drochloride was found to have antiarrhythmic activity in cats. It appears to have a potency similar to that of procaine amide hydrochloride. Replacement of the chloro substituent in 1-*n*-propyl-4-*p*-chlorobenzoylpiperazine hydrochloride by a hydrogen, methoxy, or amino group, or the *n*-propyl group by other alkyl groups abolished the activity.

5. A receptor for antiarrhythmic agents has been proposed to consist of an anionic site and a flat area. The anionic site is believed to be approximately 4.5–5.0 Å. away from the center and 3.0–3.5 Å. above the plane of the flat area.

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Keyphrases

Antiarrhythmic agents
Procaine amide analogs—synthesis
Pharmacological activity—procaine amide analogs
Atria, guinea pig—antiarrhythmic activity determination
Structure-activity relationship–procaine amide analogs

Linear Nonisothermal Stability Studies

By M. A. ZOGLIO, J. J. WINDHEUSER, R. VATTI, H. V. MAULDING, S. S. KORNBLUM, A. JACOBS, and H. HAMOT

A method for evaluation and utilization of data from linear nonisothermal kinetics has been developed. The studies have yielded energy of activation, reaction rate, and stability predictions from a single experiment. The relatively short length of time needed to complete a study and the comparatively few analytical experiments required present a significant advantage over classical kinetic methods. Large volumes of temperature-controlled space and the need for preliminary screening studies have been eliminated in the method. The advantage of this approach over other nonisothermal kinetic methods lies in the simplicity of equipment required and the ease of analysis of concentration-time (temperature) curves. The hydrolysis of *N*-acetyl-*p*-aminophenol and procainamide hydrochloride has been followed to demonstrate the validity of the theory and the advantages of the method.

THE USE OF stability predictive methods, such as that described in this report and other approaches (1), cannot be expected to replace the formal stability program. The predictive methods should rather be employed in cutting down the number of formulations which are subjected to formal testing by eliminating poor preparations through preformulation as well as formulation screening. The procedures which can be used to predict stability have reached a reasonable degree of sophistication. The recent upsurge of pre-

Received February 13, 1968, from the Pharmacy Research and Development Department, Sandoz Pharmaceuticals, Hanover, NJ 07936
Accepted for publication September 19, 1968.
Presented to the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, Las Vegas meeting, April 1967.
The technical assistance of A. Dilatush, D. Murdock, and W. Vincek is gratefully acknowledged.

formulation testing programs will most likely increase the use of predictive methods and result in further technicological growth in the field.

The classical isothermal approach to drug stability prediction has not disappeared from the industrial pharmacy scene for two reasons. First, some of the newer methods require estimates of reaction rate through slope determinations at points on a nonlinear curve and second, time-temperature relationships in the nonisothermal studies reported in the literature to date are complex and somewhat difficult to accomplish without specially designed equipment. The slope estimate method is employed in differential thermal analysis (2). A spectrophotometric method (3), which utilizes the slope of a continuous spectrophotometric curve plotted against time while the temperature of the sample cell is raised also requires a slope estimate. The first mathematically precise nonisothermal stability study was reported by Rogers (4) and uses the time-temperature relationship.

$$\frac{1}{T} = \frac{1}{T_0} - 2.303 \log (1 + t)$$

The use of Rogers' method by Cole and Leadbeater in two recent reports (5, 6) is indicative of the value of the nonisothermal technique. Limitations to this procedure which are applicable to other programmed kinetic methods including the linear method are put forth in an excellent article by Carstensen (7). The second treatment was reported by Eriksen (8) using the time-temperature relationship.

$$\frac{1}{T} = \frac{1}{T_0} - at$$

The method described in this report involves a linear relationship of time and temperature ($t = bT + C$) Energy of activation is determined by comparing analytical data to model degradation curves. The curves are obtained through digital computer solution of equations derived in the linear nonisothermal approach. The curves, once obtained for a given temperature range, can be used in any number of experiments which consider this range so that an understanding of the mathematics involved in obtaining model curves is not necessary in employing the method. After obtaining the energy of activation a simple calculation is made from the analytical and computer data to arrive at a reaction rate or stability prediction at any desired temperature. The temperature range of the experiment may be controlled by analysis of the drug degradation during the early stages of the experiment. An important feature of the method is the concentration-time (temperature) curve. It allows a visual interpretation of the effect of temperature on the degradative rate of the formulation. In the curves it can be seen quite clearly that there are fairly prominent shoulders, especially at the higher activation energies where a drug degradation rate will begin to accelerate rapidly with increased temperature.

THEORETICAL

In order to integrate the general differential equations for zero-, first-, or second-order reactions where temperature is varying with time, one must be able to express and integrate the observed rate constant as a function of time. The expression

$$k_{obs.} = ae^{-E/RT} \qquad \text{(Eq. 1)}$$

cannot be integrated for the case where temperature is changing linearly with time. It is not possible, therefore, to obtain a concentration-time equation which would yield energy of activation and reaction rate directly for the linear case.

The approach used to handle the linear time-temperature relationship is based on the following:

Consider the theoretical drug degradation in Fig. 1. In this degradation the reaction order remains unchanged throughout the experiment. The temperature is changing linearly as a function of time.

$$t = bT + C \qquad \text{(Eq. 2)}$$

The curve for the degradation can be described by a series of slopes or rate constants each representing the instantaneous rate of change of drug concentration with time at some time (temperature). The linear method is based on the assumption that the arithmetic average of these rates of change is equal to the total degradation of the drug during the experiment divided by the time span required for the

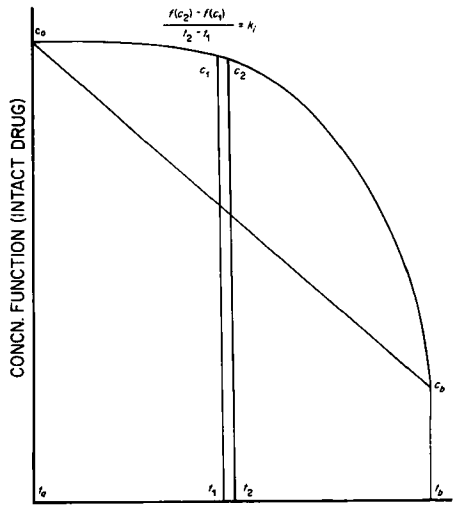

Fig. 1—Hypothetical degradation of a drug during a linear nonisothermal stability study. $t_b - t_a$ *is the total time span of the experiment. In the studies considered in this report* $b' = 1$. *Time* = b' *temperature* + *constant.*

experiment.

$$\frac{f(b) - f(a)}{b - a} = \frac{k_1 + k_2 + k_3 + \ldots k_i \ldots k_n}{n} \quad \text{(Eq. 3)}$$

If $k_i = f'(c_i)$, then the average of k over the interval (a,b) is (9):

$$\bar{k} = \frac{1}{b - a}\int_a^b f'(c)dc = \frac{f(b) - f(a)}{b - a} \quad \text{(Eq. 4)}$$

For very large n, a discrete curve should approach the smooth curve and the average of k should not differ significantly from the quantity $f(b) - f(a)/(b - a)$. The question still remains as to whether the average of k over a finite set of intervals as defined in this treatment indeed approaches $\bar{k}$ and if so how large must n be to minimize error. Both of these questions will be dealt with after formulation of the basic expressions used in applying the linear nonisothermal method.

To solve Eq. 3 for an individual rate constant, the rate constant must be expressed in terms of all other rate constants in Eq. 3. This can be done through Eq. 1. For the rate constant k_i

$$k_i = ae^{-E/RT_i} \quad \text{(Eq. 5)}$$

For the rate constant $k_{(i+1)}$

$$k_{(i+1)} = ae^{-E/RT_{(i+1)}} \quad \text{(Eq. 6)}$$

Dividing Eq. 6 by Eq. 5 yields

$$k_{(i+1)} = k_i e^{E/R\{[T_{(i+1)} - T_i]/[T_iT_{(i+1)}]\}} \quad \text{(Eq. 7)}$$

If the increment $T_{(i+1)} - T_i$ is some constant increment c then the solution for $k_{(i+2)}$ in terms of k_i becomes

$$k_{(i+2)} = k_i e^{E/R\{c/[T_{(i+1)}T_i]\}} \times e^{E/R\{c/[T_{(i+2)}T_{(i+1)}]\}} \quad \text{(Eq. 8)}$$

In this manner each rate may be expressed in terms of k_i. These expressions can be substituted for the values k_1 through k_n in Eq. 3. If $k_1 = k_i$ the following expression is obtained:

$$\frac{f(b) - f(a)}{b - a} = \frac{k_1}{n}\{1 + e^{E/R[c/(T_1T_2)]} + e^{E/R[c/(T_1T_2) + c/(T_2T_3)]} + \ldots e^{E/R[c/(T_1T_2) + c/(T_2T_3) + c/(T_3T_4) + \ldots c/(T_{(n-1)}T_n)]}\} \quad \text{(Eq. 9)}$$

The quantity in brackets can be determined using the digital computer. Since the quantity $[f(b) - f(a)]/(b - a)$ is known through experiment, rate k_1 can be calculated for a particular activation energy. In the same manner equations similar to Eq. 9 may be derived and solved for k_2 through k_n. The question as to whether the finite set of rates obtained in this manner approaches $\bar{k}$ is somewhat clarified by the following:

In dealing with a first-order reaction

$$\frac{f(c_{i+1}) - f(c_i)}{t_{(i+1)} - t_i} \quad \text{(Eq. 10)}$$

implies that $f(c) = \ln C$.

The rate constant at time zero is

$$k_0 = a^*e^{-E/RT_0} \quad \text{(Eq. 11)}$$

If this temperature is maintained for a time increment t, then the concentration at time t will be

$$C_1 = C_0 \exp(-t \cdot a^* e^{-E/RT_0}) \quad \text{(Eq. 12)}$$

If the temperature is changed to T_1 and maintained for the same increment t, after which the temperature is changed to T_2, and so on, then after n increments the concentration will be

$$C_n = C_0 \prod_{i=0}^{n-1} \exp(-t \cdot a^* \cdot e^{-E/RT_i}) \quad \text{(Eq. 13)}$$

Therefore,

$$\frac{\ln C_n - \ln C_0}{nt} = \frac{1}{nt}\left[\ln C_0 + \sum_{i=0}^{n-1} t \cdot a^* e^{-E/RT_i} - \ln C_0\right] = \frac{a^*}{n}\sum_{i=0}^{n-1} e^{-E/RT_i} = \bar{k}_n \quad \text{(Eq. 14)}$$

$\bar{k}_n$ represents the average using n discrete values.

If we consider the function:

$$\frac{a^*}{T_n - T_0} e^{-E/RT} \quad \text{(Eq. 15)}$$

then an upper Rieman sum for the function in the interval (T_0, T_n) formed by dividing the interval into n equal parts would be

$$\sum_{i=1}^{n-1}\left(\frac{T_n - T_0}{n}\right)\frac{a^*}{T_n - T_0} e^{-E/RT_i} = \frac{a^*}{n}\sum_{i=1}^{n-1} e^{-E/RT_i} \quad \text{(Eq. 16)}$$

This sum, as n approaches infinity, will tend towards:

$$\frac{1}{T_n - T_0}\int_{T_0}^{T_n} a^* e^{-E/RT}dT = \bar{k} \quad \text{(Eq. 17)}$$

such as pointed out in Eq. 4, the average of the rate constant over the integral (T_0, T_n). Hence it is reasonable to assume that for a first-order reaction arithmetic average in this treatment is adequate. The question as to how large a value of n would be to approach a smooth curve is demonstrated by testing the convergence of the bracketed expression in Eq. 9.

The reciprocal of the expression:

$$\frac{1 + \sum_{k=1}^{n-1} \exp\left[\frac{E \cdot c}{R}\sum_{i=1}^{k} T_i^{-1} T_{(i+1)}^{-1}\right]}{n} \quad \text{(Eq. 18)}$$

was evaluated for values of E at various levels of n using the values for T_0 and T_n corresponding to those used experimentally. The results, are tabulated in Table I and show rapid convergence as n approaches 384. The line of reasoning as outlined here for the first-order case can be employed for zero- and second-order reactions. For this study k_1 through k_{384} are considered and a set of 384 rates obtained for a

TABLE I—CONVERGENCE OF EXPRESSION

$$n \cdot \left\{1 + \sum_{k=1}^{n-1} \exp\left[\frac{E \cdot c}{R} \sum_{i=1}^{k} T_i^{-1} T_{(i+1)}^{-1}\right]\right\}^{-1}$$

Value of n	Value of Expression for E = 10 kcal.	E = 20 kcal.	E = 30 kcal.
8	0.322	0.0710	0.0141
16	0.292	0.0610	0.0111
48	0.271	0.0542	0.00923
96	0.267	0.0527	0.00879
192	0.267	0.0527	0.00869
384	0.267	0.0527	0.00869

particular activation energy. The rates were then used to synthesize families of model curves on a first-order or logarithmic scale. For the first-order acid-catalyzed degradation of *N*-acetyl-*p*-aminophenol and procainamide hydrochloride analytical data are superimposed over the model curves to determine activation energies (Figs. 2 and 3). The activation energy, actual experiment degradation, and computer data are then used in calculating a specific reaction rate. The reaction order is then utilized in predicting stability. The Arrhenius equation may be used with these rates to calculate rates outside of the temperature range studied.

EXPERIMENTAL

Initial experiments were carried out utilizing a temperature programmed convection oven. The equipment consisted of a convection oven (Thelco model 17) equipped with thermocouple (West program controller JSBGB-2), and saturable core reactor (West). Temperature was continuously recorded (YSI model 425C Tele-thermometer and the model G14A Varian recorder). The temperature range was arbitrarily set at 35–83° with a linear rise of 1°/hr. The accuracy of the programmed oven was found to be ±0.3°. The oven represented very

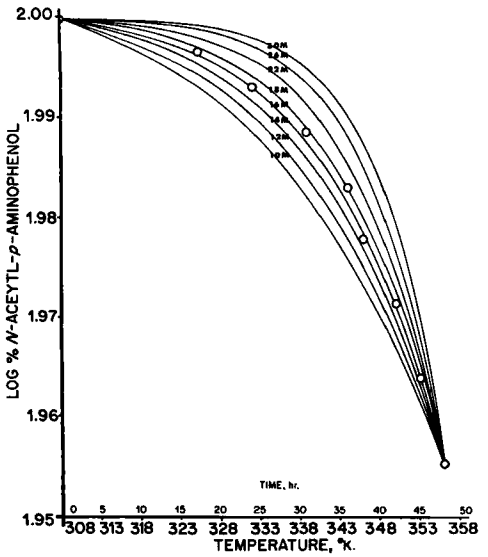

Fig. 2—Nonisothermal hydrolysis of N-*acetyl-p-aminophenol in aqueous solution (pH = 2). Temperature range, 308–356° K.;* E_a *= 17 kcal./mole;* E_a *literature = 16.69 kcal./mole.*

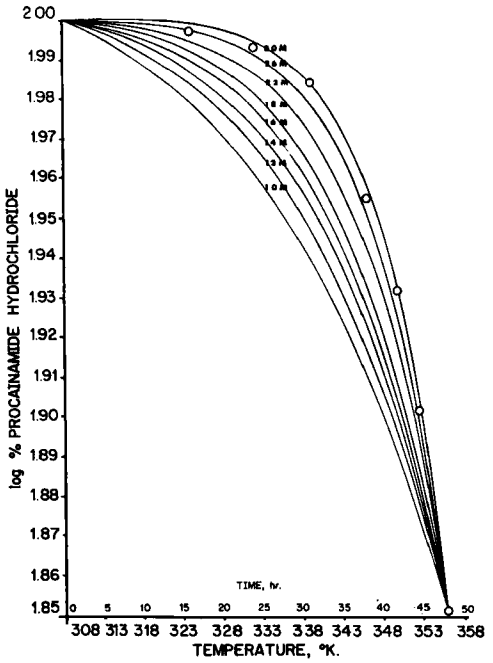

Fig. 3—Nonisothermal hydrolysis of procainamide hydrochloride in 0.934 M perchloric acid. Temperature range, 308–356°K.; E_a *= 29 kcal./mole;* E_a *literature = 13 kcal./mole.*

flexible instrumentation since the set point unit cam could be cut for a variety of temperature functions. The accuracy, however, for the linear program could be improved to ±0.1° by using a thermoregulator (Bronwill) driven by a synchronous clock motor as described by Scott (10). Temperature recording for this unit was accomplished in the same manner as for the oven.

Hydrolysis of N-Acetyl-p-aminophenol—A 0.15% solution of *N*-acetyl-*p*-aminophenol[1] in 0.2 *M* phosphate buffer (pH = 2.0) was prepared, filled into 10-ml. ampuls, and the ampuls sealed. Samples were then placed into the programmed oven and allowed to equilibrate at 35° for a period of 2 hr. A 0-hr. sample was removed and the temperature program begun. Samples were removed every 2 hr. during the first 8 hr. and analyzed for intact *N*-acetyl-*p*-aminophenol. The program of sampling was scheduled according to the results of these assays. Subsequent to the initial sampling, the ampuls were placed in a deep freeze immediately upon removal from the oven. At a convenient time the samples were quickly brought to room temperature by heating over a steam bath and analyzed for *N*-acetyl-*p*-aminophenol in the following manner.

A chromatography column (10 mm. diameter) containing 1 g. of ion-exchange resin[2] was prepared. Five milliliters of ampul solution was transferred to a 50-ml. volumetric flask and diluted to the mark with distilled water. Ten milliliters of this solution was pipeted into the chromatography column. The column was eluted with water to a volume of 100 ml. and the absorbance of the eluate determined at 243 mμ in a recording spectrophotometer (Cary model 11).

[1] Eastman Organic Chemicals, m.p. 167–168°.
[2] Rohm and Haas Co., Philadelphia, Pa.

The absorbance of the 0-hr. sample was used as the standard reading. Severely degraded samples, which distort the UV absorbance curve, were not encountered during the experiment.

Hydrolysis of Procainamide Hydrochloride (Nonisothermal)—A 0.4 % solution of procainamide hydrochloride[3] in 0.939 *N* perchloric acid solution was prepared, filled into 10-ml. ampuls, and ampuls sealed. The sampling procedure was the same as that described for *N*-acetyl-*p*-aminophenol. The programmed oven which had been used for three experiments with *N*-acetyl-*p*-aminophenol was used twice for procainamide. The third experiment with procainamide was performed using a motor-driven thermoregulator. The sampling schedule remained the same as with the oven experiments. Analysis for intact procainamide hydrochloride was accomplished in the following manner.

Four milliliters of the ampul solution was pipeted into a separator. Twenty-five milliliters of pH 9.5 buffer (sodium carbonate–sodium bicarbonate) was added. The aqueous layer was extracted with 30-ml. portions of chloroform and the extracts collected in a 100-ml. volumetric flask. The extracts were diluted to the mark with chloroform and 5.0 ml. of this solution transferred to a 50-ml. volumetric flask. This sample was diluted to the mark with chloroform and the absorbance determined at 272 mμ in a recording spectrophotometer (Cary model 11).

Hydrolysis of Procainamide Hydrochloride (Isothermal)—Hydrolysis of procainamide hydrochloride in 0.939 *N* perchloric acid was studied isothermally at constant temperatures of 35° (±0.01°), 59° (±0.01°), and 83° (±0.01°). Sampling was scheduled according to early assay results. Analytical determinations were performed in the same manner as the nonisothermal experiments.

RESULTS AND DISCUSSIONS

The analytical data for the first-order linear nonisothermal hydrolysis of *N*-acetyl-*p*-aminophenol and procainamide hydrochloride are superimposed on model degradation curves in Figs. 2 and 3. The analytical results are average results from three separate experiments and represent a total breakdown of 9.8% for *N*-acetyl-*p*-aminophenol and 28.9% for procainamide hydrochloride. The results indicate an activation energy of 17,000 cal. for *N*-acetyl-*p*-aminophenol and 29,000 cal. for procainamide hydrochloride hydrolysis.

The first activation energy is in fine agreement with the value of 16,700 cal. reported by Koshy (11). The activation energy of 29,000 cal. for procainamide hydrochloride is not in agreement with the literature value of 13,000 cal. (12). Classical isothermal techniques were employed as experimental conditions for obtaining this lower value. Reaction rates were studied at 85.3, 91.3, and 97.3°. To resolve this discrepancy, the isothermal experiment was repeated in these laboratories for the temperature range 35 to 83° and an activation energy of 27,000 cal. found (Fig. 4). The low value reported in the literature could be due to the high temperatures and narrow temperature range chosen for the study.

The information obtained through the linear non-

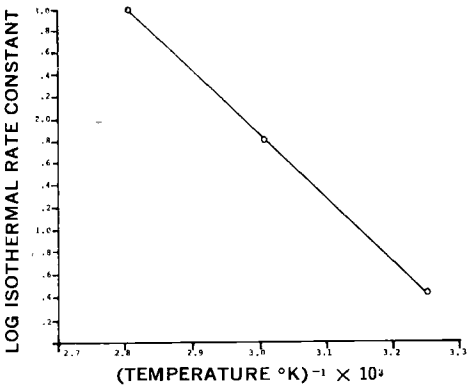

Fig. 4—Arrhenius plot showing the temperature dependency of the hydrolysis of procainamide. The energy of activation as found from this plot is 27.0 kcal./mole.

isothermal method can be used to calculate a rate constant or shelf-life prediction at some desired temperature. Using *N*-acetyl-*p*-aminophenol as an example, the following procedure would be employed in calculation of the apparent rate constant at 35°. From the left side of Eq. 9:

$$\frac{f(b) - f(a)}{b - a} = \frac{2.3 \log 100/90.2}{48}$$

$f(b) - f(a)$ represents the total first-order degradation and $b - a$ the total hours of the experiment. 90.2 is the percentage of intact drug left at the end of the experiment. From the right side of Eq. 9:

$$\frac{k_{35}\,[1 + e^{E/R[c/(T_1T_2)} + \ldots]}{n} = \frac{k_{35}\,(4278)}{384}$$

In this expression n represents the number of rate constants being considered in describing the total curve. The value of the bracketed quantity was obtained by digital computer solution of the expression with E equal to 17,000 cal. Values were generated through equations solved for k_1 through k_{384} for an activation energy range of 10–30,000 cal. in increments of 1000 cal.[4] The value for k_{35} calculated for the nonisothermal method is 1.95×10^{-4} hr.$^{-1}$. The value calculated from isothermal literature data is 2.52×10^{-4} hr.$^{-1}$. Knowing reaction order and rate, it is an easy task to integrate the appropriate rate equation to obtain a shelf-life estimation. For calculations of rates outside of the temperature range studied the Arrhenius equation may be employed.

Figures 2 and 3 are not only useful with regard to shelf-life estimation, but also present an excellent picture of the effect of temperature on reaction rate at a particular activation energy level. Analysis of these families of curves demonstrates the inadequacies of single isothermal high-temperature experiments often employed in pre formulation and formulation screening. The slopes or first derivatives at the higher temperatures can be very close yet the rates at lower temperatures widely different. If, for example, a procainamide hydrochloride solution were studied at 75° it would appear to be very unstable, whereas in fact if one examines the rates at lower

[3] The authors wish to express appreciation to the Squibb Institute of Medical Research for the generous sample of procainamide (Pronestyl).

[4] IBM 360 Fortran program will be provided to interested parties upon request.

temperature the solution is stable at these temperatures by virtue of the high activation energy of the hydrolytic reaction.

SUMMARY

Linear nonisothermal kinetic data from a single experiment can be used to obtain activation energy, reaction rate, and shelf-life prediction. The method described in this report involves a comparison of model degradation curves to experimental data for the determination of activation energy. Calculation of reaction rate and shelf-life predictions at a specific temperature are made utilizing the total experimental degradation and activation energy. The advantages of the method over the classical isothermal method lie in the use of a single experimental unit, the analysis of one set of samples, the shorter time required for completion of the experiment, and the ability to change the temperature range of the experiment without interruption of the study. The advantages over other nonisothermal methods lie in the ease of analysis of data, simplicity of time-temperature relationship, and the use of readily available laboratory equipment. The approach is limited only by assay precision, constancy of activation energy, and applicability of the Arrhenius equation. The method is proposed and is currently being evaluated for preformulation and formulation screening of ampul solutions.

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Keyphrases

Stability studies—linear, nonisothermal
Nonisothermal method—stability prediction
Kinetic equations—nonisothermal hydrolysis
Acetaminophen, procainamide HCl—test compounds
Column chromatography—separation
UV spectrophotometry—analysis

Separation and Determination of Chlorpheniramine and Its Dealkylated Metabolites from Urine

By K. ALBERT and J. J. WINDHEUSER

Application of ion-pair extraction to partition chromatographic techniques has been used effectively to separate chlorpheniramine and its dealkylated metabolites from one another in urine. Gas-liquid chromatography was employed to detect the amines. The ion-pair partition column employed selectively separated the amines prior to detection by gas chromatography. The chromatograms indicated symmetric peaks with little or no tailing typical of the secondary and primary amine metabolites. Recovery studies from urine demonstrated that at the parts-per-million level, complete recovery of chlorpheniramine and its metabolites was achieved after certain precautions against loss were taken. On the basis of these studies, an analytical procedure to separate chlorpheniramine and its metabolites from urine was developed. Recoveries of chlorpheniramine and its dealkylated metabolites were quantitative. The method offers the advantage of complete isolation of all components from one another and suggests the possibility of using ion-pair partition columns as a method to quantitatively separate other nitrogen-containing compounds from one another.

THE SEPARATION AND DETECTION of drugs from mixtures of closely related compounds is becoming more and more important. Of particular interest is the analysis of drugs and their metabolites in blood and urine in microgram quantities. This communication deals with a model system utilizing the concept of ion-pair separation of related amines from urine.

The suitability of ion-pair extraction as a process for separation and isolation of nitrogen-containing compounds in analytical samples was recently demonstrated by Higuchi *et al.* (1, 2).

Received July 19, 1968, from the School of Pharmacy, University of Wisconsin, Madison, WI 53706
Accepted for publication September 4, 1968.
Presented to the Drug Standards, Analysis and Control Section, APhA Academy of Pharmaceutical Sciences, Miami Beach meeting, May 1968.
This study was supported in part by grants from Warner-Lambert Research Institute, Morris Plains, N. J., and Smith, Kline & French Laboratories, Philadelphia, Pa.