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

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Abstract □ Using an HPLC method, the effect of pH, two buffer species (phosphate and citrate), ionic strength, and temperature on the stability of acetozolamide has been studied. The optimum pH of stability appears to be 4. The buffers and ionic strength did not affect the decomposition constant. There was a direct relationship between the activation energies and pH values, with an energy of activation (E_a) value of 16.61 kcal/mol at pH 4. The un-ionized acetazolamide is subject to specific acid–base catalysis. The K_H and K_{OH} values have been estimated to be 0.23 and 1.56 d^{-1}, respectively. These preformulation studies can be used to develop a stable oral liquid dosage form of acetazolamide.

Acetazolamide is extensively used in medicine as a diuretic. Despite its extensive use, very little information is available about the degradation of acetazolamide. The USP-NF method[1] for the quantitation of acetazolamide is based on UV spectroscopy, and therefore is not a stability-indicating assay procedure. A number of HPLC methods[2–7] have been reported to quantify acetazolamide in biological fluids. These methods were not applied to the quantitation of drug in the dosage forms, especially in the presence of its decomposition product, 5-amino-1,3,4-thiadiazole-2-sulfonamide. A stability-indicating high-performance liquid chromatography method for the quantitation of acetazolamide in pharmaceutical dosage forms has been reported by the authors.[8]

The purpose of this investigation was to study the effect of pH, phosphate and citrate buffer concentrations, ionic strength, and temperature on the stability of acetazolamide using the stability-indicating HPLC method. These preformulation studies were needed in order to develop a stable oral liquid dosage form of acetazolamide 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. Acetazolamide powder was used as received from Lederle Laboratories.

High-Performance Liquid Chromatography—A Waters ALC 202 HPLC system (Waters Associates, Milford, MA) equipped with a multiple wavelength detector (Spectroflow 770, Kratos, Ramsey, NJ) and a recorder (Omniscribe 5213-12, Houston Instruments, Austin, TX) was used. A μBondapak C18 column (30 cm × 3.9 mm i.d.) was the stationary phase. The mobile phase contained 12% methanol (v/v) and 2% acetonitrile (v/v) in a 0.02 M potassium phosphate monobasic solution in water. The flow rate was 2.0 mL/min, and the sensitivity was 0.04 AUFS at 265 nm. The chart speed was 30.5 cm/h and the temperature was ambient.

Preparation of Stock and Standard Solutions—The stock and standard solutions were prepared as described previously.[8] In the most commonly used standard solutions, the final concentrations of acetazolamide and sulfamerazine (the internal standard) were 20 and 15 μg/mL, respectively.

Preparation of Solutions for Stability Studies—All the solutions were prepared using a simple solution method and are presented in Table I. After the zero-day data (assays, physical appearances, and pH values), the solutions were stored at the appropriate temperatures in amber-colored glass bottles (dispensing bottles, Brockway Glass, Brockway, PA). The data were recorded again at the appropriate intervals.

Results and Discussion

Assay Method—The HPLC assay method is stability indicating[1] since the products of decomposition, acetic acid and 5-amino-1,3, 4-thiadiazole-2-sulfonamide, eluted with the solvent.[8]

Decomposition of Acetazolamide at Various pH Values—The decomposition of acetazolamide followed a first-order equation (Figure 1 and Table II). The optimum pH of stability was ~4 (Figure 2). Figure 2 was drawn by using the K_{obs} values at 25 ± 1 °C, which were determined using linear regression analysis.

Effect of Temperature—At higher temperatures (37, 50, 60, and 80 °C), the decomposition process also followed a first-order equation (Figure 3). From the higher temperature data at three different pH values, the energies of activation (E_a values) were determined using Arrhenius plots (Figure 4). The natural logarithm (ln) of the E_a values was directly related to pH values with the following equation:

$$\ln E_a = \ln K + 0.08286\text{pH} \quad (1)$$

where K is a constant with a value of 11.94 kcal/mol and 0.08286 is the slope of the straight line. Using the above

Table I—List of the Solutions of Acetazolamide (0.25 mg/mL) Prepared for Stability Studies

Solution No.	pH (± 0.05)	Buffering Agent (M)	Ionic Strength[a]
1	1.68	HCl (0.1)	0.3
2	3.20	Phosphate (0.1)	0.3
3	4.01	Phosphate (0.1)	0.3
4	4.98	Phosphate (0.1)	0.3
5	5.27	Phosphate (0.1)	0.3
6	5.46	Phosphate (0.1)	0.3
7	6.06	Phosphate (0.1)	0.3
8	6.86	Phosphate (0.1)	0.3
9	8.17	Phosphate (0.1)	0.3
10	7.50	Phosphate (0.05)	0.4
11	7.50	Phosphate (0.1)	0.4
12	7.50	Phosphate (0.15)	0.4
13	7.50	Phosphate (0.1)	0.6
14	7.50	Phosphate (0.1)	0.26
15	3.10	Phosphate (0.1)	0.3
16	5.85	Phosphate (0.1)	0.3
17	6.64	Phosphate (0.1)	0.3
18	6.25	Citrate (0.05)	0.28
19	6.25	Citrate (0.1)	0.56
20	6.25	Citrate (0.15)	0.84

[a] Adjusted with KCl based on the pH of the buffer solution.

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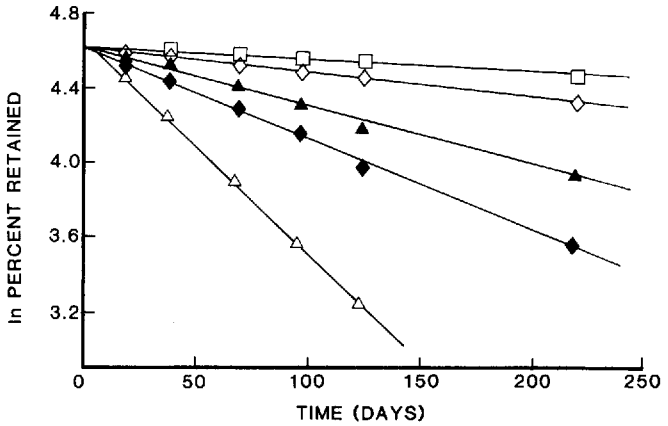

Figure 1—First-order plots of acetazolamide at different pH values. Key: (△) pH8.17; (◆) pH 1.68; (▲) pH 6.86; (◇) pH 6.06; (□) pH 5.46.

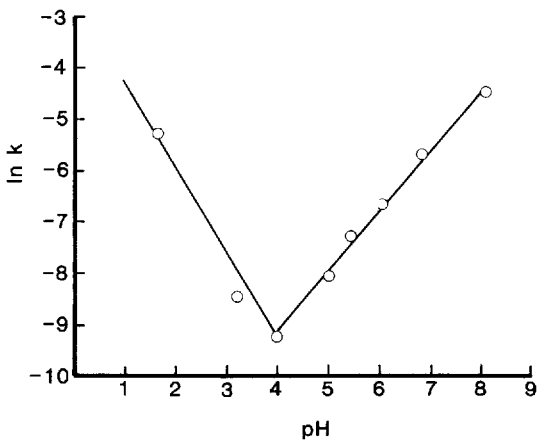

Figure 2—The pH–rate profile curve of acetazolamide from the data at 25 °C.

equation, the E_a value at pH 4 (pH value of maximum stability) was determined to be 16.61 kcal/mol.

Effect of Buffer Concentrations and the Ionic Strength—The phosphate buffer had very little effect on the K_{obs} value of

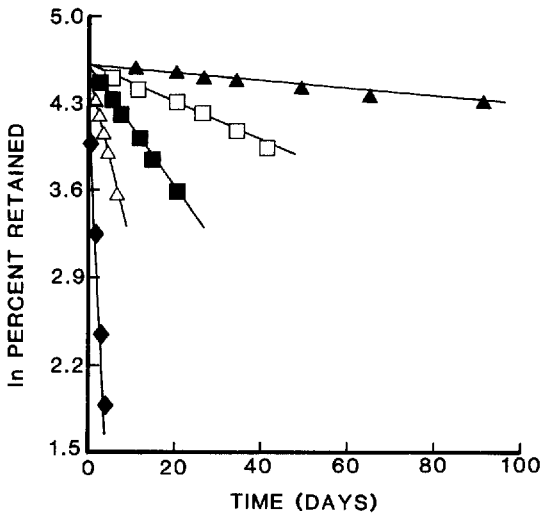

Figure 3—First-order plots of acetazolamide at higher temperatures (from solution 17, Table I). Key: (▲) 25 °C; (□) 37 °C; (■) 50 °C; (△)60 °C; (◆) 80 °C.

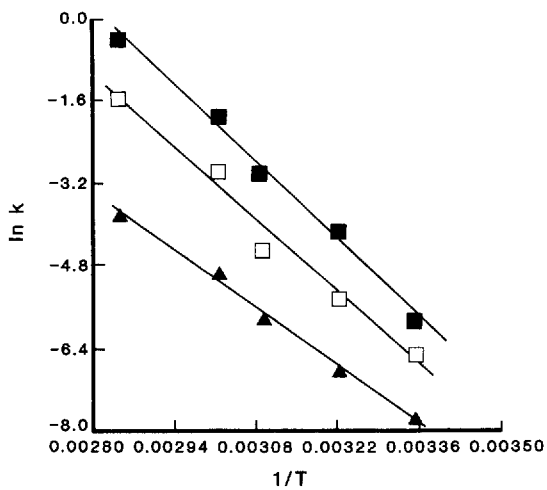

Figure 4—Arrhenius plots of acetazolamide. Key: (▲) pH 3.1; (□) pH 5.85; (■) pH 6.64.

Table II—Assay Results of Samples Stored at 25 ± 1 °C[a]

Solution No.	pH (± 0.05)	Percent Retained after Indicated Number of Days (% RSD)[b]						
		15 d	35 d	66 d	94 d	122 d	217 d	310 d
1	1.68	92.18 (2.02)	85.56 (1.17)	73.60 (0.87)	63.75 (0.85)	52.77 (1.70)	35.02 (0.45)	—
2	3.20	—	—	—	96.61 (0.45)	96.12 (0.34)	95.40 (1.17)	92.87 (0.65)
3	4.01	—	—	—	98.45 (0.28)	98.06 (0.50)	98.06 (0.64)	96.54 (0.96)
4	4.98	—	—	—	98.46 (1.10)	97.34 (1.06)	94.00 (2.78)	90.79 (1.91)
5	5.27	—	—	—	96.99 (1.62)	95.35 (2.86)	91.11 (1.77)	87.80 (1.78)
6	5.46	—	99.88 (0.69)	98.06 (0.36)	95.69 (1.37)	94.17 (1.10)	86.76 (1.16)	—
7	6.06	98.12 (0.67)	97.54 (0.14)	94.18 (1.66)	88.43 (1.82)	86.53 (2.18)	75.30 (3.49)	—
8	6.86	95.43 (0.36)	94.02 (2.93)	82.95 (2.20)	74.51 (2.05)	65.98 (2.18)	51.01 (0.46)	—
9	8.17	86.70 (0.60)	71.05 (0.99)	49.41 (0.44)	35.02 (0.57)	25.49 (0.71)	—	—

[a] All the solutions were clear throughout the study period; the pH values of solutions did not change. [b] Based on 100% at day zero; n = 6.

Table III—Assay Results of Samples with Varying Buffer Concentrations and Ionic Strengths at pH 7.5[a]

Ionic Strength	Phosphate Buffer Concentration, M	Results after Indicated Number of Days (% RSD)[b]				
		12 d	37 d	71 d	98 d	189 d
0.40	0.05	92.57	80.62	62.54	50.33	27.33
		(0.92)	(1.83)	(1.05)	(1.61)	(0.92)
0.40	0.10	91.67	79.89	60.50	48.94	25.07
		(0.34)	(1.67)	(0.93)	(0.89)	(1.07)
0.40	0.15	92.06	78.95	58.39	46.67	21.57
		(1.42)	(2.41)	(2.07)	(1.03)	(0.76)
0.60	0.10	91.58	77.70	59.42	48.12	24.31
		(0.64)	(2.13)	(1.58)	(2.32)	(2.01)
0.26	0.10	92.81	79.45	59.95	47.19	22.06
		(0.96)	(1.90)	(2.02)	(0.92)	(0.46)

[a] All the solutions were clear throughout the study period; the pH values of solutions did not change; the K_{obs} value at this pH was ~0.0071/d which did not change with change in the buffer concentration or the ionic strength. [b] Based on 100% at day zero; n = 6.

acetazolamide (Table III). The effect of citrate buffer was studied at pH 6.25 (solutions 18–20, Table I) with similar results. The K_{obs} values were similar with different ionic strengths (Table III). This indicated that it was the un-ionized acetazolamide which reacted with either H^+ or OH^-. Furthermore, the pH–rate profile curve (Figure 2) is also a typical plot of a specific acid–base catalysis. The slope was ~1.15 on the basic side of the pH (above 4) and −1.72 on the acidic side (below 4), with correlation coefficient values of 0.99 on both sides.

The hydrolysis of acetazolamide may be represented as follows:

$$K_{obs} = K_O + K_H(H^+) + K_{OH}(OH^-) \quad (2)$$

Assuming the K_O value (hydrolysis due to solvent) to be 0.0000999/d (K value at pH 4 where the hydrolysis is at its minimum), and neglecting the effect of OH^- at pH 1.68, the K_H value was estimated to be 0.23/d. Using the K_{obs} value of 0.0495/d at pH 1.68, the K_H value was estimated to be 0.23/d. Using the K_{obs} value of 0.0495/d at pH 12.5[8] and neglecting the effect of H^+, the K_{OH} value was estimated to be 1.56/d.

These preformulation studies will be useful in developing a stable (for at least 18 months) oral liquid dosage form of acetazolamide. A liquid dosage form is often required for small children and infants who cannot swallow tablets or capsules.

References and Notes

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