In normal statistical practice, k_1 is chosen to be equal to $-k_2$ to give a symmetrical confidence interval for $\mu_s - \mu_n$. However, in this instance, one is presumably interested in a symmetrical confidence interval for μ_n of the type:

$$\mu_s - \Delta \leq \mu_n \leq \mu_s + \Delta \qquad \text{(Eq. 3)}$$

where $\Delta = \{k_1 s/\sqrt{6} - (\bar{x}_s - \bar{x}_n)\} = -\{k_2 s/\sqrt{6} - (\bar{x}_s - \bar{x}_n)\}$. This relation implies that k_1 and k_2 must be chosen so that the second and third terms in this equality are equal. Thus, the confidence interval for the mean urinary excretion with the new formulation will be given as an interval which is symmetric about the corresponding mean for the standard formulation. To achieve this end, one must set:

$$2(\bar{x}_s - \bar{x}_n) = (k_1 + k_2)s/\sqrt{6} \qquad \text{(Eq. 4)}$$

and determine k_1 and k_2 accordingly.

For example, suppose in a particular crossover trial in 12 subjects that $\bar{x}_s = 11.5$, $\bar{x}_n = 10.75$, and $s = 0.75$. Then substituting in Eq. 4, one finds that $k_1 + k_2$ must equal 4.90. By examination of a tabulation of the probability integral for the t-distribution with 10 degrees of freedom[1], it can now be determined that k_1 and k_2 must be approximately equal to 6.72 and -1.82, respectively. The value of Δ can then be computed to be 1.31. With 95% confidence, it can be stated that the confidence interval $\mu_s \pm 1.31$ covers the mean μ_n or, alternatively, if μ_s is approximated by $\bar{x}_s$ (i.e., 11.5), that with 95% confidence the mean total urinary excretion of the drug for the new formulation is within 11.4% (i.e., 1.31/11.5) of the mean for the standard formulation.

The latter statement is now in a form that the clinical pharmacologist can use as the basis for a rational decision as to the clinical equivalence of the two formulations. For example, he may have decided that if the new formulation comes within 15% of the standard, it is essentially equivalent. In the numerical example given here, he would presumably decide that the new formulation is essentially equivalent to the standard even though (as one can easily verify from the numbers given) the difference between them is significant at the 0.05 level. To summarize: a final decision as to the equivalence of two formulations of a drug must involve some judgment on the part of the clinical pharmacologist or physician. To this end, it seems appropriate that the statistician's analysis should result in a statement that the former can use as the basis for his decision. If classical hypothesis testing techniques are used, however, and decisions are made on the basis of significance tests, then the decision is essentially made by the statistician and the clinical pharmacologist's judgment is bypassed. It is true that his judgment may have played an important part in designing the experiment, based on the available information concerning the inherent variability of the data. However, the judgment may be largely negated if the variability turns out,

[1] See, for example, "Biometrika Tables for Statisticians," Vol. I, E. S. Pearson and H. O. Hartley, Eds., Cambridge University Press.

in the actual trial, to be either much less or much more than anticipated.

One interesting result of the proposed approach is that if a manufacturer produces a formulation that is almost identical to the standard, a well-controlled experiment of adequate size will give small confidence intervals and a high probability of demonstrating practical equivalence. A poorly controlled experiment of inadequate size, on the other hand, will give confidence intervals so large that practical equivalence cannot be demonstrated. In the latter case, with a hypothesis-testing approach to the analysis, exactly the reverse of this situation is true.

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Determination of Meprobamate in Dissolution Studies: Shortcomings of Direct GLC and Development of a New Assay

Sir:

USP XVIII introduced dissolution tests for seven kinds of tablets and, in the case of meprobamate, a GLC procedure was specified for the determination of the drug in solution (1). The USP recommendation reads: "... the amount in solution being determined on filtered portions of the *Dissolution Medium* by means of gas chromatography, the internal standard consisting of a solution of dibutyl phthalate in anhydrous ether containing 0.4 mg. per ml." This approach was used in our laboratory and it was found that, under a variety of conditions, meprobamate cannot be accurately quantitated by direct GLC because of breakdown of the drug in the injection port. The first supplement to USP XVIII (2) revised this analytical procedure and replaced it by a colorimetric assay adapted from a method recently described by Poole et al (3). The purposes of this report are to point out the shortcomings of the USP's GLC method and to propose a specific and sensitive assay for the determination of meprobamate in solution.

The GLC method originally recommended by the USP is very similar to that proposed by Douglas et al. (4) since both utilize direct GLC of meprobamate as well as quantitation with dibutyl phthalate as an internal standard. Because the USP did not specify any GLC operating conditions, it appeared reasonable to
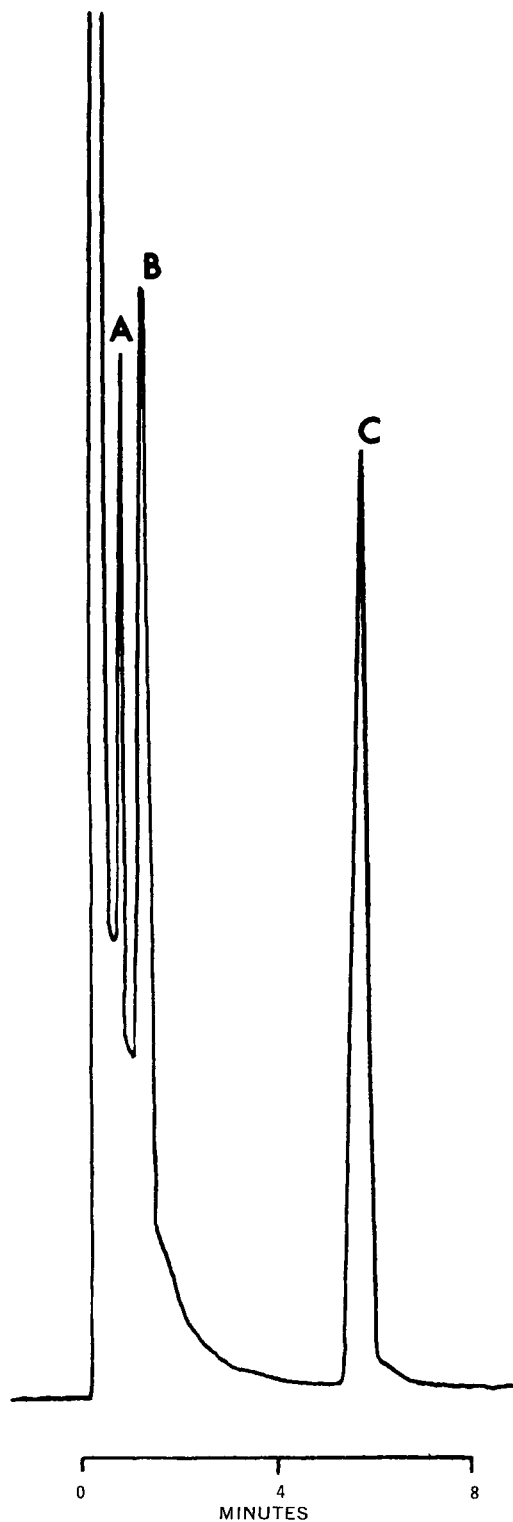
Figure 1—*Chromatogram of meprobamate (100 mcg.) obtained after injection of an ether extract using direct GLC. Key: peak A, decomposition product; peak B, meprobamate; and peak C, dibutyl phthalate.*

use those of Douglas *et al.* (4): glass column (3.8% UC-W98 methyl silicone on 80–100-mesh Diatoport S) at 180°, injection port at 275°, and detector at 225°. A typical chromatogram (Fig. 1) shows three peaks, A, B, and C, where peak C corresponds to the internal standard. From the work of previous investigators

(5–7) as well as our own results on the effect of injection port temperature on meprobamate breakdown, it is apparent that peaks B and A correspond, respectively, to unchanged meprobamate and a decomposition product. Thus, it was consistently found that a decrease in injection port temperature was accompanied by a concomitant decrease in A/B peak height ratios.

Further investigation of this phenomenon showed that the A/B peak height ratio is very dependent on injection rate, the slower injections always yielding the larger ratios. As an example, a rapid (1-sec.) followed by a slow (3-sec.) injection of an ether extract of a 200-mcg./ml. aqueous solution gave A/B peak height ratios of 3.89 and 1.64, respectively. As pointed out also by Holch and Gjaldbaek (7), these results suggest that reaction with the injection needle plays an important role in the observed decomposition. As seen in Fig. 1, the decomposition product has a very short retention time (50–60 sec.) relative to the internal standard (6 min.). Thus, under different GLC conditions (higher column temperature and/or larger solvent front), peak A can merge with the solvent peak and decomposition can be overlooked. This phenomenon was observed in our laboratory and it may well be the reason why Douglas *et al.* (4) did not find any decomposition.

Several attempts to establish a calibration curve proved unsuccessful. Table I shows the B/A and B/C peak height ratios obtained with various amounts of meprobamate after repeated injections of the ether extracts (care was taken to maintain the injection rate as constant as possible). A plot of B/C peak height ratios *versus* drug amount is not linear but parabolic. Furthermore, the coefficients of variation on repeated injections at each concentration ranged from 10.6 to 27.4%. Although the coefficients of variation decreased at the higher concentrations, they are still too high to allow an accurate determination.

The thermal decomposition of meprobamate at the injection port has been extensively documented in the past 6 years (5–10). Goldbaum and Domanski (5) found a marked increase in the meprobamate breakdown product peak with a corresponding decrease in the meprobamate peak when the injection port temperature was raised from 250 to 320° (5% SE-30 on Gas Chrom Q). Finkle (6) reported that the meprobamate breakdown product peak becomes significant at temperatures greater than 230° (2.5% SE-30 on Chromosorb G). Cardini *et al.* (9) stated that, due to decomposition, they were not able to use their GLC method for quantitative analysis. In the past year, Holch and Gjaldbaek (7) reported that meprobamate yields a total of four peaks (3.8% OV-1 on Diatoport S), three of which were identified as meprobamate, 2-methyl-2-propyl-1,3-propanediol monocarbamate, and 2-methyl-2-propyl-1,3-propanediol (I). These authors also studied the effects of injection port temperature and injection rate and obtained results very similar to our own.

Whereas some investigators (8–10) reported that decomposition precluded a quantitative determination, a few others (6, 11) attempted to minimize the breakdown and utilized direct GLC. Whether or not meprobamate can be assayed by direct GLC depends on a multitude of experimental factors (nature of solid sup-

Table I—Determination of Meprobamate by Direct GLC: B/A (Meprobamate/Decomposition Product) and B/C (Meprobamate/Internal Standard) Peak Height Ratios at Various Amounts of Meprobamate

Amount of Meprobamate, mcg.	Number of Injections	B/A Peak Height Ratio, Mean ± SD	B/C Peak Height Ratio, Mean ± SD
50	7	0.771 ± 0.203(26.33)[a]	0.544 ± 0.149(27.4)[a]
100	7	1.226 ± 0.249(20.30)[a]	1.067 ± 0.219(20.5)[a]
200	7	2.755 ± 0.573(20.8)[a]	2.522 ± 0.356(14.1)[a]
400	7	6.121 ± 1.172(19.1)[a]	5.885 ± 0.622(10.6)[a]

[a] Corresponding coefficient of variation in percent.

port and liquid phase, injection port and column temperatures, injection rate, etc.), some of which are difficult to control. Therefore, even if an investigator defines conditions whereby meprobamate can be chromatographed directly with adequate accuracy, it cannot be assumed that these conditions can be readily reproduced. It becomes apparent that the GLC procedure as originally stated in USP XVIII was inadequate since it did not specify any GLC conditions. Furthermore, in view of the large number of reports on the decomposition of meprobamate, it is surprising to find that direct GLC was selected as a reference method in USP XVIII. Finally, dibutyl phthalate is a poor choice as an internal standard since it is not structurally related to meprobamate. It is, therefore, not surprising that the USP XVIII GLC procedure was short lived. The current official procedure is a colorimetric method adapted from that of Poole et al. (3). Although this method is nonspecific (since N—H containing substances would interfere), its reproducibility was found superior to that of the USP GLC procedure.

The method proposed here involves alkaline hydrolysis of meprobamate to I followed by silylation of I with N,O-bis(trimethylsilyl)acetamide[1]; 2-methyl-2-ethyl-1,3-propanediol[2] (II) is used as an internal standard and is added directly to the dissolution sample prior to hydrolysis. After hydrolysis (10 min. at 100°), ether extraction, and evaporation, the residue is reacted with N,O-bis(trimethylsilyl)acetamide (10 min. at 60°), whereby I and II are converted to the corresponding trimethylsilyl ethers, III and IV. The latter yield symmetrical peaks with retention times of 2.5 and 3.5 min., respectively, at 115° (3% SE-30 on Chromosorb W). Table II gives peak height ratios of the silyl ethers (III/IV) for various amounts of meprobamate up to 400 mcg. A plot of the data (peak height ratio versus amount of meprobamate) yields a straight line passing through the origin (correlation coefficient $r = 0.995$). The excellent reproducibility of the method is indicated by the small coefficients of variation. The method is quite convenient for dissolution studies since it is rapid

Table II—Determination of Meprobamate after Hydrolysis and Silylation: Peak Height Ratios of III to IV at Five Different Amounts of Meprobamate

Amount of Meprobamate, mcg.	Number of Determinations	III/IV Peak Height Ratio, Mean ± SD
100	6	0.226 ± 0.00548 (2.42)[a]
150	2	0.315 —
200	6	0.416 ± 0.00510 (1.23)[a]
300	2	0.630 —
400	6	0.813 ± 0.00678 (0.834)[a]

[a] Corresponding coefficient of variation in percent.

(90 min. for a dissolution run of 10 samples with a calibration curve) as well as specific and sensitive. This approach has now been applied to the determination of meprobamate in biological fluids, and a complete report on the assay will be published shortly.

(1) "The United States Pharmacopeia," 18th rev., Mack Publishing Co., Easton, Pa., 1970, p. 402.
(2) "First Supplement to the Pharmacopeia of the United States," 18th rev., Mack Publishing Co., Easton, Pa., 1971, p. 19.
(3) J. W. Poole, G. M. Irwin, and S. Young, J. Pharm. Sci., 60, 1850(1971).
(4) J. F. Douglas, T. F. Kelley, N. B. Smith, and J. A. Stockage, Anal. Chem., 39, 956(1967).
(5) L. R. Goldbaum and T. J. Domanski, J. Forensic Sci., 11, 233(1966).
(6) B. Finkle, ibid., 12, 509(1967).
(7) K. Holch and J. C. Gjaldbaek, Dansk Tidsskr. Farm., 45, 32(1971).
(8) R. F. Skinner, J. Forensic Sci., 12, 230(1967).
(9) C. Cardini, V. Quercia, and A. Caló, Boll. Chim. Farm., 107, 300(1968).
(10) O. Cerri, ibid., 108, 217(1969).
(11) K. Holch, Dansk Tidsskr. Farm., 45, 107(1971).

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[1] TRI-SIL/BSA, Pierce Chemical Co., Rockford, Ill.
[2] K & K Laboratories, Inc., Hollywood, Calif.