Zeitschrift: Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene = Travaux de chimie alimentaire et d'hygiène

Herausgeber: Bundesamt für Gesundheit

Band: 85 (1994)

Heft: 3

Artikel: 5-Methoxypsoralen and 8-Methoxypsoralen in sun cosmetics and fragrances - analysis and market survey

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DOI: https://doi.org/10.5169/seals-982766

Mitt. Gebiete Lebensm. Hyg. **85,** 382–394 (1994)
Received 11 February 1994. Accepted 14 February 1994

5-Methoxypsoralen and 8-Methoxypsoralen in Sun Cosmetics and Fragrances – Analysis and Market Survey

Key words: Cosmetics, Bergaptene, 5-Methoxypsoralen, Methoxalen, HPLC

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Introduction

Bergaptene (5-methoxypsoralen, 5-MOP) is a natural ingredient of bergamot oil, the volatile oil obtained from Citrus bergamia, Aurantoideae. It is one of the furocoumarines, fototoxic compounds which occur in many citrus species and numerous other plants, especially from the families of the Umbelliferae, Rutaceae, Urticaceae and Papilionaceae. Furocoumarines are also present in the vegetables obtained from such plants, for example in celery, parsley and parsnip (1). Like other citrus oils, oil of bergamot has a pleasant odor. For this reason it is used in perfumery, especially colognes.

Sometimes the use of cologne gives a typical side effect, known as berloque dermatitis. Berloque dermatitis is an irregular hyperpigmentation, which occurs on regions of the skin that have been in contact with colognes and were simultaneously exposed to the sun. The cause of the dermatitis, which actually is not a dermatitis at all, could be traced to the presence of 5-MOP from the bergamot oil used to impart fragrance to the cologne. In combination with UV radiation, 5-MOP promotes melanogenesis. Besides, it causes thickening of the stratum corneum. Methoxalen (8-methoxypsoralen, 8-MOP) has similar effects.

These properties can be used in sun cosmetics and tanning accelerators to accelerate the tanning of the skin. Though furocoumarins are prohibited in the EC (Council Directive 76/768 EC, Annex II), carry-over from natural ingredients is allowed. Thus bergamot oil, which can contain up to 0.5% 5-methoxypsoralen, may be (and is) used as a source of 5-methoxypsoralen for use in sun cosmetics.

However, there is concern about the risks to human health from the application of bergaptene in sun cosmetics, since the compound has been found to be highly mutagenic and carcinogenic in combination with UV-light. When activated by long wave UV radiation (UV-A), 5-MOP reacts with the thymine bases of DNA,

forming a mono-adduct and leading to an impairment of information transmission and a risk for mutations. When not repaired by the DNA repair mechanism before cell division takes place, mutations of the daughter cells may occur, possibly leading to chronic actinic dermatosis and ultimately carcinomas (2). More intense UV-light may lead one molecule of 5-MOP (or 8-MOP) to react with two thymine bases, which, if they belong to different DNA strands, blocks cell division altogether. Exactly this reaction makes 8-MOP (and the similar but in this application rarely used 5-MOP) suitable as active agent in the photochemotherapy of psoriasis. Recently several long-term follow-up studies indicate that PUVA appears to be both a promotor of squamous cell carcinoma in patients with certain risk factors, as well as a primary carcinogen (3).

In the context of the use of 5-MOP as tanning accelerator, it should be appreciated that PUVA therapy may be less risky than application of 5-MOP in sun cosmetics, precisely while it is aimed at blocking cell division and because it is applied under controlled conditions. Such controlled conditions do not exist while sun bathing, and the formation of mono-adducts, which do not inhibit cell division, is more likely in such conditions.

Manufacturers of sunscreen products containing bergaptene claim that the induced tan subsequently provides for better UV-protection. Since UV-radiation by itself is carcinogenic too, the risk from bergaptene is claimed to be balanced by the protection provided by the more quickly obtained sun tan. The scientific evidence for this argument is still debated (4).

The EC Scientific Committee on Cosmetology, after evaluation of the available scientific data, expressed as its opinion that the maximum amount of 5-methoxypsoralen in sun tan products should not exceed 1 mg/kg. With regard to the use of bergamot oil in fragrances, the IFRA (International Fragrance Association) code of practice advises the fragrance industry to limit the amount of bergamot oil in finished products to a maximum of 0.4% (5), while for stay-on cosmetics the maximum concentration of 5-methoxypsoralen should not exceed 15 mg/kg (6).

Analysis

Methods for the determination of 5-MOP in sun cosmetics employing a variety of analytical techniques have been described in the literature. Fluorimetry of the spots obtained after TLC separation (7), GC with FID and MS detection (8), and HPLC, both with UV (1, 9) and fluorimetric detection (10) have been used. The chromatography was either reversed phase or straight phase, but was always based on isocratic elution. Similar isocratic HPLC methods have been published for the determination of 5-MOP and other furocoumarins in food and essential oils (11–13), sometimes with electrochemical detection (14). 5-MOP has been analyzed in plasma by RP-HPLC on Spherosil® C18 with fluorimetric detection (15). The detection limits reported for these methods varied between 0.1 mg/kg for the GC

methods to 1 mg/kg for HPLC with UV detection and 15 ng/ml serum for HPLC with fluorimetric detection (15).

In previous investigations of tanning accelerators the authors used isocratic RP-HPLC and UV detection, employing a C-18 column and water/methanol (47/43 (v/v)) as the mobile phase (16). The detection limit was 1 mg/kg, and the method performed trouble free in the analysis of emulsion type cosmetics. However, initial experiments with the assay in perfumeries showed, that the separation proved prone to interferences from the complicated perfume matrix. In this matrix the psoralens are accompanied by high concentrations of a large number of fragrance compounds, which regularly eluted at similar retention times as the analytes.

Though the sensitivity obtained with UV-detection (1 mg/kg 5-MOP) was judged sufficient, some preliminary experiments were performed with fluorimetric detection to investigate whether this mode of detection offered selectivity advantages. The findings of *Bettero* and *Benassi* that fragrances can be analyzed by isocratic reversed phase chromatography on a C8 column coupled to fluorimetric detection could not be confirmed in these experiments (10). In the fragrances we investigated, many peaks coinciding with 5-MOP or 8-MOP showed sufficient fluorescence to make reliable detection of these compounds impossible, and fluorimetric detection did not appear to offer any selectivity advantage. Therefore it was decided to revert to UV-diode array detection and adapt the chromatography to provide a useful system for the analysis of fragrances.

Experimental

Chemicals

Acetonitrile (ChromAR®, Promoscan, Wesel), methanol and tetrahydrofurane (Lab Chem, Dublin) were all HPLC grade. Bergaptene was obtained from Roth (Karlsruhe) and 8-methoxypsoralen from Sigma (St. Louis). Both were used as received.

The following solvent mixtures were prepared: acetonitrile/water (25/75 (v/v)) (a) and methanol/water (3/7 (v/v)) (b).

A stock solution containing approximately 250 mg/l 5-methoxypsoralen and 250 mg/l 8-methoxypsoralen was prepared by accurately weighing about 25 mg 5-methoxypsoralen and 25 mg 8-methoxypsoralen into a 100 ml volumetric flask, dissolving in methanol and making up to volume with methanol (c).

Apparatus

LC-system – HP 1090 M HPLC with ternary gradient capability and HP 1049 A diode array detector (Hewlett Packard, Waldbronn, BRD); Lichrospher® 100 RP 18 analytical column, 5 µm, 250 x 4 mm (Merck, Darmstadt).

Operating conditions: Flow rate, 1 ml/min; injection volume, 10 µl; column oven temperature 35 °C; diode array detector set at a detection wavelength of 300 Nm, bandwidth 8 Nm, reference wavelength 550, 100 Nm bandwidth.

Sample preparation

Creams and milks (emulsions)

Accurately weigh approximately 1 gram of the sample into a 25-ml conical flask with glass stopper. Add by pipette 20.00 ml methanol, heat during 5 minutes in a water bath at 60 °C and shake until a homogeneous solution is obtained. Allow to cool and filter through a membrane filter with pore size 0.45 µm.

Colognes and eaux de toilette

Accurately weigh about 0.1 g of sample into a 1.5 ml vial and add by pipette 1.00 ml methanol. Cap the vial and mix.

Calibration

Transfer by pipette 1.00 ml of standard stock solution (c) into a 100-ml volumetric flask and dilute to volume with methanol (solution (d)). Into a series of 25 ml volumetric flasks, transfer by pipette respectively 0.50 ml, 1.00 ml, 2.00 ml, 4.00 ml, 8.00 ml and 16.00 ml solution (d) and dilute to volume with methanol.

Chromatography and quantification

Because no single eluent allowed separation of the psoralens from all matrix components three alternative gradients were used:
gradient I: acetonitrile/water (25/75 (v/v)) (a) / tetrahydrofurane = 95 / 5 (v/v) during 5 min; linear ternary gradient to acetonitrile/water ((25/75 (v/v)) (a) /acetonitrile / tetrahydrofurane = 10 / 80 / 10 (v/v/v) at 20 min; subsequently constant until 30 min.
gradient II: acetonitrile/water (25/75 (v/v)) (a) during 5 min; linear binary gradient to acetonitrile/water (25/75 (v/v)) (a) / acetonitrile = 10 / 90 (v/v) at 20 min; subsequently constant until 30 min.
gradient III: methanol/water (3/7 (v/v)) (b) during 5 min; linear binary gradient to methanol/water (3/7 (v/v)) (b) / methanol = 10 / 90 (v/v) at 20 min; subsequently constant until 30 min.

Inject 10 µl of each of the calibration solutions and record the chromatograms using gradient I. A typical chromatogram of a standard solution is shown in figure 1. Record the retention times, peak heights and spectra of the peaks obtained for 5-methoxypsoralen and 8-methoxypsoralen. Construct calibration curves for both analytes.

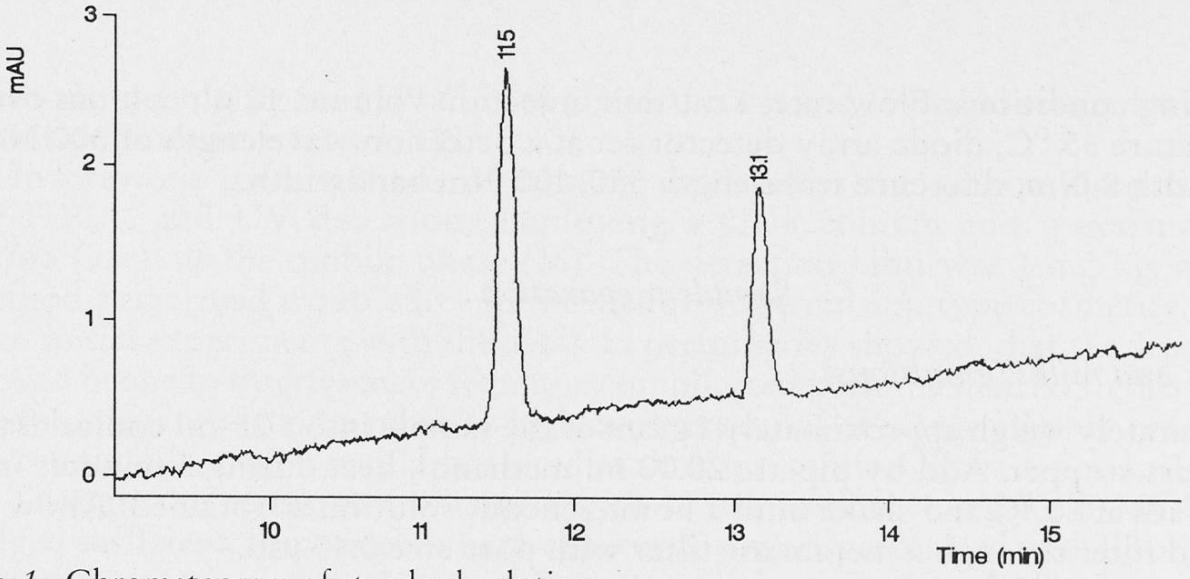

Fig. 1. Chromatogram of standard solution
Conditions as in text; gradient I. Injected: 10 µl standard solution containing 0.8 µg/ml 5-MOP and 1.6 µg/ml 8-MOP. 8-MOP: 11.5 min; 5-MOP 13.1 min

Inject 10 µl of the test solution and record the chromatogram using the same gradient as used for the calibration solutions. When peaks are obtained having the same retention time as 5-MOP or 8-MOP, record their spectra and the peak heights. Compare the spectrum with that obtained from the calibration solution, and check the peak purity. When the spectrum of the peak agrees with the one obtained in the calibration solution for the same analyte and the peak purity is satisfactory, the concentration in the test solution can be determined from the calibration graph. Repeat the procedure (calibration included) using gradient II when the identity or purity of the peaks obtained is in any way subject to doubt, and, if necessary, again with gradient III.

Results and discussion

Though the determination of 5-MOP and 8-MOP in sun cosmetics does not present difficulties, this is not true for the analysis of perfumes and similar products. Perfumes are complicated mixtures containing up to 300 ingredients, many of which show UV absorption (and fluorescense) and have polarities comparable to the analytes. Separation with a single chromatographic system therefore fails regularly, because matrix components elute at similar retention times as 5-MOP or 8-MOP. Diode array detection is necessary to control peak purity, and in these investigations samples in which 5-MOP was identified were analyzed with at least two systems for confirmation.

For most samples the best separation was obtained from gradient I; this system allowed quantification of all sun cosmetics, and allowed separation of fragrances most frequently. Typical chromatograms from both kinds of samples are shown in figures 2 and 3. Its detection limit was 1 mg/kg for 5-MOP, and calibration curves were linear up to 60 mg/kg with a correlation coefficient of 0.9999 for peak height

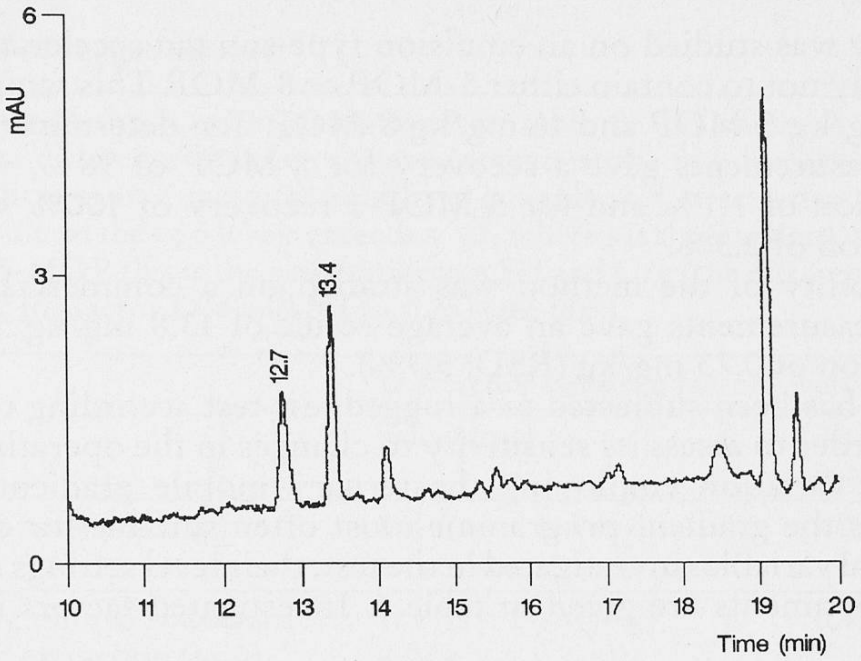

Fig. 2. Chromatogram of extract of sun cosmetic
Conditions: see text; gradient I. Sample: sun cosmetic containing 11.2 mg/kg 5-MOP; retention time 13.4 min; injected: 10 µl of the test solution

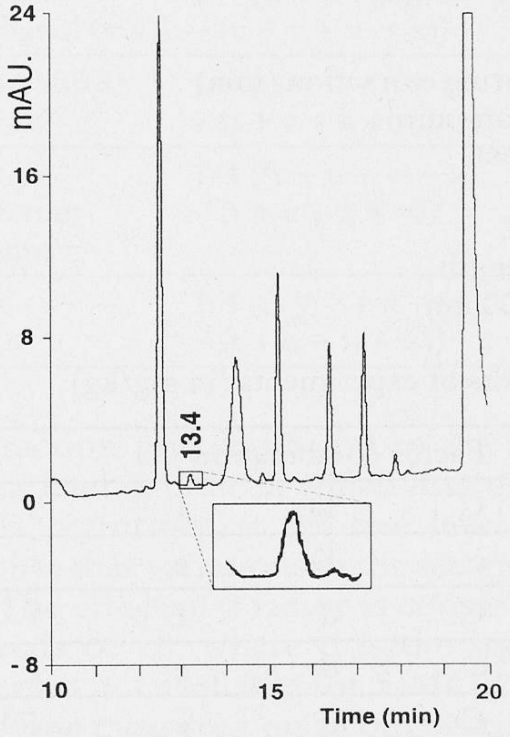

Fig. 3. Chromatogram of fragrance
Conditions: see text; gradient I. Sample: eau de toilette containing 3 mg/kg 5-MOP; retention time 13.4 min; injected: 10 µl of the test solution

measurements for 8-MOP and 0.9999 for 5-MOP. For both analytes linearity was marginally worse when area measurements were used.

The recovery was studied on an emulsion type sun tan accelerator, which was shown previously not to contain either 5-MOP or 8-MOP. This sample was spiked to levels of 8 mg/kg 5-MOP and 16 mg/kg 8-MOP. Ten determinations based on peak height measurements gave a recovery for 8 MOP of 98%, with a relative standard deviation of 1.1% and for 5-MOP a recovery of 100% with a relative standard deviation of 2.3%.

The repeatability of the method was studied on a commercial sample. Ten independent measurements gave an average result of 13.8 mg/kg 5-MOP, and a standard deviation of 0.73 mg/kg (RSD: 5.3%).

The method has been subjected to a ruggedness test according to *Youden* and *Steiner* (17) in order to assess its sensitivity to changes in the operating conditions. Tested was the variation employing the ternary mobile gradient (gradient I), because this was the gradient programme most often suitable for quantification. The experimental variables investigated in the test, their level settings and the results of the eight experiments are given in table 1. Investigated factors included both

Table 1. Ruggedness test

Experimental design, varied factors, levels and results of the experiments.

The following factors were investigated:

amount of sample (g)	A: 1	a: 1.5
heating time in waterbath during extraction (min)	B: 5	b: 10
shaking time during extraction (min)	C: 0.5	c: 1
composition extraction solvent (water/methanol (v/v))	D: 0/1	d: 1/9
column oven temperature °C	E: 40	e: 35
diode array detection wavelength band 8 nm; reference: 550, 100 nm	F: 300	f: 290
Integration mode	G: area	g: height

experimental design and results of experiments (in mg/kg):

Exp.	Factor combination							5-MOP	8-MOP
s	A	B	C	D	E	F	G	8.9407	15.438
t	A	B	c	D	e	f	g	9.1519	16.091
u	A	b	C	d	E	f	g	8.3932	15.263
v	A	b	c	d	e	F	G	8.3134	14.926
w	a	B	C	d	e	F	g	8.2559	15.266
x	a	B	c	d	E	f	G	11.146	15.576
y	a	b	C	D	e	f	G	10.949	16.149
z	a	b	c	D	E	F	g	8.1266	15.638
							$\bar{x}$	9.1597	15.543
							s	1.2180	0.4181

Table 2. Results of ruggedness test

From the results of the 8 experiments listed in table 1, the influence of each factor is calculated as the difference between the experiments at the two factor levels (see column calculation). Changes in a factor setting have a significant effect effect if the difference between the results at the two levels exceeds s. $\sqrt{2}$, where s is the standard deviation between replicates. For 5-MOP this is the case for factors F/f and G/g (the difference exceeds 1.033) and for 8-MOP for factor D/d and F/f (> 0.25) (see text).

Factor		Difference between factor levels		
		calculation	results 5-MOP	8-MOP
A or a	amount of sample (g)	1/4 ((s + t + u + v) − (w + x + y + z))	− 0.919	− 0.227
B or b	heating time in waterbath during extraction (min)	1/4 ((s + t + w + x) − (u + v + y + z))	0.428	0.098
C or c	shaking time during extraction (min)	1/4 ((s + u + w + y) − (t + v + x + z))	− 0.050	− 0.029
D or d	composition extraction solvent (water/methanol (v/v))	1/4 ((s + t + y + z) − (u + v + w + x))	0.264	*0.571*
E or e	column oven temperature (°C)	1/4 ((s + u + x + z) − (t + v + x + y))	− 0.016	− 0.129
F or f	Diode array detection wavelength, band 8 nm; reference 550, 100 nm	1/4 ((s + u + w + z) − (t + u + x + y))	*− 1.501*	*− 0.453*
G or g	integration of peak	1/4 ((s + v + x + y) − (t + u + w + z))	*1.356*	− 0.042

factors related to the extraction procedure and to the separation. Results of the ruggedness test are summarized in table 2, which lists the differences between the results of the experiments performed at the two levels for each factor. A large difference for a factor implies that variations in the setting of this factor have a large influence on the results. The effect of a factor is considered significant ($P < 0.05$) when the difference exceeds $\sigma . \sqrt{2}$, where σ is the standard deviation between replicates. Two estimates of σ are available for 5-MOP, one measured during the recovery experiments and one measured on a commercial sample. The latter, RSD 5.3% at the 13.8 mg/kg level, was felt to be the more realistic value at this concentration level. Based on this estimate of σ the detection wavelength influences the result, and there is a clear difference between the results obtained from area measurements and peak height measurements for the results of 5-MOP. Quantification based on peak height gives better repeatability, and is therefore preferred. In the determination of 8-MOP the composition of the extraction solvent and again the detection wavelength are important.

Psoralens in commercial tanning accelerators and perfumeries

The fact that some tanning accelerating cosmetics contain 5-MOP is well known (1, 2, 8, 10). From previous investigations conducted in 1984 and 1989 in our laboratory it was found that one brand in particular employed 5-MOP in many of its products, concentrations varied from 10 mg/kg up to 70 mg/kg. 5-MOP was found both in the tanning accelerators as well as in the sun protection cosmetics from this manufacturer (18).

In the present investigation a total of 119 sun cosmetics have been sampled from Dutch retail outlets during the second half of 1992 and the first half of 1993. Sampled products included accelerators, solaria cosmetics and UV protection products. Among these were all the market leaders. From the more popular brands of sun protection products more than one protection factor was selected, generally SPF 4 and 6.

Only eight products in this category contained 5-MOP, all produced by the manufacturer whose products were found to contain 5-MOP in the previous investigations. Concentrations ranged between 10 and 40 mg/kg 5-MOP. The concentration appears to increase with decreasing SPF factor claimed (see table 3). The fact that 5-MOP is found in products by this manufacturer is probably not coincidental; refined bergamot oil with negligible concentrations 5-MOP is available and the production of similar products as the above without bergaptene is feasible. Presumably the presence of 5-MOP in these products should assure a competition advantage.

Table 3. Concentrations of 5-MOP in cosmetics*

Sun cosmetics	mg/kg
Tanning sun milk skin type I SPF 7	14
tanning sun cream skin type I SPF 8	15
Bronzing milk SPF 7	12
Sun milk SPF 5	26
Sun cream SPF 10	15
Sun milk SPF 3	44
Sun milk SPF 4	42
Self tanning cream SPF 8	11

Perfumeries	
eau de toilette	2
eau de toilette	2
eau de toilette	3
eau de toilette	3
eau de toilette (imitation)	3
eau de cologne	6
eau de cologne	3
eau de cologne	2
eau de cologne	2
eau de cologne	3
eau de cologne	2

Miscelleneous	
After shave	4
Deodorant stick	18
Bergamot oil	14

* Results of determination in sun cosmetics (n = 126) and perfumeries (eaux de colognes (n = 14); eaux de toilette/parfum/parfum de toilette (n = 86); after-shaves (n = 16) and deodorants (n = 10)).

Far less is known about the occurrence and concentrations of psoralens in perfumeries. Therefore, the emphasis in this investigation was on these products, and 126 products in this category were sampled in retail stores, comprising (according to a rather arbitrary categorization) after-shaves, deodorants, colognes, eaux de toilette, eaux de perfume and perfumes.

Of these, colognes contained 5-methoxypsoralen most frequently; 6 colognes from 14 contained 5-MOP, the concentration varying between 2 mg/kg and 6 mg/kg. Of 16 after shaves only 1 contained 5-MOP in a concentration of 4 mg/kg. In the rest of the fragrances (eaux de toilette, eaux de perfume, perfumes and perfume de toilettes), totalling 86 products, 5 products contained 2–3 mg/kg 5-MOP, while in only 1 deodorant (out of 10 investigated) 5-MOP could be identified in the remarkably high concentration of 18 mg/kg. 8-MOP was identified in none of the investigated products.

Presently, further restriction of furocoumarines is being considered in the EC. A number of EC member states are concerned about the carcinogenicity of the furocoumarines and press for a 1 mg/kg limit on the presence of these compounds in cosmetics. From these investigations, it appears that with respect to the manufacturers of sun cosmetics not many companies will be affected by a complete ban on 5-MOP in cosmetics. In the fragrance industry a limit of 1 mg/kg will affect more companies, even though the concentrations found in fragrances are lower and the amounts applied are not comparable to sun cosmetics. However, it should be appreciated that fragrances are also applied to regions of the skin which are exposed to sun light and that concentrations may rise significantly after evaporation of the alcohol, considerations that should be taken into account when weighing the risk of such products and the need for regulation.

Conclusions

A large number of sun cosmetics (emulsions) and perfume products have been screened for the presence of 5-methoxypsoralen and 8-methoxypsoralen by HPLC with gradient elution and diode array detection. The method described could reliably detect concentrations of 1 mg/kg 5-MOP and 2 mg/kg 8-MOP. Its within laboratory repeatability was 0.73 mg/kg 5-MOP for a concentration of 13.8 mg/kg 5-MOP in a sun protection cream.

8-MOP could not be detected in any of the investigated products. Bergaptene (5-MOP) was found both in sun cosmetics and perfumeries, however.

In sun cosmetics the presence of 5-MOP was restricted to products from one manufacturer; the concentration varied between 10 and 40 mg/kg. In three cases the concentration exceeded the IFRA recommendation to limit the amount of 5-MOP in the finished product to 15 mg/kg.

The concentrations 5-MOP found in fragrance products were generally lower than those employed in sun cosmetics. Except for 1 deodorant, all fragrance

products remain within the maximum concentration (15 mg/kg in stay-on cosmetics) advised by the IFRA code of practice.

Summary

A method for the determination of 5-methoxypsoralen and 8-methoxypsoralen in sun cosmetics and perfumeries and its application to a large number of samples is described. The method is based on reversed phase liquid chromatography with diode array detection and uses a ternary gradient to separate the psoralens from interferences frequently caused by fragrance ingredients. Alternative gradient systems are provided in case the proposed system fails to separate the analytes.

The method allowed the determination of 1 mg/kg 5-methoxypsoralen and 2 mg/kg 8-methoxypsoralen. The recovery of the method was 98% for 5-methoxypsoralen and 100% for 8-methoxypsoralen. The repeatability determined from 10 independent analyses of a commercial sample found to contain 13.8 mg/kg 5-MOP was 0.73 mg/kg (RSD 5.3%).

The method was used to analyze 119 sun cosmetics and 126 perfumes and similar products. Eight sun cosmetics contained 5-methoxypsoralen, the concentrations varying between 10 and 40 mg/kg. In 13 out of 126 perfume products 5-MOP was identified. Especially colognes frequently contained 5-MOP; in six out of the 14 investigated eaux de cologne 5-MOP was identified. The concentrations 5-methoxypsoralen in perfumeries were generally lower than in the sun cosmetics, varying between 2 mg/kg and 6 mg/kg.

Zusammenfassung

Es wird eine Methode zur quantitativen Bestimmung von 5-Methoxypsoralen und 8-Methoxypsoralen in Sonnenschutzmitteln und Parfums beschrieben. Die Methode basiert auf Hochdruck-Flüssigkeitschromatografie mit Dioden-Array-Detektion und benutzt einen ternären Gradienten zur Trennung der Psoralene.

Das Verfahren ermöglicht die Bestimmung von 1 mg/kg 5-Methoxypsoralen und 2 mg/kg 8-Methoxypsoralen bei einer Wiederfindungsrate von 99% für 5-Methoxypsoralen und 100% für 8-Methoxypsoralen. Die Wiederholbarkeit gemessen an einem Handelsmuster betrug 0,73 mg/kg bei einer Konzentration von 13,8 mg/kg 5-Methoxypsoralen (RSD 5,3%).

Mit der Methode sind 119 Sonnenschutzmittel und 126 Parfums untersucht worden. Acht der Sonnenschutzmittel enthielten 5-Methoxypsoralen in Konzentrationen zwischen 10 und 40 mg/kg. In dreizehn der 126 Parfums wurde 5-Methoxypsoralen identifiziert. Vor allem in Eaux de cologne wurde oft 5-Methoxypsoralen gefunden; in sechs der 14 untersuchten Proben war 5-Methoxypsoralen anwesend. Die Konzentrationen in Parfums waren jedoch erheblich niedriger als in Sonnenschutzmitteln; sie variierten zwischen 2 mg/kg und 6 mg/kg.

Résumé

Ce travail décrit une méthode pour l'analyse du méthoxy-5-psoralène et du méthoxy-8-psoralène dans des produits cosmétiques. La technique employée est la chromatographie liquide à haute performance à gradient d'élution associée à une détection «diode array».

Dans les conditions opératoires décrites, la limite de détection est de 1 mg/kg de 5-méthoxypsoralène et de 2 mg/kg de méthoxy-8-psoralène, avec une récupération de 99% pour le méthoxy-5-psoralène et de 100% pour le méthoxy-8-psoralène. La répétabilité, à un niveau de concentration de 13,8 mg/kg de méthoxy-5-psoralène, est de 0,73 mg/kg (RSD 5,3%) pour dix déterminations d'un produit du commerce.

La méthode a été appliquée à la détermination du 5-MOP et du 8-MOP dans 119 produits solaires et dans 126 produits de parfumerie (parfums, eaux de toilette, eaux de colognes, «after shaves») vendus sur le marché hollandais. Le 5-MOP a été identifié dans huit produits solaires à des concentrations variant de 10 mg/kg à 40 mg/kg et dans 13 produits de parfumerie, en particulier dans les eaux de cologne dont six échantillons sur 14 étaient positifs, à des concentrations plus basses variant de 2 mg/kg à 6 mg/kg.

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