REPORT TO
OFFICE OF GENERIC DRUGS

VANCOMYCIN SOLUBILITY STUDY

DIVISION OF PRODUCT QUALITY RESEARCH
OFFICE OF TESTING AND RESEARCH
CENTER FOR DRUG EVALUATION AND RESEARCH
FOOD AND DRUG ADMINISTRATION

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INTRODUCTION

The purpose of this collaborative study was to determine the solubility of vancomycin under different pH conditions. The pH conditions selected were with-in the extremes of the normal physiological pH of the human gastrointestinal tract.

The Biopharmaceutics Classification System (BCS) Guidance for Industry is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability[1]. When combined with the dissolution of the drug product, the BCS takes into account three major factors that govern the rate and extent of drug absorption from IR solid oral dosage forms: dissolution, solubility, and intestinal permeability.

According to the BCS, the solubility of *drug substances* (API) is classified as high solubility or low solubility. The solubility class boundary is based on the highest dose strength of an Immediate Release (IR) product that is the subject of a biowaiver request. A drug substance is considered highly soluble when the *highest dose strength* is soluble in *250 ml or less of aqueous media over the pH range of 1-7.5*. The high solubility boundary layer for vancomycin is based on its highest dose strength of 250 mg dissolved in 250 mL of aqueous media.

PROJECT OBJECTIVE

To determine the solubility of vancomycin over the BCS pH range of 1-7.5 according to the Biopharmaceutics Classification Guidance.

LABORATORY PROJECT

This laboratory project was conducted in the Division of Product Quality Research (DPQR) in collaboration with the Office of Generic Drugs.

GENERAL STUDY

In general, vancomycin hydrochloride was dissolved in the selected pH media in glass vials and placed in a shaking water bath at a temperature of 37C for 24 hours according to the BCS guidance to determine the aqueous solubility. After the test period, the vancomycin samples were assayed by a validated high pressure liquid chromatographic (hplc) method to determine the pH solubility profile.

BACKGROUND

Vancomycin is a tricyclic glycopeptides antibiotic used to treat gram-positive infections by inhibiting bacterial mucopeptide biosynthesis. It is produced by the growth of certain strains of streptomyces orientalis bacteria. Vancomycin is indicated for the treatment of serious infections of staphylococci. It is also indicated for penicillin allergic patients, or for patients who have failed to respond to other antibiotics.

Vancomycin

Vancomycin has a molecular formula $C_{66}H_{75}Cl_2N_9O_{24}$.
The molecular weight of vancomycin hydrochloride is 1449.3.
Vancomycin has a CAS Number: 1404-90-6

The percent elemental composition of vancomycin is:
Carbon: 54.69%, Hydrogen: 5.22%, Chlorine: 4.89%, Nitrogen: 8.7%, and Oxygen: 26.5%

Vancomycin hydrochloride

Vancomycin hydrochloride has a molecular formula $C_{66}H_{75}Cl_2N_9O_{24} \cdot HCl$.
The molecular weight of vancomycin hydrochloride is 1485.7.
Vancomycin hydrochloride has a CAS Number: 1404-93-6.
It has a white to yellowish-white color.

The percent elemental composition of vancomycin hydrochloride is:
Carbon: 53.36%, Hydrogen: 5.16%, Chlorine: 7.16%, Nitrogen: 8.48%, and Oxygen: 25.85%

Vancomycin hydrochloride solubility

The Merck Index describes vancomycin hydrochloride as soluble in water > 100 mg/mL, moderately soluble in dilute ethanol, insoluble in higher alcohols, acetone or ethers.

Shown below in figure 1 is the chemical structure of vancomycin hydrochloride.

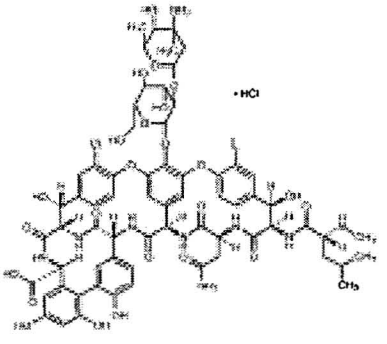


Figure 1. Vancomycin hydrochloride

Vancomycin pKa

Vancomycin is reported to have 6 pKa values: 2.18, 7.75, 8.89, 9.59, 10.4, and 12.0 (T. Takács-Novák K, Noszál B, et al 1993)

The functional groups responsible for these pK values are indicated in Figure 2 (Johnson and Yalkowsky, 2005).

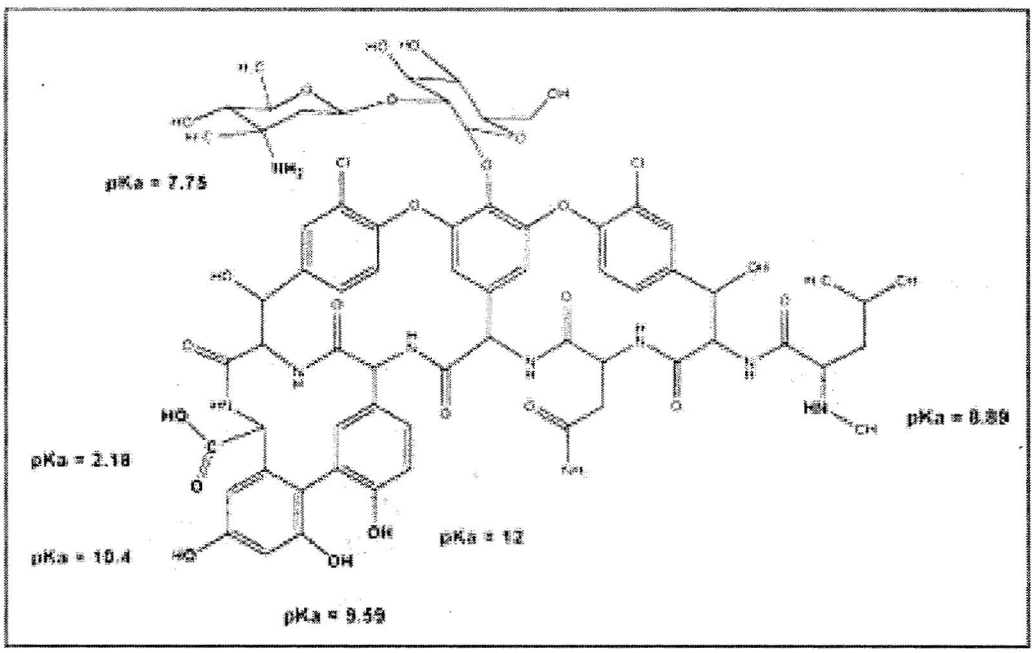


Figure 2. Vancomycin functional groups and pKa's

It has been noted in the literature that glycoside antibiotics are poorly soluble at neutral pH, but they are ionizable (Johnson and Yalkowsky, 2005). Vancomycin is an amphotheric molecule that can react with acids or bases.

MATERIALS AND METHODS

Drug Substance

Vancomycin hydrochloride, lot number 015K08251, was purchased from Sigma-Aldrich (St. Louis, MO). This material meets USP test specifications.

Chemicals and Reagents

HPLC grade acetonitrile was purchased from Burdick and Jackson (Muskegon, MI). HPLC grade monobasic and dibasic potassium phosphate, ACS grade hydrochloric and phosphoric acid and certified pH buffers (1. 3, 4, 5 and 7.5) were purchased from Fisher Scientific (Pittsburgh, PA). Filters were purchased from Millipore Corporation (Bedford, MA). Filtered 18 MOhm water was supplied in house by a Millipore Milli-Q System (Bedford, MA).

Sample and Standard Preparation

Vancomycin hydrochloride samples and standards were weighed out in diffused light. Sample and standard vials were protected from light during analytical preparation and placed in amber glass vials for analysis.

Preparation of Stock Solutions (Calibration and System Suitability Standards)

Two vancomycin standard solutions 1 and 2 (1 mg/mL) were prepared from the Sigma-Aldrich reference standard in deionized water. The stock solutions were stored in the refrigerator at about 4° C.

Preparation of Stock Solutions (Quality Control Standards)

A vancomycin standard solution 3 (1 mg/mL) was prepared from Sigma-Aldrich reference material in deionized water. The stock solution was stored at 4°C.

Preparation of Calibration Standards

Five standard solutions were prepared daily by serial dilution in mobile phase from the vancomycin stock solution 1 standard to produce nominal concentrations of 2.5, 5.0, 10, 25, 50 and 100 μg/mL of vancomycin.

Preparation of Quality Control Standards

Five quality control standard solutions were prepared daily by serial dilution in mobile phase from the vancomycin stock solution 3 to produce nominal concentrations of 2.5, 50, and 100 μg/mL of vancomycin at QC low standard (2.5 μg/mL), QC intermediate standard (50 μg/mL) and QC high standard (100 μg/mL).

Vancomycin Solubility Samples

Vancomycin hydrochloride from [redacted] lot number [redacted]) was supplied by the Office of Generic Drugs. Vancomycin hydrochloride was weighed in glass vials and aqueous media (buffer, or water) was added to create vancomycin saturated solutions at pH 1, 3, 4, 5 and 7.5. Samples were prepared in triplicate. Samples were placed for 24 hours in a shaking water bath at 37C. Samples were filtered through a 0.2 micron filter (Amicon) into glass amber vials and placed in an automated sample analyzer for HPLC analysis.

Solubility Experiments

Vancomycin would be highly soluble according to the BCS if the highest dose strength of the vancomycin equivalent to 250 mg was soluble in 250 ml of aqueous media (250mg/250 mL aqueous media) or less over the pH range of 1-7.5.

- High Solubility = 250 mg (highest dose strength) /250mL aqueous media (or less)

  A BCS solubility experiment was conducted for vancomycin at pH 1, 3, 4, 5, and 7.5

  Vancomycin solubility experiments were conducted for vancomycin in DI water

Sample Preparation

Vancomycin hydrochloride was weighed out in diffused light and added to glass vials containing pH 1.0, 3.0, 4.0, 5.0 and 7.5 buffers, respectively. The samples were prepared in triplicate according to the method outlined in the BCS guidance. The pH was adjusted following vancomycin hydrochloride addition to the buffer.

The drug substance was added to the buffer so that excess amount remains dispersed throughout the study. The vancomycin solubility samples were then incubated in a shaking water bath at 37C for 24 hours. Aliquots of the sample were carefully withdrawn, filtered with a non-adsorbing syringe filter, and diluted if necessary, and the vancomycin sample concentration was determined using high-performance liquid chromatography (HPLC).

Vancomycin solubility samples were prepared in deionized water (DI) water at a concentration of 168 mg/mL. The sample pH was not adjusted. The vancomycin solubility samples prepared in DI water were incubated in a shaking water bath at 37C for 24 hours. Aliquots of the sample were carefully withdrawn, filtered with a non-adsorbing syringe filter, and diluted if necessary, and the sample concentration was determined using high-performance liquid chromatography.

HPLC Method

The analytical method for vancomycin was developed in house by DPQR. The method buffer pH was selected based on the six know pKa values for vancomycin which include: 2.18, 7.75, 8.89, 9.59, 10.4, and 12.0.

Listed below are the general operating parameters and description of the HPLC method:

Parameter	Solubility Experiment
HPLC system	HP 1050 Series
Detection	UV @ 230 nm
Column	Phenomenex C-18 Luna (2) 250×4.6mm, 5μ
Guard column	Phenomenex C-18 Luna (2) 3.0× 4.6mm, 5μ
Mobile phase	9% ACN/ 25mM PO_4
Elution	Isocratic
pH	3.2
Flow rate	1.0 mL/min
Injection volume	50 μL

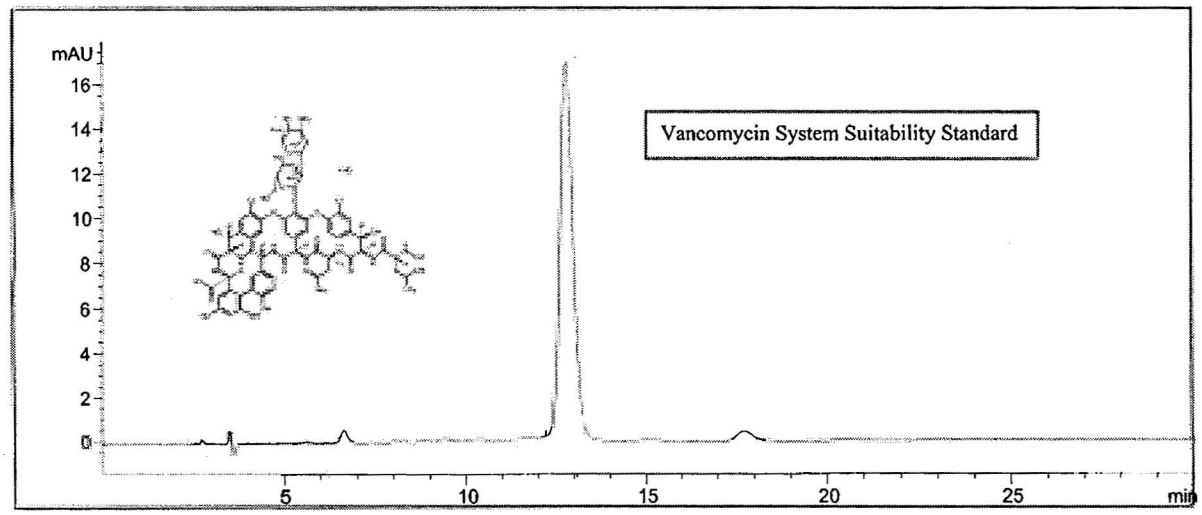


Figure 3. Chromatographic profile of vancomycin reference standard

Validation

The analytical method was validated according to USP category I for accuracy, precision, linearity, specificity and analytical range.

Validation Parameters

Validation Parameters	Acceptance Criteria
System Suitability (n = 6)	
1. Vancomycin	
Retention Time	< .25 min.
Area (RSD)	< 2.0%
k^1	> 3.0
Theorical plates	> 3,000
Symmetry	> 0.5
Linearity of Calibration Curves (n =3)	> 0.99
Accuracy	
Low QC (n = 5)	< 15 %
Intermediate QC (n = 5)	< 10 %
High QC (n = 5)	< 10 %
Precision	
Low QC (n = 5)	< 5 %
Intermediate QC (n = 5)	< 5 %
High QC (n = 5)	< 5 %
Analytical Range	2.5-100 µg/mL

Sample Calculation

A linear calibration model was generated as a weighted (1/y) least squares fit of measured peak areas to known calibration sample concentrations. The resulting weighted linear function, $y = mx + b$, was used to calculate the concentration of vancomycin for the study sample or quality control standards from assayed peak areas.

Accuracy and precision are calculated from the concentration data and the peak response of the quality control standards using the weighted linear function.

Analytical range is established by determining the accuracy, precision and linearity are acceptable over the analytical range according to the ICH Q2B.

Specificity is determined by the observation of no endogenous peaks in the sample blanks or no co-eluting peaks in the sample or calibration standards and comparison of the sample to the known reference standard.

RESULTS & DISCUSSION

HPLC Assay Method

Vancomycin is a challenging drug to assay since it a relatively large molecule with many labile groups. As a result many impurities are present in the chromatographic profile. As part of these solubility studies, a simple reverse phase isocratic elution analytical method was developed for vancomycin. Vancomycin was well resolved from numerous co-eluting peaks. The total analysis, equilibration and recovery time was 30 minutes. The analytical method was validated according to USP <1225> Validation of Compendial Methods.

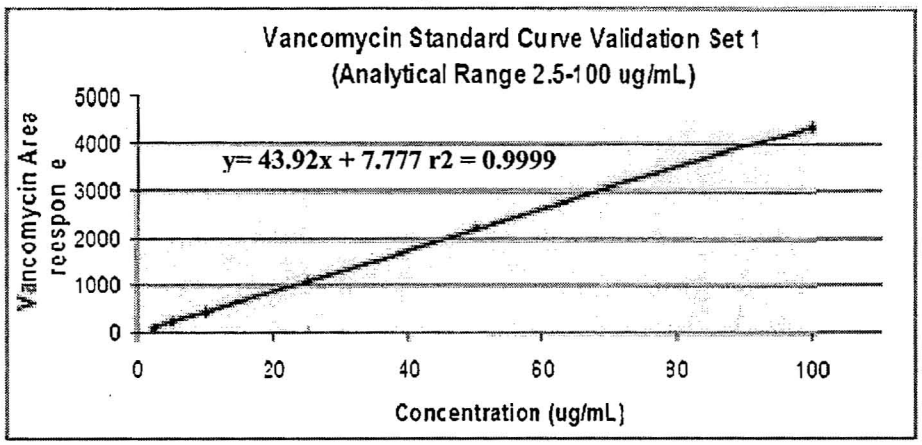
Validation Results: Solubility Study Analytical HPLC Method

Parameters	Day 1	Day 2	Day 3
System Suitability (n = 6)			
I. Vancomycin			
Retention Time (min.)	11.94 ± 0.07	12.96 ± 0.04	11.49 ± 0.05
Retention Time (RSD)	0.58%	0.29%	0.46%
Peak Area (RSD)	0.28%	0.42%	1.53%
k^1	4.68	5.17	4.47
Plates	5,750	6540	6401
Symmetry	0.83	0.83	0.81
USP Tailing	1.114	1.113	1.127
Linearity of Calibration Curves (n =2)	>0.9999	>0.9997	>0.9993
Accuracy (better than)			
Low QC (n = 5)	96.4%	99.3%	88.5%
Intermediate QC (n = 5)	105.3%	98.2%	98.4%
High QC (n = 5)	104.1%	97.3%	98.5%
Precision (better than)			
Low QC (n = 5)	3.29%	0.99%	1.21%
Intermediate QC (n = 5)	2.02%	1.73%	2.67%
High QC (n = 5)	2.58%	2.21%	1.07%
Analytical Range	2.5-100 μg/mL	2.5-100 μg/mL	2.5-100 μg/mL

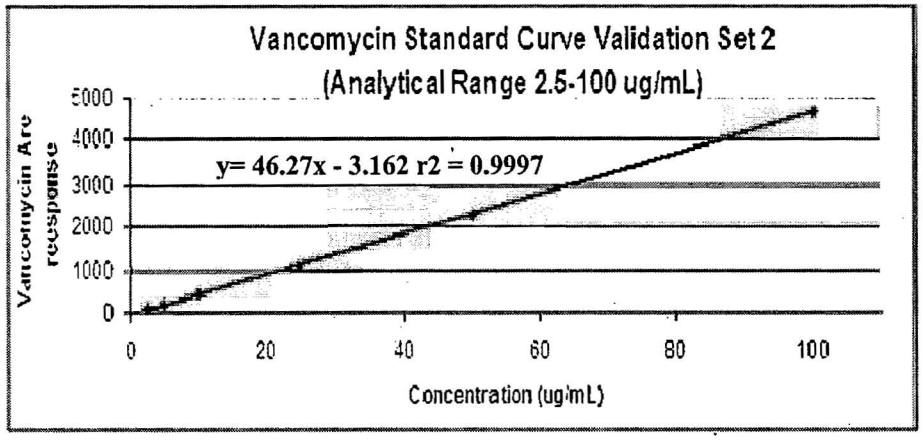
The analytical method for vancomycin was validated according to category I of the USP for assay and determined to be accurate, precise, specific and linear over the established analytical range.

Listed below are the standard curves for Day 1, 2 and 3:

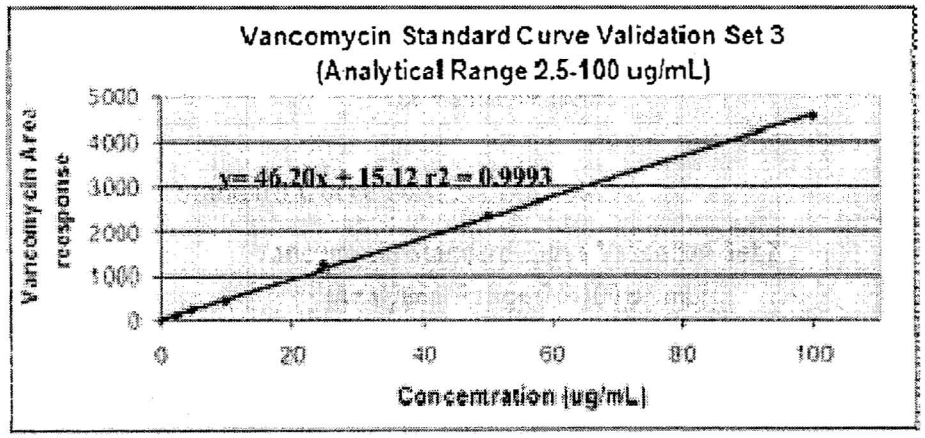
Vancomycin Standard Curve Day 1



Vancomycin Standard Curve Day 2



Vancomycin Standard Curve Day 3



Vancomycin BCS Solubility Vancomycin Sample Set (pH = 1)

Vancomycin Sample	Assayed Conc. (mg/mL)
Sample 1	139.6
Sample 2	141.1
Sample 3	140.1
Sample Mean	140.3 +/- 0.73

Vancomycin was found to be highly soluble at pH 1 at 37C for 24 hours. Vancomycin solubility is approximately 140-fold greater than the BCS high solubility boundary concentration (1 mg/ml) at pH 1 for the vancomycin sample set.

BCS Solubility Vancomycin Sample Set (pH = 3)

Vancomycin Sample	Assayed Conc. (mg/mL)
Sample 1	191.5
Sample 2	191.8
Sample 3	191.9
Sample Mean	191.7 +/- 0.2

Vancomycin was found to be highly soluble at pH 3 at 37C for 24 hours. Vancomycin solubility is approximately 190-fold greater than the BCS high solubility boundary concentration (1 mg/ml) at pH 3 for the vancomycin sample set.

BCS Solubility Vancomycin Sample Set (pH = 4)

Vancomycin Sample	Assayed Conc. (mg/mL)
Sample 1	2.97
Sample 2	3.02
Sample 3	2.96
Sample Mean	**2.98 +/- 0.03**

Vancomycin was found to be highly soluble at pH 4 at 37C for 24 hours. Vancomycin solubility is approximately 3-fold greater than the BCS high solubility boundary concentration (1 mg/ml) at the pH 4 for the vancomycin sample set.

BCS Solubility Vancomycin Sample Set (pH = 5)

Vancomycin Sample	Assayed Conc. (mg/mL)
Sample 1	9.73
Sample 2	9.35
Sample 3	9.42
Sample Mean	**9.5 +/- 0.2**

Vancomycin was found to be highly soluble at pH 5 at 37C for 24 hours. Vancomycin solubility is approximately 9-fold greater than the BCS high solubility boundary concentration (1 mg/ml) at pH 5 for the vancomycin sample set.

BCS Solubility Vancomycin Sample Set 1 (pH = 7.5)

Vancomycin Sample	Assayed Conc. (mg/mL)
Sample 1	17.7
Sample 2	17.6
Sample 3	17.4
Sample Mean	**17.5 +/- 0.2**

Vancomycin was found to be highly soluble at pH 7.5 at 37C for 24 hours. Vancomycin solubility is approximately 17-fold greater than the BCS high solubility boundary concentration (1 mg/ml) at pH 7.5 for the vancomycin sample set.

BCS Solubility Vancomycin Sample Set-Overall Data (pH 1 to pH 7.5)

Vancomycin Sample	pH 1 Conc. (mg/mL)	pH 3 Conc. (mg/mL)	pH 4 Conc. (mg/mL)	pH 5 Conc. (mg/mL)	pH 7.5 Conc. (mg/mL)
Sample 1	139.6	191.5	2.97	9.73	**17.7**
Sample 2	141.1	191.8	3.02	9.35	**17.6**
Sample 3	140.1	191.9	2.96	9.42	**17.4**
Sample Mean	**140.3 +/- 0.73**	**191.7 +/- 0.2**	**2.98 +/- 0.03**	**9.5 +/- 0.2**	**17.5 +/- 0.2**

Vancomycin was found to be highly soluble across the BCS pH range tested. Vancomycin solubility is at least 2.9-fold greater than the BCS high solubility boundary concentration of 1 mg/ml across the BCS pH range for the vancomycin sample set.

pH Profile

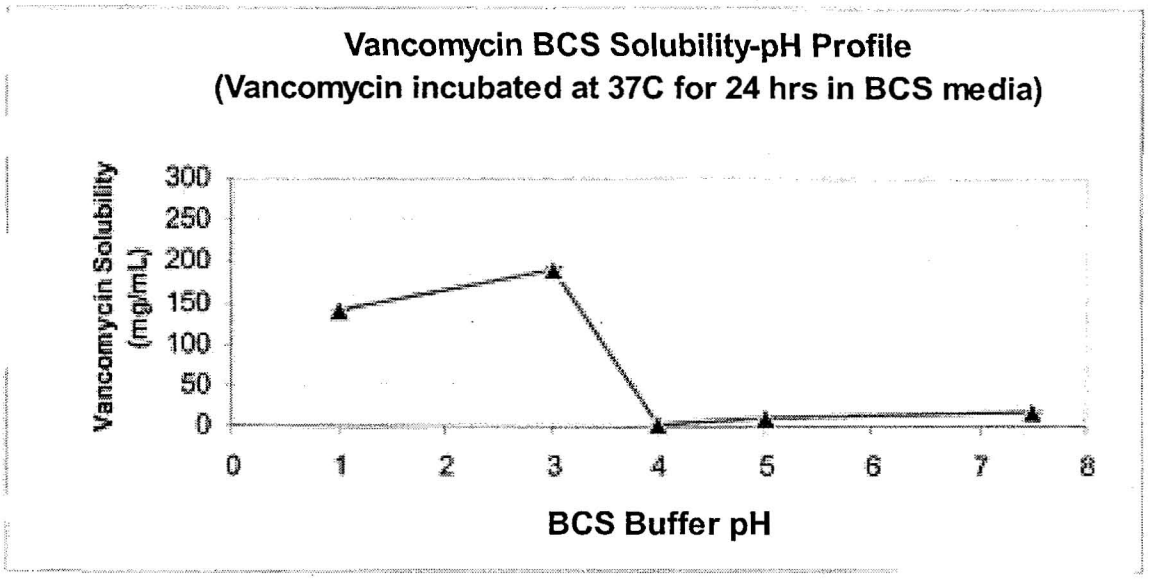


Figure 4. Vancomycin pH Profile

Water Solubility

A 164 mg/mL vancomycin solution was prepared in DI water and assayed.

Vancomycin Sample	Sample Concentration (mg/mL)
Sample 1	146.0
Sample 2	146.6
Sample 3	144.8
Sample Mean	**146.8 +/- 2.5**

Vancomycin was found to be *highly soluble in water* at approximately 145 times the high solubility boundary concentration (1 mg/mL) for vancomycin at 37C for 24 hours. These data are consistent with the Merck index which states that vancomycin hydrochloride is soluble in water in excess of 100 mg/mL.

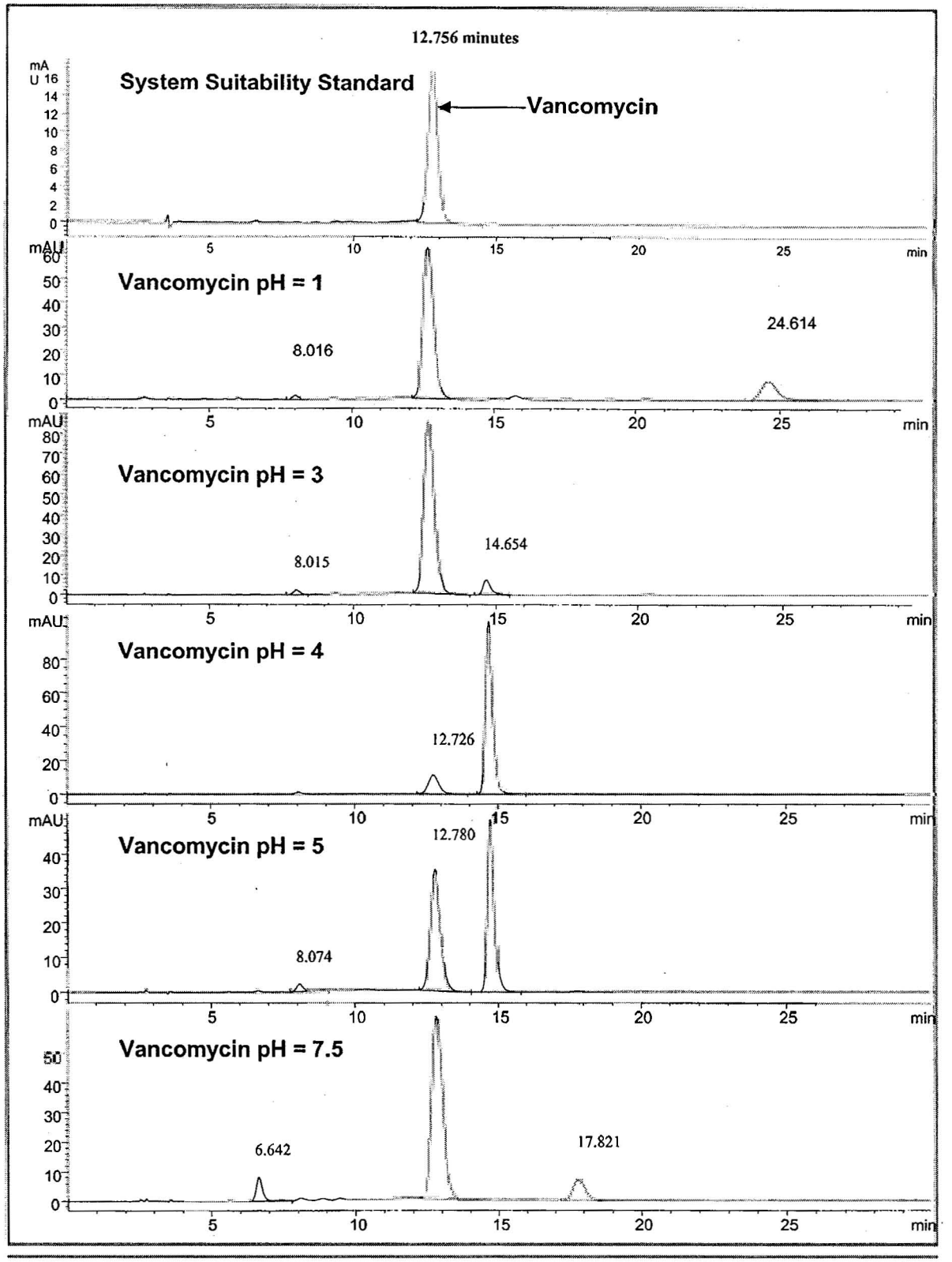


Figure 5. Vancomycin pH Chromatographic Profiles

DISCUSSION

Vancomycin was determined to have high solubility across the BCS pH range of 1-7.5. Vancomycin was determined to have solubility greater than 140 mg/mL at pH 1 and 3. Vancomycin solubility is reduced significantly, to less than 17.5 mg/mL at pH 4, 5 and 7.5.

The reduction of solubility was associated with enhanced degradation of vancomycin as observed by multiple peaks in the HPLC chromatograms (figure 5).

SUMMARY

Vancomycin solubility was determined over the BCS pH range. The pH-profile was determined and vancomycin solubility was found to be at least 2.9-fold greater than the high solubility boundary of vancomycin. **Thus, at all BCS pH levels vancomycin required less than 250 mL to dissolve the highest dose strength of 250 mg.**

- Vancomycin when dissolved in deionized water was found to have a solubility of at least 148 mg/mL.
- Vancomycin was found to have a solubility at pH 1 and 3 of 140 and 196 mg/mL respectively, or at least 140-fold greater than the high solubility boundary of vancomycin.
- Vancomycin was found to have a solubility at pH 4, 5 and 7.5 of 2.9, 9.5 and 17.5 mg/mL respectively, or at least 2.9-fold greater than the high solubility boundary of vancomycin.
- Vancomycin at pH 1, 3, 4, 5 and 7.5 would require 1.78, 1.27, 83.8, 26.3 and 14.2 ml of aqueous media, respectively to dissolve the highest dose strength of 250 mg of vancomycin.

CONCLUSION

By the method developed and validated in the Division of Product Quality Research laboratories as presented here, vancomycin hydrochloride is a high solubility compound.

REFERENCES

- <1225> Validation of Compendial methods. United States Pharmacopoeia 27/National Formulary 22.
- <621> Chromatography.. United States Pharmacopoeia 27/National Formulary 22,
- Waiver of In Vivo Bioavailability and Bioequivalence Studies for Immediate-Release Solid Oral Dosage Forms Based on a Biopharmaceutics Classification System. Guidance for Industry, August 2000.
- T Takács-Novák K, Noszál B, Tokes-Kovesdi M, Szasz G. Acid base properties and proton-speciation of vancomycin. Int. J Pharm. 1993; 89:261-263.
- Jennifer L. H. Johnson and Samuel H. Yalkowsky. Reformulation of a New Vancomycin Analog: An Example of the Importance of Buffer Species and Strength. AAPS PharmSciTech. 2006 Jan 13; 7(1).