Stability of Batanopride Hydrochloride in Aqueous Solutions

MUNIR N. NASSAR[x], CHRIS A. HOUSE, AND SHREERAM N. AGHARKAR

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Abstract □ The degradation of batanopride hydrochloride, an investigational antiemetic drug, was studied in aqueous buffer solutions (pH 2–10; ionic strength, 0.5; 56 °C) in an attempt to improve drug stability for parenteral administration. Degradation occurs by two different mechanisms depending on the pH of the solution. In acidic media (pH 2–6), the predominant reaction was intramolecular cyclization followed by dehydration to form a 2,3-dimethylbenzofuran. There was no kinetic or analytical (high-performance liquid chromatography) evidence for the formation of an intermediate; therefore, the rate of dehydration must have been very rapid compared with the rate of cyclization. In alkaline media (pH 8–10), the primary route of degradation was cleavage of the C–O alkyl ether bond. In the intermediate pH range (pH 6–8), both reactions contributed to the overall degradation. Both degradation reactions followed apparent first-order kinetics. The pH–rate profile suggests that batanopride hydrochloride attains its optimal stability at pH 4.5–5.5. Citrate buffer was catalytic at pH 3 and 5, and phosphate buffer was catalytic at pH 8. No catalytic effect was observed for the borate buffer at pH 9–10.

Batanopride hydrochloride [4-amino-5-chloro-*N*-[2-(diethylamino)ethyl]-2-[(1-methylacetonyl)oxy]benzamide hydrochloride; **1**] is a synthetic substituted benzamide that is structurally related to metoclopramide.[1,2] Compound **1** is an investigational antiemetic agent intended for use in the suppression of chemotherapy-induced nausea and vomiting.[3] The kinetics and mechanism of the degradation of **1** in aqueous solution were investigated in an attempt to develop a stable parenteral dosage form. This paper describes the effects of pH, buffers, and ionic strength (μ) on the aqueous stability of **1** and the mechanism of its degradation at pH 2–10.

Experimental Section

Materials—Batanopride hydrochloride (**1**), 4-amino-5-chloro-*N*-[2-(diethylamino)ethyl]-2,3-dimethylbenzofuran carboxamide (**2**), and 4-amino-5-chloro-*N*-[2-(diethylamino)ethyl-2-hydroxy]benzamide (**3**) were obtained from Bristol-Myers Squibb Company and used as received. Acetonitrile, 1-octanesulfonic acid sodium salt, and propylparaben were HPLC grade. Water was obtained from a Milli-Q System (Millipore Corp., Bedford, MA). All other chemicals and solvents were analytical reagent grade.

Kinetic Studies—Solutions of **1** were prepared at a concentration of 0.5 mg of base/mL in various aqueous buffers (0.01 M; pH 2–10; μ adjusted to 0.5 with sodium chloride). The solutions were transferred into 5-mL, type I flint glass ampules (Wheaton Glass Company, Millville, NJ). The ampules were sealed and stored at 56 °C. Samples were periodically removed for analysis by high-performance liquid chromatography (HPLC) and pH measurements. One milliliter of the solution of **1** was withdrawn from each ampule and combined with 10.0 mL of a stock solution of the internal standard [propylparaben at 1 mg/mL in acetonitrile:water (30:70)] and diluted to 100 mL with acetonitrile:water (30:70) prior to analysis by HPLC.

Analysis by HPLC—The modular chromatographic system consisted of a Varian model 5500 pump, a Varian model 8085 autosampler, Rheodyne 20-μL loop injector, a Kratos model 783 Spectroflow UV detector operated at 280 nm, and a Vista 402 data-processing system. A Waters μ-Bondapak C_{18} column (30 cm × 3.9 mm) was used at a flow rate of 1.5 mL/min and at ambient temperature. The mobile phase was 30:70 acetonitrile:0.005 M 1-octanesulfonic acid sodium salt in water adjusted to pH 2.7 ± 0.05 with glacial acetic acid. Propylparaben was used as the internal standard. The concentration of **1** was determined by peak area analysis. The stability-indicating nature of this assay is depicted by the chromatograms (Figure 1) of samples of **1** (0.5 mg/mL) in pH 2.0 and 9.0 buffer solutions that were forcibly degraded at 56 °C for 35 and 7 days, respectively. The degradation peaks eluted separately and were detected without apparent interference with the peak of **1**. The homogeneity of the peak of **1** was examined by comparing the UV spectrum of the eluting peak, with an on-line photodiode array detector, with that of the reference standard. No differences between the spectra were observed, indicating the absence of any degradates and/or exogenous impurity eluting under the peak. The retention times of **1** and its degradation products in this HPLC system were ~8.8, ~6.3, and ~15.7 min, respectively.

Results and Discussion

Reaction Order and Observed Rate Constants—The degradation of **1** followed apparent first-order kinetics at constant

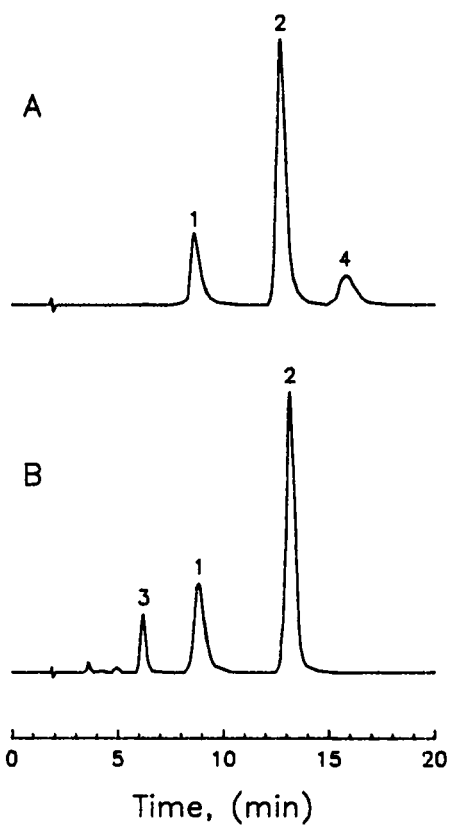

Figure 1—Chromatograms of **1** (0.5 mg/mL) in (**A**) pH 2.0 and (**B**) pH 9.0 solution when stored at 56 °C for 35 and 7 days, respectively. Key: (**1**) **1**; (**2**) internal standard; (**3**) phenolic degradation product **3**; (**4**) benzofuran **2**.

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pH, temperature, and μ at pH 2–10. This result is shown by the linearity of plots of the logarithm of concentration versus time at 56 °C (Figure 2). The buffer systems and the observed rate constants (k_{obs}) are given in Table I. The rate constants were calculated from the slopes of the semilogarithmic plots by statistical regression analysis. The regression lines were linear for all pH values (correlation coefficient, >0.99).

Effect of Buffer Concentration—The effects of buffer species and concentration on the aqueous stability of 1 were studied at pH 3.0 and 5.0 (citrate) and 8.0 (borate). For each buffer system, k_{obs} was determined experimentally as a function of buffer concentration (0.01, 0.05, and 0.1 M; Table II). Significant buffer catalysis was observed with citrate and phosphate buffers.

pH–Rate Profile—A pH–rate profile of 1 at zero buffer concentration was obtained by plotting the logarithm of k_{obs} versus the pH of solutions at constant μ of 0.5 and at 56 °C (Figure 3). Compound 1 had optimum stability at pH 4.5–5.5. In the pH range investigated, 1 existed in two forms, with the tertiary amino function being protonated or unprotonated. The pK_a (K_a is the ionization constant) of this amino group, measured potentiometrically, was 9.3. The shape of the pH–rate profile indicates that the free base and the protonated form are degraded at different rates and that degradation can be described in terms of specific-base-catalyzed reactions involving both species and a water- and acid-catalyzed reaction of the protonated compound (Scheme I). A general rate equation for the degradation of 1 as a function of pH can be written as follows:

$$k_{obs} = (k_H[H^+])\,[[H^+]/([H^+] + K_a)] + k_0\,[[H^+]/([H^+] + K_a)] + (k_{OH}[OH^-])\,([H^+]/([H^+] + K_a)] + (k'_{OH}[OH^-])\,[K_a/([H^+] + K_a)] \quad (1)$$

In eq 1, $[H^+]/([H^+] + K_a)$ and $K_a/([H^+] + K_a)$ are fractions of total compound in the protonated and free base forms, respectively, and K_a is the apparent ionization constant of the protonated tertiary amino group. The first-order rate constant k_0 refers to the water-catalyzed degradation of the protonated form, k_H is the second-order rate constant for the specific-acid catalysis of the protonated compound, and k'_{OH} is the second-order rate constant for hydroxide ion-catalyzed degradation of the unprotonated species. The second-order rate constant k_{OH} describes the specific-base-catalyzed reaction of the protonated drug or its kinetic equivalent, the uncatalyzed (water) reaction of the neutral drug.

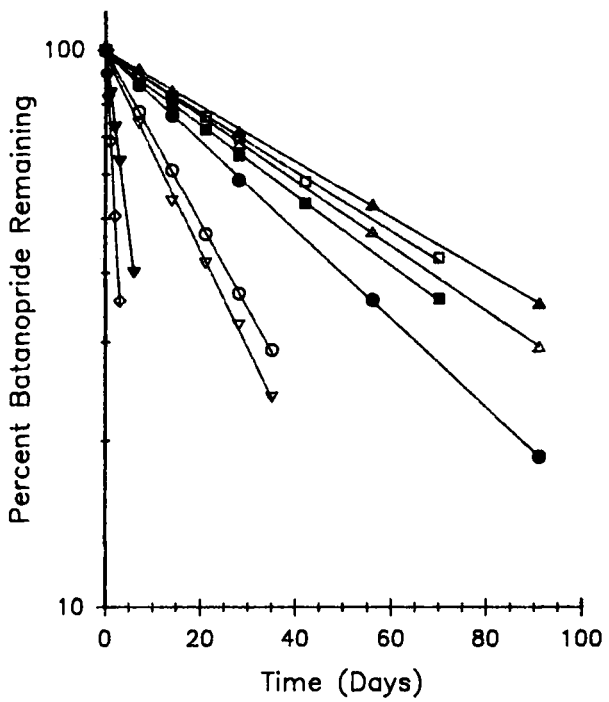


Figure 2—Apparent first-order plots for the degradation of 1 at various pH values (56 °C; μ = 0.5, NaCl). Key: (○) pH 2.0; (●) pH 3.0; (△) pH 4.0; (▲) pH 5.0; (□) pH 6.0; (■) pH 7.0; (▽) pH 8.0; (▼) pH 9.0; (◇) pH 10.0.

Table I—k_{obs} Values of Degradation of 1[a]

Buffer[b]	pH	$k_{obs} \times 10^4$, h^{-1}
Phosphate	2.0	14.70
Citrate	3.0	7.59
Citrate	4.0	5.56
Citrate	5.0	4.74
Phosphate	6.0	4.97
Phosphate	7.0	5.87
Phosphate	8.0	16.50
Borate	9.0	105.63
Borate	10.0	140.52

[a] Determined at 56 °C (μ = 0.5, NaCl). [b] Concentration of buffers, 0.1 M.

Table II—Effects of Buffer Species and Concentrations on Solution Stability of 1[a]

pH	Buffer	$k_{obs} \times 10^4$ (h^{-1})		
		0.01 M	0.05 M	0.10 M
3.0	Citrate	5.69	6.78	7.59
5.0	Citrate	3.33	4.13	4.74
8.0	Phosphate	9.42	13.17	16.50

[a] Determined at 56 °C (μ = 0.5, NaCl).

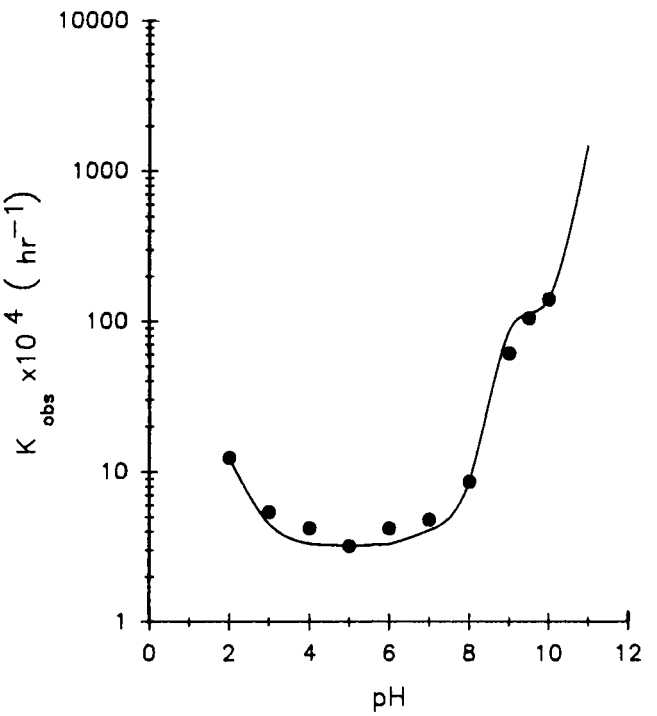
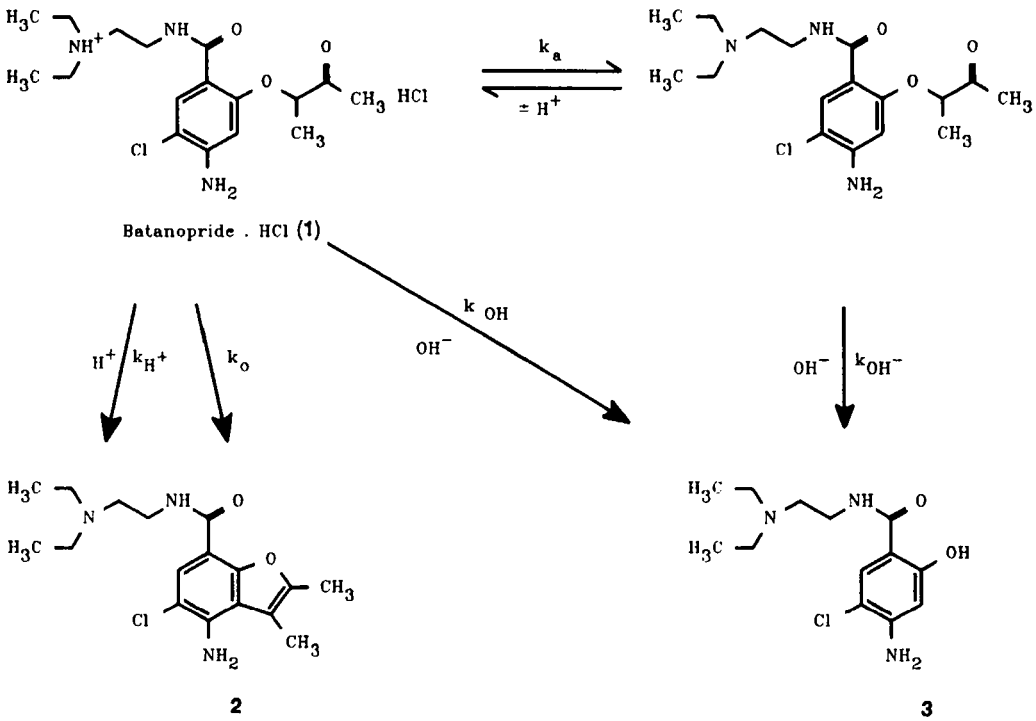


Figure 3—pH–rate profile for the degradation of 1 in aqueous solutions (56 °C; μ = 0.5, NaCl). Data represent k_{obs} values corrected for buffer catalysis.



Scheme I

Rate constants were estimated from the best fit of the pH–rate profile. The value for k_0 was $3.2 \cdot 10^{-4} \cdot day^{-1}$; the values for k_h, k_{Oh}, and k'_{Oh} were $12.4 \cdot 10^{-2}$, $8.64 \cdot 10^{2}$, and $14.9\ M^{-1} \cdot day^{-1}$, respectively. In Figure 2, the points are experimental data, and the solid line represents the theoretical curve at 56 °C calculated by substituting the microscopic constants into eq 1. The good agreement between the calculated values and the experimental data demonstrates that eq 1 adequately describes the degradation kinetics in the pH range studied. An inflection point around pH 9–9.5 was observed near the pK_a of the tertiary amino group. This phenomenon can be explained by the different degrees of catalytic effect of the hydroxide ion on the protonated and nonprotonated form of 1 in aqueous solution.

Effect of μ—The effect of μ (0.1–0.5 M) on the degradation of 1 was studied in pH 3 citrate buffer at 56 °C. The value of μ was varied by addition of sodium chloride. The k_{obs} values were 5.71×10^{-4} and $7.58 \times 10^{-4}\ h^{-1}$ at μ values of 0.1 and 0.5, respectively. These data indicate a positive kinetic salt effect.[4]

Effect of Dielectric Constant—The effect of solution polarity on the stability of 1 was evaluated with ethanol–water solutions of various compositions encompassing a calculated dielectric constant range of 37.8 to 78.5. The dielectric constant is one of several parameters used to estimate solvent polarity.[5] An increase in the stability of 1 in solution was observed as the dielectric constant (polarity) of the solution decreased (Table III). This improvement in stability is not a pH effect because all ethanol–water solutions had similar apparent pH values.

Degradation Mechanism—Degradation of 1 was found to occur by two different mechanisms depending on the pH of the

Table III—Effect of Dielectric Constant on Solution Stability of 1[a]

Solvent System	Dielectric Constant[b]	Time, weeks	pH	Amount of 1 Remaining, %
Water	78.5	4	6.2	94.9
		8	6.2	89.4
Ethanol:water (25:75)	64.9	4	6.3	98.7
		8	6.4	97.5
Ethanol:water (50:50)	51.4	4	6.3	99.5
		8	6.4	99.1
Ethanol:water (75:25)	37.8	4	6.0	99.7
		8	6.1	99.6

[a] Determined at 56 °C. [b] Calculated by the following equation[7]: dielectric constant (E) = (% v/v/100) $E_{ethanol}$ + (% v/v/100)E_{water}.

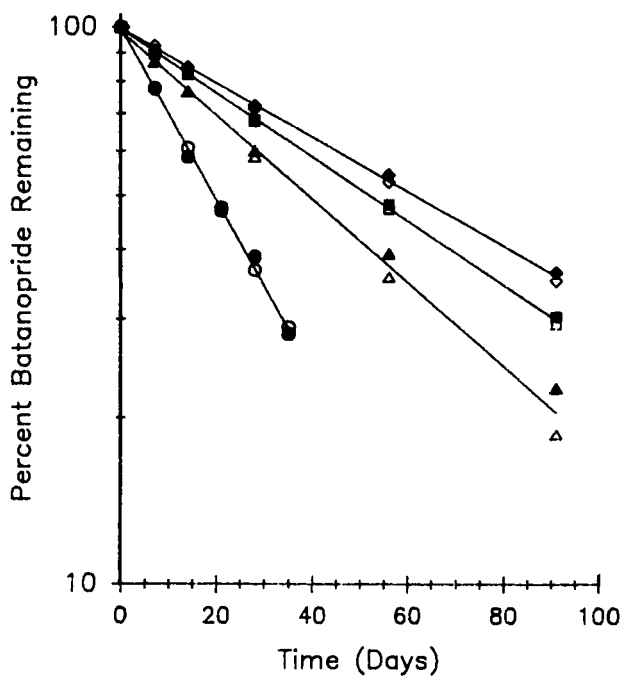


Figure 4—Disappearance of 1 at pH 2–5 and at 56 °C. Key: (●, ○) pH 2.0; (▲, △) pH 3.0; (■, □) pH 4.0; (◆, ◇) pH 5.0. Data indicated by open symbols were found by direct HPLC analysis of 1; data indicated by closed symbols were calculated from the concentration of the degradation product 2.)

solution (Scheme I). In acidic media (pH 2–6), the predominant reaction was intramolecular cyclization followed by dehydration to form a 2,3-dimethylbenzofuran (2). There was no kinetic or analytical (HPLC) evidence for the formation of an intermediate; therefore, the rate of dehydration must have been very rapid compared with the rate of cyclization. Furthermore, the rate of loss of 1 in solution and the rate of appearance of 2 were essentially the same. The residual concentrations of 1, obtained by direct analysis by HPLC, were the same as those calculated from the concentrations of 2 liberated (Figure 4). Therefore, the reaction involving the formation of 2 was responsible for the total loss of 1 in acidic media.

In alkaline media (pH 8–10), the predominant route of degradation was cleavage of the C–O alkyl ether bond to yield 3. At intermediate pH (pH 6–8), both reactions contributed to the overall degradation. Both degradation products were isolated by preparative HPLC, and their respective structures were elucidated by IR, NMR, and mass spectrometry. In addition, the degradation products 2 and 3 were synthesized, and their structures were confirmed by elemental analysis.[6]

Conclusions

The data indicate that 1 has optimum aqueous stability at pH 4.5–5.5. Citrate and phosphate buffers were catalytic and had an adverse effect on the long-term shelf-life stability of 1 in solution. In addition, the data suggest that the use of parenterally suitable solvents to lower the dielectric constant (polarity) of the solution could improve the stability of 1.

References and Notes

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