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STABILITY OF VANCOMYCIN HYDROCHLORIDE SOLUTIONS AT VARIOUS PH VALUES AS DETERMINED BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A stability-indicating HPLC assay method for the quantitation of vancomycin HCl has been developed. The developed method was used to study the effect of pH, phosphate buffer concentration and ionic strength on the stability of vancomycin HCl. The pH-rate profile curve showed 3 regions, acid-catalyzed, general base-catalyzed and the pH-independent region. The region of maximum stability was between pH 3.0 to about 5.7. On increasing the buffer concentration, phosphate ions hastened the decomposition of vancomycin HCl, the effect being more pronounced at pH 6.85 than at pH 4.43. In the pH region of 1-3, the decomposition was catalyzed by H^+ and between pH 3-7 by HPO_4^{2-}, $H_2PO_4^-$, and OH^-. An increase in the ionic strength decreased the rate of decomposition.

INTRODUCTION

Vancomycin (Fig 1) is a tricyclic glycopeptide antibiotic that is used primarily against gram positive bacteria. Despite its isolation as early as 1956, very little information is available about the physicochemical properties of vancomycin. Recently, the drug has gained importance because of its peptide structure. Geary and Schlameus investigated its use as a model for the oral delivery of peptides (1). In another study, Takacs-Novak reported the acid-base properties of vancomycin and the pH-dependent protonated species distribution (2).The USP-NF method for the quantitation of vancomycin is based on microbiological assay (3) and therefore the results can vary upto 25%. A stability-indicating high performance liquid chromatography method for the quantitation of vancomycin HCl was developed. The objective of this investigation was to study the effect of pH, phosphate buffer

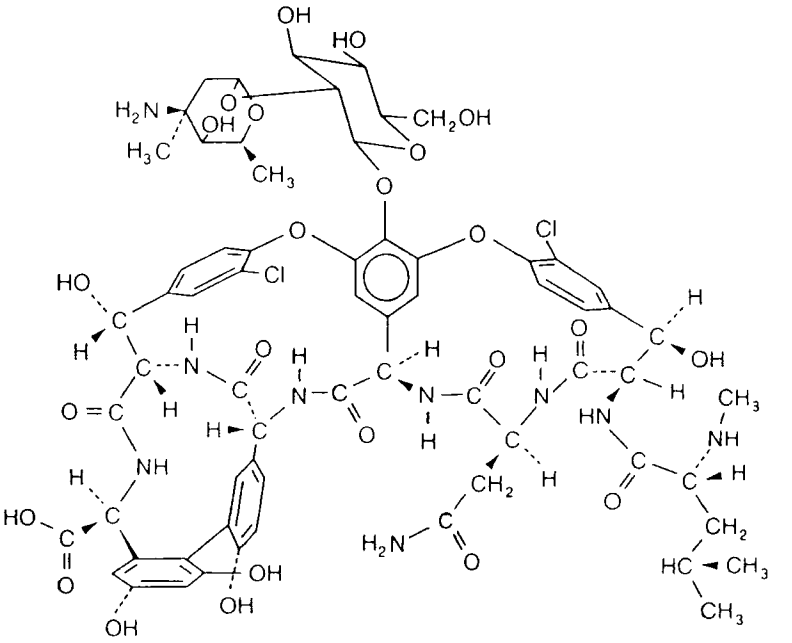

Fig 1: Structure of vancomycin

concentration and ionic strength on the stability of vancomycin HCl using a stability-indicating HPLC method.

EXPERIMENTAL SECTION

Chemicals and Reagents: All the chemicals and reagents were either USP-NF or ACS grade and used without further purification. Vancomycin HCl powder (Abbott Labs) and cefazolin sodium powder (Marsam Pharmaceuticals) were from the commercial lots.

High-Performance Liquid Chromatography: A Waters ALC 202 HPLC system (Waters Associates, Milford, MA) equipped with a universal injector (Rheodyne Model 7125), a multiple wavelength detector (Schoeffel's SF 770, Applied Biosystems) and a recorder (Omniscribe 5213-12, Houston Instruments, TX) was used. A micro C_{18} column (Whatman, 25cm x 4.5mm i.d.) was the stationary phase. The mobile phase contained 10% (v/v) acetonitrile and 0.03%v/v acetic acid in 0.02 M potassium dihydrogen phosphate in water (pH 3.9). The flow rate was 1.6 ml/min, and the sensitivity was 0.1 AUFS at 230 nm, the chart speed was 30.5 cm/h and the temperature ambient. The injection volume was 20 µl.

Preparation of Stock and Standard Solutions: The stock solutions of vancomycin HCl (1.0 mg/ml based on free vancomycin) and the internal standard, cefazolin sodium (1.0 mg/ml) in water were prepared. These solutions were further diluted with water as needed. The most commonly used standard solution contained 80 μg/ml of vancomycin and 120 μg/ml of cefazolin sodium (internal standard).

Preparation of Solutions for Stability Studies: All the solutions were prepared using a simple solution method and are listed in Table 1. After the zero-day data (assays, physical appearances, and pH values), the solutions were stored in amber-colored glass bottles at room temperature. The data were recorded again at the appropriate intervals.

Assay Solutions: Aliquots of all the stability samples were diluted with water so that the final concentration of vancomycin was 80 μg/ml (based on the label claim). The concentration of the internal standard in each solution was 120 μg/ml.

Assay Procedure and Calculations: A 20 μl quantity of the assay solution was injected into the chromatograph using the conditions described. For comparison, an identical volume of the standard solution was injected. Since the ratio of peak heights were related to the concentrations of the drug (range tested 20-160 μg/ml), the results were calculated using a simple equation:

$$\frac{(Rph)_a}{(Rph)_s} \times 100 = \text{percent of the label claim found,}$$

where $(Rph)_a$ is the ratio of the peak heights of drug to internal standard of the assay solution and $(Rph)_s$, that of the standard solution.

RESULTS AND DISCUSSION

Assay Method: The developed HPLC assay method is accurate and precise with a relative percent standard deviation of 2.5% based on 5 readings. Linearity was obtained with a correlation coefficient of 0.999 for the concentration range 20-160 μg/ml of vancomycin. The developed method appear to be stability-indicating since the decomposed peaks separated from the parent peak in the chromatogram (Figure 2A). The vancomycin HCl powder from the commercial lot was not completely pure since a small peak (# 5) also appeared in the chromatogram of a standard solution (Figure 2B).

Effect of Phosphate Concentration at Room Temperature: With increase in buffer concentration, phosphate buffer hastened the decomposition of vancomycin at pH values of 5.55 and 6.85. This phosphate catalysis was insignificant at pH 4.43 (Table 1, #1-9 and Figure 3). The slopes of the rate constants versus phosphate concentration at pH values of 4.43, 5.55 and 6.85 were 0.0037, 0.02, and 0.041

TABLE 1

List of aqueous solutions of vancomycin HCl (1.0 mg/ml) prepared for stability studies.

Solution No.	pH (±0.05)	Phosphate buffer conc. (M)	Ionic Strength[a]
1.	4.43	0.05	0.35
2.	4.43	0.10	0.35
3.	4.43	0.20	0.35
4.	5.55	0.05	0.35
5.	5.55	0.10	0.35
6.	5.55	0.20	0.35
7.	6.85	0.05	0.35
8.	6.85	0.10	0.35
9.	6.85	0.20	0.35
10.	5.55	0.1	0.10
11.	5.55	0.1	0.40
12.	5.55	0.1	0.70
13.	1.05	0.1 N HCl[b]	0.35
14.	1.3	0.05N HCl[b]	0.35
15.	2.3	0.1	0.35
16.	2.95	0.1	0.35
17.	3.35	0.1	0.35
18.	3.85	0.1	0.35
19.	4.3	0.1	0.35
20.	5.55	0.1	0.35
21.	5.8	0.1	0.35

[a] Adjusted with KCl based on pH of the buffer solution.
[b] No phosphate buffer was used.

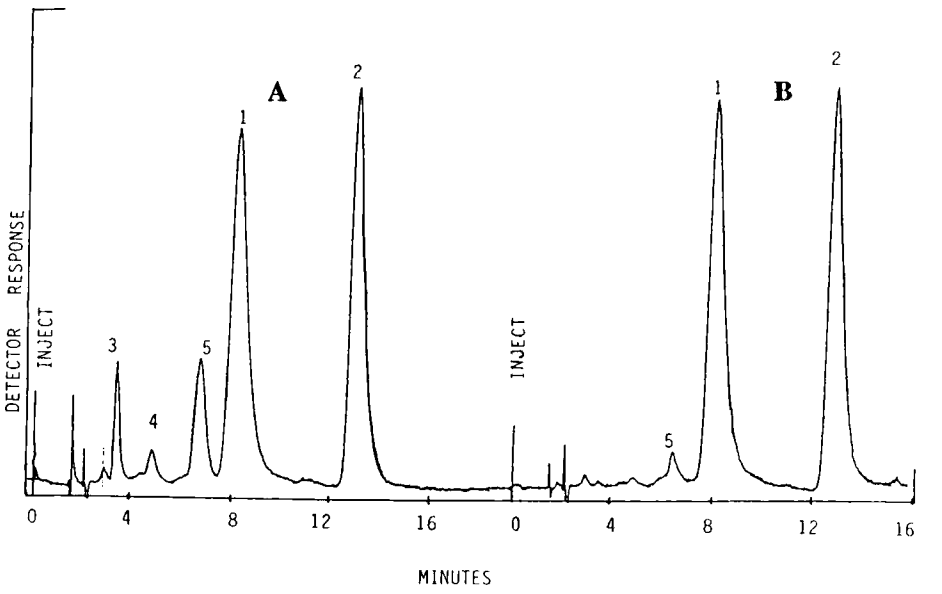

Fig 2: Sample Chromatograms; Peaks 1-5 are from vancomycin, cefazolin sodium (the internal standard), and the degradation products, respectively. Chromatogram A is from a 31 day-old degraded sample (Solution No. 12, Table 1) and B from a standard solution. For chromatographic conditions, see text.

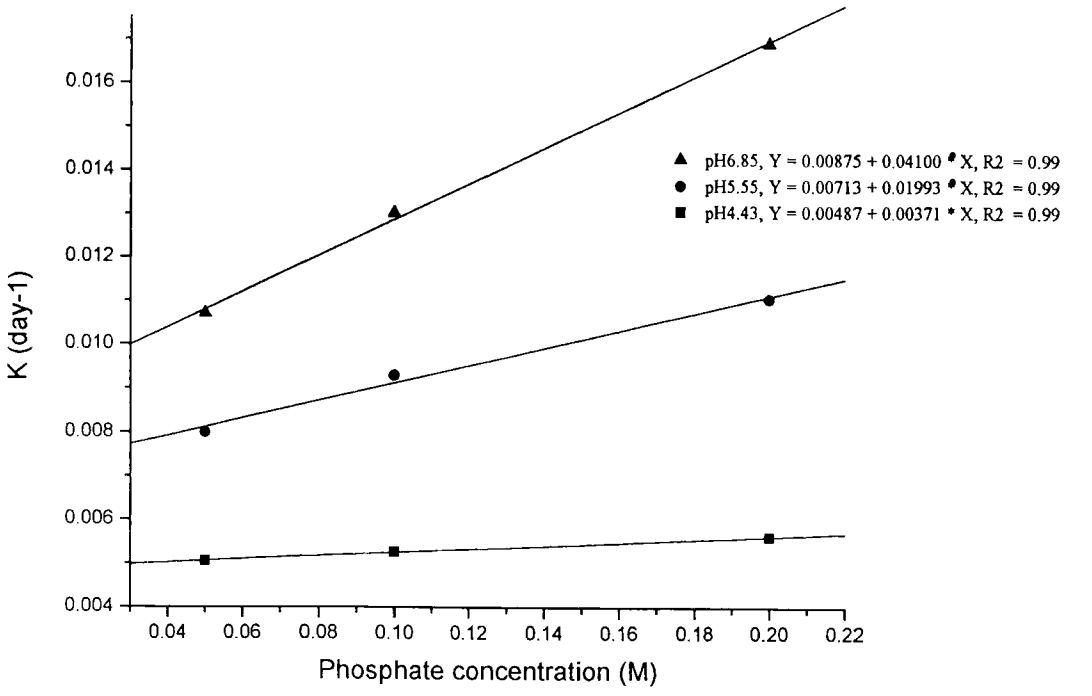

Fig 3: Effect of phosphate concentration on first order rate constants of vancomycin HCl at different pH.

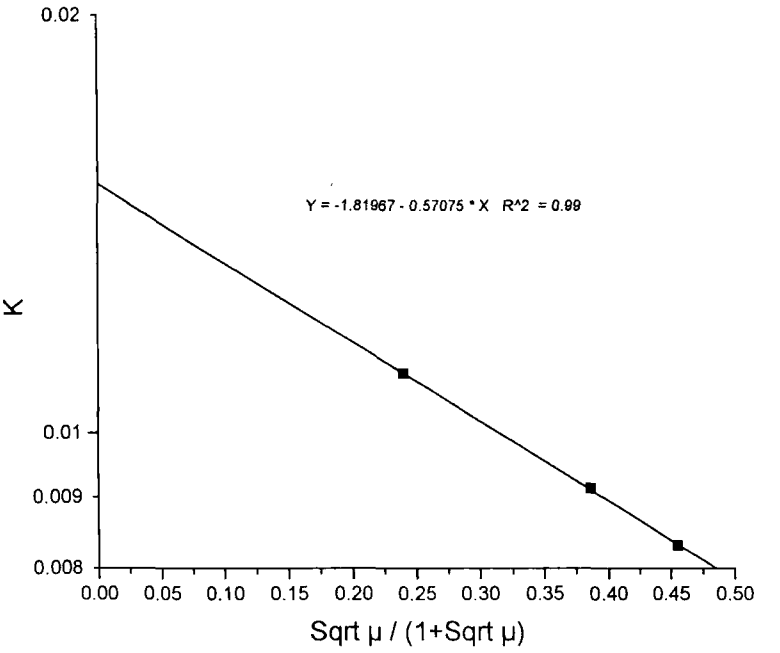

Fig 4: Effect of ionic strength using extended Debye-Huckel equation.

respectively. This indicated that the phosphate buffer effect was predominantly due to HPO_4^{2-}.

Effect of Ionic Strength at pH 5.55 at Room Temperature: From Fig. 4, it is obvious that K_{obs} value decreased with increase in the ionic strength. Therefore, the reacting ions are the protonated form of vancomycin and $OH^-/H_2PO_4^-/HPO_4^{2-}$ at pH 5.55. At this pH, vancomycin exists as a single protonated form (H_4V^+) as reported by Takacs -Novak et al. (2) and is in agreement with these results. The slope of the line of log K vs. $\sqrt{\mu}/(1+\sqrt{\mu})$ was -0.57 using the extended Debye-Huckel rule for ionic strength greater than 0.1M.

Effect of pH on the Stability of Vancomycin HCl at Room Temperature: The decomposition of vancomycin at all pH values studied followed first-order kinetics (Fig 5). The decomposition of vancomycin HCl may be represented by:

$$K_{obs} = K_0 + K_1(H^+) + K_2(H_3PO_4) + K_3(OH^-) + K_4(H_2PO_4^-) + K_5(HPO_4^{2-}) \quad (1)$$

where K_1 to K_5 are the second-order rate constants for the acid/base catalysis of vancomycin HCl and K_0 is the first order rate constant due to solvent catalysis.

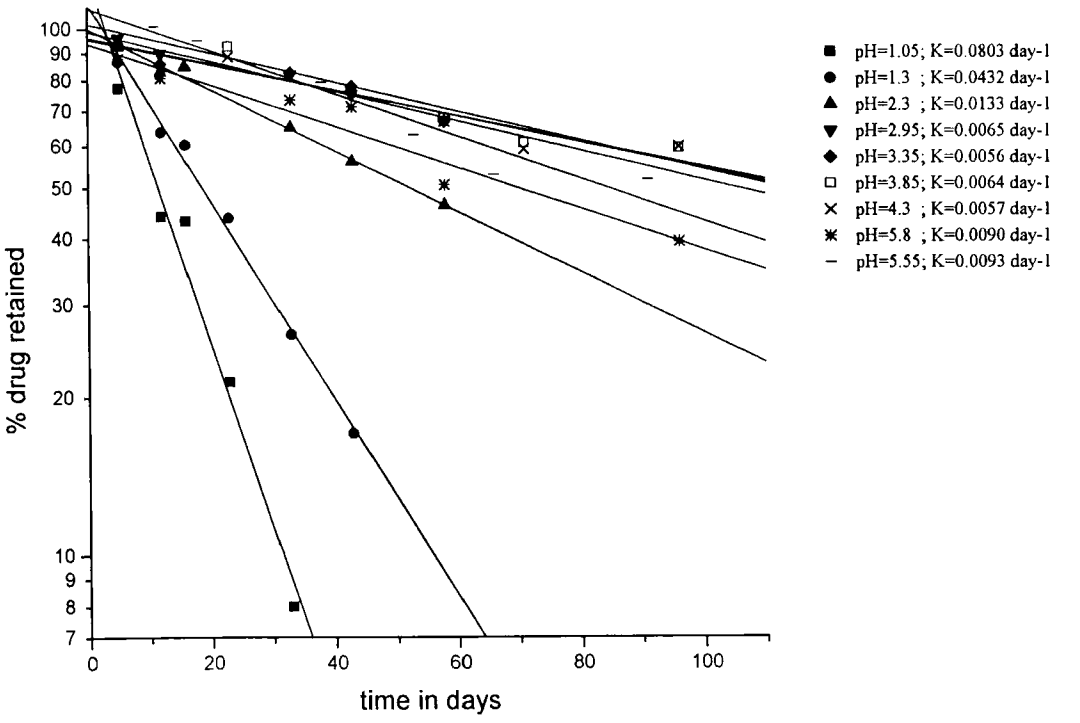

Fig 5:First order plots of vancomycin HCl degradation at various pH

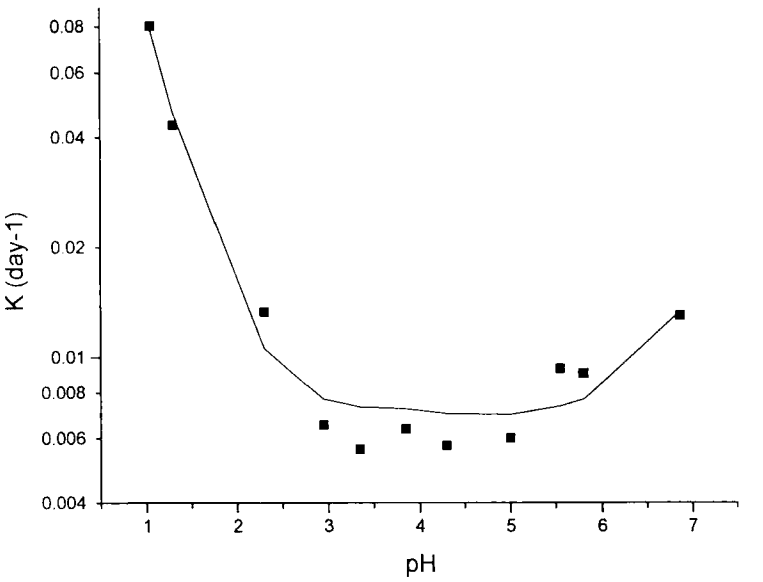

Fig 6: pH-rate profile of Vancomycin HCl. Symbols represent observed data and the line is the fitted curve using eqn 2.

Equation (1) can be rewritten as

$$K_{obs} = \frac{K_0}{D} + \frac{K_1(H^+)}{D} + \frac{K_2 M(H^+)^3}{D} + \frac{K_3 K_w}{H^+} + \frac{K_4 M(H^+)^2 K_{a1}}{D} + \frac{K_5 M(H^+) K_{a1} K_{a2}}{D} \quad (2)$$

where $D = (H^+)^3 + (H^+)^2 K_{a1} + (H^+)K_{a1}K_{a2} + K_{a1}K_{a2}K_{a3}$;
K_{a1}, K_{a2} and K_{a3} are the dissociation constants of phosphate (pK_{a1}=2.12, pK_{a2}=7.21 and pK_{a3}=12.67); M is the total phosphate concentration (0.1M); and K_w is the ionic product of water (pK_w=14).

Assume that K_0 is 0.006 day^{-1} (average K_{obs} values in the pH-independent regions where decomposition is minimum). With this value of K_0, eq 2 was fitted to the pH-rate profile curve (Fig 6) using Sigma Plot v1.01 on Windows (4). Using this program, the values were estimated to be as follows:

$K_1 = 0.81\ M^{-1}day^{-1}$
$K_2 = 2.92x\ 10^{-10}\ M^{-1}\ day^{-1}$
$K_3 = 0.005\ M^{-1}day^{-1}$
$K_4 = 0.009\ M^{-1}day^{-1}$
$K_5 = 0.213\ M^{-1}day^{-1}$

From the plot of log K_{obs} vs. pH for vancomycin HCl (Fig. 6), it is obvious that from pH 3 to about 5.7, the reaction is water catalyzed. Between pH 5.7 and 7.0, HPO_4^{2-} (K_5) had the dominating catalytic effect. Between pH 1 to 3, the reaction is acid-catalyzed. It was determined in a separate study that above pH 7.0, the decomposition of vancomycin HCl was very rapid and hence not investigated further.

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