Ministry of Environment and Energy
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Analytical Chemical Control of Chemical Substances and Chemical Preparations

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1999

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Data sheet

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Department:	Department of Environmental Chemistry
Serial title and no.:	NERI Technical Report No. 297
Publisher:	Ministry of Environment and Energy National Environmental Research Institute ©
URL:	http://www.dmu.dk
Date of publication:	November 1999. Reprinted with revision, March 2000
Referee:	Lars Carlsen
Layout:	Majbritt Pedersen-Ulrich
Please cite as:	Rastogi, S.C., Jensen, G.H., Petersen, M.R., Worsøe, I.M. & Christoffersen, C. (1999): Preservatives in skin creams. Analytical Chemical Control of Chemical Substances and Chemical Preparations. National Environmental Research Institute, Denmark. 70pp. - NERI Technical Report No. 297

Abstract: Contents of 23 selected preservatives were determined in 67 skin cream products to check whether the obligatory ingredient labelling on the products was correct, and the concentrations of the preservatives in the products were within the maximum allowed concentration for individual preservatives. The preservatives selected for the present investigation were: parabens, 2-phenoxy ethanol, benzoic acid, 4-hydroxy-benzoic acid, salicylic acid, sorbic acid, Kathon CG, methyldibromo glutaronitril,
5-bromo-5-nitro-1,3-dioxane, 2-bromo-2-nitropropane-1,3-diol, formaldehyde and formaldehyde releasers. The concentrations of target preservatives in the investigated products were within the maximum allowed concentrations of these. The Danish EPA will check the conformity of preservative labelling with the contents of the target preservatives found in the investigated products.

Keywords:	Skin creams, preservatives, formaldehyde, formaldehyde releasers, parabens, acid preservatives, methyldibromo glutaronitril, 5-bromo-5-nitro-1,3-dioxane, 2-bromo-2-nitropropane-1,3-diol, 2-phenoxy ethanol, Kathon CG, HPLC, Cosmetic Directive
ISBN:	87-7772-503-4
ISSN:	0905-815X
Number of pages:	70
Circulation:	200
Price:	DKK 50,- (incl. 25% VAT, excl. freight)

For sale at:

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Resumé

Ifølge EU's Kosmetik Direktiv/Miljø- og Energiministeriets bekendtgørelse om kosmetiske produkter er indholdsdeklaration obligatorisk på kosmetiske produkter. I nærværende undersøgelse er indholdet af 23 udvalgte konserveringsmidler (22 tilladte og 1 ikke tilladt) bestemt i 67 hudcremer for at kontrollere om indholdsdeklarationen af produkterne var korrekt, samt om indholdet af konserveringsmidlerne var indenfor den maksimalt tilladte koncentration af hvert stof. De udvalgte konserveringsmidler til undersøgelsen er: parabener, 2-phenoxyethanol, benzoesyre, 4-hydroxybenzoesyre, sorbinsyre, salicylsyre, formaldehyd/-formaldehydreleaser, 3:1 blanding af 5-chlor-2-methyl-4-isothiazolinon og 2-methyl-4-isothiazolinon (Kathon CG), 2-brom-2-nitropropan-1,3-diol (Bronopol5-brom-5-ntiropropan-1,3-dioxan (Bronidox) og methyldibromoglutaronitril. Analyserne blev udført ved EU standard analysemetoder samt andre egnede analysemetoder.

En eller flere parabener var tilstede i 86.5% (n=58) af de undersøgte produkter, 2-phenoxyethanol var tilstede i 49% (n=33) af produkterne og formaldehyd/formaldehydreleaser blev fundet i 51% (n=34) af produkterne. Kathon CG var tilstede i 3 produkter, syrekonserveringsmidler (undtagen salicylsyre) i 8 produkter, Bronopol i 5 produkter, og methyldibromoglutaronitril blev fundet i 4 produkter. Blandt udvalgte konserveringsmidler kunne benzyl paraben, salicylsyre og Bronidox ikke påvises i nogle af de undersøgte hudcremer.

Indholdet af de ovennævnte konserveringsmidler i de undersøgte hudcremer overholdt de maximalt tilladte koncentrationer i kosmetiske produkter. På grundlag af de fundende indhold af konserveringsmidler vil Miljøstyrelsen afgøre uoverenstemmelserne vedr. indholdsdeklarationerne.

Arbejdet er udført som bistandsopgave til Miljøstyrelsen

Summary

According to EU's Cosmetic Directive/Danish Statutory Order on Cosmetics, ingredient labelling on cosmetic products is mandatory. In the present investigation, 67 skin creams were analysed for the contents of 23 selected preservatives (22 permitted and 1 non-permitted) to verify whether these products complied with the Cosmetic Directive with respect to ingredient labelling as well as with respect to maximum allowed concentrations of the preservatives. The preservatives selected for the analysis were those which are/have been commonly used in the formulation of cosmetic products: parabens, 2-phenoxy ethanol, benzoic acid, 4-hydroxybenzoic acid, sorbic acid, salicylic acid, formaldehyde and formaldehyde releasers, 3: 1 mixture of 5-chloro-2-methyl-4-isothiazolin-2-one and 2-methyl-4-isothiazolin-2-one (Kathon CG), 2-bromo-2-nitropropane-1,3-diol (Bronopol), 5-bromo-2-nitro-1,3-dioxane (Bronidox) and methyldibromo glutaronitrile. The preservatives in the cosmetic products were analysed by EU standard methods, where available, or by other previously described methods employing HPLC.

One or more parabens were present in 86.5% (n=58) of the investigated products, 2-phenoxy ethanol in 49% (n=33) of the products, and formaldehyde/formaldehyde releasere were present in 51% (n=34) of the products. Kathon CG was found in 3 products, acid preservatives (except salicylic acid) in 8 products, Bronopol in 5 products, and methyldibromo glutaronitril was present in 4 products. Among the target preservatives, benzyl paraben, salicylic acid and Bronidox could not be detected in the investigated skin creams.

The contents of all of the target preservatives in the investigated products were within the maximum allowed concentration of each substance. Danish Environmental Protection Agency (DEPA) will check the conformity of preservative labelling with the contents of the target preservatives found in the investigated products.

Present work has been performed as technical support to DEPA.

1 Introduction

Ingredient labelling on cosmetic products is mandatory according to EU's Cosmetic Directive (1)/Danish Statutory Order on Cosmetic Products (2). It is important that the ingredient labelling is correct, because this can be used by consumers to avoid the use of the products containing specific chemical(s) they can not tolerate. Furthermore, dermatologists may use the ingredient labelling on cosmetic products as a guide to identify specific chemical(s) in a cosmetic product which may be cause of skin reaction in certain person(s).

Fragrances and preservatives are the main causes of allergic contact dermatitis by the use of cosmetics. The preservatives in stay-on cosmetics, for example skin creams, are more often the cause of skin reactions compared to that by wash-off cosmetic products such as shampoos. The Cosmetic Directive regulates the use of preservatives as a positive list of substances (Annex 6 of the Cosmetic Directive) which are permitted to be used in cosmetic formulations at maximum allowed concentration (MAC), for each substance. In the present investigation, contents of some selected preservatives are determined in a series of skin creams to check whether these products comply with the Cosmetic Directive with respect to both correctness of ingredient labelling and the MAC of the preservatives present. The preservatives selected for the present investigation (Table 1) were those which are/have been commonly used in the formulation of cosmetic products: parabens, 2-phenoxy ethanol, benzoic acid, 4-hydroxybenzoic acid, sorbic acid, salicylic acid, formaldehyde and formaldehyde releasers, 3: 1 mixture of 5-chloro-2-methyl-4-isothiazolin-2-one and 2-methyl-4-isothiazolin-2-one (Kathon CG), 2-bromo-2-nitropropane-1,3-diol (Bronopol), 5-bromo-2-nitro-1,3-dioxane (Bronidox) and methyldibromo glutaronitrile.

The present work has been performed as technical support to DEPA.

Table 1. Target Preservatives in the present study.

Preservative	**Maximum allowed concentration % (m/m)**	**Other regulation**
Parabens (esters of 4-hydroxy benzoic acid): Methyl paraben Ethyl paraben Propyl paraben Isobutyl paraben Butyl paraben	0.4% as 4-hydroxy benzoic acid for a paraben, 0.8% as 4-hydroxy benzoic acid for a mixture of parabens	
Benzyl paraben	*Not permitted*	
2-Penoxy ethanol	1.0	
4-Hydroxy benzoic acid	0.4	
Salicylic acid (2-Hydroxy benzoic acid)	0.5	Should not be used in products for children below 3 years, except for shampoos
Sorbic acid	0.6	
Benzoic acid	0.5	
2-Bromo-2-nitropropane-1,3-diol (Bronopol)	0.1	Avoid formation of nitrosamines
5-Bromo-5-nitro-1,3-dioxane (Bronidox)	0.1	Should only be used in wash-off products, avoid formation of nitrosamines
Methyldibromo glutaronitril	0.1	maximum 0.025% in sunscreen products
Kathon CG: 3: 1 mixture of 5-chloro-2-methyl-4-isothiazolin-2-one and 2-methyl-4-isothiazolin-2-one	0.0015	
Formaldehyde	a) 0.2 (except for the oral hygiene products) b) 0.1% (for the oral hygiene products) Concentrations expressed as free formaldehyde	Should not be used in aerosol spray products, Should be labelled “contains formaldehyde”, when the concentration in the finished product is >0.05%.
Formaldehyde releasers: Chloroacetamide Diazolidinyl urea DMDM Hydantoin Imidazolidinyl urea Methamine Paraformaldehyde Quaternium-15	*	*

* maximum allowed concentration and other regulations for formaldehyde releasers are not quoted, because they were checked on the basis of the contents of total and free formaldehyde

2 Samples

The Chemical Inspection Service of the Danish Environmental Protection Agency provided 67 samples of skin creams for the analysis of target preservatives. The samples were randomly selected from the retail outlets in Denmark. All the samples were collected in the period December 1998-January 1999. The identification of the samples analysed in the present investigation is described in Table 2.

Tabel 2. Identification of the investigated skin cream products.

NERI Reg. No.	DEPA No.	Sample name and description	Manufacturer/Importer
9-0001	425	LdB Cream Lotion Rich	Elida Faberrgé, DK
9-0002	426	Hydra-detox, Daily Moisturising Lotion	Biotherm, F
9-0003	427	Esprit de Soleil, Sunfree Tanning Skin Care Treatment	Lancôme, F
9-0004	428	Visible Difference, Perpetual Moisture	Elisabeth Arden, UK
9-0005	429	Multi-Active Day Cream	Clarins, F
9-0006	430	Tokalon Dag Creme, normal og fedtet hud	Werener Petersen, DK
9-0007	431	Anjo Face 'N' Lift, Ansigtscreme	A/S Anjo, DK
9-0008	433	Apropro, Moisturising Body Lotion	E.Tjellesen A/S, DK
9-0009	432	Nothing, Body Cream	E.Tjellesen A/S, DK
9-0010	435	Matas Fugtighedscrem, uparfumeret	Matas A/S, DK
9-0011	434	Women in Orange, Moisturizing Body Lotion	E.Tjellesen A/S, DK
9-0012	436	Elaniq, Swiss Active Care, dagcrem	Matas A/S, DK
9-0013	437	Matas Baby Lotion, uparfumeret	Matas A/S, DK
9-0014	438	Matas Carbamid fugtighedscreme 10%	Matas A/S, DK
9-0015	440	Kamille- Salve, Dr. Scheller Natur Midler	Matas A/S, DK
9-0016	439	Matas Natur Arnica Body Lotion	Matas A/S, DK
9-0017	441	Elsa Hjeronymus Fugtighedcreme, Dagcreme	Hjeronymus Cosmetic AB, SE
9-0018	442	Tiger, Muskel Massage Creme	Heigar & Co. a/s, DK
9-0019	443	Swiss Formula, Collagen-Elastin, Essential moisturiser	Cederroth, DK
9-0020	444	Swiss Formula, Aloe Vera, Hand and Body Lotion	Cederroth, DK
9-0021	445	Nivea Body, Lotion	BDF Beirsdorf, DK
9-0022	446	Nivea Visage, Moisturizing dagcreme	BDF Beirsdorf, DK
9-0023	447	Vanderbilt, Perfumed body lotion	Parfumes Vanderbilt, F
9-0024	449	Natusan pH 5.5, Body lotion	Scansellers a/s, DK
9-0025	450	Natusan Baby Hudlotion uden parfume	Scansellers a/s, DK
9-0026	452	Havre creme	FDB, DK
9-0027	453	Mini risk, Body Lotion	FDB, DK
9-0028	454	Mio Baby, bodylotion, uparfumeret	FDB, DK
9-0029	455	Sanex Bodylotion, Normal Hud	a/s Blumøller, DK
9-0030	456	Softening Lotion for dry skin	Scholl, DK
9-0031	460	Danatekt Creme	Norpharma A/S, DK
9-0032	463	Cosmea Hudlotion med parfume	Nycomed Danmark A/S
9-0033	465	Cosmea Ansigtscreme med parfume	Nycomed DAK A/S
9-0034	466	Mellisa AHA-Frugt Komplex , Urtelotion	Mellisa Natur Produkter, DK
9-0035	468	Decubal, ansigtscreme, uparfumeret	Dumex-Alpharma A/S, DK
9-0036	471	Tee Tree Oil, Active Face Cream	Australian Body Care, DK
9-0037	476	Aloe Vera Creme xtreme	Aloe Vera Group Aps, DK
9-0038	477	Allison Day Cream	Allison of Denmark
9-0039	478	Allison nature fruitcomplex, Anti Aging Day Cream	Allison A/S, DK

Table 2. Continued.

NERI Reg. No.	DE-PANo.	Sample name and description	Manufacturer/Importer
9-0040	479	Proffs Perfectly Soft Lotion	This Is it AB, SE
9-0041	480	FOB Oily Skin Masque	Hennes & Mauritz Cosmetics, DK
9-0042	481	Spa Anti Cellulite Creme	Hennes & Mauritz Cosmetics, DK
9-0043	482	RESQ Super Sparkling Body Softener	Hennes & Mauritz Cosmetics, DK
9-0044	483	DKS Impulsive Body lotion	Dansk Kosmetik Salg, DK
9-0045	484	L.O.G.G. Sport refreshing Body lotion	Hennes & Mauritz Cosmetics, DK
9-0077	448	L' Oréal Plenitude Futur.e	Capilex A/S, DK
9-0078	451	Oil of Ulay, Revtalising Day cream	Procter & Gamble, DK
9-0079	457	Prevense Enriched Night Cream	Constance Carrol Cosmetics PLC, UK
9-0080	458	Clearasil Cream	Procter & Gamble, DK
9-0081	459	Ceredal Lipogel	Preval Dermatica GmbH, DE
9-0082	461	Cliniderm Face Day cream	Nycomed, DK
9-0083	462	Cera di Cupra, Effet Anti-Age	Farmaceutci Dott. Ciccarelli SPA, I
9-0084	464	Vision vitamin Night Cream + Collagen	Nycomed Danmark A/S
9-0085	467	Imedeen Night Cream	Ferrosan A/S, DK
9-0086	469	AHAVA Moisturising Night Cream	Dead Sea Laboratories, Israel
9-0087	470	BM Regenerative Cream 3	BM Research, DK
9-0088	472	Natural sea Beauty, All Day Moisturiser	A.S. Collection, DK
9-0089	473	Vichi Cellactia Gel Corrector	A.S. Collection, DK
9-0090	474	Vichi Lumiactive Anti-Aging day cream	A.S. Collection, DK
9-0091	475	Vichi Lumineuse Sheer Radiance Tinted Moisturiser	A.S. Collection, DK
9-0092	485	Oceanus Body Lotion	The Body Shop, GB
9-0093	486	White Musk body lotion	The Body Shop, GB
9-0094	487	Carrot moisture Cream	The Body Shop, GB
9-0095	488	Aromatherapy BASE, Body Lotion	The Body Shop, GB
9-0096	489	HEMP, Elbow Grease	The Body Shop, GB
9-0097	490	Unparfumed Eder flower Eye gel	The Body Shop, GB
9-0098	491	Light Moisture Lotion	The Body Shop, GB

3 Analysis

The methods for the analyses of the target preservatives are described, in Annex 1-5. The content of CMI+MI (Kathon CG) in the skin creams was determined employing an earlier described high performance liquid chromatography (HPLC) method with UV detection at 275 nm (3, Annex 1). The contents of Bronopol, Bronidox and methyldibromo glutaronitrile in the skin creams were also determined by an earlier described HPLC method employing reductive electrochemical detection (4, Annex 2).

Total and free formaldehyde content in the skin creams were determined by the EU standard method (5, Annex 3). The total formaldehyde content determined by this method also represents the amount of formaldehyde that may be available by the permitted formaldehyde releasers, except for Bronopol and Bronidox, present in a product. The analysis is performed in 3 steps in the following sequence: identification of formaldehyde, spectrophotometric determination of total formaldehyde content in the products containing formaldehyde, and HPLC determination (employing post-column derivatization) of free formaldehyde in the products which contained ≥0.05% total formaldehyde.

EU standard methods were adapted for the determination of parabens and 2-phenoxy ethanol (6, Annex 4), and acid preservatives (7, Annex 5) in skin cream products. These methods are based on HPLC analysis of the preservatives in cosmetic products. The HPLC methods for the analyses of parabens and 2-phenoxy ethanol as well as that for acid preservatives were slightly modified, with respect to flow of the mobile phase, so as to achieve optimal separation of the target preservatives. For the analysis of parabens and 2-phenoxy ethanol, the flow of mobile phase was 0.8 ml/min; and for the acid preservatives, the mobile phase flow was 1.0 ml/min.

For all the methods, the recoveries of target substances from skin creams were determined at two relevant concentrations using an appropriate product. Moreover, repeatability of the test methods were determined by 10 consecutive analysis of standard solutions at two different levels.

As described in the original methods (Annex 1-5), the parabens, 2-phenoxy ethanol and acid preservatives were determined empolying internal standard methods, and other preservatives were quantified employing external standard methods.

In certain cases, samples spiked with 2-phenoxyethanol/methyl paraben were analysed to confirm the identification of these substances. To confirm the presence of a declared preservative in several cases, it was necessary to use the 2x recommended (in the standard methods) amount of the samples for the analysis.

4 Results and Discussion

In the present investigation contents of 23 selected preservatives were analysed in cosmetic products by previously described methods (3-7, Annex 1-5). The preservatives, in the samples were identified by comparing their HPLC retention times and 200-400 nm UV-spectra (where applicable) with the retention times and UV-spectra of the standard substances analysed under the same conditions. Performances of all of the methods for quantitative analysis, checked as described in the original methods, were found to be satisfactory:
The recoveries of all target substances, except (formaldehyde), determined at two different levels were 88-103%. Recovery of formaldehyde was not investigated, because the source of formaldehyde in the products could be various formaldehyde releasers. The detection/quantification limits of parabens were 5-30 ppm; 5 ppm for 5-bromo-5-nitro-1,3-dioxane and methyldibromo glutatronitril; 10 ppm for 4-hydroxybenzoic acid and sorbic acid; 25 ppm for 2-phenoxy ethanol, benzoic acid, salicylic acid and 2-bromo-2-nitropropane-1,3 diol; and 1ppm for formaldehyde. The calibration curves of all preservatives studied were linear (r^2 >0.995) in the investigated concentration ranges (Annex 1-5).

The typical chromatograms of the standard preservatives and the samples containing target preservatives are described in Figures 1-6. The preservatives labelled on each product and the contents of the target preservatives in the products are described in Table 3. The results have not been corrected for recoveries.

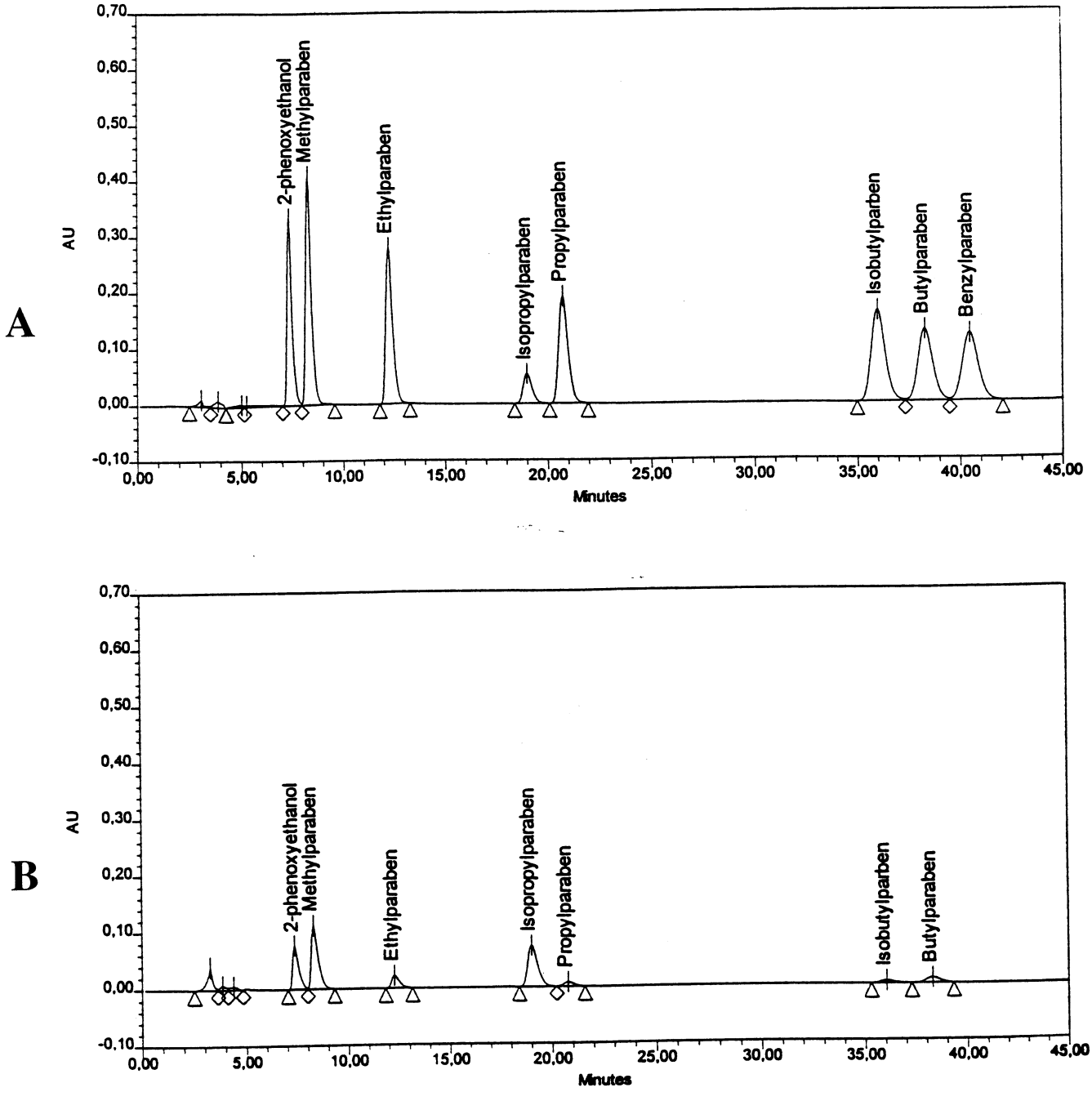
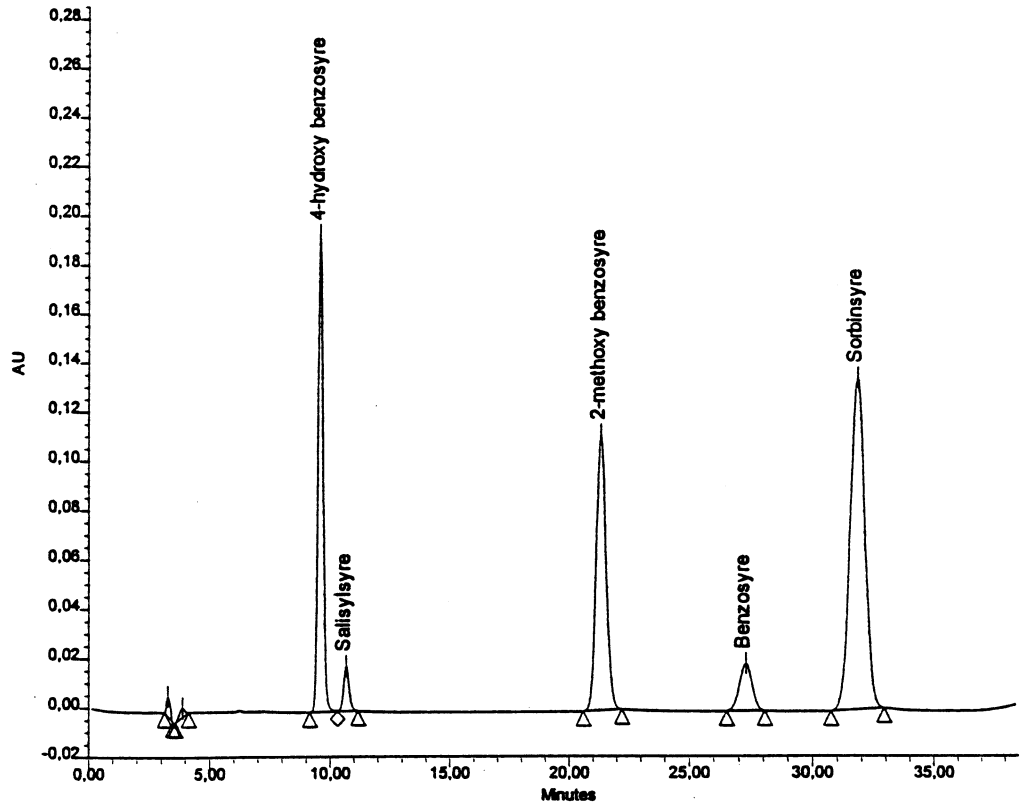


Figure 1

A: HPLC analysis of a standard mixture of parabens and 2-phenoxy ethanol. Standards: isopropyl paraben internal standard 50 µg/ml, other parabens 200 µg/ml, 2-phenoxy ethanol 1 mg/ml.

B: HPLC analysis of parabens and 2-phenoxy ethanol in the sample 9-0039/478.

A



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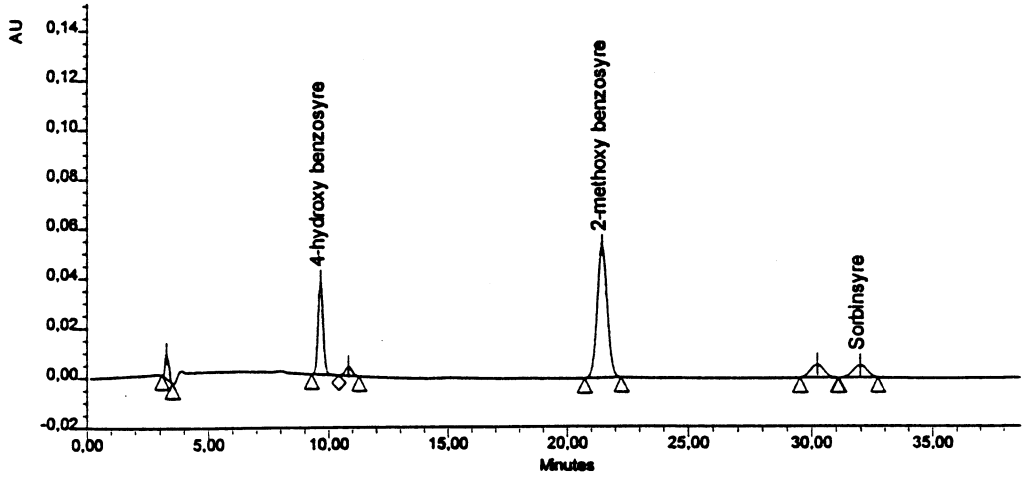
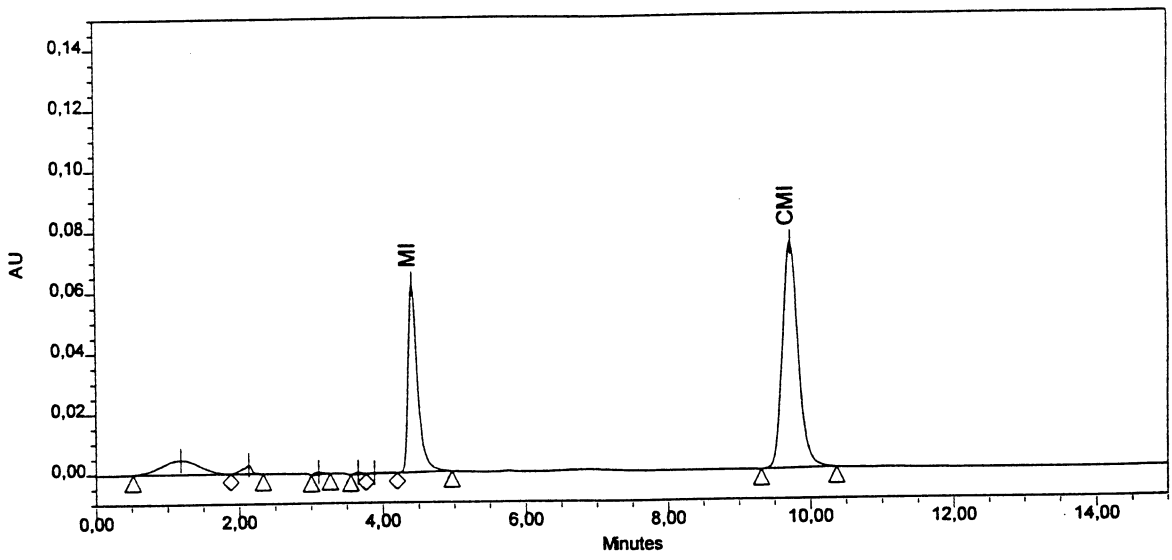


Figure 2

A: HPLC analysis of a standard mixture of acid preservatives. Standards: 2-methoxy benzoic acid internal standard 1 mg/ml, sorbic acid and 4-hydroxy benzoic acid 200 µg/ml, benzoic acid and salicylic acid 200 µg/ml.

B: HPLC analysis of acid preservatives in the sample 9-0097/490. The UV spectrum (220-400 nm) of the sample peak with retention time corresponding to salicylic acid, did not match with the UV-spectrum of standard salicylic acid.

A



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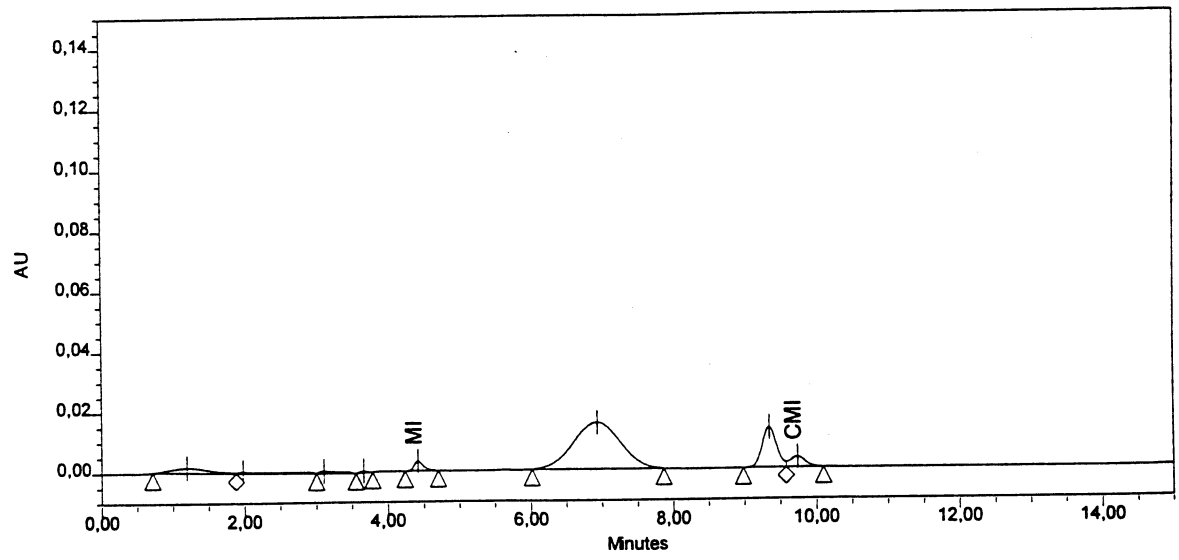
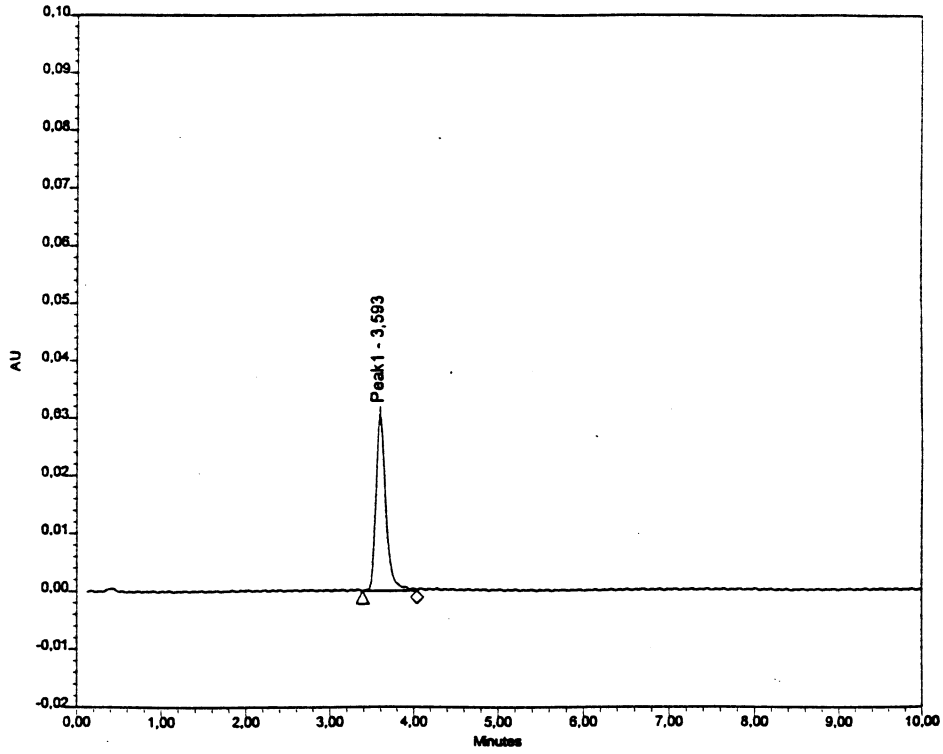


Figure 3

A: HPLC analysis of standard Kathon CG (MI+CMI). Standard Kathon CG: MI 2.9 ppm, CMI 8.7 ppm.

B: HPLC analysis of Kathon CG in the sample 90008/433.

A



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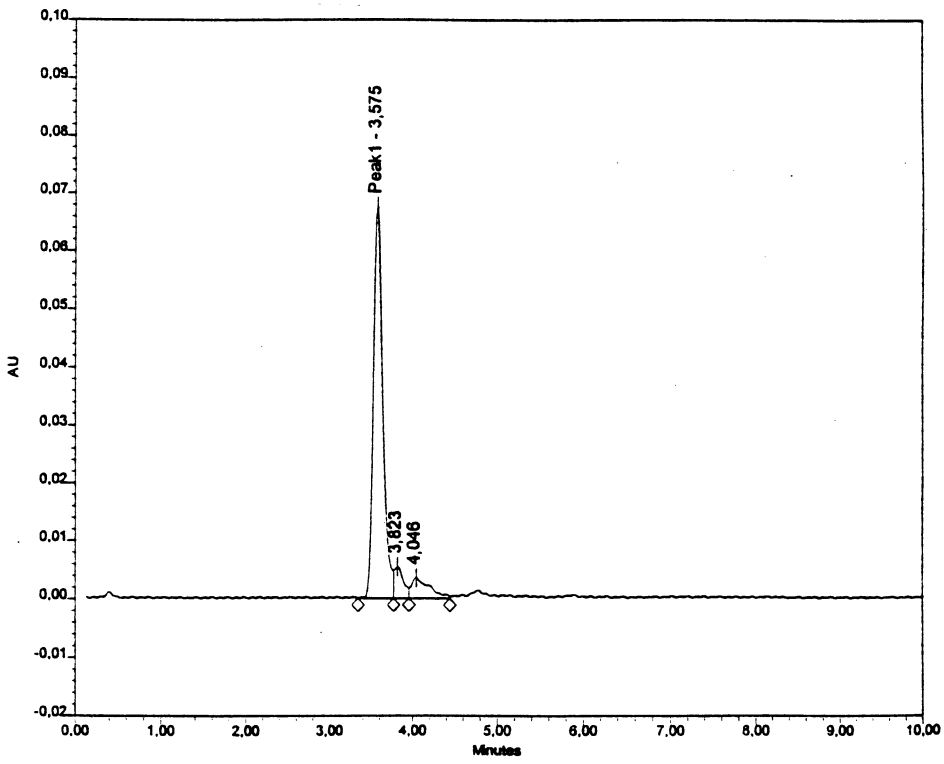
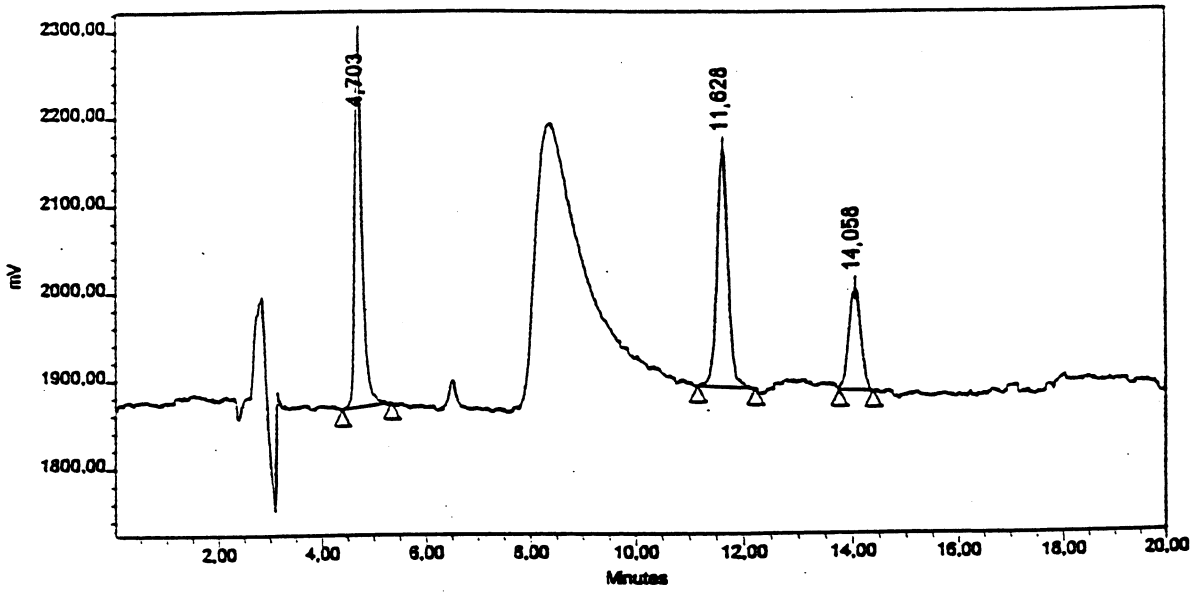


Figure 4

A: HPLC analysis of free formaldehyde. Formaldehyd standard 200 µg/ml. Peak 1: free formaldehyde.

B: HPLC analysis of free formaldehyde in the sample 9-0023/447. Peak 1: free formaldehyde.

A



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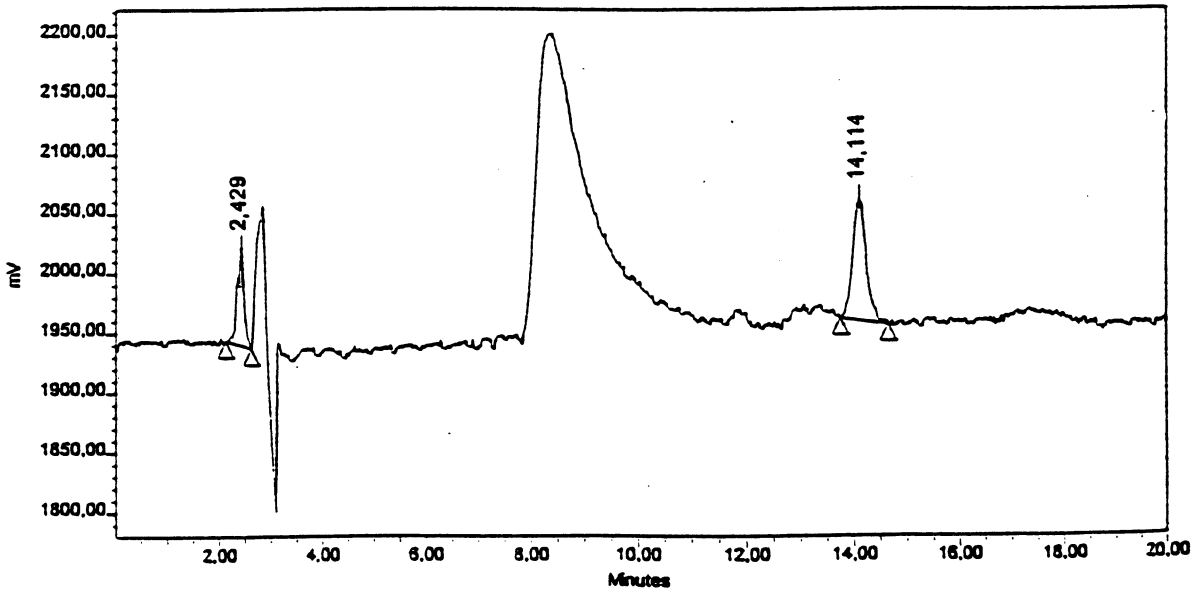
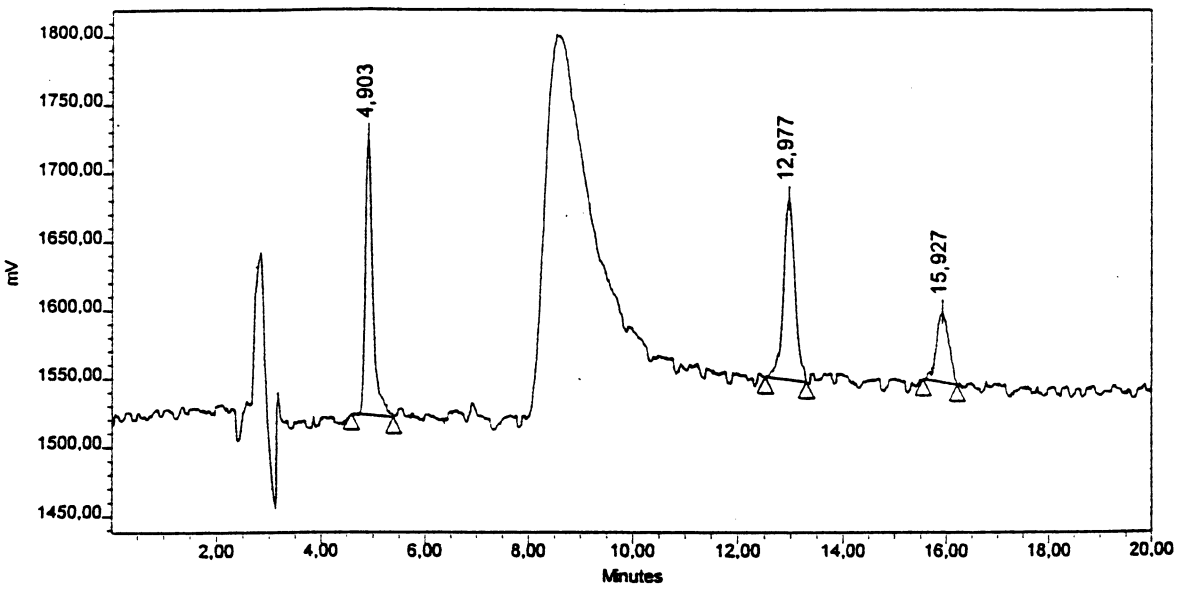


Figure 5

A: HPLC analysis of standard brominated preservatives. t_R 4.703 min: 2-bromo-2-nitropropane-1,3-diol, 80 µg/ml; t_R 11.268 min: 5-bromo-5-nitro-1,3-dioxane, 24 µg/ml; and t_R 14.058 min: methyldibromo glutaronitril 24 µg/ml.

B: HPLC analysis of brominated preservatives in the sample 9-0037/476. t_R 14.114 min: methyldibromo glutaronitril.

A



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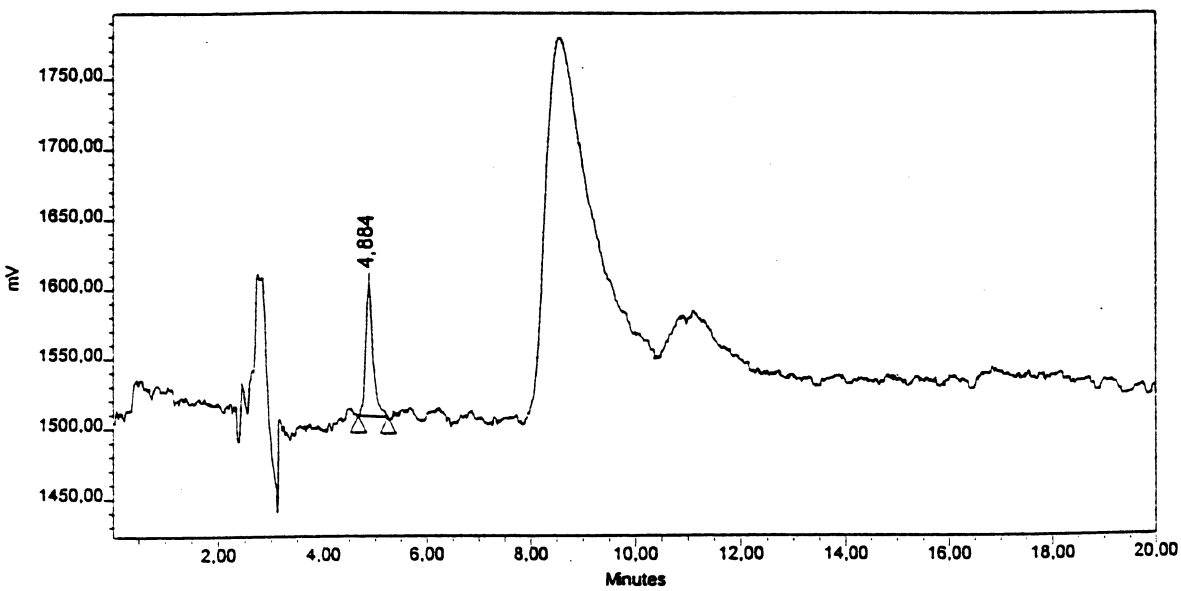


Figure 6

A: HPLC analysis of standard brominated preservatives. t_R 4.903 min: 2-bromo-2-nitropropane-1,3-diol, 32 µg/ml; t_R 12.997min: 5-bromo-5-nitro-1,3-dioxane, 9.6 µg/ml; and t_R 15.927min: methyldibromo glutaronitril 9.6 µg/ml.

B: HPLC analysis of brominated preservatives in the sample 9-0088/472. t_R 4.884 min: 2-bromo-2-nitropropane-1,3-diol.

One or more of the target parabens were present in 86.5% (n=58) of the investigated samples. Among these products, the paraben content (total of all parabens present in a product) was 0.024-0.511%. Methyl paraben (0.0074-0.4092%) was present in 85% of the sample, ethyl paraben (0.026-0.100%) in 43% of the products, propyl paraben (0.024-0.206%) in 70% of the products, isobutyl paraben (0.008-0.048) in 9% of the products, and butyl paraben (0.003-0.100%) was found in 36% of the investigated products. Benzyl paraben was not detected in any of the investigated products. The total content of all parabens present in a sample as well as the contents of individual parabens in the investigated products were within the maximum allowed concentration(s) according to the Cosmetic Directive (1).

2-Phenoxy ethanol (0.023-0.957%) was found to be present in 49% of the investigated products (n = 33). The content of 2-Phenoxy ethanol in the investigated skincreams was within the maximum allowed concentration (1.00%) of this substance in cosmetic products.

Formaldehyde by the EU standard method (5) is analysed under acidic conditions. Thus, the formaldehyde releasers 2-bromo-2-nitropropane-1,3-diol (Bronopol) and 5-bromo-5-nitro-1,3-dioxane (Bronidox) may not be detected by the EU standard method, because they release formaldehyde under basic conditions. Formaldehyde (and formaldehyde releasers, except Bronopol and Bronidox) was detected in 51% (n = 34) of the investigated products. Total content of formaldehyde in these products was 0.0001-0.0848%. Three of the products, containing ≥0.05% formaldehyde, were further analysed for the content of free formaldehyde. The free formaldehyde content in all 3 products was < 0.05%.

Kathon CG (7.25-12.2 ppm) was present in 3 of the investigated products: 9-0008/433, 9-0009/432 and 9-0011/434. The ratio of CMI:MI content in these products were 2.6:1, 2.7:1 and 1.4:1 respectively. The CMI:MI ratio in products containing Kathon CG should be 3:1. This was, however, not the case with the sample 9-0011/434. It seems that relatively high amount of CMI has metabolized in the product 9-0011/434.

Two of the investigated samples (9-0037/476, 9-0079/457) were found to contain benzoic acid (0.0026% and 0.0366% respectively); 4-hydroxybenzoic acid (0.006-0.042%) was present in 3 samples (9-0030/456, 9-0041/480 and 9-0097/490); and 4 of the investigated samples (9-0092/-485, 9-0093/486, 9-0095/488 and 9-0097/490) were found to contain sorbic acid (0.015-0.220%). Salicylic acid was not found to be present in any of the investigated products.

Four of the samples were found to contain methyldibromo glutaronitril (0.0100-0.0167%) and 5 samples contained 2-bromo-2-nitropropane-1,3-diol (0.0124-0.1096%). 5-bromo-5-nitro-1,3-dioxane was not present in any of the samples. In all the samples, except sample 9-0042/481, the contents of the brominated preservatives were within the maximum allowed concentration 0.1%. Sample no. 9-0042/481 contained 0.1096% 2-bromo-2-nitropropane-1,3-diol. As the relative standard deviation of the method is approximately 12%, this sample has been considered to be adhering to the maximum allowed concentration.

The conformity of the preservative labelling on the products with the preservative contents found the present study will be checked by DEPA

Table 3. Preservative labelling and contents of target preservatives in the investigated products.

NERI Reg.No.	DEPA No.	Preservative labelling on the product	Preservatives found	Concentration % (m/m)
9-0001	425	Methyl paraben	Methyl paraben	0.0718
		Ethyl paraben	Ethyl paraben	0.0243
9-0002	426	Phenoxyethanol	2-Phenoxyethanol	0.3782
		Methyl paraben	Methyl paraben	0.2051
		Ethyl paraben	Ethyl paraben	0.0223
		Propyl paraben	Propyl paraben	0.0405
		Butyl paraben	Butyl paraben	0.0158
9-0003	427	Phenoxyethanol	2-Phenoxyethanol	0.4053
		Methyl paraben	Methyl paraben	0.2814
		Ethyl paraben	Ethyl paraben	0.0166
		Propyl paraben	Propyl paraben	0.0636
		Butyl paraben	Butyl paraben	0.0142
9-0004	428	Methyl paraben	Methyl paraben	0.2147
		Ethylparaben	Ethyl paraben	0.1011
		Diazolidinyl urea	Formaldehyde	0.0133 (total)
9-0005	429	-	2-Phenoxyethanol	0.0450
		Methyl paraben	Methyl paraben	0.4022
		Ethyl paraben	Ethyl paraben	0.0411
		Propyl paraben	Propyl paraben	0.0258
		Butyl paraben	Butyl paraben	0.0417
		-	Formaldehyde	0.0007 (total)
		Chlorhexidin digluconate	n.a.	-
9-0006	430	Phenoxyethanol	2-Phenoxyethanol	0.3669
		Parabens	Methyl paraben	0.1757
			Ethyl paraben	0.0434
			Propyl paraben	0.0282
			Butyl paraben	0.0418
		Imidazolidinyl urea	Formaldehyde	0.0003 (total)
9-0007	431	-	2-Phenoxyethanol	0.4236
		Methyl paraben	Methyl paraben	0.0616
		Ethyl paraben	Ethyl paraben	0.0135
		Propyl paraben	Propyl paraben	0.0052
		Butyl paraben	Butyl paraben	0.0116
9-0008	433	Methyl paraben	Methyl paraben	0.2658
		Methylchloroisothiazolinone	Methylchloroisothiazolinone	8.33 ppm
		Methylisothiazolinone	Methylisothiazolinone	3.37 ppm
9-0009	432	Methyl paraben	Methyl paraben	0.2780
		Methylchloroisothiazolinone	Methylchloroisothiazolinone	6.28 ppm
		Methylisothiazolinone	Methylisothiazolinone	2.33 ppm
9-0010	435	Phenoxyethanol	2-Phenoxyethanol	0.1283
		Methyl paraben	Methyl paraben	1.1292
		Ethyl paraben	Ethyl paraben	0.0327
		Propyl paraben	Propyl paraben	0.0166
		Butyl paraben	Butyl paraben	0.0316
9-0011	434	Methyl paraben	Methyl paraben	0.2882
		Methylchloroisothiazolinone	Methylchloroisothiazolinone	4.25 ppm
		Methylisothiazolinone	Methylisothiazolinone	3.00 ppm

n.a.: not analysed, n.d.: not detected

Table 3. Continued.

NERI Reg.No.	DEPA No.	Preservative labelling on the product	Preservatives found	Concentration % (m/m)
9-0012	436	-	Formaldehyde	0.0005 (total)
9-0013	437	Methyl paraben	Methyl paraben	0.1758
		Ethyl paraben	Ethyl paraben	0.1438
		Imidazolidinyl urea	Formaldehyd	0.0332 (total)
9-0014	438	Phenoxyethanol	2-Phenoxyethanol	0.1332
		Methyl paraben	Methyl paraben	0.2044
		Ethyl paraben	Ethyl paraben	0.0052
		Propyl paraben	Propyl paraben	0.0024
		Butyl paraben	Butyl paraben	0.0035
9-0015	440	Methyl paraben	Methyl paraben	0.1504
		Propyl paraben	Propyl paraben	0.0098
		Dehydroacetic acid	n.a.	-
9-0016	439	-	-	-
9-0017	441	Phenoxyethanol	2-Phenoxyethanol	0.1567
		Methyl paraben	Methyl paraben	0.1131
		Ethyl paraben	Ethyl paraben	0.0317
		Propyl paraben	Propyl paraben	0.0154
		Butyl paraben	Butyl paraben	0.0439
9-0018	442	Methyl paraben	Methyl paraben	0.1040
		-	Propyl paraben	0.0537
		-	Formaldehyde	0.0001 (total)
9-0019	443	Phenoxyethanol	2-Phenoxyethanol	0.8025
		Methyl paraben	Methyl paraben	0.2100
		Ethyl paraben	Ethyl paraben	0.0996
		-	Formaldehyde	0.0003 (total)
9-0020	444	Phenoxyethanol	2-Phenoxyethanol	0.7478
		Methyl paraben	Methyl paraben	0.1975
		Propyl paraben	Propyl paraben	0.0800
		-	Formaldehyde	0.0009 (total)
9-0021	445	Phenoxyethanol	2-Phenoxyethanol	0.4476
		Methyl paraben	Methyl paraben	0.2829
		Ethyl paraben	Ethyl paraben	0.0199
		Propyl paraben	Propyl paraben	0.0804
		Butyl paraben	Butyl paraben	0.0172
9-0022	446	Phenoxyethanol	Phenoxyethanol	0.4092
		Methyl paraben	Methyl paraben	0.0478
		Ethyl paraben	Ethyl paraben	0.0112
		Propyl paraben	Propyl paraben	0.0059
		Butylparaben	Butylparaben	0.0124
		Methyldibromo glutaronitril	Methyldibromo glutaronitril	0.0149
		-	Formaldehyde	0.0028 (total)

n.a.: not analysed, n.d.: not detected

Table 3. Continued.

NERI Reg.No.	DEPA No.	Preservative labelling on the product	Preservatives found	Concentration % (m/m)
9-0023	447	Methyl paraben	Methyl paraben	0.0074
		Ethyl paraben	Ethyl paraben	0.0026
		Propyl paraben	Propyl paraben	0.0162
		Butyl paraben	Butyl paraben	0.0030
		Diazolidinyl urea, Quaternium 15	Formaldehyde	0.0848 (total)
				0.0458 (free)
		Hexamidine diisethionate	n.a.	-
9-0024	449	Methyl paraben	Methyl paraben	0.1498
		Ethyl paraben	Ethyl paraben	0.0402
		Propyl paraben	Propyl paraben	0.0216
		Butyl paraben	Butyl paraben	0.0502
9-0025	450	Methyl paraben	Methyl paraben	0.1572
		Ethyl paraben	Ethyl paraben	0.0424
		Propyl paraben	Propyl paraben	0.0201
		Butyl paraben	Butyl paraben	0.0409
		-	Formaldehyde	0.0059 (total)
9-0026	452	Methyl paraben	Methyl paraben	0.1375
		Propyl paraben	Propyl paraben	0.0664
		2-Bromo-2-nitropropane-1,3-diol	2-Bromo-2-nitropropane-1,3-diol	0.0124
		-	Formaldehyde	0.0179 (total)
9-0027	453	Phenoxyethanol	Phenoxyethanol	0.0601
		Methyldibromo glutatonitril	Methyldibromo glutatonitril	0.0111
9-0028	454	Phenoxyethanol	2-Phenoxyethanol	0.9565
		Methyl paraben	Methyl paraben	0.1483
		Ethyl paraben	Ethyl paraben	0.0312
		Propyl paraben	Propyl paraben	0.0185
		Butyl paraben	Butyl paraben	0.0375
		-	Formaldehyde	0.0006 (total)
9-0029	455	Phenoxyethanol	2-Phenoxyethanol	0.7086
		Methyl paraben	Methyl paraben	0.1860
9-0030	456	Methyl paraben	Methyl paraben	0.0758
		Propyl paraben	Propyl paraben	0.0958
		Imidazolidinyl urea	Formaldehyde	0.0388 (total)
		-	4-hydroxy benzoic acid	0.0062
9-0031	460	Phenoxyethanol	2-Phenoxyethanol	0.1994
		Methyl paraben	Methyl paraben	0.0735
		Ethyl paraben	Ethyl paraben	0.0163
		Propyl paraben	Propyl paraben	0.0094
		Butyl paraben	Butyl paraben	0.0189
9-0032	463	Methyl paraben	Methyl paraben	0.2294
		Propyl paraben	Propyl paraben	0.0573
9-0033	465	Methyl paraben	Methyl paraben	0.3317
		Propyl paraben	Propyl paraben	0.1592

n.a.: not analysed, n.d.: not detected

Table 3. Continued.

NERI Reg.No.	DEPA No.	Preservative labelling on the product	Preservatives found	Concentration % (m/m)
9-0034	466	-	2-Phenoxyethanol	0.4590
		-	Methyl paraben	0.0801
		-	Ethyl paraben	0.0194
		-	Propyl paraben	0.0090
9-0035	468	Methyl paraben	Methyl paraben	0.2401
		Propyl paraben	Propyl paraben	0.1249
		Benzyl alcohol	n.a.	-
9-0036	471	Methyl paraben	Methyl paraben	0.1968
		Propyl paraben	Propyl paraben	0.1587
		Imidazolidinyl urea	Formaldehyde	0.0333 (total)
9-0037	476	Phenoxyethanol	2-Phenoxy ethanol	0.0914
		Methyldibromo glutaronitril	Methyldibromo glutaronitril	0.0167
		-	Benzoic acid	0.0366
9-0038	477	Phenoxyethanol	2-Phenoxyethanol	0.4409
		Methyl paraben	Methyl paraben	0.1236
		Ethyl paraben	Ethyl paraben	0.0256
		Propyl paraben	Propyl paraben	0.0127
		-	Isobutyl paraben	0.0211
		Butyl paraben	Butyl paraben	0.0241
9-0039	478	Phenoxyethanol	2-Phenoxyethanol	0.5098
		Methyl paraben	Methyl paraben	0.1287
		Ethyl paraben	Ethyl paraben	0.0322
		Propyl paraben	Propyl paraben	0.0156
		-	Isobutyl paraben	0.0295
		Butyl paraben	Butyl paraben	0.0302
		-	Formaldehyde	0.0510 (total) 0.0106 (free)
9-0040	479	Methyl paraben	Methyl paraben	0.1607
		Propyl paraben	Propyl paraben	0.0619
		2-Bromo-2-nitropropane-1,3-diol	2-Bromo-2-nitropropane-1,3-diol	0.0203
		-	Formaldehyde	0.0022 (total)
9-0041	480	Methyl paraben	Methyl paraben	0.1608
		-	Ethyl paraben	0.0072
		Propyl paraben	n.d.	-
		-	4-hydroxy benzoic acid	0.0275
9-0042	481	Phenoxyethanol	2-Phenoxyethanol	0.2748
		Methyl paraben	Methyl paraben	0.1962
		Ethyl paraben	Ethyl paraben	0.0103
		Propyl paraben	Propyl paraben	0.0074
		Isobutyl paraben	Isobutyl paraben	0.0149
		Butyl paraben	Butyl paraben	0.0062
		2-Bromo-2-nitropropane-1,3-diol	2-Bromo-2-nitropropane-1,3-diol	0.1096
		-	Formaldehyde	0.0054 (total)
9-0043	482	Methyl paraben	Methyl paraben	0.1255
		Propyl paraben	Propyl paraben	0.1047
		Imidazolidinyl urea	Formaldehyde	0.0194 (total)

n.a.: not analysed, n.d.: not detected

Table 3. Continued.

NERI Reg.No.	DEPA No.	Preservative labelling on the product	Preservatives found	Concentration % (m/m)
9-0044	483	Phenoxy ethanol	2-Phenoxyethanol	0.0812
		Methyldibromo glutaronitril	Methyldibromo glutaronitril	0.0100
		-	Formaldehyde	0.0004 (total)
9-0045	484	Phenoxyethanol	2-Phenoxyethanol	0.3573
		Methyl paraben	Methyl paraben	0.0695
		Ethyl paraben	Ethyl paraben	0.0140
		Propyl paraben	Propyl paraben	0.0093
		Isobutyl paraben	Isobutyl paraben	0.0080
		Butyl paraben	Butyl paraben	0.0147
		-	Formaldehyde	0.0001 (total)
9-0077	448	-	2-Phenoxyethanol	0.0228
		Methyl paraben	Methyl paraben	0.2778
		Propyl paraben	Propyl paraben	0.0516
		Chlorphensin	n.a.	-
		Sodium dehydroacetate	n.a.	-
9-0078	451	Phenoxyethanol	2-Phenoxyethanol	0.4256
		Methyl paraben	Methyl paraben	0.2226
		Propyl paraben	Propyl paraben	0.1231
		Imidazolidinyl urea	Formaldehyde	0.0197 (total)
9-0079	457	Methyl paraben	Methyl paraben	0.0887
		Propyl paraben	Propyl paraben	0.1192
		Diazolidinyl urea	Formaldehyde	0.0499 (total)
				0.0234 (free)
		Sodium benzoate	Benzoic acid	0.0026
9-0080	458	Methyl paraben	Methyl paraben	0.2010
		Propyl paraben	Propyl paraben	0.0224
		-	Formaldehyde	0.0002 (total)
		Triclosan	n.a.	-
9-0081	459	-	-	-
9-0082	461	Methyl paraben	Methyl paraben	0.1942
		Propyl paraben	Propyl paraben	0.0492
		-	Formaldehyde	0.0001 (total)
9-0083	462	-	-	-
9-0084	464	Methyl paraben	Methyl paraben	0.2235
		Propyl paraben	Propyl paraben	0.0547
9-0085	467	Methyl paraben	Methyl paraben	0.2115
		Propyl paraben	Propyl paraben	0.1671
		Butyl paraben	Butyl paraben	0.0999
		Imidazolidinyl urea	Formaldehyde	0.0298 (total)
9-0086	469	Methyl paraben	Methyl paraben	0.2953
		Propyl paraben	Propyl paraben	0.2057
		Imidazolidinyl urea	Formaldehyde	0.0326 (total)
		2-Bromo-2-nitropropane-1,3-diol	2-Bromo-2-nitropropane-1,3-diol	0.0396

n.a.: not analysed, n.d.: not detected

Table 3. Continued.

NERI Reg.No.	DEPA No.	Preservative labelling on the product	Preservatives found	Concentration % (m/m)
9-0087	470	-	Formaldehyde	0.0005 (total)
9-0088	472	Phenoxyethanol	2-Phenoxyethanol	0.3711
		Methyl paraben	Methyl paraben	0.2592
		Ethyl paraben	Ethyl paraben	0.0172
		Propyl paraben	Propyl paraben	0.1674
		Butyl paraben	Butyl paraben	0.0209
		Imidazolidinyl urea	Formaldehyde	0.0220 (total)
		2-Bromo-2-nitropropane-1,3-diol	2-Bromo-2-nitropropane-1,3-diol	0.0163
9-0089	473	-	Formaldehyde	0.0003 (total)
9-0090	474	Phenoxyethanol	2-Phenoxyethanol	0.4179
		Methyl paraben	Methyl paraben	0.1742
		Chlorphensin	n.a.	-
9-0091	475	Methyl paraben	Methyl paraben	0.1622
		Propyl paraben	Propyl paraben	0.1053
		Butyl paraben	Butyl paraben.	0.0372
		Chlorphensin	n.a.	-
9-0092	485	Phenoxyethanol	2-Phenoxyethanol	0.5138
		Methyl paraben	Methyl paraben	0.3409
		Propyl paraben	Propyl paraben	0.1649
		Benzyl alcohol	n.a.	-
		Potassium sorbate	Sorbic acid	0.2193
		-	Formaldehyde	0.0003 (total)
9-0093	486	Phenoxyethanol	2-Phenoxyethanol	0.5246
		Methyl paraben	Methyl paraben	0.1383
		Ethyl paraben	Ethyl paraben	0.0296
		Propyl paraben	Propyl paraben	0.0141
		Butyl paraben	Butyl paraben	0.0319
		Cetrimonium bromide	n.a.	-
		-	Formaldehyde	0.0005 (total)
		Polysorbate	Sorbic acid	0.2193
9-0094	487	-	2-Phenoxyethanol	0.5886
		Methyl paraben	Methyl paraben	0.1972
		Propyl paraben	Propyl paraben	0.1543
		Benzyl alcohol	n.a.	-
9-0095	488	Phenoxyethanol	2-Phenoxyethanol	0.5252
		Methyl paraben	Methyl paraben	0.3028
		Propyl paraben	Propyl paraben	0.1589
		Benzyl alcohol	n.a.	-
		Potassium sorbate	Sorbic acid	0.2207
		-	Formaldehyde	0.0003 (total)
9-0096	489	Propyl paraben	Propyl paraben	0.1765

n.a.: not analysed, n.d.: not detected

Table 3. Continued.

NERI Reg.No.	DEPA No.	Preservative labelling on the product	Preservatives found	Concentration % (m/m)
9-0097	490	Phenoxyethanol	2-Phenoxyethanol	0.6711
		Methyl paraben	Methyl paraben	0.0753
		Ethyl paraben	Ethyl paraben	0.0254
		Propyl paraben	Propyl paraben	0.0097
		Isobutylparaben	Isobutylparaben	0.0152
		Butyl paraben	Butyl paraben	0.0082
		-	4-Hydroxy benzoic acid	0.0417
		-	Sorbic acid	0.0113
9-0098	491	Phenoxyethanol	2-Phenoxyethanol	0.7918
		Methyl paraben	Methyl paraben	0.2307
		Ethyl paraben	Ethyl paraben	0.0500
		Propyl paraben	Propyl paraben	0.0351
		-	Isobutylparaben	0.0481
		Butyl paraben	Butyl paraben	0.0470
		-	Formaldehyde	0.0005 (total)

n.a.: not analysed, n.d.: not detected

5 References

1. Council Directive 76/768/EEC of 27th July on the approximation of the laws of Member States relating to cosmetic products. Official Journal of EEC, No. L262, 27.9.1976, p. 169.

2. Statutory Order of the Danish Ministry of Environment and Energy, No. 303 of 18th May 1998, on Cosmetic Products.

3. Rastogi S.C. (1990) Kathon CG and cosmetic products. Contact Dermatitis 22, 155-160.

4. Rastogi S.C. and Johansen S.S. (1995) Comparison of HPLC methods for the determination of 1,2-dibromo-2,4-dicyano-butane in cosmetic products. J. Chromatogr. A 692, 53-57

5. EC Commissions Directive of 4th April 1990 (90/207/EC): IV Identification and determination of free formaldehyde. Official Journal of EC, No. L108, 28.4.1990, p. 92-101.

6. EU Commissions Directive of 2nd July1996 (96/45/EC): Identification and determination 2-phenoxy ehanol, 1-phenoxypropan-2-ol, and methyl, ethyl-, propyl-, butyl-, and benzyl-4-hydroxy-benzoate in cosmetic products. Official Journal of EC, No. L213, 22.8. 96, p. 8-15.

7. EU Commissions Directive of 7th July 1995 (95/32/EC): 1. Identification and determination of benzoic acid, 4-hydroxybenzoic acid, sorbic acid, salicylic acid and propionic acid in cosmetic products. Official Journal of EC, No. L178, 28.7. 95, p. 20-29.

Appendix 1

Method for the analysis of 2-methyl-4-isothiazolin-3-one (MI) and 5-chloro-2-methyl-4-isothiazolin-3-one (CMI) in cosmetic products

1. Scope and Field of Application:

This method is used for the determination of Kathon CG (1: 3 mixture of MI and CMI) in cosmetic products. The method is suitable for analytical control of maximum allowed concentration of Kathon CG (15 ppm) in cosmetic products.

2. Principle

The sample is suspended in methanol/0.4% acetic acid and treated with ultrasound to get a homogeneous suspension. The sample suspension is filtered and analysed by HPLC employing UV detection.

3. Reagents

3.1 Water, Millipore.
3.2 Acetic acid, analytical grade
3.3 0.4% acetic acid: 4 ml acetic acid (3.2) diluted to 1000 ml in a volumetric flask.
3.4 Acetonitrile, HPLC grade
3.5 Methanol, HPLC grade
3.7 Eluent 1: acetonitrile/methanol/0.4% acetic acid (10/10/80)
3.8 Kathon CG 1.54% from Rhom & Haas (NERI No. 2306-07): 0.385% MI and 1.155% CMI.
3.9 Stock solution of Kathon CG: Ca. 0,3 g Kathon CG is weighed accurately in a 10 ml measuring flask, and filled up to the mark with methanol/0.4% acetic acid (20/80).
Stock solution is stable for 1 week, when stored at 4°C
3.9 Calibration standard solutions: Stock solution of Kathon CG (3.9) was diluted in methanol/0.4% acetic acid (20:80) as described below:
0,25 ml stock solution in a 2 ml volumetric flask
0,25 ml stock solution in a 5 ml volumetric flask
0,25 ml stock solution in a 10 ml volumetric flask
0,25 ml stock solution in a 25 ml volumetric flask
0,25 ml stock solution in a 50 ml volumetric flask
Calibration standard solution should be prepared fresh every day.

When 0.30000 g Kathon CG is weighed for stock solution, the concentrations of MI and CMI in the calibration standard solutions will be as follows:

2 ml volumetric flask will contain 57.75 ppm Kathon CG (14.4 ppm MI and 43.3 ppm CMI)
5 ml volumetric flask will contain 23.10 ppm Kathon CG (5.8 ppm MI and 17.3 ppm CMI)
10 ml volumetric flask will contain 11.55 ppm Kathon CG (2.9 ppm MI and 8.7 ppm CMI)
25 ml volumetric flask will contain 4.62 ppm Kathon CG (1.16 ppm MI and 3.47 ppm CMI)
50 ml volumetric flask will contain 2.31 ppm Kathon CG (0.58 ppm MI and 1.73 ppm CMI)

4. Apparatus, Glass- and Plasticware

4.1 Normal laboratory glass- and plasticware

4.2 Ultrasonic bath

4.3 HPLC system with UV-detector: Waters 616 pump, Waters 717 autosamplers, Waters 996 PDA detector, Millenium 3.2.0 software for control of HPLC system and chromatographic data.

4.4 HPLC-column: Hypersil 5 (C 18), particle size 5 µm, stainless steel 250 mm x 4.6 mm, from Phenomenex, precolumn packed with C18 silica.

4.5 Sartorius SRP 25 filter, 0.45µ

5. Sample preparation

- mix the sample well before use
- weigh accurately ca. 2.00g ± 0.1g sample in a 25 ml volumetric flask, and add ca. 20 ml methanol/0.4% acetic acid (20:80)
- treat the sample by ultrasound for 10 min
- fill the measuring flask up to the mark and shake well
- filter the sample using a membrane filter (4.5)
- analyse the filtrate by HPLC for the MI and CMI content

All the samples should be prepared in duplicate. The sample extracts should be analysed within 24 hours.

6. Analysis

The samples and the calibration standards were analysed by HPLC under following conditions

Column temperature:	22°C
Run:	Isocratic
Flow:	1.0 ml/min
Eluent:	Methanol/acetonitril/0.4% acetic acid (10:10:80)
Injection volume:	25 µl
Run time:	20 min
Detection wavelength:	275 nm

For the preparation of calibration curve, all of the calibration standard solution should be analysed each day in the beginning and at the end of a run. In the sample set suitable calibration standard solution should be bracketed, i.e. suitable calibration standard solution(s) should be run after every 3-4 samples.

7. Calculation

Prepare the calibration curves for the preservatives using amounts of MI and CMI in calibration solutions and corresponding detector response (area count of chromatographic peak). From the area counts of a sample peak and the peak of a standard preservative solution, the concentrations of MI and CMI are calculated as follows:

$$\mu g/mg\ (ppm) \text{MI or CMI concentration in the sample} = \frac{AxCxE}{BxD}$$

Where
A: Area count of MI or CMI in the sample
B: Area count of MI or CMI in the standard solution
C: Concentration of MI or CMI (µg/ml) in the standard
D: Amount of the sample weighed
E: Total volume of the sample (ml)

Appendix 2

Method for the analysis of methyldibromo glutaronitrile (BCB), 2-bromo-2-nitropropane-1,3-diol (Bronopol) and 5-bromo-5-nitro-1,3-dioxane (Bronidox) in cosmetic products

1. Scope and Field of Application:

This method is used for the determination of methyldibromo glutaronitrile (BCB), 2-bromo-2-nitropropane-1,3-diol (Bronopol) and 5-bromo-5-nitro-1,3-dioxane (Bronidox) in cosmetic products. The method is suitable for the analytical control of maximum allowed concentrations of all the three substances (0.1 %) in cosmetic products.

2. Principle

The sample is suspended in 80% methanol and heated at 60°C to get a homogeneous suspension. The sample suspension is filtered and analysed by HPLC employing reductive electrochemical detection.

3. Reagents

3.1 Water, Millipore.
3.2 Methanol (Lichrosolv, gradient grade, Merck)
3.3 80% methanol: Dilute 80 ml methanol (3.2) to 100 ml with water
3.4 Acetone, HPLC grade (Fluka)
3.5 Methanol, HPLC grade
3.6 Sodium sulfate, water free (Pro analysis, Merck)
3.7 Sodium chloride (Pro analysis, Merck)
3.8 HPLC mobile phase
3.8.1 Aqueous sodium sulfate 71 g/L
3.8.2 Aqueous sodium chloride 11.6 g/L
3.8.3 Mobilphase: Transfer 400 ml acetone (3.4), 40 ml sodium sulfate solution (3.8.1) and 10 ml sodium chloride solution (3.8.2) in a 1000 ml volumetric flask, fill up to the mark with water and mix.
3.9 2-Bromo-2-nitropropane-1,3-diol (Bronopol, NERI No. 459)
3.10 5-Bromo-5-nitro-1,3-dioxane (Bronidox, NERI No. 1208)
3.11 Methyldibromo glutaronitrile (BCB, NERI No. 2263)
3.12 Stock solution of preservatives Bronopol, Bronidox and BCB
3.12.1 Accurately weigh approximately 0.2 g Bronopol in a 100 ml volumetric flask, fill up to the mark with 80% methanol (3.3) and mix
3.12.2 Accurately weigh approximately 0.2 g Bronidox in a 100 ml volumetric flask, fill up to the mark with 80% methanol (3.3) and mix
3.12.3 Accurately weigh approximately 0.2 g BCB in a 100 ml volumetric flask, fill up to the mark with 80% methanol (3.3) and mix
The stock solutions are stable for 1 week at 4°C

3.13 Calibration standard solutions

3.13.1 Transfer 10 ml Bronopol solution (3.12.1), 3 ml Bronidox solution (3.12.2) and 3 ml BCB solution (3.12.3) in a 25 ml volumetric flask and fill up to the mark with 80% methanol (3.3)

3.13.2 Transfer 1 ml. of 3.13.1 in a 2 ml volumetric flask and fill up to the mark with 80% methanol (3.3)

3.13.3 Transfer 1 ml. of 3.13.1 in a 5 ml volumetric flask and fill up to the mark with 80% methanol (3.3)

3.13.4 Transfer 1 ml. of 3.13.1 in a 10 ml volumetric flask and fill up to the mark with 80% methanol (3.3)

3.13.5 Transfer 1 ml. of 3.13.1 in a 25 ml volumetric flask and fill up to the mark with 80% methanol (3.3)

3.13.6 Transfer 1 ml. of 3.13.1 in a 50 ml volumetric flask and fill up to the mark with 80% methanol (3.3)

Calibration solutions should be prepared fresh every day.

Depending upon the amount of preservatives weighed (3.12), the calibration range for the preservatives will be:

Bronopol: approximately16-800 ppm
Bronidox: approximately 4.8-240 ppm
BCB: approximately 2.4 -120 ppm

4. Apparatus, Glass- and Plasticware

4.1 Normal laboratory glass- and plasticware

4.2 Waterbath at 60±1°C

4.3 HPLC system: Waters 616 pump, Waters 717 autosamplers, Millenium 3.2.0 software for control of HPLC system and chromatographic data, and Waters SAT/IN Modul.

4.4 Electrochemical detector from Bioanalytical System BAS LC-4B amperometric detector with Gold working electrode and Silver reference electrode

4.4 HPLC-column: Zorbax C8, particle size 5 µm, stainless steel 250 mm x 4.6 mm, from Knauer, precolumn packed with C8 silica.

4.5 Sartorius SRP 25 filter, 0.45µ

5. Sample preparation

- mix the sample well before use
- weigh accurately ca. 2.00g ± 0.1g sample in a 25 ml volumetric flask, and add ca. 20 ml 80% methanol (3.3)
- heat the mixture in a shaking waterbath (60±1°C) for 10 min
- fill the volumetric flask up to the mark and shake well
- filter the sample using a membrane filter (4.5)
- analyse the filtrate by HPLC for Bronopol, Bronidox and BCB contents.

All the samples should be prepared in duplicate. The sample extracts should be analysed within 24 hours.

6. Analysis

The samples and the calibration standards were analysed by HPLC under following conditions

Column temperature:	40°C
Run:	Isocratic
Flow:	1.0 ml/min
Eluent:	Mobile phase 3.8.3
Injection volume:	10µl
Run time:	20 min
Measuring potential:	–0.5 V (reductive)

Note: *Electrochemical detector is very sensitive to small changes in experimental parameters, it may take 2-3 hours for stabilisation of detector response after certain change made in experimental parameters. Mobile phase before use should be well sparged by He, and that should also be continuously sparged by He during the run.*

For the preparation of calibration curve, all of the calibration standard solution should be analysed each day at the start and at the end of a run. In a sample set, suitable calibration standard solutions should be bracketed, i.e. suitable calibration standard solution(s) should be run after every 3-4 samples

7. Calculation

Prepare the calibration curves for the preservatives using amounts of each preservative in calibration solutions and the corresponding detector response (area count of the chromatographic peak).

From the area counts of a sample peak and the peak of a standard preservatives, the contents of the preservative in a product is calculated as follows:

$$\%C = \frac{AxDxEx100}{BxMx1000x1000}$$

Where,
C: Concentration of a preservative
A: Area count of the preservative peak in the sample chromatogram
B: Area count of the peak of the standard preservative solution
D: Concentration (µg/ml) of the standard
M: Amount of sample (g)
E: Total volume of the sample solution

Appendix 3

'IV. IDENTIFICATION AND DETERMINATION OF FREE FORMALDEHYDE

1. PURPOSE AND SCOPE

This method describes the identification and two determination according to the presence or not of formaldehyde donors. It is applicable to all cosmetic products.

1.1. **Identification**

1.2. **General determination by pentane-2,4-dione colorimetry**

This method applies when formaldehyde is used alone or with other preservatives that are not formaldehyde donors.

Where this is not the case, and if the result exceeds the maximum permitted concentration, the following method of confirmation must be used.

1.3. **Determination in the presence of formaldehyde donors**

In the method mentioned above (1.2), during the derivization, the formaldehyde donors split and lead to results that are too high (combined and polymerized formaldehyde)

It is necessary to separate the free formaldehyde by liquid chromatography.

2. DEFINITION

The free formaldehyde content of the sample determined according to this method is expressed as percentage by mass.

3. IDENTIFICATION

3.1. **Principle**

Free and combined formaldehyde in a sulphuric acid medium turns Schiff's reagent pink or mauve.

3.2. **Reagents**

All reagents should be of analytical purity and the water has to be demineralized.

3.2.1. Fuchsin ;

3.2.2. Sodium sulphite hydrated at $7H_2O$;

3.2.3. Concentrated hydrochloric acid (d = 1,19) ;

3.2.4. Sulphuric acid, about 1M ;

3.2.5. *Schiff's reagent :*

100 mg of fuchsin (3.2.1) is weighed into a beaker and dissolved in 75 ml of water at 80 °C. After cooling, add 2,5 g of sodium sulphite (3.2.2). Make up to 100 ml.

Use within two weeks.

3.3. **Procedure**

3.3.1. Weigh 2 g of the sample in a 10-ml beaker.

3.3.2. Add two drops of sulphuric acid (3.2.4) and 2 ml of Schiff's reagent (3.2.5) . This reagent must be absolutely colourless when it is used.

Shake and leave to stand for five minutes.

3.3.3. If a pink or mauve tint is observed within the five minutes, the formaldehyde is present in excess of 0,01 % and is to be determined by the free and combined method (4) and, if necessary, by procedure (5).

4. GENERAL DETERMINATION BY PENTANE-2,4-DIONE COLORIMETRY

4.1. **Principle**

Formaldehyde reacts with pentane-2,4-dione in the presence of ammonium acetate to form 3,5-diacetyl-1,4-dihydrolutidine. This is extracted with butan-1-ol and the absorbance of the extract is measured at 410 nm.

4.2. **Reagents**

All reagents should be of analytical purity and the water has to be demineralized.

4.2.1. Anhydrous ammonium, acetate ;

4.2.2. Concentrated acetic acid, $d^{20}_4 = 1,05$;

4.2.3. Pentane-2,4-dione freshly distilled under reduced pressure 25 mm Hg 25° — it should not exhibit any absorption at 410 nm.

4.2.4. Butan-1-ol ;

4.2.5. Hydrochloric acid, 1 M ;

4.2.6. Hydrochloric acid, approximately 0,1 M ;

4.2.7. Sodium hydroxide, 1 M ;

4.2.8. Starch solution freshly prepared according to the European Pharmacopoeia (1 g/50 ml water), 2nd edition 1980, part I-VII-1-1 ;

4.2.9. 37 to 40 % w/v formaldehyde ;

4.2.10 Standard iodine solution, 0,05 M ;

4.2.11. Standard sodium thiosulphate solution, 0,1 M ;

4.2.12 *Pentane-2,4-dione reagent :*

In a 1 000 ml volumetric flask dissolve :

— 150 g ammonium acetate (4.2.1),

— 2 ml pentane-2,4-dione (4.2.3),

— 3 ml acetic acid (4.2.2).

Make up to 1 000 ml with water (pH of solution about 6,4).
This reagent must be freshly prepared ;

4.2.13. Reagent (4.2.12) without pentane-2,4-dione ;

4.2.14 *Formaldehyde-standard : stock solution*

Pour 5 g of formaldehyde (4.2.9) into a 1 000-ml volumetric flask and make up to 1 000 ml with water.

Determine the strength of this solution as follows :

Remove 10,00 ml ; add 25,00 ml of a standard iodine solution (4.2.10) and 10,00 ml of sodium hydroxide solution (4.2.7).

Allow to stand for five minutes.

Acidify with 11,00 ml of HCl (4.2.5) and determine the excess iodine with a standard sodium thiosulphate solution (4.2.11), using starch solution (4.2.8) as indicator.

1 ml of 0,05 M iodine (4.2.10) consumed is equivalent to 1,5 mg formaldehyde ;

4.2.15. *Formaldehyde-standard : diluted solution*

Dilute the formaldehyde stock solution successively 1/20 and then 1/100 with water.

1 ml of this solution contains about 1 µg of formaldehyde.

Calculate the exact content.

4.3. **Apparatus**

4.3.1. Standard laboratory apparatus ;

4.3.2. Phase separation filter, Whatman 1 PS (or equivalent) ;

4.3.3. Centrifuge ;

4.3.4. Water-bath set at 60 °C ;

4.3.5. Spectrophotometer ;

4.3.6. Glass cells with an optical path of 1 cm.

4.4. **Procedure**

4.4.1. *Sample solution*

Into a 100-ml volumetric flask weigh to within 0,001 g a quantity (in g) of the test sample corresponding to a presumed quantity of formaldehyde of about 150 µg.

Make up to 100 ml with water and mix (solution S).

(Check that the pH is close to 6 ; if not, dilute in the hydrochloric acid solution (4.2.6).)

To a 50-ml Erlenmeyer flask add :

— 10,00 ml of the solution S,

— 5,00 ml pentane-2,4-dione reagent (4.2.12).

— demineralized water to a final volume of 30 ml.

4.4.2. *Reference solution*

Possible interference due to background colour in the test sample is eliminated by the use of this reference solution :

To a 50-ml Erlenmeyer flask add :

— 10,00 ml S solution,

— 5,00 ml reagent (4.2.13),

— demineralized water to a final volume of 30 ml.

4.4.3. *Blank test*

To a 50-ml Erlenmeyer flask add :

— 5,0 ml pentane-2,4-dione reagent (4.2.12),

— demineralized water to a final volume of 30 ml.

4.4.4. *Determination*

4.4.4.1. Shake the mixtures from 4.4.1, 4.4.2 and 4.4.3. Immerse the Erlenmeyer flasks in a water-bath at 60 °C for exactly 10 minutes. Allow to cool for two minutes in a bath of iced water.

4.4.4.2 Transfer into 50-ml separating funnels containing 10 ml of butan-1-ol (4.2.4). Rinse each flask with 3 to 5 ml of water. Shake the mixture vigorously for exactly 30 seconds. Allow it to separate.

4.4.4.3. Filter the butan-1-ol phase into the measurement cells (4.3.2) through a phase-separation filter. Centrifuging (3 000 g_n for five minutes) may also be used.

4.4.4.4. Measure the absorbance A_1 at 410 nm of the extract of the sample solution from 4.4.1 against the extract of the reference solution 4.4.2.

4.4.4.5. Similarly measure the absorbance A_2 of the extract of the blank solution from 4.4.3 against butan-1-ol.

N B : All these operations must be carried out within 25 minutes from the moment when the Erlenmeyer flasks are placed in the water bath at 60 °C.

4.4.5. *Calibration curve*

4.4.5.1. Into a 50-ml Erlenmeyer flask place :

— 5,00 ml of the diluted standard solution from 4.2.15,

— 5,00 ml of the pentane-2,4-dione reagent (4.2.12),

— demineralized water to a final volume of 30 ml.

4.4.5.2. Continue as described in 4.4.4 and measure the absorbance against butan-1-ol (4.2.4).

4.4.5.3. Repeat the procedure with 10, 15, 20 and 25 ml of the diluted standard solution (4.2.15).

4.4.5.4. To obtain the zero value (corresponding to the coloration of the reagents) proceed as in 4.4.4.5.

4.4.5.5. Construct the calibration curve after subtraction of the zero value from each of the absorbances obtained in 4.4.5.1 and 4.4.5.3. Beer's Law is valid up to 30 µg formaldehyde.

4.5. **Calculations**

4.5.1. Subtract A_2 from A_1 and read off from the calibration curve (4.4.5.5) the amount C, in µg, of formaldehyde in the sample solution (4.4.1).

4.5.2. Calculate the formaldehyde content of sample (% m/m) with the aid of the following formula :

$$\text{formaldehyde content in \%} = \frac{C}{10^3 \cdot m}$$

where :

m = mass of the test portion in g.

4.6. **Repeatability** (1)

For a formaldehyde content of 0,2 % the difference between the results of two determinations in parallel carried out on the same sample should not exceed 0,005 % for determination by pentane-2,4-dione colorimetry.

If the determination of free formaldehyde leads to results greater than the maximum concentrations provided for in Directive 76/768/EEC, i.e.:

(a) between 0,05 % and 0,2 % in a non-labelled product;

(b) greater than 0,2 % in the product, whether or not labelled

the procedure described in 5 below must be applied.

5. DETERMINATION IN THE PRESENCE OF FORMALDEHYDE DONORS

5.1. **Principle**

The separate formaldehyde is transformed into a yellow lutidinic derivative by a reaction with the pentane-2,4-dione in a post-column reactor and the derivative obtained is detected by absorbance at 420 nm.

5.2. **Reagents**

All reagents should be of analytical purity and the water has to be demineralized.

5.2.1. HPLC grade water or water of equivalent quality;

5.2.2. Anhydrous ammonium, acetate;

5.2.3. Concentrated acetic acid;

5.2.4. Pentane-2,4-dione (kept at 4 °C);

5.2.5. Anhydrous disodium phosphate;

5.2.6. 85 % orthophosphoric acid (d = 1,7);

5.2.7. HPLC grade methanol;

5.2.8. Dichloromethane;

5.2.9. 37 to 40 % w/v formaldehyde;

5.2.10. Sodium hydroxide, 1 M;

5.2.11. Hydrochloric acid, 1 M;

5.2.12. Hydrochloric acid, 0,002 M;

5.2.13. Starch solution freshly prepared according to the European Pharmacopoeia (see 4.2.8);

5.2.14. Standard iodine solution, 0,05 M;

5.2.15. Standard sodium thiosulphate solution, 0,1 M;

5.2.16. *Mobile phase:*

Aqueous solution of disodium phosphate (5.2.5), 0,006 M adjusted to pH 2,1 with orthophosphoric acid (5.2.6);

5.2.17. *Post-column reagent:*

In a 1 000 ml volumetric flask dissolve:

— 62,5 g ammonium acetate (5.2.2),

— 7,5 ml acetic acid (5.2.3),

— 5 ml pentane-2,4-dione (5.2.4).

Make up to 1 000 ml with water (5.2.1).

Keep this reagent away from the light.

Conservation time: maximum three days at 25 °C.

No change in colour should be observed;

5.2.18. *Formaldehyde standard: stock solution*

Pour 10 g of formaldehyde (5.2.9) into a 1 000 ml volumetric flask and make up to 1 000 ml with water.

Determine the strength of this solution as follows:

Remove 5,00 ml; add 25,00 ml of the standard iodine solution (5.2.14) and 10,00 ml of the sodium hydroxide solution (5.2.10).

Allow to stand for five minutes.

Acidify with 11,00 ml of HCl (5.2.11) and titrate the excess standard iodine solution with standard sodium thiosulphate solution (5.2.15), using starch solution (5.2.13) as indicator.

1 ml of iodine solution (5.2.14) is equivalent to 1,5 mg formaldehyde;

(1) ISO 5725.

5.2.19. *Formaldehyde standard: diluted solution*

Dilute the stock solution to 1/100th of its initial strength in the mobile phase (5.2.16).

1 ml of this solution contrains about 37 mg formaldehyde.

Calculate the exact content.

5.3. **Apparatus**

5.3.1. Standard laboratory apparatus;

5.3.2. HPLC pump, pulsation-free;

5.3.3. Low-pressure pulsation-free pump for the reagent (or a second HPLC pump);

5.3.4. Injection valve with a 10 µl loop;

5.3.5. Post-column reactor with the following components:

+ one 1-litre three-neck flask,
+ one 1-litre flask heater,
+ two Vigreux columns with a minimum of 10 plates, two air-cooled,
+ stainless steel tube (for heat exchange) 1,6 mm — internal diameter 0,23 mm, length = 400 mm,
+ Teflon tube 1,6 mm — internal diameter 0,30 mm, length 5 m (French knitting) see Appendix 1),
+ one T-piece without any dead volume (Valco or equivalent),
+ three unions without any dead volume

Or: one post-column module Applied Biosystems PCRS 520 or equivalent fitted with a 1-ml reactor;

5.3.6. Membrane filter, pore size 0,45 µm;

5.3.7. SEP-PAK^R C_{18} cartridge or equivalent;

5.3.8. *Ready-to-use colulmns:*

— Bischoff hypersil RP 18 (type NC reference C 25.46 1805)
(5 µm, length = 250 mm, internal diameter = 4,6 mm),
— or Dupont, Zorbax ODS
(5 µm, length = 250 mm, internal diameter = 4,6 mm),
— or Phase SEP, spherisorb ODS 2
(5µm, length = 250 mm, internal diameter = 4,6 mm);

5.3.9 *Pre-column*

Bischoff K_1 hypersil RP 18 (reference K1 G 6301 1805)
(5µm, length = 10 mm, or equivalent).

5.3.10 The column and pre-column are connected by means of an Ecotube system (reference A 15020508 Bischoff) or equivalent.

5.3.11. Assemble the apparatus (5.3.5) as shown in the block diagram in Appendix 2.

The connections after the injection value must be kept as short as possible. In this case, the stainless-steel tube between the reactor outlet and the detector inlet is intended to cool the mixture prior to detection and the temperature in the detector is unknown but constant;

5.3.12. UV visible detector;

5.3.13. Recorder;

5.3.14. Centrifuge;

5.3.15 Ultrasonic bath;

5.3.16. Vibrating stirrer (vortex or equivalent).

5.4. **Procedure**

5.4.1. *Calibration curve*

This is produced by plotting peak heights as a function of the concentration of formaldehyde standard: diluted.

Prepare the standard solutions by diluting the formaldehyde reference solution (5.2.19) with the mobile phase (5.2.16):

— 1,00 ml of solution (5.2.19) diluted to 20,00 ml (about 185 µg/100 ml)
— 2,00 ml of solution (5.2.19) diluted to 20,00 ml (about 370 µg/100 ml)
— 5,00 ml of solution (5.2.19) diluted to 25,00 ml (about 740 µg/100 ml)
— 5,00 ml of solution (5.2.19) diluted to 20,00 ml (about 925 µg/100 ml)

The standard solutions are kept for one hour at laboratory temperature and must be freshly prepared.

The linearity of the calibration curve is good for concentrations between 1,00 and 15,00 µg/ml.

5.4.2. *Preparation of the samples*

5.4.2.1. Emulsions (creams, make-up base, eyeliners)

Into a stoppered 100-ml flask weigh to the nearest 0,001 g a quantity of test sample (m g) corresponding to a presumed quantity of 100 µg of formaldehyde. Add 20,00 ml dichloromethane (5.2.8) and 20,00 ml hydrochloric acid (5.2.12), accurately measured. Mix with the vibrating stirrer (5.3.16) and by means of the ultrasonic bath (5.3.15). Separate the two phases by centrifuging (3 000 g^n for two minutes). Meanwhile, wash a cartridge (5.3.7) with 2 ml methanol (5.2.7), then condition with 5 ml water (5.2.1).

Pass 4 ml of the aqueous phase of the extract through the conditioned cartridge, discard the first 2 ml and recover the following fraction.

5.4.2.2. Lotions, shampoos

Weigh into a stoppered 100-ml flask to the nearest 0,001 g a quantity of test sample (m g) corresponding to a presumed quantity of about 500 µg of formaldehyde.

Make up to 100 ml with the mobile phase (5.2.16).

Filter the solution through a filter (5.3.6) and inject or pass it through a cartridge (5.3.7) conditioned as before (5.4.2.1). All the solutions must be injected immediately after preparation.

5.4.3. *Chromatographic conditions*

— Flowrate of the mobile phase : 1 ml/min,

— Reagent flowrate : 0,5 ml/min,

— Total flowrate at the detector outlet : 1,5 ml/min,

— Injected volume : 10 µl,

— Elution temperature : In the case of difficult separations, immerse the column in a bath of melting ice : wait for the temperature to stabilize (15-20 min),

— Temperature of post-column reaction : 100 °C,

— Detection : 420 nm.

NB : The entire chromatographic system and post-column must be flushed out with water after use (5.2.1). Where the system is not used for more than two days, this flushing must be followed by flushing with methanol (5.2.7). Before reconditioning the system pass water through it to avoid recrystallization.

5.5. **Calculation**

Emulsions : (5.4.2.1) :

Formaldehyde content in % (m/m) :

$$\frac{C \cdot 10^{-6} \cdot 100}{5\ m} = \frac{C \cdot 10^{-4}}{5\ m}$$

Lotions, shampoos :

In this case the formula becomes :

$$\frac{C \cdot 10^{-6} \cdot 100}{m} = \frac{C \cdot 10^{-4}}{m}$$

where :

m = mass of the sample analysed in g (5.4.2.1),

C = formaldehyde concentration in µg/100 ml read off from the calibration curve (5.4.1).

5.6. **Repeatability** (1)

For a content of 0,05 % of formaldehyde the difference between the results of two determinations in parallel carried out on the same sample should not exceed 0,001 %.

For a content of 0,2 % of formaldehyde the difference between the results of two determinations in parallel carried out on the same sample should not exceed 0,005 %.

(1) ISO 5725.

Appendix 1

INTRUCTION FOR "FRENCH KNITTING"

ACCESSORIES REQUIRED

— One wooden bobbin :

external diameter 5 cm with a hole of 1,5 cm diameter made through the centre. Insert four steel nails (as shown in Figures 1 and 2). The distance between two nails must be 1,8 cm and they must be 0,5 cm from the hole,

— one rigid needle (of the crotchet-hook type) to loop the Teflon tube,

— 5 m of 1,6 mm Teflon tube, internal diameter 0,3 mm.

PROCEDURE

To start off the "French knitting", the Teflon tube must be threaded from the top of the bobbin to the bottom via the central hole (leaving around 10 cm of tube protruding from the bottom of the bobbin, enabling the chain to be pulled through during the knitting process) ; then wind the tube around the four nails in turn as shown in Figure 3.

The top and bottom of the French knitting will be protected by metal rings and compression screws ; take care not to crush the Teflon when pulling tight. Wind the tube around each nail for a second turn and make the 'stitches' as follows :

— lift the lower tube over the upper tube with the hook (see Figure 4). Repeat this process on each of the nails in order (1, 2, 3, 4 in an anti-clockwise direction), until 5 m or the desired length of knitting is produced.

Leave around 10 cm of tube to close the chain. Thread the tube through each of the four loops and pull gently, to close up the end of the chain.

NB : French knitting manufactured for post column reactors is available on the market (Supelco).

Schematic diagram of the bobbin

Figure 1

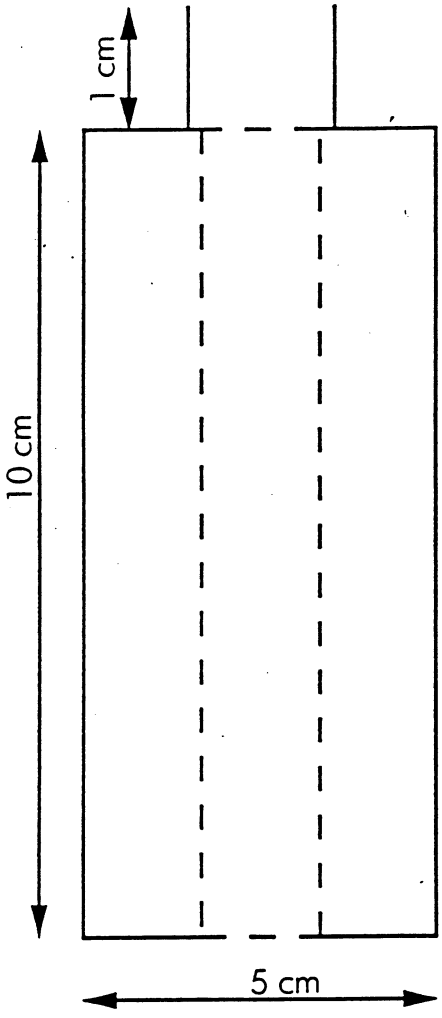


Figure 2

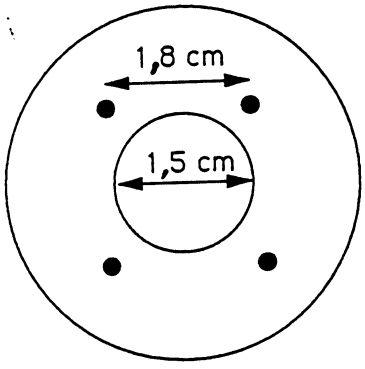
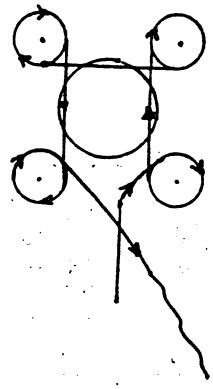
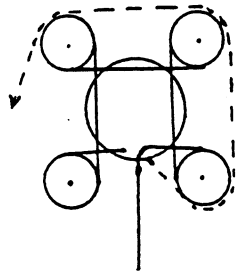


Figure 3



First tube

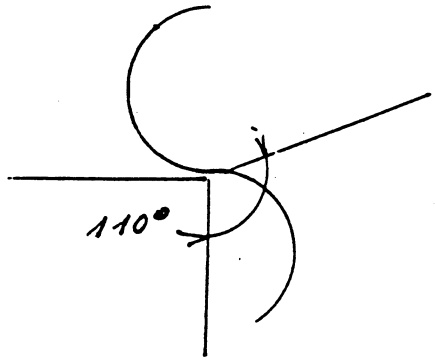
Figure 4



Second tube

To form a "stitch", lift the lower tube (unbroken line) up over the second tube (dotted line)

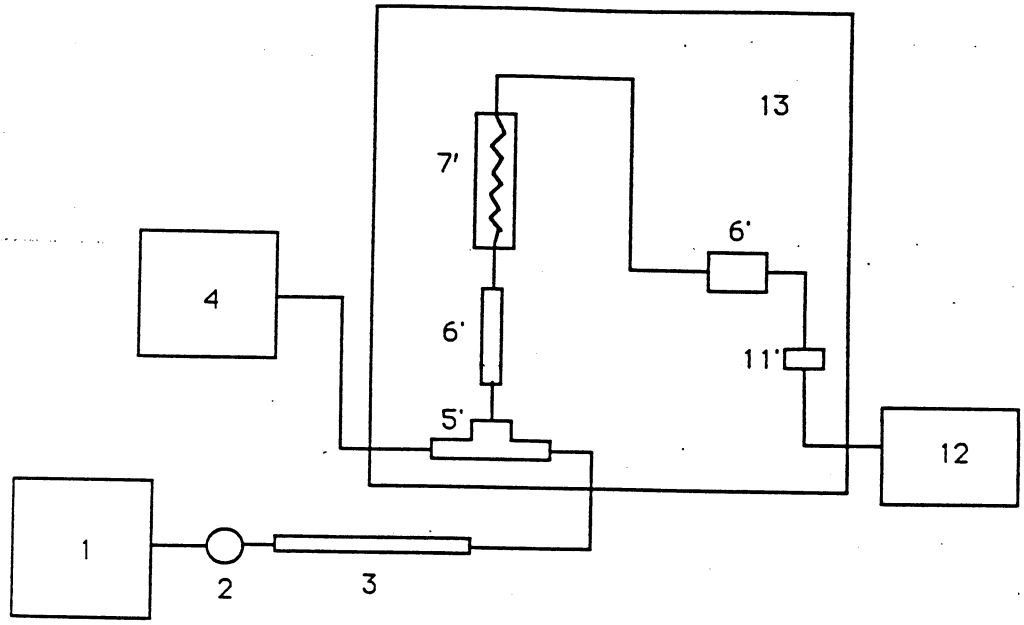
Figure 5



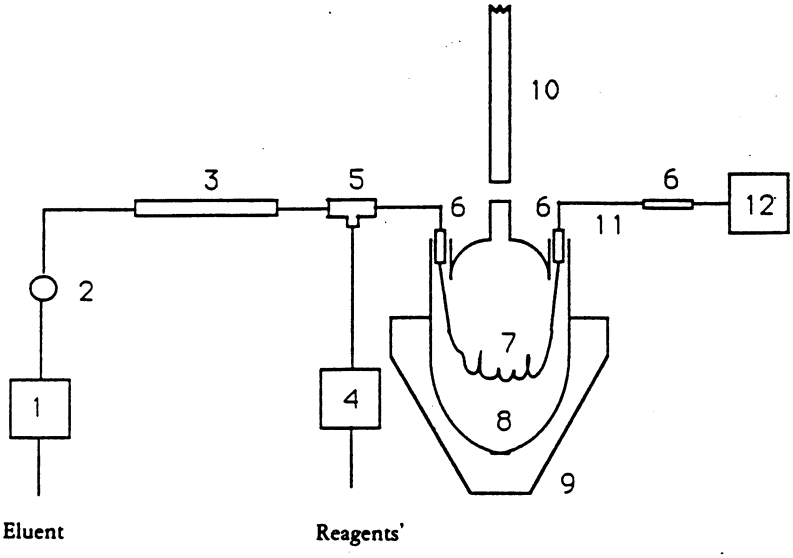
Appendix 2

1 = HPLC pump
2 = Injection valve
3 = Column with pre-column
4 = Reagent pump
5 = T-piece without dead volume
5' = T-piece (Vortex)
6-6' = Union without dead volume
7 = 'French knitting'
7' = Reactor
8 = Three-neck flask with boiling water
9 = Flask heater
10 = Coolant
11 = Stainless steel heat-exchanger tube
11' = Heat exchanger
12 = Visible UV detector
13 = PCRS 520 post-column module

5.3.5



5.3.6



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Appendix 4

IDENTIFIKATION OG KVANTITATIV BESTEMMELSE AF 2-PHENOXYETHANOL, 1-PHENOXYPROPAN-2-OL SAMT METHYL-, ETHYL-, PROPYL-, BUTYL- OG BENZYL-4-HYDROXYBENZOAT I KOSMETISKE PRODUKTER

A. IDENTIFIKATION

1. **Formål og anvendelsesområde**

Denne TLC-metode anvendes sammen med den i afsnit B beskrevne HPLC-metode til at identificere 2-phenoxyethanol, 1-phenoxypropan-2-ol samt methyl-4-hydroxybenzoat, ethyl-4-hydroxybenzoat, propyl-4-hydroxybenzoat, butyl-4-hydroxybenzoat og benzyl-4-hydroxybenzoat i kosmetiske produkter.

2. **Princip**

Konserveringsstofferne ekstraheres med acetone af den forsurede prøve af kosmetikproduktet. Efter filtrering blandes acetoneopløsningen med vand, og fedtsyrerne bundfældes i alkalisk miljø som calciumsalte. Den alkaliske acetone/vandblanding ekstraheres med diethylæter for at fjerne lipofile stoffer. Efter forsuring ekstraheres konserveringsstofferne med diethylæter. Der påføres en plet af diethylæterekstrakten på en tyndtlagsplade belagt med silicagel. Efter udvikling betragtes kromatogrammet under UV-lys og fremkaldes med Millons reagens.

3. **Reagenser**

3.1. Generelt

Alle reagenser skal være af analysekvalitet. Vand skal være destilleret vand eller vand af mindst tilsvarende renhed.

3.2. Acetone

3.3. Diethylæter

3.4. n-Pentan

3.5. Methanol

3.6. Iseddikesyre

3.7. Saltsyre, c(HCl) = 4mol/l

3.8. Kaliumhydroxidopløsning, c(KOH) = 4mol/l

3.9. Calciumchlorid (dihydrat) ($CaCl_2 \cdot 2H_2O$)

3.10. Detektionsreagens: Millons reagens

Millons reagens (Hg(II)nitrat) er en opløsning, der fås færdigfremstillet i handelen (Fluka 69820)

3.11. 2-Phenoxyethanol

3.12. 1-Phenoxypropan-2-ol

3.13. Methyl-4-hydroxybenzoat (methylparaben)

3.14. Ethyl-4-hydroxybenzoat (ethylparaben)

3.15. n-Propyl-4-hydroxybenzoat (propylparaben)

3.16. n-Butyl-4-hydroxybenzoat (butylparaben)

3.17. Benzyl-4-hydroxybenzoat (benzylparaben)

3.18. Referenceopløsninger

Af hvert af referencestofferne 3.11, 3.12, 3.13, 3.14, 3.15, 3.16 og 3.17 fremstilles en 0,1 % (m/v) opløsning i methanol.

3.19. Mobil fase

Bland n-pentan og iseddikesyre i forhold 88:12 (v/v).

4. **Apparatur**

Sædvanligt laboratorieudstyr, samt

4.1. Termostateret vandbad, 60 °C

4.2. TLC-kar (uden filtrerpapirbeklædning)

4.3. Ultraviolet lyskilde, 254 nm

4.4. Tyndtlagsplader 20 cm × 20 cm, belagt med 0,25 mm silicagel 60 F_{254}, med koncentrationszone (Merck nr. 11798, Darmstadt, eller tilsvarende).

4.5. Termostatovn med temperaturområde indtil 105 °C

4.6. Varmlufthårtørrer.

4.7. Malerulle med uldlag, længde ca. 10 cm, udv. diameter ca. 3,5 cm. Uldlagets tykkelse skal være 2-3 mm. Om nødvendigt trimmes uldlaget. (Se bemærkningen under punkt 5.2).

4.8. 50 ml reagensglas med skruelåg

4.9. Elektrisk termostatvarmeplade. Temperaturindstilling: ca. 80 °C. Varmepladen skal være dækket af en aluminiumplade på 20 × 20 cm af ca. 6 mm tykkelse for at sikre ensartet varmefordeling.

5. **Fremgangsmåde**

5.1. Prøvetilberedning

Afvej ca. 1 g prøve i et 50 ml reagensglas med skruelåg. Tilsæt 4 dråber saltsyreopløsning (3.7) og 40 ml acetone.

Til stærkt basiske kosmetikprodukter som f.eks. håndsæbe tilsættes 20 dråber saltsyreopløsning. Glasset lukkes, og blandingen opvarmes forsigtigt til ca. 60 °C for at fremme ekstraktion af konserveringsstofferne i acetonefasen, hvorpå det omrystes kraftigt i et minut.

Mål opløsningens pH ved brug af pH-indikatorpapir og indstil pH til ≤ 3 med saltsyreopløsning. Derpå omrystes igen kraftigt i et minut.

Opløsningen afkøles til stuetemperatur og filtreres gennem filtrerpapir ned i en konisk kolbe. 20 ml af filtratet overføres til en 200 ml konisk kolbe, der tilsættes 60 ml vand og blandes. Blandingens pH indstilles til ca. 10 med kaliumhydroxidopløsning (3.8) ved brug af pH-indikatorpapir.

Der tilsættes 1 g calciumchlorid dihydrat (3.9) og omrystes kraftigt. Opløsningen filtreres gennem filtrerpapir over i en 250 ml skilletragt indeholdende 75 ml dietylæter, hvorpå der omrystes kraftigt i 1 minut. Når faserne er adskilt, overføres den vandige fase til en 200 ml konisk kolbe. Opløsningens pH indstilles til ca. 2 med saltsyreopløsning ved brug af pH-indikatorpapir, hvorefter der tilsættes 10 ml dietylæter og omrystes kraftigt i et minut. Når faserne er adskilt, overføres ca. 2 ml af æterfasen til et 5 ml hætteglas.

5.2. Tyndtlagskromatografi

En TLC-plade (4.4) anbringes på den opvarmede aluminiumplade (4.9). Der påsættes 10µl af hver referenceopløsning (3.18) og 100 µl af prøveopløsningen(-erne) (5.1) på startlinjen, der afmærkes i TLC-pladens koncentrationszone.

Om ønsket kan der benyttes lufttørring til at fremskynde fordampningen af opløsningsmidlet. Fjern TLC-pladen fra varmepladen og lad den afkøle til rumtemperatur. Overfør 100 ml af den mobile fase (3.19) til et TLC-kar (4.2). Tyndtlagspladen anbringes straks i det umættede kar og udvikles ved stuetemperatur, indtil væskefronten har bevæget sig ca. 15 cm fra startlinjen. Tag pladen ud af karret og tør den med en varmlufthårtørrer.

Undersøg pladen under UV-lys (4.3), og afmærk pletterne. Anbring pladen i en termostatovn (4.5) ved 100 °C i 30 minutter for at fjerne overskydende eddikesyre. Pletterne af konserveringsstof fremkaldes derpå med Millons reagens (3.10), der påføres tyndtlagspladen ved hjælp af malerulle (4.7), således at pladen fugtes jævnt over det hele.

Bemærkning: Alternativt kan pletterne fremkaldes ved omhyggelig påføring af en dråbe Millons reagens på hver af de under UV-lys afmærkede pletter.

Estre af 4-hydroxybenzoesyre fremtræder som røde pletter, 2-phenoxyethanol og 1-phenoxypropan-2-ol som gule. Bemærk dog, at 4-hydroxybenzoesyren selv, der kan være tilstede i prøverne som konserveringsstof eller som nedbrydningsprodukt af parabenerne, ligeledes vil fremtræde som en rød plet. Se 7.3 og 7.4.

6. **Identifikation**

R_f-værdien for hver plet beregnes. Pletter fra prøveopløsningen sammenholdes med pletter af referenceopløsningen med hensyn til R_f-værdi, udseende i UV-lys og farve efter fremkaldelse og konserveringsstofferne identificeres foreløbigt. Hvis resultaterne tyder på tilstedeværelse af parabener, udføres den i afsnit B beskrevne HPLC-analyse. Ved sammenholdelse af resultaterne af TLC og HPLC bekræftes tilstedeværelsen af 2-phenoxyethanol, 1-phenoxypropan-2-ol og parabenerne.

7. **Bemærkninger**

7.1. Millons reagens bør som følge af sin giftighed påføres på en af de beskrevne måder. Påsprøjtning kan ikke anbefales.

7.2. Andre hydroxyl-forbindelser kan også give farvereaktion med Millons reagens. En tabel over farver og R_f-værdier af en række konserveringsstoffer opnået ved denne TLC-metode kan findes i: N. de Kruijf, M.A.H. Rijk, L.A. Pranato-Soetardhi og A. Schouten (1987): »Determination of preservatives in cosmetic products I: Thin layer chromatographic procedure for the identification of preservatives in cosmetic products« (J. Chromatography 410, 395-411).

7.3. R_f-værdierne i følgende tabel kan tjene som vejledning med hensyn til de værdier, der kan forventes:

Forbindelse	hR_f	Farve
4-hydroxybenzoesyre	11	rød
methylparaben	12	rød
ethylparaben	17	rød
propylparaben	21	rød
butylparaben	26	rød
benzylparaben	16	rød
2-phenoxyethanol	29	gul
1-phenoxypropan-2-ol	50	gul

7.4. Metoden kan ikke adskille 4-hydroxybenzoesyre fra methylparaben eller benzylparaben fra ethylparaben. Identifikation af disse stoffer skal bekræftes ved udførelse af den i afsnit B beskrevne HPLC-analyse og sammenholdelse af de opnåede retentionstider for henholdsvis prøve og standarder.

B. KVANTITATIV BESTEMMELSE

1. **Formål og anvendelsesområde**

Denne metode anvendes til kvantitativ bestemmelse af 2-phenoxyethanol, 1-phenoxypropan-2-ol samt methyl-4-hydroxybenzoat, ethyl-4-hydroxybenzoat, propyl-4-hydroxybenzoat, butyl-4-hydroxybenzoat og benzyl-4-hydroxybenzoat i kosmetiske produkter.

2. **Definition**

Mængden af konserveringsstof, bestemt ved denne metode, udtrykkes i masseprocent.

3. **Princip**

Prøven gøres sur ved tilsætning af svolvsyre og opslæmmes derefter i en blanding af ethanol og vand. Blandingen opvarmes forsigtigt for at smelte lipidfasen for at opnå kvantitativ ekstraktion, hvorpå den filtreres.

Indholdet af konserveringsstoffer i filtratet bestemmes ved omvendt fase HPLC med anvendelse af isopropyl-4-hydroxybenzoat som intern standard.

4. **Reagenser**

4.1. Generelt

Alle reagenser skal være af analysekvalitet henholdsvis egnede til HPLC. Vand skal være destilleret vand eller vand af mindst tilsvarende renhed.

4.2. Ethanol, absolut

4.3. 2-Phenoxyethanol

4.4. 1-Phenoxypropan-2-ol

4.5. Methyl-4-hydroxybenzoat (methylparaben)

4.6. Ethyl-4-hydroxybenzoat (ethylparaben)

4.7. n-Propyl-4-hydroxybenzoat (propylparaben)

4.8. Isopropyl-4-hydroxybenzoat (isopropylparaben)

4.9. n-Butyl-4-/hydroxybenzoat (butylparaben)

4.10. Benzyl-4-hydroxybenzoat (benzylparaben)

4.11. Tetrahydrofuran

4.12. Methanol

4.13. Acetonitril

4.14. Svovlsyreopløsning, $c(H_2SO_4) = 2 mol/l$

4.15. Ethanol/vandblanding

Bland ethanol (4.2) og vand i forholdet 9:1 (v/v).

4.16. Intern standardopløsning

Ca. 0,25 g isopropylparaben (4.8) afvejes nøjagtigt og overføres til en 500 ml målekolbe og opløses i ethanol/vandblanding (4.15). Målekolben fyldes op til mærket med ethanol/vandblanding (4.15).

4.17. Mobil fase: tetrahydrofuran/vand/methanol/acetonitril

Bland 5 rumfang tetrahydrofuran med 60 rumfang vand, 10 rumfang methanol og 25 rumfang acetonitril.

4.18. Stamopløsning af konserveringsstof

I en 100 ml målekolbe afvejes nøjagtigt ca. 0,2 g 2-phenoxyethanol, 0,2 g 1-phenoxypropan-2-ol, 0,05 g methylparaben, 0,05 g ethylparaben, 0,05 g propylparaben, 0,05 g butylparaben og 0,025 g benzylparaben, som opløses i ethanol/vandblanding, hvorefter kolben fyldes op til stregen dermed.

Opløsningen kan i køleskab holde sig i indtil en uge.

4.19. Standardopløsninger af konserveringsstof

Fyld henholdsvis 20,00 ml, 10,00 ml, 5,00 ml, 2,00 ml og 1,00 ml af stamopløsningen (4.18) i 50 ml målekolber. Hver kolbe tilsættes 10,00 ml intern standardopløsning (4.16) og 1,0 ml svovlsyreopløsning (4.14), hvorpå der fyldes op til stregen med ethanol/vandblandingen. Disse opløsninger skal være friskfremstillede.

5. Apparatur

Sædvanligt laboratorieudstyr, samt:

5.1. Termostateret vandbad, der kan holde 60 °C ± 1 °C.

5.2. HPLC-apparat med UV-detektor, bølgelængde 280 nm.

5.3. HPLC-kolonne:

Rustfrit stål, 25 cm × Ø indv. 4,6 mm (eller 12,5 cm × Ø indv. 4,6 mm), pakket med Nucleosil 5C18 eller tilsvarende (se 10.1).

5.4. 100 ml reagensglas med skruelåg.

5.5. Kogesten, carborumdumkorn, størrelse 2-4 mm, eller tilsvarende.

6. Fremgangsmåde

6.1. Prøvetilberedning

6.1.1. Prøvetilberedning uden tilsætning af intern standard

Afvej ca. 1,0 g prøve i et 100 ml glas med skruelåg. Afpipettér 1,0 ml svovlsyreopløsning (4.14) og 50,0 ml ethanol/vandblanding (4.15) i glasset. Tilsæt ca. 1 g kogesten (5.5), luk glasset og omryst det kraftigt til en homogen suspension dannes. Omryst i mindst et minut. Anbring glasset i vandbad (5.1) i 5 minutter ved 60 °C ± 1 °C for at lette ekstraktion af konserveringsstoffer over i ethanolfasen.

Afkøl straks glasset under rindende koldt vand og lad ekstraktet henstå i køleskab i en time. Filtrer ekstraktet gennem filtrerpapir. Overfør ca. 2 ml af filtratet til et 5 ml hætteglas. Ekstrakterne opbevares i køleskab, og HPLC-bestemmelsen udføres inden 24 timer.

6.1.2. Prøvetilberedning med tilsætning af intern standard

Afvej 1,0 g ± 0,1 g prøve med tre decimalers nøjagtighed (a gram) i et 100 ml reagensglas med skruelåg.

Afpipettér 1,0 ml svovlsyreopløsning og 40,0 ml ethanol/vandblanding i glasset. Tilsæt ca. 1 g kogesten og nøjagtig 10,00 ml intern standardopløsning. Luk glasset og omryst det kraftigt, indtil en homogen suspension dannes. Omryst glasset i mindst et minut. Anbring glasset i termostatvandbad 60 °C ± 1 °C i 5 minutter for at lette ekstraktion af konserveringsstoffer over i ethanolfasen.

Afkøl straks glasset under rindende koldt vand og lad ekstraktet henstå i køleskab i en time. Derpå filtreres ekstraktet med filtrerpapir.

Overfør ca. 2 ml af filtratet i et 5 ml hætteglas (prøveopløsning). Ekstraktet opbevares i køleskab, og HPLC-bestemmelse udføres inden 24 timer.

6.2. Højtryksvæskekromatografi

6.2.1. Kromatografiske parametre

— Mobil fase: tetrahydrofuran/vand/methanol/acetonitril-blanding (4.17).

— Flow af den mobile fase: 1,5 ml/minut.

— Detektorbølgelængde: 280 nm.

6.2.2. Kalibrering

Der injiceres 10 µl af hver konserveringsstof-standardopløsning (4.19). Af de opnåede kromatogrammer bestemmes forholdet mellem tophøjden for standardopløsninger af konserveringsstofferne og tophøjden for den interne standard. For hvert konserveringsstof optegnes en kurve, der viser sammenhængen mellem tophøjde-forhold og standardopløsningens koncentration.

6.2.3. Kvantitativ bestemmelse

Injicer 10 µl af prøveopløsningen uden intern standard (6.1.1) i kromatografen, og optag kromatogrammet.

Injicer 10 µl af en af standardopløsningerne af konserveringsstof (4.19), og optag kromatogrammet. De således optagede kromatogrammer sammenlignes.

Hvis kromatogrammet af prøveekstraktet (6.1.1) ikke indeholder en top med tilnærmelsesvis samme retentionstid som isopropylparaben (den anbefalede interne standard), fortsættes med injektion af 10 µl prøveopløsning med intern standard (6.1.2). Kromatogrammet optages, og tophøjderne måles.

Hvis kromatogrammet af prøveopløsningen indeholder en interfererende top med tilnærmelsesvis samme retentionstid som isopropylparaben, bør der vælges en anden intern standard.

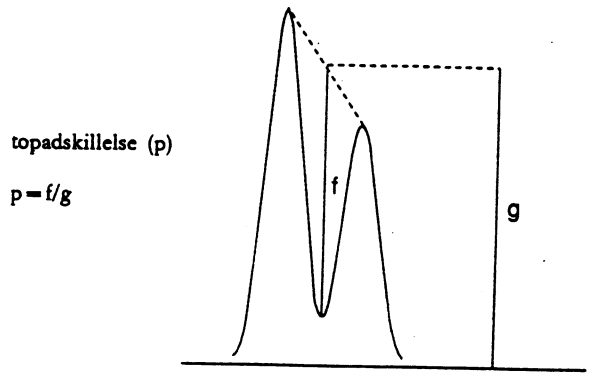
Hvis et af de undersøgte konserveringsstoffer er fraværende i kromatogrammet af prøven, kan dette konserveringsstof bruges som alternativ intern standard.

Beregn forholdet mellem tophøjderne for de undersøgte konserveringsstoffer og tophøjden for den interne standard.

Det sikres at lineær respons opnås for de standardopløsninger, der er anvendt til kaliberering.

Det sikres, at kromatogrammerne for standardopløsning og prøveopløsning opfylder følgende krav:

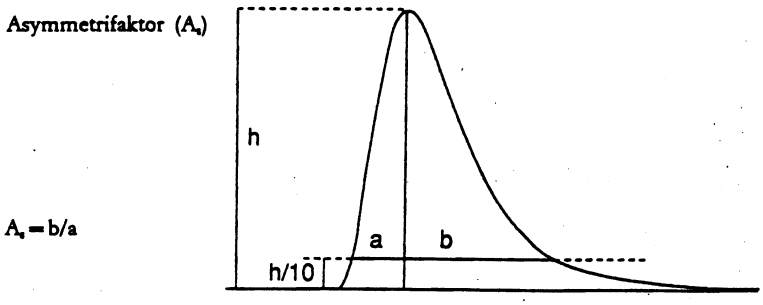
— topadskillelsen for dårligst adskilte par skal være mindst 0,90 (definition af topadskillelse er givet i figur 1).



Figur 1: Topadskillelse

Opnås den krævede topadskillelse ikke, må man enten benytte en mere effektiv kolonne eller korrigere sammensætningen af den mobile fase, således at kravet opfyldes.

— Asymmetrifaktoren A_s for samtlige toppe skal være mellem 0,9 og 1,5. (Asymmetrifaktoren er defineret i figur 2). Til optagning af kromatogrammet til bestemmelse af asymmetrifaktoren anbefales en papirhastighed på mindst 2 cm/min.



Figur 2: Asymmetrifaktor

— grundlinjen skal være stabil.

7. Beregning

Koncentrationerne af konserveringsstoffer i prøven bestemmes ved anvendelse af kalibreringskurver (6.2.2) og af forholdene mellem tophøjden for de undersøgte konserveringsstoffer og tophøjden for den interne standard. Af følgende beregnes koncentrationen, w_i i vægtprocent (% m/m), af 2-phenoxyethanol, 1-phenoxypropan-2-ol samt methyl-4-hydroxybenzoat, ethyl-4-hydroxybenzoat, propyl-4-hydroxybenzoat, butyl-4-hydroxybenzoat og benzyl-4-hydroxybenzoat:

$$\% \, w_i \, (m/m) = \frac{b_i}{200 \times a}$$

hvor:

b_i = koncentrationen (µg/ml) af konserveringsstof i prøveopløsningen, aflæst af kalibreringskurven, og

a = prøvens masse (g).

8. Repeterbarhed (1)

Se bemærkningerne i punkt 10.5.

9. Reproducerbarhed (1)

Se bemærkningerne i punkt 10.5.

10. Bemærkninger

10.1. Stationær fase

Retentionsegenskaberne af opløste stoffer i HPLC-bestemmelser er stærkt afhængige af den stationære fases type, mærke og historie. Hvorvidt en given kolonne er anvendelig til adskillelse af de undersøgte konserveringsstoffer, kan afgøres ud fra resultaterne for standardopløsningerne (se bemærkningerne under 6.2.3). Ud over det foreslåede pakningsmateriel til HPLC-kolonnen er også Hypersil ODS og Zorbax ODS fundet egnede.

Som alternativ kan man optimere sammensætningen af den mobile fase med henblik på at opnå den krævede adskillelse.

10.2. Detektionsbølgelængde

En ruggednesstest af den beskrevne metode har vist, at en ringe ændring af detektorbølgelængde kan have væsentlig indvirkning på resultaterne af bestemmelsen.

Denne parameter skal derfor være nøje kontrolleret under analysen.

(1) ISO 5725.

10.3. Interferenser

Under de beskrevne betingelser bliver mange andre stoffer, f.eks. konserveringsstoffer og tilsætningsstoffer, ligeledes elueret. Retentionstid for en lang række af de konserveringsstoffer, der er anført i bilag VI til Rådets direktiv om kosmetiske produkter, er angivet i: N. de Kruijf, A. Schouten, M.A.H. Rijk og L.A. Pranato-Soetardhi (1989): »Determination of preservatives in cosmetic products II. High-performance liquid chromatographic identification« (J. Chromatography 469, 317-398).

10.4. Til beskyttelse af HPLC-kolonnen kan en passende forkolonne anvendes.

10.5. Metoden er undersøgt i en ringtest, hvori 9 laboratorier deltog. Der analyseredes tre prøver. I følgende tabel er for hver af de tre prøver angivet gennemsnit i % m/m (m), repeterbarhed (r) og reproducerbarhed (R) for de deri forekommende konserveringsstoffer:

prøve		2-phenoxy-ethanol	1-phenoxy-propan-2-ol	methylparaben	ethylparaben	propylparaben	butylparaben	benzylparaben
vitamincreme	m	1,124		0,250	0,0628	0,031	0,0906	
	r	0,016		0,018	0,0035	0,0028	0,0044	
	R	0,176		0,030	0,0068	0,0111	0,0034	
dagcreme	m	1,196		0,266	0,076			
	r	0,040		0,003	0,002			
	R	0,147		0,022	0,004			
massagecreme	m		0,806			0,180	0,148	0,152
	r		0,067			0,034	0,013	0,015
	R		0,112			0,078	0,012	0,016

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Appendix 5

I. IDENTIFIKATION OG BESTEMMELSE AF BENZOESYRE, 4-HYDROXYBENZOESYRE, SORBINSYRE, SALICYLSYRE OG PROPIONSYRE I KOSMETISKE PRODUKTER

1. **Formål og anvendelsesområde**

Denne metode kan anvendes til identifikation og bestemmelse af benzoesyre, 4-hydroxybenzoesyre, sorbinsyre, salicylsyre og propionsyre i kosmetiske produkter. Separate procedurer benyttes til identifikation af disse konserveringsstoffer, bestemmelse af propionsyre, og til bestemmelse af benzoesyre, 4-hydroxybenzoesyre, sorbinsyre og salicylsyre.

2. **Definition**

Inhdoldet af benzoesyre, 4-hydroxybenzoesyre, salicylsyre, sorbinsyre og propionsyre bestemt efter denne metode udtrykkes som masseprocent (% m/m) fri syre.

A. IDENTIFIKATION

1. **Prinzip**

Efter ekstraktion af konserveringsstofferne med syre/base analyseres ekstraktet med TLC, idet der anvendes »on-plate« derivatisering af stofferne. Afhængig af de opnåede resultater bekræftes identifikationen ved hjælp af højtryksvæskekromatografi (HPLC) eller, hvis der er tale om propionsyre, ved hjælp af gaskromatografi (GC).

2. **Reagenser**

2.1. Generelt

Alle reagenser skal være analyserne. Vand skal være destilleret eller af mindst tilsvarende kvalitet.

2.2. Acetone

2.3. Diethylether

2.4. Acetonitril

2.5. Toluen

2.6. n-Hexan

2.7. Paraffin, flydende

2.8. Saltsyre, 4 M

2.9. Kaliumhydroxid, vandig opløsning, 4 M

2.10. Calciumklorid, $CaCl_2,2H_2O$

2.11. Lithiumkarbonat, Li_2CO_3

2.12. 2-Brom-2'-acetonaphton

2.13. 4-Hydroxybenzoesyre

2.14. Salicylsyre

2.15. Benzoesyre

2.16. Sorbinsyre

2.17. Propionsyre

2.18. Referenceopløsninger:

Der fremstilles en 0,1 % (m/v) opløsning (100 mg/100 ml) af hver af de fem konserveringsstoffer (2.13 til 2.17) i diethylether.

2.19. Derivatiseringsreagens:

0,5 % (m/v) opløsning (50 mg/10 ml) af 2-brom-2'-acetonaphton (2.12) i acetonitril (2.4). Frisk opløsning fremstilles hver uge. Opbevares i køleskab.

2.20. Katalysatoropløsning:

0,3 % opløsning af lithiumkarbonat (2.11) i vand (300 mg/100 ml). Denne opløsning skal være frisk fremstillet.

2.21. Udviklingsvæske:

Toluen (2.5)/acetone (2.2) (20 : 0,5; v/v)

2.2. Flydende paraffin (2.7)/n-hexan (2.6) (1 : 2; v/v)

3. **Apparatur**

Almindeligt laboratorieudstyr

3.1. Vandbad ved 60 °C

3.2. TLC-kar

3.3. UV-lampe, 254 og 366 nm

3.4. Tyndtlagsplader, Kieselgel 60, uden fluorescenceindikator, 20 × 20 cm, lagtykkelse 0,25 mm med koncentrationszone 2,5 × 20 cm (Merck 11845, eller tilsvarende)

3.5. Mikrosprøjte, 10 µl

3.6. Mikrosprøjte, 25 µl

3.7. Varmeovn, anvendlig for temperaturer indtil 105 °C

3.8. 50 ml reagensglas med skruelåg

3.9. Filtrerpapir, Schleicher & Shull, Weisbond nr. 5892 eller tilsvarende, diameter 90 mm.

3.10. Universal-indikatorpapir, pH 1—11

3.11. 5 ml hætteglas til prøver

3.12. Rotationsinddamper (Rotavapor eller tilsvarende)

3.13. Varmeplade

4. **Fremgangsmåde**

4.1. Prøvetilberedning

Ca. 1 g prøve afvejes i et 50 ml reagensglas med skruelåg (3.8). Der tilsættes fire dråber saltsyre, 4 M (2.8) og 40 ml acetone (2.2). Til stærkt basiske produkter som håndsæbe tilsættes 20 dråber saltsyre 4 M (2.8). Med indikatorpapir (3.10) kontrolleres, at pH er ca. 2. Glasset lukkes og omrystes kraftigt i et minut.

Hvis det er nødvendigt at fremskynde ekstraktionen af konserveringsstofferne over på acetonefasen, opvarmes blandingen forsigtigt til ca. 60 °C for at smelte fedtfasen. Opløsningen afkøles til rumtemperatur og filtreres gennem filtrerpapir (3.9) ned i en konisk kolbe. 20 ml af filtratet

overføres til en 200 ml konisk kolbe, der tilsættes 20 ml vand og blandes. pH af blandingen indstilles på ca. 10 med 4 M kaliumhydroxyd (2.9). Til pH målingen benyttes indikatorpapir (3.10).

Blandingen tilsættes 1 g calciumklorid (2.10), omrystes kraftigt og filtreres gennem filtrerpapir (3.9) over i en 250 ml skilletragt indeholdende 75 ml diethylether (2.3), og blandingen omrystes kraftigt i et minut. Lad faserne skille og aftap den vandige fase i en 250 ml konisk kolbe. Etherfasen kasseres. Den vandige fases pH indstilles til ca. 2 med 4 M saltsyre (2.8) ved brug af indikatorpapir (3.10). Derefter tilsættes 10 ml diethylether (2.3), og blandingen omrystes kraftigt i et minut. Når faserne er adskilt, overføres etherfasen til en rotationsinddamper (3.12). Den vandige fase kasseres. Etherfasen inddampes til næsten tørhed og inddampningsresten genopløses i 1 ml diethylether (2.3). Denne opløsning overføres til et hætteglas (3.11).

4.2. Tyndtlagskromatografi

For hver reference- og prøveopløsning, som skal kromatograferes, påsættes ca. 3 µl lithiumkarbonatopløsning (2.20) med en sprøjte (3.5) i lige stor afstand på startlinien af TLC-pladens (3.4) koncentrationszone, og pladen blæses tør med kold luft.

TLC-pladen anbringes på en varmeplade (3.13) opvarmet til 40 °C, for at holde pletterne så små som muligt. Med en mikrosprøjte (3.5) påsættes, på pladens startlinie nøjagtigt oven i de pletter, hvor lithiumkarbonatopløsningen påførtes, 10 µl af hver referenceopløsning (2.18) og prøveopløsningen (4.1).

Til slut påsættes ca. 15 µl derivatiseringsreagens (2.19) (2-brom-2'-acetonaphtonopløsning); igen nøjagtigt oven i de pletter, hvor reference- og prøveopløsninger samt lithiumkarbonatopløsningen påførtes.

TLC-pladen opvarmes i en ovn (3.7) ved 80 °C i 45 minutter.

Efter afkøling udvikles pladen i et TLC-kar (3.2), som på forhånd er ækvilibreret i 15 minutter (uden foring med filtrerpapir), med udviklingsvæske 2.21 (toluen/acetone), indtil væskefronten er vandret ca. 15 cm (det tager ca. 80 minutter).

TLC-pladen blæses tør med kold luft og undersøges under UV-lys (3.3.). For at forstærke fluorescencen af svage pletter kan TLC-pladen dyppes i flydende paraffin/n-hexan (2.22).

5. Identifikation

R_f-værdi af hver plet beregnes

Prøvens R_f og udseende under UV-lys sammenlignes med disse for referenceopløsningerne.

Drag en foreløbig konklusion med hensyn til identiteten af de tilstedeværende konserveringsstoffer. Udfør HPLC som beskrevet i afsnit B, eller GC som beskrevet i afsnit C, hvis tilstedeværelsen af propionsyre er påvist. Sammenlign de opnåede retentionstider for prøven med retentionstiderne af referenceopløsningerne.

Identifkation af konserveringsstofferne i prøven sker ved at kombinere resultaterne af TLC og HPLC eller GC.

B. BESTEMMELSERNE AF BENZOESYRE, 4-HYDROXYBENZOESYRE, SORBINSYRE OG SALICYLSYRE

1. Princip

Efter at være gjort sur ekstraheres prøven med en blanding af ethanol og vand. Efter filtrering bestemmes indholdet af konserveringsstoffer ved højtryksvæskekromatografi (HPLC).

2. Reagenser

2.1. Alle reagenser skal være analyserene, og hvor det er hensigtsmæssigt egnet til HPLC. Det anvendte vand skal være destilleret eller af mindst tilsvarende renhed.

2.2. Ethanol, absolut

2.3. 4-Hydroxybenzoesyre

2.4. Salicylsyre

2.5. Benzoesyre

2.6. Sorbinsyre

2.7. Natriumacetat, $CH_3COONa \cdot 3H_2O$

2.8. Eddikesyre, (ρ_4^{20} = 1,05 g/ml)

2.9. Acetonitril

2.10. Svovlsyre, 2 M

2.11. Kaliumhydroxid, vandig, 0,2 M

2.12. 2-Methoxybenzoesyre

2.13. Ethanol/vandblanding:

Ni voluminer ethanol (2.2) blandes med et volumen vand (2.1).

2.14. Intern standardopløsning:

Opløs ca. 1 g 2-methoxybenzoesyre (2.12) i 500 ml ethanol/vandblanding (2.13).

2.15. Mobil fase til HPLC:

2.15.1. Acetatbuffer: Tilsæt 6,35 g natriumacetat (2.7) og 20 ml eddikesyre (2.8) til 1 l vand og bland.

2.15.2. Den mobile fase fremstilles ved at blande ni voluminer acetatbuffer (2.15.1) med et volumen acetonitril (2.9).

2.16. Stamopløsning af konserveringsstoffer:

Afvej ca. 0,05 g 4-hydroxybenzoesyre (2.3), 0,2 g salicylsyre (2.4), 0,2 g benzoesyre (2.5) og 0,05 g sorbinsyre (2.6) nøjagtigt i en 50 ml målekolbe og fyld op til mærket med ethanol/vand blandingen (2.13). Opløsningen opbevares i køleskab og er holdbar i en uge.

2.17. Standardopløsning af konserveringsstoffer:

Af stamopløsningen (2.16) overføres henholdsvis 8,00, 4,00, 2,00, 1,00 og 0,50 ml til 20 ml målekolber. Hver kolbe tilsættes 10,00 ml intern standardopløsning (2.14) og 0,5 ml svovlsyre 2 M (2.10). Fyld op til mærket med ethanol/vandblanding (2.13). Opløsningerne skal være friskfremstillede.

3. **Apparatur**

Sædvanligt laboratorieudstyr, som ikke er nærmere specificeret, og:

3.1. Vandbad ved 60 °C

3.2. HPLC udstyr med en 10 µl injektionsloop og en UV-detektor med variabel bølgelængde.

3.3. Analytisk kolonne:

Rustfrit stål, længde 12,5—25 cm, indvendig diameter 4,6 mm, pakket med Nucleosil 5C18 eller tilsvarende.

3.4. Filtrerpapier, diameter: 90 mm, Schleicher og Schull, Weisband nr. 5892 eller tilsvarende.

3.5. 50 ml reagensglas med skruelåg

3.6. 5 ml hætteglas til prøver

3.7. Kogesten, carborundumkorn, størrelse 2—4 mm, eller tilsvarende.

4. Fremgangsmåde

4.1. Prøvetilberedning

4.1.1. Prøvetilberedning uden tilsætning af intern standard:

Ca. 1 g prøve afvejes i et 50 ml glas med skruelåg (3.5). 1,0 ml svovlsyre 2 M (2.10) og 40,0 ml ethanol/vandblanding (2.13) afpipetteres i glasset. Der tilsættes ca. 1 g carborundumkorn (3.7). Glasset lukkes og omrystes kraftigt i mindst et minut indtil en homogen suspension er dannet. Glasset anbringes i et vandbad ved 60 °C (3.1) i nøjagtigt 5 minutter for at lette ekstraktion af konserveringsstofferne over på ethanolfasen.

Glasset afkøles straks under rindende vand, og ekstraktet opbevares derefter i en time ved 5 °C. Ekstraktet filtreres gennem et filtrerpapir (3.4). Ca. 2 ml af det filtrerede extrakt overføres til et hætteglas (3.6). Ekstraktet opbevares ved 5 °C og HPLC-analysen udføres senest 24 timer efter ekstraktion.

4.1.2. Prøvetilberedning med tilsætning af intern standard:

Afvej til tredje decimal 1,0 ± 0,1 g (a gram) af prøven i et 50 ml glas med skruelåg (3.5). 1,0 ml svovlsyre 2 M (2.10) afpipetteres og derefter tilsættes 30,0 ml ethanol/vandblanding (2.13). Der tilsættes ca. 1 g carborundumkorn (3.7) og 10,0 ml intern standardopløsning (2.14). Glasset lukkes, og omrystes kraftigt i mindst et minut indtil en homogen suspension er dannet. Glasset anbringes i et vandbad (3.1) ved 60 °C i nøjagtig 5 minutter for at lette ekstraktion af konserveringsstofferne over på ethanolfasen.

Glasset afkøles straks under rindende vand, og ekstraktet opbevares derefter ved 5 °C i en time.

Ekstraktet filtreres gennem et filtrerpapir (3.4). Ca. 2 ml af det filtrerede ekstrakt overføres til et hætteglas (3.6). Ekstraktet opbevares ved 5 °C og HPLC-bestemmelse udføres senest 24 timer efter fremstilling.

4.2. Højtryksvæskekromatografi

Mobil fase: acetonitril/acetatbuffer (2.15)

Flow af den mobile fase (2.15) gennem kolonnen indstilles til 2,0 ml/min ± 0,5 ml/min.

4.2.1. Kalibrering

Der injiceres 10 µl af hver af standardopløsningerne af konserveringsstoffer (2.17) i væskekromatografen (3.2). Forholdet mellem tophøjde af de undersøgte konserveringsstoffer og af den interne standard bestemmes ved de opnåede kromatogrammer. For hvert konserveringsstof optegnes en kurve, der afbilder dette forhold mod standardopløsningens koncentration.

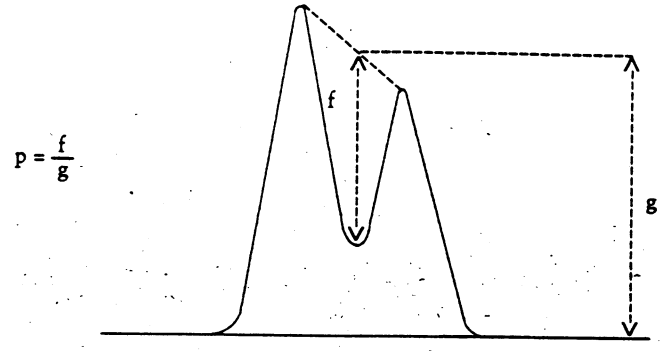
Kontrollér at der opnås lineær respons på de til kalibrering anvendte standardopløsninger.

4.2.2. Der injiceres 10 µl prøveekstrakt (4.1.1) i væskekromatografen (3.2) og kromatogrammet optages. Derefter injiceres 10 µl standardopløsning af konserveringsstoffer (2.17) og kromatogrammet optages. De opnåede kromatogrammer sammenlignes. Hvis der i kromatogrammet af prøveekstraktet (4.1.1) ikke synes at være en top med tilnærmelsesvis samme retentionstid som 2-methoxybenzoesyre (anbefalet intern standard), injiceres 10 µl prøveekstrakt tilsat intern standard (4.1.2) i kromatografen, og kromatogrammet optages.

Optræder der en interfererende top i kromatogrammet af prøveekstraktet (4.1.1) med samme retentionstid som 2-metoxybenzoesyre, vælges en mere velegnet intern standard. (Optræder et af de undersøgte konserveringsstoffer ikke i kromatogrammet, kan det pågældende stof bruges som intern standard).

Ved kromatogrammerne af en standardopløsning og et prøveekstrakt sikres, at disse opfylder følgende krav:

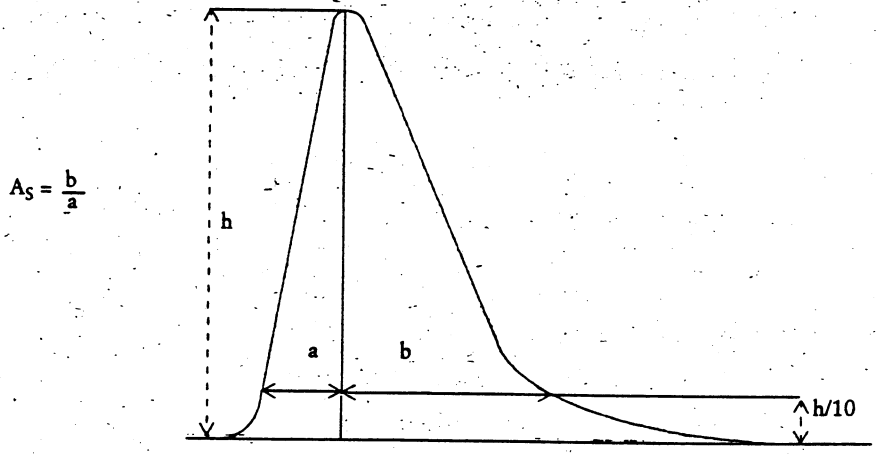
— adskillelsen af de to nærmest liggende toppe skal være mindst 0,9. (Topadskillelse er defineret i figur 1)



Figur 1 Topadskillelse (p)

Opnås den krævede adskillelse ikke, skal der enten anvendes en mere effektiv kolonne, eller sammensætningen af den mobile fase skal korrigeres, således at kravet opfyldes

— assymmetrifaktoren A_s skal for samtlige toppe være mellem 0,9 og 1,5. (Assymetrifaktoren er defineret i figur 2). For optegnelse af kromatogram til bestemmelse af asymmetrifaktor anbefales en papirhastighed på mindst 2 cm/min



Figur 2 Asymmetrifaktor (A_s)

— grundlinien skal være stabil.

5. Beregning

Benyt forholdet mellem tophøjden af det undersøgte konserveringsstof og tophøjden af 2-methoxybenzoesyre (intern standard) og kalibreringskurven til beregning af koncentrationen af syrekonserveringsstof i prøveekstraktet. Indholdet af benzoesyre, 4-hydroxybenzoesyre, sorbinsyre og salicylsyre i prøven beregnes som masseprocent (X_i) ved hjælp af formlen:

$$x_i\ \%\ (m/m) = \frac{100 \cdot 20 \cdot b}{10^6 \cdot a} = \frac{b}{500 \cdot a}$$

hvor:

b = koncentration (μg/ml) af konserveringsstoffet i prøveekstraktet, aflæst på kalibreringskurven

a = masse (g) af prøven (4.1.2).

6. Repeterbarhed (1)

Ved et indhold af 4-hydroxybenzoesyre på 0,40 % bør forskellen mellem resultaterne af to sideløbende bestemmelser udført på samme prøve ikke overstige en absolut værdi på 0,035 %.

Ved et indhold af benzoesyre på 0,50 % bør forskellen mellem resultaterne af to sideløbende bestemmelser udført på samme prøve ikke overstige en absolut værdi på 0,050 %.

Ved et indhold af salicylsyre på 0,50 % bør forskellen mellem resultaterne af to sideløbende bestemmelser udført på samme prøve ikke overstige en absolut værdi på 0,045 %.

Ved et indhold af sorbinsyre på 0,60 % bør forskellen mellem resultaterne af to sideløbende bestemmelser udført på samme prøve ikke overstige en absolut værdi på 0,035 %.

7. Bemærkninger

7.1. Resultater af en robusthedstest på metoden viste, at den anvendte mængde svovlsyre til ekstraktion af syrekonserveringsstofferne fra prøven er kritisk, og at den tilberedte prøvemængde skal holdes inden for de foreskrevene grænser.

7.2. Om ønsket kan en egnet forkolonne anvendes.

C. BESTEMMELSE AF PROPIONSYRE

1. Formål og anvendelsesområde

Denne metode kan anvendes til bestemmelse af propionsyre, maximal koncentration 2 % (m/m) i kosmetiske produkter.

2. Definition

Propionsyrekoncentration målt ved denne metode udtrykkes som masseprocent (% m/m) af produktet.

3. Princip

Efter ekstraktion af propionsyre fra produktet, udføres bestemmelse af propionsyre ved gaskromatografi, idet der bruges 2-methylpropionsyre som intern standard.

4. Reagenser

Alle reagenser skal være analyserene. Vand skal være destilleret eller af tilsvarende kvalitet.

4.1. Ethanol 96 % (v/v)

4.2. Propionsyre

4.3. 2-Methylpropionsyre

4.4. Orthofosforsyre, 10 % (m/v)

4.5. Propionsyreopløsning

Ca. 1,00 g (p gram) propionsyre afvejes nøjagtigt i en 50 ml målekolbe og fyldes op til mærket med ethanol (4.1).

4.6. Intern standardopløsning

Ca. 1,00 g (e gram) 2-methylpropionsyre afvejes nøjagtigt i en 50 ml målekolbe og fyldes op til mærket med ethanol (4.1).

(1) ISO 5725.

5. Apparatur

5.1. Sædvanligt laboratorieudstyr

5.2. Gaskromatograf med flammeionisationsdetektor

5.3. Reagensglas (20 × 150 mm) med skruelåg

5.4. Vandbad ved 60 °C

5.5. 10 ml glassprøjte med membranfilter (porestørrelse: 0,45 μm)

6. Fremgangsmåde

6.1. Prøvetilberedning

6.1.1. Prøvetilberedning uden intern standard

Ca. 1 g prøve afvejes i et reagensglas (5.3). Tilsæt 0,5 ml fosforsyre (4.4) og 9,5 ml ethanol (4.1). Glasset lukkes og omrystes kraftigt. Glasset kan om nødvendigt anbringes i et vandbad ved 60 °C i 5 minutter, således at fedtfasen tilnærmelsesvis opløses. Afkøles hurtigt under rindende vand. En del af opløsningen filtreres gennem et membranfilter (5.5). Filtratet kromatograferes samme dag.

6.1.2. Prøvetilberedning med intern standard

1 ± 0,1 g (a gram) prøve afvejes med tre decimaler i et reagensglas (5.3). Tilsæt 0,50 ml fosforsyre (4.4), 0,50 ml intern standardopløsning (4.6) og 9 ml ethanol (4.1).

Glasset lukkes og omrystes kraftigt. Glasset kan om nødvendigt anbringes i et vandbad ved 60 °C i 5 minutter, således at fedtfasen tilnærmelsesvis opløses.

Afkøles hurtigt under rindende vand. En del af opløsningen filtreres gennem et membranfilter (5.5). Filtratet kromatograferes samme dag.

6.2. Betingelser for gaskromatografi

Følgende betingelser anbefales:

Kolonne

Type	Rustfrit stål
Længde	2 m
Diameter	1/8"
Pakning	10 % SP™ 1000 (eller tilsvarende) + 1 % H_3PO_4 på Chromosorb WAW 100—120 mesh

Temperatur

Injektor	200 °C
Kolonne	120 °C
Detektor	200 °C

Bæregas

Nitrogen	
Flow	25 ml/min

6.3. Kromatografi

6.3.1. Kalibrering

Til en række 20 ml målekolber overføres med pipette henholdsvis 0,25, 0,50, 1,00, 2,00 og 4,00 ml propionsyreopløsning (4.5). Til hver målekolbe tilsættes med pipette 1,00 ml intern standardopløsning (4.6). Der fyldes op til stregen med ethanol (4.1) og blandes. Opløsninger, der tilberedes på denne måde, indeholder e mg/ml 2-methylpropionsyre som intern standard (dvs. 1 mg/ml hvor e = 1,000) og p/4, p/2, p, 2p, 4p mg/ml propionsyre (dvs. 0,25, 0,50, 1,00, 2,00, 4,00 mg/ml, hvor p = 1,000).

Injicér 1 µl af hver af disse opløsninger og tegn kalibreringskurven ved at optegne propionsyre/2-methylpropionsyre masseforholdet på x-aksen og forholdet af de tilsvarende toparealer på y-aksen.

Foretag tre injektioner af hver opløsning og udregn gennemsnitlige toparealforhold.

6.3.2. Bestemmelse

Injicér 1 µl af prøvefiltratet 6.1.1. Sammenlign kromatogrammet med kromatogrammet for en af standardopløsningerne (6.3.1). Hvis en top har omtrent samme retentionstid som 2-methylpropionsyre, ændres den interne standard. Hvis der ikke observeres interferens, injiceres 1 µl af prøvefiltratet 6.1.2, og toparealerne af propionsyre og intern standard måles.

Foretag tre injektioner af hver opløsning og beregn gennemsnitlige toparealforhold.

7. **Beregning**

7.1. På den fremkomne kalibreringskurve 6.3.1 aflæses masseforholdet (K) svarende til toparealforholdet beregnet i 6.3.2.

7.2. På grundlag af det således fremkomne masseforhold beregnes prøvens indhold af propionsyre (x) som masseprocent, idet følgende formel anvendes:

$$x\ \%\ (m/m) = K\frac{0{,}5 \cdot 100 \cdot e}{50 \cdot a} = K\frac{e}{a}$$

Hvor

K = forholdet beregnet i 7.1

e = massen i gram af den interne standard afvejet i 4.6

a = massen i g af prøven afvejet i 6.1.2

Afrund resultaterne til en decimal.

8. **Repeterbarhed** (1)

For et propionsyreindhold på 2 % må afvigelsen mellem resultaterne af to parallelle bestemmelser udført på samme prøve ikke overstige 0,12 %.

II. IDENTIFIKATION OG BESTEMMELSE AF HYDROQUINON, HYDROQUINONMONOMETHYLETHER, HYDROQUINONMONOETHYLETHER OG HYDROQUINONMONOBENZYLETHER I KOSMETISKE PRODUKTER

A. IDENTIFIKATION

1. **Formål og anvendelsesområde**

Denne metode anvendes til identifikation af hydroquinon, hydroquinonmonomethylether, hydroquinonmonoethylether og hydroquinonmonobenzylether (monobenzon) i kosmetiske produkter til hudblegning.

2. **Princip**

Hydroquinon og dets ethere identificeres ved tyndtlagskromotografi (TLC).

3. **Reagenser**

Alle reagenser skal være analyserene.

(1) ISO 5725.

KOMMISSIONENS SYVENDE DIREKTIV 96/45/EF

af 2. juli 1996

om analysemetoderne for kontrol af kosmetiske midlers sammensætning

(Tekst af betydning for EØS)

KOMMISSIONEN FOR DE EUROPÆISKE FÆLLESSKABER HAR —

under henvisning til traktaten om oprettelse af Det Europæiske Fællesskab,

under henvisning til Rådets direktiv 76/768/EØF af 27. juli 1976 om indbyrdes tilnærmelse af medlemsstaternes lovgivning om kosmetiske midler (1), senest ændret ved Kommissionens direktiv 95/34/EF (2), særlig artikel 8, stk. 1, og

ud fra følgende betragtninger:

I direktiv 76/768/EØF foreskrives en officiel kontrol af kosmetiske midler til konstatering af, om de i fællesskabsbestemmelserne fastsatte betingelser vedrørende sammensætningen af kosmetiske midler overholdes;

de nødvendige analysemetoder bør fastlægges hurtigst muligt; en række metoder er allerede blevet vedtaget med Kommissionens direktiv 80/1335/EØF (3), ændret ved direktiv 87/143/EØF (4), Kommissionens direktiv 82/434/EØF (5), ændret ved direktiv 90/207/EØF (6), Kommissionens direktiv 83/514/EØF (7), 85/490/EØF (8), 93/73/EØF (9) og 95/32/EF (10);

identifikation og bestemmelse af 2-phenoxyethanol, 1-phenoxypropan-2-ol samt methyl-, ethyl-, propyl-, butyl- og benzyl-4-hydroxybenzoat i kosmetiske midler udgør syvende etape;

de i dette direktiv fastsatte foranstaltninger er i overensstemmelse med udtalelse fra Udvalget for Tilpasning af Direktiv 76/768/EØF til det Tekniske Fremskridt —

HAR UDSTEDT FØLGENDE DIREKTIV:

Artikel 1

I forbindelse med den officielle kontrol af kosmetiske midler træffer medlemsstaterne de fornødne foranstaltninger for at sikre, at identifikation og bestemmelse af 2-phenoxyethanol, 1-phenoxypropan-2-ol samt methyl-, ethyl-, propyl-, butyl- og benzyl-4-hydroxybenzoat foretages efter de metoder, der er beskrevet i bilaget.

Artikel 2

1. Medlemsstaterne sætter de nødvendige love og administrative bestemmelser i kraft for at efterkomme dette direktiv senest den 30. september 1997. De underretter straks Kommissionen herom.

2. Når medlemsstaterne vedtager disse bestemmelser, henvises der deri til dette direktiv, eller de ledsages ved offentliggørelsen af en sådan henvisning. De nærmere regler for denne henvisning fastsættes af medlemsstaterne.

3. Medlemsstaterne meddeler Kommissionen teksten til de nationale retsforskrifter, som de udsteder på det område, der er omfattet af dette direktiv.

Artikel 3

Dette direktiv træder i kraft på tyvendedagen efter offentliggørelsen i *De Europæiske Fællesskabers Tidende.*

Artikel 4

Dette direktiv er rettet til medlemsstaterne.

Udfærdiget i Bruxelles, den 2. juli 1996.

På Kommissionens vegne

Emma BONINO

Medlem af Kommissionen

(1) EFT nr. L 262 af 27. 9. 1976, s. 169.
(2) EFT nr. L 167 af 18. 7. 1995, s. 19.
(3) EFT nr. L 383 af 31. 12. 1980, s. 27.
(4) EFT nr. L 57 af 27. 2. 1987, s. 56.
(5) EFT nr. L 185 af 30. 6. 1982, s. 1.
(6) EFT nr. L 108 af 28. 4. 1990, s. 92.
(7) EFT nr. L 291 af 24. 10. 1983, s. 9.
(8) EFT nr. L 295 af 7. 11. 1985, s. 30.
(9) EFT nr. L 231 af 14. 9. 1993, s. 34.
(10) EFT nr. L 178 af 28. 7. 1995, s. 20.

National Environmental Research Institute

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NERI's tasks are primarily to conduct research, collect data, and give advice on problems related to the environment and nature.

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Faglige rapporter fra DMU/NERI Technical Reports

1999

Nr. 288: Mere og bedre natur i landbrugslandet - dokumenteret grundlag for en ekstra indsats. Reddersen, J., Tybirk, K., Halberg, N. & Jensen, J. 109 s., 120,00 kr.

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2000

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Nr. 319: Forbrug af økologiske fødevarer. Del 2: Modellering af efterspørgsel. Af Wier, M. & Smed, S. (i trykken)

Contents of 23 selected preservatives were determined in 67 skin cream products to check whether the obligatory ingredient labelling on the products was correct, and the concentrations of the preservatives in the products were within the maximum allowed concentration for individual preservatives. The preservatives selected for the present investigation were: parabens, 2-phenoxy ethanol, benzoic acid, 4-hydroxy-benzoic acid, salicylic acid, sorbic acid, Kathon CG, methyldibromo glutaronitril, 5-bromo-5-nitro-1,3-dioxane, 2-bromo-2-nitropropane-1,3-diol, formaldehyde and formaldehyde releasers. The concentrations of target preservatives in the investigated products were within the maximum allowed concentrations of these. The Danish EPA will check the conformity of preservative labelling with the contents of the target preservatives found in the investigated products.

Ministry of Environment and Energy
National Environmental Research Institute

ISBN 87-7772-503-4
ISSN 0905-815X