## pKa Determination of Methaqualone

**Keyphrases** □ Methaqualone—spectrophotometric pKa determination □ pKa—spectrophotometric determination, methaqualone

*To the Editor:*

In a recent publication, Chatten *et al.* (1) reported the pKa value of methaqualone to be 3.56. The method used in their work was a potentiometric titration of the drug in a varying composition of acetone–water solvent systems. Methaqualone at three different molar concentrations was titrated to the half-neutralization point with standardized hydrochloric acid, and the pH was measured. The extrapolated pH values at infinite dilution of acetone were plotted against the molar concentration of the drug, and the extrapolated pH value of 3.56 from such a plot was reported as a pKa of methaqualone.

The pKa value thus obtained is questionable since the use of mixed solvents can produce anomalies in the pKa determination (2). There are two major complicating factors in the use of mixed solvents. First, the different solvating power of the two components creates new complexities; and second, the organic component contributes extra acidic and basic species to the solution. Although reproducible pH readings may be obtained in many mixed solvent systems, the pH meters are calibrated in aqueous buffers and pH values obtained under such conditions can be devoid of quantitative significance (3).

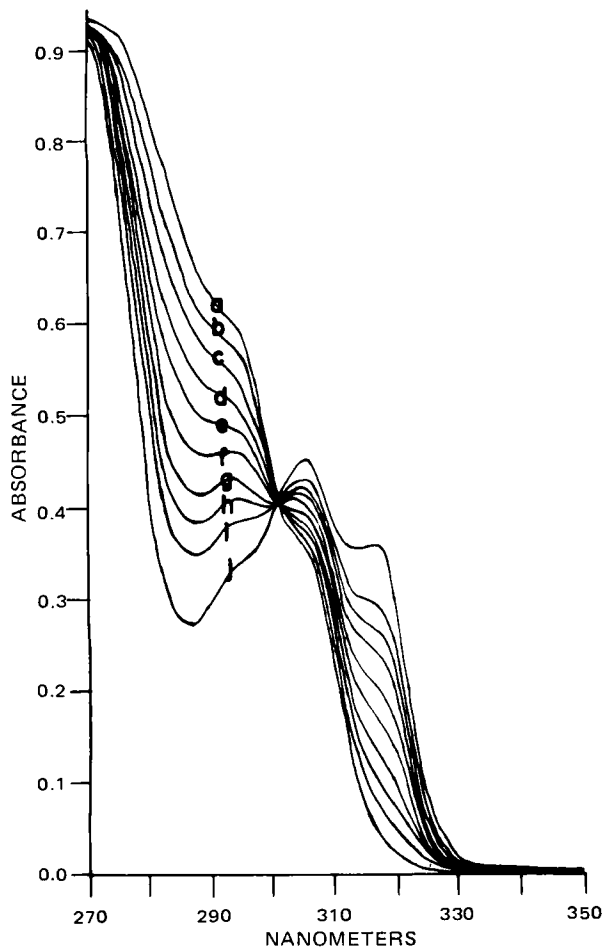


**Figure 1**—*Spectral changes of methaqualone in acidic media. Key: a, pH 1.05; b, pH 1.74; c, pH 1.96; d, pH 2.19; e, pH 2.37; f, pH 2.59; g, pH 2.79; h, pH 2.96; i, pH 3.17; and j, pH 4.56.*

**Table I—Determination of pKa of Methaqualone by Spectrophotometric Method**

| pH | Absorbance (λ 286 nm) | pKa | Absorbance (λ 316 nm) | pKa |
|---|---|---|---|---|
| 1.05 | 0.707 | — | 0.048 | — |
| 1.74 | 0.650 | 2.57 | 0.090 | 2.55 |
| 1.96 | 0.610 | 2.51 | 0.110 | 2.56 |
| 2.19 | 0.563 | 2.50 | 0.142 | 2.56 |
| 2.37 | 0.523 | 2.52 | 0.175 | 2.51 |
| 2.59 | 0.472 | 2.53 | 0.214 | 2.53 |
| 2.79 | 0.423 | 2.53 | 0.249 | 2.53 |
| 2.96 | 0.388 | 2.54 | 0.272 | 2.55 |
| 3.17 | 0.349 | 2.54 | 0.298 | 2.56 |
| 4.56 | 0.266 | — | 0.360 | — |
| | Arithmetic mean | 2.53 | Arithmetic mean | 2.54 |

For a sparingly soluble substance such as methaqualone, a UV spectrophotometric method can be used to determine the pKa value with no difficulty. In this laboratory, a spectrophotometric method was applied to measure pKa at two independent analytical wavelengths, λ 286 and 316 nm. The solution of methaqualone ($1.24 \times 10^{-4}$ *M*) was prepared in 0.1 *N* HCl to which 10 ml of 2 *M* KCl and 10 ml of 0.2 *M* $Na_2HPO_4$ were added to give a total volume of 500 ml with an ionic strength of 0.14.

Ten solutions with pH values ranging from 1.05 to 4.56 were prepared by adding a few drops of 5 *M* NaOH. The pH of each solution was measured using a pH meter[1] fitted with a glass–calomel electrode prior to absorbance reading[2]. The blank was the solvent without the sample. The spectra of 10 solutions thus obtained are shown in Fig. 1, with a well-defined isosbestic point at 301 nm. The pKa was calculated at λ 316 nm using:

$$\text{pKa} = \text{pH} + \log \frac{d_m - d}{d - d_i} \qquad \text{(Eq. 1)}$$

where $d_m$ = absorbance of the unionized species, $d$ = absorbance of the solution tested, and $d_i$ = absorbance of the ionized species.

Table I shows the data obtained from the spectral analysis of methaqualone in different pH media and the pKa value calculated from two analytical wavelengths. The protolytic constant thus determined is in disagreement with the reported value of 3.56. In our opinion, the potentiometric method for the pKa determination of methaqualone using a mixed solvent system is unreliable for reasons already discussed. Our reported pKa value is in agreement with that published previously using a spectrophotometric

[1] Brinkmann pH meter 102.
[2] Cary 14 recording spectrophotometer.

method (pKa = 2.54) at the analytical wavelength, λ 316 nm (4). At the time of preparation of this manuscript, it was found[3] that the reported pKa value of methaqualone in the Schulman *et al.* (5) study was 2.59 ± 0.006 by absorptiometric and fluorometric pH titration. This result is additional evidence of the erratic pKa value reported by Chatten *et al.* (1).

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(5) S. G. Schulman, J. M. Rutledge, and G. Torosian, *Anal. Chim. Acta*, **68**, 455(1974).

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[3] J. M. Rutledge, personal communication.

## Application of Girard Reagent T to Radioimmunological Assay of Prednisolone in Plasma

**Keyphrases** □ Prednisolone—separation from hydrocortisone with Girard reagent T, applicability to radioimmunological determination □ Hydrocortisone—reaction with Girard reagent T, separation from prednisolone, plasma □ Girard reagent T—reaction with hydrocortisone, separation from prednisolone, plasma

*To the Editor:*

Due to the cross-reactivity between endogenous hydrocortisone and prednisolone, Sullivan *et al.* (1) suppressed the production of interfering endogenous hormone by pretreating the volunteers with dexamethasone in their recent bioavailability trial of this drug. In our study, we separated prednisolone from hydrocortisone by selectively forming the water-soluble derivative of the latter and extracting the prednisolone into the organic phase.

Lederer (2) reported that the rates of reaction of a number of steroid ketones with water-soluble Girard reagent T in an acetic acid–methanol mixture established the order of keto group reactivity to be $\Delta^4$-3 ≫ 20 ≫ 11. It seems reasonable to expect that the reactivity of the $\Delta^{1,4}$-3 ketone function of prednisolone is much less than that of the $\Delta^4$-3 ketone group of hydrocortisone on the basis of resonance and steric considerations. Thus, a water-soluble hydrazone derivative of hydrocortisone can be selectively formed in the presence of prednisolone.

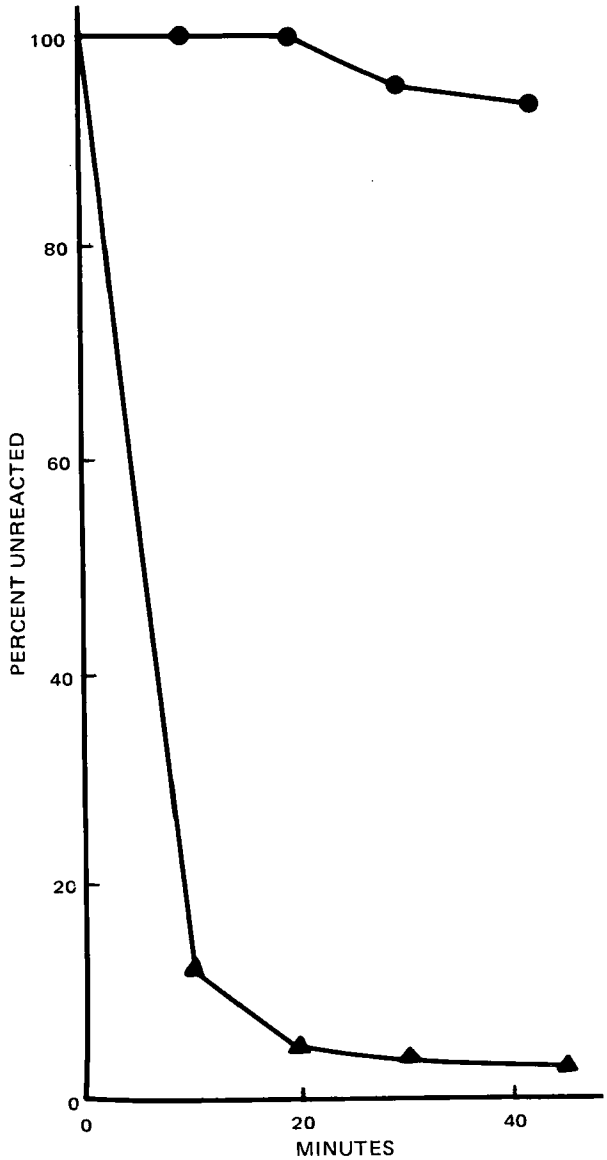


**Figure 1**—*Separation of prednisolone and hydrocortisone in plasma using Girard reagent T. Key:* ●, *prednisolone; and* ▲, *hydrocortisone.*

In our experiment, 1.0 ml of plasma was spiked with either 100,000 dpm of prednisolone (6,7-$^3$H)[1] plus 5 ng of cold carrier or 50,000 dpm of hydrocortisone (1,2-$^3$H)[2] plus 10 ng of cold carrier. Following the extraction of the drug into 5 ml of methylene chloride–ether (40:60), the dried residue was reacted with 50 μl of 10% Girard reagent T [dissolved in acetic acid–methanol (1:10)]. The reaction was stopped at 10, 20, 30, and 45 min by the addition of 0.5 ml of pH 8 phosphate buffer. The aqueous phase was extracted with 5 ml of methylene chloride–ether (40:60), the organic phase was transferred to a scintillation vial, and the solvent was evaporated under vacu-

[1] Specific activity = 40 Ci/mmole.
[2] Specific activity = 35 Ci/mmole.