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Invitro Potency Analysis of Vancomyc in Capsules by Microbiological Assay

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Abstract: *Vancomyc in, a glycopeptide antibiotic, has a narrow spectrum of activity, with action primarily against gram-positive cocci and bacilli. The drug is bacteriostatic against most enterococci and exhibits synergy when combined with an aminoglycoside. Vancomycin has been used widely in treating many susceptible infections, but the emergence of vancomycin-resistant enterococci. Now a days, It is used for the Treatment of life threatening Pseudomembranous colitis infection caused by Clostridium difficile . In this work it deals with invitro potency analysis of vancomycin capsules by microbiological assay. In the pharmaceutical industry, drug dissolution testing is routinely used to provide vitro drug release information for both quality control purposes. It is helpful for drug formulation design and post-approval manufacturing changes, stated by food and drug administration (FDA). It is followed by microbiological assay to check the potency of the drug by cylinder plate method for vancomycin (125 & 250mg) capsules against ATCC6633 Bacillus subtilis. The comparison are restricted to relationship between zone diameter measurements within plates, excluding the variation between plates. Individual plate response are normalized on the basis of the relation zone size of the standard, compared to mean zone size of a standard across the plates. In this study, the vanomycin capsules (125 & 250 mg) in raw materials, validation samples, finished products and stability samples showed the accepted product potency of 98-105%. The assay is designed in such a way that the mathematical model on which the potency equation is based can be proved to be VALID. In pharmaceutical company, before commercialization of the drug into markets, the potency of vancomycin, shelf life is predetermined and got approval of regulatory bodies and then released to commercialization to markets.*

Keywords: *Vancomyc in capsules, ATCC6633 Bacillus subtilis, Potency, Shelf life, Commercialization*.

I. Introduction

Vancomycin was first isolated in 1953 by Edmund Kornfeld (worked at Eli Lilly) from a soil sample collected from the interior jungles ofBorneo by a missionary.(shnaverson et al.,2003). The organism that produced it was eventually named Amycolatopsis orientalis.The original indication for vancomycin was for the treatment of penicillin-resistant Staphylococcus aureus.

The compound was initially called compound 05865, but was eventually given the generic name vancomycin, derived from the term "vanquish". One advantage that was quickly apparent is that Staphylococci did not develop significant resistance despite serial passage in culture media containing vancomycin. The rapid development of penicillin resistance by staphylococci led to the compounds being fast-tracked for approval by the Food and Drug Administration (FDA) in 1958. Eli Lilly first marketed vancomycin hydrochloride under the trade name Vancocin and as COVANC from Nucleus, India.(Levine and maelleringRc Jr, 2006)

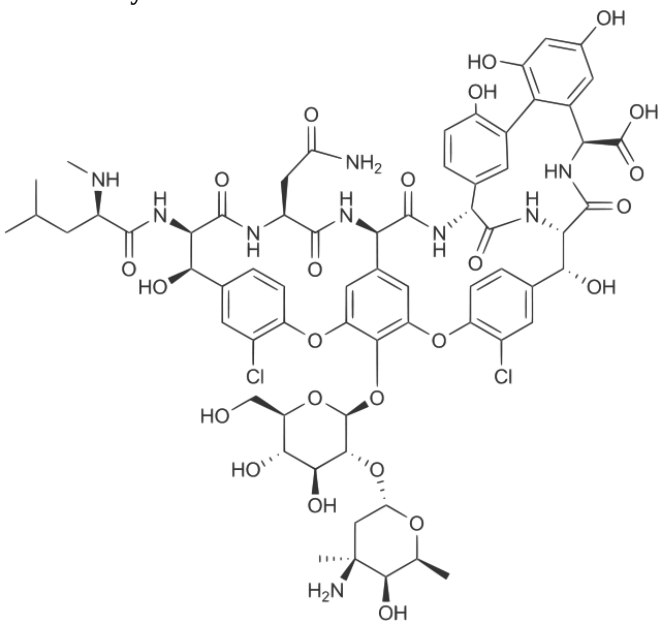
1.1 Vancomyc in Is Not First Line Drug Against Staphylococcus Aureus Infection:

It possesses poor oral bioavailability; it must be given intravenously for most infections B-Lactamase-resistant semi-synthetic penicillins such as methicillin (and its successors, Nafcillin and Cloxacillin) were subsequently developed, which have better activity against non-MRSA Staphylococci.Early trials used early impure forms of vancomycin ("Mississippi mud"), which were found to be toxic to the ears and to the kidneys. (Griffith RS, 1981)

In 2004**,** Eli Lilly licensed Vancocin to ViroPharma in the U.S., Flynn Pharma in the UK, and Aspen Pharmacare in Australia. The patent expired in the early 1980s; the FDA authorized the sale of several generic versions in the USA, including from manufacturers Bioniche Pharma, Baxter Healthcare, Sandoz, Akorn Strides and Hospira.(Approved Drug Products with Therapeutic Equivalence Evaluations)

An oral form of vancomycin was originally approved by the FDA in 1986 for the treatment of Clostridium difficile inducedpseudomembranous colitis. It is not orally absorbed into the blood and remains in the gastrointestinal tract to eradicate Clostridium difficile. This product is currently marketed by ViroPharma in the USA (orange book detail record search).

1.3 Chemical Structure Of Vancomyc in:



1.4 Mechanism Of Action:

Vancomycin acts by inhibiting proper cell wall synthesis in Gram-positive bacteria. Due to the different mechanism by which Gram-negative bacteria produce their cell walls and the various factors related to entering the outer membrane of Gram-negative organisms, vancomycin is not active against Gram-negative bacteria (except some non-gonococcal species of Neisseria).

The large hydrophilic molecule is able to form hydrogen bond interactions with the terminal D-alanyl-D-alanine moieties of the NAM/NAG-peptides. Under normal circumstances, this is a five-point interaction. This binding of vancomycin to the D-Ala-D-Ala prevents cell wall synthesis in two ways. It prevents the synthesis of the long polymers of N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) that form the backbone strands of the bacterial cell wall, and it prevents the backbone polymers that do manage to form from cross-linking with each other.(clinical pharmacology)

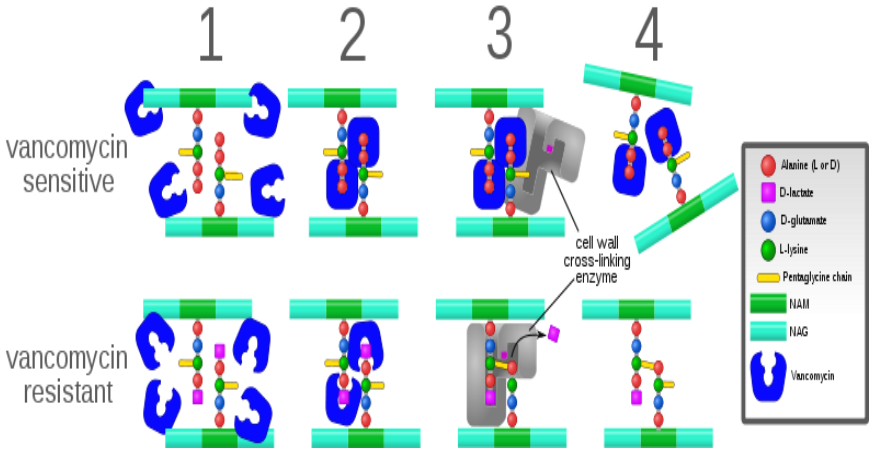


Fig-1: Mechanism of vancomycin action and resistance.

It shows only one of two ways vancomycin acts against bacteria (inhibition of cell wall cross-linking) and only one of many ways that bacteria can become resistant to it.

Vancomycin is added to the bacterial environment while it is trying to synthesize new cell wall. Here, the cell wall strands have been synthesized, but not yet cross-linked.

Vancomycin recognizes and binds to the two D-ala residues on the end of the peptide chains. However, in resistant bacteria, the last D-ala residue has been replaced by a D-lactate, so vancomycin cannot bind.

In resistant bacteria, cross-links are successfully formed. However, in the non-resistant bacteria, the vancomycin bound to the peptide chains prevents them from interacting properly with the cell wall cross-linking enzyme.

In the resistant bacteria, stable cross links are formed. In the sensitive bacteria, cross-links cannot be formed and the cell wall falls apart.

1.5 Clinical Uses

Vancomycin is indicated for the treatment of serious, life-threatening infections by Gram-positive bacteria that are unresponsive to other less-toxic antibiotics. In particular, vancomycin should not be used to treat methicillin-sensitive Staphylococcus aureus because it is inferior to penicillins such as nafcillin.(small PM & chambers HF,1990) & (Gonzalezc et al.,1999).

Additionally, oral vancomycin has recently been reported by one group to have some benefit in the treatment of primary sclerosing cholangitis, a progressive liver disease which can ultimately lead to cirrhosis and liver failure. (Davies YK & cox KM et al., 2008)

A phase 3 clinical trial is currently being conducted by the same group at the Stanford School of Medicine to test the long-term treatment of oral vancomycin for primary sclerosing cholangitis in both children and adults.

The increasing emergence of vancomycin-resistant Enterococci has resulted in the development of guidelines for use by the Centers for Disease Control (CDC) Hospital Infection Control Practices Advisory Committee.

1.6 Guidelines Restrict Use Of Vancomycin (Rossi Et Al., 2006):

Treatment of serious infections caused by susceptible organisms resistant to penicillins (methicillin-resistant Staphylococcus aureus and multi-resistant Staphylococcus epidermidis (MRSE)) or in individuals with serious allergy to penicillins

Treatment of Pseudomembranous colitis caused by the bacterium Clostridium difficile; in particular, in cases of relapse or where the infection is unresponsive to metronidazole treatment (for this indication, vancomycin is given orally, rather than via its typical, I.V. route)

For treatment of infections caused by Gram-positive microorganisms in patients with serious allergies to beta-lactam antimicrobials.

Antibacterial prophylaxis for endocarditis following certain procedures in penicillin-hypersensitive individuals at high risk.Surgical prophylaxis for major procedures involving implantation of prostheses in institutions with a high rate of MRSA or MRSE.

Early in treatment as an empiric antibiotic for possible MRSA infection while waiting for culture identification of the infecting organism. (Recommendations for Preventing the Spread of Vancomycin Resistance Recommendations of the Hospital Infection Control Practices Advisory Committee (HICPAC)

1.7 Adverse Effects:

- Vancomycin levels are usually monitored, in an effort to reduce adverse events, the value of this is not beyond debate.Peak and trough levels are usually monitored, and, for research purposes, the area under the curve is also sometimes used. Toxicity is best monitored by looking at trough values. (Lomaestro et al., 2009). Common adverse drug reactions (≥1% of patients) associated with IV vancomycin include: local pain, which may be severe and/or thrombophlebitis.
- Damage to the kidneys and to the hearing were a side-effect of the early impure versions of vancomycin, and these were prominent in the clinical trials conducted in the mid-1950s.(Levine & Maellering RC jr ,2006)
- Later trials using purer forms of vancomycin found that nephrotoxicity is an infrequent adverse effect (0.1–1% of patients), but that this is accentuated in the presence of aminoglycosides.(farber BF& Molellering RC jr, 1983)
- Rare adverse effects (<0.1% of patients) include: anaphylaxis, toxic epidermal necrolysis, erythema multiforme, red man syndrome , superinfection, thrombocytopenia, neutropenia,leucopenia, tinnitus, dizziness and/or ototoxicity.(Rossi et al.,2006)
- It has recently been emphasized that vancomycin can induce platelet-reactive antibodies in the patient, leading to severe thrombocytopenia and bleeding with florid petechial hemorrhages,ecchymoses, and wet purpura .(Drygalski A &Curtis BR,2007)

1.8 Introduction About Microbiological Assay

1.8.1 Dissolution Testing:

In the pharmaceutical industry, drug dissolution testing is routinely used to provide critical in vitro drug release information for both quality control purposes, i.e., to assess batch-to-batch consistency of solid oral dosage forms such as tablets, and drug development, i.e., to predict invivo drug release profiles. (Bai.G & Amenante, 2009)

In vitro drug dissolution data generated from dissolution testing experiments can be related to in vivo pharmacokinetic data by means of in vitro-in vivo correlations (IVIVC). A well-established predictive IVIVC model can be very helpful for drug formulation design and post-approval manufacturing changes.(Kortejavi et al.,2006)

The main objective of developing and evaluating an IVIVC is to establish the dissolution test as a surrogate for human bioequivalence studies, as stated by the Food and Drug Administration (FDA). Analytical data from drug dissolution testing are sufficient in many cases to establish safety and efficacy of a drug product without in vivo tests, following minor formulation and manufacturing changes (Qureshi and Shabnam, 2001). Thus, the dissolution testing which is conducted in dissolution apparatus must be able to provide accurate and reproducible results. (Qureshi and Shabnam, 2001).

Several dissolution apparatuses exist. In United States Pharmacopeia (USP) General Chapter <711> Dissolution, there are four dissolution apparatuses standardized and specified (United States pharmacopeia 36/ National formulary 31, 2013) They are:

- USP Dissolution Apparatus 1 - Basket (37°C)
- USP Dissolution Apparatus 2 - Paddle (37°C)
- USP Dissolution Apparatus 3 - Reciprocating Cylinder (37°C)
- USP Dissolution Apparatus 4 - Flow-Through Cell (37°C)

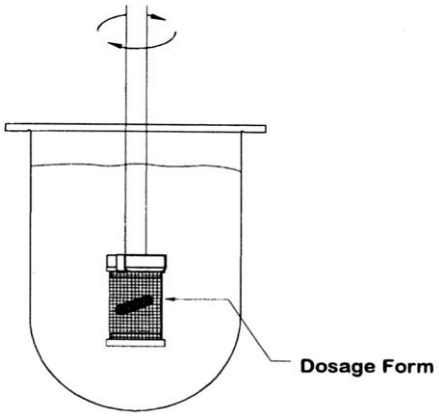


Fig-2 shows the cylindrical bowl of dissolution apparatus

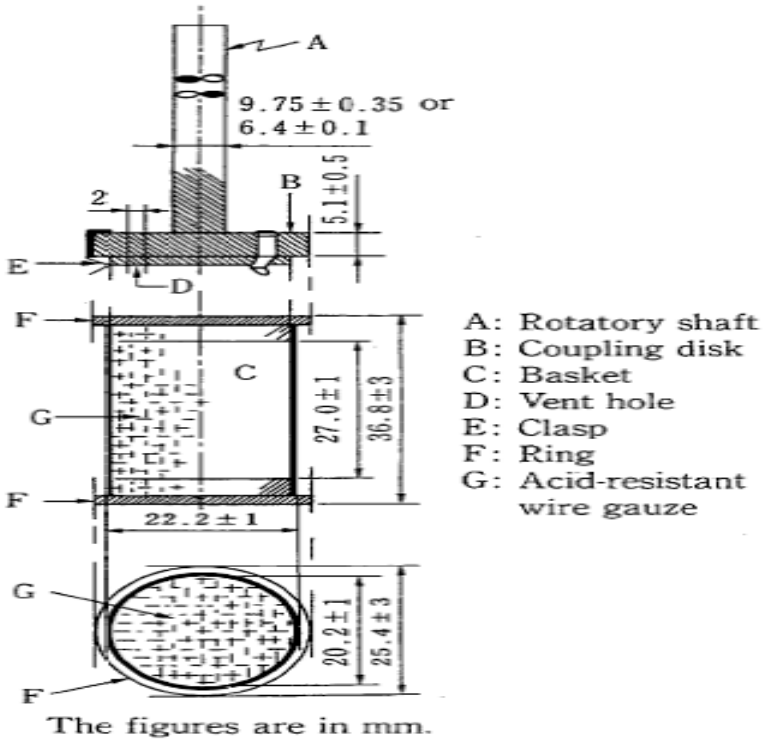


Fig-3 Shows the size and diameter of the basket in dissolution apparatus.

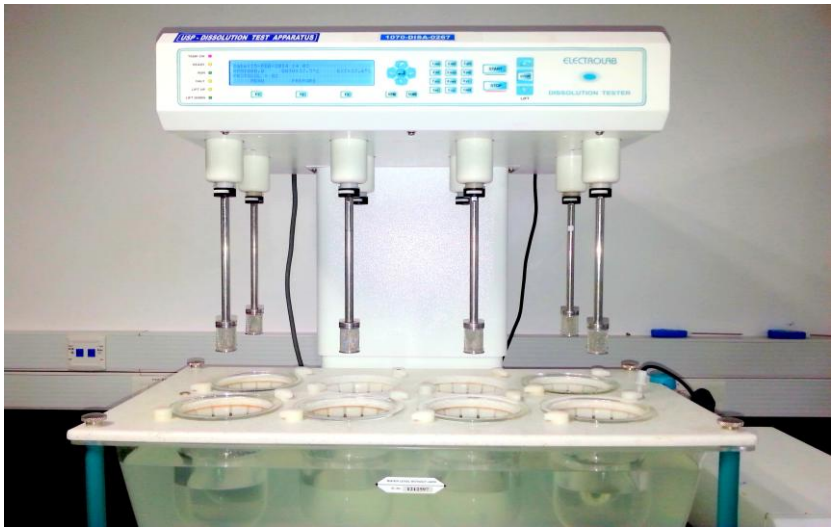


Fig- 4 Shows the digitalized dissolution apparatus.

1.8.2 Microbiological Assay:

The potency activity of antibiotics can be demonstrated by thin inhibitory effect on microorganisms under suitable methods.

A reduction in antimicrobial activity may not be adequately demonstrated by chemical method

This chapter summaries procedure for the antibiotics recognized in usp for which microbiological assay is standard analytical method.

The assay is designed in such a way that the mathematical model on which the potency equation is based can be proved to be VALID.(UNITED STATES PHARMOCOPEIA 36 /NATIONAL FORMULARY 31, 2013).

1.8.3 Principle:

The microbiological assay of an antibiotic is based upon a comparison of the inhibition of growth of microorganisms by measured concentration of the antibiotics under examination with that produced of known concentrations of a standard preparation of the antibiotic having a known activity (UNITED STATES PHARMOCOPEIA 36 /NATIONAL FORMULARY 31, 2013).

1.8.4 Microbiological Assay For Vancomycin Capsules:

1.8.4.1 Cylinder Plate Method (Cup Plate Method):

The cylinder –plate method depends upon diffusion of the antibiotic from a vertical cylinder through a solidified agar layer in a petri dish or plate to an extent such that growth of the added microorganism is prevented entirely in a zone around the cylinder containing a solution of the antibiotic

Inoculate a previously liquefied medium appropriate to the assay with the requisite quantity of suspension of the microorganism, add the suspension to the medium at the temperature between 40°C and 50°Cand immediately pour the inoculated medium into the petridishes or large rectangular plates to give a depth of 3 to 4 mm. Ensure that the layers of the medium are uniform in thickness, by placing the dishes of plates on a level surface.

Store the prepared dishes or plates or plates in a manner so as to ensure that no significant growth or death of the test organism occurs before the dishes or plates are used and that the surface of the agar layer is dry at the time of use.

Using the appropriate buffer solutions, prepare solutions of known concentrations of the standard preparation and solutions of the corresponding assumed of concentrations the antibiotic to be examined. Where directions have been given in the individual monograph for preparing the solutions, these should be followed and further dilutions made with buffer solution

Apply the solutions to the surface of the solid medium in sterile cylinders or in cavities prepared in the agar. The volume of the solution added to each cylinder or cavity must be uniform and sufficient almost to fill the holes when these are used.

When petri dishes are used, arrange the solutions of the solutions of the standard preparation and the antibiotic under the examination on each dish and so that the highest concentrations of standard and test preparations are not adjacent.

When plates are used, place the solutions in a Latin square design are used.

Leave the dishes or plates standing for 1 or 4 hours at room temperature or at 4 c as appropriate, as a period of preincubation diffusion to minimize the effects of variation in time between the applications of different solutions.

Incubate them for about 18 hours at the temperature. Accurately measure the diameters or the circular inhibition zones and calculation the results.

ANTIBIOTIC	TYPES OF ASSAY
AMPHOTERICIN	CYLINDER PLATE ASSAY
BACITRACIN	CYLINDER PLATE ASSAY
CAPREOMYCIN	CYLINDER PLATE ASSAY
CHLORTETRACYLINE	CYLINDER PLATE ASSAY
CLOXACILLIN	CYLINDER PLATE ASSAY
COLISMETHATE	CYLINDER PLATE ASSAY
COLISTIN	CYLINDER PLATE ASSAY
VANCOMYCIN	CYLINDER PLATE ASSAY
GENTAMYCIN	CYLINDER PLATE ASSAY
NOVOBIOCIN	CYLINDER PLATE ASSAY
NYSTATIN	CYLINDER PLATE ASSAY
SISOMICIN	CYLINDER PLATE ASSAY

1.8.5 Assay Design: (Cylinder Plate Method)

The comparison are restricted to relationship between zone diameter measurements within plates, excluding the variation between plates. Individual plate response are normalized on the basis of the relation zone size of the standard, compared to mean zone size of a standard across the plates. (UNITED STATES PHARMOCOPEIA 36 /NATIONAL FORMULARY 31, 2013).

II. Materials And Methods

2.1 Cleaning Of Glassware:

All the glassware used in this experiment was A grade, and were preliminarily soaked in chromic acid cleaning solution (10% potassium dichromate solution in 25% sulphuric acid) for few hours and washed thoroughly in tap water. Further it is washed in detergents, rinsed with distilled water and then dried.

2.2 Sterilization:

The glassware, media were subjected for sterilization for about 121°C for 30 minutes and 121.8°C for 22 minutes in High pressure High Vaccum sterilizer.

2.3 Material For Analysis:

The vancomycin drug in various stages from Raw material, Validation samples (Inprocess), finished products and Stability samples were provided by strides arcolab limited, Bangalore.

2.4 Materials Required For Dissolution Testing And Assay:

- Dissolution test apparatus (basket apparatus) which includes:
- Basket
- Holding rods
- Water bath
- Thermometer
- Bowl(hemispherical)
- P^H meter
- Water p^H(5-7)
- Six vancomycin capsules
- Cannula
- 20 ml syringe
- Whatman filter paperNo:42
- 25 ml, 10 ml, 50 ml volumetric flasks
- Phosphate buffer
- 5 ml pipettes
- 10 ml pipettes
- Aspirator bulbs.

2.5 Operating Conditions For Dissolution Testing:

2.5.1(Basket Apparatus)

MEDIUM	:	WATER
RPM	:	100
RUN TIME	:	45 MINUTES
VOLUME	:	900mL
TEMPERATURE	:	(37±0.5°C)

2.6 Procedure For Dissolution:

- Before dissolution p^H was checked, it was in the range of (5-7).
- Test apparatus was switched on for dissolution and wash bowls were filled with 900ml each of the dissolution media.
- The bath liquid and medium was allowed to attain the temperature of 37±0.5°C
- Six capsules were placed separately in six baskets and started the instrument
- After the specified time of 45 minutes, 20 ml of the specimen was withdrawn from a zone midway between the surface of water and the top of the rotating basket not less than 1cm from the vessel wall.
- The sample was filteredthrough the whatmann filter paper No: 42 filter
- The first 10 ml of the filtrate was discarded.
- The filtrate was collected in six separate test tubes respectively
- 3.6 ml (250mg) and 7.2 ml (125mg) of filtrate was pipetted out and diluted to 10 ml with phosphate buffer p^H 4.5(TH) (100 mcg/ml).
- 5 ml of the above filtrate was transferred into 25 ml of phosphate buffer p^H 4.5 (TL) 20 mcg/ml.

2.7 Dissoluted Filtrate:

Fig-5 shows the dissoluted filtrate after dissolution.

2.8 Glass Wares Required For Assay:

- Roux bottle
- 250 ml conical flask
- Pipettes
- Test tubes with test tube stand
- Petriplates (90mm)

2.8.1 Reagents Required:

- Monobasic potassium phosphate (AR)
- Phosphoric acid (AR)
- Potassium hydroxide (AR)
- Sodium chloride (AR)

2.8.2 Reagents Preparation:

Buffer p^H 4.5: 13.61g of monobasic potassium phosphate was dissolved in 1000 ml of water, and the p^H was adjusted with 18N phosphoric acid or 10 N potassium hydroxide to 4.5

Potassium hydroxide 10 N: 56.0g of potassium hydroxidewas dissolved and dilutedwith 1000 ml water.

Saline :(0.9% sodiumchloride)

Phosphoric acid: 3.82ml of phosphoric acid was diluted to 10 ml with water.

2.8.3 Microorganism Used:
Bacillus subtilis ATCC 6633

2.8.4 MEDIA USED:
Antibiotic assay medium No: 8
Sporulating agar antibiotic media no: 1 (seed agar)

2.8.5 Inoculum Preparation:
1. A slant of the organism was subcultured on the surface of the antibiotic media no: 8 in a test tube and incubated at 32°C to 35°Cfor 18-24 hours
2. The culturewas transferred to the slant containing sporulating media and incubated at 32°Cto 35°C for 18-24 hours and washed with 3 ml of saline.
3. The inoculum was transferred to the roux bottle containing sporulating agar and incubated for 5 days at 32°C-35°Cto get spore suspension
4. After incubation, using 50 ml of sterile water or normal saline. wash the growth which consists mainly of spores and centrifuge.
5. Re-suspend the sediment in sterile saline and stored at 8°C(stock suspension).

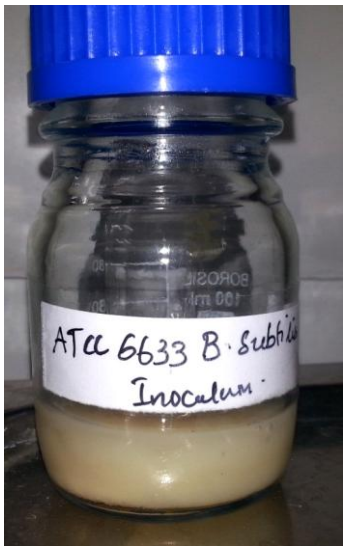


Fig- 6 shows bacillus subtilis ATCC 6633

2.8.6 Preparation Of Assay Plates:
1.0 ml of inoculum was added to 100 ml media at 45°C -50°Cand swirled to attain a homogenous suspension , 25 ml of the media was poured to each petridish. The plates were cooled for solidification.
4 cavities were punched 4 cm or 4.5cm away from each other in all the five petri plates.

2.8.7 Standard Preparation:
- Accurately weighed about 25 mg of vancomycin hydrochloride standard in 25 ml volumetric flask and diluted with sterile water mix (1000mcg/ ml) (stock solution)
- 5 ml of the solution was dissolvedwith 50 ml with phosphate buffer p^H 4.5 to get the concentration of 100mcg/ml. This is standard high(SH)
- Further 5 ml of solution (SH) was diluted to 25 ml volumetric flask with buffer p^H 4.5 +/- 0.05 to achieve standard low concentration (20 mcg/ ml) (SL).

2.8.8 Sample Preparation:
- 5 capsules were taken, cut opened and the average fill weight of capsules was found.
- Empty and the contents of the capsules was collected& mix.
- Accurately the contents of capsules equivalent 25 mg of vancomycin into 25 ml volumetric flask, diluted with sterile water to volume and mix (1000 mcg / ml) stock solution.

- 5 ml of the solution was diluted to 50 ml with phosphate buffer P^H+/- 0.05 to get the concentration of 100 mcg/ ml. This is test high (TH)
- Further 5ml of the dilution (TH) was diluted to 25 ml with buffer p^H 4.5 +/- 0.05 achieved test Low concentration (20 mcg/ml) (TL).

2.9 Procedure:

- 0.1 ml of the test and standard solution was applied in the cavities as per the design was obtained each of TH, TL, SH, SL.
- The solutions were inoculatedin five petri plates.
- The plates were kept at room temperature for 2- 4 hours at 32-35°C
- The diameters of the zones were measured using vernier calipers and carried out the calculations.

2.9.1 Calculation

$$Percentage\ of\ the\ potency = Antilog\ (2 \pm a * log\ i)$$

Where,
i= dilution factor(i.e.)1:5
log_{10}i= 0.6989

$$a = (TH + TL) - \frac{(SH + SL)}{2}(TH - TL) + (SH - SL).$$

Where,
TH=Average of zone diameters of the test high from five plates in mm
TL=Average of zone diameters of the test low from five plates in mm.
SH=Average of zone diameters of the standard high from five plates in mm.
SL=Average of zone diameters of the standard low from five plates in mm.

Percentage Of Potency

$$= Anti\ log\left\{2 \pm \left\{(TH + TL) - \frac{(SH + SL)}{2}(TH - TL) + (SH - SL)\right\} * 0.6989\right\}$$

Calculation Of Content Of Vancomycin In Mg Per Capsule:

$$\%\frac{POTENCY}{2}100 * \frac{WS}{DS} * \frac{900}{1} * \frac{10}{V} * \frac{25}{5} * \frac{P}{1000}$$

Where,
WS – Weight of standard taken in mg
DS – dilution of standard
P – potency of standard in mu/mg on asis basis
V=3.6 ml for 250 mg vancomycin hydrochloride capsules, v= 7.2 ml for 125 mg vancomycin hydrochloride capsules.

2.9.2 Calculation Of Vancomycin Dissolved In Percentage:

$$PERCENTAGE\ DISSOLVED\ IN = Vancomycin\ in\ mg\ per\ \frac{capsule}{2}\ label\ claim * 100$$

2.9.3 Calculation For Raw Materials: (Anhydrous Basis).

Vancomycin In µg/ mg = $\%\frac{POTENCY}{\square}100 * \frac{WS}{DS} * \frac{DT}{WT} * P * \frac{100}{\square}(100 - Watercontent)$

Where,

WS – Weight of hydrochloride vancomycin standard taken in mg
DS – dilution of standard preparation
P – Potency of standard in mu/mg on as anhydrous basis
WT – weight of sample taken in mg
DT – Dilution of sample preparation
WATERCONTENT = 3.4)

III. Results

In this study , raw materials, finished products, validation samples, and stability samples of vancomycin (125& 250mg) capsules are done by microbiological assay against ATCC6633 B.subtilis.
The zone diameter(mm) of two level assay of vancomycin are interpreted as follows.

3.1 Assay Plates

FIG-7: Raw Materials – Assay Plate No: 1 (TH-Test High, TL-Test low, SH- Standard High, SL-Standard low)

FIG-8: Raw Materials – Assay Plate No: 2(TH-Test High, TL-Test low, SH- Standard High, SL-Standard low)

FIG-9: Raw Materials – Assay Plate No: 3(TH-Test High, TL-Test low, SH- Standard High, SL-Standard low)

FIG-10: Raw Materials – Assay Plate No: 4(TH-Test High, TL-Test low, SH- Standard High, SL-Standard low)

FIG-11:Raw Materials – Assay Plate No:5(TH-Test High, TL-Test low, SH- Standard High, SL-Standard low)

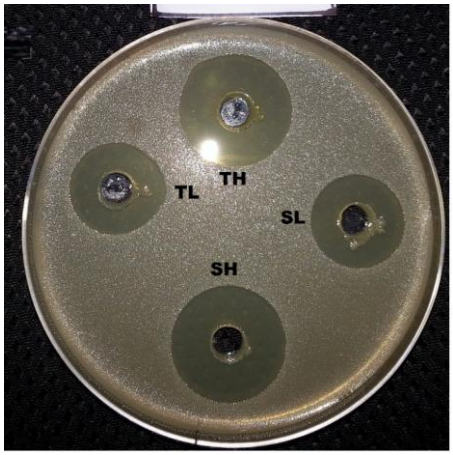


FIG-12: Validation Samples - Assay Plate No: 1 (TH-Test High, TL-Test low, SH- Standard High, SL-Standard low)

FIG-13: Validation Samples - Assay Plate No: 2 (TH-Test High, TL-Test low, SH- Standard High, SL-Standard low)

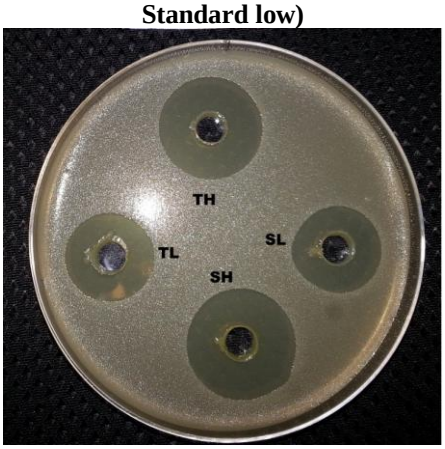


FIG-14: Validation Samples - Assay Plate No: 3 (TH-Test High, TL-Test low, SH- Standard High, SL-Standard low

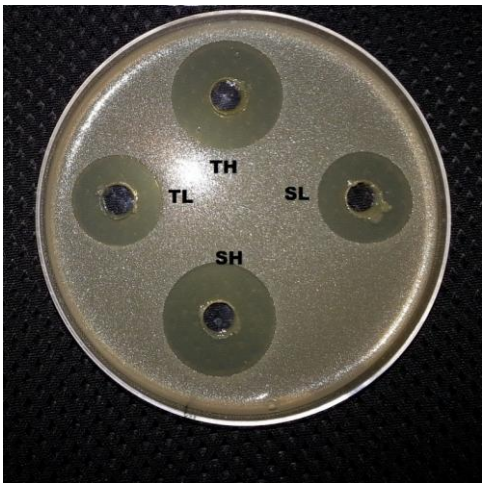


FIG-15: Validation Samples - Assay Plate No: 4 (TH-Test High, TL-Test low, SH- Standard High, SL-Standard low)

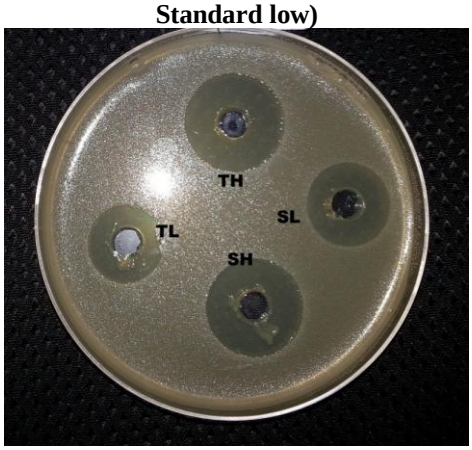


FIG-16: Validation Samples - Assay Plate No: 5 (TH-Test High, TL-Test low, SH- Standard High, SL-Standard low)

FIG-17: Finished Products– Assay Plate No: 1(TH-Test High, TL-Test low, SH- Standard High, SL-Standard low)

FIG-18:Finished Products– Assay Plate No: 2(TH-Test High, TL-Test low, SH- Standard High, SL-Standard low)

FIG-19:Finished Products– Assay Plate No: 3(TH-Test High, TL-Test low, SH- Standard High, SL-Standard low)

FIG-20:Finished Products– Assay Plate No: 4(TH-Test High, TL-Test low, SH- Standard High, SL-Standard low)

FIG-21: Finished Products –Assay Plate No: 5 (TH-Test High, TL-Test low, SH- Standard High, SL-Standard low)

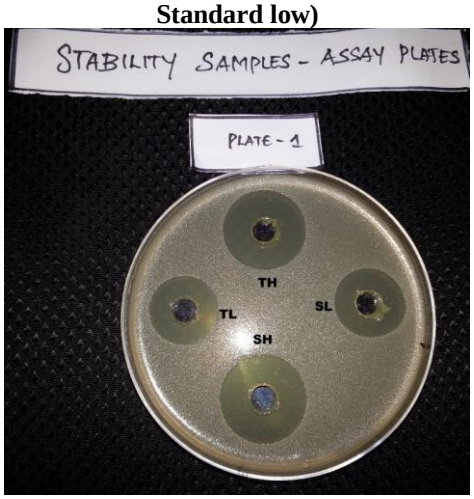


FIG-22: Stability samples –Assay Plate No: 1 (TH-Test High, TL-Test low, SH- Standard High, SL-Standard low)

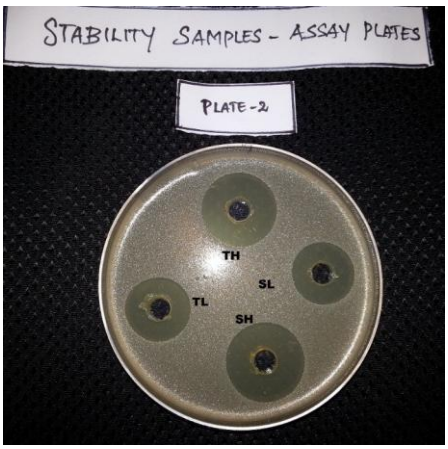


FIG-23: Stability samples –Assay Plate No: 2 (TH-Test High, TL-Test low, SH- Standard High, SL-Standard low)

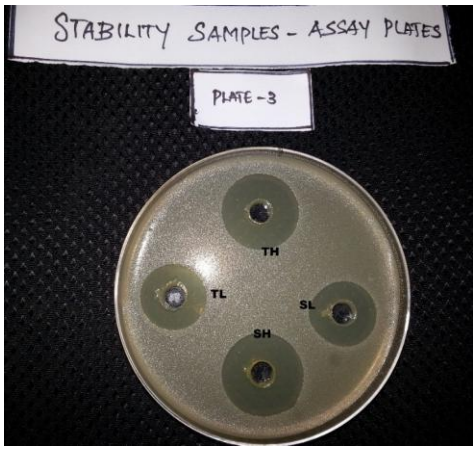


FIG-24: Stability samples –Assay Plate No: 3(TH-Test High, TL-Test low, SH- Standard High, SL-Standard low)

FIG-25: Stability samples –Assay Plate No: 4 (TH-Test High, TL-Test low, SH- Standard High, SL-Standard low)

Fig-26: Stability Samples- Assay Plate No: 5(TH-Test High, TL-Test low, SH- Standard High, SL-Standard low)

3.2 Raw Materials

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH (mm)	TL(mm)
Vanco/RM/001	Plate 1	24.25	20.33	24.26	20.35
	Plate 2	24.33	20.45	24.35	20.44
	Plate 3	24.35	20.44	24.35	20.55
	Plate 4	24.44	20.50	24.45	20.46
	Plate 5	24.55	20.66	24.60	20.31
	AVERAGE	24.33	20.47	24.40	20.41

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH (mm)	TL(mm)
Vanco/RM/002	Plate 1	24.30	20.33	24.26	20.35
	Plate 2	24.35	20.45	24.35	20.44
	Plate 3	24.35	20.44	24.35	20.55
	Plate 4	24.56	20.50	24.45	20.46
	Plate 5	24.70	20.66	24.60	20.31
	AVERAGE	24.45	20.47	24.40	20.42

3.3 Calculation

$$\%\ OF\ THE\ POTENCY = Antilog\left(2 + \frac{\square}{-} a * log\ i\right)$$

Where,
i= dilution factor (i.e.) 1:5
$\log_{10}$ i= 0.6989

$$a = (TH + TL) - \frac{(SH + SL)}{\square}(TH - TL) + (SH - SL)$$

Where,
TH=Average of zone diameters of the test high from five plates in mm
TL= Average of zone diameters of the test low from five plates in mm.
SH= Average of zone diameters of the standard high from five plates in mm.
SL= Average of zone diameters of the standard low from five plates in mm.

$$\%\ POTENCY = Anti\ log\left\{2 + \frac{\square}{-}\left\{(TH + TL) - \frac{(SH + SL)}{\square}(TH - TL) + (SH - SL)\right\} * 0.6989\right\}$$

$$Eg.\ TH = 24.40, TL = 20.42, SH = 24.45, SL = 20.47.$$

$$\left\{(TH + TL) - \frac{(SH + SL)}{\square}(TH - TL) + (SH - SL)\right\} * 0.6989 = -0.0124 * 0.6989 = -0.0086$$

$$2 + \frac{\square}{-}(TH + TL) - \frac{SH + SL}{\square}(TH - TL) + (SH - SL) =$$

$$(2 - (-0.0086)\ or\ 2 + (-0.0086) = 1.9914$$

$$ANTILOG(1.99) = 97.18\%$$

$$\%POTENCY = 97.18\%$$

$$VANCOMYCIN\ IN \frac{MICROGRAMS}{\square} mg = \%\frac{POTENCY}{\square} 100 * \frac{WS}{DS} * \frac{DT}{WT} * P * \frac{100}{\square}(100 - Watercontent)$$

Where,
WS – Weight of hydrochloride vancomycin standard taken in mg
DS – dilution of standard preparation
P – Potency of standard in mu/mg on as anhydrous basis
WT – weight of sample taken in mg
DT – Dilution of sample preparation
WATERCONTENT = 3.4

$$Vancomycin\ in\ \mu\frac{g}{mg} = \frac{97.18}{100} * \frac{25.6}{25} * \frac{5}{50} * \frac{5}{25} * \frac{25}{25.3} * \frac{50}{5} * \frac{25}{5} * 1057 * \frac{100}{100 - 3.4}$$

$$= 1057\ \mu g \frac{\square}{mg} on\ anhydrous\ basis.$$

Percentage Of Potency:

SAMPLE	PERCENTAGE OF POTENCY
Vanco/RM/ 001	97.18%
Vanco/RM/ 001	98.03%

Vancomycin In µg/Mg In Anhydrous Basis:

SAMPLE	VANCOMYCIN (MICROGRAMS) / CAPSULE
Vanco/RM/001	1057µg/mg/ capsule.
Vanco/RM/001	1012µg/mg/ capsule.

3.4 Validation Samples (S1, S2, S3) Stages:
Where,
S1=Top Layer Of Filling Capsules
S2=Middle Layer Of Filling Capsules
S3= Bottom Layer Of Filling Capsules

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
VANCO/VAL/S1/001	Plate 1	24.01	20.20	24.09	20.01
	Plate 2	24.00	20.00	24.05	20.03
	Plate 3	24.08	20.15	24.03	20.07
	Plate 4	24.10	20.14	24.06	20.02
	Plate 5	24.12	20.08	24.02	20.11
	AVERAGE	24.06	20.11	24.05	20.04
SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
VANCO/VAL/S2/001	Plate 1	24.16	20.00	24.01	20.01
	Plate 2	24.18	20.03	24.06	20.02
	Plate 3	24.12	20.05	24.00	20.05
	Plate 4	24.12	20.11	24.05	20.09
	Plate 5	24.11	20.08	24.04	20.05
	AVERAGE	24.13	20.05	24.03	20.04

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
VANCO/VAL/S3/001	Plate 1	24.04	20.05	24.18	20.16
	Plate 2	24.07	20.07	24.17	20.10
	Plate 3	24.05	20.05	24.15	20.11
	Plate 4	24.06	20.01	24.18	20.19
	Plate 5	24.05	20.06	24.10	20.10
	AVERAGE	24.05	20.04	24.15	20.13

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/VAL/S1/002	Plate 1	24.11	20.00	24.11	20.01
	Plate 2	24.12	20.04	24.13	20.03
	Plate 3	24.14	20.06	24.15	20.05
	Plate 4	24.17	20.08	24.12	20.06
	Plate 5	24.18	20.10	24.13	20.10
	AVERAGE	24.14	20.05	24.12	20.06

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/VAL/S2/002	Plate 1	24.25	20.15	24.10	20.12
	Plate 2	24.27	20.19	24.15	20.22
	Plate 3	24.20	20.17	24.12	20.22
	Plate 4	24.23	20.11	24.15	20.23
	Plate 5	24.19	20.10	24.16	20.21
	average	24.22	20.14	24.13	20.2

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/Val/S3/002	Plate 1	24.22	20.02	24.20	20.22
	Plate 2	24.23	20.04	24.22	20.22
	Plate 3	24.27	20.10	24.23	20.23
	Plate 4	24.29	20.19	24.33	20.25
	Plate 5	24.30	20.00	24.30	20.22
	AVERAGE	24.26	20.07	24.25	20.22
SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/VAL/S1/003	Plate 1	24.12	20.22	24.22	20.11
	Plate 2	24.18	20.24	24.23	20.13
	Plate 3	24.17	20.26	24.21	20.15
	Plate 4	24.16	20.29	24.25	20.13
	Plate 5	24.15	20.30	24.30	20.15
	AVERAGE	24.16	20.26	24.24	20.13

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/VAL/S2/003	Plate 1	24.22	20.21	24.11	20.01
	Plate 2	24.23	20.23	24.15	20.03
	Plate 3	24.24	20.24	24.17	20.05
	Plate 4	24.27	20.25	24.23	20.09
	Plate 5	24.23	20.27	24.25	20.10
	AVERAGE	24.23	20.04	24.18	20.05

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/VAL/S3/003	Plate 1	24.33	20.01	24.11	20.11
	Plate 2	24.34	20.03	24.13	20.12
	Plate 3	24.36	20.02	24.15	20.15
	Plate 4	24.38	20.06	24.17	20.19
	Plate 5	24.40	20.08	24.19	20.14
	AVERAGE	24.36	20.04	24.15	20.14

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/VAL/S1/004	Plate 1	24.12	20.11	24.12	20.11
	Plate 2	24.14	20.13	24.15	20.13
	Plate 3	24.18	20.17	24.19	20.15
	Plate 4	24.17	20.19	24.18	20.11
	Plate 5	24.19	20.21	24.15	20.15
	AVERAGE	24.16	20.16	24.15	20.13

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/VAL/S2/004	Plate 1	24.11	20.10	24.22	20.11
	Plate 2	24.13	20.12	24.24	20.13
	Plate 3	24.15	20.13	24.28	20.14
	Plate 4	24.16	20.14	24.30	20.12
	Plate 5	24.20	20.17	24.23	20.11
	AVERAGE	24.15	20.13	24.25	20.12

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/VAL/S3/004	Plate 1	24.22	20.11	24.12	20.22
	Plate 2	24.23	20.12	24.15	20.23
	Plate 3	24.24	20.14	24.19	20.25
	Plate 4	24.23	20.11	24.22	20.23
	Plate 5	24.26	20.13	24.21	20.21
	AVERAGE	24.23	20.12	24.17	20.22

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/VAL/S1/005	Plate 1	24.02	20.02	24.11	20.11
	Plate 2	24.04	20.05	24.13	20.13
	Plate 3	24.05	20.06	24.17	20.14
	Plate 4	24.09	20.07	24.19	20.12
	Plate 5	24.11	20.10	24.21	20.13
	AVERAGE	24.06	20.06	24.16	20.12

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/VAL/S2/005	Plate 1	24.18	20.02	24.10	20.10
	Plate 2	24.08	20.04	24.13	20.13
	Plate 3	24.11	20.10	24.17	20.15
	Plate 4	24.02	20.19	24.19	20.11
	Plate 5	24.13	20.00	24.13	20.13
	AVERAGE	24.10	20.07	24.14	20.12

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/Val/S3/005	Plate 1	24.11	20.12	24.11	20.11
	Plate 2	24.18	20.11	24.15	20.15
	Plate 3	24.21	20.12	24.19	20.17
	Plate 4	24.22	20.18	24.15	20.15
	Plate 5	24.23	20.19	24.11	20.17
	AVERAGE	24.19	20.14	24.14	20.15

Calculation:

3.5 (These Calculation Is Applicable For Validation Samples, Finished Products And Stability Samples)

$$PERCENTAGE\ OF\ THE\ POTENCY = Antilog\left(2 \pm a * log\ i\right)$$

Where,

i= dilution factor (i.e.) 1:5

$\log_{10}$ i= 0.6989

$$a = (TH + TL) - \frac{(SH + SL)}{2}(TH - TL) + (SH - SL).$$

Where,
TH=Average of zone diameters of the test high from five plates in mm
TL= Average of zone diameters of the test low from five plates in mm.
SH= Average of zone diameters of the standard high from five plates in mm.
SL= Average of zone diameters of the standard low from five plates in mm.

$$PERCENTAGE\ OF\ POTENCY = Anti\ log\left\{2 + \frac{2}{-}\left\{(TH + TL) - \frac{(SH + SL)}{2}(TH - TL) + (SH - SL)\right\} * 0.6989\right\}$$

$$(E.g.)\ TH = 24.05, TL = 20.04, SH = 24.06, SL = 20.11.$$

$$\left\{(TH + TL) - \frac{(SH + SL)}{2}(TH - TL) + (SH - SL)\right\} * 0.6989 = -0.0100 * 0.6989 = -0.00698$$

$$2 + \frac{2}{-}(TH + TL) - \frac{SH + SL}{2}(TH - TL) + (SH - SL) =$$

$$(2 - (-0.00698)\ or\ 2 + (-0.00698) = 1.9930$$

$$ANTILOG(1.9930) = 98.41\%$$

$$\%POTENCY = 98.41\%$$

3.6 CALCULATION OF CONTENT OF VANCOMYCIN IN mg PER CAPSULE:

$$\%\frac{POTENCY}{2}100 * \frac{WS}{DS} * \frac{900}{1} * \frac{10}{V} * \frac{25}{5} * \frac{P}{1000}$$

Where,
WS – Weight of standard taken in mg
DS – dilution of standard
P – Potency of standard in mu/mg on as is basis
V=3.6 ml for 250 mg vancomycin hydrochloride capsules,
v= 7.2 ml for 125 mg vancomycin hydrochloride capsules.

$$VANCOMYCIN\ IN\ \frac{mg}{CAPSULE} = \frac{98.41}{100} * \frac{25.6}{25} * \frac{5}{50} * \frac{5}{25} * \frac{900}{1} * \frac{10}{3.6} * \frac{25}{5} * \frac{1057}{1000}$$

$$= 266.28mg\ \ (for\ 250\ mg\ capsules)$$

3.7 Calculation Of Vancomycin Dissolved In %: (Specification Assay Limits)

$$VANCOMYCIN\ DISSOLVED\ IN\%\frac{2}{CAPSULE} = Vancomycin\ in\ mg\ per\ \frac{capsule}{2}\ label\ claim * 100$$

$$= 266.28\frac{mg}{2}250 * 100$$

$$= 106.5\%$$

3.8 Potency:

SAMPLE	% OF POTENCY
VANCO/ VAL/ S1/ 001	98.41%
VANCO/VAL/S2/ 001	97.81%
VANCO/VAL/S3/001	100.37%
VANCO/ VAL/ S1/ 002	99.79%
VANCO/VAL/S2/ 002	97.72%
VANCO/VAL/S3/002	97.29%
VANCO/ VAL/ S1/ 003	98.85%
VANCO/VAL/S2/ 003	99.26%
VANCO/VAL/S3/003	97.90%
VANCO/ VAL/ S1/ 004	99.17%
VANCO/VAL /S2 / 004	98.17%
VANCO/VAL/ S3 /004	99.08%
VANCO/ VAL/ S1/005	97%
VANCO/VAL/S2/ 005	98.24%
VANCO/VAL/ S3/ 005	99.72%
VANCO/ VAL/ S1/ 006	99.54%
VANCO/VAL/ S2/ 006	98.24%
VANCO/VAL/ S3 / 006	98.22%

3.9 Vancomycin In Mg/Capsule:

Sample	Vancomycin In Mg/Capsule
VANCO/ VAL/ S1/ 001	**266.28mg**
VANCO/VAL/ S2/ 001	**264.66mg**
VANCO/VAL/ S3 / 001	**271.59mg**
VANCO/ VAL/ S1/ 002	**270.02mg**
VANCO/VAL/ S2/ 002	**264.42mg**
VANCO/VAL/ S3 / 002	**263.25mg**
VANCO/ VAL/ S1/ 003	**267.48mg**
VANCO/VAL/ S2/ 003	**268.58mg**
VANCO/VAL/ S3 / 003	**264.90mg**
VANCO/ VAL/ S1/ 004	**134.17mg**
VANCO/VAL/ S2/ 004	**132.82mg**
VANCO/VAL/ S3 / 004	**134.05mg**
VANCO/ VAL/ S1/ 005	**131.23mg**
VANCO/VAL/ S2/ 005	**129.14mg**
VANCO/VAL/ S3 / 005	**134.91mg**
VANCO/VAL/ S3 / 006	**134.67mg**
VANCO/ VAL/ S1/ 006	**132.91mg**
VANCO/VAL/ S2/ 006	**132.91mg**
VANCO/VAL/ S3 / 006	**132.88mg**

3.10 Vancomycin In Percentage/ Capsule:

SAMPLE	Vancomycin %/ Capsule(Assay Limits)
VANCO/ VAL/ S1/ 001	**106.51%**
VANCO/VAL/ S2/ 001	**105.8%**
VANCO/VAL/ S3 / 001	**108.6%**
VANCO/ VAL/ S1/ 002	**108%**
VANCO/VAL/ S2/ 002	**105.7%**
VANCO/VAL/ S3 / 002	**105.3%**
VANCO/ VAL/ S1/ 003	**106.9%**
VANCO/VAL/ S2/ 003	**107.4%**
VANCO/VAL/ S3 / 003	**105.9%**
VANCO/ VAL/ S1/ 004	**107.3%**
VANCO/VAL/ S2/ 004	**106.2%**
VANCO/VAL/ S3 / 004	**107.2%**
VANCO/ VAL/ S1/ 005	**104.9%**
VANCO/VAL/ S2/ 005	**103.3%**
VANCO/VAL/ S3 / 005	**107.9%**
VANCO/ VAL/ S1/ 006	**107.7%**
VANCO/VAL/ S2/ 006	**106.3%**
VANCO/VAL/ S3/ 006	**106.3%**

3.11 Finished Products – Dissolution:

SAMPLE NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/FP/disso Jar1	Plate 1	24.41	20.20	24.14	20.04
	Plate 2	24.42	20.23	24.16	20.28
	Plate 3	24.36	20.25	24.18	20.28
	Plate 4	24.38	20.22	24.18	20.12
	Plate 5	24.40	20.21	24.20	20.14
	AVERAGE	24.34	20.2	24.21	20.17
Jar 2	Plate 1	24.22	20.11	24.01	20.01
	Plate 2	24.33	20.12	24.03	20.23
	Plate 3	24.21	20.15	24.04	20.24
	Plate 4	24.22	20.17	24.09	20.23
	Plate 5	24.39	20.19	24.10	20.25
	AVERAGE	24.27	20.14	24.05	20.19
Jar 3	Plate 1	24.36	20.11	24.11	20.04
	Plate 2	24.34	20.13	24.12	20.28
	Plate 3	24.36	20.15	24.14	20.28
	Plate 4	24.37	20.16	24.20	20.12
	Plate 5	24.38	20.18	24.22	20.14
	AVERAGE	24.36	20.14	24.15	20.17
Jar 4	Plate 1	24.10	20.12	24.23	20.10
	Plate 2	24.14	20.14	24.24	20.22
	Plate 3	24.18	20.18	24.26	20.25
	Plate 4	24.22	20.22	24.28	20.24
	Plate 5	24.24	20.28	24.30	20.28

	AVERAGE	24.17	20.18	24.26	20.21
Jar5	Plate 1	24.14	20.04	24.33	20.19
	Plate 2	24.16	20.28	24.34	20.22
	Plate 3	24.18	20.28	24.36	20.25
	Plate 4	24.18	20.12	24.38	20.24
	Plate 5	24.20	20.14	24.40	20.28
	AVERAGE	24.17	20.17	24.36	20.23
Jar6	Plate 1	24.14	20.04	24.23	20.10
	Plate 2	24.16	20.28	24.24	20.22
	Plate 3	24.18	20.28	24.26	20.25
	Plate 4	24.18	20.12	24.28	20.24
	Plate 5	24.20	20.14	24.30	20.28
	AVERAGE	24.17	20.17	24.26	20.21

3.12 Percentage Of Potency:

Finished Products(Dissolution)	Percentage Of Potency
Vanco/FP/Disso – jar 1	**97.72%**
Jar 2	**97%**
Jar3	**97%**
Jar4	**97.49%**
Jar 5	**97%**
Jar6	**97.49%**

3.13 Vancomycin In Mg/ Capsule:

Finished Products(Dissolution)	Vancomycin In Mg/Capsule
Vanco/FP/Disso – jar 1	**132.11mg**
Jar 2	**131.23mg**
Jar3	**131.23mg**
Jar4	**131.90mg**
Jar 5	**131.23mg**
Jar6	**131.90mg**

3.14 Vancomycin In Percentage/ Capsule:

Finished Products(Dissolution)	Vancomycin In %/ Capsule.
Vanco/FP/Disso – jar 1	**105.6%**
Jar 2	**100%**
Jar3	**100%**
Jar4	**105.5%**
Jar 5	**100%**
Jar6	**100%**

3.15 Finished Products- Assay

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/FP/001A	Plate 1	24.11	20.00	24.11	20.01
	Plate 2	24.12	20.04	24.13	20.03
	Plate 3	24.14	20.06	24.15	20.05
	Plate 4	24.17	20.08	24.12	20.06
	Plate 5	24.18	20.10	24.13	20.10
	AVERAGE	24.14	20.05	24.12	20.06

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/FP/002A	Plate 1	24.22	20.02	24.25	20.11
	Plate 2	24.24	20.06	24.30	20.13
	Plate 3	24.25	20.17	24.27	20.19
	Plate 4	24.22	20.12	24.29	20.21
	Plate 5	24.23	20.11	24.11	20.23
	AVERAGE	24.23	20.09	24.24	20.17

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/FP/003A	Plate 1	24.11	20.33	24.22	20.19
	Plate 2	24.12	20.32	24.24	20.21
	Plate 3	24.15	20.33	24.27	20.22
	Plate 4	24.17	20.25	24.29	20.22
	Plate 5	24.18	20.30	24.20	20.13
	AVERAGE	24.14	20.30	24.24	20.19

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/FP/004A	Plate 1	24.01	20.02	24.10	20.10
	Plate 2	24.08	20.04	24.13	20.13
	Plate 3	24.11	20.10	24.17	20.15
	Plate 4	24.02	20.19	24.19	20.11
	Plate 5	24.13	20.00	24.13	20.13
	AVERAGE	24.07	20.07	24.14	20.12
SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/FP/005A	Plate 1	24.11	20.06	24.20	20.12
	Plate 2	24.08	20.05	24.23	20.15
	Plate 3	24.12	20.10	24.27	20.17
	Plate 4	24.07	20.14	24.29	20.18
	Plate 5	24.13	20.02	24.23	20.10
	AVERAGE	24.10	20.07	24.24	20.14

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/FP/006A	Plate 1	24.12	20.02	24.22	20.20
	Plate 2	24.20	20.06	24.23	20.23
	Plate 3	24.22	20.08	24.25	20.25
	Plate 4	24.27	20.08	24.29	20.21
	Plate 5	24.29	20.07	24.23	20.23
	AVERAGE	24.22	20.06	24.24	20.22

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/FP/007A	Plate 1	24.12	20.12	24.22	20.10
	Plate 2	24.14	20.13	24.23	20.13
	Plate 3	24.11	20.13	24.27	20.15
	Plate 4	24.11	20.14	24.28	20.11
	Plate 5	24.14	20.12	24.29	20.13
	AVERAGE	24.12	20.12	24.25	20.12

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/FP/008A	Plate 1	24.22	20.11	24.11	20.11
	Plate 2	24.28	20.15	24.15	20.12
	Plate 3	24.29	20.19	24.17	20.16
	Plate 4	24.20	20.20	24.18	20.19
	Plate 5	24.23	20.22	24.20	20.21
	AVERAGE	24.24	20.17	24.16	24.19

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/FP/009A	Plate 1	24.00	20.12	24.02	20.11
	Plate 2	24.07	20.13	24.04	20.15
	Plate 3	24.15	20.15	24.05	20.17
	Plate 4	24.02	20.17	24.00	20.19
	Plate 5	24.05	20.19	24.10	20.12
	AVERAGE	24.05	20.15	24.03	20.14

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/FP/010A	Plate 1	24.00	20.12	24.31	20.11
	Plate 2	24.12	20.11	24.32	20.15
	Plate 3	24.15	20.15	24.35	20.17
	Plate 4	24.17	20.16	24.37	20.19
	Plate 5	24.13	20.19	24.39	20.21
	AVERAGE	24.11	20.14	24.34	20.16

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/FP/011A	Plate 1	24.11	20.22	24.21	20.30
	Plate 2	24.13	20.24	24.23	20.33
	Plate 3	24.19	20.26	24.27	20.35
	Plate 4	24.21	20.29	24.29	20.31
	Plate 5	24.23	20.22	24.23	20.33
	AVERAGE	24.17	20.24	24.24	20.32

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/FP/012A	Plate 1	24.21	20.18	24.20	20.20
	Plate 2	24.28	20.19	24.23	20.33
	Plate 3	24.21	20.10	24.27	20.25
	Plate 4	24.22	20.13	24.29	20.21
	Plate 5	24.23	20.20	24.23	20.23

	AVERAGE	24.23	20.16	24.24	20.24

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/FP/013A	Plate 1	24.11	20.12	24.11	20.11
	Plate 2	24.18	20.11	24.15	20.15
	Plate 3	24.21	20.12	24.19	20.17
	Plate 4	24.22	20.18	24.15	20.15
	Plate 5	24.23	20.19	24.11	20.17
	AVERAGE	24.19	20.14	24.14	20.15

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/FP/014A	Plate 1	24.11	20.15	24.30	20.22
	Plate 2	24.12	20.17	24.28	20.21
	Plate 3	24.15	20.19	24.22	20.24
	Plate 4	24.12	20.12	24.23	20.29
	Plate 5	24.18	20.15	24.25	20.20
	AVERAGE	24.13	20.15	24.25	20.23

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/FP/015A	Plate 1	24.21	20.10	24.13	20.17
	Plate 2	24.28	20.09	24.15	20.19
	Plate 3	24.30	20.19	24.19	20.15
	Plate 4	24.29	20.12	24.21	20.14
	Plate 5	24.23	20.02	24.23	20.19
	AVERAGE	24.26	20.10	24.18	20.16

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/FP/016A	Plate 1	24.11	20.11	24.14	20.19
	Plate 2	24.18	20.09	24.13	20.11
	Plate 3	24.12	20.11	24.17	20.19
	Plate 4	24.22	20.12	24.19	20.18
	Plate 5	24.23	20.01	24.13	20.12
	AVERAGE	24.17	20.08	24.15	20.15

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/FP/017A	Plate 1	24.12	20.22	24.10	20.12
	Plate 2	24.14	20.24	24.12	20.15
	Plate 3	24.15	20.26	24.12	20.17
	Plate 4	24.17	20.29	24.14	20.19
	Plate 5	24.21	20.32	24.12	20.15
	AVERAGE	24.15	20.26	24.12	20.15

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/FP/018A	Plate 1	24.11	20.12	24.20	20.00
	Plate 2	24.08	20.14	24.23	20.03
	Plate 3	24.14	20.20	24.27	20.05
	Plate 4	24.19	20.19	24.29	20.01
	Plate 5	24.23	20.20	24.23	20.03
	AVERAGE	24.15	20.17	24.24	20.02

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/FP/019A	Plate 1	24.11	20.12	24.11	20.11
	Plate 2	24.18	20.14	24.11	20.15
	Plate 3	24.11	20.10	24.15	20.17
	Plate 4	24.12	20.19	24.11	20.11
	Plate 5	24.13	20.20	24.13	20.13
	AVERAGE	24.13	20.15	24.12	20.13

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/FP/020A	Plate 1	24.11	20.12	24.22	20.10
	Plate 2	24.28	20.14	24.23	20.13
	Plate 3	24.21	20.20	24.27	20.17
	Plate 4	24.22	20.29	24.29	20.10
	Plate 5	24.23	20.20	24.23	20.15
	AVERAGE	24.21	20.19	24.24	20.13

3.16 Finished Products -Percentage Of Potency:

FINISHED PRODUCTS	PERCENTAGE OF POTENCY
Vanco/FP/001A	99.77%
Vanco/FP/002A	99.31%
Vanco/FP/003A	99.77%
Vanco/FP/004A	104.47%
Vanco/FP/005A	105.4%
Vanco/FP/006A	103.5%
Vanco/FP/007A	102.6%
Vanco/FP/008A	97.72%
Vanco/FP/009A	99.38%
Vanco/FP/010A	105.5%
Vanco/FP/011A	103.1%
Vanco/FP/012A	101.78%
Vanco/FP/013A	102.02%
Vanco/FP/014A	104.08%
Vanco/FP/015A	99.60%
Vanco/FP/016A	100.99%
Vanco/FP/017A	97.27%
Vanco/FP/018A	98.83%
Vanco/FP/019A	99.54%
Vanco/FP/020A	99.31%

3.16.1 Vancomycin In Mg/Capsule:

FINISHED PRODUCTS	VANCOMYCIN IN MG/CAPSULE
Vanco/FP/001A	134.9mg
Vanco/FP/002A	134.36mg
Vanco/FP/003A	134.9mg
Vanco/FP/004A	282.68mg
Vanco/FP/005A	285.2mg
Vanco/FP/006A	280.06mg
Vanco/FP/007A	277.62mg
Vanco/FP/008A	264.42mg
Vanco/FP/009A	270.13mg
Vanco/FP/010A	285.47mg
Vanco/FP/011A	139.49mg
Vanco/FP/012A	137.70mg
Vanco/FP/013A	138.02mg
Vanco/FP/014A	140.81mg
Vanco/FP/015A	134.75mg
Vanco/FP/016A	273.27mg
Vanco/FP/017A	263.20mg
Vanco/FP/018A	267.42mg
Vanco/FP/019A	269.34mg
Vanco/FP/020A	268.72mg

3.16.2 Vancomycin In % /Capsule:

FINISHED PRODUCTS	VANCOMYCIN IN % /CAPSULE
Vanco/FP/001A	107.9%
Vanco/FP/002A	107.4%
Vanco/FP/003A	107.9%
Vanco/FP/004A	113%
Vanco/FP/005A	114.08%
Vanco/FP/006A	112.02%
Vanco/FP/007A	111.04%
Vanco/FP/008A	105.7%
Vanco/FP/009A	108%
Vanco/FP/010A	114%
Vanco/FP/011A	111.5%
Vanco/FP/012A	110.16%
Vanco/FP/013A	110.4%
Vanco/FP/014A	112.6%
Vanco/FP/015A	107.8%
Vanco/FP/016A	109.3%
Vanco/FP/017A	105.2%
Vanco/FP/018A	106.9%
Vanco/FP/019A	107.7%
Vanco/FP/020A	107.4%

3.17 Stability Samples – Dissolution

SAMPLE NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ Tdisso Jar1	Plate 1	24.11	20.11	24.31	20.01
	Plate 2	24.15	20.12	24.33	20.03
	Plate 3	24.15	20.14	24.36	20.05
	Plate 4	24.20	20.15	24.34	20.09
	Plate 5	24.20	20.14	24.38	20.11
	AVERAGE	24.16	20.13	24.34	20.05
Jar 2	Plate 1	24.22	20.11	24.01	20.01
	Plate 2	24.33	20.12	24.03	20.23
	Plate 3	24.21	20.15	24.04	20.24
	Plate 4	24.22	20.17	24.09	20.23
	Plate 5	24.39	20.19	24.10	20.25
	AVERAGE	24.27	20.14	24.05	20.19
Jar 3	Plate 1	24.11	20.04	24.06	20.11
	Plate 2	24.12	20.08	24.04	20.13
	Plate 3	24.14	20.08	24.05	20.15
	Plate 4	24.20	20.12	24.07	20.16
	Plate 5	24.22.	20.04	24.02	20.18
	AVERAGE	24.15	20.07	24.04	20.14
Jar 4	Plate 1	24.10	20.12	24.23	20.10
	Plate 2	24.14	20.14	24.24	20.22
	Plate 3	24.18	20.18	24.26	20.25
	Plate 4	24.22	20.22	24.28	20.24
	Plate 5	24.24	20.28	24.30	20.28
	AVERAGE	24.17	20.18	24.24	20.21
Jar 5	Plate 1	24.11	20.11	24.33	20.11
	Plate 2	24.13	20.14	24.35	20.13
	Plate 3	24.13	20.14	24.44	20.16
	Plate 4	24.11	20.11	24.32	20.20
	Plate 5	24.15	20.15	24.35	20.22
	AVERAGE	24.12	20.56	24.35	20.16
Jar 6	Plate 1	24.11	20.11	24.33	20.11
	Plate 2	24.13	20.14	24.35	20.13
	Plate 3	24.13	20.14	24.44	20.16
	Plate 4	24.11	20.11	24.32	20.20
	Plate 5	24.15	20.15	24.35	20.22
	AVERAGE	24.12	20.13	24.35	20.15

Stability Sample – Dissolution
Percentage Of Potency

STABILITY SAMPLES	PERCENTAGE OF POTENCY
Vanco/ST/Disso/Jar 1	**103.75%**
Jar 2	**97%**
Jar 3	**99%**
Jar 4	**98.03%**
Jar 5	**105.1%**
Jar 6	**105.2%**

Vancomycin In Mg/Capsule:

STABILITY SAMPLES	VANCOMYCIN IN MG/CAPSULE
Vanco/ST/Disso/Jar 1	**140.3mg**
Jar 2	**131.23mg**
Jar 3	**133.94mg**
Jar 4	**132.63mg**
Jar 5	**142.1mg**
Jar 6	**142.3mg**

Vancomycin In % /Capsule:

STABILITY SAMPLES	VANCOMYCIN IN %/CAPSULE
Vanco/ST/Disso/Jar 1	**112.24%**
Jar 2	**104.9%**
Jar 3	**107.15%**
Jar 4	**106.10%**
Jar 5	**113.68%**
Jar 6	**113.84%**

3.18 Stability Samples – Assay

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/001A	Plate 1	24.21	20.10	24.13	20.17
	Plate 2	24.28	20.09	24.15	20.19
	Plate 3	24.30	20.19	24.19	20.15
	Plate 4	24.29	20.12	24.21	20.14
	Plate 5	24.23	20.02	24.23	20.19
	AVERAGE	24.26	20.10	24.18	20.16

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/002A	Plate 1	24.11	20.11	24.14	20.19
	Plate 2	24.18	20.09	24.13	20.11
	Plate 3	24.12	20.11	24.17	20.19
	Plate 4	24.22	20.12	24.19	20.18
	Plate 5	24.23	20.01	24.13	20.12
	AVERAGE	24.17	20.08	24.15	20.15

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/003A	Plate 1	24.12	20.22	24.10	20.12
	Plate 2	24.14	20.24	24.12	20.15
	Plate 3	24.13	20.26	24.12	20.17
	Plate 4	24.16	20.29	24.14	20.19
	Plate 5	24.20	20.32	24.12	20.15
	AVERAGE	24.15	20.26	24.16	20.15

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/004A	Plate 1	24.12	20.11	24.21	20.02
	Plate 2	24.09	20.13	24.22	20.04
	Plate 3	24.11	20.15	24.25	20.06
	Plate 4	24.14	20.18	24.23	20.04
	Plate 5	24.19	20.20	24.22	20.08
	AVERAGE	24.13	20.15	24.22	20.04

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/005A	Plate 1	24.22	20.12	24.15	20.21
	Plate 2	24.23	20.21	24.14	20.22
	Plate 3	24.25	20.11	24.16	20.21
	Plate 4	24.27	20.12	24.18	20.22
	Plate 5	24.21	20.22	24.20	20.25
	AVERAGE	24.23	20.15	24.16	20.22

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/006A	Plate 1	24.21	20.11	24.23	20.12
	Plate 2	24.28	20.14	24.25	20.15
	Plate 3	24.21	20.10	24.27	20.17
	Plate 4	24.22	20.19	24.28	20.12
	Plate 5	24.23	20.10	24.23	20.16
	AVERAGE	24.23	20.12	24.25	20.14

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/007A	Plate 1	24.01	20.01	24.11	20.12
	Plate 2	24.03	20.05	24.13	20.13
	Plate 3	24.05	20.07	24.15	20.15
	Plate 4	24.07	20.08	24.17	20.17
	Plate 5	24.09	20.10	24.20	20.19
	AVERAGE	24.05	20.06	24.15	20.15

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/008A	Plate 1	24.12	20.11	24.01	20.31
	Plate 2	24.13	20.13	24.03	20.30
	Plate 3	24.18	20.15	24.07	20.28
	Plate 4	24.15	20.17	24.09	20.25
	Plate 5	24.17	20.19	24.13	20.27
	AVERAGE	24.15	20.15	24.06	20.28
SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/009A	Plate 1	24.20	20.11	24.15	20.22
	Plate 2	24.22	20.13	24.14	20.27
	Plate 3	24.24	20.16	24.19	20.25
	Plate 4	24.22	20.11	24.20	20.23

	Plate 5	24.25	20.11	24.25	20.22
	AVERAGE	24.26	20.11	24.18	20.23

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/010A	Plate 1	24.11	20.13	24.31	20.12
	Plate 2	24.13	20.14	24.31	20.23
	Plate 3	24.15	20.19	24.35	20.13
	Plate 4	24.17	20.11	24.36	20.14
	Plate 5	24.20	20.20	24.40	20.15
	AVERAGE	24.15	20.15	24.34	20.15

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/011A	Plate 1	24.21	20.15	24.13	20.17
	Plate 2	24.24	20.13	24.15	20.19
	Plate 3	24.20	20.11	24.19	20.15
	Plate 4	24.29	20.12	24.21	20.14
	Plate 5	24.24	20.11	24.23	20.19
	AVERAGE	24.34	20.12	20.12	20.14

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/012A	Plate 1	24.00	20.10	24.11	20.12
	Plate 2	24.02	20.11	24.13	20.13
	Plate 3	24.05	20.12	24.15	20.15
	Plate 4	24.09	20.11	24.20	20.14
	Plate 5	24.10	20.01	24.22	20.19
	AVERAGE	24.05	20.09	24.16	20.14

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/013A	Plate 1	24.11	20.11	24.11	20.15
	Plate 2	24.13	20.05	24.15	20.19
	Plate 3	24.15	20.09	24.19	20.17
	Plate 4	24.22	20.11	24.21	20.14
	Plate 5	24.21	20.12	24.24	20.19
	AVERAGE	24.16	20.09	24.18	20.16

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/014A	Plate 1	24.01	20.11	24.11	20.15
	Plate 2	24.04	20.13	24.13	20.17
	Plate 3	24.06	20.12	24.14	20.12
	Plate 4	24.09	20.13	24.19	20.11
	Plate 5	24.11	20.14	24.22	20.11
	AVERAGE	24.06	20.12	24.152	20.13

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/015A	Plate 1	24.19	20.01	24.03	20.11
	Plate 2	24.11	20.03	24.05	20.15
	Plate 3	24.18	20.09	24.09	20.17
	Plate 4	24.22	20.02	24.01	20.14
	Plate 5	24.20	20.12	24.03	20.19
	AVERAGE	24.18	20.05	24.04	20.14

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/016A	Plate 1	24.13	20.05	24.11	20.09
	Plate 2	24.18	20.04	24.13	20.10
	Plate 3	24.15	20.05	24.14	20.11
	Plate 4	24.15	20.01	24.11	20.14
	Plate 5	24.12	20.02	24.15	20.19
	AVERAGE	24.14	20.03	24.12	20.12

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/017A	Plate 1	24.33	20.10	24.11	20.15
	Plate 2	24.28	20.19	24.13	20.11
	Plate 3	24.30	20.18	24.11	20.13
	Plate 4	24.29	20.13	24.22	20.15
	Plate 5	24.25	20.11	24.24	20.16
	AVERAGE	24.29	20.14	24.16	20.14

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/018A	Plate 1	24.09	20.01	24.11	20.16
	Plate 2	24.08	20.02	24.13	20.18
	Plate 3	24.07	20.03	24.17	20.14
	Plate 4	24.05	20.07	24.20	20.14
	Plate 5	24.04	20.09	24.22	20.18
	AVERAGE	24.06	20.04	24.16	20.16

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/019A	Plate 1	24.20	20.11	24.15	20.15
	Plate 2	24.21	20.12	24.20	20.13
	Plate 3	24.22	20.14	24.19	20.14
	Plate 4	24.25	20.15	24.19	20.14
	Plate 5	24.22	20.12	24.22	20.15
	AVERAGE	24.32	20.12	24.19	20.14

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/020A	Plate 1	24.13	20.11	24.11	20.13
	Plate 2	24.16	20.09	24.13	20.15
	Plate 3	24.13	20.11	24.15	20.16
	Plate 4	24.15	20.12	24.20	20.17
	Plate 5	24.19	20.12	24.22	20.18
	AVERAGE	24.15	20.11	24.16	20.15

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/021A	Plate 1	24.20	20.22	24.11	20.22
	Plate 2	24.22	20.24	24.13	20.23
	Plate 3	24.23	20.25	24.15	20.26
	Plate 4	24.25	20.26	24.20	20.29
	Plate 5	24.26	20.28	24.24	20.30
	AVERAGE	24.23	20.25	24.16	20.26

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/022A	Plate 1	24.21	20.10	24.13	20.11
	Plate 2	24.28	20.09	24.15	20.13
	Plate 3	24.30	20.19	24.19	20.16
	Plate 4	24.29	20.12	24.21	20.11
	Plate 5	24.23	20.02	24.23	20.18
	AVERAGE	24.26	20.10	24.18	20.12

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/023A	Plate 1	24.10	20.13	24.10	20.13
	Plate 2	24.15	20.17	24.14	20.19
	Plate 3	24.19	20.19	24.19	20.22
	Plate 4	24.22	20.11	24.21	20.25
	Plate 5	24.24	20.11	24.24	20.30
	AVERAGE	24.18	20.02	24.17	20.38

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/024A	Plate 1	24.01	20.11	24.11	20.12
	Plate 2	24.03	20.13	24.14	20.14
	Plate 3	24.05	20.17	24.18	20.13
	Plate 4	24.07	20.11	24.20	20.16
	Plate 5	24.09	20.12	24.22	20.18
	AVERAGE	24.05	20.12	24.17	20.14

SAMPLE.NO	SPECIFICATION	SH(mm)	SL(mm)	TH(mm)	TL(mm)
Vanco/ST/025A	Plate 1	24.12	20.08	24.13	20.14
	Plate 2	24.30	20.05	24.18	20.25
	Plate 3	24.30	20.18	24.13	20.20
	Plate 4	24.27	20.12	24.27	20.20
	Plate 5	24.18	20.02	24.18	20.21
	AVERAGE	24.34	20.09	24.17	20.2

3.18.2 Stability Samples - Percentage Of Potency

STABILITY SAMPLES	PERCENTAGE OF POTENCY
Vanco/ST/001A	100.91%
Vanco/ST/002A	101.69%

Vanco/ST/003A	100.39%
Vanco/ST/004A	100.60%
Vanco/ST/005A	100.19%
Vanco/ST/006A	99.80%
Vanco/ST/007A	105.6%
Vanco/ST/008A	100.83%
Vanco/ST/009A	100.79%
Vanco/ST/010A	103.7%
Vanco/ST/011A	105.9%
Vanco/ST/012A	103.27%
Vanco/ST/013A	101.76%
Vanco/ST/014A	101.92%
Vanco/ST/015A	101.6%
Vanco/ST/016A	101.3%
Vanco/ST/017A	102.5%
Vanco/ST/018A	104%
Vanco/ST/019A	97.88%
Vanco/ST/020A	101%
Vanco/ST/021A	98.6%
Vanco/ST/022A	98.83%
Vanco/ST/023A	105.8%
Vanco/ST/024A	102.85%
Vanco/ST/O25A	98.83%

3.18.3 Vancomycin In Mg/Capsule:

STABILITY SAMPLES	VANCOMYCIN IN mg/CAPSULE
Vanco/ST/001A	136.5mg
Vanco/ST/002A	137.5mg
Vanco/ST/003A	135.82mg
Vanco/ST/004A	136.10mg
Vanco/ST/005A	135.55mg
Vanco/ST/006A	135mg
Vanco/ST/007A	142.8mg
Vanco/ST/008A	136.4mg
Vanco/ST/009A	136.36mg
Vanco/ST/010A	140.30mg
Vanco/ST/011A	143.27mg
Vanco/ST/012A	139.6mg
Vanco/ST/013A	137.6mg
Vanco/ST/014A	137.8mg
Vanco/ST/015A	137.46mg
Vanco/ST/016A	137.05mg
Vanco/ST/017A	138.6mg
Vanco/ST/018A	281.4mg
Vanco/ST/019A	264.8mg
Vanco/ST/020A	273.29mg
Vanco/ST/021A	266.80mg
Vanco/ST/022A	267.42mg
Vanco/ST/023A	286.2mg
Vanco/ST/024A	278.30mg
Vanco/ST/O25A	267.42mg

3.18.3 Vancomycin In Percentage/Capsule:

STABILITY SAMPLES	**VANCOMYCIN IN PERCENTAGE/CAPSULE (ASSAY LIMIT)**
Vanco/ST/001A	109.2%
Vanco/ST/002A	110%
Vanco/ST/003A	108.6%
Vanco/ST/004A	108.88%
Vanco/ST/005A	108.44%
Vanco/ST/006A	108%
Vanco/ST/007A	114.24%
Vanco/ST/008A	109.1%
Vanco/ST/009A	109.08%
Vanco/ST/010A	112.2%
Vanco/ST/011A	114.6%
Vanco/ST/012A	111.68%
Vanco/ST/013A	110%
Vanco/ST/014A	110.24%
Vanco/ST/015A	109.9%

Vanco/ST/016A	109.6%
Vanco/ST/017A	110.8%
Vanco/ST/018A	112.56%
Vanco/ST/019A	105.92%
Vanco/ST/020A	109.3%
Vanco/ST/021A	106.7%
Vanco/ST/022A	106.9%
Vanco/ST/023A	114.4%
Vanco/ST/024A	111.3%
Vanco/ST/O25A	106.9%

Interpretation:

The potency of vancomycin in all four stages laid in between 97-106%. This present study revealed that vancomycin drug constantly maintained the observed assay specification limits in validation sample, finished product and stability sample which are in the range upto 106-114.08%

Raw material {vancomycin} potency 97-98% and vancomycin 1012-1057µg/mg in anhydrous basis.

The assay of vancomycin in % specification limits listed below:

SAMPLES	VANCOMYCIN IN % (ASSAY LIMITS)
Raw materials	>900µg/mg / capsule
Validation samples	90 – 115% (assay limits)
Finished samples	90 -115% (assay limits)
Stability samples	90 -115% (assay limits)

IV. Discussion

In this present study the % of potency of vancomycin drug (125&250mg) capsules were assessed invitro against ATCC 6633 Bacillus subtiisby microbiological assay.

The vancomycin drug was analysed in different stages raw materials, finished products, validation samples and stability samples.

This study revealed that % of potency of vancomycin in all four stages laid in between 97-106% and assay specification limits are within 106 -114.08%

- Raw materials of vancomycindrug, potency ranges from97-98% and the vancomycin % content 1012-1057 µg/mg in anhydrous basis (within specification limits)
- Validation samples were tested if there is any changes in routine processing of vancomycin there by, there is a need to check the % potency of vancomycin. It is proved that the range obtained from 97- 99.72% and follows the same specification limit of 90-115%
- Finished products had same potency 97-105.4% and assay limits 107-114.08%.and follows product specification.
- Stability samples are stored in different humidity in different chambers after commercialization. The potency range same as 97.88 -105.9% and assay specification limits of 106 -114.6% which determines the stability of the drug.

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Appendix
Composition Of Media

Antibiotic Media No: 8
Peptone- 6.0g
Yeast extract- 3.0g
Beef extract- 1.5 g
Agar -15g
Water -1000ml
P^H after sterilization – (5.9±0.1°C)
Alternatively readily available, antibiotic assay media no: 8 of (HI-Media NO M041) can be used.

Sporulating Agar Antibiotic Media No: 1 (Seed Agar):
Peptone- 6.0 g
Pancreatic digest of casein – 4.0 g
Yeast extract- 3.0 g
Beef extract- 1.5 g
Dextrose-1.0 g
Agar – 15 g
Water – 1000ml
p^H -6.6±0.1°C

To the above medium add 6.3 g of manganese sulfate (Media no: 32 as per USP)

Alternatively readily available, antibiotic assay media no: 1 of (Hi-media No: M003) can be used by adding 0.3 g of manganese sulfate.