

Stability of corticosteroid patch test preparations

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This study investigated the stability of tixocortol pivalate, budesonide, and hydrocortisone-17-butyrate (Hc-17-B) when present in a mix with petrolatum and when the corticosteroids were kept separately in petrolatum. The concentrations chosen for the corticosteroids were the same as those used in a study within the European Environmental Contact Dermatitis Research Group (EECDRG), in which 2 corticosteroid mixes (1 with a high concentration and 1 with a low concentration) and the 3 individual constituents, each at 2 concentrations, were patch tested. Ethanolic solutions of each corticosteroid, as well as 2 mixtures of these 3 corticosteroids, were also made up at corresponding concentrations. The preparations were kept at room temperature, refrigerated, and deep frozen, and repeatedly for 1 year, investigations to check stability by high performance liquid chromatography were carried out. A decrease of $\leq 20\%$ of the initial value at time 0 was used as the threshold for stability. The petrolatum preparations and the ethanolic solutions of budesonide and tixocortol pivalate were stable for at least the whole investigative period, irrespective of storage conditions, while Hc-17-B 1.0% in ethanol kept deep frozen was stable at least during the same period. The latter corticosteroid when kept at room temperature was stable for 3 months only.

Key words: corticosteroids; contact allergy; patch test preparations; tixocortol pivalate; budesonide; hydrocortisone-17-butyrate; petrolatum; ethanolic solutions; chemical stability; high performance liquid chromatography. © Munksgaard, 2000.

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Corticosteroid contact allergy is not uncommon (1). To detect it, patch testing with specific marker molecules such as tixocortol pivalate (2, 3), budesonide (4), and hydrocortisone-17-butyrate (Hc-17-B) (5) is recommended. In a multicentre study within the European Environmental Contact Dermatitis Research Group (EECDRG), patch testing with 2 corticosteroid mixes in pet. (one with a high concentration and one with a low) containing these 3 markers at 2 different concentrations has been undertaken (6). The present study was initiated to investigate the stability of tixocortol pivalate, budesonide and Hc-17-B when present in a mix with petrolatum and when each corticosteroid was kept separately in petrolatum under different storage conditions. Investigations to check stability were carried out repeatedly for 1 year.

Materials and Methods

Chemicals

Tixocortol pivalate and Hc-17-B were purchased from Chemotechnique Diagnostics, Malmö,

Sweden. Budesonide and hydrocortisone-21-butyrate (Hc-21-B) were obtained from Yamanouchi Pharma, Denmark. According to high-performance liquid chromatography (HPLC) at our laboratory, the purity of tixocortol pivalate, budesonide, and Hc-17-B was 99.6, 99.9 and 99.5%, respectively. White petrolatum (Snowwhite) USP/NF with <10 ppm α -tocopheryl acetate, added as an antioxidant, was manufactured by Apoteksbolaget, Gothenburg, Sweden. Ethanol 99.5% v/v was purchased from Kemetyl, Haninge, Sweden. The following solvents were used: methanol and *n*-heptane, both of HPLC quality, manufactured by Lab-Scan, Dublin, Ireland and Milli-Q-filtered water.

Preparation of corticosteroids in petrolatum and ethanol for storage

All preparations were made up at the Department of Occupational and Environmental Dermatology in Malmö, Sweden. The preparations in pet. corresponded to the test preparations used in the EECDRG mix study (6). Ethanolic solutions of

Table 1. Composition and final concentration of respective preparations analyzed for chemical stability; in total, 16 preparations: 2 sets of identical concentrations; 1 set where the vehicle was petrolatum (w/w) and 1 set where the vehicle was ethanol (w/v)

Preparation	Conc. (%)
mix I (high)	1.0+0.10+1.0=2.1
tixocortol pivalate	1.0
budesonide	0.10
Hc-17-B	1.0
mix II (low)	0.10+0.002+0.10=0.202
tixocortol pivalate	0.10
budesonide	0.002
Hc-17-B	0.10

each corticosteroid (tixocortol pivalate, budesonide and Hc-17-B), as well as 2 mixtures of these 3 corticosteroids, were also made up. In Table 1, the composition and concentrations of the respective preparations are given. Hc-21-B was dissolved in ethanol 99.5% v/v and used as a reference substance.

To make the corticosteroid test preparations in pet., the respective corticosteroid was accurately weighed and petrolatum was added to give the desired concentration, implying approximately 20 g for the highest concentration and 80 g for the lowest concentration. Each preparation was manually mixed for 20 min. Approximately 5 ml of each preparation was placed in disposable plastic syringes equipped with a plastic stopper for storage for 1 year in 3 different places, viz unshielded on a shelf at room temperature (20–22°C), in a refrigerator (6–8°C), and in a deep freeze (–18°C). To make the ethanolic solutions with the high concentrations, each corticosteroid was accurately weighed in a volumetric flask and ethanol 99.5% v/v was added up to 25 ml. To make the ethanolic solutions with the low concentrations, dilutions were performed. Approximately 2 ml of each ethanolic solution was placed in polypropylene vials with tightly fitted polypropylene screw caps for storage for 52 weeks under the same 3 conditions as the petrolatum preparations. Samples were drawn from each syringe/vial on one occasion only.

Sample preparation

Before HPLC analysis, an extraction procedure at room temperature was performed concerning the corticosteroid preparations in pet. About 1 g was accurately weighed and placed in a 50 ml glass tube with a screw cap and 30 ml heptane was added. The mixture was shaken vigorously for 1 min, then placed in a separating funnel and 30 ml

methanol were added. The mixture was again shaken and the methanolic phase (the lower phase) was collected in a beaker. Another 20 ml heptane and 20 ml methanol were added to the separating funnel before shaking it and removing the underphase. This was repeated once more. The 3 methanolic phases were then placed in a 100 ml volumetric flask and methanol was added up to 100 ml.

The separate corticosteroids in ethanol and the ethanolic mix solutions did not need any sample preparation before HPLC analysis.

Recovery

To estimate the extraction recovery of the separate sensitizers from the petrolatum preparations, approximately 10 mg of each corticosteroid and approximately 1g of petrolatum were accurately weighed. The corticosteroid petrolatum mixture was thoroughly stirred with a glass rod for 3–4 min. For each corticosteroid when separate, this procedure was repeated 8 ×, and for the corticosteroid mix, it was repeated 3 ×. The samples were then handled according to the extraction procedure above.

High-performance liquid chromatography (HPLC)

An isocratic HPLC system consisting of an SP Spectra Series P200 pump, an SP Spectra System UV1000 detector and an SP ChromJet integrator was used. The system was equipped with a Rheodyne 7125 injector and with a 20 µl loop. The column (4 mm i.d. × 250 mm) was packed with 5 µm Nucleosil C₁₈. The UV detector was operated at 254 nm and the flow rate was 1 ml/min. The mobile phase was methanol/water 80/20 (v/v). The detection limit was shown to be 0.05 ppm for the ethanolic solutions and 5 ppm for the petrolatum preparations, independent of the corticosteroid investigated. The intra-assay coefficient of variation for the analysis of tixocortol pivalate, budesonide, and Hc-17-B in ethanol was 0.38%, 0.23%, and 1.2%, respectively (*n*=10).

The separate corticosteroids in pet. were analyzed at time zero, at 4–6 weeks, at 14–15 weeks, at 27–28 weeks, and at 49–50 weeks. The petrolatum mix preparations were analyzed at time zero, at 7–8 weeks, at 14–16 weeks, at 28 weeks, and at 50 weeks. The separate corticosteroids in ethanol were analyzed at time zero and after that, at 1week, at 3 weeks, at 6–7 weeks, at 13–14 weeks, at 26–27 weeks, and at 48–49 weeks. The ethanolic mix solutions were analyzed at time zero, at 2–3 weeks, at 3–7 weeks, at 8–12 weeks, at 26–27 weeks, and at 48 weeks.

Calculations

Each time a sample from a test preparation was investigated, triplicate analyses were performed, and the value given for each corticosteroid is the mean value based on these triplicate analyses. If the value of a corticosteroid decreased compared to the value at time 0 for that particular corticosteroid, this decrease was allowed to be $\leq 20\%$ of the initial value at time 0, and still the corticosteroid would be regarded as stable.

Results

The extraction recovery for tixocortol pivalate, budesonide, and Hc-17-B when separate was 96.1%, 96.4% and 96.2%, respectively (mean values). The coefficient of variation for the extraction procedure was, for tixocortol pivalate, budesonide, and Hc-17-B, calculated to 3.7%, 4.4%, and 2.8%, respectively ($n=8$). Concerning the extraction procedure, when the corticosteroids were in the mix, the 3 values for tixocortol pivalate, budesonide, and Hc-17-B showed a maximal difference corresponding to ± 1.5 , ± 0.9 , and $\pm 0.8\%$ of the mean value, respectively.

For most of the corticosteroid preparations, the values of the triplicate samples showed a maximal difference corresponding to $\pm 2.5\%$ of the mean value. The preparation deviating most was tixocortol pivalate 0.10% in pet. where the difference varied between ± 3.5 to $\pm 30\%$ (in 14/15 analyses the difference exceeded $\pm 5\%$). For budesonide 0.002% in pet., the difference varied between ± 1.2 to $\pm 33\%$ (in 7/15 analyses, the difference exceeded $\pm 5\%$) and for Hc-17-B 0.10% in pet., a difference between ± 0.8 to $\pm 28\%$ was noted (in 7/15 analyses the difference exceeded $\pm 5\%$). A new test preparation with tixocortol pivalate 0.10% in pet. was made up by first dissolving the tixocortol pivalate in 0.5 ml ethanol 99.5% (v/v) before the petrolatum was added, and then the difference decreased to approximately $\pm 2.5\%$.

In Fig. 1 a-c, the stability of the respective corticosteroid when separate in petrolatum or ethanol, respectively, is shown.

All test preparations with the sensitizers separate in pet. were stable during the whole investigative period, irrespective of storage conditions, 2 examples being shown in Fig. 1a, b. Budesonide and tixocortol pivalate in ethanol were also stable under the same conditions. The deep frozen sample of Hc-17-B 1.0% in ethanol was stable during the whole time it was investigated (Fig. 1c). The value of the refrigerated sample passed the threshold of a 20% decrease at around 30 weeks

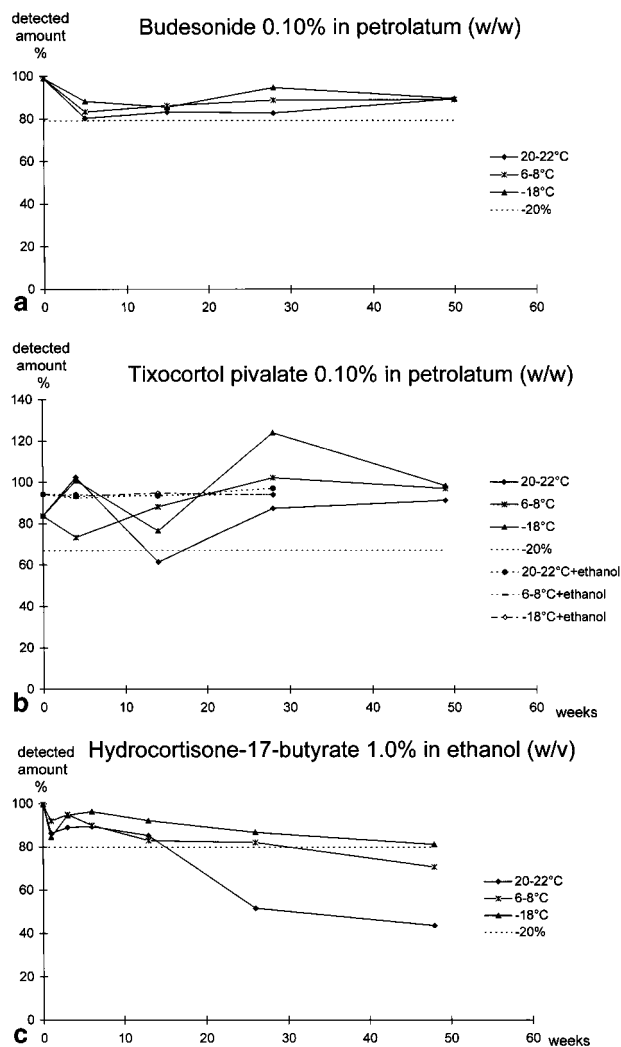


Fig. 1. (a-c) Stability of different corticosteroids in pet. or ethanol under varying storage conditions for 1 year. The dotted line represents a 20% decrease in the initial amount of sensitizer present at time 0.

and the sample kept at room temperature decreased below the 20% threshold as early as around 13 weeks (Fig. 1c). The deep frozen sample of Hc-17-B 0.10% in ethanol was stable during the whole investigative period, the sample kept at room temperature for 1 week, and the refrigerated sample for 13 weeks.

The corticosteroid mixes 2.1 and 0.202% in pet. and 2.1% in ethanol were stable during the whole investigative time and under all 3 storage conditions. Due to the decrease of Hc-17-B below 20%, the corticosteroid mix 0.202% in ethanol was stable until around week 20 at room temperature and during the whole investigative period in the other 2 storage places.

Discussion

There does not seem to be any universal agreement with respect to the residual amount of active ingredient in a test preparation that has to be present for consideration of a test preparation as being stable. The prerequisite for chemical stability for the TRUE Test (Pharmacia AS, Denmark) and patch test allergens from Hermal (Hermal, Germany) is $\pm 20\%$ of the concentration given on the label (personal communication B. Kreilgård, Pharmacia SA, Denmark and T. Schumacher, Hermal, Germany). Therefore, we also chose this limit. According to some authors (7), many compounds are quite stable for years when kept in a refrigerator at 4°C , prepared in a vehicle like pet. and stored in syringes as in this present study. Furthermore, preparations in pet. are considered to be more stable than preparations in ethanol (8), and the results of this study are in agreement with that. However, petrolatum is not recommended as the optimal patch test vehicle for Hc-17-B, as more patients in a study reacted to ethanolic preparations than to petrolatum preparations (5). For budesonide and tixocortol pivalate, either petrolatum preparations or ethanolic solutions seem to work equally well, as the outcome of testing has given equivalent patch test results (9–11). Petrolatum is more practical to handle in a standard series testing situation, once the test preparation has been formulated, though one setback, when a chemical is dispersed at a low concentration in pet., is the risk of uneven distribution. Triplicate sample figures for 0.10% tixocortol pivalate in petrolatum showed a high variation, indicating the difficulty of dispersing the substance evenly. On the other hand, when a 0.10% tixocortol pivalate preparation was made up with a very small (0.8 ml ethanol to 50 mg substance) amount of ethanol added in order to dissolve the tixocortol pivalate prior to the addition of petrolatum, there was virtually no variation. The stability of this new preparation was followed over time and was found to be stable during the study period (Fig. 1b). In a recent paper (6), we recommended that tixocortol pivalate be patch tested at 0.10% in pet., as testing with this preparation detected the same number of allergic individuals as 1.0% in pet. From a chemical point of view, either one of the 2 concentrations can be recommended, but when test preparations with concentrations from 0.10% or lower are made, we recommend that tixocortol pivalate be dissolved in a very small amount of ethanol prior to the addition of petrolatum. The results of the triplicate analyses from the low concentrations of budesonide and Hc-17-B also varied to a high extent, which is why the same procedure also was per-

formed with these. The results showed, that for Hc-17-B, there was no variation ($\pm 1\%$), but for budesonide, there was still a high difference between triplicate values ($\pm 12\%$). Consequently, it is advisable to dissolve Hc-17-B in a very small volume of ethanol prior to petrolatum being added, if a test preparation of 0.10% is made. The need to dissolve certain components in solvents before mixing them with petrolatum has been promulgated by Fregert and collaborators and also practiced since the 60s (personal communication, S. Fregert, Lund, Sweden). Nowadays, this is also done commercially with some sensitizers in pet. (personal communication, B. Niklasson, Chemotechnique Diagnostics, Sweden). There are recommendations that corticosteroid preparations dissolved in ethanol be made fresh every 6 to 8 weeks because of degradation, above all when dealing with Hc-17-B, which is said to be converted into the 21-butyric ester (12). In this study, we also saw that a new peak appeared in the chromatogram of the ethanolic solutions containing Hc-17-B after various storage periods, depending on storage conditions and the concentration of the solution, the lower concentrated solution degrading at a higher speed. This peak increased when Hc-21-B was injected into the HPLC, speaking in favour of it being Hc-21-B.

On the other hand, the ethanolic solutions of budesonide and tixocortol pivalate were stable for at least 1 year, even at room temperature. Furthermore, ethanolic solutions of Hc-17-B 1.0% were stable for at least 1 year when kept at -18°C . The sample kept refrigerated was stable for approximately 6 months and the sample at room temperature for 3 months (Fig. 1c). These results indicate that durability might be longer than has been postulated previously, but further studies are needed where handling of preparations mimicking regular use should be undertaken. Preliminary results from such a study suggest that Hc-17-B is more prone to degradation in ethanol than has been shown in this study (to be published). As it is laborious and tedious to prepare fresh test solutions every 2 months, as has been suggested, it would be worthwhile if a larger volume of the desired concentration could be prepared at one time. From this freshly made preparation, aliquots, implying a volume sufficient for 2–3 months patch testing, could be drawn, stored in vials with lids and be kept deep frozen for a period of up to 1 year. A vial could then be taken out for patch test purposes every 2–3 months and, during this time, the vial should be kept with a tightly fitting lid in a refrigerator when not being used for testing.

Tixocortol pivalate crystallized in the ethanolic solutions, especially when these were kept deep

frozen or refrigerated, so care should be taken to make sure the test substance is properly solubilized before patch testing.

When corticosteroids were in the mixes, both the petrolatum preparations and the ethanolic solutions, the stability of each corticosteroid was higher than when in the separate preparations. This was particularly conspicuous for Hc-17-B in the ethanolic mix. In the mix with the highest concentration, Hc-17-B was stable during the whole investigative period independent of storage conditions, while Hc-17-B in the ethanolic mix with the low concentration was stable for this length of time only when the mix was kept in the refrigerator and deep freeze, while at room temperature it was stable for approximately 5 months.

When analyzing budesonide in the petrolatum mixes, low values at time 0 were found. This could be due to a low recovery. Concerning the corticosteroid mix 0.202% in ethanol, budesonide had figures exceeding 100% in all 3 storage places at 3 weeks. This figure was lowered to around 100% when 2 new mix preparations were made up and analyzed after 3 weeks. The outcome of patch testing consecutive dermatitis patients with 2 corticosteroid mixes, with the same composition as in the petrolatum mixes investigated in this study, showed that around 55% of tixocortol pivalate allergy was missed (6). With regard to the results of this former study (6), one possible explanation could be degradation of tixocortol pivalate when together with budesonide and Hc-17-B in the mix. The results of the present investigation are not in agreement with this hypothesis, though, and a more likely reason might be the immunosuppressive effect of the other potent corticosteroids used in the mix.

To summarize, all the petrolatum preparations and ethanolic solutions of budesonide and tixocortol pivalate were stable for at least the whole investigative period of storage at room temperature, refrigerated and deep frozen. Hc-17-B 1.0% in ethanol was stable for at least the entire investigative time kept deep frozen and at room temperature for 3 months.

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