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Strategies for the Bioequivalence Assessment of Topical Dermatological Dosage Forms

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Abstract

The assessment of the bioequivalence of topical products not intended for absorption into the systemic circulation has presented a formidable challenge over the years. In particular, dermatological dosage forms such as creams, ointments, lotions and gels, apart from those containing topical corticosteroids, cannot readily be assessed for bioequivalence using "conventional" methodology and the only recourse to-date has been to undertake tedious, time consuming and expensive clinical end-point trials for such products. Although the human skin blanching assay (HSBA), also known as the vasoconstriction assay (VCA) has been successfully used for dermatological products containing topical corticosteroids and the methodology has found formal acceptance by a number of regulatory agencies, e.g. the US FDA amongst others, no surrogate methodology for the bioequivalence assessment of other topical dermatological products such as those containing non- steroidal anti-inflammatory drugs, anti-fungals, antibiotics and antivirals has yet found favour with regulatory agencies. Application of the HSBA, Tape Stripping (TS) and Dermal Microdialysis (DMD) for the assessment of bioequivalence is described and the theoretical basis and prognosis for each technique is presented.

Keywords: Bioequivalence; Topical; Dermatological; Human Skin Blanching Assay; Tape Stripping; Dermal Microdialysis

Introduction

Unlike the well-established approaches used for the determination of bioequivalence (BE) of oral dosage forms where the active ingredient(s) is/are intended to be absorbed into the systemic circulation, bioequivalence assessment of topical dosage forms not intended for absorption has proved to be quite difficult, daunting and extremely challenging.

Currently, apart from undertaking clinical trials in patients to assess the bioequivalence of such products, the only surrogate method which has been found to be acceptable but which is constrained to topical corticosteroids only, is the human skin blanching assay (HSBA) also known as the vasoconstrictor assay (VCA) (Guidance for industry, 1995).

The pharmacokinetic approach used for the BE assessment of systemically absorbed drugs is not considered appropriate to study the bioavailability (BA) and/or BE of topically applied drugs other than dosage forms such as transdermal patches and other products intended for local application which contain drugs intended for the systemic circulation. Apart from the analytical difficulty of measuring concentrations of drugs in the systemic circulation following topical administration where the concentrations generally reach only a fraction of the amount of drug applied to the skin surface, systemic drug concentrations are not considered to reflect the concentration in the target organ, namely, the skin.

The determination of the bioavailability of systemically absorbed products is defined as *the rate and extent to which the active ingredient or active moiety is absorbed from the drug product and becomes available at the site of action* (Kanfer and Shargel, 2008).

However, when considering products which contain active ingredient(s) not intended for systemic absorption, the US FDA, published the following statement in the US Federal Register (US Federal Register, 2009) where such products *"..... may be assessed by (surrogate) measurements intended to reflect the rate and extent to which the active ingredient or moiety becomes available at the site of action".*

Clearly, the major focus of attention for the assessment of drug products has been in the area of drugs administered extravascularly and intended to be absorbed into the systemic circulation. However, more and more attention is now being focused on the assessment of NON-ABSORBED drugs, such as topical dosage forms intended for local action.

The assessment of BE is intended to provide comparative information on the safety and efficacy of products based on their comparative BAs. These measures can involve clinical studies in patient or surrogate measures in patients or more usually in healthy human subjects. However, different approaches are required as previously explained, when considering products intended to be absorbed into the systemic circulation compared to assessing the BA/BE of topical products not intended to be absorbed. In the former instance, surrogate measures are justified by the presumption that the concentration of drug in the blood stream is in equilibrium and reflects the concentration at the site of action and a relationship between effectiveness and systemic blood concentrations of the drug is implied. Furthermore, the methodologies and the statistical assessment of data are well-established and where, in general, the regulatory requirements are based upon the maximum drug concentration in blood (C_{max}) and the extent of absorption is assessed from the area under the curve (AUC) of a plot of drug concentration versus time and falls within the prescribed limits of a confidence interval (CI) of 90% and the relative mean ratios of the test (T) and reference (R) product being within 80-125%. On the other hand, in the latter instance, surrogate measures using drug concentrations in the systemic circulation cannot be justified to assess the BE of drugs not intended to be absorbed since no relationship exists between

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effectiveness and blood concentrations of drug, and furthermore, the methodologies and assessment approaches for BE are still being developed and also the statistical assessment is yet to be defined as are specific regulatory requirements. The exception to the above is however restricted to topical dermatologic corticosteroid products only where a published FDA Guidance requires that Locke's method, which provides an exact confidence interval from untransformed data, be used(Guidance for industry, 1995).

The following Figure 1 (Flynn and Weiner, 1989) depicts differences in the types of regions targeted following the local application of topical products.

The above provides important information relating to specific types of topical products and their ultimate therapeutic target sites and intended activities.

In light of the above, consideration must be given to the meaning of the word, *"TOPICAL"* which is defined as *"belonging to a place or spot"* and topical = local.

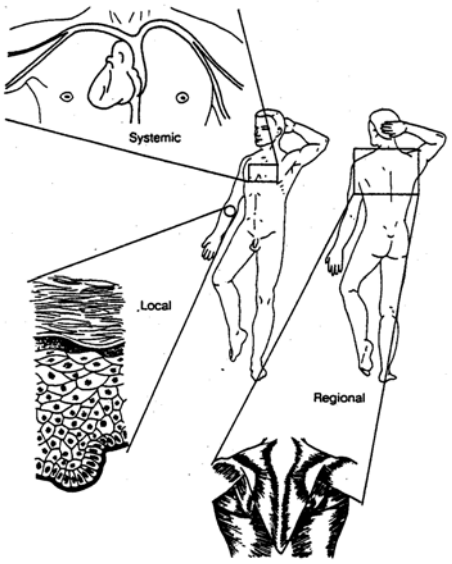
TOPICAL products for cutaneous (Dermatologic) use: These are products where the pharmacologic or other effect is confined to the surface of skin or within the skin and may or may not require percutaneous penetration and deposition.

REGIONAL products for treatment of disease or symptoms in deeper tissue: These are products where the pharmacological action is effected within musculature, vasculature, joints, synovial fluid beneath and around the application site, e.g. e.g. topical anti-inflammatory products. These dosage forms are used where more selective activity is required compared to systemic delivery and requires percutaneous absorption and deposition.

Methodologies and Assessment Approaches

Human skin blanching assay (HSBA) also known as the vasoconstrictor assay (VCA)

Whereas the original approach and methods used to assess topical products involve clinical studies in patients to assess safety and efficacy, the only well-established surrogate method which is currently generally accepted by most regulatory agencies is the Human Skin Blanching Assay (HSBA) also known as the Vasoconstrictor Assay (VCA) but restricted to the BE assessment of topical corticosteroid products. The skin blanching or "skin whitening" response used for this assessment following application of topical corticosteroid products to the skin was first observed in 1950 by Hollander et al. (1950) and applied for BA/BE assessment by McKenzie and Stoughton (1962). The use of this blanching response is an indirect measure using a perceived vasoconstrictor response following the application of a topical corticosteroid to the skin.



G.L. Flynn and N. D. Weiner in Dermal and Transdermal Drug Delivery, p. 37, 1989

Figure 1: Specific Regions Targeted for Particular Therapy Following the Local Application of Various Topical Products (Flynn and Weiner, 1989).

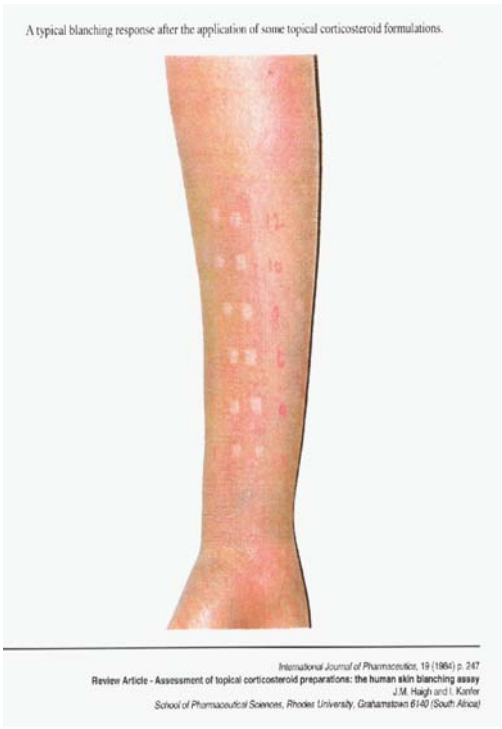


Figure 2: Typical blanching responses on the volar aspect of a human forearm following application of a topical corticosteroid product (Haigh and Kanfer, 1984).

Figure 2 shows a typical skin blanching response following the application of a topical corticosteroid to the forearm of a healthy human subject.

The method initially involved visual assessment of the blanching response using the human eye and subsequently and instrumental method of assessment was recommended by the US FDA where their guidance (Guidance for industry, 1995) issued on 2 June 1995, Topical Dermatologic Corticosteroids: *In Vivo* Bioequivalence, stated the following:

"In an era with increasingly sophisticated methods to detect changes in light, temperature, pressure and other physical and chemical changes, the use of a human observer to assess the magnitude of a pharmacodynamic effect becomes increasingly inadequate. Application of a commercially available chromameter (or colorimeter; e.g. Chroma Meter 200 or 300 model series, Minolta) to detect erythema offers the possibility of replacing subjective visual scoring in the vasoconstrictor assay with objective, quantifiable measurements. The Division of Bioequivalence currently considers the use of a chromameter to be applicable to bioequivalence studies

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based on the vasoconstrictor assay, and therefore recommends that pharmaceutical sponsors incorporate the use of a chromameter into their study designs."

Methodology

Visual assessment: The method involves application of the topical corticosteroid product to a number of skin sites and allowing the product to remain in contact with the skin for a fixed time. Excess product is then removed by gently washing and the degree of skin blanching or whitening of the skin is then assessed over a number of designated intervals of time. The visual assessment is based upon the utilization of an arbitrary intensity scale of 0 – 4 where 0 indicates no blanching and numerical increase of numbers 1 – 4 are assigned to increasing degrees of blanching observed, respectively. The blanching response can be assessed by 1 or more observers and the number of observers used is taken into account in the data processing where the total possible scores (TPS) expressed as a percentage is determined as follows:

Total possible score (TPS)

e.g. Maximum score / site	= 4
No of independent assessors	= 3
No of sites / product / arm	= S
No of subjects	= V
TPS	= 4x3xSxV
% TPS	$= \frac{\text{Actual Score}}{\text{TPS}} \times 100$

The %TPS is then plotted versus time to yield a typical blanching profile as depicted in Figure 3.

Chromameter assessment: An instrumental method involving a tristimulus colorimeter was subsequently introduced as an objective and thus "preferred" method. The Minolta chromameter, which is a portable instrument that uses tristimulus colorimetry involving reflectance spectroscopy, was adapted to measure skin blanching. This approach had subsequently been used for the objective measurement of skin color (Király and Soós, 1976; Gras et al., 1990). The chromameter functions by emitting a white light (using a pulsed xenon arc lamp) onto the chosen area of assessment and measuring the intensity of reflected light through three particular wavelength filters (analyzed at wavelengths of 450, 560, and 600 nm) or using a photodiode array in more recent instruments. The detected signal is converted into three coordinates: L^* (luminosity), a^* (the amount of green or red), and b^* (the amount of yellow or blue). These

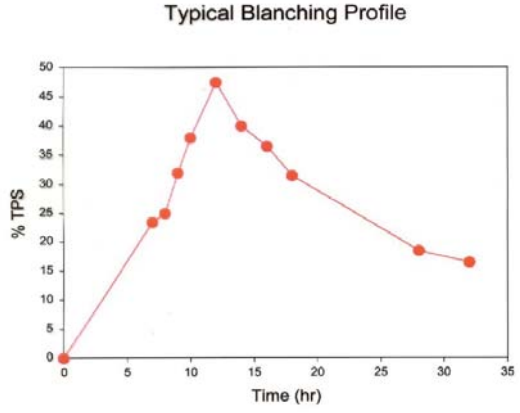

Figure 3: Typical Blanching Profile.

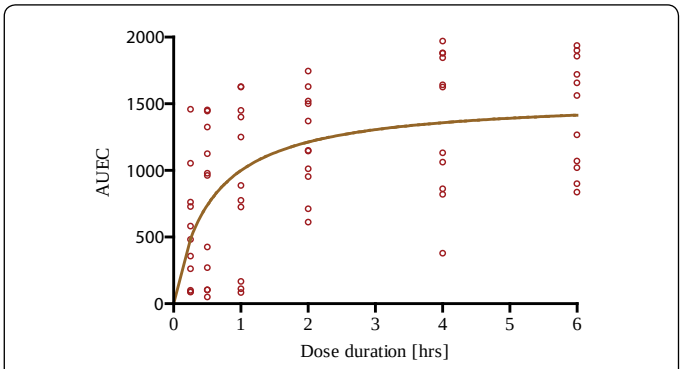

Figure 4: Data fitted to the E_{max} model to determine the dose duration.

three coordinates record color in a three-dimensional color system recommended by CIE (Commission International de l'Eclairage) (Montenegro et al., 1996; Piérarl, 1998; Taylor et al., 2006).

The skin blanching response is measured relative to the color change in the skin. As the skin blanching response develops, the skin becomes lighter and its redness fades. As the skin becomes more pale the L^* scale increases, a^* scale decreases, and b^* scale increases very slightly. It (Chan and Li Wan Po, 1992) has been shown that the L^* and a^* coordinates are more discriminative than the b^* coordinate in determining skin blanching responses, thus the latter coordinate is omitted from data analysis. However, following release of the FDA guidance, only the a-scale data has been recommended for use in the statistical analysis (Guidance for industry, 1995). This is possibly due to better correlation with visual skin blanching data found by Pershing et al. (1992). The chromameter can offer reliable and repeatable results provided that certain drawbacks are avoided such as manipulation of the measuring head of the instrument that can affect the quality of the data produced. Skin compression by the measuring head and the angle alignment of the chromameter play a role in obtaining repeatable data (Taylor et al., 2006; Schwarb et al., 1999; Waring et al., 1993). To obtain optimal results, each subject's assessment site as well as ambient temperature should ideally be controlled. It is also important for the operator to hold the chromameter head in such a way that variation in pressure is avoided (Schwarb et al., 1999). The presence of hair and variations in skin glossiness related to the amount of water and lipid on the skin surface, scarring, uneven skin tone, etc., can influence the data obtained(Fullerton et al., 1996). As a result, it is important to avoid these areas of the skin to achieve reliable and reproducible data.

Study designs

Types of studies—pilot and pivotal: The FDA Guidance recommends that two *in vivo* studies, a pilot and pivotal study, be conducted in order to determine bioequivalence between topical corticosteroid products. The pilot study utilizes a dose duration–response approach (Guidance for industry, 1995) (Figure 4) according to the E_{max} model,

$$E = E_0 + \frac{E_{max} \times D}{ED_{50} + D}$$

where E = effect elicited
E_0 = baseline effect in the absence of ligand
E_{max} = maximum effect elicited
ED_{50} = dose duration (D) at which effect is half-maximal

which controls the dose of topical corticosteroid being delivered by

comparing different times of exposure of the product on the skin (dose duration is the period of time that the formulation/product is left in contact with the skin). The development and validation of a dose–response curve (Figure 4) consisting of a plot of area under the effect curve (AUEC) versus time is therefore essential to determine ED_{50}, D1, and D2. The ED_{50} is chosen since it represents the portion of a dose–response relationship plot where the optimum discrimination of relevant differences can be detected when used in a pivotal BE study and the pilot study is usually conducted only using the reference product. Using longer dose durations may dampen the assessment of relatively small but significant differences in blanching between a test and a reference product. Furthermore, using shorter dose durations will influence the reliability and repeatability of the assessments.

The FDA Guidance (Guidance for industry, 1995) recommends that a subject must be a 'detector' in order for inclusion of their data for statistical analyses in the bioequivalence assessment. Hence, subjects' responses are expected to meet the specified minimum D2/D1 ratio of AUEC values in the pivotal study as shown in the equation below.

AUEC at D2/AUEC at D1 ≥ 1.25

where D1 = ½ED_{50} and D2= 2ED_{50}.

Comparison between visual and chromameter assessment: A pilot study (Guidance for Industry, 1998) was conducted where Dermovate® cream (containing 0.05% clobetasol propionate) was used as the reference product. The study was performed using the volar aspect of the forearms of 11 healthy human subjects. The subjects were previously screened for skin blanching response to be included in the study. Approximately 10 mg of the cream formulation was applied onto the relevant demarcated sites using dose durations of 0.25, 0.5, 1.0, 2.0, 4.0, and 6.0 hours. The blanching responses were visually assessed by three trained observers and also with a chromameter at various time intervals over a period of 26 hours after removal of the cream.

A pivotal study was subsequently conducted using the above-mentioned dose durations (Table 1) and 34 healthy human subjects were enrolled into the study. The HSBA pivotal study was implemented similarly to that of the pilot study as described previously with the exception that Dermovate® cream was utilized as both reference and test product for the determination of bioequivalence. Skin blanching was evaluated over a period of 30 hours after the removal of the applied products. The results (Figure 5) revealed that 23 subjects were found to be "detectors" in the pivotal study, but the data for all 34 subjects were included for comparison purposes.

The skin blanching profiles shown below were very similar when comparing the data between the two different assessment methods, or between "detectors" and "nondetectors" (Au et al., 2008).

This indicates that the visual and chromameter assessment methods are comparable to each other and both are equally applicable for HSBA. The inclusion of "nondetectors" data did not seem to have a significant effect on the skin blanching profiles nor on the outcomes of the comparisons for the assessment of bioequivalence.

	VISUAL		CHROMAMETER	
	Mean Ratio % (T/R)	90% CI (T/R)	Mean Ratio % (T/R)	90% CI (T/R)
Detectors (n=23)	104.6	99.3 -111.6	104.6	86.5 – 129.3
All subjects (n=34)	102.9	97.9 – 109.2	104.3	90.2 – 120.7

Abbreviations: T, test; R, reference; CI, confidence interval

Table 1: 90% confidence intervals - Locke's method visual and chromameter data Dermovate® Creams (T & R) (Au et al., 2008)

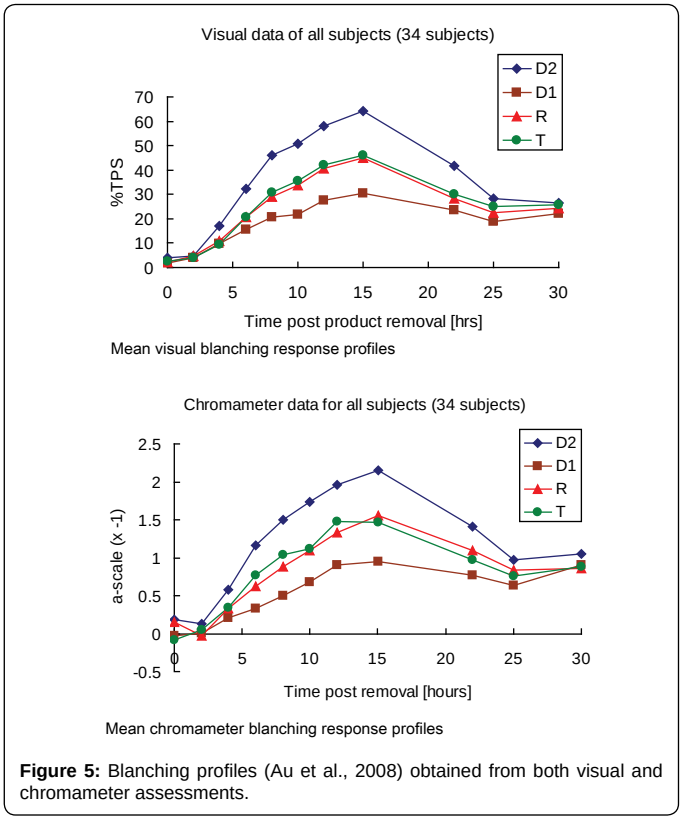

Figure 5: Blanching profiles (Au et al., 2008) obtained from both visual and chromameter assessments.

Dermatopharmacokinetic methods also known as tape stripping (TS)

The U.S Food and Drug Administration (FDA) published a guidance in 1998 (Guidance for Industry, 1998). The initial TS methodology outlining the bioavailability/bioequivalence protocol for topical formulations intended for local and/or regional activity, was published in a draft guideline but was subject to criticism which resulted in its withdrawal, mainly due to a number of limitations, in particular the sources of variability and control. TS involves sequentially removing microscopic layers (~0.5–1.0 μm thick) of SC by placing an adhesive tape strip onto the skin surface, followed by gentle pressure to ensure good contact and subsequent removal by a sharp upward movement (Figure 6) (Herkenne et al., 2007), which may be repeated 10 to more than 100 times (Surber and Davies, 2002; Surber et al., 2001).

It determines the amount of drug permeated into the *stratum corneum* and although it is a relatively painless and noninvasive (Herkenne et al., 2007) technique, disrupts the integrity of the water barrier properties of the SC (Löffler et al., 2004), which is rapidly repaired by a homeostatic response in the dermis.

Amongst the many variables that hamper the precision and reproducibility of this method is the fact that s*tratum corneum* thickness differs between each individual – hence, normalization necessary. This can be accomplished by measuring the transepidermal water loss (TEWL) which is a noninvasive bioengineering technique that describes the outward diffusion of water through the skin (Levin and Maibach, 2005). TEWL monitors the integrity of the SC water barrier function and is an indicator of skin water barrier alteration (Löffler et al., 2004; Zhai et al., 2007) with increased readings often indicating impairment of skin barrier function (Levin and Maibach, 2005). Healthy SC typically has water content of 10% to 20% and TEWL can be dramatically altered if barrier function is perturbed by

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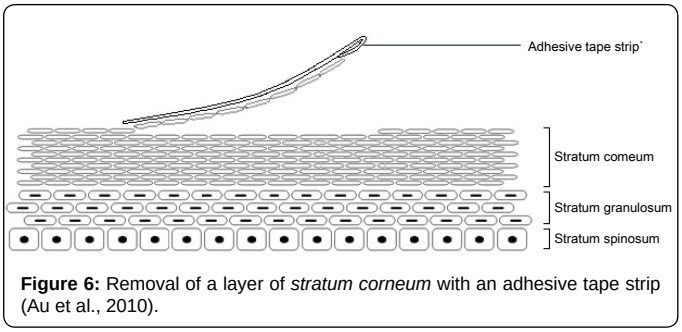

Figure 6: Removal of a layer of *stratum corneum* with an adhesive tape strip (Au et al., 2010).

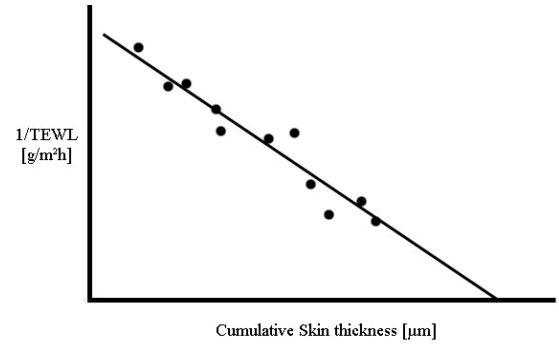

Figure 7: A plot of 1/TEWL versus cumulative skin thickness.

physical, chemical, therapeutic, and/or pathological factors (Zhai et al., 2007). Typical basal values of TEWL in adults with healthy skin are between 5 and 10 $g^{-2} \cdot h^{-1}$. Kalia et al. (2000) reported the use of TEWL in TS experiments to determine the thickness of SC in an attempt to normalize the data by incorporating the SC thickness from each subject. This involves weighing each tape strip before and after SC harvesting to determine the amount (m) of tissue removed. Each stripped amount of SC can be converted to a distance (x), which reflects thickness of the removed skin strip, ($x = m/A\rho$), where (A) is the area of the application site, (ρ) is the density of the SC, reported as ~0.88–1.42 g/cm (Anderson et al., 1991).

A plot of 1/TEWL versus cumulative skin thickness according to the relationship, $1/J = 1/TEWLx = H\text{-}x/K.D.\Delta C1$ yields a typical plot (Figure 7).

where J = flux g/m2h, H = total SC thickness, x = partial SC thickness, K = partition coefficient of water in tissue, D = water diffusivity and DC = difference in water concentration across the membrane

This equation allows the TS data to be expressed as an amount per normalized fraction of SC removed (x/L), a strategy that allows results from disparate subjects of different SC thickness to be normalized and compared. The use of TEWL with TS, however, demonstrates that the number of tape strips (i.e., 10), as suggested in the FDA Draft Guidance (Guidance for Industry, 1998) is a poor indicator of the actual amount of SC tissue removed, since no information on the relative position within the SC is known and moreover 10 tape strips fail to permit meaningful comparisons between individuals (Kalia et al., 2000). TS has been reported as being applicable to all drugs that are topically applied for local action (Wiedersberg et al., 2008). Although the TS technique is a single-point determination, it is possible to derive pharmacokinetic parameters such as AUC, C_{max} and T_{max} by sampling different sites progressively with application time, thereby providing a means to assess topical bioavailability of dermatological formulations (Pershing et al., 2003). Since the TS technique is accessible only to the SC but not the deeper tissues, for example, the viable epidermis and dermis, this technique may not be applicable to drugs that have their activity in deeper tissues.

Evaluation of the tape stripping method versus the HSBA for the assessment of BE: In order to validate the use of the TS method for the bioequivalence assessment of topical formulations, data from a TS study were compared with data generated on the same product using the HSBA. The main objectives were to determine bioequivalence of Dermovate® creams using HSBA and also tape stripping and to investigate whether tape stripping can show differences in bioavailability between the same and different topical products, i.e. the capability to measure bioequivalence or bioinequivalence.

An initial pilot TS study was undertaken to determine the ED_{50} value to determine bioequivalence using TS where dose durations of 0.5, 1, 2, 4, 6, 8, 10, and 12 hours were evaluated on the volar aspect of the forearm of six healthy human subjects. A blank site was reserved to estimate *stratum corneum* thickness for each individual using transepidermal water loss (TEWL) as and the AUC_{corr} value was determined by correcting for skin thickness (Kalia et al., 2000). The normalized skin thickness was used to compare intra and interindividual data.

Approximately 5 mg/cm² of Dermovate® cream (reference product) was applied to the relevant sites. At the end of the dose duration, the product was removed and tape stripping was commenced. The ED_{50} was found to be 2.4 hours. Two hours was chosen as the dose duration to assess bioequivalence by using TS in the pivotal studies. Scotch® tape (no. 810, 3M) was used as the adhesive tape strips for *stratum corneum* removal. A further pilot TS study was carried out to determine the number of subjects required for the pivotal TS study in order to attain an acceptable power.

This study utilized Dermovate® cream where it was compared against itself as the test and reference product. The results showed that interindividual variability (CV%) was ~14%, which indicated that approximately 30 subjects would be required to achieve a power of at least 80% (Diletti et al., 1991).

Hence a pivotal TS study was subsequently undertaken employing 30 subjects using the same Dermovate® cream as the test and reference product in the pivotal HSBA study. Upon comparing the product to itself, the results of AUC_{corr} values showed similar confidence intervals and AUCtest/AUCref ratios using untransformed (Locke's) and also log-transformed data as shown in Table 2. It is interesting to note that the results are similar to those of a previously conducted pivotal HSBA study (Au et al., 2008). Hence, the TS method is seen to be comparable to the HSBA method, as it produced the same bioequivalence outcome.

In addition, a cream and an ointment formulation were assessed against a reference cream formulation (Dovate® cream vs Dermovate®

	Mean T/R ratio (%)		90% CI	
	Untransformed (Locke's)	Log transformed	Untransformed (Locke's)	Log transformed
HSBA (n=34)				
Chromameter	104.3	-	90.2 -120.7	-
Visual	102.9	-	97.9 – 109.2	-
Tape stripping (n=7) Pilot study (AUC_{corr})	101.8	101.4	88.0 -118.3	87.4 – 17.7

Abbreviations: T, test R; reference; CI, confidence interval; HSBA, human skin blanching assay; AUC_{corr}, area under the curve of corrected tape stripping data.

Table 2: Bioequivalence Assessment Of Identical Products (Test –Dermovate® Cream, Reference – Dermovate® Cream) (Au et al., 2010).

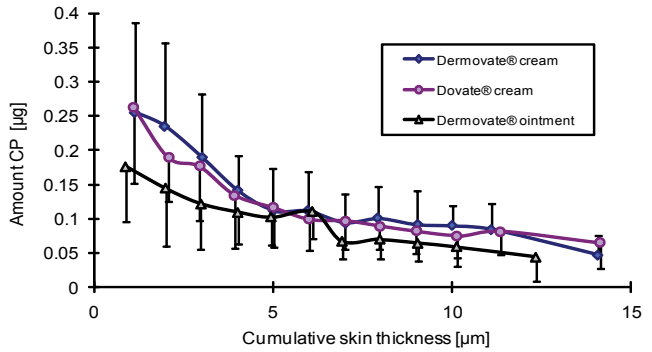

Figure 8: Bioequivalence Assessment of Topical Clobetasol Propionate Formulations Using Tape Stripping (Au et al., 2010).

	T/R ratio (%)	Confidence interval (%)
Pivotal TS Study (n=30)		
Dovate® cream vs Dermovate® cream	92.4	80.3 – 106.0
Dermovate® ointment vs Dermovate® cream	59.1	49.3 – 70.2

Reference product: Dermovate® cream
Test products: Dovate® cream and Dermovate® ointment

Table 3: Pivotal TS Studies of Clobetasol Propionate Creams and Ointment Products Using AUC_{corr} Data (Au et al., 2010).

cream and Dermovate® ointment versus Dermovate® cream). It is important to note that creams and ointments are considered not to be pharmaceutically bioequivalent and bioequivalence assessment are normally not done between these two different types of formulations. However, a bioequivalence assessment study (Au et al., 2010) was conducted to determine whether the TS method had the necessary sensitivity to determine differences between these two types of formulations, if differences do indeed exist. The figure below (Figure 8) shows the superimposed profiles using TS data and the AUC_{corr} data (Table 3) established that Dovate® cream was bioequivalent to Dermovate® cream whereas the opposite was found when comparing Dermovate® ointment against Dermovate® cream. These studies indicated that the TS method was able to determine similarities and differences between the various dosage forms studied. These data indicate that the TS method can be used as an alternative approach for the bioequivalence assessment of topical clobetasol propionate formulations. This method should therefore be equally applicable to determine bioequivalence of any topical corticosteroid formulations and also applicable with other topical dosage forms intended for local use. In addition, the application of either of the statistical methods described above for a bioequivalence assessment of topical dosage forms using the TS method may be used.

Dermal microdialysis

Microdialysis (MD) is an *in vivo* sampling technique used to measure endogenous and/or exogenous compounds in extracellular spaces (Groth et al., 2006; Groth, 1996; Plock and Kloft, 2005).Dermal Microdialysis (DMD) is a relatively new application of MD which allows continuous monitoring of endogenous and/or exogenous solutes in the interstitial fluid (ISF) of dermal tissue with minimal tissue trauma and involves the placement of small perfused membrane systems at given depths within the dermis (Figure 9). When a topical formulation is applied onto the skin and perfusate is pumped through the implanted membrane system, drug molecules from the topical formulation present in the dermal ISF diffuse (driven by the concentration gradient) into the lumen of the membrane, resulting in the presence of drug in the perfusion medium collected as dialysate. The dialysate is sampled at various intervals of time and the drug concentration in the dialysate can be determined quantitatively (Chaurasia et al., 2007).

The implantation of the membrane system in the dermis involves a relatively simple procedure, although training is imperative (Mathy et al., 2003). The implantation procedure involves initial insertion of cannnulae into the skin and subsequent threading of the DMD membranes through the cannulae within the volar aspect of the forearm of human volunteers is illustrated in Figure 9 (Tettey-Amlalo, 2009). Once the microdialysis membranes have been inserted through the cannulae and placed under the skin, the probes are connected to a microdialysis pump (Figure 10) DMD has been considered as a promising technique for the assessment of bioavailability and bioequivalence of topical formulations and has attracted a great deal of interest among research scientists, dermatologists, and the pharmaceutical industry (Groth, 1996; Shah et al., 1998a; Shah et al., 1998b; Benfeldt et al., 2007). The technique is minimally invasive and capable of producing concentration–time profiles sampled directly in the dermis, the target tissue, and is therefore suited to study the local and/or regional delivery of drugs following topical administration.

Linear membrane systems are fabricated from hollow fibers (hemodialysis cylinders) often taken from artificial kidneys. These types of membranes are not generally commercially available but are simple to manufacture in the laboratory. Their use requires entry and exit punctures by means of a guide cannula through the skin when placed in the tissue.

MD membrane systems are commonly referred to as “probes,” which comprise the permeable membranes being attached at either

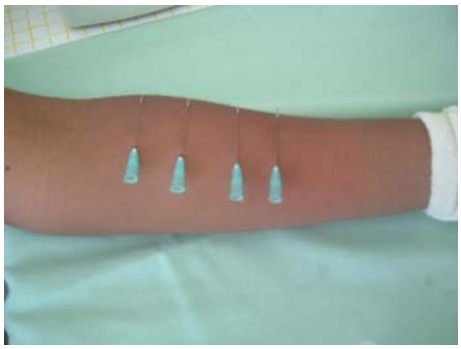

Figure 9: The implantation procedure of DMD probes within the volar aspect of the forearm of human volunteers (Tettey-Amlalo, 2009).

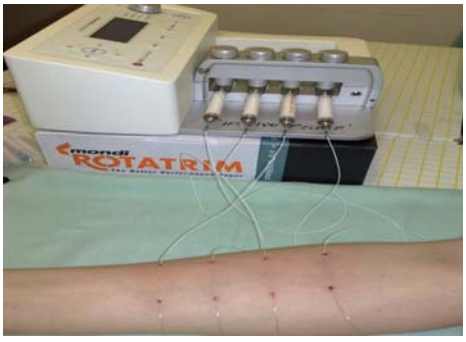

Figure 10: Microdialysis membranes inserted under the skin of a human subject and the probes connected to a microdialysis pump (Tettey-Amlalo, 2009).

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one or both ends to impermeable nylon or other inert materials such as Peek® tubing. The membrane materials used in MD probes are available in different types and pore sizes (Schnetz and Fartasch, 2001). Common substances used as membrane materials are cellulose acetate, polyacrylonitrile, polycarbonate, AN-69 copolymer (which consists of polyacrylonitrile and methylsulphonate), polyethersulphone, and cuprophan (regenerated cellulose membrane), (Anderson et al., 1991; Plock and Kloft, 2005; Schnetz and Fartasch, 2001; Zhao et al., 1995; Lönnroth and Strindberg, 1995; Stenken et al., 2001; Stenken, 1999; de Lange et al., 2000). MD membranes are porous in nature, which permits diffusion to occur within the pores of the membrane (de Lange et al., 2000).

Different membrane materials have different chemistries that can affect the recovery and/or delivery of drugs. The molecular weight cut-off (MWCO), a physical property commonly used by manufacturers of these membranes, describes the ability of a membrane to reject 90% of molecules with a specific molecular weight. Although most membrane pore sizes range from 6 to 3000 kDa, the majority of MD experiments have been conducted using membranes with MWCO of 20 kDa (Schnetz and Fartasch, 2001). It is important that the membrane as well as any other component of the MD system does not interact with the drug since this would reduce the drug concentration in the dialysate (de Lange et al., 2000). The membranes incorporated in linear probes are usually reinforced with a stainless steel guide wire during manufacture for mechanical strength.

Probe calibration

Calibration of probes may be performed *in vitro* and *in vivo*. For *in vitro* studies, the surrounding medium is referred to as the periprobe, whereas the surrounding medium for *in vivo* studies is the tissue ISF. Since MD is a dynamic technique with the perfusate continuously being pumped through the probe, equilibrium is not established and dialysate concentrations represent only a fraction of actual concentrations in the tissue ISF or in the periprobe (Brunner and Langer, 2006; Song and Lunte, 1999). The fraction obtained is referred to as extraction efficiency (*EE*), which has to be determined in order to quantitatively relate drug dialysate concentrations in either the tissue or in the periprobe. However, if the desired information from a MD experiment is the relative change in drug concentration, knowing the *in vivo EE* is not absolutely necessary. Knowledge of the *in vitro EE*, however, provides information on the reproducibility and patency of the MD probe being used (Song and Lunte, 1999). The simplest approach to calibrate a MD probe is by using a standard solution. For *in vitro* calibration studies, since the drug concentration in the periprobe is known and the perfusate contains no drug, diffusion of the drug occurs from the periprobe into and through the membrane and is collected as dialysate.

Assessment of DMD (*in vivo*) probe depth

Initially, Benfeldt et al. (1999) found increased drug concentrations in the dialysate with superficial probe insertions, but in contrast, Benfeldt and Serup (1999); Hegemann et al. (1995); Müller et al. (1997) and Simonsen et al. (2004) reported no such correlation. A probe depth of 0.6 to 1.0 mm is considered acceptable for DMD studies (Groth et al., 2006). The depth of the probe insertion, that is, the distance of the dialysis membrane within the skin to the skin surface can be measured by ultrasound imaging using a frequency of 20 MHz (Groth et al., 2006; Benfeldt et al., 1999; Benfeldt and Serup, 1999).

Composition of perfusates

Perfusates used in MD experiments vary widely in composition and P^H. Ideally, the composition, ion strength, osmotic value, and P^H of the perfusate used should be physiologically compatible with the dermis environment (Davies et al., 2000). This prevents the excessive migration of molecules into or out of the periprobe fluid due to osmotic differences. The perfusate is normally perfused at low flow rates of 1 to 10 µL/min (Brunner and Langer, 2006). Perfusates should be sterile when used in human and animal experiments.

Assessment of bioavailability/bioequivalence using DMD

In a clinical study (Tettey-Amlalo et al., 2009) involving 18 human subjects, with four probes inserted on the left volar aspect of the forearms of each subject, DMD had been successfully employed for the assessment of bioavailability of a ketoprofen topical gel formulation. The same formulation was placed on all four sites on each subject and the dialysate concentrations determined with a validated analytical UPLC-MS/MS method (Tettey-Amlalo and Kanfer, 2009). The mean dialysate concentration–time profiles are illustrated in Figure 11.

Experimental: 4 probe insertions, 4 application sites, 1 probe per site, probes were 1.5 cm apart, probes covered approximately 2 quarters of the volar aspect of the forearm of each volunteer, 18 subjects, Formulation: Fastum® gel (Tettey-Amlalo et al., 2009).

The authors reported intra- and intersubject variability of 10% and 68%, respectively. Bioequivalence was subsequently confirmed with a power of greater than 90% thereby validating DMD for the determination of topical formulations intended for local and/or regional activity (Tettey-Amlalo et al., 2009).

Conclusions

To date, the only "surrogate" measure for the assessment of bioequivalence of topical products "officially" recognised is the HSBA/VCA for topical corticosteroids. Although an instrumental method of assessing blanching is generally recommended, recent data have

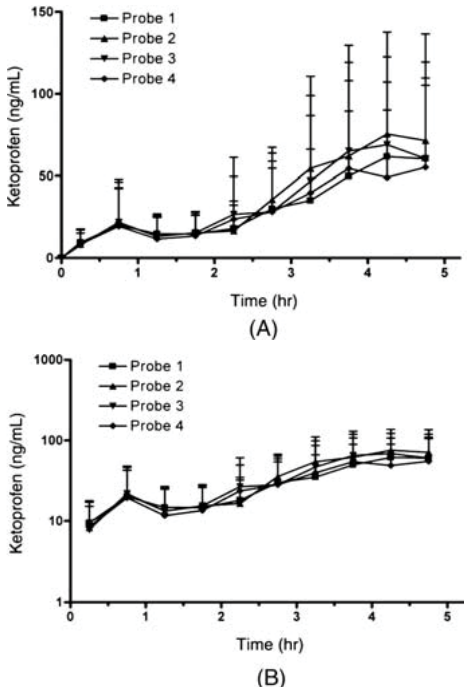

Figure 11: Mean dialysate concentration (A) and semi-log (B) time profiles.

shown that visual assessment is as good as the chromameter data for BE of topical corticosteroid products.Tape stripping data provided the same results as that of the HSBA/VCA i.e., a correlation has been demonstrated between the two methods. Hence TS has been shown to be a viable alternative BE method for the assessment of topical preparations. The application of DMD as a method for the determination of BA/BE of a topical product containing ketoprofen has been described thereby indicating that DMD has the potential for use for BE assessment of some topical products. Whereas BE of topical dosage forms not intended for absorption has proved to be quite difficult, daunting and extremely challenging, recent data using an improved TS method and also the use DMD have shown great promise for their application to assess the BA/BE of topical products.

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