



**REPORT TO
OFFICE OF GENERIC DRUGS**

**VANCOMYCIN DISSOLUTION
STUDY**

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

FOR FDA USE ONLY

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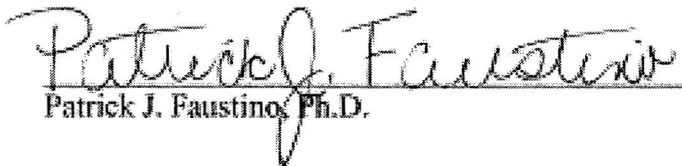
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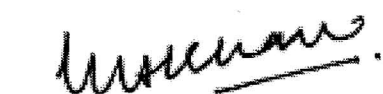
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INTRODUCTION

The purpose of this collaborative study was to determine the dissolution characteristics of oral vancomycin drug products. Vancomycin is a locally acting gastrointestinal (GI) drug product indicated for staphylococcal enterocolitis and life threatening infections by gram-positive bacteria. Vancomycin has very low gastrointestinal absorption with low systemic exposure. Therefore an *in vivo* bioequivalence study is not usually feasible. Dissolution is an *in vitro* method that can be performed relatively easily. Dissolution is an accepted regulatory approach for determining the amount of active pharmaceutical ingredient that is released following dissolution in an aqueous media. Dissolution under various GI pH conditions may provide *in vitro* evidence of its availability for pharmacological action.

The Biopharmaceutics Classification System (BCS) Guidance for Industry is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability¹. 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 dissolution of an immediate release *drug product* is considered rapidly dissolving when no less than 85% of the labeled amount of the drug substance dissolves within 30 minutes, using *US Pharmacopeia* (USP) Apparatus I at 100 rpm (or apparatus II at 50 rpm) in a volume of 900 mL or less. This time limit of 30 minutes has been established for immediate release dosage forms. However for site selective dosage forms such as vancomycin hydrochloride, the time limit for 85% dissolution has not been established so far.

PROJECT OBJECTIVE

To determine the dissolution of vancomycin drug products in various dissolution media over the BCS pH range of 1-6.8 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. The scope of this work includes dissolution at initial time only and does not include evaluation of environmental conditions and long-term storage.

GENERAL STUDY

In general, vancomycin hydrochloride (125 and 250 mg dose strength) drug product capsules were placed in a dissolution basket of a mechanically calibrated dissolution apparatus which contained selected dissolution media (BCS, USP Simulated Gastric Fluid, USP Simulated Intestinal Fluid). The dissolution water bath was maintained at a temperature of 37°C for 0-60 minutes according to the BCS guidance to determine the dissolution profiles of various vancomycin drug products (innovator or generic product samples). After the test period, the vancomycin dissolution samples were transferred from the dissolution bath to sampling vials then assayed by a validated high pressure liquid chromatographic (hplc) method to determine drug product dissolution characteristics according to the BCS guidance and USP <711>.

BACKGROUND

Vancomycin is a tricyclic glycopeptide antibiotic used to treat gram-positive infections by inhibiting bacterial mucopeptide biosynthesis. Specifically vancomycin acts by inhibiting cell wall synthesis in gram-positive bacteria. It is produced by the growth of certain strains of *Streptomyces orientalis* bacteria.

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 (14th edition) 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.

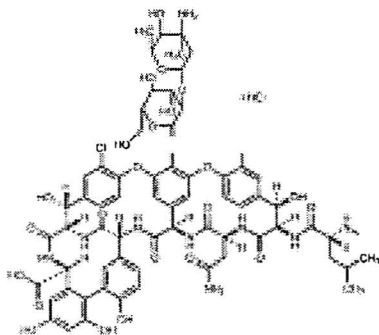


Figure 1. Vancomycin hydrochloride

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) and may undergo ionization. Vancomycin is an amphoteric molecule that can react with either acids or bases.

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

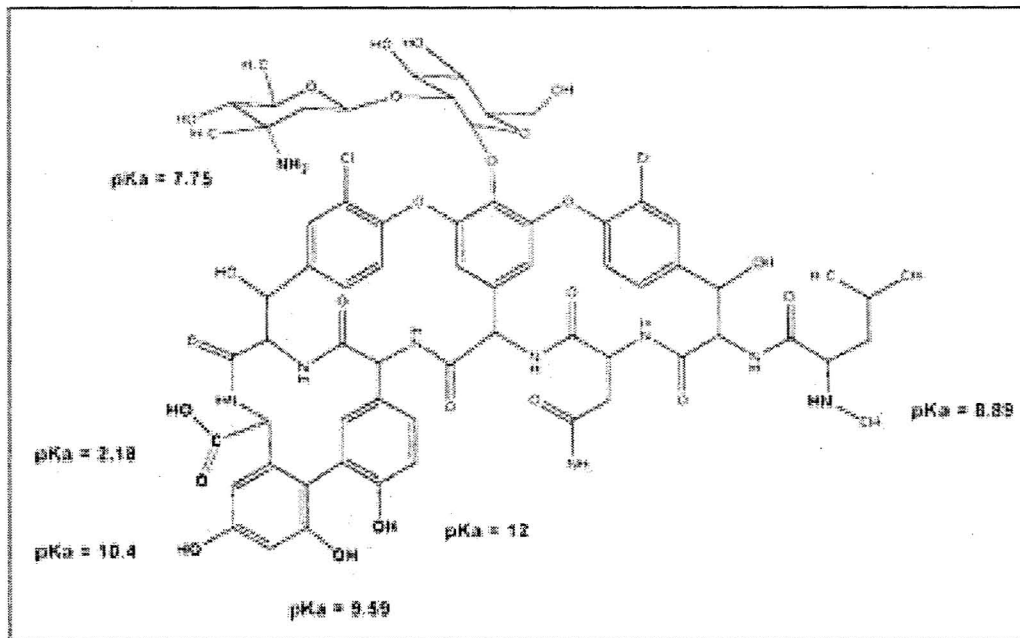


Figure 2. Vancomycin functional group pKa's

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 (4, 5 and 7) were purchased from Fisher Scientific (Pittsburgh, PA). Sample filters were purchased from Amicon-Millipore (Bedford, MA). Solvent 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 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 Drug Products

Vancomycin hydrochloride 250 mg capsules from ViroPharma lot number 431233 and 125 mg capsules lot 429915 were purchased from Washington Drug Wholesale Exchange (Savage, MD). Vancomycin hydrochloride 250 mg capsules from ViroPharma lot number A200240 and lot number 431737 were supplied by the Office of Generic Drugs. Vancomycin generic drug products were supplied by the Office of Generic Drugs.

Dissolution Apparatus

A Van Kel 7000 (Varian, Inc.) semi-automated dissolution apparatus equipped with a VK 8000 sample preparation module was used for vancomycin drug product dissolution.

Vancomycin Dissolution Samples

Vancomycin hydrochloride drug product capsules (n= 6) were carefully removed from individual blister packs and placed in dissolution Apparatus I baskets (n = 6). The baskets were mechanically lowered into the dissolution baths (n = 6) simultaneously. Approximately 5 ml of dissolution bath media was transferred from each bath (5, 10, 20, 30, 45 and 60 minutes) by an interfaced Varian VK 8000 automated sample transfer module. The samples were filtered through a 0.45 micron filter and placed in 20 mL glass tubes and automatically transferred to the system sample rack. Samples were immediately covered with Parafilm® and aluminum foil. At the end of the dissolution experiment (60 minutes) all filtered dissolution samples were manually transferred to the analytical laboratory for analysis.

HPLC Analysis

Dissolution samples were diluted in mobile phase in amber HPLC vials, capped and placed in an automated HPLC injector module. Samples were injected in an automated fashion onto the HPLC and chromatographically analyzed. Chromatographic data for the vancomycin dissolution samples was collected by the HPLC computer using Agilent ChemStation software.

Dissolution Experiments

Dissolution experiments were conducted for oral vancomycin drug products to evaluate their dissolution characteristics. Nineteen dissolution studies were conducted and are listed below:

- Study 1,2,3-BCS Dissolution media pH 1.2, 4.5 and 6.8 (ViroPharma lot 431233)
- Study 4,5-USP Dissolution media SGF pH 1.2, SIF 6.8 (ViroPharma lot 431233)
- Study 6-BCS Dissolution media pH 1.2 (ViroPharma lot A200240)
- Study 7-BCS Dissolution media pH 1.2 (—)
- Study 8-BCS Dissolution media pH 4.5 (ViroPharma lot A200240)
- Study 9-BCS Dissolution media pH 4.5 (—)
- Study 10-BCS Dissolution media pH 6.8 (ViroPharma lot A200240)
- Study 11-BCS Dissolution media pH 6.8 (—)
- Study 12-BCS Dissolution media pH 4.5 (ViroPharma lot 429915)
- Study 13-BCS Dissolution media pH 6.8 (ViroPharma lot 429915)
- Study 14-BCS Dissolution media pH 1.2 (ViroPharma lot 431737)
- Study 15-BCS Dissolution media pH 1.2 (—)
- Study 16-BCS Dissolution media pH 4.5 (ViroPharma lot 431737)
- Study 17-BCS Dissolution media pH 4.5 (—)
- Study 18-BCS Dissolution media pH 6.8 (ViroPharma lot 431737)
- Study 19-BCS Dissolution media pH 6.8 (—)
- *Study 12 and Study 13 used 125 mg capsules, all other studies used 250 mg capsules*

Dissolution Method

The dissolution method for vancomycin was carried out according to the BCS Guidance for Industry and USP general chapter <711>. USP Apparatus I at 100 rpm using the basket method was selected for the vancomycin drug product capsules according to the USP.

Listed below is the Dissolution Method:

Parameter	Dissolution Experiment
Dissolution system	Van Kel 7000
Calibration	Mechanical calibration
Basket	100 RPM
Temperature Bath	37C-calibrated thermometer verification (+/- 0.2C)
Dissolved Gasses	Vacuum degassing-manometer verification (<40%)
Media pH	pH 1.2, 4.5, 6.8
Media Type	BCS, USP SGF and SIF and USP BR
Media Volume	900 mL
Dissolution sample	Drug Product Capsule (125 or 250 mg)
Sampling time	5, 10, 20, 30, 45, 60 minutes

Listed below is the Mechanical Calibration of the Dissolution Apparatus:

Parameter	Calibration
Dissolution system	Van Kel 7000-Passed
ASTM Calibration	Mechanical Calibration
Shaft Height Test	Passed
Wobble Test	Passed
Shaft Verticality	Passed
Level Deck	Passed
Vessel Centering	Passed
Vessel Verticality	Passed
Vessel Height Test	Passed
Temperature Test	Passed
Rotation Speed Test	Passed

HPLC Method

The analytical method for vancomycin was developed in house by DPQR.

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

Parameter	Solubility Experiment
HPLC system	HP-Agilent 1050 Series
Chromatographic Software	Agilent ChemStation chromatographic software
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

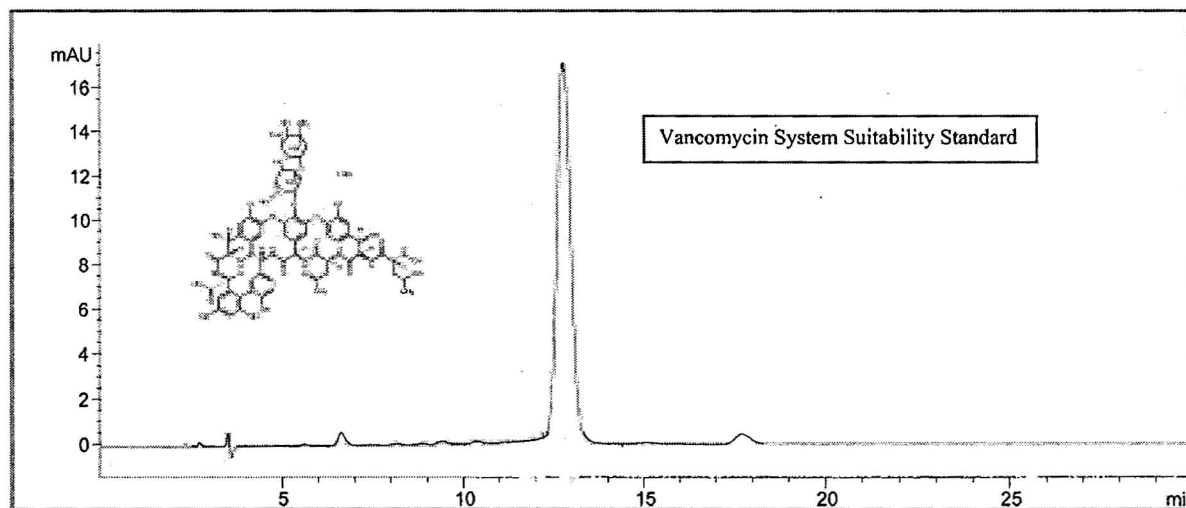


Figure 3. Chromatographic profile of vancomycin reference standard

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)	
I. Vancomycin	
Retention Time	< .25 min.
Area (RSD)	< 2.0%
k ¹	> 3.0
Theoretical 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 that 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.

Statistics

A Students t-test was used to test the null hypothesis, "*is the mean less than or equal to 85% released at 30 minutes*". The data was calculated comparing each sample dissolution mean to 85% dissolution at 30 and 45 minutes with $N-1 = 5$ degrees of freedom, one-tailed at the 95% confidence interval.

RESULTS & DISCUSSION

HPLC Assay Method

Vancomycin is a challenging drug to assay since it is 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.

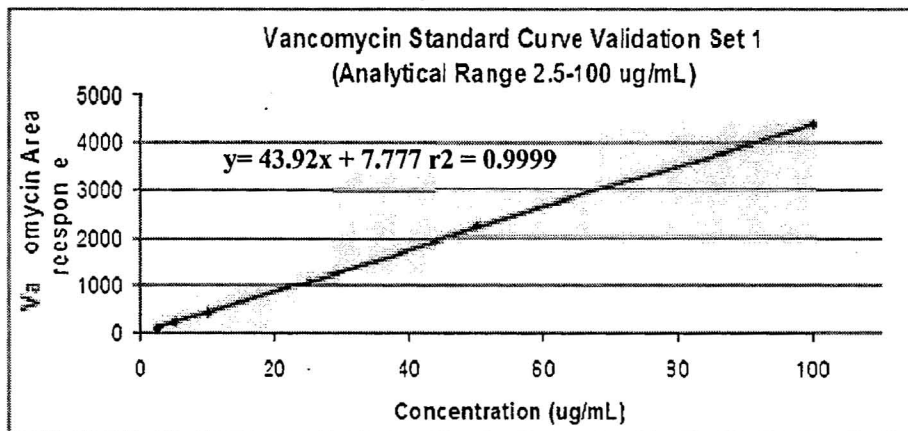
Validation Results: Dissolution Study Analytical HPLC Method

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

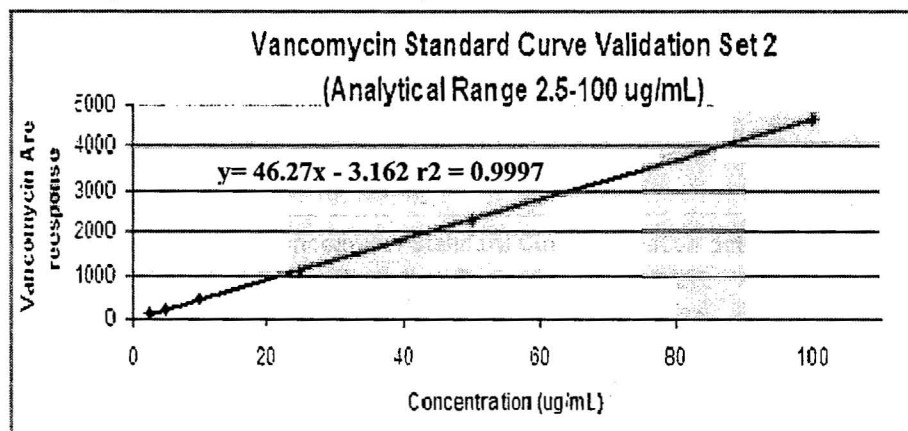
The analytical method for vancomycin was validated according to category I of the USP for assay and determined to be accurate, precise, specific and linear over the established analytical range.

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

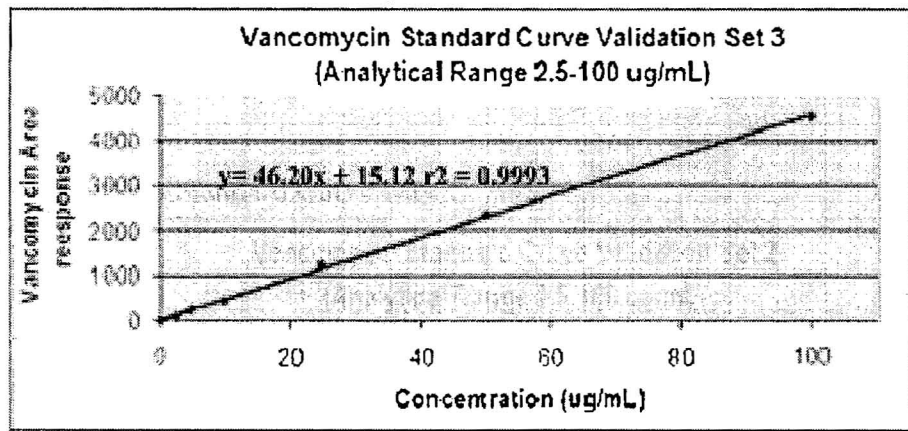
Vancomycin Standard Curve Day 1



Vancomycin Standard Curve Day 2



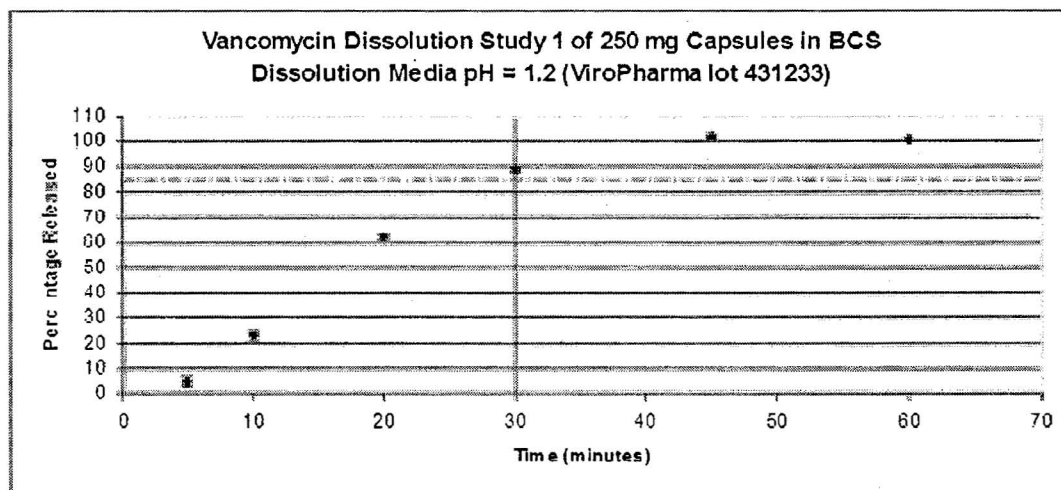
Vancomycin Standard Curve Day 3



Vancomycin Dissolution Project Data

Dissolution Study	Vancomycin Sample	Sponsor	Lot Number	Capsule Dose (mg)	Vancomycin Percentage Release (%) (average, n = 6 capsules)					
					5min	10min	20min	30min	45min	60min
1-BCS pH 1.2	Vancocin	ViroPharma	431233	250	4.6	23.1	62.4	89.1	101.7	100.9
2-BCS pH 4.5	Vancocin	ViroPharma	431233	250	1.1	7.2	36.1	71.4	101.3	105.3
3-BCS pH 6.8	Vancocin	ViroPharma	431233	250	0.8	8.7	33.4	63.3	93.2	96.9
4-USP SGF pH 1.2	Vancocin	ViroPharma	431233	250	4.5	24.2	68.5	90.2	99.0	99.5
5-USP SIF pH 6.8	Vancocin	ViroPharma	431233	250	4.2	10.7	31.9	50.1	74.9	86.9
6-BCS pH 1.2	Vancocin	ViroPharma	A200240	250	6.9	26.9	68.2	91.0	94.7	95.9
7-BCS pH 1.2	Vancomycin			250	8.0	29.3	75.9	103.5	110.5	109.9
8-BCS pH 4.5	Vancocin	ViroPharma	A200240	250	2.2	11.4	41.5	74.5	95.2	96.5
9-BCS pH 4.5	Vancomycin			250	3.2	13.0	49.8	79.8	112.7	116.0
10-BCS pH 6.8	Vancocin	ViroPharma	A200240	250	2.3	11.8	42.8	76.9	98.3	99.6
11-BCS pH 6.8	Vancomycin			250	2.9	13.1	42.9	79.6	110.4	114.5
12-BCS pH 4.5	Vancocin	ViroPharma	429915	125	0.5	9.9	44.2	84.9	95.8	96.5
13-BCS pH 6.8	Vancocin	ViroPharma	429915	125	0.9	12.7	50.9	83.6	95.3	96.4
14-BCS pH 1.2	Vancocin	ViroPharma	431737	250	4.6	25.2	67.0	89.3	94.2	93.1
15-BCS pH 1.2	Vancomycin			250	7.7	36.1	72.4	98.9	101.6	101.9
16-BCS pH 4.5	Vancocin	ViroPharma	431737	250	0.8	6.1	31.6	59.3	85.8	98.7
17-BCS pH 4.5	Vancomycin			250	1.4	9.3	38.2	69.8	100.6	104.0
18-BCS pH 6.8	Vancocin	ViroPharma	431737	250	1.2	8.1	36.3	70.4	83.3	N/A
19-BCS pH 6.8	Vancomycin			250	3.3	11.3	40.8	81.9	109.5	N/A

Vancomycin Dissolution Study 1 (BCS media pH = 1.2)



Study 1 Dissolution Data (n = 6 dissolution baths)

Vancomycin Dissolution Time	Percentage Released (%)	Standard Deviation
5 min	4.6	0.8
10 min	23.1	2.1
20 min	62.4	1.8
30 min	89.1	1.3
45 min	101.7	1.3
60 min	100.9	0.9

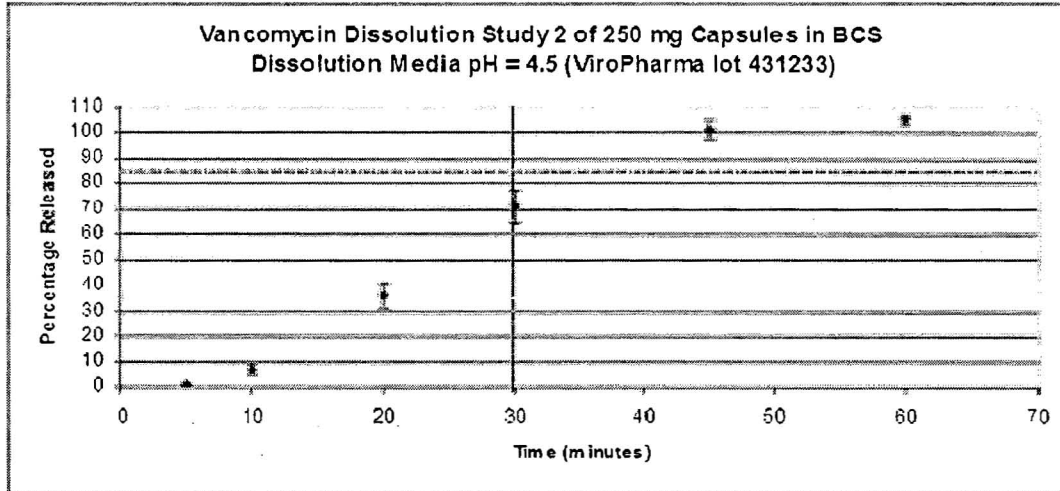
Study 1 results Vancomycin dissolution characteristics

Vancomycin 250 mg capsules were found to dissolve faster (mean data-89.1%) than 85% in 30 minutes in BCS pH 1 dissolution media.

Student t-test:

The mean at 30 minutes is 89.1, with a standard deviation of 1.3. The statistic indicates 95% confidence that the true mean is between 87.6 and 90.6. The null hypothesis that the mean dissolution at 30 minutes is less than or equal to 85% is rejected (p<0.05).

Vancomycin Dissolution Study 2 (BCS media pH = 4.5)



Study 2 Dissolution Data (n = 6 dissolution baths)

Vancomycin Dissolution Time	Percentage Released (%)	Standard Deviation
5 min	1.1	0.4
10 min	7.2	2.2
20 min	36.1	5.2
30 min	71.4	6.6
45 min	101.3	3.8
60 min	105.3	2.1

Study 2 Results Vancomycin dissolution characteristics

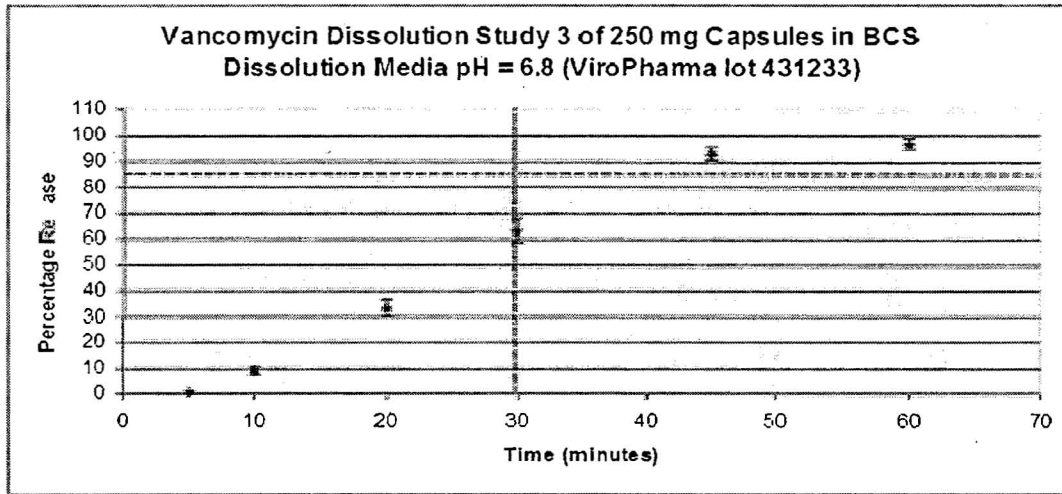
Vancomycin 250 mg capsules from ViroPharma were found to dissolve slower (mean data-71.4%) than 85% in 30 minutes in BCS pH 4.5 dissolution media.

Student’s T-test: The mean at 30 minutes is 71.4, with a standard deviation of 6.6. The statistic indicates 95% confidence that the true mean is between 63.8 and 79.8. The null hypothesis that the mean dissolution at 30 minutes is less than or equal to 85% is accepted ($p>0.05$).

Vancomycin 250 mg capsules from ViroPharma were found to dissolve faster (mean data-101.3%) than 85% in 45 minutes in BCS pH 4.5 dissolution media.

Student’s T-test: The mean at 45 minutes is 101.3, with a standard deviation of 3.8. The statistic indicates 95% confidence that the true mean is between 96.9 and 105.6. The null hypothesis that the mean dissolution at 45 minutes is less than or equal to 85% is rejected ($p<0.05$).

Vancomycin Dissolution Study 3 (BCS media pH = 6.8)



Study 3 Dissolution Data (n = 6 dissolution baths)

Vancomycin Dissolution Time	Percentage Released (%)	Standard Deviation
5 min	0.8	0.7
10 min	8.7	2.1
20 min	33.4	3.2
30 min	63.3	4.7
45 min	93.2	2.3
60 min	96.9	2.1

Study 3 Results Vancomycin dissolution characteristics

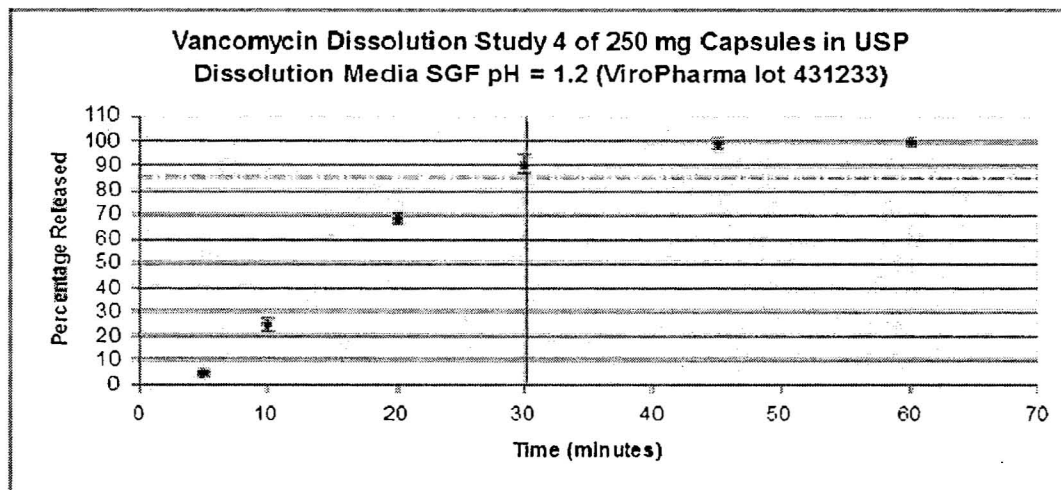
Vancomycin 250 mg capsules from ViroPharma were found to dissolve slower (mean data-63.3%) than 85% in 30 minutes in BCS pH 6.8 dissolution media.

Student t-test: The mean at 30 minutes is 63.3, with a standard deviation of 4.7. The statistic indicates 95% confidence that the true mean is between 57.8 and 68.7. The null hypothesis that the mean dissolution at 30 minutes is less than or equal to 85% is accepted ($p > 0.05$).

Vancomycin 250 mg capsules from ViroPharma were found to dissolve faster (mean data-93.2%) than 85% in 45 minutes in BCS pH 6.8 dissolution media.

Student t-test: The mean at 45 minutes is 93.2, with a standard deviation of 2.3. The statistic indicates 95% confidence that the true mean is between 90.5 and 95.8. The null hypothesis that the mean dissolution at 45 minutes is less than or equal to 85% is rejected ($p < 0.05$).

Vancomycin Dissolution Study 4 (USP SGF media pH = 1.2)



Study 4 Dissolution Data (n = 6 dissolution baths)

Vancomycin Dissolution Time	Percentage Released (%)	Standard Deviation
5 min	4.5	1.2
10 min	24.2	2.7
20 min	68.5	2.3
30 min	90.2	3.6
45 min	99.0	2.3
60 min	99.5	1.2

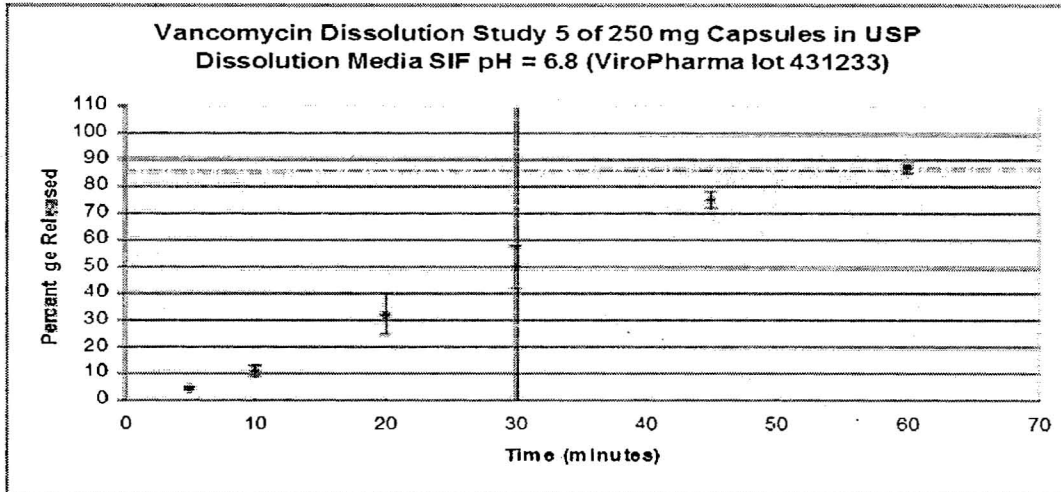
Study 4 Results Vancomycin dissolution characteristics

Vancomycin 250 mg capsules from ViroPharma were found to dissolve faster (mean data-90.2%) than 85% in 30 minutes in USP SGF pH = 1.2 dissolution media.

Student t-test:

The mean at 30 minutes is 90.2, with a standard deviation of 3.6. The statistic indicates 95% confidence that the true mean is between 86.1 and 94.3. The null hypothesis that the mean dissolution at 30 minutes is less than or equal to 85% is rejected (p<0.05).

Vancomycin Dissolution Study 5 (USP SIF media pH = 6.8)



Study 5 Dissolution Data (n = 6 dissolution baths)

Vancomycin Dissolution Time	Percentage Released (%)	Standard Deviation
5 min	4.2	0.8
10 min	10.7	2.3
20 min	31.9	7.5
30 min	50.1	7.6
45 min	74.9	3.1
60 min	86.9	1.8

Study 5 Results Vancomycin dissolution characteristics

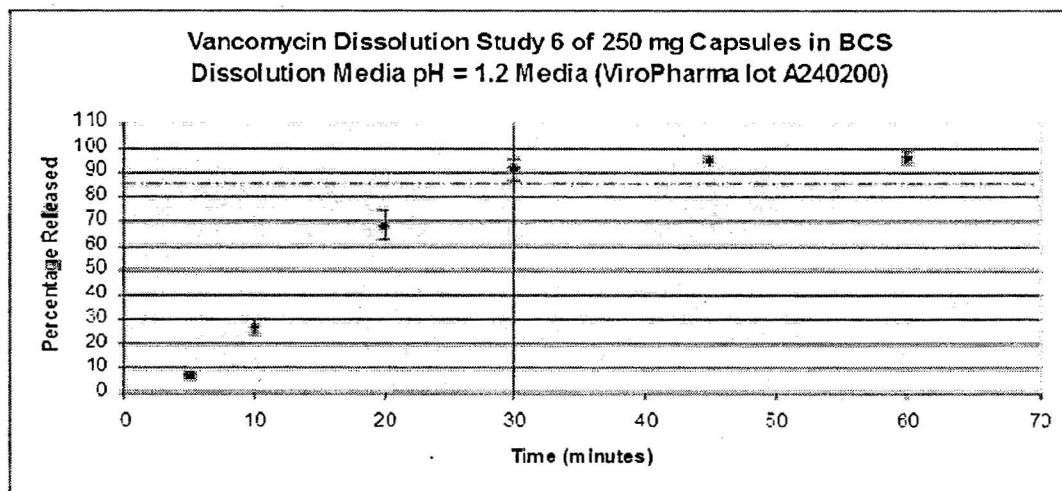
Vancomycin 250 mg capsules from ViroPharma were found to dissolve slower (50.1%) than 85% in 45 minutes in USP SIF pH = 6.8 dissolution media.

Student t-test: The mean at 30 minutes is 50.1, with a standard deviation 7.6. The statistic indicates 95% confidence that the true mean is between 41.4 and 58.8. The null hypothesis that the mean dissolution at 30 minutes is less than or equal to 85% is accepted ($p > 0.05$).

Vancomycin 250 mg capsules from ViroPharma were found to dissolve faster (mean data- 86.9%) than 85% in 60 minutes in USP SIF pH = 6.8 dissolution media, but not in 45 minutes.

Student t-test: The mean at 45 minutes is 74.9, with a standard deviation of 3.1. The statistic indicates 95% confidence that the true mean is between 71.3 and 78.5. The null hypothesis that the mean dissolution at 45 minutes is less than or equal to 85% is accepted ($p > 0.05$).

Vancomycin Dissolution Study 6 (BCS media pH = 1.2)



Study 6 Dissolution Data (n = 6 dissolution baths)

Vancomycin Dissolution Time	Percentage Released (%)	Standard Deviation
5 min	6.9	1.2
10 min	26.9	3.3
20 min	68.2	6.1
30 min	91.0	3.9
45 min	94.7	0.9
60 min	95.9	2.8

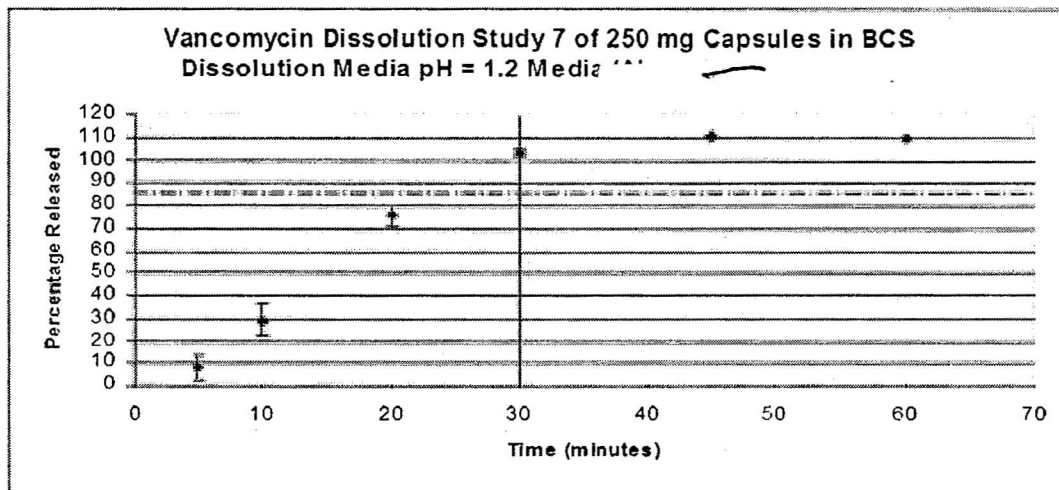
Study 6 results Vancomycin dissolution characteristics

Vancomycin 250 mg capsules from ViroPharma (lot A240200) were found to dissolve faster (mean data-91.0%) than 85% in 30 minutes in USP SIF pH = 1.2 dissolution media.

Student t-test:

The mean at 30 minutes is 91.0, with a standard deviation of 3.9. The statistic indicates 95% confidence that the true mean is between 86.5 and 95.5. The null hypothesis that the mean dissolution at 30 minutes is less than or equal to 85% is rejected (p< 0.05).

Vancomycin Dissolution Study 7 (BCS media pH = 1.2)



Study 7 Dissolution Data (n = 6 dissolution baths)

Vancomycin Dissolution Time	Percentage Released (%)	Standard Deviation
5 min	8.0	1.5
10 min	29.3	6.0
20 min	75.9	7.0
30 min	103.5	4.4
45 min	110.5	1.8
60 min	109.9	1.0

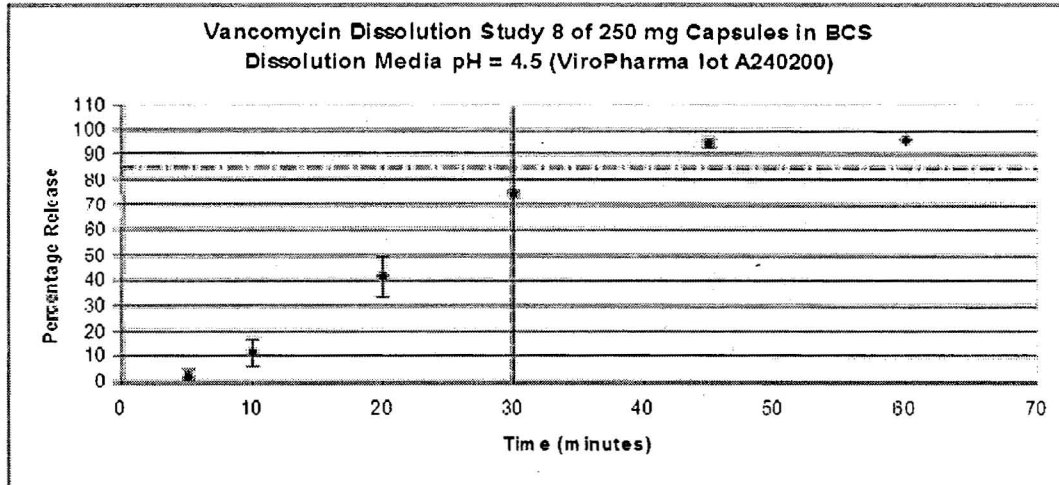
Study 7 results Vancomycin dissolution characteristics

Vancomycin 250 mg capsules from _____ were found to dissolve faster (mean data-103.5%) than 85% in 30 minutes in BCS pH = 1.2 dissolution media.

Student t-test:

The mean at 30 minutes is 103.5, with a standard deviation of 4.4. The statistic indicates 95% confidence that the true mean is between 98.4 and 108.6. The null hypothesis that the mean dissolution at 30 minutes is less than or equal to 85% is rejected (p<0.05).

Vancomycin Dissolution Study 8 (BCS media pH = 4.5)



Study 8 Dissolution Data (n = 6 dissolution baths)

Vancomycin Dissolution Time	Percentage Released (%)	Standard Deviation
5 min	2.2	0.6
10 min	11.4	1.8
20 min	41.5	5.6
30 min	74.5	7.7
45 min	95.2	1.4
60 min	96.5	1.9

Study 8 results Vancomycin dissolution characteristics

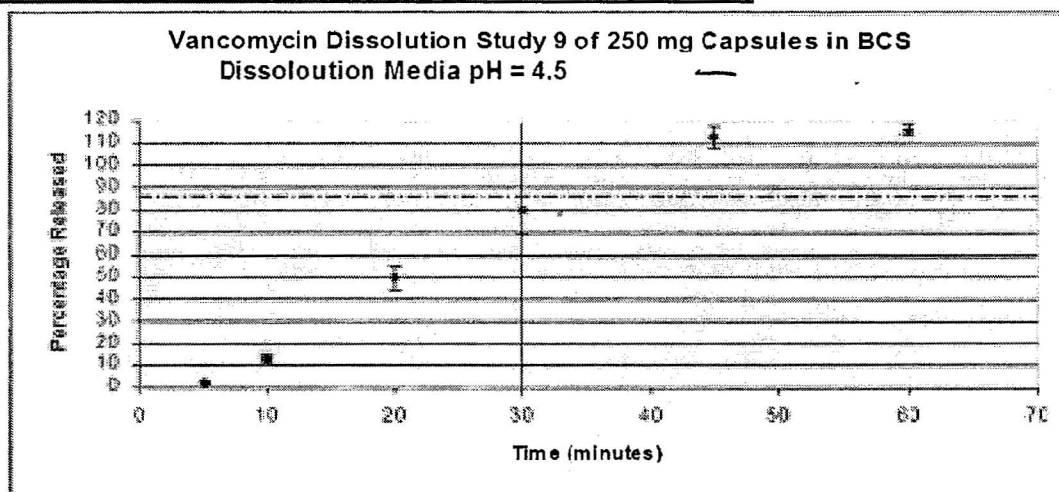
Vancomycin 250 mg capsules from ViroPharma (lot A240200) were found to dissolve slower (mean data-74.5%) than 85% in 30 minutes in BCS pH = 4.5 dissolution media.

Student t-test: The mean at 30 minutes is 74.5, with a standard deviation of 7.7. The statistic indicates 95% confidence that the true mean is between 65.7 and 83.3. The null hypothesis that the mean dissolution at 30 minutes is less than or equal to 85% is accepted ($p > 0.05$).

Vancomycin 250 mg capsules from ViroPharma (lot A240200) were found to dissolve faster (95.2%) than 85% in 45 minutes in BCS pH = 4.5 dissolution media.

Student t-test: The mean at 45 minutes is 95.2, with a standard deviation of 1.4. The statistic indicates 95% confidence that the true mean is between 93.5 and 96.8. The null hypothesis that the mean dissolution at 45 minutes is less than or equal to 85% is rejected ($p < 0.05$).

Vancomycin Dissolution Study 9 (BCS media pH = 4.5)



Study 9 Dissolution Data (n = 6 dissolution baths)

Vancomycin Dissolution Time	Percentage Released (%)	Standard Deviation
5 min	2.2	0.9
10 min	13.0	1.9
20 min	49.9	5.4
30 min	79.8	10.7
45 min	112.7	5.0
60 min	116.0	2.7

Study 9 results Vancomycin dissolution characteristics

Vancomycin 250 mg capsules from _____) were found to dissolve no faster (mean data-79.8%) than 85% in 30 minutes in BCS pH = 4.5 dissolution media.

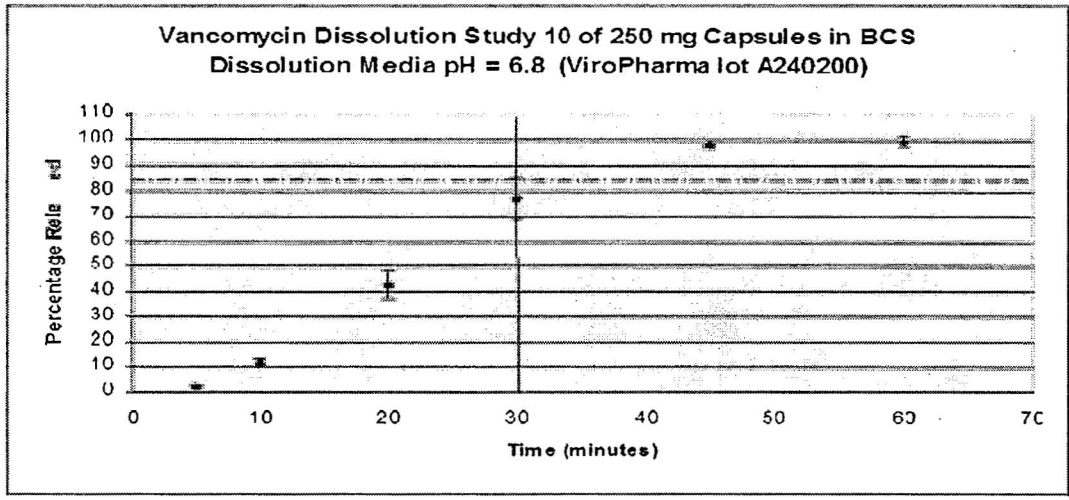
Student t-test: The mean at 30 minutes is 79.8, with a standard deviation of 10.7. The statistic indicates 95% confidence that the true mean is between 67.5 and 92.1. The null hypothesis that the mean dissolution at 30 minutes is less than or equal to 85% is accepted (p>0.05).

Vancomycin 250 mg capsules from / _____) were found to dissolve faster (mean data-112.7%) than 85% in 45 minutes in BCS pH = 4.5 dissolution media.

Student t-test: The mean at 45 minutes is 112.7, with a standard deviation of 5.0. The statistic indicates 95% confidence that the true mean is between 106.9 and 118.4. The null hypothesis that the mean dissolution at 45 minutes is less than or equal to 85% is rejected (p<0.05).

Vancomycin 250 mg capsules from _____ were found to dissolve more than **116%** in 60 minutes in BCS pH = 4.5 dissolution media.

Vancomycin Dissolution Study 10 (BCS media pH = 6.8)



Study 10 Dissolution Data (n = 6 dissolution baths)

Vancomycin Dissolution Time	Percentage Released (%)	Standard Deviation
5 min	2.3	0.6
10 min	11.8	1.9
20 min	42.9	5.8
30 min	76.9	7.9
45 min	98.3	1.5
60 min	99.6	2.0

Study 10 results Vancomycin dissolution characteristics

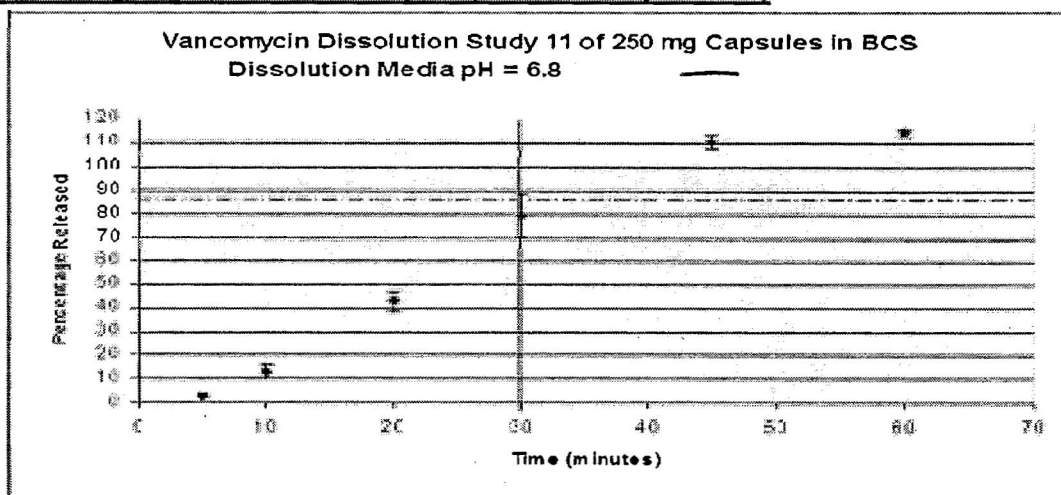
Vancomycin 250 mg capsules from ViroPharma (lot A240200) were found to dissolve slower (mean data-76.9%) than 85% in 30 minutes in BCS pH = 6.8 dissolution media.

Student t-test: The mean at 30 minutes is 76.9, with a standard deviation of 7.9. The statistic indicates 95% confidence that the true mean is between 67.8 and 85.9. The null hypothesis that the mean dissolution at 30 minutes is less than or equal to 85% is accepted ($p > 0.05$).

Vancomycin 250 mg capsules from ViroPharma (lot A240200) were found to dissolve faster (mean data-98.3%) than 85% in 45 minutes in BCS pH = 6.8 dissolution media.

Student t-test: The mean at 45 minutes is 98.3, with a standard deviation of 1.5. The statistic indicates 95% confidence that the true mean is between 95.7 and 100.0. The null hypothesis that the mean dissolution at 45 minutes is less than or equal to 85% is rejected ($p < 0.05$).

Vancomycin Dissolution Study 11 (BCS media pH = 6.8)



Study 11 Dissolution Data (n = 6 dissolution baths)

Vancomycin Dissolution Time	Percentage Released (%)	Standard Deviation
5 min	2.9	0.9
10 min	13.1	3.2
20 min	42.9	4.4
30 min	79.6	8.9
45 min	110.4	2.9
60 min	114.5	1.7

Study 11 results Vancomycin dissolution characteristics

Vancomycin 250 mg capsules from [redacted] were found to dissolve no faster (mean data – 79.6%) than 85% in 30 minutes in BCS pH = 6.8 dissolution media.

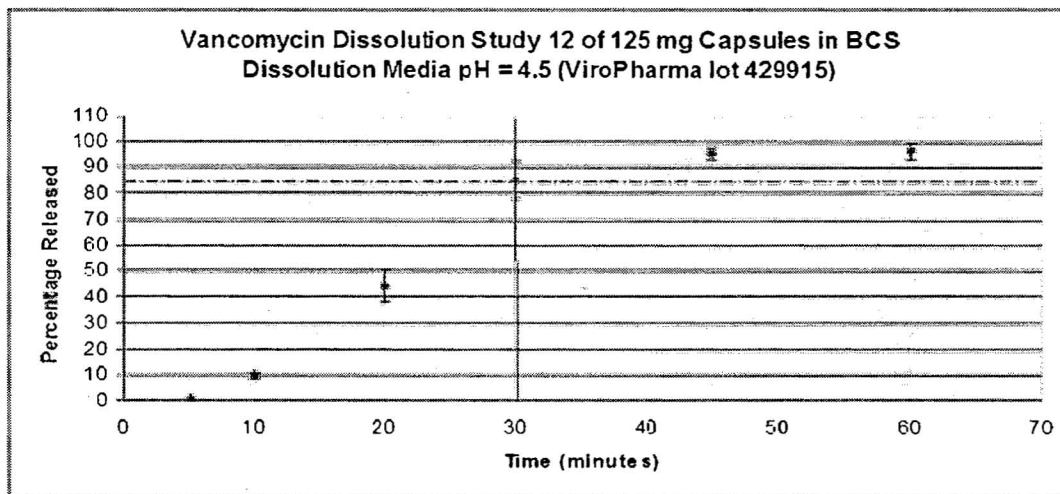
Student t-test: The mean at 30 minutes is 79.6, with a standard deviation of 8.9. The statistic indicates 95% confidence that the true mean is between 69.4 and 89.8. The null hypothesis that the mean dissolution at 30 minutes is less than or equal to 85% is accepted (p>0.05).

Vancomycin 250 mg capsules from [redacted] were found to dissolve faster than 85% in 45 minutes in BCS pH = 6.8 dissolution media.

Student t-test: The mean at 45 minutes is 110.4, with a standard deviation of 2.9. The statistic indicates 95% confidence that the true mean is between 107.0 and 113.7. The null hypothesis that the mean dissolution at 45 minutes is less than or equal to 85% is rejected (p<0.05).

Vancomycin 250 mg capsules from [redacted] were found to dissolve more than 114% in 60 minutes in BCS pH = 6.8 dissolution media.

Vancomycin Dissolution Study 12 (BCS media pH = 4.5)



Study 12 Dissolution Data (n = 6 dissolution baths)

Vancomycin Dissolution Time	Percentage Released (%)	Standard Deviation
5 min	0.5	0.2
10 min	9.9	1.5
20 min	44.2	6.5
30 min	84.9	7.2
45 min	95.8	2.2
60 min	96.5	3.0

Study 12 results Vancomycin dissolution characteristics

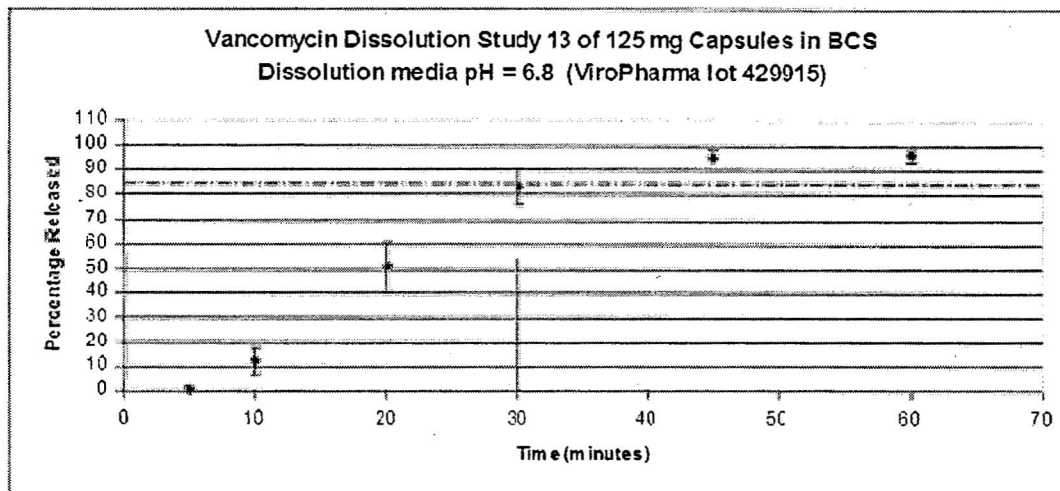
Vancomycin **125 mg capsules** from ViroPharma (lot 429915) were found to dissolve no faster than 85% (mean data-84.9%) in 30 minutes in BCS pH = 4.5 dissolution media.

Student t-test: The mean at 30 minutes is 84.9, with a standard deviation of 7.2. The statistic indicates 95% confidence that the true mean is between 76.6 and 93.2. The null hypothesis that the mean dissolution at 30 minutes is less than or equal to 85% is accepted ($p > 0.05$).

Vancomycin **125 mg capsules** from ViroPharma (lot 429915) were found to dissolve faster (mean data-95.8%) than 85% in 45 minutes in BCS pH = 4.5 dissolution media.

Student t-test: The mean at 45 minutes is 95.8, with a standard deviation of 2.2. The statistic indicates 95% confidence that the true mean is between 93.2 and 98.3. The null hypothesis that the mean dissolution at 45 minutes is less than or equal to 85% is rejected ($p < 0.05$).

Vancomycin Dissolution Study 13 (BCS media pH = 6.8)



Study 13 Dissolution Data (n = 6 dissolution baths)

Vancomycin Dissolution Time	Percentage Released (%)	Standard Deviation
5 min	0.9	0.9
10 min	12.7	5.5
20 min	50.9	9.7
30 min	83.6	7.3
45 min	95.3	2.9
60 min	96.4	2.4

Study 13 results Vancomycin dissolution characteristics

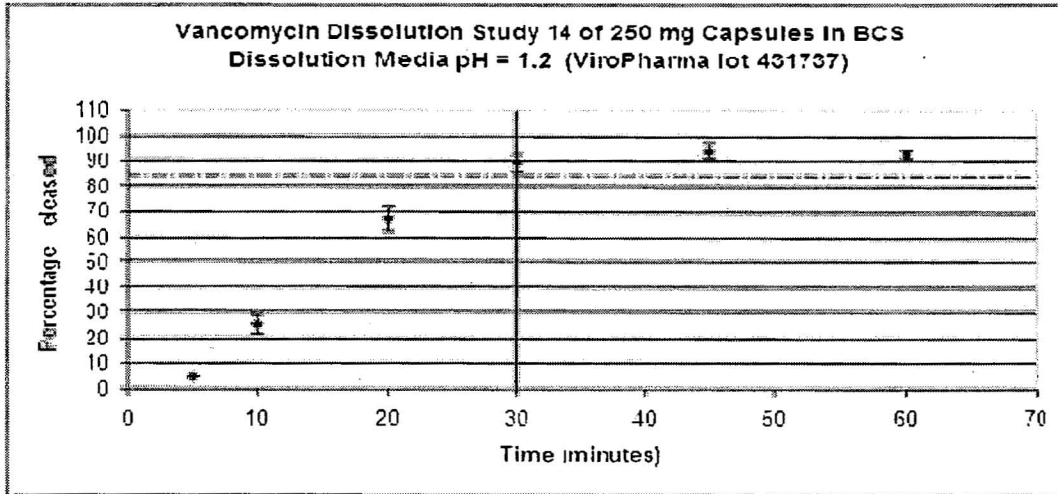
Vancomycin **125 mg capsules** from ViroPharma (lot 429915) were found to dissolve no faster (mean data-83.6%) than 85% in 30 minutes in BCS pH = 6.8 dissolution media.

Student t-test: The mean at 30 minutes is 83.6, with a standard deviation of 7.3. The statistic indicates 95% confidence that the true mean is between 75.2 and 91.9. The null hypothesis that the mean dissolution at 30 minutes is less than or equal to 85% is accepted ($p > 0.05$).

Vancomycin **125 mg capsules** from ViroPharma (lot 429915) were found to dissolve faster (mean data-95.3%) than 85% in 45 minutes in BCS pH = 6.8 dissolution media.

Student t-test: The mean at 45 minutes is 95.3, with a standard deviation of 2.9. The statistic indicates 95% confidence that the true mean is between 91.9 and 98.6. The null hypothesis that the mean dissolution at 45 minutes is less than or equal to 85% is rejected ($p < 0.05$).

Vancomycin Dissolution Study 14 (BCS media pH = 1.2)



Study 14 Dissolution Data (n = 6 dissolution baths)

Vancomycin Dissolution Time	Percentage Released (%)	Standard Deviation
5 min	4.6	0.5
10 min	25.2	3.7
20 min	67.0	4.9
30 min	89.3	3.3
45 min	94.2	2.9
60 min	93.1	1.9

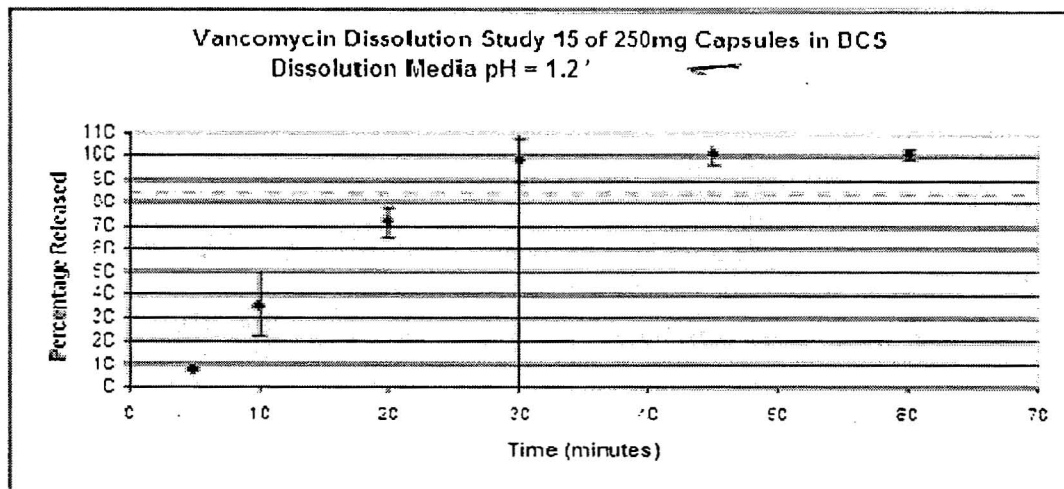
Study 14 results Vancomycin dissolution characteristics

Vancomycin **250 mg capsules** from ViroPharma (lot 431737) were found to dissolve faster (mean data-89.3%) than 85% in 30 minutes in BCS pH = 1.2 dissolution media.

Student t-test:

The mean at 30 minutes is 89.3, with a standard deviation of 6.6. The statistic indicates 95% confidence that the true mean is between 85.5 and 93.1. The null hypothesis that the mean dissolution at 30 minutes is less than or equal to 85% is rejected ($p < 0.05$).

Vancomycin Dissolution Study 15 (BCS media pH = 1.2)



Study 15 Dissolution Data (n = 6 dissolution baths)

Vancomycin Dissolution Time	Percentage Released (%)	Standard Deviation
5 min	7.7	0.8
10 min	35.9	13.7
20 min	71.9	6.4
30 min	98.4	9.3
45 min	100.9	4.1
60 min	101.3	2.1

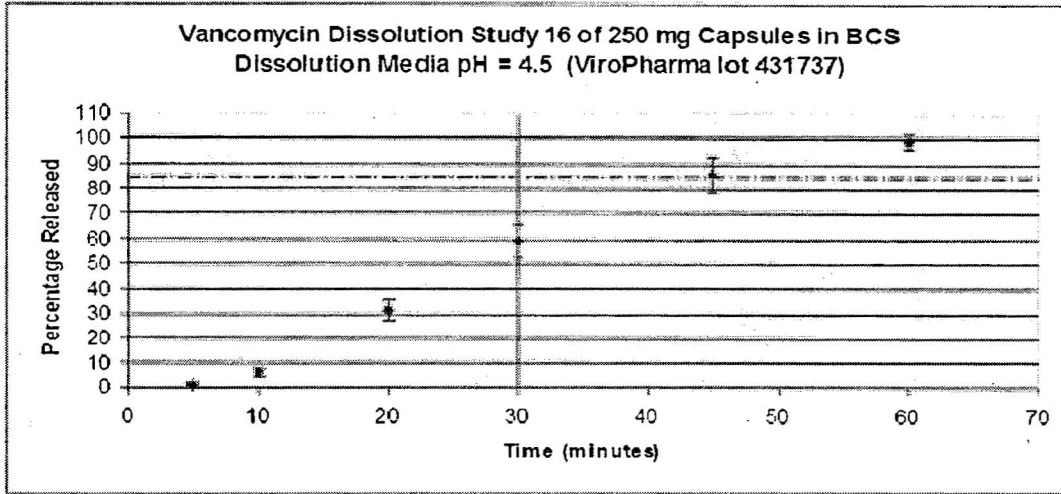
Study 15 results Vancomycin dissolution characteristics

Vancomycin 250 mg capsules from _____ are found to dissolve faster (mean data-98.4%) than 85% in 30 minutes in BCS pH = 1.2 dissolution media.

Student t-test:

The mean at 30 minutes is 98.4, with a standard deviation of 9.3. The statistic indicates 95% confidence that the true mean is between 87.7 and 109.1. The null hypothesis that the mean dissolution at 30 minutes is less than or equal to 85% is rejected (p<0.05).

Vancomycin Dissolution Study 16 (BCS media pH = 4.5)



Study 16 Dissolution Data (n = 6 dissolution baths)

Vancomycin Dissolution Time	Percentage Released (%)	Standard Deviation
5 min	0.83	0.9
10 min	6.1	1.7
20 min	31.6	3.8
30 min	59.3	6.9
45 min	85.8	6.8
60 min	98.7	3.4

Study 16 results Vancomycin dissolution characteristics

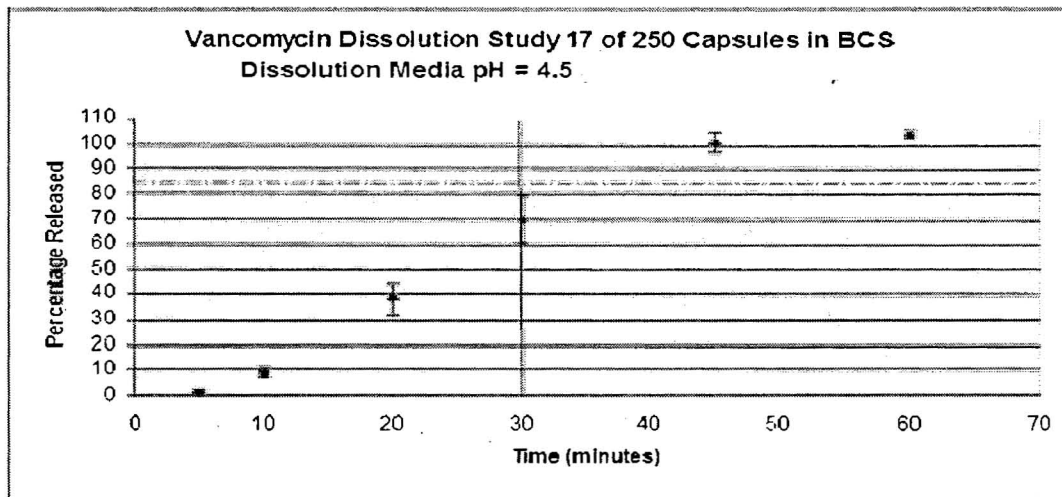
Vancomycin 250 mg capsules from ViroPharma (lot 431737) were found to dissolve slower (mean data-59.3%) than 85% in 30 minutes in BCS pH = 4.5 dissolution media.

Student t-test: The mean at 30 minutes is 59.3, with a standard deviation of 6.9. The statistic indicates 95% confidence that the true mean is between 51.3 and 67.2. The null hypothesis that the mean dissolution at 30 minutes is less than or equal to 85% is accepted ($p > 0.05$).

Vancomycin 250 mg capsules from ViroPharma (lot 431737) were found to dissolve no faster than 85% in 45 minutes in BCS pH = 4.5 dissolution media, based on the statistical data presented below.

Student t-test: The mean at 45 minutes is 85.8, with a standard deviation of 6.8. The statistic indicates 95% confidence that the true mean is between 77.9 and 93.6. The null hypothesis that the mean dissolution at 45 minutes is less than or equal to 85% is accepted ($p > 0.05$).

Vancomycin Dissolution Study 17 (BCS media pH = 4.5)



Study 17 Dissolution Data (n = 6 dissolution baths)

Vancomycin Dissolution Time	Percentage Released (%)	Standard Deviation
5 min	1.4	0.8
10 min	9.3	2.2
20 min	38.2	6.4
30 min	69.8	9.3
45 min	100.6	4.0
60 min	104.0	2.0

Study 17 results Vancomycin dissolution characteristics

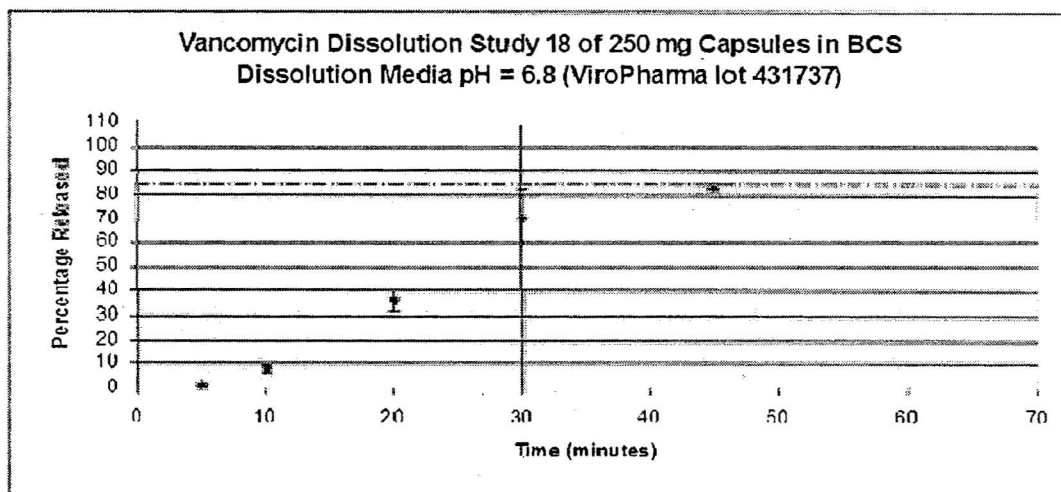
Vancomycin 250 mg capsules from [redacted] were found to dissolve slower (mean data-69.8%) than 85% in 30 minutes in BCS pH = 4.5 dissolution media.

Student t-test: The mean at 30 minutes is 69.8, with a standard deviation of 9.3. The statistic indicates 95% confidence that the true mean is between 59.1 and 80.5. The null hypothesis is that the mean dissolution at 30 minutes is less than or equal to 85% ($p > 0.05$).

Vancomycin 250 mg capsules from [redacted] were found to dissolve faster (mean data-100.6%) than 85% in 45 minutes in BCS pH = 4.5 dissolution media.

Student t-test: The mean at 45 minutes is 100.6, with a standard deviation of 4.0. The statistic indicates 95% confidence that the true mean is between 96.0 and 105.1. The null hypothesis that the mean dissolution at 45 minutes is less than or equal to 85% is rejected ($p < 0.05$).

Vancomycin Dissolution Study 18 (BCS media pH = 6.8)



Study 18 Dissolution Data (n = 6 dissolution baths)

Vancomycin Dissolution Time	Percentage Released (%)	Standard Deviation
5 min	1.2	0.7
10 min	8.1	1.7
20 min	36.3	4.3
30 min	70.4	12
45 min	83.3	1.5
60 min	N/A	N/A

Study 18 results Vancomycin dissolution characteristics

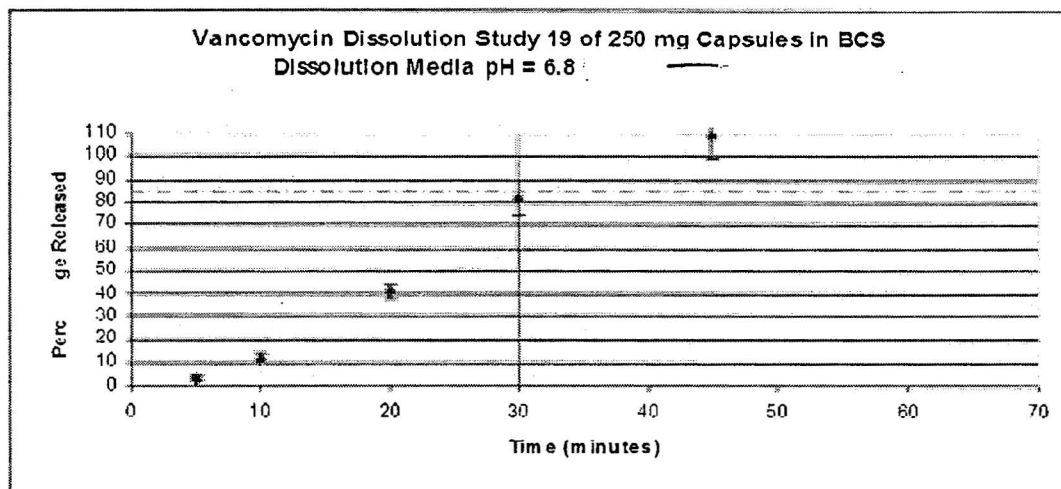
Vancomycin **250 mg capsules** from ViroPharma (lot 431737) were found to dissolve slower (mean data-70.4%) than 85% in 30 minutes in BCS pH = 6.8 dissolution media.

Student t-test: The mean at 30 minutes is 70.4, with a standard deviation of 12. The statistic indicates 95% confidence that the true mean is between 56.8 and 84.2. The null hypothesis that the mean dissolution at 30 minutes is less than or equal to 85% is accepted ($p > 0.05$).

Vancomycin **250 mg capsules** from ViroPharma (lot 431737) were found to dissolve slower (mean data-83.3%) than 85% in 45 minutes in BCS pH = 6.8 dissolution media.

Student t-test: The mean at 45 minutes is 83.3, with a standard deviation of 1.5. The statistic indicates 95% confidence that the true mean is between 81.6 and 85.0. The null hypothesis that the mean dissolution at 45 minutes is less than or equal to 85% is accepted ($p > 0.05$).

Vancomycin Dissolution Study 19 (BCS media pH = 6.8)



Study 19 Dissolution Data (n = 6 dissolution baths)

Vancomycin Dissolution Time	Percentage Released (%)	Standard Deviation
5 min	3.3	1.1
10 min	11.3	2.6
20 min	40.8	3.3
30 min	81.9	7.9
45 min	109.1	9.4
60 min	N/A	N/A

Study 19 results Vancomycin dissolution characteristics

Vancomycin 250 mg capsules from [redacted] were found to dissolve no faster (mean data-81.9%) than 85% in 30 minutes in BCS pH = 6.8 dissolution media.

Student t-test: The mean at 30 minutes is 81.9, with a standard deviation 7.9. The statistic indicates 95% confidence that the true mean is between 72.8 and 90.9. The null hypothesis that the mean dissolution at 30 minutes is less than or equal to 85% is accepted ($p > 0.05$).

Vancomycin 250 mg capsules from [redacted] were found to dissolve faster (mean data-109.1%) than 85% in 45 minutes in BCS pH = 6.8 dissolution media.

Student t-test: The mean at 45 minutes is 109.1, with a standard deviation of 9.4. The statistic indicates 95% confidence that the true mean is between 98.3 and 119.9. The null hypothesis that the mean dissolution at 45 minutes is less than or equal to 85% is rejected ($p < 0.05$).

DISCUSSION

Vancomycin drug products were determined to be rapidly dissolving at pH 1.2 in BCS dissolution media and USP SGF dissolution media at pH 1.2 where >85% was dissolved in 30 minutes.

Vancomycin drug products were found to dissolve faster than 85% in 45 minutes in BCS and USP dissolution media at pH = 4.5 and 6.8. Two exceptions to note were ViroPharma drug product lot 431233 in SIF pH 6.8 and ViroPharma product lot 431737 in BCS pH 6.8.

It should also be noted that vancomycin drug product 125 mg capsules dissolved relatively more rapidly (84.9% and 83.6%) than all other 250 mg drug product capsules (<82.2%) in pH 4.5 and 6.8 but they were still not rapidly dissolving in 30 minutes.

It should also be noted that preliminary dissolution studies conducted with USP biorelevant media at pH 4.5 (69.8%, $p > 0.9851$) and pH 6.8 (75.7%, $p > 0.9739$) with ViroPharma lot 431233 had shown the drug product was not rapidly dissolving in 30 minutes.

Application of the Student's t- test confirmed the dissolution data at the mean and only those dissolution studies that were conducted in pH 1.2 media were found to be rapidly dissolving (>85%) in 30 minutes. The Student's t- test also found that ViroPharma product lot 431737 in BCS pH 4.5 did not dissolve significantly faster than 85% in 45 minutes.

Overall vancomycin drug products at pH 1.2, pH 4.5 and pH 6.8 were found to dissolve faster than 85% in 45 minutes with note to the above listed exceptions.

CONCLUSION

Vancomycin drug product capsules were rapidly dissolving (i.e., 85% of the drug product label in 30 minutes) when the pH of the dissolution media was pH 1.2. Since the dissolution was greater than 85% in 30 minutes (pH 1.2) and less than 85% in 30 minutes (pH 4.5 and pH 6.8) as noted in the discussion section, vancomycin hydrochloride was not rapidly dissolving by the BCS Guidance. However, the products were found to dissolve greater than 85% in pH 4.5 and 6.8 with the exception of two ViroPharma lots in pH 6.8.

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