Pharmaceutical Sciences Asia

Pharm Sci Asia 2026; 53(1), 44-56

DOI:10.29090/psa.2026.01.25.7341

Research Article

Stability study of ready-to-use vancomycin prefilled injections in polypropylene syringes and low-density polyethylene bags for neonates

Jiraphong Suksiriworapong[1,2], Manita Suriyarangsee[3], Jiraporn Leanpolchareanchai[1,2], Monwarat Laohajeeraphan[3], Vimonkarn Sirisuksan[3,*]

[1] Department of Pharmacy, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand
[2] Center for Research on Extemporaneous Compounding and Pharmaceutical Technology Innovation, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand
[3] Pharmacy Department, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand

ABSTRACT

Vancomycin is extensively used to treat methicillin-resistant *Staphylococcus aureus* infections in neonates. Ready-to-use vancomycin injection solutions prefilled into sterile syringes and bags may help improve operational efficiency. Previous stability studies primarily relied on HPLC for drug content, often lacking concurrent evaluation of antibiotic potency via microbial assay, identification of degradation products, or assessment of sterility at the end of the shelf life. Furthermore, information was limited regarding head-to-head stability comparisons across different container sizes for ready-to-use vancomycin solutions. Therefore, this study aimed to evaluate the physical, chemical, and microbiological stability of a 5 mg/mL vancomycin solution in 5% dextrose by providing a head-to-head comparison across different sized containers (10-, 20-, and 50-mL polypropylene (PP) syringes and a 100-mL low-density polyethylene (LDPE) bag) while assessing both vancomycin content (HPLC) and antibiotic potency (microbial assay) and confirming sterility at the end of the shelf life. The drug solutions in all size containers had a pH range of 3.60–3.64, an osmolality of 245–249 mOsm/kg, a %labeled amount of 99–103% as determined by high-performance liquid chromatography, and an antibiotic potency of 99–101%. The vancomycin solutions were physically and chemically stable for 55 days at 5±3 °C when stored in the 10- and 20-mL PP syringes and 100-mL LDPE bag and protected from light. Under similar storage conditions, the solution in the 50-mL PP syringe had a shelf life of only 30 days at 5±3 °C owing to an exceeding limit for degradation products and impurities. All the solutions were sterile at the end of their shelf life. Therefore, these prefilled vancomycin solutions can be prepared and stored in a pharmacy service unit in a hospital for subsequent administration of the drug to neonates.

Keywords:
Vancomycin; Stability; Prefilled injection; Polypropylene syringe; Low-density polyethylene bag

1. INTRODUCTION

Vancomycin is the drug of choice for the treatment of invasive methicillin-resistant *Staphylococcus aureus* (MRSA) in neonatal sepsis, which increases morbidity and mortality risk in patients[1, 2]. The recommended dose range for neonates aged less than 3 months is 10–20 mg/kg every 8–48 h, depending on the postmenstrual age, body weight, and serum creatinine level of patients[3]. According to the recommendations of the Joint Commission on Accreditation of Healthcare Organization, American Academy of Pediatrics, and American Society

***Corresponding author:**
* Vimonkarn Sirisuksan Email: tangvimon@gmail.com

Health-System Pharmacists, ready-to-use intravenous (IV) drug admixtures can be prepared and stored in a centralized pharmacy service unit. The prepared stock provides several advantages, including overall cost savings, reduced time consumption, minimized wastage, decreased medication errors, and enhanced service efficiency. Ready-to-use drug preparations must be of good quality in terms of stability, potency, and sterility until they are administered to patients[4-6].

The stability of vancomycin has been reported under various conditions and is affected by many factors such as concentration and source of the drug, diluent type, pH, temperature, light, type, and materials of the container[7-14] as summarized in Table 1. For 5 and 10 mg/mL vancomycin solutions in 5% dextrose injection (D5W), a difference in containers resulted in different shelf lives for the drug solutions. For example, the drug solutions were stable at 24–25 °C for 24 h in a 100-mL latex elastomeric pump reservoir[7] and for 17 days in a clear 20-mL glass vial and a plastic polyvinyl chloride (PVC) bag[8]. Vancomycin solution stored in a three-piece syringe, constructed entirely from polypropylene (PP) with a rubber stopper at the inner end of the plunger, had a shelf life of 55 days, whereas vancomycin stored in a two-piece syringe, composed of a polyethylene (PE) barrel and PP plunger, had a shelf life of only 33 days[11]. The reported shelf lives of vancomycin solutions at 2–8 °C were 63 days in a clear 20-mL glass vial[8], 7 days in a PVC infusion bag[9], or 58 days in a PVC bag[10]. Different concentrations of vancomycin solution also affected their stability[12, 15]. The degradation rate of vancomycin was found to follow first-order kinetics, which depend on the initial concentration of the drug in the solution[11]. Vancomycin at 10 mg/mL in D5W and normal saline solution (NSS) stored in a PP syringe was stable for 55 and 62 days at 25 °C[11], whereas concentrated vancomycin solutions at 41.66 mg/mL in similar diluents and containers were stable for only 48 h[12]. High drug concentrations at 25, 40, 60, and 80 mg/mL in NSS were chemically stable for only 24 h[15]. However, visible particles occurred in the highest concentrated drug solution after 22 h and more than 6,000 particles with a size of ≥10 μm were found in all concentrated solutions, suggesting physical instability of the solutions. Additionally, different brands of vancomycin products result in different shelf lives of vancomycin preparations. The generic vancomycin (10 mg/mL in a D5W infusion bag) was stable for 57 days at 5±3 °C, while branded vancomycin (Vancocin®) was stable for only 43 days[16]. Another factor that affects the stability of vancomycin preparations is light. Only a few studies have investigated stability under light protection[9], and such data are thus limited. These examples illustrate the effects of concentration and source of the drug, diluent type, and container type on the stability of vancomycin solutions.

Variability in the quality of vancomycin preparations has been reported because of variable impurity profiles that may occur during production or storage[17, 18]. Different vancomycin activities of generic products compared to innovators have been observed in *in vivo* studies[19] although all tested generic products passed the United States Pharmacopeia (USP) acceptance criteria for purity and potency[17, 18]. Vancomycin may lose its sugar moieties during storage, forming aglucovancomycin and desvancosaminylvancomycin[18]. The crystalline degradation product-I (CDP-I referred to CDP-I Major, as (1.2*M*)-[-β-Asp^3]vancomycin B (3.2-*syn*-chloro[-β-Asp^3]vancomycin B, and CDP-I minor, as [-β-Asp^3]vancomycin B[20]) may also be formed in vancomycin products upon storage at high temperature[11]. Although CDP-I is typically detected in vancomycin products, it is not the most abundant compound in the prepared solution. Other degradation products include monodechlorovancomycin, demethylvancomycin, aglucovancomycin, and desvancosaminylvancomycin[21-23]. All these degradation products show less or no antibacterial activity compared to vancomycin B[18, 23]. However, some degradation products, at a certain amount, antagonize the activity of vancomycin B in the solutions, which may affect the quality of extemporaneously prepared vancomycin solutions[17]. According to the pharmacopoeia methods, the exact quantification of each impurity in the preparations could not be determined owing to the co-elution of degradation products. This variation has led to Food and Drug Administration (FDA) regulation for the evaluation of parenteral vancomycin using two assays, namely, high-performance liquid chromatography (HPLC) analysis for purity and microbial assay for potency.

Despite the fact that data on vancomycin stability are documented in the literature, the available data are limited. Most of the previous reports determined the drug content remaining using HPLC (listed in Table 1) and available data on storage with light protection is limited. Only a few reports have identified the degradation products in HPLC chromatograms[8, 9, 11, 12, 15] and possible leaching substances such as di (2-ethylhexyl) phthalate (DEPH)[9]. As previously mentioned, degradation products may affect the activity of vancomycin, and the pharmacopoeia suggests a microbial or biological assay of antibiotics for the quantification of vancomycin activity and HPLC for analysis of vancomycin content[20, 24]. Additionally, a limited number of component peaks other than the vancomycin B peak is included in the pharmacopoeia.

For more efficient service in a centralized pharmacy service unit, more information on the extended shelf life of vancomycin in accessible diluents and containers is required. Therefore, this study aimed to report the stability of a vancomycin solution in D5W

Table 1. Examples of vancomycin stability data.

Vancomycin		Diluent[a]		Type of container[b]	Light exposure[c]	Drug content analysis by	Analysis of impurities/ degradation products	Shelf life[d]	Ref. no.
Concentration (mg/mL)	Company	Type	Company						
5	Elkins-Sinn, Inc.	D5W NSS	Baxter Healthcare Corp.	100-mL Latex elastomeric pump reservoir Clear glass vial	No light protection	HPLC	No	24 h at 25 °C 14 days at 5 °C 9 weeks at -20 °C	[7]
	Eli Lilly and Company	D5W NSS	Travenol Laboratories	20-mL Clear glass vial PVC bag	No light protection	HPLC	Identify but not quantify	17 days at 24°C 63 days at 5 °C and -10 °C 17 days at 24 °C	[8]
	Qualimed Laboratories	D5W NSS	Macopharma Laboratories	PVC infusion bag PVC infusion bag	No light protection Light protection	HPLC	DEPH leaching; Identify CDP-1 but not quantify	48 h at 22 °C 7 days at 4 °C	[9]
	Lilly	D5W	Baxter Healthcare Corp.	100-mL PVC bag	ND	HPLC	No	58 days at 4 °C	[10]
	Eli Lilly and Company	DW (5-30%)	Travenol Laboratories	10-mL PP syringe	ND	HPLC	No	24 h at 4 °C 2 h at RT	[25]
10	Lilly	D5W	Baxter Healthcare Corp.	100-mL PVC bag	ND	HPLC	No	58 days at 4 °C	[10]
	Eli Lilly and Company	D5W NSS	Baxter Healthcare Corp.	5-mL Two-piece syringe (PE barrel and PP plunger) 5-mL Three-piece syringe (PP syringe and rubber stopper)	ND	HPLC	Identify CDP-1 but not quantify	D5W: 33 days at 25 °C NSS: 34 days at 25 °C Both: 84 days at 4 °C D5W: 55 days at 25 °C NSS: 62 days at 25 °C Both: 84 days at 4 °C	[11]
	Lilly Mylan	D5W	Not accessible[e]	Polyolefin infusion bag	ND	HPLC	Not accessible[e]	43 days at 5±3 °C 57 days at 5±3 °C	[16]
	Lilly	D5W	Baxter Healthcare Corp.	250-mL polyolefin bag	No light protection	HPLC	No	58 days at 4 °C and additional 72 h at RT	[26]
41.66	Mylan	D5W NSS	Baxter Healthcare Corp.	50-mL PP syringe	No light protection	HPLC	Identify degradation products but not quantify	48 h at RT	[12]
25 - 80	Sandoz	NSS	Baxter Healthcare Corp.	20- and 50-mL PP syringes	ND	HPLC	Identify degradation products but not quantify	24 h at RT	[15]

[a]D5W = 5% dextrose in water, NSS = normal saline, DW = dextrose in water; [b]PVC = polyvinyl chloride, PP = polypropylene, PE = polyethylene; [c]ND = not defined; [d]RT = uncontrolled room temperature; [e]A full paper could not be accessed.

in a variety of container sizes, which will provide flexibility for selecting the proper dose of vancomycin for a wide range of neonate body weights. Based on the recommended concentration of vancomycin that is frequently utilized in pediatric patients, a 5 mg/mL vancomycin solution with a volume range of 10–100 mL was flexibly administered to neonates with a body weight of up to 30 kg[3, 25]. The drug solution was prefilled into 10-, 20-, and 50-mL PP syringes and a 100-mL low-density polyethylene (LDPE) bag. Additionally, the stability of vancomycin preparations was assessed in terms of physical, chemical, and microbiological aspects. Chemical stability was evaluated based on antibiotic potency using a microbial assay and the contents of vancomycin B and any impurities and degradation products were evaluated using HPLC. Sterility of the solutions was investigated at the end of the study.

2. MATERIALS AND METHODS

2.1. Materials

A generic brand vancomycin hydrochloride powder for injection (500 mg/vial) was purchased from Siam Bheasach Co., Ltd., Bangkok, Thailand. Sterile water for injection (SWI) and D5W were bought from General Hospital Products. Public Co., Ltd., Pathum Thani, Thailand. Acetonitrile (ACN, HPLC grade, Honeywell Burdick & Jackson™, Honeywell Specialty Chemicals, Singapore), triethylamine (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), vancomycin standard (Sigma-Aldrich, Missouri, USA), sodium hydroxide (NaOH, CARLO ERBA Reagents S.A.S., Val de Reuil, France), 37% w/v hydrochloric acid (HCl, RCI Labscan Limited, Samut Sakhon, Thailand), and 30% w/w hydrogen peroxide (H_2O_2, Merck KGaA, Darmstadt, Germany) were used as received.

2.2. Preparation of vancomycin prefilled solution for injection

Vancomycin HCl powder (500 mg/vial) was reconstituted in 9.8 mL of SWI and mixed thoroughly until complete dissolution. The volume of the drug solution was adjusted to 10 mL using SWI. The drug solution was diluted with D5W to yield a final concentration of 5 mg/mL and then withdrawn into three sizes of PP syringes (10, 20, and 50 mL, Terumo®, Terumo Corporation, Tokyo, Japan) and a 100-mL LDPE bag. The syringe was capped with a sterile PP tip cap (MultiCap; M.E. Meditek Co., Ltd., Bangkok, Thailand).

2.3. Physical property evaluation

The vancomycin solution was evaluated in terms of its physical appearance, pH, osmolality, and optical density. The physical appearance of the preparation was visually observed on white and black backgrounds to detect any particulate matter. The pH of the drug solution was measured using a pH meter (LAQUAtwin pH-22, HORIBA, Ltd., Kyoto, Japan). A change in pH was considered acceptable if it did not differ by more than one pH unit from the initial value[26]. The osmolality of the drug solution was determined using Advanced® Osmometer Model 3250 (Advanced Instruments, Inc., Massachusetts, USA) without dilution. The clarity of the drug solution was evaluated using a UV-vis spectrophotometer (UV-2600, Shimadzu Corporation, Tokyo, Japan) at a wavelength of 600 nm.

2.4. Quantification of vancomycin

2.4.1. Drug assay by HPLC

The amount of vancomycin B in the preparation was quantified using HPLC (Agilent 1200 series, Agilent Technology, California, USA) according to a previously published protocol[20] with some modifications. In brief, the drug was eluted through Phenomenex C18 reverse phase column (250 mm × 4.6 mm, 5 µm) with a C18 guard column. The mobile phase (flow rate: 1 mL/min) consisted of a mixture of 0.2% v/v triethylamine solution pH 3.2 and ACN in gradient mode as follows: 0–13 min 9% ACN, 13–22 min 30% ACN and 22–30 min 9% ACN. The eluted drug was detected using a diode array detector at 280 nm. The HPLC condition was validated over the linear concentration range of 20–400 µg/mL with R^2 of 0.9999. The precision was less than 2% and the accuracy was in the range of 99.68–101.31%. The limit of quantitation was found to be 20 µg/mL.

2.4.2. Microbial assay

The antibiotic potency of vancomycin HCl in the prefilled solutions was also determined by a microbial assay using the cylinder-plate method according to USP 2022[24]. Five milliliters were withdrawn from each sample container, and the triplicate samples were then pooled. The pooled samples were diluted with water to a concentration of 1 mg/mL and tested against *Bacillus subtilis* ATCC 6633 at 32.0–35.0 °C for 5 days. The clear zone was measured, and antibiotic potency was calculated from the vancomycin standard curve. The results were expressed as %antibiotic potency.

2.5. Forced degradation study of vancomycin

The degradation of vancomycin was studied under forced conditions, namely, acid and base hydrolysis, thermal hydrolysis, oxidation, and photolysis. Vancomycin HCl was dissolved in the solvent and subjected to forced conditions as listed in Table 2.

Table 2. Forced degradation conditions of vancomycin

Condition	Solvent	Temperature (°C)[a]	Reaction time
Acid hydrolysis	1 M HCl	RT	15 min
Base hydrolysis	5 M NaOH	RT	60 min
Thermal hydrolysis	Water	75±5	12 h
Oxidation	30% H_2O_2	RT	2 h
Photolysis[b]	Water	RT	24 h

[a]RT = Room temperature, [b]The samples were exposed to sunlight during daytime for a cumulative 24 hours.

The drug solutions were kept in amber glass bottles, except during photolysis conditions, when they were kept in clear glass bottles. At the designated time, the solution was neutralized and diluted with the mobile phase to the final concentration of 100 µg/mL for further HPLC analysis.

2.6. Stability study

The stability of vancomycin prefilled solutions was studied for 70 days at 5±3 °C according to the International Council for Harmonization (ICH) Harmonized Tripartite Guideline and the methodological guidelines for stability studies of hospital pharmaceutical preparations[26, 27]. The preparations were evaluated for physical and chemical stabilities. Physical stability was assessed in terms of physical appearance, pH, osmolality, and clarity as previously described. HPLC analysis was performed to evaluate chemical stability, and the results were expressed as %labeled amount and %drug remaining, as shown in Equations 1 and 2, respectively. A microbial assay was also conducted to assess the %antibiotic potency and the %potency remaining was calculated according to Equation 3. The degradation products were determined by HPLC. The %total of impurities and degradation products was calculated based on the peak areas of all peaks except the main peak in the HPLC chromatograms, as stated in USP[24].

$$\%Labeled\ amount = \frac{Analyzed\ concentration\ of\ vancomycin\ B}{5} \times 100 \quad (1)$$

$$\%Drug\ remaining = \frac{Analyzed\ concentration\ of\ vancomycin\ B}{Initial\ concentration\ of\ vancomycin\ B} \times 100 \quad (2)$$

$$\%Potency\ remaining = \frac{\%Antibiotic\ potency\ at\ any\ time}{\%Antibiotic\ potency\ at\ an\ initial\ time} \times 100 \quad (3)$$

2.7. Sterility test

The sterility of the vancomycin prefilled solution for injection was evaluated according to USP[24] on day 0 and the last day at which the drug remained stable. Each sample was prepared in quadruplicate and tested.

2.8. Statistical analysis

The numerical data is expressed as the mean ± standard deviation (SD) from three measurements. One-way ANOVA or Student's t-test was used to compare the means of multiple or two groups, respectively, using IBM® SPSS® statistics software (IBM Corporation, New York, USA). If the p-value was less than 0.05, the difference was considered significant.

3. RESULTS AND DISCUSSION

3.1. Forced degradation study of vancomycin

To quantify vancomycin content, an HPLC method was developed and modified from the British Pharmacopoeia[20]. The method was validated and passed the criteria of validation method according to the ICH Harmonized Tripartite Guideline: Validation of analytical procedures: Text and methodology (Q2(R1))[28]. In addition, the forced degradation of vancomycin was performed based on two objectives. The primary objective was to confirm the specificity of the developed HPLC method. Secondly, it aimed to identify possible degradation products of vancomycin under the developed HPLC condition. The drug was further degraded under five conditions: acid hydrolysis, base hydrolysis, thermal hydrolysis, oxidation, and photolysis, which are generally encountered during the storage of liquid drug preparations. The results are summarized in Table 3, and the chromatogram traces are illustrated in Figure 1. The HPLC chromatograms demonstrate that the vancomycin peak occurred at 7.65–8.28 min and other degradation product peaks did not interfere the drug peak. This result suggests the specificity of the developed HPLC condition used for quantification of vancomycin upon stability study. Additionally, the drug was prone to degradation under all conditions.

Table 3. %Degradation of vancomycin in various forced degradation conditions

Condition	%Degradation
Acid hydrolysis	34.93%
Base hydrolysis	37.59%
Thermal hydrolysis	72.47%
Oxidation	11.17%
Photolysis	10.28%

Vancomycin was extensively degraded by thermal hydrolysis, while it underwent moderate deterioration under acid and base hydrolysis. The slight decomposition of the drug was caused by oxidation and photolysis. All tested conditions may accelerate drug degradation in solutions during storage.

3.2. Physicochemical properties of vancomycin HCl prefilled solutions

In this study, we prepared ready-to-use vancomycin HCl in D5W prefilled solutions at a concentration of 5 mg/mL, which is a common and adjustable concentration for the treatment of MRSA infections in neonates[3, 25]. It has been reported that the size of prefilled container may have an effect on the stability of the vancomycin solution for injection[11]. Hence, we prepared the drug solution and prefilled in three sizes of PP syringes (10, 20, and 50 mL) and a 100-mL LDPE bag. After preparation, the physical and chemical properties of preparations of all sizes were evaluated, and the results are shown in Figure 2 and Table 4. The solutions of all preparations were clear and colorless, and no particles were observed visually.

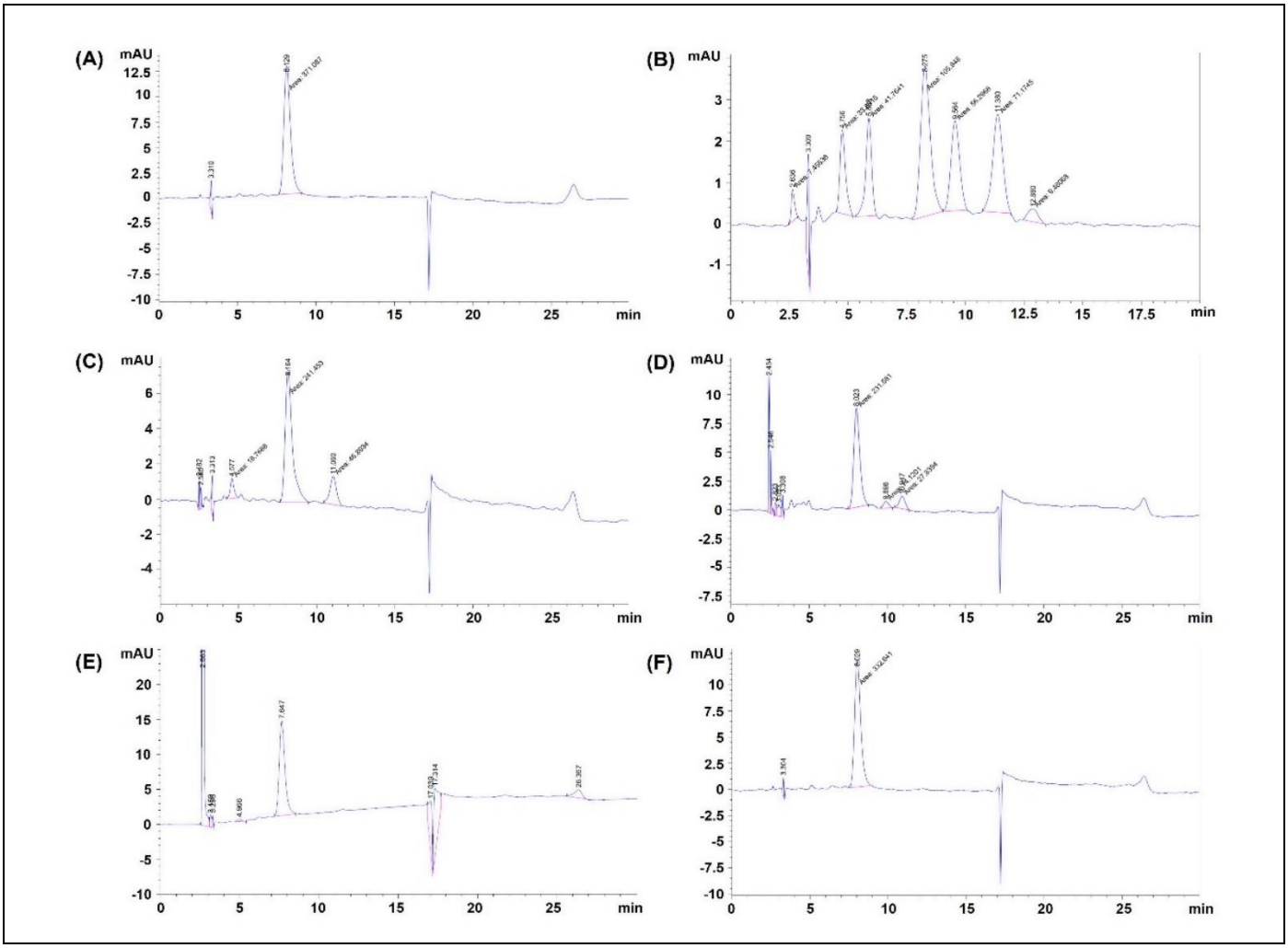

Figure 1. HPLC chromatograms of vancomycin after being subjected to forced degradation conditions. (A) Control, (B) thermal hydrolysis, (C) acid hydrolysis, (D) base hydrolysis, (E) oxidation, and (F) photolysis. The retention time of vancomycin was in the range of 7.65–8.28 min. The enlarged HPLC chromatograms are provided in the supplementary information.

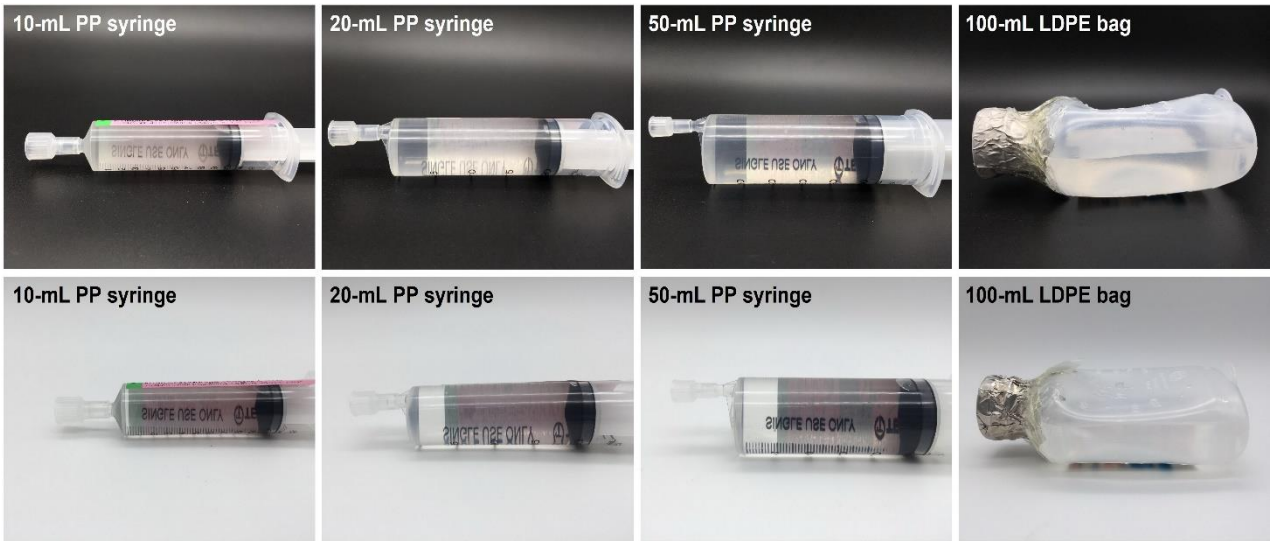

Figure 2. Examples of the appearance of ready-to-use vancomycin HCl in D5W prefilled solutions for injection (5 mg/mL) in 10-, 20-, and 50-mL PP syringes and 100-mL LDPE bag after preparation.

All preparations had a pH range of 3.60–3.64 which satisfies the requirement stated in vancomycin for injection monograph in USP[24]. Additionally, this pH range is optimal for vancomycin solutions since drug degradation is retarded over the pH range of 3.0–5.7[14].

Our results showed that the osmolality of all prepared injection solutions was within 245–259 mOsm/kg, which was near to that of blood. The intravenous administration of either hypertonic or hypotonic solutions has been reported to cause side effects in the blood and at the site of injection. Administration of hypertonic solutions with an osmolality of at least 600 mOsm/kg may cause crenation of red blood cells and pain. Meanwhile, the administration of hypotonic solutions with an osmolality of less than 150 mOsm/kg may lead to hyponatremia, hemolysis, and pain at the site of injection[29, 30]. The osmolality of blood falls in the range of 285–310 mOsm/kg[24]. It has been suggested that a solution with an osmolality less than 600 mOsm/kg and a pH close to the physiological value has a low-to-moderate risk of phlebitis when administered by intravenous infusion[30]. Therefore, all these vancomycin solutions can be administered with a low risk of adverse effects due to osmotic pressure.

The optical densities of all solutions were approximately 100%, confirming that no precipitates were formed. The analyzed vancomycin B content of all solutions was in the range of 99.9–102.7% labeled amount, which was equivalent to 4.99–5.14 mg/mL as measured by HPLC. The sum of the component peaks of freshly prepared solutions was less than 9.0%. As required in the USP, the injection products contained at least 80% of vancomycin B as the main component and not more than 9.0% of any other component based on the chromatogram peaks[24]. According to the microbial assay, all preparations contained 99.8–101.2% antibiotic potency, which passed the acceptable criteria in the USP[24]. The HPLC results were consistent with those of the microbial assays. Therefore, the stability of these solutions was further investigated.

Table 4. Characteristics (pH, osmolality, optical density, %labeled amount, %antibiotic potency, and %total of impurities and degradation products) of the prepared vancomycin HCl in D5W prefilled solutions for injection (5 mg/mL)

Samples	pH	Osmolality (mOsm/kg)	Optical density (%T)	Labeled amount (%)	Total impurities and degradation products (%)[a]	Antibiotic potency (%)
10-mL PP syringe	3.64±0.04	246.7±0.6	101.1±0.2	102.5±0.7	7.0±0.5	100.2
20-mL PP syringe	3.60±0.03*	246.3±4.7	101.1±0.1**	102.7±1.5	5.7±0.7	100.7
50-mL PP syringe	3.60±0.01*	245.3±2.9	100.9±0.0	101.2±2.2	7.1±0.2	99.8
100-mL LDPE Bag	3.62±0.02	248.7±0.6	101.2±0.0**	99.9±3.6	6.7±0.2	101.2

*,**Significantly different when compared to 10- and 50-mL PP syringes, respectively

[a]%Total of impurities and degradation products calculated based on all peaks except the main peak in HPLC chromatograms using the following equation.

$$\%\text{Total impurities and degradation products} = \frac{\text{Total peak area of all impurities and degradation products}}{\text{Total area of all peaks including vancomycin}} \times 100$$

Table 5. Appearance of ready-to-use vancomycin HCl in D5W prefilled solutions for injection (5 mg/mL) after storage at 5±3 °C for 70 days

Test \ Time (days)	10-mL PP Syringe				20-mL PP Syringe				50-mL PP Syringe				100-mL LDPE bag			
	15	30	55	70	15	30	55	70	15	30	55	70	15	30	55	70
Packaging																
Integrity[a]	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
Leakage[b]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Solution																
Color[c]	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC	CC
Particulate matter[b]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gas bubbles[b]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a]C = Complete integrity; [b]Undetectable; [c]CC = Colorless and clear.

3.3. Stability study

The stability of vancomycin in D5W in PP syringes and the LDPE bag was studied for 70 days at 5±3 °C. The appearance of all samples is summarized in Table 5. The packaging of all studied samples was completely sealed without any leakage during the study. The solutions in all containers exhibited clear colorless transparency over 70 days. Precipitation, recrystallization, and gas formation were not observed in any of the solutions.

The pH of the vancomycin solution remained almost unchanged throughout the study period (Figure 3A). At the end of the study, the solutions in 10-, 20-, 50-, and 100-mL containers had pH values of 3.64±0.03, 3.65±0.01, 3.62±0.01, and 3.64±0.01, respectively. The pH change in all containers upon storage was less than 0.2, and these values were within the pH range of maximum stability[8, 14] considering that this alteration was acceptable[26]. The literature report indicates that vancomycin degradation by water is minimal over the pH range of 3.0–5.7[14]. Conversely, decomposition is accelerated at pH ranges of 1.0–3.0 (catalyzed by acid) and 5.7–7.0 (catalyzed by dihydrogen phosphate ion). Since the pH of the solution remained constant throughout the storage period, the stability of

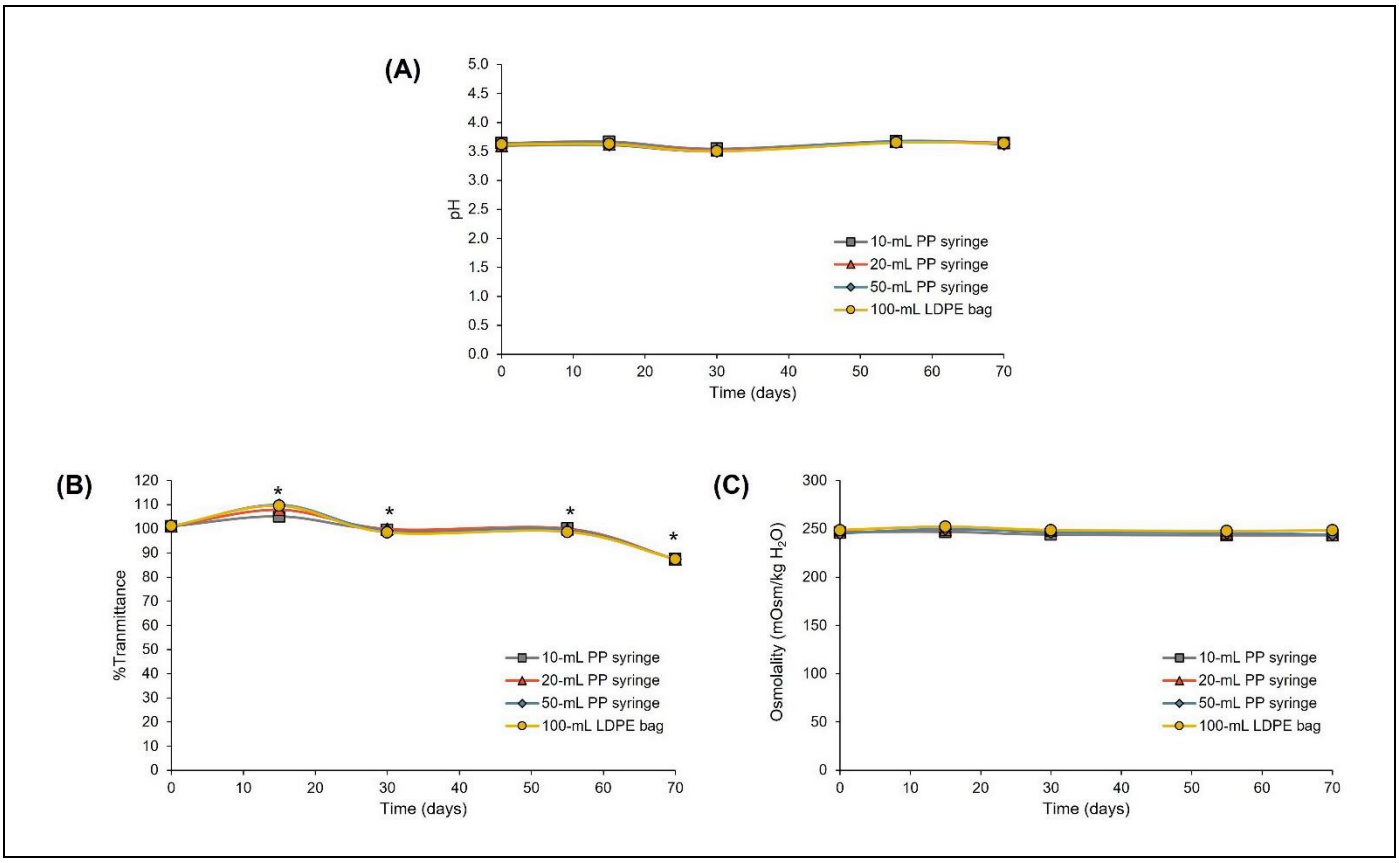

Figure 3. Stability results (A: pH, B: %transmittance, and C: osmolality) of the prepared vancomycin HCl in D5W prefilled solutions for injection (5 mg/mL) after storage at 5±3 °C for 70 days (n=3). *A significant difference of all containers was discovered when any time point was compared to day 0.

the preparations was not adversely affected by pH-catalyzed degradation.

The optical density (Figure 3B) of all containers tended to decrease over time. The %transmittance decreased to less than 90% after 55 days, although turbidity was not visually observed in all solutions. An insignificant alteration in the osmolality of all solutions was observed. At the end of the study, the osmolality of all the containers ranged from 243 to 248 mOsm/kg (Figure 3C). All container sizes had comparable pH, %transmittance, and osmolality of the solutions. Hence, the difference in container size did not affect the physical stability of the vancomycin solutions.

According to the USP vancomycin for injection monograph[24], an assessment of antibiotic potency and content of vancomycin using a microbial assay and HPLC analysis, respectively, is required. It has been well known that vancomycin products show the presence of related substances or degradation products and the quality of vancomycin products are variable due to different impurity profiles[18]. This variation led to FDA regulation for the evaluation of parenteral vancomycin using two assays, namely the HPLC assay for purity and the microbial assay for potency. Therefore, the remaining drug content over the study period was evaluated using both methods, as illustrated in Figure 4. As shown in

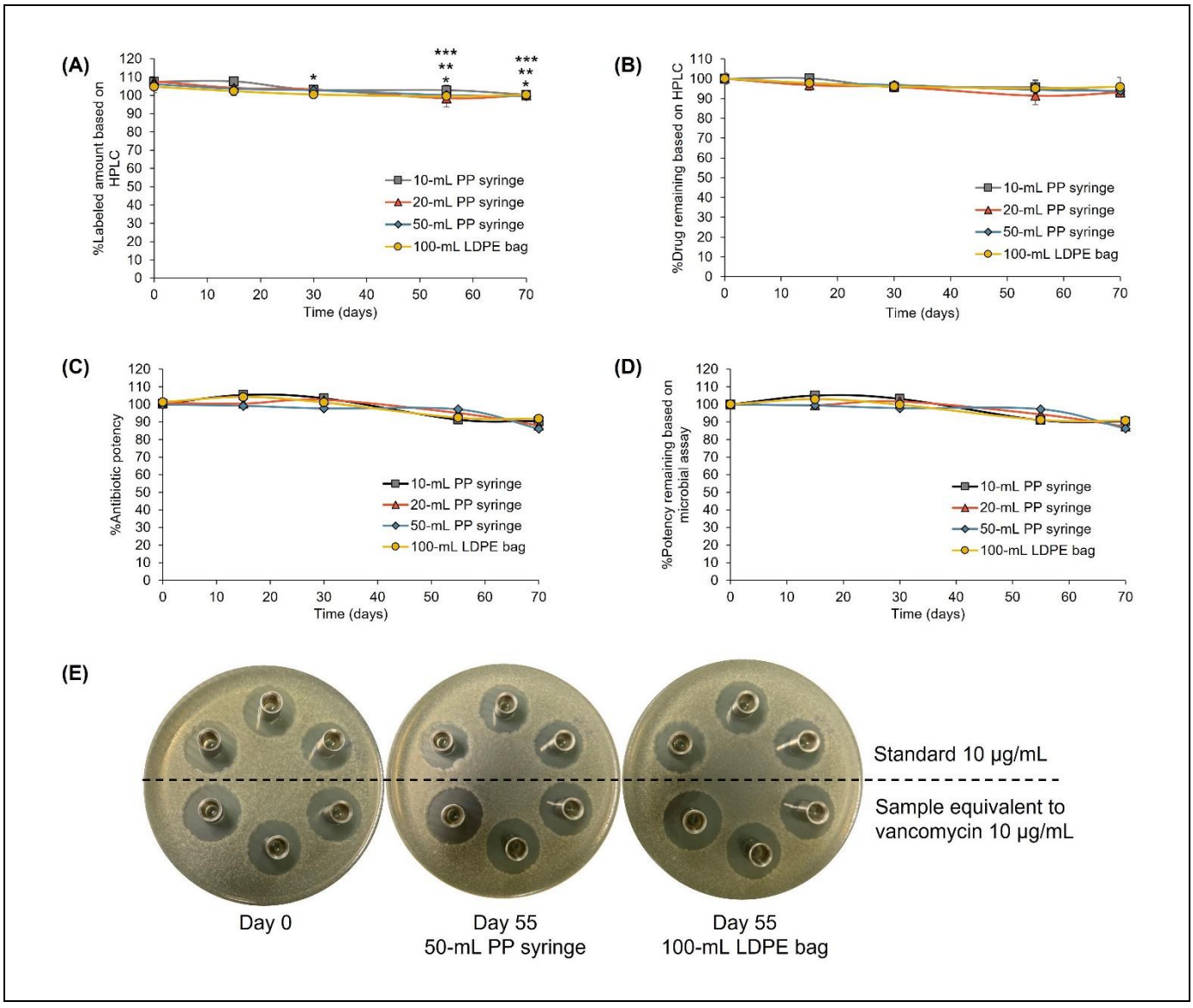

Figure 4. Percentages of labeled amount (A) and drug remaining (B) based on HPLC analysis of prefilled 5 mg/mL vancomycin solutions in D5W after storage at 5±3 °C for 70 days (n=3). Percentages of antibiotic potency (C) and %potency remaining (D) based on microbial assay (E) of prefilled 5 mg/mL vancomycin solutions in D5W after storage at 5±3 °C for 70 days (pooled triplicate samples). Examples of microbial testing images of vancomycin standard (10 µg/mL) and vancomycin solutions in a 50-mL PP syringe and a 100-mL LDPE bag (10 µg/mL) against *B. subtilis* ATCC 6633. *,**,***Significant differences of 10-, 20-, and 50-mL PP syringes were observed when compared to day 0, respectively.

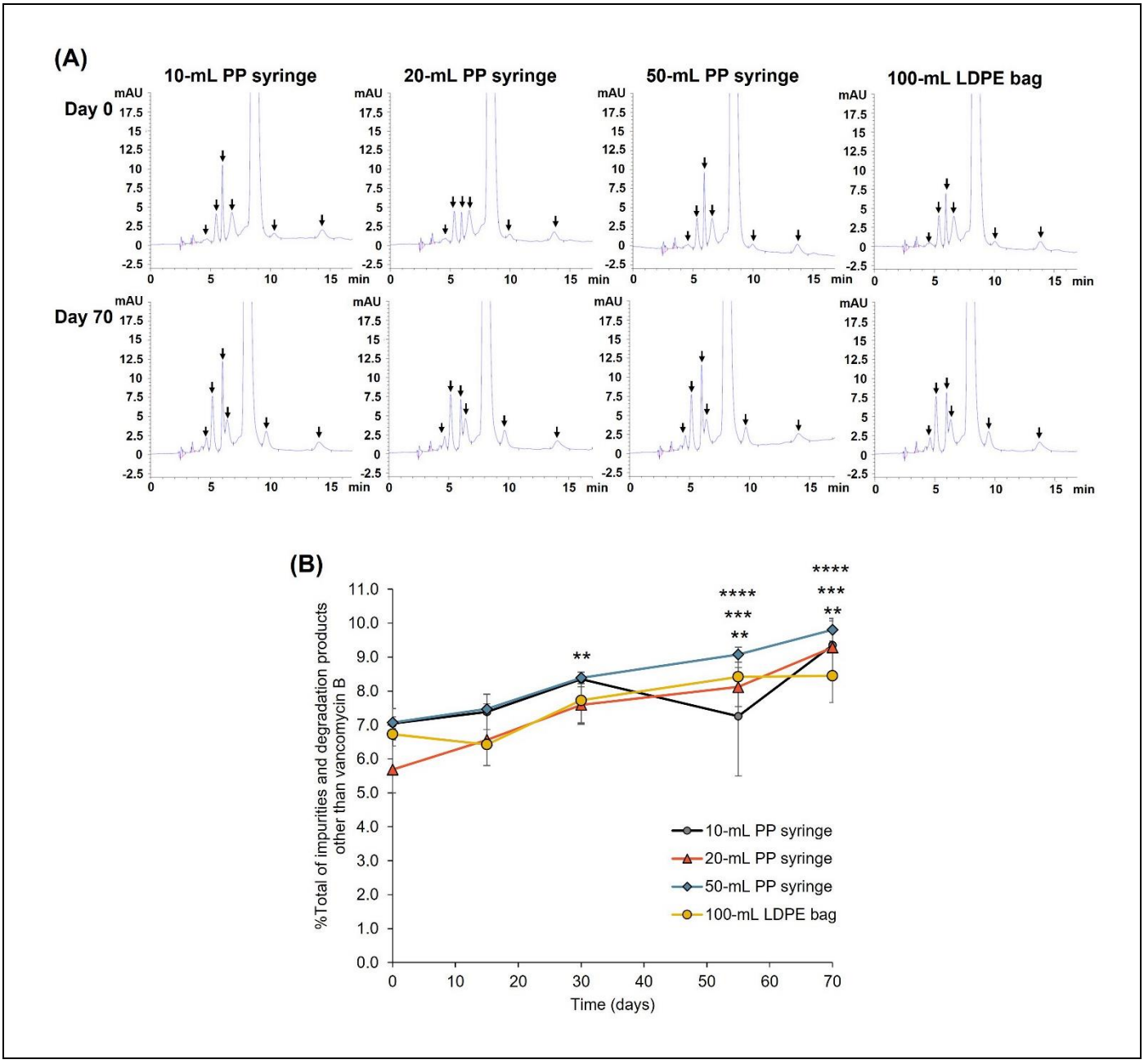

Figure 5. Examples of HPLC chromatograms (A) and %total of impurities and degradation products (B) of vancomycin solutions in all container sizes after storage at 5±3 °C for 70 days. Arrows indicate the degradation product peaks of vancomycin. **,***,****Significant differences of 20- and 50-mL PP syringes and 100-mL LDPE bag were observed when compared to day 0, respectively.

Figures 4A and B, the percentage of labeled amount and the percentage of drug remaining tended to decline after storage for 70 days. The vancomycin B content of most preparations significantly decreased on days 55 and 70 compared to day 0, except for the solution in the 100-mL container ($p < 0.05$); however, all preparations contained higher than 80% vancomycin B. Thus, all preparations passed the USP criteria for the content of the main peak. On day 70, the %drug remaining in the 10-, 20-, 50-, and 100-mL containers decreased to 93.3±0.8, 93.0±0.5, 93.9±1.9, and 95.9±4.8% of the initial preparation, respectively. Both the percentage of antibiotic potency and percentage of potency remaining based on the microbial assay remained over 90% until day 55, as shown in Figures 4C and D. As shown in Figure 4E, the diameters of the clear zones of the samples on day 55 were comparable to those on the initial day. On day 70, the percentage of antibiotic potency of the solutions in 20- and 50-mL syringes declined to less than 90%, whereas the other preparations still had a percentage of antibiotic potency of 90% or above.

The examples of HPLC chromatograms in Figure 5 show the traces of degradation products of vancomycin after storage at 5±3 °C for 70 days. The traces occurred at the retention times of 4.2–6.3 min and 9.5 min, which were similar to the degradation product peaks of vancomycin from thermal and acid hydrolysis

as shown in Figures 1B and C, respectively. The peak at 13.9–14.9 min, which appeared even on the day of preparation, corresponded to related substances in the vancomycin products. The total impurities and degradation products of all the solutions increased with storage time, as illustrated in Figure 5B. The sum of any component peaks other than vancomycin B after storage for 55 days significantly increased compared to that on the initial day of preparation ($p < 0.05$). However, we observed an increasing trend in the total impurities and degradation products related to the size of the PP syringes. Larger PP syringes, particularly the 50-mL syringe, tended to exhibit a higher %total impurities and degradation products ($p > 0.05$). The impurities in all PP syringe sizes increased to more than 9.0% on day 70; however, those in the 50-mL PP syringe was found to be 9.1±0.2% on day 55, which was over the limit of USP. However, the impurities in the 100-mL LDPE bag did not exceed the limit, even on day 70. The decrease in antibiotic potency on day 70 is related to an increase in impurities and degradation products. Previous studies have reported that precipitation does not occur in syringes with vancomycin concentrations greater than 90%[11, 31]. Thus, the reduced optical density of the vancomycin solutions in this study may have resulted from the degradation products of vancomycin after 55 days of storage, when the vancomycin B content was 90% or below. It has been reported that the degradation products in the vancomycin products possess lower or no antibacterial activity[18, 23]. Moreover, certain degradation products, in particular, CDP-I have antagonistic activity against vancomycin B[17]. CDP-I, which exists as a white floccular precipitate, has been reported as a degradation product of vancomycin upon storage at high temperature[11]. Other degradation products found in the preparations were monodechlorovancomycin, demethylvancomycin, aglucovancomycin, and desvancosaminylvancomycin[21-23]. Owing to the co-elution of related substances and degradation products in HPLC, the exact quantification of the degradation products requires other techniques with higher resolution and greater sensitivity, such as high-resolution liquid chromatography-mass spectrometry[17]. Nevertheless, vancomycin content analysis is still desired for quality control of vancomycin preparations and stability studies. In this study, we used HPLC to quantify vancomycin B and the sum of other impurities or degradation products, that may occur during storage; however, the exact amount of each impurity or degradation product could not be confirmed.

A previous study reported that different container material types gave different shelf lives of vancomycin solutions[11]. A 5 mg/mL drug solution in D5W was filled in a three-piece syringe (BD Plastipak™, constructed entirely from PP with a rubber stopper at the inner end of the plunger) and a two-piece syringe (B. Braun Medical Injekt®, composed of PE barrel and PP plunger). The drug solution in the two-piece syringe had a shorter shelf life of 33 days at 25 °C and white floccular precipitates occurred after 8 weeks of storage. Meanwhile, the shelf life of vancomycin in the three-piece syringe was 55 days and no precipitates were detected. Nevertheless, the drug solution stored at 4 °C was chemically stable for at least 84 days in both syringes. Another study revealed that concentrated vancomycin solution (41.66 mg/mL) in D5W in a PP syringe (Terumo®) was stable at ambient temperature for 48 h without protection from light[12]. The stability of 5 mg/mL vancomycin in D5W stored in a glass vial and plastic IV bag has also been investigated[8]. The study showed that the drug solution was stable for 17 days at 24 °C in the glass vial and 63 days at 5 °C and –10 °C in the PVC bag. The results of these studies showed that different types of containers affected the shelf life of vancomycin solutions. Our study revealed that drug solutions in PP syringes and LDPE bags appeared slightly turbid, as determined by the percentage of transmittance on day 70. Although the percentage of vancomycin B remaining, as analyzed by HPLC, was above 90%, the percentage of antibiotic potency of 20- and 50-mL PP syringes decreased to 88.1% and 86.1%, respectively, and their percentage of total impurities and degradation products were higher than 9.0%. All preparations were physically and chemically stable for 55 days, except for that in the 50-mL PP syringe, in which the degradation products and impurities exceeded the USP limit. Increasing the size of the PP syringe from 10 to 50 mL tentatively increased the degradation of vancomycin and decreased the antibiotic potency of the prefilled solutions. These results indicate that the different materials and container volumes may affect the physical and chemical stability of the vancomycin solution. The effects of container type and size may be more pronounced when stored at higher temperatures. In our study, a shorter shelf life of vancomycin solutions was obtained compared to a previous study[11], possibly due to the different sources of vancomycin products, diluents, and containers. Different vancomycin activities of generic products compared with those of innovators have been observed in *in vivo* studies[19]. However, all tested generic products surpassed the USP acceptance criteria for purity and potency[17, 18]. Certain drug products and differences in sources of diluent and container are known to variably affect the stability of extemporaneous preparations.

At the end of the stability study, prefilled containers were further subjected to a sterility test. All preparations passed the sterility test on days 0 and 55 when stored at 5±3 °C. Thus, this study confirmed that the 5 mg/mL vancomycin solutions in D5W prefilled in the 10-, 20-, and 50-mL PP syringes and 100-mL

LDPE bag were microbiologically stable for 55 days when stored at 5±3 °C with light protection.

Based on our results, prefilled 5 mg/mL vancomycin solutions in D5W in 10- and 20-mL PP syringes and 100-mL LDPE bag had a shelf life of 55 days when stored at 5±3 °C and protected from light. Meanwhile, the vancomycin solution in the 50-mL PP syringe could be stored under the same conditions for only 30 days.

4. CONCLUSIONS

This study illustrated the stability of 5±3 °C generic vancomycin solutions in 5% dextrose (D5W) prefilled in 10-, 20-, and 50-mL polypropylene (PP) syringes and a 100-mL low-density polyethylene (LDPE) bag. This study addressed previous knowledge gaps by providing a comprehensive, head-to-head comparison of different container sizes and simultaneously assessing physical appearance, chemical stability (vancomycin B content and total impurities by HPLC, and antibiotic potency by microbial assay), and microbiological stability (sterility) at the end of the shelf life. The key finding was that container size impacted the shelf life: the drug solutions in 10- and 20-mL PP syringes and the 100-mL LDPE bag were physically and chemically stable for 55 days, but the solution in the 50-mL PP syringe was physicochemically stable for only 30 days when stored at 5±3 °C and protected from light. All prefilled syringes and bags were confirmed to be sterile at the end of their respective shelf lives. Therefore, these prefilled vancomycin solutions can be prepared and stored as drug stocks in the pharmacy service unit, and the provided stability information, which allows for flexibility in choosing a suitable dose, will enhance the efficiency of service operations for pharmacists, nurses, and physicians. Nevertheless, the limitation of this study is that only one generic product was used, and consequently, the resulting stability cannot be universally extrapolated to other commercially available vancomycin products. Therefore, future research should involve the stability of vancomycin solutions prepared from multiple generic or brand-name products under identical conditions and the role of container size and material in accelerating vancomycin degradation.

5. ACKNOWLEDGEMENTS

This project was supported by Siriraj Research Development Fund (Managed by Routine to Research: R2R), grant number (IO): R016035038, Faculty of Medicine Siriraj Hospital, Mahidol University.

Author contribution

Conceptualization, J.S., M.S., J.L., M.L. and V.M.; Formal analysis, J.S., J.L. and V.M.; Funding acquisition, J.S. and V.M.; Investigation, J.S., M.S., J.L. and V.M.; Methodology, J.S., M.S., J.L. and V.M.; Validation, J.S., M.S., J.L. and V.M.; Writing – original draft, J.S. and V.M.; Writing – review & editing, J.S., M.S., J.L., M.L. and V.M.. All authors have read and agreed to the published version of the manuscript.

Funding

This project was supported by Siriraj Research Development Fund (Managed by Routine to Research: R2R), grant number (IO): R016035038, Faculty of Medicine Siriraj Hospital, Mahidol University.

Conflict of interest

The authors declare no conflict of interest.

Ethics approval

None to declare.

Article info:

Received September 12, 2025
Received in revised form November 27, 2025
Accepted December 27, 2025

REFERENCES

1. Jacqz-Aigrain E, Leroux S, Zhao W, van den Anker JN, Sharland M. How to use vancomycin optimally in neonates: remaining questions. Expert Rev Clin Pharmacol. 2015;8(5):635-48. doi: 10.1586/17512433.2015.1060124.
2. Dong Y, Glaser K, Speer CP. New threats from an old foe: Methicillin-resistant *Staphylococcus aureus* infections in neonates. Neonatology. 2018;114(2):127-34. doi: 10.1159/000488582.
3. Rybak MJ, Le J, Lodise TP, Levine DP, Bradley JS, Liu C, et al. Therapeutic monitoring of vancomycin for serious methicillin-resistant *Staphylococcus aureus* infections: A revised consensus guideline and review by the American Society of Health-System Pharmacists, the infectious diseases society of America, the pediatric infectious diseases society, and the society of infectious diseases pharmacists. Am J Health Syst Pharm. 2020;77(11):835-64. doi: 10.1093/ajhp/zxaa036.
4. Eiland LS, Benner K, Gumpper KF, Heigham MK, Meyers R, Pham K et al. ASHP-PPAG Guidelines for Providing Pediatric Pharmacy Services in Hospitals and Health Systems. Am J Health Syst Pharm. 2018;75(15):1151-65. doi: 10.2146/ajhp170827.
5. Stucky ER. Prevention of medication errors in the pediatric inpatient setting. Pediatrics. 2003;112(2):431-6. doi: 10.1542/peds.112.2.431.
6. van der Linden P, Douchamps J, Schmitt C, Forget D. Ready-to-use injection preparations versus conventional reconstituted admixtures: Economic evaluation in a real-life setting. Pharmacoeconomics. 2002;20(8):529-36. doi: 10.2165/00019053-200220080-00003.
7. Allen LV Jr, Stiles ML, Prince SJ, Smeeding J. Stability of 14 drugs in the latex reservoir of an elastomeric infusion device. Am J Health Syst Pharm. 1996;53(22):2740-3. doi: 10.1093/ajhp/53.22.2740.
8. Das Gupta V, Stewart KR, Nohria S. Stability of vancomycin hydrochloride in 5% dextrose and 0.9% sodium chloride injections. Am J Hosp Pharm. 1986;43(7):1729-31.

9. Khalfi F, Dine T, Gressier B, Luyckx M, Brunet C, Ballester L, et al. Compatibility and stability of vancomycin hydrochloride with PVC infusion material in various conditions using stability-indicating high-performance liquid chromatographic assay. Int J Pharm. 1996;139(1):243-7. doi: 10.1016/0378-5173(96)04623-6
10. Galanti LM, Hecq J-D, Vanbeckbergen D, Jamart J. Long-term stability of vancomycin hydrochloride in intravenous infusions. J Clin Pharm Ther. 1997;22(5-6):353-6. doi: 10.1111/j.1365-2710.1997.tb00018.x.
11. Wood MJ, Lund R, Beavan M. Stability of vancomycin in plastic syringes measured by high-performance liquid chromatography. J Clin Pharm Ther. 1995;20(6):319-25. doi: 10.1111/j.1365-2710.1995.tb00705.x.
12. Godet M, Simar J, Closset M, Hecq J-D, Braibant M, Soumoy L, et al. Stability of concentrated solution of vancomycin hydrochloride in syringes for intensive care units. Pharm Technol Hosp Pharm. 2018;3(1):23-30. doi:10.1515/pthp-2017-0031
13. Ross C, Syed B, Pak J, Jhanji V, Yamaki J, Sharma A. Stability evaluation of extemporaneously compounded vancomycin ophthalmic drops: Effect of solvents and storage conditions. Pharmaceutics. 2021;13(2). doi: 10.3390/pharmaceutics13020289.
14. Mathew M, Gupta VD. Stability of vancomycin hydrochloride solutions at various pH values as determined by high-performance liquid chromatography. Drug Dev Ind Pharm. 1995;21(2):257-64. doi: 10.3109/03639049509048108
15. Masse M, Genay S, Martin Mena A, Carta N, Lannoy D, Barthélémy C, et al. Evaluation of the stability of vancomycin solutions at concentrations used in clinical services. Eur J Hosp Pharm. 2020;27(e1):e87-e92. doi: 10.1136/ejhpharm-2019-002076 %J European Journal of Hospital Pharmacy
16. Huvelle S, Godet M, Hecq JD, Gillet P, Jamart J, Galanti LM. Long-term stability of vancomycin hydrochloride in glucose 5% polyolefin bags: The brand name versus a generic product. Int J Pharm Compd. 2016;20(5):416-20.
17. Hadwiger ME, Sommers CD, Mans DJ, Patel V, Boyne MT, 2nd. Quality assessment of U.S. marketplace vancomycin for injection products using high-resolution liquid chromatography-mass spectrometry and potency assays. Antimicrob Agents Chemother. 2012;56(6):2824-30. doi: 10.1128/AAC.00164-12.
18. Nambiar S, Madurawe RD, Zuk SM, Khan SR, Ellison CD, Faustino PJ, et al. Product quality of parenteral vancomycin products in the United States. Antimicrob Agents Chemother. 2012;56(6):2819-23. doi: 10.1128/AAC.05344-11.
19. Vesga O, Agudelo M, Salazar BE, Rodriguez CA, Zuluaga AF. Generic vancomycin products fail *in vivo* despite being pharmaceutical equivalents of the innovator. Antimicrob Agents Chemother. 2010;54(8):3271-9. oi: 10.1128/AAC.01044-09.
20. Vancomycin injection. British Pharmacopoeia. Vol II. London, United Kingdom: Medicines and Healthcare products Regulatory Agency; 2018. p. 1226-8.
21. Harris CM, Kopecka H, Harris TM. Vancomycin: structure and transformation to CDP-I. J Am Chem Soc. 1983;105(23):6915-22. doi: 10.1021/ja00361a029
22. Diana J, Visky D, Hoogmartens J, Van Schepdael A, Adams E. Investigation of vancomycin and related substances by liquid chromatography/ion trap mass spectrometry. Rapid Commun Mass Spectrom. 2006;20(4):685-93. doi: 10.1002/rcm.2364
23. Nagarajan R. Antibacterial activities and modes of action of vancomycin and related glycopeptides. Antimicrob Agents Chemother. 1991;35(4):605-9. doi: 10.1128/AAC.35.4.605.
24. The United States Pharmacopeial Convention Inc. The United States Pharmacopeia and the National Formulary (USPNF 2022). Rockville, MD: The United States Pharmacopoeial Convention; 2022.
25. Murray KL, Wright D, Laxton B, Miller KM, Meyers J, Englebright J. Implementation of standardized pediatric i.v. medication concentrations. Am J Health Syst Pharm. 2014;71(17):1500-8. doi: 10.2146/ajhp140024.
26. Brossard D, Chedru-Legros V, Crauste-Manciet S, Fleury-Souverain S, Lagarce F, Odou P, et al. Methodological guidelines for stability studies of hospital pharmaceutical preparations Part 1 : liquid preparations 2013.
27. ICH Topic Q 1 A (R2) Stability Testing of new Drug Substances and Products 2003:[20 p.].
28. ICH Harmonised Tripartite Guideline, Validation of analytical procedures: Text and methodology Q2(R1)2005:[17 p.].
29. Foster BA, Tom D, Hill V. Hypotonic versus isotonic fluids in hospitalized children: A systematic review and meta-analysis. J Pediatr. 2014;165(1):163-9.e2. doi: 10.1016/j.jpeds.2014.01.040
30. Roethlisberger D, Mahler H-C, Altenburger U, Pappenberger A. If euhydric and isotonic do not work, what are acceptable pH and osmolality for parenteral drug dosage forms? J Pharm Sci. 2017;106(2):446-56. doi: 10.1016/j.xphs.2016.09.034
31. White LO, Edwards R, Holt HA, Lovering AM, Finch RG, Reeves DS. The in-vitro degradation at 37 degrees C of vancomycin in serum, CAPD fluid and phosphate-buffered saline. J Antimicrob Chemother. 1988;22(5):739-45. doi: 10.1093/jac/22.5.739