Journal of Pharmaceutical & Biomedical Analysis
Vol. 5, No. 7, pp. 707–716, 1987
Printed in Great Britain

0731–7085/87 $3.00 + 0.00
Pergamon Journals Ltd.

Dilutions of corticosteroid creams and ointments — a stability study

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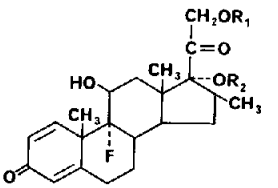
Abstract: A study was carried out on the stability of dilutions of creams and ointments of two corticosteroids, betamethasone valerate and beclomethasone dipropionate. A reversed-phase high-performance liquid chromatographic method was developed that is simple, efficient and stability-indicating in respect of the main decomposition products and has the advantage of being carried out at ambient temperature. The effect of water content of the sample solutions and the influence of large injection volumes (200–250 μl) on the resolution of the substances on the chromatogram was investigated. Use of the diluents, cetomacrogol cream (formula A) BP and white soft paraffin BP, resulted in satisfactory products in terms of chemical stability and efficacy of antimicrobial preservation.

Keywords: *Corticosteroids; creams; ointments; dilutions; reversed-phase HPLC; stability.*

Introduction

The practice of dilution of proprietary topical corticosteroid preparations has led to much controversy [1]. The preparations examined in the present study were betamethasone valerate and beclomethasone dipropionate creams and ointments; the two diluents were cetomacrogol cream (formula A) BP and white soft paraffin BP. Under certain conditions betamethasone 17-valerate (I) rearranges [2] to the 21-valerate (II) which is 15-times less potent; the rearrangement is promoted when the product is formulated in certain bases, e.g. emulsifying ointment or vehicles containing propylene

Betamethasone derivatives



I $R_1 = H$, $R_2 = CO-(CH_2)_3CH_3$
II $R_1 = CO-(CH_2)_3CH_3$, $R_2 = H$
III $R_1, R_2 = H$

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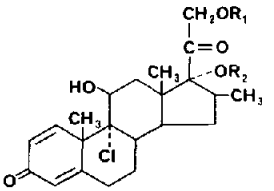
glycol and ethanolamine [3]. In aqueous solution at pH 0.5–8.0, under appropriate conditions, degradation of betamethasone 17-valerate appears to proceed entirely by rearrangement to the 21-valerate followed by hydrolysis to betamethasone alcohol (III) [4].

No reports have been traced on long-term (non-accelerated) stability studies on betamethasone valerate creams and ointments diluted with cetomacrogol cream (formula A) or white soft paraffin. Similarly, no information has been found on the stability of diluted preparations of beclomethasone dipropionate (IV); pharmacopoeial information indicates that this substance decomposes to the 17-(V) and 21-monopropionates (VI) [5].

Beclomethasone derivatives



IV $R_1, R_2 = COC_2H_5$
V $R_1 = H$, $R_2 = COC_2H_5$
VI $R_1 = COC_2H_5$, $R_2 = H$

For the selective and quantitative determination of betamethasone 17-valerate in the presence of its decomposition products, procedures based on spectrophotometry [6], thin-layer chromatography [7], densitometry [8] and high-performance liquid chromatography (HPLC) [9] have been reported. The HPLC method of the BP [5] is stability-indicating but since it is carried out at elevated temperature (60°C) it is not easy to apply routinely. The USP method is easy to apply at ambient temperature but does not appear to be stability indicating [10].

The three main aims of this work were: (a) to devise a method for the routine analysis of the corticosteroid cream and ointment dilutions that is simple and robust but also stability indicating; (b) to use this method to study the stability of these dilutions for *ca* 1 year; and (c) to apply microbial limit tests [11–14] to study the efficacy of preservation of the product during its shelf-life.

Experimental

Materials

Betamethasone 17-valerate, betamethasone 21-valerate, beclomethasone dipropionate, beclomethasone 17-propionate and beclomethasone 21-propionate used as standards were kindly provided by Glaxo Laboratories Ltd. and Allen & Hanburys Ltd. Betamethasone 17-valerate BPCRS and beclomethasone dipropionate BPCRS and betamethasone EPCRS were also used.

Absolute alcohol (Burroughs Ltd.), distilled water (sterile water for irrigation, Travenol Ltd.), hexane, methanol and acetonitrile (HPLC grade, Rathburn Chemicals Ltd.) were used. All solvents for chromatography were filtered and degassed.

The samples used were 1 in 2, 1 in 4, and 1 in 10 dilutions (m/m) of Betnovate and Propaderm ointments (Glaxo Laboratories Ltd. and Allen & Hanburys Ltd.) and 1 in 10 dilutions (m/m) of Betnovate and Propaderm creams. The original undiluted cream or

ointment (0.1% m/m for Betnovate and 0.025% m/m for Propaderm) was also examined. The diluents, cetomacrogol cream (formula A) BP and white soft paraffin BP, were tested microbiologically [11] and found to be satisfactory. The dilutions were made in the pharmacy production units of two N.W. Thames Health Authority Districts using a mixer or an ointment slab. They were packed in clean amber-glass jars of various sizes fitted with bakelite closures and stored at room temperature. Samples were randomly selected from normal stock and analysed by the routine methods of quality control; these samples were then stored in a temperature- and humidity-monitored, locked cupboard in the quality control laboratory. During the study the ranges of temperature and relative humidity were 20–26°C and 45–80%, respectively.

Apparatus and conditions

The system (Waters Associates) used for reversed-phase HPLC comprised: a 6000 solvent delivery system; a U6K injector fitted with a 2 ml loop; a 440 ultraviolet detector equipped with a Taper cell of 12.5 μl and 10-mm pathlength, operated at 254 nm with a sensitivity of 0.1 or 0.2 absorbance units full scale (a.u.f.s.); and a 730 Data Module.

A 300 × 3.9 mm i.d. stainless steel column packed with 10-μm microBondapak was used. Packing material of 10-μm size was preferred to that of 5-μm size because of the large injection volumes used [15]; the use of particles of the latter size produced no advantages in speed or resolution. The mobile phase was methanol–acetonitrile–water (49:25:26, v/v/v) delivered at a flow rate of 2.0 ml/min at 2000 psi. Quadruplicate injections (200 μl for betamethasone 17-valerate and 250 μl for beclomethasone dipropionate) were performed for each solution using an SGE (Scientific Glass Engineering Ltd.) syringe. All solutions for chromatography were filtered.

For study of the ethanol–water–hexane content of the solutions subjected to HPLC, a Pye-Unicam/Phillips PU4500 gas liquid chromatograph was used, fitted with a flame-ionisation detector and a computing integrator (4810 Spectra-Physics). The stationary phase was Porapak Q packed in a Pye-Unicam column and the operating conditions were: flow rate (Nitrogen), 45 ml/min; temperature, 180°C (injection, column) and 200°C (detector).

Solutions

Stock standard solutions (0.07% m/v) of betamethasone 17-valerate and beclomethasone dipropionate were prepared in ethanol (85%) and stored in the refrigerator. Beclomethasone dipropionate was used as internal standard for betamethasone 17-valerate and vice versa. For analysis of creams and ointments the working standard solutions (0.002% m/v and 0.0005% m/v, respectively) with 0.002% m/v or 0.0005% m/v of internal standard, were prepared in ethanol (65%) for the creams and ointments.

Stock standard solutions (0.06% m/v) of the decomposition products, betamethasone 21-valerate, beclomethasone 17-propionate, beclomethasone 21-propionate and betamethasone in absolute ethanol, were also stored in the refrigerator.

Samples of the creams and ointments equivalent to 1–1.2 mg of betamethasone 17-valerate or 0.2–0.3 mg of beclomethasone dipropionate, were dispersed in 50 ml of hexane (hot solvent for ointments). The dispersion was then extracted with 5 × 10 ml portions of ethanol (85% for the cream, 65% for the ointment) into a 50-ml volumetric flask via a cotton wool plug. The combined extracts were further diluted, with the addition of the internal standard, using ethanol (65%). All experiments were performed in duplicate.

Calibration and column efficiency

Standard solutions of betamethasone 17-valerate and beclomethasone dipropionate in ethanol (65%) produced linear graphs of peak area versus concentration over a concentration range of 0.0003–0.005% m/v. For betamethasone 17-valerate, the regression equation ($n = 9$) was: $y = 6.40 \times 10^6 x \pm 89.8$; SE of gradient = 1.39×10^2; $r = 0.99987$. The RSD at 0.00278% m/v was 1.85% ($n = 5$). For beclomethasone dipropionate, the regression equation ($n = 9$) was: $y = 5.50 \times 10^6 x + 8.2$; SE of gradient = 1.028×10^2; $r = 0.99995$. The RSD at 0.00142% m/v was 3.09% ($n = 5$).

For satisfactory resolution of betamethasone 17-valerate and the 21-derivative the number (N) of theoretical plates of the column should not be less than 4000. To determine the efficiency of the column the Waters method (CU84588 RevG/June 1980) was applied using a 0.1% w/v solution of acenaphthene with acetonitrile–water (60:40, v/v) as the mobile phase. The flow-rate was 2.5 ml/min, detection was at 280 nm, sensitivity was 1.0 AUFS and injection volume was 10 μl. The equation $N = 25 (V_R/W)^2$ (V_R = retention time; W = width of the peak at 4.4% peak height) was used for calculations of N.

Microbiology

The suspending agents were: peptone water 0.1% (Oxoid L37 with 0.1% sodium chloride) containing 3% polysorbate 80 (BDH Chemicals Ltd.); 1% polysorbate 80 (BDH Chemicals Ltd.). The culture media were: tryptone soya agar (Oxoid CM131), Sabouraud dextrose agar (Oxoid CM41) and blood agar [laboratory prepared from Columbia blood agar base (Oxoid CM331) and defibrinated horse blood (GIBCO)]. Tryptone soya broth (Oxoid CM129) and Pseudomonas selective medium [Pseudomonas agar base (Oxoid CM559) plus supplement CN(Oxoid SR102)] were used as enrichment and subculture media, respectively for *Pseudomonas aeruginosa*. Salt meat broth (Oxoid CM94) and mannitol salt agar (Oxoid CM85) were used as enrichment and subculture media, respectively, for pathogenic staphylococci. The coagulase test (Staphaurex®, Wellcome Diagnostics) was used for *Staphylococcus aureus*.

Two microbial limit tests were applied: the total viable count and the test for *Pseudomonas aeruginosa* and *Staphylococcus aureus* of the USP [10]. The method used [11] is similar to that of the European pharmacopoeia with minor modifications [12, 14]. The pour plate technique [12] was used.

For the total viable count 0.1 g of each sample was suspended in 19 ml of sterile 0.1% peptone water containing 3% polysorbate 80 at 40°C, dispersed with the aid of glass beads and agitated. One millilitre of this dispersion was added to each of 6 petri dishes; molten sterile Sabouraud dextrose agar (SDA) and tryptone soya agar (TSA) at 40–45°C (2 and 4 plates, respectively) was poured and the plates were mixed by rotating in a horizontal plane. The SDA plates were incubated at 25°C for 7 days and the TSA plates at 37°C for 3 days (2 aerobically and 2 anaerobically). In the test for *Pseudomonas aeruginosa*, 1 g of the sample was incubated in 20 ml of trypticase soya broth at 30–32°C for 18–24 h, subcultured on to the pseudomonas selective medium and incubated at 30–32°C for 48 h. In the test for *Staphylococcus aureus*, 1 g of the sample was incubated with 20 ml of salt meat broth at 37°C for 48 h, subcultured on to mannitol salt agar and incubated at 37°C for 36 h.

The same range of samples was also incubated in blood agar. Amounts of 1 g were suspended in 10 ml of 1% polysorbate 80 and mixed using a rock mixer. Quantities of 0.5 ml were dispersed on triplicate blood agar plates and incubated at 36°C for 48 h

aerobically. The plates were then examined for total viable aerobic count and also for *Staphylococcus* and *Pseudomonas* species for which blood agar is considered a medium of choice. The search for *Pseudomonas* species was based on their colonial morphology whereas the search for *Staphylococcus* species was based on their colonial morphology and on biochemical tests (Staphaurex coagulase test, Wellcome Diagnostics, ref. 1D1RZL31U, 2/85).

The samples examined included 14 dilutions of Betnovate and Propaderm creams and ointments and the corresponding original preparations and the diluents.

Results

Method of analysis

Recovery studies were carried out with measured amounts of standard solutions of betamethasone valerate and beclomethasone dipropionate added to appropriate amounts of diluents [cetomacrogol cream (formula A) BP and white soft paraffin BP], dispersed into hexane, extracted with ethanol (85% for the cream and 65% for the ointment) and assayed. All strengths of the cream and ointment dilutions of this study were represented in the above extractions. The recoveries were: 98.9 ± 0.43% (P = 0.95) for Betnovate ointment dilutions; 97.5 ± 0.65% (P = 0.95) for the Betnovate cream dilutions; 96.8 ± 1.01% (P = 0.95) for the Propaderm ointment dilutions and 100.0 ± 2.63% (P = 0.95) for the Propaderm cream dilutions.

Reproducibility studies on all the samples (original preparations and dilutions) were performed in triplicate. Because of the unavailability of normal stock at the time of the experiment, the 1 in 10 dilution of Propaderm cream was prepared in the laboratory by diluting the original cream under simulated manufacturing conditions (Table 1).

Figure 1 shows a chromatogram of a standard mixture of the substances examined. The mean retention times are: beclomethasone monoproprionate 3.22 min; betamethasone 17-valerate 3.97 min; betamethasone 21-valerate 4.48 min; beclomethasone dipropionate 5.19 min; and betamethasone 2.3 min.

Stability study

Physical and chemical stability. A monthly examination was carried out during the first 6 months and a 2-monthly examination during the remaining 6 months of the 1-year

Table 1
Results for the reproducibility study

Sample		% Labelled strength 1	2	3	Mean	Standard deviation
Betnovate ointment,	Original	102.3	101.6	101.7	101.9	0.38
Betnovate ointment,	1 in 2	99.6	98.6	98.0	98.7	0.81
Betnovate ointment,	1 in 10	92.5	92.1	90.7	91.8	0.95
Betnovate cream,	Original	103.6	102.6	103.3	103.2	0.51
Betnovate cream,	1 in 4	100.4	102.1	102.3	101.6	1.04
Betnovate cream,	1 in 10	97.1	96.6	95.8	96.5	0.66
Propaderm ointment,	Original	97.5	100.2	95.9	97.9	2.17
Propaderm ointment,	1 in 4	97.4	98.9	96.1	97.5	1.40
Propaderm ointment,	1 in 10	98.2	96.0	93.4	95.9	2.03
Propaderm cream,	Original	99.4	104.6	101.4	101.8	2.62
Propaderm cream,	1 in 10	107.3	96.8	104.0	102.7	5.37

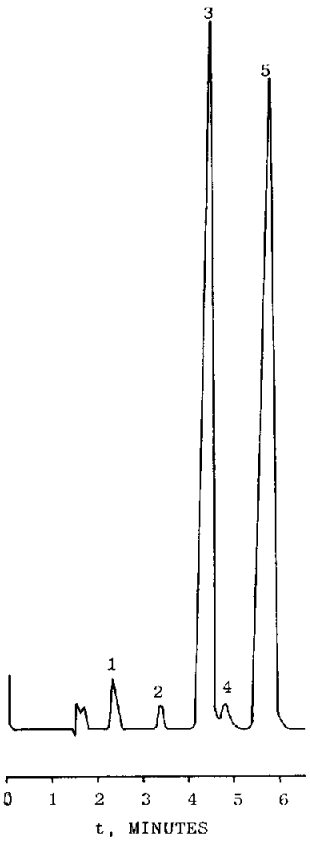


Figure 1
A chromatogram of standard solutions of betamethasone 17-valerate [3] and beclomethasone dipropionate [5] with 3% betamethasone alcohol [1], 3% beclomethasone 17-propionate [2] and 3% betamethasone 21-valerate [4].

study. The appearance of the samples was satisfactory throughout the study except for one of the original Propaderm ointments which showed separation of a small amount of liquid. All the dilutions showed little change over 12 months and, in general, their behaviour matched that of the undiluted original preparations. Figures 2–6 show graphical representations of some of the results obtained.

Microbiological stability. The Sabouraud dextrose agar and the tryptone soya agar plates produced a total viable count of less than 10 c.f.u. per g of finished product [12]. The results in the tests for *Pseudomonas aeruginosa* and *Staphylococcus aureus* were negative.

The blood agar plates produced a total viable aerobic count of less than 10^2 c.f.u. per g of product. Morphological examination of colonies showed absence of *Pseudomonas aeruginosa* but the presence of a small number of colonies of *Staphylococcus* and *Micrococcus* species on three plates. The coagulase test applied was negative for *Staphylococcus aureus*.

Discussion

Analytical method

The HPLC method is sensitive, specific, robust and easy to apply. It has also proved to be stability indicating and, carried out at room temperature, it provides a fully recorded, internally standardised analysis in 5–6 min.

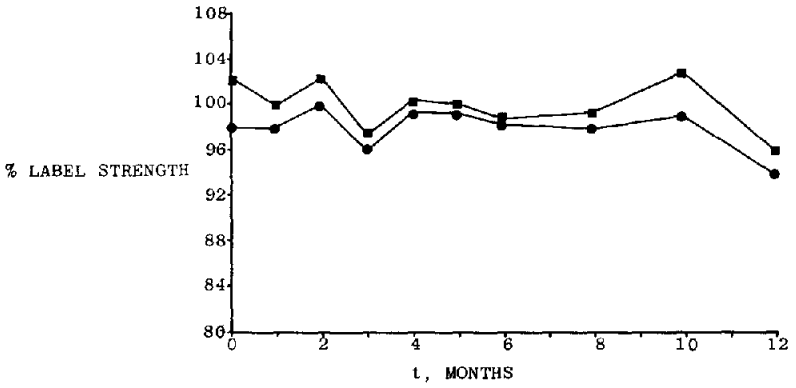


Figure 2
A graph of % label strength versus time for: (■) Betnovate ointment 1 in 2 dilution; and (●) the original ointment.

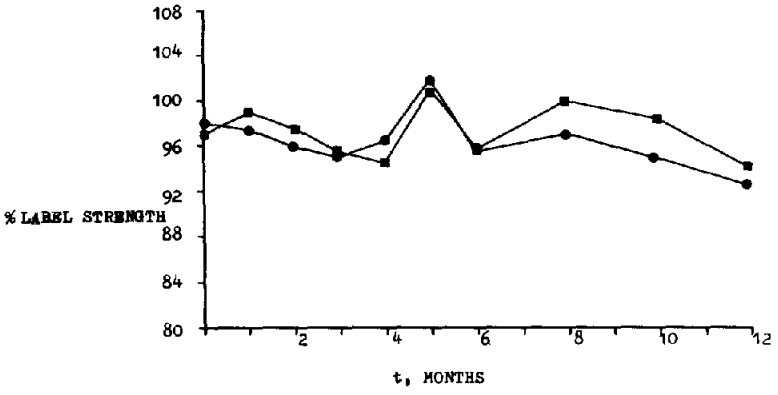


Figure 3
A graph of % label strength versus time for: (■) Betnovate ointment 1 in 10 dilution; and (●) the original ointment.

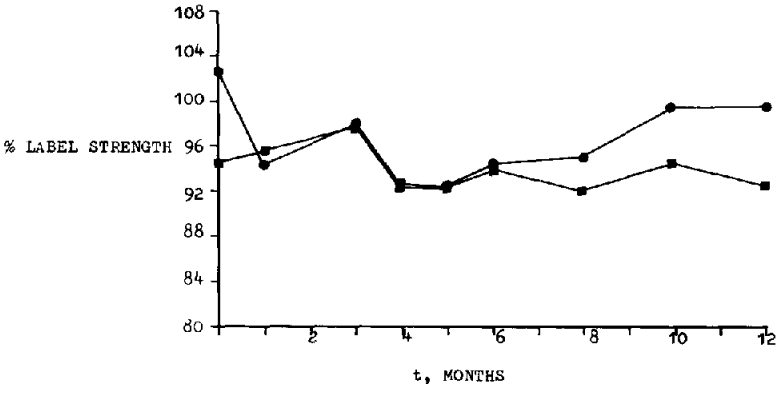


Figure 4
A graph of % label strength versus time for: (■) Propaderm ointment 1 in 2 dilution; and (●) the original ointment.

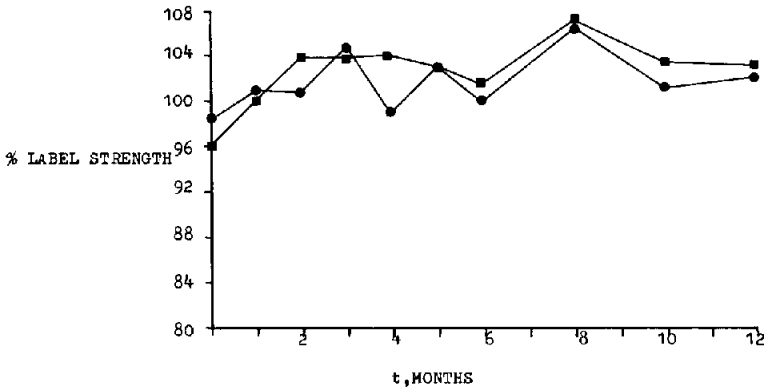


Figure 5
A graph of % label strength versus time for: (■) Betnovate cream 1 in 10 dilution; and (●) the original cream.

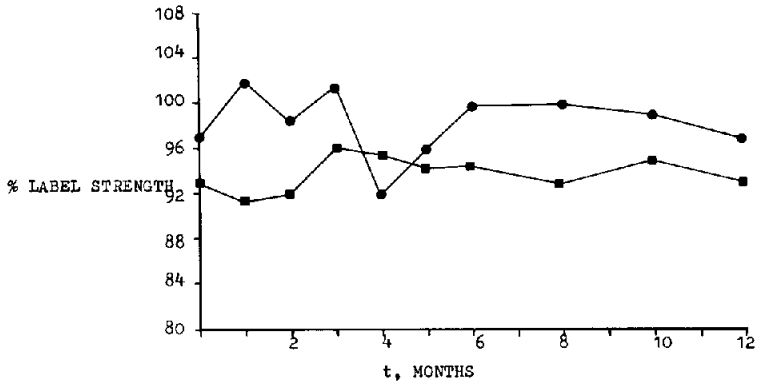


Figure 6
A graph of % label strength versus time for: (■) Propaderm cream 1 in 10 dilution; and (●) the original cream.

For chromatography of the creams, special consideration was given to the ethanol and water contents of the chromatographed solution because of the large injection volumes used [16]. If the content of ethanol (a non-eluting solvent [17] different from the mobile phase used) exceeds 80%, resolution of the chromatogram is severely affected; in contrast, if the ethanol content is only 40%, better resolution is achieved but hexane, mixed with ethanol during the extraction procedure, tends to separate out. In both cases the area count is the same although the peak height and the retention time are slightly different. In practice the proportions of ethanol and water used to dilute the final solution for chromatography will vary according to the amount of the cream initially weighed for extraction; i.e. 65% ethanol for 10 g of the 1 in 10 dilution, or 30% ethanol for 1 g of the original cream. However, restrictions described above necessitate the use of higher proportions of ethanol in the final solution for chromatography of the original cream; thus resolution of substances on the chromatogram is impaired slightly.

The ethanol–water content of the solutions subjected to HPLC was studied by gas–liquid chromatography. It was found that the chromatographed solutions of the

cream dilutions contained 65% ethanol and only a trace of hexane, whereas those of the original cream contained 74% ethanol and 3–4% hexane, the higher proportion of ethanol enhancing hexane miscibility. The discrepancy in the solvent composition between the cream dilutions and the original cream affects the chromatogram as described above. It was found that application of the non-eluting solvent concept does not seem to offer an immediate solution to the problem in a ternary mobile phase system [17]. Thus, for stability work that includes experiments on the original cream, some refinement of the method would be desirable.

The physical and chemical stability

Our 1-year stability study has shown that there is no significant decrease in the original steroid content of the diluted products manufactured in the pharmacy production units of the two N.W. Thames Regional Health Authority Districts. In the majority of cases the pattern of the results obtained for the dilution matched those of the original preparation. Generally decomposition products were not detected in dilutions where the original preparation used had at least one year shelf-life remaining. In Betnovate ointment dilutions not in this category, the 21-valerate was usually detected by the sixth month of our study, the content being less than 5% of that of the 17-valerate by the twelfth month. The same pattern of results was observed for the original creams and ointments. The Betnovate cream dilution behaved like the ointment dilutions but the content of the 21-valerate was *ca* 5% of that of the 17-valerate by the twelfth month. Propaderm ointment dilutions produced the mono-propionate derivative by the seventh month of the study, but again the proportion did not exceed 5% by the twelfth month. The same result was observed for the original ointment. In the Propaderm cream dilution studied, paradoxically, no decomposition products were observed. No clear correlation was found between decomposition and the degree of dilution or type of formulation. No evidence of betamethasone alcohol was observed in any of the Betnovate dilutions.

Microbiological stability

The microbial limit tests produced satisfactory results. The total viable counts from all media were well within the acceptable levels regionally and internationally [12, 13, 18]. The tests for *Pseudomonas aeruginosa* and *Staphylococcus aureus* were negative. The presence of *Micrococcus* and coagulase-negative staphylococcal species (e.g. *S. epidermidis*) might have been due to skin contamination. However, the use of suspending agents for the isolation of micro-organisms from semi-solid preparations has caused problems [19, 20]; more efficient dispersal systems and challenge testing are needed to reduce errors in the recoveries of micro-organisms.

Acknowledgements: We thank Glaxo Laboratories Ltd. and Allen & Hanbury Ltd. for the provision of materials and information about their analytical method. We thank Mr R. Knight and Mr M. Readwin (Medical School, Westminster Hospital) and Mr G. Jackson (Regional Quality Control Centre, Edgware General Hospital) for carrying out the microbiological work. We thank Mr D. Collis (Millipore-Waters, London) for useful information. We are grateful to Dr P. Molyneux (Macrophile Associates, London) for his critical review of this paper.

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[*First received for review 16 March 1987; revised manuscript received 16 June 1987*]