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Short communication

Stability of ricobendazole in aqueous solutions

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ABSTRACT

The chemical stability of ricobendazole (RBZ) was investigated using a stability-indicating high performance liquid chromatographic (HPLC) assay with ultraviolet detection. The degradation kinetics of RBZ in aqueous solution was evaluated as a function of pH, buffer strength and temperature. The oxidation reaction in hydrogen peroxide solution was also studied. Degradation products were analyzed by mass spectroscopy and degradation pathways are proposed. Degradation of RBZ followed pseudo first-order kinetics and Arrhenius behavior over the temperature range 24–55 °C. A V-shaped pH-rate profile over the pH range 2–12 was observed with maximum stability at pH 4.8. The shape of the pH-rate profile was rationalized by catalytic effects of various components in the solution on each RBZ species. At pH 11 the activation energy for hydrolysis was 79.5 kJ/mol, and phosphate catalysis was not observed. Oxidation occurred in hydrogen peroxide solutions and was catalyzed by the presence of copper (Cu^{2+}) ions. Ricobendazole amine and albendazole sulfone were identified by MS assay to be the degradation products of hydrolysis and oxidation respectively.

1. Introduction

Benzimidazole drugs are widely used in veterinary medicine for the prevention and treatment of parasitic diseases [1]. Their low host toxicity and broad spectrum of activity against lungworms, tapeworms and gastrointestinal (GI) nematodes have contributed to their success as anthelmintic agents. The sulfur-containing derivatives (fenbendazole, oxfendazole and albendazole) are important drugs in this class due to their high efficacy against lungworms and ability to inhibit the larval stages of most GI nematodes [2]. Ricobendazole (RBZ), also known as albendazole sulfoxide, is the active metabolite of albendazole formed by oxidation of the sulfur attached to the benzimidazole ring [3]. It is commercially available in injection products such as Bayverm PI® (Bayer Argentina) [4] and Sintyotal-R® (Biogenesis Argentina) [5].

Physicochemical properties including solubility, lipophilicity and ionization of RBZ have been reported [6] and the pharmacokinetics of RBZ has been well described [4,5,7–10]. Yet only very limited information on the stability of RBZ or other benzimidazole anthelmintics has been published [11–14]. Rose et al. [11] investigated the stability of oxfendazole, in cattle tissue on cooking and discussed its degradation pathways. Weerasinghe et al. [12,13] reported pH-dependent photodegradation of albendazole and its major metabolites, including RBZ, in aqueous solutions at pH 5, 7 and 9 under exposure to sunlight or UV light. Hernández-Luis et al. [14] investigated the effect of pH on the hydrolytic stability of several newly synthesized benzimidazole carbamates. They reported a U-shaped pH-rate profile over the pH range 1–9 for one of the compounds with the minimum hydrolysis rate at pH 5.

The objectives of the present study were to investigate the stability of RBZ in aqueous solution as a function of pH, buffer strength and temperature and the stability of RBZ in hydrogen peroxide solutions; and to identify the pathways of chemical degradation in the aqueous solutions.

2. Materials and methods

2.1. Chemicals

Ricobendazole (>99.3%) was a gift from Transchem Limited, Ambernath, India. Acetonitrile (HPLC grade) was obtained from BDH Chemicals Ltd. All other chemicals and solvents were reagent grade (BDH Chemicals Ltd., England).

2.2. Instrumentation

A stability-indicating HPLC assay method was used in this study as described previously [6]. The HPLC system comprised a SP8810 precision isocratic pump, SP8875 auto-sampler,

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Spectra 100 variable wavelength detector and Chromjet Integrator (Spectra-Physics Inc., CA). The mobile phase was a mixture of 0.02 M phosphate buffer (pH 3.0) and acetonitrile (550:200, v/v) and was pumped at a flow rate of 1.0 ml/min through a C18 Prodigy 5 μm, 4.6 mm × 250 mm column (Phenomenex®, New Zealand). The injection volume was 70 μl and the wavelength for detection was 290 nm. The assay was linear over the RBZ concentration range 0.25–10 μg/ml ($r^2 > 0.999$) and separated RBZ from the three potential degradation products [6].

2.3. Determination of degradation rate constant

The observed pseudo first-order degradation rate constants, k_{obs}, were calculated from the slopes of semi-logarithmic plots of the drug fraction remaining versus time in accordance with Eq. (1):

$$\ln\left(\frac{C_t}{C_0}\right) = -k_{obs}t \quad (1)$$

where C_0 was the initial concentration and C_t was the remaining concentration of RBZ at t time.

Solutions were monitored for at least one half-life or for a maximum of 6 months. All the RBZ solutions were protected from light during the study and experiments were performed in duplicate.

2.4. pH-degradation rate of RBZ

RBZ solutions were prepared at a concentration of 20 μg/ml (0.07 mM) by diluting a 1 mg/ml methanol stock solution with the phosphate buffers (pH 2–12). Buffers over pH range 4.8–12 were prepared by mixing 20 mM solutions of Na_2HPO_4, NaH_2PO_4 or Na_3PO_4 at appropriate ratios without adjusting ionic strength. Below pH 4, buffers were prepared from 20 mM solutions of NaH_2PO_4 with pH adjusted by addition of 1 M H_3PO_4. Solution pH was measured at room temperature (23 ± 1 °C) using a digital pH meter (Suntex SP-701, Taiwan) combined with a InLab®439/120 glass electrode (Mettler Toledo Inc.) calibrated using standard buffers at pH 4.0, 7.0 and 10.0 (BDH, England). Solutions were incubated at 55 ± 0.1 °C in a digital oven (Contherm Scientific, New Zealand). Aliquots were taken at appropriate times depending on the decomposition rate and analyzed immediately for RBZ and its degradation products after a 1:1 (v/v) dilution with Milli-Q water of room temperature.

2.5. Effects of temperature and buffer concentration on the stability of RBZ

The effect of temperature on the rate of RBZ degradation was determined at pH 11 in 0.02 and 0.1 M phosphate buffers. RBZ solutions (20 μg/ml) were prepared in the appropriate buffers as described above in Section 2.4, and stored at 24, 39, 45 and 55 °C in digital ovens (Contherm Scientific, New Zealand). Aliquots were taken at appropriate times depending on the decomposition rate and analyzed immediately for RBZ after a 1:1 dilution with Milli-Q water. The observed pseudo first-order degradation rate constants (k_{obs}), were calculated using Eq. (1). ln(k_{obs}) was plotted against $1/T$ (K) and the Arrhenius factor A, and energy of activation E_a for RBZ degradation were determined from the intercept and slope according to Eq. (2) [15] using least squares linear regression:

$$\ln k_{obs} = \ln A - \frac{E_a}{RT} \quad (2)$$

where R was the universal gas constant and T was the absolute temperature (K).

2.6. Oxidation of RBZ in hydrogen peroxide (H_2O_2) solutions

RBZ solutions (100 μg/ml) were prepared in 30% (v/v) hydrogen peroxide (H_2O_2) in water and stored at 55 °C. Aliquots were taken at appropriate times depending on the decomposition rate and were immediately diluted 10 times with Milli-Q water then analyzed by HPLC. The effects of Cu^{2+} on the rate of RBZ oxidation was investigated by addition of $CuSO_4$ (10 and 200 μM) to RBZ solutions (100 μg/ml) in 10% (v/v) H_2O_2.

2.7. Mass spectroscopy (MS) for degradation products of RBZ

Two major degradation products were observed in the degradation studies and these were collected from the HPLC effluent, stored at 4 °C then analyzed using direct infusion mass spectrometric analysis equipped with a positive ion electrospray ionization (ESI) source (Finnegan LCQ DECA Ion Trap Mass Spectrometer (MS) with Xcaliber Version 1.2 software).

2.8. Statistical analysis

Statistical analysis and least squares linear regression were performed using Minitab for Windows, version 12.1 (Minitab, Inc., PA, USA).

3. Results and discussion

3.1. pH-rate profile of RBZ

The degradation of RBZ in phosphate buffers over pH 2–12 at 55 °C resulted in appearance of one degradation product with a retention time equivalent to ricobendazole amine in the HPLC chromatogram (Product I, Scheme 1). All the correlation coefficients (r) of the semi-logarithmic plots of drug remaining versus time were >0.98 and most were >0.99, indicating the degradation of RBZ followed pseudo first-order kinetics (Fig. 1).

A V-shaped pH-rate profile of RBZ over the pH range 2–12 at 55 °C was observed with maximum solution stability at pH around 4.8 (Fig. 2). Degradation occurred more rapidly in basic than in acidic solutions, and at pH 4.8 the estimated degradation rate constant was $2.4 \pm 0.15 \times 10^{-4}$ (day^{-1}), giving an estimated half-life (t_{50}) of 96 months at 55 °C. The shape of pH-rate profile of RBZ was consistent with that reported by Hernández-Luis et al. for a benzimidazole carbamate [14]. The slope of the log k_{obs} versus pH profile was less than unity over pH 2–12, indicating the mecha-

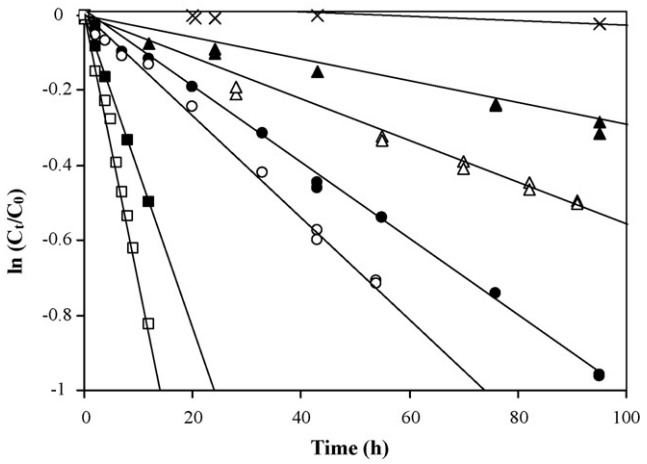
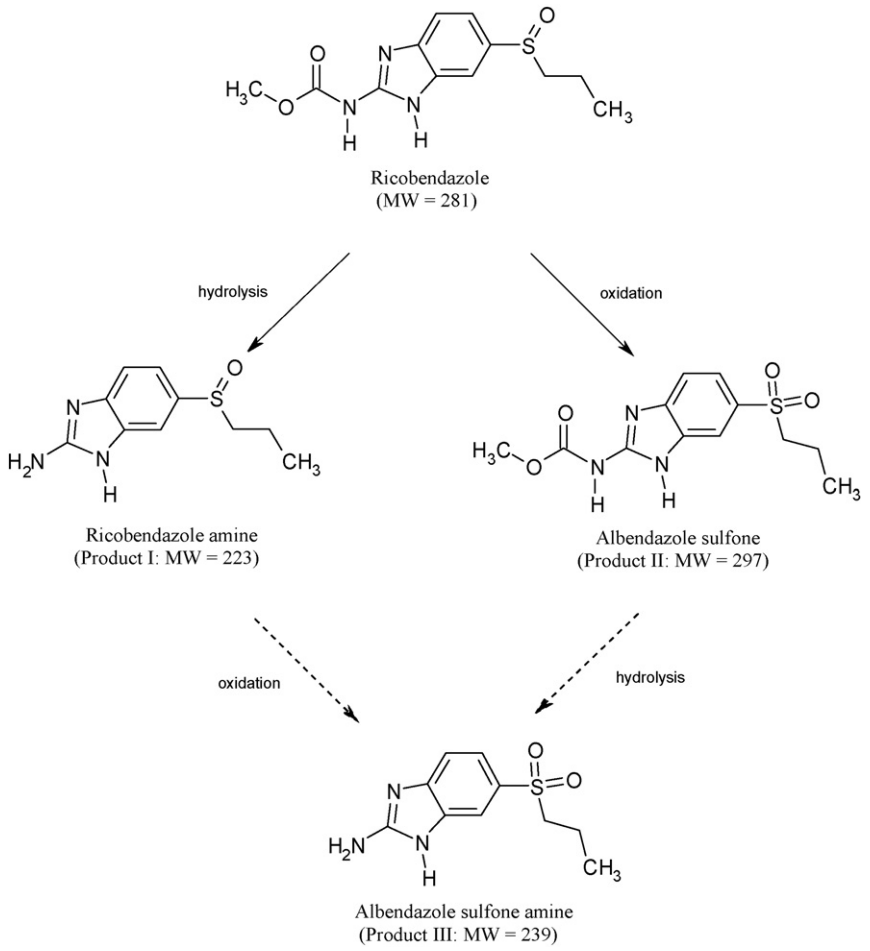


Fig. 1. First-order plots showing the degradation of RBZ at 55 °C at various pHs: (×) pH 4.8, (▲) pH 5.5, (△) pH 3, (●) pH 2, (○) pH 7.0, (■) pH 9, and (□) pH 12.



Scheme 1. Proposed degradation pathway for RBZ. Dashed lines suggest possible routes of degradation to albendazole sulfone amine.

nism of the degradation reaction was not a simple specific acid- or base-catalyzed hydrolysis [15]. It is well known that pH affects the degradation rate of many drugs and both specific and general acid- or base-catalysis of drug compounds are well reported [16–19]. Degradation rate profiles are, however, often complex for molecules such as RBZ that have multiple ionization sites within the molecule. This is because the observed degradation rate at any specified pH is composed of contributions from acid, base, water and buffer catalysis on all existing species of the drug in the solution. For each species the degradation rate (k'_{obs}) can be described by Eq. (3):

$$k'_{obs} = k_0 + k_H[H^+] + k_{OH}[OH^-] + k_{BH}[BH] + k_B[B^-] \quad (3)$$

where k_0 is the first-order constant rate for degradation of this species in water, and k_H, k_{OH}, k_{BH} and k_B are second-order rate constants for degradation of this species catalyzed by protons, hydroxyl ions, acidic form of buffer (BH) and basic form of buffer (B^-) respectively.

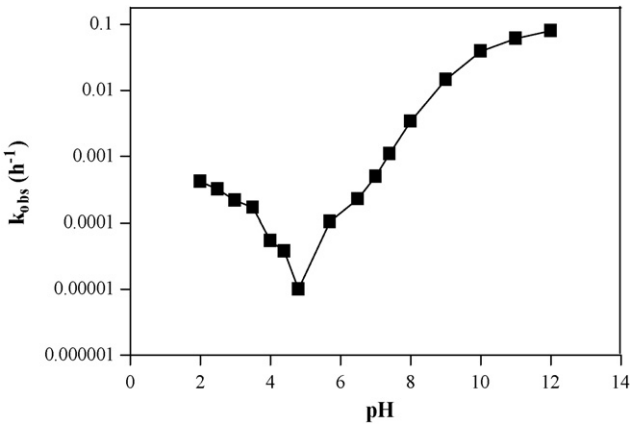


Fig. 2. pH-rate (k_{obs}, h^{-1}) profile for RBZ at 55 °C.

The previously reported amphoteric nature of RBZ (pK_a values of 3.5 and 9.8) [6] can be used to interpret the shape of the pH-degradation rate profile as general catalyzed hydrolysis; with contributions from the protonated (RBZ^+), neutral (RBZ) and deprotonated (RBZ^-) species. In the region pH 5.5–8, where RBZ exists predominately as the neutral species, an increase in pH resulted in an increase in the degradation rate with a slope of about 0.7, indicating degradation of the neutral species was catalyzed by OH^- ions as well as the other components. In the pH region around pK_a (9.8), where the fraction of neutral species decreases and k_{obs} is expected to become increasingly influenced by RBZ^- species, the slope of the pH-rate profile in this region continuously reduced, suggesting RBZ^- was less sensitive to OH^- than the neutral RBZ. This hypothesis is supported by the plateau in the pH-log k_{obs} profile over pH 11–12, where the fraction of RBZ^- is greater than 0.9. A plateau phenomenon was also observed over pH 2–3 when RBZ^+ predominated, suggesting RBZ^+ was less sensitive to H^+ than the neutral species RBZ and solvent effect was important on the stability of RBZ^+. Generally, H^+ ions have less catalytic effect on the positively charged species than on anionic species and OH^- ions have less catalytic effect on negatively charged

species than on cationic species due to the electrostatic repulsion.

For interpretation of the pH-rate profile generated at 55 °C, caution is exercised when using the pK_a values for RBZ reported previously as these were measured at 25 °C [6]. The temperature increase may result in the basic pK_a being slightly lower and acidic pK_a slightly higher compared with those at lower temperature [20]. In this study the pHs of the degradation solutions were also measured at 25 °C.

3.2. Effects of temperature and buffer concentration on the stability of RBZ

Degradation of RBZ in the buffers followed Arrhenius behavior with k_{obs} (h^{-1}) values in the range of 2.9×10^{-3} to 5.99×10^{-2} and 2.9×10^{-3} to 6.07×10^{-2} in 0.02 and 0.1 M phosphate buffer respectively over the temperature range 24–55 °C. Linear plots for Eq. (2), $\ln k_{obs} = 26.3 - 9512(1/T)$ and $\ln k_{obs} = 26.4 - 9562(1/T)$ ($r > 0.99$) were obtained for RBZ in the buffers. The energies of activation for RBZ degradation in the 0.02 and 0.1 M phosphate buffers were 79.5 ± 2.0 and 79.9 ± 1.9 kJ/mol respectively. This is within the range of 50–96 kJ/mol most commonly reported and represents an intermediate sensitivity of the degradation process to temperature [15]. There was no significant difference between the observed degradation rates in the two phosphate buffers ($P < 0.05$) which suggests that phosphate buffer had little or no catalytic effect on the hydrolysis of the RBZ at pH 11.

3.3. Oxidation of RBZ in H_2O_2

RBZ appeared relatively stable to oxidation by hydrogen peroxide. Significant degradation was observed only in 30% (v/v) H_2O_2 over 4 h at 55 °C with the appearance of a major degradation product with a retention time (R_t = 10.3 min) consistent with albendazole sulfone (Product II, Scheme 1). The apparent first-order degradation rate constant of RBZ was estimated to be 0.4 h^{-1}. The oxidation product (Product II) was not detectable in 10% (v/v) H_2O_2 at 55 °C for at least 10 h unless Cu^{2+} was added in the solution. In the presence of 10 and 200 μM Cu^{2+} the rate constants were found to be 0.7 and 1.9 h^{-1} respectively. This result suggests Cu^{2+} ions significantly accelerated the oxidation rate of RBZ. Furthermore, the hydrolysis product peak (Product I, R_t = 3.5 min) and another peak (R_t = 8.1 min) were seen in the oxidation reaction samples. The time-course of appearance of Product III suggested it could have been formed from hydrolysis of Product II or alternatively oxidation of Product I.

3.4. MS assay of degradation products of RBZ

HPLC analysis showed that RBZ in buffer solutions over pH 2–12 produced a single degradation product with a constant retention time (3.5 min). The corresponding positive ion ESI mass spectrum of this compound yielded an *m*/*e* 224, which is consistent with ricobendazole amine (MW = 223) (Fig. 3a) as a result of hydrolysis of the amine linkage in the methylcarbamate in the structure, similar to the hydrolysis reported to occur in oxfendazole [11]. ESI mass spectrum of major degradation product in the H_2O_2 solutions showed a *m*/*e* 298, which is consistent with albendazole sulfone (MW = 297) (Fig. 3b), the major metabolite of RBZ by oxidation [5,8,21,22]. The additional peak (Product III, R_t = 8.5 min) found in the H_2O_2 samples could not be collected from HPLC column for MS analysis due to its short time appearance and low yield. Given its shorter R_t than that of Product II, Product III was considered to be more hydrophilic than Product II and hence proposed to be albendazole sulfone amine formed as a result of hydrolysis of methylcarbamate group at position-2 of albendazole sulfone. Alternatively, since the degradation was carried out in the presence of H_2O_2, there could be oxidation of the ricobendazole amine (Product I) to produce the sulfone (Product III) in a mechanism similar to oxidation of ricobendazole. This compound has been reported as a metabolic product of albendazole in human [23]. The proposed degradation pathway for RBZ in aqueous solutions as illustrated in Scheme 1 was similar to our previously proposed metabolic scheme [24]; hence RBZ may undergo the same reactions *in vitro* and *in vivo*.

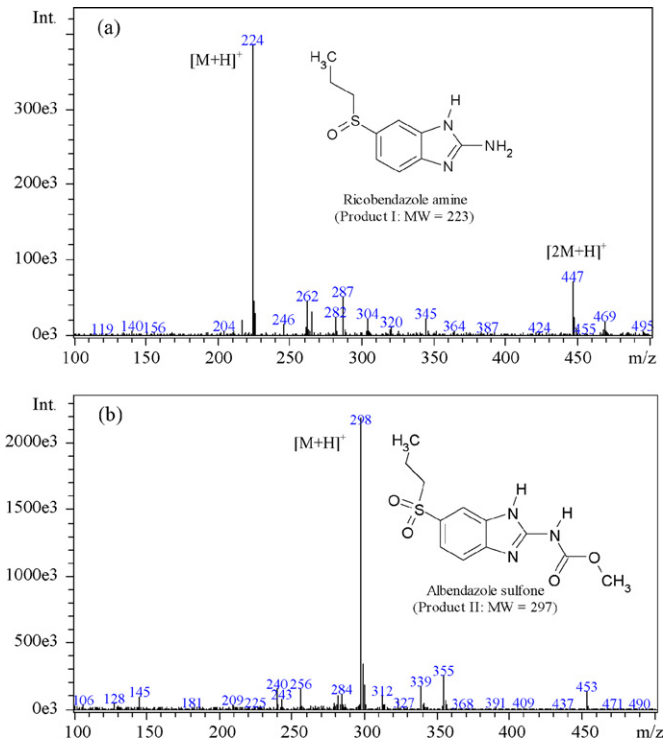


Fig. 3. Positive ion electrospray ionization mass spectra for hydrolysis Product I (a) and oxidative Product II (b) of RBZ.

In summary, the current study characterized the stability of RBZ in aqueous solutions with respect to pH, buffer concentration, temperature and oxidant H_2O_2. A V-shaped pH-rate profile can be rationalized by the different catalytic effects of various components in the solution on each RBZ species. Degradation pathways of RBZ include hydrolysis and oxidation and results provide useful information for formulation development of a stable RBZ injectable. Commercial RBZ solutions for injection have a concentration of 10% or 15% (w/v) and are formulated at low pH with additional solubilization with co-solvents. Using the degradation rate reported here it appears a solution formulated at pH 2 would have sufficient solution stability to provide an estimated shelf-life (t_{90}) of 11.7 months at 20 °C. However, stability at this pH may be improved by including an appropriate solvent in formulation, as the solvent effect on RBZ stability becomes an important factor at low pH.

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