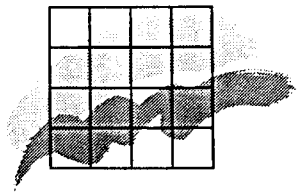

Chemical substances and chemical preparations

Investigation of selected fragrance substances in cosmetics based on natural ingredients

NERI Technical Report No. 143

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Ministry of Environment and Energy
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Data sheet

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Resumé

Visse duftstoffer i kosmetiske produkter kan fremkalde kontaktallergi. I de senere år har den store opmærksomhed omkring miljøbeskyttelse og dyrværn medført, at forbruget af naturkosmetiske produkter (N-kosmetik) er stigende. Æteriske olier, der anvendes til parfumering af N-kosmetik, kan indeholde kendte duftallergener. Et kendskab til indholdet af duftallergener i N-kosmetik er vigtigt for, at belyse en eventuel risiko for hudreaktioner ved anvendelse af disse produkter.

I nærværende undersøgelse er 42 N-kosmetik produkter (22 parfumeolier og 20 andre produkter) undersøgt for indholdet af 12 udvalgte duftstoffer inklusiv de syntetiske duftstoffer, der indgår i fragrance mix (til lappe-testning): geraniol, eugenol, isoeugenol, cinnamic alcohol, cinnamic aldehyd, hydroxycitronellal, citronellal, citral, coumarin, dihydrocoumarin, α-amylcinnamic aldehyd and α-hexylcinnamic aldehyd. Det blev påvist at 91% af parfumeolierne indeholdt 1-7 af de udvalgte duftstoffer i koncentrationer fra 0,027-7,706%. Mellem 1 og 5 af de syntetiske duftstoffer i fragrance mix blev fundet i 18/22 (82%) af de undersøgte parfumeolier.
De andre N-kosmetik produkter indeholdt 1-3 af de udvalgte duftstoffer i koncentrationen 0.0003-0.082%. Citral, citronellal og dihydrocoumarin kunne ikke påvises i nogle af de undersøgte produkter.

Resultaterne af nærværende undersøgelse viser at N-kosmetik produkter kan indeholde syntetiske duftstoffer som hydroxycitronellal og α-hexylcinnamic aldehyd. Herudover blev det også påvist at nogle af N-kosmetik produkter indeholdt relativ høj koncentration af kendte duftallergener: hydroxycitronellal, α-amylcinnamic aldehyd, coumarin, cinnamic alcohol og cinnamic aldehyd.

Arbejdet er udført som bistandsopgave til Miljøstyrelsen.

Summary

Fragrance substances have been shown to be one of the major causes of cosmetic dependent contact allergy. With the increasing awareness concerning environmental protection and animal welfare, the use of cosmetics with natural ingredients (N-cosmetics) is becoming more and more popular. The essential oils used for the formulation of N-cosmetics may, however, contain fragrance allergens. To evaluate the risk factor for contact allergy by the use of N-cosmetics, a knowledge of the contents of various fragrance allergens in these products was required.

In the present investigation, 42 N-cosmetic products (22 perfumes, and 20 other N-cosmetics) were analysed by GC-FID and GC-MS to determine contents of 12 fragrance substances, including the 7 synthetic constituents of fragrance mix used for patch testing: geraniol, eugenol, isoeugenol, cinnamic alcohol, cinnamic aldehyde, hydroxycitronellal, citronellal, citral, coumarin, dihydrocoumarin, α-amylcinnamic aldehyde and α-hexylcinnamic aldehyde. All but two perfumes (91%) contained 1 to 7 of the target fragrance substances in concentrations from 0.027-7.706%. Between 1 and 5 of the synthetic constituents of the fragrance mix were found in 18/22 (82%) of the perfumes. The remaining cosmetics (7/20, 35%) were found to contain 1 to 3 of the target fragrance substances in concentrations from 3-820 ppm (0.0003-0.0820%). Citral, citronellal and dihydrocoumarin were not detected in any of the products.

The results of the present investigation revealed that some N-cosmetic products may contain synthetic fragrance substances such as hydroxycitronellal and α-hexylcinnamic aldehyde. Furthermore, hydroxycitronellal, α-amylcinnamic aldehyde, coumarin and cinnamic aldehyde were found to be present in relative high concentration in some of the investigated N-cosmetics.

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

1 Introduction

Sensitivity to perfumes (fragrances) is a common problem. It has been demonstrated that fragrance substances are one of the major causes of cosmetics induced allergic contact dermatitis (1-3). Recently, it was demonstrated that >6% of individuals with eczema and 1-2% of the general population in Denmark were allergic to fragrance substances (4, 5). There are several thousand fragrance substances that are used in the formulation of consumer products. A fragrance may be composed of upto 300 fragrance substances. There is a need to identify the chemical nature of main fragrance ingredients responsible for causing hypersensitivity in consumers and to elucidate the cutaneous toxicology of these fragrance chemicals. Official regulation of the use of fragrance substances in consumer products, including cosmetics, is lacking.

In 1993, a joint research project with the participation of Danish Environmental Protection Agency (DEPA), The National Environmental Research Institute, Department of Environmental Chemistry and Gentofte Hospital, Department of Dermatology, Copenhagen University was started for the systematic evaluation of selected fragrance substances: analysis of fragrance substances in cosmetic products and determination of threshold concentrations of these substances to elicit skin reactions. Thus, a method has been developed for the analysis of 12 selected fragrance substances, including the synthetic constituents of fragrance mix (6, 7). An analytical investigation of cosmetics used by fragrance mix sensitized cosmetic-eczema patients has been performed to unravel that these patients were exposed to fragrance mix constituents; and contents of the selected fragrance substances in popular perfumes to evaluate the potential of these products to cause contact allergy (7). The clinical investigations employing above mentioned products have been reported recently (8, 9). Finally, skin reaction potential of selected fragrance substances in cosmetics for daily-use is under investigation (7, 10).

In the past few years, the increasing awareness concerning environmental protection as well as animal welfare has had an impact on the pattern of use of cosmetics by the general population. Thus, the use of cosmetics with natural ingredients (N-cosmetics) is getting more and more popular. As the essential oils used for perfuming of N-cosmetics may also contain well known fragrance allergens, a knowledge of contents of fragrance substances in N-cosmetics will be an aid to the management of fragrance allergy caused by these products. In the present study contents of 12 selected fragrance substances (6,10) - citral, citronellal, hydroxycitronellal, geraniol, cinnamic alcohol, cinnamic aldehyde, eugenol, isoeugenol, coumarin, dihydrocoumarin, α-amylcinnamic aldehyde, and α-hexylcinnamic aldehyde - have been determined in 42 N-cosmetic products.

Present work has been performed as a technical support to DEPA.

2 Samples

JDJ, Dermatology Department, Gentofte Hospital, selected and bought appropriate samples for the determination of the contents of the 12 target fragrance substances. In total 42 products, 22 perfumes and 20 other N-cosmetic products, were purchased from the Danish retail market and delivered to NERI for the analysis. The products represented 12 cosmetic manufacturers in Europe and in USA. The investigated samples are described in Tables 1 & 2. The manufacturers/importers of the investigated products are not disclosed in Tables 1 & 2, because the purpose of the study was to obtain a basic knowledge of concentrations of target fragrance substances in the N-cosmetics, but not to investigate any specific product.

Table 1: Identification of the perfumes investigated.

NERI - reg. no.	Scent
4-0459	Wood Musk
4-0460	Camomile
4-0461	Coconut
4-0462	L'Aird
4-0463	Tea Rose
4-0464	Mango
4-0465	Roma
4-0466	Winter Dew
4-0467	Sandalwood
4-0468	Jojoba
4-0469	Strawberry
4-0470	Vanilla
4-0471	Ananya
4-0472	Samarkand
4-0473	Primrose
4-0474	Japanese Musk
4-0475	Fuzzy Peach
4-0476	Jasmin
4-0477	Mostly Musk
4-0478	Activist
4-0479	Lavender
4-0480	Rose Musk

Table 2: Identification of the N-cosmetics (other than perfumes) investigated.

NERI - reg. no.	Product name
4-0520	Tuning Solution
4-0521	Managing Solution
4-1405	Sigøjnernatsdrøm, shampoo
4-1406	Hestekastanjegelé
4-1406	Naturlotion, citrus
4-1408	Orange bodycreme
4-1409	Natursharnpoo, Havalge og birk
4-1410	Naturlotion, rose
4-1411	Botanical Herbs Body balm, Rosemary and Hyssop
4-1412	Camille Roll-on
4-1413	Wild rose Roll-on
4-1414	Natursharnpoo, Minkolie og Protein
4-1415	Aromatic Spices Body Balm, Cinnamon and Vanilla
4-1416	Antidrying body milk with citrus extracts
4-1417	Rosenvand til ansigtet, facewater
4-1418	Urte-glandin lotion
4-1419	Lime Roll-on
4-1420	Precipitation, body lotion
4-1421	Cellulite Massage Oil
4-1422	Stress Buffer

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-FID 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 Finigan 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-amylcinnamic aldehyde 97%, α-hexylcinnamic aldehyde 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 hydroxycitronellal 95% was from Biomedicals Ltd., U.K. Active Silica 100-200 mesh was 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 analysed within 24 hours.

3.2 Sample Preparation

3.2.1 Perfumes

Perfumes were appropriately diluted in methanol for the analysis by GC-MS. Depending upon the concentrations of various target fragrance substances (as well as interfering substances) in a sample, several dilutions (from 1:10 to 1:1000, V/V) of the sample were analysed.

3.2.2 Shampoos, Creams, Lotions, Skin Tonics, Buffers and Roll On Products

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, prepared as described above, was quantitatively transferred on to the column. The initial 5 ml of the eluate was discarded. The eluate thereafter was 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 analysed within 24 h.

3.3 Analysis

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

Diluted perfumes were analysed only by the use of GC-MS. One point calibration, concentration 0.01% (w/v) of the target fragrance substances, was used for the quantification of fragrance substances in the perfumes. The concentration of target fragrance substances in the perfumes were calculated as % (w/v).

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 Descriptor:	m/z 50 - m/z 250 in 0.73 s
Library:	National Bureau of Standards

4 Results

The methods employed for the analysis of target fragrance substances in the N-cosmetics, sample preparation as well as analytical techniques GC-FID and GC-MS, were the same as reported earlier (6, 7). Thus, the identification of the target fragrance substances in sample extracts was performed employing both GC-FID and GC-MS, followed by the quantification of the fragrance substances employing GC-FID. Relatively simple GC-FID technique, however, was not suitable for the identification and hence for the quantification of target fragrance substances in perfumes because of interferences (by some other constituents of the respective products) as revealed by the GC-MS analyses of these products. Thus, for the identification of target fragrance substances with reasonable certainty (peak purity >800 and mass spectrum-match fit >900), serial dilutions of the perfumes in methanol were analysed. The dilution of a sample decreases the concentration of the target fragrance substance(s) as well as interfering substance(s), with the result that the GC-peaks of these substances are resolved (Figs. 1B-6B). However, in some cases, where the concentration of a constituent is much higher than the other, the dilution may result in undetectable amount (<1 ppm) of the substance with the lower concentration. Thus, in many cases geraniol could be identified but this substance could not be quantified. The dilution of a sample, in which a target fragrance was identified according to above mentioned criteria, was chosen for the quantification of the identified substance. Besides peak purity and spectrum-match fit, resolution of the GC-peak of the target fragrance substances from the interfering peaks was also considered for the quantification of these substances. Quantification was performed only for the reasonably resolved GC-peaks (visual observation, resolution factor not determined).

Detection limits of all of the target fragrance substances in the present study were approximately 1 ppm. The detection limits of the target fragrance substances in perfume products were though 10 ppm as these products had to be diluted, minimum 1:10, before analysis. The relative standard deviation of the determination of these substances was within 8%. To limit the load of work, quantification of target fragrance substances in perfumes has been performed employing one point calibration, concentration 0.01% (w/v) of the target fragrance substances.

The GC-MS and GC-FID chromatograms of some of the samples analysed are shown in Figs. 1-10. The contents of the target fragrance substances in the perfumes and other N-cosmetic products are reported in Tables 3 & 4. Thus, 1-7 of the target fragrance substances were found to be present in 20 of the 22 investigated perfume products. Coumarin (0.046-6.043%) was present in 11 of the products, α-hexyl cinnamic aldehyde (0.105-7.706%) in 9 products, eugenol (0.035-2.289%) and α-amyl cinnamic aldehyde (0.194-3.039%) each in 8 products and hydroxycitronellal (0.135-

6.044%) was present in 5 products (Table 2). Cinnamic alcohol and isoeugenol were found to be present respectively in 2 and 3 of the investigated perfumes. Geraniol was identified in 14 of the perfumes, but it was quantified only in one of the products. In the remaining 13 samples geraniol peak was not (properly) resolved from the large peak of linalool 2-aminobenzoate. Citral, citronellal, cinnamic aldehyde and dihydrocoumarin were not identified in any of the investigated perfume oils.

Among the other 20 N-cosmetic products, other than perfumes, 7 products were found to contain 1-3 of the target fragrance substances, concentration 0.0003-0.0820% (Table 3). Although geraniol (0.0163%) was determined in only one of the investigated products, it is not certain that other products did not contain geraniol. Many of these products were found to contain relatively high amounts of linalool 2-aminobenzoate, which interferes with the analysis of geraniol under the experimental conditions. Citral, citronellal, hydroxycitronellal and dihydrocoumarin were not found to be present in any of these 20 N-cosmetic products.

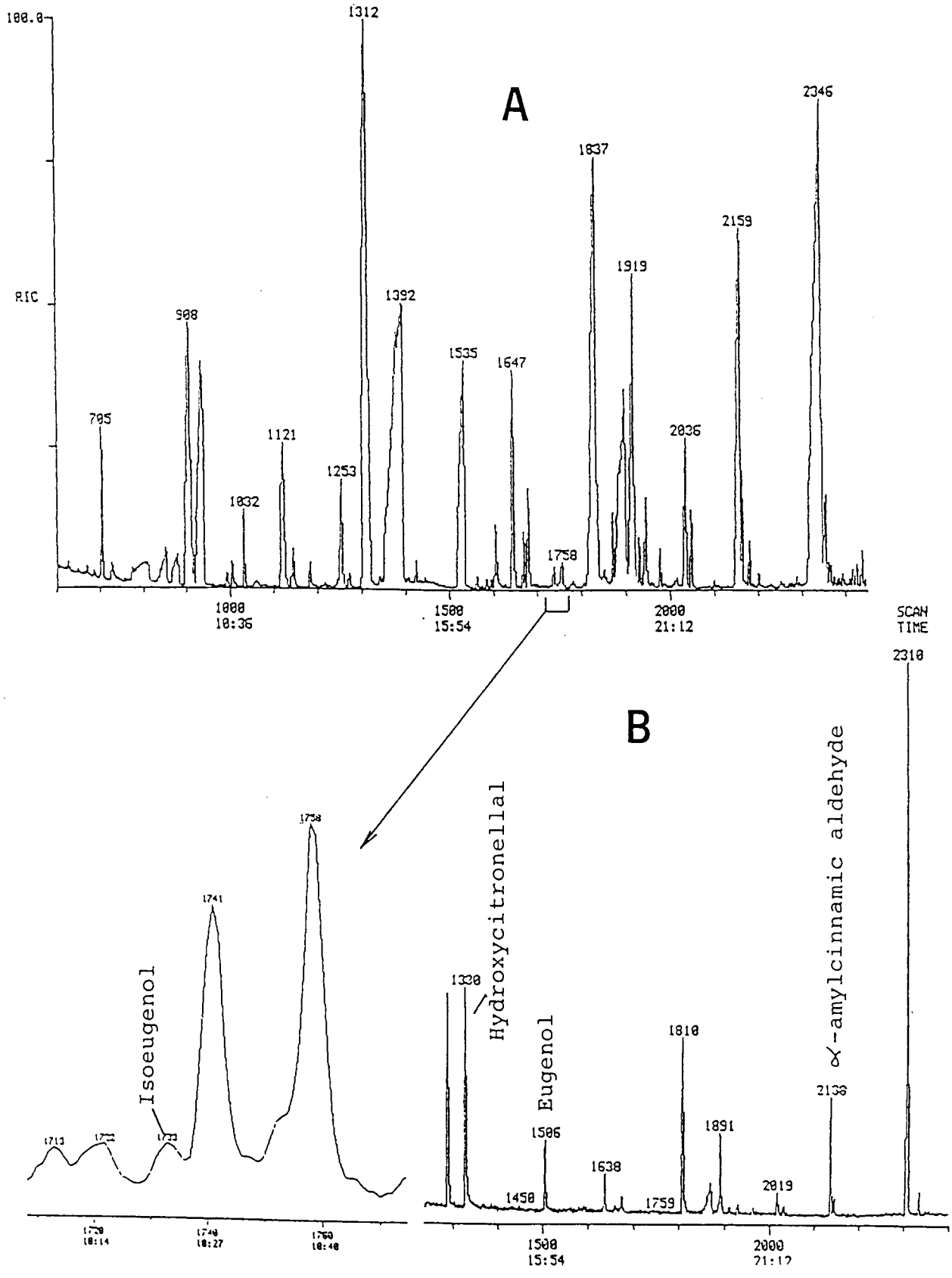

Figure 1: GC-MS analysis of sample no. 4-4062.
A - dilution 1:10, Scan 1319-1321 - geraniol.
B - dilution 1:500.

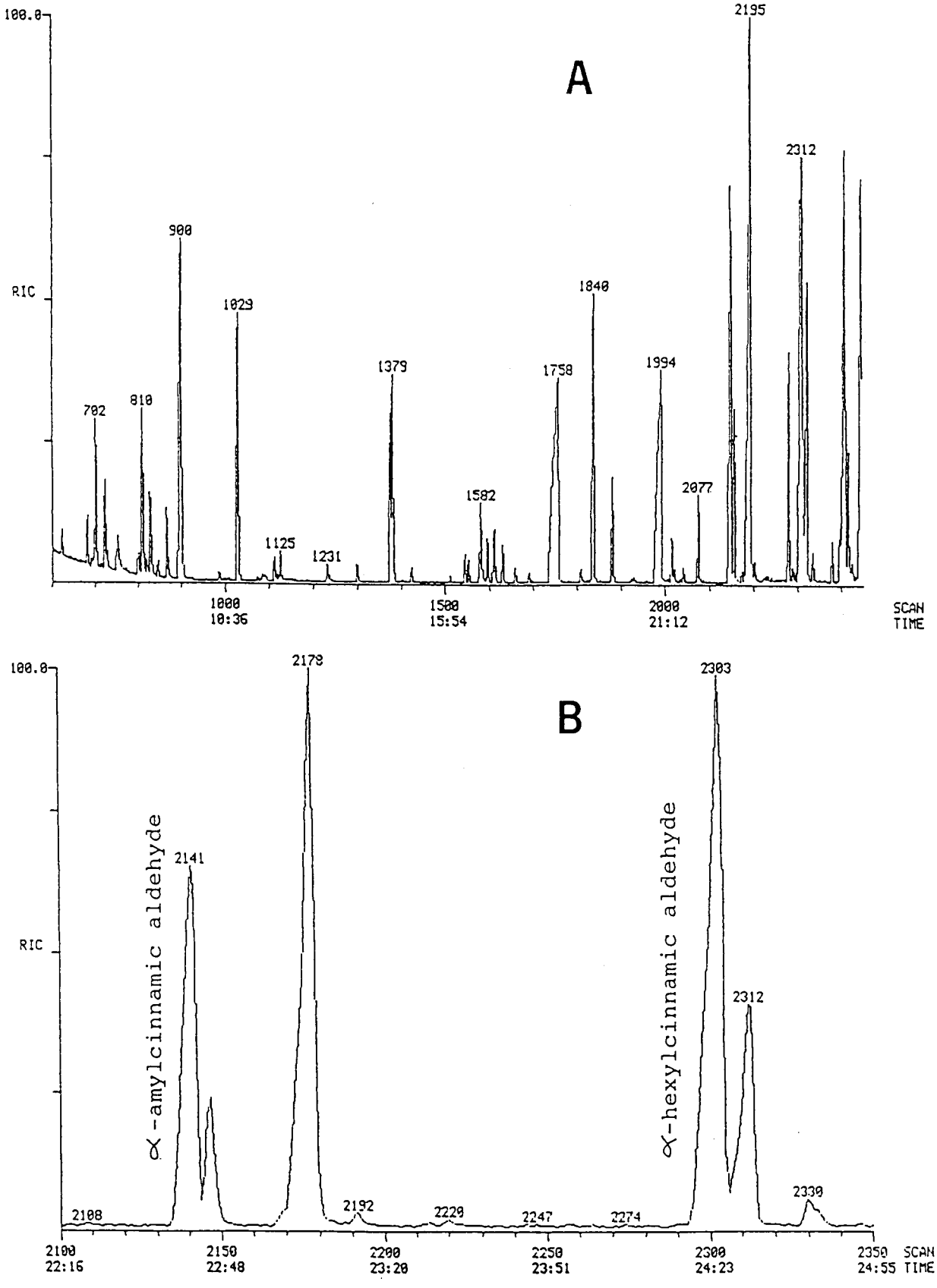

Figure 2: GC-MS analysis of sample no. 4-0464.
A - dilution 1:10, B - dilution 1:50.

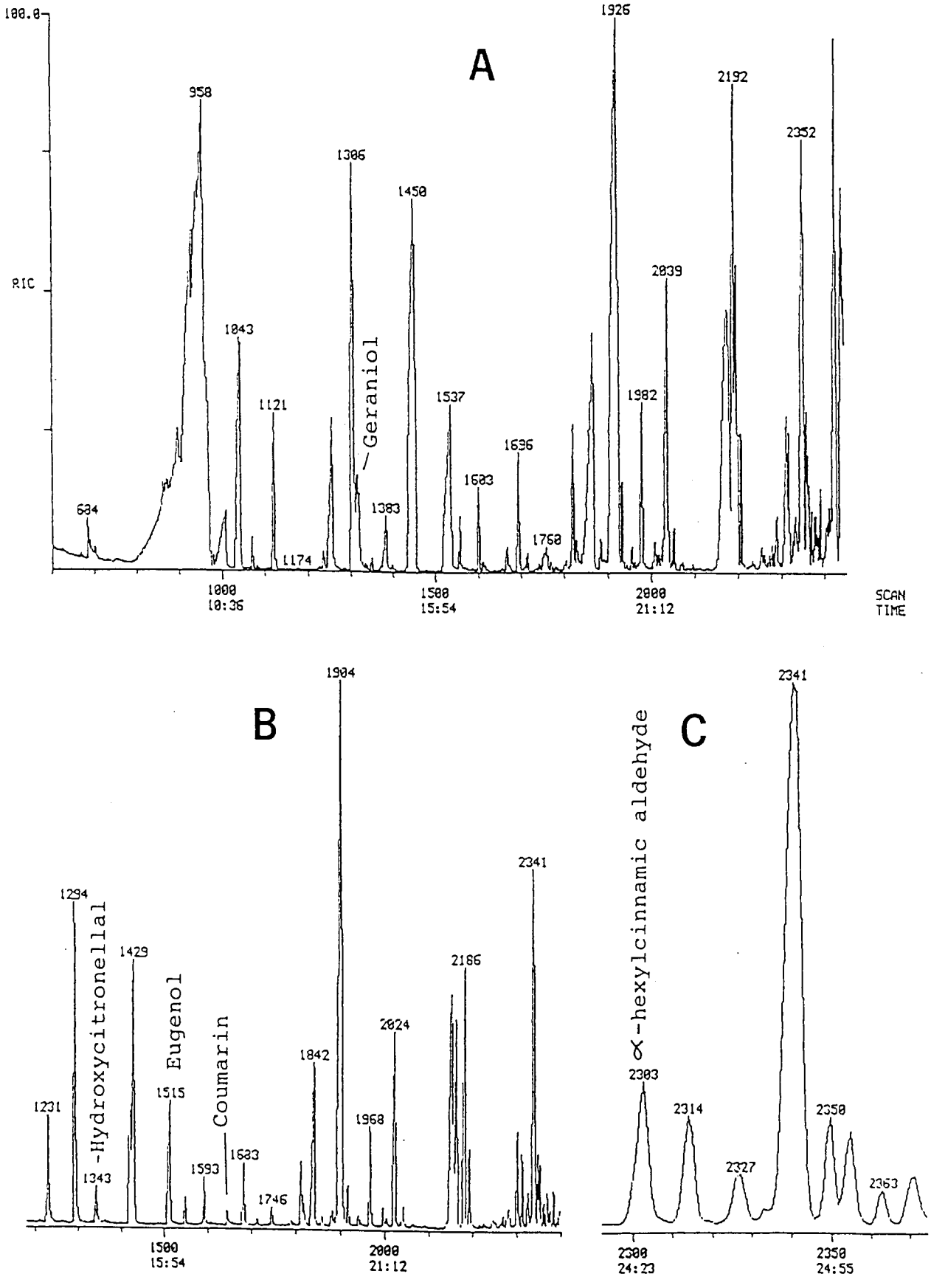

Figure 3: GC-MS analysis of sample no. 4-0465.
A - dilution 1:10, B & C - dilution 1:50.

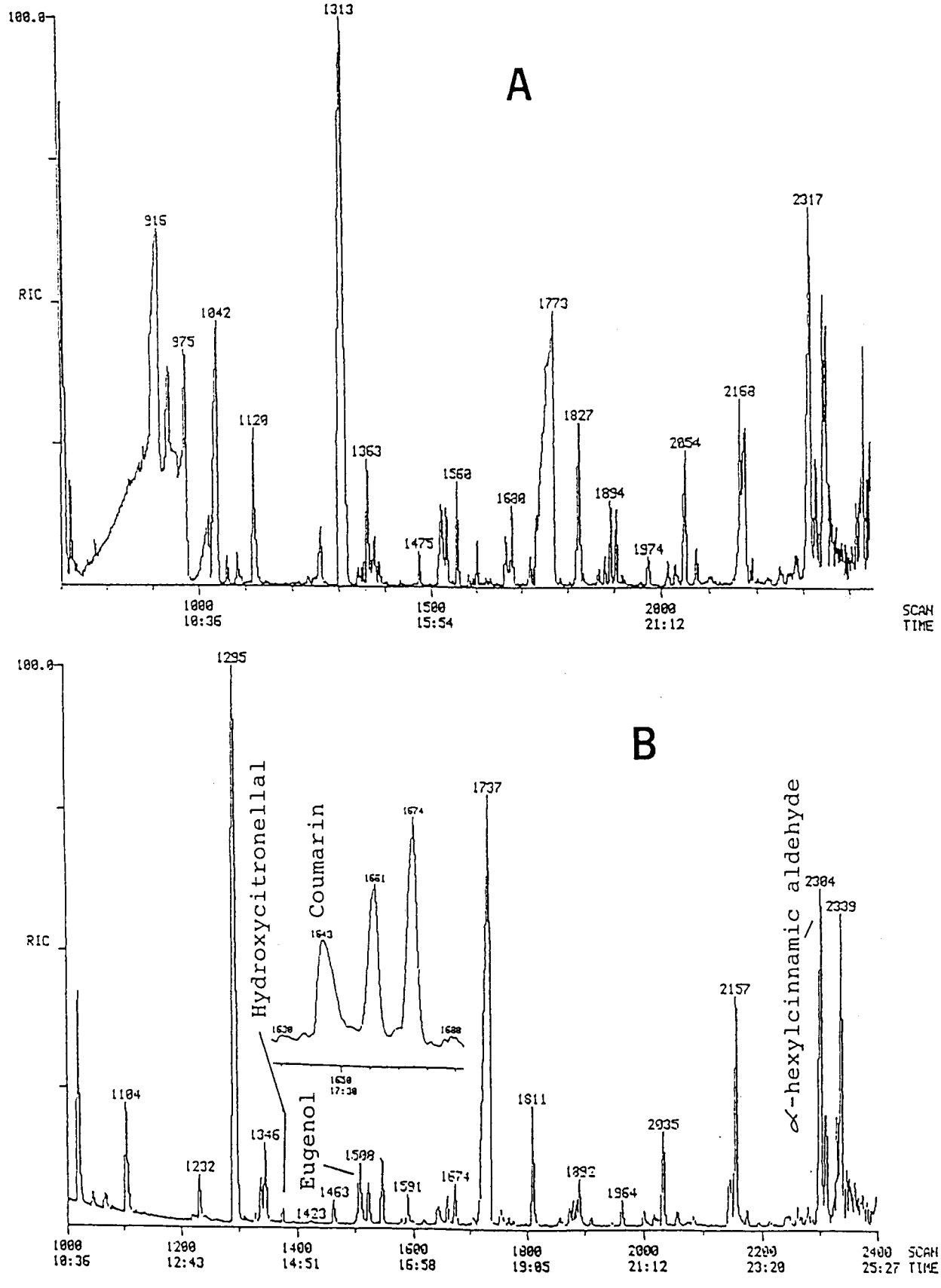

Figure 4: GC-MS analysis of sample no. 4-0472.
A - dilution 1:10, scan 1319-1322 - geraniol.
B - dilution 1:50.

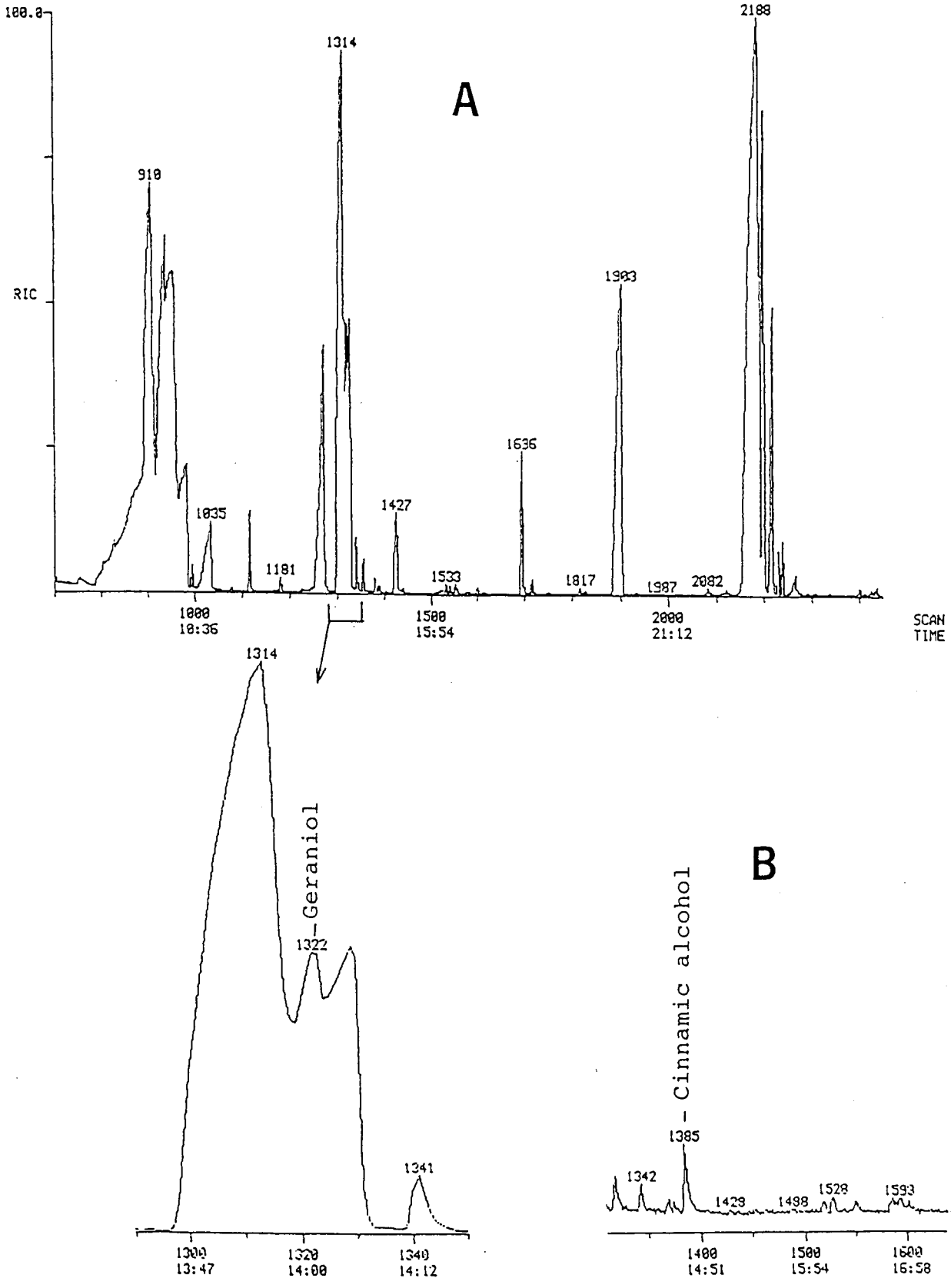

Figure 5: GC-MS analysis of sample no. 4-0473.
A - dilution 1:10, B -dilution 1:20

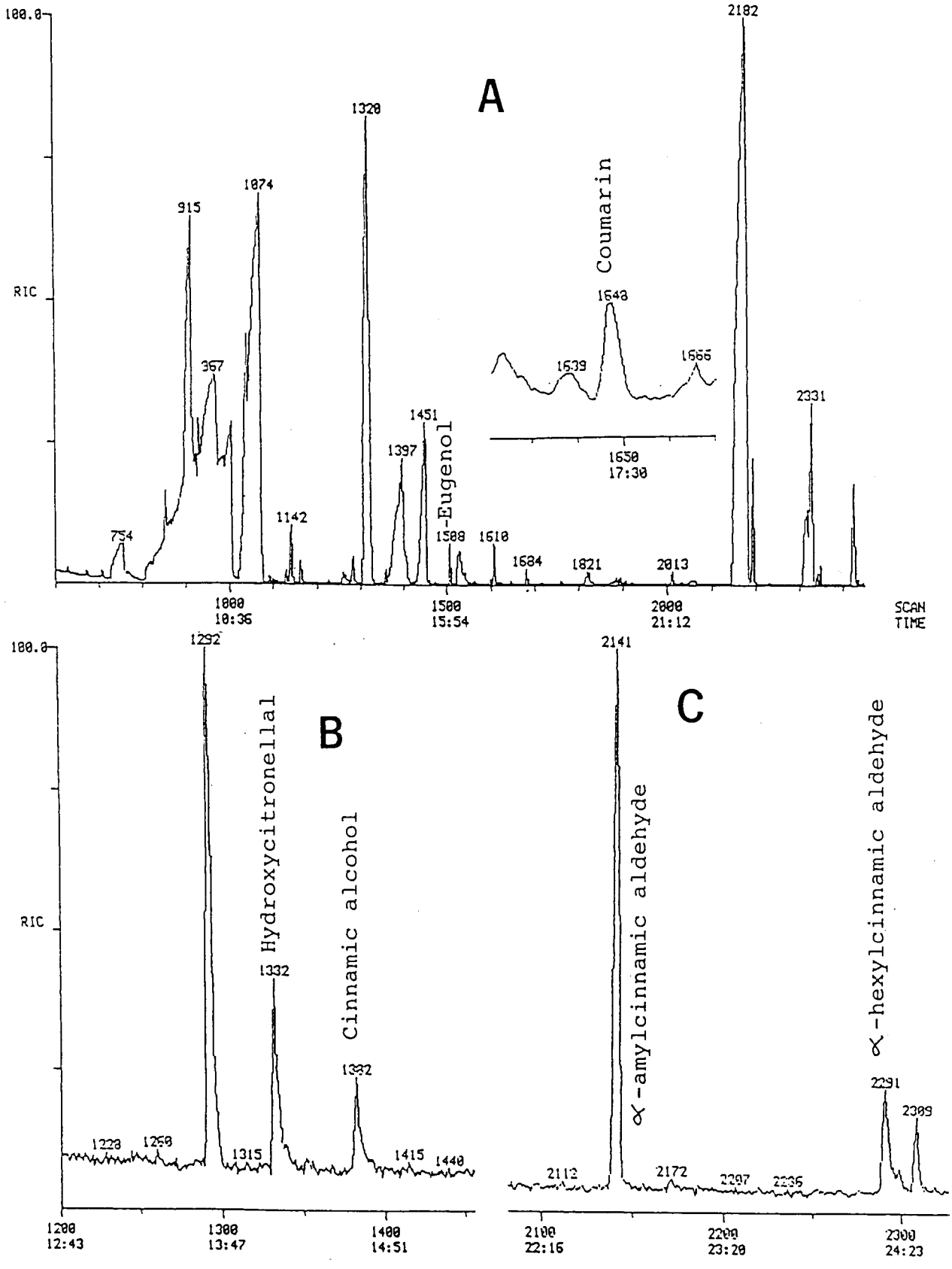

Figure 6: GC-MS analysis of sample no. 4-0476.
A - dilution 1: 10, scan 1322-1323 - geraniol.
B - dilution 1: 500, C dilution 1:1000.

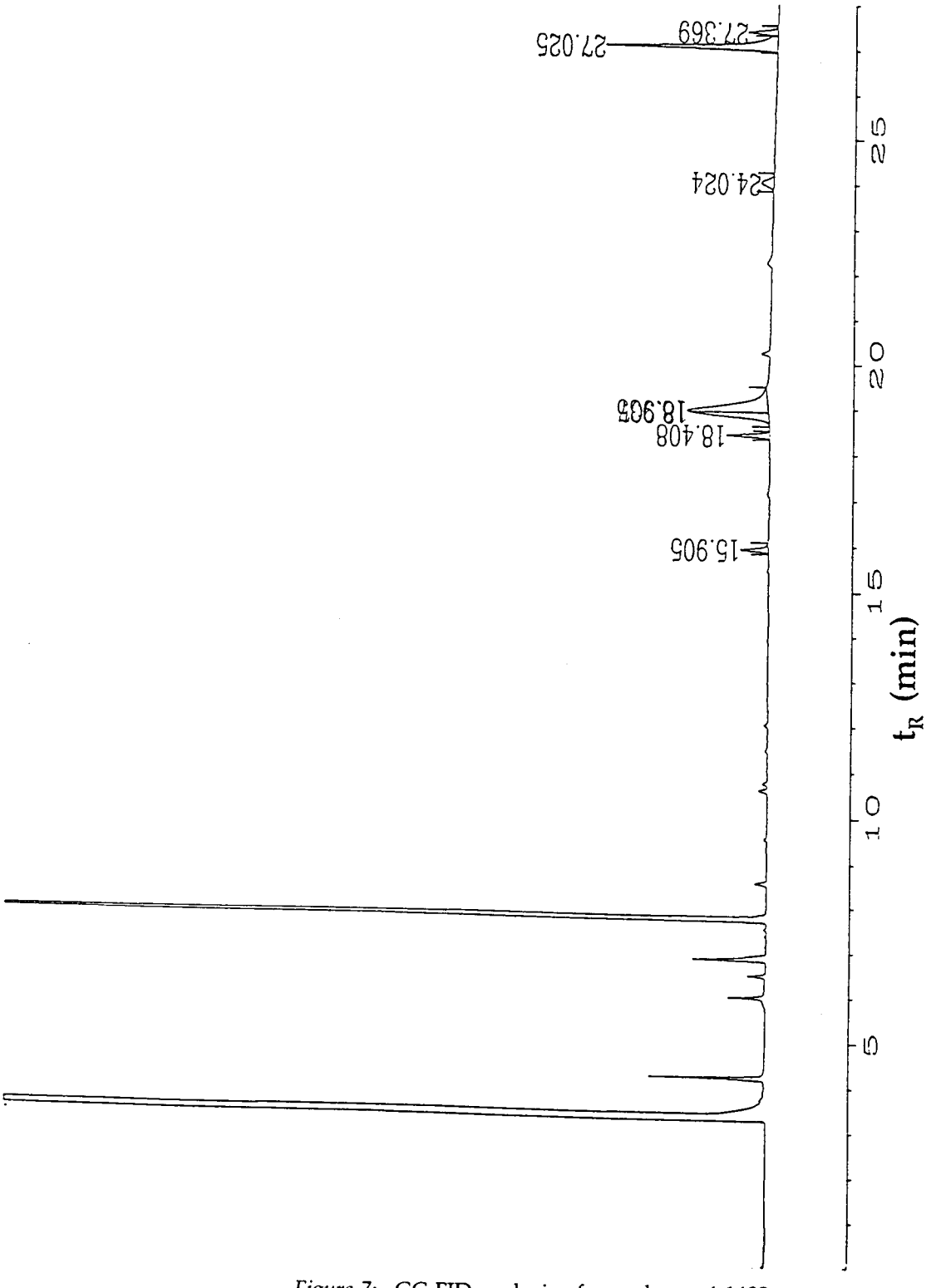

Figure 7: GC-FID analysis of sample no. 4-1408.
t_R 24.024 min - α-hexylcinnamic aldehyde.

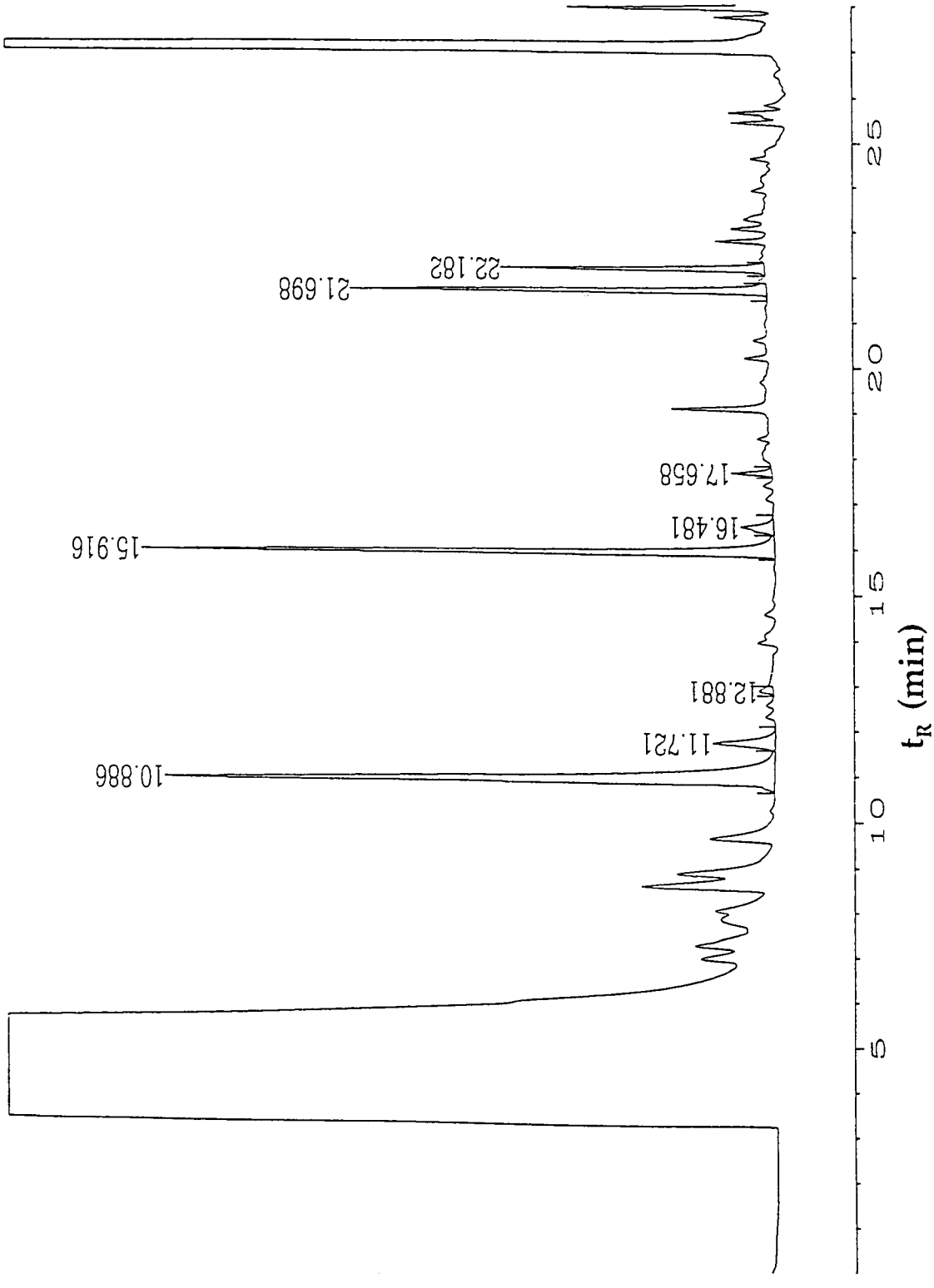

Figure 8: GC-FID analysis of sample no. 4-1415.
t_R 12.881 min - cinnamic alcohol, t_R - 16.481 min - isoeugenol,
t_R 21.698 min - α-amylcinnamic aldehyde.

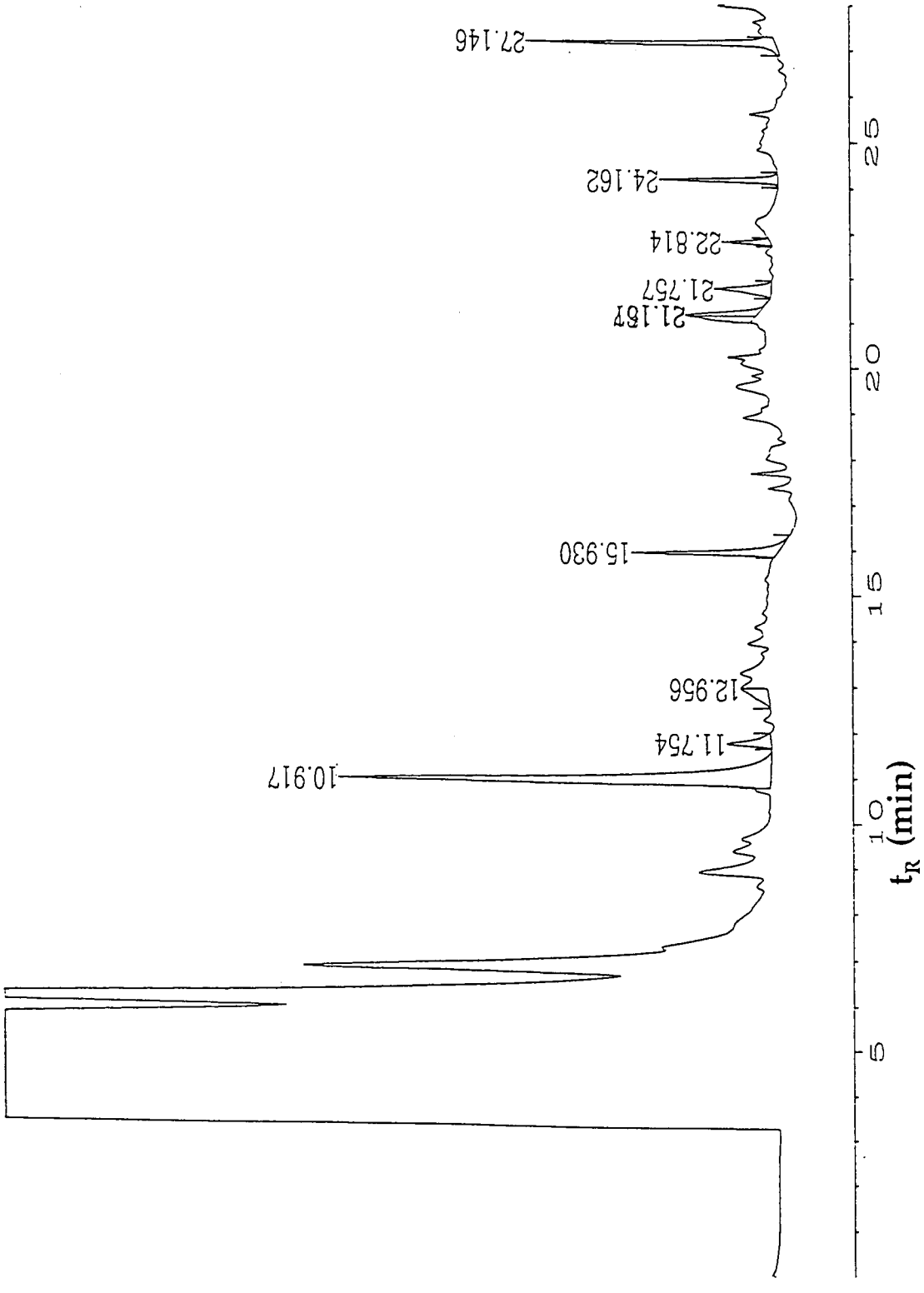

Figure 9: GC-MS analysis of sample no. 4-1418.
t_R 11.754 min - geraniol,
t_R 24.162 min α-hexylcinnamic aldehyde.

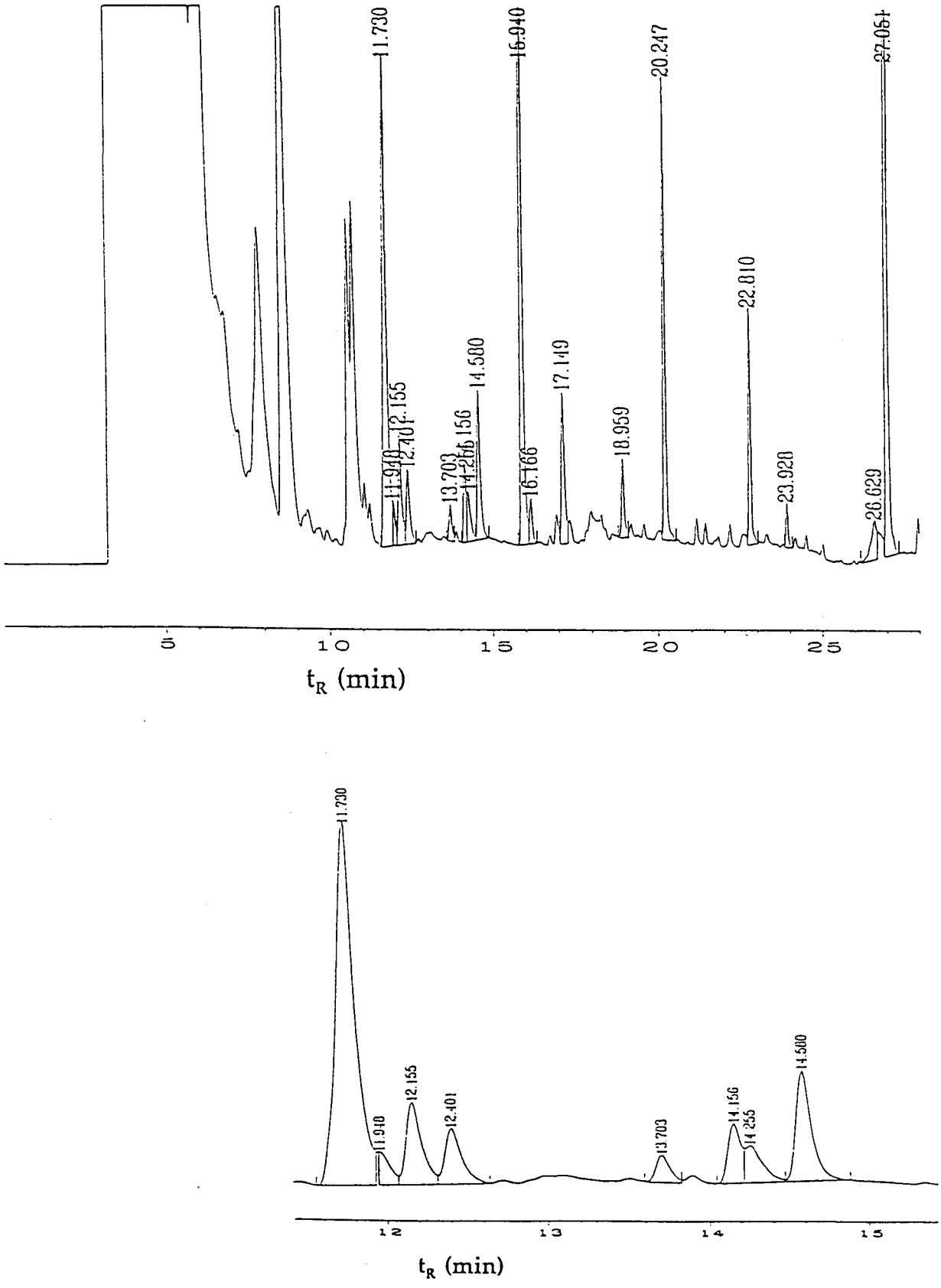

Figure 10: GC-FID chromatogram of sample no. 4-1422.
t_R 11.948 min: Cinnamic aldehyde, t_R 14.255 min: eugenol.

Table 3: Contents of fragrance substances in the perfumes.

NERI - Sample No.	Content of fragrance substances, % (w/v)							
	Geraniol	Hydroxy citronellal	Cinnamic alcohol	Eugenol	Coumarin	Isoeugenol	α-amylcinnamic aldehyde	α-hexylcinnamic aldehyde
4-0459	-	-	-	0.035	-	-	-	-
4-0460	*	-	-	-	-	-	+	+
4-0461	-	-	-	-	6.043	-	-	-
4-0462	*	6.044	-	2.006	-	0.027	2.741	-
4-0463	1.191	-	-	-	-	-	0.317	-
4-0464	-	-	-	-	-	-	2.606	3.153
4-0465	*	0.430	-	2.289	0.211	-	-	0.660
4-0466	*	-	-	-	0.192	0.139	0.289	0.105
4-0467	-	-	-	0.348	1.873	-	-	-
4-0468	*	-	-	-	-	-	-	2.196
4-0469	-	-	-	-	-	-	-	-

-: not detected, detection limit 10 ppm
+: substance present, could not be quantified due to interference
*: Quantification of geraniol was not possible due to overlapping by a large peak of linalool 2-aminobenzoate Citronellal, cinnamaldehyde, dihydrocoumarin and citral were not detected in any of the samples.

Table 3: Continued.

NERI - Sample No.	Content of fragrance substance, % (W/V)							
	Geraniol	Hydroxy citronellal	Cinnamic alcohol	Eugenol	Coumarin	Isoeugenol	α-amylcinnamic aldehyde	α-hexylcinnamic aldehyde
4-0470	-	-	-	-	-	-	-	-
4-0471	*	-	-	-	-	-	-	-
4-0472	*	0.451	-	0.821	0.315	-	-	3.039
4-0473	*	-	0.109	-	-	-	-	-
4-0474	*	0.135	0.089	0.041	0.112	-	0.194	7.706
4-0475	-	-	-	-	-	-	1.483	1.715
4-0476	*	1.158	2.101	0.169	0.155	-	3.039	1.015
4-0477	-	-	-	-	0.046	-	-	-
4-0478	*	-	-	0.293	0.754	-	-	-
4-0479	*	-	-	-	0.920	-	-	-
4-0480	*	-	-	-	1.178	-	-	-

-: not detected, detection limit 10 ppm
+: substance present, could not be quantified due to interference
*: Quantitation of geraniol was not possible due to overlapping by a large peak of linalool 2-aminobenzoate
Citronellal, cinnamaldehyde, dihydrocoumarin and citral were not detected in any of the samples.

Table 4: Contents of fragrance substances in the 20 N-cosmetic products, other than perfumes.

NERI - reg. nr.	Fragrance substance	Content % (w/w)
4-1408	α-hexylcinnamic aldehyde	0.0543
4-1410	Coumarin	0.0130
4-1411	α-hexylcinnamic aldehyde	0.0007
4-1412	Coumarin	0.0003
4-1415	Cinnamic alcohol Isoeugenol α-amylcinnamic aldehyde	0.0036 0.0127 0.0820
4-1418	Geraniol α-hexylcinnamic aldehyde	0.0163 0.0193
4-1422	Cinnamic aldehyde Eugenol	0.0745 0.0186

Geraniol identification in all the samples, except 4-1418, was not possible due to overlapping by a large peak of linalool 2-aminobenzoate.

5 Discussion

The target fragrance substances in the present investigation are the constituents of fragrance mix used for patch testing, some other well known fragrance allergens and chemically related compounds. The contents of target fragrance substances in perfumes based on natural ingredients were similar to the contents of target fragrance substances in popular perfumes (7). However, concentrations of some of the target fragrances in the perfumes based on natural ingredients were relatively high: 6.04% hydroxycitronellal, 6.04% coumarin, 3.04% α-amylcinnamic aldehyde and 2.29% cinnamic alcohol, in different products. Considering that the perfumes based on the natural ingredients are prepared from essential oils, they should not contain synthetic substances such as hydroxycitronellal and α-hexylcinnamic aldehyde. Moreover essential oils may not contain 6% coumarin or 3% α-amylcinnamic aldehyde. Thus, it may be argued that synthetic fragrance substances, including the constituents of fragrance mix, may be present in the perfumes based on natural ingredients.

Screening analysis of perfumes based on natural ingredients by GC-MS revealed that following non-target (fragrance)substances were frequently present in these products: various terpenes (carene, pinene, limonene, etc.), sesquiterpenes (caryophellene, thujopsene, etc.), citronellol, linalool, linalyl acetate, linalool 2-aminobenzoate, geranyl acetate, benzyl acetate, benzyl benzoate, piperonal, benzenemethanol, benzeneethanol, α-isomethyl ionone, cyclohexanol derivatives, dipropylene glycol, and diethyl phthalate. It should be noted that dipropylene glycol and diethyl phthalate are not natural substances.

As described above α-hexylcinnamic aldehyde has yet not been shown to be a natural substance. Thus, the presence of this substance in 3 of the 20 N-cosmetic products, other than perfumes, further support the suggestion that synthetic fragrance substances are used in the formulation of N-cosmetics. Additional support to this point of view may also come from the fact that the concentrations of some of the naturally occurring fragrance substances in the investigated products were rather high. For example, a body lotion was found to contain 0.082% α-amylcinnamic aldehyde. The body lotions may contain up to 0.5% perfume (11). Considering that the product contained 0.5% of an essential oil, then the concentration of α-amylcinnamic aldehyde in the essential oil should be 16% to achieve a final concentration of 0.08% in the product. Such a high concentration of α-amylcinnamic aldehyde is not known in any essential oil. Similarly 0.075% cinnamic aldehyde in a stress buffer product may indicate that synthetic cinnamic aldehyde was added to this preparation.

In a recent study, we have shown that the popular perfumes caused skin reactions in 6.9% fragrance mix positive eczema patients (9). As the contents of target fragrance substances in the perfumes based on natural ingredients were similar to those found in popular perfumes, it may be argued that the risk-factor for getting fragrance allergy by these two groups of products may be similar.

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