a significant problem encountered in utilizing the 75/75 Rule. Of 106 drug products tested in 18 bioavailability studies, 94 products had <40% *CV*.

The author of the original article pointed out that given a true correlation coefficient, $\rho = 0.90$, the probability of success utilizing the 75/75 Rule was 90% in bioavailability trials involving 24 subjects where the intersubject *CV* is 40% for both the test and reference drug, and the intrasubject *CV* is 30%. The probability of success of applying the 75/75 Rule will significantly increase when the inter- and intrasubject variations are reduced to <40 and 30%, respectively (Table II) (2). The proportion of 1000 studies involving as few as 12 subjects meeting the 75/75 Rule utilizing drugs with an intersubject *CV* of <40% and an intrasubject *CV* of <20% is >88%, and 98% with inter- and intrasubject *CV*s of 30 and 15%, respectively.

The application of the 75/75 Rule is only valid for drugs having a well-defined reference standard that has reproducible pharmacokinetic properties in terms of absorption and clearance. Drugs having a large coefficient of variation associated with extensive first-pass metabolism are often required to undergo multiple-dose steady-state study comparisons or other more appropriate study design as a basis of drug approval. To achieve these results, the FDA often utilizes an oral solution as a basis of comparison where the reference drug has poor bioavailability. Also, the 75/75 Rule is only applied in conjunction with a proper analysis of variance and the FDA relies on additional data analyses.

(1) J. D. Haynes, *J. Pharm. Sci.*, **70**, 673 (1981).
(2) M. C. Meyer, FDA Contract No. 223-77-3011 (Univ. of Tennessee A1975-1981 Reports).

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FDA 75/75 Rule: A Response

Keyphrases □ Bioavailability—studies involving subjects with intersubject coefficient of variation, FDA 75/75 Rule □ Bioequivalance—studies involving subjects with intersubject coefficient of variation, FDA 75/75 Rule

To the Editor:

Dr. Cabana's communication (1) refers to an article (2) that is critical on statistical grounds of the FDA Division of Biopharmaceutic's proposed 75/75 Rule for bioequivalency studies. We emphasize that the point deserving discussion here is not the rigor of the 75/75 Rule, but rather, the fatal flaws inherent in its form. The same flaws would exist even if the rule were less rigorous (50/50) or more rigorous (90/90), because it would retain the same undesirable form: the dispersion of certain ratios. We applaud the vast majority of the pharmacokinetic–bioavailability–bioequivalency regulations and guidelines as contributing to the improvement of health care; we also are glad to see that the FDA accepts the Pitman–Morgan *F*-test as the proper test for equality of test-product and reference-product variation in crossover bioavailability–bioequivalency studies. This *F*-test is described in the statistical literature as "uniformly most powerful" (3); therefore, no other test of variation in a study can have as much statistical power for detection of true differences in standard deviations. The word uniform indicates that this superiority holds for differences of all magnitudes.

In essence we agree with the communication (1) which states that the intersubject coefficient of variation (*CV*) of 40% used previously (2) is not the norm. The choice of 40% per se is not critical; however, the question is whether the results would be much different at a 35% *CV*. Such large coefficients of variation reflect the skewness of the distributions. It also should be noted that the intrasubject *CV* is 20 or 30%, common values for the error term in the analysis of variance (ANOVA).

The performance in a certain region of a proposed statistical test, such as the 75/75 Rule, generally is not very interesting to the statistician designers and the users of such a rule. The main interest in the performance of the proposed test centers on how the more variable drug products are treated by the test—whether they are treated fairly in this respect. The number of such drug products is not negligible, accounting for ≥10% (depending on a cutoff *CV* of 35 or 40%) of drugs studied, according to Dr. Cabana's Table I (1). (If other parameters for a test product have unacceptable values, they should not obscure the point under consideration.) "Are they treated fairly?" is the question addressed earlier (2) for the case of equal averages, and the answer is that they are not. For example, according to the 75/75 Rule, a test product for chlorothiazide with a variation of *AUC* values that is 50% greater than the variation of the reference product *AUC* values usually has a greater chance of being declared bioequivalent than does a test product for phenytoin with the same variation as its reference product.

The main flaw of the 75/75 Rule lies in the fact that the degree of dispersion of the ratio depends on the dispersion or both products, test and reference, without distinction. Thus, a test product which fails the 75/75 Rule in a study may do so because the reference product standard deviation is relatively large—the reference product should fail the dispersion test in that study. For example, for the 12 drugs in Dr. Cabana's Table I (1), suppose that in each study a test product always had the smallest coefficient of variation shown for that drug and the reference product had the largest coefficient of variation—both products with the same average. The unadjusted *F*-values would be, for the *AUC* columns of Table I (1): 7.9, 1.9, 4.7, 2.8, 2.4, 3.1, 3.3, 2.1, 3.4, 1.6, 3.7, 2.4, 1.8, 2.6, 5.2, 2.6. 1.8, and 5.6. Superior uniformity would be indicated for such test products but probably many would fail the 75/75 Rule falsely, because the greater variability is that of the reference products.

The statement that the 75/75 Rule "is only applied in conjunction with a proper analysis of variance" (1) implies a remedy, probably subjective, but the fatal flaws remain; the rule should be withdrawn. Furthermore, since the performance of the 75/75 Rule is affected by differences in the two mean *AUC*s (for test and reference materials),

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as well as differences in the two standard deviations, and since the means themselves are tested for equality in the ANOVA, the 75/75 Rule also might be said to place the test product in double jeopardy.

The stress placed previously (1) on a "well-defined reference standard which has reproducible pharmacokinetic properties in terms of absorption and clearance" or "an oral solution" makes us ask just where the criterion of acceptable reproducibility of a reference standard is set down?

(1) B. E. Cabana, *J. Pharm. Sci.*, **72**, 98 (1983).
(2) J. D. Haynes, *ibid*, **70**, 673 (1981).
(3) M. G. Kendall, "The Advanced Theory of Statistics," 3rd ed., vol. 2, Hafner, New York, N.Y., 1973, p. 531.

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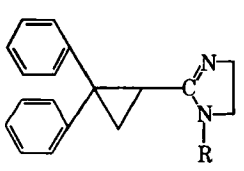
Radioimmunoassay for the New Antiarrhythmic Agent Cibenzoline in Human Plasma

Keyphrases □ Cibenzoline—new antiarrhythmic agent, radioimmunoassay in human plasma □ Antiarrhythmic agent—cibenzoline, radioimmunoassay in human plasma □ Radioimmunoassay—cibenzoline, a new antiarrhythmic agent, human plasma

To the Editor:

Cibenzoline [*dl*-4,5-dihydro-2-(2,2-diphenylcyclopropyl)-1H-imidazole] (I), a new oral antiarrhythmic agent with a novel chemical structure, is presently undergoing clinical evaluation. The present report describes the development and characteristics of a radioimmunoassay for cibenzoline which permits its quantitation directly in human plasma.

To obtain antibodies to cibenzoline, an immunogen was first prepared by covalently coupling the N_1-acetic acid derivative of cibenzoline[1] (II), as a hapten, to bovine serum albumin using a mixed anhydride procedure (1). Rabbits were immunized with the resulting conjugate, and the antiserum with the highest titer of antibodies of cibenzoline was used.



I: R = —H
II: R = CH_2CO_2H

The radioligand used for the assay was [^{3}H]cibenzoline with a specific activity of 10.8 Ci/m*M*[2]. Prior to use, radiochemical purity was established by TLC on silica gel using ethyl acetate–methanol–ammonia (80:15:5) as the solvent system.

The radioimmunoassay was carried out in 12 × 75-mm disposable glass tubes using 0.1 *M* phosphate buffered saline (pH 7.4) containing 0.1% gelatin and 0.1% sodium azide as the assay buffer. Plasma samples (0.02–0.1 ml) containing standard or unknown concentrations of cibenzoline were mixed with 0.2 ml of [^{3}H]cibenzoline in buffer (10,000 cpm) followed by 0.2 ml of diluted antiserum (1:600), and the mixture was incubated at 4° for 30 min. Then, 1 ml of a stirred suspension of a polymer-bound second antibody (goat anti-rabbit IgG)[3] was added and the tube contents were vortexed briefly and allowed to stand at 4° for 1 hr. Following centrifugation at 2000 rpm for 10 min, each supernatant was aspirated off, the pellet suspended in 0.4 ml of 1 *M* acetic acid, and mixed with 3 ml of scintillation fluid[4]. The tube was capped and radioassayed directly in a liquid scintillation counter[5] modified as described previously (2). A calibration curve was generated using a four-parameter logistic curve-fitting program for a desktop calculator[6] (3).

The logit-log calibration curve for cibenzoline was linear from 4 to 200 ng/ml using a 0.1-ml sample of plasma. Such sensitivity is adequate for the quantitation of cibenzoline following administration of therapeutic doses of the drug. The intra- and interassay coefficients of variation (n = 6) did not exceed 6.5 and 10%, respectively, over a range of 38–219 ng/ml of cibenzoline in a selection of random clinical samples. Although the antiserum was found to cross-react almost 100% with the 4,5-dehydro derivative of cibenzoline, a known metabolite of the drug in the dog[7], the specificity of the radioimmunoassay for the analysis of human plasma samples was evaluated by comparison with a specific electron-capture GLC method which was developed and utilized at another research institution[8]. For 57 clinical samples analyzed by both procedures (radioimmunoassay = y), the correlation coefficient, regression line slope, and y-intercept were 0.98, 0.93, and 16, respectively, over a range of 12–287 ng/ml. Although the slope and intercept were significantly different than 1 and 0, only 4 of the 57 highly correlated (r = 0.98) observed data points lay outside the 95% confidence limits of the fitted regression line, which indicates that the radioimmunoassay is in reasonable agreement with a specific chromatographic procedure for the quantitation of cibenzoline. It has been shown by high-performance liquid chromatography (4) that only trace amounts of the 4,5-dehydro metabolite of cibenzoline are present in human plasma, and the metabolite is separated from the parent drug in the electron-capture GLC assay.

In subjects who had received 65 mg of the drug three times a day for 6 days, the peak plasma concentrations at steady-state were ~300 ng/ml of cibenzoline.

A simple radioimmunoassay procedure with adequate sensitivity and specificity was developed for the quanti-

[1] The hapten was prepared from cibenzoline by alkylation with ethyl chloroacetate in ethanol followed by base hydrolysis and crystallized from isopropanol–ether as a partial hydrate, mp 202–204°. The MS and NMR spectra were compatible with the proposed structure.
[2] Prepared by Chemical Research Division, Hoffmann-La Roche Inc.
[3] Roche Diagnostics, Nutley, NJ 07110.
[4] Aquasol, New England Nuclear Corp., Boston, MA 02118.
[5] Packard Tri-Carb model 3255.
[6] TI-59, Texas Instruments, Lubbock, TX 79408.
[7] Data on file, Hoffmann-La Roche Inc.
[8] Personal communication, Laboratoires UPSA, Rueil Malmaison, France.

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