# THE CHEMICAL STABILITY OF BETAMETHASONE VALERATE AND FLUOCINOLONE ACETONIDE CREAM AND OINTMENT IN CETOMACROGOL AND EMULSIFYING CREAM AND OINTMENT BASES

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A mini-thesis submitted in partial fulfilment of the requirements for the degree Magister Pharmaceuticiae in the Department of Pharmaceutics, School of Pharmacy, University of the Western Cape.

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**KEYWORDS**

Betamethasone valerate

Fluocinolone acetonide

Cetomacrogol

Emulsifying

Cream

Ointment

Diluent

Degradation

Stability

Shelf Life




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ABSTRACT

**THE CHEMICAL STABILITY OF BETAMETHASONE VALERATE AND FLUOCINOLONE ACETONIDE CREAM AND OINTMENT IN CETOMACROGOL AND EMULSIFYING CREAM AND OINTMENT BASES**

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The corticosteroids are powerful anti-inflammatory molecules but their usage is hampered by adverse effects. To minimise their adverse reactions, proprietary brands of topical corticosteroids are commonly diluted with a variety of bases.

Dilution may affect the stability of the corticosteroid molecule and may result in accelerated degradation. This degradation may vary depending on the diluent base used. Such dilutions are also made in the Western Cape Department of Health public health system where unique formulations of cetomacrogol and emulsifying preparations are used as diluents. Because of the uncertainty of how these diluents would affect the steroid molecules, no accurate expiry dates could be allocated to the diluted preparations. This study set out to determine the chemical stability of betamethasone valerate and fluocinolone acetonide creams and ointments when diluted with the bases used in the public hospital system and to assign shelf lives to them.

Samples of the branded topical corticosteroid products used by the Western Cape Department of Health were diluted 1:10 and 50:50 with cetomacrogol and emulsifying cream and ointment bases. The diluted preparations were stored protected from light, moisture and air at 25°C. Samples of the preparations were assayed at intervals for their corticosteroid content using reverse phase High Performance Liquid Chromatography. These concentrations of corticosteroid provided a degradation profile of each preparation from which degradation rate constants were determined and shelf lives calculated.

The HPLC method used was sensitive, accurate and specific for the active



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ingredients studied. The retention times of betamethasone valerate and fluocinolone acetonide were 4,00 and 5,15 minutes respectively.

Betamethasone valerate was significantly more stable in the cream dilutions than in the ointment dilutions (e.g. 1:10 dilution in cetomacrogol cream $t_{90}$ = 5,46 +- months vs 1:10 dilution in cetomacrogol ointment $t_{90}$ = 1,62 +- months P = 0,0181). Conversely, fluocinolone acetonide was more stable in the ointment dilutions than in the cream dilutions but not significantly so (e.g. 1:10 dilution in cetomacrogol ointment $t_{90}$ = 12,69 +- months vs 1:10 dilution in cetomacrogol cream $t_{90}$ = 2,63 +- months P = 0,1938). In general, both corticosteroids degraded more slowly when diluted with cetomacrogol cream (e.g. 1:10 dilutions of betamethasone valerate cream $t_{90}$ = 5,46 +- months and fluocinolone acetonide cream $t_{90}$ = 2,63 +- months) than with emulsifying cream (e.g. 1:10 dilutions of betamethasone valerate cream $t_{90}$ = 3,05 +- months but not significantly so P = 0,8280 and fluocinolone acetonide cream $t_{90}$ = 0,0111 +- months extremely significantly so P<0,0001) and more slowly when diluted with cetomacrogol ointment (e.g. 1:10 dilutions of betamethasone valerate ointment $t_{90}$ = 1,62 +- months and fluocinolone acetonide ointment $t_{90}$ = 12,69 +- months) than with emulsifying ointment (e.g. 1:10 dilutions of betamethasone valerate ointment $t_{90}$ = 0,18 +- months and fluocinolone acetonide ointment 1:10 $t_{90}$ = 9,81 +- months although not significantly so P = 0,6590). Also, generally, the more concentrated dilutions were more stable than the more dilute ones (e.g 50:50 dilution of betamethasone valerate in cetomacrogol ointment $t_{90}$ = 4,33 +- months vs 1:10 dilution $t_{90}$ = 1,62 months significantly so P = 0,0140 and 50:50 dilution of fluocinolone acetonide in cetomacrogol ointment $t_{90}$ = 46,90 +- months vs 1:10 dilution $t_{90}$ = 12,69 +- months although not significantly so P = 0,6005).

Due to the rapid breakdown of the molecules it is therefore recommended that 1:10 dilutions of betamethasone valerate ointment not be made using emulsifying ointment nor should branded products of fluocinolone acetonide be diluted with emulsifying cream at all. The calculated shelf lives should be used as a guide when the various combinations of steroids and diluents are to be used.



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# DECLARATION

I declare that *The Chemical Stability of Betamethasone Valerate and Fluocinolone Acetonide Cream and Ointment in Cetomacrogol and Emulsifying Cream and Ointment Bases* is my own work, that it has not been submitted before for any degree or examination in any other university and that all the sources I have used or quoted have been indicated and acknowledged as complete references.

Michael John Titus

December 2002

Signed:.....

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# VITA

**Michael Titus** was born in the beautiful Karoo town of De Aar in the Western Cape Province of South Africa. He grew up in George on the picturesque Garden Route of the Western Cape and in Cape Town, the provincial capital. Matriculating from the Athlone High School in CapeTown, he qualified as a pharmacist at the University of the Western Cape in 1978.

He worked as a pharmacist at the Kimberley Provincial Hospital in the Northern Cape Province and for the Community Healthcare Services Organisation of the Western Cape Health Department. Later, his work at the Pre-Packing Unit of the Western Cape Health Department involved the large scale mixing of unguents for distribution to Community Healthcare Services facilities.

He is currently the pharmacy manager of the UCT Private Academic Hospital in Observatory, Cape Town.

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# CHAPTER 1

# INTRODUCTION

The corticosteroids are known to have powerful anti-inflammatory activity[1]. Consequently, they are often used to arrest and reduce inflammatory processes. Topically, they have proved of great value in the amelioration of inflammatory skin disease[2]. Adverse effects of topically applied corticosteroids, such as dermal atrophy and those resulting from systemic absorption via this route, however, limit their use[3]. To minimise such unwanted reactions, proprietary brands of topical preparations containing these steroids are commonly diluted with a variety of bases[4]. The severity of the inflammation and the sensitivity and extent of the inflamed area being treated determine the degree of this dilution.

This dilution changes the environment of the active ingredient and could compromise the therapeutic integrity of the product so diluted. Research has shown that such dilution may affect the physical[5] and chemical[6] stability of the active steroids as well as the biopharmaceutical[5] and microbiological[5] properties of the preparations.

Such dilution may thus result in an accelerated breakdown of the corticosteroid molecule. In particular, dilution with a specific base may lead to the alteration of the pH of the preparation, which, in turn, enhances the hydrolysis of some corticosteroids. For example betamethasone 17-valerate in an alkaline base medium rearranges to the more stable 21-valerate which is, however, 15 times weaker than the original form[7].

It has been proven that the degree of this chemical degradation may vary depending on the base used. Betamethasone valerate is, for example, chemically less stable in emulsifying ointment BP[6] than in cetomacrogol cream BPC



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(formula A)[8]. Similarly, hydrocortisone in a polyethylene glycol base was shown to have a shelf life of six months while it was still more than 98% intact fourteen months after dilution with cold cream USP[9, 10]. In the public hospital system of the Western Cape it is common practice, for the same reasons quoted earlier, to dilute proprietary brands of the corticosteroids fluocinolone acetonide and betamethasone valerate creams and ointments. The diluents used are cetomacrogol ointment BP, cetomacrogol cream BPC (formula A) as well as modifications of emulsifying ointment BP and aqueous cream BP (see methodology). Betamethasone valerate preparations mixed with diluents similar to those mentioned, have been the subject of extensive investigation. However, since no stability studies on fluocinolone acetonide preparations diluted with these bases could be found in literature, no stability predictions can be made for such diluted preparations. It is therefore imperative to investigate the influence of these diluents on this steroid. Since the formulae of the emulsifying ointment BP and the aqueous cream BP were slightly adapted for use in the health department hospital system, it would be helpful to include betamethasone valerate in this study and make useful comparisons.

The objective of this investigation was therefore to determine the chemical stability of betamethasone valerate and fluocinolone acetonide proprietary ointments and creams when diluted with cetomacrogol ointment and cream and emulsifying ointment and cream (aqueous cream) as used in the Western Cape department of health hospital system. From the results of the study, it was envisaged that a shelf-life could be calculated for each diluted product which is reflective of its chemical stability.

Previous studies have proven that the 17-α esters of corticosteroids undergo chemical degradation in diluents like emulsifying ointment and cream BP to the 21-α ester and betamethasone, but are not similarly affected by cetomacrogol ointment and cream bases. In particular, Betamethasone 17-valerate has been shown to undergo this degradation[6] and it is expected that fluocinolone 17-acetonide may be affected in the same way. Given that there is only a change in the proportions of the ingredients of the emulsifying cream and ointment formulae used in this study, it is



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postulated that the present investigation will reflect similar outcomes. We therefore wanted to test the hypothesis: The chemical stability of both betamethasone valerate and fluocinolone acetonide ointments and creams in cetomacrogol bases is greater than that obtained after dilution with emulsifying ointment and cream bases.


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# CHAPTER 2

# LITERATURE REVIEW

## 2.1 INTRODUCTION

Betamethasone valerate and fluocinolone acetonide are two compounds widely used as anti-inflammatories in topical preparations. They are synthetic congeners of naturally occurring steroid hormones produced by the cortex of the adrenal glands. These steroid hormones produced by the adrenal cortex i.e. adrenocorticosteroids, can be divided into three classes viz. the mineralocorticoids, some sex hormones and the glucocorticoids[11], betamethasone valerate and fluocinolone acetonide belonging to the latter class.

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## 2.2 GLUCOCORTICOID HORMONES

The major naturally occurring glucocorticoid in humans is hydrocortisone (cortisol)[12]. With other steroid hormones, these glucocorticoids share a common chemical structure (steroid skeleton) viz. the cyclopentanoperhydrophenanthrene nucleus[13].

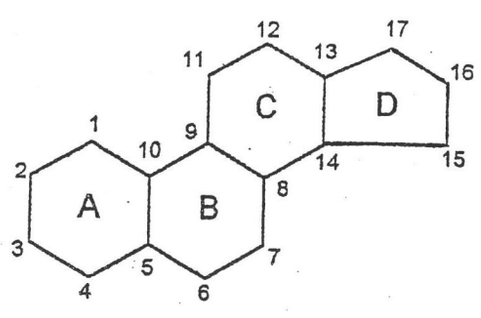


Fig. 1: Cyclopentanoperhydrophenanthrene nucleus

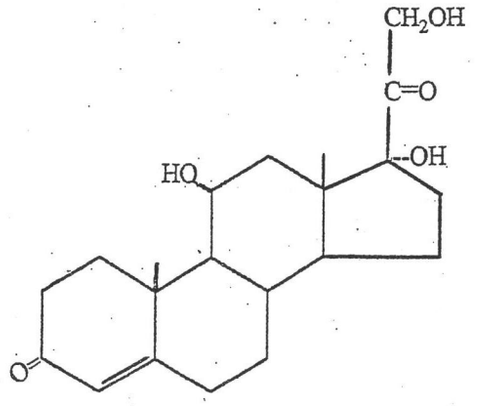


Fig. 2: Hydrocortisone



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### 2.2.1 Actions of the Glucocorticoids

The glucocorticoids affect many systems in the body because they influence the function of most cells of the body[11]. Their actions can generally be divided into two categories viz. a) anti-inflammatory and immunosuppressive effects and b) metabolic effects, negative feedback actions on the hypothalamus and anterior pituitary, and other effects[12]. As such, they stimulate the production of glucose from proteins (gluconeogenesis). This stimulates the production of insulin and may lead to the deposition of fat, particularly in the trunk, face and mesentery. They inhibit the secretion of adrenocorticotrophic hormone (ACTH) and their presence is required for the normal function of both smooth and striated muscle. They also facilitate fat absorption. The glucocorticoids also have important effects on the hematopoietic system increasing some and decreasing other blood cells[11]. Amongst these, the powerful anti-inflammatory effects of the glucocorticoids have placed them amongst the most prescribed drugs in the world[12].

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#### 2.2.1.1 Anti-inflammatory Actions

The glucocorticoids inhibit both the early and late manifestations of acute inflammation i.e. the redness, heat, pain and swelling, and the subsequent process of wound healing and repair. They effect all types of inflammation, whether it is caused by invading pathogens, by chemical or physical stimuli or by inappropriately deployed immune responses as in hypersensitivity and auto-immune disease. This suppression of inflammation at different levels of the inflammatory response occurs via a number of actions one of which has been well-researched and proven to be an anti-prostaglandin effect. However, unlike aspirin-like drugs, which block prostaglandin biosynthesis via an inhibition of the cyclo-oxygenase enzyme acting on the prostaglandin precursor arachidonic acid, the glucocorticoids act by blocking prostaglandin bio-synthesis via phospholipase $A_2$ inhibition[12].



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This most useful property of naturally occurring glucocorticoids of reversing the inflammatory process in the body has resulted in the development of many synthetic glucocorticoids.

## 2.3 SYNTHETIC COMPOUNDS

The dramatically effective results obtained with naturally occurring corticosteroids led to the synthesis of a number of chemical congeners. Use of the anti-inflammatory effect of the corticosteroids for direct application to the skin, i.e. topical use, for the treatment of inflammatory skin diseases, was first placed on a firm footing with the introduction of topical hydrocortisone in 1952[3]. This occurrence was revolutionary and dramatic. It ushered in a period described by Victor H. Witten as, "Never has there been such an exciting period in the history of dermatologic therapy!"[14]. A number of corticosteroids were synthesised , 17 in all between 1957 and 1967 and many more afterwards[15]. Manipulation of the molecule resulted in more potent products being produced. Such changes in molecular structure enhanced potency either by increasing the lipophilicity of the molecule and thus making it more permeable to the skin by increasing the corticosteroid-receptor bonding affinity or by delaying metabolism of the molecule after absorption. For example these changes include introduction of an additional double bond in the A-ring at C-1 (see fig. 1); halogenation (usually with fluorine) of the B-ring at C-9, C-6, or at both these positions; substitution at C-16 (with a methyl or hydroxyl group), esterification or acetylation at the D-ring (see fig. 1). Examples of esters commonly formed are acetates, butyrates, pivalates, propionates and valerates while acetonides are the result of acetylation[3] (see fig. 3).

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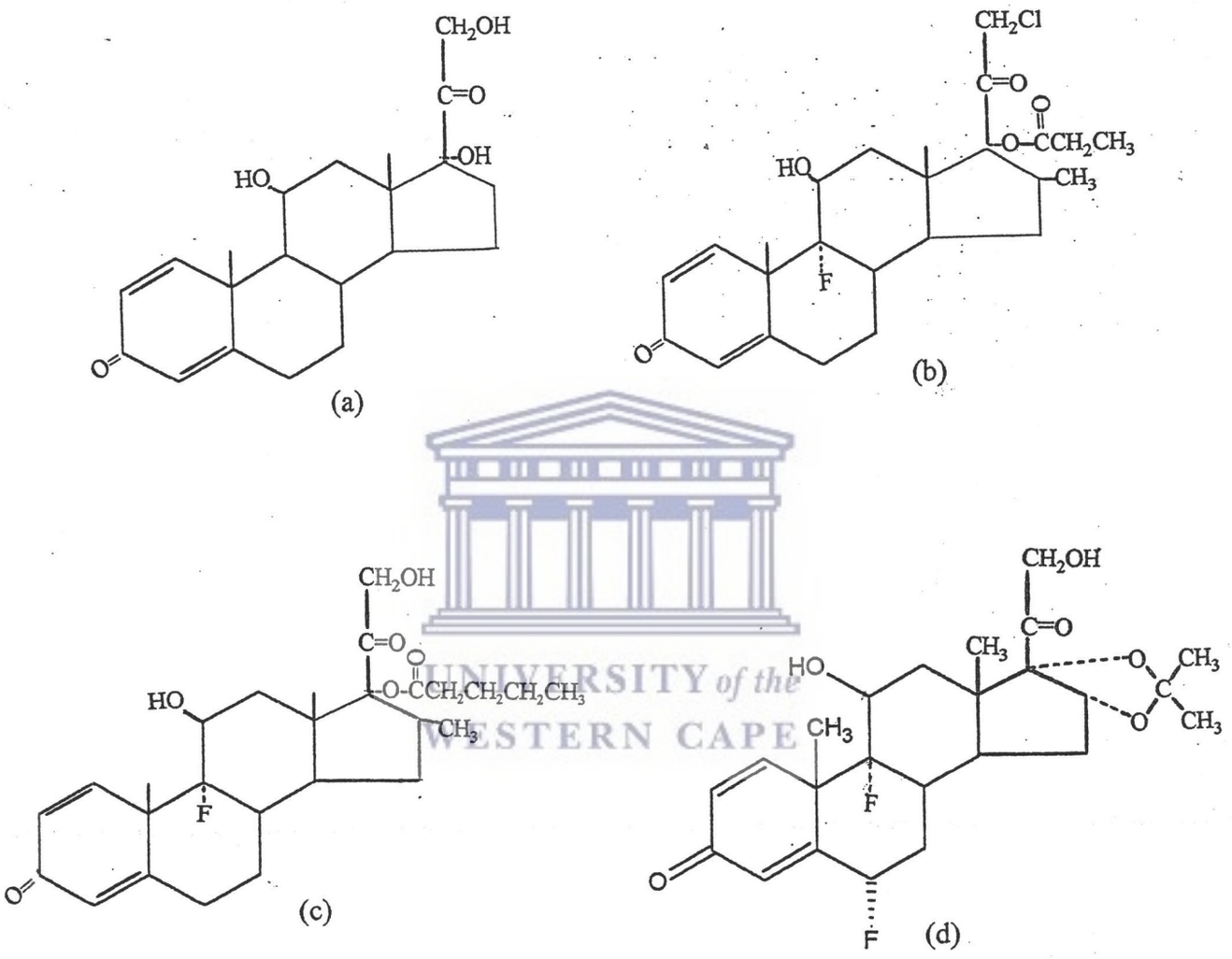


Fig. 3: Chemical structures of a) Prednisolone b) Clobatesol Propionate c) Betamethasone Valerate d) Fluocinolone Acetonide

These derivatives typically vary in their chemical stability.



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### 2.3.1 Chemical Stability of the Synthetic Glucocorticoids

As shown above, the naturally occurring glucocorticoids were chemically modified to afford advantages in terms of convenience of dosage and rapidity and duration of action. These synthetic molecules are, however, also prone to degradative chemical changes.

Being esters, the synthetic glucocorticoids can be hydrolysed back to the constituent glucocorticoid alcohol and carboxylic acid via base or acid catalysed reactions. Similarly, the 17-α-monoesters of glucocorticoids are unstable and in the presence of acid or base they may undergo acyl rearrangement to the 21-α-monoesters. In this reaction the carboxylic acid moiety of the glucocorticoid ester migrates from the C-17 position to the C-21 position. The result is a more stable but less active product. Betamethasone 17-valerate is, for example, 15 times more potent than betamethasone 21-valerate[7].

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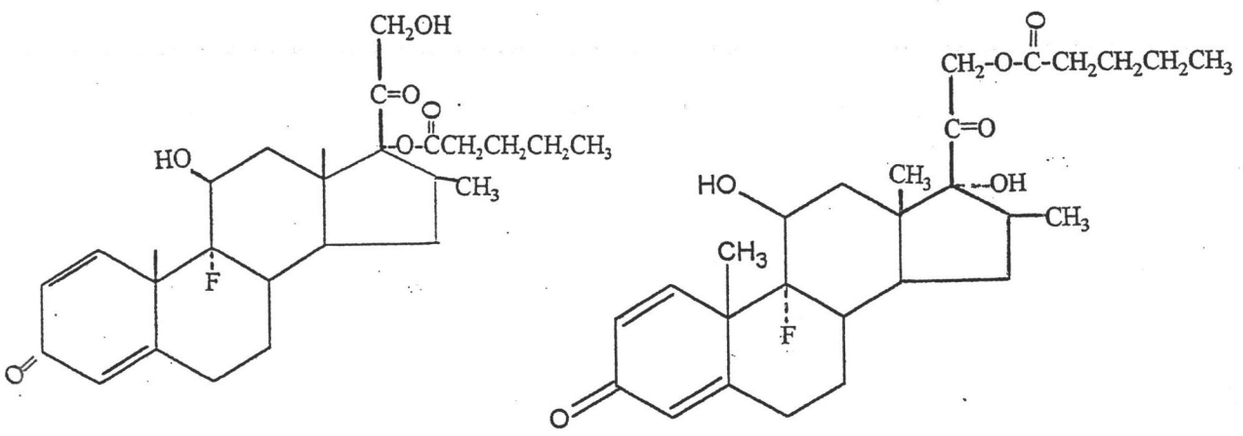


Betamethasone 17-Valerate Betamethasone 21-Valerate

Fig. 4: Rearrangement of the Betamethasone 17-Valerate Molecule



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A possible mechanism for this base catalysed reaction was proposed by Lohuizen and Verkade as well as Gardi *et al* as reported by Bundgaard and Hansen[7] as follows:

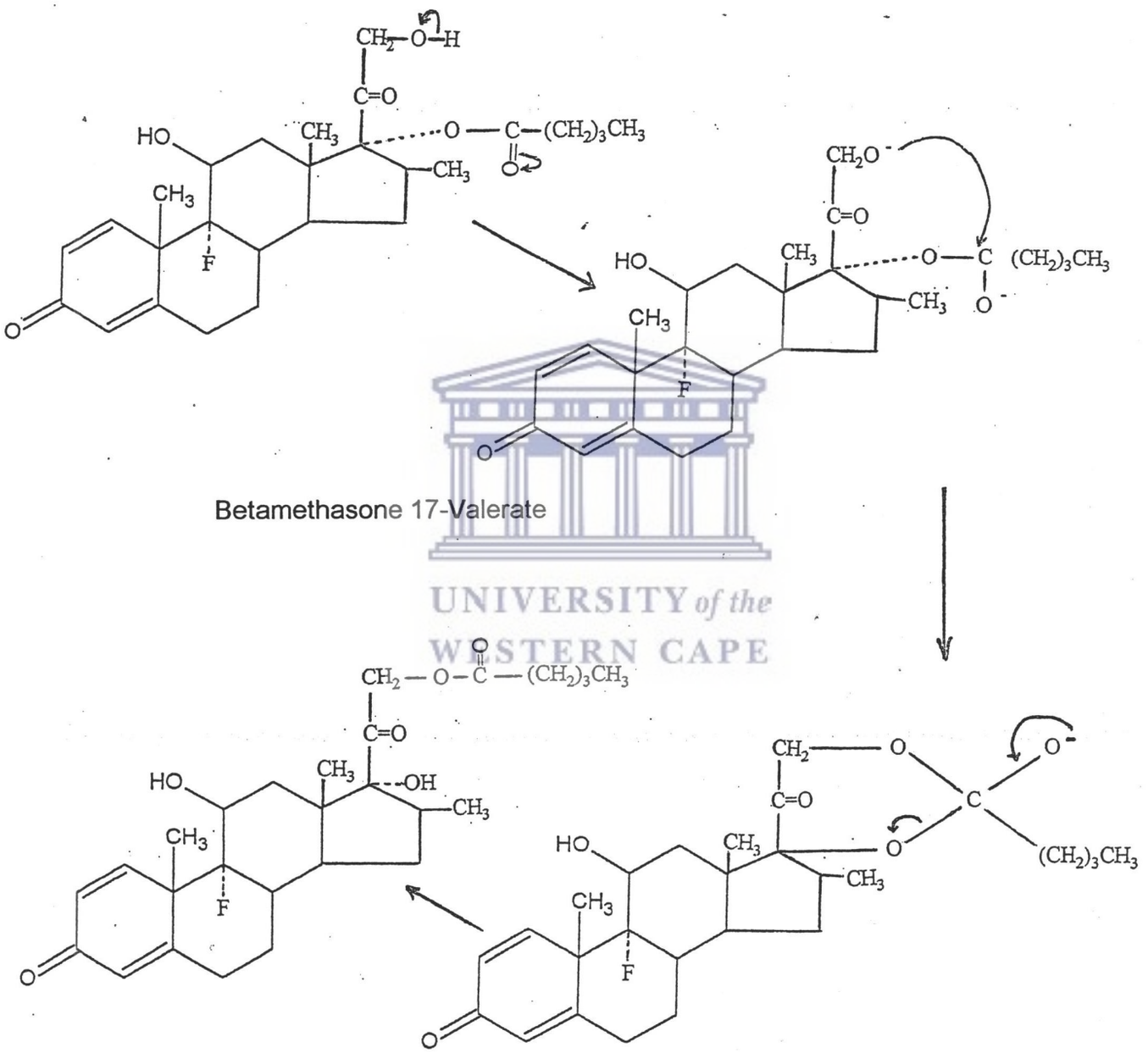


Fig. 5: Possible Mechanism for the Betamethasone 17-Valerate Rearrangement



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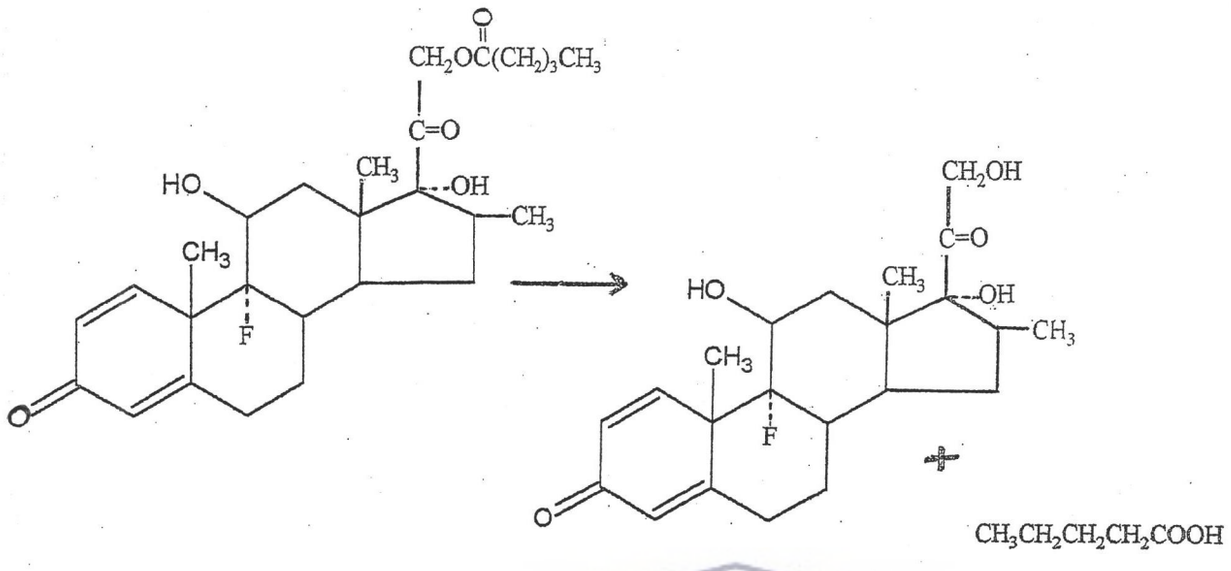
These 21-α-monoesters can then undergo further base-catalysed hydrolysis to the constituent glucocorticoid alcohol and carboxylic acid.

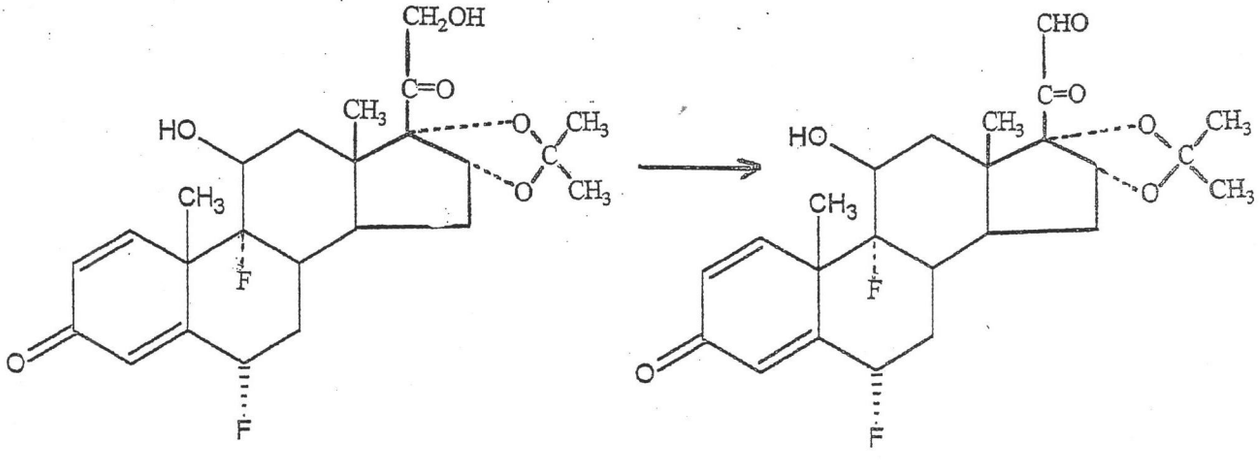


Betamethasone 21-Valerate Betamethasone Valeric Acid

Fig. 6: Hydrolysis of Betamethasone 21-Valerate into its Constituent Parts

A possible direct formation of betamethasone alcohol from the 17-valerate was found to be not significant in comparison with the rearrangement reaction[7].

Fluocinolone acetonide, like other primary alcohols, may be oxidised in the presence of oxidising agents, at the C-21 -OH to form the C-21 aldehyde[16, 17, 18]



Fluocinolone Acetonide The C-21 Aldehyde

Fig. 7: Formation of the C-21 Aldehyde from Fluocinolone Acetonide



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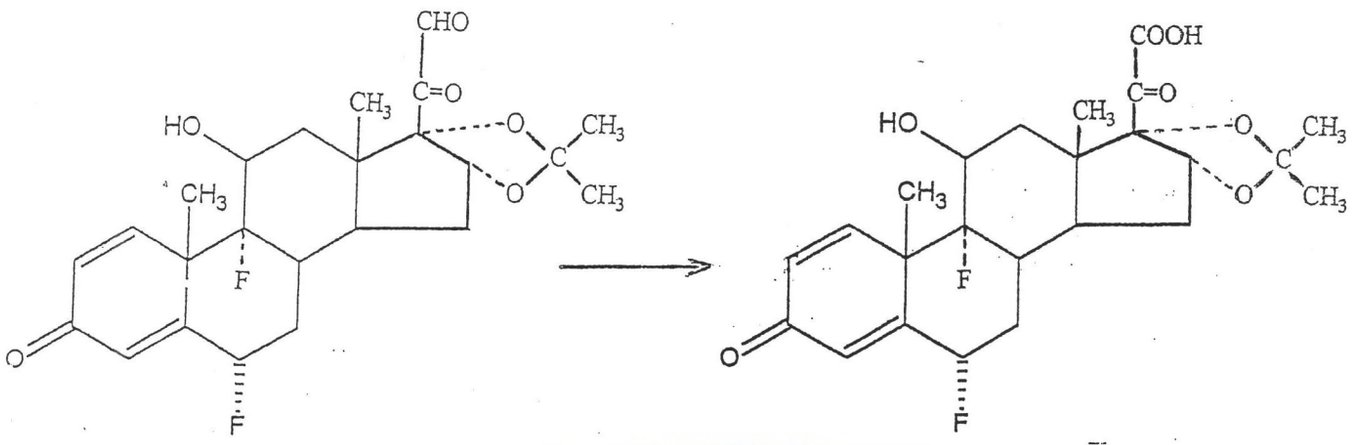
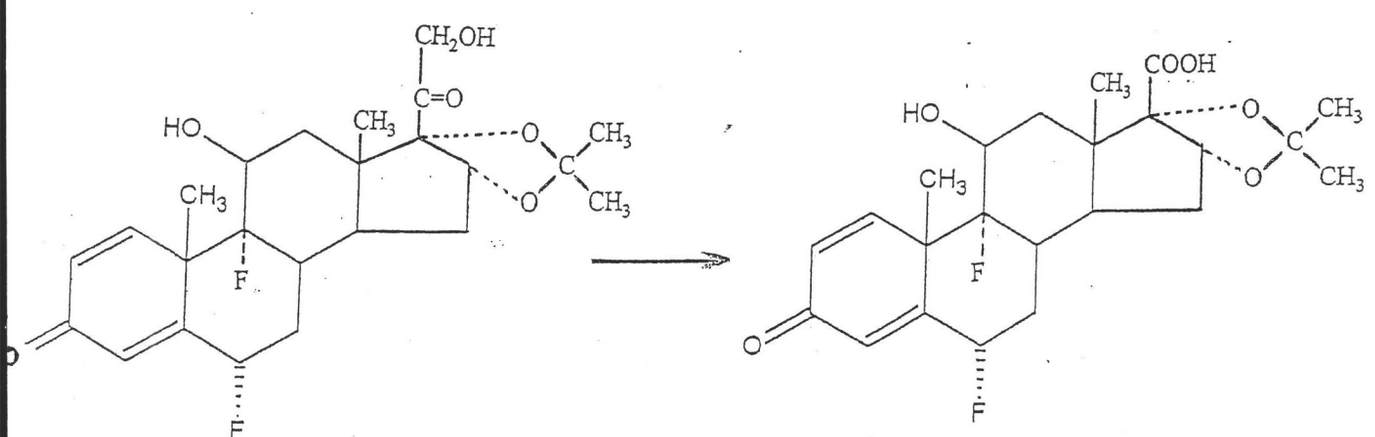
The 21-aldehyde may now be further oxidised to the 21-carboxylic acid. These oxidation reactions may also be catalysed by metal ions such as zinc[17, 18, 19].




Aldehyde Analogue Carboxylic Acid Analogue

Fig. 8: Oxidation of the Aldehyde Analogue to the Carboxylic Acid Analogue

High pH may also cause the splitting of the C-20, C-21 bond to form the C-17 etianic acid analogue which was demonstrated by Velluz *et al* using prednisolone in the presence of air and alkali[20].




Fluocinolone Acetonide The Etianic Acid Analogue

Fig. 9: Splitting of the C-20, C-21 Bond



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Further hydrolysis of the C-16, C-17 side chain may cause the loss of the cyclized acetonide group to give the tetrol derivative plus acetone[21].

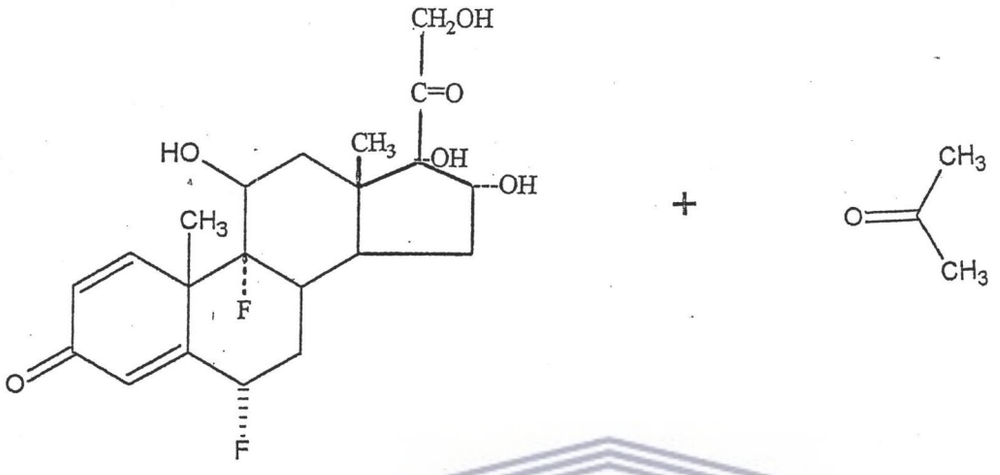


Fig. 10: Tetrol Derivative and Acetone

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All the above degradation products of fluocinolone acetonide were demonstrated to be present by Powell *et al*[21] by the use of radiopharmaceuticals in a sample of fluocinolone acetonide cream which had been stored for 26 months after mixing.

In all the above chemical reactions the influence of pH, temperature, water and other chemicals has been shown (Kenley *et al*[16] etc.). These synthetic glucocorticoids are thus unstable in particular environments and such pharmaceuticals should therefore be protected from these influences.

### 2.3.2 Adverse Effects

Very potent molecules were developed by substitution at the various positions. For example betamethasone valerate is 300 times more potent than hydrocortisone[3]. The more potent the product, the quicker anti-inflammatory relief could be obtained. Such potencies are listed, commonly using "skin-blanching" tests on live human



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subjects as criteria[22], the blanching referring to a decrease in the redness of inflamed skin by the action of the glucocorticoid. Increasing potency, however, is in many cases accompanied by an increase in adverse effects.

As mentioned, the glucocorticoids exhibit a wide spectrum of actions on the body. All these effects, except the anti-inflammatory effects, should be considered adverse reactions when treating topical inflammation. Side effects can develop locally, for example skin atrophy[23], formation of striae, purpura and haemorrhage. A condition such as psoriasis, which often requires more potent corticosteroids, may exhibit rebound flare-up upon cessation of treatment[3].

Side effects can also develop from systemic absorption via the skin. Skin absorption may be enhanced by phenomena like increased lipid solubility of the drug, application to broken skin or application under occlusion. These systemic side effects are more serious and include adrenal suppression, growth retardation, Cushing's syndrome and hypertension[2] and other effects resulting from influences on the hypothalamus and pituitary glands.

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The injudicious use of potent topical corticosteroids with the concomittant adverse effects have led to a lot of criticism. A lack of understanding of the pharmacological and clinical aspects of these preparations resulted in some confusion which was prejudicial to what was very effective medication[24]. A result of this was the production of topical steroid preparations in lower concentrations by the pharmaceutical manufacturers, as well as the extemporaneous dilution of the potent branded products.

### 2.3.3 Dilution

An attempt at minimising the adverse effects whilst maintaining the therapeutic effect is made by diluting the steroid products with a suitable base. Commonly, prescribers require 10, 20 or 50 percent of the original strength of the compound.



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This has been a very common practice for a long time and remains so today. A survey done of the prescribing patterns for topical treatment in a Family Practitioner Committee District in the United Kingdom revealed that almost 50% of all topical preparations prescribed contain steroids and of these 11,2% were for diluted products[25]. In the public health systems of the various provinces of South Africa it is also common practice to dilute branded topical corticosteroids products with suitable bases.

When large areas of the body have to be treated, the danger of systemic absorption increases. It is then safer to use less potent or the lowest effective concentration of topical steroid available[11]. Diluted topical steroids are ideally suited to treating such conditions which cover large areas such as certain forms of eczema and psoriasis. They are also utilised when more sensitive areas of the body are treated such as the face and flexures and in the treatment of young patients. Often these skin diseases take years to resolve and may even persist for life. Prescribers then often resort to diluted topical steroid preparations with an appropriate vehicle. It is also more cost effective to use diluted products when such long term treatment is required. Although dilutions of some of the original proprietary topical products are commercially available in the United Kingdom (1 in 4 and 1 in 10 dilutions of both 0,1% betamethasone valerate ointment and 0,025% fluocinolone acetonide cream)[26], this is not the case in South Africa.

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### 2.3.3.1 Vehicles Used in Dilution

A number of different bases have been used as vehicles for diluting branded corticosteroid preparations in order to alter their potency. In one study looking at the prescribing habits of family practitioners, branded preparations of betamethasone 17-valerate were ordered by prescribers to be diluted by 18 different vehicles in the dilutions: 1 in 2, 1 in 3, 1 in 4, 1 in 5, 1 in 10, 1 in 20, 1 in 100[25]. Varying degrees of success have been achieved upon dilution with regard to the integrity of the preparation and the corticosteroid. Similarly, it has become clear that the choice of



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vehicle-corticosteroid combination is important.

Topical steroid preparations occur mostly in the form of ointments, creams, gels or lotions of which the first two are used the most. Today the most popular diluents include white soft paraffin (for ointments) or a combination of emulsifying agents and white soft paraffin, liquid paraffin or water for ointments and creams, with propylene glycol often used as a solvent for the active ingredient.

White soft paraffin is bland, neutral and non-irritant and used for the dilution of ointment preparations. It is poorly absorbed percutaneously and acts as an occlusive layer when utilised as a diluent. Emulsifying agents used are either surface active agents, hydrophilic colloids (e.g. sodium carboxymethylcellulose) or finely divided solids (e.g. bentonite). The surface active agents (surfactants) are more often used and these can be either anionic, cationic or non-ionic.

Typical examples of diluents containing surface active agents are:

a) emulsifying ointment BP - has anionic surfactant,
b) aqueous cream BP - has anionic surfactant,
c) cetrimide emulsifying ointment BP - has cationic surfactant,
d) cetrimide cream BPC - has cationic surfactant,
e) cetomacrogol emulsifying ointment BP - has non-ionic surfactant and
f) cetomacrogol cream BPC (Formula A & B) - has non-ionic surfactant.

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Cationic diluents would be incompatible with anionic constituents of a preparation while anionic diluents would be incompatible with cationic ingredients. Non-ionic bases do not suffer from either of these defects. Manufacturers of topical preparations often use vehicles developed at their own research facilities. The formulae of these vehicles are mostly confidential.

Dilution of branded topical steroid preparations is, however, not without problems. Literature shows that the environmental change occurring when these products are



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diluted, may result in physical, chemical and bacteriological changes. The change in potency is also not always predictable.

### 2.3.3.2 Effects of Dilution on Potency

The potency of diluted topical steroid preparations is generally studied by means of the skin-blanching test whereby the vasoconstrictor effect of the steroids on artificially inflamed skin is used as a measure of their anti-inflammatory ability. The degree of blanching can be measured using a reflectance spectrophotometer. Boonsaner, Remon and de Rudder[27] showed that Beeler's base as a diluent reduced the skin-blanching activity of betamethasone 17-valerate less than cold cream did. Similarly, the skin-blanching test was used by Tanner and Woodford[28] to prove that cetomacrogol cream BPC (formula A) was a suitable diluent for diluting betamethasone 17-valerate. They also showed by this method, that unguentum Merck was a suitable substitute for cetomacrogol cream when diluting betamethasone 17-valerate preparations.

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It could intuitively be assumed that, when diluted, the potency of corticosteroid preparations is also reduced. This is, however, not always the case. Ryatt, Cotterril and Mehta[29] demonstrated that there was no difference in the blanching potential of Betnovate® ointment (betamethasone 17-valerate ointment) when diluted 1:4, 1:16 and 1:32 with unguentum Merck. Similarly, Gao and Li Wan Po[30] also used skin-blanching to show that diluting fluocinolone acetonide cream up to 1:10 times with a recommended base caused no significant loss of activity. This, apparently contradictory phenomenon, can possibly be explained by the fact that very specific intracellular receptors have been demonstrated with which glucocorticoids interact. This receptor-glucocorticoid binding is followed by a complex series of events starting with the movement of the steroid-receptor complex into the nucleus eventually giving rise to the biological effects noticed[31]. The number of these intracellular receptors are limited. Thus, if, even at the lower dilutions, sufficient active steroid is present to saturate those receptors, an increase in concentration



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of steroid will not produce any further enhancement in activity.

### 2.3.3.3 Microbiological Effects of Dilution

Researchers have also shown that dilution of corticosteroid preparations may compromise their ability to withstand micro-organismic attack. Manufacturers prepare their products well protected with the necessary preservatives to prevent bacterial contamination. If extemporaneous dilution of the product is not done aseptically, then bacteria may be introduced. Furthermore, dilution with an unpreserved diluent may dilute the manufacturer's preservative so that it may no longer be able to withstand bacterial challenge. Mooney[32] and Launchbury[31] refer to a drop in antibacterial activity when, upon addition of an oil to an oil in water emulsion steroid preparation, the preservative tends to partition into the oil phase and thus not be able to protect the whole product. Savin[33] has shown that *Pseudomonas aeruginosa* has the ability to actively metabolise steroids. The result of ineffectual preservation could therefore result in reduced activity of the corticosteroid or the introduction of bacteria into superficial lesions to which the preparation is being applied. On the other hand, Tanner and Woodford[28] found that the preservative system in unguentum Merck gave adequate anti-bacterial protection when used to dilute Betnovate® ointment.

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### 2.3.3.4 Physical Effects of Dilution

Diluting corticosteroid preparations may also result in physical changes occurring which could result in altering the bioavailability of the corticosteroid. Ointment preparations are often suspensions of a corticosteroid in an ointment base. Commonly propylene glycol is used to dissolve the steroid and the solution is then finely dispersed in the ointment base. Propylene glycol has also been used in creams as part of the aqueous phase. If a saturated solution of the active steroid



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in the propylene glycol is used in the preparation, then the product would have high thermodynamic activity i.e. the base provides a high concentration of steroid close to the skin. Depending on the base used, dilution of such preparations are affected differently with varying effects on the thermodynamic activity.

Improper dilution of preparations manufactured with propylene glycol can also result in undesirable products. The fine dispersion of steroid in a base is obtained by rapidly stirring and homogenising the steroid-containing propylene glycol in molten base followed by rapid cooling. When such a product is diluted by simply stirring in the desired diluent, even dispersion of the steroid may not be obtained and various parts of that product may contain differing amounts of steroid. Trituration may even cause separation of the propylene glycol[5].

Addition of a cation to a dilution prepared using an anionic emulsifier or addition of an anion to a dilution prepared by using a cationic emulsifier would cause the emulsions to crack resulting in the active ingredient partitioning to the phase in which it is most soluble. All these physical effects result in unevenly distributed active ingredient and thus an unacceptable product.

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**2.3.3.5 Effects of Dilution on Chemical Stability of Glucocorticoids**

The chemical stability of the various corticosteroid molecules in different diluent bases has also been widely researched. Fluocinolone 17-acetonide is unstable in bases that contain oxidising agents, the latter causing the formation of the 21-aldehyde and loss of the acetonide group. Compound zinc paste was thus proven to be an unsuitable diluent for both fluocinolone acetonide and fluocinonide[18]. It caused a 1:10 dilution of fluocinolone acetonide ointment to be degraded to 33,8% of its original strength within 7 days. Similarly, hydrocortisone was degraded by zinc catalysed oxidation in a zinc oxide lotion[18] and hydrocortisone 17-butyrate in a zinc oxide ointment[19]. Another study found fluocinolone acetonide ointment to have a shelf life of one month when diluted with unguentum Merck and two months when



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diluted with Lipobase[34].

Betamethasone 17-valerate is converted to the 21-valerate in diluents with an alkaline pH. Thus, a 1:4 dilution in unguentum Merck was shown to have a shelf life of 5 months after storage at room temperature[29]. However, a similar dilution of betamethasone 17-valerate in emulsifying ointment BP degraded by 60% in 6 hours[35] while in another study a 50:50 dilution in the same diluent had a shelf life of just 6 minutes[6]. Yet, a proprietary brand of the same corticosteroid with an altered formulation was found to be within label claim specifications 14 months after being similarly diluted with emulsifying ointment BP and aqueous cream BP[36]. Research also showed that Betnelan-V® cream (betamethasone 17-valerate) is more stable in Beeler's basis than in Cold Cream®[27], while cetomacrogol cream BPC (formula A) and white soft paraffin BP were declared to be "satisfactory" diluents for betamethasone 17-valerate and beclomethasone dipropionate[8].

It is thus clear that the influence of the base on the glucocorticoid is dependent on the exact combination of base and steroid. Although documents like the "*External Diluent Directory*"[37] can be used as a guide when doing extemporaneous dilutions of branded topical glucocorticoid products, no accurate predictions can be made. Also, because the emulsifying bases used in the Western Cape health department hospital system are altered versions of official BP and BPC formulae and dilution of branded topical anti-inflammatory glucocorticoid products continues to be practised by the department, it is all the more important that these diluent-glucocorticoid combinations be tested for degradation. It would serve a useful purpose to use the results of the degradation survey to calculate shelf lives for the combinations so tested.

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#### 2.3.3.5.1 Degradation Studies

Several strategies have been employed to study the degradation of glucocorticoids when they are diluted in order to gain information about the rate of degradation,



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breakdown products formed or the factors influencing breakdown[6, 7]. While the rate of chemical breakdown of each corticosteroid might be specific to each steroid-diluent combination, there are several common features in the steps taken in degradation studies of diluted steroid preparations. Preparations of such steroid-diluent combinations are typically stored under controlled conditions at ambient temperature with the removal of samples at specified times. These samples are then analysed for unchanged steroid or degradation product formed and the results used to construct degradation profiles for these combinations.

This method is, however, time-consuming. In order to reduce the time spent doing the experiments, degradation can be accelerated by subjecting preparations to drastic treatment such as the application of heat and/or the addition of acids or bases[7, 10, 16]. Results are then extrapolated to represent real-time degradation. The disadvantage of the application of heat and addition of rate-altering chemicals is that they may cause physical and chemical changes of the base, thus altering the environment of the active steroid.

Extraction of the active corticosteroid and, if needed, its breakdown products, is typically done by first dispersing an aliquot of the preparation in an organic liquid in which the base is miscible followed by extraction of the corticosteroid in an organic solvent in which the corticosteroid is soluble. The amount of corticosteroid or degradation products so extracted can then be determined by a number of analytical methods which may vary in suitability depending on the conditions of each project.

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#### 2.3.3.5.1.1 Analytical Methods

Various researchers have used different methods to assay for remaining corticosteroid. Use has been made of direct densitometry with thin layer chromatography[6], UV spectrophotometry[9], radiolabelling[21], differential pulse polarography[40], and reversed phase high performance liquid chromatography



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(HPLC)[8]. The HPLC method was used by most of the researchers in this field who described it as simple, efficient, stability-indicating, and capable of being carried out at ambient temperature. They found it to be robust with a quick turnaround time, ideal for the measurement of a large number of samples.

## 2.4 Use of Diluted Topical Glucocorticoid Preparations in the Western Cape Health System.

Dilution of branded glucocorticoid products is common in the Western Cape and, it is suspected, throughout South Africa. A telephonic survey of dermatologists in private practise in Cape Town confirmed this while it is known that the department of health of the Western Cape provincial government dispenses diluted preparations to its patients in the hospital system.

Reasons advanced for this practice are that it is ideal for application to sensitive areas and for long term use over large areas of the body because side effects are greatly reduced. Patients are also very satisfied with the emollient effect of the bases used especially those suffering from a chronic dry eczematous rash. In the health department treatment of chronic dermatoses often require the regular dispensing of large quantities of topical steroid preparations over a long period. It thus becomes very cost effective yet efficacious to use diluted products.

In the health department hospital system fluocinolone acetonide and betamethasone valerate are the glucocorticoids of which the branded products are diluted. Currently, the only diluents used for these products are cetomacrogol cream and ointment while the use of emulsifying ointment BP and aqueous cream has been stopped. The diluents required and the diluted mixtures are prepared on site according to formulae and methods as is described later in the chapter on methodology.

Typically, department hospitals prepare dilutions equivalent to 10% of the original



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strength of the branded steroid product. They are stored in opaque plastic containers at room temperature and dispensed in quantities of 30g, 50g, 100g and 500g. Expiry dates are given as three months from date of manufacture. This date is arbitrary, based only on a general impression garnered from literature.

Since the expiry dates used are not empirical, it is imperative that degradation studies be carried out using the two steroids mentioned and the bases used for dilution by the department. Proper shelf lives can then be determined for each of the combinations of glucocorticoid product and diluent. This may then enhance the cost effective use of the products.


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# CHAPTER 3

# WORK PLAN

## 3.1 OBJECTIVES

This investigation aimed at obtaining the chemical stability profiles of fluocinolone acetonide and betamethasone valerate molecules when proprietary creams and ointments containing these ingredients were diluted with cetomacrogol and emulsifying cream and ointment bases. From these degradation characteristics shelf lives for these glucocorticoid-diluent combinations were to be calculated in order to allocate the same to similar dilutions manufactured in the Western Cape health department hospital system.

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## 3.2 HYPOTHESIS

The aim of the study was to test the hypothesis that the cetomacrogol cream and ointment diluent bases would affect the active glucocorticoids less than would emulsifying ointment and aqueous cream. As a result it was postulated that the anti-inflammatory steroid preparations would be more stable when diluted in the cetomacrogol bases than in emulsifying bases.

## 3.3 STUDY APPROACH

To realise the objectives of this study, the following activities had to be undertaken: dilution of samples of the branded topical glucocorticoid products, storage and sampling of the diluted preparations, determination of the degradation rate of the glucocorticoids in the diluted preparations and the calculation of shelf lives for each



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preparation.

### 3.3.1 Dilution of the Proprietary Topical Glucocorticoid Products.

The branded topical steroids that were to be used in this study were the same ones used by the Western Cape Department of Health for dilution and distribution in its hospital system. Likewise, the diluents that were to be used were the same as those used by the department and they were also to be prepared in the same way i.e. by melting all ingredients and mixing by stirring together. Incorporation of the active ingredients into the bases was to occur by trituration with a plastic spatula on a glass slab.

### 3.3.2 Storage, Sampling and Extraction of the Diluted Preparations.

The objective of the study was to determine the effect of only the diluents on the chemical stability of the active ingredients. This meant that all other variables of the environment had to remain constant so as not to exercise any influence. The diluted preparations thus had to be stored in a controlled environment where the temperature could be maintained at a constant 25°C, protected against the influences of light, moisture and air. This, of course, precluded the use of any methods of accelerated degradation. In practice the diluted preparations would be directed to be stored below 25°C in well-closed containers impervious to light, moisture and air.

To monitor the stability of the steroids, it was decided to collect samples of the diluted preparations for up to one year. It was further decided to adjust the sampling schedule as time progressed after analysis of the initial samples based on the information obtained about each specific preparation. In addition each sample was to be extracted and processed to the assay-ready stage as soon as possible after



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its collection. It was felt that such assay-ready samples would be easier to store. The active ingredients had to be soluble in the extracting vehicle which had to have no other influence on the active ingredients.

### 3.3.3 Determination of Glucocorticoid Stability

To determine the breakdown of the glucocorticoids, the extracted active ingredient in each sample was to be assayed for total remaining glucocorticoid by means of reversed phase High Performance Liquid Chromatography (HPLC). Researchers found this to be a most suitable method of assay for the glucocorticoids[17, 34, 38] describing it as stability-indicating, robust, simple and efficient. Because a very large sample set would have to be assayed, a means of automation of the assay was also to be employed.

The glucocorticoid content of the samples was to be determined using standard techniques and the degradation profile assessed from the percentage remaining vs time data generated. From these profiles rate constants could be determined. Because the active ingredients were present in minute quantities compared to the concentration of the other ingredients of the diluent bases, it was assumed that the glucocorticoid degradation would follow pseudo first order kinetics.

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### 3.3.4 Determination of Shelf Lives

Shelf life was assumed to be the time taken for the active glucocorticoids to have degraded until 90% of the original amount of glucocorticoids remained in the diluted products. Similarly, half life was assumed to be the time taken for the glucocorticoids to have degraded in the dilutions until half of the original amount of glucocorticoids remained.



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In order to determine the shelf and half lives of each glucocorticoid preparation, use was to be made of a reaction rate equation using a degradation constant calculated for each glucocorticoid-diluent combination. Finally, to test our hypothesis, these shelf lives, obtained for the preparations using cetomacrogol or emulsifying bases, were to be compared.




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# CHAPTER 4

# METHODOLOGY

## 4.1 INTRODUCTION

The diluted preparations studied were branded samples of betamethasone valerate and fluocinolone acetonide cream and ointment diluted to 10% (1:10 w/w) and 50% (50:50 w/w) of their original strength using cetomacrogol and emulsifying cream and ointment as diluents. These mixtures were stored at ambient temperature (25°C) and protected from moisture and light. Samples were taken from each diluted preparation at various times over a period of up to one year. Each sample thus removed was immediately extracted with acetonitrile and these extracts were stored at -84°C until assayed. Each extract was assayed for active ingredient using reversed phase High Pressure Liquid Chromatography (HPLC).

The sections below provide details of the materials, equipment and procedures used in this study.

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## 4.2 MATERIALS

The following materials were used in the preparation of creams and ointments and in the assay.

Betamethasone valerate 0,1% w/w ointment and cream were: *Lenovate® ointment and cream* manufactured by *Lennon Ltd.*, Fairclough Road, Port Elizabeth, South Africa.

Fluocinolone acetonide 0,025% w/w ointment and cream were: *Cortoderm® ointment and cream* manufactured by *Lennon Ltd.*, Fairclough Road, Port Elizabeth,



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South Africa.

Betamethasone valerate powder: Authentic specimen B/N 427 distributed by the *British Pharmacopoeia Commission*, 8 Bulstrode Street, London W.1. England.

Betamethasone powder: Specimen B/N 57046 obtained from *Provincial Administration Health Department Quality Control Laboratories*, Cape Technikon, District Six, Cape Town, South Africa.

Fluocinolone acetonide powder: manufactured by *Sigma Chemical Company*, P.O. Box 14508, St. Louis, MO 63178, U.S.A.

Butyl 4-hydroxy benzoate: Batch Number 70200. Manufactured by *Riedel-de Haën* AG D-30926 Seelze, Germany.

Cetomacrogol 1000: Batch Number ET2064025LK obtained from *Croda Chemicals* Link Close, Montagu Gardens, Cape Town, South Africa.

Cetostearyl Alcohol: Batch Number A200025IM obtained from *Croda Chemicals* Link Close, Montagu Gardens, Cape Town South Africa.

Emulsifying Wax: Batch Number 832 obtained from *Barr's Pharmaceutical Industries*, Fir Street, Observatory, Cape Town South Africa.

Liquid Paraffin: Batch Number 41610. Obtained from *Lennon Ltd.* 7 Fairclough Road Port Elizabeth, South Africa.

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White Soft Paraffin: Obtained from *Barr's Pharmaceutical Industries*, Fir Street, Observatory, Cape Town, South Africa.

Chlorocresol: Obtained from *Barr's Pharmaceutical Industries*, Fir Street, Observatory, Cape Town, South Africa.

Acetonitrile: HPLC Grade. Batch Number BV554. Manufactured by *Allied Signal Inc., Burdick and Jackson*, Muskegon, MI 49442 USA.

Hexane: HPLC Grade. Batch Number BQ562. Manufactured by *Allied Signal Inc., Burdick and Jackson*, Muskegon, MI 49442 USA.



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## 4.3 PREPARATION OF MIXTURES

The following formulae and methods were used to prepare the diluent bases, the diluted glucocorticoid mixtures and the standard mixtures for the assay.

### 4.3.1 Cetomacrogol Emulsifying Ointment BP

Formula

| | |
|---|---|
| Cetomacrogol 1000 | 6% |
| Cetostearyl Alcohol | 24% |
| Liquid Paraffin | 20% |
| White Soft Paraffin | 50% |

Method - All the ingredients were melted together at 60°C and stirred until cool.
Quantity prepared: 500g

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### 4.3.2 Cetomacrogol Cream BPC (Formula A)

Formula

| | |
|---|---|
| Cetomacrogol 1000 | 1,8% |
| Cetostearyl Alcohol | 7,2% |
| Liquid Paraffin | 6,0% |
| White Soft Paraffin | 15,0% |
| Chlorocresol | 0,1% |
| Distilled Water | 69,9% |

Method - The first four of the ingredients were melted together at 60°C and stirred. The water was heated and the chlorocresol dissolved in the heated water. This was then added to the molten ingredients and stirred until cool.
Quantity prepared: 500g



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### 4.3.3 Emulsifying Ointment

| | Health Dept. Formula | BP Formula |
|---|---|---|
| Emulsifying wax | 19,35% | 30% |
| White soft paraffin | 48,40% | 50% |
| Liquid paraffin | 32,25% | 20% |

Method - The ingredients, in proportions according to the Health Department formula, were melted together at 60°C and stirred until cool.
Quantity prepared: 500g

### 4.3.4 Aqueous Cream (Hydrous Emulsifying Ointment; Emulsifying Cream)

| | Health Dept. Formula | BP Formula |
|---|---|---|
| Emulsifying wax | 5,80% | 9,00% |
| White soft paraffin | 14,52% | 15,00% |
| Liquid paraffin | 9,68% | 6,00% |
| Chlorocresol | 0,10% | 0,10% |
| Water | 69,90% | 69,90% |

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Method - The first three items, in proportions according to the Health Department formula, were melted together at 60°C and stirred. The water was heated and the chlorocresol was dissolved in the heated water. This was then added to the molten ingredients and stirred until cool.
Quantity prepared: 500g



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### 4.3.5 Diluted Glucocorticoid Preparations

The above bases were used as diluents, in proportions given below, to prepare the following glucocorticoid mixtures:

Formulae

i) Betamethasone valerate cream : cetomacrogol cream (10:90 w/w)
ii) Betamethasone valerate cream : cetomacrogol cream (50:50 w/w)
iii) Betamethasone valerate cream : emulsifying cream (10:90 w/w)
iv) Betamethasone valerate cream : emulsifying cream (50:50 w/w)

i) Betamethasone valerate ointment : cetomacrogol ointment (10:90 w/w)
ii) Betamethasone valerate ointment : cetomacrogol ointment (50:50 w/w)
iii) Betamethasone valerate ointment : emulsifying ointment (10:90 w/w)
iv) Betamethasone valerate ointment : emulsifying ointment (50:50 w/w)

i) Fluocinolone acetonide cream : cetomacrogol cream (10:90 w/w)
ii) Fluocinolone acetonide cream : cetomacrogol cream (50:50 w/w)
iii) Fluocinolone acetonide cream : emulsifying cream (10:90 w/w)
iv) Fluocinolone acetonide cream : emulsifying cream (50:50 w/w)

i) Fluocinolone acetonide ointment : cetomacrogol ointment (10:90 w/w)
ii) Fluocinolone acetonide ointment : cetomacrogol ointment (50:50 w/w)
iii) Fluocinolone acetonide ointment : emulsifying ointment (10:90 w/w)
iv) Fluocinolone acetonide ointment : emulsifying ointment (50:50 w/w)

Method - The appropriate amount of proprietary ointment or cream was weighed and added to the appropriate amount of diluent ointment or cream. The mixtures were then prepared by trituration with a metal spatula on a glass slab. Each mixture was sealed in 3 x 30g glass jars and stored in an oven set at 25°C. The door was sealed tight to prevent the passage of air, moisture and light.



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### 4.3.6 Standard Mixtures for Assay

To quantitate the amount of glucocorticoid remaining in the diluted preparations, standard assay mixtures were prepared. For these serial dilutions of betamethasone valerate and fluocinolone acetonide creams and ointments were made by diluting set amounts of the proprietary cream or ointment with each of the cetomacrogol and emulsifying creams and ointments respectively. This was done by trituration on a glass slab using a metal spatula. The resultant dilutions were: proprietary cream/ointment : diluent (50:50), (25:75), (12,5:87,5), (6,25:93,75), (3,125:96,875) w/w and undiluted amounts representing (100:0) w/w. These standard preparations were freshly prepared every time an assay was performed.

### 4.3.7 Internal Standards

For the betamethasone valerate assay, butyl 4-hydroxy benzoate (300$\mu$g/ml acetonitrile) and for the fluocinolone acetonide assay, betamethasone (110$\mu$g/ml acetonitrile) were used as internal standard solutions. These internal standards were stored at 4°C - 8°C in a refrigerator. A fresh solution was prepared every week.

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## 4.4 SAMPLING, EXTRACTION AND STORAGE

At various time intervals over a period of up to one year 200mg aliquots of each of the preparations were sampled and placed in a 3ml screw-topped glass bottle. One millilitre of hexane was added, the cap screwed on tightly and the sample dispersed in the hexane by shaking on a mechanical shaker for 3 minutes. Then 0,1ml of the respective internal standards was added. To this was added 1ml of acetonitrile and the mixture shaken for a further 3 minutes.



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The bottle was then centrifuged at 6000 rpm for 10 minutes. After centrifugation the extract was allowed to stand for 10 minutes in order to equilibrate. The bottom layer (acetonitrile extract) was then removed using a pasteur pipette, transferred to a hinge-capped Eppendorf tube and stored at -84°C.

## 4.5 HPLC ASSAY

The analysis of the glucocorticoids in the extracts was done by means of reversed phase High Performance Liquid Chromatography (HPLC). Extracts of diluted preparations were chromatographed at the same time as extracts of standard preparations.

### 4.5.1 Instrumentation

The following equipment constituted the HPLC system used in this study:

Pump - Beckman System Gold Programmable Solvent Module 126

Autosampler - Beckman System Gold Autosampler Module 507

Column - Hypersil 5, C8, 150mm x 4,6mm

Detector - Beckman System Gold Diode Array Detector Module 168

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### 4.5.2 Parameters

The following experimental parameters were employed:

| Parameter | Betamethasone Valerate | Fluocinolone Acetonide |
|---|---|---|
| Mobile Phase (acetonitrile:water) | 40:60 | 26:74 |
| Flow Rate | 1,5ml/min | 1,5ml/min |
| Injection Volume | 20$\mu l$ | 20$\mu l$ |
| Detection Wavelength | 240nm | 240nm |



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The column was kept at ambient temperature and all solutions used for chromatography were filtered and degassed by bubbling helium gas through them.

### 4.5.3 Determination of Concentration

From the standard mixtures done with each HPLC run, standard curves were drawn of the ratio of the peak heights of the active ingredient (betamethasone valerate or fluocinolone acetonide respectively) over internal standard versus the concentration of the active ingredient for each of the standard preparations. The concentration of each assay sample was obtained by using the peak height ratio for the sample and reading off its concentration on the standard curve.

## 4.6 STABILITY INDICATING TEST

In order to show that the active glucocorticoids were indeed breaking down chemically when subjected to particular stressors and that the system could measure this degradation, 1mg fluocinolone acetonide was dissolved in 20ml water and heated in 5M sodium hydroxide at 80°C for one hour. 1ml Samples were removed every 5 minutes, neutralised with hydrochloric acid and after the addition of betamethasone (internal standard), immediately injected onto the chromatograph. A graph was then drawn of the ratio of peak heights of the glucocorticoid over the peak heights of the internal standard versus time. Since the peak height of the glucocorticoid was directly proportional to its concentration, a drop in the ratio over time would indicate a drop in concentration of the steroid. This would confirm that the glucocorticoid had been degrading and that degradation could be monitored by the HPLC method that was used.



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## 4.7 DATA ANALYSIS

An average degradation-time profile for each diluted preparation was obtained by drawing a graph of the average concentration of steroid remaining versus time. Using linear regression analysis on the log concentration (expressed as a percentage) against time data, the slope was calculated and used to determine the pseudo first order rate constant according to the following equation:

$$\log [GC] = \log[GC]_0 - \frac{k_1 t}{2,303}$$

where [GC] is the concentration of glucocorticoid at a particular time *t*, $[GC]_0$ is the concentration of the glucocorticoid at the time of dilution and t is the time that has elapsed since the preparation of the dilution. $-k_1 / 2,303$ is the slope of the straight line and $k_1$, the pseudo first order rate constant.

Similarly, the half life, $t_{½}$, being the time taken for the concentration of the glucocorticoid to become 50% of its original strength, was calculated using the formula:

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$$t_{½} = \frac{0,693}{k_1}$$

Also, the shelf life, i.e. the time taken for the concentration of the active ingredient to become 90% of its original strength, (i.e. 10% decomposition has occurred) was calculated using the formula:

$$t_{90} = \frac{0,105}{k_1}$$



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## 4.8 TESTING THE HYPOTHESIS

The hypothesis to be tested was the following: The chemical stability of both betamethasone valerate and fluocinolone acetonide ointments and creams diluted in cetomacrogol bases is greater than that obtained after dilution with emulsifying ointment and cream bases. In order to test this hypothesis, comparisons of the shelf lives ($t_{90}$) and rates of degradation ($k_1$) of the active ingredients were drawn between the proprietary products when diluted:

- in cetomacrogol bases and in emulsifying bases.
- in cream bases and in ointment bases.
- 1:10 and 50:50.




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# CHAPTER 5

# RESULTS AND DISCUSSION

## 5.1 HPLC ASSAY CHARACTERISTICS

### 5.1.1 Specificity

At the start of each set of HPLC experiments chromatograms were obtained using authentic samples of the active steroids and the internal standards concerned in order to confirm their respective retention times. An extract of the diluent base concerned was also chromatographed each time to check for interfering peaks. In the case of cetomacrogol and emulsifying creams which are preserved with chlorocresol, chlorocresol was also chromatographed each time to check its peak position.

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The retention time of betamethasone valerate and fluocinolone acetonide and their respective internal standards are shown in Table 1 and figures 12 & 13. The method proved to be very specific for betamethasone valerate and fluocinolone acetonide. The peaks of the two principle elements and their internal standards were well separated and the peaks were well defined and symmetrical. The retention times of these peaks correlated well with those obtained from authentic samples of each substance.

#### 5.1.1.1 Other Peaks

Chlorocresol eluted close to the peaks of fluocinolone acetonide and butyl 4-hydroxybenzoate but the peaks were individually still measurable.



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A small unidentified peak appeared at four minutes and six seconds in the extract of fluocinolone acetonide cream in cetomacrogol cream starting from day 66. Its peak height ratio increased over time indicating a slow increase in concentration of the substance over time.

Similarly, a small unidentified peak appeared at 4 minutes and 30 seconds in the extract of betamethasone valerate ointment in cetomacrogol ointment from day 20. Its peak height ratios also indicated an increase in concentration of the substance over time.

The extracts of betamethasone valerate in both cetomacrogol cream and emulsifying cream gave sharp and well-developed peaks at 1 minute and 30 seconds. The peak height ratios remained constant over time and thus did not indicate a change in the concentration of the substances causing the peaks.

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**5.1.2 Sensitivity, Accuracy, Reproducibility, Recovery, Stability Indicating**

The method was very sensitive for the active ingredients studied. It detected 0,03$\mu$g fluocinolone acetonide and 0,125$\mu$g betamethasone valerate (Table 1). Corresponding quantities required to be detected in 1:10 dilutions in order to accurately determine shelf life were 0,090$\mu$g fluocinolone acetonide and 0,36$\mu$g betamethasone valerate, respectively.

The method allowed for accurate measurements and good inter-assay reproducibility as reflected by the relative standard deviations for peak height ratio of steroid to internal standard calculated for a number of assays for both steroids (betamethasone valerate n=14, fluocinolone acetonide n=7 Table 1).

The diluted ointments and creams were prepared by mixing with a spatula on a glass slab. A separate experiment was consequently done in order to assess how well the preparations were mixed. If the active ingredients were evenly distributed



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throughout the preparations, sampling from different parts of the jar would have no influence on the outcome of the analyses.

Aliquots of preparation were sampled from four different parts of a jar of fluocinolone acetonide ointment diluted 50:50 with cetomacrogol ointment. These samples were analysed for fluocinolone acetonide content and the concentrations compared. Results (table 22) showed that the active ingredient was evenly distributed throughout the jar. The position in the jar at which samples of preparation were removed was thus not expected to be a variable in the study.

A recovery analysis was not done. Such an analysis would have given an indication of the suitability and efficiency of the extraction method employed. However, the extraction method used has proven efficiency[17, 34, 35]. The cream and ointment samples were first dispersed in hexane which assisted in removing most of the hydrocarbon fraction originating from the diluent bases and commercial preparations. This facilitated the extraction of the active ingredients with acetonitrile. In a separate experiment it was determined that betamethasone valerate and fluocinolone acetonide were soluble in acetonitrile and insoluble in hexane[footnote1]. Complete extraction could thus have been expected.

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Previous experiments have shown the ability of the method to indicate the chemical degradation of both betamethasone valerate and fluocinolone acetonide[8,16]. Confirmation that the method was stability indicating was shown by assaying fluocinolone acetonide extracts prior to and after drastic treatment of the extracts in an environment known to cause chemical breakdown of the molecule. The extracts were heated at 80°C in 5M sodium hydroxide. There was a progressive decrease in the concentration of fluocinolone acetonide over an hour of drastic treatment (Fig. 11).

---

[1] Fluocinolone acetonide soluble one in less than 183 of acetonitrile and insoluble one in more than 7683 of hexane. Betamethasone valerate soluble one in less than 33,3 of acetonitrile and insoluble one in more than 6850 of hexane.



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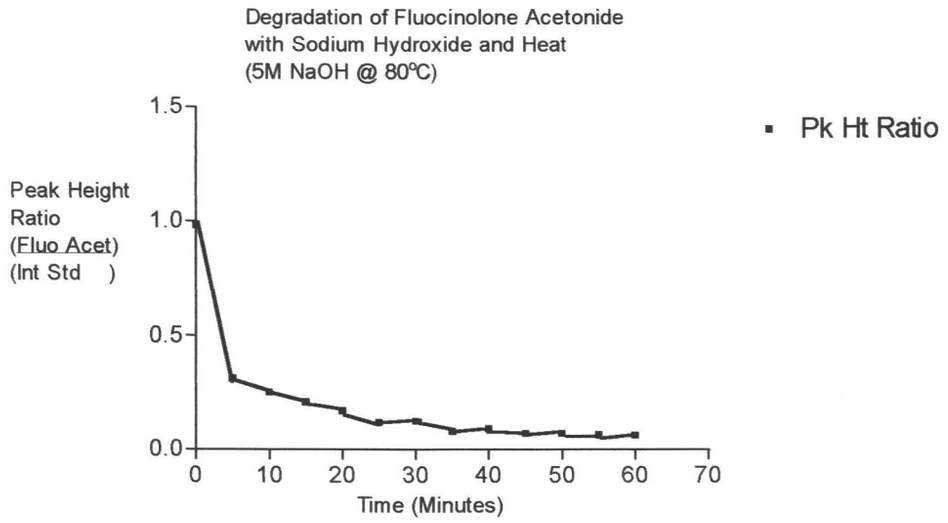


Fig.11 Graph Showing Change in Peak Height Ratios vs Time for Fluocinolone Acetonide in 5M NaOH at 80°C.

| | Betamethasone Valerate | Butyl 4-OH Benzoate | Fluocinolone Acetonide | Betamethasone |
|---|---|---|---|---|
| Retention Time (min.) | 4,00 | 2,54 | 5,15 | 3,55 |
| Detection Limits ($\mu$g) | 0,125 | | 0,03 | |
| Coefficient of Relative Variation | 12,5:87,5 dilution = ±5,89% lowest 3,24% highest 10,49% n=14<br><br>50:50 dilution =±8,27% lowest 3,61% highest 15,64% n=14 | | 12,5:87,5 dilution =±2,74% lowest 2,25% highest 3,22% n=7<br><br>50:50 dilution =±5,75% lowest 1,23% highest 10,26% n=7 | |

Table 1: Retention Times, Detection Limits and Coefficients of Relative Variation of Betamethasone Valerate and Fluocinolone Acetonide and their Internal Standards.



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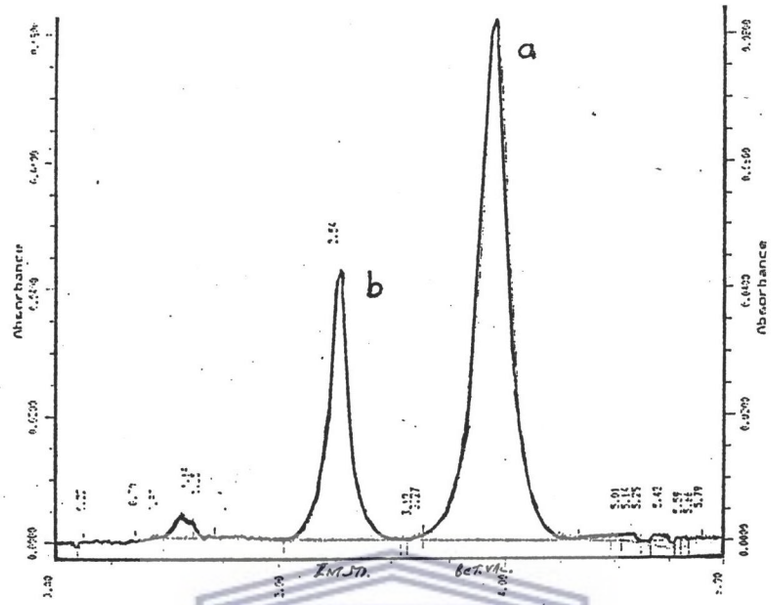


Fig. 12: Chromatogram showing a) Betamethasone valerate b) Butyl 4-hydroxybenzoate (Internal Standard) peaks.

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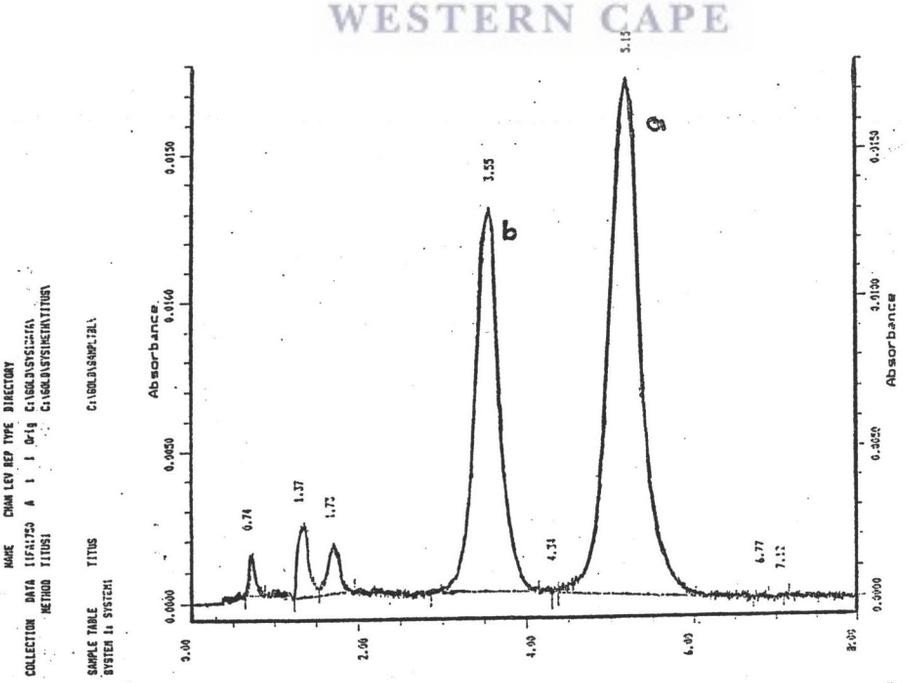


Fig. 13: Chromatogram showing a) Fluocinolone Acetonide and b) Betamethasone (Internal Standard) peaks.



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## 5.2 DEGRADATION ANALYSIS

The average degradation-time profiles of the betamethasone valerate and fluocinolone acetonide dilutions are shown in figures 14 (a) to (h) and 15 (i) to (p), respectively. They reflect semi-log concentration vs time data for the degradation of each of betamethasone valerate and fluocinolone acetonide 1:10 and 50:50 dilutions in emulsifying and cetomacrogol creams and ointments. The curves are linear regressions, the slopes of which give approximate indications of degradation rates.

The scatter of the points about the regression line is generally small with few exceptions. An assumption of 1st order kinetics appears thus to have been appropriate. However, in two cases each of both glucocorticoids, the average degradation curves bent towards the X-axis after insertion of the last data point.

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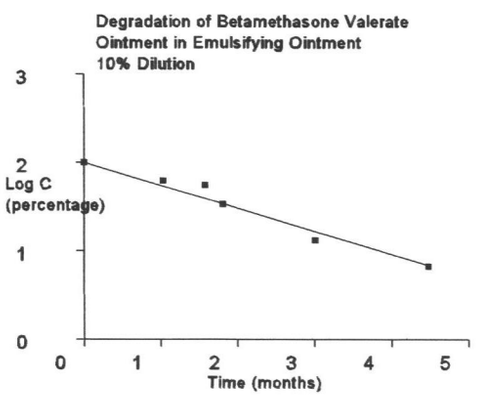
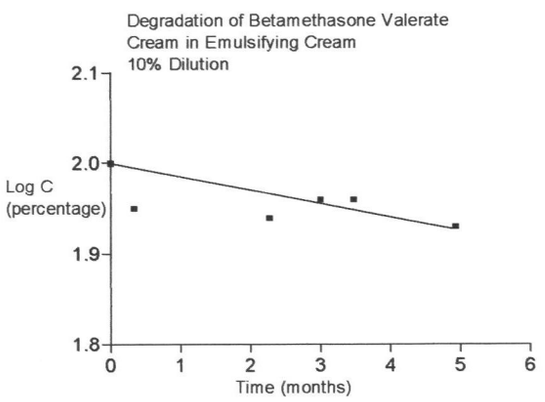
### 5.2.1 Betamethasone Valerate

The following are the average degradation profiles of the betamethasone valerate preparations:




(a)




(b)



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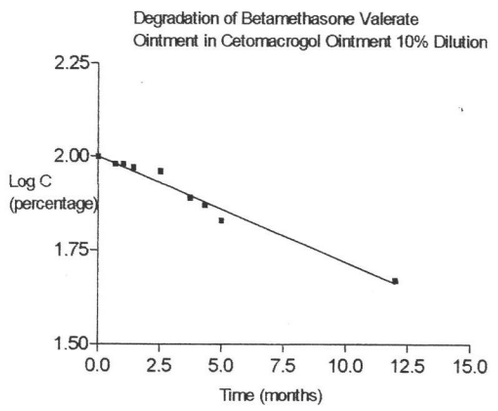
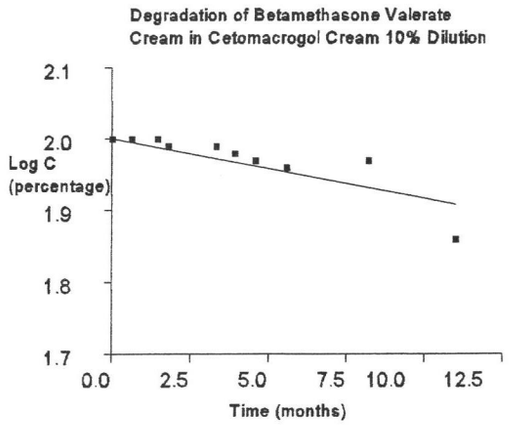
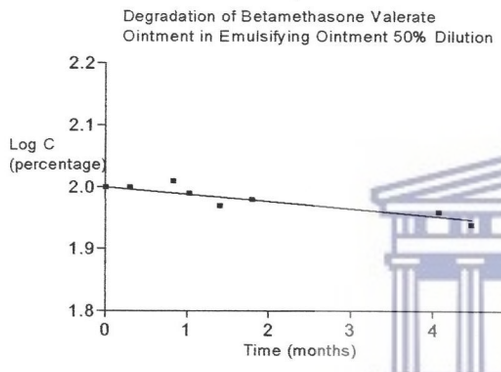


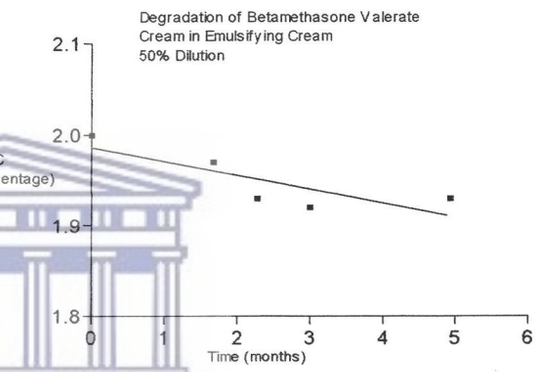

(c)




(d)




(e)




(f)

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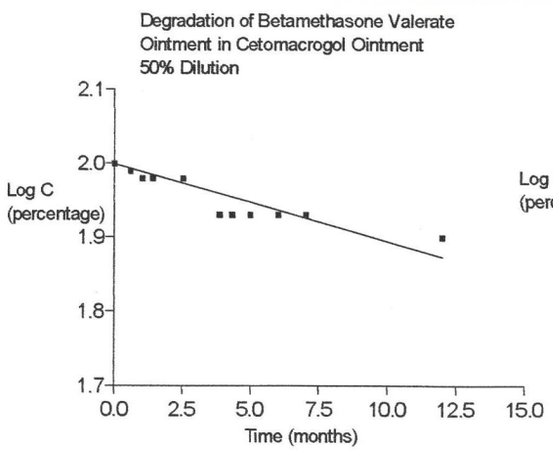
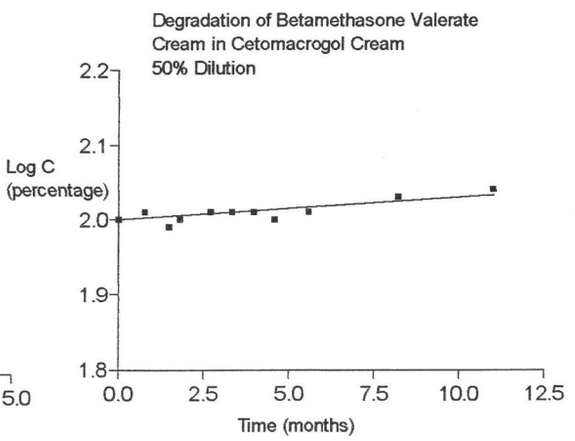



(g)




(h)

Fig. 14 Degradation - time profiles of the Betamethasone Valerate preparations



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The profiles of the dilutions in emulsifying base reflect data of up to 5 months while those dilutions in cetomacrogol base reflect data of up to twelve months. Clear trends can, however, be discerned. Betamethasone valerate creams diluted in cream bases appeared to be more stable than betamethasone valerate ointments diluted in ointment bases. The profiles also indicate that the cetomacrogol cream dilutions degraded more slowly than the emulsifying cream dilutions while the cetomacrogol ointment dilutions degraded more slowly than the emulsifying ointment dilutions. Furthermore, the 50:50 dilutions were generally more stable than the 1:10 dilutions.

Table 2 summarises the results of the statistical analysis comparing the different dilutions of the proprietary preparations of betamethasone valerate in the various bases.

| Betamethasone Valerate dilutions in: | Rate Constant ($k_1$) (months$^{-1}$) | | Half Life (months) | | Shelf Life (months) | |
|---|---|---|---|---|---|---|
| | 1:10 dilution | 50:50 dilution | 1:10 dilution | 50:50 dilution | 1:10 dilution | 50:50 dilution |
| Cetomacrogol Cream | $2,10x10^{-2}$ SD=$1,32x10^{-2}$ | no degrad. | 47,37 SD=30,98 | no degrad. | 7,18 SD=4,69 | no degrad. |
| Emulsifying Cream | $2,81x10^{-2}$ SD=$0,30x10^{-2}$ | $4,00x10^{-2}$ SD=$0,64x10^{-2}$ | 24,77 SD=2,64 | 17,57 SD=2,81 | 3,75 SD=0,40 | 2,66 SD=0,42 |
| Cetomacrogol Ointment | $7,60x10^{-2}$ SD=$3,14x10^{-2}$ | $2,09x10^{-2}$ SD=$0,67x10^{-2}$ | 10,81 SD=5,64 | 35,75 SD=10,96 | 1,64 SD=0,86 | 5,42 SD=1,66 |
| Emulsifying Ointment | $59,81x10^{-2}$ SD=NONE | $3,29x10^{-2}$ SD=$0,43x10^{-2}$ | 1,16 SD=NONE | 21,24 SD=2,81 | 0,18 SD=NONE | 3,22 SD=0,42 |

Table 2: Reaction Rate Constants, Half Lives and Shelf Lives of diluted preparations of Betamethasone Valerate.



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### 5.2.1.1 Comparison of Dilutions in Cetomacrogol vs Emulsifying Diluents

The cetomacrogol cream dilutions were found to be more stable than the emulsifying cream dilutions while the cetomacrogol ointment dilutions were more stable than the emulsifying ointment dilutions. This concurs with previous research[8,6,35] which showed cetomacrogol to be a suitable diluent while the betamethasone valerate molecule degraded to its less active analogue when proprietary samples of betamethasone valerate creams and ointments were mixed with emulsifying ointment and cream BP. They have shown this breakdown to be catalysed by the relatively higher alkalinity of the diluting bases.

Emulsifying cream and ointment BP have a pH of about 8,9[6]. This is most probably more basic than the proprietary corticosteroid ointment and cream preparations used in this investigation. The betamethasone 17-valerate molecule could therefore have undergone base-catalysed acyl migration. This chemical degradation was shown to occur via acyl migration in the presence of a basic medium where betamethasone 17-valerate rearranges to the less active betamethasone 21-valerate[7].

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The 21-monoester of betamethasone may also have undergone further hydrolysis to form the betamethasone alcohol.

There are, however, differences in the results of the current survey and those of other researchers. Ryatt *et al*[35] showed a more than 60% loss of betamethasone valerate concentration within 6 hours of diluting betamethasone valerate ointment with emulsifying ointment. This prompted their recommendations that betamethasone valerate preparations should not be diluted with emulsifying cream or ointment but suggested the use of cetomacrogol cream or ointment for that purpose.

The current study has also found the cetomacrogol diluents to be suitable for extemporaneous dilution of commercial betamethasone valerate preparations. Of



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these the 1:10 cetomacrogol ointment preparation had the shortest shelf life viz. 7,03 weeks while the 50:50 cetomacrogol cream dilution did not degrade during the period of investigation.

However, the current experiments showed shelf lives for the emulsifying cream and ointment preparations much longer than those found by the other researchers mentioned. The current experiments found a 1:10 dilution in emulsifying ointment to have a shelf life of 5,4 days compared to a 60% loss within 6 hours demonstrated by Ryatt *et al*. Likewise a 50:50 dilution used in the current survey had a half life of 21,24 months in comparison with 41 minutes found by Yip and Li Wan Po[6]. This seemingly contradictory findings are probably due to the changes in the formulae of both the sample of proprietary betamethasone valerate preparation and the emulsifying diluents used.

The above assumption can be clarified as follows. Smith *et al*[39] noted that "appreciable changes" had been made to the formulation of the proprietary preparation of betamethasone valerate in the United Kingdom, Betnovate®, in January 1982. This was confirmed by the manufacturer, Glaxo Pharmaceuticals who stated that the change had been made in order to improve certain characteristics of the product and to have the "same formulation as that available in overseas markets." All results thus published from experiments performed in the United Kingdom and in countries who drew their stock from there, had used the "old formula" Betnovate®. These results were also used to draw up the "External Diluent Directory" of the National Pharmaceutical Association in the United Kingdom which is commonly used as a guide to suitable diluents for proprietary corticosteroid preparations. Hence this directory advises that Aqueous Cream BP is unsuitable as a diluent for Betnovate® cream.

Using the "old formulation" Betnovate® preparations, Smith *et al* found results of dilution with emulsifying cream and ointment similar to researchers who had published their findings prior to January 1982, showing degradation within hours of mixing. However, upon using the "new" formulation, they found that the stability of a 25% dilution in emulsifying ointment had improved, giving more than 90%



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active ingredient remaining after 8 weeks.

Similarly, A.D. Magnus *et al*[36] conducted experiments on the degradation of Betovate® cream in aqueous cream BP and emulsifying ointment BP and other diluents respectively in South Africa in 1981. Presumably, the Betnovate® cream used was the "overseas markets" formulation to which the United Kingdom preparations had been changed, as referred to by the manufacturer. Accordingly, Magnus *et al* found these dilutions to be within label claim 14 months after dilution.

The "new formulation" of Betnovate® preparations is thus more stable after dilution with emulsifying diluents and reporting of chemical stabilities should stipulate the "vintage" of the product used.

The other probable reason for the improved stability of the proprietary preparations of betamethasone valerate cream and ointment in the current experiments could be the change in the formula of the emulsifying cream and ointment used by the Western Cape Department of Health hospital system. The Health department formulae each contain 35% less emulsifying wax and 61% more liquid paraffin than the BP formulae of these preparations. In both formulae the relative concentrations of white soft paraffin are virtually the same (difference = 3.3%) and Yip and Li Wan Po[6] have shown that a proprietary preparation of betamethasone valerate in white soft paraffin is very stable (half life of 471,2 days). It is therefore reasonable to conclude that the degradation of the betamethasone valerate preparation in emulsifying cream and ointment is due to the emulsifying wax content. It could therefore be expected that the betamethasone valerate cream and ointment preparations would be more stable in the health department formulations of emulsifying cream and ointment, as was discovered.

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### 5.2.1.2 Comparison of Cream vs Ointment Dilutions

Betamethasone valerate cream diluted with either cetomacrogol or emulsifying cream appeared to be more stable than betamethasone valerate ointment diluted with either cetomacrogol or emulsifying ointment. The only exception was the 50:50



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emulsifying cream dilution which had the shortest shelf life of the 50:50 dilution range and is out of step with what appears to be a pattern. This relatively greater stability of betamethasone valerate preparations in cream dilutions over ointment dilutions concurs with the findings of other research[8, 26].

### 5.2.1.3 Comparison of 1:10 vs 50:50 Dilutions

With the exception of the 50:50 dilution in emulsifying cream again, the results show a tendency for the 50:50 dilutions to be chemically more stable than the 1:10 dilutions. This is probably due to the fact that, whatever diluent ingredient has precipitated the degradation, such ingredient is present in greater concentration in the 1:10 preparations because they contain more base than in the 50:50 preparations. This concurs with the findings of other research[6]. If the assumption made above, that the degradation of the betamethasone valerate molecule in the emulsifying ointment dilution is due to the emulsifying wax content, then the decrease of concentration of the emulsifying wax in the 50:50 dilution gave rise to a more stable product. This improvement in stability also occurs in the cetomacrogol mixtures, albeit to a lesser degree (Table 2).

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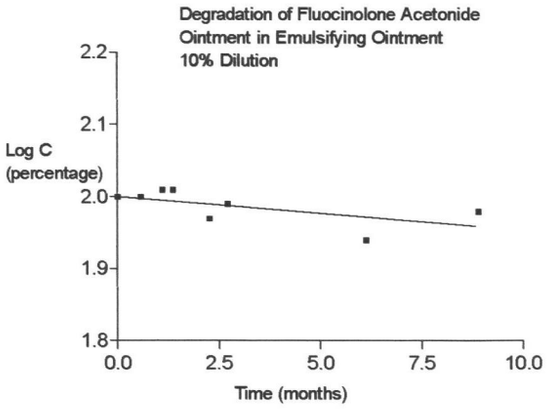
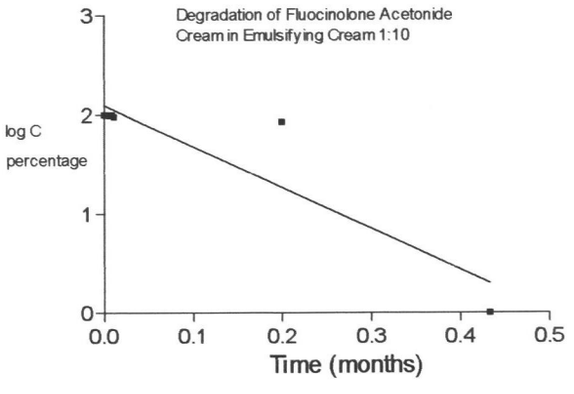
### 5.2.2 Fluocinolone Acetonide

The following are the average degradation profiles of the fluocinolone acetonide dilutions:




(i)




(j)



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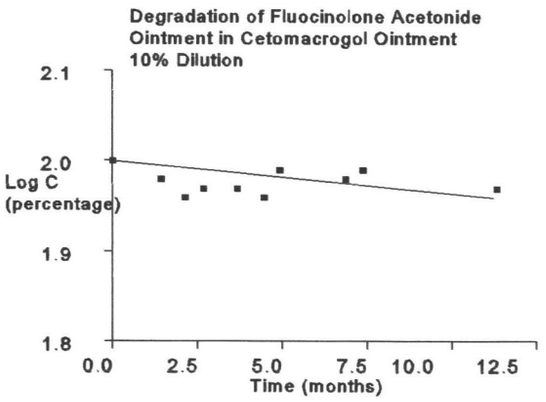
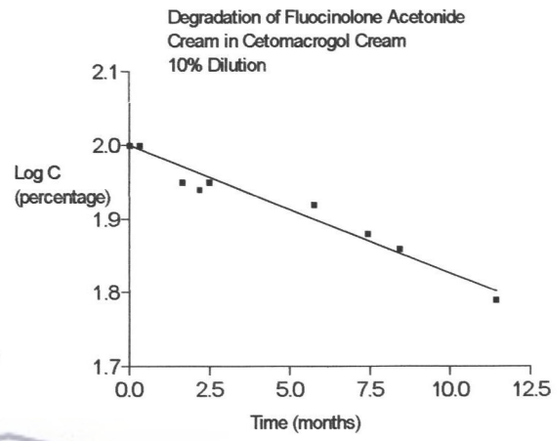



(k)




(l)

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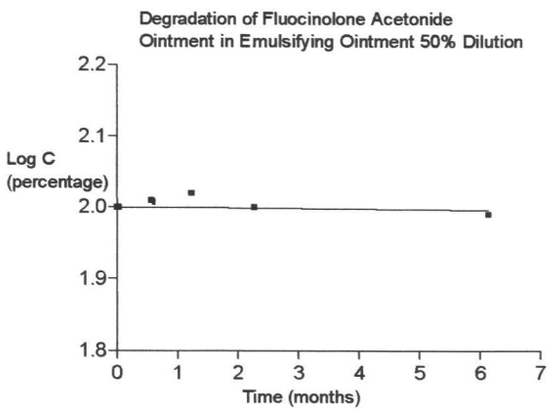


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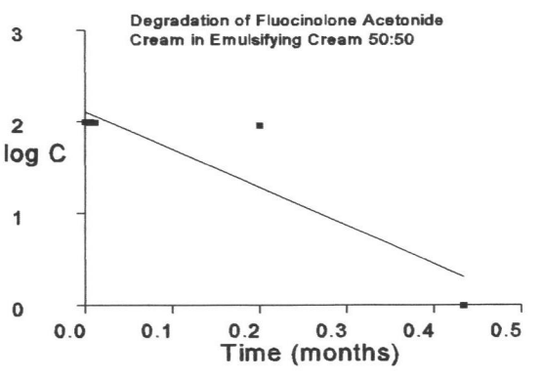


(n)



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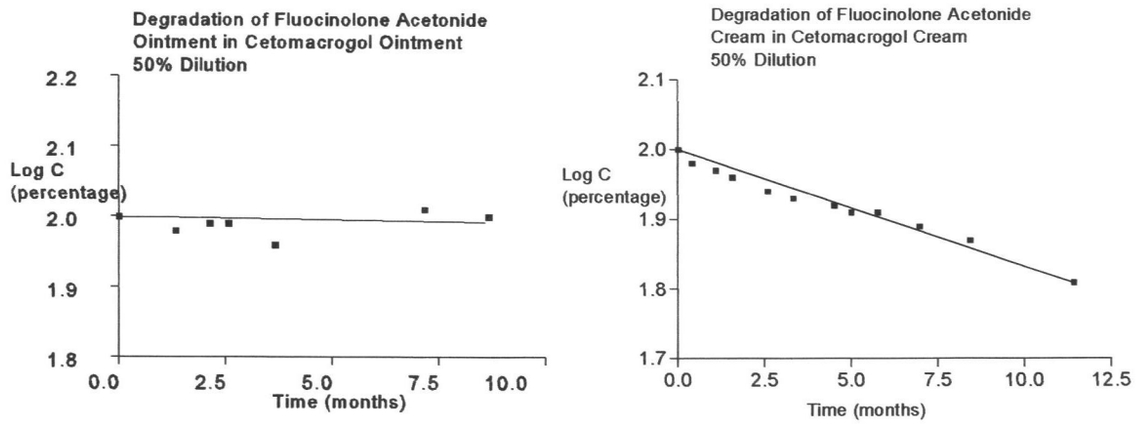


(o) (p)

Fig. 15 Average Degradation - Time profiles of Fluocinolone Acetonide preparations

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The profiles of the fluocinolone acetonide dilutions in the emulsifying bases reflect data of up to 6 months while those of the dilutions in cetomacrogol bases reflect data taken over 12 months. Again, clear trends could be discerned. Preparations of fluocinolone acetonide ointment diluted with ointment bases seemed to have longer shelf lives than the creams diluted with cream bases. Cetomacrogol cream dilutions appeared to be more stable than emulsifying cream dilutions and 50:50 dilutions tended to be more stable than 1:10 dilutions. Significantly, dilutions in emulsifying cream showed rapid degradation.

The average degradation curves of both the 1:10 and 50:50 dilution emulsifying cream preparations bent towards the X-axis after insertion of the last data point.



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Table 3 summarises the results of the statistical analysis comparing the different dilutions of the proprietary preparations of fluocinolone acetonide in various bases.

| Fluocinolone Acetonide dilutions in: | Rate Constant ($k_1$) ($months^{-1}$) | | Half Life (months) | | Shelf Life (months) | |
|---|---|---|---|---|---|---|
| | 1:10 dilution | 50:50 dilution | 1:10 dilution | 50:50 dilution | 1:10 dilution | 50:50 dilution |
| Cetomacrogol Cream | $4,02x10^{-2}$ SD=$0,29x10^{-2}$ | $3,87x10^{-2}$ SD=$1,88x10^{-2}$ | 17,29 SD=1,27 | 21,03 SD=9,98 | 2,62 SD=0,19 | 3,19 SD=1,52 |
| Emulsifying Cream | $954 \times 10^{-2}$ SD=$5,66x10_{-2}$ | $963x10^{-2}$ SD=$26,54x10^{-2}$ | 0.070 SD=0,0007 | 0,072 SD=0,001 | 0,0111 SD=0 | 0,0110 SD=0 |
| Cetomacrogol Ointment | $2,07x10^{-2}$ SD=$0,95x10^{-2}$ | no degradation | 41,40 SD=25,77 | no degradation | 6,27 SD=3,91 | no degradation |
| Emulsifying Ointment | $2,50x10^{-2}$ SD=$2,29x10^{-2}$ | $0,69x10^{-2}$ SD=$0,19x10^{-2}$ | 47,95 SD=44,00 | 104,92 SD=25,29 | 7,27 SD=6,67 | 15,90 SD=3,83 |

Table 3: Reaction Rate Constants, Half Lives and Shelf Lives of diluted preparations of Fluocinolone Acetonide.

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### 5.2.2.1 Comparison of Creams vs Ointments

The results summarised in Table 3 indicate that the proprietary fluocinolone acetonide preparations were more stable when diluted in the ointment bases than in the cream base. The ointment formulations showed shelf lives upward of six months while the cream formulations had shelf lives below 3,2 months. The shelf lives of the emulsifying cream formulations were as low as 8 hours. This phenomenon would seem to agree with the findings of Kenley *et al*[16] that fluocinolone acetonide degradation in cream samples is confined to an aqueous environment that is largely unperturbed by the non-aqueous constituents of the cream base. The ointment bases used in the experiments were entirely anhydrous



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while the cream bases consisted of 70% water.

**5.2.2.2 Comparison of 1:10 vs 50:50 Dilutions**

Generally the 50:50 ointment dilutions were more stable than the 1:10 ointment dilutions. In the case of the cetomacrogol ointment mixture, the 50:50 dilution showed no degradation during the period of the investigation while the 1:10 dilution had a shelf life of 6,27 months. The 50:50 emulsifying ointment dilution had a shelf life longer than twice that of its 1:10 counterpart. Again, this is expected since the possible degradation causing agents in the ointment base would be present in lower concentration in the 50:50 dilutions than in the 1:10 dilutions.

In the case of the cream dilutions, the difference in the rates of degradation between the 50:50 and 1:10 dilutions was slight. The 50:50 cetomacrogol cream dilution was barely more stable than the 1:10 dilution (3,19 months vs 2,62 months) while both the emulsifying cream dilutions proved to be very unstable, having virtually the same short shelf life (8 hours).

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If this degradation in the cream dilutions were due to water catalysed hydrolysis, then the similarity in the degradation profiles of the 1:10 and 50:50 dilutions is probably due to the fact that, at those amounts of water present, the concentration of water molecules can be taken to be the same. The reaction rate of the hydrolysis would thus be independent of the amount of water present.

**5.2.2.3 Comparison of Dilutions in Cetomacrogol and Emulsifying Diluents**

As shown in chapter 2.3.1 fluocinolone acetonide may be degraded to the C-17 etianic acid analogue in acid or alkaline medium. Emulsifying ointment and cream have a more alkaline pH than the cetomacrogol emulsifying bases[6]. Such breakdown was thus expected to occur more in the former than in the latter systems. Results show this to be generally true.

The 50:50 cetomacrogol ointment dilution did not degrade over the period of



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investigation while the same dilution in emulsifying ointment had a shelf life of 15,9 months. Similarly, the shelf lives of 2,63 months and 3,19 months for the 1:10 and 50:50 cetomacrogol cream dilutions respectively, contrast sharply with the almost 8 hours apiece for both the emulsifying cream dilutions. An exception to this trend was that the 1:10 dilution in emulsifying ointment had a shelf life one month longer than its cetomacrogol ointment counterpart.

Fluocinolone acetonide preparations were thus generally more stable in the cetomacrogol diluents than in the emulsifying diluents. It is, however, noteworthy that the 50:50 emulsifying ointment dilution is more than twice as stable than the 1:10 cetomacrogol ointment dilution.

| **Betamethasone Valerate Preparations** | Shelf Life (months) in Cetomacrogol Diluents | Shelf Life (months) in Emulsifying Diluents |
|---|---|---|
| 1:10 Cream Dilution | 7,18 | 3,75 |
| 50:50 Cream Dilution | no degradation | 2,66 |
| 1:10 Ointment Dilution | 1,64 | 0,18 |
| 50:50 Ointment dilution | 5,42 | 3,22 |
| **Fluocinolone Acetonide Preparations** | | |
| 1:10 Cream Dilution | 2.62 | 0,0111 |
| 50:50 Cream Dilution | 3.19 | 0,0110 |
| 1:10 Ointment Dilution | 6,27 | 7.27 |
| 50:50 Ointment Dilution | no degradation | 15,90 |

Table 4: Shelf Lives ($t_{90}$) of Betamethasone Valerate and Fluocinolone Acetonide Preparations in Cetomacrogol and Emulsifying Diluents.

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# CHAPTER 6

## CONCLUSION

The objective of this investigation was to determine the chemical stability of proprietary brands of the glucocorticoid steroids betamethasone valerate and fluocinolone acetonide cream and ointment preparations after dilution with cetomacrogol and emulsifying cream and ointment bases respectively.

Noting the ongoing common practice of prescribers of medication to request the dilution of these branded products in the aforementioned bases, the study further aimed at using the data generated by the experiments performed to assign shelf lives to these dilutions.

Previous research showed that emulsifying cream and ointment BP bases caused rapid degradation of betamethasone valerate products while cetomacrogol bases supplied a more stable environment for dilution. Despite a slight modification of the emulsifying bases tested, structural molecular similarities between betamethasone valerate and fluocinolone acetonide thus prompted the hypothesis that similar outcomes would be obtained when the two branded products were diluted with the specific diluents.

In order to achieve these objectives, diluted samples of these steroid products were prepared and stored at ambient temperature. Aliquots withdrawn from these preparations at specified intervals over a period of up to a year, were assayed by High Performance Liquid Chromatography for remaining glucocorticoid. The data obtained were then used to calculate shelf lives for each of the diluted products.

The results obtained partly supported the hypothesis that the dilutions of preparations of both the active ingredients in cetomacrogol bases would be more stable than those in emulsifying bases. The following conclusions could be drawn from these results.



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With reference to the particular branded samples of betamethasone valerate and fluocinolone acetonide used in the investigation and the cetomacrogol and emulsifying cream and ointment bases manufactured by the Western Cape health department hospital system in South Africa:

1. In general, the branded preparations of glucocorticoids were more stable in cetomacrogol cream than in emulsifying cream and more stable in cetomacrogol ointment than in emulsifying ointment. The hypothesis was, however, not always upheld when comparing cetomacrogol cream with emulsifying ointment and cetomacrogol ointment with emulsifying cream dilutions.

2. Betamethasone valerate cream dilutions were found to be more stable than ointment dilutions. Conversely, fluocinolone acetonide ointment dilutions were more stable than cream dilutions.

3. In general, a decrease in the concentration of the degradative environment, resulted in increased stability of the glucocorticoid molecule i.e. the 50:50 dilutions were generally more stable than the 1:10 dilutions.

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4. A reduction in the emulsifying wax content of the emulsifying cream and ointment BP was associated with a more stable environment for the dilution of betamethasone valerate *cream* and fluocinolone acetonide *ointment* preparations respectively.

When dilutions are made in the bases under investigation, due regard should be had for the shelf lives as calculated. The diluted preparations made by the health department can now be assigned shelf lives as indicated by Table 4. We recommend that 1:10 dilutions of betamethasone valerate should not be made using emulsifying ointment. Likewise should fluocinolone acetonide products not be diluted by any degree with emulsifying cream.



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## General

The cost of medicines is a cardinal consideration with regard to the access of the population to essential drugs. Since it has been shown that reduced concentration does not always mean reduced potency[27, 30] and in order to avoid the sometimes deleterious effect occurring with extemporaneous dilution of the branded products, it is recommended that pharmaceutical companies make available suitably diluted betamethasone valerate and fluocinolone acetonide preparations at concomitantly reduced prices.

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# TABLES

| Betamethasone Valerate Cream in Cetomacrogol Cream 1:10 Dilution | | | | | | | |
|---|---|---|---|---|---|---|---|
| Name | Time | Peak Heights | | | Concentration Remaining | | |
| Sample 1 | Days | Steroid (a) | Int. Std. (b) | Ratio (a/b) | g/100g | Percentage | Log % |
| VBV82 | 0 | 41,00 | 84,0 | 0,49 | 0,0103421 | 100 | 2.00 |
| VBV30A | 100 | 12,90 | 8,05 | 1,60 | 0,0101929 | 99 | 2.00 |
| VBV53A | 137 | 12,35 | 8,10 | 1,52 | 0,0095570 | 92 | 1.96 |
| VBV104A | 167 | 12,50 | 8,20 | 1,52 | 0,0095570 | 92 | 1.96 |
| VBV152A | 246 | 12,95 | 8,40 | 1,54 | 0,0096983 | 94 | 1.97 |
| Sample 2 | | | | | | | |
| VBW21A | 0 | 2,80 | 5,90 | 0,47 | 0,0118920 | 100 | 2.00 |
| VBW63A | 54 | 2,70 | 6,65 | 0,41 | 0,0105566 | 89 | 1.95 |
| VBW114A | 100 | 2,80 | 6,10 | 0,46 | 0,0116694 | 98 | 1.99 |
| VBW138A | 137 | 2,80 | 6,40 | 0,44 | 0,0112243 | 94 | 1.97 |
| Sample 3 | | | | | | | |
| VBX43A | 0 | 3,40 | 7,90 | 0,43 | 0,0106349 | 100 | 2.00 |
| VBX95A | 44 | 3,60 | 7,70 | 0,47 | 0,0115871 | 109 | 2.04 |
| VBX143A | 118 | 3,55 | 8,30 | 0,43 | 0,0106349 | 100 | 2.00 |
| VBX177A | 330 | 3,15 | 6,95 | 0,45 | 0,0080629 | 76 | 1.88 |
| Sample 4 | | | | | | | |
| VBY47A | 0 | 2,55 | 5,50 | 0,46 | 0,0123039 | 100 | 2.00 |
| VBY70A | 19 | 2,55 | 5,85 | 0,44 | 0,0118490 | 96 | 1.98 |
| VBY100A | 44 | 2,50 | 5,70 | 0,44 | 0,0118490 | 96 | 1.98 |
| VBY148A | 118 | 2,50 | 6,00 | 0,42 | 0,0113941 | 93 | 1.97 |
| VBY178A | 330 | 2,55 | 5,50 | 0,46 | 0,0083304 | 68 | 1.83 |

Table 6 : Sampling Time, Peak Height and Concentration of Corticosteroid Remaining for Betamethasone Valerate Cream in Cetomacrogol Cream 1:10 Dilution

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| Betamethasone Valerate Ointment in Cetomacrogol Ointment 1:10 Dilution | | | | | | | |
|---|---|---|---|---|---|---|---|
| Name | Time | Peak Heights | | | Concentration Remaining | | |
| Sample 1 | Days | Steroid (a) | Int. Std. (b) | Ratio (a/b) | g/100g | Percentage | Log % |
| VIBV1 | 0 | 39,50 | 87,50 | 0,45 | 0,0105476 | 100 | 2.00 |
| VIBV20A | 76 | 5,60 | 12,10 | 0,46 | 0,0089282 | 85 | 1.93 |
| VIBV62A | 130 | 5,50 | 14,10 | 0,39 | 0,0072998 | 69 | 1.84 |
| VIBV89A | 150 | 4,70 | 14,00 | 0,34 | 0,0061366 | 58 | 1.76 |
| Sample 2 | | | | | | | |
| VIBW31A | 0 | 9,50 | 14,10 | 0,67 | 0,0147765 | 100 | 2.00 |
| VIBW54A | 31 | 9,20 | 14,30 | 0,64 | 0,0138575 | 94 | 1.97 |
| VIBW105A | 76 | 8,55 | 14,40 | 0,59 | 0,0127087 | 86 | 1.93 |
| VIBW131A | 112 | 6,80 | 14,10 | 0,48 | 0,0101814 | 69 | 1.84 |
| VIBW153A | 150 | 6,00 | 14,00 | 0,43 | 0,0090326 | 61 | 1.79 |
| Sample 3 | | | | | | | |
| VIBX44A | 0 | 6,75 | 14,05 | 0,48 | 0,0099768 | 100 | 2.00 |
| VIBX96A | 43 | 6,65 | 14,00 | 0,48 | 0,0099768 | 100 | 2.00 |
| VIBX120A | 76 | 6,70 | 13,80 | 0,49 | 0,0102025 | 102 | 2.01 |
| VIBX144A | 112 | 5,65 | 13,95 | 0,41 | 0,0086224 | 86 | 1.93 |
| Sample 4 | | | | | | | |
| VIBY48A | 0 | 6,44 | 13,35 | 0,48 | 0,0097262 | 100 | 2.00 |
| VIBY71A | 21 | 5,95 | 13,60 | 0,44 | 0,0090527 | 93 | 1.97 |
| VIBY125A | 76 | 6,20 | 13,60 | 0,46 | 0,0092772 | 95 | 1.98 |
| VIBY149A | 112 | 5,45 | 13,65 | 0,40 | 0,0079302 | 82 | 1.91 |
| VIBY182A | 360 | 4,55 | 14,05 | 0,32 | 0,0045845 | 47 | 1.67 |

Table 7: Sampling Time, Peak Height and Concentration of Corticosteroid Remaining for Betamethasone Valerate Ointment in Cetomacrogol Ointment 1:10 Dilution



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| Betamethasone Valerate Cream in Emulsifying Cream 1:10 Dilution | | | | | | | |
|---|---|---|---|---|---|---|---|
| Name | Time | Peak Heights | | | Concentration Remaining | | |
| Sample 1 | Days | Steroid (a) | Int. Std. (b) | Ratio (a/b) | g/100g | Percentage | Log % |
| VIIBV26A | 0 | 3,60 | 8,10 | 0,44 | 0,0110326 | 100 | 2.00 |
| VIIBV32A | 10 | 3,30 | 6,95 | 0,47 | 0,0095682 | 87 | 1.94 |
| VIIBV106A | 68 | 3,50 | 7,45 | 0,47 | 0,0095682 | 87 | 1.94 |
| VIIBV129A | 104 | 3,35 | 6,55 | 0,51 | 0,0104371 | 95 | 1.98 |
| VIIBV154A | 148 | 3,35 | 7,40 | 0,45 | 0,0091337 | 83 | 1.92 |
| Sample 2 | | | | | | | |
| VIIBW85A | 0 | 3,45 | 7,70 | 0,45 | 0,0088939 | 100 | 2.00 |
| VIIBW99A | 10 | 3,60 | 8,50 | 0,42 | 0,0082386 | 93 | 1.97 |
| VIIBW147A | 90 | 3,65 | 8,95 | 0,41 | 0,0080202 | 90 | 1.95 |

Table 8: Sampling Time, Peak Height and Concentration of Corticosteroid Remaining for Betamethasone Valerate Cream in Emulsifying Cream 1:10 Dilution

| Betamethasone Valerate Ointment in Emulsifying Ointment 1:10 Dilution | | | | | | | |
|---|---|---|---|---|---|---|---|
| Name | Time | Peak Heights | | | Concentration Remaining | | |
| Sample 1 | Days | Steroid (a) | Int. Std. (b) | Ratio (a/b) | g/100g | Percentage | Log % |
| VIIIB35A | 0 | 6,45 | 13,75 | 0,47 | 0,0085592 | 100 | 2.00 |
| VIIIB58A | 31 | 4,65 | 14,05 | 0,33 | 0,0053509 | 62 | 1.79 |
| VIIIB83A | 47 | 4,30 | 13,85 | 0,31 | 0,0048574 | 57 | 1.76 |
| VIII107A | 54 | 3,15 | 14,05 | 0,22 | 0,0028830 | 34 | 1.53 |
| VIII130A | 90 | 2,20 | 13,95 | 0,16 | 0,0011555 | 14 | 1.15 |
| VIII155A | 134 | 1,40 | 13,90 | 0,10 | 0,0005721 | 7 | 0.85 |

Table 9: Sampling Time, Peak Height and Concentration of Corticosteroid Remaining for Betamethasone Valerate Ointment in Emulsifying Ointment 1:10 Dilution



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| Betamethasone Valerate Cream in Cetomacrogol Cream 50:50 Dilution | | | | | | | |
|---|---|---|---|---|---|---|---|
| Name | Time | Peak Heights | | | Concentration Remaining | | |
| Sample 1 | Days | Steroid (a) | Int. Std (b) | Ratio (a/b) | g/100g | Percentage | Log % |
| VBV82 | 0 | 217 | 89 | 2,44 | 0,0516676 | 100 | 2.00 |
| VBV30B | 100 | 14,20 | 1,80 | 7,89 | 0,0545608 | 106 | 2.03 |
| VBV53B | 137 | 14,20 | 1,90 | 7,47 | 0,0515935 | 100 | 2.00 |
| VBV104B | 167 | 14,20 | 1,90 | 7,47 | 0,0515935 | 100 | 2.00 |
| VBV152B | 246 | 14,20 | 1,80 | 7,89 | 0,0545608 | 106 | 2.03 |
| Sample 2 | | | | | | | |
| VBW21B | 0 | 4,20 | 1,80 | 2,33 | 0,0532879 | 100 | 2.00 |
| VBW63B | 54 | 4,50 | 2,10 | 2,14 | 0,0490593 | 92 | 1.96 |
| VBW114B | 100 | 4,35 | 1,85 | 2,35 | 0,0537330 | 101 | 2.00 |
| VBW138B | 137 | 4,30 | 1,95 | 2,21 | 0,0506172 | 95 | 1.98 |
| Sample 3 | | | | | | | |
| VBX43B | 0 | 4,10 | 1,75 | 2,34 | 0,0561010 | 100 | 2.00 |
| VBX66B | 23 | 4,20 | 1,60 | 2,62 | 0,0627661 | 112 | 2.05 |
| VBX95B | 44 | 4,25 | 1,80 | 2,36 | 0,0565770 | 101 | 2.00 |
| VBX119B | 81 | 4,30 | 1,70 | 2,53 | 0,0606238 | 108 | 2.03 |
| VBX177B | 330 | 4,00 | 1,65 | 2,42 | 0,0607729 | 108 | 2.03 |
| Sample 4 | | | | | | | |
| VBY74B | 0 | 3,75 | 1,75 | 2,14 | 0,0505159 | 100 | 2.00 |
| VBY70B | 23 | 3,85 | 1,90 | 2,03 | 0,0480139 | 95 | 1.98 |
| VBY100B | 44 | 3,80 | 1,85 | 2,05 | 0,0484688 | 96 | 1.98 |
| VBY123B | 81 | 3,95 | 1,80 | 2,19 | 0,0516531 | 102 | 2.01 |
| VBY148B | 119 | 3,80 | 1,85 | 2,05 | 0,0484688 | 96 | 1.98 |
| VBY178B | 330 | 3,75 | 1,70 | 2,21 | 0,0551541 | 109 | 2.04 |

Table 10: Sampling Time, Peak Height and Concentration of Corticosteroid Remaining for Betamethasone Valerate Cream in Cetomacrogol Cream 50:50 Dilution



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| Betamethasone Valerate Ointment in Cetomacrogol Ointment 50:50 Dilution | | | | | | | |
|---|---|---|---|---|---|---|---|
| Name | Time | Peak Heights | | | Concentration Remaining | | |
| Sample 1 | Days | Steroid (a) | Int. Std. (b) | Ratio (a/b) | g/100g | Percentage | Log % |
| VIBV2 | 0 | 95,00 | 43,50 | 2,10 | 0,0508937 | 100 | 2.00 |
| VIBV20B | 76 | 13,15 | 6,00 | 2,19 | 0,0494074 | 97 | 1.99 |
| VIBV62B | 130 | 13,50 | 7,40 | 1,82 | 0,0421956 | 83 | 1.92 |
| VIBV113B | 181 | 12,80 | 6,50 | 1,97 | 0,0440567 | 87 | 1.94 |
| VIBV137B | 211 | 14,10 | 7,10 | 1,99 | 0,0445220 | 88 | 1.94 |
| Sample 2 | | | | | | | |
| VIBW31B | 0 | 14,20 | 6,80 | 2,18 | 0,0492402 | 100 | 2.00 |
| VIBW54B | 31 | 14,30 | 7,20 | 1,99 | 0,0448748 | 91 | 1.96 |
| VIBW105B | 76 | 14,00 | 7,10 | 1,97 | 0,0444153 | 90 | 1.95 |
| VIBW153B | 150 | 14,00 | 7,05 | 1,99 | 0,0448748 | 91 | 1.96 |
| Sample 3 | | | | | | | |
| VIBX44B | 0 | 14,00 | 6,55 | 2,14 | 0,0474469 | 100 | 2.00 |
| VIBX96B | 43 | 14,05 | 6,55 | 2,15 | 0,0476727 | 101 | 2.00 |
| VIBX120B | 76 | 14,05 | 6,40 | 2,20 | 0,0488013 | 103 | 2.01 |
| VIBX144B | 116 | 13,95 | 7,70 | 1,81 | 0,0399980 | 84 | 1.92 |
| VIBX181B | 360 | 14,10 | 9,75 | 1,45 | 0,0348192 | 73 | 1.86 |
| Sample 4 | | | | | | | |
| VIBY48B | 0 | 13,90 | 6,45 | 2,16 | 0,0474426 | 100 | 2.00 |
| VIBY71B | 18 | 13,85 | 7,10 | 1,95 | 0,0434015 | 92 | 1.92 |
| VIBY101B | 43 | 13,85 | 6,95 | 1,99 | 0,0445240 | 94 | 1.97 |
| VIBY125B | 76 | 14,00 | 6,90 | 2,03 | 0,0445240 | 94 | 1.97 |
| VIBY149B | 116 | 13,90 | 7,95 | 1,75 | 0,0384625 | 81 | 1.91 |
| VIBY182B | 360 | 14,05 | 9,15 | 1,54 | 0,0372273 | 79 | 1.90 |

Table 11: Sampling Time, Peak Height and Concentration of Corticosteroid Remaining for Betamethasone Valerate Ointment in Cetomacrogol Ointment 50:50 Dilution



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| Betamethasone Valerate Cream in Emulsifying Cream 50:50 Dilution | | | | | | | |
|---|---|---|---|---|---|---|---|
| Name | Time | Peak Heights | | | Concentration Remaining | | |
| Sample 1 | Days | Steroid (a) | Int. Std. (b) | Ratio (a/b) | g/100g | Percentage | Log % |
| VIIBV26 | 0 | 4,15 | 1,65 | 2,52 | 0,0630045 | 100 | 2.00 |
| VIIBV32 | 10 | 4,50 | 1,80 | 2,50 | 0,0536672 | 85 | 1.93 |
| VIIBV82 | 50 | 5,20 | 1,75 | 2,97 | 0,0638774 | 101 | 2.00 |
| VIIBV106 | 68 | 4,60 | 1,85 | 2,49 | 0,0534500 | 85 | 1.93 |
| VIIBV154 | 148 | 4,55 | 1,80 | 2,53 | 0,0543190 | 86 | 1.93 |
| Sample 2 | | | | | | | |
| VIIBW85 | 0 | 3,85 | 1,60 | 2,41 | 0,0517071 | 100 | 2.00 |
| VIIBW99 | 10 | 3,8 | 1,90 | 2,00 | 0,0427513 | 83 | 1.92 |
| VIIBW124 | 50 | 4,00 | 1,90 | 2,11 | 0,0451541 | 87 | 1.94 |
| VIIBW147 | 90 | 3,95 | 2,00 | 1,98 | 0,0423144 | 82 | 1.91 |

Table 12: Sampling Time, Peak Height and Concentration of Corticosteroid Remaining for Betamethasone Valerate Cream in Emulsifying Cream 50:50 Dilution

| Betamethasone Valerate Ointment in Emulsifying Ointment 50:50 Dilution | | | | | | | |
|---|---|---|---|---|---|---|---|
| Name | Time | Peak Heights | | | Concentration Remaining | | |
| Sample 1 | Days | Steroid (a) | Int. Std. (b) | Ratio (a/b) | g/100g | Percentage | Log % |
| VIIIBV35 | 0 | 14,05 | 6,65 | 2,11 | 0,0490332 | 100 | 2.00 |
| VIIIBV58 | 31 | 14,10 | 6,95 | 2,03 | 0,0470589 | 96 | 1.98 |
| VIIIBV107 | 54 | 14,10 | 7,10 | 1,99 | 0,0460717 | 94 | 1.97 |
| VIIIBV155 | 134 | 14,15 | 7,60 | 1,86 | 0,0428634 | 87 | 1.94 |
| Sample 2 | | | | | | | |
| VIIIBW49 | 0 | 13,90 | 7,25 | 1,92 | 0,0465373 | 100 | 2.00 |
| VIIIBW59 | 9 | 13,85 | 7,00 | 1,98 | 0,0479793 | 103 | 2.01 |
| VIIIBW84 | 25 | 14,00 | 6,90 | 2,03 | 0,0491811 | 106 | 2.03 |
| VIIIBW108 | 42 | 14,00 | 7,95 | 1,76 | 0,0426917 | 92 | 1.96 |
| VIIIBW156 | 122 | 14,00 | 8,15 | 1,72 | 0,0417303 | 90 | 1.95 |

Table 13: Sampling Time, Peak Height and Concentration of Corticosteroid Remaining for Betamethasone Valerate Ointment in Emulsifying Ointment 50:50 Dilution



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| Fluocinolone Acetonide Cream in Cetomacrogol Cream 1:10 Dilution | | | | | | | |
|---|---|---|---|---|---|---|---|
| Name | Time | Peak Height | | | Concentration Remaining | | |
| Sample 1 | Days | Steroid (a) | Int. Std. (b) | Ratio (a/b) | g/100g | Percentage | Log % |
| IFA781 | 0 | 77 | 42 | 1,83 | 0,0023737 | 100 | 2.00 |
| IFA861 | 10 | 11,50 | 121,00 | 0,10 | 0,0024134 | 102 | 2.01 |
| IFA3 | 66 | 0,90 | 4,40 | 0,20 | 0,0023169 | 98 | 1.99 |
| IFA16 | 75 | 303,00 | 1430,00 | 0,21 | 0,0024043 | 101 | 2.00 |
| IFA102 | 173 | 1,10 | 5,80 | 0,19 | 0,0022294 | 94 | 1.97 |
| IFA163 | 343 | 5,00 | 44,00 | 0,11 | 0,0014803 | 62 | 1.79 |
| Sample 2 | | | | | | | |
| IFC41 | 0 | 372,00 | 1131,00 | 0,33 | 0,0043060 | 100 | 2.00 |
| IFC93 | 50 | 328,00 | 1243,00 | 0,26 | 0,0033002 | 77 | 1.89 |
| IFC117 | 75 | 301,00 | 1161,00 | 0,26 | 0,0033002 | 77 | 1.89 |
| IFC161 | 223 | 5,00 | 24,00 | 0,21 | 0,0029303 | 68 | 1.83 |
| IFC173 | 253 | 7,00 | 47,00 | 0,15 | 0,0028967 | 67 | 1.83 |

Table 14: Sampling Time, Peak Height and Concentration of Corticosteroid Remaining for Fluocinolone Acetonide Cream in Cetomacrogol Cream 1:10 Dilution

| Fluocinolone Acetonide Cream in Emulsifying Cream 1:10 Dilution | | | | | | | |
|---|---|---|---|---|---|---|---|
| Name | Time | Peak Height | | | Concentration Remaining | | |
| Sample 1 | Days | Steroid (a) | Int. Std. (b) | Ratio (a/b) | g/100g | Percentage | Log % |
| IIIFA901A | 0 | 9,00 | 13,00 | 0,69 | 0,00262170 | 100 | 2.00 |
| IIIFA902A | 2hrs | 10,50 | 15,00 | 0,70 | 0,00266392 | 102 | 2.01 |
| IIIFA903A | 4hrs | 10,00 | 15,00 | 0,67 | 0,00253712 | 97 | 1.99 |
| IIIFA904A | 6hrs | 11,00 | 15,50 | 0,71 | 0,00270627 | 103 | 2.01 |
| IIIFA905A | 8hrs | 9,50 | 14,50 | 0,66 | 0,00249484 | 95 | 1.98 |
| IIIFA951A | 6day | 13,00 | 15,00 | 0,87 | 0,00228535 | 87 | 1.97 |
| IIIFA952A | 13day | 0,00 | 0,00 | 0,00 | 0,00000000 | 0 | 0.00 |
| Sample 2 | | | | | | | |
| IIIFA901B | 0 | 11,00 | 15,00 | 0,73 | 0,00279084 | 100 | 2.00 |
| IIIFA902B | 2hrs | 11,00 | 15,00 | 0,73 | 0,00279084 | 100 | 2.00 |
| IIIFA903B | 4hrs | 11,00 | 15,00 | 0,73 | 0,00279084 | 100 | 2.00 |
| IIIFA904B | 6hrs | 11,00 | 15,50 | 0,71 | 0,00270627 | 97 | 1.99 |
| IIIFA951B | 6day | 13,50 | 15,00 | 0,90 | 0,00238514 | 85 | 1.93 |
| IIIFA952B | 13day | 0,00 | 0,00 | 0,00 | 0,00000000 | 0 | 0.00 |

Table 15: Sampling Time, Peak Height and Concentration of Corticosteroid Remaining for Fluocinolone Acetonide Cream in Emulsifying Cream 1:10 Dilution



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| Fluocinolone Acetonide Ointment in Cetomacrogol Ointment 1:10 Dilution | | | | | | | |
|---|---|---|---|---|---|---|---|
| Name | Time | Peak Height | | | Concentration Remaining | | |
| Sample 1 | Days | Steroid (a) | Int. Std. (b) | Ratio (a/b) | g/100g | Percentage | Log % |
| IIFA80' | 0 | 81,25 | 43,50 | 1,87 | 0,0023916 | 100 | 2.00 |
| IIFA17 | 80 | 346,00 | 1253,00 | 0,28 | 0,0022747 | 95 | 1.98 |
| IIFA52 | 134 | 289,00 | 1011,00 | 0,29 | 0,0023709 | 99 | 2.00 |
| IIFA73 | 148 | 271,00 | 933,00 | 0,29 | 0,0023709 | 99 | 2.00 |
| IIFA127 | 206 | 273,00 | 1001,00 | 0,27 | 0,0021786 | 91 | 1.96 |
| IIFA164 | 340 | 18,00 | 70,00 | 0,26 | 0,0022556 | 94 | 1.97 |
| Sample 2 | | | | | | | |
| IIFB14 | 0 | 482,00 | 1240,00 | 0,39 | 0,0033000 | 100 | 2.00 |
| IIFB61 | 64 | 359,00 | 1115,00 | 0,32 | 0,0026593 | 81 | 1.91 |
| IIFB88 | 80 | 347,00 | 947,00 | 0,37 | 0,0030438 | 92 | 1.96 |
| IIFB112 | 110 | 331,00 | 918,00 | 0,36 | 0,0030030 | 91 | 1.96 |
| IIFB136 | 134 | 266,00 | 870,00 | 0,31 | 0,0025632 | 78 | 1.89 |
| Sample 3 | | | | | | | |
| IIFC42 | 0 | 472,00 | 1389,00 | 0,34 | 0,0024593 | 100 | 2.00 |
| IIFC94 | 43 | 486,00 | 1415,00 | 0,34 | 0,0024593 | 100 | 2.00 |
| IIFC118 | 80 | 469,00 | 1491,00 | 0,31 | 0,0022553 | 92 | 1.96 |
| IIFC142 | 110 | 463,00 | 1511,00 | 0,31 | 0,0022553 | 92 | 1.96 |
| IIFC166 | 221 | 38,00 | 131,00 | 0,29 | 0,0025403 | 103 | 2.01 |

Table 16: Sampling Time, Peak Height and Concentration of Corticosteroid Remaining for Fluocinolone Acetonide Ointment in Cetomacrogol Ointment 1:10 Dilution

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| Fluocinolone Acetonide Ointment in Emulsifying Ointment 1:10 Dilution | | | | | | | |
|---|---|---|---|---|---|---|---|
| Name | Time | Peak Height | | | Concentration Remaining | | |
| Sample 1 | Days | Steroid (a) | Int. Std. (b) | Ratio (a/b) | g/100g | Percentage | Log % |
| IVFA12B | 0 | 413,00 | 1484,00 | 0,28 | 0,0024689 | 100 | 2.00 |
| IVFA23B | 17 | 438,00 | 1552,00 | 0,28 | 0,0024689 | 100 | 2.00 |
| IVFA34B | 33 | 447,00 | 1552,00 | 0,29 | 0,0026000 | 105 | 2.02 |
| IVFA57B | 68 | 432,00 | 1608,00 | 0,27 | 0,0023761 | 96 | 1.98 |
| IVFA81B | 81 | 401,00 | 1336,00 | 0,30 | 0,0026543 | 108 | 2.03 |
| IVFA158B | 184 | 349,00 | 1323,00 | 0,26 | 0,0022834 | 93 | 1.97 |
| IVFA169B | 267 | 43,00 | 128,50 | 0,33 | 0,0023599 | 96 | 1.98 |
| Sample 2 | | | | | | | |
| IVFB78 | 0 | 4,00 | 13,10 | 0,31 | 0,0028484 | 100 | 2.00 |
| IVFB116 | 41 | 4,20 | 13,05 | 0,32 | 0,0029434 | 103 | 2.01 |
| IVFB140 | 68 | 3,65 | 12,95 | 0,28 | 0,0025632 | 90 | 1.95 |
| IVFB170 | 184 | 33,00 | 104,00 | 0,32 | 0,0022702 | 80 | 1.90 |

Table 17: Sampling Time, Peak Height and Concentration of Corticosteroid Remaining for Fluocinolone Acetonide Ointment in Emulsifying Ointment 1:10 Dilution



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| Fluocinolone Acetonide Cream in Cetomacrogol Cream 50:50 Dilution | | | | | | | |
|---|---|---|---|---|---|---|---|
| Name | Time | Peak Height | | | Concentration Remaining | | |
| Sample 1 | Days | Steroid (a) | Int. Std. (b) | Ratio (a/b) | g/100g | Percentage | Log % |
| IFA783 | 0 | 39,00 | 64,00 | 0,61 | 0,0145259 | 100 | 2.00 |
| IFA981 | 33 | 1,90 | 1,90 | 1,00 | 0,0140946 | 97 | 1.99 |
| IFA28 | 100 | 5,90 | 4,55 | 1,30 | 0,0119330 | 82 | 1.91 |
| IFA51 | 135 | 5,00 | 3,65 | 1,37 | 0,0125450 | 86 | 1.93 |
| IFA102 | 173 | 7,75 | 5,50 | 1,41 | 0,0128947 | 89 | 1.95 |
| IFA126 | 209 | 5,15 | 4,00 | 1,29 | 0,0118456 | 82 | 1.91 |
| IFA163 | 343 | 15,00 | 23,00 | 0,65 | 0,0093105 | 64 | 1.81 |
| Sample 2 | | | | | | | |
| IFB1 | 0 | 2218,00 | 1949,00 | 1,14 | 0,0159436 | 100 | 2.00 |
| IFB11 | 12 | 1845,00 | 1845,00 | 1,00 | 0,0139322 | 87 | 1.94 |
| IFB24 | 33 | 21,50 | 21,50 | 1,00 | 0,0139322 | 87 | 1.94 |
| IFB135 | 150 | 1153,00 | 1397,00 | 0,83 | 0,0116001 | 73 | 1.86 |
| IFB162 | 253 | 16,00 | 17,50 | 0,91 | 0,0130806 | 82 | 1.91 |
| Sample 3 | | | | | | | |
| IFC41 | 0 | 1547,00 | 1160 | 1,33 | 0,0186734 | 100 | 2.00 |
| IFC93 | 47 | 1555,00 | 1272 | 1,22 | 0,0170930 | 92 | 1.96 |
| IFC117 | 78 | 1676,00 | 1312 | 1,28 | 0,0179550 | 96 | 1.98 |
| IFC173 | 253 | 8,00 | 15,50 | 0,52 | 0,0114874 | 62 | 1.79 |

Table 18: Sampling Time, Peak Height and Concentration of Corticosteroid Remaining for Fluocinolone Acetonide Cream in Cetomacrogol Cream 50:50 Dilution

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| Fluocinolone Acetonide Cream in Emulsifying Cream 50:50 Dilution | | | | | | | |
|---|---|---|---|---|---|---|---|
| Name | Time | Peak Height | | | Concentration Remaining | | |
| Sample 1 | Days | Steroid (a) | Int. Std (b) | Ratio (a/b) | g/100g | Percentage | Log % |
| IIIFA921A | 0 | 57,00 | 48,00 | 1,19 | 0,0137354 | 100 | 2.00 |
| IIIFA922A | 2 hrs | 51,50 | 48,50 | 1,06 | 0,0125156 | 91 | 1.96 |
| IIIFA923A | 4 hrs | 58,50 | 53,00 | 1,10 | 0,0128910 | 94 | 1.97 |
| IIIFA924A | 8 hrs | 56,00 | 54,00 | 1,04 | 0,0123280 | 90 | 1.95 |
| IIIFA951A | 6days | 62,00 | 51,00 | 1,22 | 0,0117479 | 86 | 1.93 |
| IIIFA952A | 13days | 0,00 | 0,00 | 0,00 | 0,0000000 | 0 | 0.00 |
| Sample 2 | | | | | | | |
| IIIFA921B | 0 | 46,00 | 45,50 | 1,01 | 0,0120465 | 100 | 2.00 |
| IIIFA922B | 2 hrs | 52,00 | 48,50 | 1,07 | 0,0126095 | 105 | 2.02 |
| IIIFA924B | 8 hrs | 59,00 | 53,50 | 1,10 | 0,0128910 | 107 | 2.03 |
| IIIFA951B | 6days | 58,5 | 51,00 | 1,15 | 0,0111042 | 92 | 1.96 |
| IIIFA952B | 13days | 0,00 | 0,00 | 0,00 | 0,0000000 | 0 | 0.00 |

Table 19: Sampling Time, Peak Height Ratios and Concentration of Corticosteroid Remaining for Fluocinolone Acetonide Cream in Emulsifying Cream 50:50 Dilution



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| Fluocinolone Acetonide Ointment in Cetomacrogol Ointment 50:50 Dilution | | | | | | | |
|---|---|---|---|---|---|---|---|
| Name | Time | Peak Height | | | Concentration Remaining | | |
| Sample 1 | Days | Steroid (a) | Int. Std. (b) | Ratio (a/b) | g/100g | Percentage | Log % |
| IIFB14 | 0 | 1589 | 1198 | 1,33 | 0,01236900 | 100 | 2.00 |
| IIFB38 | 40 | 1651 | 1279 | 1,29 | 0,01198450 | 97 | 1.99 |
| IIFB61 | 64 | 1575 | 1129 | 1,40 | 0,01304200 | 105 | 2.02 |
| IIFB165 | 260 | 99.5 | 77,5 | 1,28 | 0,01193605 | 96 | 1.98 |
| Sample 2 | | | | | | | |
| IIFC42 | 0 | 2385 | 1372 | 1,74 | 0,01197572 | 100 | 2.00 |
| IIFC118 | 77 | 2400 | 1491 | 1,61 | 0,01107205 | 92 | 1.93 |
| IIFC142 | 110 | 2424 | 1542 | 1,57 | 0,01082015 | 90 | 1.95 |
| IIFC174 | 260 | 54 | 37 | 1,44 | 0,01232737 | 103 | 2.01 |
| Sample 3 | | | | | | | |
| IIFD46 | 0 | 2524 | 1438 | 1,76 | 0,01211167 | 100 | 2.00 |
| IIFD122 | 77 | 2337 | 1421 | 1,64 | 0,01129597 | 93 | 1.97 |
| IIFD146 | 110 | 2213 | 1633 | 1,36 | 0,00939268 | 78 | 1.89 |
| IIFD172 | 215 | 130,5 | 77 | 1,69 | 0,01355398 | 112 | 2.05 |

Table 20: Sampling Time, Peak Height Ratio and Concentration of Corticosteroid Remaining for Fluocinolone Acetonide Ointment in Cetomacrogol Ointment 50:50 Dilution

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| Fluocinolone Acetonide Ointment in Emulsifying Ointment 50:50 Dilution | | | | | | | |
|---|---|---|---|---|---|---|---|
| Name | Time | Peak Height | | | Concentration Remaining | | |
| Sample 1 | Days | Steroid (a) | Int. Std. (b) | Ratio (a/b) | g/100g | Percentage | Log % |
| IVFA12C | 0 | 2093 | 1471 | 1,42 | 0,01306412 | 100 | 2.00 |
| IVFA13C | 1 | 2007 | 1428 | 1,41 | 0,01294820 | 99 | 2.00 |
| IVFA23C | 17 | 2260 | 1561 | 1,45 | 0,01331915 | 102 | 2.01 |
| IVFA34C | 37 | 2266 | 1573 | 1,44 | 0,01322641 | 101 | 2.00 |
| IVFA57C | 68 | 2207 | 1621 | 1,36 | 0,01248451 | 96 | 1.98 |
| IVFA158C | 184 | 1890 | 1340 | 1,41 | 0,01294820 | 99 | 2.00 |
| Sample 2 | | | | | | | |
| IVFB78 | 0 | 12,94 | 8,67 | 1,49 | 0,01417576 | 100 | 2.00 |
| IVFB79 | 1 | 12,75 | 8,50 | 1,50 | 0,01415992 | 100 | 2.00 |
| IVFB116 | 37 | 13,20 | 8,00 | 1,65 | 0,01558575 | 110 | 2.04 |
| IVFB140 | 68 | 13,40 | 8,65 | 1,55 | 0,01463520 | 103 | 2.01 |
| IVFB170 | 184 | 128 | 81 | 1,58 | 0,01356816 | 96 | 1.98 |

Table 21: Sampling Time, Peak Height Ratio and Concentration of Corticosteroid Remaining for Fluocinolone Acetonide Ointment in Emulsifying Ointment 50:50 Dilution



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| Fluocinolone Acetonide Ointment in Cetomacrogol Ointment 50:50 Dilutio | | | | | | |
|---|---|---|---|---|---|---|
| Position | Name | Peak Height | | | Concentration g/100g | Standard Deviation |
| Group A | | Steroid (a) | Int. Std. (b) | Ratio (a/b) | | |
| Top Left | ATL | 92,00 | 72,00 | 1,28 | 0,0098249 | 0.000624067 |
| Top Right | ATR | 137,00 | 95,00 | 1,44 | 0,0111332 | |
| Bottom Left | ABL | 128,50 | 96,00 | 1,34 | 0,0103155 | |
| Bottom Right | ABR | 134,00 | 94,00 | 1,43 | 0,0110514 | |
| Group B | | | | | | |
| Top Left | BTL | 134,50 | 93,50 | 1,44 | 0,0111332 | 0.000362632 |
| Top Right | BTR | 138,00 | 93,50 | 1,48 | 0,0114603 | |
| Bottom Left | BBL | 137,00 | 98,00 | 1,40 | 0,0108061 | |
| Bottom Right | BBR | 138,00 | 100,00 | 1,38 | 0,0106426 | |
| Group C | | | | | | |
| Top Left | CTL | 124,00 | 79,50 | 1,56 | 0,0121144 | 0.000313131 |
| Top Right | CTR | 135,00 | 86,50 | 1,56 | 0,0121144 | |
| Bottom Left | CBL | 136,00 | 89,50 | 1,52 | 0,0117873 | |
| Bottom Right | CBR | 127,00 | 86,00 | 1,48 | 0,0114603 | |
| Group D | | | | | | |
| Top Left | DTL | 131,00 | 84,00 | 1,56 | 0,0121144 | 0.000486066 |
| Top Right | DTR | 131,00 | 87,00 | 1,51 | 0,0117056 | |
| Bottom Left | DBL | 135,00 | 95,00 | 1,42 | 0,0109696 | |
| Bottom Right | DBR | 132,00 | 90,00 | 1,47 | 0,0113785 | |

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Table 22: Variation in Concentration of Fluocinolone Acetonide Ointment in Cetomacrogol Ointment Sampled at Different Positions in the Container



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| Betamethasone Valerate Cream in Cetomacrogol Cream 1:10 Dilution | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Time | | Percentage Remaining | | | | Average | Standard | Log |
| Days | Months | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Percentage | Deviation | Percentage |
| 0 | 0,00 | 100 | 100 | 100 | 100 | 100,00 | 0,00 | 2,00 |
| 19 | 0,63 | 100 | 96 | 104 | 96 | 99,00 | 3.83 | 2,00 |
| 44 | 1,47 | 100 | 91 | 109 | 96 | 99,00 | 7.62 | 2,00 |
| 54 | 1,80 | 99 | 89 | 109 | 96 | 98,25 | 8.30 | 1,99 |
| 100 | 3,33 | 99 | 98 | 103 | 94 | 98,50 | 3.70 | 1,99 |
| 118 | 3,93 | 95 | 96 | 100 | 93 | 96,00 | 2.94 | 1,98 |
| 137 | 4,57 | 92 | 94 | 98 | 90 | 93,50 | 3.42 | 1,97 |
| 167 | 5,57 | 92 | | 95 | 87 | 91,30 | 4.04 | 1,96 |
| 246 | 8,20 | 94 | | | | 94,00 | | 1,97 |
| 330 | 11,00 | | | 76 | 68 | 72,00 | 5.66 | 1,86 |

| Betamethasone Valerate Ointment in Cetomacrogol Ointment 1:10 Dilution | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Time | | Percentage Remaining | | | | Average | Standard | Log |
| Days | Months | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Percentage | Deviation | Percentage |
| 0 | 0,00 | 100 | 100 | 100 | 100 | 100,00 | 0,00 | 2,00 |
| 21 | 0,70 | 94 | 95 | 100 | 93 | 95,50 | 3.11 | 1,98 |
| 31 | 1,03 | 92 | 94 | 100 | 92 | 94,50 | 3.79 | 1,98 |
| 43 | 1,43 | 90 | 91 | 100 | 93 | 93,50 | 4.51 | 1,97 |
| 76 | 2,53 | 85 | 86 | 102 | 95 | 92,00 | 8.04 | 1,96 |
| 112 | 3,73 | 73 | 69 | 86 | 82 | 77,50 | 7.85 | 1,89 |
| 130 | 4,33 | 69 | 64 | 82 | 79 | 73,50 | 8.43 | 1,87 |
| 150 | 5,00 | 58 | 61 | 78 | 76 | 68,25 | 10.21 | 1,83 |
| 360 | 12,00 | | | | 47 | 47,00 | 0,00 | 1,67 |

| Betamethasone Valerate Cream in Emulsifying Cream 1:10 Dilution | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Time | | Percentage Remaining | | | | Average | Standard | Log |
| Days | Months | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Percentage | Deviation | Percentage |
| 0 | 0,00 | 100 | 100 | | | 100,00 | 0,00 | 2,00 |
| 10 | 0,33 | 87 | 93 | | | 90,00 | 4.24 | 1,95 |
| 68 | 2,27 | 87 | | | | | | 1,94 |
| 90 | 3,00 | 91 | 90 | | | 90,50 | 0.71 | 1,96 |
| 104 | 3,47 | 95 | 89 | | | 92,00 | 4.24 | 1,96 |
| 148 | 4,93 | 83 | 89 | | | 86,00 | 4.24 | 1,93 |

| Betamethasone Ointment in Emulsifying Ointment 1:10 Dilution | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Time | | Percentage Remaining | | | | Average | Standard | Log |
| Days | 0,00 | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Percentage | Deviation | Percentage |
| 0 | 0,00 | 100 | | | | 100 | | 2,00 |
| 31 | 1,03 | 62 | | | | 62 | | 1,80 |
| 47 | 1,57 | 57 | | | | 57 | | 1,75 |
| 54 | 1,80 | 34 | | | | 34 | | 1,53 |
| 90 | 3,00 | 14 | | | | 14 | | 1,13 |
| 134 | 4,47 | 7 | | | | 7 | | 0,83 |

Table 23: Sampling Time, Percentage Corticosteroid Remaining and Standard Deviation for Betamethasone Valerate 1:10 Diluted Preparations



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| Betamethasone Valerate Cream in Cetomacrogol Cream 50:50 Dilution | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Time | | Percentage Remaining | | | | Average | Standard | Log |
| Days | Months | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Percentage | Deviation | Percentage |
| 0 | 0,00 | 100 | 100 | 100 | 100 | 100,00 | 0,00 | 2,00 |
| 23 | 0,77 | 101 | 97 | 112 | 95 | 101,25 | 7.59 | 2,01 |
| 44 | 1,47 | 103 | 94 | 101 | 96 | 98,50 | 4.20 | 1,99 |
| 54 | 1,80 | 103 | 92 | 103 | 98 | 99,00 | 5.23 | 2,00 |
| 81 | 2,70 | 105 | 98 | 108 | 102 | 103,25 | 4.27 | 2,01 |
| 100 | 3,33 | 106 | 101 | 108 | 99 | 103,50 | 4.20 | 2,01 |
| 119 | 3,97 | 103 | 101 | 108 | 96 | 102,00 | 4.97 | 2,01 |
| 137 | 4,57 | 100 | 95 | 108 | 97 | 100,00 | 5.72 | 2,00 |
| 167 | 5,57 | 100 | | 108 | 99 | 102,30 | 4.93 | 2,01 |
| 246 | 8,20 | 106 | | 108 | 104 | 106,00 | 2.00 | 2,03 |
| 330 | 11,00 | | | 108 | 109 | 108,50 | 0.71 | 2,04 |

| Betamethasone Valerate Ointment in Cetomacrogol Ointment 50:50 Dilution | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Time | | Percentage Remaining | | | | Average | Standard | Log |
| Days | Months | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Percentage | Deviation | Percentage |
| 0 | 0,00 | 100 | 100 | 100 | 100 | 100,00 | 0,00 | 2,00 |
| 18 | 0,60 | 99 | 96 | 100 | 92 | 96,75 | 3.59 | 1,99 |
| 31 | 1,03 | 99 | 91 | 101 | 93 | 96,00 | 4.76 | 1,98 |
| 43 | 1,43 | 98 | 91 | 101 | 94 | 96,00 | 4.40 | 1,98 |
| 76 | 2,53 | 97 | 90 | 103 | 94 | 96,00 | 5.48 | 1,98 |
| 116 | 3,87 | 87 | 89 | 84 | 81 | 85,25 | 3.50 | 1,93 |
| 130 | 4,33 | 83 | 91 | 84 | 80 | 84,50 | 4.65 | 1,93 |
| 150 | 5,00 | 85 | 91 | 83 | 81 | 85,00 | 4.32 | 1,93 |
| 181 | 6,03 | 87 | 91 | 82 | 80 | 85,00 | 4.97 | 1,93 |
| 211 | 7,03 | 88 | 89 | 80 | 80 | 84,25 | 4.92 | 1,93 |
| 360 | 12,00 | | 85 | 73 | 79 | 79,00 | 6.00 | 1,90 |

| Betamethasone Valerate Cream in Emulsifying Cream 50:50 Dilution | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Time | | Percentage Remaining | | | | Average | Standard | Log |
| Days | Months | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Percentage | Deviation | Percentage |
| 0 | 0,00 | 100 | 100 | | | 100,00 | 0,00 | 2,00 |
| 10 | 0,33 | 85 | 83 | | | 84,00 | 1.41 | 1,92 |
| 50 | 1,67 | 101 | 87 | | | 94,00 | 9.90 | 1,97 |
| 68 | 2,27 | 85 | 85 | | | 85,00 | 0,00 | 1,93 |
| 90 | 3,00 | 85 | 82 | | | 83,50 | 2.12 | 1,92 |
| 148 | 4,93 | 86 | | | | 86,00 | 0,00 | 1,93 |

| Betamethasone Valerate Ointment in Emulsifying Ointment 50:50 Dilution | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Time | | Percentage Remaining | | | | Average | Standard | Log |
| Days | Months | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Percentage | Deviation | Percentage |
| 0 | 0,00 | 100 | 100 | | | 100,00 | 0,00 | 2,00 |
| 9 | 0,30 | 99 | 103 | | | 101,00 | 2.83 | 2,00 |
| 25 | 0,83 | 97 | 106 | | | 101,50 | 6.36 | 2,01 |
| 31 | 1,03 | 96 | 100 | | | 98,00 | 2.83 | 1,99 |
| 42 | 1,40 | 95 | 92 | | | 93,50 | 2.12 | 1,97 |
| 54 | 1,80 | 94 | 95 | | | 94,50 | 0.71 | 1,98 |
| 122 | 4,07 | 91 | 90 | | | 90,50 | 0.71 | 1,96 |
| 134 | 4,47 | 87 | | | | 87,00 | 0,00 | 1,94 |

Table 24: Sampling Time, Percentage Corticosteroid Remaining and Standard Deviation for Betamethasone Valerate 50:50 Diluted Preparations



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| Fluocinolone Acetonide Cream in Cetomacrogol Cream 1:10 Dilution | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Time | | Percentage Remaining | | | | Average | Standard | Log |
| Days | Months | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Percentage | Deviation | Percentage |
| 0 | 0,00 | 100 | 100 | | | 100,00 | 0,00 | 2,00 |
| 10 | 0,33 | 102 | 97 | | | 99,50 | 3.54 | 2,00 |
| 50 | 1,67 | 100 | 77 | | | 88,50 | 16.26 | 1,95 |
| 66 | 2,20 | 98 | 77 | | | 87,50 | 14.85 | 1,94 |
| 75 | 2,50 | 101 | 77 | | | 89,00 | 16.97 | 1,95 |
| 173 | 5,77 | 94 | 71 | | | 82,50 | 16.26 | 1,92 |
| 223 | 7,43 | 85 | 68 | | | 76,50 | 12.02 | 1,88 |
| 253 | 8,43 | 79 | 67 | | | 73,00 | 8.49 | 1,86 |
| 343 | 11,43 | 62 | | | | 62,00 | 0,00 | 1,79 |

| Fluocinolone Acetonide Ointment in Cetomacrogol Ointment 1:10 Dilution | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Time | | Percentage Remaining | | | | Average | Standard | Log |
| Days | Months | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Percentage | Deviation | Percentage |
| 0 | 0,00 | 100 | 100 | 100 | | 100,00 | 0,00 | 2,00 |
| 43 | 1,43 | 98 | 88 | 100 | | 95,30 | 6.43 | 1,98 |
| 64 | 2,13 | 97 | 82 | 95 | | 91,30 | 8.14 | 1,96 |
| 80 | 2,67 | 96 | 94 | 92 | | 94,00 | 2.00 | 1,97 |
| 110 | 3,67 | 99 | 91 | 92 | | 94,00 | 4.36 | 1,97 |
| 134 | 4,47 | 100 | 79 | 94 | | 91,00 | 10.82 | 1,96 |
| 148 | 4,93 | 100 | | 95 | | 97,50 | 3.54 | 1,99 |
| 206 | 6,87 | 92 | | 101 | | 96,50 | 6.36 | 1,98 |
| 221 | 7,37 | 93 | | 102 | | 97,50 | 6.36 | 1,99 |
| 340 | 11,33 | 94 | | | | 94,00 | 0,00 | 1,97 |

| Fluocinolone Acetonide Cream in Emulsifying Cream 1:10 Dilution | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Time | | Percentage Remaining | | | | Average | Standard | Log |
| Days/hrs | Months | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Percentage | Deviation | Percentage |
| 0 | 0,000000 | 100 | 100 | | | 100,00 | 0,00 | 2.000 |
| 2 Hours | 0,002778 | 102 | 100 | | | 101,00 | 1.41 | 2.004 |
| 4 Hours | 0,005555 | 97 | 100 | | | 98,50 | 2.12 | 1.993 |
| 6 Hours | 0,008333 | 103 | 97 | | | 100,00 | 4.24 | 2.000 |
| 8 Hours | 0,011110 | 95 | 96 | | | 95,50 | 0.71 | 1.980 |
| 6 Days | 0,199987 | 87 | 85 | | | 86,00 | 1.41 | 1.934 |
| 13 Days | 0,433306 | 0 | 0 | | | 0,00 | 0,00 | 0.000 |

| Fluocinolone Acetonide Ointment in Emulsifying Ointment 1:10 Dilution | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Time | | Percentage Remaining | | | | Average | Standard | Log |
| Days | Months | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Percentage | Deviation | Percentage |
| 0 | 0,00 | 100 | 100 | | | 100,00 | 0,00 | 2,00 |
| 17 | 0,57 | 100 | 101 | | | 100,50 | 0.71 | 2,00 |
| 33 | 1,10 | 104 | 103 | | | 103,50 | 0.71 | 2,01 |
| 41 | 1,37 | 103 | 103 | | | 103,00 | 0,00 | 2,01 |
| 68 | 2,27 | 96 | 90 | | | 93,00 | 4.24 | 1,97 |
| 81 | 2,70 | 108 | 89 | | | 98,50 | 13.44 | 1,99 |
| 184 | 6,13 | 93 | 80 | | | 86,50 | 9.19 | 1,94 |
| 267 | 8,90 | 96 | | | | | 0,00 | 1,98 |

Table 25: Sampling Time, Percentage Corticosteroid Remaining and Standard Deviation for Fluocinolone Acetonide 1:10 Diluted Preparations



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| Fluocinolone Acetonide Cream in Cetomacrogol Cream 50:50 Dilution | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Time | | Percentage Remaining | | | | Average | Standard | Log |
| Days | Months | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Percentage | Deviation | Percentage |
| 0 | 0,00 | 100 | 100 | 100 | | 100,00 | 0,00 | 2,00 |
| 12 | 0,40 | 99 | 87 | 99 | | 95,00 | 6.93 | 1,98 |
| 33 | 1,10 | 97 | 87 | 94 | | 92,70 | 5.13 | 1,97 |
| 47 | 1,57 | 94 | 85 | 92 | | 90,30 | 4.73 | 1,96 |
| 78 | 2,60 | 86 | 80 | 97 | | 87,70 | 8.62 | 1,94 |
| 100 | 3,33 | 82 | 79 | 93 | | 84,70 | 7.37 | 1,93 |
| 135 | 4,50 | 86 | 75 | 88 | | 83,00 | 7.00 | 1,92 |
| 150 | 5,00 | 88 | 73 | 82 | | 81,00 | 7.55 | 1,91 |
| 173 | 5,77 | 89 | 75 | 78 | | 80,70 | 7.37 | 1,91 |
| 209 | 6,97 | 82 | 79 | 71 | | 77,30 | 5.69 | 1,89 |
| 253 | 8,43 | 76 | 82 | 62 | | 73,30 | 10.26 | 1,87 |
| 343 | 11,43 | 64 | | | | 64,00 | 0,00 | 1,81 |

| Fluocinolone Acetonide Ointment in Cetomacrogol Ointment 50:50 Dilution | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Time | | Percentage Remaining | | | | Average | Standard | Log |
| Days | Months | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Percentage | Deviation | Percentage |
| 0 | 0,00 | 100 | 100 | 100 | | 100,00 | 0,00 | 2,00 |
| 40 | 1,33 | 97 | 96 | 96 | | 96,30 | 0.58 | 1,98 |
| 64 | 2,13 | 105 | 93 | 94 | | 97,20 | 6.66 | 1,99 |
| 77 | 2,57 | 105 | 92 | 93 | | 96,70 | 7.23 | 1,99 |
| 110 | 3,67 | 103 | 90 | 78 | | 90,30 | 12.50 | 1,96 |
| 215 | 7,17 | 98 | 100 | 112 | | 103,30 | 7.57 | 2,01 |
| 260 | 8,67 | 96 | 103 | | | 99,50 | 4.95 | 2,00 |

| Fluocinolone Acetonide Cream in Emulsifying Cream 50:50 Dilution | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Time | | Percentage Remaining | | | | Average | Standard | Log |
| Days/hrs | Months | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Percentage | Deviation | Percentage |
| 0 | 0,000000 | 100 | 100 | | | 100,00 | 0,00 | 2,00 |
| 2 Hours | 0,002778 | 91 | 105 | | | 98,00 | 9.90 | 1,99 |
| 4 Hours | 0,005555 | 94 | 106 | | | 100,00 | 8.49 | 2,00 |
| 8 Hours | 0,011110 | 90 | 107 | | | 98,50 | 12.02 | 1,99 |
| 6 Days | 0,199987 | 86 | 98 | | | 92,00 | 8.49 | 1,96 |
| 13 Days | 0,433306 | 0 | 0 | | | 0,00 | 0,00 | 0,00 |

| Fluocinolone Acetonide Ointment in Emulsifying Ointment 50:50 Dilution | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Time | | Percentage Remaining | | | | Average | Standard | Log |
| Days | Months | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Percentage | Deviation | Percentage |
| 0 | 0,00 | 100 | 100 | | | 100,00 | 0,00 | 2,00 |
| 17 | 0,57 | 102 | 104 | | | 103,00 | 1.41 | 2,01 |
| 37 | 1,23 | 101 | 110 | | | 105,70 | 6.36 | 2,02 |
| 68 | 2,27 | 96 | 103 | | | 99,70 | 4.95 | 2,00 |
| 184 | 6,13 | 99 | 96 | | | 97,70 | 2.12 | 1,99 |

Table 26: Sampling Time, Percentage Corticosteroid Remaining and Standard Deviation for Fluocinolone Acetonide 50:50 Diluted Preparations



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