**Table I—Average Percentage of Dimenhydrinate in Laboratory-Made Mixtures and in Commercial Samples by Proposed Method and BP Method**

| Mixture (M) (1:1) | Proposed Method Mean, %[a] | ±SD | BP Method Mean, %[a] | ±SD |
|---|---|---|---|---|
| Dimenhydrinate + aspirin ($M_1$) | 99.32 | 0.82 | 98.48 | 1.06 |
| Dimenhydrinate + acetaminophen ($M_2$) | 99.76 | 0.36 | 98.93 | 0.31 |
| Dimenhydrinate + meprobamate ($M_3$) | 98.88 | 0.39 | 98.70 | 0.65 |
| Dimenhydrinate + phenylephrine ($M_4$) | 99.76 | 0.37 | 98.82 | 0.08 |
| Dimenhydrinate + tolbutamide ($M_5$) | 99.32 | 0.31 | 99.71 | 0.19 |
| Commercial tablet[b] $T_1$ | 101.86 | 0.64 | 101.16 | 0.80 |
| Commercial tablet[b] $T_2$ | 100.53 | 0.47 | 100.44 | 0.48 |

[a] Average of four determinations. [b] Each tablet contained 50 mg of dimenhydrinate.

The effect of pH (1.60–11.20) on the absorbance of the colored precipitate (in acetone) was studied. Hydrochloric acid was used to vary the pH from 4.10 to 1.60, and dilute ammonia solution was used to vary it from 4.10 to 11.20. However, absorbance was not affected within a pH range of 2.85–8.30.

The stoichiometric balance was determined by Job's method of continuous variation (9) and a slope-ratio method (10). In Job's method, the maximum absorbance was obtained when $V_1/(V_1 + V_2) = 0.33$, which indicated the formation of the dimenhydrinate–reinecke salt precipitate in a 1:2 molar ratio. In the slope-ratio method, the $slope_1$ and $slope_2$ values were 0.0027586 and 0.0014210 $ml^{-1}$, respectively. Hence, $slope_1/slope_2$ for the precipitate was nearly equal to 2, thereby suggesting a 1:2 molar ratio.

Reinecke salt had a solubility in water of 1 in 52. Therefore, 50 ml of a saturated aqueous solution of reinecke salt was added to a 20-ml aliquot of a methanolic solution of dimenhydrinate to establish the 1:2 molar ratio requirement for maximum precipitation of dimenhydrinate.

**Table II—Recovery Experiments by Proposed Method**

| Mixture or Tablet | Quantity of Dimenhydrinate Added, g | Corresponding Concentration of Dilution Measured, g % | Mean, %[a] | ±SD |
|---|---|---|---|---|
| $M_1$ | 0.600 | 0.096 | 99.36 | 0.40 |
| $M_2$ | 0.025 | 0.075 | 99.12 | 0.66 |
| $M_3$ | 0.020 | 0.072 | 99.01 | 0.76 |
| $M_4$ | 0.055 | 0.093 | 99.36 | 0.12 |
| $M_5$ | 0.035 | 0.081 | 98.69 | 0.52 |
| $T_1$ | 0.005 | 0.033 | 99.93 | 1.17 |
| $T_2$ | 0.010 | 0.036 | 99.41 | 0.64 |

[a] Average for four determinations.

After the optimal experimental conditions for the maximal precipitation of dimenhydrinate–reinecke salt were established, the Beer's plot was obtained by employing the stock solution of the dimenhydrinate–reinecke salt precipitate in acetone. It obeyed Beer's law in the concentration range of 200–1000 μg/ml. With the least-squares method, the calibration curve from the data obtained can be described by the following regression equation: $A = 0.0045 + 1.905C$, where the regression coefficient is 0.999.

Five laboratory-made mixtures and two commercial tablet samples containing dimenhydrinate were subjected to analysis by the proposed method and the BP method (1) (Table I).

The activity of phenylephrine was checked directly with the reagent under identical experimental conditions and was found not to react.

To justify the repeatability of the proposed method, known amounts of pure dimenhydrinate were added to preanalyzed samples of mixtures and dosage forms and reassayed by the proposed method (Table II).

The presence of either pure drugs, *i.e.*, aspirin, acetaminophen, meprobamate, phenylephrine, and tolbutamide, or tablet adjuncts like starch, lactose, talc, and magnesium stearate in the quantities used caused no detectable interference in the proposed method. The analyses of two commercial products and the efficient percentage recoveries and significant reproducibility obtained suggest the feasibility of utilizing the proposed method for the quantitation of dimenhydrinate.

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# Characteristics of Equilibrium Reaction of Zolazepam


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Received September 18, 1980, from **Analytical Research and ‡Product Development, Warner-Lambert Pharmaceutical Research Division, Warner-Lambert Company, Morris Plains, NJ 07950.* Accepted for publication November 17, 1980.



**Abstract** □ The equilibrium reaction of zolazepam, a pyrazolodiazepinone, was studied and analyzed using the approach used previously for other pyrazolodiazepinone derivatives. The intrinsic ring closure equilibrium constant for this reaction was ~100 times larger than that observed for pyrazolodiazepinones studied previously. This study illustrates that the diazepinone ring can dominate in equilibrium mixtures formed at pH values far below the pKa of the corresponding open form.



**Keyphrases** □ Zolazepam—analysis of equilibrium reaction □ Structure–activity relationships—equilibrium reaction of zolazepam □ Pyrazolodiazepinones—equilibrium reaction of zolazepam □ Equilibrium—analysis of zolazepam in reaction


The characteristics of the equilibrium reaction of certain pyrazolodiazepinones were reported previously (1). This study was an extension of that work and concerned the equilibrium behavior of zolazepam, 4-(*o*-fluorophen-


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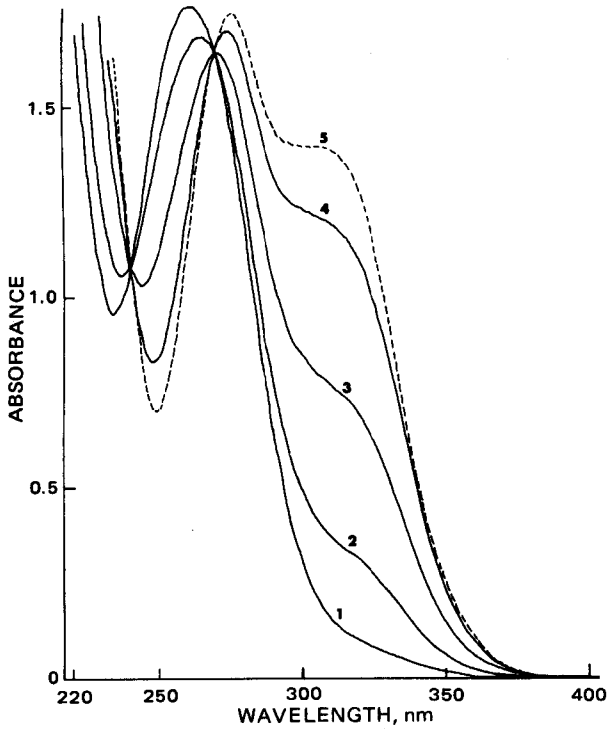



**Figure 1**—*Sequential spectra obtained for the equilibration of II at pH 3.70, 27°. Spectra 1, 2, 3, and 4 were obtained after 1, 7, 24, and 265 hr, respectively. Spectrum 4 corresponds to complete equilibration. Spectrum 5 is what would be obtained if conversion of II to I were complete.*

yl) - 6,8 - dihydro-1,3,8-trimethylpyrazolo[3,4-*e*][1,4]diazepine-7(1*H*)-one hydrochloride (I). While the nature of the reaction that occurred here was similar to that described previously, quantitative aspects differed.

## EXPERIMENTAL

**Reagents**—All chemicals used for the 0.1 and 0.05 *M* citrate and phosphate buffers of known pH values were reagent grade and were used without further purification. The hydrochloric acid solutions were prepared from prestandardized volumetric solutions[1]. The solvents, including hydrochloric acid solutions and buffers of varying pH used for kinetic studies, were adjusted to an ionic strength of 0.3 with potassium chloride. 5-(*N*-Methylaminoacetamido)-1,3-dimethyl-4-*o*-fluorobenzoylpyrazole sulfate (IIc) was used as received[2].

**Instrumentation**—The pH measurements were made using direct reading, digital pH meters[3]. UV spectra were obtained using a recording UV spectrophotometer[4].

**Equilibrium Studies**—Approximately 0.05 mEq of IIc was weighed accurately and transferred quantitatively to a 25-ml volumetric flask, using 1 ml of water to dissolve the compound. The reaction was initiated by diluting to volume with thermally equilibrated (27°) buffer with an ionic strength of 0.3 (potassium chloride). After mixing, the volumetric flasks were placed immediately in a constant-temperature water bath (27°). At appropriate intervals, 2-ml aliquots were removed, diluted to 25 ml with 0.1 *N* HCl, and mixed.

The UV spectra of the resulting solutions were obtained immediately following their preparation over 400–230 nm, using 1-cm silica cells and 0.1 *N* HCl as a blank. Samples were removed, and their spectra were obtained until redundancy was reached. The fraction of total II at equilibrium, $f_{II_T}$, was calculated by the formula: $(0.85A_i - A_{310})/0.781A_i$, where $A_i$ and $A_{310}$ are the absorbances at 271 (isosbestic point) and 310 nm, respectively. In this formula, $0.85A_i$ represents the theoretical absorbance at 310 nm for complete conversion from II to I, and $0.781A_i$ represents the expected absorbancy difference at 310 nm for complete conversion of II to I.

[1] Acculute, Anachemia Chemicals Ltd.
[2] Dr. H. Dewald, Chemistry Department, Parke-Davis/Warner-Lambert Co.
[3] Orion model 701.
[4] Cary model 11 or 14.

## RESULTS AND DISCUSSION

Typical sequential UV spectra obtained during equilibration are shown in Fig. 1. The starting material was the open form of I, 5-(*N*-methylaminoacetamido)-1,3-dimethyl-4-*o*-fluorobenzoylpyrazole (II). When II was the reactant, the spectra changed irrespective of the solution pH. When I was the starting material, changes in spectra with time were seen only at pH values of ≤6.0. As seen in Fig. 1, the spectrum of II changed to a spectrum closely resembling the spectrum of I. Isosbestic points at 240 and 271 nm were clear and sharp. At a given pH, the same redundant spectrum was produced irrespective of whether I or II was the starting material. The terminal spectra produced at pH values of ≤6.0 corresponded to the spectrum expected for mixtures of I and II containing up to 18% of II and 82% of I. The quantitative composition of equilibrium mixtures formed at various pH values is given in Table I.

The fraction of the open form ($f_{II_T}$) present at equilibrium for these systems is given by (1):

$$f_{II_T} = \frac{K_a^{IIb} + a_{H^+}}{K_a^{IIb} + a_{H^+} + K_a^{IIb}K^{*\prime}(K_a^{Ib} + a_{H^+})} = \frac{(II_T)}{(I_T) + (II_T)} \quad \text{(Eq. 1)}$$

where $I_T = (Ia) + (Ib)$, $II_T = (IIa) + (IIb)$, and:

$$K_a^{Ib} = \frac{(Ia)(H^+)}{(Ib)} \quad \text{(Eq. 2)}$$

$$K_a^{IIb} = \frac{(IIa)(H^+)}{(IIb)} \quad \text{(Eq. 3)}$$

In Eq. 1, $f_{II_T}$, $K_a^{IIb}$, and $K_a^{Ib}$ are experimentally determined known quantities (Tables I and II). Equation 1 can be rearranged so that the intrinsic ring closure constant $K^{*\prime}$ is in terms of these known quantities:

$$K^{*\prime} = \frac{(K_a^{IIb} + a_{H^+})(1 - f_{II_T})}{K_a^{IIb}(K_a^{Ib} + a_{H^+})f_{II_T}} = \frac{(Ib)}{(IIa)(H^+)} \quad \text{(Eq. 4)}$$

The value of $K^{*\prime}$ for this system was determined using Eq. 4 together with the values of $f_{II_T}$ given in Table I. The resulting values of $K^{*\prime}$ are given in Table III. As seen, the intrinsic ring closure constant for this system was ~100 times greater than it was for the ripazepam system studied previously (1).

When the values of $K^{*\prime}$, $K_a^{Ib}$, and $K_a^{IIb}$ given in Tables II and III are substituted in Eq. 1 and the values of $f_{II_T}$ are generated, the solid line shown in Fig. 2 is obtained. The calculated values correlate well with those determined by spectroscopic analysis of the equilibrium mixtures.

**Table I—Composition of Equilibrium Mixtures of I and II in Terms of $f_{II_T}$**

| pH | $f_{II_T}$[a] |
|---|---|
| 3.70 | 0.18 |
| 4.26 | 0.15 |
| 4.83 | 0.09 |
| 5.35 | 0.04 |
| 6.04 | 0.01 |

[a] These values were determined by analysis of the redundant, terminal UV spectrum obtained when equilibration was complete.

**Table II—Dissociation Constants**

| Compound | pKa | $K_a$, *M* |
|---|---|---|
| Ib[a] | 4.69 | $2.04 \times 10^{-5}$ |
| IIb[b] | 7.52 | $3.02 \times 10^{-8}$ |

[a] Determined spectrophotometrically. [b] Determined titrimetrically.

**Table III—Calculation of $K^{*\prime}$ Using Eq. 4**

| pH | $K^{*\prime}$, $M^{-1}$ |
|---|---|
| 3.70 | $1.70 \times 10^8$ |
| 4.26 | $1.48 \times 10^8$ |
| 4.83 | $1.13 \times 10^8$ |
| 5.35 | $1.14 \times 10^8$ |
| 6.04 | $1.45 \times 10^8$ |
| Average | $1.38 \times 10^8$ |

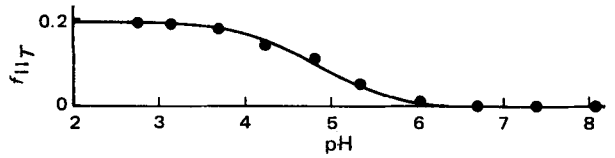


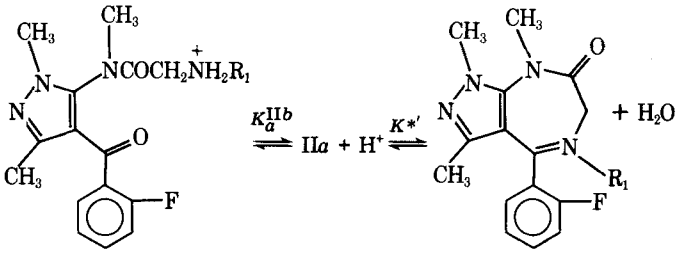

**Figure 2**—*Plot of* $f_{II_T}$ versus *pH*.

As indicated previously, at pH values below the pKa values of open and closed forms, Eq. 1 reaches a limit:

$$\lim f_{II_T}^{a\,H^+\to\infty} = \frac{1}{1 + K^{*\prime}K_a^{IIb}} \qquad \text{(Eq. 5)}$$

As seen from Eq. 5, the maximum concentration of the open form (II) that can exist in these systems depends not only on the pKa of the open form ($K_a^{IIb}$) but also on the value of the ring closure constant ($K^{*\prime}$). If, as is the case here, the value of $K^{*\prime}$ is very large ($1.38 \times 10^8\ M^{-1}$), then the maximum concentration of the open form at equilibrium is small irrespective of the solution's pH. The ring form then dominates. This situation is best understood by considering the reaction shown in Scheme I. An increase in hydrogen-ion activity serves not only to convert II to its unreactive protonated form but also to promote dehydration.

The marked increase in the value of the ring closure constant observed here may be attributed to a methyl group in position 4 of the diazepinone ring (1). Also important is the electronic distribution around the imino carbon atom (position 8). Zolazepam is a stronger base than ripazepam by a factor of 10 (1). This finding suggests that electron withdrawal from this carbon is less here than in the previous system. Attack by water would be hindered, and the ring form would be stabilized.




IIa: $R_1$ absent, no charge
IIb: $R_1$ = H, charge +1
IIc: IIa · $H_2SO_4$

Ia: $R_1$ absent, no charge
Ib: $R_1$ = H, charge +1

*Scheme I*

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# Improved Assay for Mixtures of Citrate and Citric Acid in Systemic Alkalizer Solutions

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Received May 30, 1980, from the *College of Pharmacy, University of Cincinnati–Medical Center, Cincinnati, OH 45267.* Accepted for publication November 4, 1980.

**Abstract** □ A modification of the USP method for the assay of systemic alkalizer solutions containing mixtures of citrate and citric acid is presented; it involves two titrations and ion-exchange chromatography. A diluted sample is titrated with 0.02 *N* NaOH to find the free citric acid content. The eluate from cation-exchange chromatography of an equal volume of diluted sample is titrated with 0.02 *N* NaOH to assay for total citric acid. Subtracting the results of the first titration from the second provides the citrate content. Synthetic mixtures of potassium citrate–citric acid, potassium citrate–sodium citrate–citric acid, and sodium citrate–citric acid were prepared and assayed. The method was applied to commercially available preparations. The proposed method eliminates some significant errors of the compendial method, and the accuracy and reproducibility are equal or better than those obtained with the compendial method. Theoretically, the result obtained by the compendial method does not necessarily give the citrate content.

**Keyphrases** □ Citrate–citric acid mixtures—improved assay of systemic alkalizer solutions □ Citric acid–citrate mixtures—improved assay of systemic alkalizer solutions □ Alkalizer—improved assay of citrate–citric acid mixtures

The compendial method for the assay of mixtures of citrate and citric acid solutions is a combination of an electrometric and visual titration (1). Direct application of this compendial method for the assay of citrate and citric acid to recently available commercial systemic alkalizer solutions such as potassium citrate–citric acid oral solution (I), potassium citrate–sodium citrate–citric acid oral solution (II), and sodium citrate–citric acid oral solution (III) is not practical since the preparations are generally colored and are assayed in undiluted form. Moreover, the titrants used are 1 *N* in strength. Since these difficulties allow for many sources of error, a more appropriate assay is desired for the quality control of these commercial preparations.

Alkali salts of organic acids have been analyzed by passing an aqueous solution of the salt through an ion-exchange column followed by titration of the eluate with a base (2, 3). The ion-exchange principle is included in the analysis of commercial systemic alkalizer solutions containing mixtures of citrate and citric acid. The method is simple, and the reproducibility and accuracy are equal or better than those obtained with the USP method.

## EXPERIMENTAL

**Reagents and Chemicals**—Citric acid monohydrate[1], potassium citrate monohydrate[1], sodium citrate dihydrate[1], a sulfonated polystyrene copolymer strong cation-exchange resin in the hydrogen form[2] (medium porosity), a 0.02 *N* NaOH volumetric solution obtained by quantitative dilution of 1 *N* NaOH (4), and phenolphthalein indicator solution were used. All other chemicals were reagent grade and were used without purification.

[1] Fisher Scientific Co., Fair Lawn, N.J.
[2] Rexyn 101 (H), Fisher Scientific Co., Fair Lawn, N.J.

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