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Full evaporation dynamic headspace in combination with selectable one-dimensional/two-dimensional gas chromatography–mass spectrometry for the determination of suspected fragrance allergens in cosmetic products

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ABSTRACT

Suspected fragrance allergens were determined in cosmetic products using a combination of full evaporation–dynamic headspace (FEDHS) with selectable one-dimensional/two-dimensional GC–MS. The full evaporation dynamic headspace approach allows the non-discriminating extraction and injection of both apolar and polar fragrance compounds, without contamination of the analytical system by high molecular weight non-volatile matrix compounds. The method can be applied to all classes of cosmetic samples, including water containing matrices such as shower gels or body creams. In combination with selectable ¹D/²D GC–MS, consisting of a dedicated heart-cutting GC–MS configuration using capillary flow technology (CFT) and low thermal mass GC (LTM–GC), a highly flexible and easy-to-use analytical solution is offered. Depending on the complexity of the perfume fraction, analyses can be performed in one-dimensional GC–MS mode or in heart-cutting two-dimensional GC–MS mode, without the need of hardware reconfiguration. The two-dimensional mode with independent temperature control of the first and second dimension column is especially useful to confirm the presence of detected allergen compounds when mass spectral deconvolution is not possible.

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1. Introduction

According to EU Directive 2003/15/EC [1], 24 suspected allergen compounds have to be labeled on fragranced products when the concentrations of these allergens exceed certain specified values (10 ppm for “leave-on” products and 100 ppm for “rinse-off” products). Fragranced products, including lotions, perfumes, detergents, shampoo's, gels, and other cosmetics, highly differ in fragrance constituents, concentrations and complexity. Moreover, the “matrices” range from apolar to polar and from volatile to non-volatile. Due to the large variety of solute/matrix combinations, different analytical methods are applied for the determination of allergen compounds. We suggested to classify the different fragranced products in 4 classes, including simple fragrance mixtures (class I), complex fragrance mixtures (class II), fragrances in non-volatile matrices (class III) and aqueous based fragranced products (class IV) [2]. For the determination of allergen compounds in (not too complex) fragrance mixtures (e.g. eau-de-toilette), not containing non-volatile matrix compounds (class I), simple dilution and liquid injection combined with one-dimensional gas

chromatography–mass spectroscopy (GC–MS) operated in scan mode or in selected ion monitoring (SIM) mode is mostly appropriate [3–7]. For more complex samples (class II = complex mixtures of fragrance compounds, e.g. some perfumes) two-dimensional heart-cutting GC–MS (GC–GC–MS) can offer a solution for selective determination of the target solutes if one-dimensional GC–MS does not result in sufficient chromatographic or mass spectral resolution [2,8–10]. This is especially the case when low levels of target solutes co-elute with highly abundant other fragrance solutes, and mass spectral deconvolution cannot be applied due to lack of specific ions. Also comprehensive GC (GC × GC) was applied for the analysis of perfume allergens [11–17], although, in our opinion, the potential of GC × GC in perfume and essential oil analysis is mainly related to sample comparison (defect detection, alteration, ...) and group type separation, rather than to target compound analysis [18,19].

The most important draw-back of heart-cutting GC–GC–MS is the need for a specific instrumental configuration that is often not flexible and not easily reconfigured back into a one-dimensional system that is still the workhorse in many laboratories performing routine QC analysis on perfume allergens. A typical GC–GC–MS configuration consist of a monitoring FID to detect the effluent of the first dimension column, while only the second dimension column is connected to MS. A new approach of two-dimensional

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Table 1

List of 31 allergen compounds analyzed, CAS numbers, target ion/qualifiers and their retention time (in min) for the ¹D separation using the DB-Wax capillary column and for the ²D separation using the Rtx-5 column.

No.	Compound	CAS number	Retention time ¹ D (min)	Retention time ² D (min)	Target ion	Qualifiers
1	Limonene	5989-27-5	3.64	19.97	68	93, 67
2	Linalool	78-70-6	6.29	20.54	93	71, 80
3	Phenyl acetaldehyde	122-78-1	6.92	20.09	91	92, 120
4	Methyl 2-octynoate	111-12-6	6.96	21.32	95	123, 79
5	Estragol	140-67-0	7.11	21.28	148	147, 117
6	Neral	106-26-3	7.19	21.59	69	94, 109
7	Geranial	141-27-5	7.51	21.81	69	84, 94
8	Methyl 2-nonynoate	111-80-8	7.60	22.05	79	137, 100
9	Citronellol	106-22-9	7.71	21.52	69	67, 81
10	Geraniol	106-24-1	8.20	21.69	69	68, 93
11	α-Isomethyl ionone	127-51-5	8.24	23.21	135	150, 107
12	Benzyl alcohol	100-51-6	8.38	20.04	108	79, 107
13	Hydroxy citronellal	107-75-5	8.75	21.96	59	71, 43
14	Methyl eugenol	93-15-2	9.12	22.75	178	147, 163
15	Cinnamaldehyde	104-55-2	9.30	21.84	131	132, 103
16	Lilial	80-54-6	9.32	23.56	189	147, 204
17	Eugenol	97-53-0	9.94	22.40	164	149, 103
18	Amyl cinnamaldehyde	122-40-7	10.40	24.30	202	129, 115
19	Anisyl alcohol	105-13-5	10.50	21.92	138	109, 137
20	Cinnamyl alcohol	104-54-1	10.52	22.09	92	91, 134
21	Farnesol 1	3790-71-4	10.72	24.60	69	93, 81
22	Isoeugenol	97-54-1	10.82	23.05	164	149, 131
23	Farnesol 2	106-28-5	10.87	24.73	69	93, 81
24	Hexyl cinnamaldehyde	101-86-0	10.88	24.89	129	115, 216
25	Coumarin	91-64-5	11.37	22.94	118	146, 89
26	Lylal 1	-	11.38	24.38	105	107, 79
27	Lylal 2	31906-04-4	11.44	24.42	136	93, 79
28	Amyl cinnamyl alcohol	101-85-9	11.67	24.53	133	115, 204
29	Benzyl benzoate	120-51-4	12.16	25.00	105	91, 212
30	Benzyl salicylate	118-58-1	13.06	25.55	91	228, 65
31	Benzyl cinnamate	103-41-3	16.40	26.71	91	131, 192

heart-cutting GC was presented by Sasamoto and Ochiai [20,21]. This selectable ¹D/²D GC–MS configuration is based on the combination of capillary flow technology (CFT) and low thermal mass (LTM) GC. The main advantage is the high flexibility as no hardware configuration has to be changed to perform alternating analyses in ¹D–GC–MS and ²D–GC–GC–MS, since both the first dimension effluent and the second dimension effluent are monitored with the same MS detector. In this way, both sample classes I and II can be analyzed for allergens using the same instrumental configuration.

Liquid injection of a diluted sample can however not be applied for sample classes III and IV, that consist of matrices containing non-volatile constituents such as waxes (e.g. lipstick) or detergents (e.g. soaps, shower gels) [2]. These matrices can also contain water and the fragrance compounds (and potential allergens) are present at lower concentration compared to perfumes or essential oils. For the analysis of class III samples, typically containing high molecular weight/non-volatile matrix compounds, selective sample introduction using a programmed temperature vaporizing (PTV) inlet with a polydimethylsiloxane (PDMS) packed liner in combination with automated liner exchange was proposed [22]. For the analysis of aqueous based matrices (or matrices that can be diluted in water = class IV), stir bar sorptive extraction (SBSE) was used [2]. Alternative approaches for the determination of allergens in cosmetic samples include matrix solid phase dispersion [23] and gel permeation chromatography [24]. These techniques were applied to isolate the fragrance fraction from the (high molecular weight) matrix.

In this work, an alternative approach based on full evaporation dynamic headspace sampling (FEDHS) is proposed. Full evaporation headspace analysis was introduced by Markelov and Guzowski [25] as a method for the analysis of volatile and semi-volatile analytes to overcome matrix effects. Headspace equilibration of a small liquid sample (<100 µL, depending on vapor pressure) in a

standard (20 mL) headspace vial is done at relatively high temperature (100 °C or higher), followed by headspace injection. The main advantage is the non-discriminating injection of solutes covering a relatively large boiling point range, while non-volatile material (salts, detergents) are not recovered [26–29].

In FEDHS, the aliquot of the sample introduced in the headspace vial can be increased and the volatile fraction is quantitatively evaporated, removed from the vial (“purged”) and analyzed (eventually after concentration on a thermal desorption tube) [30]. Only the non-volatile material remains in the vial avoiding contamination of the sample inlet or capillary column. This FEDHS method, applied to the determination of suspected allergens in cosmetics, is completely automated and eliminates the need for additional sample preparation, while calibration can be done using external standardization (low matrix effects).

2. Experimental

2.1. Reagents, standards and samples

The target solutes (listed in Table 1) were obtained as p.a. grade chemicals (Aldrich, Bornem, Belgium) or supplied by Robertet (Grasse, France) or Firmenich (Geneva, Switzerland). The total set of analytes consists of 31 compounds, including the 24 allergens indicated in EC directive 2003/15/EC (some regulated compounds consist of more than one isomer, e.g. citral = neral + geranial; farnesol = 2 isomers; Lylal = 2 isomers) plus some compounds that are often monitored in addition to the regulated compounds (phenyl acetaldehyde, estragol, methyl eugenol, methyl 2-nonynoate). Individual stock solutions at 10 mg/mL level were prepared in acetone (pesticide grade, Merck ChromaSolv, VWR, Leuven, Belgium). From the individual stock solutions, calibration mixtures were prepared at 10, 20, 40, 100, 200 and 400 ng/µL concentration levels by further

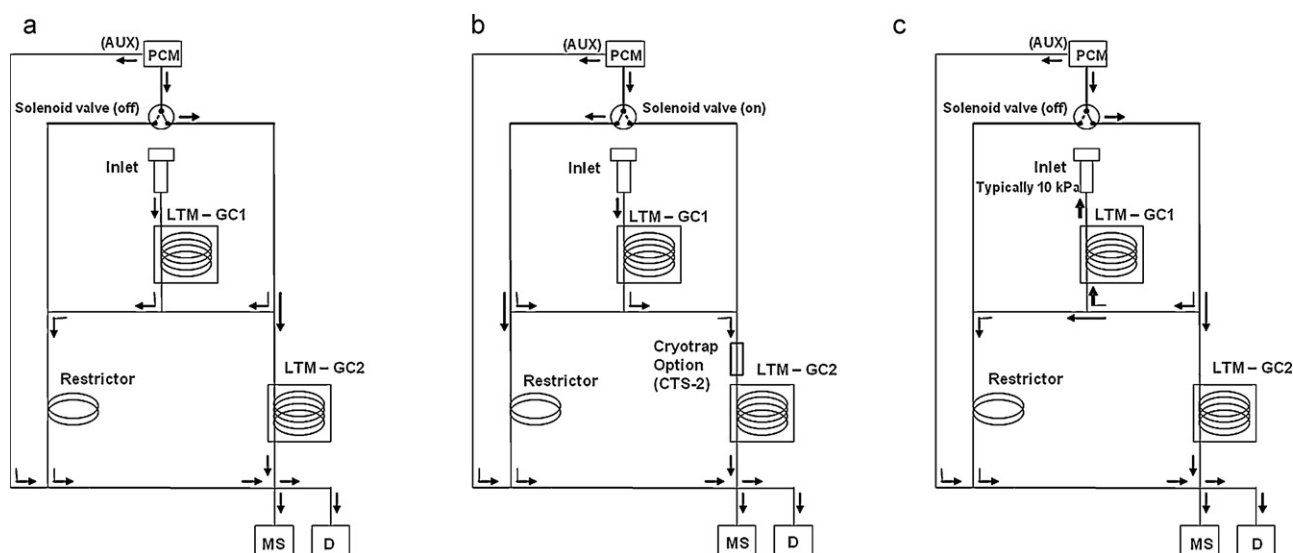


Fig. 1. Schematic flow diagrams of the selectable $^1\text{D}/^2\text{D}$ GC–MS configuration. (a) ^1D GC–MS analysis (flow column 1 to detector); (b) heart-cutting (flow column 1 to cold trap/column 2); (c) ^2D GC–MS analysis and ^1D GC back flush.

dilution in acetone. Commercial perfume samples and a cosmetic product (body cream) were obtained from a supermarket. Perfume samples were diluted 10 times in acetone.

2.2. Instrumentation

An overview of the selectable $^1\text{D}/^2\text{D}$ GC–MS configuration is shown in Fig. 1. The system consist of an Agilent 7890 GC equipped with a split/splitless inlet, a flame ionization detector (FID) and a 5975C MSD (Agilent Technologies, Wilmington, DE, USA). A

capillary flow technology (CFT) Deans switch device and a three way purged CFT splitter (Agilent Technologies) are housed in the main GC oven. The first and second dimension column are installed in two low thermal mass (LTM, Agilent Technologies) modules incorporated in the GC oven door. In addition, a programmed temperature vaporizer (PTV) inlet (Gerstel GmbH, Mullheim, Germany) is adopted as secondary inlet port. Deactivated fused silica capillaries are used to connect the LTM columns to the inlets, CFT devices and detectors. The operation of the selectable $^1\text{D}/^2\text{D}$ GC–MS configuration was described elsewhere [20,21] and can be summarized

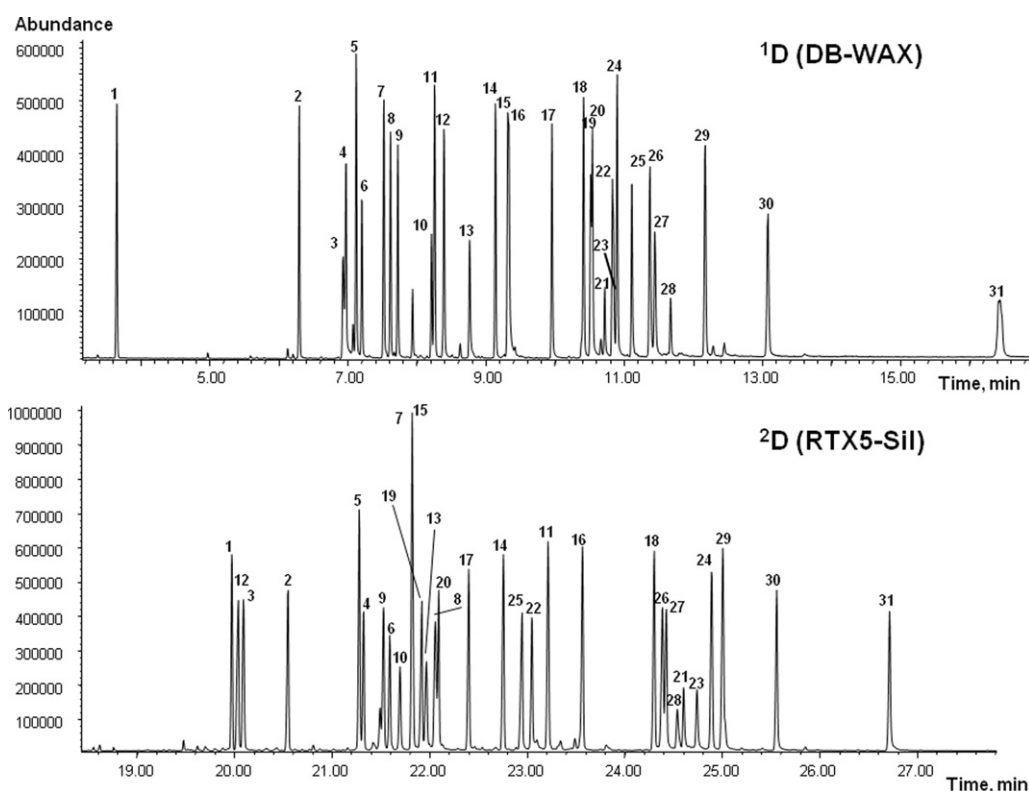


Fig. 2. Separation of the 31 allergen compounds on the polar DB-Wax ^1D column (top) and on the apolar Rtx-5Sil column after complete transfer from first dimension (bottom).

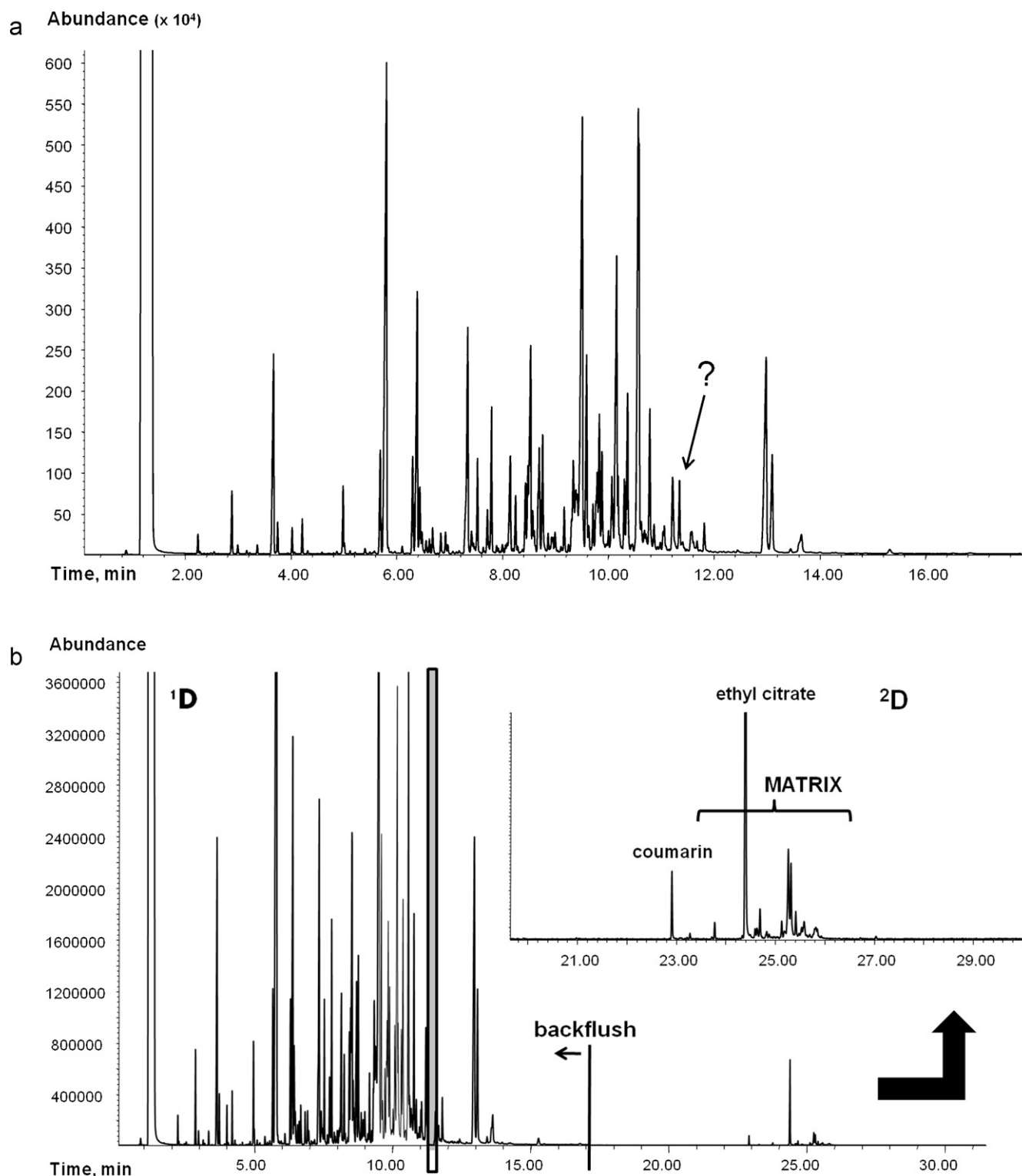


Fig. 3. (a) 1^{D} GC-MS chromatogram (TIC) of perfume sample 1 (? indicates coumarin elution window). (b) Heart-cutting 2^{D} GC-MS analysis for the determination of coumarin. Heart-cut window and back-flush initiating times are indicated in the chromatogram. Separation obtained in 2^{D} is given in zoom insert.

as follows: The sample is injected (as liquid from split/splitless injection port or as gas from thermal desorption unit, see below) into column 1 (LTM-GC1). Using the Deans switch device the effluent from the first dimension column can either be diverted to the three-way splitter via a restrictor capillary or to the second dimension column (LTM-GC2). In the first mode, the effluent is also sent to the MS detector and FID detector (1:1 split between

detectors). This instrument mode, shown in Fig. 1a, is equivalent to a one-dimensional GC-MS method. In the second mode, the effluent from the first column (typically one or more heart-cut fractions) is collected in the (cold) second dimension column (Fig. 1b). After this transfer, the first dimension column is back-flushed by lowering the pressure at the inlet (10 kPa, Fig. 1c). An additional cryo-option at the inlet of the second dimension column (CTS 2,

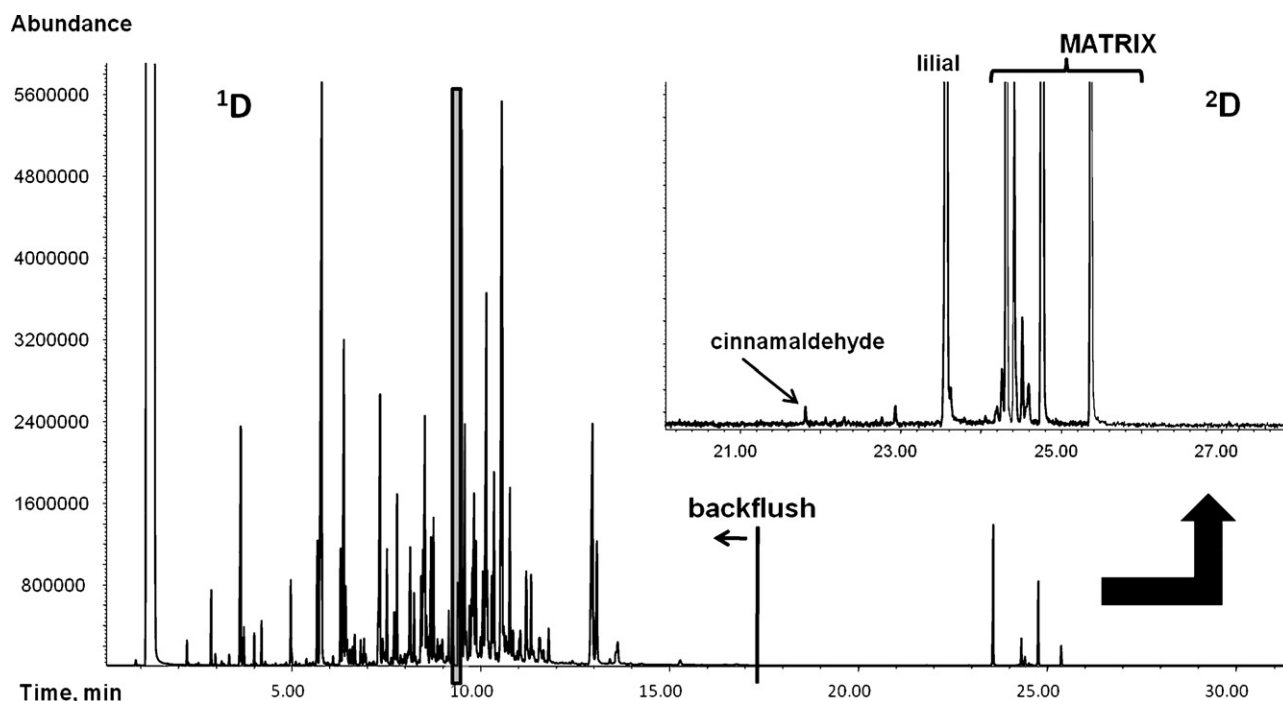


Fig. 4. Heart-cutting ^2D GC-MS analysis for the determination of cinnamaldehyde and linal. Heart-cut window and back-flush initiating times are indicated in the chromatogram. Separation obtained in ^2D is given in zoom insert.

Gerstel, see Fig. 1b) can be used to efficiently focus the most volatile fractions. After completion of the back-flush, the cryo-trap and LTM-GC2 are programmed and the eluting fraction is also monitored by parallel FID and MSD detection. The FID was not used in the present work, but could be applied for quantification or could be replaced by an olfactory port (for organoleptic evaluation). By creating different acquisition methods, an automated sequence can be programmed with sequential one-dimensional and heart-cutting two-dimensional methods without any changes in the hardware configuration.

For the analysis of allergens, a polar DB-Wax capillary column (10 m $\times$ 0.18 mm ID $\times$ 0.18 μ m df, Agilent Technologies) was selected as the first dimension and an apolar Rtx-5 (5% phenyl methyl siloxane) capillary column (10 m $\times$ 0.18 mm ID $\times$ 0.18 μ m df, Restek, Bellefonte, PA, USA) was used in the second dimension. Both columns can be programmed independently as they are installed into two separate LTM modules mounted on the main GC oven door, which itself is kept at constant high temperature (250 $^{\circ}$ C). Compounds, heart-cut from the first dimension are cryo-focused using the CTS-2 cold trapping device at -150° C in the restriction (1 m $\times$ 0.18 mm) that connects the Deans switch CFT with the second dimension column. The analytical conditions are summarized in Table 2.

Automated dynamic headspace sampling (DHS) is performed using a DHS device mounted on an MPS-2 autosampler (Gerstel). Small aliquots of the sample are placed in a 20 mL headspace vial. The headspace is purged at 80 $^{\circ}$ C during 14.5 min with 70 mL/min helium (total purge volume = 1000 mL = 50 times vial volume) and the compounds are trapped at 40 $^{\circ}$ C on a glass thermal desorption (TDU) tube filled with Tenax adsorbant. Afterwards, this TDU tube is thermally desorbed at 260 $^{\circ}$ C (7 min) in splitless mode in a thermal desorption unit (TDU) and the compounds are cryo-focused into an empty baffled PTV liner at -100° C. Injection from the PTV is done in split (1/50 mode) by programming at 720 $^{\circ}$ C/min to 280 $^{\circ}$ C. In case highest sensitivity is required, also splitless injection could be applied, but to our experience this is not needed in most cases.

3. Results and discussion

3.1. Separation of allergen compounds using ^1D GC-MS and ^2D GC-MS

In a first set of experiments, liquid injection in a split/splitless inlet was used for calibration mixtures and perfume samples (class I and II) to evaluate the $^1D/^2D$ GC-MS approach.

Fig. 2 (top) shows the separation of the 31 allergen compounds in the first dimension DB-Wax column. Compound identification and retention times in the first dimension separation are included in Table 1. Not all allergen compounds are chromatographically separated. Three co-eluting solute pairs and one triplet are observed. Cinnamaldehyde/linal 1 (15–16), anisyl alcohol/cinnamyl alcohol (19–20) and coumarin/lyral 1 (25–26) are not separated in the

Table 2
Analytical parameters.

General ^1D parameters	
Inlet (liquid injection)	Split/splitless, split ratio = 1/20 340 kPa He, constant pressure 250 $^{\circ}$ C injection temperature Back-flushing (at 17.3 min, inlet pressure = 10 kPa)
Main GC oven	250 $^{\circ}$ C (constant)
Column 1	DB-Wax (LTM, 10 m $\times$ 0.18 mm i.d. $\times$ 0.18 μ m thickness)
Oven program (LTM 1)	40 $^{\circ}$ C, 1 min – 20 $^{\circ}$ C/min – 250 $^{\circ}$ C, 20 min
Deans switch	264 kPa, constant pressure
Three way purged splitter	34 kPa, constant pressure, MS/FID detection (split = 1/2)
MS	Scan-mode (35–400 m/z)
^2D parameters	
Column 2	Rtx-5 (LTM, 10 m $\times$ 0.18 mm i.d. $\times$ 0.18 μ m thickness)
Oven program (LTM 2)	40 $^{\circ}$ C, 18 min – 20 $^{\circ}$ C/min – 270 $^{\circ}$ C, 2 min
CTS-2 cryofocusing	250 $^{\circ}$ C, ($x - 1.77$) min – 25 $^{\circ}$ C/s – -150° C, 17.5 – ($x - 1.77$) min – 25 $^{\circ}$ C/s – 250 $^{\circ}$ C, 1 min with x = initial time first heart-cut

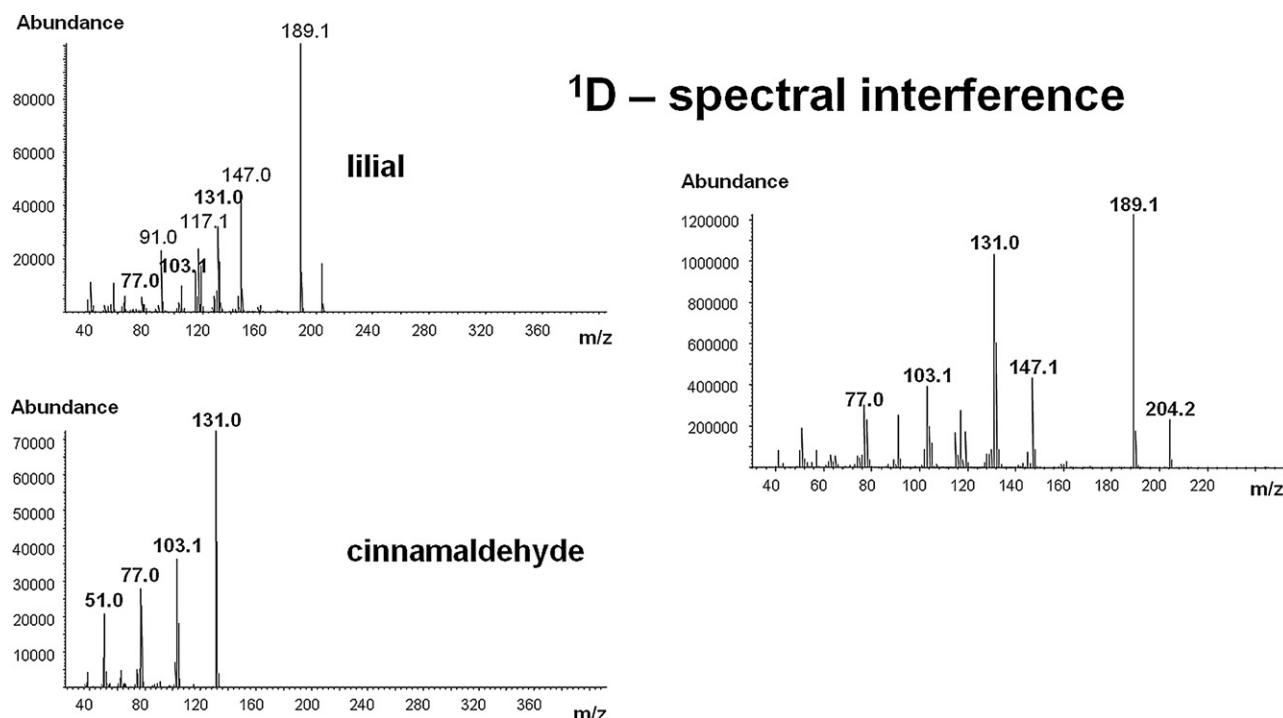


Fig. 5. Mass spectra obtained for co-eluting cinnamaldehyde and linal in ¹D-GC-MS (right) and after separation in ²D-GC-GC-MS (left).

first dimension. Isoeugenol, farnesol 2 and hexyl cinnamaldehyde (22–23–24) also elute together. Some of these co-eluting compounds can be resolved using mass spectral deconvolution (e.g. compounds 19 and 20), but in other cases, such as for cinnamaldehyde and linal, spectral interference is observed. Moreover, when real perfume samples are analyzed, it can be expected that additional co-elutions with other fragrance solutes will occur.

In cases where mass spectral deconvolution is not effective due to non-selective ions (spectral interference), a two-dimensional separation can be applied. The relative elution of the target allergen compounds on the second dimension Rtx-5Sil column is also shown in Fig. 2 (bottom). Retention times for all allergen compounds after the second dimension separation are indicated in Table 1. These retention times are the sum of first and second dimension separation, since this chromatogram is obtained by using a complete heart-cut of all 31 allergen compounds (heart-cut window: 3.5–17 min), cryo-focussing between the columns and re-injection in the second column. All co-eluting target solutes (3 pairs, one triplet) are now well separated, illustrated for instance by the excellent separation between solutes 22 (isoeugenol), 23 (farnesol 2) and 24 (hexyl cinnamaldehyde).

Of course, when applying ²D GC-GC-MS for real samples, only these fractions showing co-elution and mass spectral interference of allergens with matrix or other target solutes will be heart-cut and further analyzed in the second dimension. It is important to note that the proposed selectable ¹D/²D GC-MS configuration allows us to select the time regions for the heart-cuts using the MS trace, instead of using a monitor FID, as often applied in classical two-dimensional configurations [2,8–10]. Especially for real samples (with potentially retention time shifts due to overloading matrix compounds), mass spectrometric detection in the first dimension is an advantage. After elution of the last solute of interest (benzyl cinamate, 31), the compounds (typically low-volatile matrix solutes) not eluted from the first dimension column are back-flushed by decreasing the inlet pressure (10 kPa). This back-flush is performed while the second dimension separation is done, so no extra analysis time is required.

3.2. Analysis of perfume samples using selectable ¹D/²D GC-MS

The workflow that is applied for real samples consists first of a one-dimensional GC-MS analysis. A typical chromatogram showing the ¹D GC-MS chromatogram obtained for a commercial perfume sample is shown in Fig. 3a. The target solutes present in this sample can be identified and quantified using appropriate calibration (also performed in ¹D GC-MS mode), eventually also using MS deconvolution. For solutes where co-elution is occurring and mass spectral deconvolution is not possible, the heart-cut windows are defined and a second run is performed in ²D GC-GC-MS mode. This is illustrated for coumarin, coeluting around 11.4 min (see “?” in Fig. 3a) with other fragrance compounds in the first dimension separation. The ²D GC-GC-MS separation of the same sample is shown in Fig. 3b. The coumarin elution window is heart-cut and transferred to the second dimension, while the first dimension column is back-flushed (back-flush at 17 min). The separation obtained in the second column appears as a second part of the chromatogram. A zoom on that part (20–30 min total elution time) is also given in Fig. 3b (insert).

Coumarin, an allergen compound frequently present at relatively low concentration level, is clearly detected (22.94 min) and fully separated from interfering matrix compounds (i.e. ethyl citrate). This compound can also be quantified using appropriate calibration (done in ²D GC-GC-MS mode). From the same sample, also a heart-cut was made for cinnamaldehyde and linal (time segment 9.28–9.38 min). As shown in Fig. 4, the second dimension chromatogram shows the presence of cinnamaldehyde (21.84 min) and linal (23.56). Cinnamaldehyde was only present at trace level (11 ppm, slightly above regulated level) in the perfume sample and could only be confirmed in this second dimension trace, due to mass spectral interference from linal at much higher level (669 ppm). Fig. 5 compares the mass spectrum acquired at elution of linal during the first dimension separation (right) with the mass spectra of linal and cinnamaldehyde acquired during two-dimensional separation (left). The main ions for cinnamaldehyde identification are

Table 3

Figures of merit.

No.	Solute	Calibration (100 ppm)				Sample 1		Sample 2		Sample 3	
		RSD (%)		Linearity		Conc. (ppm)		Conc. (ppm)		Conc. (ppm)	
		¹ D	² D	¹ D	² D	¹ D	² D	¹ D	² D	¹ D	² D
15	Cinnamaldehyde	1.4	4.1	0.9999	0.9991	110	21.7	–	–	–	–
16	Lilial	1.6	1.2	0.9999	0.9997	6690	0.7	5220	1.7	–	–
25	Coumarin	1.3	1.3	0.9999	0.9999	–	–	–	–	240	2.3
26	Lylal 1	2.6	3.8	0.9977	0.9970	–	–	–	–	2920	1.4
27	Lylal 2	3.0	1.8	0.9993	0.9983	–	–	–	–	2580	0.7

RSD (%) was determined in calibration samples at 100 ppm level ($n=6$), and in samples ($n=6$).Linearity was determined in a concentration range from 10 to 400 ng/ μ L.

Conc. (ppm) in samples is determined using an external standard calibration curve.

present in the spectrum from lilial and therefore chromatographic resolution was required.

3.3. Validation of the determination of allergen compounds in perfume samples using selectable ¹D/²D GC–MS

The selectable ¹D/²D GC–MS approach for the determination of allergen compounds in perfume samples was validated using calibration mixtures and three perfume samples. The figures of merit are summarized in Table 3. First the repeatability of peak areas ($n=6$) was determined for the target compounds using ¹D GC–MS and ²D GC–GC–MS. From the RSD (%) results it is clear that equivalent repeatability is obtained in both modes with RSD % values below 5%. This indicates that heart-cutting two-dimensional analysis using a more complex selectable ¹D/²D GC–MS configuration results in similar performance as ¹D GC–MS analysis. The

results for the repeatability tests obtained here for both ¹D and ²D analysis are also in agreement with previously published results [2].

Linearity was determined in a range from 10 to 400 ng/ μ L, using ¹D GC–MS and ²D GC–GC–MS analysis. The correlation coefficients (r^2) are excellent ($r^2 > 0.995$) in all cases. No significant differences were observed between ¹D GC–MS and ²D GC–GC–MS mode.

Table 3 also includes measured concentrations and repeatability (RSD% for $n=6$) for the target compounds detected in three perfume samples using the ²D GC–GC–MS mode. RSD (%) is typically below 5%. Only for coumarin in sample 1 (concentration = 11 ppm) a higher value (21.7%) is obtained.

These data however clearly indicate that the selectable ¹D/²D GC–MS approach can be used for accurate quantification and no significant influence from the matrix on the repeatability of the determination of allergen compounds can be observed.

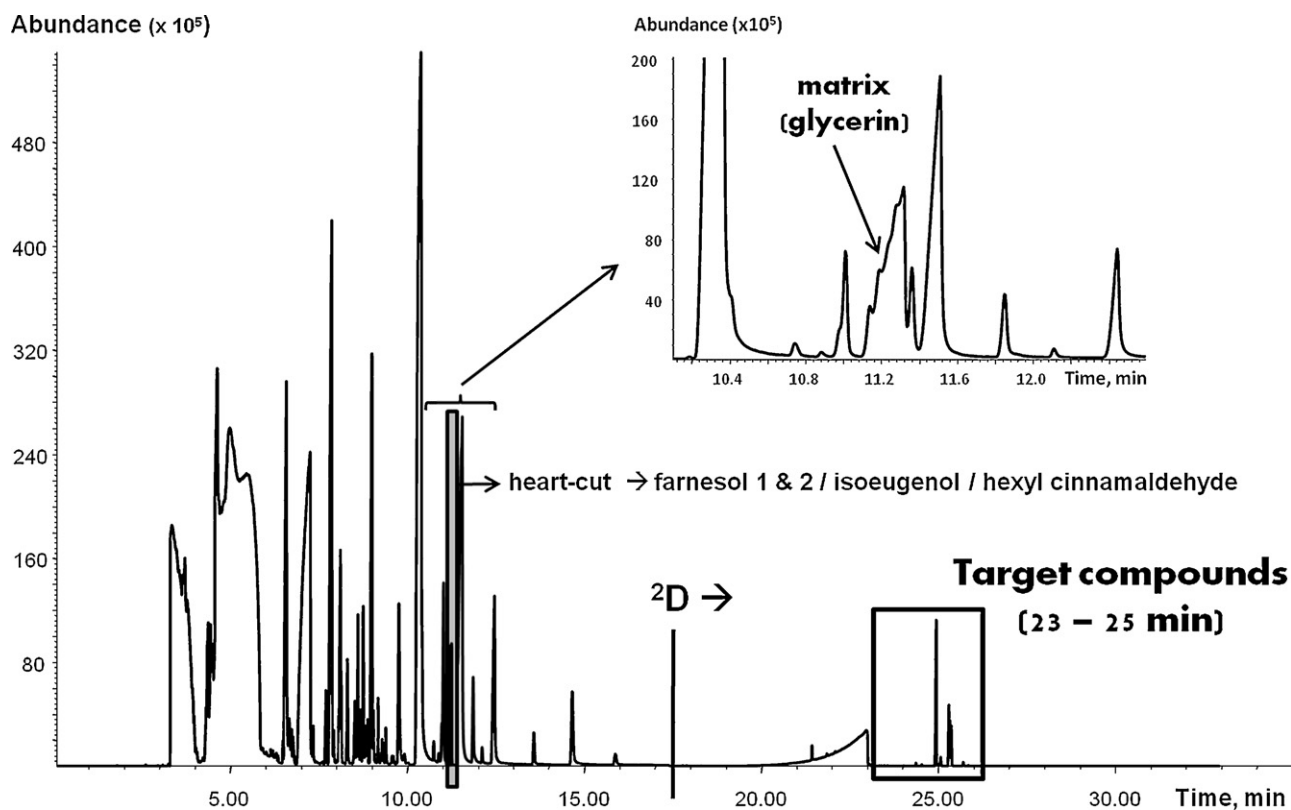


Fig. 6. FEDHS-²D GC–GC–MS analysis of body cream sample – Heart-cutting of fraction containing farnesol 1 and 2, isoeugenol and hexyl cinnamaldehyde. Zoomed area (top right) indicates area of possible coelution of farnesol 1 and 2, isoeugenol and hexyl cinnamaldehyde with glycerin in first dimension separation. Glycerin elutes at 20–23 min in second dimension. Hexyl cinnamaldehyde is detected at 24.92 min after second dimension separation.

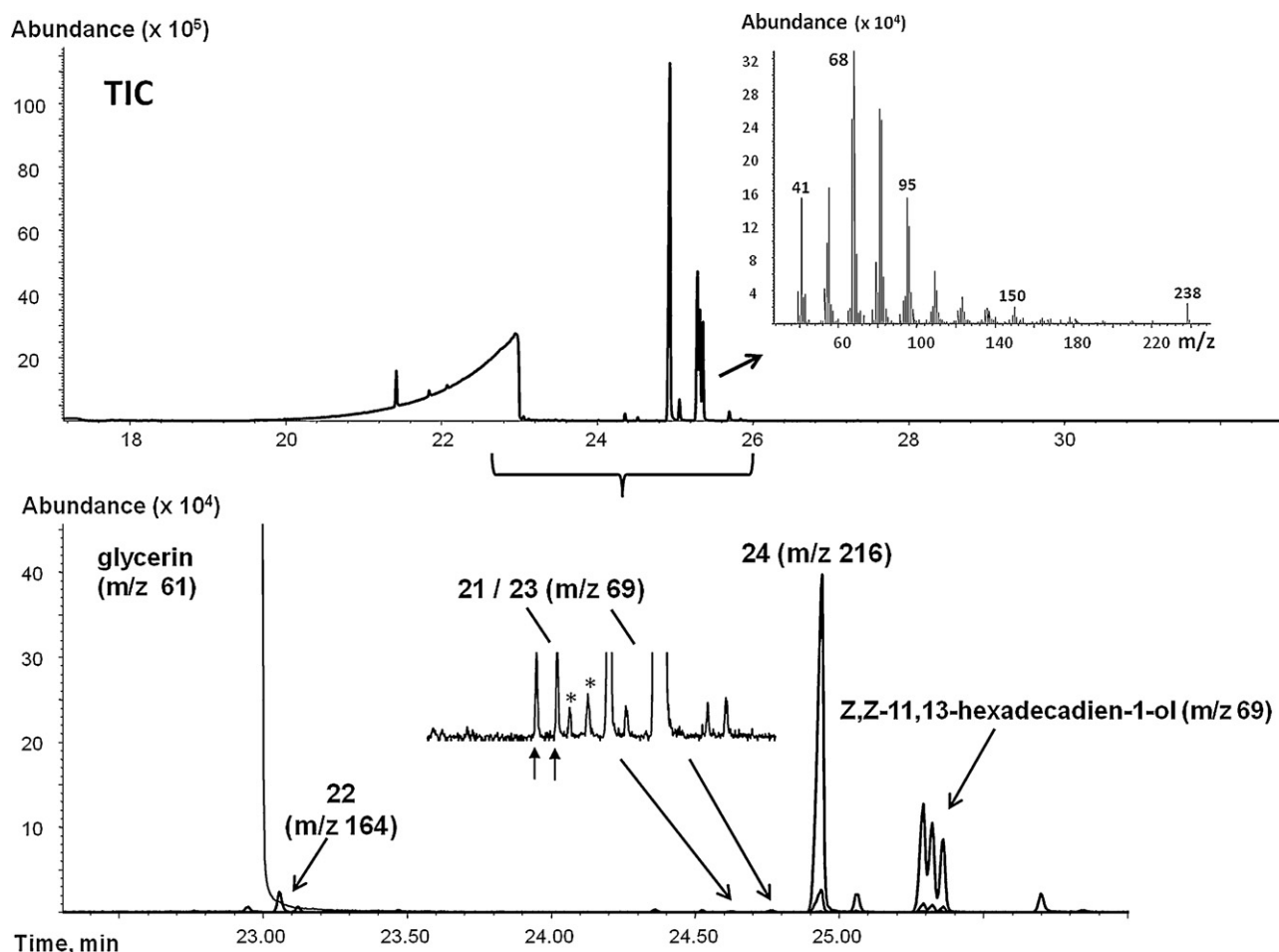


Fig. 7. FEDHS- ^2D GC-MS analysis of body cream sample spiked with 1 ppm farnesol – Second dimension separation of fraction containing farnesol 1 and 2, isoeugenol and hexyl cinnamaldehyde. Glycerin elutes at 20–23 min in second dimension (top). Extracted ion chromatograms show the presence of isoeugenol (compound 22, ion 164), hexyl cinnamaldehyde (compound 24, ion 216), farnesol 1 (compound 21, ion 69) and farnesol 2 (compound 23, ion 69). Also the presence of methylidihydrojasmonate (arrows in insert) and hexadecadienol (MS in insert) is observed.

3.4. FEDHS and selectable $^1\text{D}/^2\text{D}$ GC-MS for the analysis of cosmetic samples containing non-volatile solutes

Class III cosmetic samples, such as lipstick or class IV samples, such as shower gels and body creams, cannot be analyzed by sample dilution and direct liquid injection since the sample matrix will contaminate the analytical system and the presence of water (class IV samples) results in peak distortion [2]. By using FEDHS the sample is completely evaporated in a headspace vial at 80 °C. Volatile and semi-volatile compounds are purged using a large volume (about 50 times vial volume) and trapped on a Tenax filled thermal desorption tube, maintained at 40 °C. This high purge volume together with an elevated trap temperature of 40 °C (compared to 20–25 °C for conventional DHS) minimizes water enrichment on the adsorbant. Using this approach, it was observed that the obtained profiles of fragrance constituents (including all allergen target compounds) are similar to profiles obtained by liquid split/splitless injection and that no discrimination based on polarity or volatility is observed. This is an important advantage compared to previously described SBSE sample preparation whereby extraction is based on octanol–water partitioning coefficients, resulting in higher recoveries for apolar solutes (limonene, benzyl benzoate) than for polar solutes (benzyl alcohol, cinnamyl alcohol) [2].

Calibration of the FEDHS analysis was done by spiking aliquots of calibration solutions in headspace vials. Repeatability was typically around 5% (RSD% for $n=6$), which is slightly higher compared

to direct liquid injection (RSD's below 5%). Of course, the FEDHS method includes a fully automated headspace sample enrichment and thermal desorption GC-MS which obviously can explain these slightly higher values.

During method optimization, a breakthrough test was performed (comparison of standard solution analyzed by liquid injection with standard solution analyzed via FEDHS). As already described above, good recoveries (>80%) were obtained and no discrimination was observed between limonene (most volatile) and benzyl cinnamate (less volatile, latest eluting compound). Also good correlation was obtained between a sample analyzed by FEDHS and direct liquid injection. Of course, depending on the sample matrix, additional testing can be required to optimize FEDHS conditions for certain cosmetic samples.

The FEDHS- $^1\text{D}/^2\text{D}$ GC-MS method was applied to a body cream sample placed in a 20 mL headspace vial. Fig. 6 presents the complex ^1D GC-MS chromatogram. Different matrix compounds such as propylene glycol, glycerin, etc. overload the chromatogram. Glycerin elutes in the same elution window as the allergen compounds farnesol 1, isoeugenol, farnesol 2 and hexyl cinnamaldehyde (see zoom top right Fig. 6). It is important to note that in this analysis, the target allergen compounds elute significantly later (ca. 0.45 min) during the ^1D GC-MS analysis compared to the direct liquid injection due to matrix effect of the body cream constituents (solvent effect of highly abundant glycols). This example clearly illustrates the advantage of using MS detection also after the first

dimension. Without MS, correct peak allocation and heart-cut window selection would be difficult. Moreover, using this first analysis, both isoeugenol and hexyl cinnamaldehyde could already be identified and quantified without the need of an additional analysis, since selective ions are available. However, it was not possible to identify and quantitate both farnesol isomers due to matrix interference. These allergen compounds, if present, are only detected at low concentration and their mass spectra contain low abundant and non-selective fragment ions [10]. Therefore, a second analysis was performed in ^2D GC–GC–MS mode with a heart-cut on the farnesol fraction (11.1–11.4 min). Fig. 6 also shows the heart-cut taken from the ^1D chromatogram and the subsequent ^2D separation. The highly fronting peak eluting from 20 to 23 min is identified as glycerin and is now fully separated from the allergen compounds. The presence of isoeugenol (23.05 min, not shown) and hexyl cinnamaldehyde (24.92 min), could be confirmed using respectively ions 164 and 216 and they could be quantified at 3 ppm and 89 ppm, respectively. Farnesol 1 and 2 were not detected in the sample as the second dimension chromatogram showed no peaks at the expected retention times, 24.60 min and 24.73 min, respectively.

Fig. 7 presents the same analysis of the body cream sample spiked with 1 ppm of farnesol. The top chromatogram shows the ^2D GC–MS trace from a heart-cut (11.1–11.4 min; same as in Fig. 6). All four target allergen compounds were separated from the matrix compounds (mainly glycerin). The EIC traces, i.e. $m/z = 69$, $m/z = 216$ and $m/z = 164$ are shown in the bottom chromatogram of Fig. 7. The EIC trace with $m/z = 69$ is enlarged for the time window where both farnesol isomers elute (peaks marked with *). Farnesol 1 and 2 are detected near the LOD level and this analysis confirms that farnesol is not present in the body cream sample. Another fragrance compound, identified as methylidihydrojasmonate (two isomers indicated with an arrow) was detected in the extracted ion chromatograms at $m/z = 69$. In addition, also a cluster of isomers elutes around 25.3 min. These compounds were identified (using the NIST library) as isomers of hexadecadien-1-ol, an unsaturated long-chain alcohol. The presence of these compounds in the extracted ion chromatogram ($m/z = 69$) clearly illustrate that 2D–GC–GC–MS can be needed to confirm the absence or presence of certain allergens in cosmetic products, especially in cases where only low mass, non-selective target ions are present in the spectra.

4. Conclusion

Selectable $^1\text{D}/^2\text{D}$ GC–MS was successfully applied for the determination of allergen compounds in perfume samples by liquid injection after dilution. The flexibility of the system, i.e. no hardware changes are needed when switching between ^1D GC–MS analysis and ^2D GC–GC–MS analysis, is an important advantage. Heart-cutting windows can be easily selected using the MS trace of the first dimension separation and the second dimension

separation can be used for confirmation and quantification of co-eluting target compounds.

The combination of full evaporation dynamic headspace with selectable $^1\text{D}/^2\text{D}$ GC–MS is especially useful for cosmetic samples containing non-volatile matrix compounds such as waxes, detergents, etc. Non-discriminating injection of the fragrance fraction is possible, while inlet, column and detector contamination are eliminated.

FEDHS in combination with selectable $^1\text{D}/^2\text{D}$ GC–MS makes the qualitative and quantitative determination possible of all allergen compounds in the 4 classes of cosmetic samples using a single instrumental configuration.

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