TOPICAL CORTICOSTEROID BIOEQUIVALENCE TESTING : COMPARISON OF CHROMAMETER AND VISUAL DATA.

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ABSTRACT

The major criticism of the human skin blanching assay is the subjective nature of grading the response. Recently the American FDA released a Guidance document for topical corticosteroid bioequivalence testing. The guidelines require the use of a chromameter as a reliable method to estimate skin blanching. The purpose of this study was to evaluate the recommendations of this document for appropriateness by comparing visual and chromameter data. The visually-assessed blanching assay methodology routinely practised in our laboratories was modified to comply with the specifications of the Guidance.

The preliminary trial indicated that the training period that is required for a novice to be classified as an experienced observer is not a major problem.

The major trend that emerged from the pilot study was that visual assessment was better than the chromameter. Longer dose durations were found to be more discriminatory than shorter durations. The visual data were best described by the sigmoid E_{max} model and the chromameter data were best described by the simple E_{max} model.

The pivotal results indicated that the D_2/D_1 criterion to determine sample size of "acceptable blanchers" produced only few subjects suggesting that the validity of this criterion requires extensive investigations. The estimates of the Locke's confidence interval method were similar to those for the general simple formula. However, due to undefined parameters of the Locke's method in the Guidance, the validity of the Locke's method requires evaluation. The chromameter b-scale parameter was the least sensitive in estimating skin blanching whereas the a- and L-scale parameters produced similar results. Poor correlation between visual and chromameter was noted indicating that the visual method is still the best method.

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CHAPTER ONE

INTRODUCTION

Dermatology has undergone a major revolution since the development of topical corticosteroid therapy (1). It was the introduction of hydrocortisone acetate (compound F) (2) more than four decades ago which has been described as one of the most important contribution to medical therapeutics this century (3). This is because up to that time dermatological (especially eczematous) conditions had been poorly managed and since then the use of topically applied corticosteroids has had a substantial influence on the treatment of various skin disorders (1,4). The extensive usage of topical corticosteroid therapy has also stimulated researchers to study the pharmacological properties of these drugs and the various possible methods of assessing their potency and biopharmaceutical properties.

1.1. PHARMACOLOGY

The basic structure to which all topical corticosteroids are related is a phenathrene molecule fused with a five-membered ring structure giving rise to a cyclopentanoperhydrophenanthrene nucleus which is depicted in figure 1. The main emphasis in the initial stages of topical corticosteroid development was on the alteration of the molecule, in an attempt to produce moieties of higher intrinsic topical effect and lower mineralocorticoid side effects (5,6). The introduction of a 1:2 double bond into the molecule led to an increase in the corticoid anti-inflammatory activity and a decrease in the mineralocorticoid side effects (5,7). Schlagel et al. (8) reported that the ability of the corticosteroid to penetrate the stratum corneum to produce an anti-inflammatory effect is affected by its structure. It has been found (9) in general that the acetates of topical corticosteroids are more efficient anti-inflammatory agents than

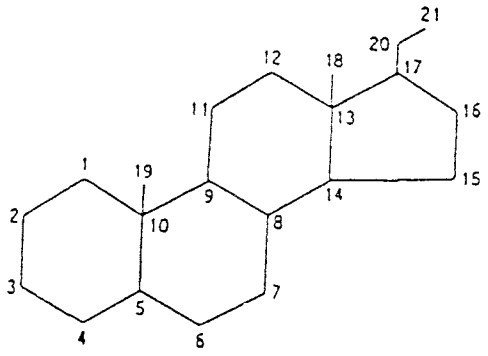


Figure 1 The basic corticosteroid nucleus

the parent alcohols possibly because of poor penetration of alcohols. However, the relationship between the structure of the molecule and its ability to penetrate the stratum corneum is not that clear. This is because the experiments of Stoughton et al. (10), found that fluocinolone acetonide penetrates the skin to a greater extent than fluocinolone alcohol with the latter being more pharmacologically effective. Another example is betamethasone 17-valerate which has been found (10) to be more pharmacological active than its parent alcohol yet the two molecules have similar penetration characteristics.

Topical corticosteroids are indicated for a wide variety of dermatological diseases (11). Examples of dermatological problems that usually respond well to topical corticosteroids include psoriasis (especially of the face and flexures), seborrhoeic dermatitis, atopic eczema, allergic contact dermatitis and primary irritant dermatitis. Other skin problems for which topical corticosteroids may be used are nail disorders, discoid lupus erythematosus and psoriasis of the palms, soles, elbows and knees. For the least responsive dermatoses such as keloids, hypertrophic scars and acne cysts, intralesion injection of topical corticosteroids is required or more potent corticosteroids may be used (11).

1.1.1. Mode of action

The therapeutic effectiveness of topical corticosteroids is based primarily on their anti-inflammatory activity, antipruritic and vasoconstrictive actions (12). Although topical corticosteroids do act indirectly in suppressing the blood vessels, this mode of action does not appear to be the main mechanism in the treatment of dermatoses. This is because Nakamura et al. (13) showed that the concentration of the intravenously injected corticosteroid was much higher in the inflamed skin than in normal skin suggesting that the agents act directly in the cells of the lesions of dermatoses, not indirectly through their action on blood vessels. Though definite explanations of the effects of corticosteroids on endogenous mediators of inflammation such as histamine, kinins, lysosomals, and prostaglandins await further experimental clarifications (11), it is currently believed (1) that the main mode of topical anti-inflammatory activity is through the inhibition of an enzyme called phospholipase A_2. The process of producing prostaglandins, leukotriens and other pro-inflammatory hydroxy fatty acids from arachidonic acid is regulated by this enzyme. When topical corticosteroids are applied in the skin, lipocortin molecule which is an antiphospholipase A_2 protein is synthesized within ribosomes during normal process of protein synthesis. The production of mediators which are responsible for inflammation will be suppressed because the lipocortin molecule will attack the phospholipase A_2 enzyme. The action of lipocortin on the enzyme will also result in the inhibition of fibrin and collagen deposition and hence cell mitosis and maturation is delayed. In addition autolysis through lysosomes activity will also be suppressed. The antimitotic effects of topical corticosteroids on human epidermis may account for an additional mechanism of action in psoriasis and other dermatologic diseases associated with increased cell turnover (14). It is probably for this reason why the steroids have such diverse use.

1.1.2. Side effects

Levine et al. (15) and Dahl et al. (16) reported that the incidence and severity of side effects becomes significant when one attempts to increase the effectiveness of corticosteroid therapy, whether by enhanced bioavailability or by increasing the inherent potency of the drug (17). The bioavailability of a steroid molecule can be enhanced through improvement of drug delivery system or by occlusion. The side effects of topical corticosteroids depend on various factors such as the skin site to which the formulation is applied, the frequency of the application and duration of usage, and individual patient variations. Side-effects are likely to develop when the corticosteroid is applied to certain anatomical sites such as the face, scrotum and vulva (17,18). More frequent application of the steroids may lead to increased side effects (17,18,19). Many corticosteroids have a reservoir effect (section 1.4.) in the skin and a decreased frequency of application may decrease the development of tachyphylaxis (17). Application of a potent steroid for a few days is unlikely to produce any serious side effects, but over a longer period, serious side effects may develop. Children and patients with renal failure are more susceptible to side effects than adults (18,20).

The side effects of topical corticosteroids may be local or systemic. The adverse local effects include the following: atrophy which is the thinning of epidermis and dermal tissue presenting as depressed, shiny, often wrinkled with prominent telangiectases and a tendency to develop purpura and ecchymosis, steroid rosacea with persistent erythema, telangiectatic vessels, pustules, and papules in the face, perioral dermatitis, acne cysts, hypopigmentation, hypertrichosis, and allergic contact dermatitis. The microbial infections on the skin may manifest as a result of topical corticosteroids suppressing the inflammatory response (1,18), hence interfering with the defence mechanism of the body. Systemic side effects of topical corticosteroids are

of a serious nature. Suppression of the hypothalamic pituitary-adrenal axis (HPA) which manifests as oedema, cushingoid features, hyperglycaemia and glycosuria is a homeostatic negative feedback responding to the high systemic corticosteroid concentrations following extensive topical absorption (1,15,16). Iatrogenic Cushing's syndrome involving several diverse organ systems, may result from prolonged exposure to potent topical corticosteroids and may cause growth retardation in children (20). Metabolic side effects include protein catabolism resulting in muscle and skin atrophy and may lead to osteoporosis. The increased glucose production and decreased glucose utilization induce hyperglycaemia and may lead to diabetes mellitus (1,20).

1.2. PERCUTANEOUS ABSORPTION

Topical corticosteroids usually have their site of action at a region below the skin stratum corneum (SC) and therefore have to penetrate the SC to be effective (5,21,22). The experiments of Leiferman et al. (23) in an attempt to ascertain the location and distribution of receptors demonstrated that higher amounts of corticosteroid receptors are found in the upper dermis layer. Ostrenga et al. (24) and Barry (25) showed that the rate limiting step is the SC. If, however, the skin is damaged and the stratum corneum is absent, the vehicle becomes the rate limiting step. It is well known that the route of skin penetration followed by an exogenous molecule may follow other paths than across the intact SC (trans- or intercellular) but also via the hair follicles and adjoining sebaceous glands or via the eccrine sweat glands. Although the SC is widely acknowledged as the main barrier to percutaneous absorption, it is also regarded as the main pathway for drug penetration (26). In the past, doubt has been cast upon the actual significance of the follicular pathway based on the fact that the orifices of hair follicles occupy only about 0.1% of the total skin surface area. A review paper by Laer et al. (27) indicated that recent reports, however, have

suggested that in addition to the transepidermal route, hair follicles and sebaceous glands may contribute significantly to topical or transdermal drug delivery. Despite these recent developments, appropriate models and quantitative methods must still be developed in order to truly ascertain the significance of follicular delivery (27).

1.2.1. The process of percutaneous absorption

The process of percutaneous absorption is a solution-diffusion phenomenon (28). The process, however, is not well understood and attempts to describe it have been made by Barry et al. (28), Schaefer et al. (29) and recently by The European Centre for Ecotoxicology and Toxicology of Chemicals (CDEA) (30). For a drug to be absorbed through the skin, the drug must first diffuse through the outer surface of the skin, the SC. Drugs which are not in solution on the surface of the SC must first undergo a process of dissolution before partitioning in the SC. Among other factors, the extent of the movement of drug into the SC is influenced by its solubility in the SC (30-33). The drug will partition between the two phases, that is, dissolved drug on the surface of the SC or in solution in a vehicle or formulation and the stratum corneum, establishing an equilibrium (thermodynamic activity equal in both phases). Once in the SC the drug will diffuse from the high concentration in the outer SC down to the lower layers of SC where the concentration is less. Some of the drug molecules entering the SC might bind to SC components and not be available to diffuse further.

At the base of the SC the drug must undergo a partition step from the SC into viable epidermis. The rate of diffusion through this layer towards the capillary network immediately below the dermo-epidermal junction depends on the nature of the drug. During all these phases, the drug may be metabolised or prevented from diffusing further by binding. At the base of the epidermis the diffusing drugs (parent and any metabolites) will partition

between the epidermis and the dermis before entering the systemic circulation. The process of percutaneous absorption can be summarised as follows: (a) Partitioning into the major barrier, the SC, (b) Diffusion of molecules through the SC (c) Partitioning from the SC to the underlying viable epidermis (d) Distribution among the vascular (capillary beds), non-vascular hydrophillic epidermal/dermal tissue and lipid domains and (e) Dynamic dermal vascular perfusion effects.

1.2.2. Factors influencing percutaneous absorption

There are numerous factors which affect percutaneous absorption and therefore the efficacy and blanching ability of topical corticosteroids. The amount of formulation and dose duration are some of the factors that affect topical absorption and are discussed in section 1.6. concerning the United States Food and Drug Administration (FDA) Guidance.

State of skin and hydration

The age of the skin is an important biological factor that affects penetration of topical corticosteroids (19). It is usually assumed that the skin of the foetus, the young and the elderly is more permeable than adult tissue (20,25). Children are more susceptible to the toxic effects of drugs because of their greater surface area per unit body weight (19). Penetration of the drug is greatly enhanced when the skin is broken, inflamed or abraded (34,35). Disease of the skin commonly alters its normal appearance. If the skin site is thickened with corns or calluses or as in ichthyosis, drug permeation should be expected to decrease (19,34,36).

Occlusion with plastic film has been shown to increase the penetration of topical corticosteroids (36). It is recognised that the degree of hydration of SC affects its permeability to corticosteroids and their transport through the skin (40). The SC has been found to be capable of absorbing large quantities of

water, causing it to swell and then increase in suppleness (37). The occlusive nature of ointment formulations has been found to increase skin hydration compared to cream formulations resulting in increased corticosteroid absorption (36,38,39). A new product recently launched here in South Africa (fluticasone propionate) has its cream form in a concentration of 0.05%, and its equivalent-strength ointment application is 0.005% (17). Possible explanations for increase in absorption of drugs are that hydration opens up the compact structure of the stratum corneum due to water diffusing from underlying epidermal layers or from the accumulation of perspiration after occlusion (38,40). Haigh and Kanfer (40) reported that although occlusion with plastic film also causes an increase in skin temperature, the practical importance of temperature effects in topical therapy is likely to be of minor importance. Occlusion increases the temperature by preventing evaporation and a decrease in the loss of heat by radiation. A publication of Bucks et al. (41) reported that occlusion does not uniformly enhance penetration of topical corticosteroids in vivo. The reasons for these results are unexplained. This phenomenon could be associated with a decrease in the partitioning potential between vehicle and the hydrated SC.

Anatomical site

Variations in drug permeability depend on the thickness and nature of the SC and the density of skin appendages (19). Feldmann and Maibach (32) demonstrated that the absorption of hydrocortisone was greatest in the regions of the head followed by the forearm and was the least in the areas of foot. Barry et al. (25) reported that the absorption of corticosteroid was less in the palms and soles regions than the other parts of the body. The reason given was that the SC in the palms and soles areas was much thicker than the other tested regions. Flexures and delicate skin areas allow increased percutaneous absorption of topical corticosteroids and therefore an increased potential for

side effects (18,20,25). However, the absorption rate varies widely for a specific drug molecule passing through similar skin sites in different volunteers; the most permeable regions in some individuals compared with the least permeable sites in others (19).

Vehicle effects

In recent years the nature of the semisolid drug delivery base has received considerable attention (42). This is because the vehicle is important in delivering the drug to exert its pharmacological effect (7). The nature of the vehicle has a profound effect on the rate of release of the topical corticosteroid from the formulation and its passage through the SC. The ideal vehicle should be cosmetically acceptable, physically stable, physiologically inert and provide an environment in which the drug is stable and from which drug in low concentrations can be readily absorbed (7,24,39,43,44). The most common pharmaceutical vehicles for topical corticosteroids used in clinical practice are the ointments, creams, lotions and gels. The extent to which the corticosteroid is released from these vehicles depends on the solubility of the drug in the vehicle, the particle size of the drug, the occlusivity of the vehicle and the presence of penetration enhancers.

It is usually assumed that the different types of topical corticosteroid formulations would show similar topical availability if these contain the same label concentration of the same corticosteroid. However, this has been found not to be the case (38,45). Smith et al. (38) conducted a comprehensive study using four different types of formulations, each containing 0.12% betamethasone 17-valerate, in occluded and unoccluded mode of application. The four formulations used were cream, lotion, ointment and scalp application. The researchers found that topical drug availability was greatest from the scalp application in both application modes, lowest from the ointment formulation

on occlusion whereas the lotion formulation showed greatest increase in topical drug availability on occlusion, even though the drug delivery would have been expected to be equivalent. The scalp application is normally applied to the skin which is reportedly more permeable than that of other anatomical sites (46). It is therefore possible that significant localized, and possibly systemic side effects may develop after repeated use of this scalp formulation. The cream and lotion formulations appeared to benefit the most when application sites were occluded as drug availability was higher than in unoccluded application sites. In contrast, drug delivery from the ointment was found to be least affected by additional external occlusion of the application sites. Similar findings with other topical corticosteroids (37,47) have been reported where in general, scalp applications, ointments and gels have better topical availability than lotions in the unoccluded mode.

Inclusion of penetration enhancers in the formulation of a base to increase corticosteroid delivery to the skin is currently receiving more attention in the scientific literature (42). There are miscellaneous penetration agents that have been investigated, for example, urea, dimethyl sulphoxide (DMSO), dimethylacetamide (DMA), urea, azone, propylene glycol, salicylic acid, resorcinol and numerous pyrrolidone derivatives (48,49). Some of these enhancers are already in clinical practice such as urea and propylene glycol. Each agent has characteristic effects on the skin and vehicle, for example, urea is believed to increase absorption of some corticosteroids due to its keratolytic activity and hydration of the SC.

1.3. THE MECHANISM OF BLANCHING

One of the properties of the topical corticosteroids is their ability to induce a blanching response after topical application. The blanching response is important because assessment of topical corticosteroid formulations is based on this property (section

1.5). However, the mechanism of how this corticosteroid-induced blanching is produced has not been fully elucidated (40,50,51). Fritz and Levine (52) demonstrated that the vasoconstrictive effect of noradrenaline in the mesoappendix of adrenalectomised rats does not occur unless the cortical extract is applied topically, suggesting that corticosteroids may support vascular tone by potentiating the pressor effects of adrenaline. A later report by Juhlin (53) showed no evidence of such potentiation in the vascular reaction of normal skin treated with fluocinolone acetonide. The latest findings into the investigations of a mechanism of vasoconstrictor action of topical corticosteroid were reported by Ahluwalia et al. (50). The researchers used betamethasone 17-valerate in rats and measured the blood flow using the improved laser Doppler velocimetry. The effect of a corticosteroid was compared with the vasodilator stimulus induced by local heating to the surface of the skin. Angiotensin converting enzyme inhibitors (captopril and enalapril) were also used for comparison purposes. Their results suggested that betamethasone 17-valerate may be acting in a manner similar to that of the angiotensin converting enzyme inhibitors to produce an inhibition of the flow responses in the skin and that this effect may be brought about by interfering with the action of vasodilator peptide(s) or protein(s). Although laser Doppler velocimetry is one of the techniques widely employed to measure both basal and stimulus-induced changes in cutaneous blood flow (54), other workers (55) have questioned its usefulness of measuring blood flow in an attempt to assess corticosteroid-induced skin blanching response. The more recent publication by Noon et al. (56) using the improved laser Doppler velocimetry indicated its imprecision in topical corticosteroid assays.

1.4. CORTICOSTEROID SKIN RESERVOIR

Knowing that topical corticosteroids can produce a blanching response even after some days following removal of the drug from the application site, the existence in the skin of a reservoir

for these drugs was first suggested by Malkinson and Ferguson (57). The researchers occluded the skin application sites for 16 hours following initial application of a corticosteroid to the skin and on removal of the occlusion material blanching was visible but faded after 10 to 16 hours. The application sites were subsequently re-occluded for 2 to 3 days and on removal of the occlusion, skin blanching was again observed. In addition the investigators noted that for some volunteers, blanching response up to 15 days after initial topical corticosteroid application was still visible. The experiments of Feldmann and Maibach (32) suggested that the deeper layers in the skin are implicated as well as the stratum corneum and that the subcutaneous fat may also be involved. Kukita et al. (58) reported that the reservoir for corticosteroids was located mainly in the horny layer although the hair follicles and sebaceous glands are also implicated. The mechanism of this reservoir is yet to be explained (50). Barry and Woodford (59) have disputed the existence of the reservoir for topical corticosteroids. They assessed blanching response using a multiple dosage regimen but could not establish the reservoir effect for more potent corticosteroids and it was again not possible to demonstrate a reservoir produced by hydrocortisone preparations. Wallace et al. (60) determined hydrocortisone concentrations from the skin samples of subjects in whom blanching response was assessed. The epidermal skin concentration of hydrocortisone was significantly greater in those subjects who exhibited blanching suggesting that an epidermal reservoir exists. The literature appears to show a general agreement among scientists (32,50,57,58,60) about the existence of a corticosteroid skin reservoir.

1.5. BIOEQUIVALENCE TESTING

Technological advances in analytical methodology and statistical development for the past 20 years have contributed significantly

in refining the science of bioequivalence testing (61). In recent years the design, performance and evaluation of bioequivalence studies have received major attention from the regulatory agencies, the pharmaceutical industry and academia (62). The best method to show that a test formulation is therapeutically equivalent to a reference formulation is to conduct clinical trials. To perform these trials for every new generic product is time consuming, costly and inconvenient (63). Clinical trials are laborious (40,63), and often lack sensitivity (64). Other bioassays that have been used for topical corticosteroids assessment include ultraviolet irradiation methods, stripping techniques, antigranuloma assays and cytological studies (65,67). These bioassays have not gained popularity over the years because they tend to be more tedious and more painful than the blanching assay or require the use of animals (65).

Of all the in vivo methods currently in use, the human skin blanching assay is the most frequently used bioscreening technique for assessing topical corticosteroid availability and potency (38,40,56,57,64-66,69,70). This is because the assay is non-invasive, simple, convenient and reproducible (40,51). The assay is based on the ability of corticosteroids to produce blanching or vasoconstriction in the microvasculature of the skin. This property relates to the amount of drug penetrating the SC from a delivery vehicle and, thus, is a basis for comparison of topical drug availability from two pharmaceutically equivalent topical corticosteroid formulations (9,71). Excellent correlation between the degree of blanching and clinical efficacy of the topical corticosteroid has already been established (6,72,73). Although many issues have been resolved concerning bioequivalence testing of drugs in general, some concerns remain regarding the experimental design and data analysis (61) of some protocols for particular group of drugs. For example, there is a major concern with regard to the human skin blanching assay

because various experimental protocols (9,40,74-76) have been implemented for assessing and comparing topical corticosteroid availability in the last 35 years. The major criticism of the blanching bioassay is that it requires the use of experienced observers to subjectively determine and rank the degree of skin pallor (blanching) induced by the topical application of a formulated product (51,64,66,69,77,78). Visual assessment has been deemed unacceptable for grading the corticosteroid-induced skin blanching response by a number of workers (64,66,77,78,), despite direct correlation between visual data and instrumental data (66,69,77). There is currently a need to develop an internationally accepted standard protocol that will solve the problems which arise in attempting to compare the results from multi-investigator and multi-center studies. In an attempt to address this issue, the United States Food and Drug Administration (FDA) recently released a Guidance document entitled "Topical Dermatologic Corticosteroids: In Vivo Bioequivalence" (section 1.6.). One of the main aims of the Guidance is to replace the subjective visual methodology with an objective instrumental method for the quantitation of drug-induced skin blanching intensity (64).

1.5.1. General features

The objective for bioequivalence testing is to test if the two products being compared exhibit the same therapeutic effect and safety profile. Bioequivalence testing originated from the concept of bioavailability which was developed in the early 1960's when it was discovered that the same active drug ingredient, in the same dose, but formulated in either similar or different products, might not have the same therapeutic and/ or toxicological properties (79). This finding prompted numerous efforts to establish definitions, guidelines, experimental protocols, specification for analytical methods, calculation of specific pharmacokinetic parameters and statistical procedures for bioavailability studies. These efforts have been extended to

bioequivalence where bioavailability of two similar products are compared. Bioavailability is defined as the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the systemic site of action. In bioequivalence testing the current practice requires that the rate of absorption is assessed by determining the maximum concentration (C_{max}) and time to reach maximum response (t_{max}), and the extent of absorption is calculated by comparing the area under the curve (AUC) values after dosing with test and reference formulations. Elze et al (80) reported that the rate and extent of absorption described above should be replaced by more adequate concept of the similarity of concentration/time profiles. The two medicinal products should be considered bioequivalent if their concentration/time profiles are so similar that they are unlikely to produce clinically relevant different and/or adverse effects. In order to quantify the similarity, suitable shape characteristics and appropriate bioequivalence limits are needed (81). The classical 90% Confidence Interval is the current acceptable statistical evaluation to declare bioequivalence for most drugs intended for systemic action (79).

The usual procedure for conducting a bioavailability test of two formulations, is to collect plasma and/or urine samples from each subject in a trial following administration of a dose of the drug (82). For topical corticosteroids, this procedure is not practically possible because the blood circulation removes the drug from the site of action (83) unlike drugs that are administered for their systemic action where the general blood circulation distributes the drug to the target tissue. Polano et al. (84) suggested that bioavailability for topical corticoids should be referred to as the difference between the amount of drug that is applied to the skin and the amount that reaches the site of action. Barry et al. (85) reported that comparative bioavailability (BA) between two formulations (bioequivalence) as assessed by the blanching assay should be expressed as follows:

$$Bioavailability = \frac{score\ achieved\ by\ the\ test\ product}{score\ achieved\ by\ the\ most\ active\ formulation} \quad \text{Equ. (1)}$$

which may be compared to the usual relationship in pharmacokinetics where

$$Bioavailability = \frac{amount\ systemically\ available\ from\ a\ dosage\ form}{amount\ systemically\ available\ from\ the\ optimum\ dosage\ form} \quad \text{Equ. (2)}$$

Slightly different values of bioavailability could be obtained if the scores referred to above are the percentage total possible score values or the area under the curve values (85).

For topical corticosteroids which have been assessed visually, the rate and extent of drug absorption has been assessed by close examination of the blanching profiles, the AUC values and by using appropriate statistical analysis such as Chi-squared or analysis of variance (ANOVA) (40). The profiles and AUC values indicate the blanching abilities of test and reference formulations (65). The profiles also provide an indication of the rate of onset and duration of action of preparation, as well as indicating the maximum blanching response and time to reach peak maximum response (65,71).

1.5.2. Visual method

Since the inception of the human skin blanching assay in 1962 (9), assessment of topical corticosteroid formulations including bioequivalence has been successfully performed using the visual method. However, despite the success some researchers (64,66,77,78) still comment that the visually-assessed human skin blanching assay is too subjective. Criticisms about the subjective method are that subtle differences in blanching responses are difficult to assess (86) and attempts to estimate and assign numerical value to the degree of skin blanching may lead to invalid conclusions (87). Other workers (64,66,77) have suggested that the subjectivity of the visual assessment to grade

blanching intensity induced by the topical corticoid is influenced by the person evaluating the blanching response, by the environment in which the blanching response is evaluated and by the biological and physicochemical differences in skin among the test population. These points are not valid based on the research that has already been done and are discussed below.

Method of assessment

Considering the problem of grading or ranking, McKenzie and Stoughton (9) in the initial experiments made no attempts to grade the blanching response which was recorded as present or absent, that is, a simple yes or no evaluation of whether or not blanching is present at each application site. The disadvantage of this method is that it gives no indication of the degree of observed blanching response. It may therefore be useful in experiments in which corticosteroids elicit only a weak blanching response or where the minimum effective concentration of corticoid is needed to be ascertained (65,88). Sarkany et al. (89) and Barret (90) appeared to be first group of workers that attempted to grade the blanching response using an arbitrary 0-3 intensity scale where 0 represents no blanching, 3 represents intense blanching and 1 and 2 represents the intermediate grades of blanching. Another method that has been used involves a direct comparison of the observed blanching or absence thereof at different application sites (40,65). For this method, the observers record that one site exhibits more blanching than the other, both sites exhibit equal blanching or that no blanching is present at either site. This method is useful for the direct comparison of different formulations applied as pairs (40). The disadvantage with this method occurs when one preparation elicits very slightly more blanching than the other . This is because the statistical analyses are likely to show significant differences whereas the graded response analysis would show non-significant differences (65). Of all the three methods, the 0-4 graded scale is the most sensitive (40) and has been successfully

used by many workers(38,45,51,65,66,69,91).

Observer training

It has been reported (66,77) that interpretation and scoring of the blanching may vary among the investigators although it has already been repeatedly (38,40,42,56,65,69,71) stated that experienced observers are able to grade blanching response without difficulty. Other workers (51) reported that subjective assessment should be performed with care and training of observers is essential. At face value, the necessity of having experienced observers for recording the intensity of blanching may seem a disadvantage of the visual blanching methodology especially if one assumes that gaining the experience requires a lengthy training period. In most reports, observations of the blanching response appear to have been made by researchers themselves, although there have been reports in which some observations were made by the volunteers (92,93). The experiments of Gruvstad and Bengtson (92) showed that the scores recorded by the volunteers were lower than those of experienced observers although these differences did not influence the results or conclusions drawn from such experiments. Others (94) have reported that the inexperienced reader always over-reads skin blanching compared with the experienced investigator. Meyer (65) analyzed data from two consecutive blanching trials and found that the differences in the AUC values calculated from the scores recorded by the inexperienced observer were smaller than the pooled AUC values calculated from the three experienced observers. It was also found that the blanching profiles plotted from the results obtained by the learner in his second trial were smoother than for the first trial. In addition, the rank order of the AUC values of the different topical corticosteroid preparations by the inexperienced observer was the same as for the pooled results of the experienced observers in the second trial which was not the case in the first trial. Their results indicated that the experience gained by the inexperienced

observer in two trials was sufficient to include him as the third experienced observer in subsequent trials. These conclusions were reinforced by the results reported by Smith et al. (71) in which three consecutive blanching trials were conducted where a learner-observer was included for training purposes. The learner recorded lower scores than the pooled results of two experienced observers. The interesting part was that even in the first attempt at scoring, the rank order assessment of the two preparations and the magnitude of the response difference between the preparations were the same for the learner and the experienced observers. In the second trial, the rank order and the response difference were very close to those observed by the experienced observers. The results in trial 3 indicated that the learner was sufficiently skilled to be classified as an experienced observer. It should be noted that this training procedure may, however, take longer times in laboratories where observations are not made at several reading times over an extended period. In the experiments of Smith et al. (71) described above, three trials would make 8640 observations and this could be theoretically accomplished in 6 days and hence, the training period is not as lengthy as may be initially assumed. Although it may take less time to teach a novice to use an optical instrument for estimating blanching response, a period of familiarization in the trial before readings may be generated with confidence is also required. Thus, the short period required to train visual observer does not appear to detract significantly from using the observer to subjectively grade blanching response.

Biological differences among subjects

There is little work that has been done concerning the biological and physico-chemical differences in the skin among test sample with regard to the human skin blanching assay. There is only one extensive work by Meyer (65) on the analyses of blanching responses in individual volunteers. The results demonstrated the

expected inter-volunteer biological variability and the investigator suggested that the subjects selected for the trial should represent a wide range of blanching response (poor, average and good blanchers) for visual assessment. This is because it has been observed that it is difficult to differentiate any differences in blanching response at adjacent sites if only good blanchers are used. In order to overcome the physiological variations in the vasoconstrictive response to corticosteroids at different forearm locations in an individual, it was suggested that a large number of application sites are required along the entire length of the forearm. However, the application sites should not be near the wrist and elbow regions. The biological and physico-chemical differences in the skin among test population due to skin disease or presence of scars and blemishes, can be minimized by inclusion of volunteers whose forearms do not present these features (64). No sex-related differences in blanching responses were found (65) and therefore both females and males can be used in the trial. In addition, Meyer (65) reported that there was no significant difference in the blanching response between left and right arm in agreement with other results (64,69). The assay usually uses healthy, Caucasian men and women who have been pre-screened for positive blanching response using a standard corticosteroid product (95). The subjects to be used should not have received corticosteroids for at least 6 weeks prior to the study. The stated age of the volunteers that have been used in the blanching assay has varied from 12 to 74 years (75,96,97).

Environmental conditions

Pershing et al. (66) suggested that the environmental conditions in which the blanching response is evaluated may also influence the interpretation of the response due to temperature, humidity, lighting and position of the skin site during assessment of the blanching response. The environmental variables such as ambient temperature and humidity is minimized in the trial by processing

all the volunteers concurrently at intervals of five minutes each. There is little work that has been done concerning the effect of temperature and humidity in the assessment of blanching response. The work by Meyer (65) indicated that temperature and humidity appears not to affect the blanching response although definite conclusions could not be drawn. Barry et al. (98) and Poulsen et al. (99) have described lighting conditions under which the blanching response was assessed. Their results showed that the best conditions were the use of fluorescent lighting with no oblique light source from a window or incandescent side lamp. Other workers (100) have assessed the blanching response under good natural light and obtained reliable results. It has been reported (98,99) that provided the lighting conditions are adequate and remain standard for each observer, the overall results of the comparative blanching response should not be affected. With respect to the positioning of the arms, it has been suggested (98,101) that they should be held horizontally or slightly upwards to prevent blood vessels enlarging when the arms hang downward so that skin blanching assay is not affected.

All the problems and solutions of the visual assessment discussed above have been described in detail by others (40,65,71) in an attempt to produce an optimized visual blanching methodology. This methodology has been demonstrated to be sensitive, accurate and reproducible for comparing the bioavailability and potency of topical corticosteroids.

1.5.3. Instrumental methods

Quantitative assessment of the intensity of vasoconstriction would allow more advanced statistical analysis of the data than is currently possible with the qualitative, visual rankings (51,95). Towards this goal, scientists have evaluated a number of instrumental techniques with which the assessment of skin pallor following controlled corticoid application may be carried out (95). Most of the techniques that have been developed are

insensitive (51), for example, reflectance spectrometry (102-107), laser-Doppler velocimetry (55,108), combination of laser-Doppler with reactive hyperaemia (109), thermography (110) and tristimulus colorimetry (111,112). While some of the reflectance spectrometry techniques have been promising, other methods especially those of the laser-Doppler velocimetry (LDV) and thermography have been less successful.

Photoelectric plethysmography

One of the earlier techniques to be used in the blanching assay was the photoelectric plethysmographic instrument which attempts to estimate the volume of blood in the vessels at the skin site where the corticosteroid formulation has been applied (113). The instrument failed to show significant reduction in the blood flow when the blanching response was clearly visible with the naked eye after topical application of an alcoholic solution of betamethasone 17-valerate. The researchers concluded that it is not sensitive enough to measure skin blanching.

Laser-Doppler velocimetry (LDV)

Amantea et al. (55) used the LDV technique to measure the effects of steroids upon human skin blood flow. Despite the appearance of blanching easily seen by the naked eye after topical application of fluocinolone cream, the LDV voltage output was not significantly different from the same skin site prior to corticosteroid application. The researchers, therefore, decided to investigate the effects of topical corticosteroid treatment following topical administration of methyl nicotinate, a powerful vasodilator. The LDV could not record any effect produced by the topical steroid. The investigators concluded that LDV did not provide a clear-cut system with which the blanching response of topical steroids could be investigated. Smith et al. (95) compared the skin blanching induced by betamethasone 17-valerate and hydrocortisone cream assessed visually and also by LDV. The visual assessment data were similar to those observed previously

and clearly demonstrated significant differences between the response profiles of the two corticosteroids in both application modes. In contrast, the LDV assessment yielded relatively imprecise data that did not demonstrate any significant differences between the two formulations in either application mode. The authors concluded that the LDV was not a viable alternative to visual assessment in topical corticosteroid bioassays. Bisgaard et al. (109) have, however, been successful by combining LDV with induced reactive hyperaemia which was attained by arterial occlusion with a blood pressure cuff on the upper arm. The instrument was found to show the expected rank order of potency in agreement with other results (66). Other workers (55), have, however questioned the usefulness of reactive hyperia in assessing blanching response.

Reflectance spectrometry (RS)

Using the improved reflectance spectroscopic technique, Andersen et al. (114) endeavoured to explain why previous laser Doppler experiments, in the assessment of corticosteroid-induced skin blanching have failed. The authors explained that since skin blanching was predominantly due to vasoconstriction and only the most potent steroid caused a significant decrease in oxyhaemoglobin and blood flow, a moderate vasoconstriction, although easily seen by the naked eye, may not be detected by the flow-dependent laser Doppler. The reason given was that LDV detects the Doppler shift, caused by moving erythrocytes near the surface of the skin and thereby measures the cutaneous blood flow in the dermis whereas the RS measures the fraction of incident polychromatic light not absorbed by the skin chromophores in the epidermis or upper dermis. Decreased venous blood may therefore be easily seen visually and measured by the RS but cannot be detected by LDV. The RS technique described above showed good results similar to those found visually whereas the results from the LDV were not reliable. A more recent paper by Noon et al. (56) compared the improved LDV, RS and a perfusion imager to

assess skin blanching response induced by beclomethasone dipropionate cream at three different concentrations. Using the visual score, blanching was detected at all concentrations of steroid. Neither LDV or a perfusion imager detected vasoconstriction at any concentration. By contrast, the RS successfully recorded blanching response but only at two concentrations. The researchers concluded that the human eye remains the most sensitive tool to measure skin blanching.

Thermography

Attempts have also been made to measure temperature changes of corticosteroid-treated skin (110). Sommerma (115) and Kiraly and Soos (111) found that the differences between normal and corticosteroid-treated skin were too small to be accurately measured by thermography. Aiache et al (110) have found a decrease in skin temperature on the site where the corticosteroid was applied but there was no correlation between temperature and the blanching assay.

It is clear from the brief review of the instrumental methods described above that they are not sufficiently accurate for estimation of the blanching response compared with the visual method.

Chromameter

Recently, the technique of tristimulus colorimetry (using the Minolta Chromameter CR200) has been employed with some success (51,66,77,116,117). It should be noted that some of these authors were using the instrument not to evaluate blanching response induced by a topical corticoid. For example, Babulak et al. (116) obtained excellent correlation ($r = 0.97$) between chromameter and visual data when estimating erythema produced by soap preparations. Smith et al. (95) commented that the use of chemicals in attempting to quantify the corticosteroid-induced skin blanching which itself is not yet fully understood merely

complicates the bioassay and is therefore unwarranted. There is only one published paper (111) which clearly found that the chromameter offered no advantage over the visual data. All other available papers (66,69,77,116) indicated good correlation between visual and chromameter data. Although Chan and Li Wan Po (51) proposed that the chromameter should form the basis of an internationally accepted standard procedure for measuring the blanching response, they based this proposal without comparing their data with the visually-assessed data. However, they also suggested that a rigorous statistical analysis comparison of the performance of the naked-eye assessment and chromameter would be worthwhile. In addition, they noted that the sensitivity of the chromameter to be able to discriminate between corticosteroids remains to be tested. Waring et al. (69) conducted a study on the performance of the Minolta Chromameter CR200 in assessing the blanching response produced by topical corticosteroids. The authors suggested that the use of the chromameter requires careful development and more validation before its use in the bioequivalence testing of corticosteroid preparations. The instrument has not been validated yet to replace the visual method despite the FDA Guidance (section 1.6.) advocating its use. The development and validation of the chromameter is therefore very important if the instrument is to be used in the future for assessing bioequivalence testing of topical corticosteroids. The advantages of the instrument are that it is portable (69) and that the ease of obtaining data makes efficient data handling essential for the derivation of discriminative parameters and their subsequent statistical analysis (51). However, its precision in measuring the skin blanching response needs evaluation.

The Minolta CR-200 chromameter is a small, portable, lightweight tristimulus colour analyzer for measuring reflected light colour. It contains a flexible, hand-held probe (the aperture of the probe is 8mm in diameter) which is used to measure skin

blanching. A pulsed Xenon arc (PXA) lamp in a mixing chamber provides diffuse, even lighting over the skin surface. Six high-sensitive silicon photocells, filtered to match the Standard Observer Response proposed by the Commission Internationale de L' Eclairage (CIE) (118) are used by the meter's double-feedback system to measure both incident and reflected light. This double-beam feedback system is used to ensure that lighting remains consistent for each measurement. When the light emitted from the Xenon lamp strikes the silicon photocells, light energy is converted into electrical signals and is sent to the microprocessor where it is adjusted for the illuminant condition desired and then converted into coordinates for the chosen chromaticity or colour space as described below: Chromaticity may be measured in either Y_{xy} or L-, a- and b-scale coordinates; colour difference in ± change in Y_{xy}, ± change in (L-, a- and b-scale). Data can be converted between coordinate systems or between chromaticity and colour difference measuring modes by the meter. The meter can be calibrated to both a standard white reflective plate and a user-selected calibration surface. The measuring head has a choice of two CIE illuminant conditions: Illuminant C (6774K) and Illuminant D_{65} (6504K) which provide lighting conditions that closely approximate average daylight. For the purposes of the research in this thesis, the L-, a- and b-scale parameters will be described. The L-scale parameter expresses the relative brightness of colour ranging from black to white; the a-scale relates to redness or greenness and the b-scale is the colour range from blue to yellow. Theoretically, a change in the skin colour as the vasoconstriction response changes in intensity, will be indicated by a change in these indices.

The operation of the chromameter is such that is calibrated using the white calibration plate provided by the manufacturer before the experiment. The desired lighting condition is chosen. The aperture of the probe is rested lightly on the skin site.

Readings taken through the measuring head are processed by the built-in microcomputer and presented digitally (as three numbers, that is, the L-, a- and b- scale) on the custom-designed liquid crystal display and can be transferred to a separate computer through the data-output terminal.

1.6. THE FDA GUIDANCE: TOPICAL DERMATOLOGIC CORTICOSTEROIDS: IN VIVO BIOEQUIVALENCE

The Guidance provides guidelines on bioequivalence testing of topical corticosteroids with a view to standardize the human skin blanching assay. It is a general guidance intended to apply to topical corticosteroids of all potency groups. Based on the available literature concerning some of the issues in the Guidance, these points require critical evaluation. Most of these critical issues in the Guidance will be investigated in this thesis.

1.6.1. Measurement of blanching response

The Guidance states that in an era with increasingly sophisticated methods to detect changes in light, temperature, pressure and other physical and chemical changes, the use of human observers to assess the magnitude of a pharmacodynamic effect becomes increasingly inadequate. Earlier discussion of visual and chromameter methods indicated that the naked eye is still the best method whereas the chromameter needs validation. The Guidance, therefore, fails to advocate the use of chromameter only as providing adequate data. In addition, although the chromameter a-scale parameter is recommended for use, other workers (69,119) who have used the instrument have found good results with the chromameter L-scale parameter. One report by Caron et al. (119) found better results with the L-scale values than with the a-scale values. The sensitivity of the chromameter parameters, therefore warrant investigations (51,69,70).

The proposed methodology includes a pilot dose duration-response

study to determine the appropriate dose duration for use in the subsequent pivotal bioequivalence study. The performance of a pilot study involves the use of non-linear pharmacodynamic models to determine the appropriate parameters which are essential in the pivotal study. As with all bioanalytical methods, this methodology of the human skin blanching assay proposed by the FDA requires careful validation before it can be adopted as a standard protocol.

1.6.2. Pilot study

The aim of the pilot study is to provide the dose-response information required to determine the parameters ED_{50}, D_1 and D_2 to be used in the subsequent pivotal bioequivalence study. The study also provides an estimation of the proportion of subjects expected to meet the minimum D_2/D_1 criterion although the validity of this ratio has not been proven. This criterion is discussed in the pivotal study (section 1.6.3.). ED_{50} is the dose duration equal to approximately half-maximal response and D_1 and D_2 values are dose durations which correspond to approximately 33% and 67%, respectively, of the maximal response. It has been suggested (64,120) that this is the sensitive portion of the dose duration response curve even though there is no published evidence to support the superiority of the ED_{50} value over the reliable 6 hours dose duration (38,40,45,51,65,66,69,71) when used in topical corticosteroid bioequivalence assessment.

Since the determination of bioequivalence in the pivotal study is entirely dependent on the results of the pilot trial, the manner in which the pilot study is performed and analyzed is critical. Any error in the assessment of results from the pilot study would affect assessment of bioequivalence in the pivotal study and consequently may lead to invalid conclusions being drawn.

Dose duration

The suggested dose durations for use are 0.25, 0.5, 0.75, 1, 1.5, 2, 4 and 6 hours. These dose durations should be equally divided between the two arms. One of the problems about this recommendation is that each dose duration will be used for only one site per arm. In a normal visual bioequivalence study there would be at least 12 sites per person per product, when comparing two products at any one dose duration (40,45,71). The collection of smaller data sets can be a problem in assessing the results. It is normally good scientific procedure to collect as much data as possible so that the results can be generated with confidence. Results where a small sample was used have been found to be misleading and hence were treated with caution (61). This is substatianted by the review literature of Chen et al. (121) concerning regulatory aspects in bioequivalence trials who concluded that there was low statistical power of detecting differences for various drugs almost in all studies which were conducted using small samples.

Another problem about the recommendation is the use of short dose durations (0.25 - 2 hours) in the study. Based on the pilot data as in the Guidance, it appears as if the ED_{50} of which bioequivalence testing will be carried out is probably expected to be obtained around these short durations. The FDA assumes that maximum blanching response is expected to occur at 6 hours after application and hence differentiation between the test and reference product at the plateau of response may be inaccurate. However, research has shown that maximum response does not occur at 6 hours dose duration (122). Magnus et al. (122) visually assessed the effect of dose duration on the blanching response in the occluded mode of application. The formulation used was a commercially available betamethasone 17-valerate cream and the occlusion times used were 2, 4, 6, 8, 10, and 12 hours. The results of the study showed that the observed blanching increased with an increased occlusion time, a plateau effect being reached

at 10 hours. The 10-hours occlusion maximum may represent the maximum blanching ability of the corticosteroid or it may be that the 10-hours occlusion time causes maximum hydration of the stratum corneum. As a result of this report, most workers (38,40,45,51,65,66,69,71) in this field have used 6 hours dose duration with success. Researchers who have used the chromameter at 6 hours (51,66,69,77) also reported good correlation with the visual scores in discriminating different corticosteroid formulations. The paper by Clarys et al. (70) using the chromameter found discrimination between different Halcinonide concentrations with only the 2 hours application period. With longer application times (12 and 18 hours), a similar blanching was recorded for all Halcinonide concentrations. It should be noted, however, that the investigators used a single observation time on which to base their results. The objection to the use of single observation times is that single-point comparisons cannot yield important information such as the maximum response, duration of response and AUC values which are important in assessing bioequivalence of topical corticosteroids (71). Another problem with single point measurements is that it has often been found (45,74,76,92,94,123) that a corticosteroid formulation can produce a relatively high blanching response in the early part of the trial and can show a rapid decline in response at later times and vice-versa. The use of a single point assessment may, therefore, result in invalid conclusions because the profiles of two topical corticosteroid formulations may be coincident at certain times while deviating at other times. Other workers (77) also showed that drug uptake into the SC occurred up to 2 hours dose duration. Dose duration greater than 2 hours demonstrated no further uptake of drug by the SC. Their results suggested that dose application periods greater than 2 hours may be inappropriate for testing bioequivalence of topical corticoid products. However, the authors used skin stripping methods which are not currently accepted by the FDA (64) in the assessment of topical corticosteroids.

Amount of topical corticosteroid

The Guidance does not have guidelines concerning the amount of the corticosteroid used. The implication is that researchers may use any amount of corticoid and the results would be expected to be valid. However, various investigators (75,122,124) have found that increasing the concentration of the topical corticoid in a common vehicle increased the degree of blanching. Magnus et al. (122) demonstrated that the observed blanching response increased when 1.6 to 4.8 mg of betamethasone 17-valerate cream was applied over 7 x 7 mm application site, but over 4.8 mg did not increase the response further. As a result of this, many published papers (38,40,42,45,71,96,122,125) have successfully reported the dose of approximately 3 mg over 7 x 7 mm application site as a standard procedure. Adams et al. (126) mentioned that a study conducted near the plateau of response does not show potential differences in drug delivery between two products. The implication is that if any amount of corticosteroid is used in accordance with the FDA guidelines, researchers may conduct their experiments at a blanching plateau response and hence the results that they will obtain may be invalid. The explanation for the attainment of a plateau could be that at the concentration level of 4.8 mg of cream per 7 x 7 mm site, the maximum blanching response of the corticosteroid has been reached, probably as a result of saturation of corticoid receptors (122).

Another problem is that there are no guidelines concerning the size and shape of the skin sites where the amount of corticoid will be applied. With respect to the size and shape, investigators (74,94) have commented that smaller square sites (7mm X 7mm) provide better observing conditions than larger, round sites. A possible disadvantage of circular test sites is that the circular corticoid-induced blanching area may be confused with the nonspecific discolourations of the skin (72,73,92) for visual assessment although this would probably be better for the chromameter.

Blanching response assessment.

The Guidance states that the readings should be taken before drug application (time zero values), at 0, 2, 4, 6, 19, and 24 hours after product removal, and also at two untreated control sites each time the reading is taken. This applies for the staggered application and synchronized removal method, that is, applying the formulation at different times but removing them simultaneously. The visual data do not need untreated control sites because visual assessment involves comparing the treated site with the surrounding untreated skin area. If the synchronized application, staggered removal method is used, the observation times should be 6, 8, 11, 24 and 28 hours after drug application. It is not clear why there are 6 observation times for one method and 5 observation times for another. In normal visual blanching trials, 10 observation times are used. Smith et al. (38,71,95) and others (40,65,76) commented that bioequivalence studies of topical corticosteroid formulations cannot be made without analysis of the full blanching profile following multiple readings over prolonged observation times. Whether the use of 5 or 6 observation times as in the Guidance will produce similar results to the use of 10 or more observations still require investigations.

Data treatment.

The zero-time readings (chromameter values at the sites before drug application) are subtracted from the raw chromameter data at each site (treated and control sites). The mean values of the corrected control sites on the same arm are subtracted from the corrected treated site values. The AUC value is then determined using the trapezoidal rule. Pershing et al. (66) reported that no significant differences in diurnal skin colour were observed in the blanching trial between anatomical locations on the same arm or between left and right forearms using the chromameter. The implication therefore is that chromameter zero-time readings are not necessary to correct colour changes that may occur during

the trial. Waring et al. (69) reported that inclusion of the zero-time value is a redundant arithmetical manipulation which does not impact on the final result. It would therefore be interesting to compare the blanching results with and without zero-time readings.

Pharmacodynamic modelling

The Guidance stipulates that the AUC values determined at each dose duration for all the subjects (minimum 12) should be fitted to an appropriate model. However, the AUC values should be fitted based on the assumption of a nonlinear mixed effects population model or based on non-linear least squares regression techniques, that is, pooling individual observations from all subjects (naive pooled data method). Although various models are available to express the relationship between the drug dose and effect, the FDA suggests that the E_{max} model or the related sigmoid model may be used to analyze the results according to the following equation:

$$E = E_o + \frac{E_{max} \cdot D}{ED_{50} + D} \qquad \text{Equ. (3)}$$

Where E is the effect, E_0 is the baseline effect E_{max} is the maximum effect and ED_{50} is the dose (D) at which the effect is half-maximal (ED_{50}).

The use of pharmacodynamic modelling as stipulated in the FDA Guidance is a new concept with regard to the human skin blanching assay. The problem with this recommendation is that the guidelines do not specify a model to be used and, therefore, different workers may probably obtain different results with the use of different models. Hence, the application of a specific pharmacodynamic model for assessing the bioequivalence of topical corticosteroids requires investigation. There are no published papers in the literature concerning the usefulness and suitability of pharmacodynamic modelling in the blanching assay.

More recently Marston et al. (127) used three different models for the chromameter data in accordance with the FDA guidelines and concluded that the different modelling approaches may result in clinically significant differences in population estimates.

The Guidance states that a primary issue requiring resolution in the application of the blanching assay to assess bioequivalence is to establish whether at the strengths of the formulation to be used in the assay, the microvasculature of the skin responds linearly. The justification of using pharmacodynamic modelling to assess linearity of the response is questionable. This is because if visual data are used to justify linearity through pharmacodynamic modelling as in the Guidance, the results may not be reliable. Visually-assessed data consists of a series of grades and is not numerical in the statistical sense, that is, a grade of two is not two times a grade one intensity. It appears as if the Guidance is attempting to compare the blanching trials with oral bioequivalence studies, hence the recommendation of pharmacodynamic models. In blanching trials, a response intensity is estimated and the products are dosed simultaneously whereas in oral studies, the amount of drug is determined by a suitable instrument and the products are dosed sequentially. Therefore, the accuracy, linearity and precision of the instrument from one dosing period to the next must be assured.

Pharmacodynamic models are designed to relate the drug effect to drug concentration or time. The objective of a pharmacodynamic model is to minimize the overall difference between the observed experimental data and the calculated data predicted by the model. The main problem that has been associated with non-linear pharmacodynamic modelling is the choice of the correct model. It is recommended (128) that the selection of a model should include careful calculation of initial estimates, repeated analyses with different sets of initial parameter and careful evaluation of the program output. After selecting and describing an appropriate

model, the investigator may need to select a weighting scheme for the data to be analyzed. Weighting schemes are especially useful when there is a wide range of observed values (128,129). The weighting schemes are also important when there is a significant error or uncertainty in the observed data (128). When the coefficient of variation (CV) about a series of data points is constant, that is, ±5% error in each data point, a weighting scheme can be estimated as follows:

$$Standard\ deviation = Observed\ value \times CV \qquad \text{Equ. (4)}$$

$$Variance = Observed\ value^2 \times CV^2 \qquad \text{Equ. (5)}$$

With a single data set, the CV^2 can be ignored. However, when fitting more than one data set simultaneously, this value is useful. Thus the weight would be

$$Weight \propto \frac{1}{Observed\ value^2} \qquad \text{Equ. (6)}$$

The weighted sum of the squared residuals (WSS) or any other objective functions such as the Akaike's Information Criterion (AIC) are the major statistical parameters provided by any of the non-linear programs (128). The most commonly used parameter is the AIC value which is calculated by the following equation:

$$AIC = N \ln WSS + 2 \times p \qquad \text{Equ. (7)}$$

where N is the number of data sets, WSS is the weighted sum of squared residuals between the observed and calculated data and p is the number of estimated parameters. The AIC value is calculated for each model. The model producing the lowest value is considered to be the better model (129,130).

The correlation coefficient (r) and the coefficient of determination (r^2), are often provided by most mathematical programmes. Both terms indicate closeness of fit between the

observed and predicted values, with a value of one representing a perfect fit. Most programmes provide the final best fit estimates with an indication of the uncertainty in these values. The uncertainty in the values may be expressed as a standard deviation, confidence interval and coefficient of variation. The lower these estimates, the better the model. The overall conclusions with regard to the use of models is that the performance of any model should be tested using different parameters such as the AIC, SD and r values to ascertain the precision of a model in describing the observed data. The results of the chosen model can also be validated by describing the observed data with other reliable methods (128).

1.6.3. Pivotal bioequivalence study

The aim the pivotal study is to document in vivo bioequivalence between the test and reference product using the dose durations determined from the pilot study. Forty to sixty subjects must be used in the study. The D_1 and D_2 values estimated from the pilot study are used to select volunteers that blanch sufficiently for inclusion in the statistical analysis of bioequivalence assessment. The criterion for selection of volunteers is such that:

$$\frac{AUC\ at\ D_2}{AUC\ at\ D_1} \geq 1.25 \qquad \text{Equ. (8)}$$

where AUC at D_2 or D_1 is the average AUC value of both arms at particular dose duration. Only individuals who meet this criterion are described as good blanchers and therefore must be included in the data analysis. No explanations are given in the Guidance concerning the validity of using this criterion. In addition there are no published reports in the literature which justified the use of this criterion in the blanching assay. One would therefore question how the 1.25 value was obtained. In addition, if the results of the criterion suggest that there is only one good blancher or none, do we carry out a statistical analysis based on only one individual or even few subjects?

Until such questions are answered, the Guidance fails to provide guidelines for which bioequivalence testing of topical products can be carried out with confidence.

The three dose durations determined from the pilot study are ED_{50} D_1 and D_2 which have been described already. Eight sites per arm for each volunteer are used: two sites are for test product at ED_{50} dose duration, the other two sites are for reference product at ED_{50} dose duration, one site for reference formulation at D_2 dose duration, one site for reference formulation at D_1 dose duration and the remaining two are untreated control sites. The problems of using few sites (D_2 and D_1) in assessing the blanching response have already been previously discussed.

The data are treated in the same way as explained for the pilot study except that no pharmacodynamic modelling is involved in the pivotal study. After determining the AUC values at each dose duration for an individual subject, the D_2/D_1 criterion is used to determine the number of "acceptable" or good blanchers. The AUC values of the "acceptable" blanchers at a dose duration of ED_{50} value are the appropriate data for statistical analysis. It should be noted that the current FDA Guidance does not have guidelines on the statistical treatment of visually-assessed data.

The problem concerning the FDA guidelines is that the statistical method recommended to determine the significance of the results is Locke's method (131). This is because despite this recommendation, there are no published papers in the literature which reported the use of the Locke's method in the blanching assay. Over the years, the statistical tests that have been used to assess bioequivalence have been the null hypothesis tests such as the Student's t-distribution tests, analysis of variance (ANOVA), etc. These tests simply assess whether the two products being compared in a trial are bioequivalent or not. Fluehler et

al. (132) and others (133) have suggested that these tests do not indicate how close the mean biavailability indicator (AUC) of test product ($\overline{X}_T$) is compared to the mean biavailability indicator of reference product ($\overline{X}_R$). In other words they do not indicate whether the difference in the $\overline{X}_T$ and $\overline{X}_R$ values obtained for the sample is representative of the population. Hauck et al. (134) criticized the tests by saying that the statistical procedures that establish whether the two products are equal or not address the equality issue rather than the bioequivalence concept. The reason given was that in practice it is recognized that no two formulations will result in bioavailability profiles which are exactly alike. It is probably for the above-mentioned criticisms that the FDA recommends the use of Locke's method which attempt to indicate if the results in the sample are best estimates for the population by using the concept of confidence interval limits. Locke's method is fully described in chapter 3.

Locke's method has been developed to be applicable for two period crossover experiments (131). In such experiments, each subject is randomly assigned to either the sequence of reference/test (RT) formulation or the sequence of test/reference formulation (TR). This means that the subjects within sequence RT receive the reference formulation at the first dosing period and after sufficient time to allow the drug to be completely metabolized and/or excreted from the body, the subjects then cross over to receive the test formulation at the second dosing period. It has been reported (131,136,137) that in addition to the effects of a drug in the body, the mean bioavailability of the two formulations will be affected by the effects of dosing the subjects at different times (period effects) and the effects of dosing the subjects in a sequence form at different times (sequence effects). However, Locke's method as in the FDA Guidance does not involve these period and sequence effects and this aspect is appropriate for the human skin blanching assay because the two products are applied at the same time in any one

trial and in any order. Hsu et al. (79) recently reported good results using Locke's method but for orally administered drugs. With respect to the blanching assay, most workers who have used the chromameter to quantify the blanching response induced by the topical corticosteroid have used either ANOVA or student's t-distribution tests (56,66,69,70,77,138,139). Locke's method, therefore, requires evaluation if it is to be used with confidence in the blanching assay.

CHAPTER TWO

METHODOLOGY AND EVALUATION OF RESULTS.

2.1. THE HUMAN SKIN BLANCHING ASSAY METHODOLOGY

The methodology of the human skin blanching assay described by Smith et al. (71) was performed for the first experiment, that is, the preliminary trial for novice observer training. The same methodology was used for all subsequent trials but modified in order to comply with the FDA guidelines (64).

Novice Observer Training Trial

A total of twelve healthy male and female Caucasian volunteers were selected from a panel of pharmacy students who were known to elicit a blanching response to a standard preparation (Betnovate cream containing 0.12% betamethasone 17-valerate). The screening of volunteers involved applying Betnovate cream to the forearm for six hours. After this period, the residual formulation was washed with soap and distilled water and patted dry with a tissue. After washing, the corticosteroid-induced blanching response was assessed an hour later. The volunteers who were used in this trial included those who were called poor, average and good blanchers. This is because it has been observed in our laboratories that there are no significant differences in blanching intensities at adjacent sites. The inclusion of average and poor responders allows the differentiation between responders that may not be noticed in the good blanchers (65). Other workers (64) have mentioned that the data of individuals whose blanching response is difficult to distinguish at adjacent sites are unreliable and therefore their data must not be included in the statistical analysis. Volunteers were not included in the trial if they used any form of systemic medication or had used topical corticosteroids for at least one month prior to the investigation or their arms had scars or blemishes. All the subjects were aware of the purposes of the

study and the methodology employed. They were also assured that only topical corticosteroids of known safety were to be applied on their forearms and that they could withdraw from the trial at any time, should they wish. A written informed consent was obtained from each subject and ethical approval was obtained from the Rhodes University Ethical Standards Committee in compliance with the Declaration of Helsinki (1964) and its subsequent amendments. Volunteers presented themselves for the application of formulations at given times between 7:30 a.m. and 8:30 a.m. to give an average application time of 8:00 a.m. All the volunteers in the trial were processed sequentially on the same day at intervals of approximately five minutes, in order to minimize any possible effects of environmental variables such as temperature and humidity. The subjects were processed in the same building for the whole trial. They were required to remain in the building housing our laboratories during the processing period and observation times with the exception of attending lectures, laboratory sessions and having meals. In addition, they were told to continue with their normal activities, but were required not to partake in any heavy exercises, were requested not to bath or shower or consume alcohol for the duration of the trial.

The preparations used were Dermovate lotion (Genpharm, Canada), an experimental lotion (each containing 0.025% clobetasol 17-propionate) and Placebo lotion. Four different application charts (Appendix B) were used in the trial which ensured that no preparation was applied twice to the same area on the forearms of any subject. The random allocation of the application charts containing the pattern, as well as coding of the preparations were performed by a person not directly involved in the grading of blanching response to prevent the unconscious recognition of a pattern by the observers during the assessment of pallor (40), which may occur if the same pattern was used for each volunteer.

Six adhesives labels (Chevron; 25mm x 38mm, Waltons Stationery, South Africa), from which two independent 7mm x 7mm square holes had been punched, were applied to the flexor area of each forearm to demarcate a total of 12 application sites per arm of each volunteer. The areas adjacent to the wrist and elbow were avoided because it has been shown (69,140) that they may influence the blanching response induced by a topical corticosteroid. All the application sites on both arms for each volunteer were unoccluded but the sites were protected by a Perspex frame designated to allow a free flow of air and held in place by Micropore surgical tape (3M Medical Products Division, South Africa). The unoccluded mode of application was chosen because most topical corticosteroids in clinical practice are used in this mode. Another reason for not occluding the sites was that occlusion of application sites has been shown to mask the delivery effects of some bases in which the corticosteroid was incorporated (38). Since three lotions were used and twelve sites were dermacated, each formulation occupied four sites per arm. The containers of the lotions were thoroughly shaken before use. Three μl of each lotion, which was equivalent to 2.4mg of formulation (141), was applied to each application site using a micropipette. Each formulation was evenly spread on the site by the tip of the micropipette. The formulations, protective covers and demarcating labels remained on the skin for exactly six hours after initial application. After the six hour period, the protective covers and adhesive labels were gently removed from each arm to minimize erythema that may be produced and any residual formulation was washed from the application sites with soap and distilled water and the area was patted dry with a tissue. Erythema that may be produced usually subsides within 30 minutes (40).

For the purposes of this trial, five observers (two novice and three experienced) were used to grade the blanching response. Novice 1 had not participated in or observed a skin blanching

trial previously and novice 2 had participated in blanching trials as a volunteer (at least twice) but had not observed previously. The three experienced observers have been assessing blanching responses for at least the past 8 years. Assessment of the blanching response was made using a five point grading scale (0-4) where

0 = no blanching
1 = slight blanching
2 = more intense blanching
3 = general even and distinct blanching
4 = marked and very intense blanching.

The skin blanching intensity was assessed independently by each observer at 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, and 28 hours after product application. The response was assessed at each observation time and the arms of each volunteer were held horizontally on a desk directly in front of each of the observer under standard overhead fluorescent lighting conditions.

Most researchers have drawn conclusions from the topical corticosteroid blanching assays based on the following parameters, the blanching profiles, area under the curve (AUC) values and one or more forms of statistical analysis. The profiles are usually produced by plotting the percentage of total possible score (%TPS) values vs time in hours after product application. The above-mentioned parameters were used to evaluate the results in this study where the statistical analysis used was the Chi-squared test. It should be noted that all the blanching profiles plotted here and for subsequent trials reported time in the x-axis in hours after product application. This was done for comparative purposes because other workers (42,45,122,123,125,140) have reported their results in the similar way.

2.1.1. Calculation of percentage total possible score (%TPS.)

The %TPS is calculated as follows:

$$Total\ Possible\ Score\ (\%TPS) = B \times O \times S \times V \quad \text{Equ. (9)}$$

where B = the maximum blanching score attainable for any one site (usually 4).

O = the number of observers (usually 3).

S = the number of sites to which each preparation is applied on each arm (usually four when three formulations are simultaneously evaluated).

V = the number of volunteers (usually 12 - 15)

The actual score (AS) for each preparation at each observation interval equals the sum of the graded scores (0-4) recorded by all the observers for the formulation, and hence, the percentage total possible score is given by:

$$\%TPS = \frac{AS}{TPS} \times 100 \quad \text{Equ. (10)}$$

From the blanching profile, the trapezoidal rule is used to calculate the AUC values. The parameters used to obtain this value are the %TPS values and the time in hours after product application. The AUC is therefore reported in terms of %TPS and time in hours.

2.1.2. Calculation of the area under the curve (AUC) value

The formula used to calculate the AUC value is as follows:

$$Area = 0.5(\%TPS_1 + \%TPS_2) \times (T_2 - T_1) \quad \text{Equ. (11)}$$

where $\%TPS_1$ is the response at observation time 1 (T_1) and $\%TPS_2$ is the response at later observation T_2.

2.1.3. Statistical analysis

Chi-squared analysis was chosen in this trial because it has been successfully used by a number of workers who visually assessed blanching responses induced by a topical corticosteroid (42,45,65,67,122,125,140-142). In this method of analysis chi-squared values are obtained by comparing the significant difference in the intensity of observed blanching for any two preparations or two dose durations by using the 0-4 scoring scale previously described. Since this scoring is used, a two-by-five contingency table is obtained to compare the responses of the two preparations at each time interval. The Chi-squared values of > 9.49 signify real differences in the observed responses based on the 95% level of significance, four degrees of freedom.

The graded blanching responses for the trials performed in our laboratories were originally processed manually by counting the responses to obtain data for statistically analysis and construction of blanching profiles. This was time consuming and as a result a computer programme was written for the Wang 2200 Basic Desk Top Mini-Computer (Wang Laboratories, U.S.A.) and was later converted for use on an Apple II-Plus Personal Computer (Apple Computer Inc., U.S.A.). A new software package has since been compiled (141) and is currently available in our laboratories. This new software was used in this trial and in all subsequent trials.

2.2. THE FDA-RECOMMENDED PILOT BLANCHING TRIAL

The reproducibility of the results from the FDA-recommended pilot study methodology was assessed by conducting two identical pilot trials which were performed eight months apart. The methodology of the human skin blanching assay described above was used here but modified in accordance with the recommendations and objectives of the FDA pilot study guidelines. The guidelines stipulate certain criteria for the subjects (minimum 12) who have to be used in the pilot dose-response study.

The subject's inclusion criteria are as follows:

1. Healthy subjects.
2. Subjects demonstrating blanching response to topical corticosteroids.
3. A written informed consent from each subject.
4. Willingness to follow study restrictions which are (a) No use of creams, emollients, or similar products on the forearms for 24 hours prior to and throughout the study (b) No bathing or showering during the periods of drug application and assessment of skin blanching (c) No strenuous exercise for the whole blanching trial.

The subject's exclusion criteria are as follows:

1. Individuals who require shaving ventral forearms to insure consistent dose on the skin surface.
2. Clinically significant hypertension or circulatory disease.
3. Individuals smoking within one week prior to the study.
4. Caffeine intake greater than 500 mg per day prior to or during the study.
5. Adverse reactions to topical or systemic corticosteroids.
6. Clinically significant history of alcoholism or drug abuse.
7. Any current or past medical condition, including active dermatitis or any dermatologic condition, which might significantly affect pharmacodynamic response to the administered drug.
8. Use of any vasoactive (constrictor or dilator) medication, prescription for OTC, that would modulate blood flow. Examples of such drugs include nitroglycerin, antihypertensives, antihistamines, NSAID's, aspirin, and OTC cough/cold products containing antihistamines and/or either phenylpropanolamine or phentolamine.
9. Any obvious difference in skin colour between the arms.
10. The use of topical corticosteroids on ventral forearms within one month prior to the study.

A total of twelve volunteers who met the above-mentioned criteria were used. Each volunteer was given a schedule sheet showing the times at which the formulations were applied to specific sites and the times at which the protective covers and dermacating labels were removed. They were requested to come to the laboratory immediately after washing their sites for estimating the blanching response visually and also by the use of chromameter. Each volunteer was also told to come 10 minutes before the first formulation is applied to take the chromameter readings (zero time values) at each designated site. Zero time chromameter values were taken only for pilot trial 2.

The two formulations chosen for comparison were Betnovate cream (Glaxo, South Africa), and Lenovate cream (Lennon, South Africa) each containing 0.12% betamethasone 17-valerate. These creams were bought shortly before the first trial and were appropriately stored for use in the second pilot trial. Betnovate cream was chosen as a reference (R) drug because it is normally used as the standard formulation for initially screening of the responders and has been utilised before in a number of trials performed in our laboratories (45,122,140,143) and others (66,138). The test (T) product was Lenovate cream. A total of twelve application sites per arm demarcated from the adhesive labels as previously described were used in both trials. For the investigators, a schedule product application sheet was designed and is shown in appendix B. This sheet shows the application charts, the times at which each volunteer should come to the laboratory for product application, the names of the preparations to be used in the study which were coded by the person not involved in the visual assessment of blanching response (the observer) and the sites and arm at which each formulations were applied on the skin for given duration. The dose durations used were 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 5, 6, 7, 8, and 10 hours. It should be noted that the recommended dose durations for the pilot study by the FDA are from 0.25 to 6 hours. In addition, the guidelines require that

there must be one site per arm for any one dose duration. The 7, 8 and 10 hour dose durations were included in the trial for comparisons purposes. The Guidance stipulates that the dose durations should be randomized between the left arm and the right arm, that is, both long and short dose durations should be equally divided between the two arms. However, in this study the formulations for the 0.25 to 2 hour periods were applied on the left arm and from the 4 to 10 hours duration, were applied on the right arm. This was done so that the removal of protective covers, adhesive labels and washing of residual formulation from the application sites at appropriate time should be easy to avoid accidentally washing the adjacent sites that were not supposed to be washed at that particular time, otherwise this would affect assessment of blanching response. For this reason, the cotton-tipped buds were used during washing procedure (distilled water) to minimize the chance of accidentally washing the sites that were not supposed to be washed. The volunteers were also told to remove the protective covers and labels with care. Although the relatively shorter dose durations were on one arm and the longer dose durations were on the other arm, the arm should not be expected to influence the blanching response. This is because it has been reported in the current FDA Guidance (64) and also by others (65,66) that there is no significant difference in the corticosteroid-induced skin blanching between the left arm and the right arm.

The first gram of the formulation was discarded so as to minimise any possible interaction between the closure and the formulation. Each formulation was applied to each designated site by means of 1ml disposable tuberculin syringes which were filled immediately prior to use in order to minimize any possible interaction between the corticosteroid and the matrix of the syringe barrel. The needles were of constant size and were cut to 5mm in length in order to facilitate the extrusion of the formulated product. Although the dose of formulation is not stipulated in the

Guidance, four stripes (7mm) of each formulation (equivalent to approximately 3.2mg) were applied to each designated site. This is the dose of formulation normally used in our laboratories (40,45,122,140,143) and others (92,94,144) have reported the results of blanching assays using the same amount. For the 0.25 to 6 hour dose durations, the formulations were applied at different times (staggered application) but were removed simultaneously (synchronized removal). The formulations were applied at the same times (synchronized application) but were removed at different times (staggered removal) for the 7, 8 and 10 hour dose durations. It should be noted that the 6 hour dose duration in this case can be used for either synchronized or staggered product application method. All the application sites were unoccluded as in the previous trial (section 2.1.). After specified contact times, the protective covers and adhesive labels were removed and any residual formulation was washed from the application site with distilled water and cotton-tipped buds. The sites were patted dry with a tissue.

In addition to the active drug sites recommended by the FDA, two untreated control sites on the flexor area of the forearm for the chromameter data are also required although the position of these sites is not given. The untreated control sites are required to correct any diurnal colour change that may occur in the skin unrelated to drug exposure. In this study, three untreated control sites per arm were used. The positions of these untreated control sites on one arm were as follows: the mid-left arm area, the mid-right arm area and the site about 5cm away from the elbow region (figure 2).

For visually-assessed data, the untreated control sites were not needed (section 1.6.). The Guidance also requires investigators to take the chromameter readings at the application sites before any drug application (zero time values). The experiments of Waring et al. (69) has led them to suggest that the subtraction

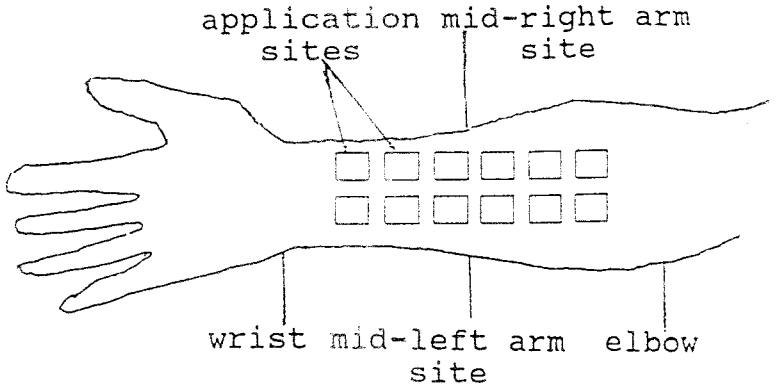


Figure 2: A representative of the human skin forearm.

of the chromameter values for the untreated control sites from that for the treated application sites can be employed with much confidence and is likely to be more valid than simply subtracting the zero time readings from the treated application sites. It would therefore appear as if the inclusion of the zero time readings as suggested by the FDA is not necessary and hence, it would not affect the overall conclusions drawn from the blanching assays even if the zero-time values are excluded. It was therefore decided not to take the zero time chromameter readings in pilot trial 1. However, the zero time readings were taken in pilot trial 2. The aim was to compare the results of blanching profiles with and without zero time values as to ascertain the effect of this factor on the results obtained.

2.2.1. Blanching response assessment

Assessment and treatment of visual data (blanching profiles, AUC values and Chi-squared statistical analysis) were the same as that described previously in section 2.1. except that in this pilot study blanching response was independently graded by three experienced observers. The two experienced observers were those used in the previous study and the third one was the trained observer who was called novice 2 in the same experiment. This observer was used as an experienced observer because his results were found to be very similar to those of the experienced

observers (section 3.1.). The observation times used were at the time of product removal and at 1, 2, 3, 4, 5, 6, 10, 11, 12, 19, 22, 24 and 26 hours after product removal for all dose durations. These observation periods equate to the 7, 8, 9, 10, 11, 12, 16, 17, 18, 25, 28, 30 and 32 hours after product application for the 6 hours dose duration. Table 1. in Appendix B lists the observation periods for other dose durations.

For the chromameter measurements of which the a-scale parameter must be used in accordance with the Guidance, skin blanching was assessed for pilot trial 1 at the time of product removal and at intervals corresponding to visual data. For pilot trial 2, the response was assessed before any drug application (time zero values), at the time of product removal and at intervals the same as for pilot trial 1. The mean of the three untreated control sites values was subtracted from the readings at the treated application site to give values referred in this study as the corrected (CC) values for pilot trial 1. For trial 2, the zero-time values were subtracted from all the sites (treated and untreated) to give values referred as the baseline corrected (BC). The mean of the three untreated control sites BC values were subtracted from the treated application site BC values to yield values referred to as the baseline-treated corrected (BTC) readings. In order to compare the results of blanching profiles for pilot trial 1 and 2 in an identical manner, the CC values were also generated in trial 2. The CC and BTC values versus time in hours after product application were plotted to produce blanching profiles of which the area under the curve (AUC) values can be determined using the trapezoidal rule. The Guidance, does not have any plotting procedures concerning the CC and BTC values as a function of time. The CC and BTC values were therefore plotted with respect to time in hours after product application. Student's t-test was used to compare the significant difference in the mean blanching response between any two formulations or two dose durations. This statistical test was chosen because it

has been previously used for the chromameter data (56,69,138) and for other optical instrumental data (95,139). The FDA recommends using the calculated AUC values in pharmacodynamic models.

2.2.2. Pharmacodynamic modelling

Since no specific modelling procedure is stipulated in the Guidance, the observed visual and chromameter a-scale AUC values (arithmetic mean of 12 subjects) at each dose duration (0.25 to 6 hours) were used in the Pcnonlin computer modelling programme (PCNONLIN V4.2, SCI Software, Kentucky, USA) (129). The non-linear pharmacodynamic models which were investigated for appropriateness were the simple E_{max} (Model 101, Equ. 12) and the Sigmoid E_{max} model (Model 105, Equ. 13):

$$E = \frac{E_{max} \cdot D}{D + ED_{50}} \qquad \text{Equ. (12)}$$

$$E = \frac{E_{max} \cdot D^{\gamma}}{D^{\gamma} + ED^{\gamma}_{50}} \qquad \text{Equ. (13)}$$

Both models describe the blanching response in terms of the estimated maximum AUC (E_{max}) and the estimated dose duration (D) required to produce half-maximal AUC (ED_{50}). For the sigmoid model, γ is the slope factor that describes the shape of the curve.

Performance of the model between the simple E_{max} and sigmoid E_{max} model was based on consideration of goodness of fit criteria: standard error of estimates, correlation coefficients (r) between observed and predicted data and the Akaike's Information Criterion (AIC). These parameters were chosen because they have been previously described as sensitive indicators in selecting the best model that describes the experimental data (130,145,146).

2.3. THE FDA-RECOMMENDED PIVOTAL BLANCHING TRIAL

The human skin blanching assay described earlier was modified to comply with the recommendations for the pivotal study methodology as stipulated in the Guidance. A minimum number of forty subjects are required for the pivotal study. A total of 40 volunteers who met the inclusion and exclusion criteria as in the pilot study were used in this pivotal study. Since it was not practically possible to process all 40 volunteers in a single day, three identical trials were conducted at weekly intervals. The first trial comprised of 12 volunteers whereas the second and third trial had 14 subjects each. In an attempt to standardise the conditions from one trial to the another, all volunteers were processed in the same building at the same place under fluorescent lighting conditions during the period of drug application and assessment of blanching response. All the coding of preparations, application of formulations to the designated sites and chromameter readings were done by the same person in each trial. Similar to the pilot study, each volunteer was given a schedule sheet showing the times at which the formulations were applied to specific sites and the times at which the protective covers and dermacating labels were removed.

The dose durations suggested by the FDA to be used for pivotal study should be approximately equal to the E_{D50}, D_1 and D_2 values determined from the pilot trial. In this study, a total of 14 application sites were applied to the flexor area on the forearm of each volunteer. A schedule product application sheet and application charts similar to those for the pilot study used in all the three pivotal trials is presented in Appendix B. The application pattern recommended by the FDA is that D_2 site on one arm should be complimentary to D_1 site on the other arm (Table 1). Similarly the R site on one arm is complimentary to T site on the arm and the same applies to the untreated site (UNT).

Table 1. A representative application chart sequence for a particular subject as proposed by the FDA.

ELBOW REGION

LEFT ARM	RIGHT ARM
D_1	D_2
T	R
UNT	UNT
R	T
UNT	UNT
T	R
D_2	D_1
R	T

WRIST REGION

It is not clear why the location of the application site for a reference formulation at D_2 dose duration on arm should always correspond with the location of application site for a test formulation in another arm considering that the same guidance (64) and others (65,66) have reported that there is no significant difference in the blanching response between left and right arm. For the pivotal study conducted in this thesis, four application patterns were designed such that the dose durations were equally randomized between the two arms in any order.

In accordance with the FDA guidelines, the test and reference products should each occupy a specific number of sites per arm. Betnovate cream and Lenovate cream each had to occupy two sites per arm at the 2 hour (ED_{50} value) dose duration. Betnovate cream occupied one site at 1 hour (D_1 value) dose duration and one site at 4 hour (D_2) dose duration per arm. The 3 and 6 hour dose durations were also used in this study for comparative purposes. Betnovate cream and Lenovate cream each occupied two sites per arm at both 3 and 6 hour dose durations. The staggered application, synchronized removal method was used where the formulations were applied at different times but removed simultaneously. In contrast to the pilot study, the dose durations in all the three pivotal trials were randomized between the left and right arm. The reason was that all application sites were washed at the same time and therefore there was no

problem of erroneously washing the sites with water when they were not supposed to be washed.

The observation times used were the same as for the pilot trials except that a 5 hour (6 p.m.) reading after product removal was not recorded in this pivotal study. The 6 p.m. reading was not taken because the subjects had to go home for meals because dinner was not provided by the investigators during this period. The method of visual blanching response assessment and data treatment was exactly the same as in the pilot study. Similarly the chromameter response measurements and data treatment were the same as for pilot trial 2 where zero time readings were also taken. The exception was that for the pivotal study, the area under the curve (AUC) values for each subject were determined whereas the mean AUC values for all subjects for the pilot study were calculated in accordance with the FDA guidelines.

For the pivotal study, pharmacodynamic modelling of AUC values is not required. Instead, the AUC values for an individual subject were used to select the good blanchers using the D_2/D_1 ratio criterion which has already been described in section 1.6. The AUC values at the 2 hour (ED_{50} value) dose duration of "acceptable" blanchers only, had to be used to test bioequivalence between Betnovate and Lenovate cream using the FDA-recommended Locke's statistical method. The description of how this statistical method works is not provided in the current FDA Guidance. However, a worked example with formulae based on their pivotal study data is given in the FDA document. Based on the data obtained from this pivotal study, the same formulae as used in the Guidance without description of some of the parameters involved in the equations were also tested to assess bioequivalence between Betnovate and Lenovate cream to ascertain if the method can be useful for describing the results of the blanching assay. Furthermore, the Locke's method was also used to test bioequivalence at the 6 hour dose duration by using the

AUC values of "acceptable" blanchers and also by using the AUC values of all volunteers (total of 40) for the whole pivotal study. Although this is not recommended by the FDA Guidance, the blanching profiles and AUC values were produced and assessed as previously described for the mean of all subjects for one trial and student's t-distribution and chi-squared tests were used as appropriate statistical methods.

CHAPTER THREE

RESULTS AND DISCUSSION

3.1. THE PRELIMINARY TRIAL FOR NOVICE OBSERVER TRAINING

Most researchers (51,66,77,138,147,148) have raised concern on the need to use experienced assessors to subjectively determine and rank the degree of skin blanching induced by topical corticosteroids. It would, therefore, appear as if the training period required for experienced observers is a major problem in the blanching assay. Despite criticisms on the need to train observers, little work up to date has been done to investigate the period that is required to train the novice observers. There are only two published reports (65,71) which showed that it takes at least two trials for a learner to gain sufficient practice to be classified as an experienced observer. Considering little work that has been done in this field, it was therefore decided to investigate the training aspect of a learner to see if the results are similar or different compared to those of the experienced observers. The investigation is also important because although there are promising new instruments such as the chromameter to quantify skin blanching, they have not been found to be better than the visual method (56,69,95,111). Moreover, the latest report by Noon et al. (56) showed that the visual method is still the best method to assess skin blanching.

For the objectives of this trial, two different learners were used; novice 1 who had not participated in or observed a skin blanching trial previously and novice 2 who had participated in blanching trials as a volunteer (at least twice) but had not observed previously. The idea of using novice 2 as a learner was to investigate if the training of subjects who have been exposed to a blanching trial without assessing the results would require the same period of training as any learner who has not participated in blanching trial before. If the results of the

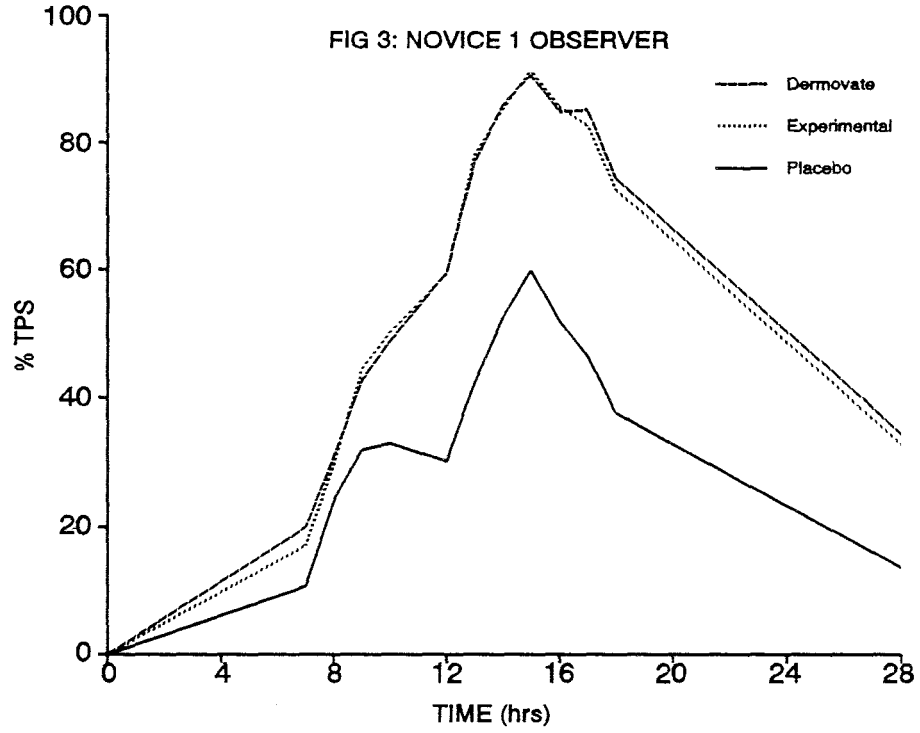
novice 2 are found to be very similar to those of the experienced observers, it may therefore be concluded that the training of a learner is not a major problem because the volunteers can easily be trained to be experienced observers. The three experienced observers have been observing blanching response for at least the past 8 years.

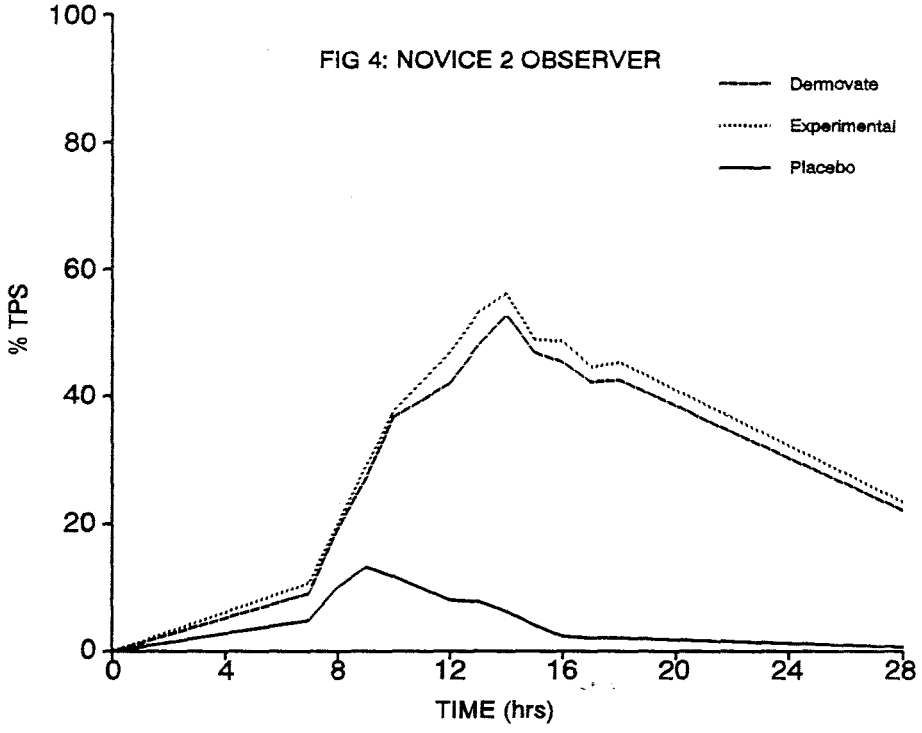
Figures 3-5 show the blanching response profiles for novice and experienced observers. Table 2. shows the area under the blanching curve (AUC), maximum blanching response (R_{max}) values and time to reach maximum blanching response (t_{max}).

TABLE 2: THE AREA UNDER THE CURVE (AUC), MAXIMUM BLANCHING RESPONSE (R_{max}) AND TIME TO REACH MAXIMUM BLANCHING RESPONSE (t_{max}) VALUES FOR NOVICE AND EXPERIENCED OBSERVERS

NOVICE 1	**AUC**	**R_{max}**	**t_{max} (hours)**
Dermovate	1323.0	90.6	15
Experimental	1295.6	91.4	15
Placebo	724.2	59.9	15
NOVICE 2			
Dermovate	780.1	52.9	14
Experimental	836.6	56.3	14
Placebo	110.9	13.3	9
EXPERIENCED			
Dermovate	934.7	58.4	15
Experimental	949.3	59.8	16
Placebo	73.6	6.2	16

In general, the response profiles demonstrated that the learners, especially novice 1 recorded scores higher than those of experienced observers for each preparation. It is clear from the AUC values and the graphs that novice 1 showed that the experimental lotion elicited slightly greater blanching response than Dermovate lotion. In contrast the results of Novice 2 demonstrated that Dermovate lotion elicited slightly greater blanching response than experimental lotion. The pooled results of three experienced observers, however, showed that the response





Figures 3-4: Visual blanching profiles for novice 1 and 2 respectively.

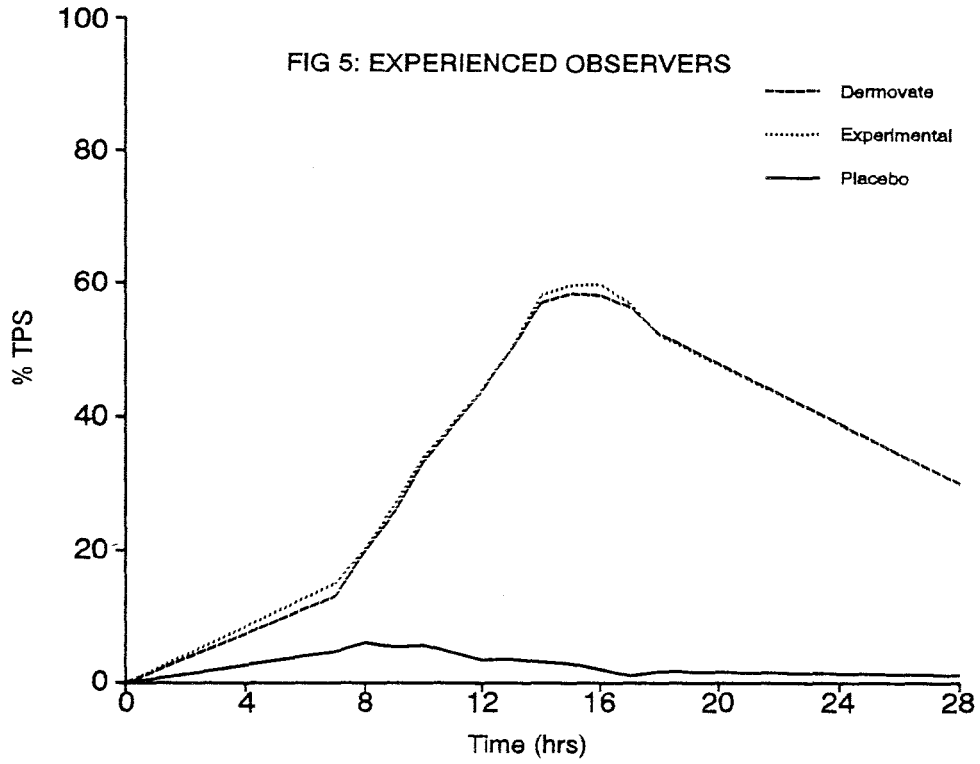


Figure 5: Visual blanching profile for experienced observers.

elicited by Dermovate lotion was similar to the response induced the by the experimental lotion. Despite these differences, both learners and experienced observers noted no statistically significant differences when comparing the response profiles between Dermovate and the experimental lotion. In addition, all observers were able to show significant statistical differences between placebo and the medicated lotions. It is interesting to note that even in the first attempt for these learners to assess skin blanching, they were able to draw the same statistical conclusions as the experienced observers. The placebo does not contain any drug and therefore it would be expected that the blanching response generated by the formulation, should, theoretically be negligible. Novice 1 detected much greater skin blanching response induced by the placebo than that recorded by novice 2. In contrast, the response noted by the experienced observers was negligible. These results, therefore, imply that novice 1 could not assess a skin blanching response as precisely as the other observers. The t_{max} values for each preparation

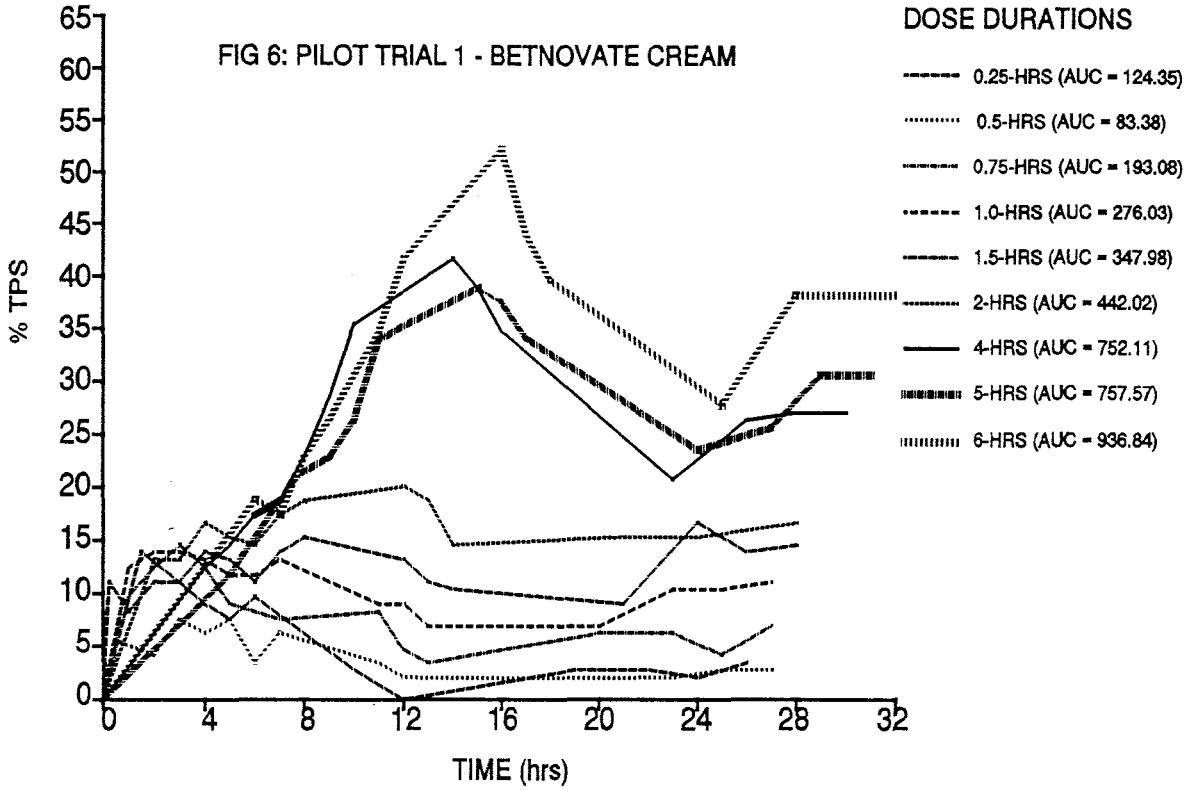
recorded by novice 2 and the experienced observers do not differ much from each other with the exception of t_{max} value of Placebo recorded by novice 1. Since the results of novice 2 were clearly in closer congruity with those of the experienced observers than were the results from novice 1, it was concluded that the training period of the learner who has participated in the blanching trial before as a volunteer, but has not assessed skin blanching response, would be less than the period of training for the learner who has never been involved in blanching trials as a subject or observer. It should also be noted that though novice 1 results differed from those of other observers in most respects, the same statistical conclusions were drawn by all the observers implying that the training of this kind of learner is not a major problem.

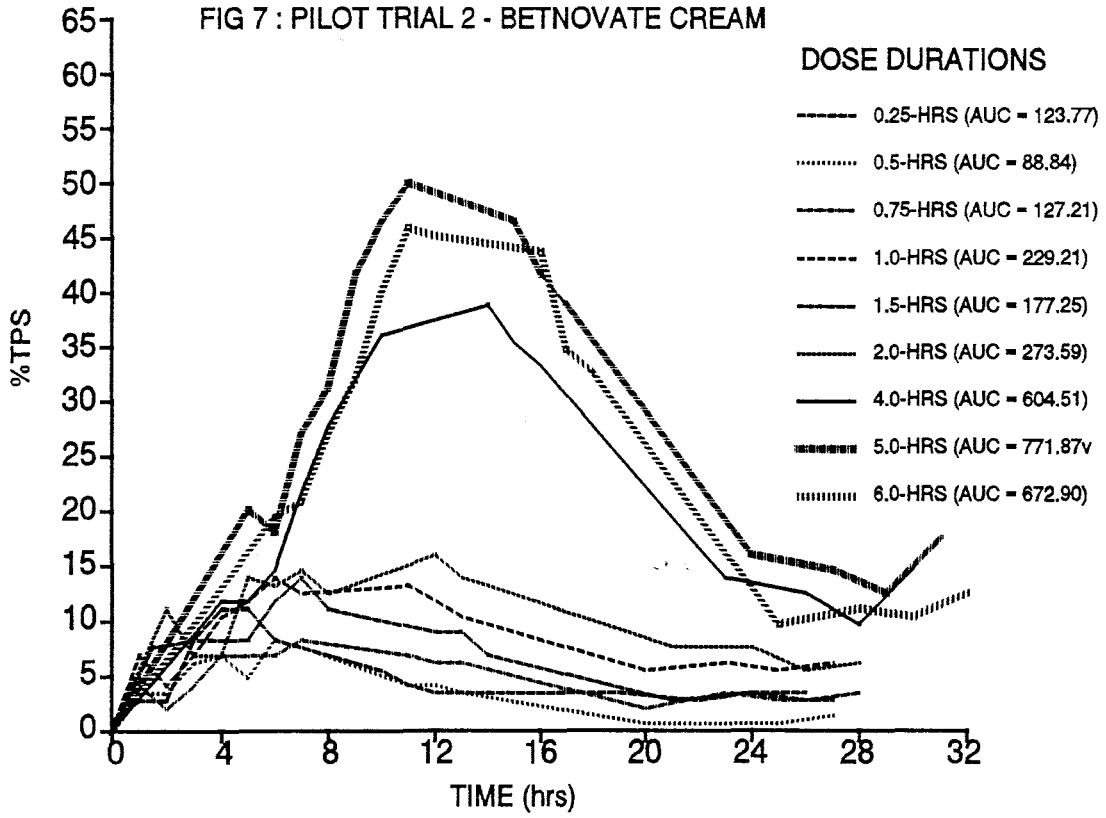
3.2. THE PILOT TRIAL

The main objectives of this study were to evaluate the pilot dose-response methodology as recommended in the FDA Guidance in terms of the following (a) the comparison of visual and chromameter a-scale data (b) the comparison of blanching responses at shorter and longer dose durations and (c) the suitability of using pharmacodynamic E_{max} models to describe skin blanching data. Two identical pilot trials were conducted and the methodology has already been described in chapter two.

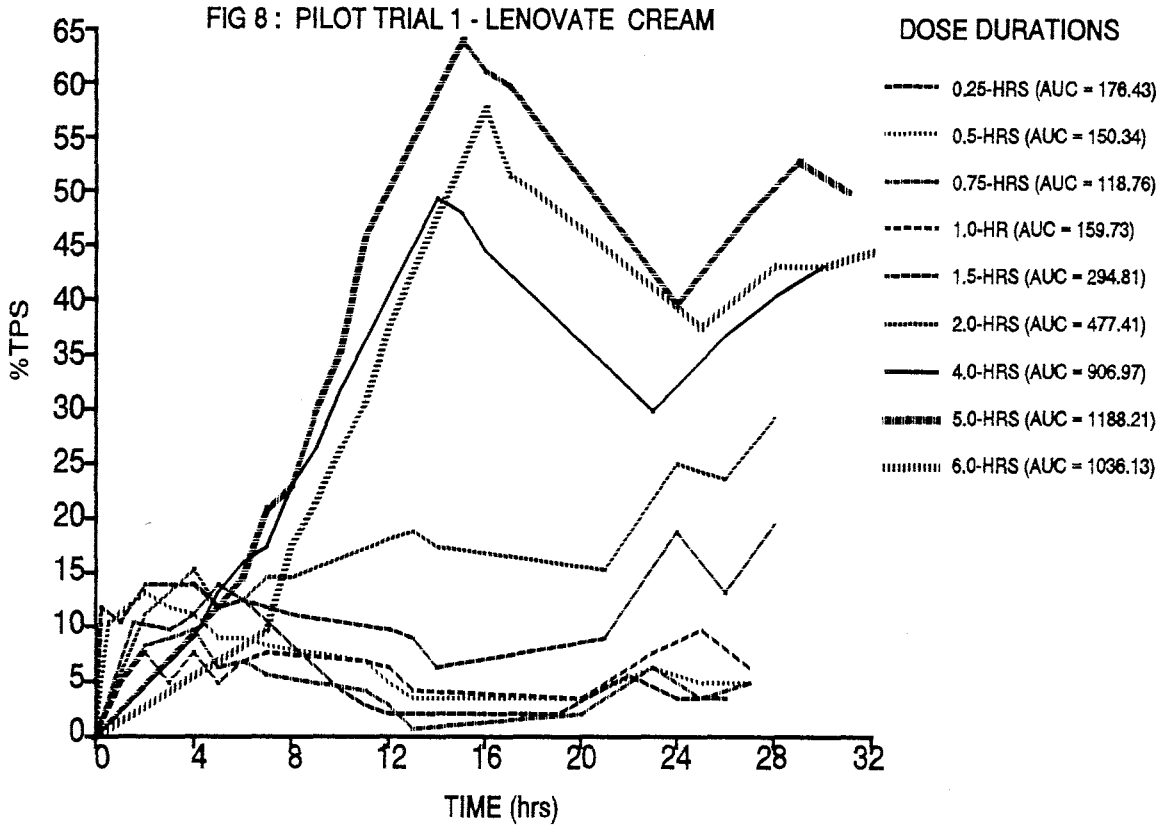
3.2.1. Assessment of visual blanching profiles.

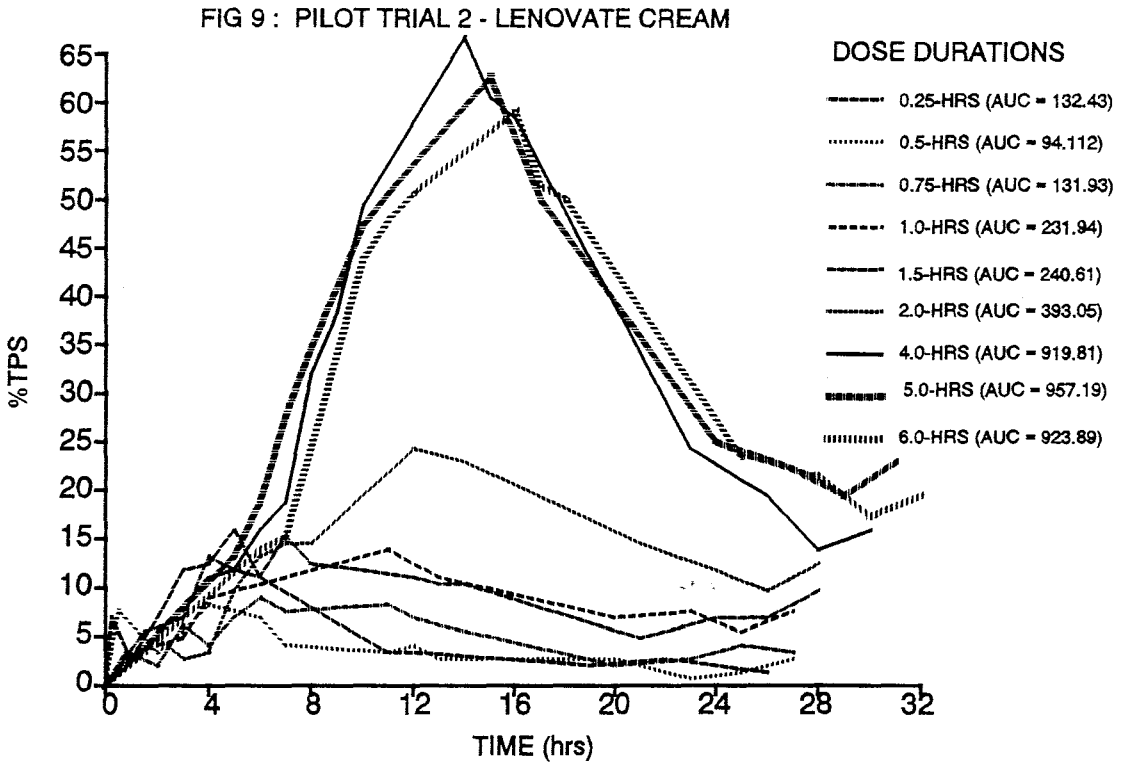
Figures 6-9 represent the visual blanching response profiles for Betnovate cream and Lenovate cream for both pilot trials. The AUC values at various dose durations are shown in the figures. The shapes of the blanching response profiles for Betnovate and Lenovate cream in pilot trial 1 are generally the same as in the pilot trial 2. For all visual curves in both pilot trials, the response profiles at longer dose durations (4 to 6 hours) show that the blanching increases to an obvious maximum at 12-16 hours after product application and then decreases. These response





Figures 6-7: Visual blanching profiles for Betnovate cream for both pilot trials 1 and 2 .





Figures 8-9: Visual blanching profiles for Lenovate cream for both pilot trials 1 and 2.

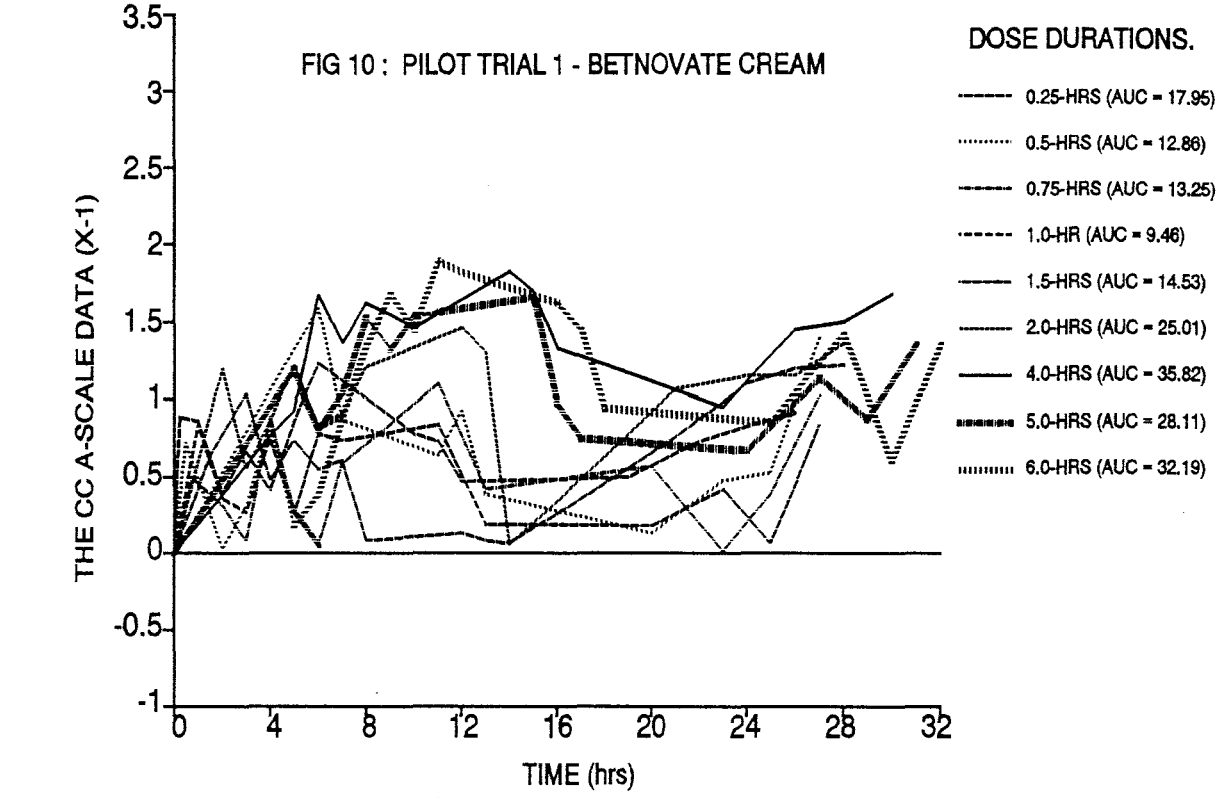
profiles at longer dose durations are similar to the results which have been obtained in previous studies using the same corticosteroid (122,149). In contrast, the response profiles at shorter dose durations (0.25 - 2 hours) are generally flat throughout the period of observation with the exception of Lenovate cream at 2 hours dose duration in pilot trial 2. The blanching profiles observed here are not as smooth as reported previously (140,149). The profiles generally show that the skin blanching increases as dose duration increases, as would be expected from the previous studies (122). From the work of Magnus et al (122) who showed that an increase in dose duration resulted in an increase in blanching, it was anticipated that the rank order of AUC values expected in this study would be as follows: 6 > 5 > 4 > 2 > 1.5 > 1 > 0.75 > 0.5 > 0.25 hours dose duration. The expected rank order of AUC values at very short dose durations (0.25 to 1 hour) was not as expected in both pilot trials, however the overall blanching response at these dose durations was so low that little significance can be applied to the specific rank order. This is because the low blanching response elicited was not intense enough to be distinguishable at 0.25 or 1 hour dose durations. Assessing topical corticosteroid formulations at these short dose durations may, therefore, lead to unreliable data for which invalid conclusions may be drawn. Probably that is why there were generally no statistically significant differences at any observation time when comparing the blanching response between Betnovate cream and Lenovate cream for the 0.25 to 2 hour dose durations in pilot trials 1 and 2. In contrast, significant differences between the two creams at peak blanching responses were noted for the 5 and 6 hour dose durations in both pilot studies.

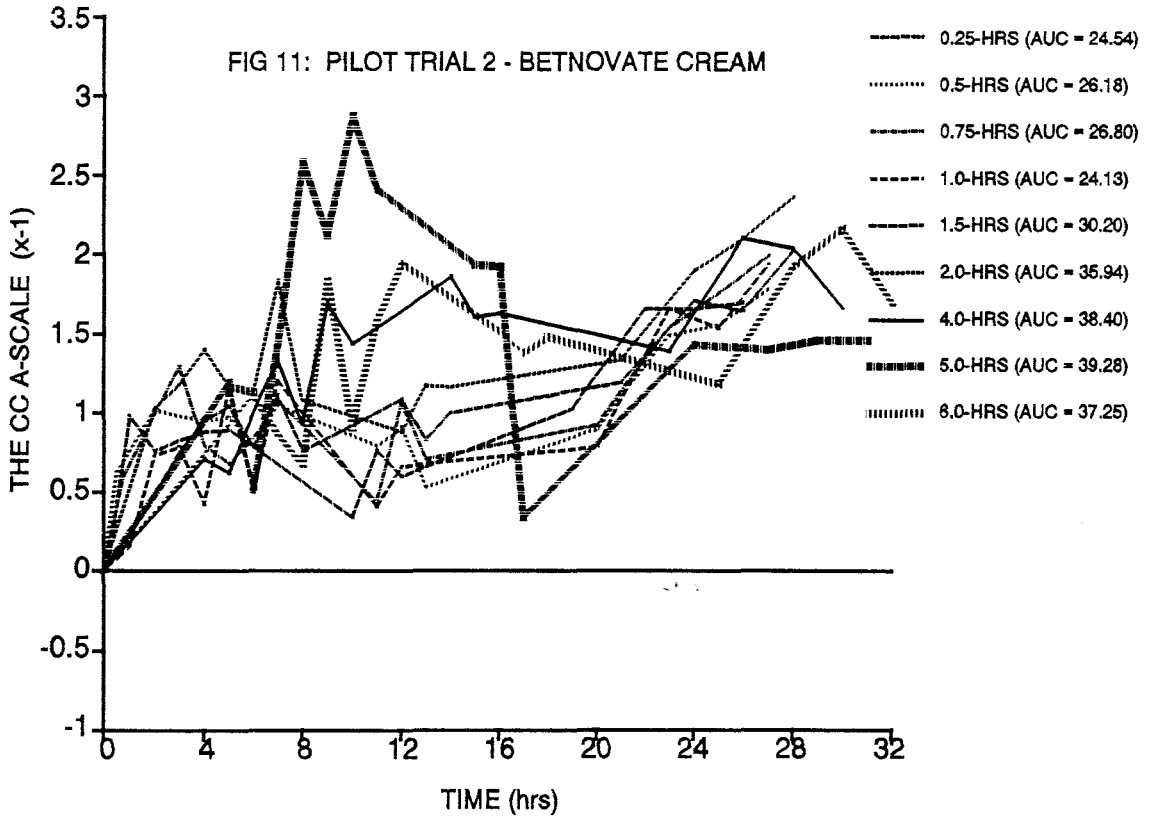
Generally, it is interesting to note that visual results in pilot study 1 are very similar to the results in pilot trial 2. However, at the 4 hour dose duration significant differences were

noted at peak blanching response in pilot study 2 but were not observed in pilot trial 1. The AUC values show that the greatest blanching response was found with the 5 hour dose duration for Lenovate cream in both trials. Considering Betnovate cream, the greatest response was found at the 6 hour dose duration in pilot trial 1 and at the 5 hour dose duration in pilot trial 2. These results are generally in contrast with the previous reports (122) that have shown that a 6 hour dose duration produced a greater blanching response than a 5 hour dose duration. It should be noted that in the previous report more application sites were used whereas only one site per arm at any one dose duration was used for the pilot trials. The unexpected result for the 5 and 6 hour dose durations is probably due to the use of a small data set in the methodology. One may conclude that there is a possibility for attaining a plateau effect (maximum blanching response) at the 5 hour dose duration. This possibility is unlikely because there was lower skin blanching at the 6 hour dose duration than at the 5 hour dose duration. If the plateau effect was attained, it would have been expected that the blanching response at the 6 hour dose duration would be similar to that at 5 hours and therefore the AUC values would have been slightly different. A previous report (122) using the same corticosteroid showed that the maximum blanching response occurred at a 10 hour dose duration. The possible explanations for the plateau effect have already been discussed in section 1.4.

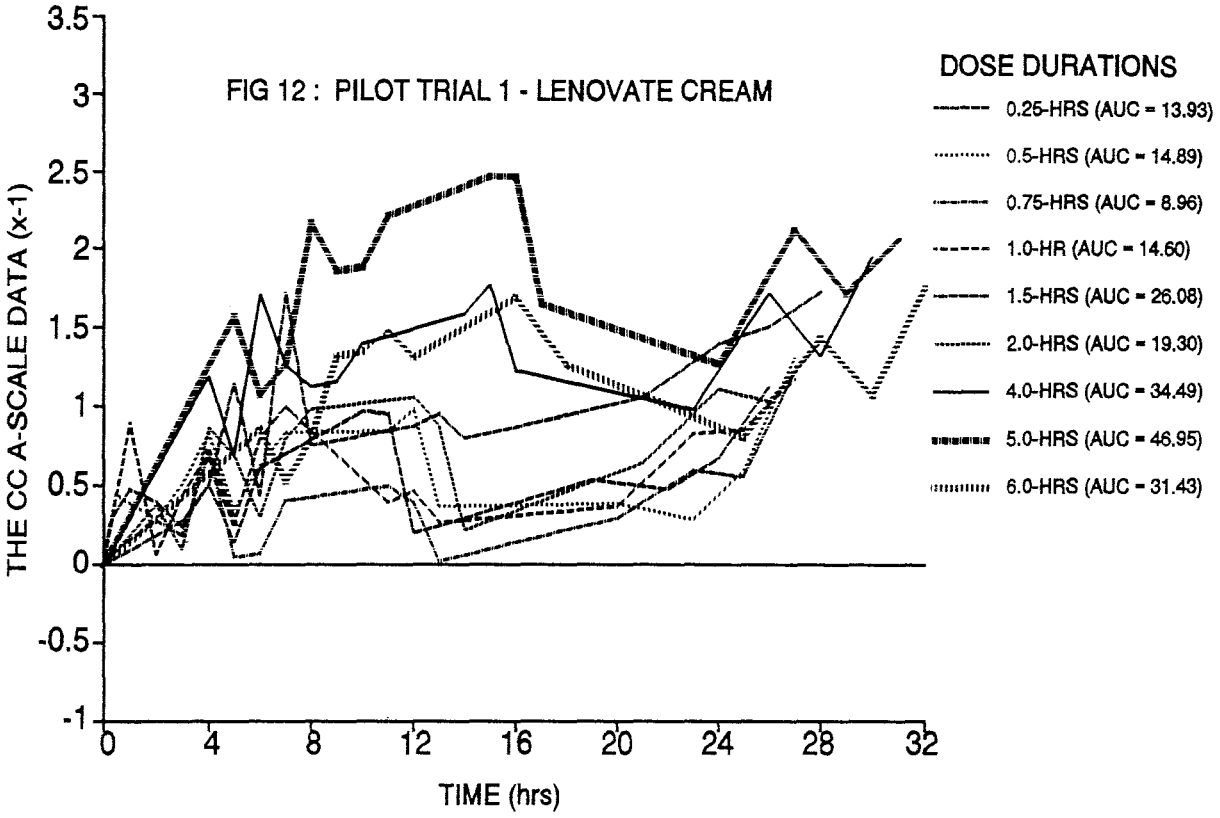
3.2.2. Assessment of chromameter blanching profiles

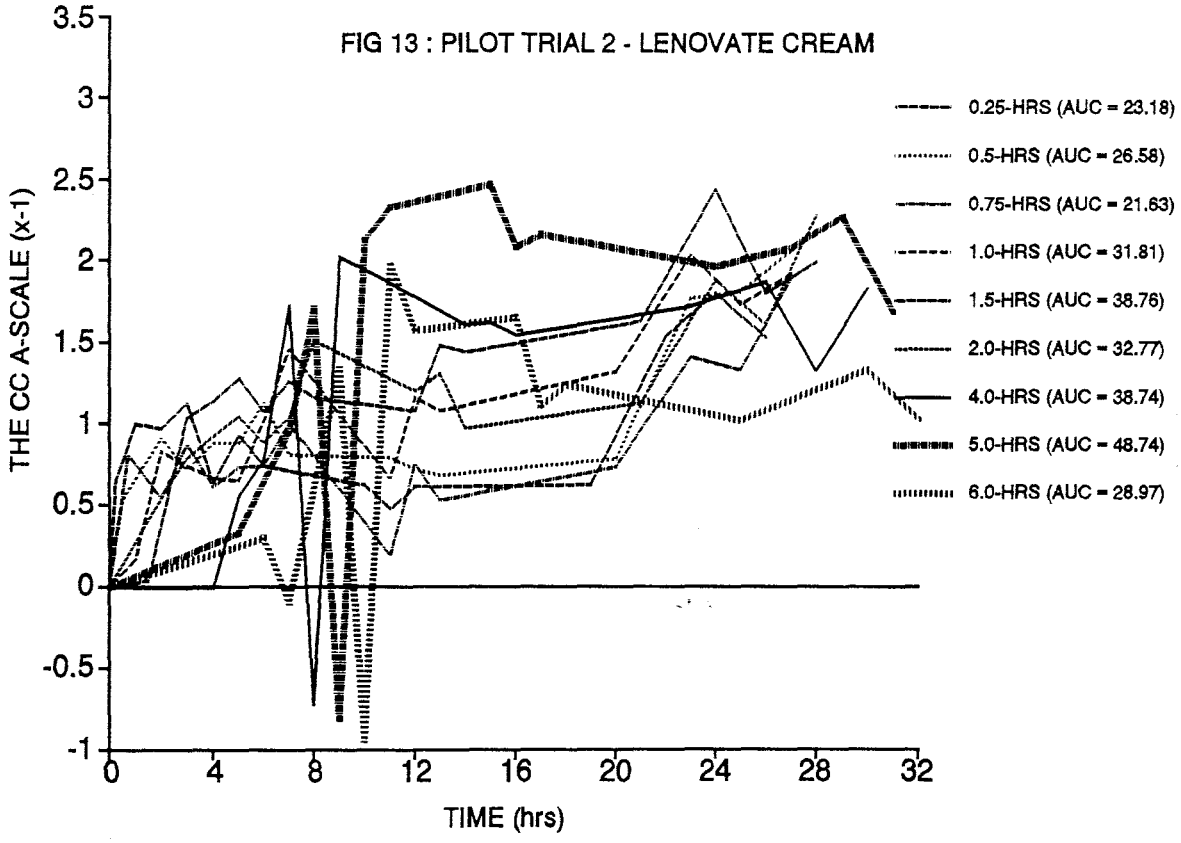
Figures 10-13 represent the chromameter blanching response profiles and AUC values for Betnovate and Lenovate cream plotted from the a-scale data for which zero time readings were not included (CC values, section 2.2.1.). The area under the curve (AUC) value is a measure of drug availability in the skin (61). The AUC values in pilot trial 1 for both Betnovate and Lenovate





Figures 10-11: Chromameter blanching profiles for Betnovate cream for both pilot trials 1 and 2.





Figures 12-13: Chromameter blanching profiles for Lenovate cream for both pilot trials 1 and 2.

creams do not show the expected rank order of AUC values with respect to an increase in dose duration as previously described. For the pilot trial 2, the rank order of AUC values was as expected with the exception of the 6 hour dose durations for both formulations, with 1 hour for Betnovate cream and with 0.75 and 1.5 hour dose durations for Lenovate cream. It is not clear from these results why the chromameter was able to measure greater skin blanching response for 0.75 hours than for the 0.25 hour dose duration but failed to measure greater response for the 1 hour duration compared to the 0.75 hours duration. In addition, the instrument failed to show the expected rank order of AUC values for both creams in pilot trial 1. These results may imply that the instrument is not capable of measuring skin blanching sufficiently. Apart from the instrument being probably insensitive, the instrumental probe which measures the response is designed such that it is difficult for an investigator to hold it as stationary as possible. When taking readings, the probe had to be moved from one site to another since a total of 24 sites per volunteer were used in the trial. It was extremely difficult to hold the probe motionless at a constant distance from the skin site at each observation time. This problem was experienced in all the trials conducted in this study and could undoubtedly have led to variation of the applied pressure from the probe to the skin site. It is difficult to estimate the extent of this variability from these pilot trials conducted because attempts were made to hold it as stationary as possible. However, Smith et al. (95) mentioned that any applied pressure from the probe on the stratum corneum will slow blood perfusion through that skin area, and hence inaccurate data would be generated. Although some workers (51,112) have raised similar opinions, only one study has been conducted to date (69) which showed the effects of applied pressure on the skin. The researchers measured different applied force from the chromameter

probe on the forearm of ten volunteers without any application of formulated product. The forces were measured by simply application of weights to the measuring head of the chromameter. Their results demonstrated that the colour of the skin as measured by the chromameter was sensitive to the force applied. The a-scale values were found to be more variable than the other parameters of the instrument even when a small amount of force was applied to the skin. The investigators concluded that variation in the applied force even to an extent of 5% is likely to provide erroneous results in the blanching assay. In an attempt to minimize the problem of handling the probe by the investigators, the authors suggested the use of a stand assembly that may be appropriate to support the chromameter for the measuring process thus enabling contact with the skin to be simply controlled, so that the minimum possible pressure is applied to the test site. However, the authors did not explain how the contact of the probe with the skin site would be simply controlled when using such a stand assembly. The current FDA Guidance does not have any recommendations concerning handling of the probe or distance of the chromameter probe from the skin. Some workers (95) suggested the use of adhesive tape or glue on the measuring head so that the probe should be fixed at a constant distance from the skin site and therefore be completely motionless during the assessment. Other researchers (51) have advocated the attachment of a pre-moulded foam casing to the measuring head, so that the sites can be monitored from a focal distance of 1 cm. The precision of using these procedures have not been investigated and, therefore, require analysis.

In congruity with visually-assessed results, it is clearly shown from the chromameter blanching profiles and the AUC values that the greatest response occurred at the 5 hour dose duration in both pilot trials. The possibility of the blanching plateau effect at the 5 hour dose duration can be ruled out as explained previously. One could speculate that the sensitivity of the

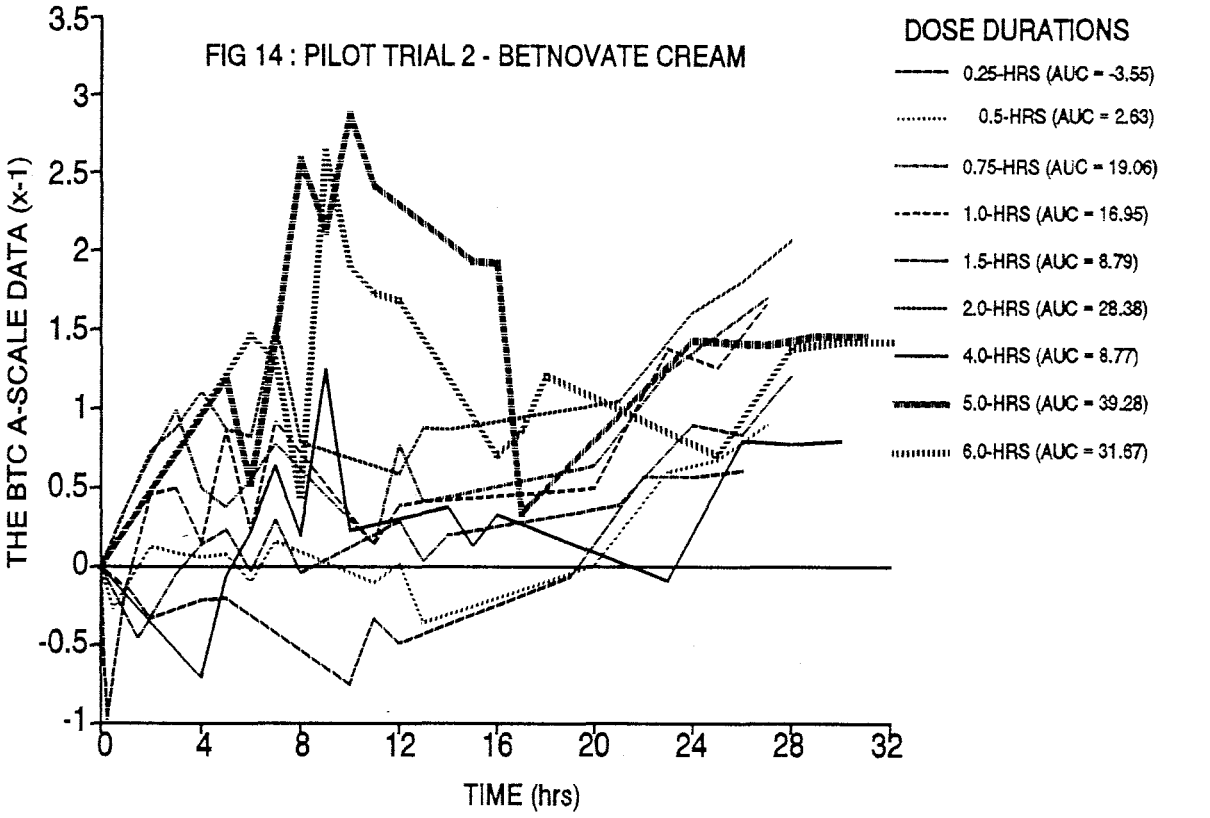
chromameter a-scale parameter at longer dose durations is low considering the FDA which assumes that the instrument may not be capable of measuring the response at about 6 hours dose durations. However, this speculation could be ruled out for various reasons. Firstly, the visual data was able to show similar results in this study and this does not mean that the naked eye cannot accurately estimate skin blanching at 6 hours dose duration. In fact most researchers have reported their results with precision at 6 hours (38,45,66,69,71,141) for the visual method. Secondly, another reason is because other workers have shown good differentiation of different topical corticosteroid formulations with the chromameter at 6 hours (51,66,69,77) and at 8 hours (77) dose duration suggesting that the chromameter is sensitive to skin blanching even at these dose durations.

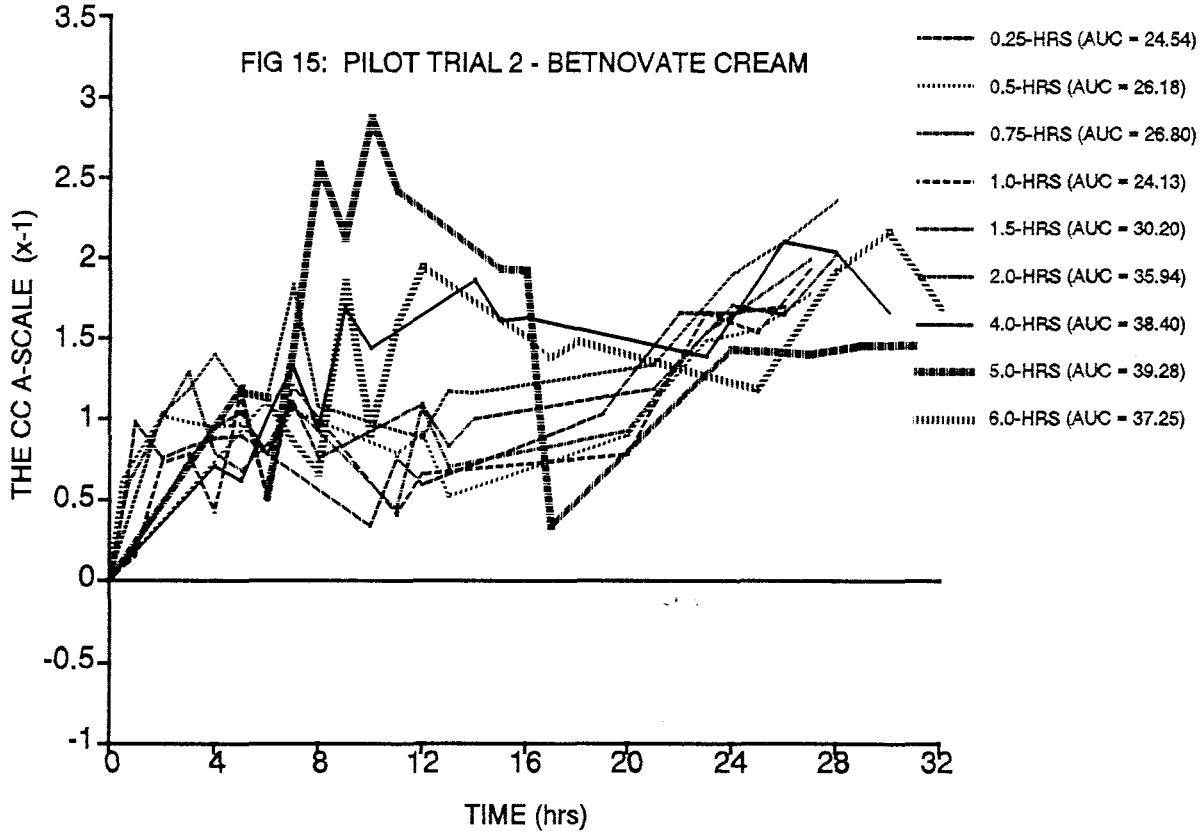
The chromameter profiles are difficult to interpret compared to the visually-assessed data especially at short dose durations (0.25 - 1.5 hours). The results at lower durations may be implying that the response was so small that the instrument was unable to measure it accurately hence it would not be able to distinguish skin blanching elicited at 0.25 and 1 hour dose durations. In contrast to the visually-assessed data, no significant differences were noted between Betnovate and Lenovate cream at any dose duration at all observation times. Since no two formulations can be identical despite having the same corticosteroid in the same concentration, one would have expected subtle differences to be noticed using the instrument. The visual method was able to demonstrate these differences at the 5 and 6 hour dose durations. The accuracy of the chromameter in measuring skin blanching, therefore, appears to be questionable. However, it should be noted that these chromameter results were obtained using the a-scale data which did not incorporate the chromameter values before any drug application on the skin sites (zero time readings). The FDA believes that incorporation of the

zero time values in the chromameter data manipulation is more accurate than using the a-scale data without inclusion of zero time readings. The reason given is that inclusion of zero readings is necessary because it allows correction of skin colour unrelated to drug exposure which can influence skin blanching and hence can interfere with the measurement of blanching.

As zero time readings were taken before any drug application in pilot trial 2, it is therefore important to compare the blanching profiles with and without zero time values to ascertain if this factor would influence the conclusions to be drawn from the blanching assays. The Betnovate blanching profiles of which the zero time values were included are presented in figure 14 where the response is shown as the baseline-treated corrected values (BTC). Figure 15 also shows the Betnovate blanching profiles produced from the corrected a-scale data without zero time readings (CC) from pilot trial 2.

Considering Betnovate cream, the shapes of the blanching profiles for the BTC and CC data are generally similar. All the profiles at longer dose durations (4-6 hours) showed that skin blanching increased at earlier times until reaching peak response around 10 to 15 hours after product application, and then the response decreased before beginning to increase again at later observation times. The possible explanation for an increase of the skin blanching response at later times after product application could be that the corticosteroid was held on the skin reservoir (section 1.4.). Both BTC and CC data showed that there were no statistical significant differences between Betnovate and Lenovate creams at any dose duration. In addition both data sets demonstrated that the greatest response occurred at the 5 hour dose duration and the blanching profiles at shorter dose durations (0.25-1.5 hours) were generally flat at most observation times except at later times. Despite these similarities between the two sets of data, some negative AUC

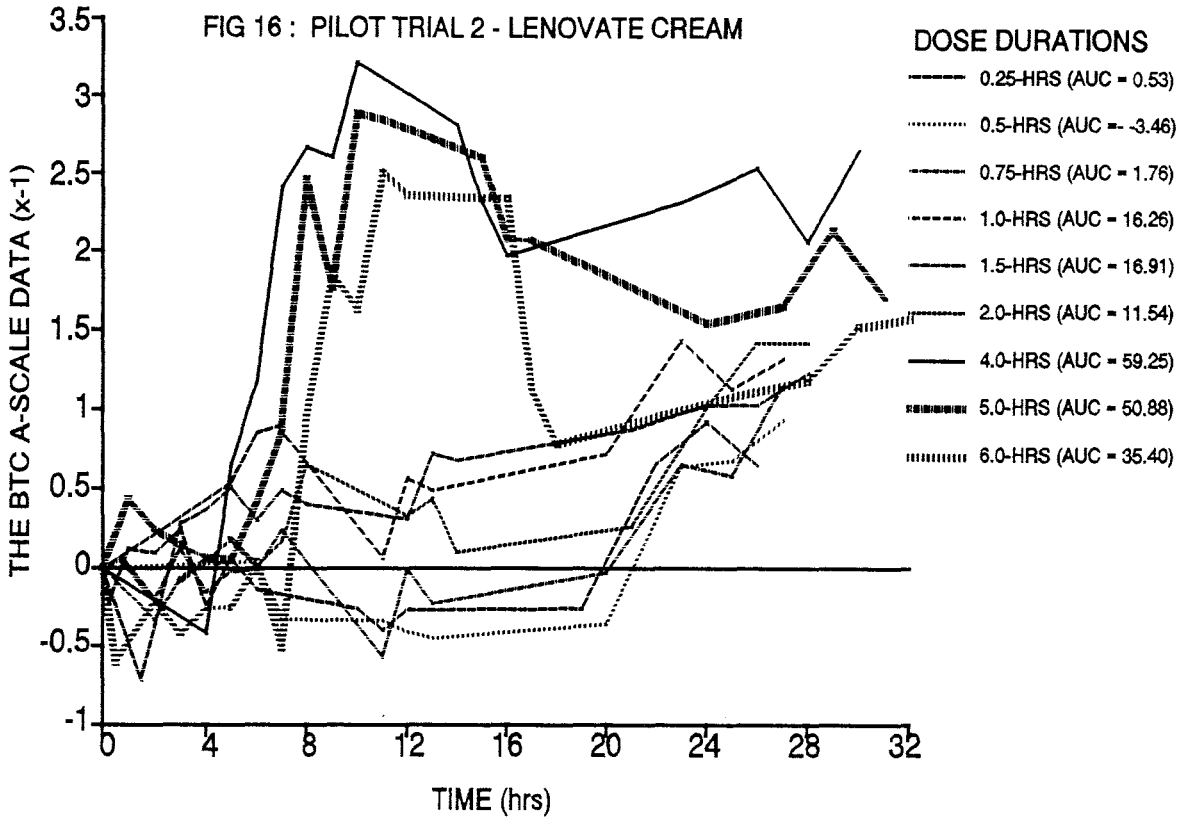


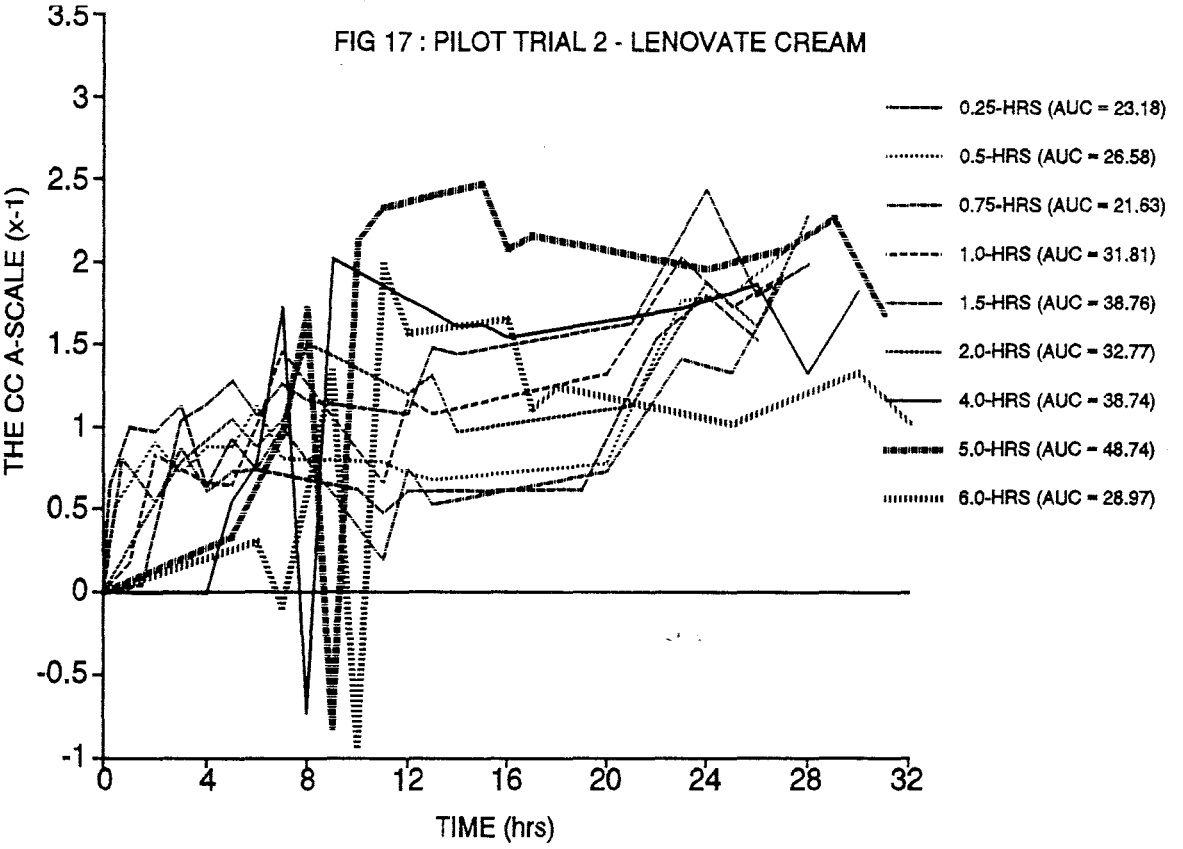


Figures 14-15: The Chromameter BTC and CC blanching profiles for Betnovate cream in pilot trial 2.

values were noted for the BTC data which were not recorded for the CC data. However, the negative AUC values were recorded for the lower dose durations (0.25 and 1.5 hours) which have been found to be unreliable as explained before. Other researchers (64) have also mentioned that lower dose durations usually lead to inaccurate data. The results of the pilot trial BTC data in the FDA Guidance did not find a mixture of positive and negative AUC data, but only negative AUC values. Another difference noted between the two data sets in pilot trial 2 was that the rank order of AUC data with respect to increase in dose duration was not as expected with the BTC data but the expected rank order was generally found with the CC data.

For Lenovate cream (figures 16 and 17), the profiles and the AUC values for the 4 to 6 hours dose durations clearly show greater blanching response than shorter dose durations for the BTC data. In contrast the response profiles and the AUC values for the CC data do not show huge difference between the longer and shorter dose durations with the exception of the 5 hours dose duration. The BTC profiles and AUC values show that the greatest response occurred at 4 hours dose duration whereas it was noted at 5 hours dose duration for the CC data. Despite these differences, both data sets demonstrated no statistical differences in the blanching response between Betnovate and Lenovate cream at all observation times. These statistical results obtained for the Lenovate cream were the same as for the Betnovate formulation. The implication of these results may be that the inclusion of zero time values does not influence the overall statistical conclusions to be drawn when comparing different topical corticosteroid formulations because the same statistical analyses are obtained even if the zero time data are excluded. However, the CC data appear to be more accurate than the BTC data because of the correct expected rank of AUC values for Betnovate cream. Furthermore, since statistical analyses could not be the only parameter from which definite conclusions can be drawn, it was





Figures 16-17: The Chromameter BTC and CC blanching profiles for Lenovate cream pilot trial 2.

decided to compare the modelling of CC and BTC data.

3.2.3. Pharmacodynamic modelling

The arithmetic means of AUC values of all subjects at 0.25 to 6 hours dose durations were used for the PCNONLIN modelling software. The AUC data used in the modelling are shown in figures 6 to 9 for visual data. For the chromameter data the AUC values used are shown in figures 10 to 16 although those on the graphs were multiplied -1 so that they should be easily compared with the visual data. The initial estimates (E_{max}, ED_{50} and gamma) that were used in the model definition to obtain reliable estimates are shown in table 3.

Table 3: Parameter estimates that were used to model the AUC data for the chromameter a-scale and visual data using PCNONLIN simple E_{max} model 101 and sigmoid E_{max} model 105.

Parameters (Chromameter)	Simple E_{max} Model				Sigmoid E_{max} Model					
	Betnovate		Lenovate		Betnovate			Lenovate		
	E_{max}	ED_{50}	E_{max}	ED_{50}	E_{max}	ED_{50}	Gamma	E_{max}	ED_{50}	Gamma
Initial Estimates	-50	2	-50	2	-50	2	10	-50	2	10
Lower Estimates	-100	0	-100	0	-100	0	0	-100	0	0
Upper Estimates	20	6	20	6	20	6	1000	20	6	1000
(Visual)										
Initial Estimates	1000	3	1000	3	1000	3	10	1000	3	10
Lower Estimates	0	0	0	0	0	0	0	0	0	0
Upper Estimates	3000	6	3000	6	3000	6	1000	3000	6	1000

The above-mentioned initial parameter values used in the modelling software were estimated depending on the dose durations and the calculated values used. The lower and upper limits, therefore, of ED_{50} values used were 0 and 6 hours respectively. However, the 12 and 18 hours ED_{50} values as upper limits were tried in circumstances where unrealistic final parameters were obtained. The lower and upper limits for gamma value used were 0 and 10 respectively because a smaller gamma value (sigmoid model) is usually obtained (129). For the E_{max} AUC value, the

lower limit for the visually-assessed data used was zero because none of the observed AUC values were negative. The upper limit of E_{max} depended on the calculated AUC value. Since most of the calculated chromameter a-scale AUC values were negative and one value was positive, the lower limit was a negative value depending on the calculated value and a positive upper limit was set to be around 20. Owing to the range of the values obtained, the chromameter a-scale and visual AUC values were also tested with and without weighting factors (section 1.6.) for modelling purposes. The modelling software used had two options for weighting schemes, that is, a weighting factor of -1 and a weighting factor of -2. A weighting factor of -1 means that, for fitting purposes, the weight associated with each AUC value is approximately equal to 1/AUC value. The weighting factor of -2 means that the weight associated with each AUC value is approximately equal to $1/AUC^2$ value. It has been reported that scaling of weights provides increased numerical stability especially when dealing with very small observed values (128, 129). The best results were found with a weighting factor of -1 in both pilot trials 1 and 2 for visually-assessed and chromameter data. The best results were found without a weighting factor for the visual data in pilot trial 2.

Modelling of visual data

The results in table 4 clearly show that the E_{max} (AUC) and ED_{50} (hours) values predicted by the models were very close to the calculated values suggesting suitability and good performance of the models used in these pilot studies in describing the experimental data. This was confirmed by the high correlation coefficient ($r > 0.95$) between the predicted and observed AUC values in tables 5 and 6. Attempting to fit the visual data of pilot trial 1 to a simple E_{max} model resulted in the parameter approaching the upper limit (5.99 hours). When the upper limits of ED_{50} value were widened to be 12 and 18 hours, the ED_{50} value obtained was greater than 6 hours which is obviously unrealistic

since the upper dose duration used in the blanching trial was 6 hours. Any ED_{50} value obtained which was close to the upper

Table 4: Comparison between calculated and predicted estimates.

Parameters (Chromameter) (hours)	PILOT TRIAL 1 Simple E_{max} Model		PILOT TRIAL 2 Sigmoid E_{max} Model	
	Betnovate	Lenovate	Betnovate	Lenovate
Calculated E_{max}	-35.82	-46.95	-39.28	-59.25
Predicted E_{max}	-40.49	-48.34	-33.84	-96.30
Calculated ED_{50}	2.00	2.50	2.50	2.00
Predicted ED_{50}	1.71	1.94	1.43	5.99
(Visual)				
Calculated E_{max}	936.84	1188.21	771.87	957.19
Predicted E_{max}	875.92	1128.11	1501.09	1261.94
Calculated ED_{50}	3.00	2.50	2.50	2.50
Predicted ED_{50}	3.23	3.25	5.99	2.80

The predicted E_{max} (AUC) and ED_{50} (hours) values shown above for the visual data in pilot trial 1 are for the sigmoid model since the simple E_{max} model was found to produce unreliable results (Table 5 and 6).

limit was regarded as unrealistic because it does not represent the duration at which the response is half-maximal by definition. Despite similar correlation coefficients for the simple and the sigmoid E_{max} models, the standard error and AIC values for the sigmoid model are clearly much lower than that for the simple model suggesting that the visual data are best described by the sigmoid model. For pilot trial 2, fitting the data to a simple model was unsuccessful for both formulations (table 6). For Betnovate cream, the data fitted a sigmoid E_{max} model resulting in the unrealistic parameter close to the upper limit (5.99 hours). For Lenovate cream, fitting the data to a sigmoid E_{max} model produced a reliable estimate of ED_{50} which was 2.8 hours and was close to the calculated value of 2.5 hours from the blanching profiles in figure 9. From these results, it was concluded that in pilot trial 2, the visual data are best described by the sigmoid model. Based on the visual data obtained in both pilot trials, the dose duration that would be selected according to the

Guidance for the pivotal study bioequivalence testing is an ED_{50} value of 3 hours for either Betnovate cream or Lenovate cream as the reference drug.

Modelling of chromameter data (when zero time readings were excluded. CC data)

The Lenovate a-scale data in pilot trial 1 (table 5) fitted to a sigmoid model produced an ED_{50} value of 5.92 which was close to the upper limit. This value was unexpected because the predicted and calculated E_{max} values were close to each other and, therefore, one would expect the predicted ED_{50} value to be close to the calculated value which was 2 hours. Further attempts to fit these data to the sigmoid model by widening the upper limit to 12 and 18 hours yielded similar goodness to fit criteria (AIC and r values) to those obtained for the simple E_{max} model, but with an unrealistic fitted estimate for the ED_{50}. Similar fitting procedures were attempted for the Betnovate cream data using the sigmoid model with no success. If one assumes that a reasonable fit of the data is indicated by a standard error of estimate that is as small a percentage as possible of the estimated parameter, then it is clear that the simple E_{max} model produces a better solution for the chromameter results than the sigmoid model. Based on these results, it was decided that the a-scale data were best fitted to the simple E_{max} model for pilot trial 1.

In pilot trial 2, the results in table 6 show that the modelling of Betnovate and Lenovate AUC data to a simple E_{max} model resulted in similar ED_{50} value obtained which was 0.25 hours. This value predicted by the model was smaller than the calculated value of 2.5 or 2 hours from the graphs in figures 11 and 13. Despite high correlation ($r = 0.99$) between the experimental and predicted AUC data, and slightly lower AIC values for Betnovate cream for the sigmoid model in table 6, the ED_{50} value obtained

Table 5: Parameter estimates obtained for pilot trial 1.

Parameters (Chromameter) (The CC data)	Simple E_{max} Model		Sigmoid E_{max} Model	
	Betnovate	Lenovate	Betnovate	Lenovate
E_{max} (AUC)	-40.49	-48.34	*	-73.02
Standard Error	12.04	13.41	*	162.91
ED_{50} (hours)	1.71	1.94	*	5.92
Standard Error	1.01	1.12	*	36.79
Gamma	-	-	*	0.68
Standard Error	-	-	*	0.73
Correlation(r)	0.82	0.86	*	0.86
AIC	53.12	54.93	*	55.78
(Visual)				
E_{max} (AUC)	1767.62	1912.31	875.92	1128.11
Standard Error	401.07	964.80	101.31	107.75
ED_{50} (hours)	5.99	5.99	3.23	3.25
Standard Error	2.03	4.34	0.38	0.30
Gamma	-	-	7.23	7.19
Standard Error	-	-	1.83	1.46
Correlation(r)	0.99	0.97	0.99	0.99
AIC	91.11	106.60	72.91	71.91

Table 6: Parameter estimates for pilot trial 2.

Parameters (Chromameter) (The CC data)	Simple E_{max} Model		Sigmoid E_{max} Model	
	Betnovate	Lenovate	Betnovate	Lenovate
E_{max} (AUC)	-37.38	-37.92	-76.82	-43.62
Standard Error	2.66	4.20	164.42	27.64
ED_{50} (hours)	0.22	0.23	5.67	0.26
Standard Error	0.08	0.13	83.06	0.49
Gamma	-	-	0.29	0.59
Standard Error	-	-	0.42	0.97
Correlation(r)	0.83	0.69	0.92	0.70
AIC	46.51	54.91	41.90	56.55
(Visual)				
E_{max} (AUC)	1437.29	*	1501.09	1261.94
Standard Error	489.54	*	1766.99	374.27
ED_{50} (hours)	5.99	*	5.99	2.80
Standard Error	3.41	*	12.02	1.24
Gamma	-	*	1.12	1.75
Standard Error	-	*	0.57	0.59
Correlation(r)	0.96	*	0.99	0.99
AIC	100.02	*	100.59	101.96

The star (*) symbol means that the model used was unsuccessful to generate any results. The dash (-) symbol means that the model is designed not to calculate such parameters.

which was 5.67 was unreliable as it was close to the upper estimate. When the AUC data for Lenovate cream where fitted to a sigmoid model, the ED_{50} value of approximately 0.25 hours was obtained which was similar to the value found when the same data were fitted to a simple E_{max} model.Despite similar ED_{50} estimates, the AIC value was slightly higher for the sigmoid E_{max} model than for the simple E_{max} model although the same correlation coefficient was obtained. The standard error estimates of the ED_{50} and E_{max} values obtained for the sigmoid model were much greater than for the simple E_{max} model suggesting that the chromameter data were fitted best to a simple E_{max} model in pilot trial 2.

Modelling of chromameter data (when zero time readings were included. BTC data)

The AUC data calculated from the corrected a-scale data when zero time values were included (BTC) and the AUC data calculated from corrected a-scale data without zero time values (CC) from pilot trial 2 were compared using the simple and sigmoid E_{max} models. The estimates predicted by the models are shown in table 7. Considering Betnovate cream using the BTC data, it was difficult to distinguish whether the sigmoid or simple E_{max} model best described the a-scale data, due to similarity of the predicted AIC and r values between the two models. In addition to the

Table 7: Parameter estimates for the chromameter values when zero values were included in the pilot trial 2 (BTC data).

Parameters (Chromameter) (The BTC data)	Simple E_{max} Model		Sigmoid E_{max} Model	
	Betnovate	Lenovate	Betnovate	Lenovate
E_{max} (AUC)	-41.72	-96.29	-33.84	-50.88
Standard Error	20.78	75.39	26.67	14.38
ED_{50} (hours)	2.29	5.99	1.43	2.11
Standard Error	2.64	7.83	2.33	0.83
Gamma	-	-	1.29	2.99
Standard Error	-	-	1.79	2.61
Correlation(r)	0.74	0.89	0.74	0.91
AIC	63.51	66.36	65.52	65.11

the standard error estimate of E_{max} value was smaller for the simple E_{max} model than when using the sigmoid E_{max} model. However, the standard error estimate of the ED_{50} value was smaller for the sigmoid E_{max} model than when using simple E_{max} model. Conclusions on the performance of the best model could, therefore, not be drawn. For Lenovate cream, the AIC and r values were slightly better for the sigmoid model than for the simple E_{max} model. The standard error estimates, however, were much greater for the simple E_{max} model suggesting that the BTC data were best described by the sigmoid E_{max} model. These results were corroborated by the ED_{50} estimates because the value predicted from the model which was 2.11 was similar to the calculated value of 2 hours when using the sigmoid model in table 4. For the simple model the ED_{50} value was 5.99 hours whereas the calculated value was 2 hours.

For the CC data, it has already been described previously that the simple E_{max} model best fitted the experimental data. Based on the CC data, the dose duration that would be selected according to the Guidance for the pivotal study bioequivalence testing is an ED_{50} value of 0.25 hours for either Betnovate cream or Lenovate cream as the reference drug. For the BTC data, bioequivalence testing in the pivotal study would imply the use of ED_{50} value of 2 hours for Lenovate cream as the reference drug. Assuming that Betnovate cream BTC data were also best described by the sigmoid model from the results in table 7, the ED_{50} value of 1.5 hours would be utilized. The modelling of CC and BTC data revealed that the inclusion of zero time readings would result in different ED_{50} estimates compared to the data where such readings are excluded. However, the question remains whether bioequivalence testing at 0.25 or 2 hours would result in the same conclusions or not. From the results of the pilot trials described earlier, the visual and chromameter methods demonstrated no significant differences between Betnovate and Lenovate cream at all observation times for these dose durations.

The reason is probably because there is insufficient blanching response to distinguish the performance differences of the two formulations at 0.25 to 2 hours dose durations. It was only the visual method which was able to show subtle differences at 5 and 6 hours dose durations. Since longer dose durations may be more discriminatory, it remains to be proven whether short dose durations can be used successfully for bioequivalence assessment. This aspect becomes more important if one considers the clinical use of topical corticosteroids which would typically have skin-contact times in excess of six hours. Ideally, bioequivalence should, as far as possible, parallel normal clinical dosage regimens. The best results obtained from the modelling of chromameter and visual data are summarized in tables 8 and 9.

Table 8: A summary of the best results obtained from the pharmacodynamic modelling for chromameter data.

Parameters (Chromameter) (The CC data)	PILOT TRIAL 1 Simple E_{max} Model		PILOT TRIAL 2 Simple E_{max} Model	
	Betnovate	Lenovate	Betnovate	Lenovate
E_{max} (AUC)	-40.49	-48.34	-37.38	-37.92
Standard Error	12.04	13.41	2.66	4.20
ED_{50} (hours)	1.71	1.94	0.22	0.23
Standard Error	1.01	1.12	0.88	0.13
Correlation(r)	0.82	0.86	0.83	0.69
AIC	53.12	54.93	46.51	54.91

Table 9: A summary of the best results obtained from the pharmacodynamic modelling for visual data.

Parameters (Visual)	PILOT TRIAL 1 Sigmoid E_{max} Model		PILOT TRIAL 2 Sigmoid E_{max} Model	
	Betnovate	Lenovate	Betnovate	Lenovate
E_{max} (AUC)	875.92	1128.11	1501.09	1261.94
Standard Error	101.31	107.75	1766.99	374.27
ED_{50} (hours)	3.23	3.25	5.99	2.80
Standard Error	0.38	0.30	12.02	1.24
Gamma	7.23	7.19	1.12	1.75
Standard Error	1.83	1.46	0.57	0.59
Correlation(r)	0.99	0.99	0.99	0.99
AIC	72.91	71.91	100.59	101.96

It has been reported that many drug concentration-effect

relationships are described by the nonlinear sigmoid E_{max} model and the same model is frequently used to model the clinical pharmacodynamics of drugs (151). It is interesting to note that the visual data follows a sigmoid dose-response relationship normally seen in pharmacological systems while the chromameter data follows a more geometric pattern in both pilot trials. This does not necessarily imply that the visual assessment is better than the instrument because the instrument may be measuring some parameters not apparent to the eye.

In summary, the major trend which emerges from the two pilot trials is that the precision of the chromameter does not appear to be as good as the visual data obtained from experienced observers. The chromameter results showed poor trial-to-trial reproducibility suggesting that the visual method remains the most sensitive tool which monitors the corticosteroid-induced blanching more accurately than the chromameter. A recent publication corroborates these findings (56). The expected rank order of AUC values at the 0.25-1.5 hours dose durations in both trials were not observed, suggesting that the use of short dose durations in the FDA pilot study may lead to the generation of unreliable data from which invalid conclusions may be drawn. The inclusion of zero time values resulted in the same statistical conclusions as the data without zero time readings from the analysis of blanching profiles. However, the modelling of both data sets produced different estimates implying that the ED_{50} value which will be used in the pivotal bioequivalence study will be different depending upon whether the zero time values were included or not. The effect of excluding zero time readings may therefore, be a worrying aspect for the objectives of the pilot study. Since the smaller ED_{50} estimates were obtained, whether these different estimates will produce similar or different results in the pivotal study remains to be investigated. Assessment of response between Betnovate and Lenovate cream at the 0.25 to 1.5 hours dose durations demonstrated no statistical

differences. Based on experience for the visual assessment of corticosteroid induced-skin blanching in this laboratory (65,67,68,141,152) the low degree of blanching elicited at shorter application times may not be intense enough or sufficiently discriminatory to show up differences in the drug delivery potential of similar formulations. This may lead to errors in assigning bioequivalence to formulations that are not equivalent simply because the methodology is not sufficiently precise to distinguish between the products at lower blanching intensity.

The Guidance does not specify a non-linear pharmacodynamic model for use in data analysis. Therefore, the use of different models and weighting factors will, almost certainly, produce different results. This being the case, the potential exists for the inappropriate selection (purposefully or inadvertently) of a model definition that may favour delivery dynamics from a particular formulation. The recent findings by Marston et al. (127) who used three different models in their pilot study data concluded that the different modelling approaches may result in clinically significant differences in population estimates. Therefore, it is possible that the pivotal bioequivalence study may be conducted at significantly different dose durations simply based on the pharmacodynamic model chosen in the pilot data analysis.

Even though the predicted ED_{50} values for visual and chromameter data are different, the estimated E_{max} values were close to the calculated values suggesting that visual and chromameter data can be fitted to different E_{max} models. However, a worrying aspect of the pharmacodynamic modelling of the a-scale data is that similar goodness of fit criteria were obtained when different upper ED_{50} values are set in the model definition, even though the model-predicted ED_{50} values from these definitions are unrealistic. It was evident for both data sets that model selection should not be

only be based on goodness of fit criteria (correlation coefficient, AIC, standard error of estimate) but also on parameter estimates that are realistic for the data collected. The standard error estimate was found to be more sensitive than the other parameters in selecting the best model that described the observed experimental data.

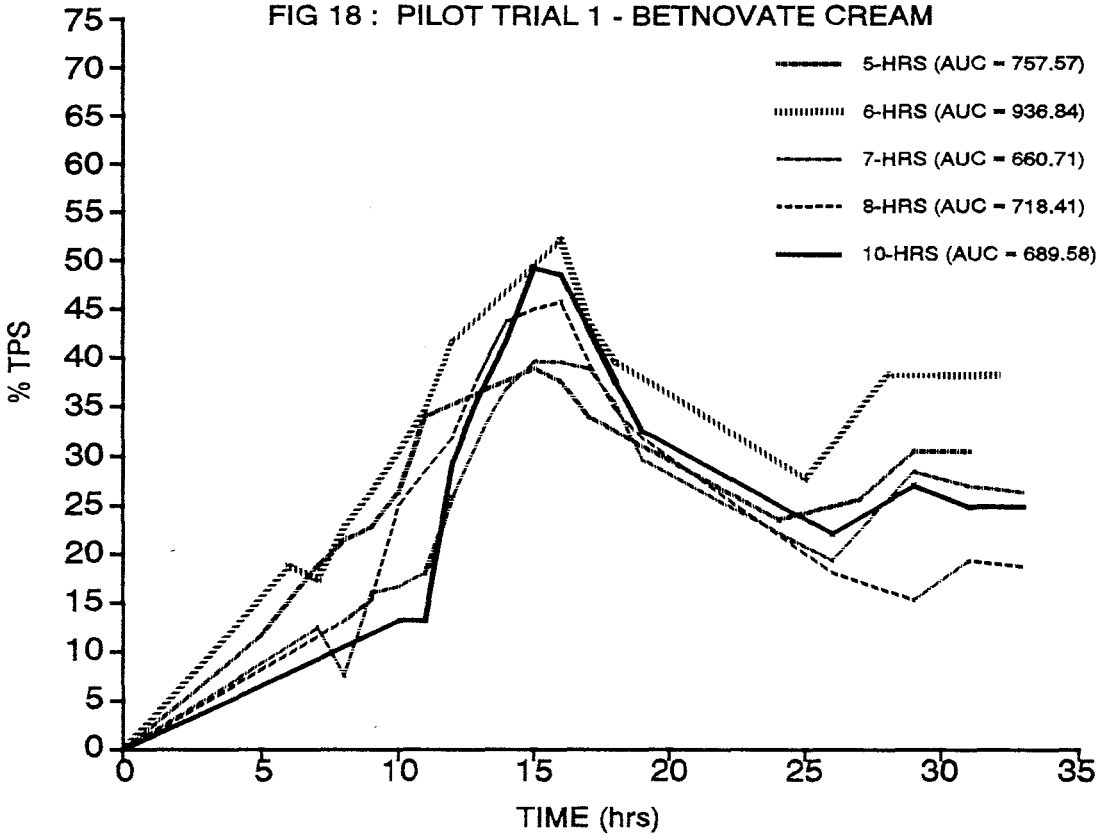
The purpose of the Guidance is to standardize the methodology for assessing corticosteroid formulations by use of an objective instrumental procedure. However, the variability allowed in the Guidance in terms of the mass of formulation applied to the skin, anatomical skin sites along the forearm to be utilised, chromameter probe manipulations and methods of data modelling employed, make it highly probable that different results will be obtained if the same study were to be performed by different investigators.

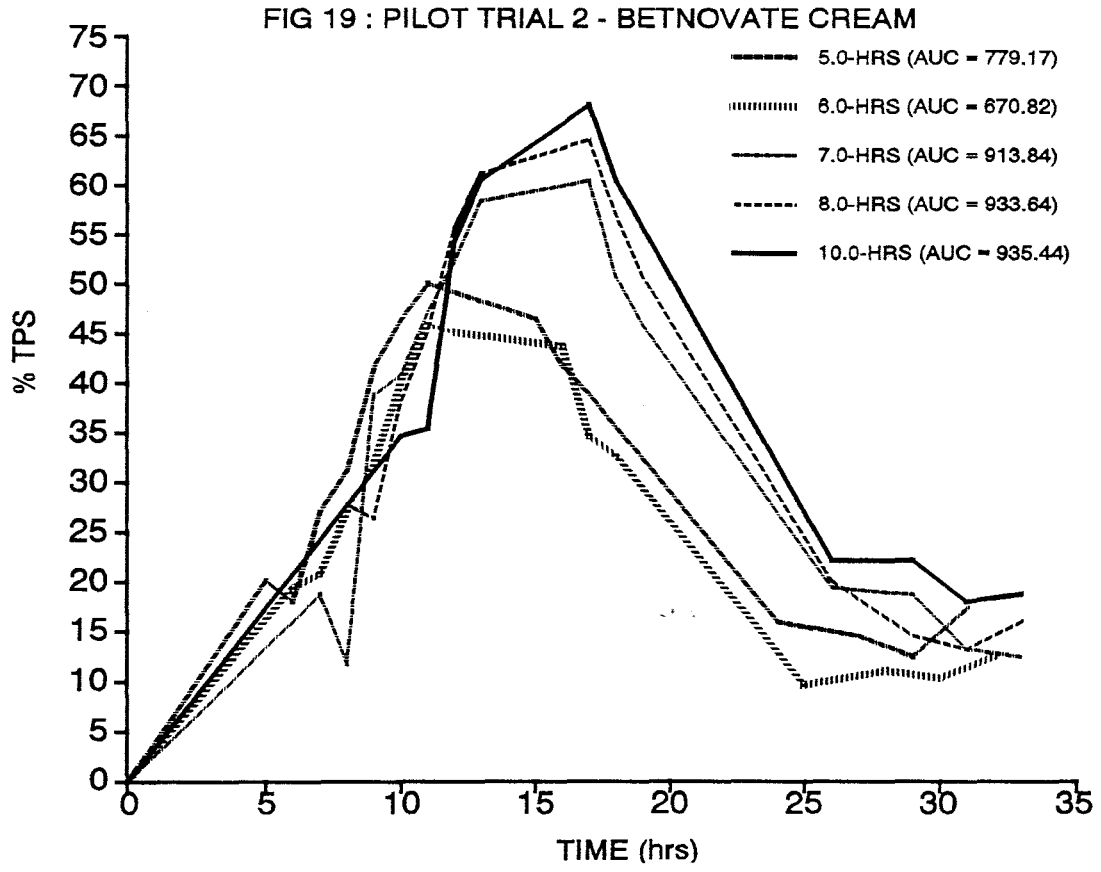
3.2.4. The effect of longer application time on blanching

Visual data

The results discussed earlier from the two pilot trials appeared to indicate that greatest blanching was occurring at the 5 hour dose duration. It was therefore decided to compare the blanching responses at 5, 6, 7, 8 and 10 hours after application to establish the dose period in which maximum blanching response occurs. This was also important considering that the Guidance assumes that the maximum response induced by the topical corticosteroid occurs around 6 hours application time.

Figures 18-19 depict the visual blanching profiles and AUC values at various dose durations for Betnovate cream. Considering Betnovate cream, the visual response profiles and AUC values for pilot trial 1 do not clearly show any pattern on the degree of response with respect to an increase in dose duration. In contrast, the results of pilot trial 2 demonstrated that an increase in dose duration was accompanied by an increase in the

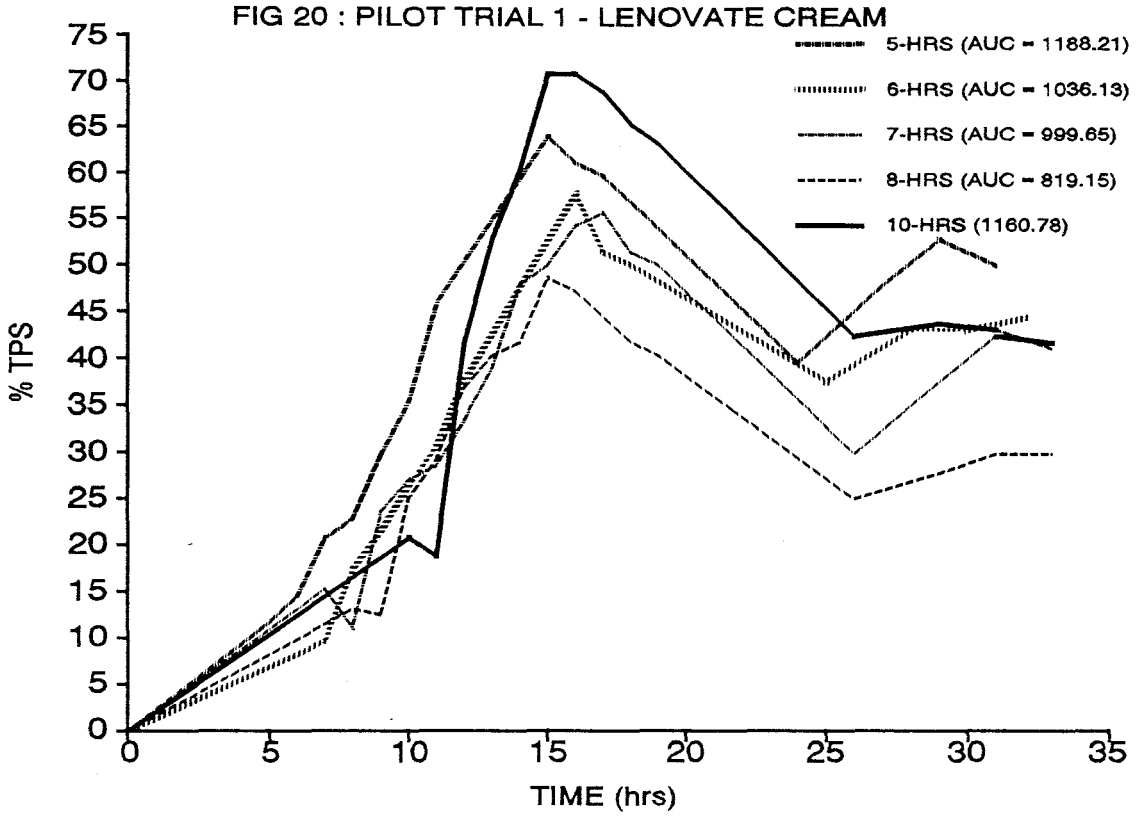


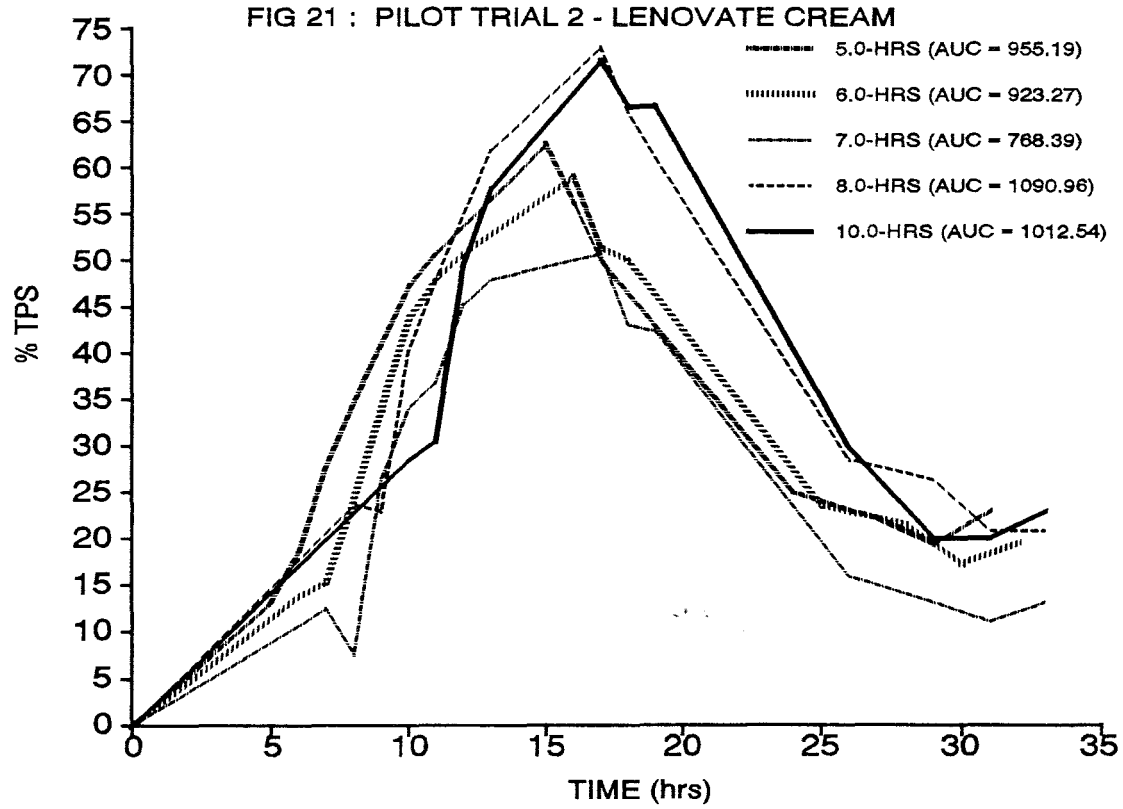


Figures 18-19: Visual blanching profiles from 5 to 10 hours dose durations for Betnovate formulation for pilot trials 1 and 2.

degree of response. However, significant differences in blanching response were noted between 5 and 7, and 6 and 8 hours dose durations but few statistical significant differences were found between 8 and 10 hours duration for both pilot trials. The implication of these results is that the degree of skin blanching at 10 hours dose duration was similar to that at 8 hours duration. This was corroborated by the AUC values as it was clearly obvious that the difference in the AUC values obtained between 8 and 10 hours was very small especially in pilot trial 2. These results, therefore, suggest that a blanching plateau effect (maximum blanching response) was being attained at 8 hours dose duration. Montenegro et al. (138) in a recent publication reported similar results. The investigators used betamethasone 17-valerate cream and found that visual assessment was able to show no significant differences in the blanching response at 8, 10 and 24 hours. However, the workers did not compare the blanching response between 5 hours and the longer dose durations to establish if the blanching plateau was not obtained at 5 hours dose duration. In contrast, the experiments of Magnus et al. (122) showed attainment of maximum blanching response at 10 hours using the same corticosteroid. The researchers did compare the blanching response between shorter and longer dose durations. They noted significant differences for the 4, 6, 8 and 10 hours but few were found between the 10 and 12 hours dose durations.

For Lenovate cream (figures 20 and 21), the response profiles and AUC values show that skin blanching was greatest at the 5 hour dose duration in pilot trial 1. Despite this response, the difference in the AUC values of 5 and 10 hours duration was not that much implying that skin blanching was similar at these periods. It was not clear why the response decreased when the dose duration was increased from 5 to 8 hours and the blanching increased again at 10 hours dose duration. One could speculate an interaction of the corticosteroid with a skin reservoir (section 1.4.) at 5 to 8 hours periods. At 10 hours duration,





Figures 20-21: Visual blanching profiles from 5 to 10 hours dose durations for Lenovate formulation for pilot trials 1 and 2.

some of the drug molecules which were held by the reservoir were probably released, and hence blanching increased. The significance of comparing the blanching response at shorter and longer dose durations, therefore, becomes apparent because if the 10 hour application time was not used in the experiment, one could have probably concluded that the plateau effect was being achieved at 5 hours dose duration since there were no significant statistical differences in the blanching response between the 5 and 6 hour dose durations. These results could also explain why the FDA assumes that a blanching plateau effect occurs at about 6 hours. It is possible that this assumption came into being because they did not assess blanching response at longer dose durations. The statistical analyses demonstrated no significant differences in the skin blanching between 8 and 10 hours suggesting that the blanching plateau effect was not being attained at 8 hours duration in contrast with the results for Betnovate formulation. For pilot trial 2, the greatest response was found at 8 hours dose duration although the response profiles and AUC values at 8 and 10 hours were similar. Statistically significant differences were noted between 7 and 8 hours but few were found between 8 and 10 hours suggesting the attainment of maximum blanching response at 8 hours duration in congruity with the results for Betnovate cream in pilot trial 1.

Chromameter data

The chromameter profiles are shown in figures 22-25. The chromameter response profiles for both formulations do not show any clear pattern in the response with an increase in dose duration in both pilot trials. For Betnovate using the AUC values, the greatest response was found at 8 hours in pilot trial 1 and at 10 hours in pilot trial 2. Although skin blanching was greatest at the 5 hour dose duration for Lenovate cream in both pilot trials, an increase in dose duration from 5 hours resulted in the corresponding increase in the AUC value especially in pilot trial 2. These results may imply that maximum blanching

FIG 22 : PILOT TRIAL 1 - BETNOVATE CREAM

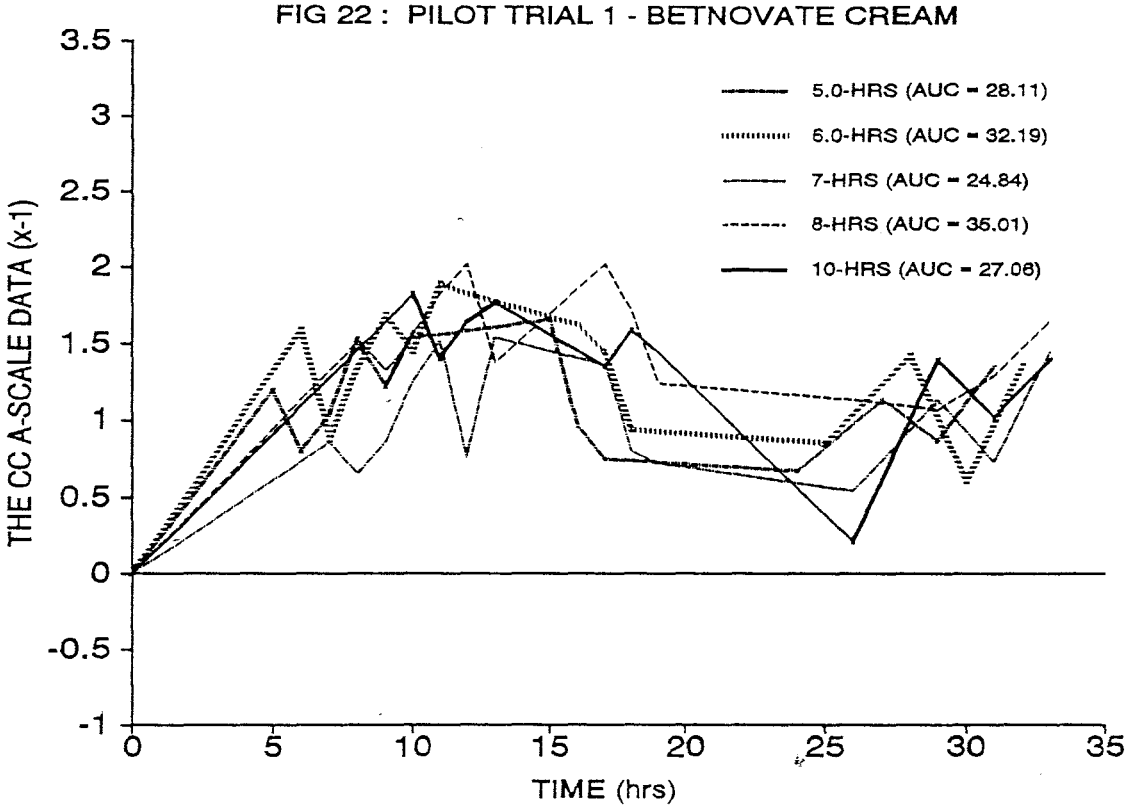
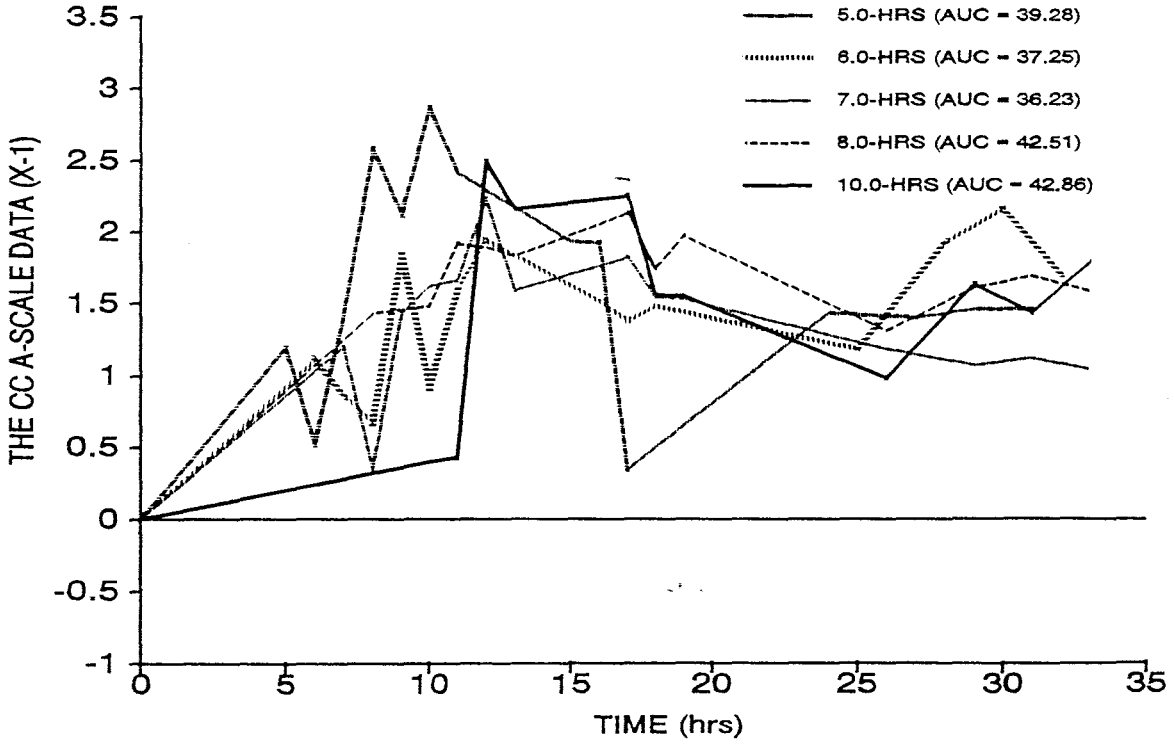
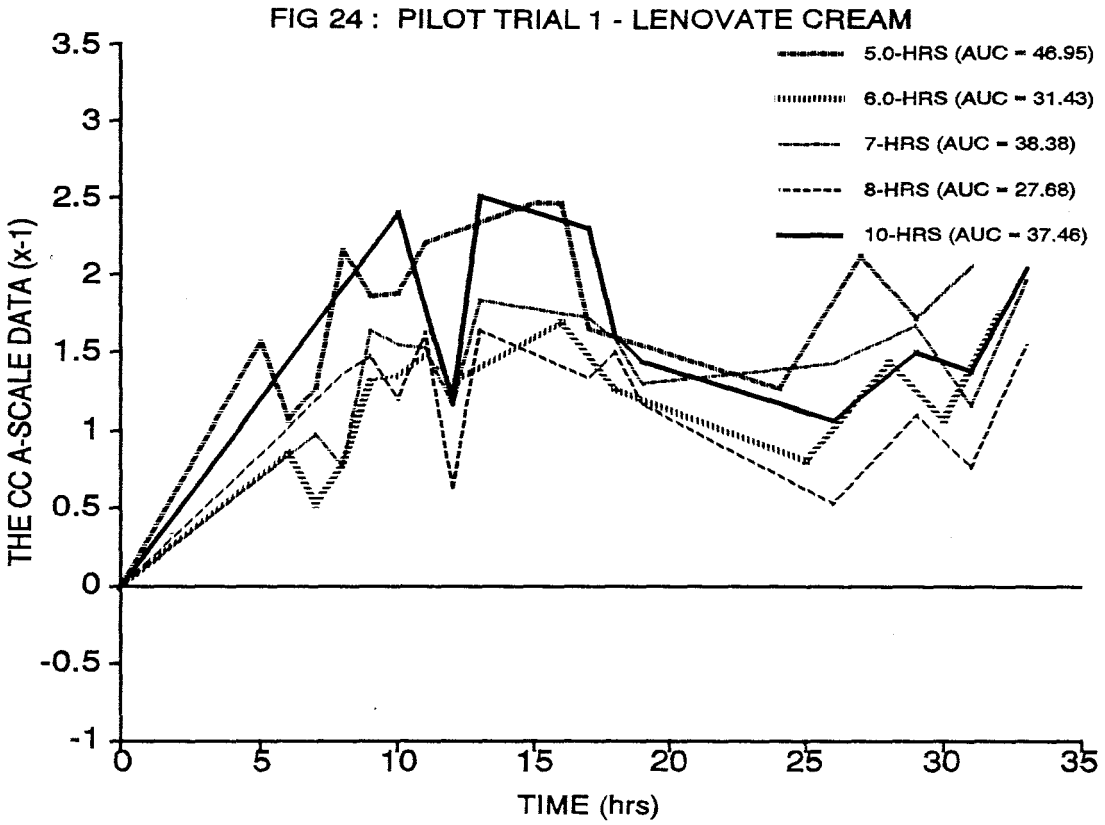
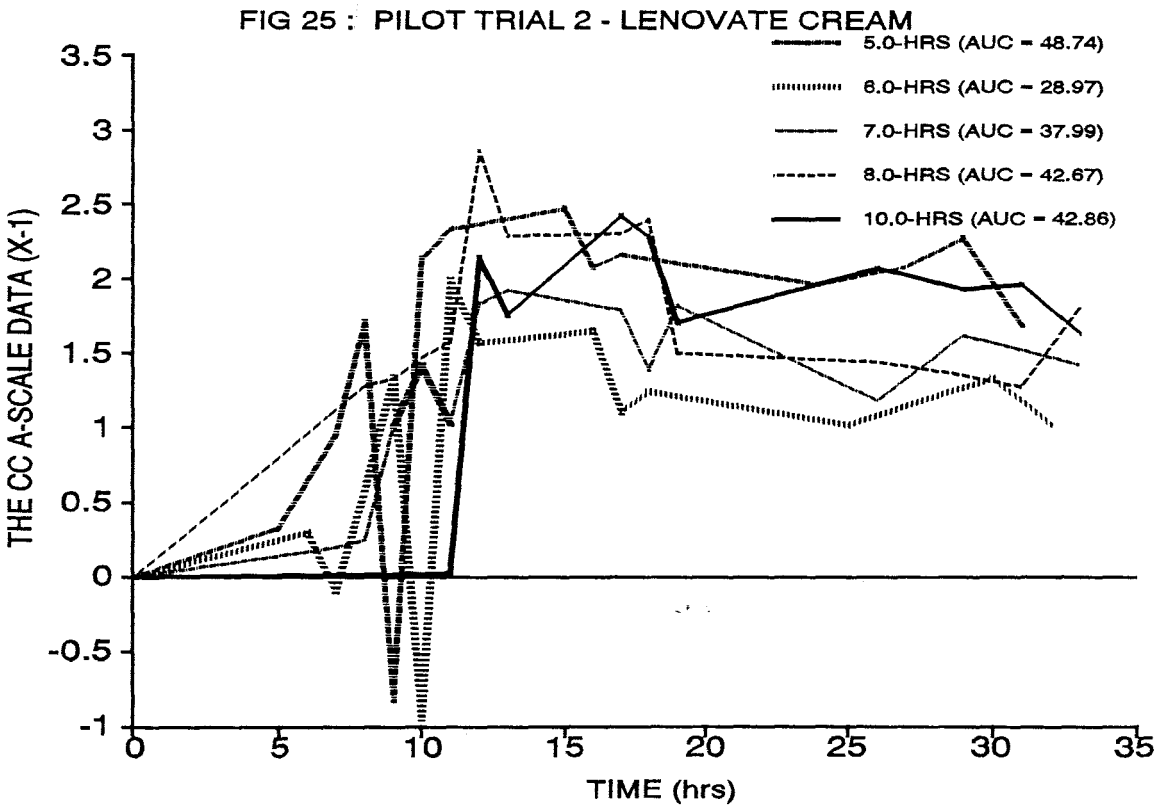


FIG 23 : PILOT TRIAL 2 - BETNOVATE CREAM



Figures 22-23: Chromameter blanching profiles from 5 to 10 hours dose durations for Betnovate cream in pilot trials 1 and 2.





Figures 24-25: Chromameter blanching profiles from 5 to 10 hours dose durations for Lenovate cream in pilot trials 1 and 2.

response does not occur at the 5 hour dose duration. It is interesting to note that although the comparison of 5 hours with longer dose durations was not done in the experiments of Montenegro et al (138), it was reported using the chromameter that no significant differences were found between skin blanching at 8 and 10 hours. For both Betnovate and Lenovate creams, no differences were noted in the blanching responses between 5 and 7, 6 and 8, 8 and 10 hours for pilot trials 1 and 2. Based on these results it was decided to see if the chromameter would be able to distinguish skin blanching that occurs at the 5 and 10 hour dose durations. For these durations, significant differences were generally not found at all observation times. Only two data points in pilot trial 1 and one data point in pilot trial 2 were found to show statistical significant differences. These data points were blanching response assessed at observation times made immediately after product removal from the skin sites. It was observed during the trials that immediately after washing the application sites, the arms of the volunteers were whitish in appearance. Kaidbey et al. (153) have noticed similar observation and reported that the whitish appearance could be due to hydration of the skin. It is therefore likely that the chromameter was measuring the white colour on the skin not induced by the corticosteroid but the colour dependent on the hydration of the skin. The Guidance should not recommend measuring or observing skin blanching immediately after washing the application sites, otherwise erroneous data may be generated. Since the chromameter was unable to distinguish blanching responses at 5 and 10 hours, its precision in measuring topical corticosteroid-induced blanching is questionable.

In summary, the visual and chromameter results indicated that the maximum blanching response does not occur at the 5 or 6 hour dose durations as is assumed by the FDA. The assumption of getting maximum response at the 6 hour dose duration appears therefore not to be valid. Apart from excellent differentiation of topical

corticosteroids that has been found at the 6 hour dose duration, this application time has other advantages in that it is convenient and does not cause undue discomfort to the subjects (40). Because of the above-mentioned reasons, it is proposed that the FDA should review the suggestion that topical corticosteroids should be assessed at the 6 hour dose duration.

3.3. THE PIVOTAL TRIAL

3.3.1. Bioequivalence testing using the FDA-recommended Locke statistical method

The current statistical methods of determining bioequivalence testing are those that show that the estimates determined in the sample are best representative of the population (79). The null hypothesis statistical methods which test a straightforward "yes" or "no" statement on differences between drug formulations are believed not to be the appropriate methods because they do not directly indicate the extent of the differences between two formulations. With this view in mind, Westlake et al. (135) proposed the concept of confidence intervals which attempts to show directly if the data obtained in the sample are best applicable to the population. In bioequivalence studies, if the ratio of the means of the test ($\overline{X_T}$) to the reference formulation ($\overline{X_R}$) of the chosen measure of bioavailability (AUC) lies between the given limits r_1 and r_2,

$$r_1 \leq \frac{\overline{X_T}}{\overline{X_R}} \leq r_2 \qquad \text{Equ. (14)}$$

then the formulations are said to be bioequivalent. The choice of r_1 and r_2 is made on medical and regulatory grounds depending on sufficient data that are available concerning bioequivalence studies. For example, the r_1 may be 0.8 and the r_2 may be 1.2 which implies that the AUC of the test formulation should not differ by more than ±20% from that of the standard formulation if the two products are bioequivalent.

Although there are many bioequivalence testing methods that use confidence limits, the currently accepted statistical test is the Classical 90% Confidence Interval (79). However, Locke's method (131) recommended by the FDA for assessing topical corticosteroid bioequivalence has been described (79) as a reliable test similar to the Classical method when assessing bioequivalence of orally administered drugs. Using Locke's method, a 90% confidence interval should be calculated for the ratio of the average AUC response due to test product to the average AUC response due to the reference product. A 90% Confidence Interval means that it is 90% certain that the population mean will fall within the interval calculated for the sample mean. However, the Guidance has not yet provided recommendations on the equivalence limits to accept bioequivalence data although it recognizes that limits wider than 0.80 - 1.25% as standard may be necessary depending on evaluation of the data submitted to the agency. Normally there are two criteria that are used to declare bioequivalence using Locke's method:

1. Level of assurance which is usually 90%.
2. Range of confidence interval limits which is 0.80 and 1.20.

If the calculated CI limits for the mean ratio of $\overline{X_T}/\overline{X_R}$ for the sample is not within the 0.80 - 1.20 range, it means that the two products will be declared not bioequivalent at the 90% level of significance. This criterion was used in all the three pivotal trials. The use of Locke's method requires that the average value of ratio of $\overline{X_T}/\overline{X_R}$ must be calculated from the total number of subjects for that sample rather than first calculating the ratio of $\overline{X_T}/\overline{X_R}$ on individual subjects and then calculating the average value. The use of a statistical test that calculates the average value by the latter method may be more appropriate because it takes into account the intra-subject variability for both test and reference formulation which is often observed in clinical trials (154). A simple general confidence interval formula (155) which calculates the mean $\overline{X_T}/\overline{X_R}$ ratio of individual subjects was also used for comparisons purposes.

The formulae used in this thesis were exactly the same as in the Guidance. The calculation of the 90% confidence interval (CI) involves determining the sample means, sample variances and sample covariance for "acceptable subjects" determined from the D_2/D_1 criterion described in chapter one.

The formulae for the sample means are presented in Equ. (15) and (16) respectively,

$$\bar{X}_T = \frac{1}{n}\sum X_{Ti} \qquad \text{Equ. (15)}$$

$$\bar{X}_R = \frac{1}{n}\sum X_{Ri} \qquad \text{Equ. (16)}$$

The formulae for the sample variances are as follows:

$$\hat{\sigma}_{TT} = \frac{\sum (X_{Ti} - \bar{X}_T)}{n - 1} \qquad \text{Equ.(17)}$$

$$\hat{\sigma}_{RR} = \frac{\sum (X_{Ri} - \bar{X}_R)}{n - 1} \qquad \text{Equ.(18)}$$

The formula for the covariance of both reference and test formulation data sets is as follows:

$$\hat{\sigma}_{TR} = \frac{\sum (X_{Ti} - \bar{X}_T) \;.\; (X_{Ri} - \bar{X}_R)}{n - 1} \qquad \text{Equ.(19)}$$

where n is the number of "acceptable blanchers". The subscript R and T refer to reference drug formulation (Betnovate cream) and test drug formulation (Lenovate cream) respectively.

The 90% CI can only be calculated if the two products are assumed to be bioequivalent through the calculation of the following parameter which is not defined in the Guidance. The parameter (G) is given by the following equation:

$$G = \frac{t^2 . \hat{\sigma}_{RR}}{n . \bar{X}_R} \qquad \text{Equ. (20)}$$

where t value is the value obtained from the normal Student's t-distribution test at 0.95 percentile for n-2 degrees of freedom.

The Guidance stipulates that if G < 1, the two products are bioequivalent and therefore a 90% proper confidence interval is needed. Conversely, if G ≥ 1, the two products are not bioequivalent and hence a confidence interval is not required. It should be noted that even though the G parameter appears to be a measure of bioequivalence in accordance with the Guidance, the acceptance of bioequivalence is only confirmed by the calculation of 90% CI limits. If the calculated G value is less than 1 and hence bioequivalence is assumed, the 90% confidence interval limits can now be calculated using the following formula:

$$90\%CI = \left(\frac{\overline{X}_T}{\overline{X}_R} - \frac{G.\hat{\sigma}_{TR}}{\hat{\sigma}_{RR}}\right) \mp \frac{t}{\overline{X}_R}\left(\frac{\hat{\sigma}_{RR}.K}{n}\right) \qquad \text{Equ. (21)}$$

where K is given by the following equation:

$$K = \left(\frac{\overline{X}_T}{\overline{X}_R}\right)^2 + \frac{\hat{\sigma}_{TT}(1 - G)}{\sigma_{RR}} + \frac{\hat{\sigma}_{TR}}{\hat{\sigma}_{RR}}\left(\frac{G.\hat{\sigma}_{TR}}{\hat{\sigma}_{RR}} - 2.\frac{\overline{X}_T}{\overline{X}_R}\right) \qquad \text{Equ. (22)}$$

A simple general formula used for calculation of confidence interval limits is as follows:

$$90\%CI = \frac{\overline{X}_T}{\overline{X}_R} \pm \frac{t(0.95)s}{\sqrt{n}} \qquad \text{Equ. (23)}$$

where s is the sample standard deviation, n is the number of data items and t is Student's t-distribution test value at the 95% level of significance for n-1 degrees of freedom.

Seven subjects were found to be "acceptable blanchers" according to the D_2/D_1 criterion. The data of these seven subjects were compared with the data of all volunteers used for the pivotal study, that is, 40 subjects. The objective was to see if the size of the sample would influence bioequivalence testing. The G values and 90% confidence interval limits (CI) obtained in accordance with Locke's method are summarized in table 10. The results obtained for the general formula are presented in table 11. In general, although the calculated confidence interval limits for the Locke's method are not the same as those for the general simple method, both statistical tests have shown that

Betnovate and Lenovate creams are not bioequivalent. The exceptions were for the 6 hour dose duration for visual data and at 2 and 3 hours for the chromameter data. At the 6 hour dose duration, Locke's method demonstrated that the two products are bioequivalent since the calculated confidence interval limits were within the standard 0.80 and 1.20 CI range. Although this was not found with the general method, the CI limits for the Locke's method do not differ much from those of the simple formula.

Table 10: The calculated confidence interval (CI) and parameter G values from the FDA-recommended Locke's method at various dose durations.

DOSE DURATION (hours) Chromameter	n = 7		n = 40	
	G value	90% CI	G value	90% CI
2	0.72	0.72 - 4.57	1.28	*
3	0.55	1.07 - 6.80	2.46	*
6	0.15	0.39 - 1.26	0.14	0.21 - 0.72
Visual				
2	0.29	1.01 - 1.32	0.06	1.22 - 1.45
3	0.21	1.41 - 1.99	0.05	1.36 - 1.69
6	0.13	0.83 - 1.17	0.03	1.03 - 1.26

Table 11: The calculated confidence interval (CI) for the general simple formula at various dose durations.

DOSE DURATION (hours) Chromameter	n = 7 95% CI	n = 40 95% CI
2	0.33 - 1.97	0.07 - 1.03
3	2.61 - 6.44	0.64 - 1.76
6	0.40 - 1.69	0.72 - 19.52
Visual		
2	1.05 - 1.30	1.34 - 1.93
3	1.70 - 12.09	1.68 - 3.97
6	0.85 - 1.27	1.20 - 1.62

n is the number of subjects used.
CI is the calculated confidence interval limits of the mean (AUC) ratio of test formulation (Lenovate cream) to reference formulation (Betnovate cream).
G is the value that determines whether bioequivalence should be assumed to exist or not.
* is the symbol which denotes that confidence interval could not be calculated because G value was greater than 1 and hence bioinequivalence was assumed.

It is also worth emphasizing that although the FDA has not provided the standard CI limits, it recognizes that the 0.80 to 1.25 range may be used depending on the data submitted to the agency. The implication of the above-mentioned criterion is that even if it was used in this study, the results for the general formula will also indicate that the two products are bioequivalent because the calculated CI are still within the standard 0.80 to 1.25 limits. Not much can be said about the "G" parameter because its significance is not specified in the cited literature. The parameter, however, appears to be affected by the number of subjects used in the statistical analysis for the chromameter data because the "G" values were greater than 1 for a bigger sample size of 40 subjects at the 2 and 3 hour dose durations. However, definite conclusions could not be drawn because the visually-assessed data were not affected by the sample size. Since the "G" value is incorporated in the calculation of the confidence interval, bioequivalence testing may also be affected if the size of the sample can have an influence in the determination of the parameter.

Locke (131) reported that the two formulations can be incorrectly declared bioequivalent if the number of subjects is too small. This view was also expressed by Westlake et al. (135) who commented that a trial of small sample size will give confidence intervals so large that the practical bioequivalence between the two formulations cannot be demonstrated. It has also been reported (135) that subtle differences between the two formulations will be detected as significant if the trial is sufficiently well controlled and the number of subjects employed is large enough. This aspect should have been obvious in the results of this pivotal study even using the "G" parameter because a bigger sample size was used. It is not clear why the "G" parameter demonstrated subtle differences for the chromameter data but not for the visual data. In addition, the differences were noted not at the 6 hour dose duration but at lower dose

durations for which their data have been found to be questionable from the earlier results of the pilot trials.

It would be interesting to investigate if the relatively larger sample size using the Locke's method would give similar results to the smaller sample size. The problem which is likely to be encountered with such an investigation is that one cannot estimate how many "acceptable blanchers" will be determined using the FDA recommended criterion because there are no guidelines on the minimum number of subjects that should be obtained. Steinijans et al. (62) reported that the probability of erroneously concluding bioequivalence can be controlled by sample size calculations. Considering the seriousness of the problem, Liu et al. (156) and Hauschke et al. (157) have developed formulae for sample size calculations so that any trial undertaken should use a certain minimum number of subjects that will produce results that reflect the population at large. Having used a minimum number of 40 subjects as stipulated in the Guidance for the pivotal study, the FDA therefore should provide guidelines on the minimum number of "acceptable" subjects for statistical analysis so that the criterion can be used with confidence.

Theoretically, the ratio of the mean AUC value of test product to that of reference formulation ($\overline{X_T}/\overline{X_R}$) should be 1 if the two products are 100% bioequivalent. In order to test whether the results of the general method and Locke's statistical test obtained in this study could be reliable, the $\overline{X_T}/\overline{X_R}$ values were calculated for each subject. The subjects for which the $\overline{X_T}/\overline{X_R}$ values were close to 1 were used as an appropriate sample size. Those whose values were within the 0.85 to 1.15 were selected. Because of the variability of the instrumental data, it was decided to use the results of visual data. Twelve subjects were found to be appropriate. It was also decided to consider the AUC values at the 6 hour dose duration because earlier results

indicated better results of visual assessment are obtained at this dose duration than shorter durations.

The average $\overline{X_T}/\overline{X_R}$ value obtained by Locke's method was 1.01 and the value found with the general method was 1.02. The deduction from this result is that there is no difference in the topical drug availability between Betnovate and Lenovate creams using either method. However, the confidence interval limits between the two methods were not that close compared to the mean ratio value. The CI values were 0.89 and 1.15 for the Locke's formula whereas they were 0.94 and 1.08 for the general formula. It is interesting to note that even though the CI values are different, the same conclusions of bioequivalence testing would have been drawn if the 0.80 to 1.20 criterion was used. However, whether the slight difference in the statistical methods would significantly influence the bioequivalence assessment still remains to be investigated. From the results in table 10 and 11, it is not clear why the CI limits for the general formula at 3 hours (visual) and at 6 hours (chromameter) are not similar to those for the Locke's method. Whether the method of determining the $\overline{X_T}/\overline{X_R}$ value of individual subjects before determining the average value for the sample as is performed by the general method has a significant influence in the final results remains to be investigated with respect to CI determination. Due to intra- and inter-subject variability, the method of calculating individual ratios described above is now considered to be more appropriate than just determining the average from total subjects in bioequivalence trials (154).

In summary, despite some minor differences noted between the two statistical methods used, the same conclusions were drawn using the 0.80 - 1.20 CI criterion. The Locke's method showed slightly different results for the smaller and bigger sample size for the chromameter data ("G" parameter) although the reliability of the results is questionable because no references are available for

the validity of the method in the blanching assay. The same test did not show different results ("G" values < 1) regardless of the sample size or different dose durations for the visual data. It would be interesting to conduct studies using different corticoids to test the ability of the "G" parameter in showing whether the two products could be assumed bioequivalent or not, that is, whether the "G" values obtained will be less than 1 (bioequivalence) or not. In addition the use of Confidence Interval limits in blanching assays as stipulated in the Guidance requires investigation. Another issue that warrants extensive investigations is the use of D_2/D_1 criterion because it produced only seven "acceptable subjects" out of 40 volunteers for the chromameter data which is advocated to generate reliable data. Using the ratio of $\overline{X_R}/\overline{X_T}$ for individual subjects, only one subject out of these seven "acceptable subjects" had the ratio value close to 1 suggesting that this is the only individual whose data can be used with confidence. It should be noted that if the D_2/D_1 criterion was applicable to the visual data for which there are no recommendations in the current Guidance, the data in Appendix E would indicate that 30 subjects would have been found to be "acceptable" blanchers. It is not explained in the Guidance why the criterion cannot be used for the visual data. From this result of visual data, it may appear that probably the criterion is useful but the chromameter was unable to generate reliable data.

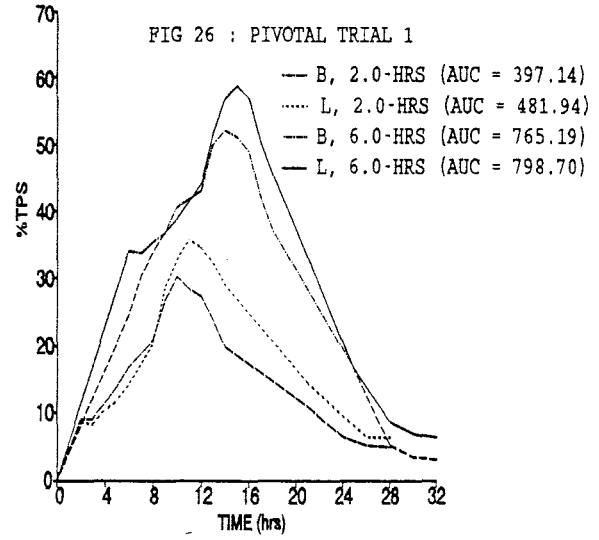
3.3.2. Bioequivalence testing using the Chi-squared analyses and Student's t-distribution test

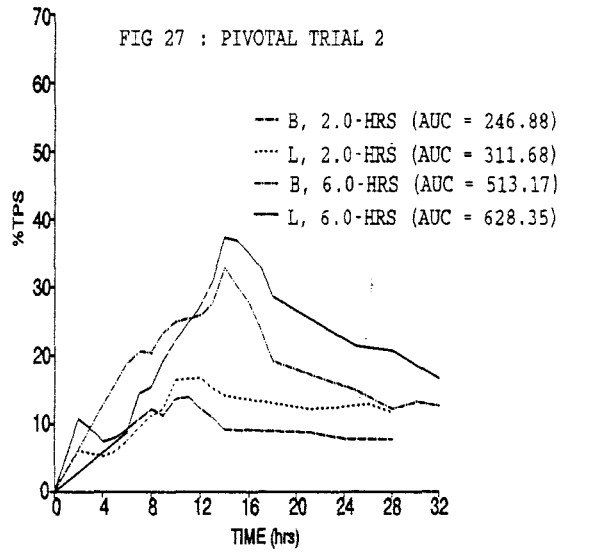
The rejection of a two-sided hypothesis such as the Student's t-distribution tests by Schuirmann (158) led other scientists (62,135,156) to favour the 90% CI statistical methods. However, a recent paper by Steinijans et al. (62) reported that the t-tests and nonparametric methods are still valid even when the intra-subject variability for test and reference formulation is not the same. He also pointed out that it is possible for the

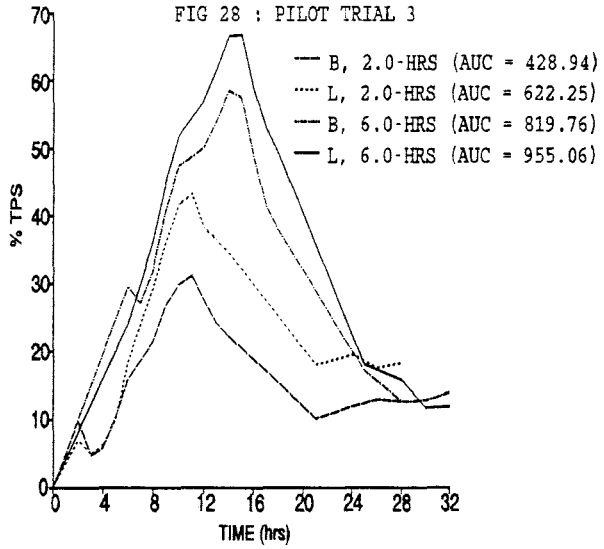
drug products to have different variabilities and still be called bioequivalent using the CI methods. The analysis of variance (ANOVA) methods which also involve the null hypothesis procedures are still valid as reported recently by Hu et al. (159). The objective of this investigation was to compare the results obtained previously for the Locke's method with those obtained using the Chi-squared analysis (visual data) and Student's t-distribution data (chromameter data).

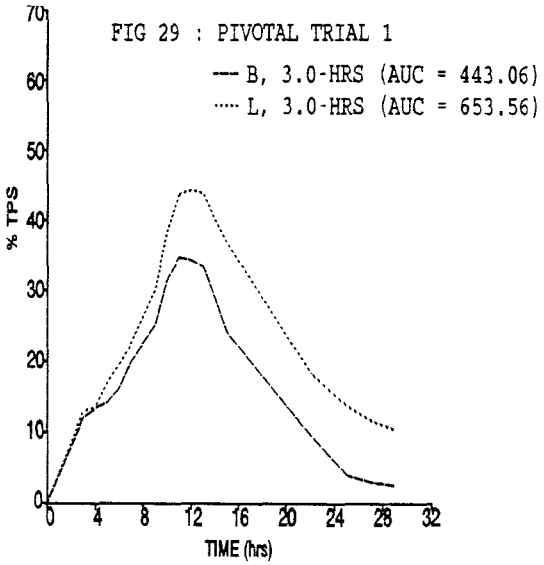
Considering the visual blanching profiles in figures 26-31, these clearly show that Lenovate cream elicited a greater blanching response than Betnovate cream in all the pivotal trials at the 2 and 6 hour dose durations. These differences were shown to be significant (Chi-squared analysis) at peak blanching responses (12 to 16 hours after product application) for the 6 hour dose duration, suggesting that Betnovate and Lenovate creams are bioequivalent. These results are consistent with those found earlier in the two pilot trials. No significant differences were noted at any observation time at the 2 hour dose duration implying that the two products are bioequivalent in all three pivotal trials. The consistency of visual results imply confidence and reliability in the visual assessment. The 3 hour dose duration demonstrated differences at most observation times between the two formulations. The profiles also clearly show the differences in the response between the two formulations. It should be noted that the 3 hour dose duration is the ED_{50} value obtained earlier from the modelling of visual data. The implication of these results is that the two products will be declared not bioequivalent at this ED_{50} value.

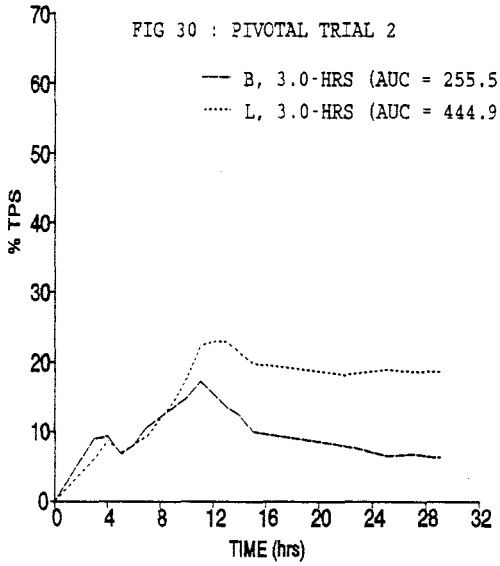
In contrast to the visual results, the chromameter AUC values and response profiles in figures 32 to 37 showed that Betnovate cream elicited a greater blanching response than Lenovate cream at the 6 hour dose duration in all three pivotal trials. The BTC data rather than the CC data (section 2.2.) were used for plotting the

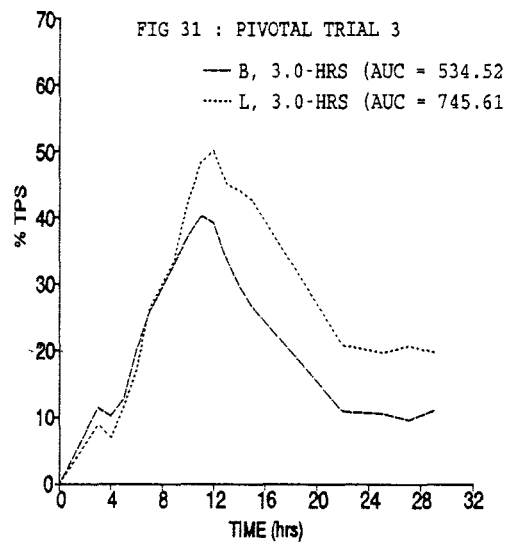




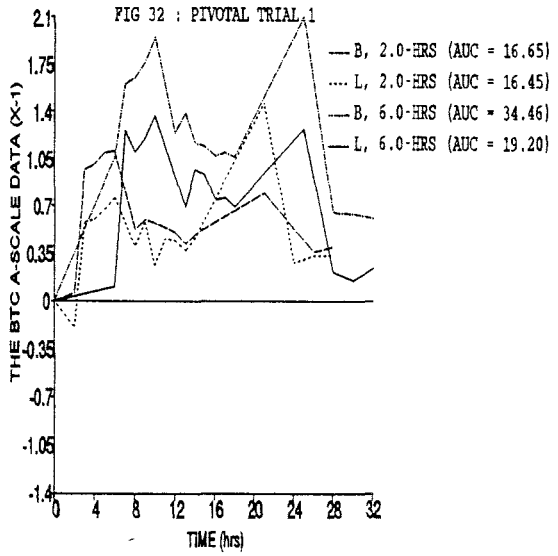


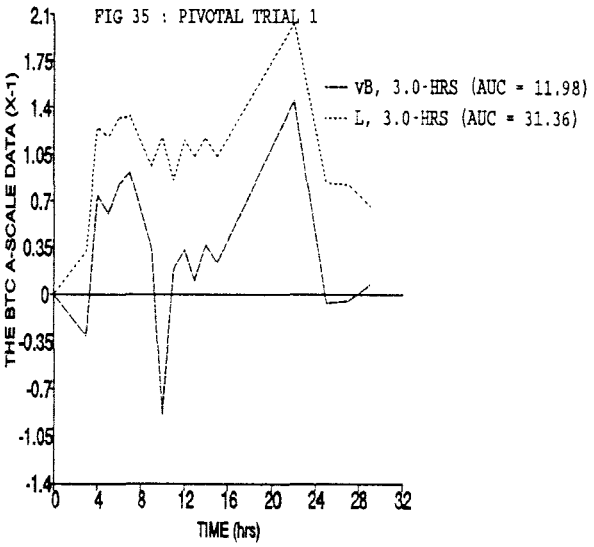


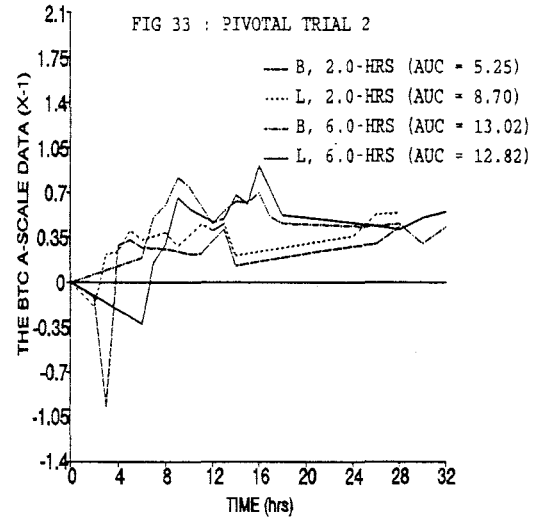


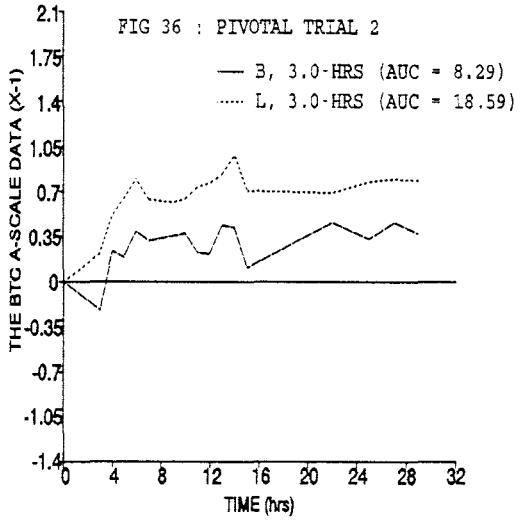


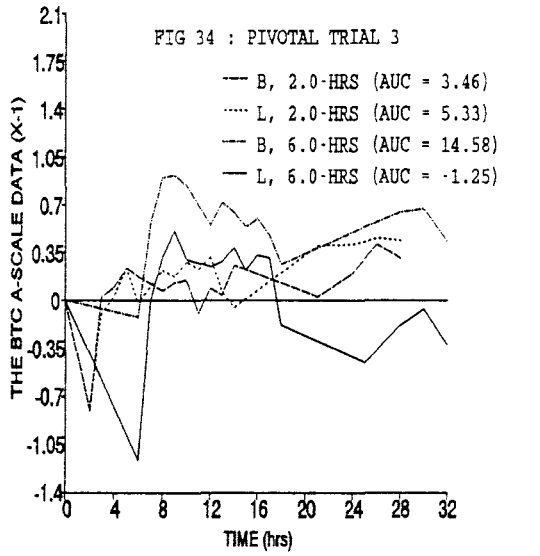
Figures 26-31: Visual blanching profiles between Betnovate(B) and Lenovate (L) cream at 2, 3 and 6 hours for all pivotal trials.

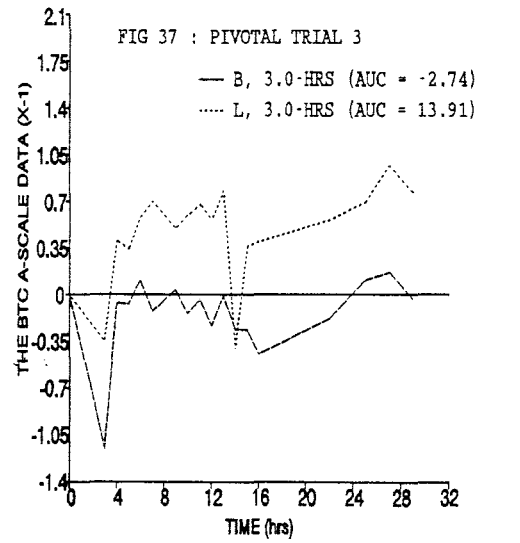












Figures 32-37: Chromameter blanching profiles between Betnovate(B) and Lenovate(L) cream at 2, 3 and 6 hours for all pivotal trials.

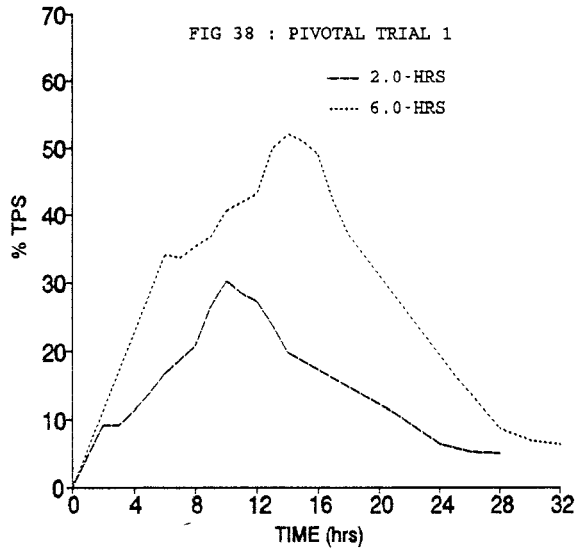
graphs because they are recommended to be more appropriate in the Guidance. Student's t-distribution test showed these differences to be significant at most observation times for pivotal trial 1 and 3 suggesting that the two products are not bioequivalent. No statistically significant differences were noted in pivotal trial 2 implying that the formulations are bioequivalent. These results are corroborated by the blanching profiles and AUC values which are very close at the 2 and 6 hour dose durations in figure 33. These results demonstrate the inability of the instrument to show subtle differences between the two formulations. The implication of these results is that it is extremely difficult to rely on the data generated by the instrument because the results are different when identical or similar trials are conducted. Since the pilot trials were not identical as compared to the pivotal trials although they were similar, one would wonder if the intra-subject and inter-subject variability, and probably environmental conditions could have contributed to the difference in the result. However, if this was the case, these variables should have affected the visual method as well. Visual assessment not only show similar results to those in the pilot trials but also in all the three pivotal trials. It is interesting to note that the response profiles for the visual (figures 29-31) and chromameter data (figures 35-37) at the 3 hour dose durations clearly demonstrate the superiority of Lenovate cream over Betnovate formulation. These differences were confirmed to be significant by statistical analysis although not in pivotal trial 2 for the chromameter data. It would therefore appear as if these results imply that the chromameter can measure skin blanching response accurately at 3 hours dose duration.

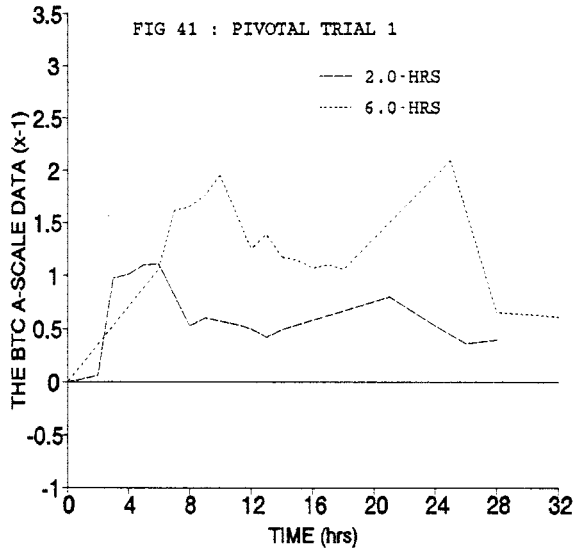
3.3.3. Assessment of sensitivity of chromameter parameters in assessing blanching response

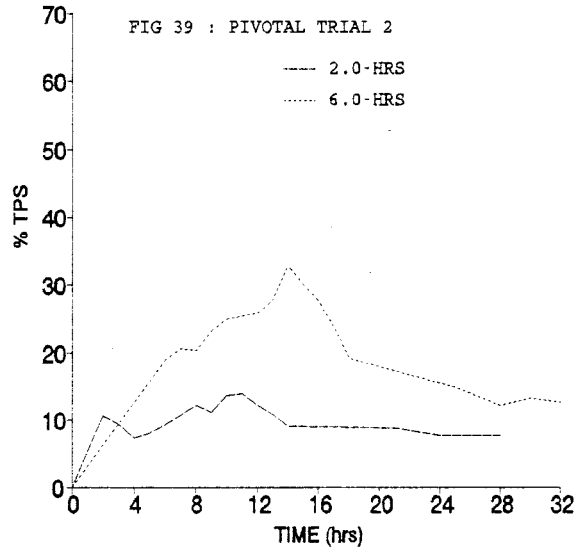
The purpose of this investigation was to compare the ability of each of the chromameter parameters (L-, a- and b-scale) to

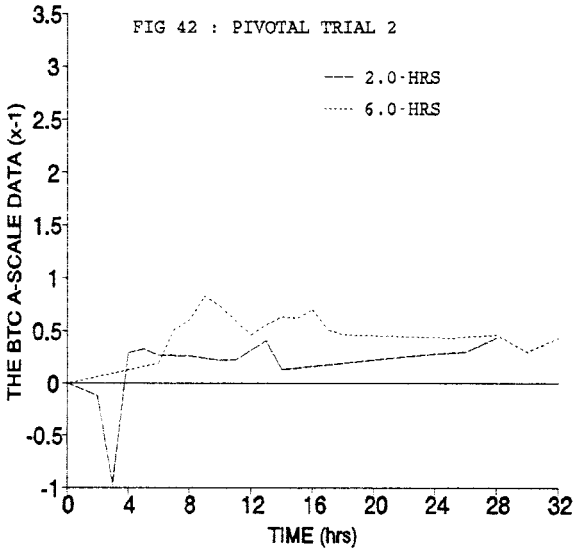
measure the skin blanching response. Validation of a chromameter needs a rigorous statistical analysis of correlation between visual and chromameter data (51). This aspect was also investigated in this study. Although the FDA Guidance stipulates the use of the a-scale parameter, others (119) have demonstrated a high correlation between the visual and chromameter L-scale. The objectives of this investigation were accomplished by comparing blanching responses at two dose durations using Betnovate cream in each of the three pivotal trials conducted. The idea was to find the method which can best distinguish blanching responses between 2 and 6 hour dose durations. Spearman's rank correlation coefficients were used as a measure of correlation between visual and chromameter data. The visual and control site corrected L-scale (BTC) values produced a positive plot and as a result, the control site corrected a- and b-scale data were multiplied by -1 to produce a positive plot for comparison purposes.

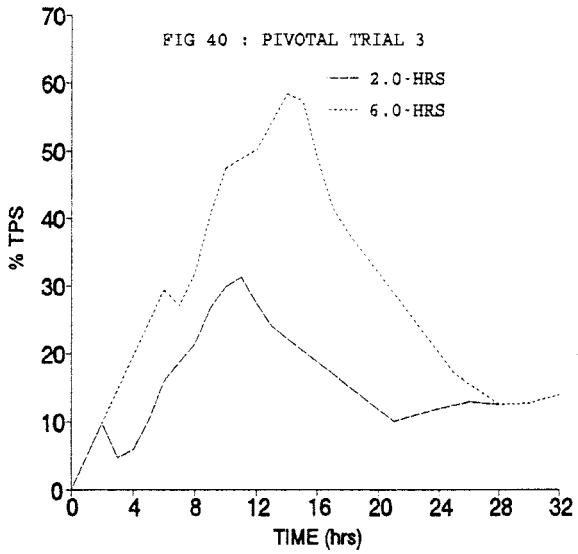
The visual and chromameter blanching profiles are depicted in figures 38 to 49. The AUC values are shown in table 12. For the visually-assessed data, the AUC values and response profiles clearly demonstrate superior skin blanching at 6 hours compared to the 2 hours dose duration in all pivotal trials. Chi-squared analysis showed significant differences at most observation times between those two dose durations. The results for the L-scale data are similar to those for the a-scale and therefore only the a-scale values are reported here (figures 33-49). The minor difference is that the profiles and the AUC for the a-scale data generally show clear differentiation between the 2 and 6 hour dose durations more than for the L-scale data. In all the pivotal trials, especially pivotal trial 1, the a-scale AUC values and response profiles showed greater response at the 6 hour compared to the 2 hour dose duration. The differences were, however, statistically significant at most observation times in pivotal trial 1 and 3. Few significant differences were noted in

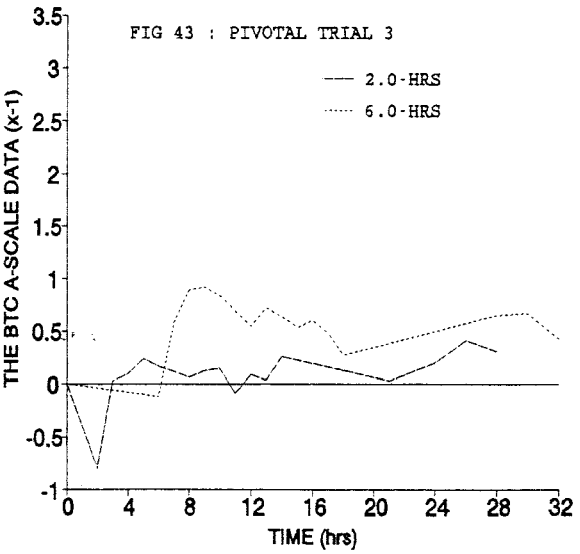




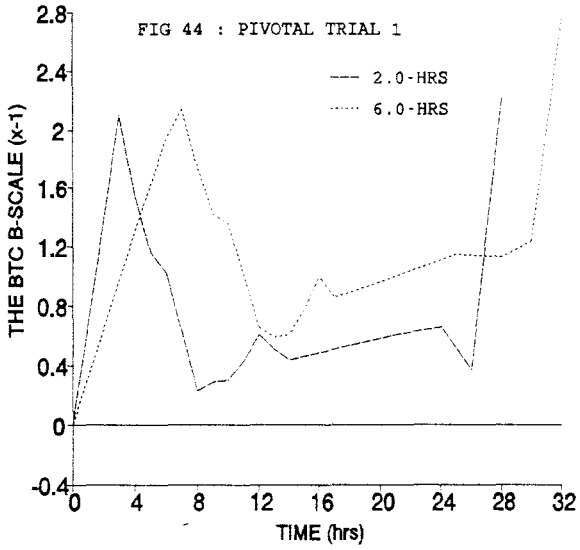


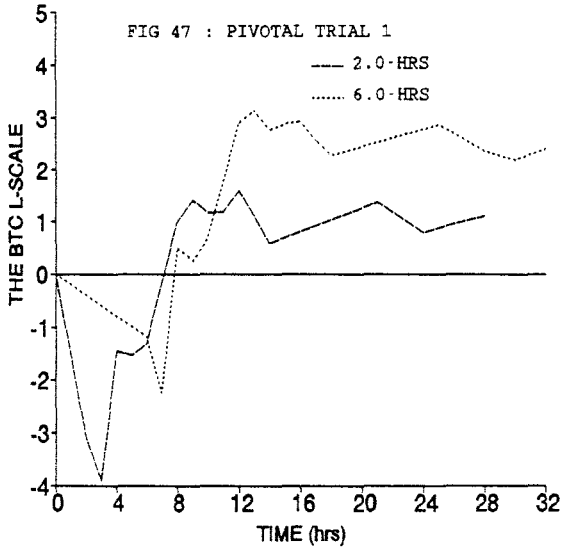


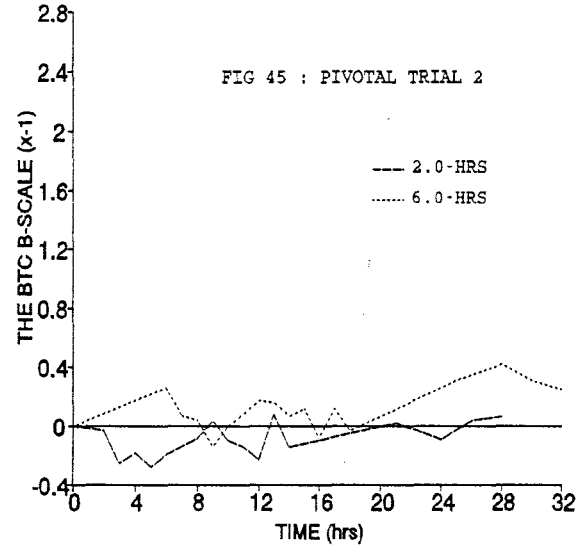


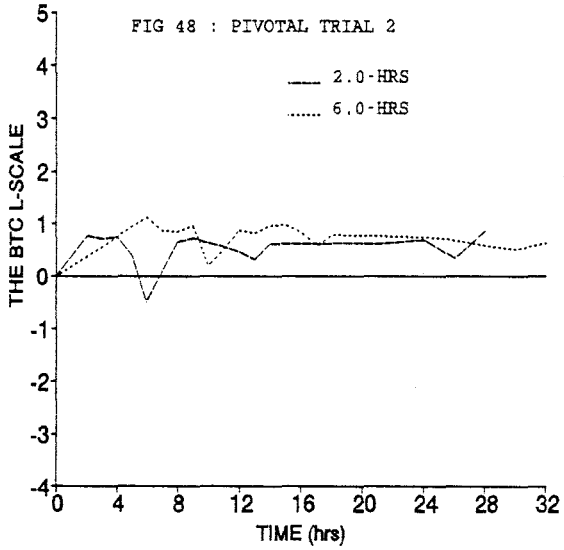


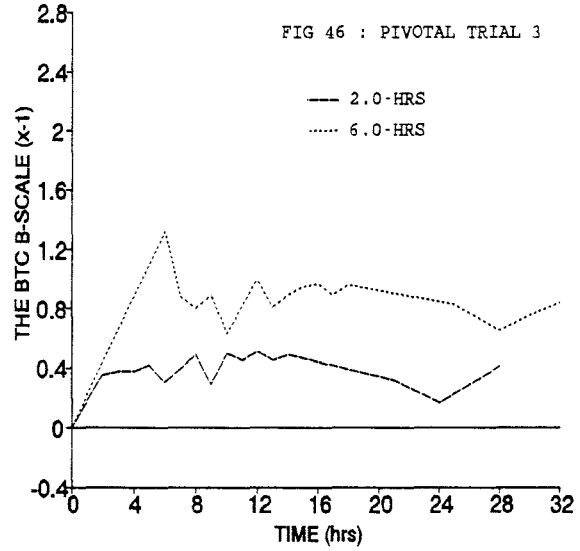
Figures 38-43: The visual and a-scale blanching profiles for Betnovate cream at 2 and 6 hours dose duration.

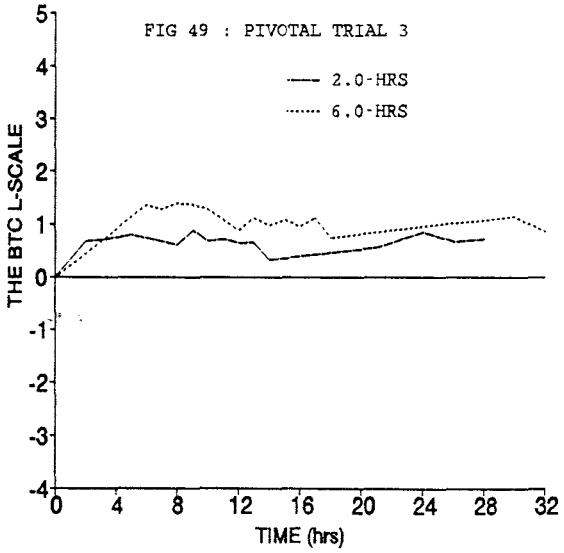












Figures 44-49: The b- and L-scale blanching profiles for Betnovate cream at 2 and 6 hours dose duration.

pivotal trial 2 and this is corroborated by the profiles which show that there was a small difference in skin blanching despite the drug being left on the skin for a longer period. It is interesting to note that both visual assessment and a-scale parameter measurement could clearly distinguish blanching between the two dose durations although it was much clearer for the visual method. One would therefore assume that at longer dose durations, the sensitivity of the a-scale in quantifying the blanching response probably decreases. This assumption, however,

Table 12: The AUC values for Betnovate cream at 2 and 6 hours dose durations.

AUC values (At 2 hours)	PIVOTAL TRIAL 1	PIVOTAL TRIAL 2	PIVOTAL TRIAL 3
Visual	397.14	246.88	428.94
a-scale	16.61	5.25	3.46
b-scale	18.93	-2.02	9.72
L-scale	11.03	13.96	16.51
(At 6 hours)			
Visual	765.19	513.17	829.76
a-scale	34.46	13.02	14.58
b-scale	30.51	-4.71	22.16
L-scale	53.76	18.80	26.89

may not be valid considering the recent reports by Montenegro et al. (138) who performed the experiments at 8 hours and reported reliable results. The researchers found that the a-scale value readings from the chromameter were able to distinguish blanching response at 0.5 hours and the other time points (8, 10 and 24 hours). In addition, Pershing et al. (77) also used the 8 hour application time and found that the a-scale was able to differentiate five commercial 0.05% betamethasone diproprionate formulations. Although the b-scale AUC values showed greater response at 6 hours, especially in pivotal trial 1 and 3, no statistically significant differences were noted in all pivotal trials except in trial 3 suggesting that the b-scale was not

sensitive enough to distinguish skin blanching between the 2 and 6 hour dose durations. Additionally, there were no marked changes in the b-scale responses (almost zero) throughout the observation times in pivotal trial 2. The insensitivity of the b-scale in estimating blanching has been found by others (51,66).

3.3.4. Correlation between chromameter and visual data

Based on the results from the two pilot trials conducted earlier and also because a 6 hours application time has been used extensively by other workers (40,45,51,122,142), the correlation between visual and chromameter data was determined using the Spearman's Rank Correlation Test. This test was chosen for three reasons. Firstly, because the test is applicable to the data which cannot be measured numerically, but which can be placed in rank order (160). For visual assessment, the skin blanching response was ranked using the grading scale and therefore, the test was appropriate for the visual data. Secondly, the test can also be used for the data generated instrumentally. This is because the test uses the ranked values but not the actual values. In this case, for the test to be applicable, the numerical values that are measured are placed in the rank order, given ranks and then the test can be used on the ranked values. With respect to the blanching assay, the response was measured numerically by the chromameter and hence, the test was appropriate. Thirdly, the test was also chosen because other workers (77) have reported reliable results using the same test when comparing visual and chromameter data. The Spearman's formula which is used to determine the correlation coefficient is given by the following equation:

$$r_s = 1 - \frac{6\ S\ (X - Y)^2}{n_p\ (n_p^2 - 1)} \qquad \text{Equ. (24)}$$

Where r_s = Spearman's correlation coefficient

X = numerical values of ranks of items called x

Y = numerical values of ranks of items called y

n_p = number of pairs of items

A perfect positive correlation and negative correlation between the two items of comparison, for example, between visual and chromameter data, are indicated by an r_s value of +1 and -1 respectively. In order to know whether the correlation (r_s) between the two items is a real one or simply due to chance, the following formula for the calculation of the critical ratio t is used:

$$t = r_s \frac{n - 2}{1 - r_s^2} \qquad \text{Equ. (25)}$$

If the calculated value of t is greater than the tabular value at the 5% probability level using the normal Student's t-distribution test table, the correlation is probably real or significant. The probability of correlation is insignificant or is not real if the calculated value of t is less than or equal to the tabular value at the 5% probability level.

The correlation coefficients for Betnovate and Lenovate cream are presented in table 13. There was generally poor correlation ($r < 0.7$) between the L-scale and visual data for both formulations. In addition, a negative correlation was noted in pivotal trial 3 for Betnovate cream and in trials 2 and 3 for Lenovate cream. This was corroborated by the blanching profile in figure 49 for which the Betnovate L-scale data at 6 hours were small values and the profiles were generally flat. In contrast, the visual graphs in figure 40 show a completely different profile for which the response increases up to maximum blanching at about 14 hours after product application and then decreases. It should be noted that although there were negative correlation coefficients for Betnovate cream which were not found for the Lenovate cream for the chromameter data, the blanching profiles for the Lenovate cream were not plotted. This is because the chromameter data were found to be variable especially with the Lenovate cream and therefore it was decided to plot the Betnovate cream data. A negative correlation was not expected because the L-scale

measures brightness of the skin colour in congruity with visual assessment. One would have expected positive values for this parameter in agreement with the results of other workers (51,69,77). The results clearly demonstrate that the L-scale is not a good parameter for measuring skin blanching. However, if one considers the average of the three trials, the L-scale is probably better than the a-scale because better results were obtained in two trials whereas with the latter, it was better than the former only in one trial. Since three similar pivotal trials were conducted and the results showed poor correlation in all of them, the conclusions from this study can be generated with confidence.

Table 13: Spearmans's rank correlation coefficients (r_s) at 6 hours dose duration between visual data and control site-corrected chromameter parameter data.

Parameters	PIVOTAL TRIAL 1	
	Betnovate cream	Lenovate cream
L-scale	0.55	0.31
a-scale	-0.28	-0.10
b-scale	0.77	0.40
Parameters	PIVOTAL TRIAL 2	
L-scale	0.44	-0.16
a-scale	-0.71	-0.30
b-scale	0.50	0.23
Parameters	PIVOTAL TRIAL 3	
L-scale	-0.13	-0.11
a-scale	-0.11	0.27
b-scale	-0.41	-0.69

This is in contrast to a previous report (119) which demonstrated good results for the L-scale which appeared to be better than the recommended a-scale parameter although the conclusions were drawn from only one study.

For the b-scale, poor correlation was also noted although the r_s value ($r = 0.77$) for Betnovate cream in pivotal trial 1 was the best one obtained in the study. Surprisingly, there were two correlation coefficients of different signs using the same parameter. One was a positive correlation described above and

another was a negative correlation coefficient of -0.69 in pivotal trial 3 for Lenovate cream. This result clearly demonstrates that one cannot rely on the b-scale to measure skin blanching in agreement with other workers (51,69,77). All the correlation coefficients were statistically significant at 95% level of significance except for a value of -0.11 in pivotal trial 3. These results mean that the correlation coefficients can be described with confidence although the data with a coefficient of -0.11 are questionable. It is possible that imprecise data could have been obtained as a result of slight variation of the applied pressure from the sensor on the skin surface because it was extremely difficult to hold the sensor as motionless as possible despite many attempts. Another possible explanation closely related to the pressure effects is the slight variation of the distance between the skin surface and the chromameter data that could have contributed to the data obtained.

3.4. SUMMARY, CONCLUSIONS AND FUTURE AREAS OF RESEARCH.

The preliminary trial indicated that the training period that may be required to train a learner to be classified as an experienced observer is not a major problem in the assay. The results showed that the training period for a subject who has participated in the trial before as a volunteer (at least two trials) but not as an observer may be less than a learner who has never participated in the trial before or observed blanching responses. Even at first attempt, both learners had the same statistical conclusions as the experienced observers. The training period for a novice will depend on the number of observation times that are used to assess skin blanching. It will obviously take longer for a learner to be classified as an experienced observer if the blanching assay methodology uses few observation times. Based on few published papers and on the results obtained, the period of training may take only two blanching trials using at least 10 observation times.

The pilot results indicated that, despite using two similar topical corticosteroid formulations, longer dose durations were found to demonstrate subtle differences in skin blanching in contrast to the shorter dose durations for the data assessed visually. The implication of the results is that errors in assigning bioequivalence to similar formulations is likely to occur if shorter dose durations are used in the pivotal bioequivalence study. Improvements in the discriminatory power of shorter and longer dose durations could be done by assessing topical corticosteroids from different potency groups. Such investigations will not only demonstrate the significance of the appropriate dose duration essential in the assay, but will also test the accuracy and precision of the chromameter in measuring skin blanching. The visual profiles were not as smooth as previously reported and it was difficult to interpret the chromameter profiles. In addition, some unexpected results were noted at longer dose durations using both methods of assessment. The anomalous results obtained could be attributed to the use of a smaller data set because only one application site per arm for any one dose duration was used in accordance with the FDA guidelines. Invalid conclusions may, therefore, be drawn from such studies and therefore it is proposed that the FDA should recommend the use of more application sites. The problem experienced with the chromameter in all the trials performed in this study was that it is extremely difficult if not impossible to hold the probe as motionless as possible when taking measurements. Since other workers have shown that even a small pressure that can be applied to the skin as a result of probe manipulations may influence skin blanching. It is therefore proposed that the improvement in the use of the instrument should consider appropriate handling of the probe on the skin. This aspect will be related closely to the distance of the chromameter from the skin. Taking measurements at variable distances may probably contribute to the imprecision of the instrument. Research therefore is required to investigate this aspect. The

suggestion would be to mark the chromameter probe using sellotape, glue or any material that may stick to the sensor so that the distance between the skin surface and the probe is fixed throughout the study. Taking the chromameter readings before any drug application (zero values) did not affect the statistical conclusions drawn from the pilot trials. However different estimates of ED_{50} parameter were obtained when the zero readings were not taken. Since the main aim of the pilot study is to determine the ED_{50} value, inclusion or exclusion of the zero time data would be an important feature. Although different ED_{50} estimates were obtained, they were all smaller numbers, that is, less than 3 hours dose durations. It remains to be evaluated whether bioequivalence assessment at these shorter dose durations would be reliable.

From the investigation of non-linear pharmacodynamic models, visual data were found to follow a sigmoid-response pattern which is usually seen in many pharmacological systems, whereas the chromameter data followed a geometric pattern. This does not necessarily imply that visual assessment is more accurate than the chromameter because the instrument may be measuring some parameters not apparent to the naked eye. However, when the chromameter data which included the readings taken before any drug application on the skin sites (zero readings), the data were best described by a sigmoid model for Betnovate cream but not for Lenovate cream. The estimates of the ED_{50} values were different between the chromameter data when zero values were included and the chromameter data when zero values were excluded. These results imply that the inclusion of zero readings as recommended by the FDA may affect bioequivalence assessment than data when the zero values were excluded. However, the worrying aspect was that both data sets yielded lower ED_{50} estimates which must be used to assess bioequivalence in the pivotal study. The full benefit of shorter dose durations requires investigations. The results obtained here could be a function of the models used.

Since the FDA does not have guidelines on the specific models for use, it is proposed that recommendations are required so that results can be easily compared among different investigators. The modelling software used here was such that the weighting schemes had to be used due to variability of the data especially for the chromameter data. The usefulness of the weighting schemes for a specific modelling software is another aspect that may need investigations. Such investigation will be important because it will not only validate the use of a particular weighting scheme, but will add knowledge to the usefulness of the non-linear pharmacodynamics in the assay.

The problem experienced with pharmacodynamic modelling of the a-scale data was that similar goodness of fit criteria were obtained when different upper ED_{50} values were set in the model definition, even though the model-predicted ED_{50} values from these definitions were unrealistic. It was evident for both data sets that model selection should not be only be based on goodness of fit criteria (correlation coefficient, AIC, standard error of estimate) but also on parameter estimates that are realistic for the data collected. The standard error estimate was found to be more sensitive than the other parameters in selecting the best model that described the observed experimental data.

The pilot trial results also indicated that the maximum blanching response was not attained at 6 hours as it is probably assumed by the FDA. Statistical analysis demonstrated no significant responses at 8 and 10 hours suggesting attainment of maximum blanching response was occurring at 8 hours. However, this was found for one formulation whereas for the other product, few significant differences were noted and therefore definite conclusions could not be drawn. The instrument was found to distinguish between skin blanching at the 2 and 6 hour dose durations but could not differentiate differences at 5 and 10 hours implying its inability to measure skin blanching compared

with the visual method. Investigations are therefore necessary to determine the ability of the instrument to measure skin blanching response at longer dose durations.

Seven "acceptable blanchers" were obtained out of 40 in accordance with the D_2/D_1 criterion stipulated in the Guidance. This is worrying because of objections to the use of smaller sample size by many researchers. The validity of this criterion in the assay is questionable because a smaller data set was obtained from the blanchers who were known to be good blanchers when assessed visually. In addition, using a theoretical value of 1 for the ratio of bioavailability of test product to that for the reference formulation on the assumption of 100% certain that the two products are bioequivalent, only one subject out of these 7 "acceptable" blanchers was found to be a good blancher because his ratio was close to 1. Little significance can be attributed to the Locke's method because of the undefined parameters that are incorporated in the equations such as the "G" and "K" parameters. Furthermore, there are no available reports concerning the applicability of the method in the assay. Some of the results determined showed bioequivalency using the "G" parameter, regardless of the sample size using all dose durations studied. The confidence interval limits, however, revealed that such bioequivalence is not accepted. The implication is that if the ED_{50} value in the pivotal study was considered to be either the 2, 3 or 6 hour duration, one would therefore conclude that all the results obtained in the study showed that the two products are not bioequivalent at the 2, 3 and 6 hour dose durations. Whether the "G" values or the confidence interval limits are suitable or not to determine the bioequivalence of topical corticosteroids requires investigations. The general simple confidence interval formula was found to yield values similar to those for the Locke's method. In contrast to Locke's method, the simple formula involves the calculation of the ratio of the mean bioavailability of test product to reference product

of individual subjects which takes into account the variability of the subject. Further investigations may be necessary to establish the best statistical methods which employ confidence interval limits.

Both the Student's t-test and Chi-squared analysis demonstrated similar statistical results at the 2 and 6 hour dose durations but differed at the 3 hour dose duration. Reliable results have already been reported with the use of either statistical method and the difference may probably be attributed to the precision of the method of estimating skin blanching. The visual method was found to be consistent in all the pivotal trials whereas the chromameter only showed similar statistical results in two pivotal trials. It would be, however, worthwhile to conduct investigations between two different topical corticosteroids or between placebo and a topical corticoid formulation to establish the sensitivity of the instrument and visual observations especially at the 2 or 3 hour dose durations since these were the ED_{50} values obtained from the pilot trials. The blanching profiles, AUC values and statistical analyses for visual and chromameter data at the 3 hour dose duration generally demonstrated superiority of Lenovate cream over Betnovate cream. It would appear as if the 3 hour dose duration may be the period for which the chromameter data are reliable. This aspect needs further investigations.

Why the instrument was able to show similar results in pivotal trial 1 and 3 but failed to demonstrate the same statistical analysis in pivotal trial 2 is open to speculation. Despite the variables such as intra- and inter-subject variability that may have contributed to the results, visual data under the same conditions were consistent. One could speculate that the reason why the instrument failed to show similar results with those found in the pilot trials could be that it is not as sensitive as the visual method. The results suggested that the chromameter

data from any one trial may be unreliable and therefore conclusions to be drawn from such study should be treated with caution.

Although all the chromameter parameters were able to show that skin blanching was greater at 6 hours compared to the 2 hours, the chromameter a-scale profiles were similar to those for the L-scale and their data were found to be slightly better than the b-scale in clearly differentiating the intensity of response. Despite the profiles for the a- and L-scale parameters generally being similar, the correlation coefficients for the L-scale on average suggest that it is slightly better than the a-scale parameter. Poor correlation coefficients between chromameter and visual data were noted. The major trend that emerged from this thesis is that visual method is still the best method for estimating skin blanching. The chromameter still needs careful evaluation if it is to be used with confidence in the blanching assay.

APPENDIX A

This appendix contains all the formulations used in the study.

FORMULATIONS USED IN THIS STUDY.

Betnovate cream (0.12% betamethasone 17-valerate) Glaxo (Pty) Ltd., Manchester Road, Wadeville, SA.

Dermovate lotion (0.05% clobetasol propionate) Genpharm Inc., Advance Road, Ontario, Canada.

Experimental lotion (0.05% clobetasol propionate) Genpharm Inc., Advance Road, Ontario, Canada.

Lenovate cream (0.12% betamethasone 17-valerate) Lennon Limited, Fairclough Road, Port Elizabeth, SA.

Placebo lotion Genpharm Inc., Advance Road, Ontario, Canada.

APPENDIX B

This appendix contains the application charts used for all the trials.

APPLICATION PATTERNS USED IN THE PRELIMINARY TRIAL.

CHART 1

SITE NUMBER	PRODUCT	PRODUCT	SITE NUMBER
1	A	B	2
3	C	A	4
5	B	C	6
7	A	B	8
9	C	A	10
11	B	C	12

CHART 2

SITE NUMBER	PRODUCT	PRODUCT	SITE NUMBER
1	B	C	2
3	A	B	4
5	C	A	6
7	B	C	8
9	A	B	10
11	C	A	12

CHART 3

SITE NUMBER	PRODUCT	PRODUCT	SITE NUMBER
1	C	A	2
3	B	C	4
5	A	B	6
7	C	A	8
9	B	C	10
11	A	B	12

CHART 4

SITE NUMBER	PRODUCT	PRODUCT	SITE NUMBER
1	C	B	2
3	A	C	4
5	B	A	6
7	C	B	8
9	A	C	10
11	B	A	12

Formulations A, B and C were Dermovate, Experimental and Placebo lotions respectively.

APPLICATION PATTERNS USED IN THE PILOT TRIALS.

CHART 1

SITE NUMBER	PRODUCT	DOSE DURATION	PRODUCT	SITE NUMBER
1	A	10/2	B	2
3	B	8/15	A	4
5	A	7/10	B	6
7	B	6/0.75	A	8
9	A	5/0.5	B	10
11	B	4/0.25	A	12

CHART 2

SITE NUMBER	PRODUCT	DOSE DURATION	PRODUCT	SITE NUMBER
1	A	8/1.5	B	2
3	B	7/1.0	A	4
5	A	6/0.75	B	6
7	B	5/0.5	A	8
9	A	4/0.25	B	10
11	B	10/2	A	12

CHART 3

SITE NUMBER	PRODUCT	DOSE DURATION	PRODUCT	SITE NUMBER
1	A	7/1.0	B	2
3	B	6/0.75	A	4
5	A	5/0.5	B	6
7	B	4/0.25	A	8
9	A	10/2	B	10
11	B	8/1.5	A	

CHART 4

SITE NUMBER	PRODUCT	DOSE DURATION	PRODUCT	SITE NUMBER
1	A	6/0.75	B	2
3	B	5/0.5	A	4
5	A	4/0.25	B	6
7	B	10/2	A	8
9	A	8/1.5	B	10
11	B	7/1.0	A	12

In chart 1, a dose duration of 10/2 means that the formulation next to 10 (Product A) was applied on the application site (designated number 1) for 10 hours. Similarly, the formulation next to 2 (Product B) was applied to site designated 2 on the right arm. The same applies to other dose durations for all charts.

APPLICATION PATTERNS USED IN THE PIVOTAL TRIALS.

CHART 1

SITE NUMBER	PRODUCT	DOSE DURATION	PRODUCT	SITE NUMBER
1	A	2	B	2
3	B	6/1	A	4
5	A	3	B	6
7	B	6	A	8
9	A	3	B	10
11	B	2	A	12
13	A	4/6	A	14

CHART 2

SITE NUMBER	PRODUCT	DOSE DURATION	PRODUCT	SITE NUMBER
1	A	3	B	2
3	B	6	B	4
5	A	3	B	6
7	B	2	A	8
9	A	4/6	A	10
11	A	2	B	12
13	B	6/1	A	14

CHART 3

SITE NUMBER	PRODUCT	DOSE DURATION	PRODUCT	SITE NUMBER
1	A	3	B	2
3	B	2	B	4
5	A	4/6	A	6
7	A	2	B	8
9	B	6/1	A	10
11	A	3	B	12
13	B	6	A	14

CHART 4

SITE NUMBER	PRODUCT	DOSE DURATION	PRODUCT	SITE NUMBER
1	A	4/6	A	2
3	A	2	B	4
5	B	6/1	A	6
7	A	3	B	8
9	B	6	A	10
11	A	3	B	12
13	B	2	A	14

PRODUCT APPLICATION SHEET: PILOT TRIALS

TIME	VOLUNTEER	PRODUCT	SITES	ARM
7:30	1	A	1,4,5,8	L
		B	2,3,6,7	
7:35	2	A	1,4,5,12	L
		B	2,3,6,11	
7:40	3	A	1,4,9,12	L
		B	2,3,10,11	
7:45	4	A	1,4,5,8	L
		B	2,3,6,7	
7:50	5	A	1,4,9,12	L
		B	2,3,10,11	
7:55	6	A	1,8,9,12	L
		B	2,7,10,11	
8:00	7	A	1,4,5,12	L
		B	2,3,6,11	
8:05	8	A	1,4,5,8	L
		B	2,3,6,7	
8:10	9	A	1,8,9,12	L
		B	2,7,10,11	
8:15	10	A	1,4,9,12	L
		B	2,3,10,11	
8:20	11	A	1,8,9,12	L
		B	2,7,10,11	
8:25	12	A	1,4,5,12	L
		B	2,3,6,11	
8:30	1	A	9	L
		B	10	
8:35	2	A	8	L
		B	7	
8:40	3	A	5	L
		B	6	
8:45	4	A	9	L
		B	10	
8:50	5	A	5	L
		B	6	
8:55	6	A	4	L
		B	3	
9:00	7	A	8	L
		B	7	
9:05	8	A	9	L
		B	10	
9:10	9	A	4	L
		B	3	
9:15	10	A	5	L
		B	6	
9:20	11	A	4	L
		B	3	
9:25	12	A	8	L
		B	7	
9:30	1	A	12	L
		B	11	
9:35	2	A	9	L
		B	10	
9:40	3	A	8	L
		B	7	
9:45	4	A	12	L
		B	11	
9:50	5	A	8	L
		B	7	
9:55	6	A	5	L
		B	6	
10:00	7	A	9	L
		B	10	
10:05	8	A	12	L
		B	11	
10:10	9	A	5	L
		B	6	

Product application sheet for pilot trials ***(Continued)***

TIME	VOLUNTEER	PRODUCT	SITES	ARM
10:15	10	A	8	L
		B	7	
10:20	11	A	5	L
		B	6	
10:25	12	A	9	L
		B	10	
11:30	1	A	12	R
		B	11	
11:35	2	A	1	R
		B	2	
11:40	3	A	12	R
		B	11	
11:45	4	A	9	R
		B	10	
11:50	5	A	1	R
		B	2	
11:55	6	A	9	R
		B	10	
12:00	1	A	1	R
		B	2	
12:00	7	A	8	R
		B	7	
12:05	8	A	8	R
		B	7	
12:05	2	A	4	R
		B	3	
12:10	9	A	1	R
		B	2	
12:10	3	A	1	R
		B	2	
12:15	10	A	8	R
		B	7	
12:15	4	A	12	R
		B	11	
12:20	11	A	12	R
		B	11	
12:20	5	A	4	R
		B	3	
12:25	12	A	9	R
		B	10	
12:25	6	A	12	R
		B	11	
12:30	7	A	9	R
		B	10	
12:30	1	A	4	R
		B	3	
12:35	8	A	9	R
		B	10	
12:35	2	A	5	R
		B	6	
12:40	3	A	4	R
		B	3	
12:40	9	A	4	R
		B	3	
12:45	10	A	9	R
		B	10	
12:45	4	A	1	R
		B	2	
12:45	1	A	5	R
		B	6	
12:50	5	A	5	R
		B	6	
12:50	11	A	1	R
		B	2	
12:50	2	A	8	R
		B	7	

Product application sheet for pilot trials (continued).

TIME	VOLUNTEER	PRODUCT	SITES	ARM
12:55	12	A	12	R
		B	11	
12:55	6	A	1	R
		B	2	
12:55	3	A	5	R
		B	6	
1:00	7	A	12	R
		B	11	
1:00	4	A	4	R
		B	3	
1:00	1	A	8	R
		B	7	
1:05	8	A	12	R
		B	11	
1:05	5	A	8	R
		B	7	
1:05	2	A	9	R
		B	10	
1:10	9	A	5	R
		B	6	
1:10	6	A	4	R
		B	3	
1:10	3	A	8	R
		B	7	
1:15	7	A	1	R
		B	2	
1:15	10	A	12	R
		B	11	
1:15	4	A	5	R
		B	6	
1:15	1	A	9	R
		B	10	
1:20	11	A	4	R
		B	3	
1:20	8	A	1	R
		B	2	
1:20	5	A	9	R
		B	10	
1:20	2	A	12	R
		B	11	
1:25	3	A	9	R
		B	10	
1:25	6	A	5	R
		B	6	
1:25	9	A	8	R
		B	7	
1:25	12	A	1	R
		B	2	
1:30	10	A	1	R
		B	2	
1:30	7	A	4	R
		B	3	
1:30	4	A	8	R
		B	7	
1:35	11	A	5	R
		B	6	
1:35	8	A	4	R
		B	3	
1:35	5	A	12	R
		B	11	
1:40	12	A	4	R
		B	3	
1:40	9	A	9	R
		B	10	
1:40	6	A	8	R
		B	7	

Product application sheet for pilot trials *(Continued)*.

TIME	VOLUNTEER	PRODUCT	SITES	ARM
1:45	10	A	4	R
		B	3	
1:45	7	A	5	R
		B	6	
1:50	8	A	5	R
		B	6	
1:50	11	A	8	R
		B	7	
1:55	12	A	5	R
		B	6	
1:55	9	A	12	R
		B	11	
2:00	10	A	5	R
		B	6	
2:05	11	A	9	R
		B	10	
2:10	12	A	8	R
		B	7	

PRODUCT APPLICATION SHEET: PIVOTAL TRIALS

TIME	VOLUNTEER	PRODUCT	SITES	ARM
7:30	1	A	8,14	L
		B	3,7	L
		A	4,10	R
		B	3,13	R
7:35	2	A	4,10	L
		B	3,13	L
		A	8,14	R
		B	3,7	R
7:40	3	A	6,14	L
		B	9,13	L
		A	4,10	R
		B	3,13	R
7:45	4	A	8,14	L
		B	3,7	L
		A	6,14	R
		B	9,13	R
7:50	5	A	6,14	L
		B	9,13	L
		A	8,14	R
		B	3,7	R
7:55	6	A	2,10	L
		B	5,9	L
		A	6,14	R
		B	9,13	R
8:00	7	A	4,10	L
		B	3,13	L
		A	2,10	R
		B	5,9	R
8:05	8	A	8,14	L
		B	3,7	L
		A	2,10	R
		B	5,9	R
8:10	9	A	2,10	L
		B	5,9	L
		A	8,14	R
		B	3,7	R
8:15	10	A	6,14	L
		B	9,13	L
		A	2,10	R
		B	5,9	R

Product application sheet for pivotal trials *(Continued)*.

TIME	VOLUNTEER	PRODUCT	SITES	ARM
8:20	11	A	2,10	L
		B	5,9	L
		A	4,10	R
		B	3,13	R
8:25	12	A	4,10	L
		B	3,13	L
		A	6,14	R
		B	9,13	R
8:30	13	A	8,14	L
		B	3,7	L
		A	4,10	R
		B	3,13	R
8:35	14	A	4,10	L
		B	3,13	L
		A	8,14	R
		B	3,7	R
9:30	1	A	13	L
		A	9	R
9:35	2	A	9	L
		A	13	R
9:40	3	A	5	L
		A	9	R
9:45	4	A	13	L
		A	5	R
9:50	5	A	5	L
		A	13	R
9:55	6	A	1	L
		A	5	R
10:00	7	A	9	L
		A	1	R
10:05	8	A	13	L
		A	1	R
10:10	9	A	1	L
		A	13	R
10:15	10	A	5	L
		A	1	R
10:20	11	A	1	L
		A	9	R
10:25	12	A	9	L
		A	5	R
10:30	13	A	13	L
		A	9	R
10.35	14	A	9	L
		A	13	R
10:30	1	A	5,9	L
		B	6,10	L
		A	1,5	R
		B	2,6	R
10.35	2	A	1,5	L
		B	2,6	L
		A	5,9	R
		B	6,10	R
10:40	3	A	1,11	L
		B	2,12	L
		A	1,5	R
		B	2,6	R
10:45	4	A	5,9	L
		B	6,10	L
		A	1,11	R
		B	2,12	R
10:50	5	A	1,11	L
		B	2,12	L
		A	5,9	R
		B	6,10	R

Product application sheet for pivotal trials *(Continued)*.

TIME	VOLUNTEER	PRODUCT	SITES	ARM
10:55	6	A	7,11	L
		B	8,12	L
		A	1,11	R
		B	2,12	R
11:00	7	A	1,5	L
		B	2,6	L
		A	7,11	R
		B	8,12	R
11:05	8	A	5,9	L
		B	6,10	L
		A	7,11	R
		B	8,12	R
11:10	9	A	7,11	L
		B	8,12	L
		A	5,9	R
		B	6,10	R
11:15	10	A	1,11	L
		B	2,12	L
		A	7,11	R
		B	8,12	R
11:20	11	A	7,11	L
		B	8,12	L
		A	1,5	R
		B	2,6	R
11:25	12	A	1,5	L
		B	2,6	L
		A	1,11	R
		B	2,12	R
11:30	13	A	5,9	L
		B	6,10	L
		A	1,5	R
		B	2,6	R
11:30	1	A	1,12	L
		B	2,11	L
		A	8,11	R
		B	7,12	R
11:35	14	A	1,5	L
		B	2,6	L
		A	5,9	R
		B	6,10	R
	2	A	8,11	L
		B	7,12	L
		A	1,12	R
		B	2,11	R
11:40	3	A	4,7	L
		B	3,8	L
		A	8,11	R
		B	7,12	R
11:45	4	A	1,12	L
		B	2,11	L
		A	4,7	R
		B	3,8	R
11:50	5	A	4,7	L
		B	3,8	L
		A	1,12	R
		B	2,11	R
11:55	6	A	3,14	L
		B	4,13	L
		A	4,7	R
		B	3,8	R
12:00	7	A	8,11	L
		B	7,12	L
		A	3,14	R
		B	4,13	R
12:15	10	A	4,7	L
		B	3,8	L

Product application sheet for pivotal trials *(Continued)*.

TIME	VOLUNTEER	PRODUCT	SITES	ARM
12:05	8	A	1,12	L
		B	2,11	L
		A	3,14	R
		B	4,13	R
12:10	9	A	3,14	L
		B	4,13	L
		A	1,12	R
		B	2,11	R
		A	3,14	R
		B	4,13	R
12:20	11	A	3,14	L
		B	4,13	L
		A	8,11	R
		B	7,12	R
12:25	12	A	8,11	L
		B	7,12	L
		A	4,7	R
		B	3,8	R
12:30	13	A	1,12	L
		B	2,11	L
		A	8,11	R
		B	7,12	R
12:30	1	A	4	L
		A	14	R
12:35	14	A	8,11	L
		B	7,12	L
		A	1,12	R
		B	2,11	R
12:35	2	A	14	L
		A	4	R
12:40	3	A	10	L
		A	14	R
12:45	4	A	4	L
		A	10	R
12:50	5	A	10	L
		A	4	R
12:55	6	A	6	L
		A	10	R
1:00	7	A	14	L
		A	6	R
1:05	8	A	4	L
		A	6	R
1:10	9	A	6	L
		A	4	R
1:15	10	A	10	L
		A	6	R
1:20	11	A	6	L
		A	14	R
1:25	12	A	14	L
		A	10	R
1:30	13	A	4	L
		A	14	R
1:35	14	A	14	L
		A	4	R

Table 1. Assessment periods at which blanching response was observed for various dose durations (DD).

DD (hrs)	Assessment Period (Hours after product application)															
0.25	1	2	3	4	5	6	7	8	9	10	11	12	19	22	24	26
0.5	2	3	4	5	6	7	8	9	10	11	12	13	20	23	25	27
0.75	2	3	4	5	6	7	8	9	10	11	12	13	20	23	25	27
1.0	2	3	4	5	6	7	8	9	10	11	12	13	20	23	25	27
1.5	3	4	5	6	7	8	9	10	11	12	13	14	21	24	26	28
2.0	3	4	5	6	7	8	9	10	11	12	13	14	21	24	26	28
4.0	5	6	7	8	9	10	11	12	13	14	15	16	23	26	28	30
5.0	6	7	8	9	10	11	12	13	14	15	16	17	24	27	29	31
6.0	7	8	9	10	11	12	13	14	15	16	17	18	25	28	30	32
7.0	8	9	10	11	12	13	14	15	16	17	18	19	26	29	31	33
8.0	-	9	10	11	12	13	14	15	16	17	18	19	26	29	31	33
10.0	-	-	-	11	12	13	14	15	16	17	18	19	26	29	31	33

All the observation times shown above as hours after product application for each dose duration equate to 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 19, 22, 24 and 26 *hours after product removal.*

APPENDIX C

VISUAL DATA.

This appendix is only for visually-assessed data. The tables contain all the raw data collected at each reading time, the actual score (AS) AND %TPS for various formulations at particular dose duration (DD). Chi-squared values for comparisons of formulations or response between dose durations are also presented. The underlined chi-squared values are statistically significant ($P < 0.05$). For the Preliminary Trial, the observation times are hours after product application since only a 6 hr dose duration was used. The observation times for all other trials at various dose durations are presented as hours after product removal for comparisons purposes.

2. THE AS AND %TPS VALUES FOR DIFFERENT LOTIONS RECORDED BY NOVICE 1 OBSERVER. ***(preliminary trial).***

TIME (hrs)	DERMOVATE AS	%TPS	EXPERIMENTAL AS	%TPS	PLACEBO AS	%TPS
7	77	20.1	66	17.2	41	4.8
8	119	31.0	114	29.7	93	6.2
9	164	42.7	171	44.5	123	5.6
10	188	49.0	193	50.3	127	5.7
12	229	59.6	228	59.4	116	3.5
13	295	76.8	300	78.1	163	3.6
14	330	85.9	327	85.2	202	3.2
15	348	90.6	351	91.4	230	3.0
16	326	84.9	329	85.7	200	2.0
17	327	85.2	318	82.8	179	1.0
18	286	74.5	279	72.7	145	1.8
28	133	34.6	127	33.1	53	1.2

3. THE AS AND %TPS VALUES FOR DIFFERENT LOTIONS RECORDED BY NOVICE 2 OBSERVER.

TIME (hrs)	DERMOVATE AS	%TPS	EXPERIMENTAL AS	%TPS	PLACEBO AS	%TPS
7	35	9.1	41	10.7	19	4.9
8	74	19.3	77	20.1	39	10.2
9	104	27.1	111	28.9	51	13.3
10	141	36.7	145	37.8	45	11.7
12	161	41.9	180	46.9	31	8.1
13	184	47.9	204	53.1	30	7.8
14	203	52.9	216	56.3	24	6.3
15	180	46.9	188	49.0	16	4.2
16	174	45.3	187	48.7	9	2.3
17	162	42.2	171	44.5	8	2.1
18	163	42.4	174	45.3	8	2.1
28	85	22.1	90	23.4	3	0.8

4. THE AS AND %TPS VALUES FOR DIFFERENT LOTIONS RECORDED BY ALL EXPERIENCED OBSERVERS. *(preliminary trial)*

TIME (hrs)	DERMOVATE AS	%TPS	EXPERIMENTAL AS	%TPS	PLACEBO AS	%TPS
7	153	13.3	175	15.2	55	4.8
8	230	20.0	234	20.3	71	6.2
9	298	25.9	311	27.0	64	5.6
10	386	33.5	396	34.4	66	5.7
12	506	43.9	509	44.2	40	3.5
13	578	50.2	579	50.3	42	3.6
14	658	57.1	671	58.2	37	3.2
15	673	58.4	688	59.7	34	3.0
16	670	58.2	689	59.8	23	2.0
17	649	56.3	656	56.9	12	1.0
18	604	52.4	601	52.2	21	1.8
28	348	30.2	349	30.3	14	1.2

5. THE CHI-SQUARED VALUES FOR DERMOVATE (D) v EXPERIMENTAL (E) AND DERMOVATE v PLACEBO (P) RECORDED BY NOVICE AND ALL EXPERIENCED OBSERVERS.

TIME (hrs)	NOVICE 1 D v E	D v P	NOVICE 2 D v E	D v P	EXPERIENCED D v E	D v P
7	3.3	12.6	1.7	1.7	4.5	54.5
8	2.7	12.7	1.0	17.5	0.2	102.4
9	0.6	13.8	1.1	26.0	1.1	180.0
10	2.7	24.9	1.1	40.9	0.7	223.6
12	2.3	62.3	1.7	67.4	1.3	345.3
13	1.9	76.4	3.9	74.3	1.8	363.7
14	0.9	91.7	3.4	91.8	2.7	403.4
15	0.3	94.0	2.8	101.3	1.6	398.5
16	1.5	80.2	5.9	113.5	4.6	414.1
17	2.3	96.5	0.9	119.2	4.5	424.9
18	0.9	74.0	1.5	108.0	3.5	397.3
28	1.3	56.0	1.8	75.0	1.3	286.5

6. THE CHI-SQUARED VALUES FOR EXPERIMENTAL v PLACEBO RECORDED BY NOVICE AND ALL EXPERIENCED OBSERVERS.

TIME (hrs)	NOVICE 1 E v P	NOVICE 2 E v P	EXPERIENCED E v P
7	6.0	9.9	75.0
8	7.1	21.3	104.6
9	16.0	32.8	189.0
10	32.9	44.9	234.4
12	54.4	77.8	339.0
13	77.5	93.2	346.2
14	91.0	103.1	391.1
15	98.5	111.0	395.9
16	82.3	122.9	410.8
17	89.3	122.4	428.2
18	69.8	122.1	368.4
28	47.1	90.0	284.0

PILOT TRIAL 1.

7. THE AS AND %TPS VALUES FOR BETNOVATE CREAM AT 0.25 - 1 HR DOSE DURATIONS.

TIME (hrs)	0.25 AS	%TPS	0.5 AS	%TPS	0.75 AS	%TPS	1 AS	%TPS
0	16	11.1	8	5.6	13	9.0	18	12.5
1	12	8.3	6	4.2	18	12.5	20	13.9
2	16	11.1	11	7.6	21	14.6	20	13.9
3	16	11.1	9	6.3	18	12.5	19	13.2
4	13	9.0	11	7.6	13	9.0	17	11.8
5	11	7.6	5	3.5	12	8.3	17	11.8
6	14	9.7	9	6.3	11	7.6	19	13.2
10	4	2.8	5	3.5	12	8.3	13	9.0
11	2	1.4	3	2.1	7	4.9	13	9.0
12	0	0	2	1.4	5	3.5	10	6.9
19	4	2.8	2	1.4	9	6.3	10	6.9
22	4	2.8	3	2.1	9	6.3	15	10.4
24	3	2.1	4	2.8	6	4.2	15	10.4
26	5	3.5	4	2.8	10	6.9	16	11.1

8. THE AS AND %TPS VALUES FOR BETNOVATE CREAM AT 1.5 - 5 HRS DOSE DURATIONS.

TIME (hrs)	1.5 AS	%TPS	2 AS	%TPS	4 AS	%TPS	5 AS	%TPS
0	20	13.9	19	13.2	18	12.5	17	11.8
1	16	11.1	19	13.2	21	14.6	22	15.3
2	20	13.9	24	16.7	25	17.4	27	18.8
3	19	13.2	22	15.3	27	18.8	31	21.5
4	16	11.1	21	14.6	33	22.9	33	22.9
5	20	13.9	25	17.4	41	28.5	38	26.4
6	22	15.3	27	18.8	51	35.4	49	34.0
10	19	13.2	30	20.1	60	41.7	56	38.9
11	16	11.1	27	18.8	56	38.9	54	37.5
12	15	10.4	21	14.6	50	34.7	49	34.0
19	13	9.0	22	15.3	30	20.8	34	23.6
22	24	16.7	22	15.3	38	26.4	37	25.7
24	20	13.9	23	16.0	39	27.1	44	30.6
26	21	14.6	24	16.7	39	27.1	44	30.6

9. THE AS AND %TPS VALUES FOR BETNOVATE CREAM AT 6 - 10 HRS DOSE DURATIONS.

TIME (hrs)	6 AS	%TPS	7 AS	%TPS	8 AS	%TPS	10 AS	%TPS
0	27	18.8	18	12.5	19	13.2	19	13.2
1	25	17.4	11	7.6	22	15.3	19	13.2
2	33	22.9	23	15.6	36	25.0	42	29.2
3	38	26.4	24	16.7	41	28.5	52	36.1
4	44	30.6	26	18.1	46	31.9	60	49.3
5	50	34.7	37	25.7	55	38.2	54	43.1
6	60	41.7	57	39.6	57	39.6	47	32.6
10	70	52.1	56	38.9	50	34.7	32	22.2
11	63	43.8	51	35.4	46	31.9	39	27.1
12	57	39.6	43	29.9	26	18.1	36	25.0
19	40	27.8	28	19.4	22	15.3	36	25.0
22	55	38.2	41	28.5	28	19.4		
24	55	38.2	39	27.1	27	18.8		
26	55	38.2	38	26.4				

10. THE AS AND %TPS VALUES FOR LENOVATE CREAM AT 0.25 - 1 HR DOSE DURATIONS. *(pilot trial 1).*

TIME (hrs)	0.25 AS	%TPS	0.5 AS	%TPS	0.75 AS	%TPS	1 AS	%TPS
0	17	11.8	15	10.4	6	4.2	7	4.9
1	15	10.4	19	13.2	12	8.3	11	7.6
2	20	13.9	17	11.9	13	9.0	7	4.9
3	20	13.9	16	11.1	14	9.7	11	7.6
4	20	13.9	13	9.0	9	6.3	7	4.9
5	17	11.8	12	8.3	10	6.9	10	6.9
6	18	12.5	12	8.3	8	5.6	11	7.6
10	6	4.2	10	6.9	6	4.2	10	6.9
11	4	2.8	7	4.9	4	2.8	9	6.3
12	3	2.1	5	3.5	1	0.7	6	4.2
19	3	2.1	5	3.5	3	2.1	5	3.5
22	8	5.6	9	6.3	9	6.3	11	7.6
24	5	3.5	7	4.9	5	3.5	14	9.7
26	5	3.5	7	4.9	7	4.9	9	6.3

11. THE AS AND %TPS VALUES FOR LENOVATE CREAM AT 1.5 - 5 HRS DOSE DURATIONS.

TIME (hrs)	1.5 AS	%TPS	2 AS	%TPS	4 AS	%TPS	5 AS	%TPS
0	15	10.4	16	11.1	13	9.0	17	11.8
1	14	9.7	19	13.2	19	13.2	21	14.6
2	16	11.1	22	15.3	23	16.0	30	20.8
3	20	13.9	17	11.8	25	17.4	33	22.9
4	18	12.5	18	12.5	33	22.9	43	29.9
5	17	11.8	21	14.6	38	26.4	51	35.4
6	16	11.1	21	14.6	46	31.9	66	45.8
10	16	9.7	26	18.1	71	49.3	92	63.9
11	13	9.3	27	18.8	69	47.9	88	61.1
12	9	6.3	25	17.4	64	44.4	86	59.7
19	13	9.0	22	15.3	43	29.9	57	39.6
22	27	18.8	36	25.0	53	36.8	69	48.0
24	19	13.2	34	23.6	58	40.3	76	52.8
26	28	19.4	42	29.2	62	43.1	72	50.1

12. THE AS AND %TPS VALUES FOR LENOVATE CREAM AT 6 - 10 HRS DOSE DURATIONS.

TIME (hrs)	6 AS	%TPS	7 AS	%TPS	8 AS	%TPS	10 AS	%TPS
0	12	8.3	22	15.3	19	13.2	30	20.8
1	14	9.7	16	11.1	18	12.5	27	18.8
2	25	17.4	34	23.6	36	25.0	60	41.7
3	31	21.5	39	27.1	42	29.2	76	52.8
4	38	26.4	41	28.4	53	36.8	99	68.8
5	44	30.6	48	33.3	58	40.3	94	65.3
6	54	37.5	56	38.9	64	44.4	91	63.2
10	83	57.6	80	55.6	60	41.7	61	42.4
11	74	51.4	74	51.4	58	40.3	63	43.8
12	72	50.0	72	50.0	36	25.0	62	43.1
19	54	37.5	43	29.9	40	27.8	59	41.0
22	62	43.1	54	37.5	43	29.9		
24	62	43.1	61	42.4	43	29.9		
26	64	44.4	60	41.7				

12. **THE CHI-SQUARED VALUES FOR BETNOVATE CREAM v LENOVATE CREAM AT 0.25 - 6 HRS DOSE DURATIONS.** *(pilot trial 1).*

TIME (hrs)	0.25	0.5	0.75	1	1.5	2	4	5	6
0	0.1	6.1	4.1	4.7	4.8	0.6	3.1	0.5	8.4
1	0.5	8.3	4.1	3.0	2.2	1.6	1.0	6.8	4.8
2	2.8	2.1	3.4	9.0	1.1	1.0	0.9	9.0	2.3
3	0.9	3	1.4	3.9	0.7	1.7	2.1	14.4	2.1
4	2.7	0.3	1.1	6.3	1.7	1.1	2.1	13.1	1.8
5	2.1	4.7	0.3	2.9	0.5	0.8	0.8	9.7	3.5
6	0.9	1.2	1.1	3.7	1.5	1.8	6.9	12.9	5.0
10	0.5	0.1	2.1	2.3	1.2	1.9	7.4	14.0	6.4
11	0.7	2.0	1.4	1.4	1.1	1.7	3.8	13.8	11.3
12	3.1	1.6	2.9	2.1	1.6	6.0	3.3	14.6	8.6
19	0.2	1.4	3.0	2.0	2.0	1.2	3.3	8.4	8.3
22	1.6	1.4	0	0.7	1.5	4.4	10.1	12.1	6.2
24	0.6	3.6	2.1	0.1	0.6	3.8	12.0	14.7	10.3
26	0.00	1.0	1.1	2.8	1.1	6.6	12.9	12.3	6.6

14. **THE CHI-SQUARED VALUES FOR BETNOVATE CREAM COMPARING BLANCHING RESPONSE BETWEEN VARIOUS DOSE DURATIONS.**

TIME (hrs)	5/6	6/7	7/8	5/7	6/8	8/10
0	3.7	33.8	11.0	19.0	33.8	1.0
1	4.9	8.2	10	22.3	28.2	22.3
2	6.9	3.6	22.3	6.6	22.3	22.3
3	11.2	5.9	5.7	3.1	1.0	22.3
4	15.0	10.4	10.2	8.7	3.7	38.3
5	6.3	5.0	3.8	10.4	1.1	15.2
6	4.9	7.8	2.9	9.4	2.4	15.2
10	12.7	6.6	1.9	7.2	8.3	2.9
11	12.9	8.9	1.4	6.3	12.2	3.4
12	12.0	6.4	1.5	3.2	8.2	1.5
19	5.1	7.0	1.2	3.7	6.0	4.1
22	14.6	10.5	6.5	2.5	25.2	2.9
24	15.6	10.8	3.7	0.6	18.5	1.5
26	5.9	8.6	5.5	3.5	16.1	5.4

15. **THE CHI-SQUARED VALUES FOR LENOVATE CREAM COMPARING BLANCHING RESPONSE BETWEEN VARIOUS DOSE DURATIONS.**

TIME (hrs)	5/6	6/7	7/8	5/7	6/8	10/8
0	1.6	13.0	20.7	20.6	13.0	22.3
1	2.8	24.0	14.4	24.0	22.3	18.9
2	4.7	2.6	22.3	7.9	22.3	33.8
3	0.3	5.1	8.4	3.6	2.0	29.3
4	2.3	5.8	0.5	5.8	5.6	3.9
5	3.0	3.5	1.0	3.8	10.5	3.0
6	5.9	4.7	0.7	4.6	4.1	4.3
10	2.1	7.9	3.7	1.7	8.0	14.0
11	3.3	10.0	5.2	15.2	9.0	12.1
12	2.9	1.1	2.5	8.3	4.3	12.6
19	0.2	6.0	4.4	10.8	4.3	11.2
22	1.2	3.4	7.1	9.4	6.6	10.0
24	6.6	5.1	8.5	8.5	8.0	
26	5.5	3.7	5.6	4.0	9.2	

PILOT TRIAL 2

16. **THE AS AND %TPS VALUES FOR BETNOVATE CREAM AT 0.25 - 1 HR DOSE DURATIONS.**

TIME (hrs)	0.25		0.5		0.75		1	
	AS	%TPS	AS	%TPS	AS	%TPS	AS	%TPS
0	2	1.4	5	3.5	7	4.9	10	6.9
1	4	2.8	5	3.5	3	2.1	6	4.2
2	4	2.8	9	6.3	6	4.2	10	6.9
3	12	8.3	10	6.9	10	6.9	15	10.4
4	16	11.1	7	4.9	10	6.9	17	11.8
5	16	11.1	12	8.3	10	6.9	20	13.9
6	12	8.3	11	7.6	12	8.3	18	12.5
10	8	5.6	6	4.2	10	6.9	19	13.2
11	6	4.2	6	4.2	9	6.3	17	11.8
12	5	3.5	5	3.5	9	6.3	15	10.4
19	5	3.5	1	0.7	3	2.1	8	5.6
22	4	2.8	1	0.7	5	3.5	9	6.3
24	5	3.5	1	0.7	4	2.8	8	5.6
26	5	3.5	2	1.4	4	2.8	9	6.3

17. **THE AS AND %TPS VALUES FOR BETNOVATE CREAM AT 1.5 - 5 HRS DOSE DURATIONS.**

TIME (hrs)	1.5		2		4		5	
	AS	%TPS	AS	%TPS	AS	%TPS	AS	%TPS
0	11	7.6	16	11.1	17	11.8	29	20.1
1	12	8.3	10	6.9	17	11.8	26	18.1
2	12	8.3	10	6.9	21	14.6	39	27.1
3	12	8.3	20	13.9	31	21.5	45	31.3
4	17	11.8	19	13.2	40	27.8	60	41.7
5	20	13.9	21	14.6	46	31.9	67	46.5
6	16	11.1	18	12.5	52	36.1	72	50.0
10	13	9.0	23	16.0	56	38.9	67	46.5
11	13	9.0	20	13.9	51	35.4	60	41.7
12	10	6.9	19	13.2	48	33.3	56	38.9
19	4	2.8	11	7.6	20	13.9	23	16.0
22	5	3.5	11	7.6	18	12.5	21	14.6
24	4	2.8	8	5.6	14	9.7	18	12.5
26	5	3.5	9	6.3	21	14.6	25	17.4

18. **THE AS AND %TPS VALUES FOR BETNOVATE CREAM AT 6 - 10 HRS DOSE DURATIONS.**

TIME (hrs)	6		7		8		10	
	AS	%TPS	AS	%TPS	AS	%TPS	AS	%TPS
0	28	19.4	27	18.8	40	27.8	34	23.6
1	30	20.8	17	11.8	38	26.4	33	22.9
2	39	27.1	56	38.9	55	38.2	58	40.3
3	46	31.9	59	41.0	65	45.1	69	47.9
4	58	40.3	68	47.2	80	55.6	79	54.9
5	66	45.8	75	52.1	88	61.1	89	61.8
6	65	45.1	84	58.3	93	64.6	105	72.9
10	63	43.8	87	60.4	82	56.9	95	66.0
11	50	34.7	73	50.7	73	50.7	88	61.1
12	47	32.6	66	45.8	29	20.1	41	28.5
19	14	9.7	28	19.4	21	14.6	38	26.4
22	16	11.1	27	18.8	19	13.2	30	20.8
24	15	10.4	19	13.2	23	16.0	30	20.8
26	18	12.5	18	12.5				

19. THE AS AND %TPS VALUES FOR LENOVATE CREAM AT 0.25 - 1 HR DOSE DURATIONS. *(pilot trial 2).*

TIME (hrs)	0.25		0.5		0.75		1	
	AS	%TPS	AS	%TPS	AS	%TPS	AS	%TPS
0	10	6.9	11	7.6	5	3.5	5	3.5
1	4	2.8	5	3.5	3	2.1	6	4.2
2	10	6.9	8	5.6	9	6.3	7	4.9
3	17	11.8	12	8.3	6	4.2	13	9.0
4	18	12.5	11	7.6	10	6.9	14	9.7
5	23	16.0	10	6.9	13	9.0	15	10.4
6	16	11.1	6	4.2	11	7.6	16	11.1
10	7	4.9	5	3.5	12	8.3	20	13.9
11	5	3.5	6	4.2	10	6.9	18	12.5
12	5	3.5	4	2.8	9	6.3	16	11.1
19	3	2.1	4	2.8	3	2.1	10	6.9
22	4	2.8	1	0.7	4	2.8	11	7.6
24	3	2.1	2	1.4	6	4.2	8	5.6
26	2	1.4	4	2.8	5	3.5	11	7.6

20. THE AS AND %TPS VALUES FOR LENOVATE CREAM AT 1.5 - 5 HRS DOSE DURATIONS.

TIME (hrs)	1.5		2		4		5	
	AS	%TPS	AS	%TPS	AS	%TPS	AS	%TPS
0	8	5.6	7	4.9	16	11.1	19	13.2
1	10	6.9	4	2.8	17	11.8	27	18.8
2	19	13.2	5	3.5	23	16.0	40	27.8
3	17	11.8	14	9.7	27	18.8	50	34.7
4	16	11.1	19	13.2	46	31.9	59	41.0
5	22	15.3	21	14.6	55	38.2	68	47.2
6	18	12.5	21	14.6	71	49.3	73	50.7
10	16	11.1	35	24.3	96	66.7	90	62.5
11	15	10.4	34	23.6	87	60.4	81	56.3
12	15	10.4	33	22.9	84	58.3	72	50.0
19	7	4.9	21	14.6	35	24.3	36	25.0
22	10	6.9	17	11.8	28	19.4	32	22.2
24	10	6.9	14	9.7	20	13.9	28	19.4
26	14	9.7	18	12.5	23	16.0	33	22.9

21. THE AS AND %TPS VALUES FOR LENOVATE CREAM AT 6 - 10 HRS DOSE DURATIONS.

TIME (hrs)	6		7		8		10	
	AS	%TPS	AS	%TPS	AS	%TPS	AS	%TPS
0	20	13.9	18	12.5	34	23.6	41	28.5
1	22	15.3	11	7.6	33	22.9	44	30.6
2	35	24.3	38	26.4	58	40.3	71	49.3
3	49	34.0	49	34.0	69	47.9	83	57.6
4	63	43.8	53	36.8	79	54.9	103	71.5
5	69	47.9	65	45.1	89	61.8	96	66.7
6	73	50.7	69	47.9	105	72.9	96	66.7
10	85	59.0	73	50.7	95	66.0	43	29.9
11	74	51.4	62	43.1	88	61.1	29	20.1
12	72	50.0	61	42.4	41	28.5	29	20.1
19	34	23.6	23	16.0	38	26.4	33	22.9
22	31	21.5	19	13.2	30	20.8		
24	25	17.4	16	11.1	30	20.8		
26	28	19.4	19	13.2				

22. **THE CHI-SQUARED VALUES FOR BETNOVATE CREAM v LENOVATE CREAM AT 0.25 - 6 HRS DOSE DURATIONS.** ***(pilot trial 2).***

TIME (hrs)	0.25	0.5	0.75	1	1.5	2	4	5	6
0	6.4	6.4	0.4	2.0	1.1	5.2	1.0	2.8	1.8
1	0	0	1.0	0	0.3	3.2	0.5	0.7	2.7
2	3.2	0.1	0.8	1.1	2.9	2.1	3.3	2.3	6.0
3	2.2	0.3	1.3	0.4	2.2	2.2	1.8	3.0	1.5
4	2.1	1.4	0	0.3	0.1	3.0	2.3	5.2	8.1
5	4.3	0.3	0.7	2.3	0.3	5.2	1.8	0.4	3.3
6	2.3	1.3	0.3	3.5	3.1	4.0	10.9	3.5	7.6
10	4.4	0.1	0.5	1.9	0.5	8.5	16.6	6.7	5.8
11	0.1	0	0.1	1.8	0.1	5.9	13.0	10.9	4.9
12	1.5	0.1	0	1.1	0.8	9.1	15.4	5.3	16.1
19	1.1	1.9	2.0	0.4	2.2	3.4	4.8	3.9	8.7
22	0	0	0.2	0.2	3.2	1.5	3.3	3.5	6.3
24	1.0	0.4	2.7	0.8	3.2	3.1	1.5	6.1	3.1
26	1.4	0.7	1.1	1.3	5.4	6.3	0.6	5.7	3.0

23. **THE CHI-SQUARED VALUES FOR BETNOVATE CREAM COMPARING BLANCHING RESPONSE BETWEEN VARIOUS DOSE DURATIONS.**

TIME (hrs)	5/6	6/7	7/8	5/7	6/8	8/10
0	0.5	23.7	0	25.7	23.7	0
1	2.7	4.0	9.3	20.0	20.6	1.0
2	4.2	10.7	9.7	5.3	1.2	25.7
3	0.4	8.9	4.2	9.1	9.6	56.8
4	9.3	8.4	1.6	6.3	12.1	9.5
5	2.6	4.5	2.2	2.8	10.1	4.2
6	3.2	10.3	2.2	11.8	11.2	0.8
10	1.6	8.7	1.1	6.5	10.4	1.3
11	4.1	10.6	4.8	8.5	11.1	6.5
12	3.4	10.6	1.6	3.9	11.8	0.3
19	2.4	6.8	6.7	5.3	6.2	3.4
22	0.9	5.7	1.2	2.6	1.9	2.0
24	0.3	1.2	1.1	1.2	1.0	1.5
26	1.4	4.5	4.5	6.0	2.7	1.1

24. **THE CHI-SQUARED VALUES FOR LENOVATE CREAM COMPARING BLANCHING RESPONSE BETWEEN VARIOUS DOSE DURATIONS.**

TIME (hrs)	5/6	6/7	7/8	5/7	6/8	8/10
0	0.1	16.7	0	15.0	16.6	0
1	0.9	3.2	9.2	12.0	16.6	20.0
2	5.0	1.1	6.0	3.2	7.2	49.1
3	0.7	0.1	4.6	1.4	5.9	13.5
4	3.6	4.9	10.3	0.8	4.4	2.3
5	1.1	0.9	14.7	2.6	9.6	1.3
6	3.2	4.7	10.8	2.8	4.8	4.2
10	0.6	4.3	15.3	6.2	7.3	5.9
11	1.7	2.2	16.0	3.5	10.3	3.7
12	6.2	6.0	14.7	5.3	8.3	0.4
19	0.3	3.3	7.1	2.2	3.1	5.9
22	0.5	1.7	7.9	1.5	4.3	2.7
24	4.6	5.2	4.6	1.8	8.5	4.3
26	2.8	7.1	5.4	7.8	2.7	

25. THE AS AND %TPS VALUES FOR BETNOVATE CREAM AT 1 - 4 HRS DOSE DURATIONS. *(pivotal trial 1).*

TIME (hrs)	1		2		3		4	
	AS	%TPS	AS	%TPS	AS	%TPS	AS	%TPS
0	27	9.4	53	9.2	69	12.0	68	23.6
1	23	8.0	53	9.2	77	13.4	57	19.8
2	28	9.7	66	11.4	82	14.2	65	22.6
3	36	12.5	80	13.9	93	16.2	71	24.7
4	38	13.2	97	16.8	115	20.0	78	27.1
6	57	19.8	120	20.8	145	25.2	90	31.3
7	68	23.6	153	26.6	182	31.6	113	39.2
8	79	27.4	174	30.2	200	34.7	120	41.7
9	77	26.7	163	28.3	198	34.4	117	40.6
10	70	24.3	157	27.3	193	33.5	113	39.2
11	66	22.9	137	23.8	165	28.7	95	33.0
12	58	20.1	114	19.8	139	24.1	81	28.1
19	29	10.1	63	10.9	55	9.6	37	12.9
22	29	10.1	37	6.4	23	4.0	24	8.3
24	21	7.3	30	5.2	17	3.0	18	6.3
26	20	6.9	29	5.0	15	2.6	13	4.5

26. THE AS AND %TPS VALUES FOR LENOVATE CREAM AT 2 - 6 HRS DOSE DURATIONS.

TIME (hrs)	2		3		6	
	AS	%TPS	AS	%TPS	AS	%TPS
0	50	8.7	75	13.0	141	24.5
1	49	8.5	78	13.5	175	30.4
2	60	10.4	99	17.2	194	33.7
3	68	11.8	113	19.7	212	36.8
4	82	14.2	129	22.4	223	38.7
6	117	20.3	174	30.2	255	44.3
7	165	28.6	221	38.4	298	51.7
8	189	32.8	253	43.9	328	56.9
9	206	35.8	256	44.4	338	58.7
10	199	34.5	253	43.9	327	56.8
11	185	32.1	230	39.9	290	50.4
12	167	29.0	212	36.8	262	45.5
19	83	14.1	106	14.4	94	16.3
22	55	9.5	79	13.7	30	5.2
24	37	6.4	67	11.6	20	3.5
26	37	6.4	60	10.4	18	2.6

27. THE AS AND %TPS VALUES FOR BETNOVATE CREAM AT 6 HRS DURATION AND CHI-SQUARED VALUES BETWEEN 2 AND 6 HRS DURATIONS.

TIME (hrs)	AS	%TPS	BETNOVATE 2/6	LENOVATE 2/6
0	196	34.0	76.7	41.7
1	194	33.7	64.7	62.3
2	204	35.4	60.5	65.4
3	212	36.8	57.8	75.8
4	234	40.6	64.4	79.9
6	249	43.2	53.7	60.1
7	288	50.0	47.4	60.4
8	300	52.1	37.8	48.0
9	294	51.0	36.9	37.0
10	282	49.0	33.7	43.1
11	242	42.0	26.3	39.5
12	213	37.0	25.7	33.2
19	95	14.5	10.5	16.8
22	50	8.7	5.8	8.8
24	40	6.9	2.0	6.2
26	37	6.4	1.8	7.6

28. THE AS AND %TPS VALUES FOR BETNOVATE CREAM AT 1 - 4 HRS DOSE DURATIONS. *(pivotal trial 2).*

TIME (hrs)	1		2		3		4	
	AS	%TPS	AS	%TPS	AS	%TPS	AS	%TPS
0	20	6.0	72	10.7	61	9.1	34	10.1
1	13	3.0	63	9.4	63	9.4	42	12.5
2	10	3.6	49	7.3	46	6.8	38	11.3
3	12	4.8	54	8.0	55	8.2	50	14.9
4	16	3.9	62	9.2	72	10.7	53	15.8
6	13	4.5	82	12.2	92	13.7	54	16.1
7	15	7.4	75	11.2	100	14.9	60	17.9
8	25	5.1	92	13.7	116	17.3	70	20.8
9	17	5.1	94	12.2	104	15.5	65	19.3
10	17	4.5	82	12.2	91	13.5	61	18.2
11	15	4.8	73	10.9	83	12.4	55	16.4
12	16	4.2	61	9.1	67	10.0	39	11.6
19	14	3.9	59	7.8	54	8.0	35	10.4
22	13	5.1	52	7.7	44	6.5	32	9.5
24	17	4.8	52	7.7	46	6.8	29	8.6
26	16	4.2	52	7.7	43	6.4	29	8.6

29. THE AS AND %TPS VALUES FOR LENOVATE CREAM AT 2 - 6 HRS DOSE DURATIONS.

TIME (hrs)	2		3		6	
	AS	%TPS	AS	%TPS	AS	%TPS
0	41	6.1	41	6.1	59	8.8
1	38	5.7	58	8.6	98	14.6
2	35	5.2	47	7.0	104	15.5
3	40	6.0	55	8.2	130	19.4
4	51	7.6	64	9.5	149	22.2
6	75	11.2	97	14.4	183	27.2
7	82	12.2	119	17.7	208	31.0
8	111	16.5	151	22.5	251	37.4
9	112	16.7	155	23.1	248	36.9
10	113	16.8	155	23.1	235	35.0
11	102	15.2	143	21.3	221	32.9
12	95	14.1	133	19.8	192	28.6
19	82	12.2	123	18.3	144	21.4
22	84	12.5	128	19.0	140	20.8
24	87	12.9	126	18.8	125	18.6
26	78	11.6	126	18.8	112	16.7

30. THE AS AND %TPS VALUES FOR BETNOVATE CREAM AT 6 HRS DOSE DURATION. THE CHI-SQUARED VALUES BETWEEN 2 AND 6 HRS DOSE DURATIONS.

TIME (hrs)	BETNOVATE		BETNOVATE 2/6	LENOVATE 2/6
	AS	%TPS		
0	127	18.9	24.7	5.1
1	139	20.7	35.6	28.3
2	137	20.4	48.0	35.6
3	157	23.4	51.3	41.0
4	168	25.0	54.9	46.9
6	174	25.9	33.1	43.2
7	186	27.7	40.1	50.3
8	221	32.9	43.2	53.7
9	202	30.1	37.3	57.3
10	186	27.7	41.4	50.0
11	160	23.8	37.1	47.4
12	129	19.2	28.4	45.2
19	100	14.9	19.1	29.4
22	82	12.2	15.9	22.9
24	89	13.2	13.9	11.1
26	85	12.7	13.1	8.9

31. THE AS AND %TPS VALUES FOR BETNOVATE CREAM AT 1 - 4 HRS DOSE DURATIONS. *(pivotal trial 3).*

TIME (hrs)	1 AS	%TPS	2 AS	%TPS	3 AS	%TPS	4 AS	%TPS
0	29	8.6	66	9.8	77	11.5	71	21.1
1	9	2.7	32	4.8	69	10.3	56	16.7
2	16	4.8	40	6.0	86	12.8	70	20.8
3	22	6.5	69	10.3	136	20.2	98	29.2
4	28	8.3	107	15.9	173	25.7	123	36.6
6	52	15.5	144	21.4	223	33.2	138	41.1
7	65	19.3	180	26.8	250	37.2	146	43.5
8	71	21.1	201	29.9	271	40.3	160	47.6
9	76	22.6	210	31.3	263	39.1	162	48.2
10	63	18.8	184	27.4	226	33.6	140	41.7
11	61	18.2	162	24.1	199	29.6	122	36.3
12	57	17.0	149	10.1	178	26.5	109	32.4
19	28	8.3	68	11.9	73	10.9	43	12.8
22	27	8.0	80	12.9	71	10.6	30	8.9
24	29	8.6	87	13.1	65	9.7	30	8.9
26	42	12.5	85	12.3	75	11.2	37	11.0

32. THE AS AND %TPS VALUES FOR LENOVATE CREAM AT 2 - 6 HRS DOSE DURATIONS.

TIME (hrs)	2 AS	%TPS	3 AS	%TPS	6 AS	%TPS
0	47	7.0	60	8.9	161	24.0
1	33	4.9	47	7.0	201	29.9
2	43	6.4	77	11.5	243	36.2
3	66	9.8	115	17.1	302	44.9
4	124	18.5	177	26.3	349	51.9
6	197	29.3	228	33.9	384	57.1
7	241	35.7	287	42.7	414	61.6
8	280	41.7	326	48.5	448	66.7
9	292	43.5	337	50.1	449	66.8
10	257	38.2	303	45.1	396	58.9
11	245	36.5	296	44.0	357	53.1
12	232	34.5	286	42.6	330	49.1
19	121	18.1	140	20.8	121	18.0
22	131	19.5	132	19.6	106	15.8
24	118	17.6	139	20.7	79	11.8
26	123	18.3	133	19.8	80	11.9

33. THE AS AND %TPS VALUES AT 6 HRS DURATION AND CHI-SQUARED VALUES BETWEEN 2 AND 6 HRS DOSE DURATIONS.

TIME (hrs)	BETNOVATE AS	%TPS	BETNOVATE 2/6	LENOVATE 2/6
0	198	29.5	80.2	66.7
1	182	27.1	101.5	117.6
2	215	32.0	120.1	151.3
3	274	40.8	118.5	152.1
4	319	47.5	92.4	122.8
6	338	50.3	73.9	82.0
7	364	54.2	68.3	79.8
8	393	58.5	68.4	71.5
9	386	57.4	61.2	61.7
10	329	49.0	42.3	42.8
11	279	41.5	33.0	38.1
12	254	37.8	28.7	30.6
19	115	17.1	14.6	3.1
22	85	12.7	0.3	4.2
24	86	12.8	0.2	11.2
26	94	14.0	6.4	16.4

34. THE CHI-SQUARED VALUES FOR BETNOVATE CREAM v LENOVATE CREAM AT 2 - 6 HRS DOSE DURATIONS. ***(all pivotal trials).***

TIM hrs	2	3	6	2	3	6	2	3	6
	PIVOTAL TRIAL 1			PIVOTAL TRIAL 2			PIVOTAL TRIAL 3		
0	0.7	1.8	11.3	10.1	5.1	40.6	4.3	4.7	6.5
1	4.3	1.0	2.3	8.3	0.4	9.0	3.6	6.5	2.6
2	1.1	5.8	1.8	3.4	0	9.1	4.5	3.6	7.2
3	1.5	6.7	3.3	3.1	1.4	4.5	2.7	10.4	3.5
4	3.6	4.7	3.8	1.4	0.6	3.4	1.8	10.3	15.8
6	9.8	10.8	13.2	2.4	5.3	3.6	9.9	10.4	9.2
7	4.0	12.3	9.8	5.2	5.9	2.5	9.0	7.8	19.1
8	4.3	12.1	7.1	5.8	7.8	3.8	12.1	9.9	17.3
9	7.6	13.5	7.9	2.9	13.8	8.7	14.9	10.7	21.5
10	6.6	11.7	11.7	7.6	15.7	14.0	14.4	13.5	14.9
11	6.6	19.0	12.4	9.7	15.2	15.1	16.7	20.7	22.0
12	9.9	19.1	6.2	6.5	18.5	18.4	19.2	21.8	27.13
19	5.2	37.7	7.2	7.3	36.1	18.0	18.9	27.4	.5
22	4.0	37.4	8.2	12.2	59.2	24.9	19.0	22.4	5.5
24	1.3	37.4	8.2	11.6	58.0	10.3	7.2	29.1	1.0
26	2.4	32.2	7.8	6.6	62.0	7.5	15.1	19.6	7.3

APPENDIX D

CHROMAMETER DATA.

This appendix is only for raw data generated by the chromameter. All the raw data are the average of 12 subjects unless specified. The application patterns and product application schedule sheets used were the same as for the visual data. The observation times for all trials at various dose durations are presented in hours after product removal for comparison purposes. It should be noted that for the preliminary trial for novice observer training, the chromameter was not used. The chromameter results presented here are only for the pilot and pivotal trials.

PILOT TRIAL 1

35. THE CHROMAMETER a-scale VALUES FOR BETNOVATE CREAM AT 0.25 - 2 HRS DOSE DURATIONS.

TIME (hrs)	0.25	0.5	0.75	1	1.5	2
0	8.64	8.81	9.03	8.61	8.58	8.15
1	8.27	8.76	8.58	8.60	8.65	8.20
2	8.00	8.32	8.45	8.52	8.43	8.21
3	8.70	8.62	8.61	8.56	8.43	8.29
4	9.23	9.54	9.37	9.32	9.18	8.56
5	8.88	9.02	8.98	8.75	8.79	8.47
6	8.66	8.71	8.93	8.83	8.91	8.47
10	9.24	9.27	9.37	9.12	9.03	8.49
11	9.01	8.99	8.93	9.20	8.65	8.39
12	8.54	8.64	8.68	9.20	8.36	8.34
19	7.43	7.89	7.37	7.93	7.30	6.93
22	7.81	8.09	7.96	8.23	7.53	7.36
24	7.53	7.77	7.88	8.28	7.16	7.28
26	7.76	7.61	7.48	7.99	7.37	7.40

36. THE CHROMAMETER a-scale VALUES FOR BETNOVATE CREAM AT 4 - 10 HRS DOSE DURATIONS.

TIME (hrs)	4	5	6	7	8	10
0	8.83	8.38	7.93	8.71	7.81	7.68
1	8.25	8.11	8.03	8.28	7.84	8.19
2	7.68	8.03	7.72	8.29	7.99	7.89
3	8.41	8.40	8.05	8.56	8.14	8.12
4	8.46	8.88	8.68	8.64	8.01	8.40
5	7.77	7.96	7.58	8.59	8.33	8.09
6	8.12	7.99	7.64	8.18	7.73	7.75
10	7.88	8.09	8.08	8.39	7.98	7.61
11	7.91	8.71	8.21	9.09	7.78	7.43
12	7.77	8.39	8.43	8.47	7.10	7.61
19	7.13	7.44	7.43	7.69	7.74	7.54
22	7.44	7.92	7.42	7.68	7.34	
24	6.88	7.97	7.60	7.90	7.28	
26	7.44	7.38	7.53	7.41		

37. THE CHROMAMETER a-scale VALUES FOR LENOVATE CREAM AT 0.25 - 2 HRS DOSE DURATIONS. *(pilot trial 1).*

TIME (hrs)	0.25	0.5	0.75	1	1.5	2
0	9.42	9.65	9.33	9.62	8.65	9.86
1	9.07	9.35	8.58	9.68	9.04	9.86
2	8.95	8.98	8.69	9.28	8.82	9.31
3	9.18	9.54	8.51	9.72	8.98	9.56
4	9.81	10.03	9.37	9.88	9.68	10.02
5	9.35	9.49	8.93	9.53	8.96	9.26
6	8.97	9.43	8.78	9.27	9.23	9.44
10	9.88	9.87	9.30	10.24	9.49	9.73
11	9.27	9.51	9.07	9.78	9.23	9.54
12	9.28	9.56	8.91	9.65	8.53	9.28
19	8.18	8.44	7.93	8.68	7.57	8.29
22	8.72	8.78	8.06	8.73	7.81	8.21
24	8.34	8.48	8.04	8.38	7.42	9.03
26	8.29	8.16	7.76	8.48	7.64	8.08

38. THE CHROMAMETER a-scale VALUES FOR LENOVATE CREAM AT 4 - 10 HRS DOSE DURATIONS.

TIME (hrs)	4	5	6	7	8	10
0	8.38	7.99	8.71	8.79	7.84	7.58
1	8.22	7.84	8.38	8.33	7.59	8.21
2	7.96	7.81	8.26	7.79	8.36	7.80
3	8.61	7.78	8.53	8.73	8.63	7.39
4	8.86	8.34	8.78	9.03	8.34	7.51
5	8.26	7.63	7.99	8.45	8.02	7.99
6	8.09	7.28	8.20	8.35	8.37	7.77
10	8.16	7.21	8.06	8.71	8.20	6.91
11	7.92	7.49	8.13	8.88	8.02	7.31
12	8.03	6.86	8.11	8.52	7.70	7.25
19	7.13	6.83	7.41	7.25	7.71	6.89
22	7.03	7.13	7.28	7.80	7.87	
24	7.32	6.68	7.53	7.73	7.43	
26	6.98	7.12	7.13	7.46		

39. THE STUDENT'S t-DISTRIBUTION TEST VALUES FOR BETNOVATE CREAM v LENOVATE CREAM AT 0.25 - 6 HRS DOSE DURATIONS.

TIME (hrs)	0.25	0.5	0.75	1	1.5	2	4	5	6
0	1.02	0.70	0.45	0.19	0.34	2.50	0.24	0.67	1.35
1	0.78	0.81	0.16	0.58	0.44	1.39	0.65	0.61	1.10
2	1.22	0.22	1.00	0.15	0.06	0.00	1.59	0.14	1.51
3	0.73	0.09	0.50	1.48	0.43	1.06	0.58	0.74	1.04
4	1.47	0.12	0.46	0.36	0.61	1.00	0.19	1.05	0.26
5	0.55	0.26	0.47	0.21	0.56	0.93	0.36	0.71	0.83
6	0.19	0.36	0.63	0.43	0.07	2.84	0.07	0.97	0.99
10	1.28	0.18	1.13	2.00	0.92	1.77	0.71	1.57	0.35
11	0.460	1.23	0.43	0.02	0.35	1.09	0.04	2.74	0.11
12	.052.	0.12	0.06	1.04	0.52	0.17	0.12	1.70	0.59
19	13	0.19	0.80	0.87	0.62	1.62	0.05	1.05	0.47
22	0.55	0.16	0.04	1.12	0.98	0.01	0.48	0.27	0.70
24	1.450	0.16	0.85	1.95	0.86	0.43	0.57	1.56	1.00
26	.75	0.00	0.48	0.39	0.67	0.51	0.56	1.47	1.61

The total degrees of freedom for comparisons of any two data sets are 22.

40. THE STUDENT'S t-DISTRIBUTION TEST VALUES FOR BETNOVATE CREAM COMPARING BLANCHING BETWEEN VARIOUS DOSE DURATIONS. *(pilot trial 1).*

TIME (hrs)	5/6	6/7	7/8	5/7	6/8	8/10
0	0.64	1.97	1.94	1.56	0.08	0.48
1	0.51	0.85	1.35	0.31	0.59	0.18
2	0.31	0.99	1.14	0.61	0.47	0.11
3	0.59	1.25	1.28	0.55	0.54	0.59
4	0.10	0.43	0.03	0.36	0.38	0.15
5	0.52	1.75	0.85	1.09	0.92	0.32
6	0.54	0.60	0.79	0.04	0.17	0.92
10	0.26	0.75	0.60	0.63	0.11	2.09
11	0.76	1.41	1.13	0.70	0.09	0.03
12	0.31	0.30	0.83	0.05	0.50	0.24
19	0.45	0.56	0.92	1.01	0.48	1.27
22	0.18	0.05	0.05	0.15	0.10	
24	0.03	0.31	2.01	0.20	2.01	
26	1.29	1.97		0.87		

41. THE STUDENT'S t-DISTRIBUTION TEST VALUES FOR LENOVATE CREAM COMPARING BLANCHING RESPONSE BETWEEN VARIOUS DOSE DURATIONS.

TIME (hrs)	5/6	6/7	7/8	5/7	6/8	10/8
0	1.50	0.03	0.87	1.23	0.98	1.35
1	1.19	0.25	1.38	0.79	1.70	0.65
2	1.36	0.75	1.45	2.53	0.86	0.76
3	1.30	1.00	0.40	1.11	0.35	2.04
4	1.00	0.38	0.56	0.55	0.38	1.44
5	1.07	0.73	0.97	1.50	0.48	0.20
6	1.45	0.51	0.69	1.01	0.18	0.32
10	1.64	0.23	0.37	1.23	0.19	0.56
11	1.98	0.60	0.56	1.39	0.03	0.38
12	1.06	0.07	1.60	1.15	1.45	2.81
19	0.92	1.36	0.87	0.45	0.37	2.29
22	0.94	1.30	1.77	0.17	0.27	
24	0.99	0.99	0.00	0.26	0.99	
26	1.04	1.11		0.22		

The total degrees of freedom for comparisons of any two data sets are 22.

PILOT TRIAL 2

42. THE CHROMAMETER a-scale VALUES FOR BETNOVATE CREAM AT 0.25 - 2 HRS DOSE DURATIONS.

TIME (hrs)	Control	0.25	0.5	0.75	1	1.5	2
0	9.02	9.98	8.57	10.35	9.18	9.85	8.63
1	10.25	8.77	7.88	9.38	8.02	9.25	7.68
2	8.43	8.87	8.05	9.02	8.22	8.68	7.38
3	8.33	8.85	7.78	10.00	8.23	8.87	7.40
4	10.44	9.03	7.45	10.03	7.47	8.73	7.40
5	9.15	9.53	8.68	10.20	8.62	9.57	6.72
6	8.01	8.85	7.65	9.08	7.43	8.95	7.27
10	8.07	9.65	7.78	10.48	7.85	8.58	7.18
11	8.93	9.97	8.13	10.47	8.13	9.60	7.87
12	8.32	9.68	8.47	10.00	8.17	8.97	7.07
19	8.07	8.43	7.48	9.48	7.12	8.38	6.48
22	9.07	7.83	7.67	8.63	6.82	8.05	7.13
24	7.51	6.98	6.60	8.40	6.48	7.58	5.72
26	8.52	8.22	6.50	8.22	6.50	7.65	6.25

43. THE CHROMAMETER a-scale VALUES FOR BETNOVATE CREAM AT 4 - 10 HRS DOSE DURATIONS. *(pilot trial 2).*

TIME (hrs)	4	5	6	7	8	10
0	10.05	7.12	10.87	8.77	10.59	8.22
1	10.27	7.15	10.53	5.37	8.56	5.52
2	9.55	6.48	9.95	6.65	9.80	6.28
3	10.12	5.83	10.03	6.57	9.45	5.87
4	9.97	6.25	10.18	6.13	9.73	6.37
5	10.07	6.42	10.18	6.73	9.25	6.63
6	9.78	6.52	9.88	6.48	9.12	6.67
10	9.63	6.75	9.63	6.10	9.35	6.63
11	9.67	6.68	10.05	7.23	8.83	5.83
12	10.27	7.30	9.85	6.48	9.07	5.37
19	9.17	6.30	9.40	6.12	8.48	6.00
22	8.73	6.47	8.73	6.90	7.87	
24	8.12	5.47	8.20	5.73	8.53	
26	8.77	6.22	8.88	5.48		

44. THE CHROMAMETER a-scale VALUES FOR LENOVATE CREAM AT 0.25 - 2 HRS DOSE DURATIONS.

TIME (hrs)	Control	0.25	0.5	0.75	1	1.5	2
0	9.02	8.57	9.93	8.12	10.32	10.22	9.57
1	10.25	8.00	9.22	8.53	8.98	8.08	8.97
2	8.43	7.78	8.82	7.60	8.60	7.82	8.65
3	8.33	7.60	9.17	7.80	9.08	7.62	9.22
4	10.44	8.10	9.32	7.72	9.22	7.83	9.30
5	9.15	8.58	9.45	8.83	9.50	7.98	9.70
6	8.01	8.08	9.08	7.50	8.18	7.40	8.60
10	8.07	7.77	9.30	8.15	8.85	7.13	8.48
11	8.93	9.00	10.12	7.87	9.08	7.78	9.72
12	8.32	8.23	9.70	8.25	8.72	7.45	9.58
19	8.07	7.40	8.48	7.22	8.40	6.57	8.77
22	9.07	7.38	7.38	7.32	7.47	6.52	7.80
24	7.51	6.30	7.38	6.43	7.33	6.30	7.52
26	8.52	7.17	7.52	6.45	7.45	6.72	7.43

45. THE CHROMAMETER a-scale VALUES FOR BETNOVATE CREAM AT 4 - 10 HRS DOSE DURATIONS.

TIME (hrs)	4	5	6	7	8	10
0	3.58	10.92	8.08	10.37	8.16	5.98
1	3.38	10.12	7.77	5.25	5.88	4.23
2	3.03	9.45	7.03	7.53	7.18	5.25
3	2.62	9.73	6.33	9.45	7.22	5.18
4	7.57	9.77	6.70	9.25	6.45	5.17
5	8.43	9.87	6.88	9.05	5.80	5.20
6	7.98	9.73	6.37	9.45	6.12	5.62
10	8.28	9.22	6.17	8.87	5.65	4.52
11	8.13	8.90	7.30	9.17	6.13	5.07
12	8.15	9.17	7.23	9.82	6.42	4.22
19	6.95	8.73	6.53	8.80	6.12	5.03
22	8.07	8.88	6.62	8.58	5.88	
24	6.52	8.10	5.82	8.02	6.10	
26	7.82	8.60	6.03	8.75		

46. **THE CHROMAMETER a-scale VALUES TAKEN AT CONTROL SITES AND APPLICATION SITES FOR ALL DOSE DURATIONS (DD) FOR BETNOVATE CREAM BEFORE DRUG V APPLICATION (ZERO READINGS).** ***(pilot trial 2).***

	DOSE DURATION (HRS)						
FORMULATION	Contr	0.25	0.5	0.75	1	1.5	2
BETNOVATE	10.51	9.94	8.06	10.04	8.96	9.95	8.93
LENOVATE	10.51	8.74	9.65	8.28	10.45	8.85	9.74

	DOSE DURATION (HRS)						
FORMULATION	Contr	4	5	6	7	8	10
BETNOVATE	10.51	10.39	8.28	10.72	8.77	10.59	8.22
LENOVATE	10.51	9.82	11.00	8.38	10.37	8.16	5.98

47. **THE STUDENT'S t-DISTRIBUTION TEST VALUES FOR BETNOVATE CREAM v LENOVATE CREAM AT 0.25 - 6 HRS DOSE DURATIONS.**

TIME (hrs)	0.25	0.5	0.75	1	1.5	2	4	5	6
0	0.55	0.43	0.33	0.31	0.31	0.25	0.85	0.87	1.25
1	0.22	0.73	1.16	0.19	0.19	0.42	0.97	0.09	1.33
2	0.46	0.91	1.36	0.39	0.39	0.35	1.62	0.11	0.39
3	0.47	0.59	0.94	0.09	0.09	0.40	1.14	0.95	0.71
4	0.04	0.55	0.57	0.91	0.91	0.40	1.31	0.87	0.46
5	0.28	0.27	0.84	0.19	0.19	0.24	1.36	1.01	0.18
6	0.16	0.81	0.67	0.07	0.07	0.57	0.22	1.58	0.19
10	0.47	0.47	0.99	0.07	0.07	0.03	0.26	0.47	0.49
11	0.07	1.03	0.84	0.16	0.16	0.94	0.52	1.20	0.11
12	0.23	0.29	1.12	0.09	0.09	0.58	0.94	1.12	0.02
19	0.21	0.53	0.78	0.28	0.28	0.62	1.15	1.70	0.12
22	0.08	0.05	0.68	0.04	0.04	1.03	0.78	0.67	1.01
24	0.31	0.01	1.13	0.14	0.14	0.23	1.20	0.10	1.09
26	0.05	0.05	0.76	0.37	0.37	1.49	1.05	2.11	0.58

49. **THE STUDENT'S t-DISTRIBUTION TEST VALUES FOR BETNOVATE CREAM v LENOVATE CREAM AT 0.25 - 6 HRS DOSE DURATIONS.**
(Without zero values)

TIME (hrs)	0.25	0.5	0.75	1	1.5	2	4	5	6
0	0.73	0.12	0.27	0.02	0.31	0.83	1.56	0.71	0.57
1	0.01	0.34	1.04	0.13	0.53	0.58	0.09	0.01	0.78
2	0.49	0.77	0.58	0.07	0.49	0.89	0.29	0.11	0.07
3	0.06	0.33	0.35	0.49	0.49	0.19	0.54	0.22	0.46
4	0.13	0.13	0.11	1.10	0.48	0.35	0.77	0.72	0.01
5	0.93	0.78	0.24	0.23	0.24	0.77	0.37	0.18	0.44
6	0.55	0.34	0.16	1.15	1.01	0.66	0.48	0.64	0.27
10	0.11	0.46	0.46	0.18	0.14	0.35	0.24	1.51	0.01
11	0.44	0.56	0.37	0.52	1.39	0.17	0.01	0.54	0.06
12	0.38	0.14	0.44	1.01	0.56	0.24	0.12	1.48	0.67
19	0.20	0.24	0.30	0.46	2.11	0.24	0.38	0.50	0.02
22	0.03	0.30	0.21	0.17	0.40	0.01	0.35	1.35	1.07
24	0.01	1.16	0.78	0.36	1.51	0.56	0.89	1.30	1.30
26	0.36	0.48	0.18	0.03	0.05	0.17	0.21	0.46	0.68

The total degrees of freedom for comparisons of any two data sets are 6.

50. THE STUDENT'S t-DISTRIBUTION TEST VALUES FOR BETNOVATE CREAM COMPARING BLANCHING RESPONSE BETWEEN VARIOUS DOSE DURATIONS. *(pilot trial 2).*

TIME (hrs)	5/6	6/7	7/8	5/7	6/8	10/8
0	0.46	2.08	0.34	1.84	1.96	
1	0.67	1.38	0.28	1.04	1.32	0.52
2	0.02	1.65	0.42	1.31	1.40	0.67
3	0.27	2.86	0.16	2.10	3.27	0.88
4	0.08	4.47	1.31	3.88	3.41	1.35
5	0.57	4.58	1.14	3.97	5.34	1.20
6	0.15	4.40	0.18	4.08	4.12	0.64
10	0.09	2.77	0.93	3.14	2.67	0.61
11	0.46	5.60	0.16	4.07	2.82	0.13
12	0.74	4.29	2.37	4.55	2.68	0.29
19	0.23	4.19	1.89	4.15	2.03	0.94
22	0.75	5.89	0.26	9.54	5.91	0.69
24	1.01	9.97	2.63	5.12	6.42	
26	0.60	6.34				

51. THE STUDENT'S t-DISTRIBUTION TEST VALUES FOR LENOVATE CREAM COMPARING BLANCHING RESPONSE BETWEEN VARIOUS DOSE DURATIONS.

TIME (hrs)	5/6	6/7	7/8	5/7	6/8	10/8
0	0.56	0.33	0.01	0.98	0.35	0.69
1	1.37	0.21	0.03	1.59	0.22	0.35
2	0.83	0.34	1.40	2.05	0.91	0.87
3	0.84	1.48	0.09	4.20	1.58	0.13
4	0.84	3.70	3.64	4.53	5.51	1.32
5	0.93	4.80	0.01	9.18	3.51	1.29
6	0.93	2.63	0.29	4.07	2.84	0.37
10	1.24	4.48	0.99	9.07	3.98	0.85
11	2.51	3.17	0.49	4.55	6.28	0.90
12	1.34	3.04	1.56	8.51	2.37	1.47
19	1.14	2.49	1.18	3.06	3.33	0.02
22	1.41	3.26	1.20	4.96	2.71	
24	1.53	3.20	0.61	7.07	3.28	
26	0.69	1.84		5.26		

The total degrees of freedom for comparisons of any two data sets are 6.

52. THE CHROMAMETER a-scale VALUES FOR BETNOVATE CREAM AND LENOVATE REAMS AT 1 - 6 HRS DOSE DURATIONS. *(pivotal trial 1).*

TIME (hrs)	1	2	3	4	6	2	3	6
	BETNOVATE CREAM					LENOVATE CREAM		
0	8.68	8.81	9.25	8.81	7.61	9.02	8.41	8.70
1	9.05	7.76	8.08	7.83	6.93	8.24	7.34	7.41
2	7.98	7.76	8.25	7.60	6.92	8.32	7.46	7.61
3	8.05	7.58	7.92	7.58	6.71	8.14	7.21	7.41
4	7.57	7.47	7.75	7.55	6.43	7.98	7.12	7.16
6	7.83	8.19	8.45	8.13	7.28	8.49	7.62	7.74
7	8.21	8.54	10.11	8.30	7.55	8.62	7.81	8.38
8	8.56	8.42	8.89	8.45	7.63	8.69	8.00	7.96
9	8.62	8.58	8.87	8.55	7.78	8.74	7.83	8.13
10	8.50	8.49	8.96	8.71	7.72	8.62	7.81	8.17
11	8.57	8.45	8.58	8.36	7.58	8.68	7.56	8.06
12	8.32	8.74	9.08	9.07	7.97	8.92	8.06	8.48
19	8.80	8.81	8.25	8.05	7.31	8.20	7.45	8.29
22	7.89	7.76	8.44	8.24	7.44	8.04	7.33	8.44
24	7.70	8.10	8.59	8.45	7.62	8.17	7.50	8.55
26	7.79	8.05	8.46	8.32	7.65	8.16	7.65	8.63

53. THE CHROMAMETER L-scale VALUES FOR BETNOVATE CREAM AND LENOVATE CREAMS AT 1 - 6 HRS DOSE DURATIONS. *(pivotal trial 1).*

TIME (hrs)	1	2	3	4	6	2	3	6
	BETNOVATE CREAM					LENOVATE CREAM		
0	57.51	56.38	55.31	54.48	57.57	55.45	57.53	55.03
1	56.21	54.86	52.95	54.08	56.39	54.35	55.55	52.26
2	58.65	58.00	57.72	55.98	59.21	56.32	59.49	57.06
3	59.08	58.24	56.18	56.71	59.29	57.18	58.96	57.86
4	60.00	59.03	58.08	58.71	60.28	57.92	60.39	58.44
6	62.18	61.55	60.99	61.17	62.74	60.27	62.86	61.31
7	62.35	61.98	60.99	61.26	62.97	60.73	63.04	61.39
8	62.03	61.83	60.87	61.36	62.70	60.54	62.69	61.58
9	63.32	61.74	60.91	61.02	62.72	60.22	62.90	61.44
10	61.83	62.14	60.71	60.89	62.75	60.54	62.92	61.30
11	62.10	61.82	60.84	60.84	62.54	8.68	63.39	61.01
12	59.71	61.45	60.75	60.46	62.41	8.92	62.38	61.15
19	62.14	61.66	60.84	60.56	62.43	8.20	62.81	60.82
22	62.43	61.54	60.86	60.49	62.39	8.04	62.60	60.62
24	62.06	61.79	60.60	60.76	62.29	8.17	62.78	60.43
26	62.24	61.74	60.91	60.90	62.32	8.16	62.86	60.53

54. THE CHROMAMETER b-scale VALUES FOR BETNOVATE CREAM AND LENOVATE CREAMS AT 1 - 6 HRS DOSE DURATIONS.

TIME (hrs)	1	2	3	4	6	2	3	6
	BETNOVATE CREAM					LENOVATE CREAM		
0	13.77	14.43	14.95	14.51	13.64	14.73	13.71	15.07
1	13.44	13.95	14.27	14.55	13.69	14.32	13.17	14.47
2	14.30	14.80	15.60	15.20	14.39	14.98	14.15	15.77
3	14.48	15.07	15.26	15.31	14.59	15.05	14.16	16.00
4	14.72	15.24	17.68	15.83	14.70	15.32	14.56	15.99
6	14.99	15.96	16.61	16.52	15.31	15.85	15.02	17.03
7	15.22	16.03	16.47	16.56	15.51	15.95	15.17	17.04
8	15.16	15.91	16.51	16.51	15.39	16.05	15.14	17.13
9	14.99	15.85	16.47	16.40	15.28	15.81	14.95	16.95
10	15.19	15.81	16.46	16.51	15.21	15.96	15.05	16.91
11	15.15	15.85	16.52	16.49	15.28	16.16	15.06	16.97
12	15.23	15.77	16.40	16.36	15.11	15.89	14.87	16.74
19	15.26	15.95	16.54	16.35	15.20	19.40	15.15	16.64
22	14.99	15.64	16.46	16.20	14.95	15.75	14.77	16.39
24	14.84	15.77	16.31	16.14	14.68	15.78	14.74	16.34
26	14.92	15.51	16.16	16.08	14.76	15.74	14.89	16.26

55. THE CHROMAMETER a-scale VALUES FOR BETNOVATE CREAM AND LENOVATE CREAMS AT 1 - 6 HRS DOSE DURATIONS. *(pivotal trial 2).*

	1	2	3	4	6	2	3	6
	BETNOVATE CREAM					LENOVATE CREAM		
0	8.57	9.21	9.59	9.58	8.58	9.37	8.90	9.43
1	7.85	9.82	8.89	9.12	8.07	8.67	8.39	8.72
2	7.71	8.51	8.94	8.77	7.93	8.64	8.20	8.54
3	7.58	8.61	8.81	8.64	7.82	8.57	8.16	8.35
4	7.69	8.54	8.76	8.68	7.77	8.51	8.18	8.28
6	7.74	8.58	8.77	8.96	8.09	8.49	8.23	8.48
7	7.94	8.84	9.01	9.00	8.22	8.85	8.45	8.67
8	8.47	9.38	9.64	9.45	8.65	9.25	8.89	8.93
9	8.73	9.73	9.98	9.92	9.00	9.51	9.16	9.35
10	8.83	9.66	9.79	9.94	8.97	9.59	9.13	9.06
11	7.81	8.55	8.85	9.00	8.13	8.55	8.00	8.25
12	7.68	8.51	8.80	8.80	7.92	8.44	7.97	8.10
19	7.26	8.24	9.54	8.41	7.76	8.19	7.81	8.05
22	7.62	8.40	8.64	8.85	7.92	8.36	7.91	8.22
24	7.58	8.18	8.37	8.36	7.94	8.04	7.73	7.93
26	7.59	8.22	8.55	8.62	7.93	8.18	7.85	8.15

56. THE CHROMAMETER L-scale VALUES FOR BETNOVATE CREAM AND LENOVATE CREAMS AT 1 - 6 HRS DOSE DURATIONS. *(pivotal trial 2).*

TIME (hrs)	1	2	3	4	6	2	3	6
	BETNOVATE CREAM					LENOVATE CREAM		
0	64.97	62.96	62.42	62.17	63.88	62.90	63.83	62.51
1	64.91	63.05	62.49	62.40	62.79	63.07	63.75	62.67
2	65.12	63.10	62.47	62.67	63.72	63.07	63.67	62.80
3	64.71	62.35	61.83	62.08	63.45	62.52	63.24	62.33
4	63.21	62.89	62.19	62.34	62.91	62.84	63.59	62.68
6	64.79	62.80	62.29	62.20	63.54	62.96	63.53	62.67
7	64.93	62.89	62.40	62.43	63.52	62.90	63.65	62.72
8	64.52	62.48	62.00	61.97	63.35	62.62	63.51	62.75
9	64.89	72.21	62.10	62.09	63.66	62.75	63.75	62.84
10	64.63	61.90	62.03	61.80	6.43	62.55	63.42	62.73
11	64.62	62.49	62.01	62.01	63.28	62.797	63.66	62.82
12	64.96	62.87	62.39	62.04	63.55	0.08	63.71	62.92
19	64.99	62.31	62.39	62.43	63.42	62.306	63.72	62.75
22	64.90	62.85	62.34	62.14	63.26	3.15	63.71	62.72
24	64.54	62.49	62.15	61.94	63.15	62.67	71.60	71.10
26	64.98	62.98	62.20	62.35	63.28	62.90	63.39	62.41

57. THE CHROMAMETER b-scale VALUES FOR BETNOVATE CREAM AND LENOVATE CREAMS AT 1 - 6 HRS DOSE DURATIONS.

TIME (hrs)	1	2	3	4	6	2	3	6
	BETNOVATE CREAM					LENOVATE CREAM		
0	14.18	15.23	15.89	15.66	14.66	15.28	14.57	15.88
1	14.43	15.46	15.88	16.04	14.87	15.16	14.65	15.90
2	14.60	15.49	16.08	16.09	15.01	15.46	14.80	16.05
3	14.55	15.52	16.04	16.24	15.05	15.47	14.78	16.10
4	14.42	15.40	16.04	16.07	14.96	15.33	14.72	16.06
6	14.39	15.49	16.02	16.11	14.95	15.42	14.81	16.08
7	14.40	15.20	15.92	15.88	14.82	15.30	14.59	15.84
8	14.10	14.96	15.55	15.62	14.51	14.91	14.38	15.69
9	13.66	14.65	15.26	15.23	14.11	14.60	14.06	15.44
10	13.90	14.78	15.33	15.45	14.40	14.65	14.18	15.47
11	14.56	15.34	16.03	16.16	15.03	15.46	14.90	16.13
12	14.74	15.71	16.081	16.17	17.55	15.53	14.99	16.28
19	14.67	15.34	5.90	15.91	14.80	15.31	14.76	15.83
22	14.27	15.44	16.03	16.07	14.63	15.33	14.79	16.00
24	14.20	15.26	15.75	15.70	14.72	15.03	14.65	15.80
26	14.40	15.32	15.82	16.01	14.89	15.25	14.66	15.76

58. THE CHROMAMETER a-scale VALUES FOR BETNOVATE CREAM AND LENOVATE CREAMS AT 1 - 6 HRS DOSE DURATIONS. *(pivotal trial 3).*

TIME hrs	1	2	3	4	6	2	3	6
	BETNOVATE CREAM					LENOVATE CREAM		
0	9.06	9.70	10.04	9.60	10.21	9.58	9.18	9.85
1	8.45	8.75	8.81	8.81	8.05	8.77	8.26	8.56
2	8.08	8.58	8.71	8.38	7.57	8.47	8.20	8.06
3	8.18	8.48	8.58	8.27	7.65	8.32	8.03	7.94
4	8.23	8.50	8.77	8.42	7.66	8.51	7.88	8.05
6	8.31	8.58	8.55	8.51	7.96	8.27	8.10	8.09
7	8.19	8.68	8.88	8.84	8.01	8.54	8.07	8.24
8	8.39	8.74	10.87	8.71	8.14	8.38	8.04	8.23
9	8.57	9.03	9.17	8.98	8.27	8.54	8.28	8.57
10	8.62	8.90	8.97	8.71	8.20	8.51	9.97	8.42
11	8.71	9.00	9.26	8.86	8.38	8.80	9.21	8.52
12	8.41	8.91	9.04	8.63	8.22	8.53	8.10	9.76
19	7.75	8.05	8.29	8.19	7.51	7.62	7.49	8.35
22	8.12	8.51	8.57	8.46	7.96	8.17	7.90	8.71
24	8.15	8.31	8.56	8.33	7.95	8.10	7.71	8.57
26	8.15	9.15	8.48	8.40	7.99	7.97	7.68	8.64

59. THE CHROMAMETER L-scale VALUES FOR BETNOVATE CREAM AND LENOVATE CREAMS AT 1 - 6 HRS DOSE DURATIONS. *(pivotal trial 3).*

TIME hrs	1	2	3	4	6	2	3	6
	BETNOVATE CREAM					LENOVATE CREAM		
0	64.34	63.81	63.03	63.21	64.61	63.67	64.45	63.20
1	64.26	63.85	63.34	63.30	64.55	63.77	64.48	63.70
2	64.32	62.75	63.28	63.22	64.75	63.81	64.49	63.71
3	64.42	63.89	63.21	63.36	64.74	63.86	64.56	63.83
4	68.79	63.83	63.30	63.23	79.98	63.79	64.59	63.80
6	64.14	63.77	63.37	63.15	64.18	63.88	64.40	63.69
7	64.38	64.02	63.47	63.32	64.51	63.90	64.65	63.88
8	63.99	63.74	63.31	63.09	64.25	63.84	64.57	63.69
9	63.86	63.65	63.09	62.91	64.14	63.57	64.26	63.49
10	63.81	63.63	63.18	62.98	64.17	63.86	64.43	63.63
11	63.85	63.52	63.07	62.94	64.16	73.02	64.51	63.53
12	63.98	63.61	63.11	63.11	64.22	72.55	64.63	63.58
19	62.59	64.21	63.59	63.43	63.81	63.076	64.83	63.60
22	64.16	63.91	63.43	63.11	64.34	3.97	64.55	63.28
24	61.46	63.68	63.07	63.07	64.26	63.82	64.30	62.94
26	64.56	64.10	63.47	63.69	64.55	64.02	64.76	63.34

59. THE CHROMAMETER b-scale VALUES FOR BETNOVATE CREAM AND LENOVATE CREAMS AT 1 - 6 HRS DOSE DURATIONS.

TIME (hrs)	1	2	3	4	6	2	3	6
	BETNOVATE CREAM					LENOVATE CREAM		
0	14.38	15.38	15.80	15.63	14.37	15.22	14.52	15.83
1	14.51	15.37	15.93	16.02	14.741	15.33	14.71	15.98
2	14.66	15.46	16.01	16.08	4.89	15.29	14.82	16.12
3	14.73	15.54	16.05	16.07	14.92	15.39	14.83	16.22
4	14.86	15.59	16.24	16.14	15.06	15.57	14.85	16.25
6	14.84	15.42	16.00	16.01	14.90	15.31	14.88	16.10
7	14.80	15.62	16.16	16.04	15.03	15.43	14.83	16.28
8	14.74	15.34	16.05	15.89	14.82	15.24	14.81	16.14
9	14.67	15.32	16.00	15.99	14.73	15.33	14.65	16.06
10	14.40	15.32	16.211	16.01	14.79	15.45	14.94	16.20
11	14.59	15.46	6.54	16.08	14.91	15.44	14.83	16.11
12	14.83	15.57	16.28	16.14	14.96	15.52	14.90	16.19
19	14.99	15.84	16.11	16.39	15.23	15.87	15.14	16.32
22	14.87	15.73	16.09	16.11	15.15	15.75	15.05	16.01
24	14.68	15.52	16.15	16.18	14.88	15.57	14.78	15.96
26	14.77	15.46	16.54	16.02	14.89	15.60	14.96	15.80

60. THE MEAN CONTROL SITE CHROMAMETER L-, a- AND b-scale VALUES.

TIME (hrs)	PIVOTAL TRIAL 1			PIVOTAL TRIAL 2		
	L	a	b	L	a	b
0	58.66	9.51	16.20	61.58	9.38	15.86
1	58.20	9.38	16.38	61.72	9.16	15.85
2	58.63	9.41	16.76	61.73	9.11	15.95
3	58.95	9.32	16.65	61.33	9.23	15.86
4	59.52	9.23	16.68	61.60	9.69	15.87
6	59.74	9.36	16.60	61.56	9.14	16.06
7	59.75	9.78	16.74	61.55	9.38	15.84
8	59.84	9.64	16.63	61.10	10.90	15.50
9	59.73	9.77	16.69	61.52	10.21	15.14
10	59.73	9.63	16.84	61.48	10.23	15.23
11	59.28	9.52	16.78	61.57	9.23	16.09
12	60.04	9.88	16.63	61.60	8.93	16.21
19	59.47	10.26	16.98	61.59	8.75	16.05
22	59.93	8.94	16.71	61.54	8.97	15.98
24	60.00	9.11	16.55	61.42	8.86	15.94
26	59.82	9.10	18.15	61.47	8.83	16.01

61. **THE MEAN CONTROL SITE CHROMAMETER L-, a- AND b-scale VALUES.** *(pivotal trial 3).*

TIME (hrs)	PIVOTAL TRIAL 3 L	a	b
0	62.24	9.37	15.94
1	62.30	9.23	15.97
2	62.25	9.04	16.04
3	69.53	9.13	16.14
4	62.27	9.09	16.04
6	62.23	9.05	16.19
7	62.31	9.23	16.15
8	62.24	9.29	16.09
9	68.53	9.72	16.06
10	62.17	9.39	16.10
11	68.29	9.38	16.10
12	62.43	9.04	16.24
19	62.81	8.53	16.39
22	62.22	9.11	16.12
24	62.12	9.15	16.00
26	62.11	8.94	16.11

62. **THE MEAN CHROMAMETER VALUES TAKEN AT CONTROL SITES AND APPLICATION SITES FOR ALL DOSE DURATIONS (DD) FOR BETNOVATE CREAM BEFORE DRUG APPLICATION (ZERO READINGS).**

DD (hrs)	PIVOTAL TRIAL 1 L	a	b	PIVOTAL TRIAL 2 L	a	b
Control	59.78	9.41	16.83	62.26	8.97	16.09
1	60.31	8.68	16.31	64.72	8.12	14.73
2	60.60	8.76	16.41	61.66	8.63	15.42
3	60.63	8.84	16.43	62.93	8.89	15.61
4	60.81	8.81	16.57	63.09	8.62	15.60
6	59.87	8.56	16.19	63.39	8.36	15.18

THE MEAN CHROMAMETER VALUES TAKEN BEFORE DRUG APPLICATION (ZERO READINGS) AT CONTROL SITES AND APPLICATION SITES FOR ALL DOSE DURATIONS (DD) FOR LENOVATE CREAM. *(Continued).*

DD (hrs)	PIVOTAL TRIAL 1 L	a	b	PIVOTAL TRIAL 2 L	a	b
Control	59.78	9.41	16.83	62.26	8.97	16.09
2	59.55	8.92	16.35	63.01	8.71	15.41
3	60.29	8.62	16.16	63.18	8.65	15.15
6	60.69	8.70	16.57	62.95	8.68	15.70

63. **THE MEAN CHROMAMETER VALUES TAKEN AT CONTROL SITES AND APPLICATION SITES FOR ALL DOSE DURATIONS (DD) FOR BETNOVATE CREAM BEFORE DRUG APPLICATION (ZERO READINGS).** *(pivotal trial 3).*

DD (hrs)	PIVOTAL TRIAL 3 L	a	b
Control	62.84	8.87	15.98
1	63.23	8.53	15.56
2	63.71	8.40	15.77
3	63.74	8.39	15.57
4	63.59	8.45	15.76
6	63.61	8.30	15.75

63. **THE MEAN CHROMAMETER VALUES TAKEN BEFORE DRUG APPLICATION (ZERO READINGS) AT CONTROL SITES AND APPLICATION SITES FOR ALL DOSE DURATIONS (DD) FOR LENOVATE CREAM.** ***(pivotal trial 3).***

DD (hrs)	PIVOTAL TRIAL 3		
	L	a	b
Control	62.84	8.87	15.98
2	63.70	8.33	15.67
3	63.72	8.34	15.60
6	63.65	8.15	15.81

64. **THE STUDENT'S t-DISTRIBUTION TEST VALUES FOR BETNOVATE CREAM v LENOVATE CREAM AT 2, 3 AND 6 HRS DOSE DURATIONS (DD) FOR EACH TRIAL FOR THE a-scale PARAMETER.**

TIME (hrs)	PIVOTAL TRIAL 1 DD (hrs)			PIVOTAL TRIAL 2 DD (hrs)			PIVOTAL TRIAL 3 DD (hrs)		
	2	3	6	2	3	6	2	3	6
0	1.41	2.28	2.53	0.15	1.43	1.64	0.04	3.19	0.15
1	1.56	1.98	1.14	1.18	1.02	1.69	0.67	2.30	2.69
2	3.08	3.18	1.83	0.23	2.35	1.57	0.34	2.04	2.64
3	1.74	1.52	1.78	0.24	1.57	0.44	0.01	3.28	2.41
4	1.87	1.61	1.77	0.21	1.48	0.75	0.72	4.87	2.58
6	1.39	2.27	1.23	0.44	1.02	0.04	0.66	2.10	1.97
7	0.63	0.39	2.08	0.12	1.06	0.34	0.13	3.02	2.40
8	0.39	2.06	0.47	0.41	1.88	0.29	0.37	3.17	1.15
9	0.86	3.08	1.05	0.28	1.80	0.03	0.92	3.60	1.37
10	0.81	3.01	0.91	0.10	1.65	0.75	0.68	3.22	0.91
11	0.37	3.65	1.20	0.14	2.11	0.66	0.11	1.91	0.65
12	0.76	2.78	1.45	0.28	3.16	0.46	0.67	2.84	1.55
19	0.45	0.47	0.68	0.24	0.88	0.07	1.05	2.70	4.27
22	2.13	5.21	4.06	0.19	2.38	0.43	0.60	2.53	3.58
24	0.68	3.96	2.59	0.80	1.67	0.99	0.05	3.95	4.37
26	0.54	2.39	2.86	0.38	1.56	0.59	0.41	2.75	2.46

65. **THE STUDENT'S t-DISTRIBUTION TEST VALUES FOR BETNOVATE CREAM COMPARING BLANCHING RESPONSE BETWEEN 2 AND 6 HRS DOSE DURATIONS FOR EACH PIVOTAL TRIAL.**

TIME (hrs)	a-scale PIVOTAL TRIALS			L-scale PIVOTAL TRIALS			b-scale PIVOTAL TRIALS		
	1	2	3	1	2	3	1	2	3
0	3.31	0.96	2.29	1.90	0.45	1.11	1.28	0.40	3.09
1	2.00	1.31	2.80	1.96	0.18	1.01	0.09	1.12	1.99
2	2.52	1.96	4.68	1.73	0.23	1.05	0.33	0.58	1.77
3	1.87	1.63	3.68	1.76	0.77	0.94	0.49	0.19	1.80
4	2.43	1.93	2.82	2.01	0.31	0.99	0.66	0.69	1.25
6	2.39	0.75	2.61	2.28	0.12	0.59	0.84	0.51	1.66
7	2.28	1.50	3.08	2.48	0.05	0.43	0.58	0.16	1.98
8	1.31	1.83	2.39	2.19	0.40	0.60	0.60	0.38	1.33
9	2.57	1.25	2.53	2.36	0.71	0.70	0.68	0.83	1.92
10	1.60	1.39	1.72	1.54	0.45	0.67	0.78	0.09	1.19
11	2.30	0.32	1.84	2.09	0.18	0.90	0.82	0.18	1.58
12	1.76	2.18	2.26	2.18	0.10	0.79	0.89	0.17	1.77
19	1.01	0.68	1.85	1.72	0.29	0.84	0.95	0.70	1.82
22	0.59	0.61	2.02	2.06	0.66	0.39	0.29	1.36	1.80
24	1.06	0.04	1.47	1.60	0.11	0.85	1.73	0.38	1.98
26	0.87	0.02	0.43	1.76	0.82	0.26	0.29	0.62	1.57

The total degrees of freedom for comparisons of any two data sets are 14.

APPENDIX E

THE LOCKE'S EXACT CONFIDENCE INTERVAL METHOD

This appendix contains the corrected chromameter a-scale AUC values of all subjects [40] used in the three pivotal trials at 1 hour (D_1), 2 hours (ED_{50}), 3, 4 and 6 hours (D_2). The chromameter AUC values which are underlined are only for subjects known as the "acceptable blanchers" in accordance with the D_2/D_1 ratio. Visual AUC values were also included at 2, 3 and 6 hours.

66. THE CHROMAMETER a-SCALE AUC VALUES AT DOSE DURATIONS EQUAL TO D_1 AND D_2, AND THE RATIO OF AUC AT D_2 TO AUC AT D_1 OF ALL 40 SUBJECTS.

SUBJECT	AUC AT D_1	AUC AT D_2	RATIO OF D_1/D_2
1	-2.58	-42.25	16.41
2	-13.03	-1.28	0.10
3	-11.4	47.93	-0.69
4	-13.96	1.27	-0.09
5	-22.87	-38.74	1.69
6	-9.60	-12.20	1.27
7	-13.57	-16.44	1.21
8	-5.04	-24.84	4.93
9	-104.32	-98.14	0.94
10	3.18	-5.69	-1.79
11	-27.02	-20.64	0.76
12	1.31	30.56	23.36
13	-27.93	13.74	-0.36
14	-4.09	-10.47	2.59
15	-3.94	-18.44	4.68
16	-40.10	-63.83	1.59
17	23.30	12.15	0.52
18	-16.12	-1.82	0.11

66. THE CHROMAMETER a-SCALE AUC VALUES AT DOSE DURATIONS EQUAL TO D_1 AND D_2, AND THE RATIO OF AUC AT D_2 TO AUC AT D_1 OF ALL 40 SUBJECTS. ***(Continued)***

SUBJECT	AUC AT D_1	AUC AT D_2	RATIO OF D_1/D_2
19	25.47	5.59	0.22
20	6.28	13.38	2.13
21	-27.00	4.25	-0.16
22	-26.52	-0.24	0.01
23	-18.06	-6.53	0.36
24	66.98	18.93	0.28
25	58.61	42.51	0.73
26	-19.53	34.22	-1.75
27	-19.36	-20.36	1.05
28	11.60	-8.55	-0.74
29	1.38	7.98	5.77
30	-5.22	26.16	-5.01
31	1.95	-9.06	-4.64
32	11.84	2.57	0.22
33	10.95	20.90	1.91
34	-13.07	6.36	-0.49
35	8.77	22.37	2.55
36	13.39	-51.18	-3.82
37	22.83	22.16	0.97
38	-11.44	1.23	-0.11
39	-31.10	-19.90	0.64
40	11.84	-14.28	-1.21

67. THE CHROMAMETER a-SCALE AUC VALUES FOR BETNOVATE CREAM AND LENOVATE CREAM OF ALL 40 SUBJECTS AT A DOSE DURATION EQUAL TO 2 AND 3 HOURS.

DD (hrs) SUBJECT	BETNOVATE CREAM 2	3	LENOVATE CREAM 2	3
1	-6.38	-20.38	-21.78	-30.21
2	-22.58	9.00	-12.35	-15.28
3	-3.14	4.61	-6.68	-23.14
4	-15.10	-6.50	-21.43	-27.65
5	24.53	-23.64	-33.88	-35.57
6	20.83	10.45	-1.33	-43.55
7	-52.20	-25.98	-19.63	-37.83
8	-17.35	1.93	-16.59	-29.24
9	-97.48	-79.23	-87.69	-131.90
10	10.57	5.85	3.67	3.71
11	-9.69	10.88	-2.04	-4.99
12	16.61	-7.21	18.83	-3.44
13	2.58	-14.65	-18.31	-8.01
14	-29.19	-28.28	-8.47	-36.79
15	-26.63	-26.02	-21.67	-13.00
16	-51.14	-46.35	-64.49	-52.59
17	19.95	1.41	-8.59	9.54
18	-19.59	-14.79	-24.44	-28.15
19	4.18	7.22	9.95	-0.16
20	4.11	9.00	-9.70	-2.27
21	14.28	-23.25	-21.70	-26.40
22	-24.38	4.25	3.75	-39.32
23	-2.29	-26.91	7.88	-22.06
24	30.21	24.83	25.80	-0.73
25	29.13	32.28	15.06	-1.68
26	6.34	16.99	4.30	-23.40

67. THE CHROMAMETER a-SCALE AUC VALUES FOR BETNOVATE CREAM AND LENOVATE CREAM OF ALL 40 SUBJECTS AT A DOSE DURATION EQUAL TO 2 AND 3 HOURS. ***(Continued)***

DD (hrs) SUBJECT	BETNOVATE CREAM 2	3	LENOVATE CREAM 2	3
27	9.22	-5.13	-13.33	-27.96
28	-25.70	-22.40	-12.19	8.95
29	-1.00	24.61	-4.14	-20.47
30	31.80	0.19	14.22	2.55
31	-18.39	-6.29	-20.44	-26.59
32	18.17	11.85	-12.03	7.45
33	13.64	10.00	20.14	-11.63
34	-13.47	22.95	-20.83	-26.22
35	5.57	28.67	27.02	4.95
36	-38.87	11.50	-27.33	-31.42
37	33.46	13.37	23.52	16.12
38	5.10	-14.64	-19.00	-36.27
39	-12.88	-16.98	-35.18	-33.74
40	-9.47	-1.96	-9.28	-20.81

68. THE CHROMAMETER a-SCALE AUC VALUES FOR BETNOVATE CREAM AND LENOVATE CREAM OF ALL 40 SUBJECTS AT A DOSE DURATION EQUAL TO 6 HOURS.

SUBJECT	BETNOVATE	LENOVATE	SUBJECT	BETNOVATE	LENOVATE
1	-41.63	-31.31	6	-50.90	-35.50
2	-20.89	-22.29	7	-33.68	-32.62
3	-12.98	6.53	8	-39.39	-73.07
4	-32.80	-17.85	9	-132.84	-15.15
5	-41.50	-7.25	10	3.20	-3.99

68. THE CHROMAMETER a-SCALE AUC VALUES FOR BETNOVATE CREAM AND LENOVATE CREAM OF ALL 40 SUBJECTS AT A DOSE DURATION EQUAL TO 6 HOURS. *(Continued).*

SUBJECT	BETNOVATE	LENOVATE	SUBJECT	BETNOVATE	LENOVATE
11	-11.53	-3.99	26	-5.53	-7.96
12	0.07	16.06	27	-37.33	-11.21
13	-19.22	-12.58	28	-23.88	-25.44
14	-8.02	-22.75	29	-18.37	3.72
15	-18.07	-20.07	30	8.96	21.00
16	-68.61	-89.48	31	-16.73	5.16
17	16.74	8.64	32	0.48	18.65
18	-8.35	-10.42	33	1.35	14.66
19	0.28	9.29	34	-24.77	13.51
20	9.42	-8.89	35	1.54	16.62
21	-34.78	-8.73	36	-19.98	-19.66
22	-14.19	-13.74	37	6.21	25.68
23	-32.04	-27.12	38	-29.93	0.91
24	1.25	27.50	39	-28.35	-11.89
25	24.55	14.45	40	-13.10	-37.46

69. THE AVERAGE CHROMAMETER a-SCALE AUC VALUES FOR ONLY "ACCEPTABLE BLANCHERS" AT 2 HRS DOSE DURATION.

SUBJECT	BETNOVATE CREAM	LENOVATE CREAM
1	-6.38	-21.78
5	-24.53	-33.88
6	20.83	-1.33
8	-17.35	-16.59
14	-29.19	-8.47
15	-26.63	-21.67
16	-51.14	-64.49

70. THE VISUAL AUC VALUES FOR BETNOVATE CREAM AND LENOVATE CREAM OF ALL 40 SUBJECTS AT A DOSE DURATION EQUAL TO 2 AND 3 HOURS.

DD (hrs)	BETNOVATE CREAM		LENOVATE CREAM	
SUBJECT	2	3	2	3
1	17.19	37.24	25.09	74.22
2	30.82	41.93	30.21	36.89
3	1.13	3.99	0.17	6.34
4	7.73	6.77	7.99	35.24
5	85.33	67.44	85.76	86.46
6	68.32	52.34	88.11	87.85
7	37.5	35.33	35.50	61.98
8	48.09	40.80	59.03	69.88
9	70.23	80.90	97.14	94.97
10	16.49	22.40	24.83	41.40
11	1.91	1.65	6.60	12.15
12	12.41	52.17	21.09	46.35
13	5.13	30.51	8.26	45.09
14	9.90	0.37	10.12	9.82
15	7.51	13.02	8.78	23.36
16	72.92	56.55	75.97	75.74
17	9.75	10.71	20.46	17.49
18	8.33	18.23	36.31	46.06
19	6.92	12.95	8.93	25.30
20	3.35	11.76	6.10	16.44
21	10.57	2.98	9.16	3.72
22	6.03	6.03	9.52	18.75
23	18.68	10.19	16.74	33.63
24	22.69	20.39	38.62	50.63
25	54.91	53.50	59.97	70.91
26	11.09	8.41	4.99	8.04

71. **THE AVERAGE VISUAL AUC VALUES FOR BETNOVATE CREAM AND LENOVATE CREAM OF ALL 40 SUBJECTS AT A DOSE DURATION EQUAL TO 2 AND 3 HOURS.** ***(Continued)***

DD (hrs) SUBJECT	BETNOVATE CREAM 2	3	LENOVATE CREAM 2	3
27	19.49	27.53	25.52	27.53
28	14.06	38.69	30.80	38.69
29	20.16	5.73	13.24	5.73
30	11.16	19.87	15.99	19.87
31	5.13	17.93	12.20	17.93
32	27.46	15.48	43.75	15.48
33	6.40	1.41	36.31	1.41
34	53.13	44.94	72.77	44.94
35	4.09	16.96	19.79	16.96
36	84.30	100.45	88.62	100.45
37	42.44	44.12	53.13	44.12
38	27.46	43.68	40.25	43.68
39	54.09	80.88	74.26	80.88
40	59.15	76.86	95.61	76.86

72. **THE VISUAL AUC VALUES FOR BETNOVATE CREAM AND LENOVATE CREAM OF ALL 40 SUBJECTS AT A DOSE DURATION EQUAL TO 6 HOURS.**

SUBJECT	BETNOVATE	LENOVATE	SUBJECT	BETNOVATE	LENOVATE
1	64.84	54.77	6	117.36	94.36
2	44.70	80.73	7	70.40	62.15
3	11.81	4.69	8	84.03	94.36
4	17.80	36.81	9	114.24	97.92
5	94.18	71.14	10	41.41	80.73

73. **THE VISUAL AUC VALUES FOR BETNOVATE CREAM AND LENOVATE CREAM OF ALL 40 SUBJECTS AT A DOSE DURATION EQUAL TO 6 HOURS.** ***(Continued).***

SUBJECT	BETNOVATE	LENOVATE	SUBJECT	BETNOVATE	LENOVATE
11	14.41	24.39	26	13.17	21.28
12	90.02	111.46	27	21.58	83.78
13	33.56	53.42	28	45.16	72.77
14	14.81	23.14	29	74.03	40.18
15	38.10	44.94	30	51.41	59.15
16	96.50	109.82	31	27.60	50.52
17	11.76	41.96	32	54.84	39.51
18	23.29	56.70	33	10.34	29.39
19	39.81	29.91	34	93.45	87.28
20	15.33	43.45	35	21.88	32.37
21	15.70	22.25	36	106.10	106.18
22	27.98	36.16	37	62.43	73.29
23	45.46	31.62	38	65.40	71.58
24	51.79	33.33	39	90.40	88.17
25	85.94	80.36	40	103.87	112.87

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