ACKNOWLEDGMENTS AND ADDRESSES

Received August 20, 1973, from the *Division of Pharmaceutical Sciences, Mead Johnson Research Center, Mead Johnson and Company, Evansville, IN 47721*

Accepted for publication October 15, 1973.

The author thanks Mr. David C. Jordan, Technical Information Services, Mead Johnson Research Center, for programming the calculations of dissolution profiles for this report. The helpful criticisms of Mr. Robert A. Lipper were greatly appreciated.

Colorimetric Determination of Guanazole in Plasma and Blood

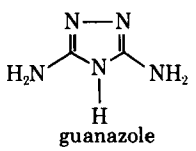
C. DAVE[x] and L. CABALLES

Abstract □ A simple colorimetric procedure is described for determination of guanazole (3,5-diamino-1,2,4-triazole), a new antileukemic drug. The test is based upon diazotizing guanazole in water or in trichloroacetic acid extract obtained from plasma or serum, coupling the diazonium salt with diphenylamine, and extracting the azo dye with isopentyl alcohol. The product has a λ_{max} at 485 nm, is stable for several days, and obeys Beer's law over a wide concentration range. An amount as low as 5 nmoles in 2 ml can be determined under the conditions described. Under the same condition, 3-amino-1,2,4-triazole gave a yellow product in isopentyl alcohol (λ_{max} 450 nm) with a sensitivity 20 times less than that with guanazole, whereas 1*H*-1,2,4-triazole gave no color at all. Recoveries of guanazole added to fetal calf serum and dog plasma were within 95–105%. Following a single intravenous dose to a rat, blood levels of guanazole, evaluated by the colorimetric procedure, were comparable to those obtained measuring concurrently administered ^{14}C-labeled compound by standard tracer techniques. A half-life of 68 min was thus observed by both methods. The sensitivity and reproducibility of the assay, together with the relative lack of interference from other substances like urea and amino acids, make the procedure suitable for the determination of plasma levels of drug in small samples.

Keyphrases □ Guanazole in plasma and blood—colorimetric analysis □ Colorimetry—analysis, guanazole in plasma and blood

Guanazole (3,5-diamino-1,2,4-triazole, NSC-1895, mol. wt. 99.1), reportedly synthesized by Pellizzari in 1894 (1), is a new antileukemic agent which is therapeutically effective against mouse leukemia L-1210 (2) and human acute myelocytic leukemia (3, 4). Its antileukemic actions appear to be related to its ability to inhibit ribonucleoside diphosphate reductase, a key enzyme involved in deoxynucleotide synthesis during the DNA-synthetic phase of the cell cycle (5).

A sensitive colorimetric procedure was developed to study the pharmacokinetics of guanazole in patients with acute myelocytic leukemia (6). The rationale for the present methodology is based upon the fact that the primary amino groups of 1,2,4-triazole are diazotizable (7). The diazotized product in the case of guanazole was coupled with diphenylamine, resulting in the formation of a red dye which could be readily extracted with isopentyl alcohol.



guanazole

EXPERIMENTAL

Chemicals and Reagents[1]—The purity of labeled and unlabeled guanazole was determined by TLC on silica gel precoated plastic sheets without fluorescent indicator[2] using the following three solvent systems (v/v): (*a*) methanol–dioxane (2:1), (*b*) water–dioxane (1:10), and (*c*) water–methanol (1:10).

Chromatograms were developed in a TLC chamber[3] for 30–45 min. Spots were visualized by placing the dried chromatogram in iodine vapors, and the R_f values for guanazole were found to be 0.31, 0.73, and 0.61. Both labeled and unlabeled compounds migrated as a single spot on the chromatogram with each solvent system.

For a standard colorimetric assay, the following reagents were prepared: (*a*) acid mixture, 1.5% (v/v) sulfuric acid in acetic acid; (*b*) nitrite reagent, 1% (w/v) $NaNO_2$ in water; and (*c*) diphenylamine reagent, 10% (w/v) diphenylamine in the acid mixture reagent.

Protein Precipitation—Blood plasma or serum samples were generally diluted five- to 10-fold with 5% (w/v) trichloroacetic acid, mixed, and allowed to stand on ice for 10 min. The precipitates were centrifuged and the supernate was used for analysis. In samples with low guanazole content, plasma was diluted only with an equal volume of 10% trichloroacetic acid, the precipitate was washed once with one volume of 5% trichloroacetic acid, and the supernates were combined.

Standard Colorimetric Procedure—To a 2-ml aliquot containing 0.5–50 μg guanazole, 0.1 ml of the acid mixture reagent was added and cooled in ice. Nitrite reagent (0.1 ml) was added and mixed rapidly, and the mixture was kept in ice for 10 min for optimal diazotization. Diphenylamine reagent (0.1 ml) was added and mixed rapidly, and the mixture was left for 15 min at room temperature for maximum color development. The azo dye was extracted with 3.0 ml of isopentyl alcohol by gently inverting the tube several times. Centrifugation, although not required in the authors' experience, may be carried out if an emulsion forms. The organic solvent phase was transferred to another test tube containing about 1 g of anhydrous sodium sulfate, mixed, and allowed to stand for 5 min. The absorbance of the solvent layer was then read[4] in a 1-cm cell at 485 nm.

Since trichloroacetic acid extracts from control plasma samples

[1] Guanazole, as well as guanazole uniformly labeled with ^{14}C (51 μCi/mg), was generously provided by Dr. J. F. Holland, Mount Sinai Hospital, New York, N.Y. 3-Amino-1,2,4-triazole and 1*H*-1,2,4-triazole were obtained from Eastman Kodak, Rochester, N.Y. Fetal calf serum was purchased from Grand Island Biologicals, Grand Island, N.Y. All other chemicals were analytical grade from Fischer Chemical Co.

[2] Eastman Kodak, Rochester, N.Y.

[3] Gelman.

[4] Beckman model DB-G spectrophotometer.

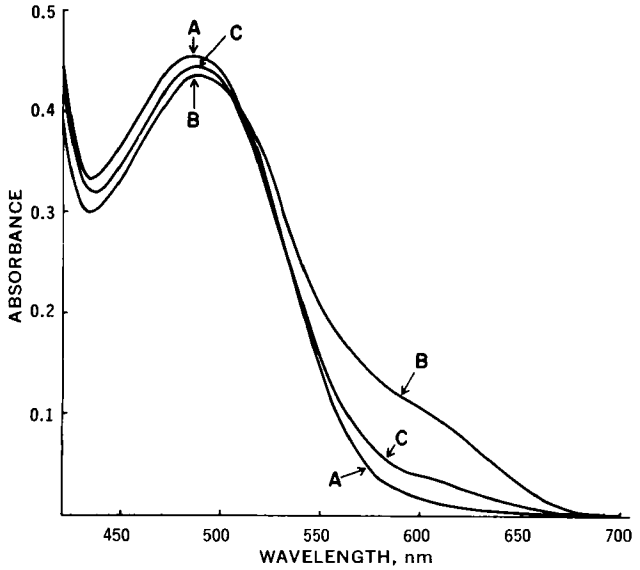


Figure 1—*Spectrum of azo dye product from guanazole. Azo dye solution in isopentyl alcohol was obtained from 4.5 μg guanazole dissolved in 2 ml water (A), from 2 ml of 5% trichloroacetic acid (B), or from 2 ml of 5% trichloroacetic acid extract of 0.5 ml fetal calf serum containing 9 μg/ml guanazole (C). The spectra A, B, and C were recorded on a dual-beam spectrophotometer (Cary model 14) using the isopentyl alcohol layer from the corresponding blank reagent mixtures in the reference cell.*

gave blank readings similar to the ones obtained with 5% trichloroacetic acid alone, the absorbance values of the samples were read against reagent blanks obtained with 2.0 ml of 5% trichloroacetic acid alone.

Blood Levels of Guanazole in Rat—Charles River female rats (200–250 g) were injected intravenously with 60 mg guanazole containing 0.5 μCi of ^{14}C-labeled compound in 0.5 ml water. At various time intervals after guanazole administration, 0.1 ml blood was withdrawn from the tail vein into heparinized graduated capillary tubes[5] through a surgical incision which was then sealed

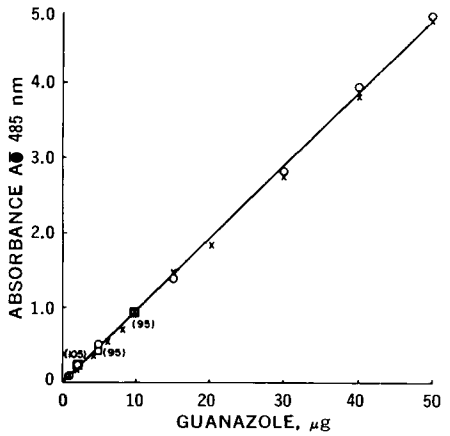


Figure 2—*Standard curve for determination of guanazole. The color of the azo dye from guanazole in 2 ml water (○) or 5% trichloroacetic acid (×) was extracted in 3 ml isopentyl alcohol as described in the text. Extracts with absorbance greater than 1.0 were diluted sufficiently with isopentyl alcohol. Absorbances of color from 0.2-, 0.5-, and 1.0-ml samples of dog plasma containing 10 μg/ml guanazole are also plotted (□), and the percentage recoveries are indicated by the corresponding numbers in parentheses.*

[5] Clay-Adams, Inc., N.J.

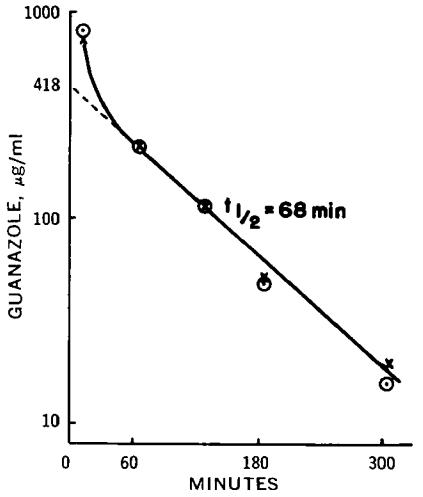


Figure 3—*Levels of guanazole in rat blood. After a single intravenous injection of 0.5 μCi/60 mg of ^{14}C-labeled guanazole, blood samples were withdrawn and analyzed for guanazole by the colorimetric procedure (○) and by radioactivity measurements (×).*

with flexible collodion. For subsequent samples, it was usually possible to reopen the same wound by removing the collodion seal. The content of the capillary tube was emptied in nine volumes of 5% trichloroacetic acid and centrifuged at 1000×*g* for 5 min. Aliquots from the supernate were analyzed by the colorimetric procedure and by measuring radioactivity.

Determination of ^{14}C-Radioactivity—Aliquots of trichloroacetic acid supernate from plasma or blood were directly transferred to glass counting vials, and water was added to make 1 ml. To a glass vial, 10 ml of toluene counting fluid containing 0.6% 2,5-diphenyloxazole, 0.06% 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene, and 15% (v/v) Biosolv-3[6] was added and mixed, and the vial was counted in a liquid scintillation counter[7]. The counts were corrected for quenching by automatic external standard ratio method (8, 9).

RESULTS

Spectrum and Standard Curve—As shown in Fig. 1, a spectrum with a symmetrical peak at 485 nm was obtained with azo dye derived from guanazole in the absence of trichloroacetic acid. In the presence of 5% trichloroacetic acid, a shoulder appeared at 590 nm in addition to the peak at 485 nm. In the case of trichloroacetic acid extract obtained from fetal calf serum, the 590-nm shoulder was reduced considerably, and the spectrum of the resulting azo dye was essentially similar to that obtained in the absence of trichloroacetic acid.

A typical linear regression curve passing through the origin (correlation coefficient = 0.9999) was obtained for the colorimetric assay of guanazole as shown in Fig. 2. This was in excellent agreement with Beer's law at least up to about 1.5 absorbance units. For absorbance greater than 1.5, the azo dye solution was diluted sufficiently with isopentyl alcohol and read at 485 nm, and the absorbance value of the original solution was calculated from the absorbance value of the dilute solution and the dilution factor. It is also evident from Fig. 2 that trichloroacetic acid did not noticeably affect the color intensity at 485 nm, the λ_{max} of the azo dye. The recovery of guanazole from dog plasma was also in excellent agreement with the different amounts of guanazole added to each sample as indicated in Fig. 2. Similar recoveries (95–105%) were also obtained with fetal calf serum (Fig. 1), rat blood (Fig. 3), and human plasma (data not shown).

Data obtained for colorimetric assay of guanazole over several months were statistically analyzed on a programmable calculator[8] using the least-squares method (Table I). Values of reagent blank

[6] Beckman Instruments Inc.
[7] Packard.
[8] Wang 700B.

Table I—Reproducibility of the Colorimetric Assay of Guanazole

Experiment	Range Analyzed, μg	Reagent Blank[a], A	Regression Reaction[b], $Y = SX + C$	Correlation Coefficient
1	0–14 (8)[c]	0.018	$= 0.0949X - 0.0033$	0.9999
2	0–12 (7)	0.009	$= 0.1032X - 0.0065$	0.9996
3	0–12 (6)	0.018	$= 0.1033X - 0.0078$	0.9999
4	0–50 (9)	0.022	$= 0.0977X - 0.0384$	0.9998
5	0–15 (7)	0.013	$= 0.0958X + 0.0090$	1.0000
	Mean ± SE	0.016 ± 0.002	$= 0.0989\ (\pm 0.0018)X - 0.0094\ (\pm 0.0078)$	

[a] Obtained with 5% trichloroacetic acid. [b] Y = absorbance, S = slope, X = amount of guanazole in micrograms, and C = constant intercept to y-axis. [c] Numbers in parentheses denote number of samples containing different amounts of guanazole analyzed.

with 5% trichloroacetic acid are around 0.02 absorbance unit in most cases. With 10% trichloroacetic acid as blank, this value nearly doubled; whereas in the absence of trichloroacetic acid, the value was below 0.01 absorbance unit. Values obtained from the intercept were nearly zero in all the cases, in agreement with Beer's law. Values for the slope and the correlation coefficient obtained over time were also in agreement with each other (Table I).

Conditions for Reaction with Nitrite Reagent—As shown in Fig. 4, the optimal diazotization, as monitored by azo dye formation, occurred between 10 and 15 min at 0–4° in the presence of 0.1 ml nitrite reagent to 3.0 ml of reaction mixture containing guanazole. With a lower concentration of nitrite reagent (0.075–3.0 ml), it took longer to develop color of equal intensity. At higher concentrations of nitrite reagent (from 0.2–0.4 to 3.0 ml), diazotization occurred very rapidly, although the product appeared unstable.

Condition for Reaction with Diphenylamine Reagent—As shown in Fig. 5, the color intensity of the azo dye obtained with 1–5 μg guanazole increased with increasing concentration of diphenylamine and reached a plateau value at a ratio of about 0.1 ml of 10% diphenylamine to 3.1 ml of the diazotized mixture. Although the color intensity obtained with 15% diphenylamine solution was slightly higher, for practical purposes 0.1 ml of diphenylamine reagent was used for the color development. As shown in Table II, the color intensity reached a plateau value after 10 min; variation of temperature between 0 and 37° did not significantly affect the intensity of color. Therefore, the coupling reaction with diphenylamine was carried out at room temperature (23°) for 15 min.

Color Reaction with Other Congeners—When 100 μg 3-amino-1,2,4-triazole was tested for color development under the conditions described for the standard procedure, an orange-red-colored product was obtained which turned yellow on extraction with isopentyl alcohol and gave an absorption peak (0.67 absorbance unit) at 450 nm (Fig. 6) instead of the 485-nm peak for guanazole. At 485 nm, the dye from 3-amino-1,2,4-triazole had a color intensity 60% that of its own at 450 nm and about 5% of that obtained with guanazole. Again, in similarity with the product from guanazole, this dye was also found to be very stable for at least 2 days and the development of color was quite reproducible. The parent compound, 1*H*-1,2,4-triazole, did not give any absorbance in the visible spectrum.

Interference with Other Chemicals—The standard procedure was run in the presence and absence of 5 μg guanazole with 2 mg urea, 100 mg sucrose, or 1 ml tissue[9] culture medium (10) containing, among other nutrients, about 1 mg amino acids/ml. In all cases, the blank readings were less than 0.03 absorbance unit and the recovery of guanazole was within 5% the actual amount added.

Levels of Guanazole in Rat Blood—A typical time course of guanazole level in rat blood is shown in Fig. 3 after a single intravenous injection of the drug. The blood levels determined with the ^{14}C-radioactivity and colorimetric procedures tallied reasonably well. An initial rapid distribution phase was followed by a slower first-order elimination process with a half-life ($t_{1/2}$) of 68 min regardless of the technique used.

DISCUSSION

The newly developed colorimetric procedure was found to be sensitive and specific for guanazole and was adapted to drug level determination in physiological fluids. While this manuscript was in preparation, another method utilizing the Bratton-Marshall (11) diazotization reaction and coupling was reported (12) for guanazole determination. Among the advantages of the present procedure are its reproducibility, its sensitivity, and the relative lack of interference by constituents in the blood or tissue culture media containing fetal calf serum. The high solubility of the azo

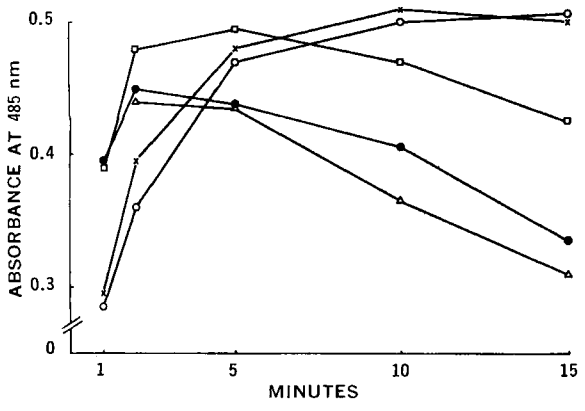


Figure 4—*Effect of time for diazotization and of concentration of nitrite reagent on azo dye formation. At time = 0, 0.075 (○), 0.10 (×), 0.20 (□), 0.3 (●), or 0.4 (△) ml of nitrite reagent was added to an acidified solution of guanazole (5.0 μg in 3.0 ml) on ice. At different time intervals thereafter, the color was developed with diphenylamine reagent, extracted, and read as described in the text.*

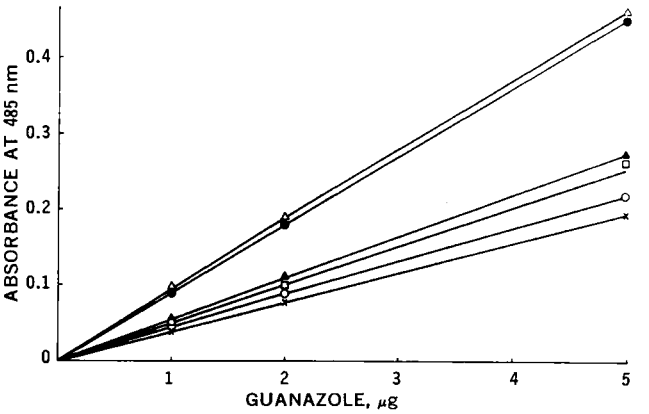


Figure 5—*Effect of varying concentration of diphenylamine on intensity of color. To the 3.1-ml diazotized mixture, prepared as described in the text, 0.1 ml of 1 (×), 2 (○), 3 (□), 4 (▲), 10 (●), or 15 (△) % diphenylamine in the acid mixture was added, kept for 15 min at 23°, extracted, and read as described in the text.*

[9] RPMI 1640.

Table II—Effect of Time and Temperature on the Coupling Reaction between Diazotized Guanazole and Diphenylamine[a]

Amount of Guanazole, μg	Reaction with Diphenylamine for					
	5	10	15 min	15 min		
	at 23°			at 0°	23°	37°
5	0.469	0.500	0.500	0.485	0.500	0.520
10	—	—	—	0.980	1.000	1.030

[a] Absorbance of blank = 0.018. All values are in absorbance units. Diazotization was carried out with nitrite reagent as described in the text.

dye into isopentyl alcohol permits the use of a small volume of solvent for dye extraction, thereby increasing the sensitivity of the assay. As has been shown, there is an excellent agreement between the colorimetric method and that using ^{14}C-radioactivity.

The optimization of the diazotization reaction with nitrous acid was a crucial step in the colorimetric reaction for guanazole, this being consistent with similar findings reported for 3-amino-1,2,4-triazole (13). The choice of diphenylamine as a coupling agent was based upon the rationale that coupling of one or two diphenylamine residues with hydrophobic aromatic rings to a guanazole molecule would render the azo dye lipid soluble and extractable with water-immiscible hydrophobic solvents. The azo product obtained using 1-aminonaphthol-2,4-disulfonic acid was not extractable in isopentyl alcohol.

Since the isopentyl alcohol–water partition coefficient of the azo dye appears to be high and since the miscibility of isopentyl alcohol and water is quite low, the intensity of color in the organic phase is dependent on the total guanazole content or color produced in the aqueous mixture rather than on the drug concentration *per se*. Thus, by altering the proportions of isopentyl alcohol to the aqueous mixture, it should be possible to increase the sensitivity of the test. Indeed, when 1 ml of isopentyl alcohol was used to extract the color obtained with 2.5 μg guanazole in 3.2 ml aqueous mixture, an absorbance of 0.710 was obtained, using 1-cm path length narrow cells, instead of the expected 0.729 or 0.243 × 3 when 3 ml of the solvent was used for extraction. Extraction of the azo dye with organic solvent may be responsible not only for a decreasing interference by other nonspecific absorbing materials in the reaction mixture but may also impart remarkable stability to the color developed. Different plasma or serum proteins were found to prevent to a variable extent the optimal diazotization with nitrous acid as well as the coupling with diphenylamine. Thus, it was necessary to remove proteins with agents like trichloroacetic acid. Other protein-denaturing agents were not tested. Normal acid-soluble constituents in plasma or serum having amino groups, *i.e.*, amino acids and urea, did not interfere at the concentrations tested, either with blank readings or with the test samples.

Since guanazole is excreted unchanged in mice, rat, dog (14), and man (6, 12), no interference is expected from metabolites during the colorimetric determination of the drug in plasma or serum from patients undergoing treatment. Indeed, the procedure was already applied satisfactorily to the determination of the levels of guanazole in patients undergoing therapy (6). The same procedure was also satisfactorily applied to the determination of guanazole in rat tissues[10].

The fact that the color of the azo product of guanazole is distinct from that of 3-amino-1,2,4-triazole indicates some degree of specificity of the reaction with guanazole. The lack of reactivity of 1*H*-1,2,4-triazole is consistent with the expectation that a primary amino group is indeed required for the color reaction to occur. It seems from the results that both 3- and 5-primary amino groups of guanazole participate in diazotization reaction. In contrast, 3-amino-1,2,4-triazole, having only one primary amino group, can form an azo dye with only one diphenylamine molecule. This being a smaller system of conjugated olefinic bonds, it would form a smaller chromophore-absorbing maximum at 450 nm than that seen with azo dye from guanazole (λ_{max} 480 nm).

[10] Unpublished data of the authors.

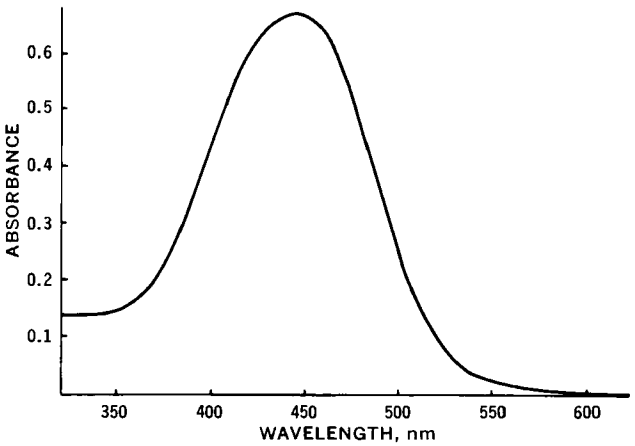


Figure 6—*Spectrum of azo dye product from 3-amino-1,2,4-triazole. The azo dye solution obtained from 3-amino-1,2,4-triazole (100 μg in 2 ml water) was recorded on a spectrophotometer (Cary model 14).*

This postulate is consistent with the observed ability of the primary amino group at 3- and 5-positions in 1,2,4-triazole to form diazonium compounds (7).

3-Amino-1,2,4-triazole, a herbicide (15, 16), and an inhibitor of the enzyme catalase (13, 17), could be estimated by several methods (13, 15) including the one involving azo dye formation (13). Without any modification, the presently described procedure could also be utilized for the determination of 3-amino-1,2,4-triazole at 450 nm with a sensitivity of 50–100 nmoles. This sensitivity is comparable to that of a recently described procedure (13). Optimization of diazotization and coupling reactions, however, might further increase the sensitivity of the color reaction for 3-amino-1,2,4-triazole using the present method.

In conclusion, a sensitive colorimetric procedure has been described for the determination of guanazole in plasma and blood. The assay may be useful for monitoring drug levels in plasma as well as in other tissue fluids.

REFERENCES

(1) E. Lieber and G. B. L. Smith, *Chem. Rev.*, **25**, 213(1939).

(2) R. B. Livingston and S. K. Carter, in "Guanazole, NSC 1895: Clinical Brochure," National Cancer Institute, Bethesda, Md., 1969.

(3) D. Yakar, J. F. Holland, R. R. Ellison, and A. Freeman, *Cancer Res.*, **33**, 972(1973).

(4) J. S. Hewlett, G. P. Bodey, C. A. Coltman, E. J. Freireich, A. B. Haut, and K. B. McCredie, *Clin. Pharmacol. Ther.*, **14**, 271(1973).

(5) R. W. Brockman, S. Shaddix, W. R. Laster, Jr., and F. M. Schabel, Jr., *Cancer Res.*, **30**, 2358(1970).

(6) C. Dave, J. Mattern, and D. Higby, *Proc. Amer. Ass. Cancer Res.*, **14**, 341(1973).

(7) E. Hoggarth, in "Chemistry of Carbon Compounds—Heterocyclic Compounds," vol. IVA, E. H. Rodd, Ed., Elsevier, New York, N.Y., 1957, p. 439.

(8) J. H. Parmentier and F. E. L. Haal, *Int. J. Appl. Radiat. Isotopes*, **20**, 305(1969).

(9) J. W. Davies and T. C. Hall, *Anal. Biochem.*, **27**, 77(1969).

(10) G. E. Moore, *J. Amer. Med. Ass.*, **199**, 519(1967).

(11) A. C. Bratton and E. K. Marshall, Jr., *J. Biol. Chem.*, **128**, 537(1939).

(12) N. Gerber, R. Seibert, D. Desiderio, R. M. Thompson, and M. Lane, *Clin. Pharmacol. Ther.*, **14**, 264(1973).

(13) B. B. L. Agrawal and E. Margoliash, *Anal. Biochem.*, **34**, 505(1970).

(14) M. A. Hahn and R. H. Adamson, *Pharmacologist*, **12**, 447(1970).

(15) K. A. Sund, *J. Agr. Food Chem.*, **4**, 57(1956).

(16) E. W. Hauser and J. Thompson, *ibid.*, **2**, 680(1954).

(17) E. Margoliash, A. Novogradsky, and A. Schejter, *Biochem. J.*, **74**, 339(1960).

ACKNOWLEDGMENTS AND ADDRESSES

Received August 24, 1973, from the *Department of Experimental Therapeutics, Roswell Park Memorial Institute, New York State Department of Health, Buffalo, NY 14203*

Accepted for publication October 4, 1973.

Supported by Core Program Grant CA-13038 from the National Cancer Institute, U.S. Public Health Service, and Institutional General Research Support Grant 61124 from the U.S. Public Health Service.

[x] To whom inquiries should be directed.

Evaluation of Mathematical Models for Diffusion from Semisolids

JAMES W. AYRES and PAUL A. LASKAR[x]

Abstract □ Models used in the description of diffusion processes were applied to drug release from semisolids. Two models, an exponential model for finite systems and a nonexponential model for semi-infinite and infinite systems, are evaluated for their suitability in describing literature data for release of substances from semisolids. The regression lines calculated for these data are evaluated using analysis of variance and examination of residuals. Evaluation of these models using these data indicates that the exponential model derived for finite systems is applicable in all the literature cases while the nonexponential model has restricted applicability.

Keyphrases □ Diffusion, drugs from semisolids—exponential and monoexponential models evaluated using literature data, regression lines calculated □ Semisolids—evaluation of mathematical models used to describe drug diffusion □ Ointments—evaluation of mathematical models used to describe drug diffusion □ Drug diffusion from semisolids—exponential and monoexponential models evaluated using literature data, regression lines calculated

Diffusion of drug agents from different solid and semisolid vehicles has been the subject of numerous reported investigations. During work in this laboratory with the diffusion of local anesthetics through semisolid dosage forms, it became necessary to evaluate mathematical models commonly used to describe diffusion. Several authors (1–3) described diffusion models which quantitate the diffusion process under various experimental conditions. For example, point, plane, and spherical sources of diffusion in finite, semi-infinite, and infinite systems in one, two, or three dimensions have been described. The terms finite, semi-infinite, and infinite are descriptive terms which locate the boundaries to diffusion in the x direction when a drug diffuses from a dosage form bounded between the planes $x = 0$ and $x = j$, while the semi-infinite system extends from a bounding plane at $x = 0$ to $x = +\infty$ and the infinite svstem extends to infinity in the $+x$ and $-x$ directions (3). In the case of diffusion in one dimension for a finite system, the desired solution for the amount diffused is given by the equations (4, 5):

$$M_t = M_\infty\left[1 - \frac{8}{\pi^2}\sum_{m=0}^{\infty}\frac{1}{(2m+1)}\exp\left(-(2m+1)^2 D\pi^2 t/l^2\right)\right] \quad \text{(Eq. 1)}$$

where:

M_t = amount diffused up to time t
M_∞ = amount diffused to time ∞
D = diffusion coefficient
t = time
l = thickness of membrane or layer

$$Q_{0,t} = C_0AH\left\{1 - \frac{8}{\pi^2}\sum_{m=0}^{\infty}\left[\frac{1}{(2m+1)^2}\exp\left(-\frac{(2m+1)^2\pi^2 Dt}{4H^2}\right)\right]\right\} = C_0AH\left\{1 - \frac{8}{\pi^2}\left[\exp\left(-\frac{\pi^2 Dt}{4H^2}\right) + \frac{1}{9}\exp\left(\frac{-9\pi^2 Dt}{4H^2}\right) + \ldots\right]\right\} \quad \text{(Eq. 2)}$$

where:

$Q_{0,t}$ = amount diffused between time = 0 and t
C_0 = initial concentration in phase from which the substance diffuses
A = area over which diffusion occurs
H = thickness of diffusing region
D = diffusion coefficient
t = time

It should be noted that $1 + (1/9) + (1/25) + \ldots = 8/\pi^2$. A model for semi-infinite and infinite systems (6) was suggested by Higuchi (7) as a simplified model for percutaneous absorption from an ointment. This model has the form:

$$Q = (2A - C_s)\left[\frac{Dt}{1 + 2(A - C_s)/C_s}\right]^{1/2} \quad \text{(Eq. 3)}$$

where:

Q = amount absorbed at time t per unit area of exposure
A = concentration of drug expressed in units per cubic centimeters
C_s = solubility of drug in units per cubic centimeter in external phase of ointment
D = diffusion constant of drug in external phase

Equation 3 may take the form (6) used in the analysis of diffusion of medicaments from semisolid dosage forms (8–11):