

Determination of suspected allergens in cosmetic products by headspace-programmed temperature vaporization–fast gas chromatography–quadrupole mass spectrometry

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Abstract In the present work, a strategy for the qualitative and quantitative analysis of 24 volatile compounds listed as suspected allergens in cosmetics by the European Union is reported. The list includes benzyl alcohol, limonene, linalool, methyl 2-octynoate, β -citronellol, geraniol, citral (two isomers), 7-hydroxycitronellal, anisyl alcohol, cinnamal, cinnamyl alcohol, eugenol, isoeugenol (two isomers), coumarin, α -isomethyl ionone, lilial®, α -amylcinnamal, lyral®, α -amylcinnamyl alcohol, farnesol (three isomers), α -hexyl cinnamal, benzyl cinnamate, benzyl benzoate, and benzyl salicylate. The applicability of a headspace (HS) autosampler in combination with a gas chromatograph (GC) equipped with a programmable temperature vaporizer (PTV) and a quadrupole mass spectrometry (qMS) detector is explored. By using a headspace sampler, sample preparation is reduced to introducing the sample into the vial. This reduces the analysis time and the experimental errors associated with this step of the analytical process. Two different injection techniques were used: solvent-vent injection and hot-split injection. The first offers a way to improve sensitivity at the same time maintaining the simple headspace instrumentation and it is recommended for compounds at trace levels. The use of a liner packed with Tenax-TA® allowed the compounds of interest to be retained during the venting process. The signals obtained when hot-split injection was used allowed quantification of all the compounds according to the thresholds of the European Cosmetics Directive. Monodimensional gas chromatography coupled to a conventional quadrupole mass

spectrometry detector was used and the 24 analytes were separated appropriately along a run time of about 12 min. Use of the standard addition procedure as a quantification technique overcame the matrix effect. It should be emphasized that the method showed good precision and accuracy. Furthermore, it is rapid, simple, and—in view of the results—highly suitable for the determination of suspected allergens in different cosmetic products.

Keywords Allergens · Headspace analysis · Programmed temperature vaporization · Cosmetic products

Introduction

Twenty-six chemicals used in cosmetic products are suspected as being responsible for skin reactions. Following the European Cosmetic Directive [1], an indication of the presence of potential allergens in cosmetic products is required if a limit of 0.01% (100 mg/kg) for rinse-off and 0.001% (10 mg/kg) for leave-on products is exceeded. Of these 26 suspected allergens, 24 are volatile compounds and can be analyzed by gas chromatography. The other two are natural extracts (oak and tree moss). A list of the analytes studied is given in Table 1.

The range of matrices and concentrations at which these suspected allergens must be determined is very extensive and hinders the analysis of all compounds with a single method [2]. Additionally, samples that contain volatile or semivolatile solutes at a very broad concentration range or samples that contain non-volatile materials such as natural extracts are good examples of the varying complexity of the samples.

In most cases, the preparation of samples of liquid products consists of a single dilution step. However, in

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Table 1 Target compounds, SIM windows, retention times and m/z ions selected for template generation

Compound	SIM window	t _R (min)	m/z selected
Benzyl alcohol (No 1)	1	5.798	79, 108
Limonene (No 2)		5.811	68 , 93
Linalool (No 3)	2	6.225	71 , 93
Methyl 2-octynoate (No 4)	3	6.880	95 , 123
β-citronellol (No 5)	4	7.035	41 , 69
Geraniol (No 6)	5	7.212	41, 69
Geranial -isomer 1 (No 7)		7.238	41, 69
Geranial -isomer 2 (No 7')	6	7.424	41, 69
7-hydroxycitronellal (No 8)		7.499	43, 59
Anisyl alcohol (No 9)		7.518	109, 138
Cinnamal (No 10)		7.556	103, 131
Cinnamyl alcohol (No 11)	7	7.667	92 , 134
Eugenol (No 12)	8	7.970	103, 164
Isoeugenol -isomer 1 (No 13)		8.279	131 , 164
Isoeugenol -isomer 2 (No 13')		8.562	131 , 164
Coumarin (No 14)	9	8.673	118, 146
α-isomethylionone (No 15)	10	8.764	135 , 150
Lilial ® (No 16)	11	8.976	147, 189
α-amylcinnamal (No 17)	12	9.710	91, 117
Lylal ® (No 18)		9.811	93, 136
Farnesol -isomer 1 (No 19)		9.830	41, 69
α-amylcinnamyl alcohol (No 20)		9.861	91, 133
Farnesol -isomer 2 (No 19')		9.871	41, 69
Farnesol -isomer 3 (No 19'')	13	10.01	41, 69
α-hexylcinnamal (No 21)	14	10.43	117, 129
Benzyl cinnamate (No 22)		10.52	91 , 131
Benzyl benzoate (No 23)	15	10.69	91, 105
Benzyl salicylate (No 24)	16	11.82	65, 91

(Ions in bold were used for quantitation)

products containing high-molecular weight or non-volatile compounds, such as shampoos or creams, a special sample clean-up step is required in order to prolong the life of the liner and the capillary column and to avoid contamination problems between injections. To remove unwanted matrix components from the target substances, different methods have been proposed, such as size-exclusion chromatography (SEC) [3] and automated liner exchange (ALEX) [2], in which the liner is automatically replaced after each injection into a gas chromatograph. Another alternative that does not involve any additional sample treatment and does not lead to an increase in analysis time is the use of a HS sampler. In headspace analysis, a liquid or solid sample is placed in a sealed vial for equilibration at high temperature. Then, a known aliquot of the gas phase is transferred to the analytical instrument, avoiding the injection of non-volatile compounds and minimizing the matrix effect.

Solid-phase microextraction (SPME) [4] and HS-SPME [4, 5] have sometimes been used to improve sensitivity. However, this step prolongs analysis time and increases the errors associated with these steps of the analytical process.

The use of a programmed temperature vaporizer (PTV) inlet to inject the samples is an attractive option that allows different modes of injection, including hot-split and solvent-vent injection. This latter setup is a preconcentration technique in the injection port that offers benefits in terms of simplicity and automation possibilities, together with very good sensitivity [6, 7].

Monodimensional gas chromatography–mass spectrometry (GC-MS) [2–4, 8–14] and gas chromatography–flame ionization detection (GC-FID) [11] are commonly employed in the analysis of major and minor components of cosmetic samples. Unfortunately, the occurrence of co-elution is frequent in this type of analysis and it prevents GC-FID from overcoming the difficulty of quantifying compounds co-eluted with other analytes. The huge complexity of some samples means that MS spectra are rarely pure-compound spectra. In this case, mass spectral deconvolution methods can be of significant assistance [15, 16]. Some research groups have explored the possibilities of comprehensive two-dimensional GC×GC-FID [17–19], GC×GC-MS [18, 20–22], multidimensional GC-FID [19], and dual column

GC-MS [23] as other alternatives. However, these methods often require longer analysis times than those employed here. A review focused on analytical methods for allergenic fragrance-related substances in cosmetics have been recently published [24].

In this work, we propose a new method with quantitation limits lower than the threshold values indicated by the European Cosmetic Directive for the determination of 24 suspected allergens in cosmetic products. Two different injection techniques were used. The first is proposed for samples with very low concentrations of analytes and is based on solvent-vent injection. The second technique (hot-split injection) is recommended when there is a high concentration of matrix components or analytes. In both cases, fast gas chromatography with a conventional quadrupole MS detector in the selected ion-monitoring acquisition mode (HS-PTV-GC-MS (SIM)) is used. This is the first application of HS-PTV devoted to the identification and quantification of all those allergens related to fragrances declared to cause contact allergies when applied to the skin.

Experimental

Chemicals

Propyl acetate and the 24 suspected allergens (Table 1) were supplied by Sigma-Aldrich (Steinheim, Germany). Purities of the reference compounds are at least 95% except for cinnamal (93%) α -isomethylionone (85%), and α -amylcinnamyl alcohol (85%).

Standard solutions and samples

Stock solutions of the 24 suspected allergens (concentrations ranging from 6.7 to 13.5 g/L) were prepared in propyl acetate. Working solutions and calibration standards were prepared by appropriate dilutions of the stock solutions in propyl acetate. To perform these measurements, 20 μ L of standard solution was placed in 10-mL glass vials (Agilent Technologies, DE, Germany) sealed with Teflon/silicone septa (Agilent Technologies, Germany). Each sample was analyzed in triplicate (three vials; one injection per vial).

Three perfumes, two anti-hair loss products, a post-depilation mousse, a cream deodorant, and three different cream samples (body, sun, and hand creams) were analyzed. The samples were purchased from Spanish supermarkets. To perform the measurements of liquid samples (perfumes and anti-hair loss products), 10 μ L of the raw or diluted sample plus 10 μ L of the solvent (propyl acetate) or solvent-allergen solution was placed in 10-mL glass vials sealed with Teflon/silicone septa. For the

mousse, deodorant, and cream samples, a portion of 1 g was weighed into a centrifuge tube and 2 mL of solvent (propyl acetate) were added. The tube was closed and shaken vigorously for 5 min using a vortex device. To perform the measurements, 10 μ L of the vortex-homogenized mixture plus 10 μ L of solvent or solvent-allergen solution was placed in 10-mL glass vials sealed with Teflon/silicone septa. Each sample was analyzed in triplicate.

HS-PTV-fast GC-MS measurements

HS sampling was performed with a PAL autosampler (CTC Analytics AG, Zwingen, Switzerland). This sampler is equipped with a tray for 32 consecutive samples and an oven with positions for six sample vials. Oven temperature was kept at 130 °C and the equilibration time was set at 10 min. The injected volume was 5.0 mL (syringe at 140 °C).

All experiments were carried out with a PTV inlet (CIS-4; Gerstel, Baltimore, MD, USA). The three liners (71 mm $\times$ 2 mm) assayed were an empty baffled liner and two liners packed with glass wool or with a chemical sorbent (Tenax-TA[®]) designed to trap organics. Hot-split injection and solvent-vent injection were assayed. In the optimized method, a liner packed with Tenax-TA[®] was used.

When hot-split injection was used, the injector temperature was kept at 275 °C throughout the analysis time (split ratio 1:5, split flow 7.0 mL/min). In the solvent-vent injection mode, the injector venting temperature was 50 °C. The venting flow was adjusted to 125 mL/min and the venting pressure to 5.0 psi (34474 Pa). After 0.50 min, the split valve was closed and the liner was flash-heated at a rate of 12 °C/s to 275 °C. The analytes were transferred from the liner to the capillary column (1.5 min). The split valve was then opened (split flow 125 mL/min) and the liner temperature was held at 275 °C for 5.00 min. Cooling was accomplished with liquid CO₂.

To perform the gas chromatographic measurements, an Agilent 6890 GC device equipped with a low-polarity DB-VRX capillary column (20 m $\times$ 0.18 mm $\times$ 1 μ m) from Agilent J&W was used. The initial oven temperature was 50 °C for 1.00 min; this was increased at a rate of 24 °C/min to 240 °C and then held for 3.00 min. The total chromatographic run time was 11.92 min. The carrier gas was helium N50 (99.995% pure; Air Liquide) and flow rate was 1.5 mL/min.

The detector was a quadrupole mass spectrometer (HP 5973N) equipped with an inert ion source. It was operated in electron-impact mode using an ionization voltage of 70 eV. The ion source temperature was 230 °C, and the quadrupole was set at 150 °C. The analyses were performed in the scan and SIM modes.

The scan detection mode was only used for the identification of the compound in the samples. The *m/z*

range was 25–285 amu, and the abundance threshold value was set to 0. The different compounds were identified by comparison of the experimental spectra with those of the NIST'05 database (NIST/EPA/NIH Mass Spectral Library, version 2.0).

SIM detection mode was used for the quantification of the compounds in the sample and the m/z ratios used were the most abundant ones of the compounds studied (ions in bold in Table 1). Sixteen SIM windows were used and the ions were acquired with a dwell-time of 10 ms.

Data analysis

Data collection was performed with Enhanced ChemStation software [25] from Agilent Technologies.

Results and discussion

Preliminary study of HS-PTV-fast GC-MS data

In the present work, 24 compounds suspected of being skin sensitizers were studied in ten cosmetic products. The list of analytes includes eight alcohols (benzyl alcohol, linalool, β -citronellol, geraniol, anisyl alcohol, cinnamyl alcohol, α -amylcinnamyl alcohol, and farnesol), eight carbonyl compounds (geranial, 7-hydroxycitronellal, cinnamal, α -isomethylionone, lilial, α -amylcinnamal, lyral, and α -hexylcinnamal), four esters (methyl 2-octynoate, benzyl cinnamate, benzyl benzoate, and benzyl salicylate), one lactone (coumarin), two phenols (eugenol and isoeugenol), and one cyclic terpene (limonene).

Initially, different volumes (5–40 μL) of a laboratory-prepared solution containing 5 mg/L of each analyte in propyl acetate were placed in the vial (all experiments were carried out using conditions described in the “Experimental” section). The increase in peak area with sample volume was linear up to a volume of 20 μL . For volumes higher than 20 μL , linearity was lost and the increase of signal with volume was much lower. In light of the results, a sample volume of 20 μL was selected.

A comparative study of the signals obtained for all the compounds was performed using three types of liners: an empty baffled liner and two liners packed with glass wool or Tenax-TA[®], respectively. Each of them was found to exhibit a different analyte absorption capacity. Moreover, with each type of liner two injection modes were assayed: hot-split (split ratio 1:5) and solvent-vent. A standard solution containing the 24 analytes at a concentration level of 5 mg/L was analyzed (five replicates) using the different liners and the two injection modes (all liners were checked using both injection modes). The highest signals were obtained when the solvent-vent injection mode was used.

The signals were of similar intensities for the three liners studied. However, the standard deviation for all experiments was smaller when the Tenax-TA[®]-filled liner was used. In view of these results, the Tenax-TA[®] liner was used.

Figure 1a shows the total ion chromatogram (scan mode) obtained on analyzing a solution containing all the analytes when the solvent-vent injection mode was used (experimental conditions are described in the “Experimental” section). Retention times and peak shapes were similar when hot-split injection was used. After 11.92 min, most compounds appeared separated, and eight analytes (Nos. 1, 2, 8, 9, 18, 20, and two isomers of No. 19) were seen in three partially overlapping groups (bI–bIII in Fig. 1): two groups (bI and bII) of two compounds each, and one group (bIII) with four analytes.

The partial overlapping of some of the compounds seen in Fig. 1a did not prevent their individual chromatographic determination. The determination of all the allergens was possible using extracted ion chromatograms (Fig. 1, parts 1bI, 1bII, and 1bIII), as may be seen for the pairs benzylalcohol (No. 1)/limonene (No. 2) and hydroxycitronellal (No. 8)/anisyl alcohol (No. 9). The same was the case of the set of the four compounds lyral (No. 18)/farnesol isomer 1 (No. 19)/amylcinnamylalcohol (No. 20)/farnesol isomer 2 (No. 19'). Farnesol isomer 3 (No. 19''), which appeared completely separated from the other compounds, is also included in Fig. 1bIII.

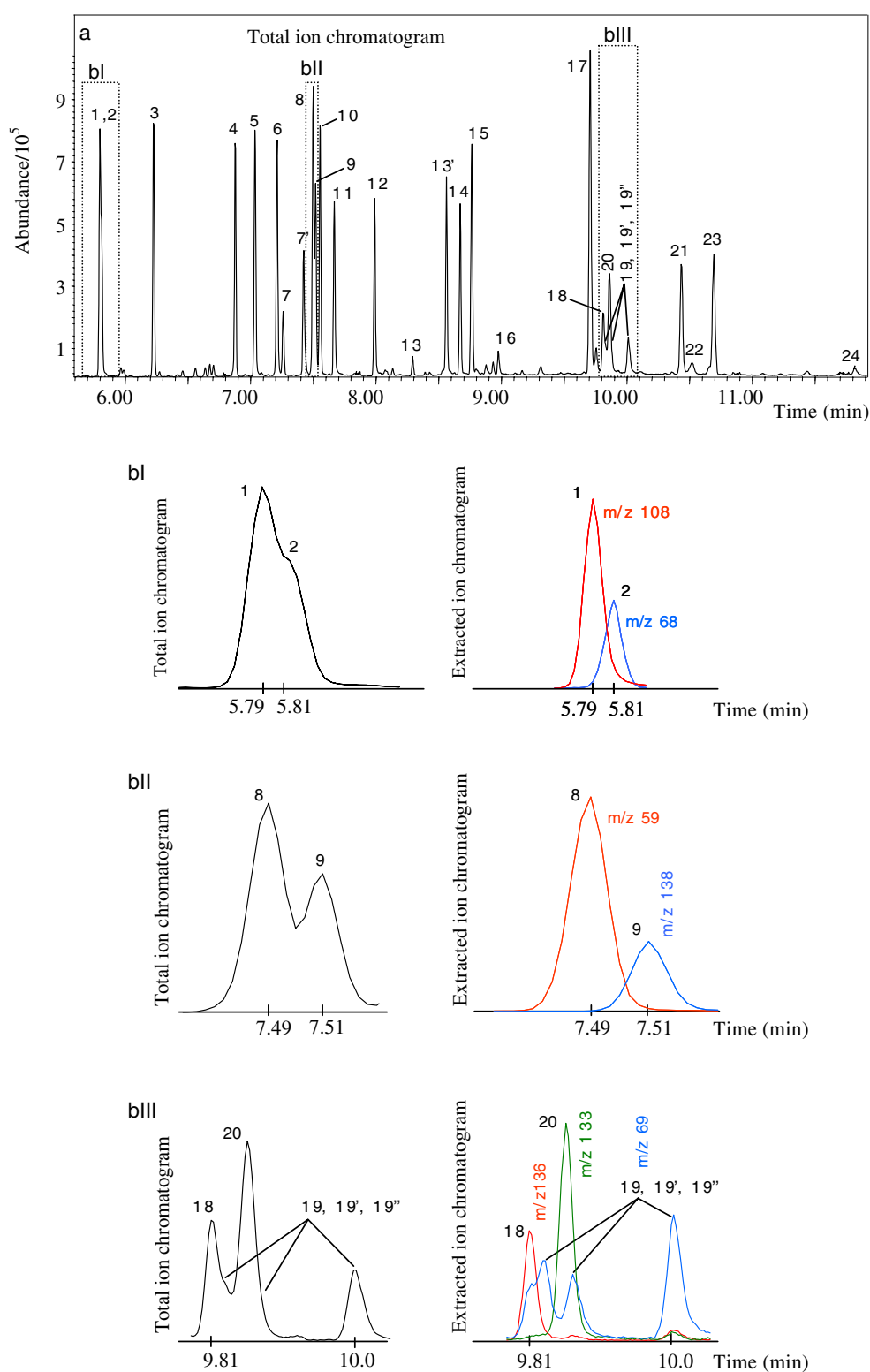
An easy and rapid way to obtain an overall image of the mass spectrum of each compound in the sample consists of representing the different m/z variables recorded against the time of analysis. In this way, the information contained in a chromatogram can be visualized by using contour figures with time and mass/charge ratio axes. Figure 2a corresponds to the figure of the information contained in the chromatogram shown in Fig. 1a. Thus, it is possible to visualize all the information contained in the chromatogram (signal intensity, retention time and the mass spectrum) in a single figure [26, 27].

From plot 2a, it is possible to establish specific zones for each of the allergens studied. The zones used to identify the analytes are marked with red circles in Fig. 2b. Finally, it is possible to compile a template containing the columns t_R (column 3) and m/z (column 4) from Table 1 as coordinates. Use of this template allows rapid identification of the suspected allergens in the samples. Figure 2c shows the template generated.

HS-PTV-fast GC-MS method evaluation

In fast GC, the usual value [28] for peak widths at half height ($W_{1/2}$) is 0.2–3 s, and typical run times range from 1 to 10 min. The ($W_{1/2}$) of all the suspected allergens studied was lower than 3 s (most of them had a $W_{1/2}$ of less than

Fig. 1 **a** Total ion chromatogram for a solution containing the 24 suspected allergens in propyl acetate at 5 mg/L. (*bl*)–(*bIII*), extracted ion chromatograms corresponding to zones *bl*–*bIII*, respectively, of Fig. 1a. The compounds are labeled with numbers according to Table 1



0.9 s) and the chromatographic run time was 11.92 min. Thus, this case is a fast GC application for the compounds studied.

The calibration models were constructed using six uniformly distributed concentration levels and a blank (Table 2); triplicate measurements were made for each

calibration standard for the two injection modes (solvent-vent and hot-split). The analytical signals used for the calibration models (SIM mode) were the areas of the extracted ion chromatograms for the ions depicted in bold in Table 1. All calibrations showed good linear behavior

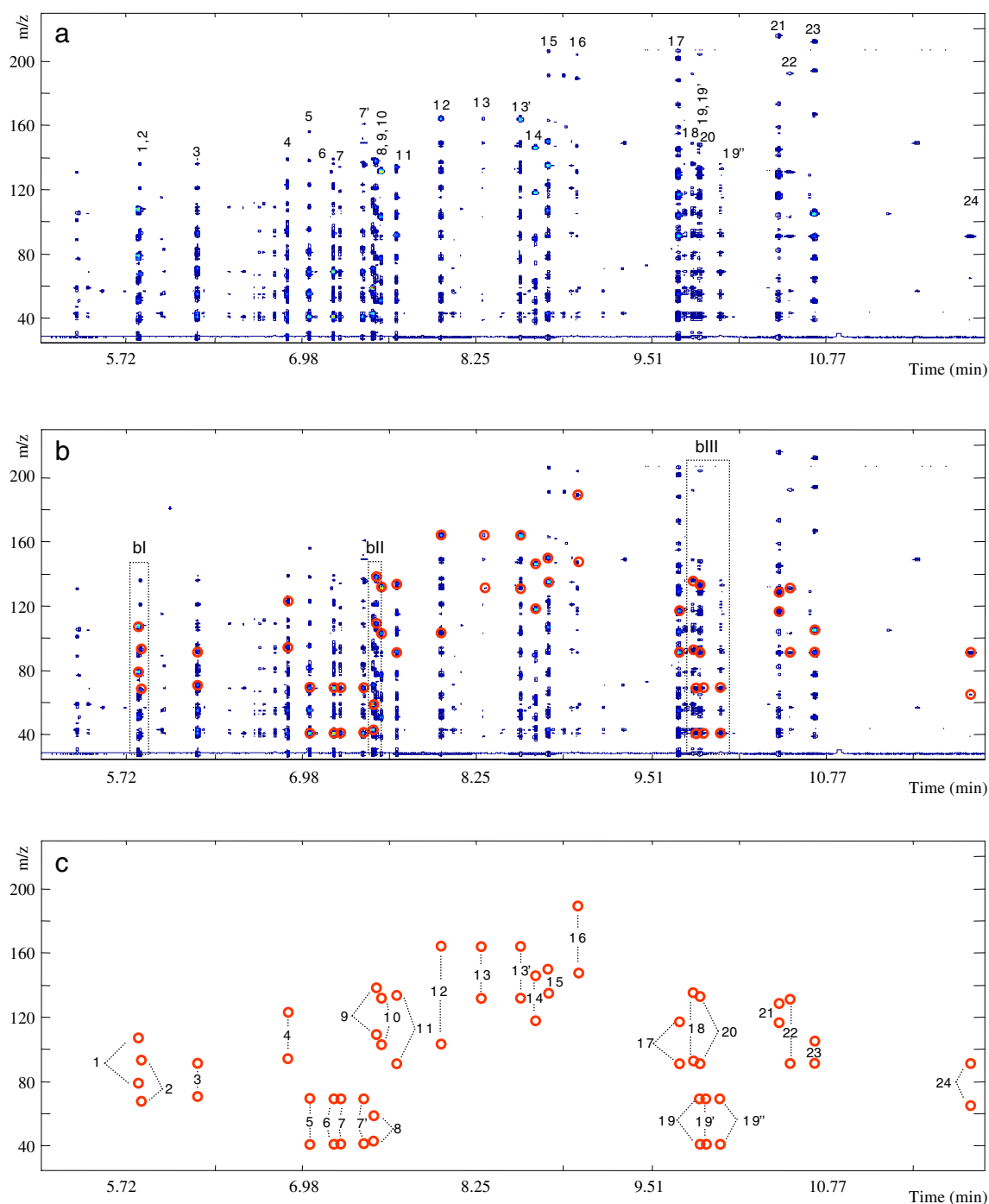


Fig. 2 **a** Contour surfaces with time and m/z ratio axes for a solution containing the 24 analytes studied in propyl acetate at 5 mg/L. **b** Zones selected (red circles) for each suspected allergen studied. **c**

Template generated for the identification of compounds. The compounds are labeled with numbers according to Table 1

and the values of the coefficient of determination (R^2) can be seen in Table 2. The intercept included zero in all cases, and the models generated did not show lack of fit. The quantification of compounds with isomers (Nos. 7, 13, and 19) was performed using the total area corresponding to the sum of the individual areas of each isomer. Table 2

summarizes the analytical characteristics of the two proposed injection modes. The slopes for the calibration models in solvent-vent injection mode were always higher (between threefold for benzyl cinnamate (No. 22) and 37-fold for β -citronellol (No. 5)) than those obtained with hot-split injection.

Table 2 Analytical characteristics of the method proposed for the quantification of 24 suspected allergens

Compound	Solvent-vent injection			Hot-split injection (1:5)		
	Range (mg/L)	Slope	R^2	Range (mg/L)	Slope	R^2
1	0.0620–639	381±15	0.9911	0.0620–124	16.1±0.6	0.9941
2	0.0490–505	131±4	0.9939	0.0980–980	26.9±0.8	0.9957
3	0.0506–521	181±4	0.9975	1.27–10.1	15.1±0.4	0.9962
4	0.0549–565	108±2	0.9985	0.0549–110	7.8±0.3	0.9941
5	0.0490–338	227±12	0.9815	1.22–9.79	6.1±0.1	0.9794
6	0.0523–361	156±5	0.9936	1.31–10.4	5.0±0.1	0.9828
7 ^a	0.101–104	223±5	0.9972	2.53–20.2	20.3±0.6	0.9961
8	0.0523–540	631±17	0.9958	0.0523–10.5	29±1	0.9932
9	0.0678–698	147±4	0.9956	1.66–13.2	8.9±0.4	0.9903
10	0.0585–602	424±8	0.9975	0.0585–11.7	27.3±0.7	0.9966
11	0.0660–680	113±2	0.9966	1.68–13.5	8.8±0.5	0.9859
12	0.0633–652	216±5	0.9962	1.58–12.7	16.5±0.6	0.9940
13 ^a	0.0637–657	50±1	0.9956	1.59–12.7	4.3±0.1	0.9942
14	0.0679–699	212±5	0.9967	0.0679–10.1	20.6±0.3	0.9985
15	0.0474–488	412±7	0.9978	0.0474–7.11	36.9±0.6	0.9981
16	0.285–293	33.8±0.8	0.9959	0.285–28.5	3.46±0.06	0.9980
17	0.0564–198	135±2	0.9905	0.0564–8.47	19.7±0.3	0.9966
18	0.291–250	76.3±0.7	0.9978	7.27–30.3	5.00±0.09	0.9949
19 ^a	0.303–261	48±1	0.9858	7.57–31.2	3.7±0.1	0.9927
20	2.06–982	72±3	0.9848	2.86–11.8	2.84±0.06	0.9939
21	0.0543–559	129±5	0.9843	1.36–5.59	14.5±0.2	0.9974
22	0.313–162	40±2	0.9776	0.321–48.1	12.0±0.2	0.9906
23	0.0664–571	333±14	0.9889	0.0664–6.84	47±1	0.9918
24	– ^b	– ^b	– ^b	0.345–35.5	41±2	0.9796

^a Quantification was performed with the total area including all the isomers

^b It was not possible to obtain a suitable calibration curve due to the strong interaction of this compound with the filling of the liner

When the solvent-vent injection mode was used, it was not possible to obtain a suitable calibration curve for benzyl salicylate (No. 24) owing to the strong interaction of this compound with the filling of the liner (Tenax-TA®). Desorption was incomplete even when the maximum recommended temperature for this type of material (300 °C) was used, leading to a clear memory effect.

Repeatability ($n=10$) was evaluated (Table 3) at a level corresponding to an S/N ratio of approximately 3 and was seen to be satisfactory, with an RSD below 12% for all compounds, except benzyl salicylate (No. 24, RSD 20 %) when solvent-vent injection mode was used. The low reproducibility for this latter compound was due to the previously described effect. For hot-split injection, the RSD was equal or below 10% for all analytes. In many cases, the RSD was lower than 6%.

The detection limit (DL) was estimated using the following equation:

$$DL = \frac{3.3\sigma}{S}$$

where σ is the standard deviation of the peak response for ten replicates ($n=10$), corresponding to standard solutions

that provided an S/N ratio of approximately 3; S is the slope of the calibration curve, and 3.3 is Student's " t for $n-1$ degrees of freedom (99% probability)". The DLs calculated are shown in Table 3, and when solvent-vent injection was used values as low as 0.0011 mg/L for benzyl alcohol (No. 1) were obtained. The DLs were between two- and 24-fold higher when the hot-split injection mode was used, except for benzyl cinnamate (No. 22); for this latter compound, the DLs were similar with both injection modes.

The quantification limit (QL) was estimated using the following equation:

$$QL = \frac{10\sigma}{S}$$

For all the suspected allergens, the QLs (Table 3) are well below the stipulated cut-off levels (0.01% for "rinse-off" and 0.001% for "leave-on" cosmetics) with both injection modes.

In view of the results, choice of the injection mode will depend on the concentration of the allergens in the sample. For high concentrations, hot-split injection is recommended and for lower concentration levels, solvent-vent injection will provide improved sensitivity for most compounds owing to the preconcentration effect in the liner.

Table 3 Figures of merit of the method proposed for the quantification of 24 suspected allergens

Compound	Solvent-vent injection			Hot-split injection (1:5)		
	RSD (%)	DL (mg/L)	QL (mg/L)	RSD (%)	DL (mg/L)	QL (mg/L)
1	5.1	0.0011	0.0033	4.6	0.0099	0.029
2	4.3	0.0031	0.0094	3.6	0.010	0.030
3	3.6	0.0057	0.017	4.0	0.027	0.082
4	5.7	0.0020	0.0062	5.8	0.012	0.038
5	8.4	0.0054	0.016	3.5	0.11	0.34
6	9.5	0.0059	0.018	4.8	0.14	0.44
7 ^a	5.9	0.0016	0.0049	6.2	0.014	0.042
8	10	0.0055	0.017	2.3	0.014	0.042
9	8.5	0.013	0.041	4.3	0.15	0.46
10	3.0	0.0016	0.0049	4.6	0.0064	0.019
11	5.3	0.0093	0.028	3.1	0.12	0.37
12	6.1	0.0055	0.017	8.9	0.034	0.10
13 ^a	4.6	0.014	0.043	5.2	0.17	0.52
14	8.4	0.0070	0.021	6.4	0.021	0.063
15	11	0.0052	0.016	5.1	0.013	0.039
16	2.6	0.020	0.061	10	0.069	0.21
17	3.3	0.0059	0.018	3.3	0.0099	0.030
18	5.0	0.030	0.090	3.9	0.091	0.28
19 ^a	11	0.094	0.29	3.6	0.35	1.1
20	7.3	0.021	0.066	4.3	0.22	0.66
21	4.0	0.0078	0.024	7.7	0.026	0.078
22	6.1	0.089	0.27	5.8	0.097	0.29
23	3.2	0.0042	0.013	2.1	0.0086	0.026
24	20	— ^b	— ^b	4.5	0.030	0.090

^a Quantification was performed with the total area including all the isomers

^b It was not possible to obtain a suitable calibration curve due to the strong interaction of this compound with the filling of the liner

The quantification limits reported in other studies are higher than those obtained here: SCE-GC-MS [3] (3–4 mg/kg), GC-MS [8, 14] (0.9–7.1 mg/kg and 10 mg/L, respectively), MDGC-FID [19] (1 mg/L, six studied analytes), and dual column GC-MS [23] (<4 mg/kg). Some contributions have reported similar and lower quantification limits: GC×GC-FID [18] (33–73 µg/L), GC×GC-MS [18] (9–89 µg/L) and SPME-GC-MS [4] (0.004–0.909 µg/L). However, the analysis time required for the two methods cited above, based on comprehensive two-dimensional GC×GC, was higher than 55 min. The method including SPME needs 30 min for the GC run time plus 25 min for sample equilibration and extraction. Other proposed methodologies [2, 9, 17, 19, 21, 22] require between 29 and 95 min for the temperature program to be completed and to ensure complete elution of the compounds in the sample injected. Furthermore, some methods [3] require additional sample preparation steps, and this increases the analysis time. As far as we know, there is only one method [8] employing a lower analysis time (5 min) than that proposed here (11.92 min). However, the quantification limits of this fast method (0.9–7.1 mg/kg) are close to the maximum residue limit for leave-on cosmetic products (10 mg/kg).

Analysis of cosmetic products

Following the strategy described above for the rapid identification of compounds, ten cosmetic products were analyzed. Initially, all the samples were analyzed using the solvent-vent injection mode in order to check the presence of compounds at very low concentration levels. In all of them, the identification of some suspected allergens was positive and the characteristic zones of these compounds in the template used showed a signal. Table 4 shows the analytes detected and identified in the products. In the ten samples studied, the suspected allergens most frequently found were limonene and linalool.

The quantitative determination of fragrance allergens was carried out in four cosmetic products: two perfumes (samples A and B), a post-depilation mousse, and a body cream. In all samples, the suspected allergens found were present in concentrations high enough to be determined by the hot-split injection mode.

Perfume samples

The total ion chromatogram of perfume A (scan mode) is shown in Fig. 3a. Ten compounds were detected: limonene,

Table 4 Suspected allergens identified with HS-Fast GC-MS in the cosmetic products tested. Solvent-vent injection was used

Cosmetic product	Compounds
Perfume A	Limonene, linalool, β -citronellol, geraniol, geranial isomer 1, geranial isomer 2, 7-hydroxycitronellal, eugenol, lylal, benzyl benzoate
Perfume B	Limonene, linalool, cinnamal
Perfume C	Limonene, linalool, coumarin, lylal, benzyl benzoate
Anti-hair loss product A	Limonene, linalool
Anti-hair loss product B	Limonene, linalool
Post-depilation mousse	Linalool, eugenol, α -isomethylionone, α -amylcinnamal, α -hexylcinnamal
Body cream	Limonene, linalool, β -citronellol, coumarin, α -isomethylionone, lilyal, α -hexylcinnamal, benzyl benzoate
Deodorant	Limonene, linalool, eugenol, coumarin, α -isomethylionone, lilyal, α -hexylcinnamal, benzyl benzoate
Sun cream	Limonene, linalool, β -citronellol, geraniol, 7-hydroxycitronellal, cinnamal, eugenol, coumarin, α -isomethylionone, lilyal, lylal, α -hexylcinnamal, benzyl benzoate
Hand cream	Limonene

linalool, β -citronellol, geraniol, geranial (two isomers), 7-hydroxycitronellal, eugenol, lylal, and benzyl benzoate. The corresponding contour plot is shown in Fig. 3b, on which the previously generated template had been superimposed. Figure 4 shows the extracted ion chromatograms in two different zones (a and b) where some of the analytes studied (geranial isomer 1 and eugenol) overlap with other major

compounds (linalyl acetate and geranyl acetate). The partial overlapping of some of the compounds does not prevent their individual chromatographic separation. The determination of the allergens is possible using extracted ion chromatogram, as may be seen in Fig. 5.

In order to check the possible existence of a matrix effect in the analysis of the perfume samples, a study was

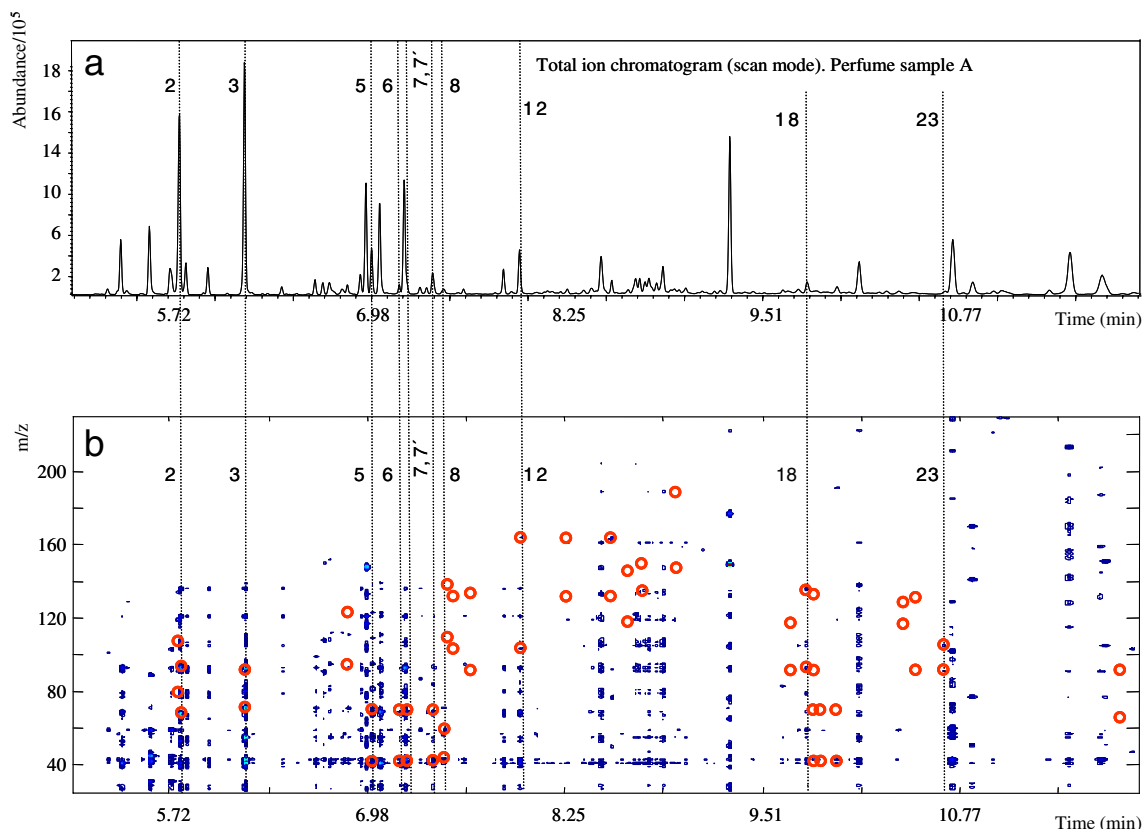


Fig. 3 **a** Total ion chromatogram (scan mode) for perfume sample A. **b** Contour surfaces for perfume sample A with the template generated for the identification of the compounds. The compounds are labeled with numbers according to Table 1

Extracted ion chromatograms (scan mode). Perfume sample A

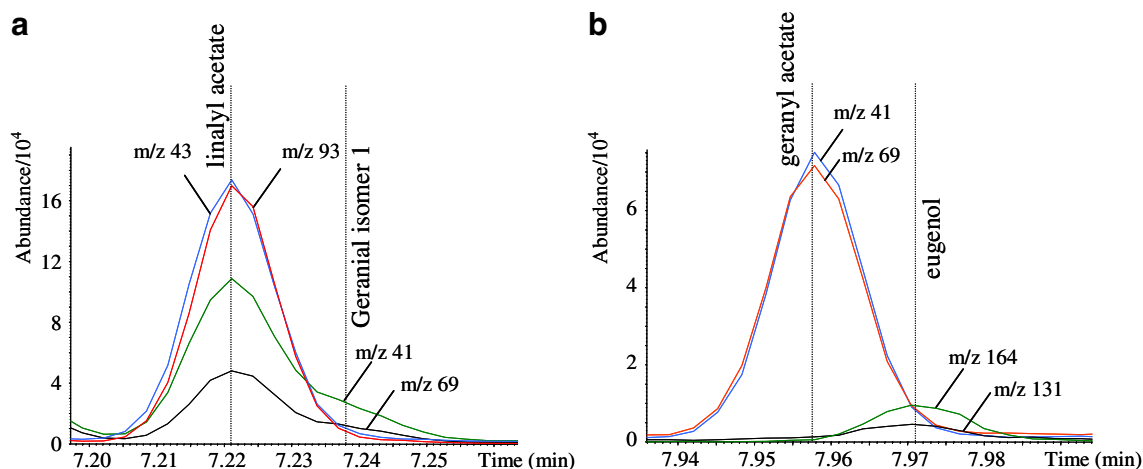
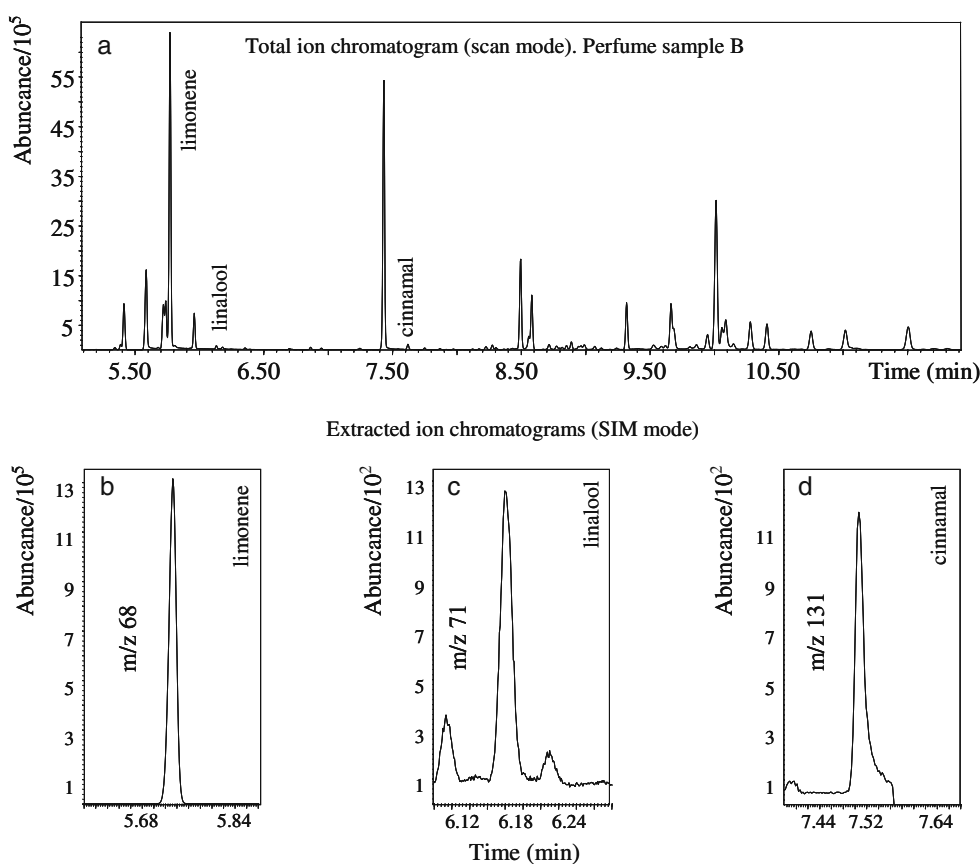


Fig. 4 Extracted ion chromatograms (scan mode) corresponding to compounds **a** geranial isomer 1 and **b** eugenol, which overlap with other major compounds in perfume sample A

undertaken in which the signals obtained in three different samples were measured. The first was a solution of the ten analytes detected (perfume A) in propyl acetate; the second was perfume sample A diluted in propyl acetate, and the third one contained the ten analytes of the first solution and perfume sample A, both at the same proportions as above. If no matrix effect occurs, the sum of signals corresponding

to the different analytes in solution 1 and sample 2 should be similar to the signal generated by those analytes in sample 3. In this case, the sum of signals was always lower than the signals corresponding to sample 3 and the matrix effect elicited an increase in the analytical signal. This effect has been reported previously [29] as the enrichment of analytes due to the solvent effect. A similar

Fig. 5 a Total ion chromatogram (scan mode) for perfume sample B. Extracted ion chromatograms (SIM mode) for **b** limonene, **c** linalool, and **d** cinnamal



matrix effect was found in perfume B. In view of the results, quantification was performed with the standard addition method.

Due to the signal intensities of the identified compounds, hot-split injection was used for the determination of them (split ratio 1:50). The concentration range for the standard additions set (five concentration levels) was as follows: limonene (0–2940 mg/L), linalool (0–3038 mg/L), β -citronellol (0–1820 mg/L), geraniol (0–1960 mg/L), geranial (0–1605 mg/L), 7-hydroxycitronellal (0–105 mg/L), eugenol (0–150 mg/L) lylal (0–2490 mg/L) and benzyl benzoate (0–2453 mg/L). All samples were measured in triplicate. The variables used in the calibrations were the area under the curve of the compounds in the extracted ion chromatograms from Table 1 (ions in bold). In all cases, calibrations with no lack of fit were obtained, with R^2 equal to or greater than 0.99. The concentrations obtained and their confidence intervals (95% probability) for the suspected allergens in perfume A are shown in Table 5.

Table 5 Concentrations and confidence intervals (95% probability) for the suspected allergens detected in the different cosmetic products

Cosmetic products	Compounds	HS-PTV-fast GC-MS Hot-split injection
Perfume A	Limonene	$(4.7 \pm 0.4) \times 10^3$ mg/L
	Linalool	$(3.3 \pm 0.2) \times 10^3$ mg/L
	β -citronellol	$(5.3 \pm 0.3) \times 10^2$ mg/L
	Geraniol	$(1.8 \pm 0.2) \times 10^2$ mg/L
	Geranial ^a	$(6.0 \pm 0.2) \times 10^2$ mg/L
	7-Hydroxycitronellal	83 $\pm$ 8 mg/L
	Eugenol	93 $\pm$ 5 mg/L
	Lylal	$(1.38 \pm 0.05) \times 10^3$ mg/L
	Benzyl benzoate	$(3.4 \pm 0.9) \times 10^2$ mg/L
Perfume B	Limonene	$(3.3 \pm 0.1) \times 10^3$ mg/L
	Linalool	16.9 $\pm$ 0.7 mg/L
	Cinnamal	23 $\pm$ 2 mg/L
Post-depilation mousse	Linalool	96 $\pm$ 5 mg/kg
	Eugenol	80 $\pm$ 8 mg/kg
	α -Isomethylionone	45 $\pm$ 8 mg/kg
	α -Amylcinnamal	40 $\pm$ 3 mg/kg
	α -Hexylcinnamal	56 $\pm$ 3 mg/kg
Body Cream	Limonene	17 $\pm$ 2 mg/kg
	Linalool	$(1.6 \pm 0.2) \times 10^2$ mg/kg
	β -Citronellol	4.1 $\pm$ 0.5 mg/kg
	Coumarin	3.5 $\pm$ 0.2 mg/kg
	α -Isomethylionone	28 $\pm$ 2 mg/kg
	Lilial	10 $\pm$ 1 mg/kg
	α -Hexylcinnamal	80 $\pm$ 5 mg/kg
	Benzyl benzoate	15 $\pm$ 2 mg/kg

^a Quantification was performed with the total area including all the isomers

Three analytes (limonene, linalool and cinnamal) were detected in perfume sample B when the template was superimposed over its contour plot. Figure 5a shows the total ion chromatogram of the sample (scan mode). Figure 5b–d shows the extracted ion chromatograms (SIM mode) for limonene (m/z 68), linalool (m/z 71) and cinnamal (m/z 131), respectively. A split ratio of 1:50 was employed for quantification.

The concentration range for the standard additions set (five concentration levels) was as follows: limonene (0–1999 mg/L), linalool (0–142 mg/L), and cinnamal (0–164 mg/L). All samples were measured in triplicate. The variables used in the calibrations were the area under the curve of the compounds in the extracted ion chromatograms from Table 1 (ions in bold). In all cases, calibrations with no lack of fit were obtained, with R^2 equal to or greater than 0.999. The concentrations obtained and their confidence intervals (95% probability) of the suspected allergens in perfume B are shown in Table 5.

Mousse and cream samples

Using the compound identification strategy described above, five suspected allergens were identified in the post-depilation mousse (linalool, eugenol, α -isomethylionone, α -amylcinnamal, and α -hexylcinnamal). All the compounds were properly quantified when the hot-split injection was used (split ratio 1:5).

To check the possible existence of a matrix effect in the analysis of these samples, the same study described above for the perfume samples was undertaken. For the post-depilation mousse, the sum of signals was always higher than the signals corresponding to the sample containing the analytes in propyl acetate plus those present in the mousse. A similar matrix effect was found in cream sample. In view of the results, quantification was performed with the standard addition method. The matrix effect in the four studied samples produced a signal change for all analytes between 0.62-fold and 1.7-fold in comparison with those in propyl acetate.

The concentration range for the standard additions set (five concentration levels) was as follows: linalool (0–70.0 mg/L), eugenol (0–40.5 mg/L), α -isomethylionone (0–40.8 mg/L), α -amylcinnamal (0–49.7 mg/L), and α -hexylcinnamal (0–40.0 mg/L). In all cases, calibrations with no lack of fit were obtained and an R^2 value equal to or greater than 0.99 was observed. The concentrations obtained and their confidence intervals (95% probability) of the suspected allergens in the post-depilation mousse are shown in Table 5.

The last studied sample was a body cream in which eight compounds were identified: limonene, linalool, β -citronellol, coumarin, α -isomethylionone, lilial, α -hexylcinnamal, and benzyl benzoate. A split ratio 1:5 was used for quantification.

The concentration range for the standard additions set (five concentration levels) was as follows: limonene (0–24.6 mg/L), linalool (0–101 mg/L), β -citronellol (0–24.5 mg/L), coumarin (0–24.5 mg/L), α -isomethylionone (0–23.8 mg/L), lylial (0–19.8 mg/L), α -hexylcinnamal (0–50.6 mg/L), and benzyl benzoate (0–26.6 mg/L). In all cases, calibrations with no lack of fit were obtained, with an R^2 equal to or greater than 0.99. The concentrations obtained and their confidence intervals (95% probability) for the suspected allergens in the body cream are shown in Table 5.

Recovery studies were carried out to determine the accuracy of the method. Accuracy was determined by adding a known amount of each analyte to the samples and analyzing three replicates with the standard additions method. Table 6 shows the recovery rates for perfume sample A, obtained by comparison of the amount added and the predicted value. For the compounds detected in perfume sample A, the recovery rates were obtained by comparison of the amount added and the difference between the results obtained with and without the addition. They ranged from 80% to 115%, which can be considered acceptable. Similar results were obtained for all samples with recovery values between 84% and 117%. In all cases, RSD was lower than 7%.

Conclusions

The proposed methodology has proved to be useful for the rapid detection and quantification of suspected allergens in different type of cosmetic products. The information contained in the low-resolution chromatogram is sufficient for the identification of the compounds present in the samples. Plotting the chromatograms by contour surfaces with time and m/z axes allows rapid visualization of the results and detection of the allergens present in a sample.

The use of a HS-PTV combination to introduce the sample provided satisfactory results. The volatiles of the sample were analyzed without interference from the non-volatile matrix. An important advantage of the methodology used here is that no complex treatment of the sample is required, thus minimizing the creation of analytical artifacts and the errors associated with this step of the analytical process. Standard addition method was used for quantification.

The proposed method based on different injection techniques into monodimensional gas chromatography–quadrupole mass spectrometry revealed good precision and accuracy. The solvent-vent injection mode was used to analyze compounds at very low concentrations, which are not detectable with other conventional techniques. The

Table 6 Accuracy of the method

Compound	Added concentration (mg/L)	Recovery rate (%) perfume A
Benzyl alcohol (No 1)	1.11	99
Limonene (No 2)	0.882	95
Linalool (No 3)	0.861	103
Methyl 2-octynoate (No 4)	0.988	92
β -citronellol (No 5)	0.881	96
Geraniol (No 6)	0.941	87
Geranial ^a (No 7+7')	0.101	101
7-hydroxycitronellal (No 8)	0.943	95
Anisyl alcohol (No 9)	1.22	103
Cinnamal (No 10)	1.05	92
Cinnamyl alcohol (No 11)	3.43	90
Eugenol (No 12)	1.14	97
Isoeugenol ^a (No 13+13')	1.15	83
Coumarin (No 14)	1.22	97
α -isomethylionone (No 15)	0.853	101
Lilial [®] (No 16)	5.13	101
α -amylcinnamal (No 17)	1.02	88
Lylal [®] (No 18)	0.291	94
Farnesol ^a (No 19+19'+19'')	10.6	80
α -amylcinnamyl alcohol (No 20)	5.94	102
α -hexylcinnamal (No 21)	0.978	113
Benzyl cinnamate (No 22)	5.63	102
Benzyl benzoate (No 23)	1.19	115
Benzyl salicylate (No 24)	10.1	90

^a Quantification was performed with the total area including all the isomers

use of a liner packed with Tenax-TA® allowed the analytes of interest to be retained during the solvent process. Split injection was employed when the analytes were present in the samples at high concentration levels. Benzyl salicylate cannot be determined with solvent-vent injection mode when a Tenax-packed liner is used due to the strong interaction of this compound with the filling of the liner at 50 °C. However, this compound was quantified satisfactorily using hot-split injection mode (275 °C) with a Tenax-packed liner.

In this work, solvent-vent injection was used to detect suspected allergens in the cosmetic products. After that, quantification was performed using hot-split injection due to the high signal intensity of the detected compounds.

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References

1. Directive 2003/15/EC of the European Parliament and of the Council of 27 February 2003 amending Council Directive 76/768/EEC on the approximation of the laws of the Member States relating to Cosmetic Products (2003) Off J Eur Union L66:26–35
2. David F, Devos C, Joulain D, Chaintreau A, Sandra P (2006) *J Sep Sci* 29:1587–1694
3. Niederer M, Bollhalder R, Hohl Ch (2006) *J Chromatogr A* 1132:109–116
4. Lamas JP, Sanchez Prado L, García Jares C, Llompert M (2009) *Anal Bioanal Chem* 394:1399–1411
5. Bieri S, Marriott PJ (2006) *Anal Chem* 78:8089–8097
6. Pérez Pavón JL, del Nogal SM, Fernández Laespada ME, Moreno Cordero B (2008) *J Chromatogr A* 1202:196–202
7. Pérez Pavón JL, del Nogal SM, Fernández Laespada ME, Moreno Cordero B (2009) *Anal Bioanal Chem* 394:1463–1470
8. Mondello L, Sciarrone D, Casilli A, Tranchida PQ, Dugo P, Dugo G (2007) *J Sep Sci* 30:1905–1911
9. Chaintreau A, Joulain D, Marin C, Schmidt CO, Vey M (2003) *J Agric Food Chem* 51:6398–6403
10. Rastogi SC (1995) *J High Resolut Chromatogr* 18:653–685
11. Rastogi SC, Johansen JD, Frosch P, Menné T, Bruze M, Lepoittevin JP, Dreier B, Andersen KE, White IR (1998) *Contact Dermat* 38:29–35
12. Rastogi SC, Johansen JD (2008) *Contact Dermat* 58:278–281
13. Rastogi SC, Johansen JD, Menné T, Frosch P, Bruze M, Andersen KE, Lepoittevin JP, Wakelin S, White IR (1999) *Contact Dermat* 41:84–88
14. Bassereau M, Chaintreau A, Duperrex S, Joulain D, Leijts H, Loesing G, Owen N, Sherlock A, Schippa C, Thorel PJ, Vey M (2007) *J Agric Food Chem* 55:25–31
15. Luan W, Sandy C, Szelewski M (2008) Agilent Technologies (Application Brief)
16. Asten A (2002) *Trac-Trend Anal Chem* 21:698–708
17. Shellie R, Marriott P, Chaintreau A (2004) *Flavour Fragr J* 19:91–98
18. Cordero C, Bicchi C, Joulain D, Rubiolo P (2007) *J Chromatogr A* 1150:37–49
19. Dunn MS, Vulic N, Shellie RA, Whitehead S, Morrison P, Marriott PJ (2006) *J Chromatogr A* 1130:122–129
20. Debonneville C, Thomé MA, Chaintreau A (2004) *J Chromatogr Sci* 42:450–455
21. Debonneville C, Chaintreau A (2004) *J Chromatogr A* 1027:109–115
22. Mondello L, Casilli A, Tranchida PQ, Dugo G, Dugo P (2005) *J Chromatogr A* 1067:235–243
23. Leijts H, Broekmans J, Pelt L, Mussinan C (2005) *J Agric Food Chem* 53:5487–5491
24. Chaintreau A (2007) In: Salvador A, Chisvert A (eds) *Analysis of cosmetics products*. Elsevier, Amsterdam
25. Enhanced ChemStation, MSD ChemStation E0000202 Agilent Technologies, 2007, CA, USA
26. Pérez Pavón JL, del Nogal SM, García Pinto C, Fernández Laespada ME, Moreno Cordero B (2006) *Anal Chem* 78:4901–4908
27. Pérez Pavón JL, del Nogal SM, Fernández Laespada ME, García Pinto C, Moreno Cordero B (2007) *J Chromatogr A* 1141:123–130
28. Matisova E, Dömötöróvá M (2003) *J Chromatogr A* 1000:199–221
29. Engewald W, Teske J, Efer J (1999) *J Chromatogr A* 856:259–278