., Favoino, O., Dugo, P., Dugo, G., *J. Sep. Sci.* 2004, 27, 1149–1156.
- [13] Mastovská, K., Lehotay, S. J., *J. Chromatogr. A* 2003, 1000, 153–180.
- [14] Blumberg, L. M., Klee, M. S., *Anal. Chem.* 1998, 70, 3828–3839.