Pharmaceutical Research, Vol. 26, No. 2, February 2009 (© 2008)
DOI: 10.1007/s11095-008-9742-9

Research Paper

Improved Bioequivalence Assessment of Topical Dermatological Drug Products Using Dermatopharmacokinetics

Berthe N'Dri-Stempfer,[1,4] William C. Navidi,[2] Richard H. Guy,[3] and Annette L. Bunge[1,5]

Received August 6, 2008; accepted October 2, 2008; published online October 22, 2008

Purpose. A dermatopharmacokinetic (DPK) approach, in which drug levels in the stratum corneum (SC) are measured as a function of time post-application and post-removal of the product using tape-strip sampling *in vivo* in humans, has been considered for the comparative assessment of topical bioavailability. Its application to-date has been limited by contradictory results and concerns that variability in the method necessitates large numbers of treatment sites and volunteers. The objective of this study was to test whether a revised protocol could better assess bioequivalence.
Methods. A blinded study of three 1% econazole nitrate cream products, for which the SC is the site of action, was conducted to examine several modifications to the DPK methodology. In addition to protocol changes designed to reduce experimental variability, bioequivalence was assessed at a single uptake time and a single clearance time measured in duplicate in each subject.
Results. Conclusive determinations of bioequivalence were achieved with only four treatment sites per product in each of 14 volunteers, which was less than one-third the number required in a previous DPK investigation.
Conclusions. Comparative bioequivalence can be assessed conclusively with fewer treatment sites in fewer subjects with robust methods that should be less sensitive to inter-laboratory differences.

KEY WORDS: dermatopharmacokinetics; econazole; skin; stratum corneum; tape stripping; topical drug bioequivalence.

INTRODUCTION

The United States Food and Drug Administration (FDA) is seeking an alternative to a clinical efficacy study for the assessment of bioequivalence of topical drug products. In 1998, a draft guidance (1) was released proposing a dermatopharmacokinetic (DPK) approach, in which drug levels in the stratum corneum (SC) are measured as a function of time post-application and post-removal of the product using tape-strip sampling *in vivo* in humans. According to the guidance, the SC at the exposed site is removed by the sequential application and removal of 12 pieces of adhesive tape, of which the first two are discarded and the remaining ten are combined and quantified for drug. Topical products that produce comparable SC drug amount *versus* time profiles would be considered bioequivalent (BE) just as oral products are judged BE if they produce comparable plasma concentration *versus* time curves. The FDA guidance specified that topical product performance be quantified by the time integration of the drug amount in the stripped SC (AUC), the maximum amount per unit area in the SC (Q_{max}) and the time at which Q_{max} is first observed (T_{max}) (1). To develop the kinetic profiles, the guidance indicated that the amount of drug in the SC should be determined in no less than eight sites: at least four sites exposed to the product for different exposure periods, and four sites exposed to drug for the longest exposure period followed by four different clearance periods.

In May 2002, the FDA withdrew the DPK guidance (2) in part because a comparison study of a reference tretinoin gel with a bio-inequivalent product produced contradictory results (3–5). Further concerns were that the method: (a) may not accurately reflect therapeutic effectiveness if the target tissue is not the SC and penetration through another pathway (e.g., hair follicles) is important, or if the skin barrier function is perturbed by disease, and (b) might be so difficult and/or complex that the time and cost involved in setting up and validating the technique would be unacceptable. In response to these concerns, the FDA initiated studies to identify and evaluate sources of variability in the method, and to optimize the test procedure to minimize the number and complexity of the required tests while producing the required information for making a regulatory decision. Because the DPK method, which measures bioavailability in the SC directly, has not yet been validated for drugs acting on deeper tissues, these studies have been restricted to drugs that primarily target the

[1] Chemical Engineering Department, Colorado School of Mines, 1500 Illinois Street, Golden, Colorado 80401, USA.
[2] Mathematical and Computer Science Department, Colorado School of Mines, Golden, Colorado 80401, USA.
[3] Department of Pharmacy & Pharmacology, University of Bath, Bath, BA2 7AY, United Kingdom.
[4] *Present address*: Array BioPharma, Longmont, Colorado, USA.
[5] To whom correspondence should be addressed. (e-mail: abunge@mines.edu)

SC; specifically, the anti-fungal agents (6,7). The goals were to develop a DPK protocol that is reproducible within and between laboratories, that minimizes the number and complexity of the required tests, and that can be set up in any testing laboratory with reasonable scientific skill.

Based on results of a series of preliminary studies, which are described elsewhere (6), together with a re-analysis of the earlier tretinoin DPK data (8,9), five improvements to the original methodology of the 1998 guidance were proposed: (a) improved cleaning of excess drug from each test site at the end of the uptake period, (b) determination and inclusion of drug from the first two tape strips in the reported total amount taken up into the SC, (c) an increase in the number of tape strips collected combined with a method to ensure reliable collection of nearly all the SC, (d) improved control of the tape strip sampling area within the drug application area (to avoid edge effects), and (e) a procedure for analyzing the tape strips that can provide quantitative information on the potential uncertainty in the determination. The rationale for these five modifications are illustrated in the research described here.

Because the quantity of SC collected on the first two tape strips is highly variable, the amounts of absorbed drug present in these samples can also show considerable variability. Consequently, if drug found in the first two tape strips is not included in the analysis, the estimate of the total amount absorbed may be unreliable. The FDA's rationale for discarding the first two tapes was that they might include residual drug left behind by ineffective cleaning. Clearly, the best solution to this potential problem is to effectively clean the site and then include drug from all tape strips. Instead of using multiple cotton swabs or Q-tips to clean residual drug, as recommended in the guidance (and as used in the earlier tretinoin study (10)), we have shown that commercially available alcohol swabs are convenient and effective at cleaning the econazole cream products. The time needed for this procedure (≤30s) is so short that the amount of drug either removed from within the SC, or driven into the barrier, is likely to be negligible.

To reduce variability and to improve reproducibility, DPK determinations should be made on a similar fraction of the SC that is collected in a way that is independent of the subject, the operator, or the type of adhesive tape used. Ideally, as much of the drug in the SC as possible must be measured as the depth to which a drug penetrates varies in time. There is substantial evidence that the quantity of SC collected by a fixed number of tape strips is highly variable (11–13) and that often a significant fraction of the SC is not collected (13–15). Variability in tape adhesives, investigators carrying out the procedures, and site-to-site variation in cohesiveness of the SC within or between subjects all affect the quantity of SC collected on each tape strip. Methodologies to standardize application pressure do not resolve this problem (13,14).

It makes more sense, therefore, to require the number of tape strips collected from each site to be based on a reliable indicator that the SC has been nearly completely removed. Here, an eight-fold increase in transepidermal water loss (TEWL) was used to signal that most of the SC had been collected. Alternative metrics, such as spectrophotometric analysis of the tapes would also work (16,17); gravimetric determination (12,13,18,19) or protein quantification (20), are also available, but are more labor intensive and cannot be used in real time to judge that a sufficient number of tape strips has been collected.

Control of the SC sampling area at each treatment site ensures that: (a) the same area is stripped each time, and (b) artifacts are not introduced by edge effects at the limits of the application area. The latter problem has been identified (5) as the reason why conflicting results were obtained in the comparative tretinoin gel studies reported earlier (3,4). Unlike these and other earlier DPK investigations (3,4,10,21), in which the tape-stripped area was larger than the area of application of the formulation, we have restricted the treated and tape-stripped areas by rigid templates as illustrated in Fig. 1.

Drug levels in the SC collected on the tapes are often low, making reliable quantification difficult. Usually, drug levels in the tapes are quantified following extraction into solvent and analysis of the resulting solution by high performance liquid chromatography (HPLC) or gas chromatography (GC). To ensure that the maximum number of assay measurements provide results that are above the limit of

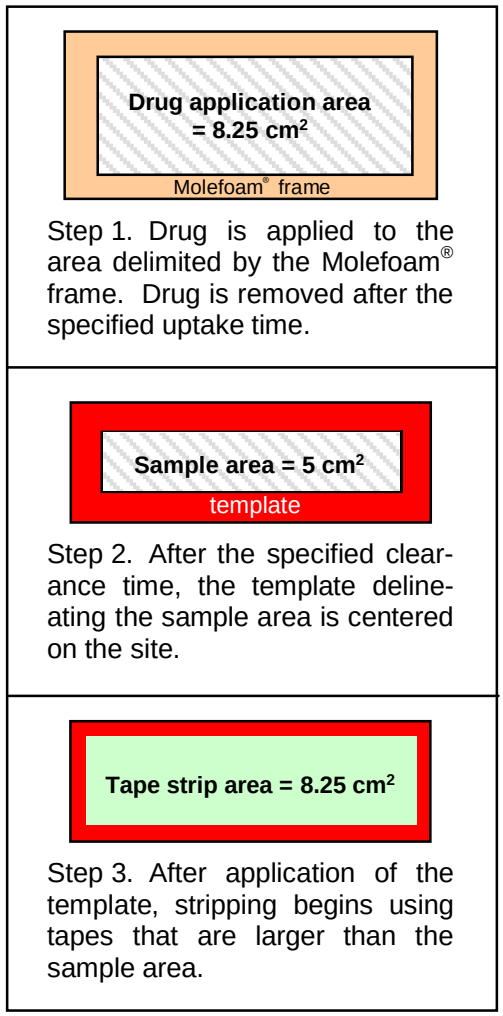

Fig. 1. Schematic diagram illustrating the drug application and tape stripping protocols.

reliable quantification (LOQ) or limit of detection (LOD) for the drug, a useful strategy is to divide the collected tape strips into a few groups for extraction into the least volume of solvent possible. The number of groups and the number of tapes in each group are chosen to improve drug detection and reduce the fraction of drug that is below LOQ and LOD. By judiciously choosing the tape groups, the drug determination from at least one group of tapes for each site is then above the LOQ. For any groups that are below LOQ, the maximum possible amount of drug below this value can be assigned; ideally, this would represent less than 10 to 20% of the amount drug measured in the extracts above the LOQ. In all cases, the first two tape strips are analyzed separately to ascertain the amount of drug that would not have been included in procedures following the 1998 FDA guidance. Depending on the total number of tapes collected, the remaining tapes are divided into two to four groups that are expected to contain similar amounts of SC as determined in a pilot study (6). Because the amount of SC decreases as tape number increases, the number of tapes in each group increases with tape number.

The present study examines three econazole nitrate creams. Preliminary DPK measurements were performed to identify suitable times for uptake and clearance. Because absorption and clearance of the anti-fungal drug were slow, and the amounts recovered in the SC tape strips were low, the eight time-point profile suggested by the FDA draft guidance (1) was impractical, particularly in light of the need to study all three formulations in all subjects (a requirement meaning that at least 1month had to elapse between treatments to allow for full recovery of the tape-stripped skin sites). Instead, the SC was exposed to drug for one uptake time of 6h, after which sites were cleaned and then sampled either immediately or after a clearance period of 17h. To study three different products at one uptake and one clearance time required six sites, and allowed six additional sites to be used to duplicate the determinations. The latter approach offered the potential advantage that statistical power would be increased enough to reduce the number of subjects required for a conclusive BE assessment.

The rationale for this simplified approach was based upon a re-analysis of the comparative study, which followed the DPK guidance, of three tretinoin gels (10,21). This re-assessment (9) found that the conclusion of bioequivalence from measurements at only one uptake and one clearance time was identical to that from the analysis of the entire DPK experiment. Furthermore, differences in bioequivalence for clearance and uptake, which were not detectable by the original analysis, could be discerned with the new approach.

MATERIAL AND METHODS

Materials

All solvents (acetonitrile, methanol and tetrahydrofuran) were from Mallinckrodt Chemicals (Phillipsburg, New Jersey) and either liquid chromatography or UV-spectrophotometric grade. Econazole nitrate and miconazole nitrate salts (100% and 99.5% purity, respectively) were purchased from Sigma-Aldrich (St. Louis, MO). Ammonium carbonate and potassium phosphate monobasic (both ACS certified) were from Fisher Chemicals (Fair Lawn, NJ) and phosphoric acid (Baker analyzed reagent with 85.5% purity) was from JT Baker Chemical Company (Phillipsburg, NJ). Deionized water was polished with a Millipore MILLI-Q reagent water system (Bedford, MA).

Three 1% (*w*/*v*) econazole nitrate creams were supplied by the FDA. The reference listed drug (RLD), Spectazole® from Ortho (now Johnson & Johnson) was compared to approved generic products (i.e., the therapeutic equivalence is listed as AB in the FDA Orange Book (22)) from Clay-Park (Bronx, NY) and Taro (Hawthorne, NY). It is evident from the package inserts that the Clay-Park product is qualitatively (i.e., Q1) equivalent but that the Taro product is not. Before beginning the study, the identity of the three drug products was blinded by placing approximately 10g of each product into identical 0.5-oz ointment tubes (Total Pharmacy Supply, Arlington, TX), which were sealed and labeled as A, B and C. At the end of the study, it was revealed that A was Clay-Park, B was Ortho, and C was Taro.

Drug Application and Cleaning

Fourteen volunteers (ten men and four women, 12 white and 2 Asian, 20 to 52 years old with a mean age of 26), with no history of dermatological disease, participated in the study, which was approved by the FDA Research Involving Human Subjects Committee (RIHSC). Informed consent was obtained from each subject.

One hour before drug was applied, the forearms of the volunteer were cleaned with a gentle soap solution (Purpose, Johnson & Johnson, Skillman, NJ), rinsed thoroughly with warm water and dried. Each treatment site (8.25cm^2 in area) was demarcated with a rectangular-shaped frame (dimensions of the inside hole were 1.5cm × 5.5cm) cut from self-stick adhesive Molefoam® (Dr. Scholl's® Molefoam® padding, Schering-Plough HealthCare Products Inc.), as illustrated in Fig. 1, and applied to the ventral forearm with the long dimension oriented across the forearm and a 3.1-cm distance (center-to-center) between sites. Drug was applied to a maximum of six sites on each arm, located so that the study area was at least 5cm above the wrist and a minimum of 0.5cm below the bend in the arm at the elbow. This requirement was satisfied in all but the smallest volunteer. A few tape strips were collected from one additional site, located above the wrist and below the drug application site closest to the wrist, for use in preparing the blank samples for the drug analysis.

Approximately 37.5mg of econazole nitrate cream was applied to a finger tip cut from a laboratory glove (N-Dex nitrile glove, Best Manufacturing, Menlo, GA), which was placed on the index finger of the applicator's gloved hand. Drug was distributed as evenly as possible over the demarcated area, providing approximately 4.5mg of product per square centimeter of exposed skin area, which corresponded to 45μg cm^{-2} of econazole nitrate. The actual amount delivered was determined by weighing the finger tip before and after drug application. A rectangular shaped piece (6cm × 2cm) of Teflon mesh (PTFE Mesh ET-8500, InterNet Incorporated) was then placed on top of the Molefoam

frame and held onto the skin with a non-occlusive, flexible dressing (Tegaderm® transparent strips, 3M, Minneapolis, MN). Skin contact with the Tegaderm® tape was prevented by the Teflon mesh, which was lifted above the skin surface by the Molefoam® template.

Drug was applied for 6 hours, after which the dressing and the Molefoam® frame were removed and residual drug cleaned from the skin by quickly and gently wiping the skin three times with a fresh alcohol swab wipe (isopropyl alcohol 70% *v/v*, Triad Disposable Inc. or H&P Industries Inc.) followed by gentle drying with a clean gauze pad (Dynarex® non-sterile gauze sponge, Dynarex Corporation). Because the total cleaning time of less than a 0.5min is much shorter than the approximately 12-h lag time for this drug to penetrate the SC, a minimal amount of the dermally absorbed drug should be removed by this procedure. Cleaned sites were either tape stripped immediately or re-covered with a clean Teflon mesh held on top of a fresh Molefoam® frame (positioned at the same location as the original frame) with Tegaderm® tape. After 17h, these dressings were removed and the sites tape stripped. To allow time for cleaning and tape stripping of each site, the individual drug applications were staggered.

For the convenience of the volunteers, sites that were tape stripped immediately after drug removal (i.e., uptake sites) were assigned to one forearm and sites that were tape stripped 17h after drug removal (i.e., the clearance sites) were assigned to the other arm. Volunteers were allowed to choose the arm assignments and all but one volunteer chose the right arm for the uptake sites. The three drug products were randomly assigned to the three sites on the upper forearm (site numbers 1 to 3 and 7 to 9 for uptake and clearance samples, respectively) and to the three sites on the lower forearm (site numbers 4 to 6 and 10 to 12 for uptake and clearance samples, respectively).

Tape Stripping of Skin

To obtain adequate amounts of drug for chemical analysis, custom-sized adhesive tape strips (1.5 × 5.5cm) were used that are larger than those commercially available. It was demonstrated independently that (a) the prepared tape strips (Scotch™ packaging tape, Series 3850, 3M, St. Paul, MN) did not lose mass when rubbed during application to the skin surface, (b) drug was readily extracted from SC that adhered to the tape, and (c) adhesive or other components of the tape did not interfere with chemical analysis of the drug in the tape extracts (6). Before tape stripping began, a rectangular-shaped template frame constructed from a thin Teflon® sheet (0.762mm thick), demarcating a 1cm × 5cm rectangular-shaped hole, was centered within the drug application site, as illustrated in Fig. 1, and held in place with a Velcro fastener that was attached to the ends of the template. By this means, all tape strips were applied to the same location on the test site and that edge effects near the Molefoam® template were minimized. Depending on the location of the test site, the shape of the volunteer's arm, and how tightly the template was attached, there was some small but noticeable bulging of the stripped site through the template hole. This could lead to tape strip collection from an area that was slightly larger than $5cm^2$, thereby contributing to the sampling variability. It was shown in the pilot study, during which the amounts of SC on the tapes were quantified, that the adhesive was released completely from the Teflon® template.

The adhesive tape strips were applied to the skin with vigorous rubbing by the gloved operator and then removed quickly. The process was repeated on a given site using at least 12, but not more than 30 tape strips. Tape strips were removed from the skin by pulling the narrow end in alternating directions for successive strips. The exact number of tapes that were applied was determined by the measurement of transepidermal water loss (TEWL) before the first tape strip and after a set number of tape strips using the AquaFlux® evaporimeter (Biox System Ltd., London, UK). Tape stripping was stopped if TEWL reached eight times the baseline value; however, no more than 30 strips were ever removed. Prior determinations of TEWL have shown that an eight-fold increase in the measurement indicates nearly complete removal of the SC (23). Compared with methods that specify the use of a fixed and a smaller number of tape strips, this TEWL-based procedure more reliably ensures nearly complete collection of the SC from each treatment site. As a result, this procedure is less sensitive to the effects of the formulation on the amount of SC removed per strip. In a pilot study, (7) the mass of skin removed by each tape strip was quantified by weighing tape strips before and after application to the skin (Mettler Toledo AX26DR Delta Range semi-microgram balance interfaced to PC for data collection). From these measurements, SC collection on the tape strips was determined to be reproducibly complete. As a result, adjustments of the measured drug amounts for differences in the amount of SC collected was not necessary and, in this study therefore, the SC on the tape strips was not quantified.

To confirm that the drug products had no significant effect on TEWL, the baseline value taken immediately after drug removal was compared with that determined on an adjacent site to which no drug had been applied. Generally, the differences observed were negligible. Because each TEWL determination takes approximately 1min, measurements were made so as to minimize the total number of evaluations per site. TEWL was first measured after twelve tape strips, and then following each subsequent four. Stripping was stopped as soon as TEWL reached, or surpassed, eight times the baseline value, or after a total of 30 tape strips, whichever occurred first. TEWL measurements for all sites are provided elsewhere (7).

Determination of Drug Amount from Tape Strips

Drug amounts below the LOQ or LOD are problematic in a BE assessment method that compares drug amounts determined at specified times instead of AUC. For this reason, the analytical procedure was designed to maximize drug detection while also providing quantitative information on the potential uncertainty in the determination, meaning the fraction of the measured drug amount that might be present, but determined poorly or not at all. This was accomplished by dividing the tape strips into groups that could be extracted in the solvent volume required to extract only one strip without adversely affecting the extraction efficiency. The goal was to divide the collected tapes into as

few groups as possible for reliable solvent extraction of drug to yield an amount in excess of the LOQ.

Chemical was assayed on all tapes; no tapes were discarded. The first two tape strips were combined and analyzed separately from the remaining strips. The remaining strips were combined into 2 to 4 groups of sequentially ordered tapes that, based on measurements in a pilot study, were estimated to contain ~750μg of SC.(6) Because the amount of skin collected decreases with increasing tape strip number, the number of tapes combined into a group increased with tape strip number. The total number of groups and the number of tapes in each group varied with the total number of tapes collected as listed in Table I.

Each group of tapes were placed into a 2-mL borosilicate vial (Clear SepCap Vials, 12 × 32mm, National Scientific Company) and extracted by gentle shaking with 1mL of methanol for approximately 1h (Roto-Shake Genie, Scientific Industries set to rotating motion at speed 8). After shaking, the samples were refrigerated for approximately 20 hours and then transferred to a 2-mL HPLC vial for analysis. Blank samples for each subject were prepared by following this same extraction procedure on tape strips collected from a separate ventral forearm site that was not exposed to the drug product. To squeeze several tapes into a small volume, each tape in the group was rolled separately into a cylinder of decreasing diameter. The smaller diameter cylinders were then slipped inside the larger ones. As no more than five tapes could be combined in these concentric cylinders, any remaining tapes were rolled into small diameters and squeezed between the concentric tape rolls.

A separate analysis of tapes containing known amounts of drug and SC was conducted to evaluate the efficiency of the extraction procedure (7). Good drug recovery was observed when as many as 8 tapes were combined (7). The recovery was 80–90% for groups containing four or more tapes, and the variability increased when the number of strips was greater than six. We expect that these results represent the worst case, because the econazole was not distributed as uniformly on these doped tape strips as it would be on tapes collected from the drug treatment sites of study volunteers. Also, most of the drug is usually present in the first two or three groups, which contain a smaller number of tapes (see Table I). A complete listing of the drug amounts determined for each group of tape strips is provided elsewhere (7).

Table I. Number of Consecutively Collected Tape Strips Combined into Groups for Drug Extraction as a Function of the Total Number of Tapes Collected

	Tape Group Number				
Total number of tapes	1	2	3	4	5
12	2	5	5	–	–
16	2	4	5	5	–
20	2	5	6	7	–
24	2	6	8	8	–
28	2	5	6	7	8
30	2	5	7	8	8

The amount of drug per unit area in the SC (Q) was calculated as the sum of drug in all groups of the tape strip samples normalized by the sample area (A) as follows:

$$Q = \frac{1}{A} \sum_{N=1}^{N_{\text{group}}} C_{\text{drug},N} V_{\text{extract},N} \quad (1)$$

where $V_{\text{extract},N}$ is the volume into which the Nth group of tape strips was extracted, N_{group} is the number of tape groups, and $C_{\text{drug},N}$ is the concentration of drug measured in the extract from the Nth group provided that $C_{\text{drug},N}$ was larger than the LOD. Measurements of the total drug mass larger than the LOQ were compared with estimates of the mass of drug on any tape groups that were below either the LOD or the LOQ. The magnitude of the potential uncertainty in the measured total drug mass was then estimated by calculating the amount of drug that could have been in groups of tapes determined to be below the LOD or LOQ.

HPLC Analysis

Econazole in the methanol extracts of the tape strips (10μL injection volume) was quantified by HPLC equipped with a diode array detector set at 220nm and operated at room temperature (typically, 23°C) using a SB-C18 acid resistant Stable Bond® analytical column (5mm pore size, 250mm long, 4.6mm diameter) and a guard column (4.6mm by 12.5mm) of the same packing (both from Agilent Technologies, Palo Alto, CA), and an acidic (pH 2.0) mobile phase of acetonitrile/0.1M potassium phosphate monobasic/phosphoric acid at a ratio of 46:53:1 (on a volume basis) pumped at a flow rate of 1mL min^{-1}. These conditions produce a pressure of about 130 bars and a retention time of approximately 8min for econazole nitrate.

For each experiment, the HPLC was calibrated using six standards, chosen to bracket the expected concentrations of the extract. Calibration standards were injected in triplicate; all samples were analyzed in duplicate (7). Following standard procedures (24–26), the LOD and LOQ were determined to be 44.3ng mL^{-1} and 147ng mL^{-1} (7). When expressed on the basis of skin sample area, the LOD or LOQ for a group of tapes was 8.86ng cm^{-2} and 29.4ng cm^{-2}, respectively, for a 5-cm^2 area and extraction into 1mL of methanol.

Assessing Drug Amount in Each Product

The amount of econazole nitrate was determined in the three commercial products following published procedures (27). Approximately 0.6g of the product was placed into the bottom of a 60-mL volumetric flask (Saybolt Viscosity, Class A, Wilmad-LabGlass, Ref. LG-8194-100, Buena, NJ), mixed with 1.2mL of tetrahydrofuran and shaken until completely dissolved. Miconazole nitrate (0.05mg mL^{-1}) in 12mL methanol was added as an internal standard. After mixing, further methanol was introduced to give a total volume of 60mL, after which the solution was mixed thoroughly and then filtered through a 0.2μm Teflon filter (Pall™ Life Sciences).

Samples (10μL injection volume) were analyzed under ambient conditions (~23°C) by a modification of the proce-

dure used for the tape-strip extracts. The mobile phase was a filtered aqueous solution of ammonium carbonate (0.1% *w/v*) mixed with methanol and THF in the ratio of 20:78:2 by volume. This was pumped at 2mL min^{-1} through a Zorbax Eclipse XDB-C18 analytical column (5mm pore size, 250mm long, 4.6mm diameter) and a guard column of the same packing (4.6mm by 12.5mm; Agilent Technologies, Palo Alto, CA). The retention times were about 6.0 and 10.0min for econazole nitrate and miconazole nitrate, respectively. Run time was 12min. Calibration curves were based on the absorbance peak areas of econazole nitrate or the peak-area ratio of econazole nitrate to miconazole (the internal standard). The efficiency of econazole extraction from the cream products was assessed by adding econazole nitrate to the drug-free cream base provided by the FDA (Placebo CPL R&D lot RX054) to give a nominal concentration of 1% w/v and analysis following the procedure described above.

Data Analysis

Because the distribution of dermal absorption measurements have been shown to be log normal (28–30), all quantities derived from the measured drug amounts were calculated in terms of the parameter $y_{ijk} = \log Q_{ijk}$, where Q_{ijk} is the amount of drug in the SC determined in the kth replicated site treated with product i on subject j. For K replicated measurements of each product in each subject, the mean log transformed amount of drug is:

$$\overline{y}_{ij.} = \frac{1}{K}\sum_{k=1}^{K}\overline{y}_{ijk} \tag{2}$$

The mean value and the upper and lower 90% confidence intervals were calculated for each product in all subjects for three quantities: (a) the amount (Q_i) of drug in the SC, (b) the ratio (R_i) of the amount of drug in the SC (Q_i) to the amount from the reference product (Q_{ref}), and (c) the ratio (W_i) of the amount of drug after clearance ($Q_i|_{\text{clearance}}$) to the amount before clearance ($Q_i|_{\text{uptake}}$). Both Q_i and R_i are calculated for 6h of uptake or 17h of clearance. For all three quantities, the mean value ($\overline{z}_{i..}$) and standard deviation (s_i) for product i averaged over J subjects is determined for the appropriate log transformed quantity $\overline{z}_{ij.}$ as follows:

$$\overline{z}_{i..} = \frac{1}{J}\sum_{j=1}^{J}\overline{z}_{ij.} \tag{3}$$

$$s_i^2 = \frac{1}{J-1}\sum_{j=1}^{J}\left(\overline{z}_{ij.} - \overline{z}_{i..}\right)^2 \tag{4}$$

The projected half-width of the 90% confidence interval for the population mean of product i ($\delta_{i,90\%}$) is then determined from Eq. (5):

$$\delta_{i,90\%} = \frac{s_i \cdot t_{0.05,J-1}}{\sqrt{J}} \tag{5}$$

where $t_{0.05,J-1}$ is the t-value of the two-tailed student distribution for a probability of 0.1 and $J - 1$ degrees of freedom. For each product i, the sample geometric mean for the untransformed quantity Z_i and the lower and upper 90% confidence bounds (i.e., $Z_{i,\text{lower}}$ and $Z_{i,\text{upper}}$, respectively) for the population geometric mean are calculated as follows:

$$Z_i = 10^{\overline{z}_{i..}} \qquad Z_{i,\text{lower}} = 10^{\left(\overline{z}_{i..} - \delta_{i,90\%}\right)} \qquad Z_{i,\text{upper}} = 10^{\left(\overline{z}_{i..} + \delta_{i,90\%}\right)} \tag{6}$$

To calculate the amount of drug Q_i, $\overline{z}_{ij.} = \overline{y}_{ij}$. To calculate the ratio of the amount of drug from product i and the reference product R_i, $\overline{z}_{ij.} = \overline{y}_{ij.} - \overline{y}_{rj.}$, where r designates the reference product. The ratio of the amount of drug after clearance to the amount before clearance W_i is calculated using $\overline{z}_{ij.} = \overline{y}_{ij.}\big|_{\text{clearance}} - \overline{y}_{ij.}\big|_{\text{uptake}}$. When quantities are reported for the three products combined, the procedure was to calculate the average of the log-transformed value for all products within a subject and then to average across all subjects.

Bioequivalence Evaluation

We assessed bioequivalence of each of the two generic products to the reference listed drug (i.e., Spectazole® from Ortho) for mean values of the drug amount in the SC measured after 6 hours of uptake or 17h of clearance in each subject using the Schuirmann two one-sided hypothesis test (for which the alternative hypothesis is the one of equivalence), which was applied to the ratio of average log-transformed responses (31,32). Traditionally, products have been considered bioequivalent if the 90% confidence interval for the ratio of the population geometric means are contained completely within the 0.8 to 1.25 interval (22). Occasionally, this requirement has been relaxed. For example, the 1998 draft guidance from FDA describing DPK assessment for topical bioequivalence specified that the 90% confidence interval for the ratio of the maximum amount of drug measured in the SC ($Q_{\max}$) only had to fall within the 0.7 to 1.43 interval to be considered bioequivalent. Following the traditional FDA criteria, products 1 and 2 are considered: (a) bioequivalent if $R_{i,90\%,\text{upper}}$ and $R_{i,90\%,\text{lower}}$ are both contained completely within the 0.8 to 1.25 interval, (b) non-bioequivalent if $R_{i,90\%,\text{upper}}$ and $R_{i,90\%,\text{lower}}$ are both outside the 0.8 to 1.25 interval, and (c) inconclusive if neither the bioequivalence nor non-bioequivalence criterion is satisfied.

Following this procedure, bioequivalence was assessed separately for measurements of drug uptake (i.e., drug in the SC was determined immediately after cleaning the treatment site, i.e., R_i was calculated using $\left(\overline{y}_{ij.} - \overline{y}_{rj.}\right)\big|_{\text{update}}$) and drug clearance (i.e., drug in the SC was determined some time after the cleaning the treatment site, i.e., R_i was calculated using $\left(\overline{y}_{ij.} - \overline{y}_{rj.}\right)\big|_{\text{clearance}}$). Also, bioequivalence was calculated for the sum of the logarithms of the amount of drug in the SC after uptake and clearance (i.e., R_i was calculated using $\left(\overline{y}_{ij.}\big|_{\text{uptake}} + \overline{y}_{ij.}\big|_{\text{clearance}}\right) - \left(\overline{y}_{rj.}\big|_{\text{uptake}} + \overline{y}_{rj.}\big|_{\text{clearance}}\right)$).

Estimated Lag Times

The lag time for clearance was estimated for each product from the ratio (W_i) of the amount of drug in the SC

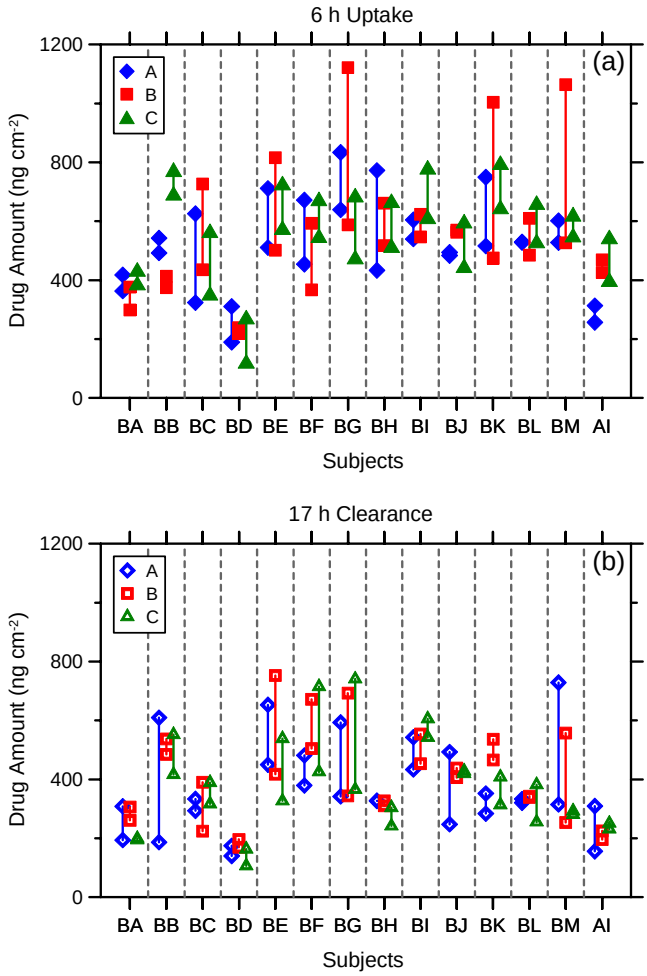

Fig. 2. Drug amounts per unit area of the duplicate determinations in each subject for three econazole products measured after: **a** 6 h of uptake, or **b** 17 h of clearance.

after the 17 hour clearance time to the amount of drug in the SC after no clearance time. For each of the three drug products, experimental values of W_i were compared with the theoretical equations for W, provided in the Appendix, to derive estimates of the lag time for penetration through the SC. In these calculations, an initial estimate of t_{lag} is determined from the following re-arrangement of Eq. (A3):

$$t_{lag} \cong -\frac{t - t_o}{6 \log_{10} W} \tag{7}$$

where t_o is the time at which drug was removed (i.e., 6h in this study) and $(t - t_o)$ is the clearance time (17 hours). As discussed in the Appendix, Eq. (7) only applies for $t_o > 1.2$. If this criterion is not meant, then a trial-and-error solution of the slightly more complicated expression, Eq. (A4), is required.

RESULTS

Drug Amount in Each Product

The amounts of econazole nitrate in each of the three formulations were not significantly different from each other (one-way ANOVA, $P = 0.62$). None of the three amounts differed significantly from 1% (90% confidence intervals are 0.996 ± 0.075% for Ortho, 1.014 ± 0.080% for Clay-Park, and 0.969 ± 0.069 for Taro).

Drug Amount in the Tapes and Bioequivalence

The detailed experimental results for the three econazole creams measured in fourteen subjects are provided elsewhere (7). The total amounts of drug measured in the tape strips from duplicate treatment sites for each product in each volunteer are shown in Fig. 2. Table II lists the mean total amounts of drug measured for each product in each subject after 6h of uptake or 17h of clearance. Overall, only about 1% of the applied drug was recovered in the SC 6h after application, and this was reduced by approximately 30% after

Table II. Means and Lower and Upper 90% Confidence Intervals for the Amount of Drug in the SC after 6 h Uptake ($Q_i|_{uptake}$), or 17 h Clearance ($Q_i|_{clearance}$), Following Application of Three Econazole Products; The Ratio of The Amounts After Clearance to Uptake (W_i) and the Lag Time for Penetration Through the SC (t_{lag}) are also Given

Quantity		Product[a]			
		A	B	C	All
$Q_i\|_{uptake}$ (ng/cm^2)	Mean	489	518	531	512
	Lower CI	423	441	450	443
	Upper CI	565	607	627	593
$Q_i\|_{clearance}$ (ng/cm^2)	Mean	338	376	339	350
	Lower CI	287	316	278	296
	Upper CI	397	446	414	415
W_i	Mean	0.690	0.726	0.638	0.684
	Lower CI	0.638	0.625	0.566	0.628
	Upper CI	0.747	0.843	0.720	0.744
t_{lag} (hours)	Mean	12.6	13.9	11.0	12.4
	Lower CI	11.0	10.7	9.2	10.7
	Upper CI	14.8	19.8	13.7	14.7

[a] *A* Clay-Park, *B* Ortho, and *C* Taro

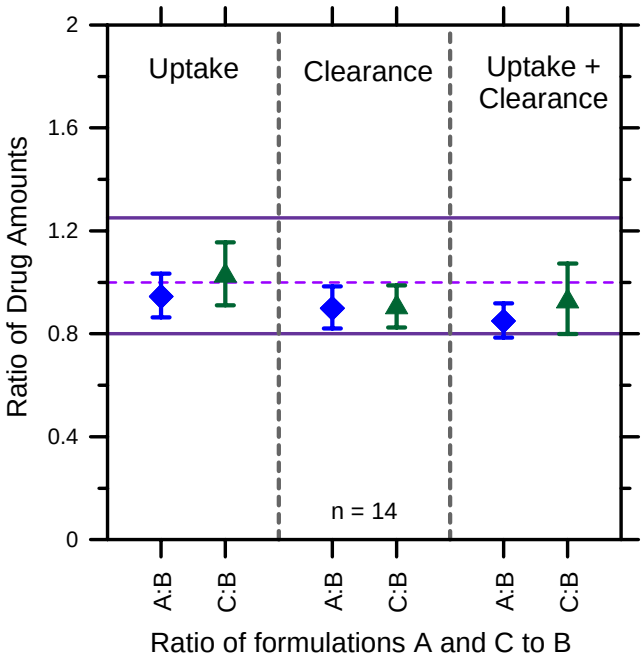

Fig. 3. Bioequivalence assessment of the generic econazole creams (*products A* and *C*) compared with the reference listed drug (*product B*) measured in 14 volunteers. Bioequivalence was evaluated using the ratio of the log-transformed amount of drug in the SC (mean± 90% confidence interval) after 6 h of uptake or 17 h of clearance. Traditionally, to be considered bioequivalent, the 90% confidence interval of the ratio must fall entirely within the indicated 0.8 to 1.25 interval.

17h of clearance. The ratios of drug amounts (mean ± 90% confidence interval) for products A or C compared with product B (the RLD) after uptake, clearance, or uptake and clearance combined are shown in Fig. 3 and Table III.

The variability in the amount of drug in skin for each of the three products is large (Table II). It is evident from Fig. 2 that the subject-to-subject variability is larger than the within subject variability, supporting the approach of comparing products within each subject. It is also evident that duplication improved the outcome. As a result, with studies from only 14 volunteers, the BE assessment (relative to the traditional BE confidence interval of 0.8 to 1.25) is conclusive for both products A and C (i.e., Clay-Park and Taro, respectively) for uptake and clearance analyzed separately, and almost conclusive for uptake and clearance combined (Table III, Fig. 3).

Clearance Rates and Lag Time Estimates

The mean values and 90% confidence intervals of the clearance-to-uptake ratio (W) for each product averaged over all volunteers are listed in Table II. From these values, the lag time (t_{lag}) to clear econazole from the SC has been estimated using t_o = 6 hours and ($t - t_o$) = 17h in either Eq. (A4) or Fig. 6 (in this case, $t_o/(6t_{lag})$ is too small for Eq. (7) to apply). On average, W was 0.684 for all products in all subjects, from which t_{lag} was estimated to be ~12.4h. The calculated lag times are consistent with the BE results, which showed that the mean clearance rates for both generic products (A and C) were faster than that for the reference listed product.

A challenge of the simplified BE methodology described here is to choose appropriate uptake and clearance periods. These should logically be related to the estimated lag time, but guidelines for the selection of sensible values for t_o/t_{lag} and $(t_o - t_{lag})/t_{lag}$ still need to be developed. Nevertheless, the analysis presented here illustrates how t_{lag} and the uptake and clearance ratios, t_o/t_{lag} and $(t_o - t_{lag})/t_{lag}$, can be experimentally quantified.

Number of Tape Strips and Efficiency of Drug Collection

Each site was tape stripped between 12 and 30 times. Tape stripping stopped at less than 30 tape strips if the TEWL measured at the site equaled or exceeded eight times the baseline rate. The number of tape-strips required was not influenced by the formulation applied suggesting that any differences in the vehicles studied in this work did not significantly influence the amount of SC removed per strip. In Fig. 4, the maximum, minimum and average number of tape strips collected are shown for each subject after 6h of uptake or 17h of clearance for all formulations combined. The number of tape strips collected was variable both within and between subjects. In the clearance phase for two subjects, the minimum and maximum numbers of tape strips required were 12 and 30. The mean number of tape strips collected was more than 15 in all but two subjects. There was no clear difference in the number of tape strips required after 17h of clearance compared with 6h of uptake alone.

Tape strips were combined into groups for solvent extraction and the groupings were selected to improve drug detection and to reduce the fraction of the total amount of drug that might be present but determined poorly (i.e., below the LOQ and LOD). The extracts of tapes collected immediately after 6 hours of uptake were below the LOD from only two of the 14 volunteers. After 17h of clearance,

Table III. Bioequivalence Assessment of the Generic 1% Econazole Creams (Products A and C, from Clay-Park and Taro, Respectively) Compared with the Reference Listed Drug (Product B from Ortho) Determined in 14 Volunteers

	Product A			Product C		
BE Metric	Mean	Lower CI	Upper CI	Mean	Lower CI	Upper CI
Uptake	0.945	0.863	1.034	1.026	0.911	1.156
Clearance	0.899	0.821	0.984	0.903	0.825	0.988
Uptake and Clearance	0.850	0.785	0.919	0.926	0.799	1.073

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 with or without 17 hours clearance

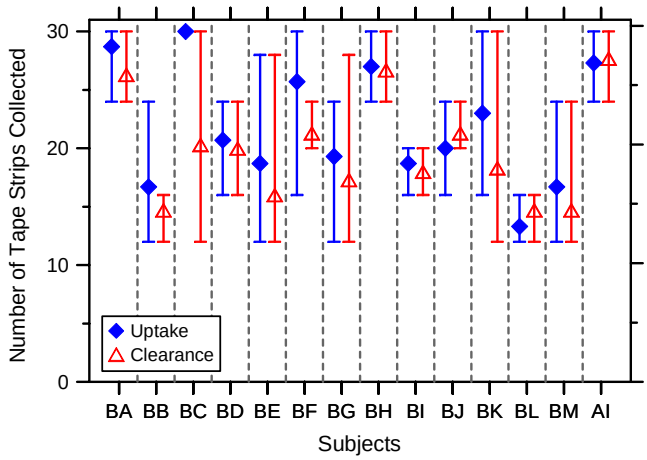

Fig. 4. Number of tape strips collected from each subject after 6 h of uptake (*filled diamonds*) or 17 h of clearance (*open triangles*). The symbols designate the arithmetic mean number of tape strips collected for the duplicated sites treated with the three formulations (i.e., six sites). The upper and lower limits respectively designate the largest and smallest number of tape strips that were collected from the six sites in each subject.

samples from three volunteers were below the LOD. Maximum and minimum estimates of the percentage of drug that was less than the LOQ (based on the amount of drug greater than LOQ at each site) are listed in Table IV for uptake and clearance separately. The maximum estimates were calculated assuming that all samples with determinations below the LOQ and LOD contained the maximum amount of drug (i.e., 147 and 41ng cm^{-2} for LOQ and LOD, respectively). The minimum estimates were calculated assuming all samples below the LOQ and LOD contained the minimum amount of drug (i.e., 41 and 0ng cm^{-2} for the LOQ and LOD, respectively). The actual percentage of drug less than the LOQ is bounded by the minimum and maximum estimates. In Table IV, the mean, high and low values of the maximum and minimum estimates are listed for the six test sites after 6h of uptake sampled either immediately or after 17h of clearance. As expected, the percentage of drug below LOQ is higher after 17h of clearance. On average, after 6h of uptake between 1.6 and 5.5% of the drug in the collected tape strips was less than the LOQ; after 17h of clearance, between 3.5 and 12.4% of the drug was less than the LOQ.

Amount of Drug in First Two Tape Strips

Almost half of the amount of drug greater than the LOQ was collected in the first two tape strips (Fig. 5). In the 84 sites sampled after 6h of uptake (i.e., six sites in 14 volunteers), the first two tape strips contained 43.2 ± 10.4% (mean ± 1 standard deviation) of the drug amount greater than the LOQ. After 17h of clearance, the amount of drug in the first two tape strips decreased, but the percentage of the drug amount increased to 52.6 ± 9.4%.

DISCUSSION

Any estimate of the mass ratio for two products is subject to both inter- and intra-subject variability. Inter-subject variability arises from differences in physiologic characteristics but is not influenced by the experimental method. Intra-subject variability results from site-to-site variations in forearm skin and from experimental error. As the magnitude of site-to-site variation is almost certainly much smaller than that introduced by experimental factors, it follows that intra-subject variability can be reduced by improving the experimental method. Such improvements can substantially increase the power of BE tests and reduce, thereby, the number of subjects needed to obtain a definitive comparison. The results of this econazole study substantiate this line of thinking: 14 subjects and 56 treatment sites per product were required to establish BE, an outcome that compares very favorably with the earlier tretinoin investigation (10,21), which followed the 1998 FDA guidance document (1) to also evaluate a reference product and two generics, and involved 49 subjects and 392 treatment sites.

Further, in the tretinoin study (10,21), tapes 3–10 were combined and extracted as a single group; hence, there were eight chemical analyses required for each product on each subject (i.e., 392 analyses = 49 volunteers multiplied by 8 analyses/volunteer). In this econazole investigation, tapes from each site were divided into three to five groups, with an average of 4.13. The average number of analyses, therefore, was 16.5 per subject for each product (i.e., 4.13 analyses multiplied by 4 sites/product), and 231 in total for each product (i.e., 14 subjects multiplied by 16.5 analyses/product). So, although, the revised protocol required more analyses per subject, the reduced experimental variability of the new method allowed conclusive BE assessment with 40% fewer chemical analyses and one-third as many volunteers.

Table IV. Ratios (in Percent) of the Estimated Amount of Drug Below LOQ to the Amount Above LOQ

	Uptake			Clearance		
Estimate of the amount < LOQ[a]	Mean	Highest	Lowest	Mean	Highest	Lowest
Maximum	5.5	11.1	2.1	12.4	21.6	6.3
Minimum	1.6	3.2	0.6	3.5	4.3	1.0

[a] Maximum and minimum estimates for the amount less than the LOQ were calculated using the maximum and minimum values, respectively for the LOQ and LOD. The maximum estimate used the maximum values of 147 and 41 ng/cm^2 for LOQ and LOD, respectively; the minimum estimate used the minimum values of 41 and 0 ng/cm^2 for LOQ and LOD, respectively.
For each subject, and for both uptake and clearance, maximum and minimum estimates of the six ratios, representing three products with duplicate measurements, were calculated. The table presents maximum and minimum estimates of the average over all subjects of the mean, the lowest, and the highest of the six ratios for both uptake and clearance.

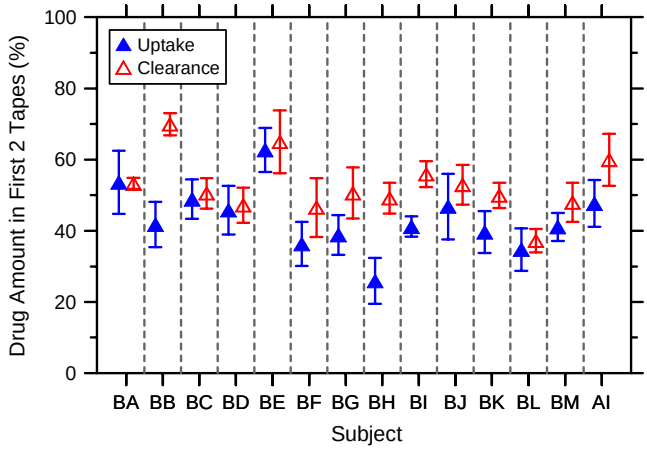

Fig. 5. Percentage of the amount of drug greater than the LOQ that was in the first two tape strips (arithmetic mean±1 standard deviation for duplicate determinations of the three products) after 6 h of uptake alone and followed by 17 h of clearance.

These latter observations are reinforced by a recent simulation exercise (8) that compared data from the two-time method presented here with those from the AUC approach that was used in the earlier tretinoin investigation (10,21). Assuming products to be bioequivalent if both confidence intervals were contained in the 0.80 to 1.25 interval, it was shown that the two-time method could achieve the same accuracy with less than 40% as many volunteers. This improved efficiency was demonstrated for both BE and non-BE products, and is due both to the optimized experimental protocol and to the replication of measurements. Both approaches are specific, in that they rarely misclassify BE products as non-BE or vice versa. The advantage of the two-time method, as demonstrated here, is that it is much less likely to produce inconclusive results.

The degree of variability in the data presented here has recently (8) been compared qualitatively to that from the tretinoin study (10,21). The variances across subjects of the measurements taken at any given time were considerably higher in the earlier work. Given that there is no reason to expect any difference in inter-subject variability between the two studies, it may be concluded that the method used in the econazole experiments reduced the variability. One of the more significant improvements to the protocol was undoubtedly the inclusion of drug from the first two tape strips in the reported total amount. Even with the improved cleaning procedure to remove excess drug from the test sites, the proportion of the total amount of drug greater than the LOQ, that was collected in the first two tape strips, remained considerable: based on the 90% confidence intervals, from 26 to 61% for sites measuring uptake and from 37 to 68% in sites measuring clearance.

Several research questions remain to be answered before any method for determining bioavailability, including an improved DPK protocol, can be used to confidently assess topical BE. Firstly, it is critical to demonstrate that: (a) the proposed method can correctly identify non-bioequivalent products, and (b) different laboratories using an optimal method reach the same conclusions regarding BE of equivalent and inequivalent products.

Secondly, an appropriate BE criterion (i.e., the 90% confidence interval within which the log normal data must lie to be BE) needs to be specified. The chosen confidence interval needs to be narrow enough to minimize the likelihood of false positives (i.e., that non-BE products are evaluated as BE), but sufficiently wide to avoid false negatives (i.e., BE products are evaluated as either not BE or inconclusive). To experimentally assess the 0.8 to 1.25 criterion requires several studies of products that are either known to be BE or not BE. A logical starting point might be to assess the 0.8 to 1.25 criterion with mathematical simulations of BE and non-BE situations using estimates of variability based upon the experimental results described here.

Thirdly, guidelines, with which to select uptake and clearance times, need to be developed. The choice of clearance time is the easier of the two and can be specified relative to the drug's estimated lag time for penetrating the SC. This might be determined in a pilot study following procedures similar to those outlined above. Although further research is needed to properly specify the appropriate range, a reasonable proposal would be one to five times the lag time. In this econazole study, for example, the clearance time used was approximately 1.4 times the lag time. A recommended uptake time is less clear. In this study, the value was chosen based on experimental observations that the drug amount in the SC increased only slowly after 6h (~1/2 the lag time). More pragmatically speaking, a longer uptake time would have complicated the scheduling of volunteer participation. Alternatively, as two therapeutically equivalent products should produce the same drug levels in the SC when used as prescribed, an appropriate "uptake time" might be after repeated applications, following the prescribed frequency of dosing, such that steady-state drug levels in the SC are established. It is worth noting that the lag times for most dermatological drugs (like econazole) are likely to be >12h; it follows that the FDA guidance recommendation, that the longest drug uptake time should result in steady state being achieved, is simply impractical unless the product is reapplied.

Other issues include examination of different cleaning methods for removing drug from the skin surface, and the effect of applied dose; i.e., is the revised DPK approach sensitive to the impact of applying either different amounts of a given (constant concentration) product per area, or the same amount per area of formulation using products containing different concentrations of active.

Finally, a regulatory-worthy DPK method must also include specifications for the type of tape employed. The material should meet the following requirements: (a) drug should be easily solvent-extractable from the SC adhered to the tape, and (b) the adhesive or other extractable components of the tape should not interfere with the analytical method used to quantify drug in the solvent extract. The possibility of allergy to the tape adhesive exists and must be assessed; however, because the contact time with the tape used for stripping is relatively short, there is perhaps more concern about the adhesives used on the materials employed to protect treatment sites during the experiment.

CONCLUSIONS

Several modifications to the FDA guidance (1) for the use of DPK to evaluate the bioequivalence of topical

products were evaluated in a study comparing an innovator and two generic econazole creams. The number of sampling times was reduced to only two (one after an uptake time of 6h and the other following a clearance period of 17h) and the measurements were duplicated in each subject. In addition, the protocol was changed to reduce experimental variability by ensuring that most of the drug in the SC was collected, thereby enabling conclusive determinations of bioequivalence with only 14 volunteers and four sites per product assessed. Specifically, three modifications in the method were made, with the supplemental goal of significantly reducing laboratory-to-laboratory variability: (a) transepidermal water loss (TEWL) measurements were made to assess the fraction of the SC removed by the tape-stripping procedure, (b) a new cleaning procedure reduced variability by improving removal of residual drug before tape stripping, and (c) drug present on all tape strips was included in the comparison of the amounts taken up into the SC from different products. Furthermore, the collected tape strips were analyzed for drug in groups selected both to improve analytical sensitivity and to provide an estimate of the amount of drug that is quantified poorly.

ACKNOWLEDGMENTS

We acknowledge support from the FDA. No endorsement from the FDA or US government should be inferred.

APPENDIX

The amount of drug within the SC varies with time (t) relative to the lag time for diffusion through the SC (t_{lag}) during the uptake and clearance periods as described previously (9). If drug is removed from the skin surface at time t_o, then the amount of drug in the SC at a given time (t) is described by Eqs. (A1) and (A2) for uptake (i.e., $t < t_o$) and clearance (i.e., $t > t_o$), respectively:

$$\frac{Q}{Q_o} = \frac{1}{2} + \frac{4}{\pi^2}\sum_{n=0}^{\infty}\frac{\exp\left[-(2n+1)^2\pi^2 t \Big/ \left(6t_{lag}\right)\right]}{(2n+1)^2} \qquad \text{for } t \le t_o \tag{A1}$$

$$\frac{Q}{Q_o} = 2\sum_{n=0}^{\infty}\frac{(-1)^n \exp\left[-\lambda_n^2 (t-t_o)/6t_{lag}\right]}{\lambda_n}\left(\frac{1}{\lambda_n^2} - 2\sum_{m=1}^{\infty}\frac{\exp\left[-m^2\pi^2 t_o/(6t_{lag})\right]}{m^2\pi^2 - \lambda_n^2}\right)$$
$$\text{where } \lambda_n = \frac{2n+1}{2}\pi \qquad \text{for } t > t_o \tag{A2}$$

In these equations, Q is the total amount of drug per area in the SC, $Q_o = K \times C_v \times L$, where K is the SC-vehicle partition coefficient, C_v is the drug concentration in the vehicle and L is the thickness of the SC), and t_o is the time at which drug was removed. In deriving Eqs. (A1) and (A2) it is assumed that (9): (a) drug absorption into the SC is slow compared with release from the vehicle, (b) drug transport in the SC is Fickian with a constant diffusion coefficient in a pseudo-homogeneous membrane of thickness L, (c) drug concentration in the vehicle applied to the skin surface is constant, and (d) sink conditions (i.e., drug concentration is zero) exist at the inner boundary of the SC.

The predicted variation in W as a function of clearance time ($t - t_o$) relative to t_{lag} is shown in Fig. 6. The curves in this figure were derived by dividing the amount in the SC (i.e., Q calculated from Eq. (A2) for a time after drug was removed from the SC surface (i.e., $t>t_o$) by the amount in the SC at the time drug was removed (i.e., at time $t=t_o$, Q was calculated from Eq. (A1)). Strictly, these results only apply if the skin layers beneath the SC provide little resistance to clearance and the diffusion coefficient and partition coefficient in the SC do not change significantly as the drug clears (9).

As shown in Fig. 6, W is insensitive to the time that drug is cleaned from the surface (t_o) as along as t_o is greater than about 1.2 times t_{lag}. Furthermore, the logarithm of W is linear with respect to time and represented reasonably well by the equation:

$$\log_{10} W \cong -(t - t_o)/\left(6t_{lag}\right) \tag{A3}$$

Significantly, 1.2 t_{lag} is about half the time required to reach steady state, which occurs at approximately 2.4 t_{lag} (33).

When $t_o < 1.2\ t_{lag}$, $\log_{10}W$ remains mostly linear in time; the slope is the same as that when $t_o>1.2\ t_{lag}$. However, the intercept at $t=t_o$ is larger than 1, and it increases as t_o decreases relative to t_{lag}. Linear regression of the results shown in Fig. 6 for $(t_o)<1.2\ t_{lag}$ yields Eq. (A4):

$$\log_{10} W = -1.072\,(t - t_o)/(6\,t_{lag}) + \log_{10}\left(0.978 - 0.133\ \log_{10}\left[t_o/(6\,t_{lag})\right]\right) \tag{A4}$$

which should be used when $0.06\ t_{lag}<t_o<1.2\ t_{lag}$. But, if $t_o <\sim 0.1\ t_{lag}$, Eq. (A4) should not be used if $(t-t_o)<0.3\ t_{lag}$, as

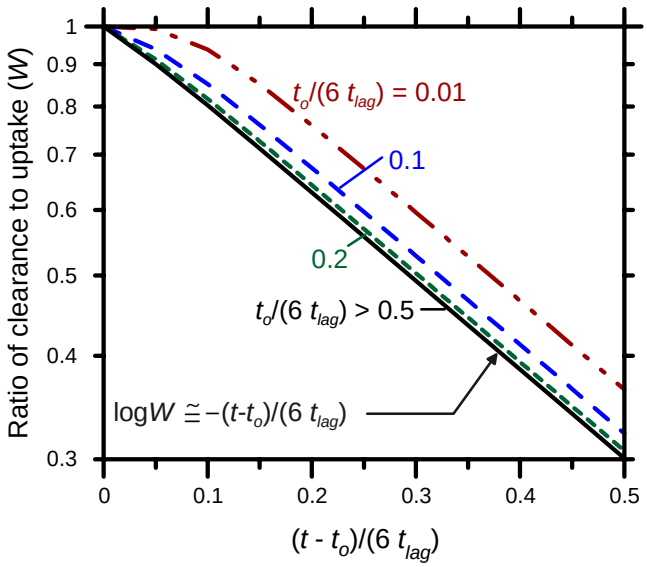

Fig. 6. Logarithm of the calculated amount of drug in the SC at time t after cleaning the skin surface at time t_o, normalized by the mass of drug in the SC at t_o, plotted as a function of normalized time after drug removal, i.e., $(t-t_o)/(6\ t_{lag})$. Curves are shown for different values of the time at cleaning (t_o) normalized by t_{lag}. The logarithm of the drug mass ratio is linear with respect to $(t-t_o)/(6\ t_{lag})$ and essentially insensitive to the cleaning time (i.e., $t_o/(6\ t_{lag})$) provided that $t_o>\sim 1.2\ t_{lag}$.

the assumption of linearity with $(t-t_o)$ no longer applies. The calculated results shown in Fig. 6, or provided by Eqs. (A3) or (A4), can be used to derive estimates for t_{lag} from experimental measurements of *W*.

REFERENCES

1. US FDA. Guidance for Industry: topical dermatological drug product NDAs and ANDAs-*in vivo* bioavilability, bioequivalence, *in vitro* release, and associated studies. Draft Guidance, June 1998, U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), (1998).
2. US FDA. Guidance for industry on special protocol assessment; availability. *Fed. Regist.* **67**:35122 (2002).
3. L. K. Pershing. Bioequivalence assessment of three 0.025% tretinoin gel products: Dermatopharmacokinetic *vs.* Clinical Trial Methods, Transcribed presentation to the *Advisory Committee for Pharmaceutical Sciences Meeting, Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA)*, Rockville, MD, November 29, 2001; presentation slides available at http://www.fda.gov/ohrms/dockets/ac/01/slides/3804s2_02_Pershing/index.htm; transcript of presentation available at http://www.fda.gov/ohrms/dockets/ac/01/transcripts/3804t2_01_Morning_Session.pdf pp. 31–47
4. T. J. Franz. Study #1, Avita Gel 0.025% *vs* Retin-A Gel 0.025%, Transcribed presentation, *Advisory Committee for Pharmaceutical Sciences Meeting, Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA)*, Rockville, MD, November 29, 2001; presentation slides available at http://www.fda.gov/ohrms/dockets/ac/01/slides/3804s2_03_franz.pdf transcript of presentation available at http://www.fda.gov/ohrms/dockets/ac/01/transcripts/ 3804t2_01_Morning_Session.pdf pp. 47–61
5. D. P. Conner. Differences in DPK Methods, Transcribed presentation to the *Advisory Committee for Pharmaceutical Sciences Meeting, Center for Drug Evaluation and Research (CDER), Food and Drug Administration (FDA)*, Rockville, MD, November 29, 2001; presentation slides available at http://www.fda.gov/ohrms/dockets/ac/01/slides/3804s2_05_conner/index.htm; transcript of presentation available at http://www.fda.gov/ohrms/dockets/ac/01/transcripts/3804t2_01_Morning_Session.pdf, pp 71–75
6. A. L. Bunge, B. N'Dri-Stempfer, W. C. Navidi, and R. H. Guy. Dermatopharmacokinetics: improvement of methodology for assessing bioequivalence of topical dermatological drug products, Revised Final Report, Award No. D3921303, Submitted to Department of Health and Human Services, Food and Drug Administration, Colorado School of Mines, Golden, CO, September 2, 2006.
7. A. L. Bunge, B. N'Dri-Stempfer, W. C. Navidi, and R. H. Guy. Therapeutic Equivalence of Topical Products, Final Report, Award No. 223-04-3004, Submitted to Department of Health and Human Services, Food and Drug Administration, Colorado School of Mines, Golden, CO, January 30, 2007 (Revision submitted June 2008).
8. W. Navidi, A. Hutchinson, B. N'Dri-Stempfer, and A. L. Bunge. Determining bioequivalence of topical dermatological products by tape-stripping. *J. Pharmacokin. Pharmacodyn.* Article in press, doi:10:1007/s10928-008-9091-7 (2008).
9. B. N'Dri-Stempfer, W. C. Navidi, R. H. Guy, and A. L. Bunge. Optimizing metrics for the assessment of bioequivalence between topical drug products. *Pharm. Res.* **25**:1621–1630 (2008) Medline. doi:10.1007/s11095-008-9577-4.
10. L. K. Pershing. Final Report to Food and Drug Administration (FDA): Dermatopharmacokinetic Bioequivalence Study on Three Tretinoin Gel, 0.025% Products, University of Utah, Salt Lake City, 2000.
11. L. K. Pershing, J. L. Corlett, and J. L. Nelson. Comparison of dermatopharmacokinetic *vs.* clinical efficacy methods for bioequivalence assessment of miconazole nitrate vaginal cream, 2% in humans. *Pharm. Res.* **19**:270–277 (2002) Medline. doi:10.1023/A:1014486716823.
12. Y. N. Kalia, I. Alberti, N. Sekkat, C. Curdy, A. Naik, and R. H. Guy. Normalization of stratum corneum barrier function and transepidermal water loss *in vivo*. *Pharm. Res.* **17**:1148–1150 (2000) Medline. doi:10.1023/A:1026474200575.
13. L. M. Russell, S. Wiedersberg, and M. B. Delgado-Charro. The determination of stratum corneum thickness—an alternative approach. *Eur J Pharm Biopharm* **69**:861–870. doi:10.1016/j.ejpb.2008.02.002 (2008).
14. C. Pellanda, E. Ottiker, C. Strub, V. Figueiredo, T. Rufli, G. Imanidis, and C. Surber. Topical bioavailability of triamcinolone acetonide: effect of dose and application frequency. *Arch. Dermatol. Res.* **298**:221–230 (2006) Medline. doi:10.1007/s00403-006-0677-x.
15. I. Jakasa, M. M. Verberk, A. L. Bunge, J. Kruse, and S. Kezic. Increased permeability for polyethylene glycols through skin compromised by sodium lauryl sulphate. *Exp. Dermatol.* **15**:801–807 (2006) Medline. doi:10.1111/j.1600-0625.2006.00478.x.
16. H.-J. Weigmann, U. Lindemann, C. Antoniou, G. N. Tsikrikas, A. I. Stratigos, A. Katsambas, W. Sterry, and J. Lademann. UV/VIS absorbance allows rapid, accurate, and reproducible mass determination of corneocytes removed by tape stripping. *Skin Pharmcol. Appl. Skin Physiol.* **16**:217–227 (2003) Medline. doi:10.1159/000070844.
17. U. Lindemann, H.-J. Weigmann, H. Schaefer, and W. Sterry. Evaluation of the pseudo-absorption method to quantify human stratum corneum removed by tape stripping using protein absorption. *Skin Pharmcol. Appl. Skin Physiol.* **16**:228–236 (2003) Medline. doi:10.1159/000070845.
18. L. K. Pershing, S. Bakhtian, C. E. Poncelet, J. L. Corlett, and V. P. Shah. Comparison of skin stripping, *in vitro* release, and skin blanching response methods to measure dose response and similarity of triamcinolone actonide cream strengths from two manufactured sources. *J. Pharm. Sci.* **91**:1312–1323 (2002) Medline. doi:10.1002/jps.10147.
19. F. Pirot, Y. N. Kalia, A. L. Stinchcomb, G. Keating, A. Bunge, and R. H. Guy. Characterization of the permeability barrier of human skin *in vivo*. *Proc. Nat. Acad. Sci. USA*. **94**:1562–1567 (1997) Medline. doi:10.1073/pnas.94.4.1562.
20. F. Dreher, A. Arens, J. J. Hostynek, S. Mudumba, J. Ademola, and H. I. Maibach. Colorimetric method for quantifying stratum corneum removed by adhesive-tape stripping. *Acta. Derm. Venereol. (Stockh)*. **78**:186–189 (1998). doi:10.1080/00015559 8441495.
21. L. K. Pershing, J. L. Nelson, J. L. Corlett, S. P. Shrivastave, D. B. Hare, and V. P. Shah. Assessment of dermatopharmacokinetic approach in the bioequivalence determination of topical tretinoin gel products. *J. Am. Acad. Dermatol.* **48**:740–751 (2003) Medline. doi:10.1067/mjd.2003.175.
22. US FDA. Approved drug products with therapeutic equivalence evaluations (Electronic Orange Book), U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Office of Pharmaceutical Science, Office of Generic Drugs, http://www.fda.gov/cder/orange/obannual.pdf (2007).
23. Y. N. Kalia, F. Pirot, and R. H. Guy. Homogeneous transport in a heterogeneous membrane: Water diffusion across human stratum corneum *in vivo*. *Biophys. J.* **71**:2692–2700 (1996).
24. US EPA. Assessing Values to Non-Detected/Non-Quantified Pesticide Residues in Human Health Food Exposure Assessments, Guidance Document: Office of Pesticide Programs, (March 23, 2000), Washington, DC, http://www.epa.gov/pesticides/trac/science/trac3b012.pdf, 2000.
25. V. R. Meyer. *Practical high-performance liquid chromatography*. Wiley, West Sussex, England, 1994.
26. J. Corley. Best practice in establishing detection and quantification limits for pesticide residues in foods. In P. W. Lee (ed.), *Handbook of Residue Analytical Methods for Agrochemicals, Volumes 1–2*, Wiley, West Sussex, England, 2003.
27. R. Christinat, and H. W. Zulliger. Stability indicating HPLC-method for the determination of econazole nitrate in cream and lotion formulations. *Arzneimittel. forschung/Drug Res.* **34**:551–553 (1984).
28. P. A. Cornwell, and B. W. Barry. Effects of penetration enhancer treatment on the statistical distribution of human skin permeabilities. *Int. J. Pharm.* **117**:101–112 (1995). doi:10.1016/0378-5173(94)00341-2.

29. A. C. Williams, P. A. Cornwell, and B. W. Barry. On the non-Gaussian distribution of human skin permeabilities. *Int. J. Pharm*. **86**:69–77 (1992). doi:10.1016/0378-5173(92)90032-W.
30. G. B. Kasting, T. G. Filloon, W. R. Francis, and M. P. Meredith. Improving the sensitivity of *in vitro* skin penetration experiments. *Pharm. Res*. **11**:1747–1754 (1994) Medline. doi:10.1023/A:1018915416930.
31. D. J. Schuirmann. A comparison of the two one-sided tests procedure and the power approach for assessing the equivalence of average bioavailability. *J. Pharmacokinet. Biopharm*. **15**:657–680 (1987) Medline. doi:10.1007/BF01068419.
32. G. E. P. Box, and D. R. Cox. An analysis of transformations. *J. R. Stat. Soc., Series B*. **26**:211–252 (1964).
33. A. L. Bunge, R. L. Cleek, and B. E. Vecchia. A new method for estimating dermal absorption from chemical exposure. 3. Compared with steady-state methods for prediction and data analysis. *Pharm. Res*. **12**:972–982 (1995) Medline. doi:10.1023/A:1016298012408.