

Presence of formaldehyde in topical corticosteroid preparations available on the Swedish market

ANTHONY TEIK-JIN GOON, BIRGITTA GRUVBERGER, LENA PERSSON, MARLÈNE ISAKSSON AND MAGNUS BRUZE

Department of Occupational and Environmental Dermatology, SE-205 02 Malmö, Sweden

The aim of this study was to investigate the formaldehyde content of topical corticosteroid preparations available on the Swedish market. 73 samples were analysed with the chromotropic acid (CA) method for semi-quantitative formaldehyde determination and 30 samples with a high-performance liquid chromatographic method. These included 24 ointments, 28 creams, 1 lotion, 6 liniments, 2 gels, 9 solutions, 2 mousses and 1 oral paste. Formaldehyde was found in 5 creams and 1 ointment. Sources of formaldehyde in these preparations were discussed. Isopropanol was identified as a probable source of yellow discoloration, leading to false-negative results with the CA method.

Key words: chromotropic acid; corticosteroids; formaldehyde; high-performance liquid chromatography; isopropanol. © Blackwell Munksgaard, 2003.

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Formaldehyde is a well-documented contact allergen and is present in many products in daily use, including cosmetics, shampoos, topical medicaments and dishwashing liquids. A semi-quantitative method, based on the reaction of formaldehyde with chromotropic acid (CA), can be relatively easily performed to detect formaldehyde release from samples (1). A more accurate quantitative method is a high-performance liquid chromatographic method, with 2,4-dinitrophenylhydrazine (2,4-DNPH) as a derivatizing agent (2).

In this study, we aimed to investigate the formaldehyde content of 73 topical corticosteroid preparations available on the Swedish market in the autumn of 2002.

Materials and Methods

All topical corticosteroid preparations obtainable from the Swedish market at the time of this study were investigated. Unopened samples of these preparations were purchased from the Malmö University Hospital pharmacy. These included 24 ointments, 28 creams, 1 lotion, 6 liniments, 2 gels, 9 solutions, 2 mousses and 1 oral paste. These samples were then opened and the contents analysed for formaldehyde release using the CA method (1). High-performance liquid chromatography (HPLC) was performed on all samples for

which formaldehyde content had been demonstrated by the CA method to quantify the amount of formaldehyde (2). The high-performance liquid chromatographic method was also performed on all samples that had caused discoloration of CA, to look for hidden formaldehyde content. All analyses of samples, standards and blanks were performed in duplicate.

Chromotropic acid method for semi-quantitative formaldehyde determination

To 25-ml glass jars with ground-in stoppers, we added 1.0 ml of standard solutions of formaldehyde (40.0 µg/ml, 20.0 µg/ml, 10.0 µg/ml and 2.5 µg/ml). A jar with 1.0 ml of purified water was used as a blank. For the analysis of the samples, 1.0 g of each sample was weighed and placed in a jar. In each of these jars (standard, blank and sample) was placed a 1.0-ml glass tube containing 0.5 ml of the reagent, freshly prepared CA in concentrated sulfuric acid (4 mg/ml). The jars were kept in the dark and the colour observed after 2 days. A violet colour of the reagent appears if formaldehyde is present in a sample, and this would be compared with the violet colour of the reagents in the jars with standard solutions to get a semi-quantitative estimation of formaldehyde content. Sometimes, a yellowish discoloration develops.

To find out whether isopropanol could cause discoloration of CA and mask the colour change to formaldehyde, another small experiment was performed. The method was as outlined above but modified as follows. One jar containing 1.0 ml of purified water was used as a blank. To jars containing 1.0 ml of purified water, we added the following: 40 μ l of formaldehyde (1000 μ g/ml), 20 μ l of isopropanol, 40 μ l of isopropanol, 20 μ l of isopropanol + 40 μ l of formaldehyde (1000 μ g/ml) and 40 μ l of isopropanol + 40 μ l of formaldehyde (1000 μ g/ml). Small tubes containing the reagent were then placed in each of these jars.

High-performance liquid chromatography quantitative determination of formaldehyde

1 g of accurately weighed sample was dissolved in 9.0 ml of tetrahydrofuran (THF):water (9:1). Standard solutions of formaldehyde, 4 μ g/ml, 6 μ g/ml and 10 μ g/ml, were prepared. 1.0 ml of each of these standard solutions was added to 9.0 ml of THF:water (9:1). A blank solution consisting of 1.0 ml of purified water and 9.0 ml of THF:water (9:1) was also prepared.

1.0 ml of each of these diluted standard, blank or sample solutions was added to 0.4 ml of 0.1% 2,4-DNPH, stirred for 60 s in a vortex mixer and allowed to stand for 2 min at room temperature. This solution was then stabilized by adding 0.4 ml of 0.1 M phosphate buffer (pH 6.8), 0.7 ml of 1 M sodium hydroxide solution and 2.5 ml of acetonitrile. Aliquots of 20 μ l were injected into the HPLC system.

The HPLC system used was from Thermo Separation Products (San Jose, CA, USA). This system consisted of a Spectra Series P200 gradient pump, a Spectra System UV1000 detector and a SP4400 ChromJet integrator. The system was equipped with a Rheodyne 7125 injector with a 20- μ l loop. The column (4.6 mm internal diameter $\times$ 250 mm) was packed with Nucleosil C₁₈ (5 μ m). The mobile phase was acetonitrile:water (60:40). The flow rate was 1 ml/min.

Results

The results of CA and high-performance liquid chromatographic analyses are summarized in Table 1. The preparation with the highest content of formaldehyde was Flutivate 0.05% creme (GlaxoSmithKline AB, Mölndal, Sweden), with a content of >40 μ g by the semi-quantitative CA method and 178 p.p.m. by the high-performance liquid chromatographic method. Apolar 0.1% salva (Alpharma AB, Stockholm, Sweden), MILDISON lipid 1% kräm (Yamanouchi Pharma AB, Malmö, Sweden), Cortimyk kräm (CCS, Clean

Chemical Sweden AB, Borlänge, Sweden), Daktacort kräm (Janssen-Cilag AB, Sollentuna, Sweden) and Pevisone kräm (Janssen-Cilag AB) were also found to contain smaller amounts of formaldehyde by the CA method and by the high-performance liquid chromatographic method (5–11 p.p.m.).

Of 24 ointments tested, only Apolar 0.1% salva was found to contain small amounts of formaldehyde (9 p.p.m.). Of 28 creams tested, 5 were found to contain formaldehyde, with a range of 5–178 p.p.m. The cream with the highest content of formaldehyde was Flutivate 0.05% creme. Formaldehyde was not demonstrated in any of the lotions, liniments, gels, solutions or the oral paste. 16 samples had given a definite yellow discoloration – 7 solutions, 4 liniments, 4 creams and 1 ointment. Furthermore, 2 more ointments had also showed a slight tinge of yellow discoloration.

In the smaller experiment with CA, we found yellow discoloration in the jars where only isopropanol had been added, the yellow colour being darker in the jar with 40 μ l compared with the jar with 20 μ l. In the jars containing isopropanol and 40 μ g of formaldehyde, the jar with 20 μ l of isopropanol showed some violet colour (lighter than that in the control), with some yellow discoloration, while the jar with 40 μ l of isopropanol showed only a deep yellow discoloration. The control jar with only 40 μ g of formaldehyde had shown the expected violet colour.

Discussion

The CA method for qualitative formaldehyde determination was first described in 1959 by Blohm (3). In this study, we have modified the method to make it a semi-quantitative analysis by comparing the intensity of the violet colour of the reagents with that of 4 standard solutions of varying concentrations. However, the CA method for determination of formaldehyde is known to give false-negative results due to discoloration (4). Formaldehyde does not give a violet colour with the reagent when substances causing discoloration are present. Isopropanol is one of the possible sources of discoloration in many samples, and it can also mask the violet colour. Our study aimed to determine the formaldehyde content of the samples studied but did not attempt to investigate whether the formaldehyde was evenly distributed within their containers.

The detection of formaldehyde in corticosteroid creams was first reported in 1980 (5). In that report, 16 of 20 creams gave positive reactions but none gave discoloration. No formaldehyde was found in 7 corticosteroid ointments. Formaldehyde was found in polyethylene glycols (carbowaxes

Table 1. Formaldehyde content in topical corticosteroid preparations available on the Swedish market, tested with the chromotropic acid (CA) method and a high-performance liquid chromatographic method

Trade name	Active ingredients	Formulation	CA analysis*	HPLC (µg/g)
Flutivate 0.05% creme†	FP	Cream	>40 µg	178
Daktacort kräm	H, MN	Cream	~2.5 µg	11
Apolar 0.1% salva	D	Ointment	<2.5 µg	9
Pevisone kräm	TA, EN	Cream	~2.5 µg	7
Mildison lipid 1% kräm	H	Cream	~10 µg	5
Cortimyk kräm	H, MN	Cream	~2.5 µg	5
Emovat 0.05% creme	CB	Cream	<2.5 µg	<5
Fucidin-hydrocortison creme	HA, FU	Cream	<2.5 µg	<5
Locoid lipid 0.1% kräm	HB	Cream	<2.5 µg	<5
Kenacort-T 0.1% kräm‡	TA	Cream	Yellow	<5
Kenacutan creme‡	TA, HQ	Cream	Yellow	<5
Kenacombin novum kräm	TA, N	Cream	Yellow	<5
Elocon 0.1% fet krem	MF	Cream	Yellow	<5
Ficortril 1% liniment	H	Liniment	<2.5 µg	<5
Betnovat 1 mg/ml liniment§	BV	Liniment	Yellow	<5
Betnovat med neomycin liniment§	BV, NS	Liniment	Yellow	<5
Celeston valerat 0.1% liniment§	B17αV	Liniment	Yellow	<5
Locoid 0.1% kutan opløsning §	HB	Liniment	Yellow	<5
Colifoam 100 mg/dos rektalskum	HA	Mousse	~10 µg	<5
Ficortril 1% salva	H	Ointment	<2.5 µg	<5
Elocon 0.1% salve	MF	Ointment	Yellow	<5
Flutivate 0.05% salve	FP	Ointment	Slightly yellow	<5
Hydrokortison 1% ACO salva	H	Ointment	Slightly yellow	<5
Diproderm 0.05% kutan lösning§	BD	Solution	Yellow	<5
Diprosalic kutan lösning§	BD, SA	Solution	Yellow	<5
Celeston valerat 0.1% lösning§	B17αV	Solution	Yellow	<5
Dermovat 0.5 mg/ml ⁻¹ lösning§	CP	Solution	Yellow	<5
Uniderm 1% lösning§	H	Solution	Yellow	<5
Elocon 0.1% kutan lösning§	MF	Solution	Yellow	<5
Betnovat 1 mg/ml lösning§	BV	Solution	Yellow	<5
Diproderm 0.05% creme	BD	Cream	Negative	ND
Betnovat 0.1% creme	BV	Cream	Negative	ND
Betnovat med chionoform creme	BV, CQ	Cream	Negative	ND
Betnovat med neomycin creme	BV, NS	Cream	Negative	ND
Celeston valerat med chionoform creme	B17αV, CQ	Cream	Negative	ND
Celeston valerat med gentamicin kräm	B17αV, GS	Cream	Negative	ND
Celeston valerat comp kräm	B17αV, NS	Cream	Negative	ND
Dermovat 0.05% creme	CP	Cream	Negative	ND
Apolar oily 0.1% krem	D	Cream	Negative	ND
Ibaril 0.25% creme†	DM	Cream	Negative	ND
Synalar 0.025% kräm	FA	Cream	Negative	ND
Hydrokortison 1% CCS kräm	H	Cream	Negative	ND
Uniderm 1% kräm	H	Cream	Negative	ND
Locoid 0.1% creme	HB	Cream	Negative	ND
Celeston valerat 0.1% creme	B17αV	Cream	Negative	ND
Fenuril-Hydrokortison creme	H	Cream	Negative	ND
Diprolen 0.05% gel	BD	Gel	Negative	ND
Synalar 0.025% gel	FA	Gel	Negative	ND
Ibaril med salicylsyra kutan opløsning	DM, SA	Liniment	Negative	ND
Locoid crelo 0.1% lotion	HB	Lotion	Negative	ND
Bettamousse kutanskum	BV	Mousse	Negative	ND
Diproderm 0.05% salve	BD	Ointment	Negative	ND
Diprolen 0.05% salva	BD, SA	Ointment	Negative	ND
Diprosalic salva	BD	Ointment	Negative	ND
Betnovat 0.1% salva	BV	Ointment	Negative	ND
Betnovat med chionoform salva	BV, CQ	Ointment	Negative	ND
Betnovat med neomycin salva	BV, NS	Ointment	Negative	ND
Celeston valerat 0.1% salva	B17αV	Ointment	Negative	ND
Dermovat 0.05% salva	CP	Ointment	Negative	ND
Emovat 0.05% salva	CB	Ointment	Negative	ND
Synalar 0.025% salva	FA	Ointment	Negative	ND
Hydrokortison 1% CCS salva	H	Ointment	Negative	ND
Uniderm 1% salva	H	Ointment	Negative	ND
Terracortril salva	H, OT	Ointment	Negative	ND
Locoid 0.1% salva	HB	Ointment	Negative	ND
Kenacort-T 0.1% salva	TA	Ointment	Negative	ND

Table 1. Continued

Trade name	Active ingredients	Formulation	CA analysis*	HPLC (µg/g)
Kenacutan salva	TA, HQ	Ointment	Negative	ND
Xyloproct rektalsalva	HA, L	Ointment	Negative	ND
Scheriproct N rektalslava	PC, CH	Ointment	Negative	ND
Proctosedyl rektalsalva	H, CH, FS	Ointment	Negative	ND
Kenacort-T 0.1% munhålepasta	TA	Oral paste	Negative	ND
Ficortril 1% lösning	H	Solution	Negative	ND
Kenacort-T comp lösning	TA, SA	Solution	Negative	ND

Corticosteroids: B17 α V = betamethasone 17 α -valerate; BD = betamethasone dipropionate; BV = betamethasone valerate; CB = clobetasone butyrate; CP = clobetasol propionate; D = desonide; DM = desoximetasone; FA = fluocinolone acetonide; FP = fluticasone propionate; H = hydrocortisone; HA = hydrocortisone acetate; HB = hydrocortisone butyrate; MF = mometasone furoate; PC = prednisolone caproate; TA = triamcinolone acetonide. Other active ingredients: CH = cinchocaine hydrochloride; CQ = clio. The intensity of the violet colour of the reagent solution from each sample was compared with reference reagent solutions containing 2.5 µg, 10 µg, 20 µg and 40 µg of formaldehyde.

†Contains isopropyl myristate.

‡Contains isopropyl palmitate.

§Contains isopropanol.

and macrogol) and their derivatives contained in the creams. Auto-oxidation of polyethylene glycols and their derivatives produces formaldehyde (6, 7), and heat, which is often used in the sterilization of corticosteroid creams and ointments, increases this degradation. Surfactants used as emulsifiers in oil-in-water preparations can also produce formaldehyde due to oxidation during storage and handling of these products (8).

In the past, it had been a common practice in Sweden to prescribe only ointments to formaldehyde-allergic patients, as it had been thought that ointments were unlikely to contain formaldehyde. In this study, we have found that small amounts of formaldehyde can also be present in ointments (1/24, 4.2%), but whether the amounts found are clinically relevant has not been determined. The presence of formaldehyde is more likely to be found in creams (5/28, 17.9%).

In this study, the preparation with the highest content of formaldehyde was Flutivate 0.05% creme. From product labelling information, the most likely source of formaldehyde in this cream was 'imidurea' (imidazolidinyl urea), a well-known formaldehyde-releasing preservative. However, this may not be the only source of formaldehyde in this cream.

In one of the reports mentioned earlier (4), the presence of isopropanol in certain products was thought to be a potential source of discoloration of CA. In our smaller experiment with the CA method, we demonstrated that isopropanol was indeed a source of yellow discoloration of CA. In addition to that, the presence of isopropanol in a sample containing formaldehyde can also mask the violet colour. In our study, none of the 18 samples which had given a yellow discoloration by the CA method was found to contain formal-

dehyde by the high-performance liquid chromatographic method. This is in sharp contrast to the findings in the report, including topical corticosteroid preparations, by Fregert et al. (9).

From the ingredient labelling of the samples which had given a definite yellow discoloration, all 11 solutions and liniments were found to contain isopropanol, while 2 creams (Kenacort-T 0.1% kräm (Bristol-Myers Squibb AB, Bromma, Sweden) and Kenacutan creme (Bristol-Myers Squibb AB)) contained isopropyl palmitate, which could undergo hydrolysis in the presence of water, releasing isopropanol.

The cause of the definite yellow discoloration in the remaining 3 preparations (2 creams and an ointment) was not obvious from the labelling. The 2 ointments which had given a slight tinge of yellow discoloration had no obvious cause on the labelling. Both of these were also not found to contain formaldehyde by the high-performance liquid chromatographic method.

2 creams had isopropyl myristate on the labelling, Ibaril 0.25% creme (Aventis Pharma AB, Stockholm, Sweden) and Flutivate 0.05% creme. The former did not give any discoloration of CA, while the latter was the sample in which the highest formaldehyde content was detected. None of the other samples had isopropanol or any other isopropyl esters on their labelling.

In this study, we have demonstrated that the CA method is a rough estimate of formaldehyde content when compared with the used high-performance liquid chromatographic method. However, it is a much simpler method and can be conducted in laboratories without HPLC facilities. One of the major drawbacks of this method is the discoloration that frequently occurs, especially when analysing solutions and liniments,

many of which contain isopropanol or ingredients which have the potential to form isopropanol in the preparation. All the 3 solutions and 1 liniment which had not given a yellow discoloration of the CA did not contain isopropanol on the labelling, while all those that did give the yellow discoloration had isopropanol in their labelling. This is further circumstantial evidence of the role of isopropanol and isopropyl esters as the cause of yellow discoloration of CA. The potential sources of yellow discoloration from the other creams and all the ointments have not been found.

Another source of formaldehyde contamination may be the packaging. Water-based preparations in plastic tubes coated with melamine- or carbamide-formaldehyde resin can take up formaldehyde. This has been reported in cosmetics (10) and chemicotechnical preparations (11) but not in topical medicaments.

The amounts of formaldehyde found in the products that we have tested may not be sufficient to induce sensitization or to elicit an allergic contact dermatitis on intact normal skin of formaldehyde-sensitive individuals but may very well maintain, or even aggravate, a pre-existing dermatitis in previously formaldehyde-sensitized subjects. Repeated open application testing (ROAT) or use testing of formaldehyde-sensitized subjects and controls, performed in a double-blind study, will be necessary to determine the elicitation thresholds of contact allergy to formaldehyde in these products. This may be difficult due to the suppression of the allergic response by the corticosteroids in these preparations.

The threshold concentration for a positive patch test reaction in occluded patch testing to formaldehyde on healthy normal skin in formaldehyde-sensitive patients has been reported to be 250 p.p.m. (12). In the same study, definite positive reactions in ROAT were not seen when performed on healthy normal skin, but it may be that the exposure time of 1 week was too short.

To elucidate the clinical relevance of formaldehyde hypersensitivity in a patient using a corticosteroid preparation containing formaldehyde, the corticosteroid preparation should be tested in a situation in which it would normally be used, i.e. on skin with dermatitis. This would also increase the formaldehyde bioavailability in the skin. This methodology has been used in a previous study, where the clinical relevance of budesonide allergy was investigated with repetitive use testing on experimental allergic contact dermatitis from nickel in subjects hypersensitive to budesonide (13).

In another study of ROAT on elicitation of allergic contact dermatitis in isoeugenol-allergic individuals (14), the time until an isoeugenol-

allergic individual reacted in a ROAT depended on individual sensitivity as well as on exposure concentration; for lower concentrations of the allergen or lower degrees of sensitivity, allergic contact dermatitis may develop only after several weeks of exposure. A negative ROAT after 7 days may therefore be a false negative.

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Address:
Anthony Teik-Jin Goon
National Skin Centre
1 Mandalay Road
Singapore 308205
Singapore
Tel: +65 62534455
Fax: +65 62533225
e-mail: agtj_2000@yahoo.com