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Evaluation of the Proposed FDA Pilot Dose-Response Methodology for Topical Corticosteroid Bioequivalence Testing

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Purpose. The American FDA has recently released a Guidance document for topical corticosteroid bioequivalence testing. The purpose of this study was to evaluate the recommendations of this document for appropriateness. The new specifications require a dose-vasoconstriction response estimation by the use of a Minolta chromameter in a preliminary pilot study to determine the parameters for use in a pivotal bioequivalence study.

Methods. The visually-assessed human skin blanching assay methodology routinely practiced in our laboratories was modified to comply with the requirements of the pilot study so that visual and chromameter data could be compared. Two different cream formulations, each containing 0.12% betamethasone 17-valerate, were used for this comparison.

Results. Visual data showed the expected rank order of AUC values for most dose durations whereas the chromameter data did not show similar results. The expected rank order of AUC values for both chromameter and visual data was not observed at very short dose durations. In fitting the data to pharmacodynamic models, equivalent goodness of fit criteria were obtained when several different parameter estimates were used in the model definition, however the visual data were best described by the sigmoid E_{max} model while the chromameter data were best described by the simple E_{max} model.

Conclusions. The E_{max} values predicted by the models were close to the observed values for both data sets and, in addition, excellent correlation between the AUC values and the maximum blanching response (R_{max}) ($r > 0.95$) was noted for both methods of assessment. The chromameter ED_{50} values determined in this study were approximately 2 hours for both preparations. At this dose duration the instrument would not be sensitive enough to distinguish between weak blanching responses and normal skin for bioequivalence assessment purposes.

KEY WORDS: human skin blanching assay; pilot dose-response study; betamethasone 17-valerate cream; pharmacodynamic modelling; chromameter.

INTRODUCTION

Over the past three decades, topical availability and potencies of corticosteroid formulations have been assessed visually using the human skin blanching assay (1). Despite its widespread use, researchers have adopted different experimental protocols to assess topical corticosteroid availability (2,3). Haigh and Kanfer (3) and Smith *et al.* (4) have refined the methodology so that the technique is reliable and reproducible for the assessment of topical corticosteroid formulations, provided multiple, trained observers are utilized. This, however, is considered to be too subjective by other workers (5–9). The American Food and Drug Administration (FDA) has recently released a Guidance document (6) which attempts to standardize the technique so that any assessment of bioequivalence of topical corticosteroids will be precise and accurate if the specified methodology is strictly adhered to. The Guidance as it presently stands comprises two distinct sections. Firstly, a pilot dose-response study is required, based on a dose duration method which is conducted solely with a reference listed drug. Although the dose of formulation to be applied topically is not specified in the Guidance, the objective of this 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. 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 (6, Section II, p3) 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 when used in topical corticosteroid bioequivalence assessments. It is interesting to note that the FDA proposes the use of this parameter which has yet to be proven of relevance to the pharmacological effect being measured in this bioassay. Secondly, a pivotal study is required to compare the in vivo response of the test product with a reference product using a dose duration which is approximately the same as the ED_{50} value determined from the pilot study. The manner in which the pilot study is performed and analyzed is critical since the protocol for the pivotal study depends entirely on the results of the pilot study.

The main objectives of this study were to evaluate the pilot dose-response methodology as recommended in the Guidance in terms of the following: (a) the comparison of visual and chromameter 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. The visual assessment method, in particular, has been an area of debate in recent years (5–10), and has been deemed unacceptable (5,9) for grading the corticosteroid-induced skin blanching response, despite direct correlation between visual and instrumental data (11), and between visual data and clinical efficacy (4). It has been reported (5,10) that the use of the chromameter provides an objective and quantitative method for evaluating the intensity of skin blanching induced by topical corticosteroids. There are few published reports describing the effect of dose duration on the blanching activity of the same corticosteroid (12,13). Therefore, a comparison of visual and chromameter data, as well as the effect of various dose durations on the blanching response, are necessary for evaluation of the Guidance. The use of pharmacodynamic models for corticosteroid bioequivalence testing, as suggested in the Guidance, is a new concept with regard to the human skin blanching assay. Since there is no specific recommendation in the Guidance concerning choice of model, the selection and use of a particular pharmacodynamic fitting procedure requires investigation.

MATERIALS

The two cream formulations chosen for comparison were Betnovate (Glaxo, South Africa) and Lenovate (Lennon, South

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Africa) each containing 0.12% betamethasone 17-valerate. These two products were chosen because they have been used repeatedly as standard formulations in our laboratory and a database of results exists for these creams (1,4,14). A Minolta CR-200b Chromameter (Minolta Corporation, Ramsey, N.J.) was used. The instrument objectively records colour in terms of hue, light value and saturation. These parameters are described as the L-scale, which expresses the relative brightness of colour ranging from black to white; the a-scale, which is the colour hue related to redness or greenness and the b-scale, which is the colour range from blue to yellow. The colour of any surface can be quantitated by a combination of the three values. Theoretically, a change in these indices should reflect a change in skin colour.

METHODS

The methodology of the visual human skin blanching assay routinely practiced in our laboratories (15) was modified to comply with the specifications of the pilot dose-response study. The Guidance stipulates that there should be only one site per person for each dose duration (6, Section IV, p11). Twelve healthy male and female Caucasian volunteers with normal forearm skin and who had been pre-screened for positive blanching response in accordance with the Guidance requirements were selected. Ethical approval was obtained from the Rhodes University Ethical Standards Committee in compliance with the Declaration of Helsinki (1964) and its subsequent amendments. All subjects had previously taken part in similar studies and written informed consent was obtained from each subject. All volunteers were processed 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.

Five adhesive labels, from which two 7 mm × 7 mm squares had been punched, were applied to the flexor aspect of both forearms to demarcate a total of 10 application sites per arm of each volunteer. Four stripes (7 mm) of each formulation (equivalent to approximately 3.2 mg) were applied in a double-blind, randomized manner to each designated site and were spread using a glass rod. This is the dose of formulation normally used in this laboratory for bioequivalence testing. The formulations were applied at different times (staggered application) but removed simultaneously (synchronized removal) thus remaining in contact with the application sites for 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 5 and 6 hours. Both arms of each volunteer were left unoccluded but were protected by porous Perspex frames. After the specified contact times, the protective covers and adhesive labels were removed and the application sites were then gently washed with soap and distilled water using cotton-tipped buds and patted dry (6, Section IV, p12).

Visual Assessment of Blanching Response

Response assessment was made independently by three experienced observers at 0, 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 6, 7, 8, 9, 10, 11, 12, 16, 17, 18, 25, 28, 30, and 32 hours after product application for the 6-hour dose duration. Table I lists the observation periods for the other dose durations. Responses were graded using a 0 to 4 scale where 0 = no blanching, 1 = slight blanching, 2 = more intense blanching, 3 = general even and distinct blanching and 4 = marked and very intense blanching. Visual scoring does not require separate untreated control site correction since the assessment comprises a comparison of the treated site with the surrounding unmedicated skin. The percentage of the total possible score (%TPS) was calculated (15) and plotted against time in hours after product application to produce blanching profiles for both formulations.

Chromameter Assessment of Blanching Response

The instrument was calibrated using the white calibration plate (CD-A43) immediately before the study. Blanching responses at all application sites were assessed using the a-scale parameter at the time of product removal and thereafter at intervals corresponding to the visual observations (Table I). In addition, readings were also taken at three untreated control sites on the forearm at each reading time to correct any diurnal colour change that may occur on the skin unrelated to drug exposure. The average of these readings was subtracted from the reading taken at each drug application site to yield a control site-corrected value. Zero-time chromameter values were not recorded prior to drug application since it has been shown (5,16) that no significant differences in diurnal skin colour are observed between anatomical locations on the same arm or between left and right forearms. Inclusion of the zero-time value is, therefore, a redundant arithmetical manipulation which does not impact on the final result. This was an intentional deviation from the Guidance since, theoretically, one could argue the rationale for subtracting any correction values from the chromameter readings of medicated sites, as one is attempting to obtain an absolute value of the skin colour at each observation time and monitor the change in this colour as the skin blanching progresses. The mean control site-corrected values were plotted versus time after application to conform with normal bioequivalence data reporting, since plotting procedures are not stated in the Guidance.

Statistical Analysis of Data

The trapezoidal rule was used to calculate the area under the blanching curve (AUC) for each dose duration for the visual and chromameter data. Chi-squared analyses were performed on the visual data and student's t-distribution tests were per-

Table I. Assessment Periods at Which Blanching Response was Observed for Various Dose Durations (DD)

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

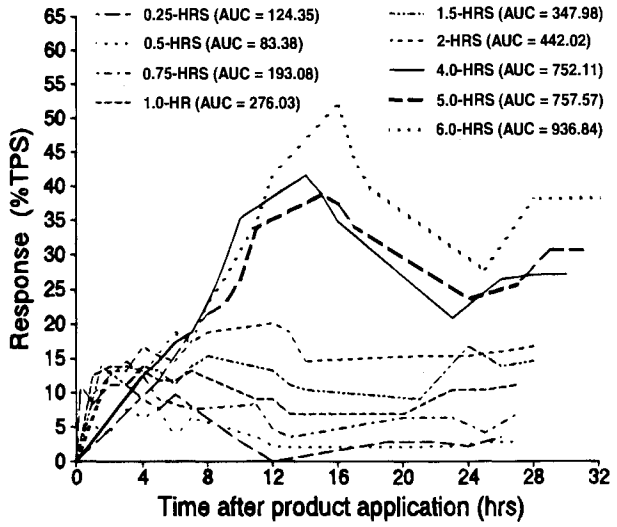

Fig. 1. Visual blanching response profiles for Betnovate cream.

formed on the chromameter data ($p < 0.05$). Determination of E_{max} and ED_{50} values was carried out using an appropriate model (PCNONLIN V4.2, SCI Software, Lexington, KY).

RESULTS AND DISCUSSION

Assessment of Visual Blanching Profiles

Figures 1 and 2 represent the blanching response profiles for Betnovate and Lenovate creams plotted as %TPS versus time after product application at various dose durations. The profiles, up to the 2-hour dose duration, show little drug-induced vasoconstriction activity. In contrast, the profiles for the 4-, 5- and 6-hour dose durations show that the blanching peaks at 14–16 hours after product application. These response profiles are similar to the results which have been obtained in previous studies using the same corticosteroid formulations (1,14). However, the blanching profiles observed here are not as smooth as reported previously (1,4,14), almost certainly

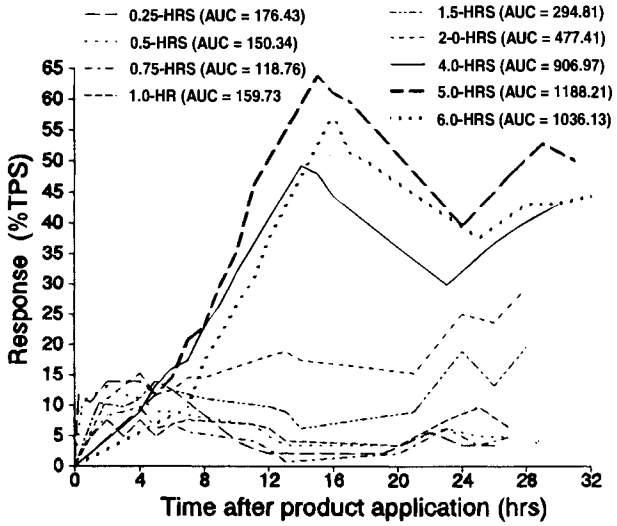

Fig. 2. Visual blanching response profiles for Lenovate cream.

because of the single application site per volunteer for each dose duration. The response profiles generally show that skin blanching increases as the dose duration increases, as would be expected from previous studies (12). This trend is, however, more obvious at longer dose durations; the rank order of AUC values at very short dose durations (0.25, 0.5, 0.75 hours) is not as expected. The greatest blanching response was found with the 5-hour dose duration for Lenovate cream. This result was not expected as previous results have shown that a 6-hour dose duration produces a greater skin blanching response than a 5-hour dose duration (12). The anomalous result for the 5- and 6-hour dose durations for the Lenovate cream is probably due to the use of the small data set. When comparing the response of Betnovate with Lenovate, statistically significant differences were only found at maximal response for the 5- and 6-hour dose durations.

Assessment of Chromameter Blanching Profiles

Figures 3 and 4 represent the blanching response profiles for Betnovate and Lenovate creams plotted as control site-corrected a-scale data versus time after product application at various dose durations. In contrast to the sample data presented in the Guidance, all the chromameter results recorded in this study were negative and were, therefore, multiplied by -1 to create a positive plot for comparison with the visual data. This data is clearly different from the sample data presented in the Guidance (6, Appendix III). It should be noted that the FDA data were corrected for pre-application baseline values. In congruity with the visual results, the chromameter profiles show greater blanching responses for the 2- to 6-hour dose durations than for the shorter dose durations, with maximal response for Lenovate cream at the 5-hour dose duration. The profiles, however, are clearly not as uniform as the visual data, and do not show the expected blanching response rank order, in either the maximum response or AUC values for various dose durations. There were no statistically significant differences between the two formulations at any observation time for any dose duration. The reason why the profiles for the chromameter data are not as uniform as those for the visual data, and do not show the expected blanching response rank order, is open to speculation. If more application sites per volunteer for each dose duration were used, then the precision of both the chromameter and visual data would be improved.

Pharmacodynamic Modelling

Since no specific modelling procedure is stipulated in the Guidance (6, Section III, p4), both the simple E_{max} (Equation 1) and sigmoid E_{max} (Equation 2) models were investigated for appropriateness.

$$E = \frac{E_{max} \cdot D}{D + ED_{50}} \quad (1)$$

$$E = \frac{E_{max} \cdot D^{\gamma}}{D^{\gamma} + ED_{50}^{\gamma}} \quad (2)$$

Both models describe the blanching response (arithmetic means of all-subject data AUC values as listed in Figures 1–4) 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. Appropriate model selection 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). The chromameter and visual data were weighted for modelling purposes because of the wide range of AUC values and the best results were found with a weighting factor of -1. This means that the weighting associated with each AUC value is approximately equal to 1/AUC. The model attempts to produce the best line fit of AUC versus dose duration data (17) from which the E_{max}, ED_{50}, D_1 and D_2 values may be estimated. Modelling of the chromameter data to determine an ED_{50} value showed that these data were best described by the simple E_{max} model (Table II). Attempts to fit the data to the sigmoid model using a realistic upper estimate for the ED_{50} of 6 hours resulted in the parameter approaching this limit (5.99 hours). Further attempts to fit these data to the sigmoid model by widening the upper limit to 12 and 18 hours yielded similar goodness of fit criteria to those obtained for the simple E_{max} model, but with an unrealistic fitted estimate for the ED_{50}. Furthermore, the computer programme was unable to produce parameter estimates for Betnovate cream using the sigmoid model despite attempts to fit the data by widening the parameter limits. 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 (Table II). Furthermore, the predicted and observed E_{max} values (Figures 3 and 4) are similar. However, there is little distinction between the models when one considers the correlation and AIC values.

Table II. Parameter Estimates for the 'a' Scale Chromameter and Visual Data Obtained from PCNONLIN Simple E_{max} Model 101 and Sigmoid E_{max} Model 105

	Simple E_{max} model		Sigmoid E_{max} model	
Parameters	Betnovate cream	Lenovate cream	Betnovate cream	Lenovate cream
(chromameter)				
E_{max} (hours)	−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} (hours)	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

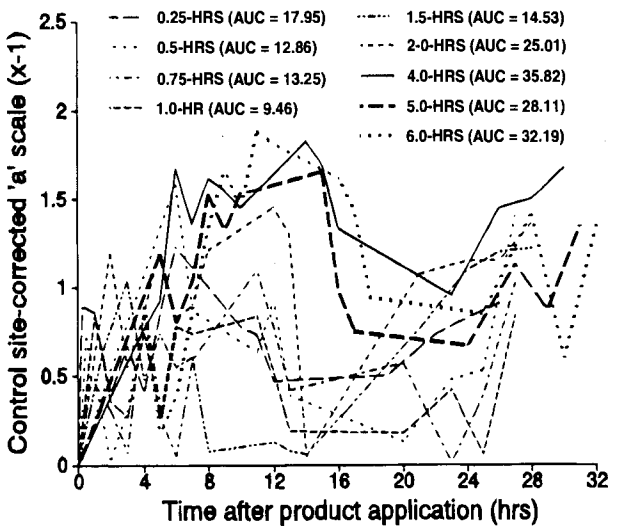

Fig. 3. Chromameter blanching response profiles for Betnovate cream.

The results show that the ED_{50} value for Betnovate cream is different from that of Lenovate cream by approximately 0.2 hours. This result was not unexpected because although both formulations contain the same drug in the same concentration, the vehicles in both formulations are almost certainly different. Based on the chromameter ED_{50} values obtained in this study, the dose durations that would be selected according to the Guidance for the pivotal study are: an ED_{50} value of 1.5 hours, a D_1 value of 0.75 hours and a D_2 value of 3 hours for Betnovate cream as the reference formulation and an ED_{50} value of 2 hours, a D_1 value of 1 hour and a D_2 value of 4 hours for Lenovate cream as the reference formulation. Attempting to fit the visual data to a simple E_{max} model using upper estimates for ED_{50} of 6, 12 and 18 hours resulted in unrealistic fitted estimates for the ED_{50}. Despite similar correlation coefficients for the simple and the sigmoid E_{max} models, the AIC values for the sigmoid model are lower than those for the simple model. This is corroborated by examination of the standard error of estimates for the E_{max} and ED_{50} parameters which are

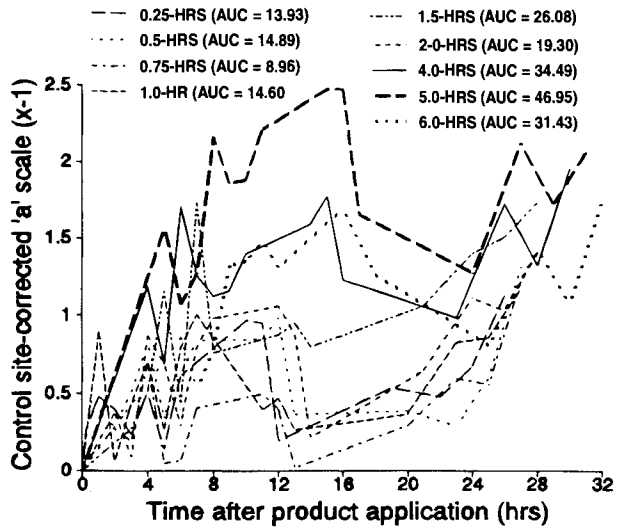

Fig. 4. Chromameter blanching response profiles for Lenovate cream.

lower than 12% for the sigmoid model and substantially greater for the simple model. These criteria suggest that the visual data are best described by the sigmoid model. Based on the ED_{50} values obtained, the dose durations that would be selected for a pivotal study are: an ED_{50} value of 3 hours, a D_1 value of 1.5 hours and a D_2 value of 6 hours for either formulation when used as the reference product. In comparing the predicted and observed ED_{50} values (Figures 1 and 2), 3 hours is an appropriate estimate. Similarly, the E_{max} values predicted by the sigmoid model are close to the observed values.

It is interesting to note that the visual data follows the normal sigmoid dose-response relationship seen in pharmacological systems, while the chromameter data follows a more geometric pattern. This does not necessarily mean that the visual data is more accurate as the instrument may be measuring parameters of vasoconstriction that are not apparent on visual examination. In addition, excellent correlation between the AUC values and the maximum blanching response (R_{max}) values were noted for visual data ($r = 0.99$) for both formulations, and also for chromameter data ($r = 0.97$ for Betnovate cream, $r = 0.96$ for Lenovate cream). Although the Guidance recommends the use of AUC values in modelling, these results show that the use of R_{max} values may also be applicable.

CONCLUSIONS

Even though the predicted ED_{50} values for visual and chromameter data are different, the estimated E_{max} values were close to the observed 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 values are 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. If unmedicated baseline values had been subtracted from the chromameter data as recommended in the Guidance then both positive and negative results may have been obtained depending on the absolute values of the untreated site chromameter readings. Such values would make modelling even more problematic and the observed dose-response relationship and the estimated parameters less reliable. The Guidance does not specify a particular 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 favor delivery dynamics from a particular formulation.

The profiles of the chromameter data are not as uniform as those of the visual data, and do not show the expected blanching response rank order for either the maximum response or AUC values for various dose durations. We maintain that the visual method is more sensitive and monitors the induced blanching more accurately than the chromameter. A recent publication (16) corroborates this contention. The subjectivity of visual observation methodology implies that there will be inter-laboratory variability of the absolute blanching values recorded for a specific formulation. However, rank order comparisons between formulations have been shown to be reproducible (15), even though there are inherent problems with the statistical analysis of the nonparametric visual results.

The visual data for short dose durations (0.25, 0.5, and 0.75 hours) in this study were found to give an unexpected rank order of AUC values. In addition, the visual curves were not as smooth as previously reported and some anomalous results were observed because of the small data set. Having only one application site per subject does not take into account the variability in blanching response at different forearm sites (18). It is therefore suggested that the use of more application sites per person per product at any dose duration may increase the precision of the results. In addition, a hand-held probe cannot be precisely regulated with regard to distance from the skin or hand vibration, further contributing to the imprecision of the results. Experience in our laboratory has indicated that positioning, skin contact and alignment of the probe are more reliable when self assessment is performed by the subject. If this had been found by the FDA to be problematic then the Guidance should suggest multiple readings of the same application site or replicate application sites. In addition, 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 assessments should, as far as possible, parallel normal clinical dosage regimens.

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 this document in terms of the mass of formulation applied to the skin, anatomical skin sites along the forearm to be utilized, 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.

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