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## **New Developments in the Methodology Available for the Assessment of Topical Corticosteroid-Induced Skin Blanching**

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Since the publication of the previous edition of this book (1) there have been considerable developments and controversy in the field of topical corticosteroid bioequivalence assessment. There has been considerable discussion in the literature concerning the use of the Minolta chromameter for the measurement of corticosteroid-induced skin blanching (2), as it is believed this instrument would produce more objective results than the visual grading procedure. These efforts culminated in the release of a guidance document (3) from the Food and Drug Administration (FDA) detailing the procedures to be followed for the determination of topical corticosteroid bioequivalence using the chromameter. Since the promulgation of this document there have been challenges on the validity and scientific merit of the documented procedures (4), and recently the FDA itself conceded that it may be necessary

to redefine some of the protocol evaluations (5). This chapter attempts to define the current standing of the two methods of response assessment.

## I. INTRODUCTION

The human skin blanching assay (1) is unique in the field of bioequivalence testing of topical products in that the assessment methodology relies upon the production of a side effect of localized vasoconstriction and the consequent blanching (whitening) of the healthy skin following the topical application of corticosteroids. Since it has been shown (6) that the intensity of the induced blanching is directly proportional to the clinical efficacy of the corticosteroid, this procedure is particularly convenient as the induced response may be used as an indicator of the potency of a new corticosteroid drug moiety or the success of the topical vehicle delivery system in bioequivalence evaluations. In the past 15 years it has become patently obvious that formulations that are pharmaceutical equivalents (the same drug in the same type of formulation at the same concentration) may have markedly different clinical efficacy simply because of the differing potential of the compounded vehicle to release the drug to the stratum corneum. Considerable effort has, therefore, been applied to the research of delivery vehicle optimization and maximization of the thermodynamic leaving potential of drugs in topical formulations (7). Allied to this research effort has been the need to develop analytical systems capable of discriminating between the subtle drug delivery potentials of very similar formulations—especially for topical bioequivalence testing. Fortunately for corticosteroid products, this has been relatively facile because of the blanching phenomenon.

## II. VISUAL ASSESSMENT

The human skin blanching assay is routinely practiced in laboratories throughout the world, as it is a valuable tool for the assessment of the topical availability of corticosteroid formulations (8). Since the degree of blanching is directly proportional to the clinical efficacy, it follows that if two formulations containing the same corticosteroid in the same concentration are being compared, the formulation that produces the greatest degree of blanching will be clinically most efficacious (9). If different corticosteroids are being compared, the one that produces the highest degree of blanching will be the more potent, and this has led to the production of the corticosteroid formulation potency ranking tables, all of which are based on results obtained from the visually applied blanching assay.

This blanching effect has been successfully estimated over the last 35 years (10) by subjective visual assessments using an arbitrary grading scale.



This system has substantial merit as the human eye is an excellent discriminator of very small differences between colors, and numerous publications have documented the successful practical application, sensitivity, and remarkable reproducibility of the visual blanching methodology (11,12). The ability to parallel this color discrimination is still to be refined in optical analytical instruments, especially as the eye-brain combination has the ability to accurately and simultaneously assess skin color differences between the application site and surrounding (unmedicated) skin. To date this has required the manual subtraction of one value from another using instrumental measurements. Furthermore, the global visual assessment subconsciously accounts for skin factors such as inherent skin pigmentation, hirsutism, and mottling, parameters that cannot easily be accounted for with instrumental measurements.

The methodology of the visual assay protocol was documented at length in the previous edition of this book (1), and only subsequent information is discussed here. This assay procedure is subjective in nature since the assessments are performed by the human eye. We have reported at length on the accuracy and reproducibility of this procedure (11,12) and have shown that, provided the assay is performed by trained observers, very similar results are obtained on repeated evaluation, and intertrial reproducibility was shown to be excellent (13). We routinely use three trained observers to assess the degree of skin blanching, because of the subjective nature of the observations, and normally 12 volunteers with at least three (and often more) application sites on each forearm being dosed with the same formulation.

To assess the reproducibility of the assay, three identical trials at 8-week intervals were mounted (12) comprising 18 volunteers, three observers, and two commercially available formulations (formulations A and B) containing the same concentration of the same steroid and each occupying six sites per arm. After a 6-h application period the formulations were removed from the skin and the blanching was estimated at intervals over an extended period of time. Since each formulation was applied to 12 sites per volunteer, it follows that at each time interval the blanching produced by a particular formulation was estimated 648 times. It can be seen from the area under the curve (AUC) values in Table 1 that, for each trial, each observer placed the two formulations in the same rank order, thus verifying the reproducibility of this assay procedure.

A further refinement of the assay protocol has been designed in our laboratories. A retrospective reanalysis of the data obtained from various blanching trials (14) showed quite clearly that the degree of blanching produced by the same formulation varies depending upon the position on the forearm which it occupies. Maximum blanching occurs in the middle of the forearm, with a slight reduction closer to the elbow and a dramatic reduction

**Table 1** Area Under the Blanching Curves for Two Pharmaceutically Equivalent Formulations Determined by Three Independent Observers in Each of Three Replicate Trials

Observer	Trial	Formulation A	Formulation B
1	1	803	619
	2	781	674
	3	767	624
2	1	815	656
	2	888	784
	3	799	639
3	1	731	558
	2	686	616
	3	605	516

closer to the wrist. For this reason we have suggested that more than one site on each forearm should be demarcated for each formulation (preferably a minimum of three sites) and these should be spread over the whole length of the forearm. This finding is especially troublesome when assessed in terms of the FDA guidance protocol, which makes no stipulation of the number or positioning of application sites. In the light of the preceding discussion, this would clearly produce incorrect results.

### III. CHROMAMETER ASSESSMENT

The use of the eye to estimate corticosteroid-induced skin blanching has been criticized (15) due to the subjectivity of visual assessments, which does not allow for interlaboratory comparison of results. It has been suggested (3,15) that the chromameter should be used to make these measurements, as it is an objective method that quantifies the reflectance of a xenon light pulse in terms of three indices, the a-scale (red-green), the b-scale (yellow-blue), and the L-scale (light-dark). These three values define a point in three-dimensional space that characterizes the color of the measured surface.

The FDA guidance suggests the use of the chromameter to measure skin blanching in a complex protocol of pilot and pivotal trials with multiple correction (baseline and unmedicated-site values) of the a-scale values only. The a-scale values were chosen as they are the only set of values that appear to show appreciable changes over the period of time during which blanching is measured. One of the many contentious issues in the guidance document that have been reported (4,16) is the requirement that the data from the pilot

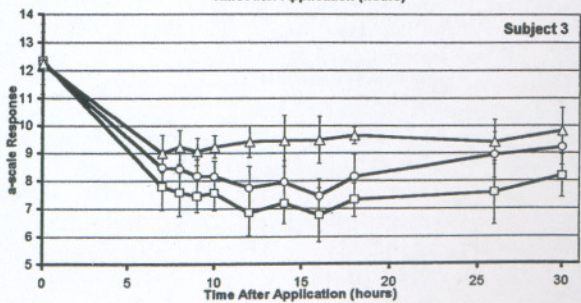
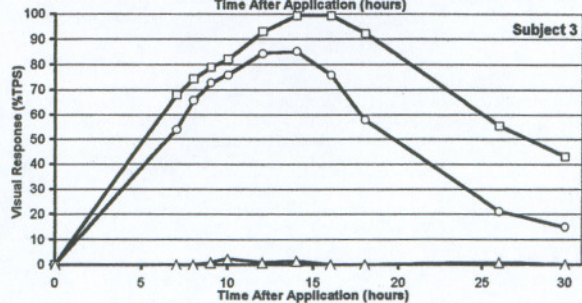
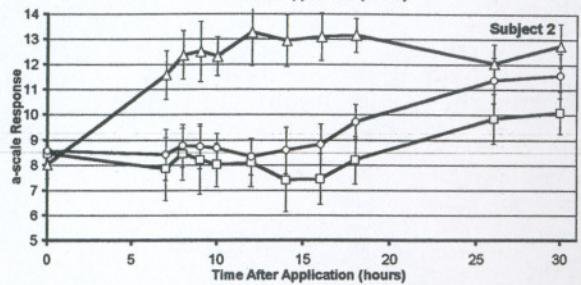
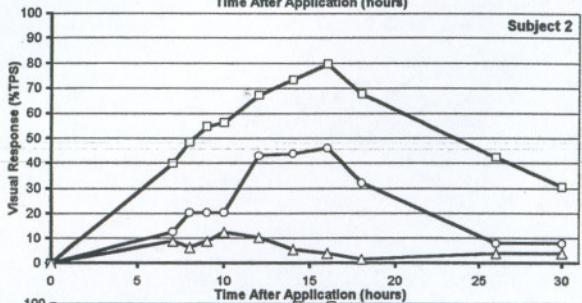
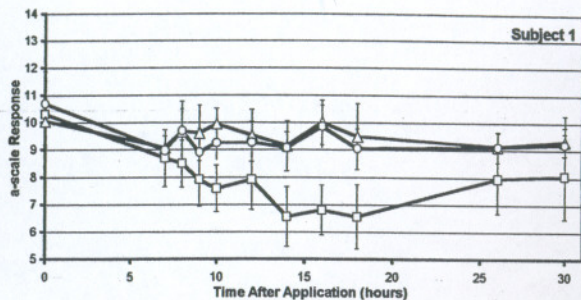
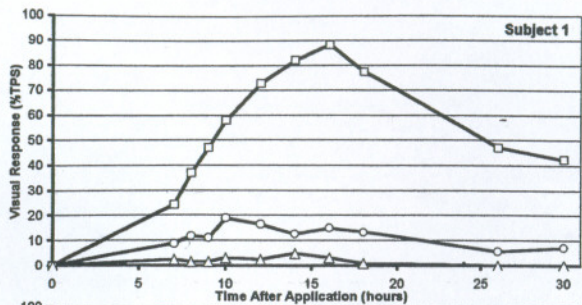


investigation be modeled by suitable software to produce an effective dose at 50% response and establish a 33–67% range of response. The latter is used in the assessment of the results from the pivotal trial to exclude all subjects whose response does not fall within this range. This in itself does not seem logical, as no screening procedure is applied to the pilot study volunteers in order to ensure that they are a typical subsection of the population of responders. The inclusion/exclusion criteria that may be produced from an inappropriately-selected group of volunteers for the pilot study may skew the “acceptable” results generated in the pivotal study.

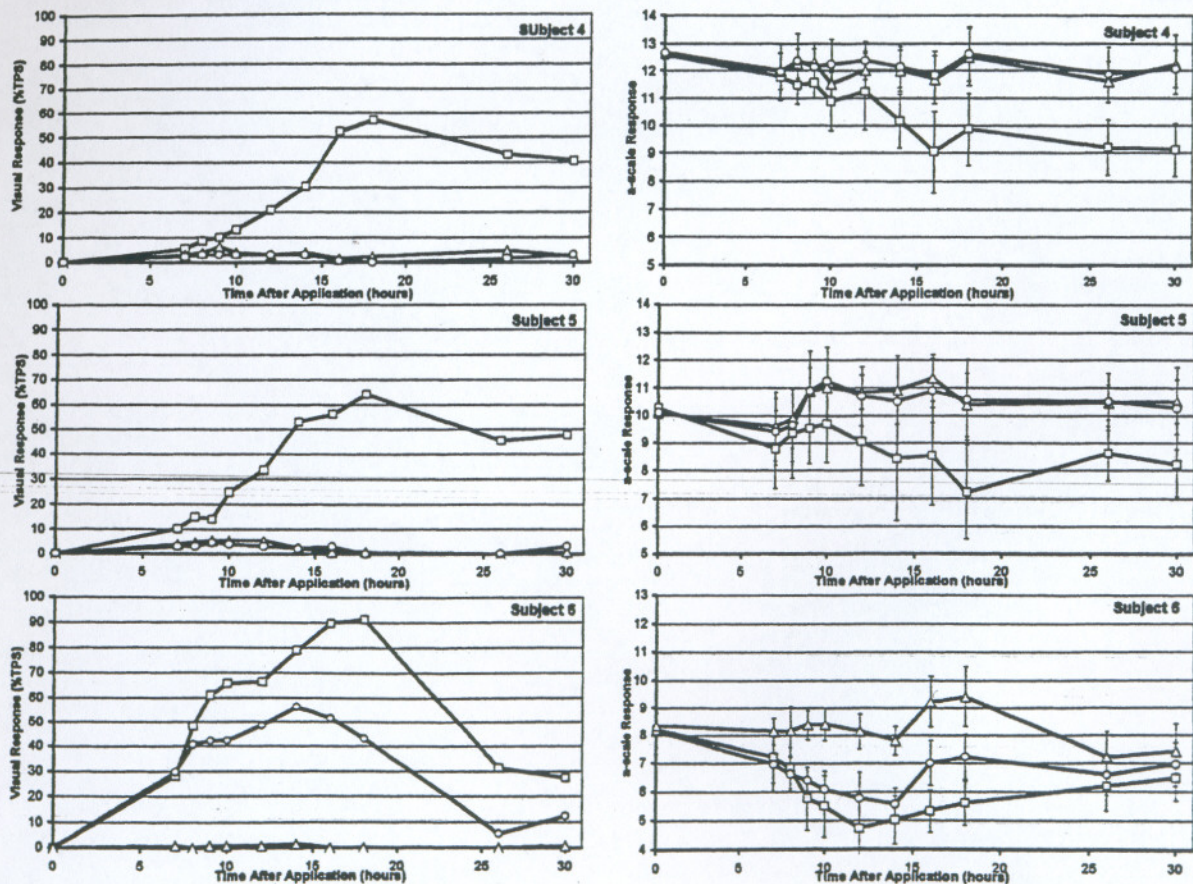
There are, similarly, several theoretical problems with the modeling procedure for the topical drug delivery data, since the exact dose of drug generating a specific response cannot be determined. The amount of formulation applied to the skin is a constant, known mass (arbitrarily chosen by the investigator), but the mass of drug that penetrates the skin and reaches the site of action is variable depending on the biological characteristics of the skin of each volunteer. In addition, the skin contact time of the dose of formulation is also left to the discrimination of the investigator, further confounding the concept of dose in the dose-response relationship. The guidance document suggests varying the skin contact time of the applied dose of formulation to generate different doses for modeling purposes; the relationship between skin contact time and the mass of drug penetrating the skin (especially when assessed in terms of stratum corneum reservoir formation) has not been sufficiently evaluated. Recent developments in the field of skin stripping methods for the determination of mass of drug in the skin may be beneficial in this regard. Moreover, the results of data modeling exercises are only as good as the raw data generated in the experimentation. In light of this discussion, there has been some comment on the accuracy and precision of the data generated by the chromameter for modeling purposes (4).

Our experiences with the chromameter (both the Minolta 200 and 300 models) have demonstrated (4,16) that the results obtained are far from objective. For the measurement of homogeneous, planar surface colors, the results obtained with the chromameter are reproducible and accurate and, therefore, objective. However, when it is applied to the manual measurement of the color of human skin, several problems arise:

1. The pressure of the head of the chromameter applied by the investigator to the skin of the volunteer can change the color of the skin due to vasocompaction.
2. The presence of hairs, moles, and skin mottling on the forearm can give artifactual readings.
3. The angle at which the chromameter is presented to the skin by the investigator can cause different values to be recorded at the same site.







**Figure 1** Individual corticosteroid-induced skin blanching data (visual and uncorrected a-scale chromameter) for six subjects using a typical study protocol (□ clobetasol propionate, ○ betamethasone 17-valerate, △ unmedicated control sites).

4. The chromameter is a hand-held instrument; thus the operator could become fatigued, as several hundred readings are taken per hour in a topical availability trial involving a full panel of subjects.

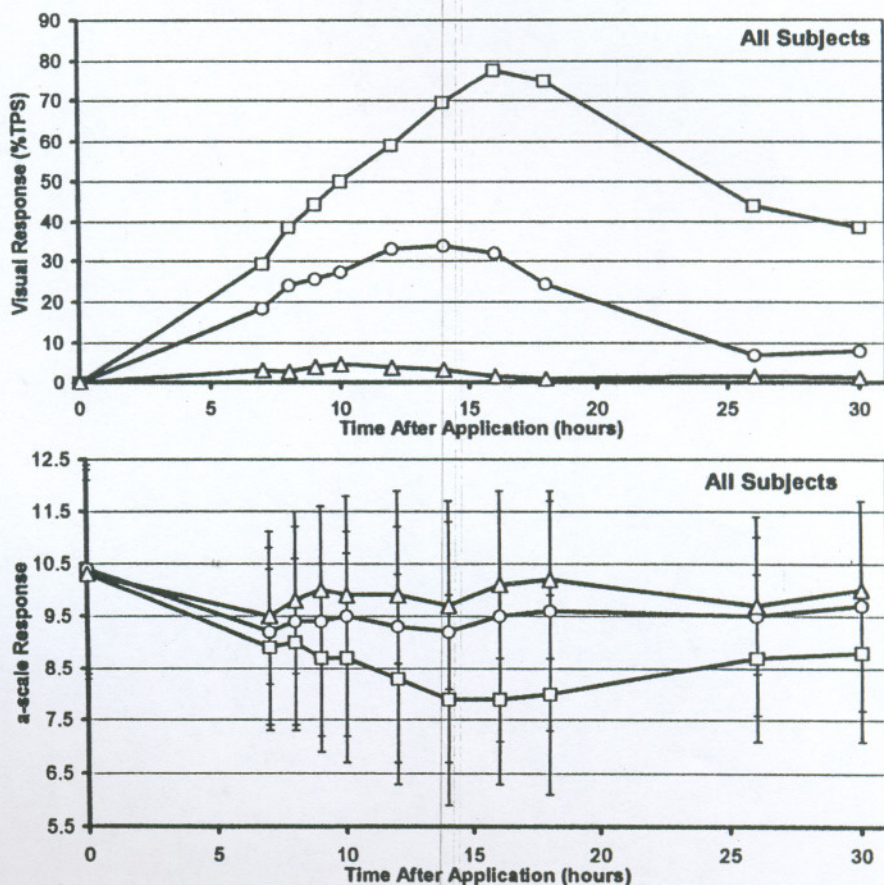
The inherent objectivity of the instrumental computations is reduced in overall value by the subjective manipulation of the chromameter head by the investigator; these are problems that would not arise if the instrument were being used in a nonbiological environment.

The results from six subjects in a typical blanching trial evaluation are depicted in Fig. 1. Here the blanching response profiles of two corticosteroid formulations from different potency groups (Dovate, 0.05% clobetasol propionate, Pharmacia-Lennon, South Africa; and Betnovate, 0.1% betamethasone 17-valerate, Glaxo-Wellcome, South Africa) were recorded by visual observation using four independent observers and by chromameter assessment. The visual profiles for each volunteer are typical, from our experience, and show a clear maximal response for the more potent formulation and, for four of the six subjects depicted, a lesser response for the lower potency formulation. There is negligible response recorded at the unmedicated (control) sites for all volunteers, and subjects four and five also show negligible response for the lower potency formulation. What is immediately apparent from the a-scale chromameter data is that there is less of a clear distinction between the control, lower, and higher potency formulation responses, although the rank order of response is appropriate. Especially troublesome is that the precision of the mean data values is such that there is no clear statistical difference between the responses of these two formulations from different potency groups. The period of peak blanching (12–14 h after initial application) is crucial in the determination of bioequivalence by visual observation. If we examine the profiles of the typical responders (subjects 1–3 and 6), there is no statistical difference between the data points of the two formulations at this region of peak response. This result is mirrored even for the “atypical” subjects four and five. In a blind assessment, one might conclude from these results that the two formulations are equivalent, whereas the visual methodology (and clinical usage) has proved these products to be of different potency. One may also debate the merits of attempting to model data with this degree of imprecision, especially when the maximal response of the most potent formulation produces a response profile that is only marginally different to that produced from untreated skin. These concerns reiterate those of a previous publication (4). We have observed that the differences between a-scale values for individual volunteers are so large that meaningful results are difficult, if not impossible, to obtain from commercially available modeling packages.

If one returns to the question of inclusion criteria for subjects, clearly only four of these six volunteers are typical responders and yet the guidance



document does not make any provision for the exclusion of subjects from the pilot study pool. If this had been part of a pilot study pool, then all the results would have been used to determine the 50% effective dose and the 33–67% range. The mean data for all six subjects are shown in Fig. 2, which reinforces the discussion already presented: There is clear distinction between the products in the visual assessment, and in the chromameter assessment no statistical difference between the high-, moderate-potency, and unmedicated responses. Since the visual grading is based on an arbitrary



**Figure 2** Mean corticosteroid-induced skin blanching data (visual and uncorrected a-scale chromameter)  $\pm$  1 standard deviation for six subjects using a typical study protocol (□ clobetasol propionate, ○ betamethasone 17-valerate, △ unmedicated control sites).

0-4 scale, standard deviation analysis of the visual means is inappropriate, but if this is conducted by assuming the scale is approximately linear, then very good precision is obtained about each point with clear statistical difference between the three profiles (16).

A further problem with the FDA guidance is the way in which it is suggested that the a-scale values should be corrected for both baseline and untreated site values. We have shown (16) that irrespective of the manner in which these values are corrected, the shapes of the blanching profiles remain essentially unchanged. More important from the view of determining bioequivalence, although some of the a-scale blanching profiles do show some similarity to the visually obtained curves, the standard deviations observed for the a-scale values are so large that there is overlap of the means of treated and untreated sites at all observation times.

The FDA guidance document suggests the use of a-scale values only. It seems to us that since the chromameter produces three values for the full definition of any color, it should be possible to utilize all three values for more accurate definition of skin color and therefore more appropriate conclusions to be drawn. We have measured the Euclidean distances in three-dimensional space (16) between untreated sites and treated sites, and plots of these values show greater similarity to the visually obtained blanching profiles than do the individual profiles of the a-, b-, and L-scale values. However, even the Euclidean distance plots of the reported initial assessment display unacceptably large standard deviations with overlapping of mean error bars of the treated and untreated sites at most observation points. Nevertheless, this mode of data analysis is worthy of further investigation.

#### IV. CONCLUSION

We concede that the visual assessment of corticosteroid-induced skin blanching is subjective. However, the proven reproducibility and accuracy of this assay procedure indicate that it still has a useful role to play in the assessment of topical corticosteroid availability and, especially, in providing the standard to which objective techniques must equate or surpass. The caveat that must be applied here is that the visual assay has to be performed correctly by experienced personnel. The most important factors in this regard are:

1. More than one observer should be used. In our laboratories we routinely use three or four observers.
2. The observers must be trained. We have found (17) that it takes inclusion in three full training trials before an observer can be



considered reliable and consistent for inclusion into a topical availability trial.

3. More than one site per arm per formulation must be used, and these sites must be spread over the whole length of the forearm, avoiding wrist and elbow. We use three, four, or six sites per arm per formulation, depending on the trial structure.
4. Readings must be taken over a period of time after removal of the formulations to allow the construction of a blanching profile. We have often noted that a formulation that produces high blanching in the early part of a trial will produce lower AUC values than a formulation that produces lower blanching early in the trial.

We agree that it would be preferable to have an objective method for the measurement of corticosteroid-induced skin blanching, and we are working toward the realization of this idea. We believe that the use of the chromameter is a step in the right direction, but we are not sure that this is the ideal instrument, used in the currently prescribed manner, for making these measurements. There is still much validation that has to be performed before this technique can achieve global acceptance.

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