Design and Modeling Considerations for Skin Permeation Studies

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Abstract

Transdermal Drug Delivery Systems (TDDS), also referred to as patch products, are becoming a more common drug delivery form since their convenience and dosing properties make them an attractive alternative to oral and other forms of drug delivery. In vitro skin permeation or skin diffusion analytical methods are commonly used to assess product quality, changes on stability, formulation and manufacturing effects. We present a novel application of a randomized nested block design that permits an unambiguous decomposition of the total variability into its component parts of skin donor, pieces nested within skin and residual error. With such a design, changes in the skin permeation profile on stability for a drug product lot are appropriately estimated and tested. We provide a case study to illustrate the design and associated statistical modeling.

Key Words: Skin permeation, mixed effects modeling, nested design

1. Introduction

Transdermal Drug Delivery Systems (TDDS) are an easy to use, painless and convenient way to deliver a therapeutically active compound. TDDS are formulated with a pressure-sensitive adhesive that assures the adhesion of the patch to the skin. The choice of excipients produces slow delivery of the pharmacologically active compound through the intact skin leading to a constant systemic absorption rate and a stable plasma concentration profile. For these reasons, patient compliance may be improved and side effects may be minimized. As such, there is good potential for many more compounds to be formulated as TDDS to add to the 25 products currently marketed in the US. There are requirements on the active compound's chemical and physical properties that restrict its delivery through the skin, a dynamic biological matrix, however it is beyond the scope of this paper to delve deeply into these technical aspects. Rather, the focus of this paper is to elucidate the value of a statistical design in unambiguously assessing the variance components associated with skin donor for an in vitro skin permeation or skin diffusion analytical method. This is important, since the development of a TDDS as a drug product must satisfy regulatory rules for marketing, which includes chemical and physical characterization for quality control purposes requiring validated analytical methods.

Franz type or In-Line Cell systems have been commonly used to perform in vitro skin permeation studies to assess product quality, changes on stability, formulation and manufacturing effects. Briefly, the method requires placing a sample of skin (human, animal) or artificial membrane as a permeation barrier between donor and receptor compartments. Drug passes into the receptor compartment and its concentration is measured at predetermined intervals. Experimental factors that can influence the

permeation results such as stirring speed and temperature are studied closely during method development and are optimized during early validation studies. These non-biologic factors have received a fair amount of discussion through the compendial references (USP, 2009). It is also well known that when human or animal skin is used, incipient biological variability between skin donors also has a large effect. Although factors contributing to the overall variability of the measurement system are well known, we have found no references to address how to properly design skin permeation studies in such a way as to account for the incipient variability attributable to skin donors in a way that can be regarded as statistically appropriate.

In this paper, we address this need by proposing a statistical design and modeling strategy that leads to unambiguous estimates of variability attributable to skin donors, as well as pieces within skin. Using a similar strategy, it would also be possible to extend the design to more closely look at variability attributable to site of skin sample (e.g. breast, abdomen, arm) and other factors such as race or age of the donor. In the following sections, we discuss our modeling strategy, provide a case study by way of example that sets forth a statistical design capable of providing unambiguous estimates of variability and end with a conclusion section

2. Modeling Strategies

2.1 Nonlinear Model

Henning et al (2009) developed an extension of Fick's second law of diffusion to describe the in vitro permeation profile over permeation time:

$$Q(t) = AK_{lip/aq}lc_0\left[\frac{D}{l^2} - \frac{1}{6} - \frac{2}{\pi}\sum_1^\infty\left(\frac{-1^n}{n^2}\right)\exp\left(-Dn^2\pi^2 t/l^2\right)\right]$$

where

$Q(t)$ = the total mass of drug in receptor compartment at permeation time t,

A = the area available for diffusion,

$K_{lip/aq}$ = partition constant,

l = length of the diffusion pathway,

c_0 = drug concentration in the donor compartment,

D = diffusion coefficient,

n = number of stratum corneum sheets.

The Henning model is conceptually clear and very attractive from the point of view of deriving a mechanism based model. It permits characterization of the entire permeation profile starting at permeation time 0. On the other hand, the translation of the Henning model into a fully mixed effects statistical model would encounter difficulties in accounting for random components attributable to skin donor differences. This is because the diffusion coefficient (D) appears in both the pre-exponential constant and the exponential term. Specialized software would be required and the model would be sensitive to the choice of time point for measurements and number of skin donors and number of pieces from each skin donor. These present obstacles to both the design and

modeling aspects of implementing the Henning model as a statistical tool to assess important effects attributable to the formulation and stability time.

2.2 Linear Model

A mixed effects linear model that captures the important sources of variability has only a few fixed and random terms accounting for process, permeation time and skin donor effects. The model is fit to the linear phase of the permeation profile. A specific mathematical form will be given in section 4 while discussing the case study. The advantages of a standard linear mixed effects model besides its ease of use and the connections to the statistical design, is that it allows for random components to be fit in a natural way. Another attractive feature is that standard statistical software is capable of fitting such a model with very little difficulty, although in some cases, the interpretation of the random components may be problematic.

The linear model is not immediately connected to a mechanistic model and has the disadvantage of not being able to account for the early curvature of the permeation profile. However, we believe that despite the descriptive nature of the model, and the small loss of information, the estimated model parameters have good statistical properties and are sufficient to serve as clear indicators of product quality, changes on stability, formulation and manufacturing effects. The results of the model can also readily be related to parameter estimates arising from *In Vitro In Vivo* correlation studies.

3. Case Study – Assessing Stability Changes in Skin Permeation

It is of interest to study the stability properties of a TDDS formulation with respect to skin permeation. We applied statistical design principles to balance the variance components associated with skin donors and pieces within skin donor, requiring that independence be preserved across stability time points.

3.1 Stability Study Design

The following design parameters underlay the requirements for the allocation of skin donors and pieces to lot-stability time combinations:

i. 3 lots of Compound A
ii. 5 stability time points per lot.

Due to the essential objective of the study, to estimate fixed and random effects, and the limited supply of skin donors, the allocation of skin donors to factor combinations of lot-stability time point were governed by the following restrictions:

i. skin donors: females at various ages, skin samples from various sites (abdomen, arm, breast), not controllable a priori,
ii. up to 9 pieces nested within donor, number not controllable a priori,
iii. 2 different skin donors and 6 pieces for each lot at each stability time point,
iv. independence of skin donors across stability times, meaning a skin donor can only be allocated to a single lot-stability time combination.

The randomization principle is an important aspect of a statistical design. It states that skin donors and pieces within each skin donor are randomly allocated to the fixed effect combinations. In addition, the allocation is carried out according to a structured allocation or blocking such that the variance components of skin donor and pieces within the same

skin donor are orthogonalized. The importance of doing this is that it leads to unambiguous estimates of the variance components, and confers statistical validity on the estimates of the fixed parameter estimates. Once the sampling frame was defined, the random allocation of skin donors and pieces nested within skin donor followed a scheme partially described in Table 1.

Table 1: Skin Donor and Piece Allocation Scheme

Skin Sample Donor Sampling Frame	*Available Pieces*	*Random Allocation of Donors and Piece(skin) to Lots*		
		LOT 1	*LOT 2*	*LOT 3*
D1 ….			1,2,5 (T=15mo)	
D24	1-5			2,3,6 (T=18mo)
D25	1-6	1,3,5 (T=18mo)		
D26	1-6			2,3,7 (T=18mo)
D27	1-7		2,4,5 (T=18mo)	
D28	1-6	1,2,4 (T=18mo)		
D29	1-5		1,3,5 (T=18mo)	
D30	1-7			1,2,3 (T=24mo)
D31	1-3			1,3,4 (T=24mo)
D32 …D36	1-4			

3.2 Permeation Study Design

In vitro permeation data were collected for each skin piece, at 8, 24, 32, 48 and 72 hours. The profiles were measured at predefined stability time points (6, 9, 12, 15, 18, 24 months). Figure 1 graphs the observed 24 to 72hr profiles across stability time points for LOT 1, for each skin/piece combination (data masked for reporting purposes).

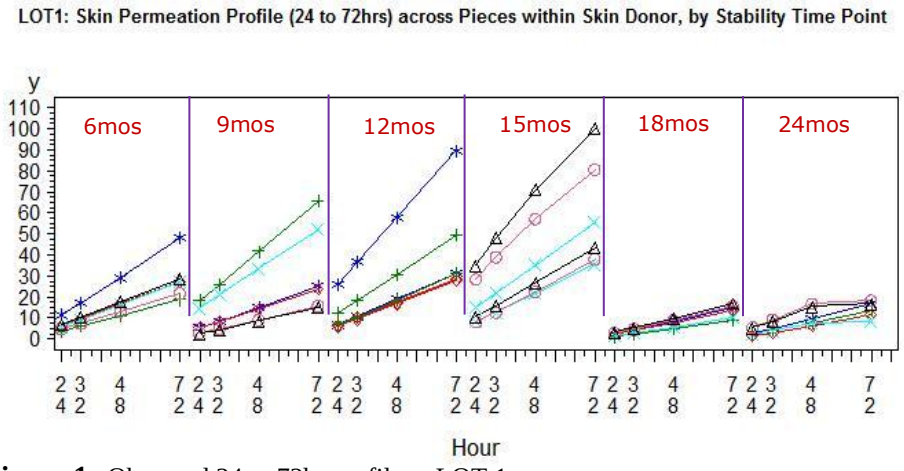

Figure 1: Observed 24 to 72hr profiles - LOT 1

3.3 Mixed Effects Model

The permeation profile was characterized for two key responses:

a. the start of the linear phase (steady state) of the permeation profile (24 to 72 hr) as a reflection of extent of permeation,
b. the rate of permeation over the linear phase of the profile.

The changes on stability for the 2 responses were estimated using standard linear mixed effects modeling that estimated fixed lot/stability time-specific initial, fixed lot/stability time-specific permeation rate and random skin donor and piece effects to both the initial and permeation rate, assuming correlated initial and permeation rate random effects. The model follows:

$$y_{l(m(jk)),i} = (A_{0,jk} + \alpha_{m(jk)} + \lambda_{l(m(jk))}) + (B_{jk} + \beta_{m(jk)} + \delta_{l(m(jk))}) \times T_{l(m(jk)),i} + \varepsilon_{l(m(jk)),i}$$

where

$y_{l(m(jk)),i}$ = i-th hour permeation for the l-th piece within the m-th skin donor, j-th lot and k-th stability time point,

$A_{0,jk}$ = mean skin permeation for the j-th lot and k-th stability time point at initial,

$\alpha_{m(jk)}, \beta_{m(jk)}$ = random effects of the m-th skin donor within the j-th lot and k-th stability time point on initial and slope, with 0 means and covariance matrix $\begin{bmatrix} \sigma_\alpha^2 & \sigma_{\alpha\beta} \\ \sigma_{\alpha\beta} & \sigma_\beta^2 \end{bmatrix}$,

$\lambda_{l(m(jk))}, \delta_{l(m(jk))}$ = random effects of the l-th piece within the m-th skin donor, j-th lot and k-th stability time point on initial and slope, with 0 means and covariance matrix $\begin{bmatrix} \sigma_\lambda^2 & \sigma_{\lambda\delta} \\ \sigma_{\lambda\delta} & \sigma_\delta^2 \end{bmatrix}$,

B_{jk} = fixed permeation rate for the j-th lot and k-th stability time point,

$T_{l(m(jk)),i}$ = i-th hour covariate for the l-th piece within the m-th skin donor, the j-th lot and k-th stability time point,

$\varepsilon_{l(m(jk)),i}$ = residual error, distributed as $N(0, \sigma_e^2)$.

PROC MIXED in SAS software was used for the statistical analysis (SAS Institute, Inc., 2009).

3.4 Modeling Results

Based on the mixed effects model parameter estimates representing the individual stability time points, a linear contrast for change over stability time was estimated for both the 24hr skin permeation and hourly skin permeation rate parameters. Table 2 lists the estimates of average yearly change for LOT 1, as an example. The average stability profile indicated that the lots are decreasing slightly in permeation rate and extent as they age, but none of the changes on stability were statistically significant. The results set the stage for future studies to use these estimates to consider sensitivity and power in carrying out additional confirmatory studies.

Table 2: Estimates of Average Yearly Change – LOT 1

	Yearly Change	*se*	*p-value*
24hrs Skin Permeation	-51	150	0.735
Hourly Skin Permeation Rate	-3.4	3.5	0.334

Table 3 lists the random components associated with rate of change and extent of permeation at start of linear phase. Skin donor differences accounted for ~75% of total variability (for both initial and slope), while pieces within skin accounted for ~25% of total variability (for both initial and slope). The random components associated with rate of change and extent of permeation at start of linear phase had a correlation of 0.99, for both skin donor and pieces within skin donor. This very high correlation suggests that not much additional information is gained by collecting additional data beyond the 24 hr. point. Future studies could consider a simplified design as a consequence.

Table 3: Estimates of the Between and Within Skin Donor Variability *Variance (%Total)*

Initial (24hrs)		*Permeation Rate*		*Residual*
Skin	*Piece*	*Skin*	*Piece*	
51600 (74%)	*18300 (26%)*	*34 (75%)*	*12 (25%)*	36

3. Conclusion

A randomized nested block design orthogonalizing fixed and random effects is useful for studying skin permeation from TDDS. The analytical measurements from such an experiment can be easily analyzed statistically with a mixed effects model with random coefficients in both intercept and slope terms to estimate formulation or stability time effects as well as random effects associated with skin donor and pieces. The recommendation given by Henning et al (2009) to weight the statistical analysis is naturally accounted for with the mixed model in a completely appropriate way through a random coefficients model so the need for weighting is eliminated. The quantification of these variance components from a properly designed study such as this sets the stage for designing future studies where required sensitivity to treatment differences can be easily accommodated.

The major source of variability is skin donor, constituting 75% of the total variability in the skin permeation assay system. The high correlation between permeation at start of steady state and rate of change suggests a simpler experimental design and protocol could be followed in estimating meaningful effects leading to savings in time and resources.

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