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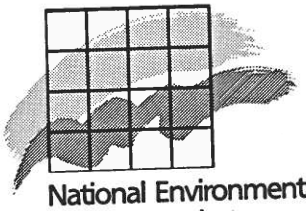

National Environmental
Research Institute

Chemical substances and chemical preparations

Chromatographic analysis of some fragrances in cosmetics and toiletries

NERI Technical Report No. 106

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Resumé

Parfumer kan fremkalde kontakt dermatitis i mennesker ved anvendelse af parfumeholdige kosmetika og toiletartikler. På nærværende tidspunkt er indholdet af parfumer i kosmetika og toilet artikler ikke reguleret. Herudover er oplysninger, vedrørende parfumestoffer som fremkalder kontakt dermatitis ved anvendelse af parfumeholdige produkter, sparsomme. Der ønkedes en oversigt over inholdet af parfumestoffer i kosmetika og toiletartikler, der anvendes af en større del af befolkningen. I øjeblikket er der ingen metode til rutineanalyser af parfumer i kosmetika og toiletartikler. 10 parfumestoffer der er kendt for at fremkalde kontakt dermatitis samt et beslægtet kemisk stof blev udvalgt til udvikling af en analysemetode.

I nærværende arbejde er en gaskromatografisk (GC) og en GC-massespektrometrisk (GC-MS) metode udviklet til analyser af 11 parfumestoffer: kanelalkohol, kanelaldehyd, eugenol, hydroxycitronellal, α-amylkanelaldehyd, geraniol, isoeugenol, coumarin, dihydrocoumarin, citral og citronellal. Prøver uden compleks matrix, for eksempel eau de toilette blev analyseret ved GC uden nogen prøvetilberedning. Til GC analyse blev parfumestoffer fra sæbe og vaskepulver ekstraheret i ethylacetat ved at omryste en vandig opløsning af prøven med opløsningsmidlet. Parfumer fra andre prøver blev ekstraheret ved oprensning en methanolisk opløsning/suspension af prøverne ved silicagel søjlekromatografi. Genfindingen af parfumestoffer fra shampooer, cremer, lotion, læbstift, ansigtspudder, aftershave, deodorant spray, eau de toilette og sæbe fandtes til 80% - 116%. Repeterbarhed (r) af analysemetoden til bestemmelse af parfumestoffer i produkter var indenfor 5%. Kalibreringskurver af alle undersøgte stoffer er lineære i det undersøgte koncentrationsområde: 0,005% - 0,50%. Det blev også påvist at opløsninger af parfumestoffer var stabile i op til 3 dage. Ved nærværende metode kunne enklte parfume stoffer i koncentrationen 10-25 ppm i et produkt detekteres. Men kvantitativ bestemmelse af parfumestoffer i et produkt var kun muligt når disses koncentrationer var ≥0,01% (100 ppm).

Metoderne der er udviklet i nærværende arbejde blev anvendt til analyser af de 11 parfume stoffer i 32 tilfældigt valgte prøver af kosmetika og toiletartikler. Da der ikke blev konstateret problemer ved ovenævnte analyser, blev det konkluderet at metoderne beskrevet i nærværende rapport kan anvendes til rutineanalyser af parfumestoffer i kosmetika og toiletartikler. Herudover er det blevet påvist at metoderne kan også anvendes til analyser af visse parfumestoffer i opvaskmidler og vaskepulver.

Arbejdet er udført som bistandsopgave til Miljøstyrelsen.

Summary

Perfumes (fragrances) are one of the major causes of allergic contact dermatitis in humans due to use of cosmetics and toiletries. At present there is no official regulation of the perfumes cosmetics and toiletries. There is only sparse information concerning fragrance substances which cause allergic contact dermatitis, due to the presence of these substances in cosmetics and toiletries. Therefore, a survey of content of fragrances in commonly used cosmetics and toiletries was required. However, no analytical methods were available for a routine analysis of fragrances in cosmetics and toiletries. 10 fragrance substances which are known to cause allergic contact dermatitis and a chemically related fragrance substance were selected for the developement of an analytical method.

In the present work, a gas chromatographic (GC) and a GC-mass spectrometric (GC-MS) method are developed for the analysis of the selected 11 fragrance substances: cinnamyl alcohol, cinnamyl aldehyde, eugenol, hydroxy citronellal, α-amyl cinnamaldehyde, geraniol, isoeugenol, coumarin, dihydrocoumarin, citral, and citronellal. Samples without complex matrix, for example, eau de toilette were analysed directly by GC without pretreatment. For GC analysis, fragrances from soap and powder detergent were extracted in ethyl acetate by shaking an aqueous sample solution with the organic solvent. Perfumes from other samples were extracted by silica gel column chromatography of the methanolic solution/suspenion of the samples. The recovery of fragrances from shampoos, lotions, creams, deodorant sprays, eau de toilette, aftershave, face powder, lipstick and soap were found to be 80% - 116%. The repeatabilities (r) of the quantitation of fragrances by the present method were within 5%. Calibration curves of the fragrances analysed by GC were found to be linear in the investigated concentration range 0.005% - 0.50%. Fragrance solutions were found to be stable up to 3 days only. With the present method, it was possible to detect 10 - 25 ppm of a single fragrance substance present in a product. However, quantitation was only possible when a fragrance substance was present in concentration ≥0.01% (100 ppm) in a product.

The methods developed have been applied for the analysis of fragrances in 32 randomly selected cosmetics and toiletries samples. Since no problems were encounterd during the analysis of above mentioned products, it was concluded that the methods described in the present report can be used for the routine analysis of fragrances in cosmetics and toiletries. Furthermore, it is demonstrated that the methods described can also be applied for the analysis of some selected fragrances in dishwasher and laundry products.

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

1 Introduction

The use of fragrances (perfumes) in cosmetics and toiletries is associated with pleasure and some times with therapeutics. Some fragrance substances, for example, citral and cinnamaldehyde are also used as flavouring agents in food. Various household products, for example, laundry products, dishwasher products, all-purpose cleaners, air freshners, and WC cleaners are also perfumed. Perfumes, in general, are not active ingradients of consumer products, but their use in consumer products is to give consumers good feelings and/or to mask odour(s) from other ingradient(s) in a product.

To date, several thousands of perfumes are known. All known perfumes are organic compounds or mixtures of organic compounds, derived from natural sources or produced synthetically. Not all perfumes can be used in all types of products, because the perfume used in the formulation should not interfere with the functional activity of a product and it should also be stable in the product. Recently described guidelines for perfuming various types of consumer products (1, 2) indicate that these products may contain from a single fragrance up to a mixture of 50-60 (or even more) fragrance substances in various concentrations. The recommended concentration of perfumes in certain types of consumer products (1, 2) is described in Table 1.

Perfumes are considered to be low toxic substances. However, acute toxicity tests have revealed that certain perfumes may be moderately toxic at high concentrations (3). Subchronic and chronic neurotoxicity and teratogenicity of certain fragrance substances have also been described. For example, acetyl ethyl tetramethyl tetralin (4) and musk ambrette (5) have been shown to be neurotoxic, and citral to be teratogenic (6, 7). The well established adverse health effects of perfume exposure are skin sensitization and allergic contact dermatitis including photosensitization and phototoxicity (3, 8-15). Thus, several cases of skin sensitization due to the use of perfumed cosmetics and toiletries have been reported in the past 25 years (16-40). Allergic contact dermatitis caused by a mixture of fragrances may some times be due to a reaction product(s) of some substances in the mixture - *compound allergy* (41). Skin sensitization property of some perfumes may be inhibited in the presence of some other fragrance substances (42) - the so called *quenching* phenomenon.

Perfumes get into the environment primarily by evaporation and from 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.

Perfumes are considered to be one of the major causes of allergic.

Table 1: Recommeded content of perfumes in consumer products (1,2).

Product type	Recommended perfume content, % (w/w)
Toilet waters and colognes	0.5 - 30.0
Bath oils	3.0 - 5.0
Soaps	0.5 - 2.0
Luxury soaps	up to 5.0
Shower and bubble bath products	0.5 - 4.0
Liquid and dry shampoos	0.5 - 1.0
Hair conditioners	0.3 - 0.5
Hair sprays	0.1 - 0.5
Hair lotions	0.2 - 0.4
Deplitaroies	up to 1.0
Facial make-up and powders	0.5 - 1.0
Lip care products	up to 1.0
Talc	0.2 - 1.0
Eye make-up	minimum
Cream and body lotions	0.3 - 0.5
Deodorants and antiperspirants	1.0 - 2.0
Powders and sprays for pretreatment of laundry	0.2 and above
Powdered heavy-duty detergents	0.1 - 0.4
Light-duty detergents	0.08 - 0.2
Liquid detergents	up to 1.0
Special detergents for coloured textiles	0.3 - 0.4
Fabric softners	0.25 - 0.5 (and above)
Dishwasher products	0.1 - 0.5
All-purpose cleaner	0.1 - 0.3
Air freshners	0.5 - 2.0
WC cleaners	up to 5.0
Urinal stones	up to 1.0

contact dermatitis as a result of the use of cosmetics and toiletries (33, 35, 40). Unlike many other allergic substances (preservatives, colours, UV-filters etc.), content of perfumes in cosmetics and toiletries is not regulated by the Danish regulations on cosmetics (43). According to the last ammendment (44) of European Union's Cosmetic Directive 76/768/EEC (45), perfume containing cosmetic products should be labelled "contains perfumes", but listing of perfume ingredients on the product is not required. Thus, in no country is there any requirement for approval for either ingradients 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 ingradients on consumer products. However, perfume industries are self-regulating the use of perfumes in consumer products, including cosmetics and toiletries. This is carried out by joint efforts of the two international organizations of perfume industries: the International Fragrance Association (IFRA) and the Research Institute of Fragrance Materials (RIFM). On the basis of toxicological research on more than 1300 fragrance substances, IFRA has produced a list of restricted fragrance substances which is described in Table 2 (3, 46). Eventhough industries' organizations have been functioning for 27 years, perfume allergy still prevails.

The reason for lack of regulation on perfumes may be that there are too many (>5000) of them, and reliable toxicological data on many of the the fragrance substances is lacking. The situation is complicated by the fact that many perfumes are mixtures of several single fragrance substances, and they may produce compound allergy. For an optimal regulation of perfumes, systematic investigations are needed to unreveal 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 presence of these substances in the respective products. The data compiled on single substances, thus, can be used for the regulation of perfumes. Continuing efforts in this direction may lead to a complete database on production, use and toxic effects of perfumes. A step in this direction has been taken by Danish Environmental Protection Agency (DEPA) in cooperation with Copenhagen County Hospital at Gentofte and National Environmental Research Institute (NERI). NERI's contribution in the project will be to provide the data on the content of some selected fragrance substances in consumer products, specifically in cosmetics and toiletries, and in dishwasher and laundry products.

An analytical method for the identification and quantitation of the selected 11 fragrance substances (Table 3) in consumer products was needed. The analytical method required should be sensitive for identification and determination of fragrances, in the concentration range that are used in the formulation of various types of cosmetics and toiletries, and it should be suitable for the routine analysis of fragrances. Although gas chromatography with flame ionization detection (GC-FID) and GC-mass spectrometry (GC-MS) have occasionally been used for the identification of

Table 2. Fragrance materials restricted by IFRA (28)

Substance	*Recommendation*[a]	*Reason*
Acetylethyltetramethyltetralin (AETT)	Prohibited	Neurotoxicity
5-Acetyl-1,1,2,3,3,6-hexamethylindane	Restricted	Phototoxicity
Acetyl isovaleryl	Prohibited	Sensitization
Acetylated vetiver oil	Preparation	Sensitization by some samples
Alantroot oil (Elecampane oil)	Prohibited	Sensitization
Allyl esters	Restricted	Irritation
Allyl heptine carbonate	Restricted	Sensitization
Amylcyclopentenone (2-Pentyl-2-cyclopenten-1-one)	Restricted	Sensitization
Angelica root oil	Restricted	Phototoxicity
Anisylidene acetone (4-(*p*-Methoxyphenyl)-3-buten-2-one)	Prohibited	Sensitization
Benzylidene acetone (4-Phenyl-3-buten-2-one)	Prohibited	Sensitization
Bergamot oil	Restricted	Phototoxicity
Bitter orange oil expressed	Restricted	Phototoxicity
p-tert.-Butylphenol	Prohibited	Sensitization and depigmentation
Carvone oxide	Quenching[c]	Sensitization
Cassia oil	Restricted	Sensitization
Cinnamic alcohol	Restricted	Sensitization
Cinnamic aldehyde	Quenching[c]	Sensitization
Cinnamic aldehyde–methyl anthranilate Schiff base	Quenching[c]	Sensitization
Cinnamon bark oil, Ceylon	Restricted	Sensitization
Citral	Quenching[c]	Sensitization
Costus root oil, absolute and concrete from *Saussurea lappa* Clarke	Prohibited	Sensitization
Cumin oil	Restricted	Phototoxicity
Cyclamen alcohol (3-(4-Isopropylphenyl)-2-methylpropanol)	Prohibited[b]	Sensitization
Diethyl maleate	Prohibited	Sensitization
Dihydrocoumarin	Prohibited	Sensitization
2,4-Dihydroxy-3-methylbenzaldehyde	Prohibited	Sensitization
4,6-Dimethyl-8-*tert.*-butylcoumarin	Prohibited	Photosensitization
Dimethyl citraconate	Prohibited	Sensitization
Ethyl acrylate	Prohibited	Sensitization
Ethyl heptine carbonate	Restricted	Sensitization
Farnesol	Specifications	Sensitization by impurities
Fig leaf absolute	Prohibited	Phototoxicity and Sensitization
trans-2-Heptenal	Prohibited	Sensitization
Hexahydrocoumarin	Prohibited	Sensitization
trans-2-Hexenal	Restricted	Sensitization
trans-2-Hexenal diethyl acetal	Prohibited	Sensitization
trans-2-Hexenal dimethyl acetal	Prohibited	Sensitization
α-Hexylidene cyclopentanone	Restricted	Sensitization
Hydroabietyl alcohol	Prohibited	Sensitization
Hydroquinone monoethyl ether	Prohibited	Depigmentation
Hydroquinone monomethyl ether	Prohibited	Depigmentation
Hydroxycitronellal	Restricted	Sensitization

Table 2. Continued.

Substance	*Recommendation*[a]	*Reason*
Isoeugenol	Restricted	Sensitization
6-Isopropyl-2-decalol	Prohibited	Sensitization
Lemon oil cold pressed	Restricted	Phototoxicity
Lime oil expressed	Restricted	Phototoxicity
Marigold oil and absolute (Tagetes oil and absolute)	Restricted	Phototoxicity
Menthadienyl-7-methyl formate	Restricted	Sensitization
7-Methoxycoumarin	Prohibited	Photosensitization and sensitization
α-Methylanisylidene acetone (1-(4-Methoxyphenyl-1-penten-3-one)	Prohibited	Sensitization
6-Methylcoumarin	Prohibited	Photosensitization
7-Methylcoumarin	Prohibited	Photosensitization
Methyl crotonate	Prohibited	Sensitization
4-Methyl-7-ethoxycoumarin	Prohibited	Photosensitization
6-Methyl-3,5-heptadienone	Restricted	Sensitization
Methyl heptine carbonate	Restricted	Sensitization
p-Methylhydrocinnamic aldehyde	Restricted	Sensitization
Methyl *N*-methylanthranilate (Dimethylanthranilate)	Restricted	Phototoxicity
3-Methyl-2(3)-nonene nitrile	Restricted	Sensitization
Methyl octine carbonate	Restricted	Sensitization
Musk ambrette	Prohibited[b]	Neurotoxicity and photosensitization
Nitrobenzene	Prohibited	Acute toxicity
Nootkatone	Specifications	Sensitization by impurities
Oakmoss absolute and resinoid (concrete)	Restricted	Sensitization
1-Octen-3-yl acetate (Amylvinylcarbinyl acetate)	Restricted	Sensitization
Opoponax	Preparations	Sensitization by some samples
Oils from Pinacea family	Specifications	
Pentylidene cyclohexanone	Prohibited	Sensitization
Perilla aldehyde	Restricted	Sensitization
Peru balsam (exudation from *Myroxylon pereirae* (Royle) Klotzsh	Prohibited	Sensitization
Phenylacetaldehyde	Quenching[c]	Sensitization
Propylidene phthalide	Restricted	Sensitization
Pseudoionone (2,6-Dimethylundeca-2,6,8-trien-10-one)	Prohibited[b]	Sensitization
Pseudomethylionones	Prohibited[b]	Sensitization
Rue oil	Restricted	Phototoxicity
Safrole, isosafrole and dihydrosafrole	Prohibited[b]	Chronic toxicity
Savin oil	Specifications	Sensitization by some samples
Sclareol	Specifications	Sensitization by some samples
Styrax American and Asian	Preparation	Sensitization by some samples
Verbena absolute (*Lippia citriodora*)	Restricted	Sensitization
Verbena oil (*Lippia citriodora*)	Prohibited	Phototoxicity and sensitization

[a] For more information such as the reasons for the recommendations, the limits of restrictions, etc., see the IFRA Code of Practice.[1]
[b] Special exemptions exist.
[c] Quenching means the substance should only be used with a quenching agent.

perfumes, no method is available for the sample preparation prior to GC analysis of perfumes in consumer products. Moreover, no single GC method is described, so far, for the analysis of the selected fragrance substances. In the present work, an analytical method is developed for the sample preparation followed by GC-FID and GC-MS analyses of fragrance substances in cosmetics and toiletries. As the knowledge of perfumes used in laundry and dishwasher products was also required, the applicability of the analytical methods was evaluated for the analysis of fragrances in such products as well.

The work has been carried out as a technical support to DEPA.

2 Target Fragrance Substances

The choice of fragrance substances to be analysed was based on the results of studies that have been performed to evaluate contact sensitization potential of various fragrance raw materials (8-10, 47, 48), and also on the basis of the results of some important experimental research and epidemiological studies demonstrating presensence of perfume allergens in cosmetics (13, 16, 19, 32, 33, 37, 49-52). Only fragrance substances that have been described repeatedly to cause contact sensitization in humans have been selected for the development of analytical method in the present work. Perfumes from natural sources which contain several fragrances, for example, Balsam of Peru, oak moss, etc., were ignored, because it is not practical to determine contents of these substances in consumer products. No criteria for the environmental pollution or ecotoxicology were taken into account, while selecting the perfume ingradients for the present study. All in all, 11 fragrance substances (Table 3) were chosen for the present study. The first 7 substances (Table 3): cinnamyl alcohol, cinnamaldehyde, eugenol, hydroxy citronellal, α-amyl cinnamaldehyde, geraniol and isoeugenol are part of European Standard Fragrance Mixture that is used for the detection of skin sensitization by perfumes in humans. Besides these 7 substances, European Standard Fragrance Mixture also contains oak moss. It is considered that testing with European Standard Fragrance Mixture may reveal 70-80% cases of perfume allergy in humans (34). Three other fragrance substances substances included in the present study: coumarin, dihydrocoumarin and citral have also been shown to cause skin sensitization (17, 19, 20, 24, 51, 53). Citronellal was included because its chemical structure is related to hydroxycitronellal, citral and geraniol.

Table 3: Target fragrance substances for the developement of an analytical method.

Fragrance substance	Synonym	CAS registry no.
Cinnamyl alcohol	3-phenyl-2-propen-1-ol	104-54-1
Cinnamaldehyde (trans)	3-phenyl-2-propenal	14371-10-9
Eugenol	2-methoxy-4-(2-propenyl) phenol	97-53-0
Hydroxy citronellal	3,7-dimethyl-7-hydroxy-6-octenal	107-75-5
α-Amyl cinnamaldehyde	2-phenylmethylene heptanal	122-40-7
Geraniol	3,7-dimethyl-2,6-octadien-1-ol	106-24-1
Isoeugenol	2-methoxy-4-(1-propenyl) phenol	97-54-1
Coumarin	2H-1-benzopyran-2-one	91-64-5
Dihydrocoumarin	3,4-dihydro 2H-1-benzo-2-pyranone	119-84-6
Citral (cis- and trans-, geranial and neral)	3,7-dimethyl-2,6-octadienal	5392-40-5
Citronellal	3,7-dimethyl-6-octenal	106-23-0

3 Samples

To develop an analytical method for fragrances in cosmetics and toiletries, 18 randomly selected products were used (Table 4). A laundry product and a dishwasher product was also used for the method developement (Table 4). Following method developement, analysis of selected fragrances was performed in 32 products (Table 5).

Table 4. Products used for the developement of a method for the analysis of fragrances.

Sample No.	Product category
1-0355	Shampoo and balsam 2:1
1-0385	Body cream
1-0402	Shampoo
2-0013	Deodorant spray
2-0435	Skin lotion
2-0555	Baby shampoo, without perfume
2-0834	Handcream
2-0874	Skin lotion, without perfume
2-0900	Night cream
2-0948	Cream deodorant
2-0969	Hand cream
2-0971	Shampoo
2-0976	Shampoo
2-1010	Lipstick
2-1013	Face powder
3-1103	Bar-soap
3-1192	Dishwasher
3-1193	Powder (granular) detergent for woolen laundry
3-1716	Eau de toilette
3-1717	Eau de toilette

Table 5. Products analysed for fragrance content.

Sample No.	Product category
1-0367	Moisturising lotion
1-0378	Shampoo for coloured hair
1-0402	Hair shampoo
1-0410	Dusch gel
1-0415	Saloon shampoo and balsam 2 i 1
1-0416	Body lotion
1-0440	Body shampoo
1-0484	Dusch gel
1-0486	Hair balsam
1-0516	Shower gel
1-0519	Cream bath gel
1-0851	Hair shampoo
1-0904	Body lotion
1-0905	Body lotion
1-0948	Cream deodorant
1-0957	Face cleanser
2-0011	Deodorant spray (aerosol)
2-0013	Deodorant spray (aerosol)
3-1167	Shampoo and dusch
3-1168	Cream shampoo
3-1185	Day cream
3-1187	Day cream
3-1192	Dishwasher
3-1409	Hair shampoo
3-1711	Aftershave
3-1712	Aftershave
3-1713	Deodorant spray (pump spray)
3-1714	Eau de toilette
3-1715	Eau de toilette
3-1716	Eau du toilette
3-1717	Eau du toilette
3-1718	Eau du toilette

4 Experimental

4.1 Materials

4.1.1 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 in GC and HP 3396 integrator 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 0.12 µm., from Chrompack, The Netherlands (Cat. No. 7770).

4.1.2 Glasware

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

4.1.3 Chemicals

Eugenol 99%, isoeugenol 98%, geraniol 98%, dihydrocoumarin 99%, cinnamyl alcohol 98%, α-amyl cinnamaldehyde 97% and citral (mixture of cis and trans isomers, geranial and neral) 95% were from Aldrich, Germany; cinnamaldehyde (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 analytical grade were from E. Merck, Germany. All the chemical were used as obtained.

4.1.4 Reference solutions

10% (w/v) stock solutions of all the fragrances were prepared in methanol. The solutions were stored at 4°C for maxium 3 days. Calibration standards 0.005%, 0.01%, 0.02%, 0.05%, 0.10% and 0.50% 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.

4.2 Sample Preparation

4.2.1 Sample Preparation without Internal Standard

4.2.1.1 Eau de Toilette, Aftershave and Deodorant Sprays

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

Deodorant spray products in aerosol cans were taken out of the cans as described before (54). 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.

4.2.1.2 Shampoos, Creams, Lotions, Lipstick and Face powder
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 (15 min for lipstick). The solution/homogeneous suspension thus obtained was immedetiately cooled to room temperature (20°C). The fragrance substances from the solution/suspension were extracted as described below.

A 20 cm x 1.8 (i.d.) cm 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. First 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 analysed within 24 h.

4.2.1.3 Bar-soap, Dishwaher and Powder detergent
Dishwasher sample for the fragrance analysis was treated exactly as shampoos, lotions etc. (4.2.1.2).

The soap sample was scraped to thin flakes. Approximately 1 g of the flakes were accurately weighed in a 50 ml conical flask. The sample was suspended in 10 ml distilled water and dissolved by heating for 5 min at 60°C. The solution was quickly cooled to room temperature. 10.00 ml ethyl acetate was added and the mixture was vigrously shaked for 2 min. The aqueous and organic phases were allowed to separate. The organic phase was centrifuged for 5 min at 3500 rpm. The clear organic phase was transferred into autosampler vials and analysed within 24 h.

Powder detergent was treated as the bar-soap.

4.2.2 Sample preparation with Internal Standard

GC-screening analyses of fragrance substances present in the diluted samples/sample extracts were performed (see 4.3 Analysis). One of the target fragrance substance, which was not present in a sample, was used as an internal standard. The concentration of internal standard in diluted samples/sample extract was kept at 0.02%. Appropriate amount of internal standard was mixed with the diluted sample/sample extract, before making up to the final volume.

4.3 Analysis

A mixture of fragrances containing all target fragrance substances at concentration 0.05% was analysed 10 times by GC-FID to check the repeatability of the GC-method (4.3.1). Calibration standards 0.005% - 0.50% were analysed by GC-FID to prepare calibration curves.

To determine recovery of the fragrance substances from various types of cosmetics and toiletries, samples described in Table 4 were spiked with target fragrance substances to concentration 0.45%. Spiked and non-spiked samples were treated as described above (4.1.1) and analysed by GC-FID.

For the determination of the fragrance substances in cosmetics and toiletries (Table 5), first a qualitative analysis of the diluted samples/sample extracts was performed by GC-FID. Thereafter, the confirmation of the GC-FID identified substances was performed by GC-MS (4.3.2) screening of the samples/sample extracts. Quantitative analysis of the identified substances was performed by GC-FID. All the samples were analysed in duplicate.

4.3.1 Conditions for GC-FID

GC column:	CP-Sil 5CB 50 m x 0.32 mm (i.d.)
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

4.3.2 Conditions for GC-MS

GC as described in 4.3.1

MS

Interface:	Direct to ionsource, 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

5 Results

A number of preliminary GC-experiments, employing 0.10% of fragrance solutions, were performed to establish optimal conditions for GC analysis of target fragrance substances. By the GC method thus established (4.3.1), all of the target substances including the isomers of citral were resolved from each other (Fig. 1). The GC method was then applied to establish optimal conditions for the analysis of target fragrance substances by GC-MS. A reconstituted ion chromatogram of target fragrance substances under the optimal GC-MS conditions (4.3.2) is shown in Fig. 2. The detection limits of target fragrance substances by the GC-method were 2-5 ppm, and the detection limits of all the target fragrance substances by GC-MS method were < 1 ppm.

Retention times (t_R) and relative t_R (relative to t_R of citronellal) of the investigated fragrance substances under the optimal GC conditions are described in Table 6. Coefficients of variation (C_v) of the GC-relative t_R of all the fragrance substances except cinnamyl alcohol, over a period of 3 weeks, were found to be ≤ 0.1% (Table 6). The C_v of relative t_R of cinnamyl alcohol was 0.43%.

Tabel 6. GC retention times (t_R), relative t_R of and coefiicients of variation of the fragrance substances.

Fragrance substance	t_R (min) mean ± s.d.	Realtive t_R reltive to citronellal	% C_v
Cinnamyl alcohol	11.094 ± 0.048	1.356	0.430
Cinnamaldehyde (trans)	10.454 ± 0.008	1.278	0.077
Eugenol	12.365 ± 0.013	1.511	0.104
Hydroxy citronellal	10.544 ± 0.010	1.289	0.095
α-Amyl cinnamaldehyde	19.292 ± 0.013	2.358	0.065
Geraniol	10.037 ± 0.009	1.227	0.090
Isoeugenol	14.473 ± 0.011	1.769	0.073
Coumarin	14.283 ± 0.015	1.746	0.105
Dihydrocoumarin	13.918 ± 0.012	1.701	0.088
Citral (trans)	9.849 ± 0.010	1.204	0.101
Citronellal	8.182 ± 0.008	1.000	0.095

Figure 1. Chromatogram of a mixture of fragrances (0.02%) analysed by GC-FID. Peak no. 1-citronellal, 2-citral (trans), 3-geraniol, 4-cinnamaldehyde (trans), 5-hydroxy citronellal, 6 - citral (cis), 7-cinnamyl alcohol, 8-eugenol, 9-dihydro-coumarin, 10-coumarin, 11-isoeugenol, 12-α-amyl cinnamaldehyde. The major peak is the solvent peak and the other peaks represent impurities in the reference fragrances.

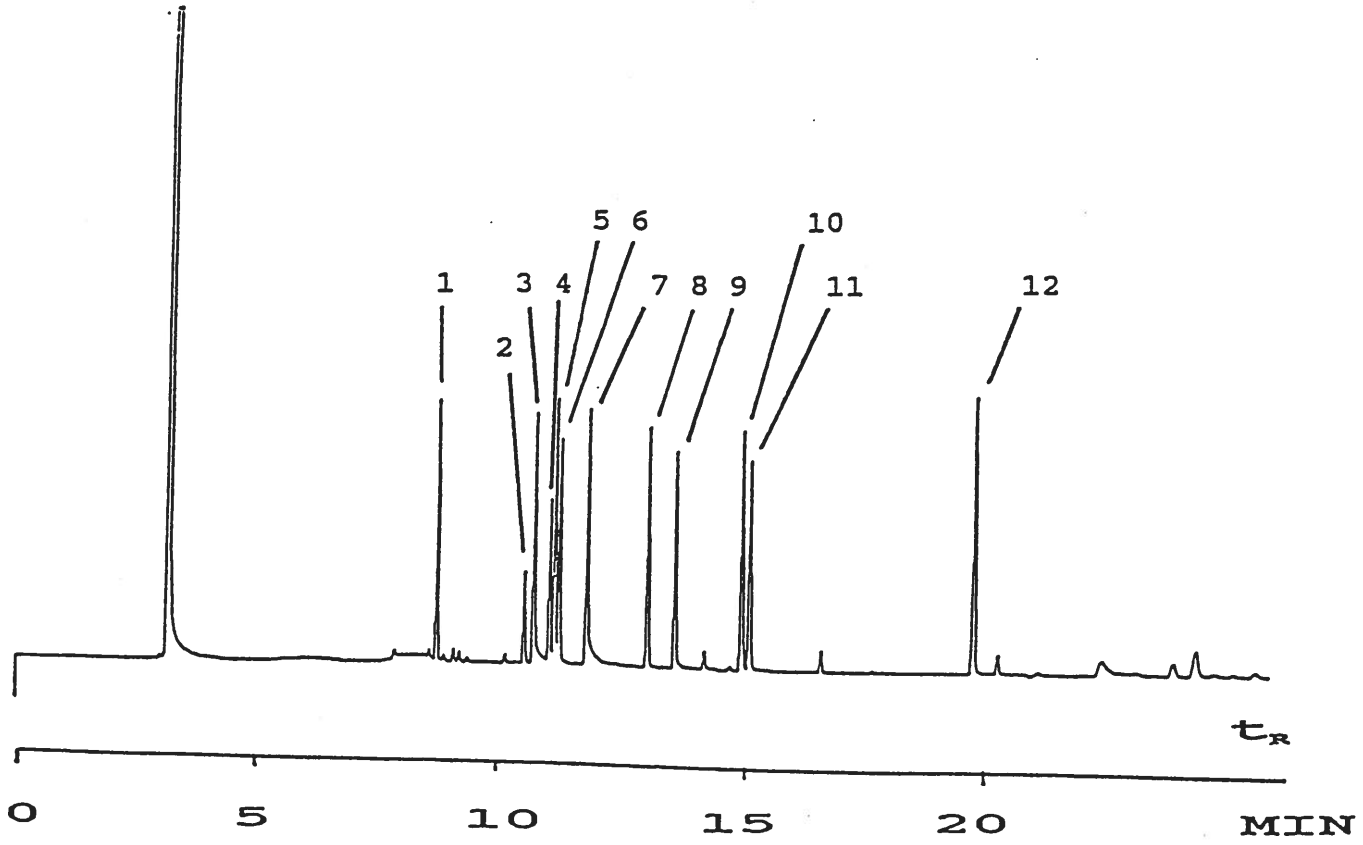
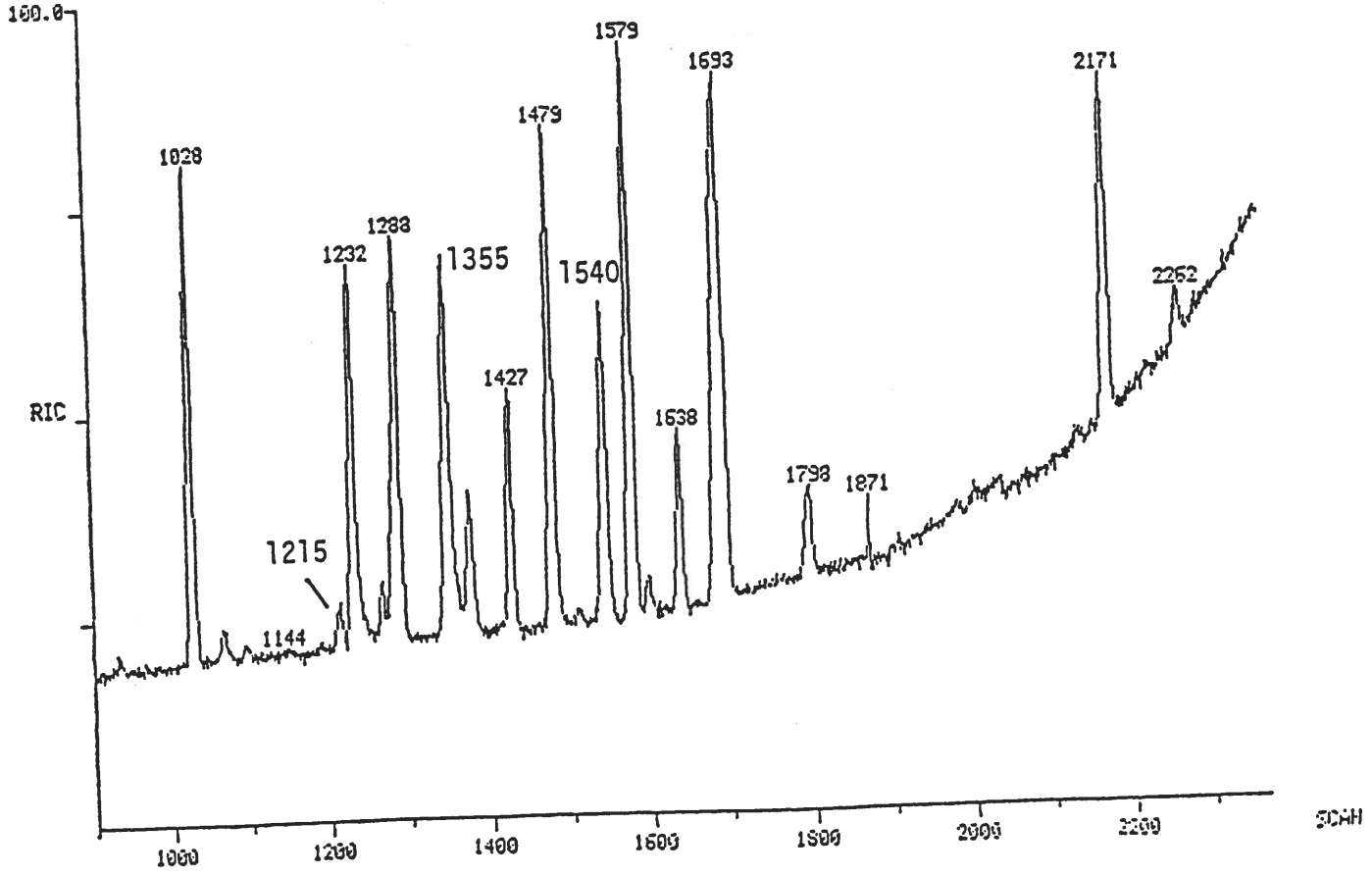


Figure 2. Reconstituted ion chromatogram (RIC) of the standard fragrances (0.02%) analysed by GC-MS. Scan no. 1028-citronellal, 1215-citral (trans), 1232-geraniol, 1288-cinnamaldehyde (trans), 1355-hydroxycitronellal, 1427-citral (cis), 1479-cinnamyl alcohol, 1540-Eugenol, 1579-dihydro-coumarin, 1693-isoeugenol + coumarin (1688), and 2171-α-amyl cinnamaldehyde. Other peaks represent impurities in the reference fragrances.



Replicate analyses of the mixture of fragrances, containing 0.05% of each fragrance, revealed that the repeatability (r) of the GC-FID method to be within 5% for all the investigated fragrances, except for geraniol and cinnamyl alcohol. The repeatability for geraniol and cinnamyl alcohol analysis by GC-FID were found to be 7% and 11% respectively.

GC was also employed to test the stability of the fragrance solutions. A mixture containing 0.02% of all the fragrance substances, prepared from freshly prepared stock solutions (10%) of the fragrances, was transferred into several GC-vials. The capped GC-vials were stored at 4°C and GC-FID analysis was performed on days 1, 2, 3 and 7. It was revealed that concentration of all fragrance substances, except α-amyl cinnamaldehyde, in the vials reduced constantly. In one week the concentrations of fragrances were reduced to 65-75%. The diluted solutions of α-amyl cinnamaldehyde were rather stable, variation was <4%, which is within the repeatability of the method. The concentration of fragrances in stock solutions stored at 4°C were found to be stable for 3 days, α-amyl cinnamaldehyde stock solution was stable up to one week. On the fourth day, the concentrations of fragrances in stock solutions were reduced by 10-20%. The stability of the mixture of diluted fragrace solutions was also tested after storing them at -18°C. It was found that the diluted solutions of fragrances (concentration 0.02%) in closed GC-vials stored at -18°C were stable up to 3 days, variation less than 5%.

Freshly prepared calibration standards were analysed by GC-FID for the preparation calibration curves. Calibration curves for all the investigated fragrances were linear in the concentration range 0.005% - 0.50%. Calibration curves of the fragrances in concentration range 0.005-0.1% are shown in Figs. 3A-3K. The correlation coefficients (r^2) for all of the calibration curves were ≥0.995 (Table 7).

To establish optimal condtions for sample preparation, preliminary experiments were performed employing 2 shampoos, 1 skin lotion and 1 cream sample. Attempts to extract fragrance substances in an organic solvent (ethyl acetate, isooctane) from these products or from the aqueous suspensions of the products failed. Alcoholic suspensions/solutions of the products after centrifugation or filteration through membrane filters did not result in clear solutions for GC analysis. Threfore, it was considered to purify fragrances from the samples by column chromatography employing alumina/silica gel as adsorbent. Use of alumina as stationary phase for column chromatography did not result in purification of fragrances from alcoholic suspensions/solutions of the cosmetics, because virtually nothing was retained by the column. Silica gel on the other hand was found to be suitable for the purification of fragrances from alcoholic solutions/suspensions of the shampoos and lotions. The conditions for the purification of fragrances from shampoos, lotion and cream samples were optimized by changing following parameters: alcohol type (ethanol/methanol), amount of sample (1g/2g), volume of alcohol used for suspending the sample (5 ml/10ml), time of heating of the sample suspension

Figure 3A

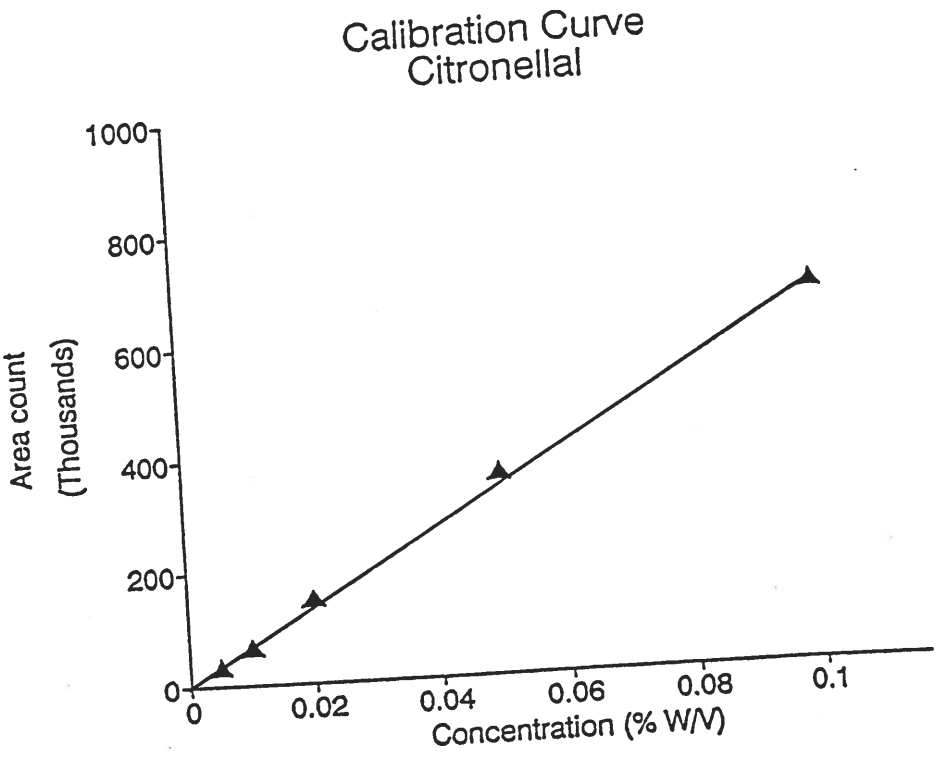


Figure 3B

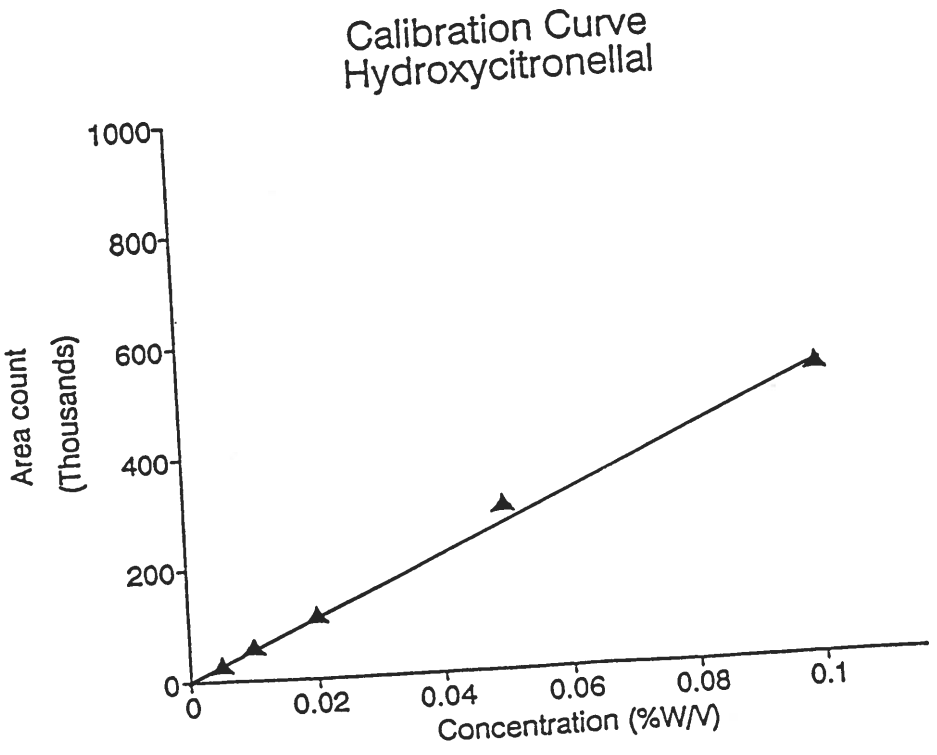


Figure 3C

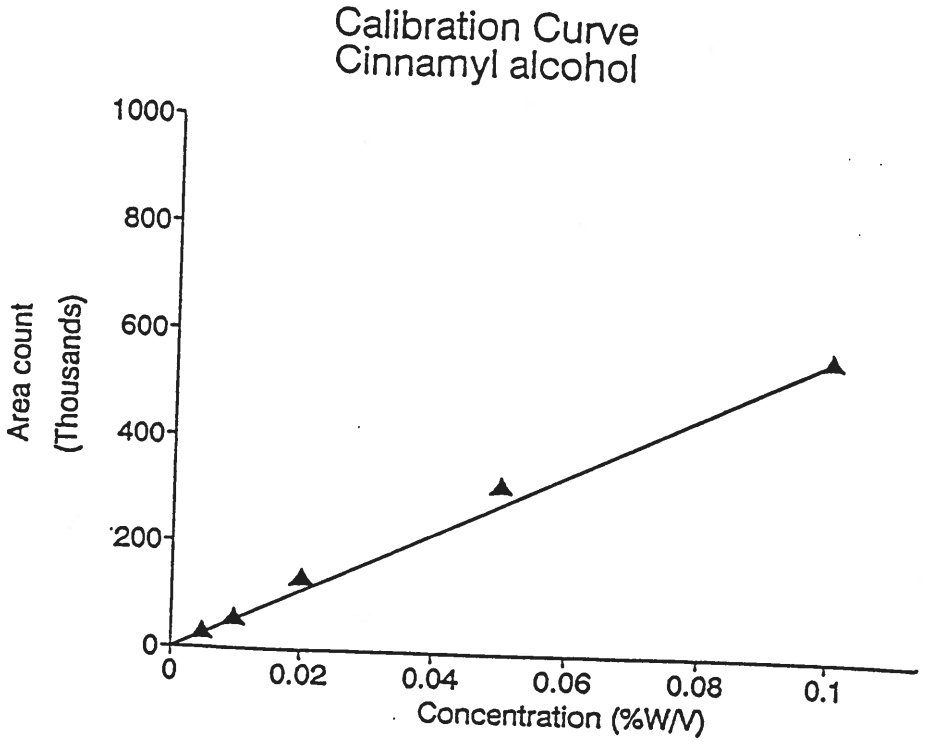


Figure 3D

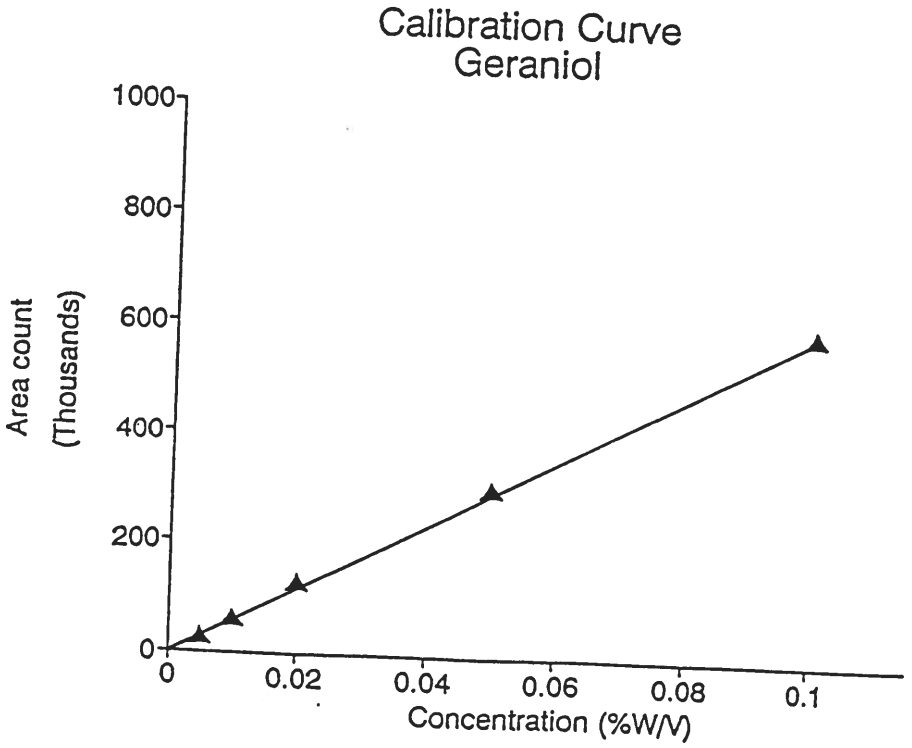


Figure 3E

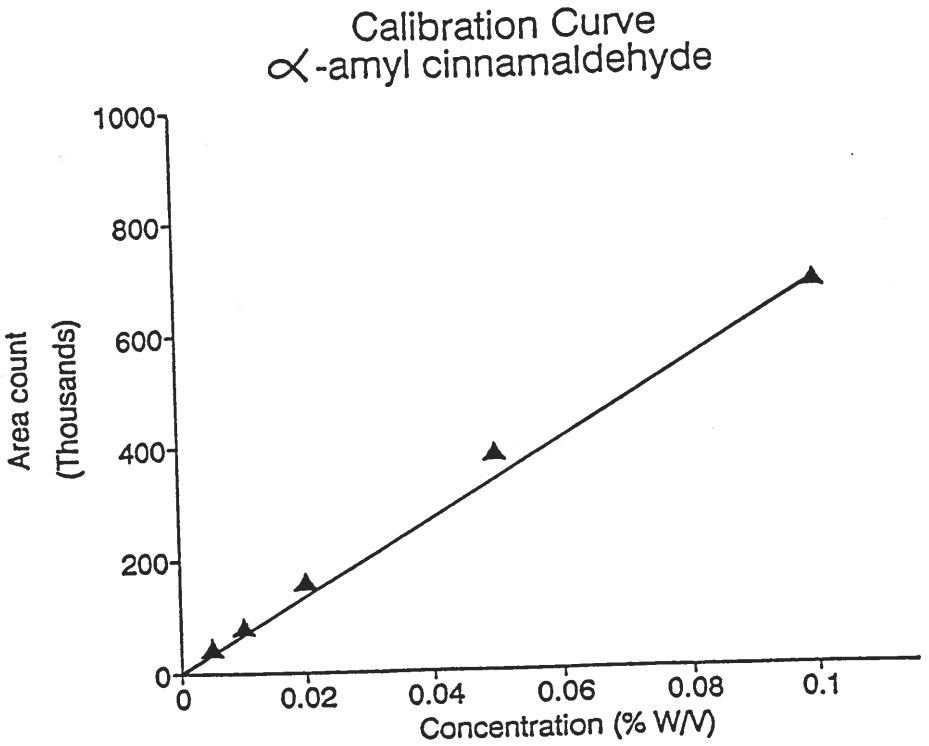


Figure 3F

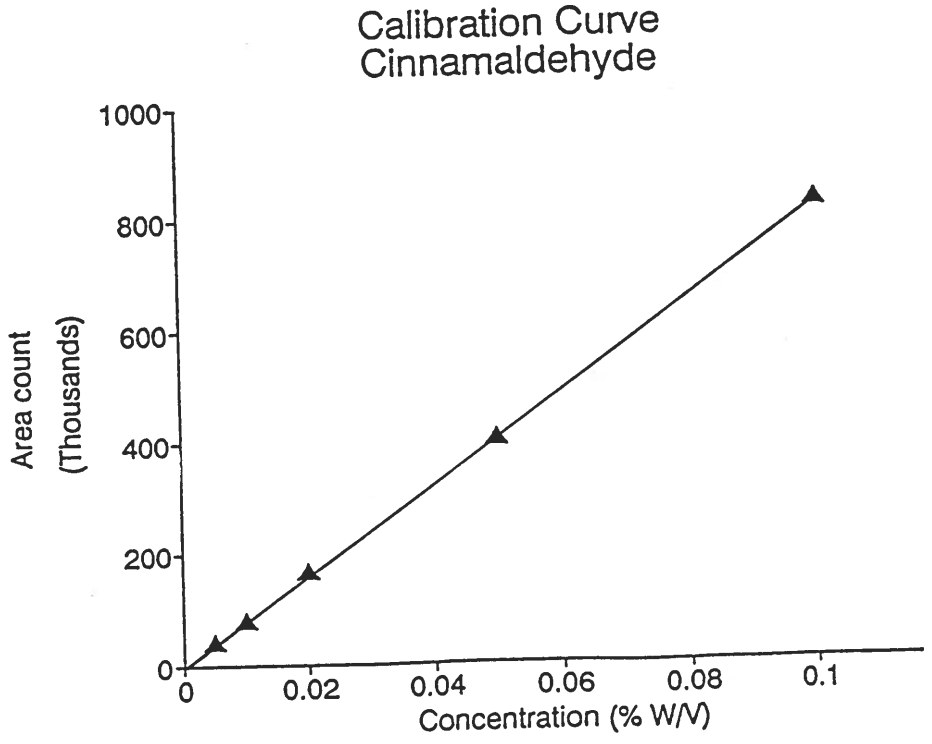


Figure 3G

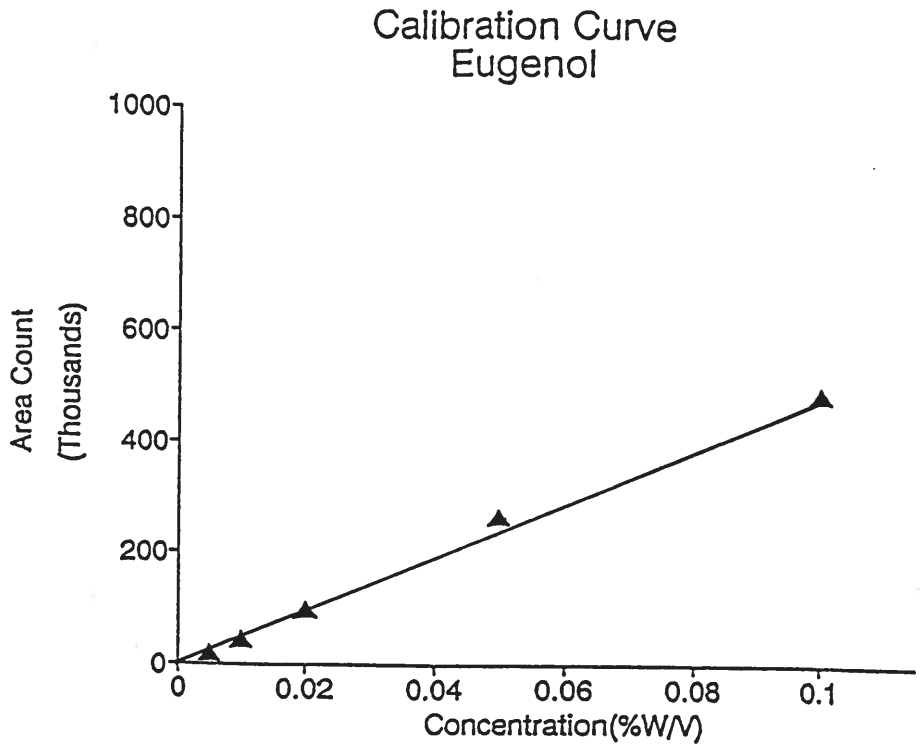


Figure 3H

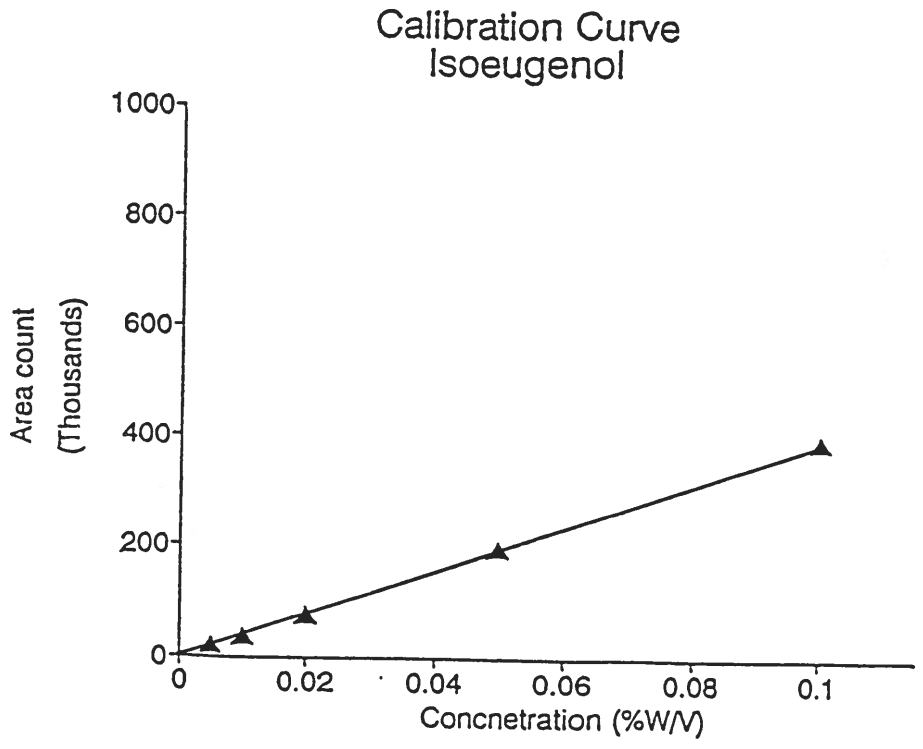


Figure 3I

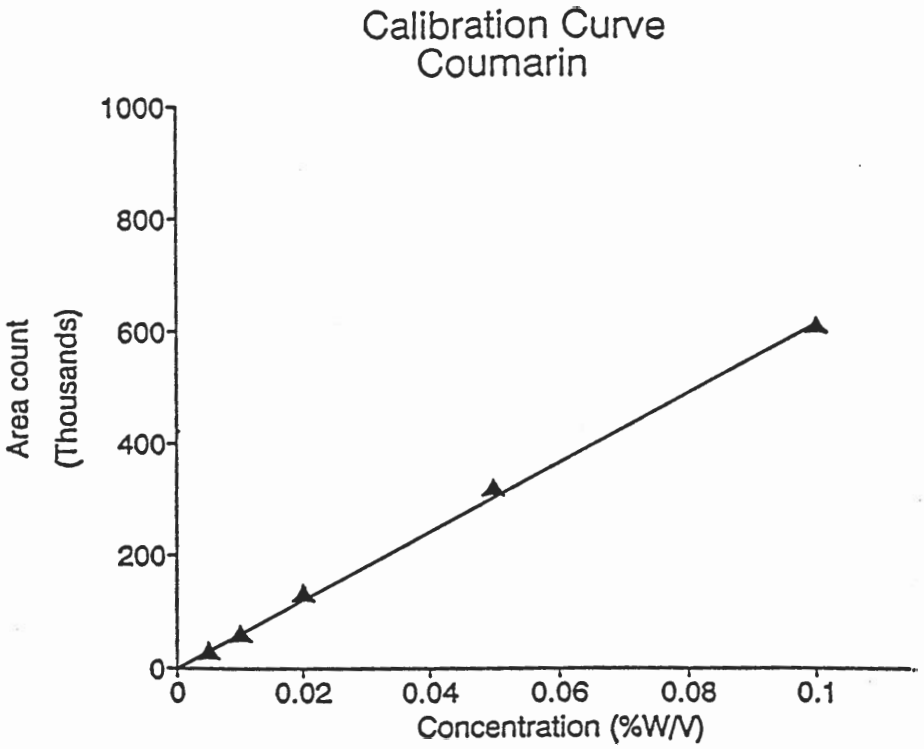


Figure 3J

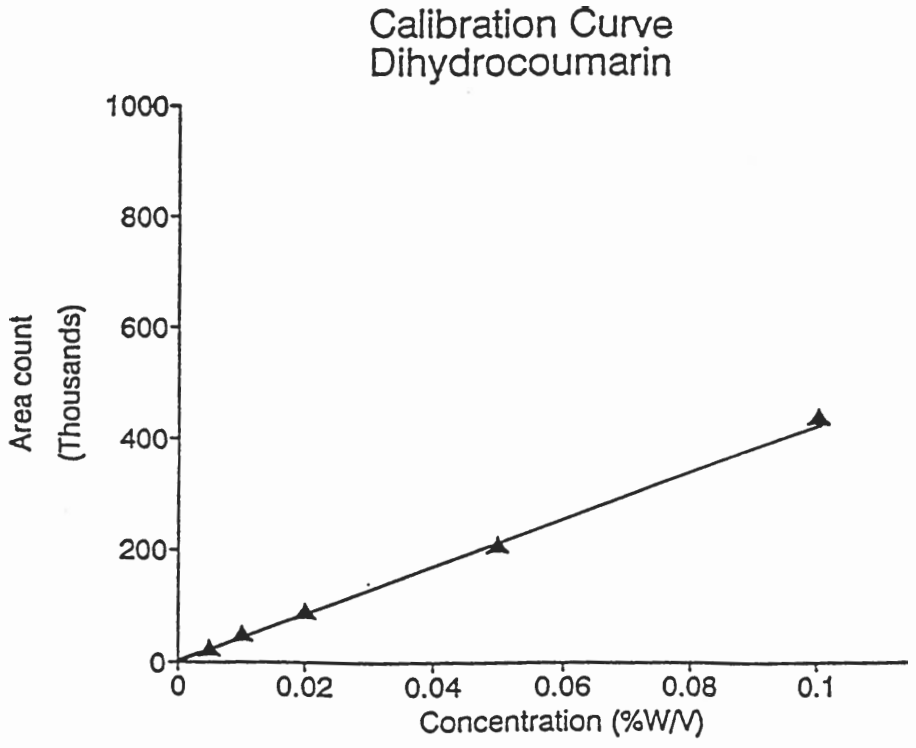


Figure 3K

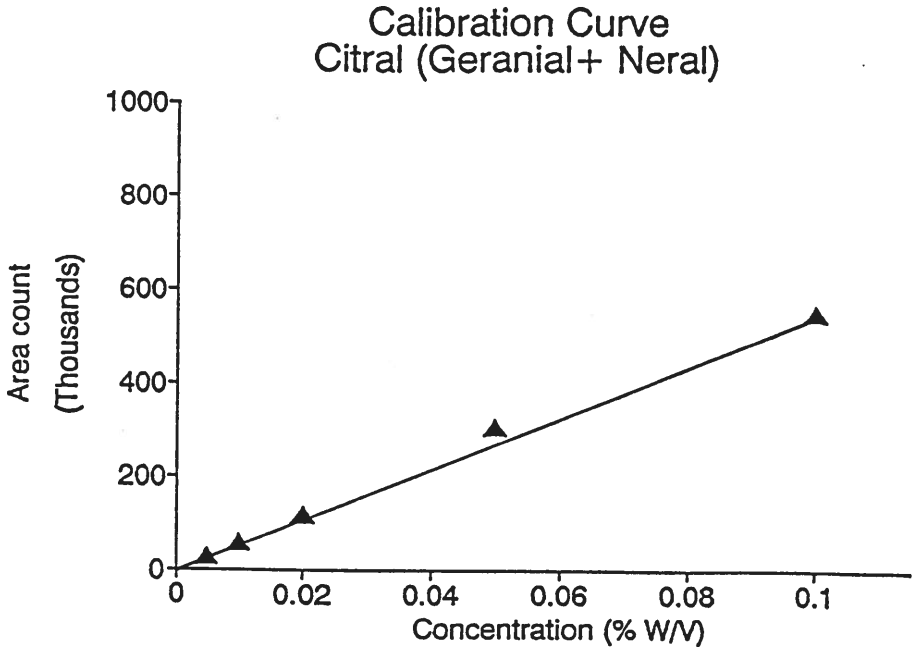

Calibration Curve
Citral (Geranial+ Neral)
Area count
(Thousands)
1000
800
600
400
200
0
0
0.02
0.04
0.06
0.08
0.1
Concentration (% W/V)

Table 7: Correlation coefficient (r^2) for concentration of fragrances and GC response.

Fragrance standard	r^2 Concn. 0.005-0.10%	r^2 Concn. 0.005-0.50%
Cinnamyl alcohol	0.9949	0.9945
Cinnamaldehyde	0.9999	0.9999
Eugenol	0.9972	0.9990
Hydroxycitronellal	0.9957	0.9992
α-Amyl cinnamaldehyde	0.9956	0.9988
Geraniol	0.9997	0.9985
Isoeugenol	0.9998	0.9981
Coumarin	0.9990	0.9999
Dihydrocoumarin	0.9986	0.9980
Citral (geranial + neral)	0.9958	0.9996
Citronellal	0.9983	0.9998

Table 8: Recoveries of fragrance standards from silica gel column. Number of experiment = 8)

Fragrance standard (concentration 0.45%)	% Recovery range
Cinnamyl alcohol	83 - 102
Cinnamaldehyde	79 - 107
Eugenol	83 - 97
Hydroxycitronellal	87 - 105
α-Amyl cinnamaldehyde	82 - 96
Geraniol	84 - 108
Isoeugenol	83 - 112
Coumarin	84 - 112
Dihydrocoumarin	87 - 116
Citral (geranial + neral)	80 - 94
Citronellal	83 - 114

at 60°C (5min/10min/15min), use of activated and unactivated silica gel for column chromatography, height of the silica gel column (5cm/6cm/7cm), and the spike-level of fragrances (0.045% and 0.450%). Under the optimal conditions described in 4.2.1.2 the recovery of all the substances was found to be 79-116% from the blanks (fragrance mixture treated without any cosmetics/toiletry sample, Table 8)) and 85-114% from the spiked samples (results not shown). Moreover, it was revealed that recovery was not affected by changing the followings: amount of sample from 1 g to 2 g, silica gel activated overnight at 110°C to unactivated silica gel for column chromatography, column height from 6 cm to 7 cm and heating time from 10 min to 15 min. It was also revealed that recoveries of the fragrance substances were better from the sample suspensions in methanol than those in ethanol.

The method established for sample preparation of shampoos, cream and lotion was also found to be suitable for the sample preparation a face powder and a dishwasher product. For the analysis of fragrances in a lipstick, it was though necessary to heat the sample with methanol for 15 min. The above mentioned method of sample preparation, however, was not applicable for the bar-soap and powder (granular) detergent for laundry. Attempts were then made to extract fragrances in an organic solvent from the aqueous solution of bar-soap spiked with 0.45% of all of the investigated fragrance substances. The experimental conditions described in 4.2.1.3 were found to be optimal for the extraction of fragrances from bar-soap. This sample preparation method was also applicable for the analysis of fragrances in the powder detergent.

Analyses of fragrances in liquid samples, without a complex matrix, for example, eau de toilette, deodorant sprays and aftershave lotions, were performed by GC without involving any sample preparation step. Practical experiences have though revealed that dilution of these samples in methanol, 1:2, 1:5 or 1:10, may be necessary for the analysis of fragrances which were present in relatively higher concentrations.

Employing the above mentioned methods recoveries of the all the 11 target fragrances substances have been determined in 20 randomly selected samples (Table 4): 5 shampoos, 7 creams and lotions, 1 face powder, 1 deodorant spray (in aerosol can), 2 eau de toilette, 1 lipstick, 1 dishwasher and in 1 powder detergent. All the samples, except eau de toilette and deodorant samples, were spiked to 0.45% with the fragrance substances and analysed by GC-FID. Eau de toilette and deodorant samples were spiked only with 5 of the investigated fragrance substances (Table 9B), because GC-screening of these products revealed the presence of relatively major peaks with t_R similar to other fragrance substances. The recoveries of all the investigated fragrances from the samples were found to be ≥ 80%, except for the recoveries of citral, cinnamaldehyde, and dihydrocoumarin from bar-soap and powder detergent samples (Tables 9A & 9B). The recoveries of the citral, cinnamaldehyde and dihydrocoumarin from powder deter-

Table 9A: Recoveries of fragrances from cosmetic products spiked with 0.45% of each of the standard fragrance substances.

Fragrance Substance	% Recovery Shampoos (n = 5)	% Recovery Creams (n = 7)	% Recovery Face powder	% Recovery Lipstick	% Recovery Bar-soap
Cinnamyl alcohol	83 - 105	88 - 112	92	89	83
Cinnamaldehyde	84 - 112	85 - 111	91	91	37
Eugenol	88 - 109	92 - 117	92	107	94
Hydroxycitronellal	93 - 114	88 - 106	90	87	84
α-Amyl cinnamaldehyde	92 - 107	91 - 104	91	97	92
Geraniol	96 - 116	92 - 112	91	91	90
Isoeugenol	86 - 107	88 - 106	91	113	94
Coumarin	95 - 115	91 - 113	98	86	87
Dihydrocoumarin	94 - 108	89 - 109	111	97	8
Citral	80 - 95	80 - 92	93	92	40
Citronellal	82 - 98	81 - 100	91	88	100

Table 9B: Recoveries of fragrances from cosmetics and other consumer products spiked with 0.45% of each of the standard fragrance substances.

Fragrance Substance	% Recovery Deodorant spray	% Recovery Eau de toilette (n = 2)	% Recovery Dishwasher	% Recovery Washing Powder for woolen laundry
Cinnamyl alcohol	102	98	n.d.	90
Cinnamaldehyde	n.d.	n.d.	96	48
Eugenol	n.d.	n.d.	99	91
Hydroxycitronellal	n.d.	n.d.	114	93
α-Amyl cinnamaldehyde	n.d.	n.d.	98	91
Geraniol	96	96	100	94
Isoeugenol	100	91	102	82
Coumarin	n.d.	n.d.	99	89
Dihydrocoumarin	104	92	95	44
Citral	n.d.	n.d.	109	65
Citronellal	90	94	103	93

n.d.: recovery not determined because samples were not spiked with the respective fragrance substances.

gent were 65%, 47% and 44% respectively. The recoveries of citral and cinnamaldehyde from the bar-soap were 39 and 37% respectively. Dihydro-coumarin, however, was not possible to recover from the soap, recovery only 8%.

The methods developed in the present work, were applied for the analysis of fragrances in 32 products (Table 5). All the samples were appropriately treated and analysed for fragrances by GC-FID and GC-MS. In general, no problems were identified in adapting the procedures established for the analysis of fragrances (4.1 - 4.3). GC-FID and GC-MS chromatograms of some of the investigated samples are shown in Figs. 4 - 10. When the GC-MS screening showed the presence of a target fragrance substance that was not identified by GC-FID, 2 g sample was used for the GC analysis. The identification of fragrances in the samples was performed on the basis of the GC- relative t_R and the mass spectra of the fragrance substances. The identification by MS was considered to be correct - some samples showed GC-peaks with the same t_R, as one of the target fragrance substances, but they were not confirmed by MS. One of the fragrance substances which was not found to be present in a sample, as revealed by GC-FID/GC-MS screening, was chosen as an internal standard for the quantitative analysis of fragrances in the sample - for our purpose citronellal was found to be quite suitable because this substance was found to be present in one product (dishwasher) only.

For practical reasons, for example, relatively fast analyis, quantitation of fragrances was performed by GC-FID. The results of quantitative analysis are described in Table 10. In some samples, it was not possible to perform quantitation of fragrance substances identified by GC-MS, because of the lower senstivity of GC-FID compared to that of GC-MS. The concentrations of these fragrances, described as "+" in Table 10, in the respective samples are less than 0.01%.

Figure 4. Analysis of fragrances in a body shampoo (1-0440) by GC-FID. t_R 13.912-dihydrocoumarin, 14.220-coumarin. (Eugenol in the sample was identified only by GC-MS).

Figure 5. Analysis of fragrances in a dusch gel (1-0484) by GC-FID. t_R 10.115-geraniol, 10.515-cinnamaldehyde, 14.002-dihydrocoumarin, 14.365-coumarin, 19.380-α-amyl cinnamaldehyde.

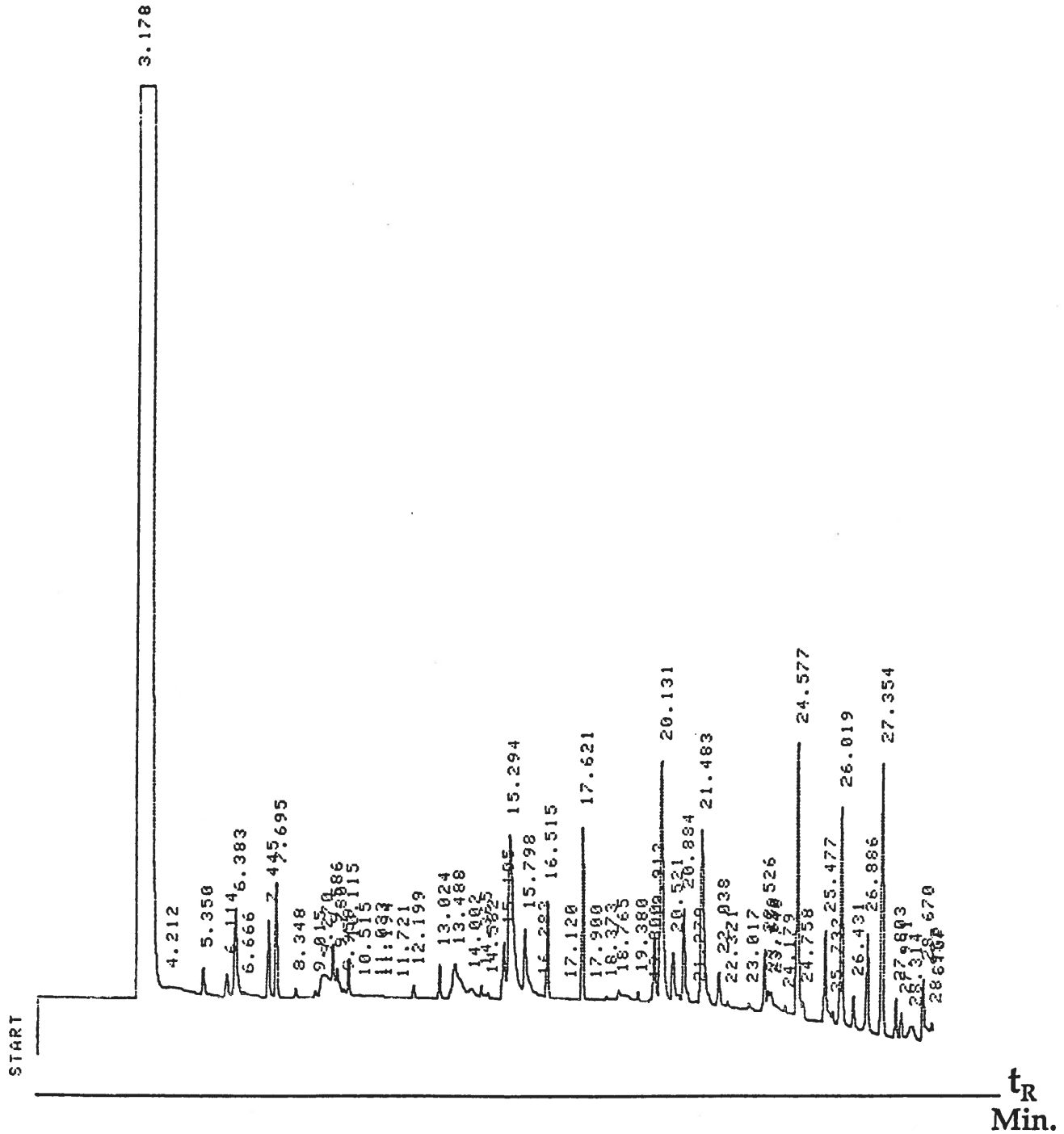


Figure 6. Analysis of fragrances in a body lotion (1-0904) by GC-FID. t_R 8.179-internal standard (citronellal), 10.031-geraniol, 10.530-hydroxy citronellal, 11.110-cinnamyl alcohol, 12.355-eugenol, 14.260-coumarin, 14.469-isoeugenol. (Cinnamaldehyde in the sample was identified only by GC-MS).

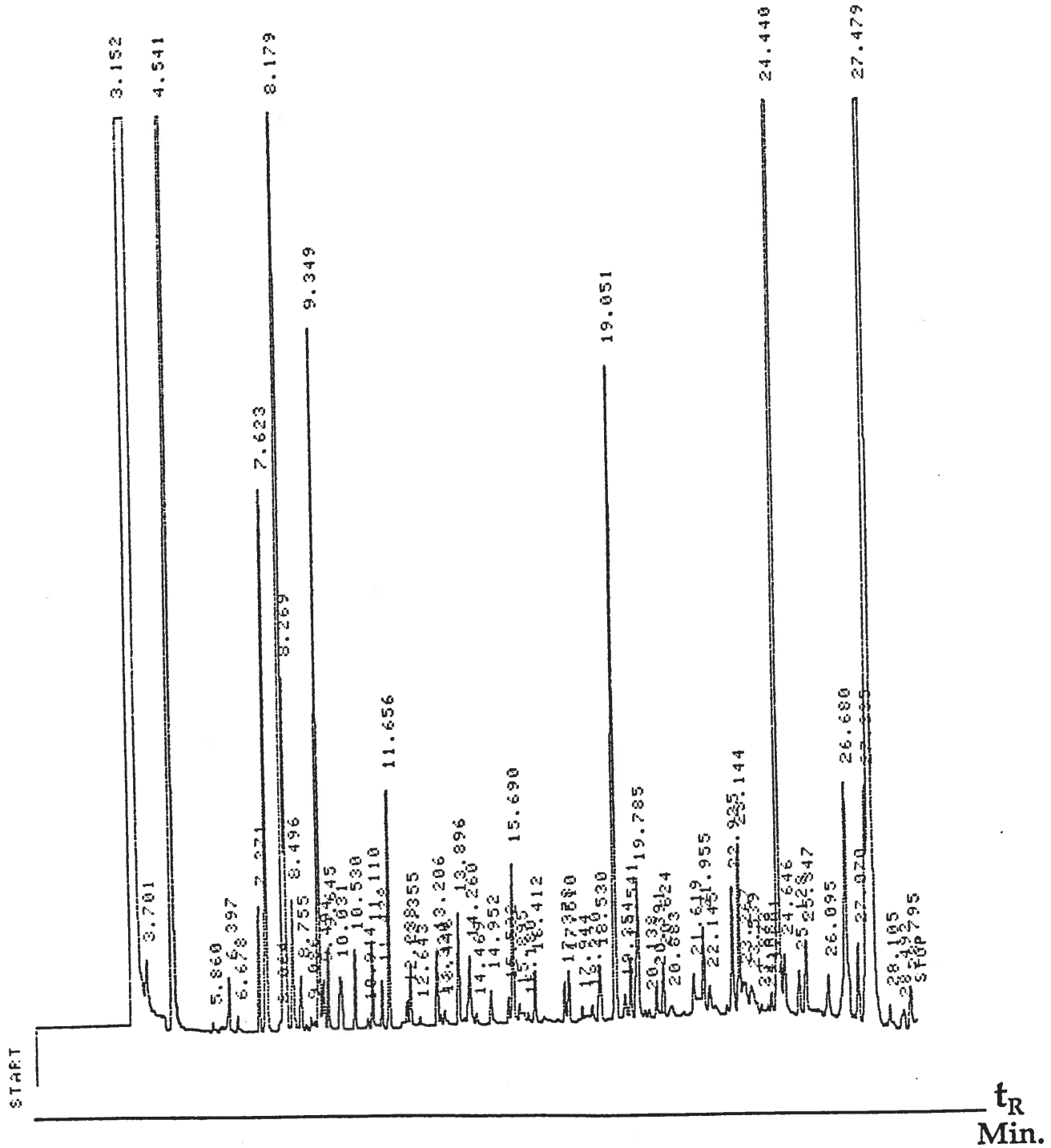


Figure 7. Analysis of fragrances in an eau de toilette (3-1714) by GC-FID. t_R 8.185-internal standard (citronellal), 10.099-geraniol, 10.548-hydroxy citronellal, 11.124-cinnamyl alcohol, 12.371-eugenol.

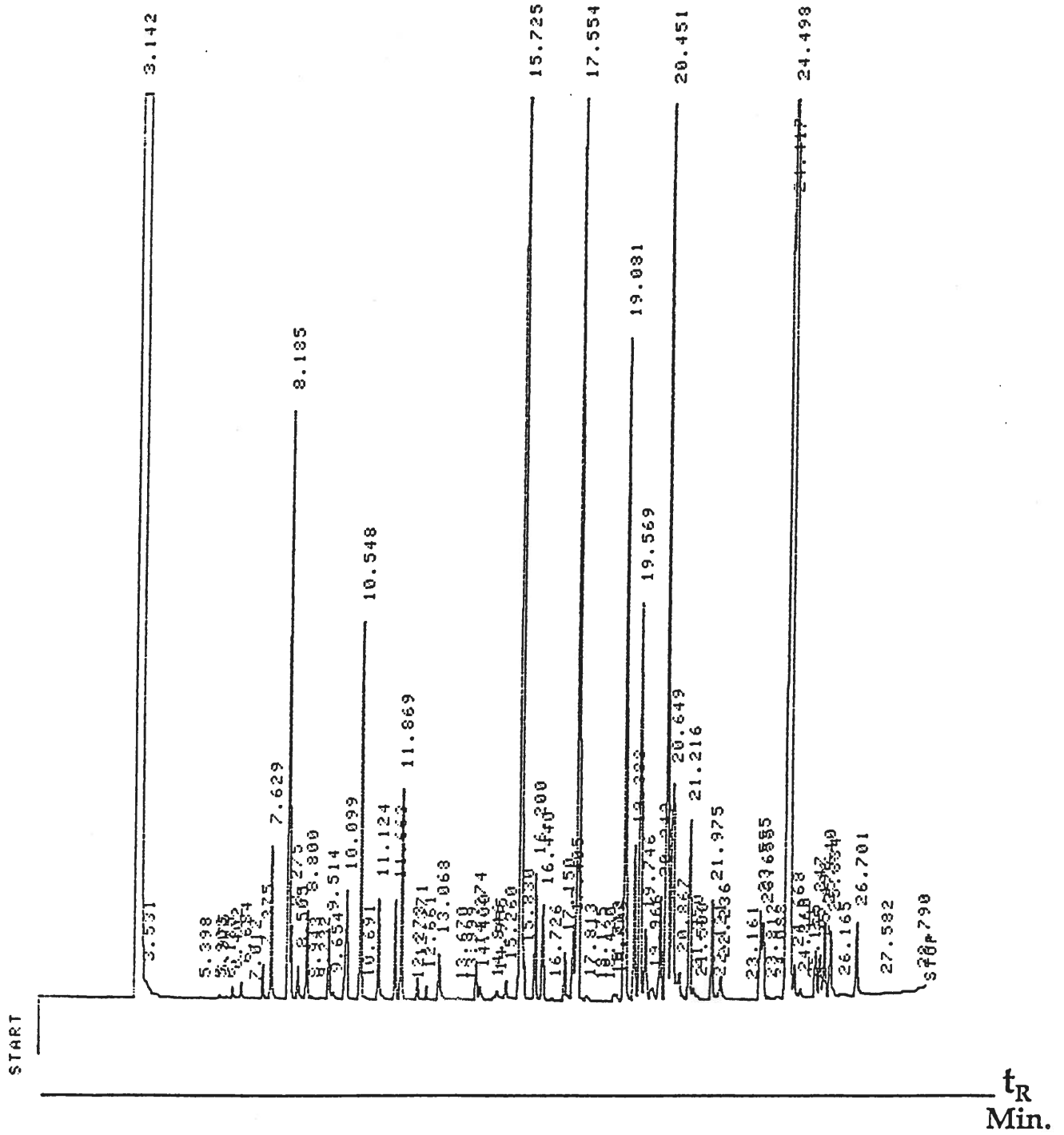


Figure 8. Analysis of fragrances in a deodorant spray (2-0011) by GC-FID. t_R 9.838-citral, 10.530-hydroxy citronellal, 14.262-coumarin. (Cinnamyl alcohol in the sample was identified by GC-MS only, see Figure 9).

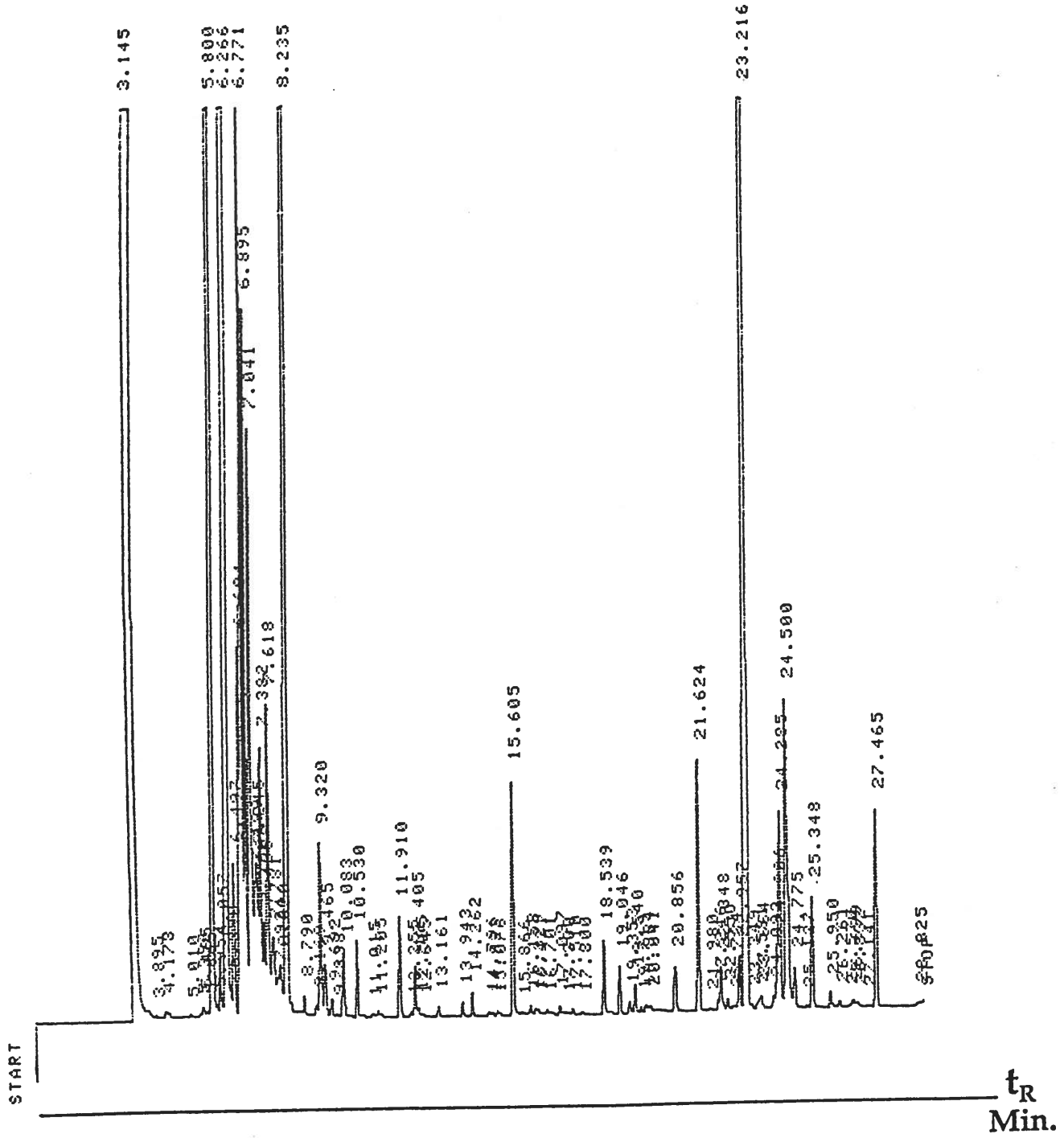


Figure 9. Analysis of fragrances in a deodorant spray (2-0011) by GC-MS. Scan no. 1261-citral (trans), 1282-hydroxy citronellal, 1351-cinnamyl alcohol, 1678-coumarin.

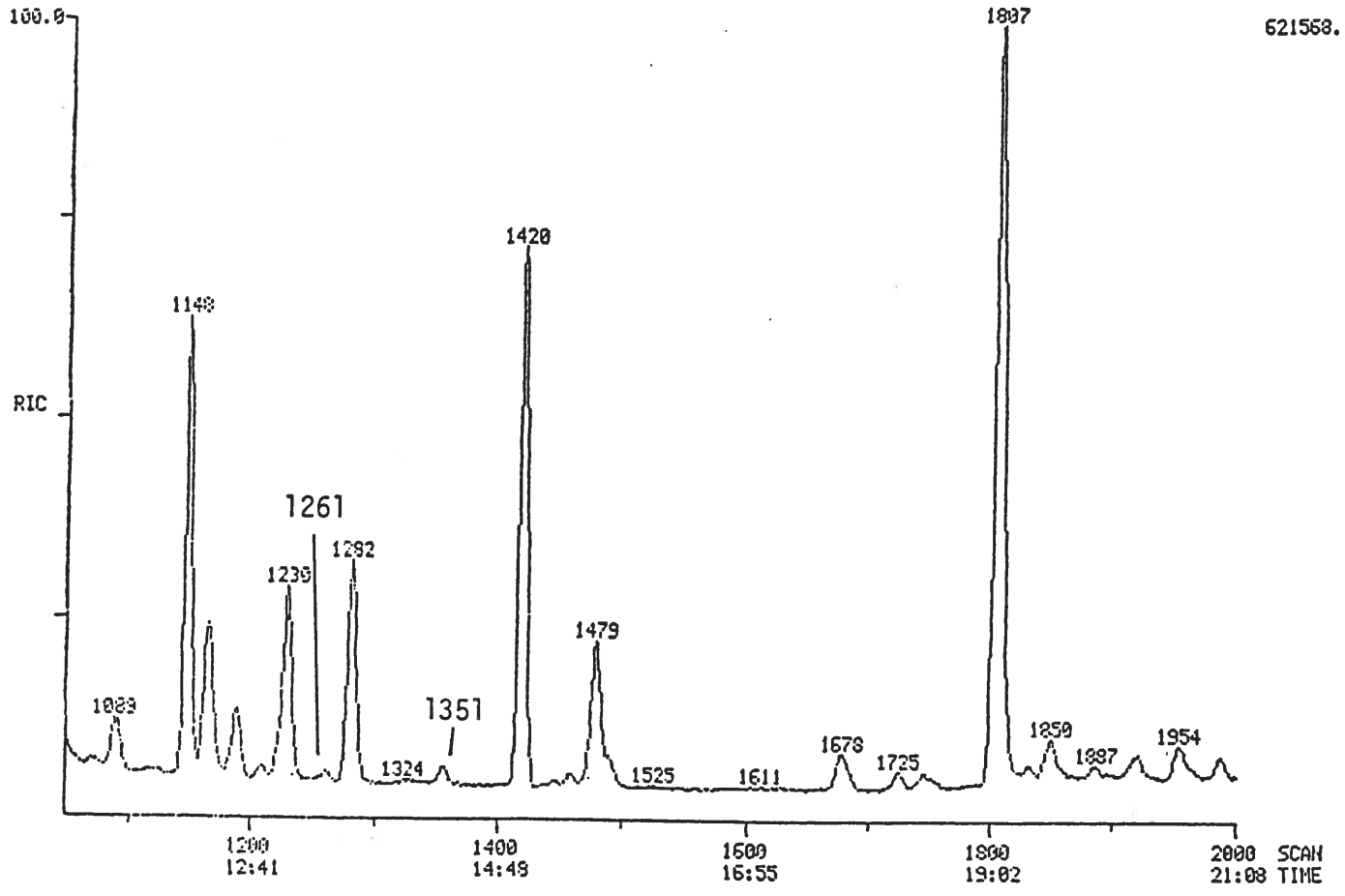


Figure 10. Analysis of fragrances in a aftershave lotion (3-1711) by GC-MS. Scan no. 794-cinnamyl alcohol, 901-eugenol, 1058-dihydrocoumarin. A new CP-Sil-5CB column was used which resulted in faster elution of compounds compared to that by old column.

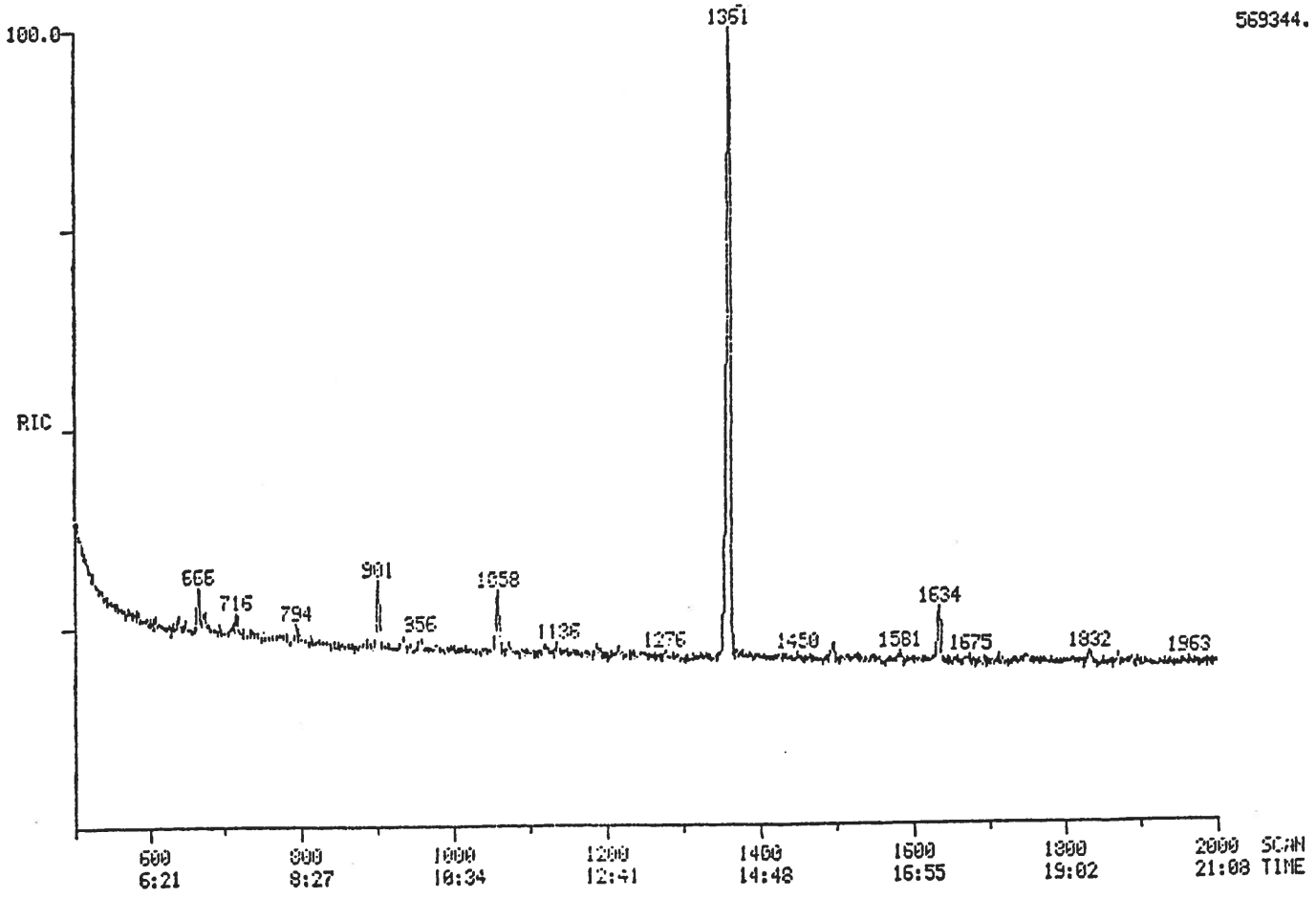


Table 10. Content of fragrances in the investigated products.

Sample No.	Cinnamyl alcohol % (w/w)	Cinnamalde-hyde % (w/w)	Eugenol % (w/w)	Hydroxy citronellal % (w/w)	α-Amyl cinnamaldehyde % (w/w)	Geraniol % (w/w)	Isoeugenol % (w/w)	Coumarin % (w/w)	Dihydro-coumarin % (w/w)	Citral % (w/w)	Citronellal % (w/w)
1-0367	-	-	-	+	-	-	-	-	-	+	-
1-0378	+	+	+	-	+	+	-	-	-	-	-
1-0402	+	-	+	-	-	+	-	+	+	-	-
1-0410	-	+	-	-	-	-	-	0.035	0.110	-	-
1-0415	+	+	+	-	0.079	0.010	-	-	-	+	-
1-0416	-	-	+	0.026	-	0.042	-	0.036	-	-	-
1-0440	-	-	+	-	-	-	-	+	0.047	-	-
1-0484	-	+	-	-	0.022	0.042	-	0.016	0.055	-	-
1-0486	+	-	-	-	-	0.009	-	-	-	-	-
1-0516	0.021	+	-	+	0.048	0.031	-	-	-	-	-
1-0519	0.028	-	-	0.031	-	0.018	-	+	0.036	-	-
1-0851	-	-	0.025	-	0.023	0.028	-	0.076	-	+	-
1-0904	0.054	+	0.039	0.053	-	0.028	+	0.054	-	-	-
1-0905	-	-	0.069	-	-	0.075	+	-	-	-	-
1-0948	-	-	+	-	-	+	-	-	-	-	-
1-0957	-	-	-	-	-	+	-	+	-	-	-

Table 10. Continued.

Sample No.	Cinnamyl alcohol % (w/w)	Cinnamalde-hyde % (w/w)	Eugenol % (w/w)	Hydroxy citronellal % (w/w)	α-Amyl cinnamaldehyde % (w/w)	Geraniol % (w/w)	Isoeugenol % (w/w)	Coumarin % (w/w)	Dihydro coumarin % (w/w)	Citral % (w/w)	Citronellal % (w/w)
2-0011	+	-	-	0.123	-	-	-	0.033	-	+	-
2-0013	-	-	+	+	0.053	-	+	-	-	-	-
3-1167	-	-	-	-	-	+	-	+	-	-	-
3-1168	0.036	-	-	0.036	-	+	-	-	+	-	-
3-1185	+	-	+	0.014	+	+	0.022	0.065	+	-	-
3-1187	-	-	+	-	+	+	+	+	-	-	-
3-1192	-	-	-	-	-	+	+	+	-	-	+
3-1409	+	+	+	-	+	+	+	+	+	-	-
3-1711	0.019	-	0.060	-	-	-	-	-	+	-	-
3-1712	-	-	-	0.027	-	0.064	-	-	+	-	-
3-1713	-	-	-	+	+	0.042	-	-	-	-	-
3-1714	0.167	-	0.042	0.661	-	0.218	-	-	-	-	-
3-1715	0.201	-	0.042	0.246	-	0.076	0.261	-	-	-	-
3-1716	-	-	0.233	-	-	-	0.021	-	-	-	-
3-1717	-	-	+	-	-	0.194	+	-	-	-	-
3-1718	-	-	0.037	-	-	0.210	-	-	-	-	-

6 Discussion

To develop a method for the analysis of fragrances in cosmetics and toiletries, it was necessary to concentrate on some fragrance substances and also on some commonly used product categories. Fragrance substances which have been shown to cause allergic contact dermatitis were selected for the present work (see 2. Target Fragrance Substances). Citronellal was included because its chemical structure is similar to some of the fragrances selected. The product categories chosen for the method developement were those which are commonly used by the general population, i.e. soap, shampoo, cream and lotion, deodorant spray, lipstick, face powder, and eau de toilette. A dishwasher and a powder detergent for the laundry was included because a method was also needed for the investigation of fragrances in these products.

The target fragrance substances in the present study are relatively polar compounds with boiling points 47°C - 299°C. A relatively polar GC-column may be preferred for the analysis of polar compounds. However, commercially available polar GC-columns are not suitable for use at high temperatures (>250°C). A relatively non-polar GC-column CP-Sil-5CB chosen for the GC analysis of fragrances in the present work was suitable for the fragrance analyis, temperature program 140°C - 300°C. Under the optimal GC-FID conditions established in the present work, all the target fragrances were found to resolve from each other and the run time 29 min was considered to be reasonable for routine analysis. The detection limits of fragrances 2-5 ppm was also considered to be reasonable, as the method was applicable for the analysis of a product containing approximately 0.01% (100 ppm) of a fragrance substance, diluted to 4 ppm during sample preparation (as described in Table 1, the recommended perfume concentration in various types of products is ≥0.10%). The GC-MS method was more sensitive than GC-FID method, the detection limits of fragrances being <1 ppm. Thus, the fragrances present at concentration level 10-25 ppm in the products, diluted to 0.5 - 1.0 ppm during sample preparation, could be identified employing GC-MS. Selective ion monitoring employing GC-MS will be required for the identification of fragrances at lower concentrations in the products. Identification of fragrance substances was based on their relative t_R in GC as well as on the their mass spectra. For practical reasons GC-FID was preferred rather than GC-MS for quantitative analysis of fragrances.

The repeatability of the method (within 5%) for all the target fragrance substances except for geraniol (7%) and cinnamaldehyde (11%) were acceptable, considering that the method involves analysis of 11 substances most of which are susceptible to oxidation by atmospheric oxygen. Stability of the fragrance substances indicated that stock solutions (10%) should not be used after they were had been stored for 3 days at 4°C. Diluted fragrance solutions in air-tight vials should also not be stored more than 3 days at -18°C. The stabilities of stock fragrance

solutions stored at -18°C have not been checked in the present work. GC-chromatograms of the fragrance solutions stored at -18°C did not show any major peak other than the peaks for original fragrances. Thus, the possible reason for loss of fragrances from their stock/diluted solutions may be due to their preferential evaporation from the fragrance solutions in methanol. It was also observed that α-amyl cinnamaldehyd was stable for one week. A comparatively higher C_v of relative t_R (0.43) and repeatability of determination (11%) of cinnamyl alcohol may be associated with the unstability of this substance.

The recoveries of fragrances form blanks (fragrance solutions without a sample) and from the samples spiked with the fragrances were found to be similar (Table 8, 9A & 9B). Therefore, it was not necessary to run blanks with each set of experiments and the content of fragrances in the samples were calculated on the basis of the GC-response factors of the untreated fragrance solutions. It is, however, recommended to run regularly a blank, to check the stability of the method. Although the boiling point of citronellal is 47°C, its recovery (82-103%) from the investigated products was found to be similar to the recoveries of other fragrances from the products. A rather great variation (80-116%) in recoveries of various fragrance substances may be due to their uncontrolled and relatively fast evaporation during the sample preparation which involves heating at 60°C followed by coloumn chromatography or shaking with ethyl acetate. Thus, the results of quantitative analysis of fragrances will have an uncertainity of approximately ± 20%. This uncertainity in quantitation may, however, not influence the toxicity evaluation of the fragrances. It should be noted that the concentration of a fragrance(s) in a product may also reduce with the increasing age of the product, unless a stablizer for the fragrance is used in the formulation. It was not possible to investigate this phenomenon in the present work as the samples with known formulations were not available.

The method thus established in the present work was applied for the analyses of target fragrances in 32 products described in Table 5. During the analyses of fragrances in these products, no problems were encountered. Thus, it is considered that the present method can be used for routine analysis of fragrances in cosmetics, toiletries, dishwashers and detergents for laundry.

Eventhough the present study involves relatively small number of randomly chosen samples, the results *may* indicate that cinnamaldehyde, isoeugenol, citral and citronellal are not commonly used in the formulation of cosmetics and toiletries (Table 10). Eugenol and geraniol were more commonly present in the investigated samples. The results of the present work also showed that one of the prohibited substances in IFRA list (Table 2), dihydrocoumarin was identified in 10 of the 32 investigated products.

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