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EXPERT REVIEW

Bioequivalence for Topical Products—An Update

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ABSTRACT With the exception of topical glucocorticoids, demonstrating bioequivalence between generic and reference topical products entails conducting clinical end-point trials which are both lengthy and expensive. Considerable effort has been channeled towards development and validation of alternative approaches to demonstrate bioequivalence of topical and transdermal products. The critical opportunity pathways identified by the FDA for the industry for topical bioequivalence include the following surrogate methods: *in vitro* studies, dermatopharmacokinetic method, dermal microdialysis and near infrared spectroscopy. This review provides an update of recent advances in these methodologies.

KEY WORDS bioequivalence · dermatopharmacokinetics · *in vitro* diffusion · microdialysis · near infra red · tape stripping · topical

INTRODUCTION

There is a growing realization amongst health policy makers and increasingly even amongst the general public that the cost of prescription drugs makes up a significantly large percentage of health care costs. The potential cost savings that could accrue with the use of generic drugs is significant. The analysis of Intercontinental Marketing Services (IMS) data on the Office of Generic Drugs (OGD) website shows how increased competition from generic drugs affects price (1). More than five generic competitors caused the drug price to fall to 25% of the brand price. However, generic competition is low for topical drug products due to the difficulty in demonstrating bioequivalence of the generic drugs. With the exception of topical glucocorticoids, demonstrating bioequivalence between generic and reference topical products entails conducting clinical end-point trials which are both lengthy and expensive. The only surrogate method approved by the FDA, to date, is the vasoconstrictor assay used for topical glucocorticoids (2). The methodological details of the vasoconstrictor assay have been reviewed (3), and improvements to the method have been discussed elsewhere (4,5). Considerable effort has been channeled towards development and validation of alternative approaches to demonstrate bioequivalence of other topical and transdermal products. The critical opportunity pathways identified by the FDA for the industry with regards to topical bioequivalence include the following surrogate methods: *in vitro* studies, dermatopharmacokinetic method (DPK), dermal microdialysis (DMD) and near infrared spectroscopy (NIR) (6). While some other alternative methods, such as skin biopsy (7,8) and suction blister (9–13), have been developed, they are considerably invasive and have failed to gain popularity. Methods considered minimally invasive—DPK, DMD and non-invasive—spectroscopy and *in vitro* method are under serious consideration. The procedural details and issues related to DPK and DMD methodologies have been extensively reviewed by Herkenne *et al.* (14). This review provides an update of recent advances in these methodologies as well as an overview of *in vitro* and spectroscopic methods.

IN VITRO DIFFUSION

The *in vitro* diffusion study set up for topical products consists of either excised human/animal skin or synthetic membrane mounted between donor and receptor chambers. Test formulations are applied to the skin or

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membrane surface facing the donor chamber. Receptor fluid samples are collected through a side-arm sampling port at defined intervals and are analyzed for drug content. *In vitro* diffusion cells have historically been used to 1) screen formulations to select promising candidates, 2) elucidate location/mechanism of action of permeation enhancers (15–17) and 3) demonstrate equivalence after post-approval changes to the product. *In vitro* dissolution systems have been widely used for oral drug products to develop correlations between *in vitro* and *in vivo* data in order to optimize formulations. An *in vitro-in vivo* correlation (IVIVC) has been defined by the Food and Drug Administration (FDA) as "a predictive mathematical model (which can be non-linear) describing the relationship between an *in-vitro* property of a dosage form and an *in-vivo* response" (18). The United States Pharmacopoeia (USP) defines IVIVC as "the establishment of a rational relationship between a biological property or a parameter derived from a biological property produced by a dosage form and a physicochemical property or characteristic of the same dosage form" (19). The parameter derived from the biological property is generally AUC, C_{max} or the total amount of drug absorbed, while the physicochemical property is the rate or extent (cumulative release) of drug dissolution or release *in vitro* profile. The main goal behind establishing IVIVC is to use the *in vitro* test as a surrogate for human studies and thus to minimize the number of bioequivalence studies performed during the initial approval process and during the scaling-up and post-approval changes.

The excised human skin model has been used in the field of transdermal products, and a good IVIVC has been established especially for rate of absorption (20–24). In the "Critical Opportunities Pathway," the FDA identifies the *in vitro* diffusion study combined with rheological testing to demonstrate bioequivalence of qualitatively and quantitatively (Q1Q2) equivalent drug products (6). Recently, Franz *et al.* reported use of excised human skin to specifically demonstrate bioequivalence of topical products (25). The authors used the finite dose method, where the physiological conditions are duplicated in the *in vitro* set up (26). In this technique, the skin integrity was tested with tritiated water. The model was validated by comparing *in vitro* absorption of twelve organic compounds with their *in vivo* absorption reported by Feldmann and Maibach (27). Good correlation was obtained for chloramphenicol, phenol urea, nicotinamide, acetylsalicylic acid, salicylic acid, benzoic acid and dinitrochlorobenzene. However, four- to ten-fold differences between *in vitro* and *in vivo* data were obtained for hippuric acid, nicotinic acid, thiourea and caffeine. These were attributed to the discrepancies in the protocols rather than the lack of sensitivity of the model. When the differences in the protocols were reconciled by using abdominal skin, a non-occlusive covering, a skin wash at 24 h for *in vitro* studies and extended urine collection until complete excretion of the drugs in *in vivo* experiments, good IVIV correlation was obtained. The authors further tested the validity of the model by comparing vehicle effect on absorption for caffeine formulation. The model proved the sensitivity by showing similar reduction in absorption of caffeine from water-based gels *versus* petrolatum and ethylene glycol (EG) gels. Similar results were noted for testosterone and benzoic acid formulations. The utility of the *in vitro* model was further demonstrated by showing a good correlation between results from an un-occluded finite dose *in vitro* study and *in vivo* absorption study for a volatile organic compound—benzene.

The validated *in vitro* model was then tested for its utility as a surrogate for clinical studies by comparing the *in vitro* diffusion data generated on seven approved generic products (five glucocorticoids and two tretinoin products) and the corresponding reference products with the clinical data (Tables I and II). All five glucocorticoid products—alclometasone dipropionate 0.05% cream and ointment, halobetasol propionate 0.05% cream and ointment and mometasone furoate 0.1% ointment—had been previously shown to be bioequivalent to the respective Reference Listed Drugs (RLDs) by vasoconstrictor assay with 90% confidence intervals within 0.80–1.25. By using *in vitro* diffusion method, the authors showed that the test/reference ratio for the total *in vitro* absorption was within the same limit. The exception was mometasone ointment, for which the test/reference ratio in the *in vitro* testing was found to be 0.63. The authors attributed the lack of IVIV correlation to the insufficient number of skin sections employed in the *in vitro* testing. The authors noted the remarkable similarity between the rates of absorption obtained for the generic and the RLD formulation in the *in vitro* study. Similar results were observed for two therapeutically equivalent generic tretinoin gels, 0.01% and 0.025% and the respective RLDs, when tested for total absorption, maximum rate of absorption and time of maximum rate of absorption, with the exception of maximum rate of absorption for 0.025% tretinoin gel, for which the 90% confidence interval fell between 0.95 and 1.27.

The *in vitro* technique is relatively simple and inexpensive. It is also possible to collect different sections of the skin (epidermis, dermis) at different time points during the experiment to determine concentration of drug in the tissue as a function of time. However, there are several shortcomings of the *in vitro* method. The obvious limitation of the model is lack of live tissue, underlying supportive structure, metabolic activity and general circulation. Reasonable estimates cannot be obtained for drugs which are metabolized in the skin or distributed through the blood. Other issues include utilization of the full skin, which yields low

Table I IVIV Comparison of Five Generic Glucocorticoid Products (Test) *Versus* the Corresponding Reference Products (Reproduced from Ref (25) with Permission)

	In vitro absorption[a], ng/cm^2/48h			*In vivo* VC assay[a], negative $AUEC_{0-24\ h}$		
	Test	Reference	Test/Reference	Test	Reference	Test/Reference
Alclometasone cream	4.52	4.39	1.03	18.5	16.8	1.10
Alclometasone ointment	66.95	70.0	0.96	16.0	17.4	0.92
Halobetasol cream	110.4	96.9[b]	1.14	33.1	30.7	1.08
Halobetasol ointment	246.7	256.3	0.96	28.6	28.5	1.00
Mometasone ointment	213.4	338.7	0.63	13.7	12.3	1.11

[a] Listed numbers are mean values.

[b] Average of three reference lots, none of which were used in the VC study. In all other comparisons identical lots of test and reference products were used in both the *in vivo* and the *in vitro* studies.

absorption values for poorly water soluble drugs, making the comparison between test and reference product difficult or even erroneous. Since drugs enter the systemic circulation *in vivo* at the top of the dermis, the full aqueous dermal compartment acts as an artificial barrier in the *in vitro* set-up for poorly water-soluble molecules. This problem could be avoided by using either dermatomed skin, where the thickness of the dermal compartment is reduced, or isolated epidermis, where the dermal layer is eliminated. It is also important to maintain the sink conditions by including solubilizing agents in the receptor fluid. Replacing the normal saline with more lipophilic physiological solutions such as rabbit serum or bovine serum albumin can improve percutaneous absorption of lipophilic drugs. Non-ionic surfactants such as Volpo-20 and ethanolic solutions have also been shown to be effective for hydrophobic drugs yielding results more predictive of *in vivo* absorption. However, minimal effect on the skin barrier properties from these receptor solutions is desired and should be demonstrated (28–30).

Table II *In Vitro* Comparison of the Primary Endpoints for Test and Reference Tretinoin Gels (Reproduced from Ref (25) with Permission)

	Test	Reference	Test/Reference	90% CI[a]
0.01% tretinoin gel				
AUC	3.00	2.97	1.02	97.06–107.46
J_{max}	0.55	0.57	1.04	92.53–115.05
T_{max}	3.60	3.57	1.04	92.23–116.37
0.025% tretinoin gel[b]				
AUC	3.49	3.47	1.03	95.14–110.45
J_{max}	0.91	0.88	1.11	95.08–127.88
T_{max}	3.66	3.72	0.98	97.26–99.52

[a] 90% CIs for the ratio of the means (Test/Reference) of the listed parameters

[b] Lots of test and reference material used were identical to those used in the clinical study

The agency currently does not accept *in vitro* release testing as a surrogate for *in vivo* bioavailability (BA) or bioequivalence (BE) data. It also discourages sponsors to use this technique to compare different formulations across manufacturers. However, *in vitro* release testing using a semi-synthetic membrane is accepted as a "useful test to assess product sameness under certain scale-up and post-approval changes (SUPAC)," as it is believed to collectively reflect any differences due to several physicochemical properties such as solubility, particle size of the drug and rheological properties of the vehicle (31). Accepted for this purpose are level 2 changes, those that could significantly affect formulation performance. Examples include changes in components and composition (>5% and <10% excipient amount, change of supplier or technical grade of a structure-forming excipient and change in particle size distribution of the drug substance), manufacturing equipment (equipment of different type or design or operating principles), manufacturing process, change in batch size beyond a factor of ten times the size of biobatch and manufacturing site. *In vivo* BE testing is required for any changes beyond level 2, for example, a change in crystalline structure of the drug substance if it is in suspension. Since, for qualitatively and quantitatively equivalent products (Q1Q2), the differences between the brand and generic products are essentially level 2 changes, *in vitro* release testing was suggested as a possible surrogate method to demonstrate bioequivalence (6). However, regulatory guidance regarding experimental design and acceptance criteria is lacking.

SKIN STRIPPING

Skin stripping is also known as the dermatopharmacokinetic (DPK) method, in which drug concentration in the stratum corneum is measured as a function of time by sequentially removing layers of the stratum corneum by tape-stripping. The rationale behind this method is that the

topically applied drug must pass through the first barrier, the stratum corneum (SC), in order to be available to the underlying tissues, and in some instances get absorbed into the systemic circulation, analogous to the orally absorbed drugs which must enter into the blood stream in order to be available at the site of action (32). Thus, if the drug level in the SC is assayed over a period of time, the SC concentration-time profiles obtained from two products can be effectively compared for bioequivalence. This assumption was supported by the studies of Rougier *et al.* in which good correlation was shown between the concentration of drug in the SC at 30 min and the total amount absorbed in the systemic circulation over 4 days for compounds with widely different physicochemical properties (33). This method was under serious consideration by the FDA, and a draft guidance on the method was released in 1998 (34). As outlined in the guidance, to evaluate drug absorption and elimination, a specific area of the SC is allowed to equilibrate with the drug product for a specific amount of time, after which the drug product is removed from the skin, and the SC tissue is collected by successive application and removal of twelve tapes. The first two tapes are discarded, and the rest are combined and analyzed to determine the drug level in the SC at a specific time during the uptake or elimination phase to generate the SC concentration time profiles and the time integrated parameters, such as area under the curve (AUC), maximum concentration (C_{max}) and time to maximum concentration (T_{max}). The guidance was withdrawn later in May 2002, when contradictory results were obtained from two independent laboratories during method validation using tretinoin gel (35–37). Nevertheless, skin stripping is still being evaluated as a tool for bioavailability and bioequivalence assessment of topical products (38). Recently, N'Dri-Stempfer *et al.* proposed several refinements to the original methodology (39). The authors argued that the first two tapes which were to be discarded as per the FDA guidance should be included in the analysis for reliable determination of the drug in the SC. The FDA's recommendation was based on the assumption that the residual drug product could be collected in the first two tapes, thus resulting in an overestimation of the drug level in the SC. The authors proposed to resolve this obvious limitation by removing the product quickly and effectively from the application site by using commercially available alcohol swabs. The authors also stressed the importance of collecting almost all the SC for better reproducibility of results and reduced variability due to subject-to-subject variation, operator, type of tape used and site of SC collection. The FDA guidance required use of a fixed number of tapes, which could result in incomplete and variable SC collection. To circumvent this problem, the authors used an eight-fold increase in trans-epidermal water loss (TEWL) as an indicator to ensure nearly complete removal of SC. Another important drawback of the study design used in the method validation was stripping of larger skin area than the area of formulation application, which resulted in edge effects, one of the reasons for study failure as mentioned by the advisory committee (35–37). The authors addressed this issue by stripping a smaller central area of the larger application area using rigid templates of specific size (Fig. 1). To obtain results mostly above limit of quantification or limit of detection, the collected tapes were divided into 3–5 groups, assigning the first two tapes to the first group to evaluate their effect on data variability. The details of this procedure can be found in the original paper (39). In a separate study, the authors reanalyzed the tretinoin data (40,41) and showed that one uptake and one clearance time could be used rather than four uptake and four clearance times used in the original protocol (42,43). The simplified study design permitted at least

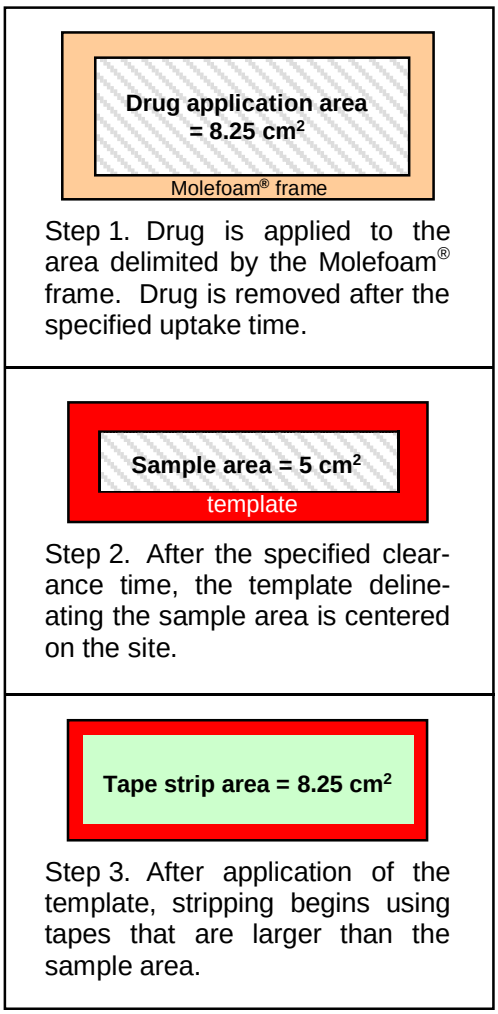

Fig. 1 Schematic diagram illustrating the drug application and tape stripping protocols (Reproduced from Ref. (39) with permission).

duplicate measurements for test and reference products, thus reducing intrasubject variability—a factor considered a major contributor to the overall variability.

With the improved design as described above, three econazole nitrate creams (two approved generics *vs.* one RLD) were evaluated in 14 volunteers in a blinded study using a two-point method—one uptake time of 6 h and a clearance period of 17 h. Each formulation was applied to four sites in each volunteer. The formulations were removed after 6 h of application. The SC was collected after 6 h (drug uptake) from two sites and 17 h (clearance) from the other two sites. All formulations were tested in duplicate in all volunteers for both time points; thus, each volunteer served as his or her own control, improving statistical power. The total number of sites used in this study was 168 (3 products X 4 sites X 14 subjects) compared to 1,176 in tretinoin study (3 products X 8 sites X 49 subjects).

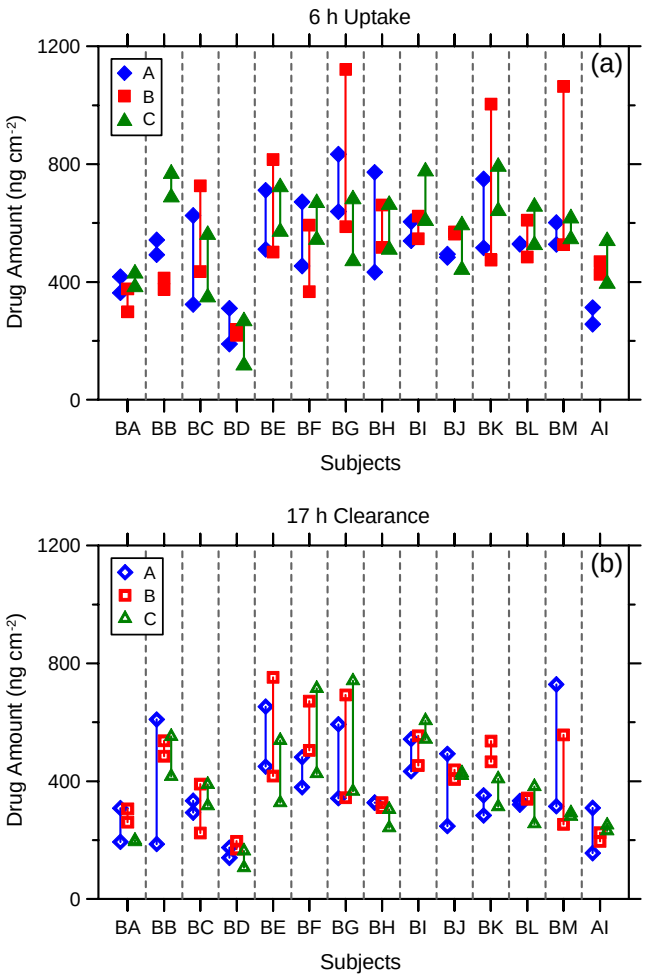

Fig. 2 Bioequivalence assessment of the generic econazole creams (Product A and C) compared with the RLD (Product B) measured in 14 volunteers. Bioequivalence was evaluated using the ratio of the log-transformed amount of drug in the SC (mean ± 90% confidence interval) after 6 h of uptake and 17 h of clearance (Reproduced from Ref. (39) with permission).

In the draft guidance for DPK studies the FDA had relaxed the bioequivalence criteria to 70–143% compared to the traditional limits of 80–125%. It was found that both the approved generic formulations were within the limits of the traditional acceptance criteria for total drug uptake and clearance, failing only slightly when the uptake and clearance were combined (Fig. 2). The calculated lag times of 12.4 h were consistent with the clinical bioequivalence data.

In another independent study by Au *et al.* refinements to the original DPK methodology were made with respect to dosage application, duration of product contact with the application site, removal of excess formulation from the skin, control of temperature and relative humidity of the environment during the study and normalization of the drug penetration data with skin thickness (44). The authors stressed the importance of considering the stratum corneum thickness from each individual subject to reduce variability. For example, the SC thickness on the forearm varies from 5 to 20 μm in normal humans (45). Hence, the total amount of SC tissue removed from different individuals with the same number of strips may vary. Thus, to reduce inter-subject as well as intra-subject variability (between sites within a subject), it is important to determine the total SC removed during the experiment. In this study where DPK methodology was compared with VC assay to assess BE of topical clobetasol propionate cream and ointment formulations, the optimum contact time for the drug product with the skin was determined to be 2 h by using a sigmoidal dose–response model (46). The authors showed that results from DPK method were comparable to those obtained from VC assay (5). The authors also showed the importance of including SC thickness in data analysis to obtain more discriminatory results. The ointment formulation was found to be bioequivalent to the cream formulation when the SC thickness determined using transepidermal water loss (TEWL) (47) was not taken into account, whereas the two formulations were shown to be non-bioequivalent when the data were corrected for SC thickness (Fig. 3).

The DPK method is a useful technique, particularly for drugs for which the site of action is the SC (48–57). A significant advantage of this method is that both the test and the reference product can be tested in the same individuals; thus, each subject serves as a control for himself/herself, reducing intra-subject variability and requiring fewer subjects for the same statistical power. The improved study design employed in the study of econazole nitrate cream (39) yielded bioequivalent results in just 14 subjects and 56 treatment sites per product compared to 49 subjects and 392 treatment sites per product used in the tretinoin study. Supportive conclusions were also drawn in

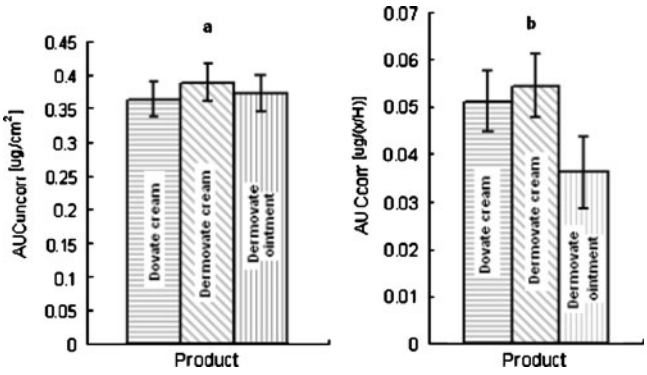

Fig. 3 Comparison between the use of the AUC_{uncorr} and AUC_{corr} values of the different formulations obtained from tape stripping. AUC values with SEM ($n = 30$) (Reproduced from Ref (44) with permission).

another study by the same authors where data from the two point method were compared with those from the AUC approach used in the tretinoin study (43). The two-time-point method required 40% fewer subjects, yielding results with the same accuracy and reliability as the AUC method. The lesser degree of variability found in this approach compared to the tretinoin study was attributed to the better study design, particularly the analysis of the first two strips. In the clobetasol formulations study, the number of subjects required for adequate statistical power was determined to be 32 from a pilot study (44).

While the improvements made to the original study design seem promising, these findings have not been replicated by independent laboratories. Another drawback of this approach lies in the obvious challenge that for each drug, guidelines have to be developed for uptake and clearance times or product-skin contact times. However, it should be noted that FDA approved VC assay method also requires a pilot study with the RLD product alone in order to determine 'dose duration' to be used later in the pivotal study and to select responders (subjects demonstrating adequate vasoconstriction). The responders are included in a pivotal study. In pivotal *in vivo* bioequivalence study, test and reference products are compared using data from detectors (subjects whose pharmacodynamic responses meet a specified minimum value) only (2). The guidance recommends including 40–60 evaluable subjects (those who meet both responder and detector criteria), which requires screening of much larger size of population, thus requiring far more subjects than the DPK as well as DMD methodology discussed below. For DPK methodology, the uptake and clearance times could be determined from a pilot study by assessing several uptake and clearance times or developing a sigmoidal dose–response curve (46) in a limited number of subjects. For some drugs, repeated applications may be required to obtain quantifiable amounts in the SC. Lastly, this approach has yet to be standardized in diseased skin.

MICRODIALYSIS

This technique was introduced in 1994 by Ault *et al.* (58) and further explored by Groth (59), Cross (60) and Benfeldt (61). Methodological details have been reviewed extensively in recent reviews (62–65). The method involves placing an ultrathin hollow fiber, called a probe, in the dermis. The probe is semi-permeable and perfused with a sterile buffer at a slow rate using a microdialysis pump. The probe serves as an artificial vessel, allowing exchange of small diffusible molecules from the extracellular fluid into the probe and vice versa. Thus, similar to oral absorption experiments, this method can provide concentration-time profiles allowing pharmacokinetic measurements. Biomarkers produced in response to topically applied drugs have also been sampled using this technique (66). It can also be used in diseased, perturbed skin (62,67). Test and reference formulations can be tested simultaneously in each volunteer from several sampling sites. This is of critical importance, since it reduces inter-subject variability—a factor that has a considerable impact on the overall coefficient of variation, thus reducing the total number of subjects required to establish bioequivalence for topically applied drugs (64,68). In the lidocaine bioequivalence study (69), discussed in detail below, Shah *et al.* showed that with DMD methodology, 27 subjects are required to establish bioequivalence with 90% confidence interval and 80–125% bioequivalence limits when two probes are used per formulation. The number of subjects reduced to 18 when three probes are used per application site (Table III). McCleverty *et al.* estimated the components of variance on data obtained from a DMD study conducted on eight human volunteers using methyl salicylate formulations. The authors estimated the subject number to be 20 to declare bioequivalence with 80% power and within 80–125% confidence limits when both test and reference formulations are tested in the same volunteers from duplicate sampling sites (68).

The microdialysis method is beleaguered with several technical difficulties. Recoveries are low for highly lipophil-

Table III Bioequivalence (BE) Study Size Estimates (Reproduced from Ref (69) with Permission)

Probability (%)	Limits of variation	Two probes per area	Three probes per area
BE study with two formulations in each subject			
90	80–125%	27	18
BE study with one formulations in each subject			
90	80–125%	985	962

Number of subjects required for BE determinations of topical formulations in healthy human volunteers, based on intra-individual and inter-individual variabilities. 90% probability and limitation of variability between 80 and 125% correspond to the current criteria for BE determination.

ic and highly protein-bound drugs. This problem can be overcome to some extent by using solubilizers such as albumin, Intralipid®, Encapsin® (70,71), cyclodextrins and cosolvents like ethanol, propylene glycol and dimethylsulfoxide (72). Recoveries of lipophilic drugs can also be affected due to drug binding to the probe material (73). Probe calibration is necessary for quantitative measurements, as complete drug recovery is not possible (74). Perfusion rate and sampling times need to be optimized for acceptable drug recovery. Sensitive analytical methods are required due to low drug levels in the perfusate. Selection of tissue-compatible perfusion fluid that can also solubilize the drug of interest can be challenging. It is an invasive technique causing tissue trauma and inflammatory response (75). It requires special training of laboratory personnel in order to achieve probe insertion at a consistent depth in the skin. Although a correlation between probe depth and drug recovery is debated (69,76,77), Holmgaard *et al.* argue that data will be affected depending upon the probe insertion site, e.g. superficial dermis *vs.* subcutaneous tissue, and a thorough investigation of this issue is needed (62). Finally, the most serious drawback of this method is the large variability resulting in coefficients of variation between 50 and 100%. Recently, Shah *et al.* reported comparison of lidocaine absorption from cream and ointment using DMD and DPK methods (69). In eight healthy human volunteers, two sites on one forearm were sampled using four DMD probes over 5 h. On the other arm, tape stripping was performed on two sites, 30 and 120 min after product application. Both methods showed higher absorption from the cream formulation, thus showing that both formulations were nonbioequivalent. A higher coefficient of variation (41–42%) was noted for DMD method, compared to the DPK method (15–25%). In the DMD method, analysis of variance attributed intra-subject variability of 19% between probes, 20% between the two penetration areas and 61% to inter-subject variability. The findings were supported by another study where Tettey-Amlalo *et al.* investigated the use of DMD technique for bioequivalence assessment of ketoprofen topical gel formulation (76). In this study, the same formulation was applied to four sites on the volar surface of the forearms. The sites were sampled using four DMD probes, at 30 min intervals over 5 h. Two sites were treated as test and two as reference. The areas under the curves obtained from the concentration-time profiles for test and reference sites were compared for bioequivalence. The intra-subject variability was found to be 10% between probes, and inter-subject variability was 68%. However, results from this study should be interpreted with caution since only one formulation was tested. With different formulations, the variabilities could be different. In this study, C_{max} values could not be clearly established, and only AUC values were used to determine bioequivalence. The authors pointed out that for topical corticosteroid products, only area under the effect curve (AUEC) is used for bioequivalence assessment by VC assay (2). The authors further argue that C_{max} is used in bioequivalence assessment of systemically available drugs and may not be a suitable parameter for those not intended for systemic absorption.

Compared to skin stripping, microdialysis, although conceptually appealing, is a relatively less explored technique. It has been used to measure dermal concentration of topically applied drugs and delivery systems (78–80) and, in some instances, to compare it after oral absorption (81,82). Increased drug permeation through perturbed skin has also been demonstrated using DMD methodology (77,83,84). However, microdialysis as a surrogate method to demonstrate bioequivalence has not been fully explored. Limited data are available in humans to enable comparison with other techniques and demonstrate its usefulness for bioequivalence purposes.

NEAR IR

Raman spectroscopy and Near Infra-Red (Near IR) spectroscopy represent advanced non-invasive *in vivo* techniques for real-time determination of diffusion of drugs and chemicals into human skin (14,85,86). The prerequisite for the spectroscopic technique is that the molecule of interest should possess distinct spectral features of sufficient intensity to be able to differentiate from the skin spectrum, thus limiting its universal use.

Confocal Raman spectroscopy has been used *in vivo* to identify the molecular structure of the skin, level of skin hydration and effect of moisturizing agents on skin hydration (87–89) and to study penetration of certain chemicals like urea and dimethylsulfoxide into the skin (90,91). It has also been used to show relative effects of different classes of penetration enhancers on topical delivery of retinol (92,93). Although its non-invasive nature and possibility of real-time profiling of drugs penetrating into the skin make this technique attractive, a major drawback of Raman spectroscopy is that it is semi-quantitative. It allows relative measurements rather than absolute determination of drug concentration in the skin.

Near IR is a relatively new non-invasive, quantitative technique which is still in its infancy. Initially, attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) method was developed by Guy *et al.* (94–96) to quantify the drug in tape strips in order to avoid the tedious and lengthy drug extraction procedure. However, tape stripping was still required. It was shown that if tape stripping was performed after short application times, drug diffused faster than tape stripping, thus perhaps losing

crucial information (96). This ceases to be an issue if application times are long enough; however, pilot experiments are needed to determine the optimum exposure time before tape stripping commences. In order to circumvent this issue, Medendorp *et al.* explored the possibility of using NIR spectrometry for *in vitro* quantification of econazole nitrate and 4-cyanophenol in hairless guinea pig skin (97). The authors later used NIR spectrometry for *in vitro* quantification of econazole nitrate and estradiol in human skin (98). The rationale behind using the NIR is the fact that NIR waves can penetrate the skin up to several centimeters depending upon the wavelength, and when combined with linear multivariate statistics, NIR spectroscopy can be used to quantify an analyte as it diffuses through the skin. Additionally, net analyte signal (NAS) calibration offers the advantage of one-point calibration. Thus, when a set of pure spectra for each component and the matrix of spectra (spectra for the placebo formulation and the skin) are available, the net analyte signal can be calculated for the compound of interest. The detailed mathematical equations for this method can be found elsewhere (98). The authors used the NIR technique to quantify dermal absorption of econazole nitrate from a saturated solution prepared in propylene glycol and 1% cream and estradiol from a solution prepared in ethanol, using cadaver human skin *in vitro*. The skin samples were allowed to equilibrate with the formulations for 2 h, after which the skin samples were washed to remove the drug products. The skin samples were immediately analyzed using NIR on both the epidermal and dermal sides. After NIR analysis, the skin samples were then solvent-extracted and assayed by HPLC. The NIR method was found to be sensitive, and results correlated strongly with those from the tissue extraction/HPLC experiments with an r^2 ranging from 0.967 to 0.996, a standard error of estimate ranging from 1.98 to 5.53% and a standard error of performance ranging from 2.12 to 6.83% (Fig. 4).

The NIR technique offers several advantages over other methodologies, since it is quantitative, rapid, noninvasive and nondestructive. The inventors have indicated its possible use *in vivo* with the aid of fiber-optic probes. Recent studies have reported use of diffuse-reflectance near-infrared spectrometer with a fiber-optic probe (99,100). The study by Egawa measured urea and water contents in the human stratum corneum, *in vivo*, after the treatment of urea-containing cream (99). However, this technique is still in an exploratory phase, and its practicality for *in vivo* topical bioequivalence studies is yet to be proven.

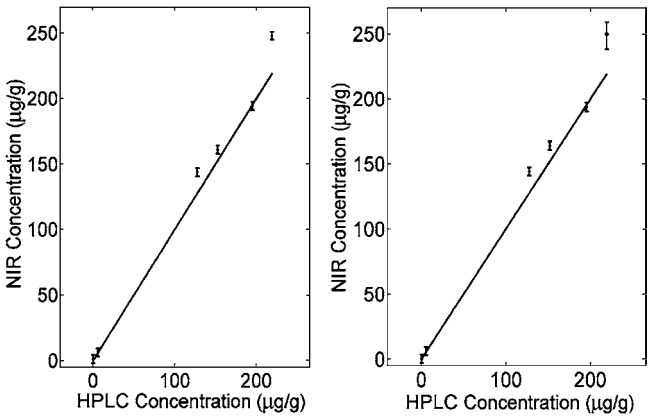

Fig. 4 NIR calibration line from human skin treated with an applied dose of either 50 mg of placebo cream or 1% econazole itrate cream, (*left*) dermis calibration and (*right*) epidermis calibration (Reproduced from Ref (98) with permission).

DISCUSSION

For most topical products, the FDA recommends a bioequivalence study with clinical endpoints, in which the outcome for efficacy is either yes or no. It has been shown that studies with continuous outcomes, for example, oral bioavailability studies, have significantly higher statistical power than those with dichotomous outcomes (101,102). Due to the dichotomous nature of results of topical bioequivalence studies and the large inter-subject variability, the number of subjects enrolled in clinical endpoint trials can be several hundred to achieve sufficient statistical power. Because of the large, costly and sometimes even insensitive clinical endpoint trials, the FDA has acknowledged the need to develop alternate methods to demonstrate bioequivalence for locally acting drug products. Surrogate bioequivalence studies for topical products are even more complicated because generally less than 1% of the applied drug gets absorbed into the skin, making the surrogate methodology less sensitive, each methodology is association with considerably higher variability, and they have inherent inter-subject variation. Given that the sites of action for topical drugs are usually more accessible than for other routes, e.g. orally administered drugs, more meaningful comparisons for these systems can be made if drug levels can be measured directly at the site of action. Since topical drug products are meant to act at various sites, a single substitute method cannot be universally employed. There is a need to develop several methods in order to provide satisfactory alternate methods for different classes of drug products. When the original DPK guidance (34) was withdrawn in 2002, one of the concerns raised by the FDA was that the DPK method measures drug permeation through the healthy SC. As a result, it may not accurately predict therapeutic equivalence of drugs penetrating through pathways other than the SC, for example, hair follicles. Also, it may not be a good indicator of bioequivalency of products when used in diseased skin where integrity of SC is compromised. Consequently, the FDA

recommended skin stripping as a surrogate method for establishing bioequivalence of drugs for which the site of action is the stratum corneum itself, for example, antifungals (42,103). For drugs which act deeper into the subcutaneous tissue, microdialysis seems a promising method in spite of the technical challenges this methodology currently faces. *In vitro* diffusion studies could also be used for drug products for which the site of action is the epidermis or dermis, e.g. anti-acne drugs. The amount of drug that gets localized in each subcompartment over a period of time could be compared for bioequivalent generics and brand to establish the validity of the proposal. Independent verification will not only increase the validity but also contribute to refinement of the technique and encourage more widespread adoption.

There are several other methods still on the horizon. Near IR is the newest of all and most attractive due to its noninvasive and nondestructive nature. It has not been tested for bioequivalence purposes yet, but definitely offers exciting opportunities for research for molecules which are 'active in the near IR region' in an *in vivo* matrix. Another spectroscopy technique that has been explored to study composition of the skin constituents and, in some instances, drug penetration is Raman spectroscopy. Although Raman spectroscopy has been used as a semiquantitative tool to measure relative drug concentrations in the skin, its noninvasive nature and the prospects of measuring real-time drug penetration make the technique appealing. If it can be used to compare relative drug penetration into the skin from two different products to establish topical bioequivalence, it can open a new avenue for research.

In addition to the substitute bioequivalence methods suggested by the FDA, several other methods have been reviewed by Guy *et al.* for different classes of drugs (14,104). The vasoconstrictor assay, where the degree of skin blanching is measured as a function of percutaneous absorption of topical corticosteroids, is recognized by the FDA as a bioequivalence method. Similar measurements could be used in assessing absorption of topical NSAIDs. Topical NSAIDs have a diminishing action on vasodilatation caused by nicotinic acid. This can be measured using a chromometer or a laser Doppler velocimeter (105–111). The authors have also mentioned measurement of transepithelial water loss (TEWL) and irritation, using methods described above, to evaluate absorption of topical retinoids (110–112).

The future challenges for all methodologies discussed herein include optimization of each methodology, method verification and validation by independent laboratories, and proving reproducibility and sensitivity of the techniques for different classes of drugs. Above all, a continuing dialogue between the scientists and the FDA is necessary to establish clear guidelines on each method.

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