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STABILITY OF HI-6 IN SOLUTION

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ABSTRACT

A stability-indicating reversed-phase high performance liquid chromatographic method was developed for the detection of HI-6 and its degradation products under accelerated degradation conditions. The degradation kinetics of HI-6 in aqueous solution over a pH range of 1.14 to 5.54 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 HI-6 was observed in the pH range 2.0 to 3.0 No effect of general acid/base catalysis of HI-6 was noted in the study. The degradation rate constants of HI-6 affected by different ionic strength systems . Irradiation with 254 nm UV light at 25 ± 0.5°C was found when compared with the light-protected controls. Incorporation of nonaqueous propylene glycol or polyethylene glycol 400 in the pH 3.10 HI-6 solution show an increase in its stability at 70 ± 0.5°C.

INTRODUCTION

HI-6 (pyridinium, 1-[[[-4(aminocarbonyl) pyridiniol] methoxyl] methyl]-2-(hydroxylimino)-dichloride, belongs to a series of bisquaternary pyridinum oximes appears to offer promise in the treatment of poisoning included by a variety of organophosphate cholinesterase inhibitors. It has been widely tested in variety of species and found effective, particularly against soman, an extremely potent, oxime-resistant organophosphate[1-9].

It antidote qualities for the treatment of organophosphate pesticide are not well known. HI-6 and other H-series oximes, apparently act in a number of ways : reactivation of acetylcholinerase, blockage of ganglia and muscarinic receptors, stimulation of vasopressor and respiratory center receptors[10-13] . However, as yet, no information concerning the chemical stability of HI-6 aqueous solution. The purpose of this investigation were to (1) develop a rapid, precise and reliable high performance liquid chromatographic (HPLC) method which show stability-indicating capacity, and (2) determine the stability of HI-6 in various pH buffer solutions and nonaqueous : water systems under controlled conditions.

EXPERIMENTAL

Materials -- HI-6 was obtained from our laboratory (National Defense Medical center, Taipei,Taiwan,R.O.C.). 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; potassium chloride; sodium hydroxide and hydrochloric acid were from E. Merck (Darmstadt, F.R.Germany). Propylene glycol was from J. T. Baker Chemical (Philipburg, NJ). Polyethylene glycol 400 was from Fisher Scientific (Fair Lawn, NJ).

Kinetic Studies -- Eight buffer solutions of varying buffer species with constant concentration (0.05M) at fixed ionic strength (I=0.5) were prepared at each specific pH (pH 1.14-2.54 hydrochloric acid, pH 3.10-5.54 acetate buffer). The HI-6 was dissolved by the above buffer solutions to achieve the concentration of $3.0X10^{-5}M$. The solutions were than sealed in the type I glass ampules and stored in a dark oven maintained at $70 \pm 0.5^{o}C$ for up to 180 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 HI-6 were determined in triplicate by the stability-indicating HPLC method.

High Performance Liquid Chromatographic Analysis -- The instrument was equipped with a single piston pump (model LC-6A, Shimadzu, kyoto, Japan) set at 305 nm and LichroCART C_{18} column 3.9mmX15cm with 5μm packing, E. Merck. The mobile phase containing 25% acetonitrile (V/V) and 80% 0.01M 1-heptanesulfonic acid sodium salt, pH was adjusted to 3.2 by acetic acid. Its flow rate was maintained at 1.0 ml/min. The absorbance of HI-6 and its degradation products were recorded using a strip-chart (model C-R6A, Shimadzu, Kyoto, Japan) at a chart speed of 0.1 cm/min. The linearity of the calibration curve of peak height versus concentration (μg/ml) for the analytical range between 2.2 to 5.6 μg/ml was excellent, with a correlation coefficient (r) of 0.999. The intra- and inter-day precision of this HPLC method at a HI-6 concentration of 22.4 μg/ml was 2.51 and 0.14% (n=3), respectively. The stability-indicating nature of this assay is depicted by the chromatogram (Figure 1) where samples of HI-6 (22.4 μg/ml) in pH 2.54 hydrochloric acid solution was degraded at $70 \pm 0.5^{o}C$ for 100 hours. The degradation products eluted separately and were detected without apparent interference with the peak of interest. The relative retention time of HI-6 is 14.5 minutes. The homogenesity of HI-6 peak was examined by performing a diode array (model SPD-M6A, Shimadzu, kyoto, Japan) spectral overlay analysis in the UV range of 191.0 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 procedures was 10 ng/ml. The stability of HI-6 at each designated storage time interval in this study was expressed as a percentage of its initial concentration (100.0% at time zero).

Buffer Effect Studies -- Three buffer solutions of different buffer concentrations (0.05, 0.1 and 0.2M) and I=0.5 were used to study the catalysis effect of buffer species on the degradation of HI-6 at each species pH and temperature ($70 \pm 0.5^{o}C$). Acetate buffer at

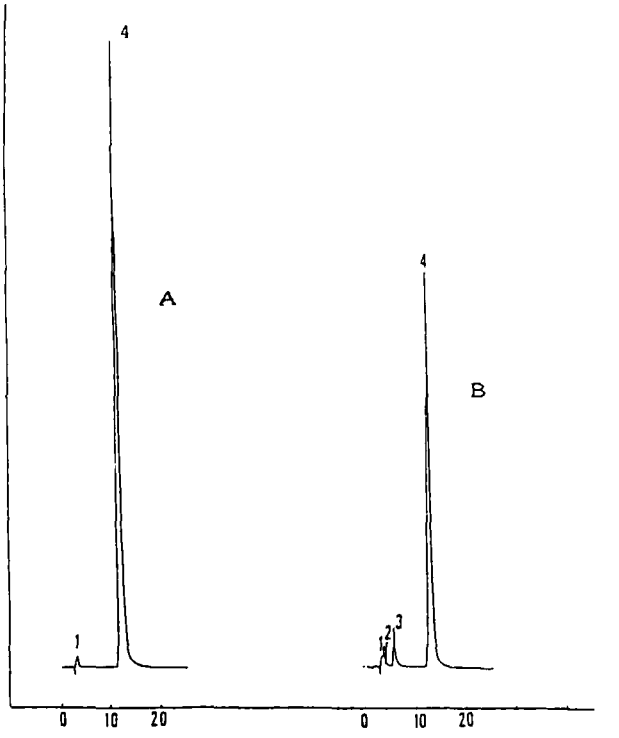

Figure 1
HPLC chromatogram of HI-6 ($3.0X10^{-5}M$) in pH 2.54 hydrochloric acid solution (A) immediately after preparation, and (B) after 100 hours of storage at $70 \pm 0.5°C$.
Key : (1) solvent; (2,3) degradation products; (4) HI-6

pH 3.10, 4.85 and 5.54 were evaluated. The final concentration of HI-6 was $3.0X10^{-5}M$.

Salt Effect Studies -- To test the effect of ionic strength on the degradation of HI=6 ($3.0X10^{-5}M$) buffer solutions of various total ionic strength (0.05, 0.3 and 0.7) with constant buffer species concentration at fixed pH were prepared. A stability study of these solutions at $70 \pm 0.5°C$ was conducted. A pH 3.10 acetate buffer with 0.05M total buffer concentration was studied.

Temperature Effect Studies -- Solutions of $3.0X10^{-5}M$ of HI-6 in 0.05M acetate buffer at pH 3.10 and I=0.5 were prepared. The temperature dependence of the degradation of HI-6 was studied at 50, 60, 70 and $90 \pm 0.5°C$.

Solvent Effect Studies -- Solutions of $3.0X10^{-5}M$ of HI-6 in different propylene glycol:water or polyethylene glycol 400:water systems were prepared. All solutions were buffered to pH 3.10 with a 0.05M acetate buffer at I=0.5. These solutions were then stored in a constant temperature oven at $70 \pm 0.5°C$.

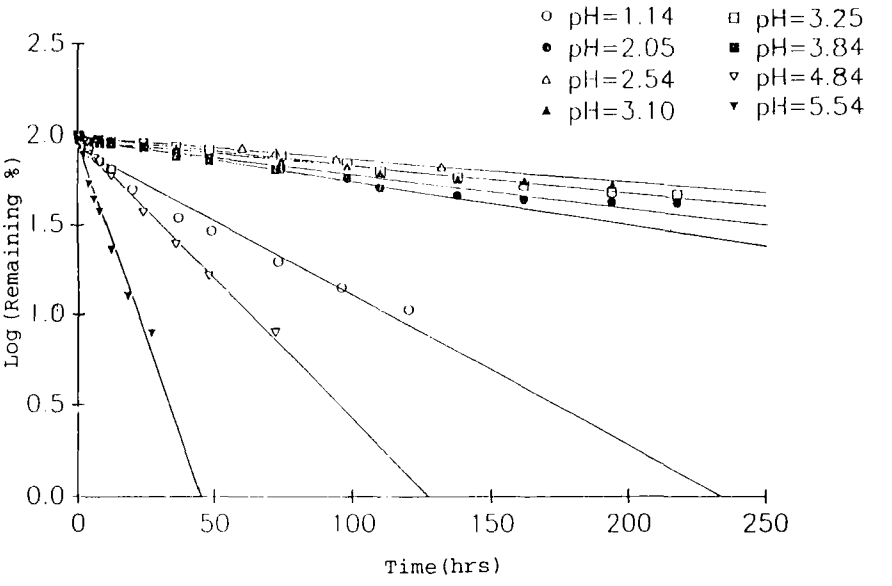

Figure 2
Pseudo-first order degradation kinetics of HI-6 in constant buffer solution (0.05M) of different pH at 70±0.5°C and I=0.5.

Table 1 -- Degradation rate constants of HI-6 in different buffer species concentration (0.05, 0.1 and 0.2M) of pH 3.10 solution under I=0.5 and storage temperature (70±0.5°C) conditions.

	K_{obs} (hr^{-1})*		
pH	0.05M	0.1M	0.2M
3.10	0.00156 ±0.00010	0.00130±0.00011	0.00149±0.00020
4.85	0.0206 ±0.0003	0.0193 ±0.0009	0.0223 ±0.0011
5.54	0.003621±0.0003	0.03924±0.0005	0.04069±0.0009

* mean±s.d. (n=3)

Photolysis- A solution of HI-6 at a concentration of $3.0X10^{-5}$M in the pH of 3.10 acetate buffer solution at I=0.5 and total buffer concentration (0.05M) were prepared. The solution was placed under a 254 nm UV light of 150 uwatt/cm intensity at a distance of 30 cm. A control group wrapped with aluminum foil to protect the solution from UV irradiation was also studied under the same conditions. The experiment was done at 25±0.5°C.

RESULTS AND DISCUSSION

Degradation Kinetics -- Stability profile for HI-6 in 0.1M buffer solution at 70±0.5°C are shown in Figure 2. The linear relationship between logarithmic % remaining and

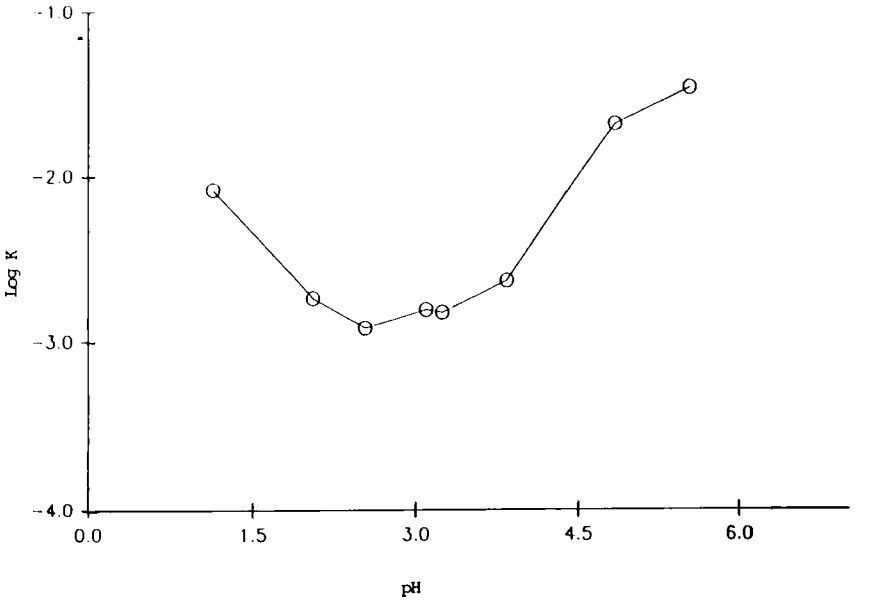

Figure 3
pH-rate profile of the degradation of HI-6 at constant buffer concentration (0.05M), I=0.5 and 70±0.5°C.

Table 2 -- Degradation rate constants or HI-6 in different ionic species concentrations (0.05, 0.3 and 0.7)of pH 3.10 solutions under constant buffer species concentration (0.05M) and storage temperature (70±0.5°C) conditions.

Ionic Strength (I)	K_{obs} (hr^{-1})*
0.05	0.001663±0.00014
0.3	0.002044±0.00021
0.7	0.002392±0.00008

*mean±s.d. (n=3)

storage time indicated pseudo-first degradation kinetics for HI-6 in aqueous solution, the degradation rate constants was determined from the slope of the semilog plot by statistical regression with correlation coefficient (r) greater than 0.98.

Buffer Species -- No significant difference was observed (Table 1) for the degradation rate constants of HI-6 under three different concentrations (0.005, 0.1 and 0.2M) of the same buffer species (acetate, phosphate) at each species pH solution over the range of 3.10 to 5.54. The general acid/base catalysis of acetate and phosphate buffers on the degradation of HI-6 was not significant.

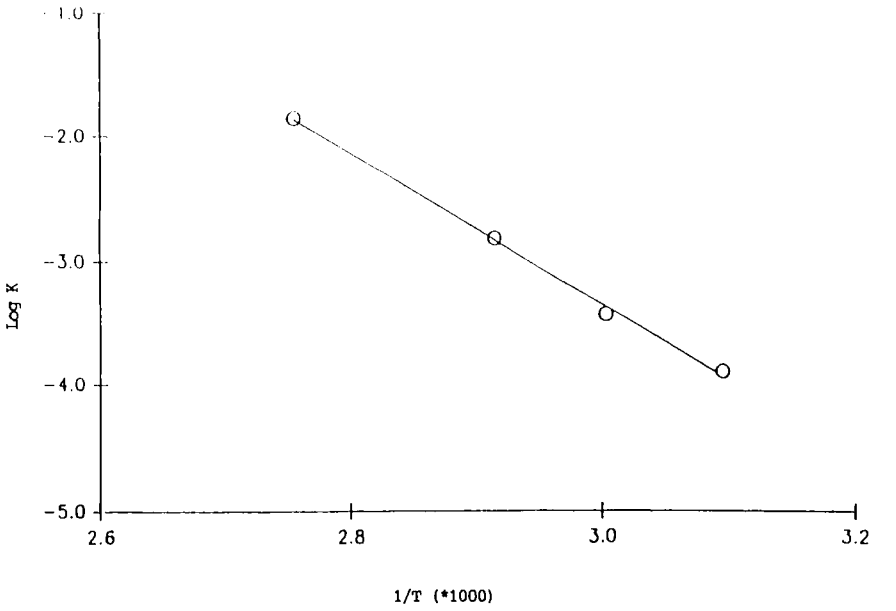

Figure 4
Arrhenius plot of the degradation of HI-6 ($3.0X10^{-5}M$) in pH 3.10 acetate buffer (0.05M) solution and at I=0.5.

Table 3 -- Stability of HI-6 in polyethylene glycol. 400:water and propylene glycol:water solvent systems at pH 3.10 and 70±0.5°C

solvent ratio	$K_{obs}(hr^{-1})$*	
	propylene glycol:water	P.E.G. 400#:water
20:80	0.0006525±0.0000013	0.0008372±0.0000011
40:60	0.0004350±0.0000017	0.0007077±0.0000020
60:40	0.0002817±0.0000009	0.0005701±0.0000012
80:20	0.0002468±0.0000018	0.0003805±0.0000019

* mean±s.d. (n=3); # polyethylene glycol 400

pH-rate profile -- The effect of pH on the degradation of HI-6 in aqueous solution under zero buffer concentration and I=0.5 at 70±0.5°C is shown as the plots of log K_{obs} versus pH and is depicted on Figure 3. In the pH range 2.0 to 3.0 under these study conditions, HI-6 was found to be more stable than in other regions.

Salt Effect -- The results of different ionic strength effects on the stability of HI-6 in the pH 3.10 acetate buffer solutions at 70±0.5°C are listed in Table 2. It was observed

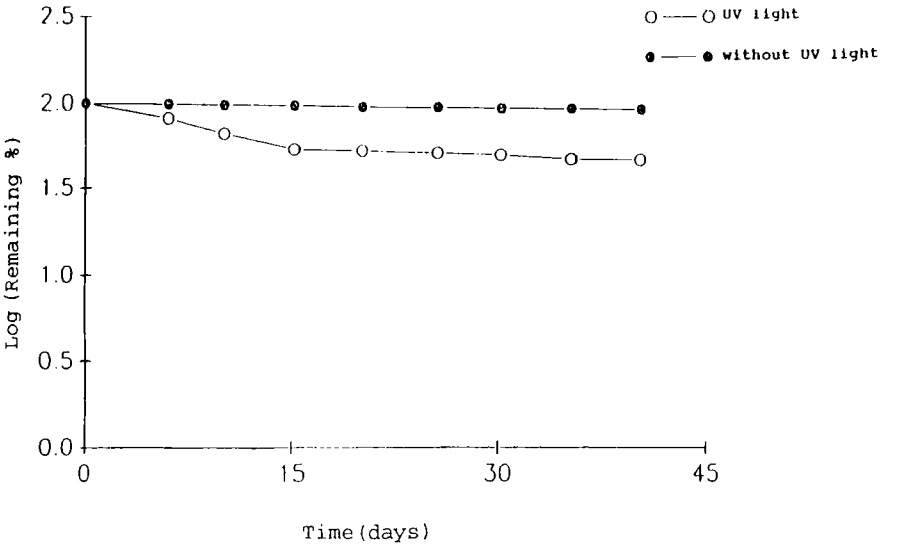

Figure 5
UV light on the stability of HI-6 in pH 3.10 acetate buffer solution (0.05M) at I=0.5 and 25 ± 0.5°C.

that the degradation rate constants of HI-6 in different ionic strength systems increased as the ionic strength increased.

Temperature Effect -- The temperature dependence of the degradation of the rate of HI-6 ($3.0X10^{-5}M$) in pH 3.10 acetate buffer (0.05M) at I=0.5 was determined by plotting the log. of degradation rate constant versus 1/temperature (°K), as seen in Figure 4. The energy of activation in this solution was determined to be 27.59 kcal/mole from the slope.

Solvent Effect -- The results of propylene glycol/polyethylene glycol 400 effects on the stability of HI-6 in the pH 3.10 acetate buffer solutions at 70 ± 0.5°C are listed in Table 3. It was observed that the degradation rate of HI-6 in propylene glycol and polyethylene glycol 400:water systems decreased as the content of propylene glycol and polyethylene glycol 400 increased. No explanation is proposed related to the degradation mechanism of HI-6 in these solvent systems due to complicated factors, such as dielectric constant, surface tension, viscosity, activity coefficient of HI-6 and its transition products, etc.

Photolytic Effect -- The stability results of HI-6 irradiation is shown in Figure 5. UV irradiation effect did accelerate the degradation processes of HI-6 in the light-expose sample in comparison with those which were light-protected under otherwise the same experimental conditions.

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