Correlation of Dissolution, Disintegration, and Bioavailability of Aminosalicylic Acid Tablets

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Abstract ☐ Rank-order correlations are demonstrated among dissolution, disintegration, and several measures of bioavailability for tablets of aminosalicylic acid and its salts. The correlation between disintegration time and the percent of the dose excreted in the urine is examined quantitatively using both linear and quadratic models. The results support the use of either disintegration or dissolution tests to control the availability of this drug and suggest control limits for these tests. The suggested limits on the disintegration tests are shorter than those currently specified in USP XVIII monographs on these items.

Keyphrases ☐ Aminosalicylic acid and aminosalicylate tablets—rank-order correlations among dissolution, disintegration, and bioavailability ☐ Dissolution, disintegration, and bioavailability of aminosalicylic acid and aminosalicylate tablets—rank-order correlations ☐ Disintegration, dissolution, and bioavailability of aminosalicylic acid and aminosalicylate tablets—rank-order correlations ☐ Bioavailability, dissolution, and disintegration of aminosalicylic acid and aminosalicylate tablets—rank-order correlations

In 1956, Chapman *et al.* (1) reported a direct relationship between *in vitro* disintegration times and the biological availability of sodium aminosalicylate tablets. More recently, Middleton *et al.* (2) compared disintegration times and dissolution rates with the biological availability of tablets of aminosalicylic acid and sodium, potassium, and calcium aminosalicylate. Middleton *et al.* concluded, however, that disintegration times did not correlate with availability while dissolution rates gave good correlation. Neither paper supported its claims with a statistical evaluation of the data.

The doubt Middleton *et al.* (2) cast upon the value of disintegration tests for monitoring drug availability from aminosalicylate tablets, plus the fact that they did not use the USP-NF dissolution apparatus and presented only very limited data on tablets of aminosalicylic acid itself (our primary concern), led to this reevaluation of the relationship between *in vivo* and *in vitro* data for this product. Data available in the literature were used along with data available in this laboratory on a variety of aminosalicylic acid formulations exhibiting a wide range of dissolution and disintegration times. Significant rank-order correlations were found between several *in vivo* and *in vitro* parameters of availability, but only the data compiled on disintegration times and urinary excretion of drug were adequate for quantitative correlation.

EXPERIMENTAL

Tablets—Uncoated tablets and tablets with various enteric coatings were used. These tablets exhibited a wide range of dissolution and disintegration behavior. Suspensions of aminosalicylic acid powder were also administered to several subjects.

Dissolution Studies—Dissolution rates for uncoated tablets were measured in the USP-NF dissolution apparatus, using 900 ml. of pH 7.5 buffer (USP) in each vessel and a stirring speed of 165 r.p.m.

Enteric-coated tablets were washed with pH 1.2 buffer for 1 hr. before placing them in the dissolution apparatus. Samples were collected at 5-min. intervals for rapidly dissolving tablets and at 20-min. intervals for all other tablets. The dissolution samples were analyzed for aminosalicylic acid using a modification of the automated analysis procedure reported by Wrightman *et al.* (3).

The results of the dissolution tests are reported as the average time required for 20% of the drug to go into solution from four or five tablets (T_{20}). This time was determined by a straight-line interpolation between the data points; in the case of enteric-coated tablets, it does not include the time spent in pH 1.2 buffer prior to the dissolution test. T_{20} was chosen rather than the more common T_{50} because several of the tablets tested dissolved so slowly that T_{50} could not be determined in a reasonable length of time; several T_{20} values were not determined for this same reason.

Disintegration—Disintegration times were determined using the USP apparatus. The disintegration times for enteric-coated tablets were determined in simulated intestinal fluid USP following 1 hr. in simulated gastric fluid USP. The disintegration times for non-enteric tablets were determined in simulated gastric fluid USP. Disks were used in both cases. The procedure differed from the USP procedure in that the tablets were observed continuously and a disintegration time for each tablet was recorded. The average of the observed disintegration times is reported rather than the time required for all the tablets to disintegrate, as specified in USP XVIII. This modification was expected to give better correlation with the clinical results, which are also reported as average plasma or urine concentrations from several subjects, each of whom ingested several tablets. In addition, a measure of tablet uniformity is available from data recorded in this manner.

Clinical Studies—All subjects were fasted overnight before receiving six 500-mg. tablets of aminosalicylic acid, which constitutes a normal therapeutic dose of this drug substance. Blood samples
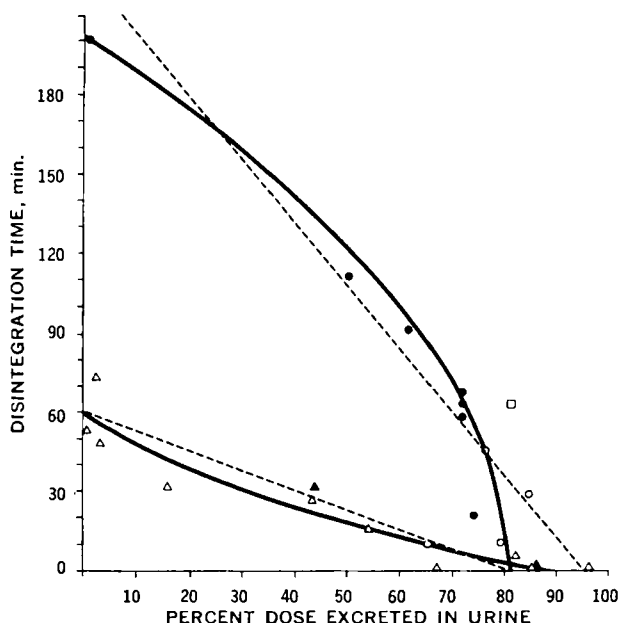
Figure 1—*Correlation between disintegration times and the percent of the dose excreted in the urine. The solid and dashed lines represent the quadratic and linear regression lines, respectively. Key: △, aminosalicylic acid data from this paper; ▲, aminosalicylic acid data (2); ○, sodium aminosalicylate data (2); □, calcium aminosalicylate data (2); and ●, sodium aminosalicylate data (1).*

Table I—Individual Levels of Drug in Plasma and Urine

Formulation	Subject	0 hr.	1 hr.	2 hr.	3 hr.	Plasma Level, mcg./ml. 4 hr.	6 hr.	8 hr.	10 hr.	12 hr.	Urine Recovery, g./12 hr.
A	1	0.0	65.0	54.0	22.0	10.2	0.6	0.0	—	0.0	2.81
	2	0.0	42.0	84.0	55.0	28.0	4.7	1.7	—	0.0	3.11
	3	0.0	4.4	2.7	2.7	34.0	19.0	5.3	—	1.0	2.32
	4	0.0	20.0	11.5	64.0	63.0	22.0	6.0	—	1.0	2.80
	5	0.0	84.0	40.0	14.7	4.7	0.0	0.0	—	0.0	2.85
	6	0.0	43.0	46.5	25.0	10.5	1.7	0.0	—	0.0	2.75
	7	0.0	67.0	47.0	22.0	7.0	1.0	0.0	—	0.0	3.19
	8	0.0	8.0	34.7	56.0	41.0	13.0	1.7	—	0.0	2.43
	9	0.0	2.0	57.0	64.0	43.5	8.0	1.7	—	0.0	2.38
	10	0.0	47.0	65.0	34.5	13.7	2.3	0.0	—	0.0	4.17
B	1	0.0	—	53.1	—	9.0	1.3	0.0	0.0	0.0	2.58
	2	0.0	—	43.5	—	16.3	3.3	2.3	0.0	0.0	2.90
	3	0.0	—	14.3	—	0.7	0.0	0.0	0.0	0.0	1.79
	4	0.0	—	52.0	—	31.4	14.0	5.0	0.0	0.0	3.00
C	1	0.0	—	43.0	—	13.3	1.6	0.0	0.0	0.0	2.57
	2	0.0	—	25.7	—	34.0	9.0	1.6	0.0	0.0	2.68
	3	0.0	—	15.7	—	5.0	12.3	0.0	0.0	0.0	1.57
	4	0.0	—	57.0	—	43.0	0.7	3.0	1.0	0.0	2.97
D	1	0.0	—	44.7	—	11.7	1.5	0.0	0.0	0.0	2.95
	2	0.0	—	39.0	—	25.5	4.3	0.0	0.0	0.0	2.26
	3	0.0	—	23.4	—	2.3	0.0	0.0	0.0	0.0	0.47
	4	0.0	—	64.0	—	32.0	8.3	2.0	0.0	0.0	2.34
E-2	1	0.0	0.0	0.0	16.0	28.0	7.3	2.0	—	0.0	2.86
	2	0.0	0.0	0.0	58.5	46.0	16.0	4.5	—	0.0	2.44
	3	0.0	0.0	0.0	4.5	13.0	2.0	1.0	—	11.5	1.02
	4	0.0	0.0	0.0	16.0	33.0	13.0	6.5	—	1.0	1.38
	5	0.0	0.0	0.0	29.0	32.0	7.5	1.0	—	0.0	2.70
	6	0.0	0.0	0.0	8.0	14.7	7.0	2.0	—	0.0	1.18
	7	0.0	0.0	0.0	45.5	48.7	7.3	1.0	—	0.0	2.70
	8	0.0	0.0	0.0	0.0	2.0	0.0	0.0	—	0.0	0.05
	9	0.0	0.0	0.0	4.0	4.0	4.0	3.3	—	1.5	1.41
	10	0.0	0.0	0.0	19.7	35.0	4.5	2.0	—	0.0	1.71
E-1	1	0.0	—	0.0	—	36.7	9.0	1.3	0.0	0.0	1.89
	2	0.0	—	20.3	—	31.3	20.0	4.3	1.2	0.0	2.56
	3	0.0	—	0.0	—	0.0	2.8	2.8	1.4	0.0	0.66
	4	0.0	—	0.0	—	12.0	16.7	2.8	0.7	0.7	1.35
F	1	0.0	—	0.0	—	0.0	2.0	6.0	—	4.0	1.20
	2	0.0	—	0.0	—	0.0	0.0	2.0	—	2.0	0.73
	3	0.0	—	8.0	—	16.0	4.0	2.0	—	0.0	1.60
	4	0.0	—	0.0	—	0.0	0.0	8.0	—	11.0	1.35
	5	0.0	—	0.0	—	24.0	30.0	6.0	—	0.0	1.60
G	1	0.0	—	0.0	—	0.0	0.0	1.6	—	0.6	0.37
	2	0.0	—	0.0	—	0.0	0.0	0.0	—	0.0	0.26
	3	0.0	—	0.0	—	0.0	0.0	0.0	—	0.0	0.21
	4	0.0	—	0.0	—	0.0	0.0	0.0	—	0.6	0.27
	5	0.0	—	0.0	—	0.0	0.3	5.7	—	7.0	1.30
H	1	0.0	—	0.0	—	0.0	0.0	1.5	—	0.6	0.18
	2	0.0	—	0.0	—	0.0	0.0	0.0	—	0.0	0.01
	3	0.0	—	0.0	—	0.0	0.0	0.0	—	0.0	0.26
	4	0.0	—	0.0	—	0.0	0.0	0.0	—	0.0	0.02
	5	0.0	—	0.0	—	0.0	0.0	0.0	—	0.0	0.03
I	1	0.0	—	0.0	—	0.0	0.0	0.0	—	0.0	0.04
	2	0.0	—	0.0	—	0.0	0.0	0.0	—	0.0	0.02
	3	0.0	—	0.0	—	0.0	0.0	0.0	—	0.0	0.02
	4	0.0	—	0.0	—	0.0	0.0	0.0	—	0.0	0.02
	5	0.0	—	0.0	—	0.0	0.0	1.5	—	2.7	0.03
J	1	0.0	—	0.0	—	0.0	0.0	0.0	—	0.0	0.02
	2	0.0	—	0.0	—	0.0	0.0	0.0	—	0.0	0.01
	3	0.0	—	0.0	—	0.0	0.0	0.0	—	0.0	0.02
	4	0.0	—	0.0	—	0.0	0.0	0.0	—	0.0	0.02
	5	0.0	—	0.0	—	0.0	0.0	0.0	—	0.0	0.05

were drawn at 0, 2, 4, 6, 8, and 12 hr. in early studies. In later studies, 1-, 3-, and 10-hr. samples were also drawn. Urine was collected for 12 hr. following drug administration.

Plasma and urine samples were analyzed by procedures adapted from Way et al. (4). The basic procedure consists of diazotization of the aminosalicylic acid followed by reaction with N-1-naphthylethylenediamine to form a rose-colored adduct whose absorbance is measured at 540 nm. This procedure measures the primary aromatic amines in the sample.

To precipitate plasma proteins, plasma samples were treated with trichloroacetic acid prior to developing the color. The reported plasma concentrations represent the sum of aminosalicylic

acid and its metabolites that have a free amino group; aminosalicyluric acid, for example, is found in plasma in low but significant amounts (4–6) and would be included in the reported concentration. The major metabolite (4–7), N-acetyl-p-aminosalicylic acid, would not be included in the reported concentrations. However, the kinetics of elimination of aminosalicylic acid and its metabolites are very nearly linear up to a plasma concentration of approximately 110 mcg./ml. (8). Since the plasma concentrations encountered in this study did not exceed 85 mcg./ml. (Table I), which is well within the linear region, the area under the plasma level–time curve as determined by this method is a suitable measure of drug availability.

Urine samples were subjected to an acid hydrolysis before

Table II—In Vivo **and** In Vitro **Availability of Aminosalicylic Acid from Tablets[a]**

Tablet	Number of Subjects	Time of Plasma Peak, hr.	Plasma Peak, mcg./ml.	Area under Plasma Curve, mcg.-hr./ml.	Percent Recovered in Urine[b]	Mean Disintegration Time, min.	T_{20}, min.
A Uncoated	10	2 ± 1	63 ± 5	177 ± 14	96 ± 18	<1	<4
B Powder	4	<2	41 ± 18	123 ± 72	85 ± 19	—	<4
C Uncoated	4	2.5 ± 1	37 ± 17	133 ± 61	82 ± 20	4.6 ± 1.3	<5
D Uncoated	4	<2	43 ± 17	129 ± 67	67 ± 35	0.5 ± 0.7	<4
E-2[c] Enteric	10	4 ± 0	27 ± 6	82 ± 16	58 ± 30	—	—
E-1[c] Enteric	4	5 ± 1	22 ± 15	82 ± 58	54 ± 27	15 ± 5	16 ± 9
F Enteric	5	8 ± 3	13 ± 11	55 ± 45	43 ± 13	26 ± 5	36 ± 11
G Enteric	5	—[d]	—[d]	—[d]	16 ± 15	32 ± 8	96 ± 32
H Enteric	5	—[d]	—[d]	—[d]	3 ± 4	48 ± 15	—[e]
I Enteric	5	—[d]	—[d]	—[d]	2 ± 4	73 ± 13	—[f]
J Enteric	5	—[d]	—[d]	—[d]	0.8 ± 0.5	53 ± 5	—[f]

[a] Ranges are ±1 SD. [b] Calculated as the percent of labeled potency of the tablet. [c] Two independent clinical trials were conducted on Tablet E. [d] Plasma level parameters could not be calculated because the plasma concentration had not passed its maximum at the time the last blood sample was drawn. The mean area under the plasma level curve up to 12 hr. was less than 8 mcg.-hr./ml. [e] A T_{20} value was not calculated because one out of four tablets showed no dissolution after 2 hr. in the pH 7.5 buffer. [f] There was no measurable dissolution after 3 hr. in the pH 7.5 buffer.

Table III—Rank-Order Correlation Coefficients for In Vivo **and** In Vitro **Availability of Aminosalicylic Acid[a]**

	Peak Plasma Level	Area under Plasma Level–Time Curve	Percent of Dose Excreted in Urine	Mean Disintegration Time	Dissolution, T_{20}
Time of plasma peak level	−0.77	−0.49	−0.71	+0.94 (0.02)	+1.00 (0.01)
Peak plasma level		+0.83 (0.1)	+0.83 (0.1)	−0.83 (0.1)	−0.77
Area under plasma level–time curve			+0.83 (0.1)	−0.60	−0.49
Percent of dose excreted in urine				−0.96 (0.01)	−0.93 (0.01)
Mean disintegration time					+0.95 (0.01)

[a] For correlations significant at the 0.1 level or below, the approximate confidence level is given in parentheses.

analysis. This procedure frees the amino group in the N-acetyl metabolite, so that the reported values are a measure of total amino-salicylic acid and metabolites and provide a good basis for comparing the relative bioavailability of aminosalicylic acid from various formulations.

DISCUSSION

The results of the in vivo and in vitro experiments are summarized in Table II. The entries in Table II are arranged in order of decreasing percentage of drug recovered in the urine, and a comparison of the columns suggests several rank-order correlations among the variables. The data suggest that longer disintegration times and T_{20}'s are associated with a greater delay before the peak plasma concentration is reached, a lowering of the peak concentration, a smaller area under the plasma concentration–time curve, and reduced amounts of drug excreted in the urine. The correlation coefficients and corresponding significance levels derived from the distribution of sums of squares of rank differences (9) are presented in Table III for these data. Several correlations are, in fact, significant, the most notable being the correlations between time of plasma peak, the percent of the dose excreted in the urine, and the disintegration and dissolution times.

Table IV summarizes the published data relating tablet disintegration time to the amount of drug excreted in the urine following the administration of tablets of aminosalicylic acid and its salts. A significant correlation between the in vivo and in vitro test results is also found with these data. However, the correlation coefficient calculated for the disintegration times and urine recoveries of the salts was only −0.72, while the correlation coefficient calculated from the data on tablets of the acid was −0.96. The lower correlation coefficient found for the salts is due to the existence of a range of disintegration times over which the bioavailability remains essentially at its maximum value (Fig. 1). A better evaluation of the correlation in this case is obtained from the regression analysis discussed below.

Although a rank-order correlation between in vivo and in vitro tests may be sufficient for establishing manufacturing controls to

assure a lower limit on the availability of drug from manufactured lots of tablets, a more quantitative correlation is desirable because it allows the data to be summarized in a simple algebraic relation and also because it allows one to estimate the reliability of predictions made from the model equation. Therefore, the correlation between disintegration time (X) and percent of the dose excreted in the urine (Y) was examined using both a linear and a quadratic model, i.e., ($Y = a_1 + b_1 X$) and ($Y = a_2 + b_2 X + c_2 X^2$), respectively.

Table IV—Summary of Availability Data on Aminosalicylate Tablets from the Literature[a]

Tablet	Percent Recovered in Urine[a]	Mean Disintegration Time, min.	Reference
Sodium salt:			
A	74	19.5	1
B	72	58.0	1
C	72	67.0	1
D	72	63.5	1
E	61.5	91.1	1
F	50.0	111.5	1
G	2.1	200.4	1
H	78.3	10	2
I[b]	84.5	28	2
J[b]	76.4	45	2
Calcium salt:			
K	81.2	63	2
Free acid:			
L	85.9	1	2
M[b]	43.7	31	2

[a] Calculated as the percent of labeled potency of the tablet. The correlation between the tabulated urine recoveries and disintegration times for the sodium and calcium salts is significant at the 0.02 level when calculated from the distribution of sums of squares of rank differences (9). The correlation coefficient is −0.720. [b] Enteric-coated tablets.

Table V—Relation of Percent of Dose Excreted in the Urine (Y) to Tablet Disintegration Time (X)

	Linear Model	Quadratic Model
Aminosalicylic acid:		
Equation	$Y = 80.8 - 1.37X$	$Y = 87.1 - 2.34X + 0.0153X^2$
Multiple correlation coefficient	-0.94	-0.96
Standard error of estimate	13.1	10.6
Aminosalicylate salt:		
Equation	$Y = 94.1 - 0.412X$	$Y = 80.5 - 0.009X - 0.00194X^2$
Multiple correlation coefficient	-0.94	-0.98
Standard error of estimate	8.3	4.8

Only disintegration times and urine levels were used in this analysis, because these parameters covered a wider range of values than the other parameters, and more data pairs were available (Tables II and IV). The quadratic model was included in the analysis, because a plot of disintegration time against the percent drug recovered in the urine (Fig. 1) suggested a curvilinear relation between the variables. A curvilinear relation would be expected on theoretical grounds as well. If the rate of release of drug from the dosage form were much faster than the rate of absorption, the drug would be maximally available; a further increase in the rate of release would have no effect on the availability of the drug. Similarly, when the release is so slow that essentially no drug is absorbed, a further decrease in the rate of release would not affect the availability. These regions of maximal and minimal availability would be expected to be joined by some smooth function, $Y = Y(X)$. In the absence of a theory defining the functional form of $Y(X)$ more precisely, a simple polynomial is a reasonable choice for use in a study of the correlation between Y and X. The results of the analyses using these models are presented in Table V, and the regression curves are plotted in Fig. 1.

As indicated in Table V, the regression analyses of the data on aminosalicylic acid and its salts were carried out separately. The experimental points in Fig. 1 suggest that the acid and its salts do not fall on the same regression curve, and subsequent statistical treatment confirmed that the difference is significant. This difference in behavior is probably not an artifact generated by interlaboratory assay differences, because the data of Middleton *et al.* (2) on aminosalicylic acid are consistent with the present results, while their data on the salts are consistent with the results of Chapman *et al.* (1). Three possible sources of this difference are: (a) differences between the acid and its salts with respect to physical–chemical properties, such as solubility or rate of dissolution of the crystals; (b) the fact that most of the slow disintegrating tablets of aminosalicylic acid were enteric coated, whereas most tablets of the salts were not; or (c) the difference in the disintegration tests used for enteric tablets as opposed to nonenteric tablets (see *Experimental* section). The available data are not sufficient to decide among these alternatives.

The multiple correlation coefficients in Table V show that 92 or 96% of the variance in the urinary excretion of drug following the administration of aminosalicylic acid or an aminosalicylate salt, respectively, can be ascribed to the effect of tablet disintegration time when the quadratic model is assumed. Only 88% of the variance can be ascribed to tablet disintegration time when the linear model is assumed.

The standard error of the estimate for the quadratic model indicates that 95% of the time the experimental urine recovery of aminosalicylic acid should lie within $\pm 21\%$ of the value predicted by the regression line. The 95% confidence limits for urine recovery values predicted for the salts of aminosalicylic acid are $\pm 10\%$. The confidence limits for the linear model are broader than those for the quadratic model for both groups of tablets. While the confidence limits are not as tight as one would like, they are reasonable in view of the large intersubject variability normally encountered in clinical studies.

The constant terms in the equations in Table V represent the maximum availability of the drug predicted by the model. The values of 87.1 and 80.5% obtained for the quadratic model are in good agreement with the values reported in the literature, which range from 70 to 85% (1, 2, 4–6, 10, 11).

CONCLUSIONS

The correlations between disintegration times and bioavailability (Table V) show that it is possible to use disintegration tests to control the availability of drug from aminosalicylate tablets. Tablets of aminosalicylic acid and sodium and potassium aminosalicylates are official in USP XVIII. The USP requires that uncoated tablets disintegrate within 30 min. and that enteric-coated tablets disintegrate within 150 min. As can be seen from Fig. 1, only the limits set for uncoated tablets of the sodium and potassium salts are adequate to ensure drug availability; tablets of the salts disintegrating within 30 min. are as available as a suspension of the drug (80.5%). The USP tests would pass uncoated tablets of aminosalicylic acid, which were only 30.4% available, and enteric-coated tablets of aminosalicylic acid and aminosalicylates, which were 0.0 and 35.5% available, respectively. The results indicate that the time limits for enteric-coated tablets should be the same as the time limit for uncoated tablets and that the limit on the disintegration time for aminosalicylic acid tablets should be reduced from 30 to approximately 10 min.

The data in Tables II and III, as well as the data of Middleton *et al.* (2), show that dissolution tests may also be used to control drug availability from tablets of aminosalicylic acid and its salts. In principle, dissolution tests are more likely to give valid comparisons of drug availability from different formulations than are disintegration tests, because availability could be limited by the rate of dissolution from the tablet fragments rather than by the rate of disintegration of the tablet. The available data indicate, however, that the possible discrepancy between dissolution and disintegration times does not, in fact, occur for typical formulations of tablets of aminosalicylic acid and its salts. Middleton *et al.* (2) probably failed to find a correlation between disintegration times and bioavailability because they studied tablets of both aminosalicylic acid and its salts, and these fall on different correlation lines. Their data were not sufficient to make the existence of two lines obvious, and the resulting distribution of points was interpreted as a lack of correlation. The open symbols in Fig. 1 represent the data of Middleton *et al.* and clearly show the apparent lack of correlation.

In the present study, a problem was encountered in attempting to correlate dissolution time with urinary excretion of drug because T_{20}'s for the least available tablets were too long to be conveniently measured, whereas the T_{20}'s for the most available tablets were too short to be measured accurately. This situation made a full regression analysis of the data impossible, although it did not obscure the rank-order correlation existing between these variables. The data in Table II show that T_{20} must be limited to 15 min. or less, when the test is conducted as described in the *Experimental* section, to ensure at least 50% drug availability.

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GLC Determination of 7-Trifluoromethyl-1-methyl-5-phenyl-1H-1,5-benzodiazepine-2,4-(3H,5H)-dione in Plasma

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Abstract □ A GLC assay method is described for the determination of 7-trifluoromethyl-1-methyl-5-phenyl-1H-1,5-benzodiazepine-2,4-(3H,5H)-dione in the plasma or serum of dogs, rabbits, or humans. After extraction with hexane from acidified plasma or serum, the compound is analyzed by GLC using a flame-ionization detector. Quantitation is accomplished using a standard curve, which was found to be linear over the 0.002–3.5-mcg. range. The analysis is sensitive to amounts of the drug above 0.1 mcg./ml. Recovery of the title compound added to human plasma averaged 98.7%, with a variation of approximately 7.1% (*RSD*). Plasma data are presented to demonstrate the utility of the method.

Keyphrases □ 7-Trifluoromethyl-1-methyl-5-phenyl-1H-1,5-benzodiazepine-2,4-(3H,5H)-dione—GLC analysis in plasma □ 1,5-Benzodiazepine compounds—GLC analysis of 7-trifluoromethyl-1-methyl-5-phenyl-1H-1,5-benzodiazepine-2,4-(3H,5H)-dione in plasma □ GLC—analysis, 7-trifluoromethyl-1-methyl-5-phenyl-1H-1,5-benzodiazepine-2,4-(3H,5H)-dione in plasma

A recently synthesized member of the benzodiazepine class of compounds, 7-trifluoromethyl-1-methyl-5-phenyl-1H-1,5-benzodiazepine-2,4-(3H,5H)-dione[1] (I), is currently being evaluated for its activity as a minor tranquilizer. It differs significantly from other benzodiazepine psychotropic agents in that the carbon and nitrogen atoms in the 4- and 5-positions of the diazepine ring are reversed, giving rise to the 1,5-benzodiazepine structure. The pharmacological action of Compound I on the CNS of various test animals has been reported (1, 2), as well as the metabolism of the drug in animals and man (3).
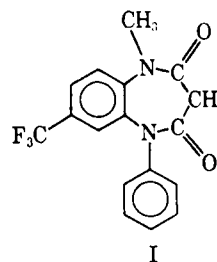
To assist in correlating pharmacological activity with blood levels of Compound I, it was necessary to develop a sensitive, specific method of analysis that would permit processing of a large number of samples in a relatively short time. Various methods have been described for the determination of benzodiazepines, notably diazepam and chlordiazepoxide, in physiological fluids. In some cases, quantitation is achieved by making use of the UV-absorbing properties of the compound (4), while in others it depends upon hydrolysis of the drug followed by colorimetric determination of a Bratton–Marshall adduct (5–8). Cathode ray polarography has been successfully applied to plasma determinations (9). The most sensitive and specific methods use GLC, either of the intact compounds or of their hydrolysis products, and electron-capture detection (4, 10–12). However, these methods require extensive, time-consuming manipulations to produce an extract clean enough for chromatography.

The method described here used GLC followed by flame-ionization detection for the determination of Compound I in plasma or serum to levels of 0.1 mcg./ml. The analysis is specific for Compound I in the presence of its known metabolites and is applicable to human or animal samples after administration of therapeutic doses.

EXPERIMENTAL

Reagents—The purity of Compound I used was 100% as determined by GLC and TLC. All other materials were analytical reagent quality. The hexane was purified further by shaking with sulfuric acid, washing with water until neutral, and redistilling

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