Preformulation Studies of Spironolactone: Effect of pH, Two Buffer Species, Ionic Strength, and Temperature on Stability

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Abstract □ Using a stability-indicating HPLC assay method, the effect of pH, two buffer species (citrate and phosphate), ionic strength, and temperature on the stability of spironolactone in 20% solution of ethyl alcohol in water has been studied. The optimum pH of stability appears to be ~4.5. On increasing the buffer concentration, both species hastened the decomposition of spironolactone. The ionic strength did not affect the stability of the drug. The energy of activation has been estimated to be ~78.8 kJ/mol at pH 4.3. The un-ionized spironolactone is subject to general acid-base catalysis. The K_h and K_{oh} values at 40 °C have been estimated to be 1.63 and 2.8×10^5 day^{-1}, respectively. The HPO_4^{-2} ion had ~10 times more catalytic effect than the $H_2PO_4^{-1}$ ion. This data will be used to develop a stable oral liquid dosage form of the drug.

Spironolactone is extensively used in medicine as a diuretic. It is often prescribed to small children, but it is difficult for small children to swallow tablets, the only dosage form available for oral use. A number of methods[1–4] have been reported for compounding oral liquid dosage forms from the tablets. No systematic study has been reported to determine the physicochemical properties of spironolactone, and to compound a stable oral liquid dosage form of spironolactone using the powder rather than the commercially available tablets.

The purpose of this investigation was to study the effect of pH, phosphate and citrate buffers, ionic strength, and temperature on the stability of spironolactone using a stability-indicating HPLC assay method. These preformulation studies were conducted to help develop an oral liquid dosage form of spironolactone which is not currently available.

Experimental Section

Chemicals and Reagents—All the chemicals and reagents were either USP-NF or ACS grade and used without further purification. Spironolactone powder was generously supplied by G. D. Searle & Company, Skokie, IL, and used as received.

High-Performance Liquid Chromatography—A Waters ALC 202 HPLC system (Waters Associates, Milford, MA) equipped with a multiple wavelength detector (Schoeffel's SF 770) and a recorder (Omniscribe 5213-12, Houston Instruments, Austin, TX) was used. A μBondapak phenyl column (30 cm × 3.9 mm i.d.) was the stationary phase. The mobile phase contained 39% (v/v) of acetonitrile in 0.01 M KH_2PO_4 aqueous buffer. The flow rate was 2.2 mL/min, the detector was set at 254 nm (0.04 AUFS), the chart speed was 30.5 cm/h, and the temperature was ambient.

Preparation of Stock and Standard Solutions—The stock solutions of spironolactone (1.25 mg/mL) and the internal standard, methyltestosterone (0.25 mg/mL) in methanol were prepared fresh every week. These solutions were diluted further with 25% (v/v) aqueous methanol containing 0.01 M KH_2PO_4 as needed. The most commonly used solution (the standard solution) contained 80 μg/mL of spironolactone and 25 μg/mL of methyltestosterone (internal standard).

Assay Solutions—All the assay solutions were diluted with an aqueous buffer solution containing 0.01M KH_2PO_4 and 25% methanol (v/v) so that the final concentration of spironolactone was 80 μg/mL (based on the label claim). The concentration of the internal standard in each solution was 25 μg/mL.

Preparation of Solutions for Stability Studies—All the solutions for stability studies were prepared by diluting a stock solution of spironolactone in ethyl alcohol (1.25 mg/mL) with an aqueous buffer solution. The final solutions contained 0.25 mg/mL of spironolactone and 20% (v/v) ethanol. All the solutions prepared are listed in Table I. After the zero-day data (assays, physical appearance, and pH values), the solutions were stored at appropriate temperatures (40, 50, and 60 °C) in amber-colored glass bottles (4-oz dispensing bottles, Brockway Glass Company, Brockway, PA). The data were recorded again at the appropriate intervals (between 2 to 156 days).

Results and Discussion

Assay Method—The HPLC assay method is stability indicating, since canrenone (the product of decomposition of spironolactone) eluted before spironolactone. The method is accurate and precise with a percent relative standard deviation of 0.9 based on five readings. The complete details of the developed method are being reported separately.[5]

Effect of pH on the Stability of Spironolactone—The decomposition of spironolactone at all pH values followed first-order law (Figure 1). The pH–rate profile curve (Figure 2) indicates that the optimum pH value of stability for spironolactone is ~4.5. For Figures 1 and 2, the data of solutions 1–7 (Table I) at 40 °C were used. Solutions 8 and 9

Table I—Solutions of Spironolactone (0.25 mg/mL) Prepared in 20% Aqueous Ethanol for Stability Studies

Solution No.	pH (± 0.05)	Buffering Agent (M)	Ionic Strength[a]
1	2.3	Phosphate (0.05)	0.20
2	3.4	Phosphate (0.05)	0.20
3	4.5	Phosphate (0.05)	0.20
4	5.2	Phosphate (0.05)	0.20
5	6.2	Phosphate (0.05)	0.20
6	7.3	Phosphate (0.05)	0.20
7	8.3	Phosphate (0.05)	0.20
8	9.2	Phosphate (0.05)	0.20
9	9.7	Phosphate (0.05)	0.20
10	4.3	Phosphate:citrate[b] (0.14)	0.29
11	4.3	Phosphate:citrate[b] (0.14)	0.38
12	4.3	Phosphate:citrate[b] (0.14)	0.63
13	4.3	Phosphate:citrate[b] (0.14)	0.88
14	4.3	Phosphate:citrate[b] (0.14)	1.13
15	5.3	Phosphate:citrate[b] (0.14)	0.67
16	4.3	Citrate (0.05)	0.30
17	4.3	Citrate (0.10)	0.30
18	4.3	Citrate (0.20)	0.30
19	4.3	Phosphate (0.05)	0.30
20	4.3	Phosphate (0.10)	0.30
21	4.3	Phosphate (0.20)	0.30

[a] Adjusted with KCl based on the pH of the buffer solution. [b] Prepared according to the directions in ref 6.

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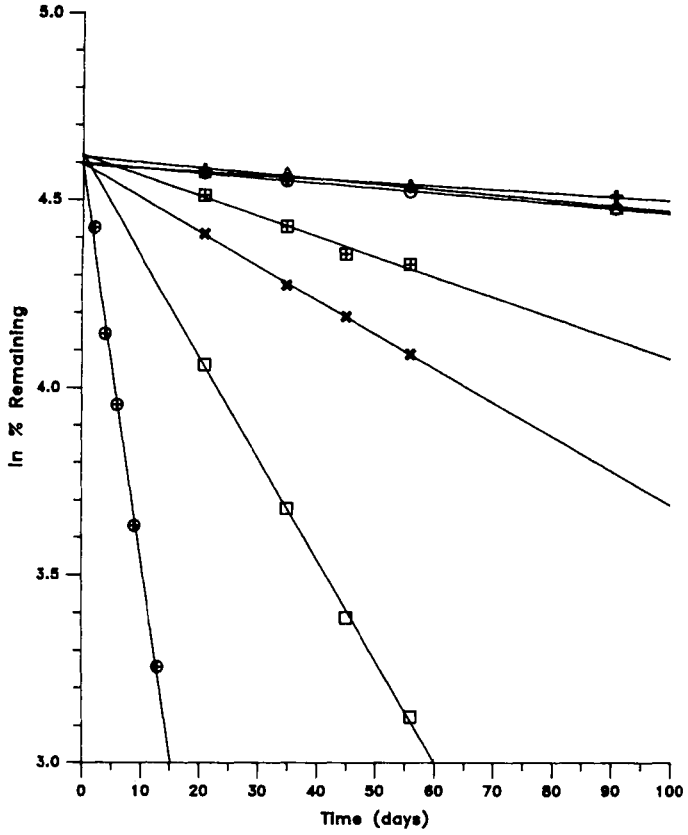

Figure 1—Effect of pH on the stability of spironolactone at 40 °C (solutions 1–7, Table I). Key: (X) pH 2.3; (△) pH 3.4; (+) pH 4.5; (○) pH 5.2; (⊞) pH 6.2; (□) pH 7.3; (⊕) pH 8.3.

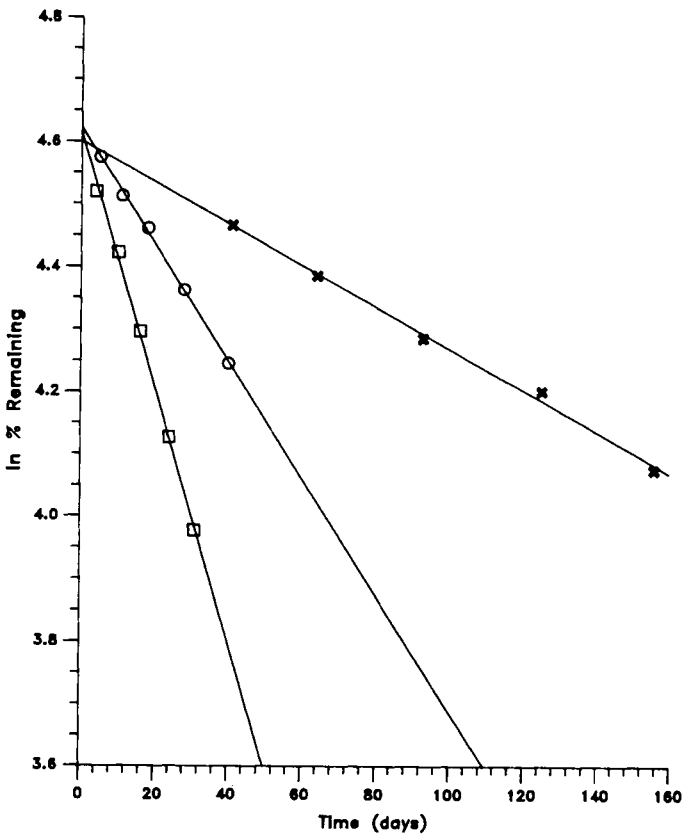

Figure 3—First-order plots of spironolactone at higher temperatures (solution 12, Table I). Key: (X) 40 °C; (○) 50 °C; (□) 60 °C.

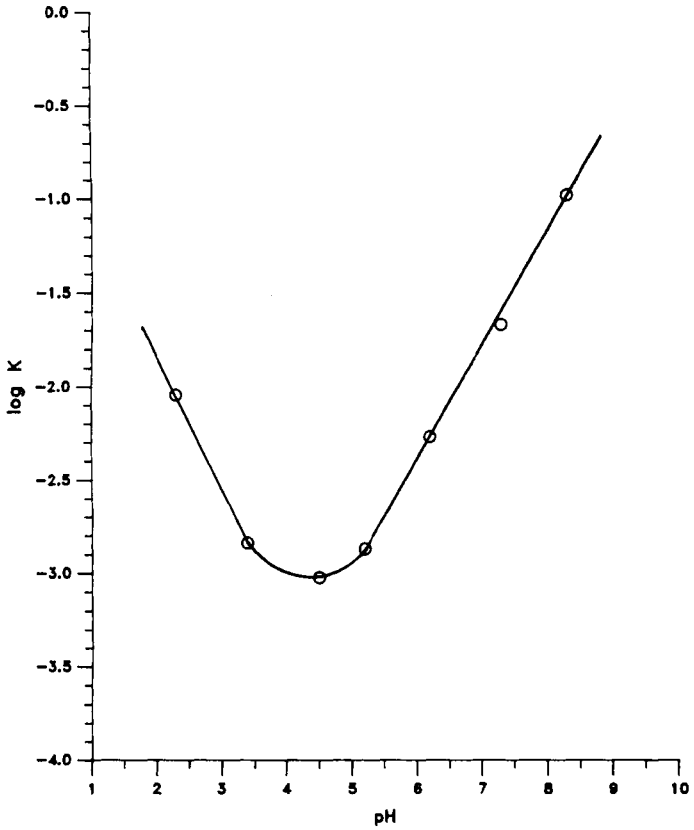

Figure 2—pH–rate profile curve of spironolactone at 40 °C (solutions 1–7, Table I).

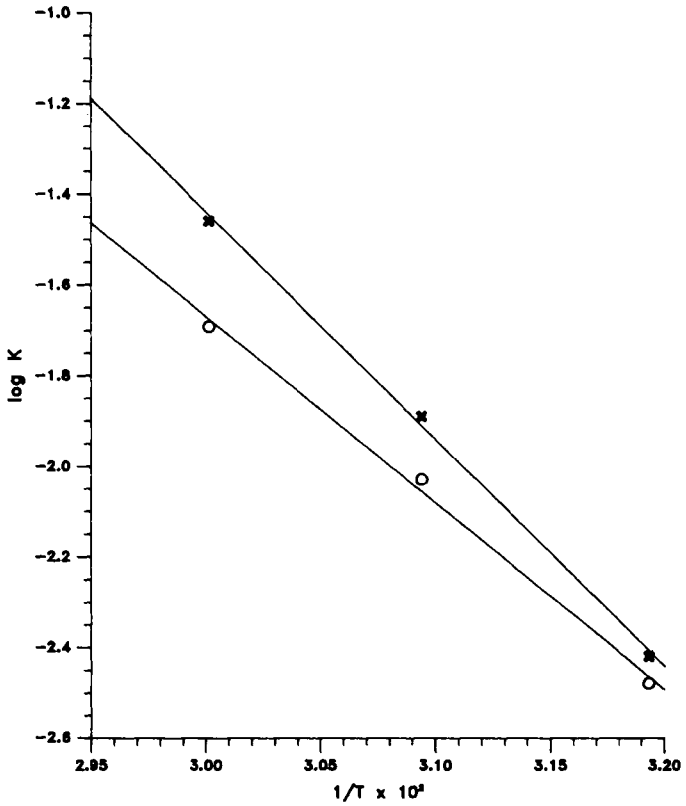

Figure 4—Arrhenius plots for spironolactone at (○) pH 4.3 and (X) 5.3 (solutions 12 and 15, Table I).

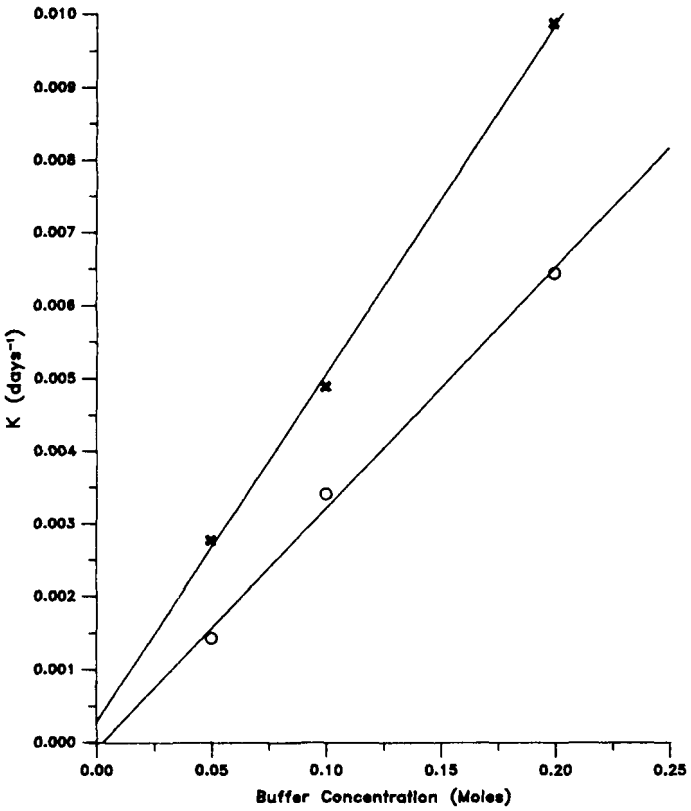

Figure 5—Effect of buffer species on the degradation constant (k) of spironolactone at pH 4.3 and 50 °C (solutions 16–21, Table I). Key: (X) citrate; (○) phosphate.

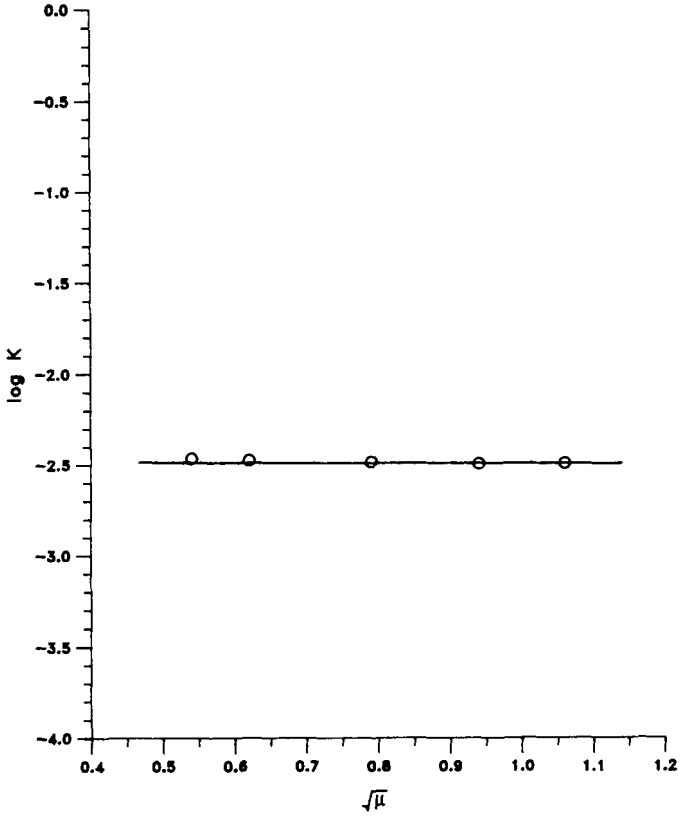

Figure 6—Effect of ionic strength on the stability of spironolactone at pH 4.3 and 40 °C (solutions 10–14, Table I).

(Table I) decomposed rapidly; therefore, they were not studied further.

Effect of Temperature—At all the higher temperatures (40, 50, and 60 °C), the decomposition followed first-order law (Figure 3) and, from the Arrhenius plot (Figure 4), the energies of activation were estimated to be 78.8 and 96.0 kJ/mol at pH 4.3 and 5.3, respectively.

Effect of Buffer Concentrations, Spironolactone Concentration, and Ionic Strength—Both phosphate and citrate buffers hastened the decomposition (Figure 5) of spironolactone with increase in the buffer concentration. Phosphate proved to be only slightly better than the citrate buffer. The slope of the phosphate curve was 0.0333 versus 0.0477 for the citrate curve. The ionic strength did not affect the decomposition constant (Figure 6). The first-order decomposition did not depend on the initial concentration of spironolactone. Since the ionic strength did not affect the K value of spironolactone, it can be assumed that the un-ionized spironolactone reacted with H^+ or OH^- ions. Therefore, the hydrolysis of spironolactone in phosphate buffer may be represented as follows:

$$K_{obs}=K_O+K_h(H^+)+K_{oh}(OH^-)+K_{H_2PO_4}(H_2PO_4^-)+$$

$$K_{HPO_4}(HPO_4^{2-}) \quad (1)$$

Preliminary investigations indicated that the phosphate buffer effect was predominantly due to HPO_4^{2-}. At pH 2.3, the effect of phosphate buffer and the OH^- ion may be neglected since the concentrations of both these ions at pH 2.3 will be very small. Therefore, eq 1 can be rearranged as follows:

$$K_{obs} = K_O + K_h(H^+) \quad (2)$$

At pH 4.5, the effect of pH and the buffer is at its minimum (Figure 2), therefore, the decomposition is predominantly catalyzed by the solvent. Therefore, the K_{obs} values at pH 4.5 (0.00095 day^{-1}) is K_O. Using the K_{obs} value at pH 2.3 (40 °C) of 0.0091 day^{-1}, and substituting 0.00095 day^{-1} for K_O in eq 2, the K_h value was estimated to be 1.63 day^{-1} at 40 °C. On modifying eq 2 by adding K_{oh} (OH^-) and again neglecting the effect of HPO_4^{2-} (since it will be only 1/11 of the total buffer concentration at pH 6.2), the K_{oh} at 40 °C was estimated to be 2.8×10^5 day^{-1} when a K_{obs} value of 0.00542 day^{-1} at pH 6.2 was used. The hydrolysis of spironolactone was predominantly subject to OH^- since the value of K_{oh} is $\sim 1.72 \times 10^5$ times more than that of K_h. These preformulation studies will help to develop a stable (for at least 18 months) oral liquid dosage form of spironolactone.

References and Notes

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