Comparison of Spironolactone Tablet Dosage Forms in Healthy Humans

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Abstract □ A single-dose, crossover, comparative study of 25- and 100-mg spironolactone tablets was undertaken in 39 healthy males. Following a total dose of 200 mg, blood samples were drawn at 0, 1, 2, 3, 4, 6, and 24 hr and a single collection of urine was taken after the initial 6 hr. The dethioacetylated active metabolite was measured in these specimens and was employed as the indicator of drug absorption and elimination. A comprehensive statistical analysis, including: *(a)* multivariate hour-by-hour comparisons with plasma aldadiene levels, *(b)* comparisons with total plasma area and total urinary metabolite level, *(c)* bivariate comparisons with peak plasma aldadiene levels, and *(d)* urinary sodium–potassium ratio responses, examines the question of the biologieal equivalence of these two spironolactone preparations. The primary discriminators for comparing the two dosage forms were found to be plasma metabolite levels at Hours 3 and 4, peak plasma metabolite level, and total 24-hr area under the plasma level curve. Moreover, a single plasma level by itself does not appear to be sufficient to test for biological equivalence, so correlations among levels should not be ignored. The mean peak plasma metabolite levels for the 25- and 100-mg formulations reached 67.8 and 61.6 μg/100 ml, respectively, each at the same mean time of 160 min.

Keyphrases □ Spironolactone—statistical comparison of two tablet dosage forms (25 and 100 mg), analysis, humans □ Statistical analysis—comparison of spironolactone tablet dosage forms (25 and 100 mg), multivariate analysis, determination of discriminators □ Dosage forms—comparison of 25- and 100-mg spironolactone tablets, humans, multivariate statistical analysis (hour-by-hour comparisons) □ Discriminators—determined for comparison of spironolactone tablet dosage forms

Spironolactone is a specific antagonist of aldosterone, modifying electrolyte metabolism only in the presence of aldosterone-like compounds (1–5) and reversing all electrolyte-regulating effects of aldosterone regardless of the tissue studied (2, 3). Spironolactone is employed in the diagnosis (6) and treatment (7) of primary hyperaldosteronism and is often combined with other diuretics (thiazides) in the treatment of hypertension. The effects from such combinations in reducing blood pressure tend to be greater than the individual responses from either drug alone (8).

Spironolactone is presently marketed in the form[1] of 25-mg tablets. Since the drug is often given in daily dosages of between 200 and 400 mg (9, 10), it was thought that a 100-mg tablet dosage form could offer a greater convenience to patients who would otherwise be taking as many as four 25-mg tablets in a single dose. The primary intent of this study was to determine whether spironolactone absorption with 100-mg tablets was equivalent to absorption of the same dose in the form of 25-mg tablets. Also, based upon elevation in the urinary sodium–potassium ratio, it was of interest to determine whether a difference in pharmacological response between these two dosage formulations could be observed in healthy subjects.

[1] Aldactone, 25-mg spironolactone tablets, 3-(3-oxo-7α-acetylthio-17β-hydroxy-4-androsten-17α-yl) propanoic acid lactone, G. D. Searle & Co.

EXPERIMENTAL

A group of 39 males with no symptoms of chronic diseases participated in this single-dose crossover study, which consisted of one 24-hr test period and then a 2-week interval followed by a second 24-hr test period. The ranges of age and body weight were 21–39 years and 56.7–106.6 kg (125–235 lb), respectively. All subjects abstained from the use of all drugs for at least 3 days and from alcohol for 24 hr prior to a test day. Each test period was initiated approximately 2 hr after a light breakfast. About 4–5 hr into the test, a light lunch was consumed in which fried or fat meats were avoided. After the subjects emptied their bladders, each subject at zero time ingested 200 mg of spironolactone in a randomized order, either 8 × 25-mg or 2 × 100-mg tablets, along with two 250-ml glasses of water. In the second test period, the randomized order of drug ingestion was interchanged in crossover fashion. An additional two glasses of water were taken midway into the first 6 hr.

During each test period at 0, 1, 2, 3, 4, and 6 hr, 10-ml blood samples were collected using heparinized, sterile, disposable needles and syringes. An additional blood sample was drawn from all subjects 24 hr after the beginning in each test period. Levels of the dethioacetylated active metabolite of spironolactone were measured in plasma and urine by a spectrofluorometric method (11), and these levels served as indicators of drug absorption and elimination.

To compare the urinary sodium–potassium ratio, each subject collected 6-hr urine samples on the day prior to the first test period and in each test period. To minimize effect of diurnal variations, all samples were collected during the same 6-hr period (between 9 am and 3 pm). Side effects were not expected and were not observed. A 2-week interval was maintained between test periods.

STATISTICAL

The statistical analysis for comparing the 25- and 100-mg tablet formulations is based on the multivariate Hotelling T^2 method (12–14). The Hotelling T^2 method, a special case of the general multivariate linear model, permits comparisons between two groups at several time points and/or with several different variables simultaneously. Rahlfs and Bedall (15) discussed the appropriateness of the general multivariate linear model and other methods for analysis of time-dependent biological data, which, of course, includes the case discussed here.

The X and Y notation is hereafter used to distinguish the subject data corresponding to the two dosage forms: I, 25-mg tablets; and II, 100-mg tablets. A bold character distinguishes a vector from a scalar component. If N denotes the total number of subjects, then let the column vectors:

$$\mathbf{X}_i = \begin{bmatrix} X_{i1} \\ \cdot \\ \cdot \\ \cdot \\ X_{ik} \end{bmatrix} \qquad \mathbf{Y}_i = \begin{bmatrix} Y_{i1} \\ \cdot \\ \cdot \\ \cdot \\ Y_{ik} \end{bmatrix}_{i=1,\ldots,N} \qquad \text{(Eq. 1)}$$

denote K measurement variables relating to the ith subject with

respect to the two dosage forms, Groups I and II, respectively.

If d_{ik} denotes the difference between the kth measurement variables for Groups I and II relating to the ith subject:

$$d_{ik} = X_{ik} - Y_{ik} \qquad \text{(Eq. 2)}$$

the mean and variance of the kth difference variable are:

$$\bar{d}_k = (1/N)\sum_{i=1}^{N} d_{ik} \qquad \text{(Eq. 3)}$$

and:

$$s_k^2 = (1/N - 1)\sum_{i=1}^{N}(d_{ik} - \bar{d}_k)^2 \qquad \text{(Eq. 4)}$$

respectively. The correlation matrix of the K difference variables is defined as:

$$R = \begin{bmatrix} 1 & r_{12} & \cdots & r_{1K} \\ . & 1 & & . \\ . & & . & . \\ . & & & . \\ r_{K1} & \cdots & & 1 \end{bmatrix} \qquad \text{(Eq. 5)}$$

where $r_{km} = (1/N - 1)\,\Sigma^{N}_{i=1}(d_{ik} - \bar{d}_k)(d_{im} - \bar{d}_m)/s_k s_m$.

Since a correlation matrix is symmetrical, ($r_{ij} = r_{ji}$), the same correlations that appear above the main diagonal also appear below the main diagonal. Hereafter, the below-diagonal correlations will not be listed.

The Hotelling T^2 statistic is defined as:

$$T^2 = \mathbf{t}^* R^{-1}\mathbf{t} \qquad \text{(Eq. 6)}$$

where $\mathbf{t}$ is the column vector whose components $t_1, \ldots, t_K$ are the individual Student t statistics corresponding to the K measurement variables; R^{-1} is the inverse of the correlation matrix R; and the asterisk denotes vector transposition. The Student t statistic, t_k, is defined by:

$$t_k = \sqrt{N}(\bar{d}_k/s_k) \qquad \text{(Eq. 7)}$$

for $k = 1, \ldots, K$.

If the inverse of the correlation matrix is given by:

$$R^{-1} = \{\alpha_{ij}; 1 \le i,j \le K\} \qquad \text{(Eq. 8)}$$

then Eq. 6 becomes:

$$T^2 = \sum_{i=1}^{K}\sum_{j=1}^{K}\alpha_{ij}t_i t_j \qquad \text{(Eq. 9)}$$

In the particular case when all correlations are zero, Eq. 9 reduces to:

$$T^2 = t_1^2 + \ldots + t_K^2 \qquad \text{(Eq. 10)}$$

The Hotelling T^2 statistic is a positive real number which relates directly to a calculated F statistic value by:

$$F_{\text{calc}(K,m-K)}(\alpha) = [(m - K)/(m - 1)K]T^2 \qquad \text{(Eq. 11)}$$

where α is the level of significance and K, $m - K$ are the two degrees of freedom. The value of $m = N$ relates to pairwise comparisons or the so-called one-sample case, whereas $m = N_1 + N_2 - 1$ is used for unpaired comparisons or the case of two samples with sample sizes N_1 and N_2. Primary attention here is focused on the pairwise case, since each subject had both dosage forms in crossover fashion.

For a specific comparison using the Hotelling T^2, if the calculated value of F is less than the corresponding tabled value of F (16), then the difference between I and II is said to be *not* significant at the appropriate level of probability α. If the calculated F is greater than the tabled F, the comparison *is* considered significantly different at the level α.

For two variables ($K = 2$), Hicks (17) gave a useful comparison between the correlated and uncorrelated cases. It is possible, for example, at a fixed significance level, for values of t_1 and t_2 not to be significant while the bivariate (t_1, t_2) *is* significant. On the other hand, either t_1 or t_2 or both, taken separately, can be significant while the bivariate (t_1, t_2) is *not* significant.

In past pharmacokinetic studies, the area under the plasma level curve has been probably the single most important variable.

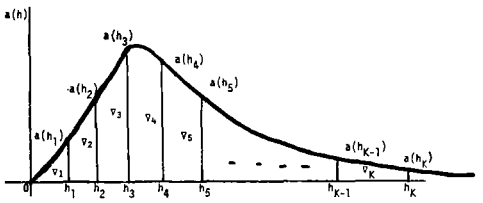

Figure 1—*Typical plasma level curve in which area is subdivided into the* **K** *subareas* $\nabla_1, \ldots, \nabla_K$.

By considering the plasma levels themselves, total plasma area, and certain other variables collectively in a multivariate framework, an assessment of the relative importance of area is obtained, as discussed in the *Results and Discussion* section. Here the role of subareas is considered.

In the multivariate approach, the area under the plasma level curve for each subject is broken up into K subareas (trapezoids), $\nabla_1, \ldots, \nabla_K$. Each subarea is determined by the difference between two successive time points, the corresponding plasma levels $a(h)$, and a straight line connecting two levels as in Fig. 1.

The K trapezoids, $\nabla_1, \ldots, \nabla_K$, are defined to be the K components of a vector which can relate to either X or Y, corresponding to the two dosage forms I and II, respectively. A Hotelling T^2 analysis can then be applied to subareas as the basic variates.

However, the obtaining of areas by straight lines implies geometrically that a linear transformation is applied to each data set, $a(h_1), \ldots, a(h_K)$. In other words, the kth trapezoid, ∇_k, is simply:

$$\nabla_k = [a(h_{k+1}) + a(h_k)]/2; \qquad k = 1, \ldots, K \qquad \text{(Eq. 12)}$$

But since the value of Hotelling T^2 remains unchanged with respect to nonsingular linear transformations (12, 13), calculations can be based on the plasma levels, $a(h_k)$, rather than the subareas, ∇_k. Moreover, for such biological comparisons, an explicit model for the plasma concentration curve is unnecessary.

In either case, using the levels, $a(h_k)$, or the areas, ∇_k, it is necessary that the time points, $h_1, \ldots, h_K$, be the same for both groups in the comparison. The choice of the number of variates, on the other hand, is often a delicate one and is decided on grounds that are only partly pharmacological and partly statistical. This question is not discussed here. However, later selection of variables or discriminators is obtained.

RESULTS AND DISCUSSION

In addition to the significance testing for differences, all 11 measurement variables, as well as subsets in the comparisons discussed here, are considered collectively, and stepwise selection of relative "best" discriminators are obtained. A set of m relative best discriminators in a set of K variables is here defined as that set of m variables, among all subsets of m variables, that accounts for the largest difference between the two groups under study. There are several accounts relating to the concepts of best discriminators and stepwise selection of discriminators (13, 14, 18, 19).

Multivariate (Hour-by-Hour) Comparisons with Plasma Metabolite Levels—As already mentioned, blood samples were obtained from each subject on each of the two dosage forms (I, 25 mg; and II, 100 mg) at the times, in hours, of $h_1 = 1$, $h_2 = 2$, $h_3 = 3$, $h_4 = 4$, $h_5 = 6$, and $h_6 = 24$. The mean values ± standard

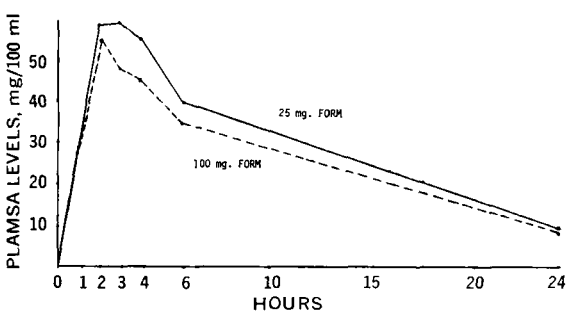

Figure 2—*Mean plasma levels of the 25- and 100-mg tablet forms.*

Table I—Mean Values of Plasma Levels at 1, 2, 3, 4, 6, and 24 hr for Two Tablet Forms

Dosage Form	1 hr	2 hr	3 hr	4 hr	6 hr	24 hr
I	27.3 ± 2.6	57.8 ± 3.7	58.4 ± 3.4	54.6 ± 2.2	39.5 ± 1.8	9.05 ± 0.53
II	27.6 ± 3.2	54.9 ± 5.6	47.7 ± 3.7	45.1 ± 2.9	34.3 ± 2.0	8.60 ± 0.58

errors of the six plasma metabolite levels in units of μg/100 ml are listed in Table I and displayed graphically in Fig. 2. (Hereafter, all mean values are listed with their corresponding standard errors in this fashion.)

The matrix R_1 of correlation coefficients of the plasma levels $X_1, \ldots, X_6$ for Form I is available in Table II, and the matrix R_2 of correlation coefficients of the levels for Form II is listed in Table III. The matrix R of correlation coefficients of differences between the six plasma levels for the two forms, I and II, and its inverse R^{-1} are found in Tables IV and V, respectively. The determinants of R_1, R_2, and R are in Table VI.

Correlated Case—For the computation of the Hotelling T^2, in addition to R^{-1}, it is necessary to have the individual pairwise Student t variables for the six levels. Their squares were found to be: $t_1^2 = 0.013$, $t_2^2 = 0.268$, $t_3^2 = 6.142$, $t_4^2 = 14.992$, $t_5^2 = 10.097$, and $t_6^2 = 0.757$.

When using the values of α_{ij} from R^{-1} in Table V, Eq. 9 becomes:

$$T^2 = (\alpha_{11}t_1^2 + \ldots + \alpha_{66}t_6^2) + 2(\alpha_{12}t_1t_2 + \ldots + \alpha_{56}t_5t_6) =$$
$$1.45t_1^2 + 2t_2^2 + 1.74t_3^2 + 3.94t_4^2 + 3.73t_5^2 + 1.13t_6^2 -$$
$$2[(0.61t_1t_2 + 0.31t_1t_3 + 0.62t_2t_3 + 0.17t_1t_4 + 0.86t_2t_4 +$$
$$0.71t_3t_5 + 2.99t_4t_5 + 0.25t_2t_6 + 0.15t_5t_6 + 0.08t_4t_6) -$$
$$(0.18t_3t_4 + 0.41t_1t_5 + 0.24t_2t_5 + 0.25t_1t_6 + 0.27t_3t_6)] \quad \text{(Eq. 13a)}$$

and now with the values of $t_1, \ldots, t_6$:

$$T^2 = 108.83 - 2[45.9 - 2.8] \quad \text{(Eq. 13b)}$$
$$= 22.63$$

For $N = 39$ and $K = 6$, F calculated by Eq. 11 is:

$$F_{calc} = 3.28 \text{ for } df_1 = 6, df_2 = 33 \quad \text{(Eq. 14)}$$

which, compared to a tabled F value (16) of about 3.4, is *not* significant at the 1% level (actually, 0.012).

Uncorrelated Case—The assumption that all correlations are zero implies, for the element of R^{-1}, that $\alpha_{ij} = 1$ for $i = j$ and $\alpha_{ij} = 0$ for $i \neq j$. In this case, calculation by Eq. 10 yields:

$$T^2 = t_1^2 + \ldots + t_6^2 = 32.27 \quad \text{(Eq. 15)}$$

or correspondingly, $F_{calc} = 4.68$, which for the same $df_1 = 6$, $df_2 = 33$, is *clearly significant* at the 1% level (actually 0.002).

Therefore, the *relative percent variation in* T^2 of the correlated result with respect to the uncorrelated one is:

$$\frac{32.27 - 22.63}{32.27} \sim 30\% \quad \text{(Eq. 16)}$$

Significance of Correlation—The significance of correlation can be seen in many ways.

From the point of view of hypothesis testing, Morrison (23, p. 113) obtains the likelihood ratio test for the complete independence of the components of a normally distributed K component vector as:

$$\chi^2 = -\left[N - 1 - \frac{(2K + 5)}{6}\right] \ln|R| \quad \text{(Eq. 17)}$$

The hypothesis of independence (*i.e.*, no correlation) is accepted if:

$$\chi^2 \leq \chi^2_{\alpha;K(K-1)/2} \quad \text{(Eq. 18)}$$

and rejected if $\chi^2 > \chi^2_{0.05;K(K-1)/2}$, where $\chi^2_{\alpha;K(K-1)/2}$ is the upper 100 α percentage point of the central χ-squared distribution with $K(K-1)/2$ degrees of freedom. If the criteria (Eq. 18) are overwhelmingly rejected, there is not only strong support for a multivariate analysis but a univariate approach may be quite misleading.

Application of Eq. 17 to R, the correlation matrix of $K = 6$ plasma level differences, yields from Table VI and for $N = 39$:

$$\chi^2 \cong -[38 - 3] \ln 0.0846$$
$$= 86.44 \quad \text{(Eq. 19)}$$

at 15 degrees of freedom. A $\chi^2 = 86.44$ compared to critical values of 25, 30.58, and 37.70 at significance levels of 5, 1, and 0.1%, respectively, is highly significant. This, therefore, gives strong motivation for a multivariate approach. Moreover, it is seen from Table VI that:

$$|R_1| \cong |R_2| \quad \text{(Eq. 20)}$$

This approximate equality not only gives support to the use of the Hotelling T^2 but pinpoints the major source of variation to be in the mean level differences.

Pursuing this latter point, the mean level differences at Hours 3, 4, and 6 are about 18, 17, and 13%, respectively; at other time points, they are essentially negligible. It is somewhat difficult to deal with these differences since the peak of a "volunteer" can occur at one of several times during 2, 3, 4, and 6 hr. However, it is later indicated that the mean peak levels for I and II only differ by about 9% and the mean time of peak occurrence was found to be 160 min for both I and II.

The significance of correlation can also be seen from the point of view of total area under the plasma level curve. As was inferred previously, the Hotelling T^2 is, in effect, a multivariate comparison of total area. Total area is broken up into six correlated subareas, which are then compared vectorily. It was previously noted (Eq. 14) that this comparison is not significant at the 1% level (actually 0.012). On the other hand, it will be seen later that the univariate comparison of total area yields a pairwise t_T =

Table II—Correlation Coefficients of Six Plasma Levels for 25-mg Tablet Form I

$$R_1 = \begin{bmatrix} 1 & 0.79 & 0.57 & 0.04 & 0.05 & 0.31 \\ & 1 & 0.72 & 0.19 & 0.24 & 0.40 \\ & & 1 & 0.40 & 0.41 & 0.42 \\ & & & 1 & 0.93 & 0.45 \\ & & & & 1 & 0.55 \\ & & & & & 1 \end{bmatrix}$$

Table III—Correlation Coefficients of Six Plasma Levels for 100-mg Tablet Form II

$$R_2 = \begin{bmatrix} 1 & 0.54 & 0.53 & 0.25 & 0.29 & 0.05 \\ & 1 & 0.70 & 0.64 & 0.53 & 0.26 \\ & & 1 & 0.56 & 0.57 & 0.21 \\ & & & 1 & 0.92 & 0.53 \\ & & & & 1 & 0.55 \\ & & & & & 1 \end{bmatrix}$$

Table IV—Correlation Coefficients of Six Plasma Level Differences between Forms I and II

$$R = \begin{bmatrix} 1 & 0.46 & 0.38 & 0.17 & 0.07 & -0.18 \\ & 1 & 0.55 & 0.54 & 0.43 & 0.08 \\ & & 1 & 0.44 & 0.46 & -0.11 \\ & & & 1 & 0.84 & 0.16 \\ & & & & 1 & 0.16 \\ & & & & & 1 \end{bmatrix}$$

Table V—Inverse Matrix of R

$$R^{-1} = \begin{bmatrix} 1.45 & -0.61 & -0.31 & -0.17 & 0.41 & 0.25 \\ & 2.0 & -0.62 & -0.86 & 0.24 & -0.25 \\ & & 1.74 & 0.18 & -0.71 & 0.27 \\ & & & 3.94 & -2.99 & -0.08 \\ & & & & 3.73 & -0.15 \\ & & & & & 1.13 \end{bmatrix}$$

Table VI—Determinants of R_1, R_2, and R

$\|R_1\|$	$\|R_2\|$	$\|R\|$
0.0106	0.0161	0.0846

Table VII—Statistics for the Pair (∇_T, U_T)

Dosage Form	$\bar{\nabla}_T$	$\bar{U}_T$	Correlation (∇_T, U_T)
I	703.4 ± 28.7	296.6 ± 19.7	0.01
II	619.8 ± 34.8	285.8 ± 21.5	0.11

2.94, which *is* significant at the 1% level (actually 0.006). One explanation for these two different results must relate to correlation.

Another view of significance of correlation, of course, relates to the *relative percent variation* in T^2 already indicated in Eq. 16. Equation 16 was computed with the pairwise t's of the six plasma levels. The t's for the levels at Hours 3, 4, and 6 are significant at α = 5% (0.017), 1% (0.001), and 1% (0.003), respectively, while the other t's at Hours 1, 2, and 24 are quite small. There is then a tendency to conclude that the effect of the set (1, 2, 24) on the overall process is not only small but also tends to dilute the role of the triplet (3, 4, 6). That this is not the case can be seen from the following.

First of all, the triplet (3, 4, 6) by itself yields a value of T^2 = 15.8. When the vector (1, 2, 24) is combined with (3, 4, 6), T^2 increases by about 43% to 22.63 (Eq. 13). This indicates that in a truly multivariate situation, t values are not necessarily indicators of the importance of variables.

Now, by making use of nonsingular linear transformations of the six plasma levels, three new variables can be found which have significant univariate t's (either at 5 or 1%). One such variable, of course, is total area which, in effect, is a sum of the six plasma levels. It was noted above that total area has a t_∇ = 2.94, which is significant at 1% (0.006). If total area replaces, say, plasma level 1, T^2 for the resulting six-variate comparison remains at 22.63 (Eq. 13) since a nonsingular linear transformation leaves T^2 fixed. However, T^2 for the uncorrelated case is T^2_{uncorr} = 40.94 and *the relative percent variation* in T^2 is now:

$$\frac{40.94 - 22.63}{40.94} = 44\% \qquad \text{(Eq. 21)}$$

This process can be continued to replace plasma levels at Hours 2 and 24 by, say, two new variables, Y and Z: Y = (level 2 + level 3 + level 4), and Z = (level 3 + level 6 + level 24). The t's of Y and Z are 2.19 and 3.11, respectively. The new six-variate vector (Y, Z, total area, level 3, level 4, and level 6) still gives T^2_{corr} = 22.63 but now T^2_{uncorr} = 54.34. The percent variation in correlation is:

$$\frac{54.34 - 22.63}{54.34} = 58\% \qquad \text{(Eq. 22)}$$

It seems clear that the correlations play a dominant role in these comparisons.

Comparisons with Total Plasma Areas and Total 6-hr Urines—Comparisons were made here using the pair (∇_T, U_T) where ∇_T in units of (micrograms per milliliter) × 24 hr denotes the total area (0–24 hr) under the plasma level curve, and U_T in milliliters relates to the total 6-hr urinary aldadiene level. The mean total plasma area, $\bar{\nabla}_T$, the mean total 6-hr urinary aldadiene level, $\bar{U}_T$, and the correlation between ∇_T and U_T for the two dosage forms are listed in Table VII.

When using a pairwise bivariate Hotelling T^2, the difference between the 25- and 100-mg dosage forms was found not to be significant at the 1% level (actually 0.021) and, moreover, ∇_T was found to be the better discriminator of the two. When the variables ∇_T and U_T were considered separately and when neglecting correlation, comparison with ∇_T showed a significant difference at the 1% level (actually 0.006), whereas with U_T no significant differences were observed. There is virtually no correlation between ∇_T and U_T for either dosage form.

Bivariate Comparison with Peak Metabolite Levels—The 2nd, 3rd, and 4th hr are generally the times of occurrence of the peak levels of individual curves. Therefore, it is of interest to make comparisons with the pair [h_m, max $a(h)$], where h_m is the time at the peak and max $a(h)$ is the peak plasma level.

Table VIII—Statistics for the Pair [h_m, max $a(h)$]

Dosage Form	Mean Time h_m	Mean max $a(h)$	Correlation h_m and max $a(h)$
I	160 ± 7.7 min	67.8 ± 3.2 μg/100 ml	−0.10
II	160 ± 7.5 min	61.6 ± 4.9 μg/100 ml	−0.35

Table IX—Statistics for Sodium and Potassium[a]

	$\bar{Na}$	$\bar{K}$	Correlation	$\overline{\text{Log (10 Na/K)}}$
I	67.9 ± 4.3	22.2 ± 1.2	0.50	1.5 ± 0.04
II	59.8 ± 4.1	18.7 ± 1.4	0.54	1.7 ± 0.2

[a] The units of sodium and potassium are mEq/6 hr.

The mean times, the mean peak levels, and the correlation between h_m and max $a(h)$ for the two dosage forms are listed in Table VIII. In general, mean peak levels are greater than the largest mean levels at specific measurement times. Consequently, the mean peak levels of 67.8 and 61.6 in Table VII are greater than the mean levels of 58.4 and 54.9 in Table I, respectively.

The mean peak level of the 25-mg dosage form is about 9% higher than that of the 100-mg form and the significant differences for the peak levels separately were found at the 5% level (actually 0.041). Since the mean times in each case are the same, a bivariate Hotelling T^2 showed the difference between the 25- and 100-mg dosage forms not to be significant at the 5% level (actually 0.124). The larger correlation, 35%, in Table VII for the 100-mg form probably relates to the slower passage of the larger tablet through the GI tract and/or to a smaller surface area relative to 4 × 25-mg tablets.

Comparisons with Urinary Sodium and Potassium—Comparisons with urinary sodium (Na) and potassium (K) data were made using a bivariate Hotelling T^2 on the pair (Na, K) and a univariate Student's t on the often used statistic log (10 Na/K). By using each statistic separately, the pair (Na, K) and the log ratio of Na/K, comparisons of the 6-hr test data were made for each dosage form with the corresponding data obtained the day prior to the testing, and no significant differences were found.

By using either the pair (Na, K) or the logarithm of the ratio Na/K, no significant differences at the 5% level were found between the two dosage forms during the test periods. The means of Na and K, the correlation (Na, K), and the mean of log (10 Na/K) for the two dosage forms are given in Table IX.

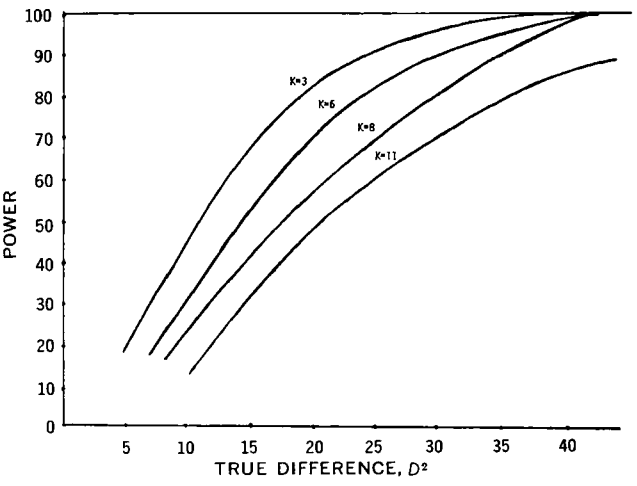

Figure 3—*Power (probability of detecting* D^2*) for Hotelling* T^2 *for* α = 1% *and* N = 39.

Table X—Correlation Coefficients of All 11 Variable Differences between Forms I and II

$\hat{R}$ =

1 hr	2 hr	3 hr	4 hr	6 hr	24 hr	Mean Time	Peak	Urine Volume	Log	Plasma Area	
1	0.46	0.38	0.17	0.07	−0.18	−0.16	0.44	0.28	0.07	0.31	1 hr
	1	0.55	0.54	0.43	0.08	−0.39	0.87	0.25	−0.09	0.71	2 hr
		1	0.44	0.46	−0.11	0.16	0.67	0.19	0.12	0.66	3 hr
			1	0.84	0.16	0.06	0.71	0.23	0.09	0.82	4 hr
				1	0.16	0.11	0.62	0.10	0.20	0.89	6 hr
					1	−0.23	−0.01	−0.12	−0.28	0.28	24 hr
						1	−0.14	0.02	−0.02	−0.01	Mean Time
							1	0.18	−0.02	0.78	Peak
								1	0.01	0.20	Urine Volume
									1	0.08	Log
										1	Plasma Area

All 11 Measurement Variables—In the previous sections, different subsets of a total of 11 measurement variables were used in the comparison of two dosage forms of spironolactone. It is now of interest to make comparisons in both the correlated (Eq. 9) and uncorrelated (Eq. 10) cases with all 11 measurement variables and to obtain some assessment of the relative importance of each variable with respect to its discrimination ability. Several accounts on the concept of best discriminator and stepwise selection of discriminators are available (13, 14, 18, 19).

The means for all 11 variables are listed in Tables I, VII, VIII, and IX, and the correlation matrix $\hat{R}$ of the 11 variable differences is given in Table X. When using the fact that $\hat{R}$ is not singular, despite redundancies in the measurement variables, the pairwise Hotelling T^2 for all 11 variables yields:

$$T^2_{corr} = 38.56 \qquad \text{(Eq. 23)}$$

while the corresponding uncorrelated Hotelling T^2 (Eq. 10) is found to be:

$$T^2_{uncorr} = 43.81 \qquad \text{(Eq. 24)}$$

The percent variation of correlated T^2 with respect to uncorrelated T^2 is about 12%.

The selection of variables were here obtained using a stepwise regression in which variables are entered by forward selection using the multiple correlation coefficient as the criterion for ordering. Selections can also be obtained based on Hotelling T^2 by selecting those variables that contribute most to T^2. Both procedures give generally consistent results. However, as is expected with different selection procedures, there are differences.

With the stepwise 11-variate regression procedure, the discriminators, in order of decreasing importance, were found to be: plasma level at Hour 4, plasma level at Hour 3, peak plasma level, total 24-hr plasma area, time of peak plasma level, urinary electrolyte ratio (log 10 Na/K), and plasma level at Hour 2. The plasma levels at Hours 1, 6, and 24 and the total 6-hr urine have about equally low discrimination power.

With the 11-variate Hotelling T^2, the corresponding order of discriminators was found to be: plasma level at Hour 4, total 24-hr plasma, plasma level at Hour 6, plasma level at Hour 3, peak plasma level, and plasma level at Hour 2. The plasma level at Hour 24 and the urinary electrolyte ratio have lesser power, while the plasma level at Hour 1, the time of peak, and the total 6-hr urine have equally low discrimination power.

There appears to be general agreement that the four key variables are plasma level at Hour 4, plasma level at Hour 3, peak plasma level, and total 24-hr plasma area. The plasma level at Hour 6 and the time of peak seem to have inversely important roles, depending upon the selection technique; the urinary electrolyte ratio, the plasma levels at Hours 1, 2, and 24, and total 6-hr urine have lesser roles of decreasing importance.

Finally, the selection procedure based on T^2 takes into account both mean and variance–covariance information, whereas it appears that the stepwise regression procedure puts less weight on the means of the variables. This accounts for the low position of time of peak, whose mean difference is zero, in the T^2 procedure. However, more study of stepwise selection procedures is needed.

Power of Hotelling T^2—Because of the presence of nonnegligible correlations, the statistical approach for comparing dosage forms is here based on a multivariate test of significance. Another approach might be based on confidence intervals. However, the use of univariate confidence intervals, as indicated by Westlake (20), appears not to be useful here since correlations are not taken into account. Using a new technique (22), tables are being prepared for obtaining multivariate (simultaneous) confidence intervals and prediction intervals that are more to the point.

The criterion often used to judge the desirability of a significance test is the power function. The method of Hotelling T^2 has not only a number of optimum properties (13), but curves of its power function can be obtained from Tang's tables (24).

For a sample of size 39 and significance level 1%, T^2 power curves for vectors of K = 3, 6, 8, and 11 components as a function of true differences, D^2 (*i.e.*, true values of Hotelling T^2), are given in Fig. 3. For each K, one can readily obtain the smallest D^2, which can be detected with a power of, say, 90%.

Other Studies—With three daily dosage regimens at 25, 100, and 200 mg of spironolactone and a placebo, Wolf *et al.* (25) investigated antihypertensive responses in 24 cases of benign, essential hypertension. Each dosage regimen was administered continuously in a randomized double-blind fashion for 6 weeks including two crossover periods. Systolic and diastolic blood pressures were significantly lower for all patients on spironolactone compared to placebo. The decreases in blood pressure were not significant at the low dosage level (25 mg), while the most efficacious response occurred with the 100-mg regimen. Treatment at the 200-mg dosage level appeared to be no more effective than at the 100-mg level.

In the present study, it is believed that absolute differences in plasma metabolite levels, particularly peak levels of 67.8 and 61.6 μg/100 ml observed for the 25- and 100-mg tablet formulations, respectively, would be clinically unimportant. This conjecture is consistent with the above-mentioned observations (25, 26) that, following a therapeutic dosage regimen with a relatively narrow dosage range, an optimum circulating level of active metabolites can be achieved and an optimal antihypertensive response of spironolactone can be realized. It is anticipated that a clinical requirement of 100 mg of spironolactone can be achieved with either four 25-mg tablets or one 100-mg tablet.

SUMMARY

A comparative study of two dosage forms of an important marketed drug was carried out using the techniques of multivariate statistical analysis. In studying the biological equivalence of dosage forms as a time-dependent process, two significant facts emerged. Correlations among measurement types should not be ignored and measurement variables should be selected according to their collective discrimination ability.

Specifically, comparisons of the 25- and 100-mg spironolactone tablet forms were examined by multivariate comparisons with: *(a)* hour-by-hour plasma metabolite levels, *(b)* total plasma area and total urinary metabolite level, *(c)* peak plasma levels, and *(d)* urinary electrolytes, sodium and potassium.

The key discriminators for comparing the two dosage forms were found to be plasma metabolite levels at Hours 4 and 3, peak plasma metabolite level, and total 24-hr area under the plasma level curve. The total 6-hr urinary metabolite level and the plasma levels at Hours 1 and 24 were relatively poor discriminators.

The mean peak plasma metabolite levels for the 25- and 100-mg formulations reached 67.8 and 61.6 μg/100 ml, respectively, at a mean time of 160 min.

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β-Hydroxyhomomethionine and β-Hydroxymethoxinine: Preparation and Separation of Diastereomers

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Abstract □ β-Hydroxyhomomethionine and β-hydroxymethoxinine were prepared by a one-step condensation of the corresponding aldehydes with cupric glycinate in an alkaline medium. The diastereoisomers of each compound were separated by means of partition column chromatography with cellulose as the immobile phase and methyl ethyl ketone–*n*-butanol–ammonia–water as the eluant. The compounds were characterized by paper chromatography in five solvent systems, chemical ionization mass spectrometry, elemental analysis, Van Slyke ninhydrin determination of α-carbonyl carbon, Van Slyke nitrous acid determination of primary amino nitrogen, IR absorption, and NMR spectra.

Keyphrases □ β-Hydroxyhomomethionine—preparation and separation of diastereomers □ β-Hydroxymethoxinine—preparation and separation of diastereomers □ Methionine analogs—preparation and separation of diastereomers of β-hydroxyhomomethionine and β-hydroxymethoxinine

In studies on β-hydroxy-α-amino acids, it was noted that certain of these amino acids in the *N*-chloroacetylated form, even as racemic mixtures of the diastereomers, inhibited the growth of certain microorganisms in systems selected for antitumor screening (1). Of the 11 *N*-acyl-β-hydroxy-α-amino acids tested, *N*-chloroacetyl-β-hydroxynorleucine showed the most promise (1).

β-Hydroxynorleucine may be considered to be a structural analog of threonine and methionine, and it is possible that its growth-inhibitory activity is attributable to its structural resemblance to those amino acids. Since *N*-acylmethionine is a better substrate of hog renal acylase than is *N*-acylnorleucine (2), it appeared that, if the growth-inhibitory action was related to this hydrolysis, a methionine analog would be a better inhibitor.

With this in mind the preparation of two methionine analogs was undertaken. The present article reports the synthesis, the isolation of the diastereomers, and identification of β-hydroxyhomomethionine[1] and β-hydroxymethoxinine[1].

EXPERIMENTAL

Preparation of β-Hydroxyhomomethionine A, B, and AB[2]—Ten grams (47.2 mmoles) of cupric glycinate (3) was dissolved in 300 ml of 0.2 *M* sodium carbonate solution. The solution was cooled to 5° and 20 ml (about 186 mmoles) of 3-methylthiopropionaldehyde[3] was added in four equal portions over 8 hr. The alkalinity of the reaction mixture was tested before and after each addition of the aldehyde, and more 0.2 *M* sodium carbonate solution was added as required to keep the system basic. The reaction mixture was then set in the coldroom at 5° with constant stirring (magnetic stirrer) overnight (18–22 hr), after which time the reaction was terminated by acidification with acetic acid. A small aliquot was removed and spotted on Whatman No. 1 chromato-

[1] β-Hydroxyhomomethionine = 2-amino-3-hydroxy-5-methylthio-*n*-valeric acid; β-hydroxymethoxinine = 2-amino-3-hydroxy-4-methoxy-*n*-butyric acid.

[2] The letters A and B are arbitrarily assigned to the diastereomers moving faster and slower, respectively, when chromatographed on paper and developed in a methyl ethyl ketone–*n*-butanol–concentrated ammonia–water (3:5:1:1) solvent system.

[3] Eastman Kodak Co., Rochester, N.Y.