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A stability-indicating assay and the preformulation characteristics of the radiosensitizer, 1,2,4-benzotriazin-3-amine 1,4-dioxide*

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Abstract: A stability-indicating LC assay was developed for the analysis of 1,2,4-benzotriazin-3-amine 1,4-dioxide and applied to the preformulation characterization of the drug. The dissociation constants of the drug were determined using UV–vis spectrophotometry. The LC method was used to determine the aqueous stability of the drug under a variety of accelerated conditions, its solubility in a variety of pharmaceutical solvents and its octan-1-ol–water partition coefficient. The preformulation data were used to develop three prototype aqueous formulations of the drug at a concentration of 0.5 mg ml^{-1} in 5% Dextrose Injection USP, phosphate buffer (pH 7.4) and phosphate buffered mannitol. The 3-month stability of those formulations at room temperature was demonstrated.

Keywords: *1,2,4-Benzotriazin-3-amine 1,4-dioxide; preformulation, stability, hydrolysis, kinetics, solubility, partition coefficients; dissociation constants; stability-indicating assay; liquid chromatography.*

Introduction

1,2,4-Benzotriazin-3-amine 1,4-dioxide (SR 4233, WIN 59075, **1**, Fig. 1) is novel radiosensitizer that is currently under clinical development in the United States and in Europe as a potential adjuvant to radiation for the treatment of various cancers. *In vitro*, **1** has a high degree of selective toxicity for hypoxic cells [1–5]. *In vivo*, **1** has significant antitumour activity when combined with either radiation [6] or with an agent that induces hypoxia in tumours [7, 8]. The title compound is reduced in hypoxic cells by two successive two-electron transfer reactions to give 1,2,4-benzotriazin-3-amine 1-oxide and 1,2,4-benzotriazin-3-amine, respectively [9–11]. The major enzyme responsible for the reduction of **1** in liver microsomes [10] and in tumour cells [12] appears to be of the P-450 class. Both reduction products are inactive [1, 11] and it has been proposed that the active species is a free-radical intermediate formed by the one-electron reduction of **1** [12, 13].

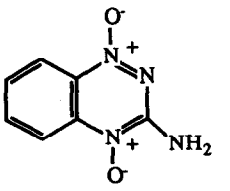

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Figure 1
Structure of 1,2,4-benzotriazin-3-amine 1,4-dioxide (**1**, WIN 59075).

1,2,4-Benzotriazin-3-amine 1,4-dioxide is a neutral, highly polar compound whose physicochemical properties are previously uncharacterized. Knowledge of the pharmaceutically relevant physicochemical properties were considered an important prerequisite to the development of a stable solution for injection. The present study was conducted to develop a stability-indicating LC assay for **1** and to apply that assay to the stability of **1** in aqueous solution. The octan-1-ol–water partition coefficient of **1** and its solubility in

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various aqueous solvents were also determined using the same stability-indicating assay.

Experimental

Chemicals and reagents

1,2,4-Benzotriazin-3-amine 1,4-dioxide (WIN 59075, **1**, Fig. 1) was obtained from Sterling Winthrop (Rensselaer, NY, USA) and was used as received. The chemicals used for the preparation of buffer solutions were reagent grade and were obtained from various sources. HPLC grade acetonitrile was obtained from Fisher Scientific. Deionized water prepared by the MilliQ System (Millipore, Woburn, MA, USA) was used throughout.

Liquid chromatography

The modular liquid chromatograph consisted of a Shimadzu SCL-6A System Controller, a Shimadzu LC-6A Solvent Delivery Pump, a Shimadzu SIL-6A Autoinjector and a Perkin–Elmer LC235 Diode Array Detector. Data were collected and reduced with an Epson Equity 1+ Microcomputer and Perkin–Elmer Omega 235 Software. Compound **1** was separated from its degradation products on an ODS Hypersil column (5 μm, 150 × 4.6 mm, i.d.) eluted isocratically with acetonitrile–phosphate buffer (0.1 M, pH 7.0)–triethylamine (92:8:0.4, v/v/v) at ambient temperature (22 ± 1°C) and a flow rate of 1.5 ml min^{-1} (Fig. 2). Under these conditions, **1** eluted from the column with a retention time of 2.79 min.

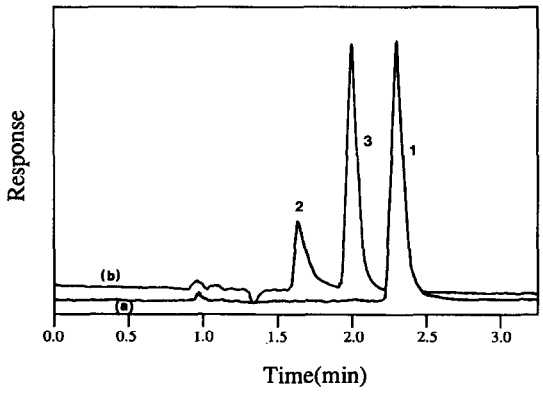

Figure 2
Analytical separation of **1** from its main degradation products of alkaline hydrolysis (**2** and **3**). Column: ODS Hypersil (5 μm, 150 × 4.6 mm, i.d.); mobile phase acetonitrile–phosphate buffer (0.1 M, pH 7.0)–triethylamine (92:8:0.4, v/v/v); temperature ambient (22 ± 1°C); flow rate of 1.5 ml min^{-1}. Key: (a) **1** (50 μg ml^{-1}) in phosphate buffer (0.1 M, pH 7.4); (b) **2** and **3** after degradation of **1** for 24 h in 0.1 M NaOH at 100°C.

The method was essentially the same as that reported previously by Shetty *et al.* [14] for the analysis of the antineoplastic drug, dacarbazine. Fixed wavelength detection of **1** using the diode array detector was at 265 nm. Peak purity and characterization of degradation products was accomplished by multi-wavelength detection over the range 200–360 nm.

Assay procedure

Solutions were assayed for **1** by diluting with mobile phase into the previously determined linear range of 25–100 μg ml^{-1}. For each assay triplicate samples were taken and each diluted sample was injected in duplicate. The results are expressed as the mean ±SD of the three determinations.

Mass spectrometry

Electron ionization (EI) mass spectra were obtained on a Nermag (Paris, France) R10-10 quadrupole mass spectrometer with a SPECTRAL 30 data system. Solutions were evaporated from the direct insertion probe at 5°C s^{-1} to 500°C. Ionization was with 70 eV energy, 200 mA emission and the source block at 250°C.

Determination of dissociation constants

The dissociation constants of **1** were determined spectrophotometrically. Five milligrams of **1** were weighed into a 100 ml volumetric flask, dissolved in an appropriate buffer and then adjusted to volume. The spectra (200–800 nm) of each solution was then obtained at ambient temperature (22 ± 1°C) using a Shimadzu 2100 UV–vis spectrophotometer.

Determination of octan-1-ol–water partition coefficients

The aqueous octan-1-ol partition coefficients of **1** were determined at 25.0 ± 0.1°C using the shake flask method. Water, phosphate buffer (pH 6.5, 7.4 and 11, μ = 0.15), acetate buffer (pH 4.5, μ = 0.15) and HCl (0.1 M, μ = 0.15) were studied as the aqueous phase. The ionic strength was adjusted in all cases with NaCl. A 50-μl aliquot of an aqueous 1-mg ml^{-1} stock solution of **1** was added to a 25-ml stoppered conical flask containing 5.00 ml of buffer-saturated octan-1-ol and 4.95 ml of octan-1-ol saturated buffer. The flasks were shaken in a water bath at 25.0 ± 0.1°C for at least 24 h until equilibration had occurred. The phases were separated by centrifugation at approxi-

mately 200*g* for 2 min. The aqueous phase was analysed directly by liquid chromatography. A 1-ml aliquot of the organic phase was evaporated to dryness and the residue dissolved in 0.5 ml of phosphate buffer (pH 7.0). The apparent partition coefficient of **1** was determined from

$$D = \frac{[\mathbf{1}]_{org}}{[\mathbf{1}]_{aq}}, \quad (1)$$

where $[\mathbf{1}]_{aq}$ is the concentration of **1** in the aqueous phase and $[\mathbf{1}]_{org}$ is the concentration of **1** in the organic phase.

Solubility measurements

Approximately 25 mg of **1** was placed in a 25-ml conical flask and 5 ml of solvent added. Duplicate flasks were prepared for each solvent tested. The flasks were shaken in a water bath (25.0 ± 0.01°C) for 48 h. When appropriate, the pH of the solution was measured at the end of the experiment and readjusted if the change was more than ±0.2 pH units. If pH re-adjustment was necessary the suspensions were shaken for a further 24 h. An aliquot of each suspension was filtered through a syringe filter (0.45 μm), diluted with phosphate buffer (pH 7.0) and analysed by liquid chromatography. The solid was recovered by vacuum filtration through a glass sinter and dried at 50°C for 24 h. In every case, the melting points (206.8°C dec) and physical appearances of the recovered solids were the same as the starting material.

Stability studies

Preliminary stability studies were conducted initially to confirm the assay was stability indicating by storing aqueous solutions of **1** (0.5 mg ml^{-1}) for 24 h in glass ampoules and then assaying by LC for the concentration of the intact drug and for the appearance of putative degradation products. The following storage conditions were used to validate the LC assay: phosphate buffer (0.1 M, pH 7.4, 100°C, dark, nitrogen atmosphere), HCl (0.1 M, 100°C, dark, nitrogen atmosphere), NaOH (0.1 M, 100°C, dark, nitrogen atmosphere), phosphate buffer (0.1 M, pH 7.4, 22°C, UV light, nitrogen atmosphere), and phosphate buffer (0.1 M, pH 7.4, 22°C, UV light, oxygen atmosphere). The effect of exposure to light was determined by placing the ampoules approximately 5 cm from a low-power germicidal UV lamp (Model GAT4/1: The Second Source, Duarte, CA, USA). Samples were protected from light, where indicated, by wrapping the ampoules in aluminum foil.

The effect of normal fluorescent room lighting and air on the stability of **1** 0.5 mg ml^{-1} in 5% dextrose injection USP, phosphate buffered (0.1 M, pH 7.4) mannitol and phosphate buffer (0.1 M, pH 7.4) was studied at room temperature (22 ± 1°C) over a period of 3 months. Controlled kinetic studies were conducted to determine the loss of **1** in 0.5 M HCl, 0.1 M phosphate buffer (pH 7.1) and 0.01–0.05 M NaOH at 60.0 ± 0.2°C (μ = 0.15 with KCl). Solutions of **1** (0.5 mg ml^{-1}) were stored in glass ampoules. A constant temperature was maintained (60.0 ± 0.2°C) by placing the ampoules in a thermostatted water bath.

Results and Discussion

Assay development and validation

Selectivity. The concentrations of **1** remaining after 24 h under a UV light at pH 7.4 (22 ± 1°C) were 98.8% with a nitrogen atmosphere and 97.9% with an oxygen atmosphere and no additional peaks were observed in the final chromatograms. This suggested that **1** was not susceptible to photolysis or oxidation. In contrast to the effects of light or oxygen, **1** was not detected after storage for 24 h at 100°C in either 0.1 M NaOH or 0.1 M HCl. Two new peaks attributed to the degradation products of **1** in 0.1 M HCl and 0.1 M NaOH were detected after 24 h. These compounds had retention times of 1.92 min (**2**) and 2.42 min (**3**) (Fig. 2). Approximately 7% of the initial concentration of **1** was detected after 24 h at 100°C in pH 7.4 buffer. Compounds **2** and **3** were the major peaks detected in the pH 7.4 solution which also contained three minor peaks eluting with retention times of 4.16, 4.51 and 12.3 min.

Precision. The precision of the method and the systems were demonstrated by repeated (n = 6) analysis of a 0.5 mg ml^{-1} solution of **1** in 5% Dextrose Injection USP and repeated (n = 6) injection of a 50 μg ml^{-1} standard solution, respectively. The RSD of the method was 0.49% (n = 6) and the RSD of the system was 0.45% (n = 6).

Recovery. The mean peak area of 4:5 dilution of the 50 μg ml^{-1} standard with mobile phase was 81.2% (n = 2).

Linearity. The linearity of the method was demonstrated by duplicate injections of **1** in mobile phase at concentrations of 25, 35, 50, 75 and 100 μg ml^{-1}. The relationship between peak area (A) and the concentration of **1** (C) satisfied the equation:

$$A = 3.01 \times 10^5 C + 491; \; r > 0.999. \quad (2)$$

Dissociation constants

The shifts in UV–vis spectra of **1** with change in pH (Fig. 3) were consistent with the presence of two dissociation steps and three Bjerrum species (**1a**, **1b** and **1c**) (Fig. 4). The most likely ionizable functional group is the primary amine, which may accept a proton at low pH (**1a**) and lose a proton at high pH (**1c**).

The change in absorbance (A_t) of **1** with hydrogen ion concentration may be described by

$$A_t = \frac{A_{1a}[H^+]^2 + A_{1b}[H^+]K_{a,1} + A_{1c}K_{a,1}K_{a,2}}{[H^+]^2 + [H^+]K_{a,1} + K_{a,1}K_{a,2}}, \quad (3)$$

where A_{1a}, A_{1b} and A_{1c}, are the absorbances of the cationic, neutral and anionic forms of the drug, respectively, and $K_{a,1}$ and $K_{a,2}$ denote the apparent dissociation constants.

At low pH the contribution of the second dissociation step to the absorbance is insignificant and equation (3) may be simplified to give equation (4) which was used for the determination of $K_{a,1}$

$$A_t = \frac{A_{1a}[H^+] + A_{1b}K_{a,1}}{[H^+] + K_{a,1}}. \quad (4)$$

The A_t–pH data were analysed according to

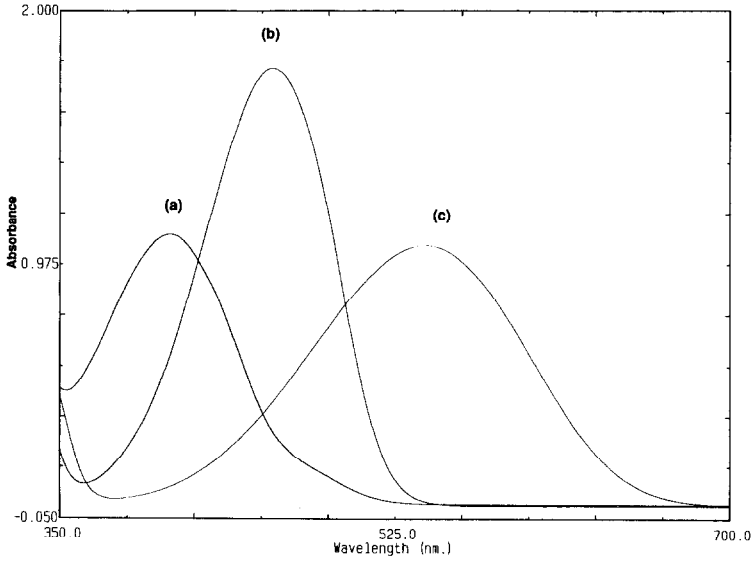

Figure 3
UV–vis spectra of **1** at (a) pH 0, (b) 7 and (c) 14.

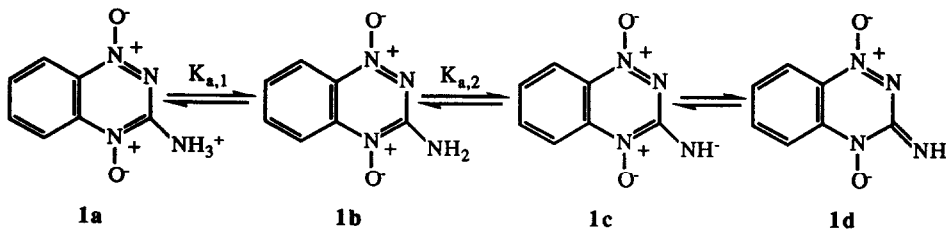

Figure 4
Proposed ionization scheme for **1**.

equation (4) by non-linear regression analysis to obtain values of $K_{a,1}$ at the three analytical wavelengths of 0.133 (407.4 nm), 0.146 (460.6 nm) and 0.138 (498.8 nm). Those values corresponded to an average value for $K_{a,1}$ of 0.139 and a $pK_{a,1}$ of 0.86.

The second pK_a value was determined in the same manner using equation (5) to obtain values of $K_{a,2}$ at three different wavelengths: 2.93×10^{-13} (421.8 nm), 2.79×10^{-13} (460.6 nm) and 2.94×10^{-13} (539.8 nm). Those values correspond to an average value for $K_{a,2}$ of 2.89×10^{-13} and a $pK_{a,2}$ of 12.54

$$A_t = \frac{A_{1b}[H^+] + A_{1c}K_{a,2}}{[H^+] + K_{a,2}} . \qquad (5)$$

Because the Bjerrum species with an overall charge of minus one could potentially exist as the two tautomers, **1c** and **1d**, the measured value of the second dissociation step actually represented an average of both the dissociation and the tautomerization steps (Fig. 4). This assumption is reasonable because no time-dependent changes in spectra were observed at high pH (8.00–13.75) indicating that both the dissociation step and the tautomerization step, if present, were rapid.

Octan-1-ol partition coefficients

The apparent partition coefficient [D, equation (1)] of **1** between phosphate buffer (pH 7.4, $\mu = 0.15$) and octan-1-ol was 0.15 at 25.0 ± 0.1°C. The partition coefficient decreased slightly in very acidic ($D = 0.12$ at pH = 1.0) and alkaline solutions ($D = 0.090$, pH = 11.0) consistent with the decreased partition coefficient of those species carrying an overall positive (**1a**) or negative (**1c**) charge. The low partition coefficient of **1** was consistent with the highly polar nature of the compound. However, the relative independence of the partition coefficient on pH may be biopharmaceutically important because it might mean that the oral bioavailability of **1** is independent of pH and thus constant down the length of the gastrointestinal tract.

Aqueous solubility

The aqueous solubility of **1** at 25.0 ± 0.1°C ranged from 1.18 mg ml^{-1} in Sodium Chloride Injection USP to 1.40 mg ml^{-1} in 5% Dextrose Injection USP (Table 1). The solubility of **1** was not substantially higher in solutions containing ethanol. The only solvents in which the solubility was significantly enhanced were 0.1 M HCl and those containing propylene glycol. The pH of 0.1 M HCl is too low to be pharmaceutically useful; however, the enhanced solubility in propylene glycol and water–propylene glycol mixtures may be useful in producing more concentrated and thus less bulky liquid formulations.

Aqueous stability

Kinetics. The assay validation experiments showed that **1** was unstable at pH 1 and 13 and a temperature of 100°C. However, experiments conducted under less extreme conditions (60°C) showed that the **1** was considerably more susceptible to base-catalysed hydrolysis than it was to acid-catalysed degrad-

Table 1
Solubility (S) of **1** in various pharmaceutical solvents at 25°C

Solvent	S^* (mg ml^{-1})	Solvent	S^* (mg ml^{-1})
Water	1.32	5% Dextrose Injection USP	1.40
0.9% Sodium Chloride Injection USP	1.18	ethanol	1.30
Ethanol–water (10:90, v/v)	1.50	ethanol–water (40:60, v/v)	2.21
Propylene glycol–water (40:60, v/v)	4.27	propylene glycol–water (10:90, v/v)	1.36
Propylene glycol	4.27	ethanol–propylene glycol–water (10:40:50, v/v/v)	3.71
HCl (0.1 M, $\mu = 0.15$)	2.40	phosphate buffer (pH 6.5, $\mu = 0.15$)	1.20
Phosphate buffer (pH 7.4, $\mu = 0.15$)	1.25	phosphate buffer (pH 11.0, $\mu = 0.15$)	1.27
Phosphate-buffered mannitol (pH 7.4, $\mu = 0.15$)	1.33	acetate buffer (pH 4.5, $\mu = 0.15$)	1.29

*Mean of two determinations.

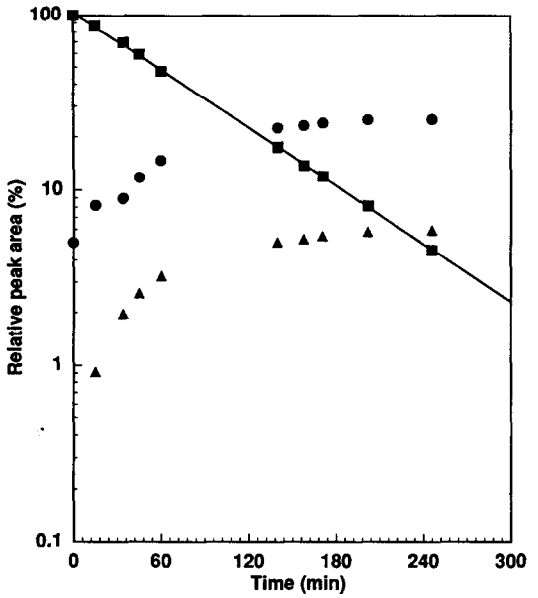

Figure 5
Loss of **1** (squares) and appearance of **2** (circles) and **3** (triangles) in 0.02 M NaOH (60.0 ± 0.2°C, μ = 0.15). The data are expressed as a percentage of the initial area of **1**.

ation. For example, the loss of **1** was 10.1% after 48 h at 60.0 ± 0.1°C in 0.5 M HCl and less than 2% degradation was seen after 48 h at 60.0 ± 0.1°C in phosphate buffer (pH 7.1). In contrast, the half life of **1** in 0.01 M NaOH at 60.0 ± 0.1°C was 110 min.

The kinetics of degradation of **1** in aqueous solutions containing 0.01–0.05 M NaOH were pseudo first order with rate of degradation increasing with increasing hydroxide concentration. The relationship between the pseudo first order rate constant for the loss of **1** (k_{obs}) was non-linear [Fig. 6(a)], consistent with a change in reactivity with change in ionization state of the drug. The relationship between k_{obs} and [OH^-] satisfied the relationship [equation (6)].

$$k_{obs} = k_{OH} f_{1b}[OH^-], \qquad (6)$$

where k_{OH} is the second order rate constant for the specific base catalysed hydrolysis of the neutral form the drug, **1b** and f_{1b} is the fraction of **1b** present. Equation (6) may be restated in terms of the base dissociation constant ($K_{b,2}$) as equation (7)

$$k_{obs} = \frac{k_{OH} K_{b2} [OH^-]}{K_{b2} + [OH^-]}. \qquad (7)$$

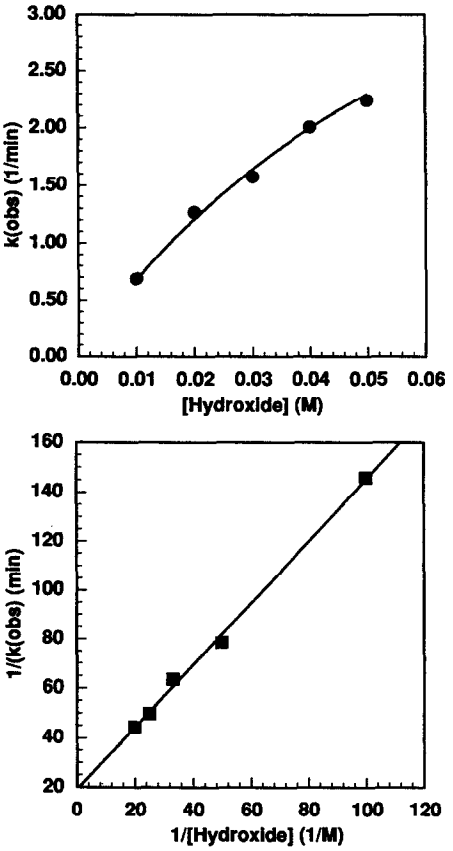

Figure 6
Relationship between the pseudo first order rate constant for the hydrolysis of **1** and the concentration of hydroxide ion at 60.0 ± 0.2°C (μ = 0.15) plotted according to equations (11) (circles) and (13) (squares). The points are experimental and the lines are theoretical.

Equation (7) may be written in its linear form [equation (8)]:

$$\frac{1}{k_{obs}} = \frac{1}{k_{OH}[OH^-]} + \frac{1}{k_{OH} K_{b2}}. \qquad (8)$$

Substituting $K_{a,2}/K_w$ for $K_{b,2}$ gives equation (9):

$$\frac{1}{k_{obs}} = \frac{1}{k_{OH}[OH^-]} + \frac{K_{a2}}{k_{OH} K_w}. \qquad (9)$$

Figure 6(b) shows the linear relationship between the reciprocal of k_{obs} and the reciprocal of the hydroxide concentration at 60.0 ± 0.1°C (μ = 0.15). The values of k_{OH} (1.32×10^{-2} M^{-1} s^{-1}) and $K_{a,2}$ (1.41×10^{-13}) were obtained from the slopes and the intercept terms of equation (9). The value of 12.85 for $pK_{a,2}$ obtained kinetically at 60°C compared

favourably with a value of 12.54 obtained spectrophotometrically at 25°C.

Product analysis and possible mechanism. Figures 2 and 5 show that the hydrolysis of **1** was accompanied by the appearance of two degradation products, **2** and **3**, which were produced in parallel. Compounds **2** and **3** were isolated from a completely degraded sample of **1** by preparative liquid chromatography (Fig. 7). Re-injection of the chromatographic fractions indicated that they were both greater than 95% pure based on peak area measurements (Fig. 7). The solvents were removed by lyophilization and the recovered solids were then analyzed by EI–MS.

Compound **2** was yellowish-orange and similar in appearance to the parent compound, **1**. The UV–vis spectra of **1** and **2** were identical, suggesting that the heterocyclic ring system of **2** was still intact. Compound **3** was a white amorphous powder and the absorption band at 460 nm present in the spectra **1** and **2** was absent, suggesting the opening of the heterocyclic ring system in the conversion of **1** to **3**. A molecular ion for **2** was observed with a *m/z* value of 179, which is one more than the parent compound, **1**. An *m/z* of 179 is consistent with 3-hydroxy-1,2,4-benzotriazine 1,4-dioxide, **2c**, which could be formed by hydroxide ion attack at C3 followed by elimination of ammonia (Fig. 8). Compound **2a** could tautomerize to **2b**, which would then protonate to give **2c** in the acidic mobile phase used in the preparative LC system. The EI- and CI–MS spectra of **3** were uninformative and the further attempts to identify the structure of **3** were unsuccessful. The ^{13}C-NMR and ^{1}H-NMR spectra of **1**, **2** and **3** were uninformative due to lack of useful protons and carbon atoms.

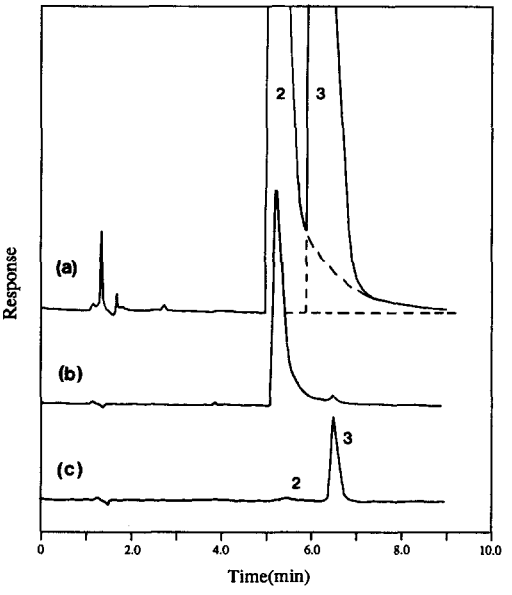

Figure 7
Preparative separation of the main products of alkaline hydrolysis of **1**. Column: ODS Hypersil (5 μm, 150 × 4.6 mm, i.d.); mobile phase acetonitrile–water–triethylamine (96:4:0.4, v/v/v); temperature ambient (22 ± 1°C); flow rate of 1.5 ml min^{-1}. Key: (a) preparative separation; (b) re-injection of peak **2**; (c) re-injection of peak **3**.

Prototype aqueous formulations

The preformulation data presented here suggested that a stable aqueous formulation of

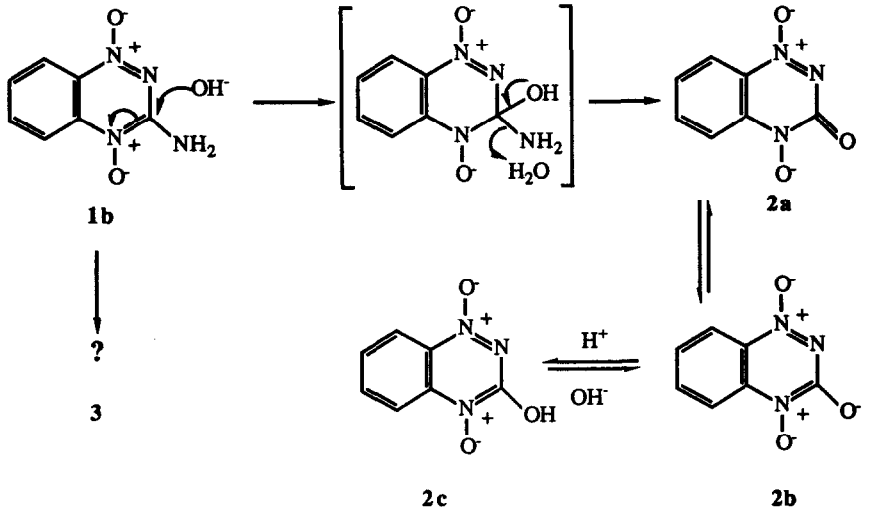

Figure 8
Proposed mechanism for the specific base catalysed hydrolysis of **1**.

Table 2
Three month stability data for **1** in various solvents systems at room temperature (22 ± 1°C)

	Conc. (SD) (mg ml^{-1})*								
	5% Dextrose			Phosphate–mannitol			Phosphate (pH 7.4)		
Time (days)	N_2 light	Air light	Air dark	N_2 light	Air light	Air dark	N_2 light	Air light	Air dark
0	0.504 (0.006)	0.504 (0.006)	0.504 (0.006)	0.501 (0.001)	0.501 (0.001)	0.501 (0.001)	0.507 (0.002)	0.507 (0.002)	0.507 (0.002)
1	0.505 (0.006)	0.492 (0.011)	0.488 (0.004)	0.497 (0.002)	0.486 (0.003)	0.502 (0.007)	0.498 (0.002)	0.496 (0.002)	0.496 (0.004)
2	0.494 (0.011)	0.496 (0.053)	0.501 (0.026)	0.497 (0.019)	0.491 (0.012)	0.488 (0.003)	0.486 (0.007)	0.501 (0.004)	0.493 (0.005)
3	0.500 (0.002)	0.503 (0.002)	0.500 (0.002)	0.493 (0.006)	0.489 (0.008)	0.495 (0.001)	0.494 (0.003)	0.494 (0.002)	0.495 (0.001)
4	0.500 (0.004)	0.499 (0.005)	0.497 (0.005)	0.498 (0.004)	0.494 (0.001)	0.489 (0.005)	0.498 (0.001)	0.498 (0.004)	0.497 (0.002)
7	0.501 (0.003)	0.500 (0.003)	0.500 (0.003)	0.496 (0.004)	0.499 (0.005)	0.493 (0.003)	0.493 (0.003)	0.498 (0.002)	0.493 (0.002)
14	0.500 (0.048)	0.501 (0.002)	0.484 (0.004)	0.494 (0.001)	0.497 (0.003)	0.491 (0.002)	0.496 (0.002)	0.493 (0.002)	0.497 (0.001)
21	0.516 0.002	0.492 (0.038)	0.502 (0.005)	0.514 (0.004)	0.517 (0.004)	0.504 (0.003)	0.521 (0.014)	0.523 (0.014)	0.506 (0.014)
28	0.503 0.004	0.501 (0.001)	0.498 (0.004)	0.495 (0.002)	0.503 (0.005)	0.496 (0.003)	0.502 (0.003)	0.499 (0.003)	0.497 (0.001)
59	0.488 (0.006)	0.498 (0.002)	0.488 (0.006)	0.511 (0.001)	0.502 (0.001)	0.496 (0.002)	0.484 (0.025)	0.503 (0.003)	0.497 (0.003)
120	0.531 (0.006)	0.430 (0.007)	0.473 (0.002)	0.491 (0.000)	0.499 (0.001)	0.491 (0.003)	0.497 (0.003)	0.498 (0.001)	0.494 (0.002)

*$n = 3$.

1 could be prepared at neutral pH. Accordingly three prototype formulations of 0.5 mg ml^{-1} **1** were prepared in 5% Dextrose Injection USP, phosphate buffered mannitol (pH 7.4) and phosphate buffer (pH 7.4). Each formulation was sealed in glass ampoules under either a nitrogen or an air atmosphere and stored at room temperature (22 ± 1°C) either in the dark or under normal, continuous room lighting for 3 months. Table 2 shows that **1** is stable for 120 days at room temperature in the three solvents systems studied and that there was no effect of light or atmospheric oxygen on the stability of the compound.

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