Journal of Chromatography A, 1217 (2010) 2599–2605

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Quantitative determination of some volatile suspected allergens in cosmetic creams spread on skin by direct contact sorptive tape extraction–gas chromatography–mass spectrometry

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ARTICLE INFO

Article history:
Available online 4 January 2010

Keywords:
Direct contact sorptive tape extraction (DC-STE)
PDMS tape
GC–MS
Cosmetic cream
Volatile suspected allergen
Skin
Quantitative analysis

ABSTRACT

This study describes a method based on direct contact sorptive tape extraction followed by on-line thermal desorption gas chromatography–mass spectrometry (DC-STE–GC–MS) to detect and quantify a group of suspected volatile allergens on the European Union (E.U.) list and a related compound on the skin (the stratum corneum) of volunteers treated with a cream of known composition fortified with the reference allergens. The following compounds were tested: citronellol, Z-citral (neral), geraniol, cinnamaldehyde, anisyl alcohol, cinnamyl alcohol, eugenol, methyleugenol, coumarin, isoeugenol, α-isomethylionone, 2-(4-tert-butylbenzyl)propionaldehyde (lilial), α-amylcinnamaldehyde, α-hexylcinnamaldehyde. Sorptive tape extraction (STE) is a sorption-based sampling technique in which a flexible polydimethylsiloxane (PDMS) tape is used to recover analytes by direct contact with the surface of a solid matrix or from the headspace in equilibrium with it. The reliability of the method was confirmed by: (i) allergen recoveries varying from 52.3% for lilial to 95.7% for neral, (ii) linearity in the range 10–150 ppm, with regression coefficient R^2 always above 0.97, (iii) repeatability of each analyte, RSD% never exceeding 10%, (iv) intermediate precision, always below 15%, and (v) LOD and LOQ in the ppb range, therefore fully compatible with E.U. prescriptions (ppm). Other parameters such as substantivity analyte, approximate permeation through skin and influence of different nature of stratum corneum on recovery were also investigated. The method was also successfully applied to five commercially available creams declared to contain some of the allergens in question spread on the skin of the same volunteers.

1. Introduction

The ever-increasing importance of volatiles as markers to characterize liquid or solid matrices has strongly stimulated the development of highly effective sample preparation techniques, mainly for vapor phase sampling. Several solventless techniques suitable for application to both liquid and vapor phases have successfully been developed, after the introduction of the first and most popular method, i.e. solid-phase microextraction (SPME) [1,2]. The newer techniques aim to offer better performance than SPME and extend the fields of application; they include in-tube sorptive extraction (INCAT, SPDE), sorptive extraction (SBSE, HSSE), solid-phase aroma concentrate extraction (SPACE), large surface area sampling (MESI, MME, STE) and liquid phase microextraction (LPME, HS-LPME). Their use in headspace sampling was recently reviewed by Bicchi et al. [3]. Most techniques are based on the high concentration capacity approach, i.e. techniques where the analytes are accumulated into a polymer by sorption or adsorption and recovered by liquid or thermal desorption on-line or off-line to gas chromatography (GC), as such or combined with MS (GC–MS).

In 2006 Sandra et al. introduced sorptive tape extraction (STE) [4], a technique whereby the analytes are accumulated by sorption on a thin flexible PDMS tape, recovered by either thermal or solvent desorption and analysed on-line by GC or GC–MS. They applied STE to study the effect of a cosmetic treatment on the composition of human skin sebum (taken as marker) through *in vivo* sampling by direct contact of the PDMS tape with the skin surface. More recently, Bicchi et al. [5] successfully applied PDMS tapes to static headspace (HS-STE) and direct contact (DC-STE) sampling at the surface of solid matrices, such as the leaves of aromatic plants and fruits, and in the fragrance field. The main advantages of PDMS tapes are high analyte recovery, due to their large surface, and especially their specific ability to sample analytes by direct contact from the surface of a solid matrix. The influence of surface on recovery was already discussed by Bruheim et al. [6] who found that, with a thin sheet of a PDMS membrane, better recoveries were obtained in shorter times and with higher sensitivity than with a thick-film PDMS-coated SPME fibre, in sampling PAHs-spiked water. This

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doi:10.1016/j.chroma.2009.12.052

Table 1
List of the suspected allergens and a related compound investigated with their CAS numbers, Log $K_{o/w}$ values and concentrations in the mother cream.

#	Compound	CAS	Log $K_{o/w}$	Concentration in mother cream (ppm)
1	Citronellol	106-22-9	3.56	183
2	Z-citral (neral)	106-26-3	3.45	227
3	Geraniol	106-24-1	3.47	194
4	Cinnamaldehyde	104-55-2	1.82	192
5	Anisyl alcohol	105-13-5	1.16	190
6	Cinnamyl alcohol	104-54-1	1.84	186
7	Eugenol	97-53-0	2.73	186
8	Methyleugenol[a]	93-15-2	3.03	187
9	Coumarin	91-64-5	1.51	203
10	Isoeugenol	97-54-1	2.65	194
11	α-Isomethylionone	127-51-5	4.84	181
12	Lilial	80-54-6	4.36	198
13	α-Amylcinnamaldehyde	122-40-7	4.33	231
14	α-Hexylcinnamaldehyde	101-86-0	4.82	173

[a] Compound not included in the E.U. list.

increased performance for both vapor phase and in-solution sampling was shown to be due to the larger ratio between surface area and extraction phase volume.

Standards of quality and safety for cosmetic and food products are becoming increasingly severe. One example is the list of 26 compounds suspected of being possible causes of contact-allergy reactions in fragrance-sensitive consumers, included in the latest E.U. legislation on cosmetics [7]. The amount of these substances must be declared on the label if it exceeds the limit of 0.001% for "leave-on" and 0.01% for "rinse-off" cosmetic products. Several methods for the determination of suspected allergens in fragrances and other cosmetic products have been reported [8–11].

Another equally important aspect concerns monitoring these compounds after application of a cosmetic formulation containing them, in particular detection and quantitation on the skin surface after cosmetic treatment, and studying skin permeation and persistence, the latter more correctly known as "substantivity" of the application [12].

This study aimed to detect and quantify 13 suspected volatile allergens and a related compound on the skin surface (i.e. the stratum corneum) after treatment with a reference cream of known composition fortified with them. This was achieved by treating volunteers with cream fortified with known amounts of a standard mixture of the compounds investigated, and with a number of commercially available creams (five) whose labels declared they contained them, and then detecting and quantifying them on the skin surface by direct contact STE, followed by on-line recovery by thermal desorption and GC–MS analysis (DC-STE–GC–MS).

2. Experimental

2.1. Chemicals, reagents and matrices

Pure standards of citronellol (1), Z-citral (neral) (2), geraniol (3), cinnamaldehyde (4), anisyl alcohol (5), cinnamyl alcohol (6), eugenol (7), methyleugenol (8), coumarin (9), isoeugenol (10), α-isomethylionone (11), 2-(4-tert-butylbenzyl)propionaldehyde (lilial) (12), α-amylcinnamaldehyde (13), α-hexylcinnamaldehyde (14) and undecane, used as internal standard (IS), were from the laboratory collection of standards. Methyleugenol was included on the list to show that the method can be extended to the quantitation of other compounds used in the cosmetic field. Table 1 lists the analytes investigated, their CAS numbers and Log $K_{o/w}$. A standard mixture of 50 mg of each of the 14 compounds under investigation (SA mixture) was prepared and stored at −20 °C until use.

PDMS tapes (length: 15 mm, width: 4 mm, thickness: 0.5 mm; area: 0.6 cm^2) were kindly supplied by Prof. Dr. Pat Sandra (Research Institute for Chromatography – Kortrijk (Belgium)).

A cream consisting of Phytocream® (SEPPIC, France) (3%), octyl octanoate (14%), glycerol (5%) and water (78%) was supplied by the Laboratory of Cosmetic Chemistry, Dipartimento di Scienza e Tecnologia del Farmaco, University of Turin (Italy) and taken as reference ("cream" for short). The cream was fortified with an amount of SA mixture suitable to achieve a final concentration of each investigated allergen of around 200 ppm (for short "mother cream"). Table 1 reports the concentrations in ppm of the analytes in the mother cream; it was then diluted with suitable amounts of unfortified cream to achieve the concentration required for each experiment.

Two volunteers (volunteer 1 and volunteer 2) underwent these experiments. Both volunteers gave their informed consent after having been informed in detail about all risks involved with the study and on how to proceed in case of adverse reaction. All procedures were performed in compliance with relevant laws and institutional guidelines.

Five commercially available creams, whose labels declare compositions similar to that of the reference cream and indicates them to contain the suspected allergens investigated (for short "commercial cream"), were also analyzed.

2.2. DC-STE skin surface sampling

A weighed amount (70 mg) of both the cream spiked with a known concentration of the investigated allergens and a related compound obtained by a suitable dilution of the mother cream and the commercial creams was spread uniformly on a precisely defined area of the back of one hand of one volunteer; a surface large enough to afford at least six non-overlapping DC-STE samplings was circumscribed (32 cm^2). The PDMS tape was rested on the treated surface of the hand for 30 min at the skin temperature. After sampling, PDMS tapes were removed from the hand, inserted into a glass tube and then introduced into a thermodesorber (TDU, Gerstel, Mülheim a/d Ruhr, Germany) from where the analytes were recovered and analyzed by GC-MS (see Section 2.3). This procedure was used to evaluate the following parameters for each investigated analyte: calibration curve and linearity, repeatability and intermediate precision, limits of detection (LOD) and quantitation (LOQ), recovery, substantivity, cream permeation, influence of nature of stratum corneum of the two volunteers on recovery, and to analyze five commercial creams.

Undecane was used as internal standard: it was homogeneously sorbed into all PDMS tapes before each experiment by suspending them in 4 mL of a standard solution of undecane in water (4 μg/mL) and stirring them for 30 min, following the method proposed by Pawliszyn for SPME [13].

Table 2
List of the target ions (in bold) and qualifiers, calibration curves, regression coefficients R^2, LOD and LOQ values of the investigated compounds analyzed by DC-STE–GC–MS.

#	Compound	Ions	Calibration curve equation	R^2	LOD (ppb)	LOQ (ppb)
1	Citronellol	**69**, 95, 81	$y = 41227x - 245189$	0.9964	40	110
2	Z-citral (neral)	**69**, 94, 109	$y = 26281x - 92035$	0.9899	140	400
3	Geraniol	**69**, 123, 93	$y = 76909x - 427211$	0.9940	50	130
4	Cinnamaldehyde	**131**, 132, 103	$y = 15144x + 164547$	0.9330	15	50
5	Anisyl alcohol	**138**, 137, 109	$y = 11676x + 31631$	0.9887	15	50
6	Cinnamyl alcohol	**92**, 134, 115	$y = 15277x - 190695$	0.9995	200	560
7	Eugenol	**164**, 103, 149	$y = 44491x - 344722$	0.9726	20	50
8	Methyleugenol[a]	**178**, 163, 147	$y = 43873x - 205330$	0.9921	15	50
9	Coumarin	**146**, 118, 89	$y = 45862x - 234345$	0.9958	35	100
10	Isoeugenol	**164**, 149, 131	$y = 27971x - 134847$	0.9996	190	500
11	α-Isomethylionone	**135**, 206, 150	$y = 82236x - 447020$	0.9945	70	200
12	Lilial	**189**, 204 147	$y = 34069x - 247767$	0.9999	50	130
13	α-Amylcinnamaldehyde	**202**, 201, 129	$y = 29270x - 210605$	0.9923	60	150
14	α-Hexylcinnamaldehyde	**216**, 215, 129	$y = 29208x - 73216$	0.9849	110	250

[a] Compound not included in the E.U. list.

2.2.1. Calibration curves, linearity and quantitation

A calibration curve was constructed for each investigated compound, by spreading a weighed amount (70 mg) of cream suitably diluted from the mother cream to obtain concentrations of about 10, 25, 50, 100 and 150 ppm of each analyte on the circumscribed surface (about 32 cm^2) of the back of one hand of volunteer 1, and then submitted to sampling with a PDMS tape (DC-STE). The sampled analytes were thermally recovered from the tape and analyzed on-line by GC–MS under the conditions reported in Sections 2.2 and 2.3.

The investigated analytes were quantitated by GC–MS operating in single ion monitoring acquisition mode (SIM) by determining the areas of at least three selected ions (one target ion and two qualifiers) for each analyte, both to confirm its identity on the basis of the quality values referred to target ion area ratios of a reference standard and to quantify it. Table 2 reports *m*/*z* target and qualifier ions used for SIM acquisition of the 14 compounds investigated. The calibration curves for each analyte investigated were calculated on the basis of the area of its target ion (normalised *versus* the undecane IS) *versus* the corresponding concentration.

2.2.2. Repeatability, intermediate precision

Weighed aliquots (70 mg) of the cream spiked with 25, 50, and 100 ppm of each investigated compound were spread uniformly on the selected surface (32 cm^2) of the back of one hand of volunteer 1, and sampled with a PDMS tape (DC-STE). The sampled analytes were recovered from the tape thermally and analyzed by GC–MS under the conditions reported in Sections 2.2 and 2.3. Each experiment was repeated six consecutive times to evaluate repeatability. Intermediate precision was determined on the 50 ppm spiked cream, analyzed every 4 weeks over a period of 3 months.

2.2.3. LOD and LOQ determination

The LOD and LOQ of each analyte was determined following Eurachem guidelines [14]. Ten blank experiments were carried out on the unspiked cream with the method described above. The LOD of each analyte was calculated from the average "peak to peak" noise values sampled in its region of elution in the chromatogram, with a coverage factor of 3. LOQ was experimentally determined by analyzing samples spiked with decreasing concentration of each analyte. LOQ was the lowest concentration to which the error for peak area determination (assignment) was ≤20%.

2.2.4. Analyte recovery, substantivity and approximate skin permeation and influence of nature of volunteers' stratum corneum on recovery

Analyte recovery was determined by spreading a weighed amount (70 mg) of the cream spiked with 50 and 100 ppm of each analyte, obtained by suitable dilution of the mother cream, uniformly on the selected surface (32 cm^2) of the back of one hand of volunteer 1, then submitted to sampling with a PDMS tape (DC-

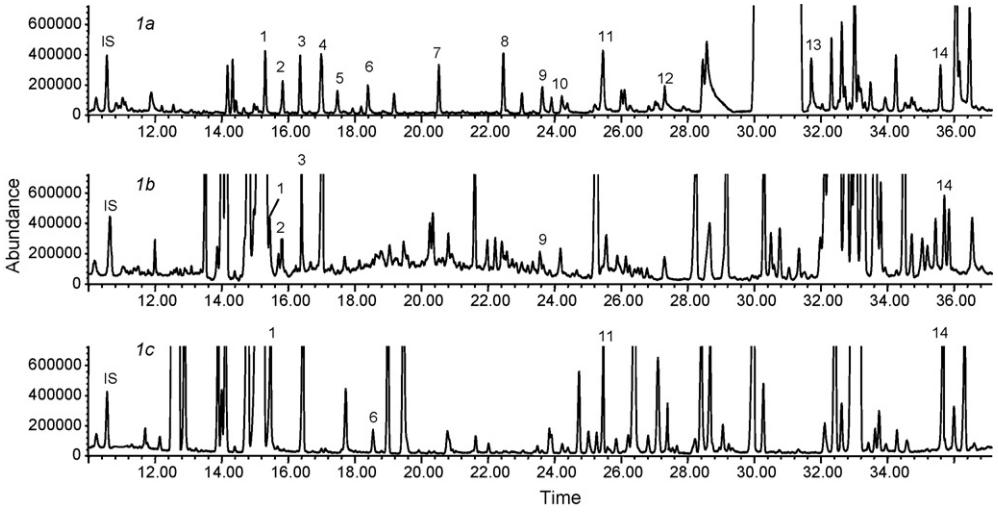

Fig. 1. TIC–GC–MS profiles after DC-STE sampling of the cream spiked with 50 ppm of each analyte (a), and of commercial cream 1 (b) and commercial cream 2 (c) (for analysis conditions and peak identification see text and tables).

Table 3
Repeatability (RSD%) of the DC-STE–GC–MS method on cream spiked with different amounts of the compounds investigated and intermediate precision.

#	Compound	Repeatability (RSD%)				Intermediate precision (RDS%)
		25 ppm	50 ppm	100 ppm	Average	50 ppm
1	Citronellol	5.7	4.4	2.4	3.4	9.0
2	Z-citral (neral)	4.8	4.4	2.8	3.3	6.5
3	Geraniol	6.0	4.7	3.7	4.2	9.2
4	Cinnamaldehyde	1.2	0.1	0.9	0.2	6.5
5	Anisyl alcohol	6.6	5.6	4.2	5.0	9.8
6	Cinnamyl alcohol	3.8	2.4	1.8	2.1	10.6
7	Eugenol	8.4	7.2	7.3	7.2	12.1
8	Methyleugenol[a]	7.9	8.1	7.2	7.2	8.2
9	Coumarin	4.2	3.7	3.8	3.3	3.2
10	Isoeugenol	8.6	7.8	6.9	7.1	14.6
11	α-Isomethylionone	10.2	9.3	8.8	9.0	8.2
12	Lilial	2.2	0.5	1.2	1.0	13.9
13	α-Amylcinnamaldehyde	8.9	7.4	6.2	7.1	2.5
14	α-Hexylcinnamaldehyde	15.4	13.4	9.4	12.2	14.2

[a] Compound not included in the E.U. list.

STE). The sampled analytes were thermally recovered from the tape and analyzed by GC–MS under the conditions reported in Sections 2.2 and 2.3. The recovery was determined by the % ratios between the absolute amount of the analyte obtained by DC-STE–GC–MS and that spiked in the cream.

The suspected allergens substantivity on the stratum corneum was measured by DC-STE sampling after 0, 20, 40 and 60 min from spreading the cream (70 mg) spiked with 50 ppm of each analyte in different positions of the surface of the back of the hand of volunteer 1 under the above conditions.

A series of experiments were also carried out under the same conditions reported above but by applying DC-STE sampling to an equivalent surface of a Pyrex glass plate spread with a known amount of the 50 ppm spiked cream instead of the back of the hand of the volunteer 1. These experiments were run to evaluate the allergen skin permeation.

The influence of different stratum corneum on recovery was evaluated by DC-STE sampling of the back of one hand of volunteers 1 and 2 treated with the same amount (70 mg) of the base cream spiked with 50 ppm of the investigated analytes spread uniformly on the selected surface (32 cm^2) and then analyzed under the above conditions.

2.2.5. Analysis of commercial creams

Five commercial creams were analyzed under the same conditions adopted for the spiked cream. The suspected allergens reported to be contained in the five commercial creams investigated are listed in Table 6.

2.3. Analysis conditions

Analyte thermal desorption was carried out with a TDU unit from Gerstel (Gerstel, Mülheim a/d Ruhr, Germany) driven by a MPS-2 multipurpose sampler (Gerstel, Mülheim a/d Ruhr, Germany) installed on an Agilent 6890 GC unit coupled to an Agilent 5973N MSD (Agilent, Little Falls, DE, USA) (Gerstel, Mülheim a/d Ruhr, Germany). For TDU the following parameters were used: desorption program: from 30 to 250 °C (5 min) at 60 °C/min; flow mode: splitless, transfer line: 300 °C. A Gerstel CIS-4 PTV injector was used to cryogenically focus the analytes thermally desorbed from the PDMS tapes. The PTV was cooled to −50 °C using liquid CO_2; injection: PTV; injection temperature: from −50 to 250 °C (5 min) at 12 °C/s. The inlet was operated in the split mode (split ratio 1:10).

Chromatographic conditions: helium was used as carrier gas at a flow rate of 1 mL/min.

Column: FSOT Mega 5-MS (d_f 0.25 μm, I.D. 0.25 mm, length 30 m) (Mega, Legnano (Milan), Italy). Temperature program: from 0 °C (1 min) to 80 °C/min at 70 °C (0 min), then to 180 °C (0 min) at 3 °C/min, then to 250 °C (5 min) at 15 °C/min.

MSD conditions: MS operated in EI mode (70 eV), full scan with a mass range from 35 to 350 amu and SIM acquisition (dwell time 40).

3. Results and discussion

Several parameters were investigated to evaluate the reliability of DC-STE–GC–MS for the purpose of quantifying a group of suspected allergens and a related compound in a cream spread on the stratum corneum, in particular: calibration curve and linearity, repeatability and intermediate precision, limits of detection (LOD) and quantitation (LOQ), recovery, substantivity on the skin, allergen skin permeation, influence of nature of stratum corneum on recovery; in addition five commercial creams were also analyzed.

Unless specified otherwise, all data are the mean of three repetitions after sampling for 30 min with PDMS tapes of the back of one hand of a volunteer spread with the cream spiked with suitable analyte concentrations, followed by thermal desorption of the recovered analytes from the tape and on-line analysis by GC–MS. Fig. 1a reports a TIC–GC–MS profile after DC-STE sampling of the cream spiked with 50 ppm of each allergen.

3.1. Calibration curves and quantitation

Table 2 reports the equations of the concentration (ppm)/normalized areas calibration curves of the investigated compounds and the corresponding regression coefficients R^2 after DC-STE–GC–MS analysis. Target ions and qualifiers of each analyte were selected as reported by Chaintreau et al. [15]. The areas of the target MS ions were used for quantitation. These results show that, in the range of concentrations considered (i.e. 10–150 ppm), the linearity was very good with R^2 always above 0.97; the only exception is cinnamaldehyde (4) whose R^2 is 0.9330. The unusual behavior of cinnamaldehyde is probably due to the irregular peak shape at the lowest spiked quantities (namely 10 and 25 ppm), that interferes with its correct area integration.

3.2. Repeatability and intermediate precision

Table 3 reports repeatability (RSD%) calculated over six determinations of the DC-STE–GC–MS analyses on the 25, 50, and 100 ppm spiked creams, the RSD% means, and the intermediate precision determined over a period of 3 months by analyzing the 50 ppm

Table 4
Average % recoveries and RDS% of the investigated allergens and a related compound and their % area reduction after DC-STE samplings at different times after application of 50 ppm spiked cream.

#	Compound	Recovery %	Recovery repeatability (RSD%)	% Area reduction		
				After 20 min	After 40 min	After 60 min
1	Citronellol	90.4	1.5	77.4	92.6	96.9
2	Z-citral (neral)	95.7	1.0	95.3	98.9	99.4
3	Geraniol	87.7	6.6	75.8	91.8	96.8
4	Cinnamaldehyde	94.8	1.0	87.4	94.3	97.8
5	Anisyl alcohol	86.3	3.0	52.6	81.5	92.4
6	Cinnamyl alcohol	70.6	2.5	61.5	87.1	95.1
7	Eugenol	90.0	5.4	53.7	77.6	92.3
8	Methyleugenol[a]	81.6	5.3	51.2	73.0	90.1
9	Coumarin	78.7	7.7	58.1	82.0	93.4
10	Isoeugenol	83.3	9.6	32.0	53.5	68.6
11	α-Isomethylionone	66.8	10.1	40.3	59.1	73.9
12	Lilial	74.0	6.5	15.4	19.1	35.8
13	α-Amylcinnamaldehyde	52.3	6.2	23.4	23.6	27.8
14	α-Hexylcinnamaldehyde	58.4	10.2	26.1	26.3	26.6

[a] Compound not included in the E.U. list.

spiked cream every 4 weeks. RSD% values were determined on the analyte areas normalized *versus* undecane (IS). The results show that the average repeatability is very good, the average value for each compound never exceeding 10%, with the exception of α-hexylcinnamaldehyde (14) (12.2%). The intermediate precision was also satisfactory since it was always below 15%, ranging from 2.5% for α-amylcinnamaldehyde (13) to 14.6% for isoeugenol (10).

3.3. Limits of detection (LOD) and quantitation (LOQ)

Table 2 reports the LOD and LOQ values calculated following Eurachem guidelines. The results show that they are very low compared to legal limits, meaning that the method enables allergens below the limits set by the E.U. legislation to be easily detected. The LOD ranged from 15 ppb for cinnamaldehyde (4), anisyl alcohol (5) and methyleugenol (8) to 200 ppb for cinnamyl alcohol (6), while the LOQ for the same compounds was 50 and 560 ppb, respectively.

3.4. Analyte recovery, substantivity and approximate skin permeation and influence of nature of volunteer's stratum corneum on recovery

Table 4 reports the recoveries of investigated compounds after 30 min sampling calculated on their amount determined by DC-STE–GC–MS *versus* the amount spiking the cream. The concentrations were calculated from the above calibration curves. The analyte recoveries were all rather high, ranging from a minimum of 52.3% for α-amylcinnamaldehyde (13) and 58.4% for α-hexylcinnamaldehyde (14) to a maximum of 95.7% for neral (2). The repeatability of recovery was also very good, being around 10% for all analytes investigated. The difference in recoveries are due to several co-occurring factors: (i) analyte solubility in PDMS, which is directly proportional to the octanol/water distribution constant $K_{o/w}$ [16], (ii) analyte partition between the cream components being it an emulsion of a hydrophobic phase in water, (iii) analyte skin permeation, and to a lesser extent (iv) analyte volatility.

The analyte substantivity on the stratum corneum was also determined. In this case, DC-STE sampling was carried out in different parts of the hand surface, spread with cream spiked with 50 ppm of the investigated compounds, at different times (0, 20, 40 and 60 min) after application. Table 4 reports the area% reduction of each investigated analyte 20, 40, and 60 min after application, measured by DC-STE–GC–MS analysis, taking values for time zero as 100%. As expected, the results varied widely, being conditioned by the same factors mentioned above for recovery, although in this case volatility assumes a bigger role as is evident from the % reductions over time. After 1 h, the most volatile compounds were almost completely absent from the skin, while content of the less volatile, such as α-amylcinnamaldehyde (13) and α-hexylcinnamaldehyde (14), remained almost constant. These experiments were also useful to define the trend of analyte decay over time. All compounds decayed exponentially, with R^2 ranging from 1 for isoeugenol (10) to 0.8997 for neral (2). Fig. 2 reports the diagrams for isoeugenol (10) and cinnamyl alcohol (6).

These data may also be useful to obtain an indication of analyte evaporation and skin permeation. Permeation through the stratum corneum was measured by running a set of experiments under exactly the same operative conditions as for substantivity, but replacing the back of the hand of the volunteer with a Pyrex glass plate. The approximate permeation percent was determined by subtracting the absolute amount of each analyte found on the

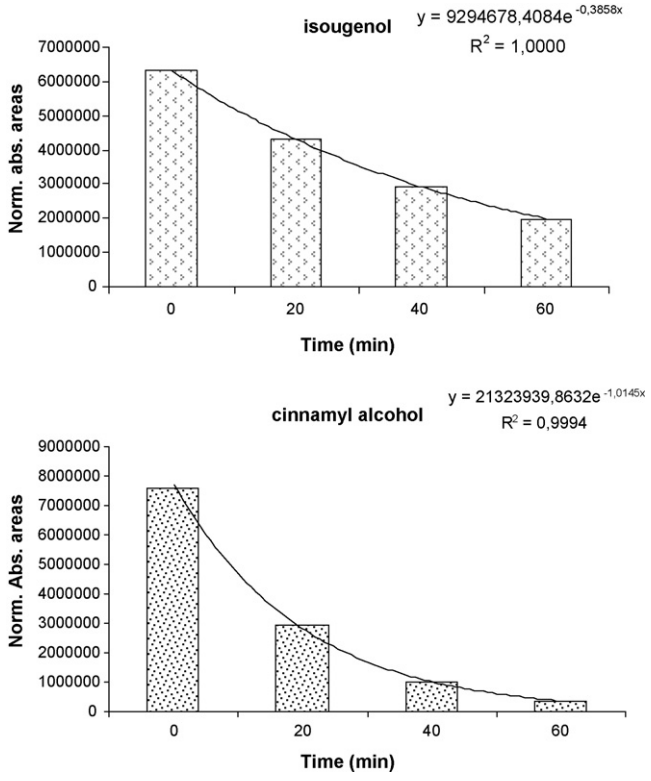

Fig. 2. Diagrams of the exponential decay over time of isoeugenol (10) and cinnamyl alcohol (6).

Table 5
Approximate permeation percentage of each compound and quantitative results after application of 50 ppm spiked cream for the two volunteers.

#	Compound	Approximately % permeation (volunteer 1)	Influence of different stratum corneum		
			Cream concentration (ppm)	Volunteer 1 (ppm)	Volunteer 2 (ppm)
1	Citronellol	12.7	46	43	43
2	Z-citral (neral)	15.6	57	52	58
3	Geraniol	12.6	48	45	44
4	Cinnamaldehyde	7.8	48	45	15
5	Anisyl alcohol	16.5	48	43	30
6	Cinnamyl alcohol	7.6	47	46	19
7	Eugenol	19.6	47	40	40
8	Methyleugenol[a]	14.5	47	43	36
9	Coumarin	12.7	51	48	50
10	Isoeugenol	8.0	48	48	40
11	α-Isomethylionone	11.1	45	42	39
12	Lilial	6.9	49	49	31
13	α-Amylcinnamaldehyde	14.6	58	53	45
14	α-Hexylcinnamaldehyde	16.1	43	39	31

[a] Compound not included in the E.U. list.

Table 6
DC-STE–GC–MS quantitative determination of the declared allergens in the five commercial creams investigated.

#	Compound	Cream 1 (ppm)	Cream 2 (ppm)	Cream 3 (ppm)	Cream 4 (ppm)	Cream 5 (ppm)
1	Citronellol	20	83	40	24	/
2	Z-citral (neral)	51	/	/	/	46
3	Geraniol	90	/	36	16	/
6	Cinnamyl alcohol	/	54	/	/	/
9	Coumarin	12	/	/	/	28
11	α-Isomethylionone	/	65	/	16	/
14	α-Hexylcinnamaldehyde	75	121	/	10	/

skin from that determined on the Pyrex glass plate, and calculating its percentage *versus* the total amount recovered from the glass plate. Table 5 reports the percentage of each analyte's approximate permeation through the stratum corneum; these were found to be relatively low, never exceeding 20%.

The influence of the nature of the stratum corneum on analyte recovery was also preliminarily evaluated. Volunteers 1 and 2 were treated with the same amount of cream spiked with 50 ppm of each compound and their hands analyzed by DC-STE–GC–MS. The results, reported in Table 5, are encouraging since the concentrations are different but quite similar with the two volunteers, and also considering that, in these preliminary experiments, important parameters, such as the different nature and condition of the stratum corneum and the difference in cream permeation between the two volunteers, were not controlled. Further experiments are under way to evaluate the method's applicability and the reliability of the results on a larger series of controlled subjects.

3.5. Analysis of commercial creams

Five commercial creams similar to the model cream, and reported to contain the investigated allergens, were analyzed under the conditions reported for spiked cream, and quantified by means of the calibration curves reported in Table 2. Table 6 shows that the investigated commercial creams contained the suspected allergens reported in the label: one of them (cream 4) in amount close to the "leave-on" E.U. limits to be declared, the others in different and variable amounts. Fig. 1b reports the TIC–GC–MS profile after DC-STE sampling of commercial cream 1 and Fig. 1c shows that of commercial cream 2.

The influence of the matrix on the results was also investigated by analyzing two of the commercial creams (cream 3 and cream 5) spiked with 50 ppm of the SA mixture and analyzing them with the DC-STE–GC–MS method described. The analytes' recoveries (not reported) were in line with those in Table 4 for the spiked cream, thus excluding interference due to the matrix effect. On the other hand, the same creams were also analyzed after a standard addition of 50 ppm of the allergens identified in them. The results were very similar to those obtained with the calibration curves showing the reliability of the method described.

3.6. General considerations

This study confirms the effectiveness and concentration capability of STE used for direct contact sampling from the skin. Its application to the analysis of suspected allergens and related compounds in creams applied to the skin is a highly reliable and sensitive method, offering high recoveries of the analytes investigated together with good repeatability and sensitivity.

The main advantages of DC-STE, in particular for applications in this field and more in general in biology, are that (i) it comprises a one-step sampling procedure not requiring any further matrix or sample manipulation, (ii) it enables several simultaneous samplings to be run by applying the required number of tapes onto the solid surface investigated, (iii) it permits sampling(s) where the biological phenomenon to be monitored takes place, and (iv) all other steps of the method (thermal desorption and GC–MS analysis) can be run automatically. On the other hand, it requires the availability of a dedicated instrumentation for on-line thermal desorption and of a multipurpose autosampler for automatic analysis.

In conclusion, DC-STE is an effective technique for sampling from solid biologically active surfaces. In particular the results reported show its efficacy in studying phenomena related to the skin, where it can be effectively applied to determine directly or indirectly skin permeability to drugs and cosmetics, to monitor both ingredients and their bioavailability, and skin markers to evaluate their local effect.

Acknowledgments

This study was carried out within the project entitled: "Sviluppo di metodologie innovative per l'analisi di prodotti agroalimen-

tari" (FIRB Cod.: RBIP06SXMR_002) of the Ministero dell'Istruzione, dell'Università e della Ricerca (MIUR) (Italy).

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