DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 20(11), 1895-1903 (1994)

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STABILITY OF MIXTOXANTRONE HYDROCHLORIDE IN SOLUTION

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ABSTRACT

A stability-indicating reversed-phase high performance liquid chromatographic method was developed for the detection of mitoxantrone HCl and its degradation products under accelerated degradation conditions. The degradation kinetics of mitoxantrone HCl in aqueous solution over a pH range of 1.18 to 7.20 and its stability in propylene glycol- or polyethylene glycol 400-based solutions were investigated. The observed rate constants were shown to follow apparent first-order kinetics in all cases. The pH-rate profile shows that maximum stability of mitoxantrone HCl was obtained at pH 4.01. No general acid or base catalysis from acetate or phosphate buffer species was observed. The catalysis rate constants on the protonated mitoxantrone imposed by hydrogen ion, water and hydroxy ion were determined to be $3.72 \times 10^{-4} min^{-1}$; $5.64 \times 10^{-5} min^{-1}$ and $1.108 \times 10^{-2} min^{-1}$, respectively. The degradation rate constants of mitoxantrone affected by different ionic strength systems. Irradiation with 254 nm UV light at $25 \pm 0.5^{o}C$ was found when compared with the light-protected controls. Incorporation of nonaqueous propylene glycol or polyethylene glycol in the pH 4.01 mitoxantrone solution shows an increase in its stability at $50 \pm 0.5^{o}C$.

INTRODUCTION

Mitoxantrone HCl 1,4-dihydroxy-5-8-bis((((2-hydroxy ethyl)amino)ethyl) amino)-9,10-anthracenedione dihydrochloride was found to possess antiviral, antibacterial, immunomodulatory and substantial antitumor activity[1-16] . It is now believed that the antitumor activity of mitoxantrone is due to its interaction with DNA topoisomerase II. Topoisomerase II is an enzyme responsible DNA helix supercoling which ultimately leads to cell death.

However, as yet, no information concerning the chemical stability of mitoxantrone HCl aqueous solution. The purpose of this investigation were to (1) develop a rapid, prescise and reliable high-performance liquid chromatographic method which would show stability-indicating capability and (2) determine the degradation linetics of mitoxantrone kinetics of mitoxantrone HCl in various pH buffer solution and nonaqueous : water systems under controlled conditions.

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EXPERIMENTAL

Materials :

Mitoxantrone was obtained from Dr.Pong Cheng (National Defense Medical Center, Taipei, Taiwan, R.O.C.). The pentobarbital sodium used as an internal standard was received from Tokyo Chemical (Tokyo, Japan). 1-heptanesulfonic acid sodium was from Sigma Chemical (Louis MO). Sodium phosphate, monobasic; Acetic acid and Sodium acetate were from Wako Pure Chemical (Tokyo, Japan). Sodium phosphate,dibasic; Acetonitrile; Ammonium Acetate; Potassium Chloride; Sodium hydroxide and Hydrochloric acid were from E.Merck (Darmstat, F.R. Germany). All materials were used as received.

Kinetic Studies :

Seven buffer solutions of varying buffer species with constant concentration (0.1M) at fixed ionic strength (I=0.5) were prepared at each specific pH (pH 1.18-2.11 hydrochloric acid, pH 3.43-4.03 acetate buffer and pH 5.54-7.20 phospahte buffer). The mitoxantrone was dissolved by the above buffer solutions to achieve the concentration of 1.0 mg/ml. Three different concentrations (0.05, 0.1 and 0.2M) of buffer species at each pH of the above solutions were prepared at constant ionic strength (I=0.5) atjusted by potassium chloride [17-19]. The solutions were then sealed in type I glass ampules and stored in a dark oven maintained at 50 $\pm$0.1°C for up to 90 hours. Samples were removed from the oven at each time interval and stored immediately in a -20°C freezer until analyzed. Samples were removed from the freezer, equilibrated to room temperature, and mixed in a vortex mixer prior to assay. The pH values were checked (Vision 6071, JENCO Electron LTD,CA) for each sample to detect any significant change of pH at each designated time. Concentrations of mitoxantrone were determined in triplicate by the stability-indicating reversible high-performance liquid chromatographic (HPLC) method. Appropriate dilutions of the samples were made before injecting into the HPLC to insure that the analytical concentrations were within the linear ramge of detection.

High performance Liquid Chromatographic Analysis :

The instrument was equipped with a dual piston pump (model LC-6A, Schimadzu, Kyoto, Japan) set at 242 nm, and a NOVA PAK C18 COLUMN (3.9 mm x 15 cm with 5 µm packing, Waters Associates). The mobile phase containing 25% acetonitrile(V/V) and 75% 0.2M ammonium acetate at pH 4.0 including 5 um 1-heptanesulfonic acid sodium salt. The flow rate of the mobile phase was maintained at 0.8 ml/min. The absorbance of mitoxantrone and its degradation products were recorded using a strip-chart recorder (Model SCI-6A, Schimadzu, Kyoto, Japan) at a chart speed of 0.3 cm/min. The linearity of the calibration curve of peak area ratio versus concentration (mg/ml) for the analytical range between 0.005 to 0.015 mg/ml was excellent, with a correlation coefficient (r) of 0.994 (Y=138.96X-0.0574). The within-day and between-day precision of this HPLC method at a mitoxantrone concentration of 0.01 mg/ml was 0.10 and 1.18% (n=4), respectively. The concentration of mitoxantrone was determined by the method of peak area ratio. The initial concentration was designated as 100%, and all subsequent concentrations were expressed as a percentage of the initial concentration. The stability-indicating nature of this assay id depicted by the chromatograms (Figure 1) where samples of mitoxantrone (1.0 mg/ml in pH 2.11 hydrochloric acid solution was degraded at 50°C for 96 hours. The degradation products eluted separately and were detected without apparent interference with the peak of interest. The relative retention times of mitoxantrone and the internal standard were 3.5 and 11.2 minutes, respectively. The homogeneity of mitoxantrone peak was examined by collecting the eluted mitoxantrone peak from several injections and performing a diode array (model SPD-M¢A, Shimadzu, Tokyo, Japan) spectral overlay analysis in the UV range of 191.0 nm to 401.0 nm. No difference (curve fit 0.99) in the UV spectral between the eluted and pure drug samples suggests the absence of any degrade or exogenous impurities eluting under the peak of interest. The sensitivity of the reported produces was 50 ng/ml.

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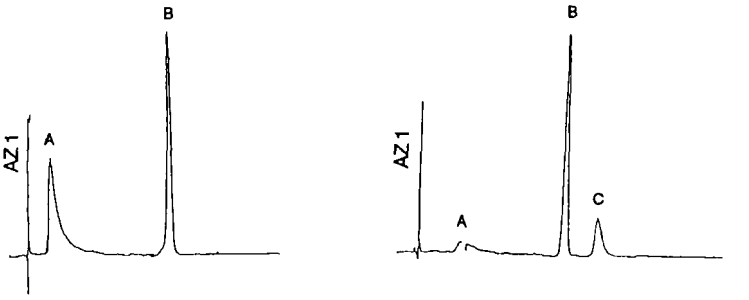

FIGURE 1

HPLC chromatogram of mitoxantrone HCl (1.0mg/ml) in pH 2.11 hydrochloric acid solution (A) immediately after preparation, and (B) after 96 hours of storage at 50 ± 0.5°C. Key: (A) mitoxantrone HCl; (B) pentobarbital sodium; and (C) degradation product.

Buffer Effect Studies :

Three buffer solutions of different total buffer concentrations (0.05,0.1 and 0.2M) and constant ionic strength (I=0.5) were used to study the catalytic effect of buffer species on the degradation of mitoxantrone at each species pH and temperature (50 ± 0.5°C). Acetate buffer at pH 3.43,4.01 and phosphate buffer at pH 5.54, 6.81 and 7.20 were evaluated. The final concentration of mitoxantrone was 1.0 mg/ml.

Salt effect Studies :

To test the effect of ionic strength on the degradation of mitoxantrone (1.0 mg/ml), buffer solutions of various total ionic strength (I=0.1,0.3,0.5, 0.7 and 0.9) with constant buffer species concentration at fixed pH were prepared. A stability study of these solutions at 50 ± 0.5°C was conducted. A pH 3.11 acetate buffer and a pH 7.20 phosphate buffer, both with 0.1M total buffer concentration, were studied.

Temperature Effect Studies :

Solutions of 1.0 mg/ml of mitoxantrone in 0.1M acetate buffer at pH 4.01 and constant ionic strength (I=0.5) were prepared. The temperature dependence of the degradation of mitoxantrone was studied at 40 ± 0.5°C, 60 ± 0.5°C and 70 ± 0.5°C.

Solvent Effect Studies :

Solutions of 1.0 mg/ml of mitoxantrone in different propylene glycol : water or polyehylene glycol 400 : water systems were prepared. All solutions were buffered to pH 4.01 with a 0.1M acetate buffer at constant ionic strength (I=0.5). These solutions were then stored in a constant temperature oven at 50 ± 0.5°C.

Photolysis :

A solution of mitoxantrone at a concentration of 1.0 mg/ml in the pH 4.01 acetate buffer solution at a constant ionic strength (I=0.5) and total buffer concentration (0.1M) were prepared. The solution was placed under a 254 nm UV light of 150 µw/cm intensity at a distance of 30 cm. A control group wrapped with aluminum foil to protect the solution from UV irridation was also studied under the same conditions. The experiment was done at 25 ± 0.5°C.

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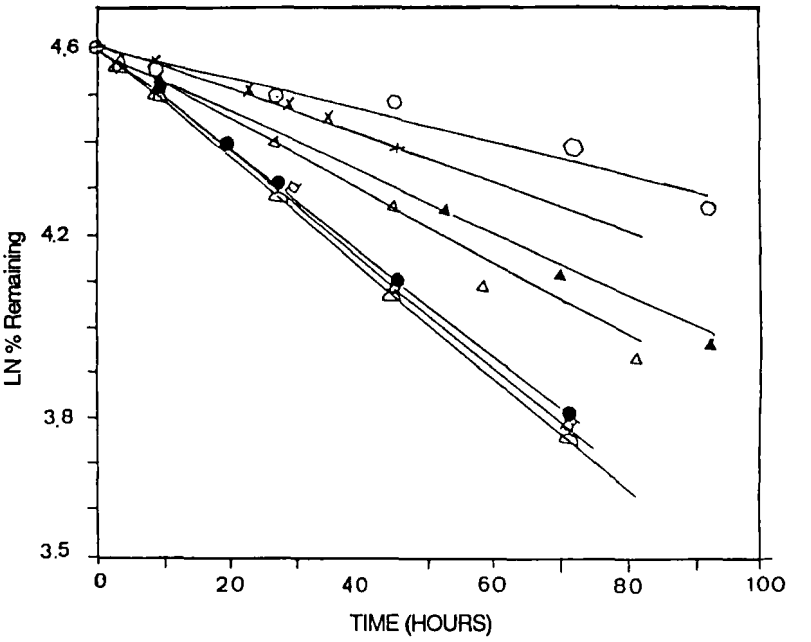

FIGURE 2

Psedo first-order degradation kinetics of mitoxantrone HCl in various buffer solutions (0.1 M) of different pH at 50 ± 0.5°C and I=0.5.
Key : (⊂)pH 1.18 ; (△)pH 2.11 ; (✕)pH 3.43; (○)pH 4.03; (▲)pH 5.54 (●)pH 6.81; (◇)pH 7.20.

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RESULTS AND DISCUSSION

Order of Reaction and Observed Rate Constants :

Figure 2 illustrates typical plots of the stability of mitoxantrone HCl in different pH buffers at 50 ± 0.5°C. The results indicate that the degradation of mitoxantrone HCl in aqueous solution follows apparent first-order kinetics at constant pH, temperature, ionic strength, and buffer species concentrations[20-25]. The rate constants were obtained from the slopes of the semilog plot by statistical regression with correlation coefficients (r) greater than 0.98.

Effect of Buffer Concentration :

No significant difference was observed (Table 1) for the degradation rate constants of mitoxantrone HCl under three different concentrations (0.05,0.1 and 0.2M) of the same buffer species (acetate, phosphate) at each species pH solution over the range of 3.43 to 7.20. The general acid-base catalysis of acetate and phosphate buffers on the degradation of mitoxantroneHCl was not significant[26,27];however, rate constants at zero buffer concentration were still determined at each pH by extrapolation from the data.

pH-Rate Profile :

A pH-rate profile of mitoxantrone HCl at zero buffer concentration was obtained by plotting the logarithmic rate constants versus pH of solutions at constant ionic strength of 0.5 and temperature of 50 ± 0.5°C(Figure 3). At

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TABLE 1

Degradation Rate Constant of Mitoxantrone HCl in Different Buffer Species Concentrations (0.05,0.1 and 0.2M) of Various pH Solution under Constant Ionic Strength (I=0.5) and Storage Temperature (50 ± 0.5°C) Conditions.

pH	K_{obs} (10^4 min^{-1})*		
	0.05 M	0.1 M	0.2 M
3.43	0.7885 ± 0.0017	0.7246 ± 0.0020	0.7586 ± 0.0015
4.01	0.5623 ± 0.0031	0.5608 ± 0.0027	0.6166 ± 0.0035
5.54	1.0476 ± 0.0029	1.0237 ± 0.0030	1.0471 ± 0.0013
6.81	1.7378 ± 0.0057	1.7380 ± 0.0041	1.7418 ± 0.0065
7.20	2.1379 ± 0.0047	2.4551 ± 0.0036	2.1022 ± 0.0016

*mean ± s.d. (n=3)

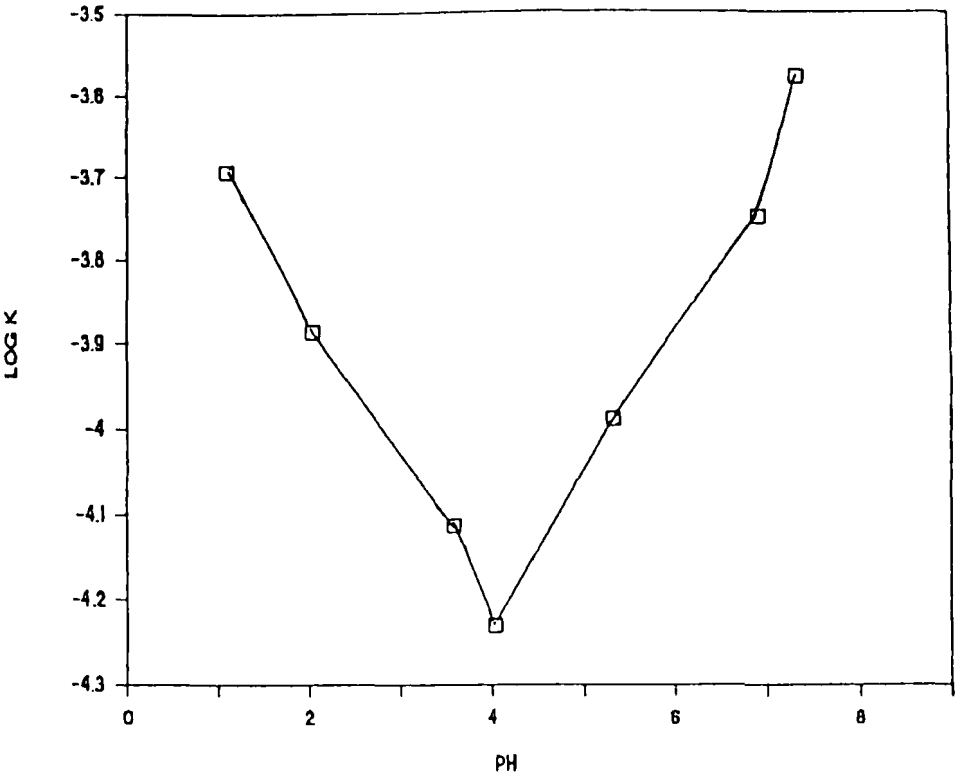

FIGURE 3

The pH-rate profile of mitoxantrone HCl in aqueous solution under zero buffer concentration and constant ionic strength (I=0.5) at 50 ± 0.5°C.

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TABLE 2

Degradation Rate Constants of Mitoxantrone HCl in Different Ionic Species Concentrations (0.1,0.3,0.5,0.7) of pH 3.11 acetate solutions under Constant Buffer Species Concentration (0.1M) and Storage Temperature ($50 \pm 0.5^{o}C$) conditions.

Ionic Strength (I)	$K_{obs} \times 10^{4}$ (min^{-1})*
0.1	0.2399 ± 0.0031
0.3	0.5539 ± 0.0017
0.5	0.6128 ± 0.0014
0.7	0.6732 ± 0.0021

* mean $\pm$ s.d. (n=3)

TABLE 3

Degradation Rate Constants of mitoxantrone HCl in Different Ionic Species Concentrations (0.3,0.5,0.7,0.9) of pH 7.20 phosphate solution under constant Buffer Species Concentration (0.1M) and Storage Temperature ($50 \pm 0.5^{o}C$) conditions.

Ionic Strength (I)	$K_{obs} \times 10^{3}$ (min^{-1})*
0.3	0.5046 ± 0.0014
0.5	0.4646 ± 0.0017
0.7	0.4351 ± 0.0025
0.9	0.3711 ± 0.0011

* mean $\pm$ s.d. (n=3)

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pH < 4.03, the degradation of mitoxantrone HCl is described primarily by the catalytic effect of specific acid. At pH 4.03, the pH independence region, it was primarily due to the catalysis of water. At pH > 4.03, catalysis of hydroxyl ion became predominant. A general hypothetical rate equation for mitoxantrone HCl degradation as a function of pH can be written as follows :

$$K_{obs} = K_o + K_{H^+} [H^+]^n + K_{OH^-}[OH^-]^m \quad \text{-------(1)}$$

where K_{obs} is the overall observed rate constant; K_o is the water catalysis rate constant; K_{H^+} is the specific acid catalysis rate constant and K_{OH^-} is the specific base catalysis rate constant, and n,m are the order reaction with respect to $[H^+]$ and $[OH^-]$ according to eq.1.,a plot of K_{obs} against hydrogen ion concentration should yield a slope of K_{H^+} and an intercept of K_o in strong acid solutions. The K_o and K_{H^+} was determined to be $K_o = 5.64 \times 10^{-5}$ min^{-1}, $K_{H^+} = 3.72 \times 10^{-4}$ min^{-1}. At high pH solution (pH4.03-7.20), a plot of ($K_{obs} - K_o$) against hydrogen ion concentration should yield $K_{OH^-} = 1.108 \times 10^{-2}$ min^{-1} .The value of n and m was detrermined to be 0.35 and 0.3009.

Effect of Salt Concentration :

The results of different ionic strength of mitoxantrone HCl in the pH 3.11 acetate and pH 7.20 phosphate buffer solutions at $50 \pm 0.5^{o}C$ are listed in Table 2,3. It was observed that the degradation rate constants of mitoxantrone HCl affected by different ionic strength systems[28] .

Effect of Temperature :

The temperature dependence of the degradation of the degradation rate of mitoxantrone HCl (1.0mg/ml) in pH 4.01 acetate buffer (0.1M) at constant ionic

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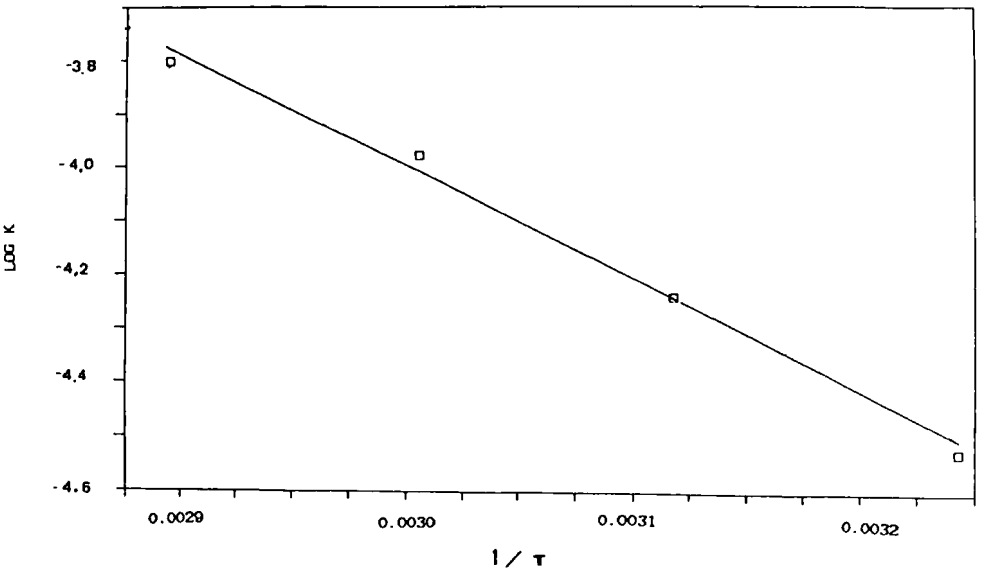

FIGURE 4

Arrhenius plot of the degradation of mitoxantrone HCl(1.0 mg/ml) in pH 4.01 acetate buffer (0.1 M) solution and at constant ionic strength of 0.5.

TABLE 4

Stability of Mitoxantrone HCl in Polyethylene Glycol 400 or propylene glycol : water Solvent Systems at pH 4.01 and 50 ± 0.5°C.

Solvent Ratio	K_{obs} (hr^{-1})*	
	propylene glycol : water	PEG 400 : water
20 : 80	0.0933 ± 0.0016	0.08287 ± 0.0021
40 : 60	0.007121 ± 0.0006	0.06878 ± 0.0003
60 : 40	0.005435 ± 0.0007	**
80 : 20	0.001353 ± 0.0004	**

* mean ± s.d. (n=3); ** not determined

TABLE 5

Effect of UV light on the Stability of Mitoxantrone HCl in pH 4.01 acetate Buffer Solution (0.1M) at I=0.5 and 25 ± 0.5°C.

Time (hr)	% Remaining*	
	light-protected	light-exposed
42	97.93 ± 4.01	96.07 ± 3.23
186	89.27 ± 2.71	60.18 ± 0.98
235	86.09 ± 2.83	55.89 ± 3.28
378	78.99 ± 2.71	52.14 ± 1.32

* mean ± s.d.(n=3)

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strength (I=0.5) was determined by plotting the log of degradation rate versus 1/temperature, as seen in Figure 4. The energy of activation in this solution was determined to be 13.4 kcal/mole from the slope[29,30]

Effect of Nonaqueous Solvents :

The effects of nonaqueous solvents on the degradation of mitoxantrone HCl using different ratios of propylene glycol : Water or polyethylene glycol : water as the media are listed in Table 4. The degradation rate constants decreased when the content of either propylene glycol or polyethylene glycol in the solution was increased. The reaction mechanism of mitoxantrone HCl degradation in solutions containing propylene glycol or polyethylene glycol is not yet known; however, the stabilization of mitoxantrone HCl degradation might be possible due to complicated factors such as dielectric constants, surface tension, viscosity, activity coefficient of mitoxantrone HCl and its reansition products, etc [31,32] .

Effect of UV light :

Table 5 shows the data for the stability of mitoxantrone HCl (1.0mg/ml) in pH 4.01, 0.1M acetate buffer solution at I=0.5 and 25 $\pm$ 0.5°C under UV (r=254nm) irradiation and light-protected conditions. UV irradiation effect did accelerate the degradation processes of mitoxantrone HCl in the light-exposed sample in comparison with the same experimental condition.

ACKNOWLEDGEMENT

This work was supported by a grant from The National Science Council, Republic of China. (NSC 78-0412-B016-59).

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