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THE USE OF RESTRICTION ERRORS IN THE DESIGN OF EXPERIMENTS

by

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ABSTRACT

By incorporating restriction errors into experimental design models, the developmental scientist is forced to consider what restrictions on randomization have been placed on his experiment whether they are inadvertent or planned. With the experimental design in mind along with the restriction errors the scientist should be able to eventually establish a very reasonable design model and analysis for that experiment.

One of the more fundamental tools of pharmaceutical research and development, particularly in the design of dosage forms, is that of the accelerated stability test (1). In general, accelerated testing done early in the development scheme is designed to screen the formulation for the effects caused by changing variables. Variables are anything associated with the formula or the manufacturing of the formula. Many developmental scientists would change only one variable at a time. While this may be sound advice occasionally, it is time consuming and could actually miss the most important effects (interactions). An investigation using multiple variable changes must be done within the framework of a properly designed experiment. Without the benefit of a proper design the many interrelationships between factors (variables) can never be successfully analyzed. Only within the framework of an appropriate experimental design can the proper relationships and interactions between the factors be extracted from the experimental data.

What is needed from an experiment is first expressed as a statistical experimental design. The experiments are then carried out according to the design. The data is gathered and analyzed according to the statistical design. An important step in the experimental design, particularly in more complex experiments, is to follow through with the design model and construct the theoretical expected mean squares (EMS) table and establish the statistical tests that can be performed from the design (2). Designing an experiment and then performing the work without

first testing the theoretical design can lead to situations where it is impossible to statistically test for the factors of interest(3). For example, if a pharmaceutical scientist wanted to find out how the compression pressure used to manufacture tablets affected the tablet's dissolution time, the following experimental design could be set up to run.

FACTORS AND LEVELS

Tablet formulations (T) A, B, C, D

Compression Pressures (P) 1, 2, 3

Weeks of Storage (W) 0, 2, 4, 12

EXPERIMENTAL DESIGN

The 12 different tablet formulations - compression pressure combinations would be randomized and a batch of each made and placed on stability. Enough tablets would be drawn at random from each batch to obtain three dissolution measurements per design cell.

DATA ANALYSIS FROM THE PROPOSED DESIGN

The resulting model for the dissolution test experiment is given below:

$$Y_{ijkl} = \mu + T_i + P_j + TP_{ij} + \delta_{(ij)} + W_k + TW_{ik} + PW_{jk} + TPW_{ijk} + \varepsilon_{(ijk)l} \qquad (1)$$

where: $i = 1,2,3,4$ $j = 2,3,3$ $k = 1,2,3,4$ $l = 1,2,3$

Y_{ijkl} = dissolution measure of the l^{th} tablet, after the k^{th}

week of storage, manufactured at the j^{th} pressure level of the i^{th} tablet formulation.

T_i = effect of the i^{th} tablet formulation (fixed).

P_j = effect of the j^{th} compression pressure (fixed).

TP_{ij} = effect of the interaction of the j^{th} compression and the i^{th} tablet formulation.

W_k = effect of k weeks of storage (fixed).

$\delta_{(ij)}$ = error terms for batch-to-batch variation per level. In this case only 1 batch per level.

TW_{ik} = effect of the interaction of the k^{th} week of storage and the i^{th} tablet formulation.

PW_{jk} = effect of the interaction of the k^{th} week of storage and the j^{th} pressure level

TPW_{ijk} = effect of the interaction of the k^{th} week of storage, the j^{th} pressure level and the i^{th} tablet formulation.

$\varepsilon_{(ijk)l}$ = random error caused by the l^{th} run of the k^{th} week of storage, of the j^{th} pressure level of the i^{th} tablet formulation.

The corresponding Analysis of Variance (ANOVA) is shown in Table I.

It is obvious that this design has no tests that can be run for T, P, and the interaction TxP. The design must be changed in order to obtain meaningful results for the analysis of the data. If the ANOVA had not been generated before the work was done, a lot of data would have been generated worthlessly.

TABLE I

ANOVA Using Equation (1)

Source	df	EMS
Tablet Formulation(T)	3	$\sigma_\varepsilon^2 + 12\sigma_\delta^2 + 36\phi(T)$
Compression Pressure(P)	2	$\sigma_\varepsilon^2 + 12\sigma_\delta^2 + 48\phi(P)$
TP	6	$\sigma_\varepsilon^2 + 12\sigma_\delta^2 + 12\phi(TP)$
δ	0	$\sigma_\varepsilon^2 + 12\sigma_\delta^2$
Storage Time (W)	3	$\sigma_\varepsilon^2 + 36\phi(W)$
TW	9	$\sigma_\varepsilon^2 + 9\phi(TW)$
PW	6	$\sigma_\varepsilon^2 + 12\phi(PW)$
TPW	18	$\sigma_\varepsilon^2 + 3\phi(TPW)$
Within (TPW)	96	σ_ε^2

The major problem with the proposed experimental design was that the design did not contain any consideration for the restriction of randomization that was imposed by the experimental design. The method of design development didn't even ask the scientist to consider such things.

To help avoid the errors developed above, the introduction of random restriction errors into the design model should be made. A restriction error component should be placed into the design model

corresponding to every restriction on randomization introduced into the design. This will force each design model to be different but will allow experimenters to delete those terms for a specific experiment if they believe the effects to be zero. The restriction error cannot be estimated from the data but it is placed in the model, with appropriate indexing, and is allowed to appear in the corresponding analysis of variance (ANOVA) as a source of variance. There are no degrees of freedom (df) and no sum of squares (SS) for the restriction error. However, since the restriction error appears in the theoretical linear model, the variance component for it does appear in the expected mean squares (EMS). This variance component in the EMS forces the experimenter to recognize it and to account for it in the F-tests. The real advantage of using restriction error is that if forces the experimenter not only to recognize the restriction on randomization he has imposed on his design in an effort to save time and money, but also to see its effects on the overall results of the experiment (4,5).

EXPERIMENT RE-DESIGN

The new experimental design randomly manufactures one batch of each of the 12 formulation-pressure combinations as before, but only one measurement of dissolution is taken per cell. The entire experiment is then repeated using another batch for each of the 12 formulation-pressure combinations. The new design model using restriction error components is shown below.

$$Y_{ijkl} = \mu + R_i + \delta_{(i)} + T_j + P_k + TP_{jk} + \text{error(a)} + \gamma_{(ijk)} + W_l + TW_{jl} + PW_{kl} + TPW_{jkl} + \text{error(b)} + \varepsilon_{(ijkl)} \quad (2)$$

where: $i = 1,2 \quad j = 1,2,3,4 \quad k = 1,2,3 \quad l = 1,2,3,4$

Y_{ijkl} = dissolution measure after the l^{th} week of storage of the k^{th} pressure level of the j^{th} tablet formulation in the i^{th} replicate.

R_i = effect of the i^{th} replicate (random).

$\delta_{(i)}$ = first restriction error (random).

T_j = effect of the j^{th} tablet formulation (fixed).

P_k = effect of the k^{th} pressure level (fixed).

TP_{jk} = effect of the interaction of the k^{th} pressure level and the j^{th} tablet formulation.

error(a) = pooled (RT_{ij}, RP_{ik} and RTP_{ijk}).

$\gamma_{(ijk)}$ = second restriction error (random).

W_l = effect of the l^{th} week of storage (fixed).

TW_{jl} = effect of the interaction of the l^{th} week of storage and the j^{th} tablet formulation.

PW_{kl} = effect of the interaction of the l^{th} week of storage and the k^{th} pressure level.

TPW_{jkl} = effect of the interaction of the l^{th} week of storage of the k pressure level and the j^{th} tablet formulation.

error(b) = pooled (RW_{il}, RTW_{ijl}, RPW_{ikl} and $RTPW_{ijkl}$).

$\varepsilon_{(ijkl)}$ = random error within the l^{th} week of storage of the k^{th} pressure level of the j^{th} tablet formulation in the i^{th} replicate.

There are two restrictions on randomization inherent in the new design which are accounted for in the model. The first restriction (δ) occurs with the two replications of the experiment being performed stepwise. The second restriction (γ) occurs within the tablet making sequence. It is easier to make all of the required tablets by varying the tablet formulations one compression pressure at a time. The pharmaceutical scientist would not expect to see any significant interaction between the replications and any of the other factors. Therefore those factors are pooled into error a = pooled (RT_{ij}, RP_{ik} and RTP_{ijk}) and error b = pooled (RW_{il}, RTW_{ijl}, RPW_{ikl} and $RTPW_{ijkl}$). The corresponding ANOVA is shown in Table II.

It is obvious that the new test design is much better. The ANOVA shows that there are 11 df with which to test T, P, and TxP.

The use of restriction error components in designing experimental tests will force the scientist to examine his design for areas where randomization has been restricted, naturally or by design, and to thereby be able to design a model for the analysis desired.

As a further illustration of the use of this design method let us suppose that a pharmaceutical scientist wishes to study the absorption of preservative from a parenteral solution into rubber closures.

Specifically the scientist is interested in the effects due to different rubber polymers, different filler concentrations and storage time.

TABLE II

ANOVA Using Equation (2)

Source	df	EMS
Replications (R)	1	$\sigma^2 + 4\sigma_\gamma^2 + 4\sigma_a^2 + 48\sigma_\delta^2 + 48\sigma_R^2$
First Restriction Error (δ)	0	$\sigma_\varepsilon^2 + 4\sigma_\gamma^2 + 4\sigma_a^2 + 48\sigma_\delta^2$
Tablet Formulation (T)	3	$\sigma_\varepsilon^2 + 4\sigma_\gamma^2 + 4\sigma_a^2 + 24\phi(T)$
Compression Pressure (P)	2	$\sigma_\varepsilon^2 + 4\sigma_\gamma^2 + 4\sigma_a^2 + 32\phi(P)$
TP	6	$\sigma_\varepsilon^2 + 4\sigma_\gamma^2 + 4\sigma_a^2 + 8\phi(TP)$
Error (a)	11	$\sigma_\varepsilon^2 + 4\sigma_\gamma^2 + 4\sigma_a^2$
Second Restriction Error (γ)	0	$\sigma_\varepsilon^2 + 4\sigma_\gamma^2$
Storage Time (W)	3	$\sigma_\varepsilon^2 + \sigma_b^2 + 24\phi(W)$
TW	9	$\sigma_\varepsilon^2 + \sigma_b^2 + 6\phi(TW)$
PW	6	$\sigma_\varepsilon^2 + \sigma_b^2 + 8\phi(PW)$
TPW	18	$\sigma_\varepsilon^2 + \sigma_b^2 + 2\phi(TPW)$
Error (b)	36	$\sigma_\varepsilon^2 + \sigma_b^2$
Within (ε)	0	σ_ε^2

FACTORS AND LEVELS

Rubber Polymer (R)	A,B
Batches (B)	1,2,3
Filler Concentration (F)	1,2,3
Mold Sheet (S)	1,2
Storage Time (t)	0,n

EXPERIMENTAL DESIGN

The eighteen polymer-batches-filler combinations would be compounded and two sheets of rubber closures molded from each combination. Each sheet of closures would be cut in half and one half used for closures at t = 0 and the other half used to supply closures at t = n.

The experiment can be illustrated as in Table III.

The dotted line indicates the mold sheets being divided into two portions for testing at the two times.

The model for this design is shown below:

$$\begin{aligned} Y_{ijklm} = {} & \mu + R_i + B_{(i)j} + \delta_{(ij)} + F_k + RF_{ik} + BF_{(i)jk} \\ & + S_{(ijk)l} + \omega_{(ijkl)} + t_m + Rt_{im} + Bt_{(i)jm} \\ & + Ft_{km} + RFt_{ikm} + BFt_{(i)jkm} + St_{(ijk)lm} \\ & + \varepsilon_{(ijklm)} \end{aligned} \qquad (3)$$

TABLE III

Rubber Closure Experimental Design

				Rubber Closure Formulations (R)											
				1						2					
				Batches (B)						Batches					
				1		2		3		1		2		3	
				Storage Time(t)											
				t_o	t_n	t_o	t_n	t_o	t_n	t_o	t_n	t_o	t_n	t_o	t_n
Filler Conc. (F)	1	Mold Sheets (S)	1												
			2												
	2		3												
			4												
	3		5												
			6												

Dashed line indicates sheet physically cut into 2 pieces.

where: $i = 1,2 \quad j = 1,2,3 \quad k = 1,2,3 \quad l = 1,2 \quad m = 1,2$

Y_{ijklm} = absorption measure after the m^{th} storage time of the l^{th} mold sheet of the k^{th} filler level of the j^{th} batch of the i^{th} rubber polymer.

R_i = effect of the i^{th} rubber polymer (fixed).

$B_{(i)j}$ = effect of the j^{th} batch in the i^{th} rubber polymer (random).

$\delta_{(ij)}$ = first restriction error.

F_k = effect of the k^{th} filler level (fixed).

RF_{ik} = effect of the interaction of the k^{th} filler level and the i^{th} rubber polymer.

$BF_{(i)jk}$ = effect of the interaction of the k^{th} filler level of the j^{th} batch of the i rubber polymer.

$S_{(ijk)l}$ = effect of the l^{th} mold sheet of the k^{th} filler level of the j^{th} batch of the i^{th} rubber polymer (random).

ω = second restriction error.

t_m = effect of the m^{th} time of storate (fixed), and so on for all other symbols.

$\varepsilon_{(ijklm)}$ = random error within the m^{th} time of storage of the l^{th} mold sheet of the k^{th} filler level of the j^{th} batch of the i^{th} rubber polymer.

The ANOVA from equation 3 is shown in Table IV. Tests can be made for the 3 main factors of rubber polymer (K), filler concentration (F) and storage time (t) as well as the RF, BF, Rt, Ft, RFt and BFt interactions.

TABLE IV

AVOVA Using Equation (3)

Source	df	EMS
R_i	1	$\sigma_\varepsilon^2 + 2\sigma_\omega^2 + 2\sigma_s^2 + 12\sigma_\delta^2 + 12\sigma_B^2 + 36\phi(R)$
$B_{(i)j}$	4	$\sigma_\varepsilon^2 + 2\sigma_\omega^2 + 2\sigma_s^2 + 12\sigma_\delta^2 + 12\sigma^2_B$
$\delta_{(ij)}$	0	$\sigma_\varepsilon^2 + 2\sigma_\omega^2 + 2\sigma_s^2 + 12\sigma_\delta^2$
F_k	2	$\sigma_\varepsilon^2 + 2\sigma_\omega^2 + 2\sigma_s^2 + 4\sigma^2_{BF} + 24\phi(F)$
RF_{ik}	2	$\sigma_\varepsilon^2 + 2\sigma_\omega^2 + 2\sigma_s^2 + 4\sigma^2_{BF} + 12\phi(RF)$
$BF_{(i)jk}$	8	$\sigma_\varepsilon^2 + 2\sigma_\omega^2 + 2\sigma_s^2 + 4\sigma^2_{BF} + 12\phi(RF)$
$S_{(ijk)l}$	18	$\sigma_\varepsilon^2 + 2\sigma_\omega^2 + 2\sigma_s^2$
$\omega_{(ijkl)}$	0	$\sigma_\varepsilon^2 + 2\phi_\omega$
t_m	1	$\sigma_\varepsilon^2 + \sigma_{St}^2 + 6\sigma^2_{Bt} + 36\phi_t$
Rt_{im}	1	$\sigma_\varepsilon^2 + \sigma^2_{St} + 6\sigma^2_{Bt} + 18\phi_{Rt}$
$Bt_{(i)jm}$	4	$\sigma_\varepsilon^2 + \sigma^2_{St} + 6\sigma^2_{Bt}$
Ft_{km}	2	$\sigma_\varepsilon^2 + \sigma^2_{St} + 2\sigma^2_{BFt} + 12\phi_{Ft}$
RFt_{ikm}	2	$\sigma_\varepsilon^2 + \sigma^2_{St} + 2\sigma^2_{BFt} + 6\phi_{RFt}$
$BFt_{(i)jkm}$	8	$\sigma_\varepsilon^2 + \sigma^2_{St} + 2\sigma^2_{BFt}$
$St_{(ijk)lm}$	18	$\sigma_\varepsilon^2 + \sigma^2_{St}$
$\varepsilon_{(ijklm)}$	0	σ_ε^2
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