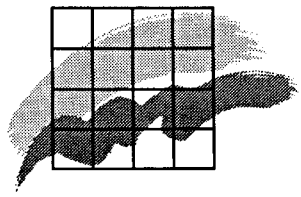

Chemical substances and chemical preparations

Contents of some skin sensitizing fragrances in selected cosmetics

NERI Technical Report No. 129

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Ministry of Environment and Energy
National Environmental Research Institute
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Data Sheet

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Contents

Resumé

Indholdet af udvalgte duftstoffer er bestemt i kosmetiske produkter med det formål, at belyse exponeringsmængden af disse stoffer ved anvendelse af kosmetik, samt at belyse disse stoffers threshold-koncentrationer, som kan give hudreaktioner hos visse personer. De kosmetiske produkter, der blev udvalgt til undersøgelse for hudreaktioner i Dermatologisk Afdeling, Gentofte Sygehus, blev analyseret for indholdet af duftstoffer. I alt 67 prøver var fordelt i 3 grupper: i) 10 af de mest populære parfumer i Europa, ii) 23 patch- og/eller use-test positive kosmetiske produkter, og iii) 17 duftstofblandinger, der anvendes til formulering af kosmetik til daglig brug, samt tilsvarende 17 kosmetiske produkter. Prøverne blev analyseret ved en tidligere udviklet metode til identifikation og bestemmelse af følgende duftstoffer: citral, citronellal, hydroxycitronellal, geraniol, cinnamylalcohol, cinnamaldehyd, eugenol, isoeugenol, coumarin, dihydrocoumarin, α-amylcinnamaldehyd, og α-hexyl cinnamaldehyd.

Det blev påvist, at alle prøver indeholdt mellem 2 og 9, af de 12 udvalgte duftstoffer i koncentrationer fra ca. 0,0001% til >1,0%. Citronellal, cinnamaldehyd og dihydrocoumarin kunne ikke detekteres i de undersøgte prøver. Parfumer og duftstofblandinger indeholdt den højeste koncentration af duftstoffer, efterfulgt af eau de parfume, eau de toilette, aftershavelotion og i shampooer og cremer. På grund af et begrænset antal prøver af hver produkt type er der ikke udført statistisk vurdering af resultaterne, hverken for anvendelse af duftstoffer i diverse produktkategorier eller exponeringsmængde af duftstoffer ved anvendelse af disse produkter. Resultaterne viser dog, at flere prøver indeholdt >0,25% hydroxycitronellal, der er den max. tilladte koncentration i kosmetika ifølge International Fragrance Research Association.

Ved analyser udført i nærværende undersøgelse var det også muligt at identificere flere ikke-udvalgte duftstoffer i kosmetiske produkter. En semi-kvantitativ bestemmelse af nogle af de meget anvendte ikke-udvalgte duftstoffer er ligeledes udført i forbindelse med nærværende undersøgelse.

Arbejdet er udført som bistandsopgave til Miljøstyrelsen.

Summary

In the present investigation, contents of selected sensitizing fragrance allergens in cosmetic products were determined with the aim to get a knowledge of use-concentrations of these substances as well as threshold concentrations of the respective substances to produce skin sensitization in susceptible persons. Cosmetic samples, selected for the investigation of skin sensitization reactions, were obtained through Dermatology Department, Gentofte Hospital. The samples represented 10 of the most popular perfumes in Europe, 23 patch- and/or use-test positive cosmetics, 17 fragrance mixtures used for the formulation of cosmetics for 'daily use' and 17 corresponding cosmetic products. All the samples were analyzed employing earlier developed GC-FID and GC-MS methods for the identification and determination of following fragrance substance: citral, citronellal, hydroxy citronellal, geraniol, cinnamic alcohol, cinnamic aldehyde, eugenol, isoeugenol, coumarin, dihydro coumarin, α-amyl cinnamaldehyde and α-hexyl cinnamaldehyde.

The results of the analysis showed that 2 to 9 of the 12 target fragrance substances, in concentrations approx. 0.0001% - > 1.0%, were present in all of the samples investigated. Citronellal, cinnamic aldehyde and dihydrocoumarin were not present in any of the samples. The contents of target fragrance substances was found to be highest in perfumes and fragrance mixtures used for the formulation of cosmetics, followed by eau de parfum, eau de toilette, aftershave lotion and shampoos and creams.
No attempt has been made to statistically evaluate the results, neither with respect to use-concentrations nor with respect to distribution of target fragrance substances in the investigated product categories. The results, however, demonstrated that many samples contained > 0.25% hydroxy citronellal, which is the maximum allowed concentration in cosmetics according to International Fragrance Research Association.

Identification of several non-target fragrance substances in cosmetics was possible by the analytical method used in the present investigation. Thus, semi-quantitative determination of some of the non-target fragrance substances, frequently present in the investigated cosmetics, has also been performed.

Present work has been performed as a technical support to Danish Environmental Protection Agency.

1 Introduction

The use of fragrances (perfumes) in cosmetics is associated with pleasure and some times with therapy. A perfumer has more than 7000 substance available to compose a perfume. All known fragrance substances are organic compounds or mixtures of organic compounds, derived from natural sources or produced synthetically. Recently described guidelines for perfuming various types of consumer products (1, 2) indicate that these products may contain from a single fragrance substance up to a mixture of 50-60 (or even more) fragrance substances in various concentrations. The recommended concentrations (0.10% - 30%) of perfumes in various categories of cosmetics are described elsewhere (1, 2).

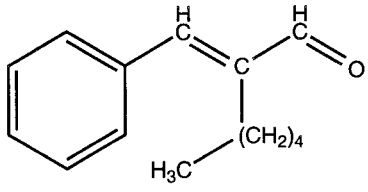
Perfumes are considered to be one of the major causes of allergic contact dermatitis as a result of the use of cosmetics (3 - 5). Earlier studies have indicated that 30-50 % cases of all cosmetic dependent skin reactions may be due to perfume ingredients (3, 4)). Approximately 2 % of Danish population has been reported to be sensitized by fragrances (6).

In no country is there any requirement for approval for either ingredients that go into fragrances or for the compound fragrances itself. There are no requirements to test fragrance materials for safety for use in consumer products and there is no requirement to list the fragrance ingredients on consumer products. However, perfume industries are self-regulating the use of perfumes in consumer products, including cosmetics. Eventhrough industries' organizations, International Fragrance Association (IFRA) and the Research Institute of Fragrance Materials (RIFM) have been supporting the industries for more than 25 years, perfume allergy still prevails.

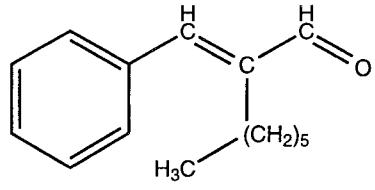
For an optimal regulation of perfumes, systematic investigations are needed to unravel the trend of use of allergic fragrance substances in various consumer products together with epidemiological studies to reveal sensitization reactions (and other toxic effects) due to the use of respective products. Thus, the data compiled on single substances can be used for regulation of perfumes. A step in this direction has been taken by Danish Environmental Protection Agency (DEPA) in cooperation with Gentofte Hospital, Copenhagen University and National Environmental Research Institute (NERI). NERI's contribution in the project is to provide data on the content of some selected fragrance substances in consumer products, specifically cosmetics and toiletries.

Recently, we developed a method for quantitative analysis of 11 fragrance substances in various types of cosmetics (7). The target fragrance substances in the previous study (7) were those which are known to cause skin sensitization in humans. In the present investigation, we have analyzed the content of these fragrance

substances in a number of products suggested by the Dermatology Department, Gentofte Hospital (J. D. Johansen): i) 10 popular perfumes, skin sensitization to which is being tested at present, ii) 23 cosmetic products which produced positive skin reactions by patch- and/or use-test in 11 eczema patients, and iii) 17 fragrance mixtures used in cosmetic formulations and the corresponding cosmetic products (cosmeticproducts for daily use). The 12 target fragrance substances for the present study (Table 1) included all of the 11 fragrance substances selected in the previous study (7) and α-hexyl cinnamaldehyde. The last mentioned substance was included in the present study, because it is chemically related to one of the target fragrance substances, α-amyl cinnamaldehyde, and that is also frequently used in relatively large amounts in perfumes (8).



α-amyl cinnamaldehyde



α-hexyl cinnamaldehyde

Present work has been performed as a technical support to Danish Environmental Agency.

Table 1: Target fragrance substances.

Fragrance substance	**Chemical Name (IUPAC)**
Cinnamyl alcohol	3-phenyl-2-propen-1-ol
Cinnamaldehyde (trans)	3-phenyl-2-propenal
Eugenol	2-methoxy-4-(2-propenyl) phenol
Hydroxy citronellal	3,7-dimethyl-7-hydroxy octanal
α-Amyl cinnamalde-hyde	2-phenylmethylene heptanal
Geraniol	3,7-dimethyl-2,6-octadien-1-ol
Isoeugenol	2-methoxy-4-(1-propenyl) phenol
Coumarin	2H-1-benzopyran-2-one
Dihydrocoumarin	3,4-dihydro 2H-1-benzo-2-pyranone
Citral	3,7-dimethyl-2,6-octadienal
Citronellal	3,7-dimethyl-6-octenal
α-hexyl cinnamaldehyde	2-phenylmethylene octanal

2 Samples

The cosmetic products selected for the investigation of skin reactions by patch- and/or use-test at Dermatology Department, Gentofte Hospital, were analyzed for the contents of the target fragrance substances. The cosmetic products were selected with the aim to elucidate followings:

i) threshold concentrations, in use-cosmetics, of the target fragrance substances to produce skin sensitization in susceptible persons,
ii) concentrations of target fragrance substances in patch- and/or use-test positive cosmetics, and
iii) concentrations of target fragrance substances in various cosmetic products used by 'general population', i.e. contents of target fragrance substances in cosmetic products for daily use.

JDJ, Dermatology Department, Gentofte Hospital, purchased the samples from the Danish retail market in the period October 1993-June 1994 and submitted them to NERI for analysis. A cosmetic manufacturer provided 17 fragrance mixtures which were used in the formulation of various types of cosmetics for daily use. The cosmetic products corresponding to the fragrance mixtures, as informed by the cosmetic manufacturer, were purchased from the retail market.

The samples analyzed in the present investigation are divided into 3 groups (sub-projects) relating to the aims i) - iii) described above. The product category and the NERI-registry no. of the products analyzed in sub-projects i) - iii) are described respectively in Tables 2 - 4. Thus, the 10 of the most popular perfumes in Europe (Table 2), as identified by Financial Times (9), were analyzed in sub-project i). The identification of perfumes by sample no. is not disclosed in Table 2, as the aim of the present investigation was to get a knowledge of use-concentrations of target fragrances in the cosmetic products, but not the investigation of any specific product. For the same reasons, the names of the products and the identification of manufacturers of cosmetic products are also not described in the Tables 3 and 4.

Table 2. The popular perfumes analyzed for the contents of target fragrance substances.

Cosmetic Products	NERI-reg.no.
Popular perfumes: YSL *Paris* (eau de parfum), YSL *Opium* (parfum), Christian Dior *Poison* (esprit de parfum), Nina Ricci *L'Air du Temps* (parfum), Cacharel *Anais Anais* (parfum), Gloria Vanderbilt *Vanderbilt* (eau de parfum), Giorgio *Beverly Hills* (eau de toilette), Lancomé *Tresor* (parfum), CC *Chanel No. 5*, Estée Lauder *Beautiful* (parfum)	Sample no. are described randomly: 4-0522, 4-0523, 4-0524, 4-0525, 4-0526, 4-0527, 4-0528, 4-0529, 4-0530, 4-0531

Table 3. Patch- and/or use-test positive cosmetics analyzed for target fragrance substances.

NERI-reg.no.	Cosmetic product	Patient no.	Product test
4-0481	Eau de parfum	1	Patch-test: +
4-0482	Body lotion	2	Use-test positive, day 4
4-0483	Eau de toilette	3	Use-test positive, day 6
4-0484	Deodorant	4	Patch-test: +, and Use-test positive, day 3
4-0485	Deodorant		Patch-test: +
4-0487	Deodorant		Patch-test: +
4-0486	Eau de toilette	5	Patch-test: +
4-0488	Eau de toilette		Patch-test: +
4-0489	Aftershave	6	Patch-test: +
4-0490	Aftershave		Patch-test: +
4-0491	Aftershave		Patch-test: +
4-0492	Aftershave		Patch-test: +
4-0493	Hair lotion		Patch-test: +
4-0494	Deodorant		Patch-test: +
4-0495	Shaving gel		Patch-test: +
4-0496	Deodorant	7	Use-test positive, day 3
4-0497	Aftershave	8	Patch-test: +
4-0498	Eau de perfume	9	Patch-test: +
4-0499	Eau de toilette	10	Patch-test: +, and Use-test positive, day 3
4-0500	Night cream	11	Patch-test: +
4-0501	Day cream		Patch-test: +
4-0502	Facial cream		Patch-test: +
4-0503	Facial cream		Patch-test: +

Table 4. Fragrance mixtures and corresponding cosmetic products analyzed for target fragrance substances.

Fragrance mixture		Corresponding cosmetic product	
NERI-reg.no.	**Fragrance mixture code**	**NERI-reg.no.**	**Product category**
4-0532	1	4-0504	Bath gel for babies
4-0538	2	4-0505	Shower gel
4-0548	3	4-0506	Volumen shampoo
4-0545	4	4-0507	Luxury shampoo
4-0533	5	4-0508	Balsam shampoo
4-0536	6	4-0509	Body lotion
4-0547	7	4-0510	Skin tonic
4-0539	8	4-0511	Carbamide moisturizing cream
4-0540	9	4-0512	Creame rinse
4-0541	10	4-0513	Deo antiperspirant
4-0535	11	4-0514	Sun lotion
4-0537	12	4-0515	Body shampoo
4-0538	13	4-0516	Deodorant stick
4-0542	14	4-0517	Deodorant stick
4-0546	15	4-0518	Cream rinse
4-0544	16	4-0519	Glycerine cream
4-0534	17	4-0575	Body lotion

3 Experimental

3.1 Materials

3.1.1 Apparatus

Hewlett Packard (HP) gas chromatograph HP 5890 with split/ splitless injector and flame ionization detector (FID) has been used for GC analysis. Autosampler HP 7673 was used for sample introduction into GC-column and HP Vectra Chemstation was used for the collection of GC-data. For GC-MS analysis, a Finnigan INCOS 50 mass spectrometer coupled to a HP 5890 gas chromatograph was used. The GC-column used was a 50 m (l) x 0.32 mm (i.d.) WCOT fused silica coated with CP-Sil 5CB, d_f 1.2 µm., from Chrompack, The Netherlands (Cat. No. 7770).

3.1.2 Glassware

Normal laboratory glassware and glass columns 20 cm x 1.8 cm (i.d.), for column chromatography, were used.

3.1.3 Chemicals

Eugenol 99%, isoeugenol 98%, geraniol 98%, dihydrocoumarin 99%, cinnamic alcohol 98%, α-amyl cinnamaldehyde 97%, α-hexyl cinnamaldehyde (99%) and citral (mixture of cis- and trans-isomers) 95% were from Aldrich, Germany; cinnamic aldehyde (trans) 98% was from Fluka, Switzerland; crystalline coumarin and citronellal 85-90% were from Sigma Chemical Co., U.S.A., and hydroxy citronellal 95% was from Biomedicals Ltd., U.K. Silica gel for column chromatography was ICN Active Silica 100-200 mesh from ICN, England. All other chemicals of analytical grade were from E. Merck, Germany. All the chemical were used as obtained.

3.1.4 Reference solutions

10% (w/v) stock solutions of all the fragrances were prepared in methanol. The solutions were stored at 4°C for maximum 3 days (7). Calibration standards 0.001%, 0.01%, 0.02%, 0.05%, and 0.10% of all the fragrances were prepared by diluting the stock solutions in methanol. These solutions were prepared from freshly prepared stock solutions and they were analyzed within 24 hours.

3.2 Sample Preparation

3.2.1 Eau de Toilette, Aftershave and Deodorants

Depending upon the concentrations of various fragrance substances, these samples were appropriately diluted in methanol, so that the concentrations of the target fragrance substances in the diluted solutions were below 0.1%.

Deodorant spray products in aerosol cans were taken out of the cans as described previously (10). If necessary, the samples/-

diluted samples were centrifuged before GC analysis. The amount of propellant and the weight of the residue, obtained by centrifugation, were recorded. These values were used in the calculation of contents of fragrance substances in the product.

3.2.2 Shampoos, Creams, Lotions, Skin Tonics and Deo Sticks
Approximately 1 g sample was accurately weighed in a 10 ml volumetric flask. A small portion of boiling chips were added to the sample and the flask was filled up to the mark with methanol. The mixture was shaken gently and then heated at 60°C for 10 min The solution/homogeneous suspension thus obtained was immediately cooled to room temperature (20°C). The fragrance substances from the solution/suspension were extracted as described below.

A 20 cm x 1.8 cm (i.d.) glass column was packed with wet silica gel (in methanol) to 7 cm. The cooled sample solution/suspension in the volumetric flask was quantitatively transferred into the column and that was allowed to pass through the column. The initial 5 ml of the eluate was discarded. The fragrances, which eluted thereafter, were collected in a 25 ml volumetric flask. The column was further eluted with additional 20 ml methanol and the eluate was collected in the same 25 ml volumetric flask. The flask was filled with methanol up to the mark. The fragrance extract was immediately transferred into autosampler vials and analyzed within 24 h.

3.3 Analysis

Qualitative analyses of the target fragrance substances in the diluted samples/sample extracts were performed by GC-FID (3.3.1) and GC-MS (3.3.2). Quantisation of the identified substances was performed by GC-FID. Calibration standards 0.001% - 0.10% were also analyzed by GC-FID to prepare calibration curves of target fragrance substances. The calibration curves were used for the calculation of the respective target fragrance substances in the samples analyzed. The concentrations of fragrance substances in the samples, treated according to 3.2.2, were calculated as % (w/w). The contents of fragrance substances in other samples were calculated as % (w/v). All the samples were analyzed in duplicate.

3.3.1 Conditions for GC-FID

Oven temperature	: 140°C to 280°C, 5°C min 1 min at 280°C
Injector	: Split, temperature 300°C
Injection volume	: 1 µl
Detector	: FID, temperature 300°C
Carrier gas	: N_2, flow 54 ml/min
Column head-pressure	: 14 psi (1.8 ml/min)
Make-up gas	: N_2, flow 29 ml/min

3.3.2 Conditions for GC-MS

GC as described in 3.3.1, except that He was used as carrier gas, column head-pressure: 20 psi.

MS

Interface	: Direct to ion source, temperature 290°C
Ionization	: 70 eV, electron impact at 175°C
Scan Descriptors	: m/z 50 - m/z 250 in 0.73 s
Library	: National Bureau of Standards

4 Results

The methods used for the analysis of target fragrance substances were the same as described in the previous report (7). In brief, the samples were diluted or extracted, followed by the analyses of fragrance substances in the diluted samples/sample extracts by GC-FID and GC-MS. Identification of the target fragrance substances was performed on the basis of their GC retention times (t_R) as well as on the basis of their mass spectra. The quantisation of the fragrances is performed by GC-FID, employing calibration curves of standard fragrance substances. The method was also found to be suitable for the analysis of α-hexyl cinnamaldehyde, which was not included in the previous study (7). The detection limit of α-hexyl cinnamaldehyde was similar to other target fragrances, approximately 1ppm. The analysis of a facial cream spiked with α-hexyl cinnamaldehyde to 0.1% revealed that the recovery of this substance was 99%. Chromatograms obtained by GC and GC-MS analysis of some of the products analyzed in the present investigation are shown in Figures 1 - 9.

The contents of the target fragrance substances in the analyzed samples are described in Tables 5 - 7. In few samples, it was not possible to determine the contents of some of the target fragrance substances identified by MS, due to the presence of an interfering peak. These results are described as "+" in Tables 5 and 6. Furthermore, the contents of substances in concentrations 1 ppm - 10 ppm have been described as <0.001% in Tables 5 and 6, because of a relatively large variation (>15%) in double determinations. As shown in Table 7, the contents of target fragrance substances in many of the samples were 1-10 ppm. Therefore, attempts were made to improve the limits of detection as well as limits of quantisation of target fragrance substances during the analysis of cosmetic products for sub-project iii). Replacement of the capillary GC-column with a new one, of the same type, resulted in approximately 0.2 ppm as the limit of detection of target fragrance substances. Moreover, substances present in concentrations at levels down to 1 ppm could be quantitated with a greater certainty (repeatability <10%).

The popular perfumes (Table 5) and the fragrance mixtures used for the formulation of cosmetics (Table 7) were, as expected, found to contain relatively high concentrations of fragrance substances. Cinnamic aldehyde, dihydro coumarin and citronellal were, however, not detected in any of the samples.

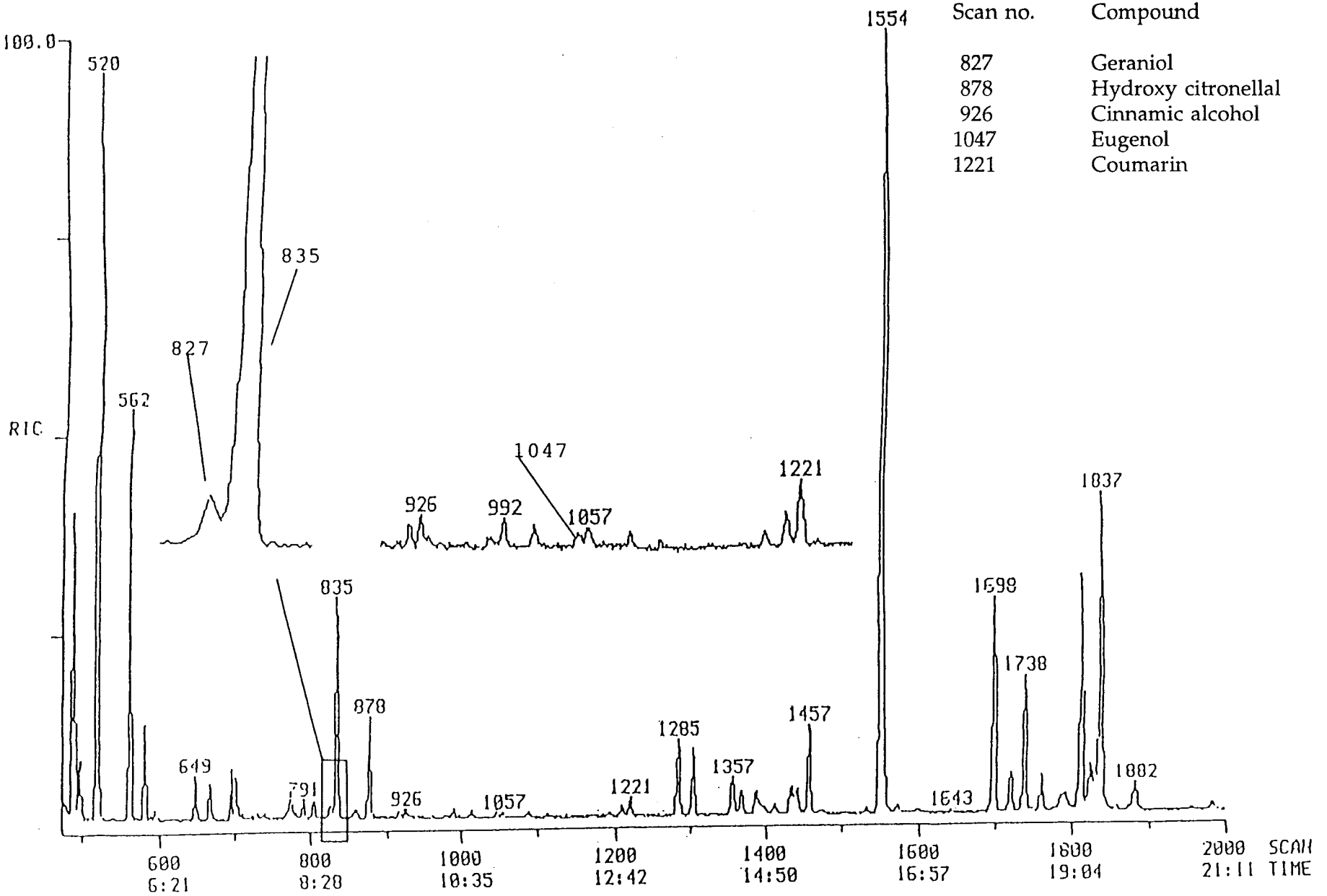
Among the 10 popular perfumes investigated, geraniol (0.08% - 0.49%), hydroxy citronellal (0.26% - 1.15%) and eugenol (0.04% - 0.89%) each were found to be present in 9 samples (Table 5). Isoeugenol (0.02% - 0.34%) was present in 7 of the investigated popular perfumes, cinnamic alcohol (0.03% - 0.79%) and coumarin (0.04% - 1.14%) each in 6 samples, α-hexyl cinnamaldehyde (0.01% - 1.65%) in 5 samples, and α-amyl cinnamaldehyde

(0.03% - 0.69%) was present in 3 of the samples. Citral was present in 2 samples, but that could not be quantitated because of the interference by an unidentified substance(s) present in these samples. All investigated popular perfumes contained 3 - 9 of the 12 target fragrance substances.

The analyses of the target fragrance substances in 23 patch- and/or use-test positive cosmetic products revealed the presence of hydroxy citronellal (<0.001% - 0.54%) in 20 products, eugenol (<0.001% - 0.22%) in 17 products, coumarin (<0.001% - 0.23%) and α-hexyl cinnamaldehyde (<0.001% - 0.66%) each in 15 products, cinnamic alcohol (<0.001% - 0.15%) and geraniol (<0.001% - 0.62%) each in 12 products, α-amyl cinnamaldehyde (<0.001% - 0.33%) in 7 products and the presence of isoeugenol (<0.001% - 0.03%) in 6 products. Citral (0.09%) was found to be present in only one of the products, an after shave lotion. Of the 12 target fragrance substances, 2 - 8 substances were found to be present in all investigated patch- and/or use-test positive cosmetics.

The contents of target fragrance substances in the 17 fragrance mixtures used in cosmetic formulations as well as in the corresponding cosmetic products are described in Table 7. Among the fragrance mixtures, 13 samples contained α-hexyl cinnamaldehyde (0.03% - 1.34%), geraniol (0.05% - 0.19%), was found in 12 samples, α-amyl cinnamaldehyde (0.01% - 0.85%) in 9 samples, cinnamic alcohol (0.02% - 0.33%) and eugenol (0.03% - 0.39%) each in 8 samples, hydroxy citronellal (0.01% - 0.24%) and coumarin (0.08% - 0.8%) each in 6 samples, and isoeugenol (0.14% and 0.17%) was present in two of the fragrance mixtures. Citral was present in 3 of the investigated fragrance mixtures, but that could not be quantitated due to the interference by chromatographic peak of an unidentified substance. All of the investigated fragrance mixtures contained 2 - 7 of the 12 target fragrance substances. The contents of various target fragrance substances in the cosmetic products, corresponding to the 17 fragrance mixtures, were from approx. 0.2 ppm (detection limit) to 81 ppm (0.0081%) (Table 7). Most of the target substances present in the fragrance mixtures were also identified in the respective cosmetic products. Thus, a positive identification of the respective fragrance substances in 48 cases, out of 67 possibilities, was observed (Table 7). The reason for unability to identify the target fragrances in remaining cases may be: i) contents of these substances in cosmetic products were below the detection limits (approx. 0.2 ppm), or ii) relatively higher loss of these substances during cosmetic formulation or sample preparation for GC analysis, or iii) the use of a different fragrance mixture for the cosmetic formulation than anticipated (informed by the manufacturer).

Figure 1: GC-MS of sample no. 4-0489



Scan no.	Compound
827	Geraniol
878	Hydroxy citronellal
926	Cinnamic alcohol
1047	Eugenol
1221	Coumarin

Figure 2: GC-MS of sample no. 4-0524

Scan no.	Compound
807 & 858	Citral
829	Geraniol
885	Hydroxy citronellal
931	Cinnamic alcohol
1054	Eugenol
1222	Coumarin
1251	Isoeugenol
1710	α-amyl cinnamaldehyde
1936	α-hexyl cinnamaldehyde

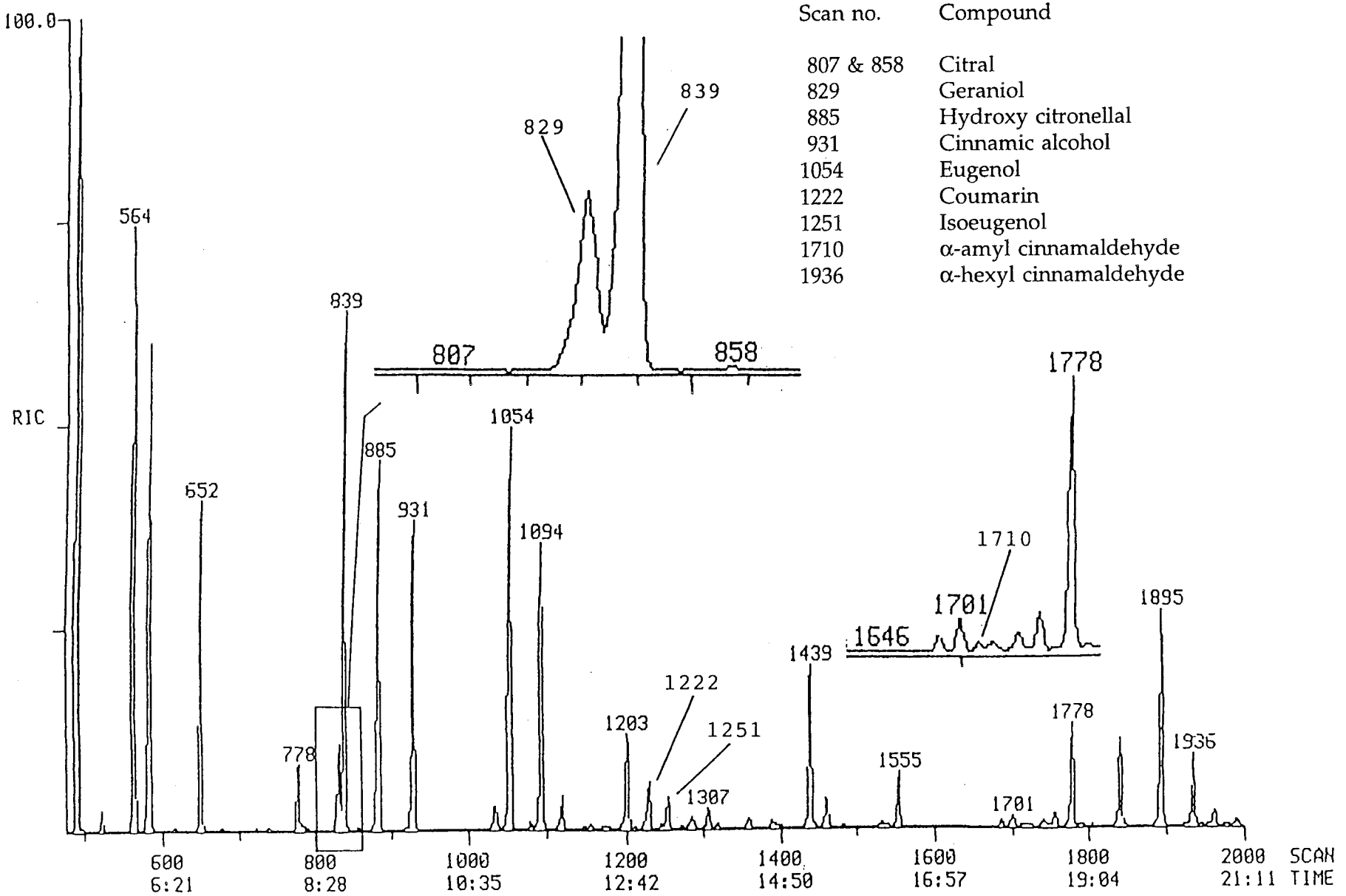


Figure 3: GC-MS of sample no. 4-0530

Scan no.	Compound
881	Hydroxy citronellal
1047	Eugenol
1249	Isoeugenol
1710	α-amyl cinnamaldehyde
1934	α-hexyl cinnamaldehyde
1249	

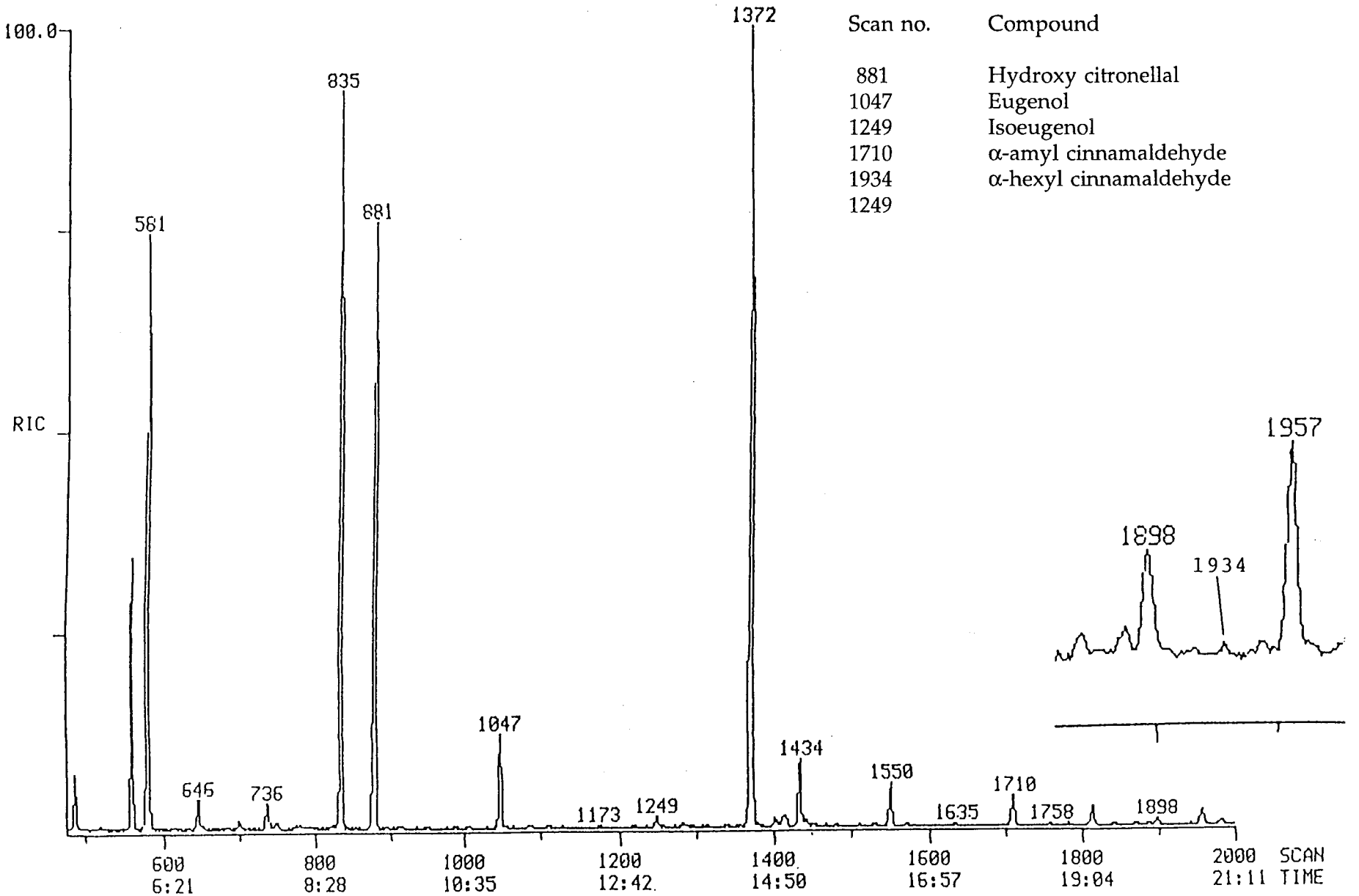


Figure 4: GC-FID of sample no. 4-0507

Retention time (min)	Compound
22.305	α-amyl cinnamaldehyde
24.768	α-hexyl cinnamaldehyde

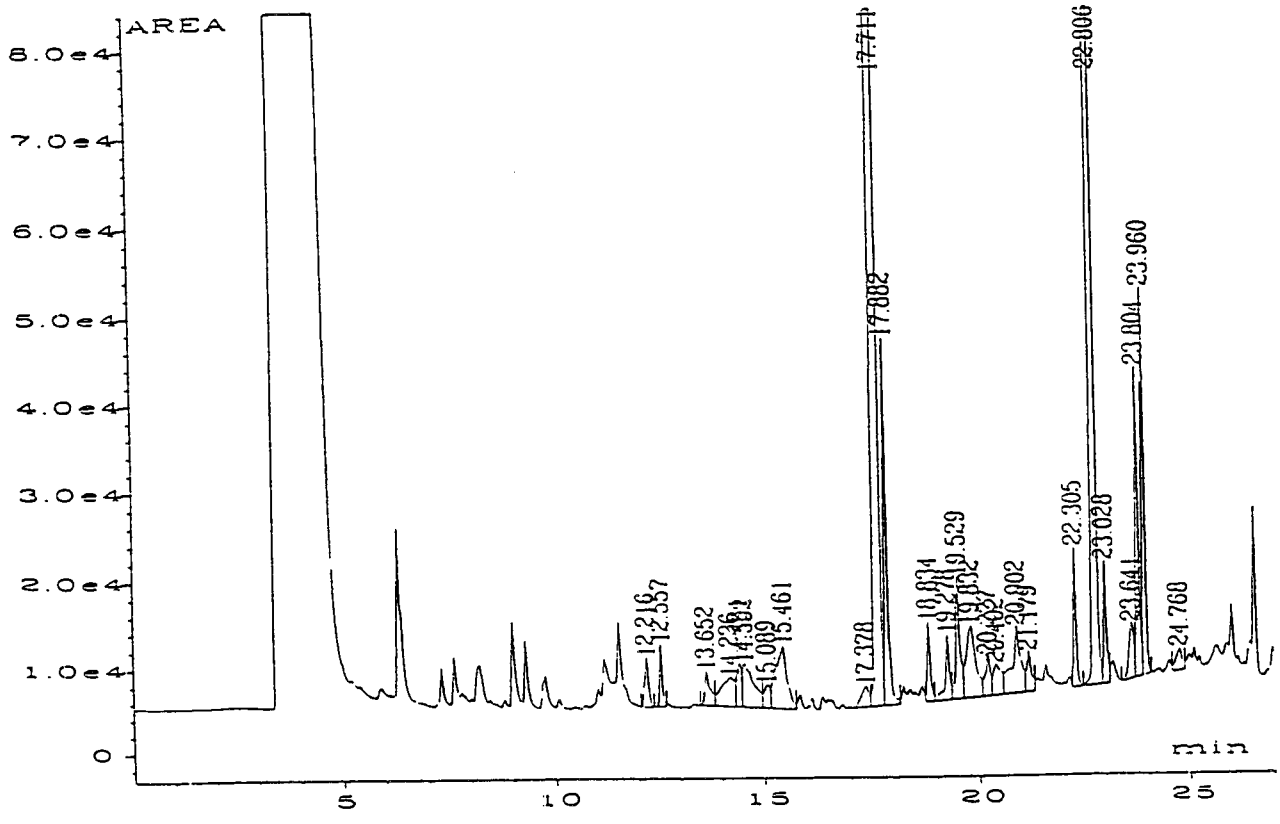


Figure 5: GC-FID of sample no. 4-0516

Retention time (min)	Compound
12.768	Hydroxy citronellal
14.820	Eugenol
17.002	Coumarin

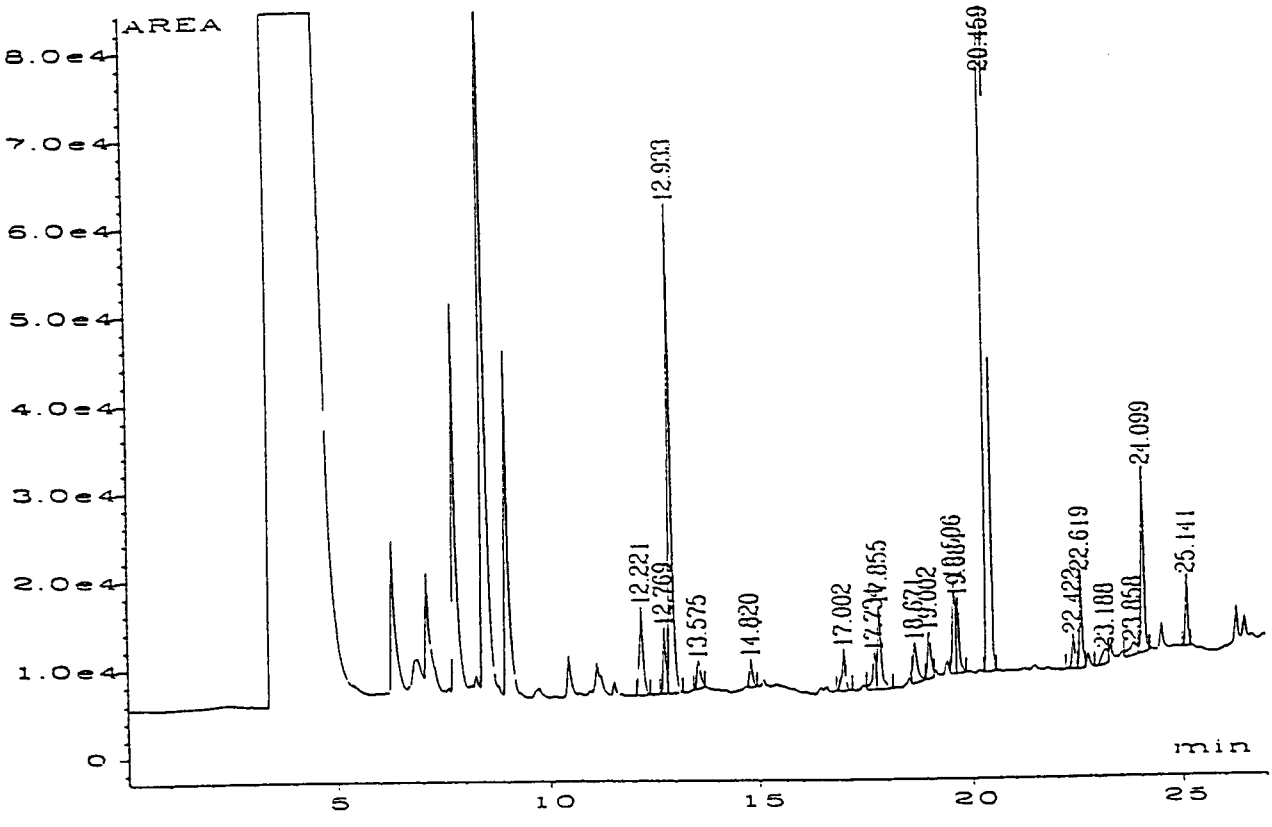


Figure 6: GC-FID of sample no. 4-0519

Retention time (min)	Compound
12.770	Hydroxy citronellal
22.357	α-amyl cinnamaldehyde

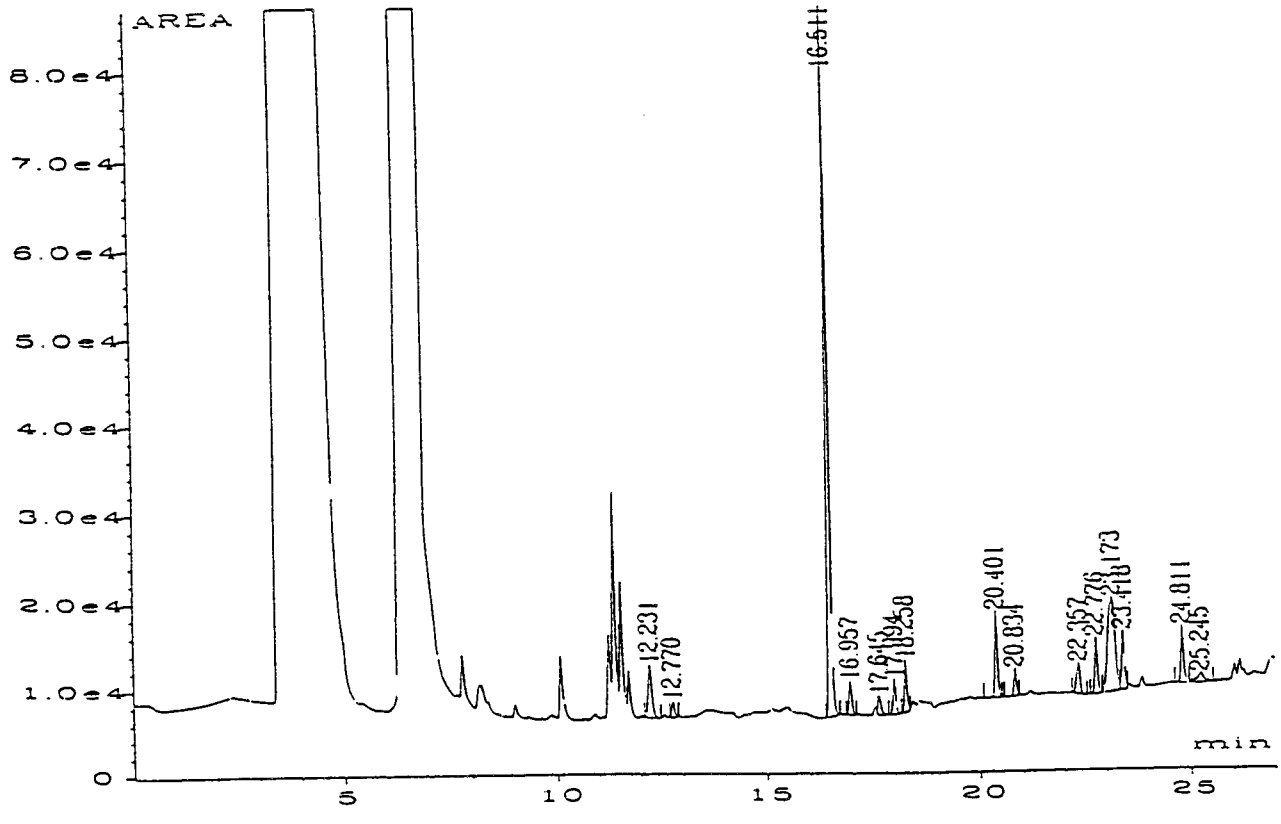


Figure 7: GC-FID of sample no. 4-0522

Retention time (min)	Compound
12.200	Geraniol
12.804	Hydroxy citronellal
13.439	Cinnamic alcohol
14.500	Citral (overlapping peak 14.535)
22.345	α-amyl cinnamaldehyde
24.794	α-hexyl cinnamaldehyde

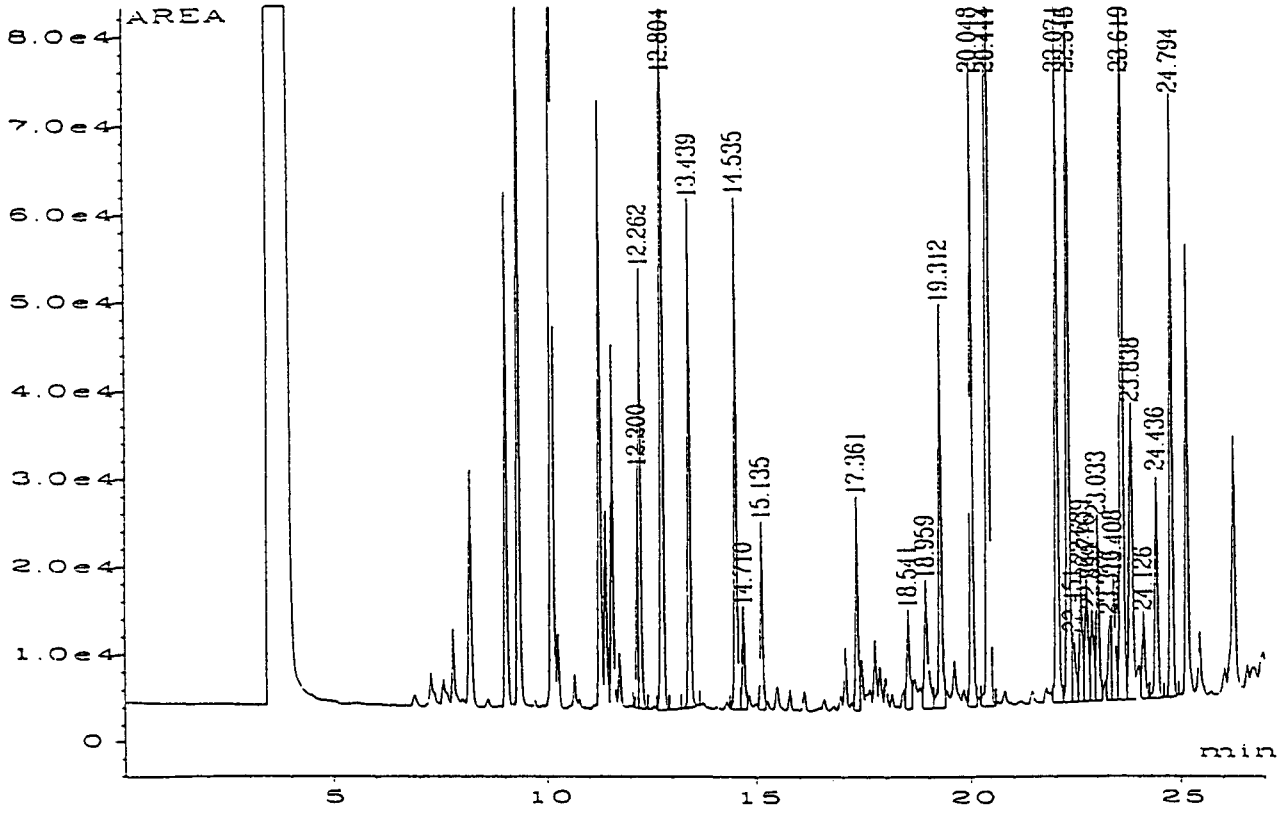


Figure 8: GC-FID of sample no. 4-0527

Retention time (min)	Compound
12.200	Geraniol
12.810	Hydroxy citronellal
13.439	Cinnamic alcohol
14.850	Eugenol
17.014	Coumarin
17.227	Isoeugenol
24.804	α-hexyl cinnamaldehyde

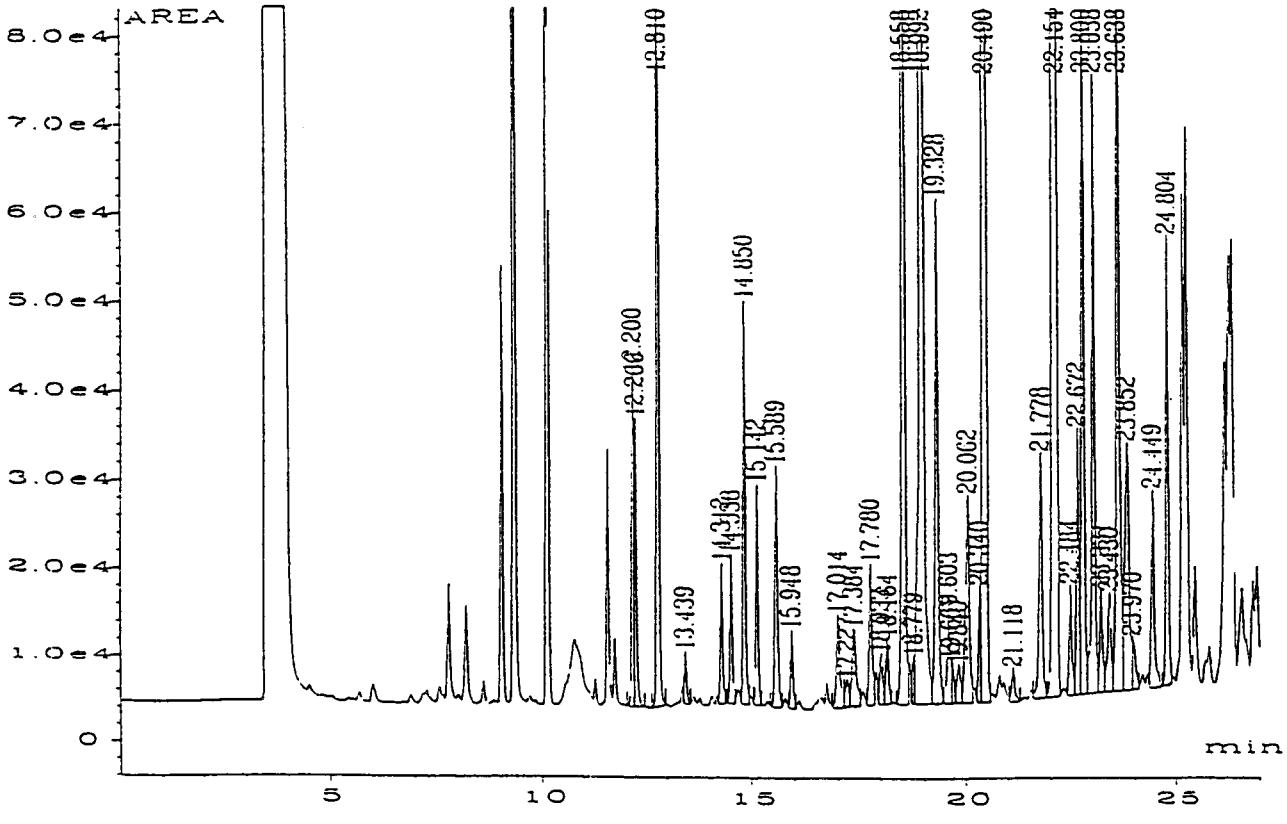


Figure 9: GC-FID of sample no. 4-0539

Retention time (min)	Compound
12.200	Geraniol (overlapping peak 12.267)
12.794	Hydroxy citronellal
13.444	Cinnamic alcohol
22.350	α-amyl cinnamaldehyde
24.840	α-hexyl cinnamaldehyde

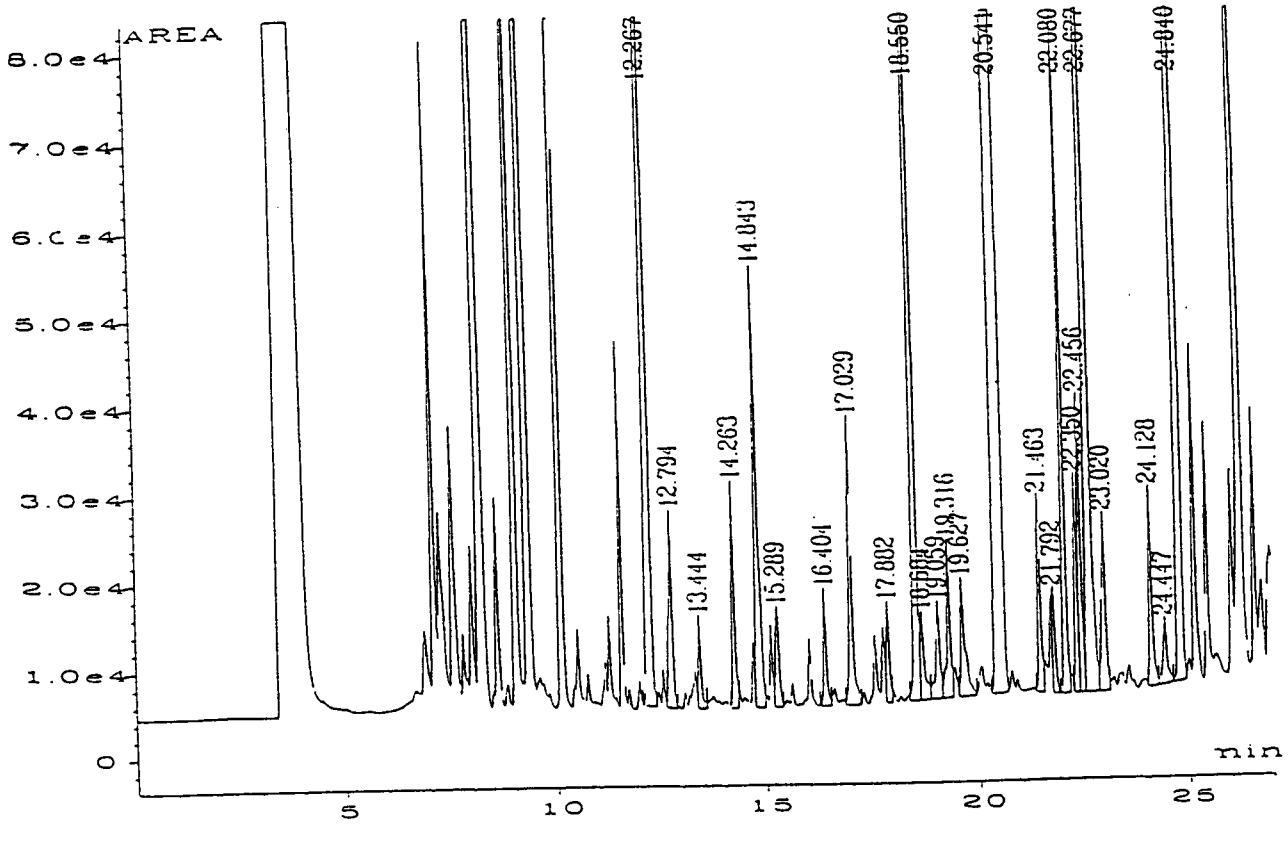


Table 5: Contents of fragrance substances in popular perfumes[1].

NERI-reg.no.	Concentration of fragrance substances, % (w/v)								
	Geraniol	Hydroxy citronellal	Cinnamic alcohol	Citral	Eugenol	Coumarin	Isoeugenol	α-amyl cinnamaldehyde	α-hexyl cinnamaldehyde
4-0522	0.1569	1.0941	0.3979	+	-	-	-	0.6954	0.4975
4-0523	0.1963	0.2546	-	-	0.4280	+	0.1125	-	-
4-0524	0.4846	1.0857	0.7910	+	0.8891	0.5820	0.3370	0.0328	0.3459
4-0525	0.0813	0.7628	0.2383	-	0.0437	0.0388	-	-	-
4-0526	0.4482	0.3636	0.0966	-	0.0487	1.1407	-	-	-
4-0527	0.1536	0.5655	0.0286	-	0.2055	0.0844	0.0465	-	0.3503
4-0528	0.1547	1.1919	-	-	0.5768	0.2758	0.2757	-	-
4-0529	0.0995	-	-	-	0.0703	-	0.0179	-	-
4-0530	-	1.1483	-	-	0.4278	-	0.1284	0.2419	0.0109
4-0531	+	0.3485	0.0581	-	0.1257	-	0.0769	-	1.6539

[1] Citronellal, cinnamic aldehyde and dihydro coumarin were not detected in any of the investigated samples.
+: substance present but not quantitated due to interference
-: not detected, detection limit 0.0001% approximately

Table 6: Contents of fragrance substances in the patch or use test cosmetics[1].

NERI-reg.no.	Concentration of fragrance substances, % (w/v)*								
	Geraniol	Hydroxy citronellal	Cinnamic alcohol	Citral	Eugenol	Coumarin	Isoeugenol	α-amyl cinnamaldehyde	α-hexyl cinnamaldehyde
4-0481	0.0431	0.5422	0.1452	-	0.0357	<0.0010	-	-	-
4-0482	0.0277	0.0294	0.0026	-	<0.0010	-	<0.0010	<0.0010	0.0118
4-0483	0.0555	0.0644	-	-	0.0066	-	-	-	0.6633
4-0484	-	0.1218	-	-	0.0295	-	-	-	-
4-0485	-	0.3133	0.0628	-	0.1723	0.1743	<0.0010	-	-
4-0486	-	-	-	-	-	-	-	<0.0010	0.2074
4-0487	-	0.0045	-	-	-	0.1454	-	-	0.0775
4-0488	0.1165	0.1110	0.0530	-	0.2218	0.0983	0.0342	0.1163	-
4-0489	+	0.0670	0.0094	-	0.0047	0.0382	-	-	-
4-0490	-	0.0084	<0.0010	-	<0.0010	-	-	-	-
4-0491	+	-	0.0046	-	0.0517	0.1393	-	-	0.1100
4-0492	-	<0.0010	-	-	-	0.0527	-	-	<0.0010

[1] Citronellal, cinnamic aldehyde and dihydro coumarin were not detected in any of the investigated samples.
+: substance present but not quantitated due to interference
-: not detected, detection limit 0.0001% approximately
*: (w/w) for 4-0482

Table 6: Continued.

NERI-reg.no.	Concentration of fragrance substances, % (w/v)*								
	Geraniol	Hydroxy citronellal	Cinnamic alcohol	Citral	Eugenol	Coumarin	Isoeugenol	α-amyl cinnamaldehyde	α-hexyl cinnamaldehyde
4-0493	-	0.0138	-	-	<0.0010	0.0252	<0.0010	<0.0010	0.0059
4-0494	<0.0010	0.1065	+	-	0.0119	+	-	-	<0.0010
4-0495	-	0.0050	-	-	0.0099	0.0086	-	-	0.0098
4-0496	-	-	-	-	-	0.2286	-	-	0.2417
4-0497	0.0741	0.1683	<0.0010	-	<0.0010	0.1770	<0.0010	<0.0010	<0.0010
4-0498	0.6202	0.3287	<0.0010	0.0935	<0.0010	<0.0010	-	-	0.6075
4-0499	+	0.1461	0.0041	-	0.0155	0.0852	0.0131	0.3259	0.0925
4-0500	0.0107	0.0036	<0.0010	-	0.0405	-	-	0.0139	-
4-0501	-	0.0063	-	-	-	<0.0100	-	-	<0.0010
4-0502	-	<0.0010	-	-	-	-	-	-	0.0047
4-0503	0.0033	0.0057	-	-	0.0030	-	-	-	-

Citronellal, cinnamic aldehyde and dihydro coumarin were not detected in any of the investigated samples.
+: substance present but not quantitated due to interference
-: not detected, detection limit 0.0001% approximately
*: (w/w) for 4-0495, 4-0500, 4-0501, 4-0502 and 4-0503

Table 7: Contents of fragrance substances in fragrance mixtures and in the corresponding cosmetic products

Fragrance mixture			Corresponding cosmetic product		
NERI-reg-.no.	Fragrance substances identified	Concentration % (w/v)	NERI-reg.no.	Fragrance substances	Concentration % (w/w)
4-0532	Geraniol α-hexyl cinnamaldehyde	0.0500 0.2980	4-0504	Geraniol α-hexyl cinnamaldehyde	0.0003 0.0004
4-0538	Hydroxy citronellal Cinnamic alcohol Eugenol Coumarin α-amyl cinnamaldehyde α-hexyl cinnamaldehyde	0.0990 0.1000 0.1028 0.0824 + 0.1773	4-0505	Hydroxy citronellal Cinnamic alcohol Eugenol Coumarin α-amyl cinnamaldehyde α-hexyl cinnamaldehyde	0.0006 0.0002 n.d. 0.0002 0.0019 0.0007
4-0548	Citral Geraniol Cinnamic alcohol Coumarin α-hexyl cinnamaldehyde	+ 0.0954 0.0610 0.2180 +	4-0506	Citral Geraniol Cinnamic alcohol Coumarin α-hexyl cinnamaldehyde	n.d. 0.0006 0.0002 n.d. 0.0006
4-0545	α-amyl cinnamaldehyde α-hexyl cinnamaldehyde	0.8462 +	4-0507	α-amyl cinnamaldehyde α-hexyl cinnamaldehyde	0.0019 0.0007
4-0533	Geraniol Cinnamic alcohol Eugenol Isoeugenol α-hexyl cinnamaldehyde	+ 0.1020 0.2740 0.1350 0.0300	4-0508	Geraniol Cinnamic alcohol Eugenol Isoeugenol α-hexyl cinnamaldehyde	n.d. n.d. 0.0005 0.0002 0.0010
4-0536	α-amyl cinnamaldehyde α-hexyl cinnamaldehyde	0.0050 +	4-0509	α-amyl cinnamaldehyde α-hexyl cinnamaldehyde	n.d. 0.0081

+: substance present but not determined due to interference
n.d.: not detected, detection limit approximately 0.2 ppm.

Table 7: Continued.

Fragrance mixture			Corresponding cosmetic product		
NERI-reg.no.	Fragrance substances identified	Concentration % (w/v)	NERI-reg.no.	Fragrance substances	Concentration % (w/w)
4-0547	Citral	+	4-0510	Citral	n.d.
	Geraniol	+		Geraniol	< 0.0001
	Hydroxy citronellal	+		Hydroxy citronellal	n.d.
	Eugenol	+		Eugenol	< 0.0001
	Coumarin	0.1327		Coumarin	< 0.0001
	Isoeugenol	0.1702		Isoeugenol	n.d.
	α-amyl cinnamaldehyde	0.0832		α-amyl cinnamaldehyde	n.d.
4-0539	Geraniol	+	4-0511	Geraniol	n.d.
	Hydroxy citronellal	0.0636		Hydroxy citronellal	n.d.
	Cinnamic alcohol	0.0163		Cinnamic alcohol	0.0005
	α-amyl cinnamaldehyde	0.0356		α-amyl cinnamaldehyde	0.0001
	α-hexyl cinnamaldehyde	0.7508		α-hexyl cinnamaldehyde	0.0007
4-0540	Geraniol	0.0695	4-0512	Geraniol	n.d.
	Eugenol	0.3878		Eugenol	< 0.0001
	α-amyl cinnamaldehyde	1.3477		α-amyl cinnamaldehyde	0.0001
	α-hexyl cinnamaldehyde	0.4078		α-hexyl cinnamaldehyde	0.0003
4-0541	Geraniol	0.1866	4-0513	Geraniol	0.0003
	Cinnamic alcohol	0.0183		Cinnamic alcohol	0.0001
	Eugenol	0.0581		Eugenol	0.0002
	Coumarin	0.4248		Coumarin	+
	α-amyl cinnamaldehyde	0.0367		α-amyl cinnamaldehyde	0.0002
	α-hexyl cinnamaldehyde	1.3407		α-hexyl cinnamaldehyde	0.0001
4-0535	Cinnamic alcohol	0.0827	4-0514	Cinnamic alcohol	0.0001
	Eugenol	0.1090		Eugenol	0.0001
	α-amyl cinnamaldehyde	0.1747		α-amyl cinnamaldehyde	0.0002
	α-hexyl cinnamaldehyde	0.3146		α-hexyl cinnamaldehyde	0.0017

+: substance present but not determined due to interference
n.d.: not detected, detection limit approximately 0.2 ppm

Table 7: Continued.

Fragrance mixture			Corresponding cosmetic product		
NERI-reg-.no.	Fragrance substances identified	Concentration % (w/v)	NERI-reg.no.	Fragrance substances	Concentration % (w/w)
4-0537	Geraniol Cinnamic alcohol Eugenol α-amyl cinnamaldehyde α-hexyl cinnamaldehyde	0.1187 0.3288 0.0252 0.0134 1.0489	4-0515	Geraniol Cinnamic alcohol Eugenol α-amyl cinnamaldehyde α-hexyl cinnamaldehyde	+ 0.0010 0.0002 n.d. 0.0044
4-0543	Geraniol Hydroxy citronellal Eugenol Coumarin	+ 0.1408 0.0563 0.8042	4-0516	Geraniol Hydroxy citronellal Eugenol Coumarin	n.d. 0.0018 0.0009 0.0008
4-0542	Geraniol Coumarin	+ 0.1437	4-0517	Geraniol Coumarin	0.0008 0.0010
4-0546	Citral Geraniol	+ +	4-0518	Citral Geraniol	n.d. n.d.
4-0544	Hydroxy citronellal Cinnamic alcohol α-amyl cinnamaldehyde	0.2444 0.0119 0.1461	4-0519	Hydroxy citronellal Cinnamic alcohol α-amyl cinnamaldehyde	0.0005 n.d. 0.0047
4-0534	Geraniol Hydroxy citronellal α-hexyl cinnamaldehyde	+ 0.0070 0.5300	4-0575	Geraniol Hydroxy citronellal α-hexyl cinnamaldehyde	n.d. n.d. 0.0021

+: substance present but not determined due to interference
n.d.: not detected, detection limit approximately 0.2 ppm

5 Discussion

An analytical method developed earlier (7) was employed for the determination of 12 target fragrance substances in selected cosmetic products. No problems were encountered during the analysis of the target fragrance substances in various types of cosmetic products in the present investigation. Furthermore, it was shown that the method was also applicable for the analysis of α-hexyl cinnamaldehyde in cosmetics. Analysis of target fragrance substances in cosmetics for daily use (sub-project iii, samples described in Table 4) required higher sensitivities of detection and quantisation compared to those for the other products. The replacement of the old column (used for approximately 6 months) with a new one of the same type (cf. Section 3.3.1), higher sensitivities of detection and quantisation of fragrance substances, 0.2 ppm and 1 ppm respectively, were achieved. To obtain higher sensitivity, a similar column to the one used before but with thinner coating of the stationary phase, CP-Sil-5CB, 50 m x 0.32 mm, d_f 0.12 µm, was also tried. By the use of this column, still higher sensitivities for detection and quantisation of fragrance substances, compared to those obtained by the use of GC-column with 1.2 µm coating, were obtained (results not shown). The use of this column required also a new temperature program for GC. As it was not appropriate to change the analysis parameters in the middle of the project, all the samples were analyzed employing the GC-method described previously (7). It is, however, recommended that CP-Sil-5CB capillary column with 0.12 µm thickness should be employed in future studies concerning GC-analysis of fragrances.

All cosmetic products analyzed in the present study were found to contain at least 2, and maximum up to 9, of the 12 the target fragrance substances. As all of the target fragrance substances except α-hexyl cinnamaldehyde are known to cause skin sensitization in humans, the selection of the target substances seems to be appropriate. It should be noted that α-hexyl cinnamaldehyde was declared safe only on the basis of a very limited study (11). However, this substance is chemically similar to the well known skin sensitizer, i.e. α-amyl cinnamaldehyde. Moreover, α-hexyl cinnamaldehyde is frequently used in relatively high concentration in cosmetic products (8).

Because of the limited number of samples in the present study, it will not be appropriate to make a statement concerning use-concentrations of fragrance substances in various categories of cosmetic products. It was, however, observed that the concentrations of target fragrance substances were highest in perfume samples, followed by eau de parfum, eau de toilette, after shave lotion and the least in shampoos and creams etc.

According to guidelines of IFRA, perfume industries organization, hydroxy citronellal in concentrations >0.25% should not be used

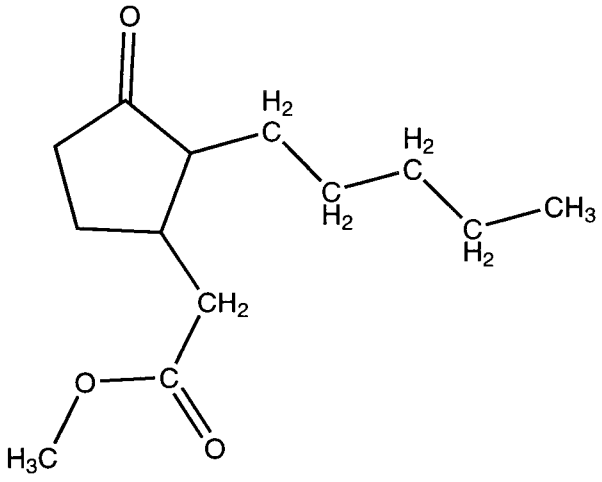
in cosmetics. However, >0.25% hydroxy citronellal was found in 8 of the 10 popular perfumes and in 3 of the 23 patch- and/or use- test cosmetic products in the present investigation. Similarly, some other restricted substances by IFRA (12), i.e. cinnamic alcohol and isoeugenol, were found in concentrations upto respectively 0.79% and 0.34% in the cosmetic investigated. Relatively high concentrations (> 0.25%) of geraniol, eugenol, coumarin, α-amyl cinnamaldehyde and α-hexyl cinnamaldehyde were also found in several of the products investigated. Cinnamic aldehyde, dihydro coumarin and citronellal were not identified in any of the investigated products.

GC analysis of the cosmetic products in the present investigation also revealed the presence of several unidentified chromatographic peaks - non-target fragrance substances (Figures 1 - 9). The GC-MS analysis offers an opportunity to identify many of these substances. The non-target substances were identified on the basis of their GC-t_R and/or on the basis of their mass spectra. In absence of t_R, a mass spectrum match with a fit of >950 and purity >900 was used as criteria for identification. Following fragrance substances were frequently found in the investigated cosmetic products (Figures 10 - 12): benzyl acetate; benzyl benzoate; linalool; linalyl acetate; linalool, 2-aminobenzoate; citronellol; citronellyl acetate; 2-aminobenzoic acid, methyl ester; piperonal; benzene- methanol and α-methy benzenemethanol, acetate; benzeneethanol (phenethyl alcohol), α,α-dimethyl benzeneethanol and its acetate; benzene propanol, α,α-dimethyl benzenepropanol and its acetate; α,α,4-trimethy-3-cyclohexene-1-methanol; 3-ethoxy benzaldehyde and 2-hydroxy-/4-hydroxy-3-methoxy benzaldehyde; 2-hydroxybenzoic acid, 2-methyl propyl ester; 1,2-dimethoxy-4-(2-propenyl) benzene, ethyl citrate; 4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one; 5-pentyldihydro-/5-heptidyldihydro-2-(3H)-furanone; terpenes (limonene, carene, cineole, etc.) sesquiterpenes ($C_{15}H_{24}$: caryophellene, farnesen, thujopsene, etc.) and sesqiterpene lactones; patchouli alcohol; santalol; Unknown 1 and Unknown 2; and unidentified substances with scan nos. 1442, 1722, 1818 & 1882.

Attempts were made to identify two of the unidentified substances, Unknown 1 and Unknown 2 (Figures 10 - 12), because these were present in over 30% of the samples analyzed. The mass spectra of Unknown 1 and Unknown 2 are shown in Figures 13 and 14. On the basis of the first five hits of best fit with the mass spectra in MS-library (Fig. 13), it is speculated that Unknown 1 may be a phenol-derivative. Note that none of the substances described in first five hits can not produce m/z 163, m/z 191 and m/z 206. It is also possible that the GC-peak of Unknown 1 is impure and it represents 2 or more substances having exactly the same retention times, because the MS-identification at various points of the GC-peak resulted in the 5 same hits except that their ranking were different.

On the basis of the results of the library search (Fig. 14), rank 1 with fit 980 and purity 427 (3-oxo-2-pentyl 1-cyclopenten-1-acetic

acid, methyl ester also known as methyl jasmonate), Unknown 2 is suggested to be dihydro methyljasmonate. Dihydromethyl jasmonate, also called Hedione®, is one of the synthetic fragrance materials which is frequently used in relatively high amounts in perfumes (13). It has not been possible to obtain authentic Hedione® as reference material for the confirmation of the identification of Unknown 2.



Hedione®

A semi-quantitative determination of some of the non-target fragrance substances, frequently present in the 10 popular perfumes and in the 5 patch- and/or use-test positive aftershave lotion, was performed. Thus, the contents of 12 non-target fragrance substances and 2 non-fragrance substances (limonene and diethyl phthalate) have been quantitated as per cent respective GC-peak areas (of the total GC-peak areas obtained by GC-FID analysis of a product). The results of semi-quantitative determination of the non-target substances are described in Tables 8 and 9. As shown in Table 8, limonen, diethyl phthalate, phenethyl alcohol, linalool and its 2-aminobenzoic acid ester, benzyl acetate, benzyl benzoate, citronellol, piperonal and Unknown 1 were present in various concentrations (0.01% - 14%) in 70 - 100% of the investigated perfumes. Methyl ester of 2-aminobenzoic acid (0.12% - 1.52%) was present in 50% of the 10 popular perfumes. Dimethyl ester of phenethyl alcohol, dimethoxy propenyl benzene and Unknown 2 each were present in concentration 0.08% - 4.4% in 3 of the perfumes. Although the number of aftershave lotion investigated are only 5, the trend of use of the non-target fragrance substances in these products was similar to that for the popular perfumes (Table 9), except that the concentrations of the non-target substances were relatively lower than in the perfumes. The detection limits of non-target substances were not elucidated in the present investigation.

Figure 10: GC-MS of sample no. 4-0488

Scan no.	Compound
585	Phenethylalcohol (benzeneethanol)
650	Benzyl acetate
668	α,α-dimethyl benzeneethanol
702	α-methyl benzenemethanol, acetate
719	3,7-dimethyl 1-octanol
776	Citronellol
827	Geraniol
836	Linalool, 2-aminobenzoate
878	Hydroxy citronellal
926	Cinnamic alcohol
982	α,α-dimethyl benzeneethanol, acetate
996	Piperonal
1049	Eugenol
1089	Geranyl acetate
1234	4-(2,6,6-trimethyl-2-cyclohexen-1-yl) 3-buten-2-one
1334	1,2-dimethoxy-4-(1-propenyl)-benzene
1374	Unknown 1
1564	Diethyl phthalate
1699	Unknown 2
1722, 1741, 1814 & 1882	Sesqui-terpenes and lactones
1775	2-hydroxybenzoic acid, 2 methylpropyl ester

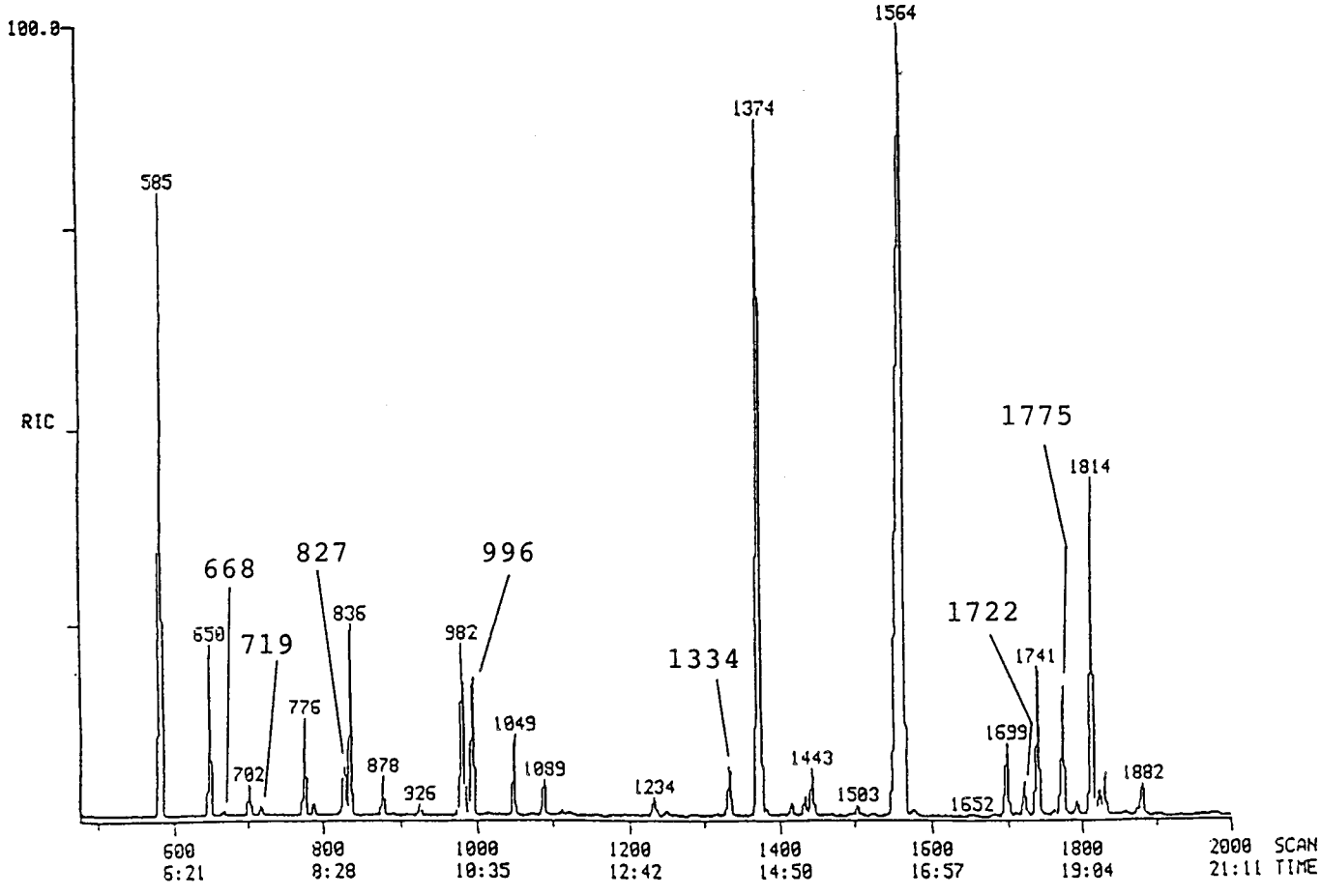


Figure 11: GC-MS of sample no 4-0522

Scan no.	Compound
490	Limonene
563	Linalool
584	Benzeneethanol
653	Benzyl acetate
741	α,α,4-trimethyl-3-cyclohexene-1-methanol (S)-
755	1α,2β,2-methyl-5-(1-methylethyl)-cyclohexanol
777	Citronellol
829	Geraniol (E)-
837	Linalool, 2-aminobenzoate
884	Hydroxy citronellal
929	Cinnamic alcohol
1019	2-aminobenzoic acid, methyl ester
1053 & 1091	Geranyl acetate (E)- & (Z)-
1274	5-heptyl dihydro-2(3H)-furanon
1372	Unknown 1
1507	5-propyle-1,3-benzodioxole
1561	Diethyl phthalate
1707	Unknown 1
1718	α-amyl cinnamaldehyde
1937	α-hexyl cinnamaldehyde
1964	Benzyl benzoate

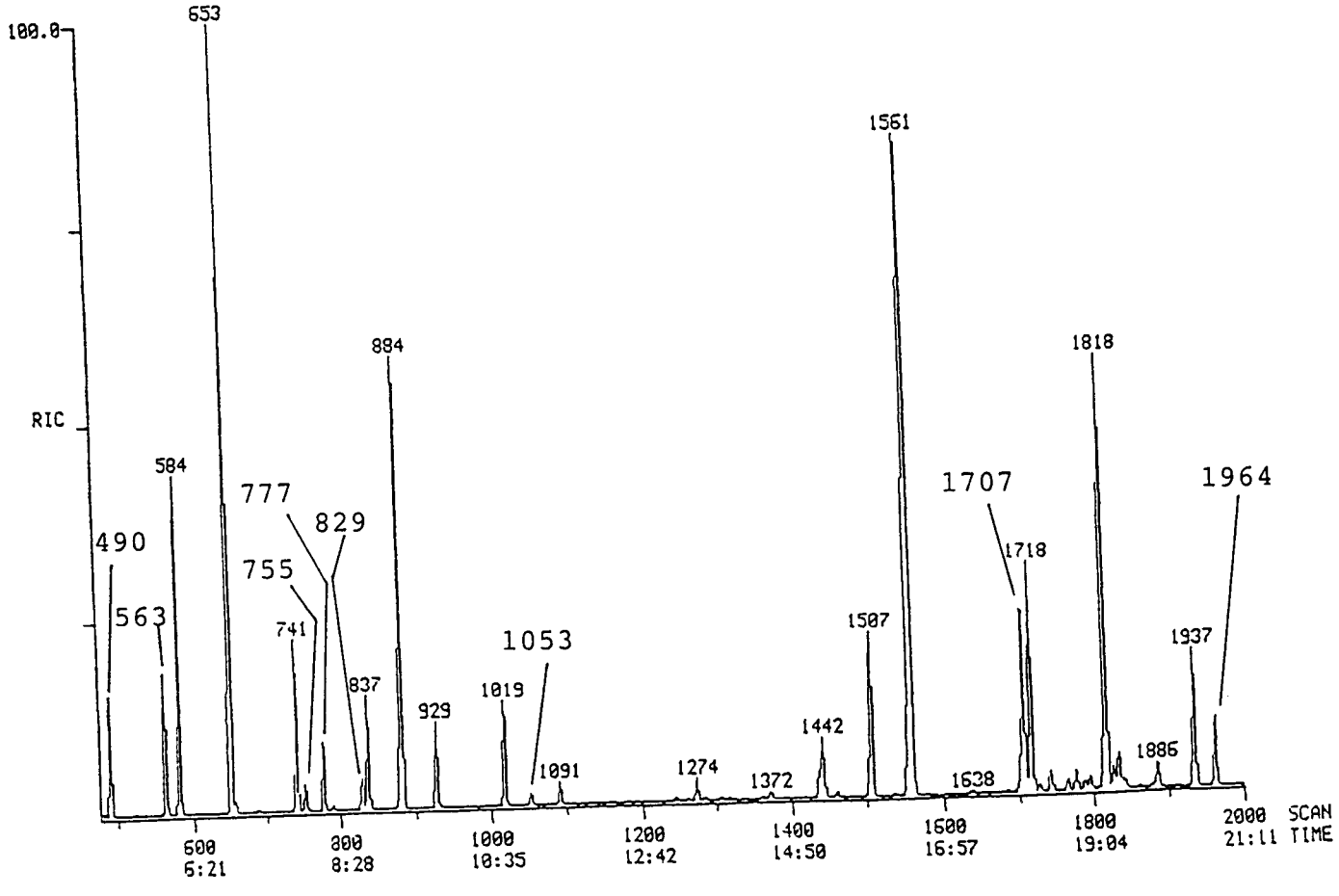


Figure 12: GC-MS of sample no. 4-0523

Scan no.	Compound
491	Dipropylene glycol
586	Benzene ethanol
651	Benzyl acetate
669	α,α-dimethyl benzeneethanol
703	α-methyl benzenemethanol, acetate
777	Citronellol
828	Geraniol
837	Linalool, 2-aminobenzoate
880	Hydroxy citronellal
984	α,α-dimethyl benzeneethanol, acetate
998	Piperonal
1051	Eugenol
1237	4-(2,6,6-trimethyl-2-chyclohexen-1-yl)-3-buteno-2-one
1337	1,2-dimethoxy-4-(2-propenyl)-benzene
1378	Unknown 1
1703	Unknown 2
1962	Benzylbenzoate

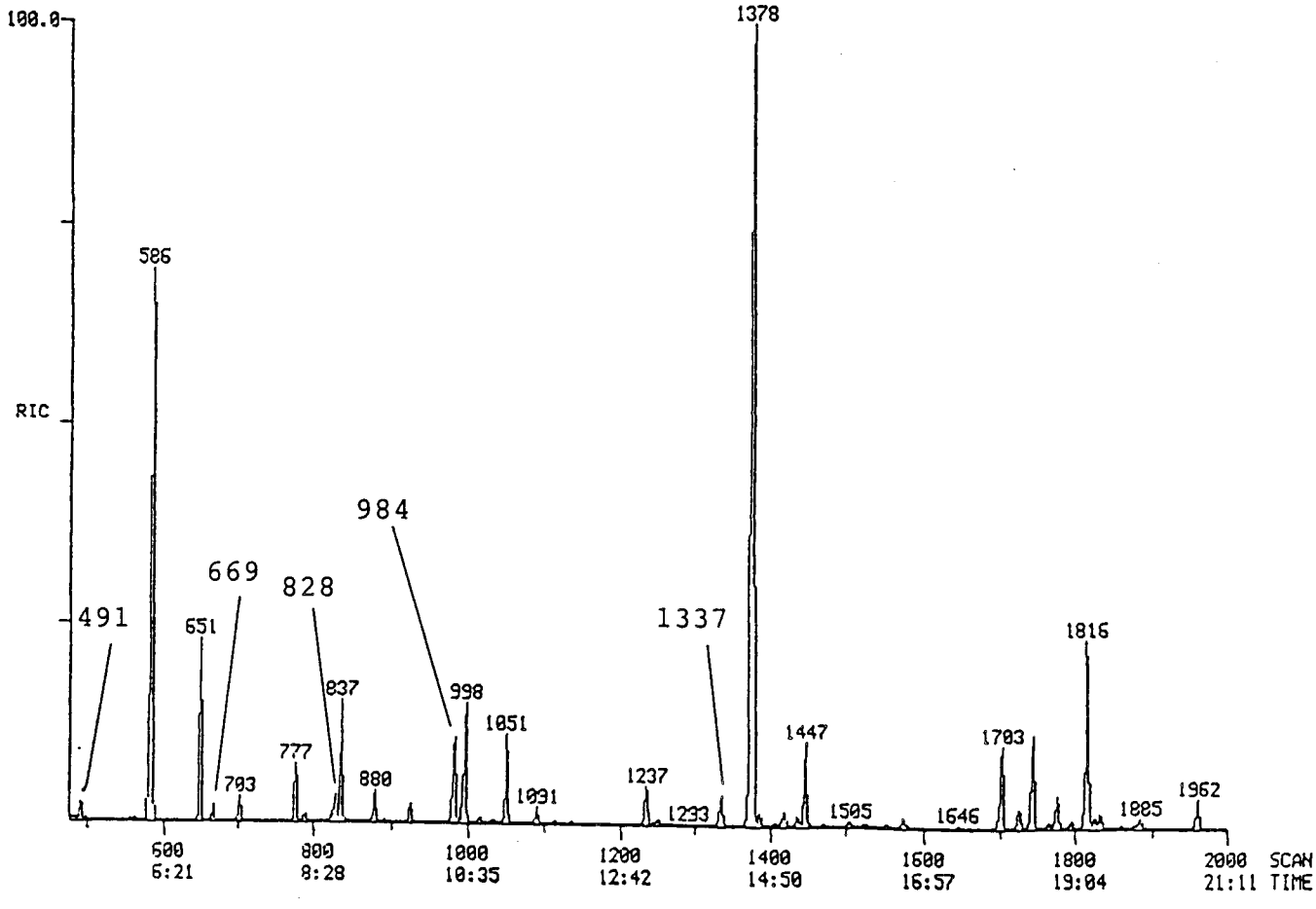
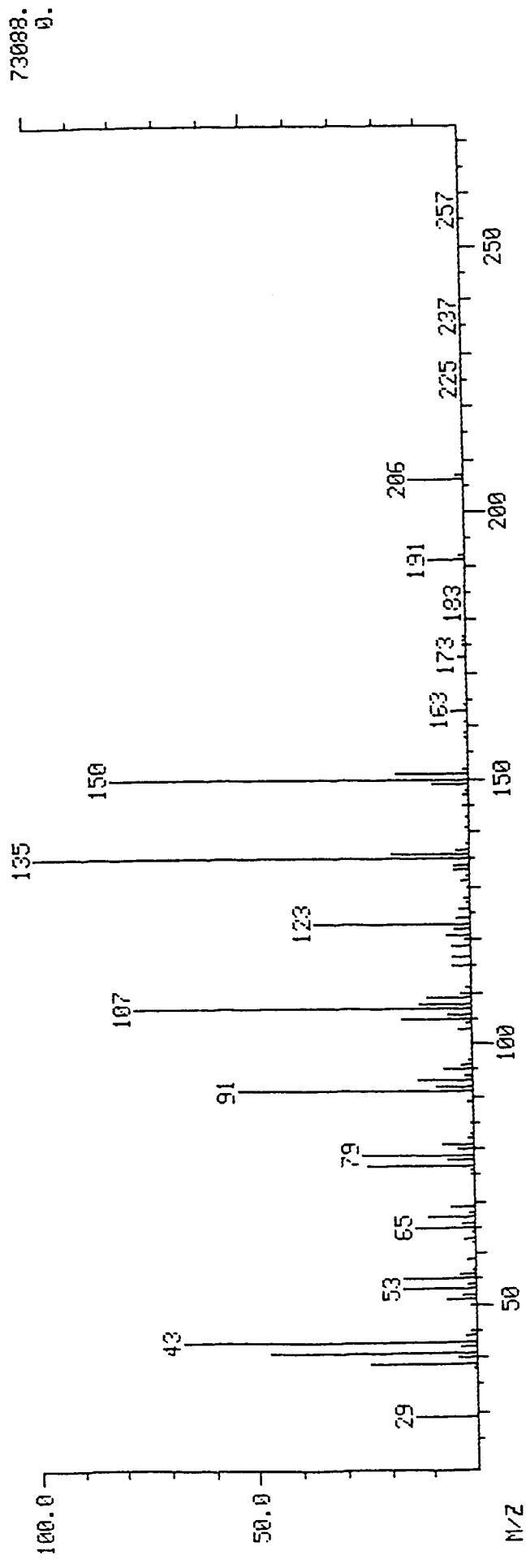


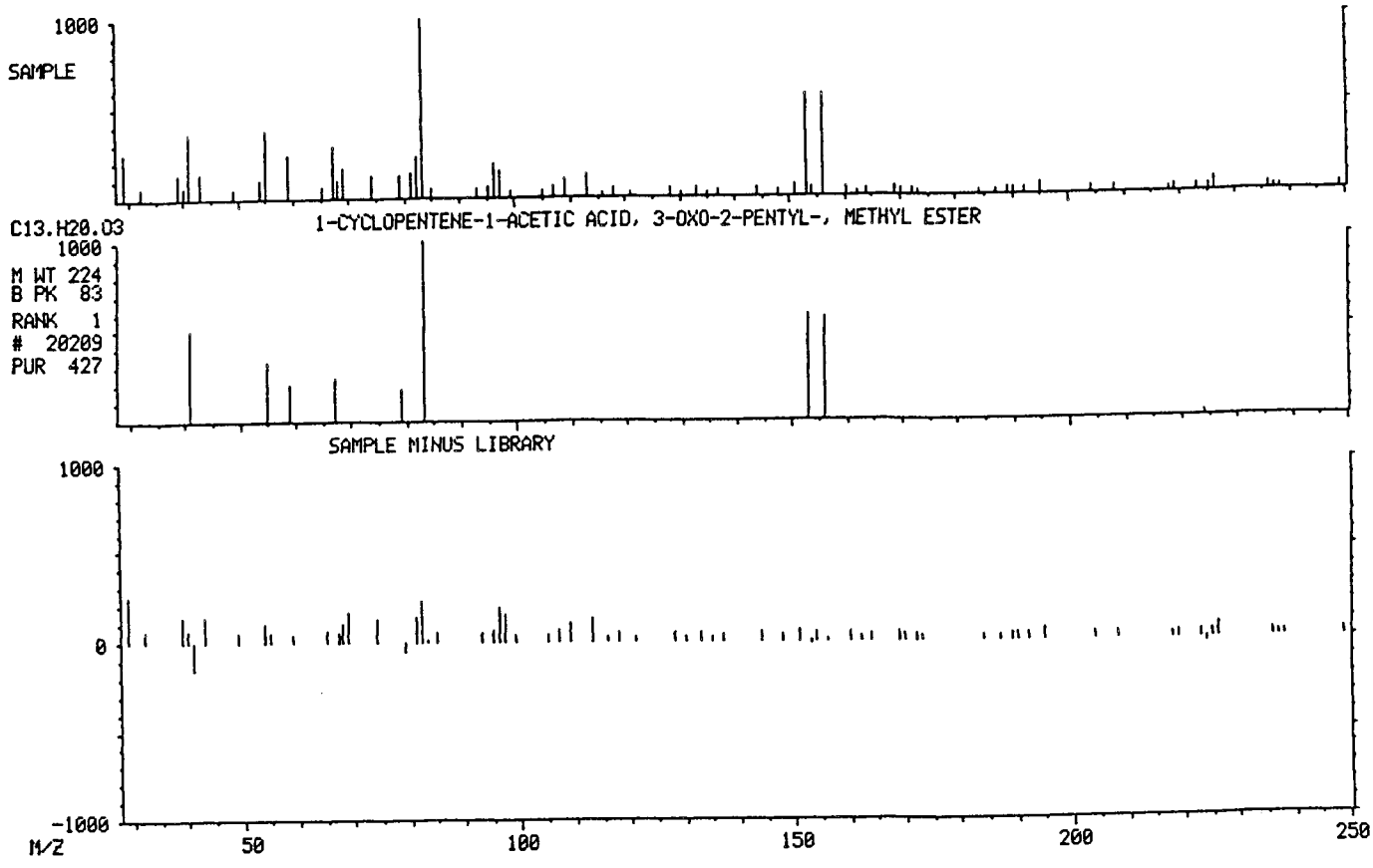
Figure 13: Mass spectrum of Unknown 1.



Rank	In.	Name	M.Wt	B.Pk	Purity	Fit	RFit
1	7430	PHENOL, 2,3,5,6-TETRAMETHYL-	150	135	640	932	640
2	7419	PHENOL, 5-METHYL-2-(1-METHYLETHYL)-	150	135	636	917	636
3	7455	BENZENE, 2-METHOXY-1,3,5-TRIMETHYL-	150	135	620	911	620
4	7449	PHENOL, 2-ETHYL-4,5-DIMETHYL-	150	135	609	920	609
5	7435	PHENOL, 3-(1,1-DIMETHYLETHYL)-	150	135	597	899	597

Rank	Formula
1	C10.H14.O
2	C10.H14.O
3	C10.H14.O
4	C10.H14.O
5	C10.H14.O

Figure 14: Mass spectrum of Unknown 2 and comparison of sample mass spectrum with the mass spectrum of the rank 1 compound suggested by library search.



Rank	In.	Name
1	20209	1-CYCLOPENTENE-1-ACETIC ACID, 3-OXO-2-PENTYL-, METHYL ESTER
2	21889	CYCLOHEXANE, 1,1'-(1,5-PENTANEDIYL)BIS-
3	17870	CYCLOHEXANE, 1,1'-(1,3-PROPANEDIYL)BIS-
4	20609	11-DODECEN-1-OL, 2,4,6-TRIMETHYL-, (R,R,R)-
5	21888	CYCLOHEXANE, 1,1'-PENTYLIDENEBIS-

Rank	Formula	M.Wt	B.Pk	Purity	Fit	RFit
1	C13.H20.O3	224	83	427	980	434
2	C17.H32	236	83	355	706	374
3	C15.H28	208	83	288	841	303
4	C15.H30.O	226	69	287	652	384
5	C17.H32	236	152	281	631	415

Table 8: Contents of non-target fragrance and non-fragrance substances in the popular perfumes.

Fragrance/ non-fragrance substance	Content* in perfumes, NERI reg. no..									
	4-0522	4-0523	4-0524	4-0525	4-0526	4-0527	4-0528	4-0529	4-0530	4-0531
Phenethyl alcohol	2.30	6.33	4.21	0.87	0.35	2.89	0.83	2.45	3.47	2.88
Linalool	1.28	0.18	3.19	0.13	4.05	0.73	3.80	1.33	1.82	0.74
Citronellol	0.88	1.13	1.09	0.45	0.66	0.19	0.40	0.18	-	-
Piperonal	-	1.47	-	1.14	0.05	0.04	2.15	2.60	-	0.05
Benzyl acetate	3.93	1.84	2.15	0.41	0.67	2.13	3.31	1.25	0.58	-
Benzyl benzoate	1.28	1.05	0.75	0.88	0.37	0.01	1.46	0.69	0.61	0.59
Linalool, 2-amino-benzoate	1.21	1.65	2.52	0.67	-	0.17	-	0.92	2.66	-
Phenethyl alcohol, dimet-hyl acetate	-	1.81	-	0.63	-	-	-	0.08	-	-
Dimethoxy propenyl benzene	-	1.20	-	0.22	0.80	-	-	-	-	-
2-aminobenzoic acid, methyl ester	1.29	0.33	-	-	-	-	1.52	0.30	-	0.12
Diethyl phthalate	4.38	0.22	1.04	7.36	0.67	0.21	1.98	2.95	-	-
Limonene	0.74	0.08	6.01	0.15	0.19	0.26	-	2.18	0.73	-
Unknown 1	0.30	8.05	0.09	14.38	1.87	0.15	0.84	4.66	4.21	0.70
Unknown 2 (evt. dihydro methyljasmonate)	2.10	1.76	-	-	-	-	4.36	-	-	-

* % GC peak area of total peak areas obtained by GC-FID analysis;
- not detected

Table 9. Content of non-target fragrance and non-fragrance substances in patch- and/or use-test aftershave lotion.

Fragrance/ non-fragrance substance	Content* in aftershave lotion, NERI reg. no.				
	4-0489	4-0490	4-0491	4-0492	4-0497
Phenethyl alcohol	0.44	-	0.28	-	0.19
Linalool	1.20	0.17	0.12	0.28	0.95
Citronellol	0.08	0.05	0.21	0.05	0.25
Piperonal	0.03	0.01	-	-	-
Benzyl acetate	0.23	-	-	-	-
Benzyl benzoate	0.05	0.08	-	-	0.14
Linalool, 2-amino-benzoate	0.78	0.04	1.94	-	0.76
2-aminobenzoic acid, methyl ester	0.03	-	-	-	-
Limonene	0.87	0.12	1.96	0.14	0.54
Diethyl phthalate	2.49	0.05	10.10	1.36	5.56
Unknown 1	0.30	0.03	0.20	0.06	0.34
Unknown 2 (evt. dihydro methyljasmonate)	-	-	1.01	0.34	-

* % GC peak area of total peak areas obtained by GC-FID analysis;
- not detected

Analyses of target fragrance substances in selected cosmetic products was undertaken with the aim that the results of the study may lead to basic knowledge about the threshold concentrations of the respective fragrance substances to cause skin sensitization in susceptible persons. The main clinical work is in progress at the Dermatology Department, Gentofte Hospital. However, the results of a part of the study have shown a correlation between the skin reactions and the concentrations of the target fragrance substances (JDJ personal communication).

The presence of non-fragrance substances diethyl phthalate and limonene in relatively high concentrations in most of the samples, may be of concern for human health. Diethyl phthalate has been shown to absorb through human skin (14) and that, in analogy with other phthalates, may also be harmful to human health. The use of limonene, as a quenching agent for some fragrance sensitizers in perfume mixtures has been recommended IFRA (12). Limonene has, however, been shown to induce hepatic microsomal monooxygenase activity (15), it can produce skin irritation and skin sensitization (16), and inhalation of limonene may affect pulmonary function (17). In a recent study, Takayama and Nagai (18) demonstrated that limonene in presence of ethanol is a potential skin penetration promoter. They suggest that, at first, d-limonene penetrates into the skin under coexistence with ethanol and that may change the barrier structure of the stratum corneum. The transfer of ethanol (and substances soluble in ethanol) to skin is thereby enhanced under the coexistence with d-limonene in the skin. The fragrance mixtures containing ethanol and limonene may thus also increase penetration of the skin sensitizers present in the mixtures. Finally, limonene and other terpenes may also be of concern from environmental point of view, because the products of atmospheric oxidation of terpenes by ozone (and other oxidants: OH-radical and NO_x) may have low vapour pressure and may accumulate as an aerosol (19).

It may be useful to have a knowledge of fragrance substances which may be persistent in the environment and/or may be toxic to environmental health. Perfumes get into the environment primarily by evaporation and being washed down the drain. Although perfumes are widely dispersed into the environment, the fate and ecotoxicity of these substances are not known. The data concerning production and use of various perfumes are not available and environmental impact assessment of perfumes has not yet been done. The data obtained in the present investigation may be useful to get a rough knowledge of the amounts of some fragrance and non-fragrance substances released into the environment through the use of cosmetics. Some of the fragrance substances, for example, nitro musks have been shown to be persistent in the environment (20 - 22). Nitro musks were, however, not included as target substances in the present investigation.

6 References

1. Streschank B. (1991): Support materials for deodorant mixtures. In *Perfumes: Art, Science & Technology* (Eds. P.M. Müller and D. Lamparsky), Chapter 11. Elsevier Science Publishers Ltd., England. pp 347-362.

2. Funesti J.K. (1991): Perfumery applications: Functional products. In *Perfumes: Art, Science & Technology* (Eds. P.M. Müller and D. Lamparsky), Chapter 12. Elsevier Science Publishers Ltd., England. pp 363-382.

3. Adam R.M. and Maibach H.I. (1985): A five year study of cosmetic reactions. *J. Am. Acad. Dermatol.* 13: 1062-1069.

4. De Groot A.C. (1987): Contact allergy to cosmetics: causative ingradients. *Contact Dermatitis* 17: 26-34.

5. Larsen W.G., Jackson E.M., Barker, M.O., Bednarz R.M., Engasser P.G., O'Donoghue M.N. and Strauss J.S. (1992): A primer on cosmetics. *J. Am. Acad. Dermatol.* 27: 469-484.

6. Nielsen N.H. and Menné T. (1992): Allergic contact sensitization in an unselected Danish population. The Glostrup allergy study, Denmark. *Acta Derm. Venerol. (Stockh)* 72:456-460.

7. Rastogi S.C. and Jensen G.H. (1994): Chemical substances and chemical preparations. Chromatographic analysis of some fragrances in cosmetics and toiletries. Natl. Environ. Res. Inst., Roskilde, Denmark. *NERI Technical report No. 106.*

8. Fenn R.S. (1989): Aroma chemical usage trends in modern perfumery. *Perfumer & Flavorist* 28: 1-10.

9. Rawsthorn A. (1993): Thrown off the Scent. *Financial Times* 4th November.

10. Rastogi S.C. (1993): Sample preparation for gas chromatographic analysis of organic solvents in aerosol cans. *Chromatographia* 36: 201-203

11. Opdyke D.L.J. (1974): Hexyl cinnmaic aldehyde. *Food Cosmetic Toxicol.* 12:915.

12. Ford R.A. (1991): The toxicology and safety of fragrances. In *Perfumes: Art, Science & Technology* (Eds. P.M. Müller and D. Lamparsky), Chapter 16. Elsevier Science Publishers Ltd., England. pp 441-463.

13. Fráter G. and Lamparsky D. (1991): Synthetic Products. In *Perfumes: Art, Science & Technology* (Eds. P.M. Müller and D. Lamparsky), Chapter 20. Elsevier Science Publishers Ltd., England. pp 533-628.

14. Mint A., Hotchkiss S.A.M. and Caldwell J. (1994): Percutaneous absorption of diethyl phthalate through rat and human skin *in vitro* *Toxic. in Vitro* 8: 251-256.

15. Reicks M.M. and Crankshaw D. (1993): Effects of d-limonene on hepatic microsomal monooxygenase activity and paracetamol-induced glutathione depletion in mouse. *Xenobiotica* 23: 809-819.

16. Joseffson C. (1993): Limonene. In Health effects of selected chemicals, *Vol.* 2. Nordic Council of Ministers, Copenhagen. pp. 105-135.

17. Falk-Filipsson A., Löf A., Hagberg M., Hjelm E.W. and Wang Z. (1993): *J. Toxicol. Environ. Health* 38: 77-88.

18. Takayama K and Nagai T. (1994): Limonene and related compounds as potential skin penetration promoters. *Drug Developm. and Ind. Pharm.* 20: 677-684.

19. Grosjean D., Williams E.L., Grosjean E., Andino J.M. and Seinfeld J.H. (1993): Atmospheric oxidation of biogenic hydrocarbons: Reaction of ozone with β-pinene, d-limonene and transcaryophyllene. *Environ. Sci. Technol.* 27: 2754-2758.

20. Rimkus G., Rimkus B. and Wolf M. (1994): Nitro musks in human adipose tissue and breast milk. *Chemosphere* 28: 421-432.

21. Rimkus G. and Wolf M. (1995): Nitro musk fragrances in biota from freshwater and marine environment. *Chemosphere*, in press.

22. Gatermann R., Hühnerfuss H., Rimkus G., Wolf M. and Franke S. (1995): The distribution of nirobenzene and other nitroaromatic compounds in the North Sea. *Mar. Pollut. Bull.*, in press.

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