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Note

Liquid chromatographic determination of clorazepate decomposition rates

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Clorazepate is a benzodiazepine-type tranquilizer available in oral dosage forms as either the monopotassium or dipotassium salt. Product information[1] indicates that aqueous solutions of clorazepate are unstable and undergo rapid decomposition. The decomposition occurs by loss of the carboxyl group at the 3-position of the benzodiazepine nucleus. This decarboxylation process has been shown to be acid catalyzed and the rate of the reaction increases as solution pH decreases[2]. Clorazepate is stable only as the salt form and the decomposition occurs following protonation of the carboxylate anion. Clorazepate salts are highly water soluble with little or no organic solvent solubility. The rapid decarboxylation of clorazepate under acidic conditions results in therapeutic plasma levels of the decomposition product, N-desmethyldiazepam, and only trace levels of the parent drug[3]. Thus, the pharmacological profile of this product is essentially that of N-desmethyldiazepam which has been shown to be a very potent benzodiazepine[4]. N-Desmethyldiazepam is also the major metabolite of diazepam in humans.

In previous studies[5], administration of clorazepate with sodium bicarbonate and other antacid preparations reduced the rate and extent of appearance of N-desmethyldiazepam in blood. Chun *et al.*[6] have shown a trend toward slower absorption for clorazepate when administered with antacids, however, no significant effect on extent of absorption as measured by the area under the plasma level-time curves was observed. In a similar study[3] the bioavailability of clorazepate determined by evaluating its conversion to and absorption as N-desmethyldiazepam, was significantly reduced in the presence of a higher pH, and the peak metabolite plasma level and the time of occurrence of this peak was reduced and delayed respectively.

The direct analysis of clorazepate by some analytical techniques[7] including gas chromatography is very difficult due to the ease of the decarboxylation reaction. Previous reports[2,3] concerning the rates of clorazepate decomposition have required extractions and the measurement of N-desmethyldiazepam levels. This report describes the result of direct liquid chromatographic measurement of clorazepate levels during decomposition studies.

EXPERIMENTAL

Equipment

The liquid chromatograph was a modular system consisting of a Waters Assoc. (Milford, MA, U.S.A.) Model 6000A pump, Model U6K injector and a Model 440 ultraviolet detector operated at 254 nm and 0.02 a.u.f.s. The column (15 cm × 4.6 mm I.D.) was packed with C_{18} chemically bonded spherical silica (5 μm), Ultrasphere ODS (Altex, Berkeley, CA, U.S.A.). All pH measurements were made using a Beckman (Fullerton, CA, U.S.A.) model 3500 digital pH meter with a combination electrode.

Reagents and chemicals

All reagents were of ACS reagent-grade quality and were used without further purification. HPLC grade methanol, mono- and dipotassium phosphate and phosphoric acid were purchased from Fisher Scientific Company (Atlanta, GA, U.S.A.). Three stock solutions of 0.2 *M* phosphate were prepared: dipotassium phosphate, 34.8 g/l; monopotassium phosphate, 27.2 g/l; and phosphoric acid, 23.1 g of 85%/l. Five 0.1 *M* phosphate buffer solutions ranging in pH from 2 to 6 at approximately one pH unit increments were prepared by mixing varying amounts of the stock solutions to obtain the desired pH and diluting with an equal volume of water.

The chromatographic mobile phase was a mixture of 0.1 *M* phosphate buffer (pH 7.46) and methanol (3:7). All aqueous solutions were prepared in double-distilled water.

Decomposition studies

Samples of clorazepate (4-mg range) were accurately weighed and transferred to 10-ml volumetric flasks. The flasks were filled to volume with 0.1 *M* phosphate buffer at the desired pH. Triplicate samples were prepared and analyzed at pH 2.00, 2.99, 4.01, 4.99 and 6.05. The addition of buffer to the clorazepate sample was recorded as time zero and the times of injection recorded until the clorazepate peak had virtually disappeared. Liquid chromatographic analysis of the solutions was accomplished by injecting 5-μl samples with a mobile phase flow-rate of 1.5 ml/min.

RESULTS AND DISCUSSION

The acid-catalyzed decomposition of clorazepate to yield N-desmethyldiazepam is illustrated in Fig. 1. The decomposition reaction is known to result in the loss of the carboxyl-group from the 3-position of the benzodiazepine ring. The reversed-phase liquid chromatographic separation of clorazepate and N-desmethyldiazepam is shown in Fig. 2. The chromatograms in Fig. 2 illustrate the results obtained for clorazepate decomposition at pH 2.99. The separation was achieved using a mobile phase of 70% methanol in 0.1 *M* phosphate buffer pH 7.46. The high mobile phase pH is required to insure that no clorazepate decomposition occurs during the analysis. The decarboxylation reaction has been shown[7] to occur at a rate rapid enough to preclude the liquid chromatographic observation of the parent molecule in an aqueous mobile phase of pH 4.6 or less and substantial on-column decarboxylation was observed at pH 5.6. The high mobile-phase pH used in this study

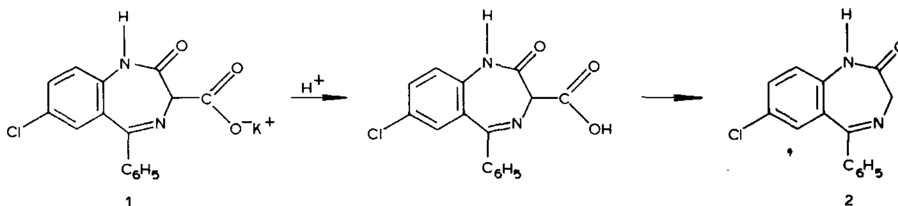


Fig. 1. Decomposition pathway for clorazepate. Structures: 1, clorazepate monopotassium; 2, N-desmethyldiazepam.

keeps the carboxyl-group in the ionic form preventing the decarboxylation. Thus, the clorazepate is being chromatographed as an ionic species and this accounts for its relatively low capacity factor (k'). Previous work[7] has confirmed the identity of peak 1 in Fig. 2 as clorazepate. The k' value for clorazepate can be increased by increasing the aqueous component of the mobile phase indicating that the ionic solute is undergoing the typical reversed-phase retention process. A similar liquid chromatographic procedure[8] for the analysis of clorazepate in pharmaceutical products made use of hydrophobic ion-pairs by adding tetrabutylammonium ion to the mobile phase. Sufficient retention was obtained in this study with the more hydrophilic phosphate counterion. Both the monopotassium and dipotassium salts of clorazepate have been shown to produce species in solution of identical chromatographic characteristics[7].

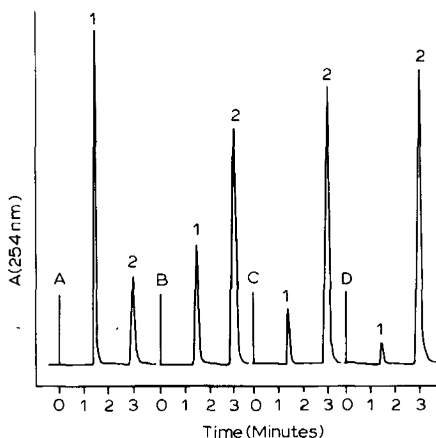
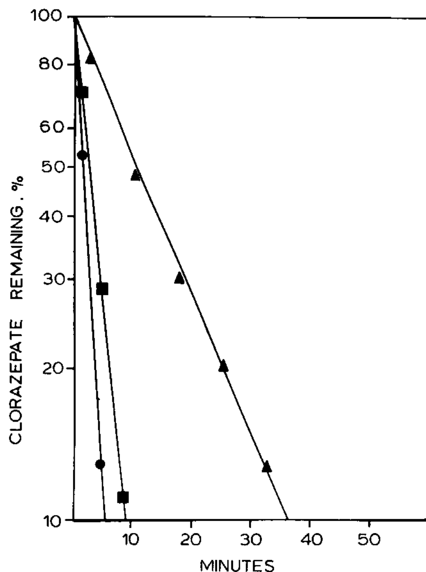


Fig. 2. Liquid chromatographic analysis of a clorazepate solution of pH 2.99. Reaction times: A, 1.43 min; B, 5.56 min; C, 9.07 min; D, 12.64 min. Peaks: 1 = clorazepate; 2 = N-desmethyldiazepam.

The rate of clorazepate decomposition was studied as a function of solution pH with the concentration of unchanged clorazepate measured by liquid chromatography. The pH and buffer capacity of the mobile phase were sufficient to inhibit the decomposition reaction upon injection. The decomposition experiments were conducted by preparing solutions of clorazepate in phosphate buffer at pH 2.00, 2.99, 4.01, 4.99 and 6.05. At least three trials were conducted at each pH level. The insolubility of N-desmethyldiazepam in water results in precipitation as the decomposition progresses and prevents the quantitation of its accumulation. Thus, no attempts were made to measure the corresponding formation rates for N-desmethyldiazepam.



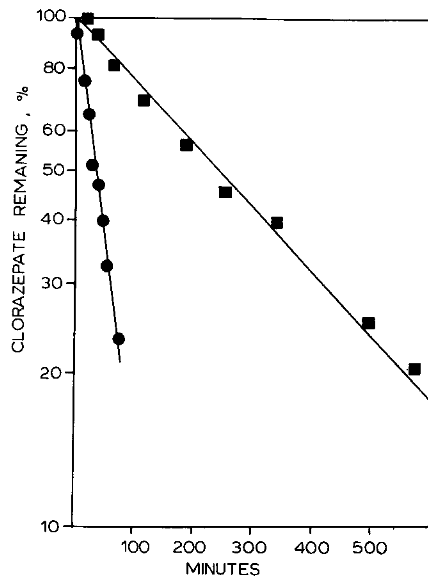


Fig. 3. Apparent first order disappearance of clorazepate in pH 2 (●), pH 3 (■) and pH 4 (▲) 0.1 *M* phosphate buffers at 22.0°C.

Fig. 4. Apparent first order disappearance of clorazepate in pH 5 (●) and pH 6 (■) 0.1 *M* phosphate buffers at 22.0°C.

Figs. 3 and 4 show the semilogarithmic plots for the percentage of clorazepate remaining as a function of time for the pH buffer systems studied. At constant pH and temperature, the degradation followed an apparent first order process. The linear relationship describing an apparent first order degradation mechanism is shown in eqn. 1.

$$\log C = \log C_{\text{initial}} - K_d t/2.303 \quad (1)$$

The terms C and C_{initial} are the concentrations of clorazepate in the buffer at time t and time zero, respectively and K_d is the apparent or observed first order degradation rate constant. Degradation kinetics were followed through at least three half-lives for all

TABLE I

CLORAZEPATE DEGRADATION RATE CONSTANTS AS A FUNCTION OF pH

Conducted at 22°C.

pH	K_d *(h^{-1}), mean ± S.D.*	*Correlation coefficient, r, mean ± S.D.*
2.00	24.94 ± (1.30)	0.993 ± (0.007)
2.99	14.95 ± (0.40)	0.982 ± (0.002)
4.01	4.02 ± (0.40)	0.990 ± (0.005)
4.99	1.29 ± (0.34)	0.987 ± (0.014)
6.05	0.17 ± (0.02)	0.994 ± (0.003)

pH systems to assure the reaction order. Final estimates of K_d were obtained by fitting the data to Eq. 2 by means of a non-linear squares method[9].

$$C = C_{\text{inital}}\ e^{-K_d t} \tag{2}$$

The mean degradation rate constants at each of the pH conditions studied are presented in Table I. Degradation half-lives for pH conditions 2 to 6 were 1.7, 2.8, 10.3, 32.3, and 241.7 min, respectively. Previous studies[2,10] employing extraction methods and analysis reported half-lives of 1.8 and 28 min at pH values of 2 and 5 for studies at 27.5°C. While it would be of interest to evaluate the decarboxylation reaction via appearance of N-desmethyldiazepam, the observed precipitation of degradation product prevented including this kinetic approach.

A profile of log K_d as a function of buffer pH for the decarboxylation of clorazepate is illustrated in Fig. 5. Over the pH range of 2 to 6, the apparent first order rate constant varied by 150-fold (24.9 to 0.17 h^{-1}). The non-linearity of the profile between pH 2 and 4 may be a consequence of protonation of the nitrogen at the 4-position of the benzodiazepine ring. Other benzodiazepines have reported pK_a values for the nitrogen at the 4-position ranging from 1.8 to 3.4 (refs. 11 and 12). Either slower or the absence of decarboxylation of the N-4 protonated form of clorazepate relative to that of the unprotonated form (pH 4–6) may offer some explanation for the noted curvature. Similar curvature in pH profiles has been noted at various temperatures[2] for this drug. While the relationship of log K_d *versus* pH appears linear from pH 4 to 6 as expected for an acid catalyzed reaction involving one drug species, the limited pH range examined does not permit its complete evaluation.

The results of this study show that degradation rates determined by direct measurement of clorazepate using liquid chromatography are very similar to those

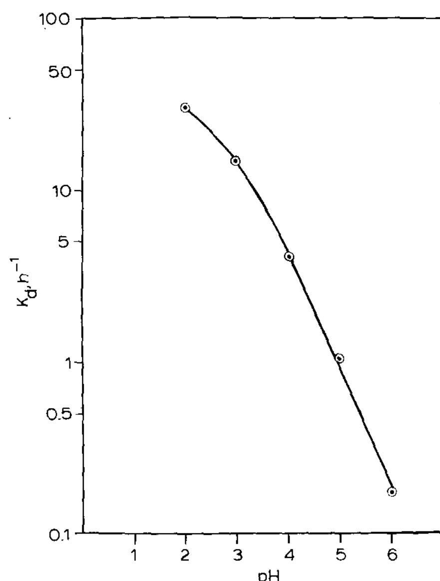


Fig. 5. The pH–rate profile of clorazepate at 22.0°C.

determined by other methods. Clorazepate, the prodrug form of N-desmethyldiazepam, has a degradation half-life of 1.7 min at pH 2.0 which increases to 241.7 min at pH 6.0.

REFERENCES

1 *Physicians' Desk Reference*, Medical Economics Company, Aradell, NJ, 1981.
2 R. Raveux and M. Briot, *Clin. Ther.*, 4 (1969) 303–311.
3 C. W. Abruzzo, T. Macasieb, R. Weinfeld, J. A. Rider and S. A. Kaplan, *J. Pharmacokin. Biopharm.*, 5 (1977) 377–390.
4 S. H. Curry, *Clin. Pharmacol. Ther.*, 16 (1974) 192–197.
5 R. I. Shader, A. Georgotas, D. J. Greenblatt, J. S. Harmatz and M. D. Allen, *Clin. Pharmacol. Ther.*, 24 (1978) 308–315.
6 A. H. C. Chun, P. J. Carrigan, D. J. Hoffman, R. P. Kershner and J. D. Stuart, *Clin. Pharmacol. Ther.*, 22 (1977) 329–335.
7 C. R. Clark and F. T. Noggle, Jr., *J. Chromatogr.*, 188 (1980) 426–430.
8 L. Elrod, Jr., D. M. Shada and V. E. Taylor, *J. Pharm. Sci.*, 70 (1981) 793–795.
9 C. M. Metzler, G. L. Elfring and A. J. McEwen, *A User's Manual for Nonlin and Associated Programs*,
10 K. Florey (Editor), *Analytical Profiles of Drug Substances*, Vol. 4, Academic Press, New York, NY, 1975.
11 K. Florey (Editor), *Analytical Profiles of Drug Substances*, Vol. 1, Academic Press, New York, NY, 1972.
12 K. Florey (Editor), *Analytical Profiles of Drug Substances*, Vol. 2, Academic Press, New York, NY, 1973.