Quality Assessment of U.S. Marketplace Vancomycin for Injection Products Using High-Resolution Liquid Chromatography-Mass Spectrometry and Potency Assays

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In response to a published concern about the potency and quality of generic vancomycin products, the United States Food and Drug Administration investigated a small sampling of the vancomycin products available in North America with regard to purity, content, and potency. To facilitate identification of impurities, a new liquid chromatography method was developed using high-resolution mass spectrometry in addition to diode array detection to characterize impurities in several commercial products. Furthermore, a microbiological assay was utilized to link the analytical profiles with an *in vitro* potency. All products tested met the quality specifications outlined in the United States Pharmacopeia (USP) (vancomycin hydrochloride for injection monograph) for impurities and potency (USP, Vancomycin hydrochloride for injection. United States Pharmacopeia and National Formulary, vol USP 34-NF 29, 2011).

Vancomycin is a glycopeptide antibiotic effective against Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* [13]. Originally developed by Eli Lilly and approved for use in 1958 by the FDA, vancomycin commonly is used as a drug of last resort for infections that have developed resistance to other antibiotics, particularly penicillins. Vancomycin is rarely used as a first-line treatment because it has to be administered intravenously, it is less effective for *Staphylococcus* strains that are not methicillin resistant, and early, less-pure forms exhibited nephrotoxicity and ototoxicity [1, 10, 11]. Even today, there are ongoing concerns regarding nephrotoxicity and its relationship to the administered dose of vancomycin [1, 10, 11].

Vancomycin is produced by fermentation of the actinobacterial species *Amycolatopsis orientalis* [9]. It is a secondary metabolite produced from a seven-module, three-protein nonribosomal peptide synthetase that enzymatically assembles the heptapeptide core structure of vancomycin from 5 nonproteogenic amino acids, β-OH-Tyr (position 2), β-OH-Tyr (position 6), 4-OH-PheGly (position 4), 4-OH-PheGly (position 5), and 3,5-$(OH)_2$-PheGly (position 7) and Leu (position 1) and Asn (position 3) [15]. This core structure undergoes oxidative cross-linking before final enzymatic tailoring adds a methylation to the amino group of Leu and two successive sugars, glucosyl and vancosaminyl moieties, to produce the final bioactive structure [15]. This compound inhibits cell wall synthesis in Gram-positive bacteria, which is its clinical mode of action [9].

When these studies were performed, six manufacturers were marketing vancomycin for injection in the United States. The innovator product from Eli Lilly is no longer manufactured. In a recent article by Vesga et al., the quality and efficacy of generic vancomycin products were assessed and called into question [20]. The authors claimed that two of the generic manufacturers' products were inferior to Eli Lilly's innovator product (which the authors had acquired before its removal from the market). The authors postulated that the differences observed were related to a specific impurity, crystalline degradation product (CDP-1) [8]. CDP-1 results from the spontaneous deamidation of asparagine in

the vancomycin B heptapeptide backbone [8], which is a known process for asparagine-containing peptides [21]. CDP-1 exists as a major and minor atropisomer after degradation. This transformation is accelerated by high temperatures and alkaline pH and results in a change in the solubility of the molecule. More specifically, this impurity has been proposed as a functional vancomycin antagonist and may affect vancomycin potency and efficacy.

In response to this report, impurity and potency studies were initiated by the United States Food and Drug Administration (FDA) to characterize the quality of U.S. marketplace vancomycin products. Many methods for the determination and characterization of vancomycin in various matrices exist in the literature [2, 4, 5, 7, 16, 17]. Significantly, both the United States Pharmacopeia and British Pharmacopoeia publish methods for the determination of vancomycin purity and potency [3, 19]. In order to assess these claims of poor purity and potency, the FDA performed impurity assays according to compendial methods on all six vancomycin products available in the U.S. marketplace, as described in the accompanying paper by Nambiar et al. [14]. However, the exact quantification of CDP-1 was not possible due to its coelution with other impurities in the compendial methods. Moreover, the UV traces in the impurity analysis often contained multiple impurities that were not identified in the USP or BP methods. In order to quantify the amount of CDP-1 present as well as to identify some of the additional impurities, the FDA developed an accurate-mass-based ultrahigh-performance liquid chromatography (UHPLC)-UV mass spectrometric (MS) method to assess the quality of these marketplace products. Finally, the microbiological
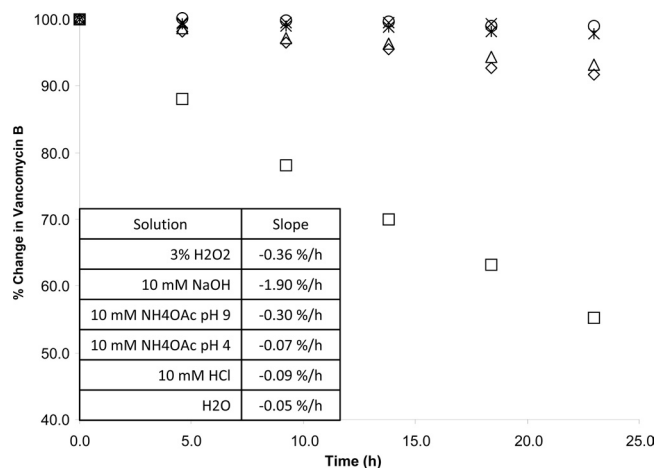
FIG 1 Degradation of vancomycin B in various solvents. ◇, 3% H_2O_2; ×, 10 mM ammonium acetate, pH 4; △, 10 mM ammonium acetate, pH 9; *, 10 mM HCl; ○, H_2O; □, 10 mM NaOH.

Solution	Slope
3% H2O2	-0.36 %/h
10 mM NaOH	-1.90 %/h
10 mM NH4OAc pH 9	-0.30 %/h
10 mM NH4OAc pH 4	-0.07 %/h
10 mM HCl	-0.09 %/h
H2O	-0.05 %/h

activities of the six vancomycin products and CDP-1 alone were assessed to further elucidate the biological significance of the characterized impurity profiles and particularly the effect of CDP-1. Collectively, these data provide a comprehensive look at the quality of the U.S. marketplace products.

MATERIALS AND METHODS

All chemicals used were from Fisher Scientific. Vancomycin for injection was purchased from the American marketplace. Vancomycin B standards were obtained from USP and Sigma-Aldrich. All MS solvents were Optima liquid chromatography (LC)-MS grade or better. CDP-1 was pre-

pared according to the literature (8, 12) and characterized by accurate-mass LC-MS.

LC-MS acquisition and analysis. An ultrahigh-pressure liquid chromatographic (UHPLC) method, based on a modification of the work of Diana et al., was used to evaluate vancomycin products (4). Using a Phenomenex Kinetex C_{18} column (100 by 2.1 mm, 1.7 μm) operating at 40°C, vancomycin B and any impurities were separated using a binary gradient where mobile phase A was 95:5 (vol/vol) H_2O-0.2 M ammonium acetate, pH 9, and mobile phase B was 65:30:5 (vol/vol/vol) H_2O-methanol-0.2 M ammonium acetate, pH 9. The flow rate was 200 μl/min, and the gradient program ramped from 50% B at 0 min to 70% B at 20 min and from 70% B to 100% B in 0.1 min and then was held at 100% B for 10 min, before reequilibrating at 50% B for 7 min. The dwell volume of this system is approximately 900 μl. The inline diode array was scanned from 200 to 400 nm in 2-nm steps, and the 280-nm-wavelength chromatogram was extracted. The injection volume was 5 μl. Eluent was infused directly into an LTQ-Orbitrap XL with electron transfer dissociation (ETD) through the ion max source operating in positive ion mode. Mass spectra (500 to 1,700 m/z) were collected in the Orbitrap with the resolution set to 30,000 at 400 m/z. Spectra were acquired in profile mode. All data analysis was performed using ThermoFisher's Xcalibur software (2.0.7). Quantification was performed using peak areas derived from extracted wavelength chromatograms (λ = 280 nm) or extracted ion chromatograms (EIC) at the m/z of interest (e.g., m/z 724.71 for vancomycin B and m/z 725.21 for CDP-1).

For validation studies, USP vancomycin was used as received. The method was evaluated with regard to specificity, linearity, accuracy, and precision. Vancomycin stock solutions were prepared using filter-sterilized water (Waters' Synergy 185 MilliQ system) at a concentration of 10 mg/ml. Working solutions were prepared by serial dilution of the stock solution. For both assay and validation, CDP-1 stock solution, 0.1 mg/ml, was prepared by dissolving CDP-1 in mobile phase B. Samples prepared for accuracy studies were prepared using the vancomycin and CDP-1 stock solutions. For assay, vancomycin samples were prepared by dissolv-
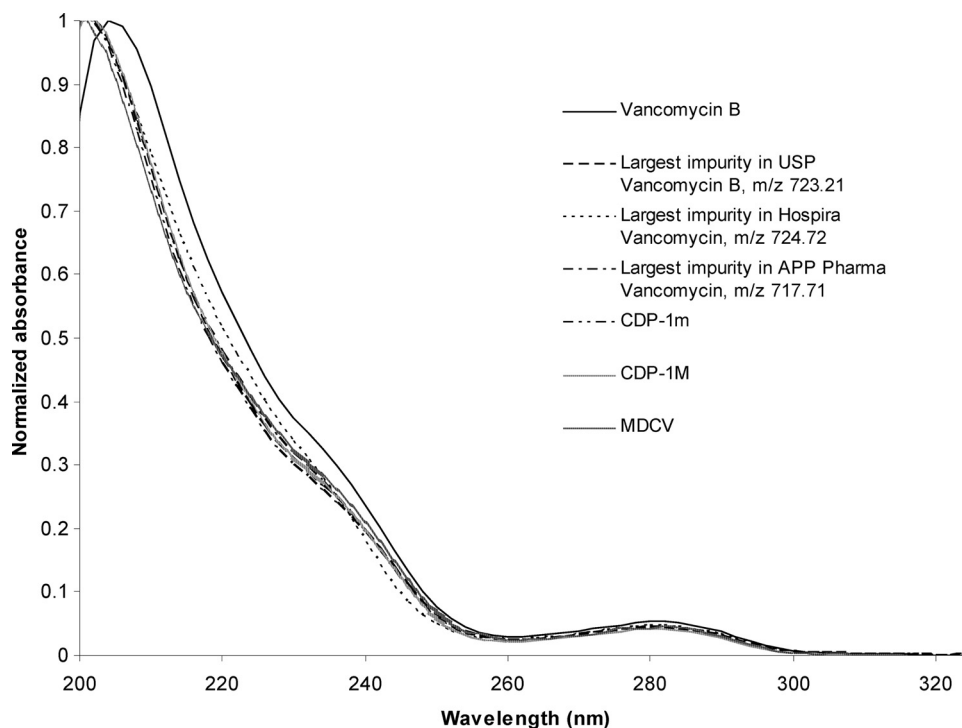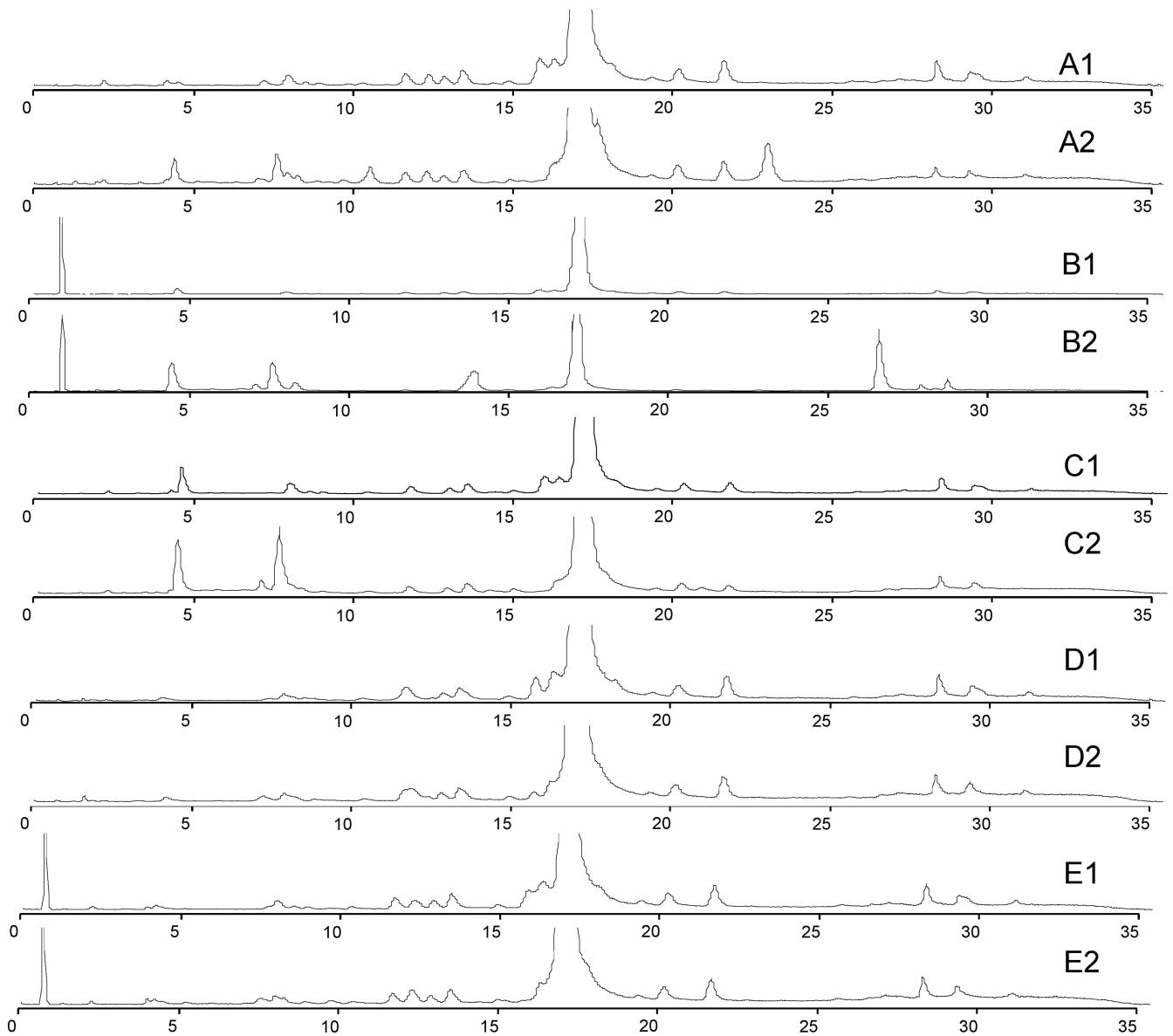

FIG 2 Normalized UV spectra of vancomycin B and some representative major (area % > 1) impurities.

FIG 3 Solution degradation of vancomycin B. (A1 and A2) VM B in 3% H_2O_2 fresh and at 24 h, respectively. (B1 and B2) VM B in 10 mM NaOH fresh and at 24 h, respectively. (C1 and C2) VM B in 0.2 M ammonium acetate (pH 9) fresh and at 24 h, respectively. (D1 and D2) VM B in 0.2 ammonium acetate (pH 4) fresh and at 24 h, respectively. (E1 and E2) VM B in 10 mM HCl fresh and at 24 h, respectively.

ing solid products in water and then diluting them to 1.0 mg/ml with water. One product was supplied as a vancomycin solution for injection (nominal concentration, 5 mg/ml), and for analysis, this solution was diluted with water to a concentration of 1.0 mg/ml. For degradation studies, a working solution was prepared using Sigma-Aldrich vancomycin B (a nonpharmaceutical preparation) at 2 mg/ml in water.

Microbiological assay. The USP method for vancomycin described in "Antibiotics—microbial assays" was used to conduct the cylinder plate potency evaluation of vancomycin HCl products (18). In brief, Difco (Lawrence, KS) antibiotic medium 8 (100 ml) was seeded with 4 ml of a stock spore suspension of *Bacillus subtilis* (ATCC 6633; Manassas, VA) and added to medium 8-coated petri dishes. The amount of spore suspension seeded was determined by trial to give a linear response with a USP vancomycin standard dilution series (data not shown). One-milligram/milliliter stock solutions of USP vancomycin HCl or vancomycin products were prepared in MilliQ H_2O. Final concentrations of 10 μg/ml

(products) and USP standard curve samples were prepared in phosphate buffer, pH 4.5. For CDP-1 spiking experiments, CDP-1 was prepared as a 10-mg/ml stock in dimethyl sulfoxide (DMSO) and then diluted in the 1-mg/ml vancomycin solutions. For studies involving CDP-1 alone, CDP-1 was diluted (1:10,000 to 1:40) from a 10-mg/ml CDP-1 DMSO stock into 1 ml of MilliQ water. DMSO was used as a solvent control for the USP vancomycin standard. The median concentration of USP vancomycin (10 μg/ml; 50 μl) was added to 3/6 cylinders on each plate in alternating positions. The remaining cylinders received either USP standard atropisomer or vancomycin product (10 μg/ml) using 3 plates per point. After a 24-h incubation at 37°C, the zones of inhibition were measured from scanned images of each plate using Image J software (NIH, Bethesda, MD). The average USP vancomycin median concentration (10 μg/ml) in each test or standard group (3 dishes; $n = 9$) and the total assay average USP vancomycin median concentration (30 dishes; $n = 90$) were used to normalize each test or standard concentration. A standard curve

TABLE 1 Identities of vancomycin impurities

Compound	Abbreviation	Formula	Exact mass	$(M + H^+)/z$, +1	$(M + 2H^+)/z$, +2
Aglucovancomycin	AGLUV	$C_{53}H_{52}Cl_2N_8O_{17}$	1,142.28275	1,143.29058	ND[a]
Desvancosaminylvancomycin	DESV	$C_{59}H_{62}Cl_2N_8O_{22}$	1,304.33557	1,305.3434	ND
Deamidated desvancosaminylvancomycin	DESV*	$C_{59}H_{61}Cl_2N_7O_{23}$	1,305.31959	1,306.32741	653.66762
Oxidized desvancosaminylvancomycin	Ox-DESV	$C_{59}H_{62}Cl_2N_8O_{23}$	1,320.33048	1,321.33831	661.17307
Deamidated oxidized desvancosaminylvancomycin	Ox-DESV*	$C_{59}H_{61}Cl_2N_7O_{24}$	1,321.3145	1,322.32233	661.66508
Didechlorovancomycin	DDCV	$C_{66}H_{77}N_9O_{24}$	1,379.50814	1,380.51597	690.7619
Monodechlorovancomycin 1 or 2	MDCV	$C_{66}H_{76}ClN_9O_{24}$	1,413.46917	1,414.477	707.74241
Deamidated monodechlorovancomycin 1 or 2	MDCV*	$C_{66}H_{75}ClN_8O_{25}$	1,414.45319	1,415.46101	708.23442
CDP intermediate	CDPi	$C_{66}H_{72}Cl_2N_8O_{24}$	1,430.40365	1,431.41148	716.20965
Vancomycin B − CH$_3$ + H	VM B − Me	$C_{65}H_{73}Cl_2N_9O_{24}$	1,433.41455	1,434.42237	717.7151
Deamidated vancomycin B − CH$_3$ + H	VM B − Me*	$C_{65}H_{72}Cl_2N_8O_{25}$	1,434.39857	1,435.38258	718.20711
CDP − CH$_3$ +H	CDP − Me	$C_{65}H_{72}Cl_2N_8O_{25}$	1,434.39857	1,435.40639	718.20711
Vancomycin B	VM B	$C_{66}H_{75}Cl_2N_9O_{24}$	1,447.4302	1,448.43803	724.72293
CDPm	CDP	$C_{66}H_{74}Cl_2N_8O_{25}$	1,448.41422	1,449.42205	725.21494
CDPM	CDP	$C_{66}H_{74}Cl_2N_8O_{25}$	1,448.41422	1,449.42205	725.21494
Oxidized vancomycin B − CH$_3$ + H	Ox-VM B − Me	$C_{65}H_{73}Cl_2N_9O_{25}$	1,449.40946	1,450.41729	725.71256
Vancomycin B + CH$_3$ − H	VM B + Me	$C_{67}H_{77}Cl_2N_9O_{24}$	1,461.44585	1,462.45368	731.73075
Vancomycin B + Cl − H	CDP + Me	$C_{67}H_{76}Cl_2N_8O_{25}$	1,462.42987	1,463.4377	732.22276
Oxidized vancomycin B	Ox-VM B	$C_{66}H_{75}Cl_2N_9O_{25}$	1,463.42511	1,464.43294	732.72038
Vancomycin B + Cl − H	VM B + Cl	$C_{66}H_{74}Cl_3N_9O_{24}$	1,481.39123	1,482.39905	741.70344
Unknown 1	Unknown 1			1,481.37827	1,482.38554	741.69641
Unknown 2	Unknown 2			1,465.38337	1,466.39064	733.69896
Unknown 3	Unknown 3			1,473.44275	1,474.45002	737.72865
Unknown 4	Unknown 4			1,467.39691	1,468.40418	734.70573
Unknown 5	Unknown 5			1,579.46797	1,580.47524	790.74126
Unknown 6	Unknown 6			1,431.43297	1,432.44024	716.72376
Unknown 7	Unknown 7			1,445.41189	1,446.41916	723.71322

[a] ND, not detected.

was determined using linear regression analysis in Excel with log USP vancomycin µg/ml on the x axis and corrected diameter (mm) on the y axis. Antibiotic potency was calculated by interpolation from this standard curve and was expressed as a percentage of the reference concentration. Briefly, the expected concentration of a sample is equal to the antilog [(corrected mean diameter − a)/b], where the corrected mean diameter is the corrected mean of a sample's zones of inhibition, a is the intercept of the regression line, and b is the slope of the regression line. The potency is then expressed as the ratio of the expected sample concentration to the reference sample concentration multiplied by 100. Potency for the six American marketplace samples tested neat, spiked with 9% CDP-1, and spiked with 25% CDP-1 was determined. The antimicrobial activity of CDP-1 was also assessed. Significance was determined between neat and CDP-1-spiked vancomycin products using a paired two-tailed t test in Excel (*, $P < 0.05$; **, $P < 0.01$).

RESULTS AND DISCUSSION

The UHPLC-MS method was suitably linear, as a strong correlation between instrument response (peak area) and vancomycin B concentration for both UV ($R \geq 0.999$) and EIC ($R \geq 0.990$) was observed over the range of 0.5 to 1.2 mg/ml, the concentration range in which all products were tested. Accuracy, expressed as the recovery, was 100.1% ± 0.5% and 97.9% ± 3.5% ($n = 18$) with UV and EIC (at m/z 724.72), respectively. Precision, expressed as the % relative standard deviation (RSD) of the average recovery, was 0.5% and 3.5% for UV and EIC, respectively. The specificity of the method was defined as baseline resolution between crystalline degradation product atropisomers (CDP-1m and CDP-1M) and vancomycin B. In cases where method optimization did not allow

TABLE 2 Summary of results for U.S. marketplace vancomycin samples

Parameter	Vancomycin assay result						
	USP	Hospira	Bioniche	Akorn Strides	APP Pharma	Sandoz	Baxter
Vancomycin B assay value (%, wt/wt)[a]	ND[c]	100.8	111.4	109.8	107.0	109.9	94.9
CDP-1 total (%, wt/wt)	ND	0.48	0.20	0.25	0.15	1.21	0.06
Largest impurity	Unknown	VM B isomer	VM B isomer	VM B isomer	VM B − Me	VM B isomer	Unknown
m/z of largest impurity ($z = 2$)	723.71266	724.72104	724.72104	724.72104	717.71280	724.72104	723.71266
Total impurities (area %)[b]	5.43	9.37	4.79	5.15	5.03	4.68	5.53
CDP-1 total (area %)[b]	0.05	0.28	0.19	0.16	0.19	0.40	0.09
Largest impurity (area %)[b]	1.23	1.03	0.97	0.95	1.12	0.95	1.24
Area % of VM B[b]	94.6	90.6	95.2	94.9	95.0	95.3	94.5

[a] When assayed quantitatively against a USP standard.

[b] Normalized area percent.

[c] ND, not determined.

TABLE 3 Impurity distributions of 6 U.S.-marketed vancomycin formulations[a]

Avg UV RT	Avg BPC RT	Identity by MS	m/z, z = 2	UV area %						
				USP	Hospira	Bioniche	Akorn Strides	APP Pharma	Sandoz	Baxter
1.6			ND		0.09		0.02		0.04	
1.9			ND		0.02					
2.4	2.5	Unknown 1	741.69641		0.70		0.02	0.12	0.08	0.13
4.1	4.2	CDP-1m	725.21282	0.02	0.18	0.11	0.09	0.11	0.31	0.03
6.8	7.0	CDP-1M	725.21295	0.03	0.10	0.08	0.07	0.08	0.09	0.06
7.4	7.5	VM B − Me	717.71326	0.03	0.19	0.03	0.09		0.52	0.02
7.6	7.7	CDP	725.21322		0.40	0.12	0.11	0.12	0.14	
	7.8	Unknown 2	733.69896	0.05	0.41	0.08	0.10	0.16	0.07	0.18
8.4	8.5	VM B − Me	717.71315		0.12	0.03	0.02	0.03		0.07
8.8			ND				0.02			
9.2	9.3	VM B − Me	717.71293		0.06			0.24		0.14
9.9	10.1	VM B − Me	717.71250		0.02			0.06		0.03
10.6	10.7	VM B − Me	717.71280	0.38	0.29	0.11	0.12	**1.12**	0.05	**1.07**
11.0	11.1	CDPi	716.20750	0.09	0.29	0.18	0.40	0.20	0.30	
11.7	11.8	MDCV	707.74020	0.06	0.16	0.05	0.07	0.10	0.16	0.05
12.2	12.3	VM B + Me	731.72869	0.18	0.52	0.17	0.09	0.25	0.02	0.38
13.0	13.1	Unknown 3	790.74126					0.08	0.02	
13.6	13.7	VM B	724.72006		0.21	0.18	0.22	0.14	0.18	0.10
14.3	14.4	Unknown 4	734.70573	0.96	0.72	0.18	0.15		0.14	0.07
14.8			ND		0.42			0.33		0.36
15.9	16.0	VM B	724.72133	94.58	90.64	95.19	94.87	94.99	95.33	94.49
18.4	18.5	VM B isomer	724.72084	0.03	0.48	0.03	0.07	0.02	0.18	0.10
18.6			ND							0.09
18.9	19.0	VM B isomer	724.72145	0.19	0.58	0.58	0.65	0.27	0.49	0.19
19.4			ND	0.05						
20.2	20.4	VM B isomer	724.72104	0.63	**1.03**	**0.97**	**0.95**	0.85	**0.95**	0.32
24.3	24.4	VM B + Cl	742.70065	0.18	0.51	0.39	0.25	0.19	0.11	0.13
25.8			ND			0.02	0.03			
26.3			ND	0.04	0.05	0.04	0.04	0.05	0.05	
26.7			ND		0.03	0.02	0.06	0.02	0.02	
26.8	27.0	Unknown 5	737.72865			0.04				
27.1	27.2	VM B + Me	731.72916	0.51	1.00	0.57	0.48	0.09	0.10	0.19
27.2			ND							0.08
27.7	27.9	Unknown 6	716.72376	0.68	0.56	0.22	0.30			0.25
28.3	28.5	Unknown 7	723.71322	**1.23**		0.39	0.46	0.15	0.21	**1.24**
28.8			ND						0.02	
29.5			ND						0.05	0.06
31.0			ND						0.01	0.02
31.4			ND		0.23	0.05	0.06	0.07	0.08	0.08
32.3		AGLUV	ND	0.09		0.15	0.21	0.18	0.23	0.04
34.2			ND						0.06	0.05

[a] Values for the largest impurities found in a product are shown in bold. ND, not determined; RT, retention time; BPC, base peak chromatogram.

for baseline resolution of other vancomycin impurities, the specificity of the method was provided by the high mass resolution of the mass spectrometer used in these studies.

Quantification of CDP-1 was less precise and accurate than that for vancomycin B. CDP-1 recovery was studied over the range of 0.1% to 1.0% (wt/wt) and averaged 128% ± 15% and 96% ± 14% (n = 24) by UV and EIC (at m/z 725.21), respectively. In order to dissolve CDP-1 at a concentration adequate to spike vancomycin B solutions for accuracy studies, mobile phase B, a weakly alkaline solution, was used. While CDP-1 is stable in this solution for at least 5 days at 8°C in 10 mM ammonium acetate, pH 9, vancomycin B is not. CDP-1 is formed from vancomycin B at a rate of approximately 2.7 µg ml^{-1} h^{-1} at 8°C in 10 mM ammonium acetate, pH 9 (Fig. 1). As a result, CDP-1 recovery from spiked vancomycin B solutions was high at low concentra-

tions using UV detection. Because the performance of the method for the quantification of CDP-1 by external standard was less accurate than desirable, the method of normalized area percentages was adopted to estimate impurities. Implicit in any normalized area percent assay is the assumption that any impurity detected provides the same instrument response as the main analyte of interest. To test this assumption, the UV spectra for vancomycin B and each impurity were normalized (to account for differences in concentration) and compared. The results for major impurities (i.e., impurities present at approximately 1%) are displayed in Fig. 2. Vancomycin B impurities produce UV spectra nearly identical to vancomycin B. As a result, vancomycin impurities with the same core aromatic structure were assumed to provide nearly identical instrument responses when analyzed using UV detection.

TABLE 4 Summary of antimicrobial potency results for marketplace vancomycin samples[a]

| | Potency of vancomycin product | | | | | | | | |
| | Neat | | | Spiked with 9% (wt/wt) CDP-1 | | | Spiked with 25% (wt/wt) CDP-1 | | |
Source	Avg	SD	% RSD	Avg[b]	SD	% RSD	Avg[b]	SD	% RSD
Sandoz Incorporated	106	5.9	5.6	102	3.8	3.7	90*	2.9	3.2
Baxter Health Care Corporation	97	7.3	7.6	98	6.4	6.6	83	7.3	8.7
Hospira Inc.	106	9.5	8.9	105	11.9	11.4	89*	1.7	1.9
APP Pharmaceutical LLC	108	2.9	2.7	103	5.7	5.5	96**	1.4	1.5
Bioniche Pharma USA LLC	112	5.6	5	99	7.5	7.6	93**	6.9	7.4
Akorn Strides LLC	109	1.9	1.7	103*	2.6	2.6	92*	5.1	5.6

[a] USP potency criterion, 90 to 115%.
[b] Significant difference between neat and CDP-1-spiked samples (*, $P < 0.05$; **, $P < 0.01$).

For impurity identity tests, a nonpharmaceutical vancomycin sample was forcibly degraded under a variety of conditions and analyzed in order to determine what types of impurities and/or degradation products might be present in pharmaceutical preparations (Fig. 3). Twenty unique masses were attributed by exact mass shifts (<4 ppm) from vancomycin B or CDP-1, and their identities are shown in Table 1. These assignments were confirmed by tandem MS data. Vancomycin and its derivatives or degradation products had a characteristic fragmentation pattern where loss of the vancoaminyl sugar was followed by the loss of the glucosyl sugar, leaving the core structure behind. In all cases, any mass shift was associated with modification of the core structure.

The forced degradation of (nonpharmaceutical) vancomycin revealed that in all cases, once vancomycin goes into aqueous solution, CDP-1 is formed, and monodechlorovancomycin (MDCV) disappears with time. The rate at which CDP-1 is formed is dependent on the solution pH, and formation is fastest in alkaline solutions. Furthermore, in addition to CDP-1, other impurities are formed and some impurities which are initially present disappear. The degradation of vancomycin B appears to be pseudo-first-order with respect to the sample solvent.

The products from the six manufacturers were analyzed with the validated method, and the results are shown in Table 2. All products met USP specifications of not more than 9% of any one impurity and not less than 80% vancomycin B in agreement with the previous analysis by the USP and British Pharmacopoeia methods, as described in the accompanying paper by Nambiar et al. (14). Variability of the impurity profiles was observed between manufacturers, including the crystalline degradation product, CDP-1, which coeluted with another impurity in some products, making precise quantitation difficult by UV. CDP-1 was typically not the most abundant impurity for freshly prepared solutions. In the marketplace samples studied, the largest single impurities were between 0.95% and 1.24% and total impurities were between 4.68% and 9.37%, well within the USP specifications of <9% and <20%, respectively. CDP-1 was determined to be less than 2% (wt/wt) in all cases.

For the U.S. marketplace samples, impurities were identified by exact mass matching to the forced degradation identifications (Table 1). In addition to the five previously described impurities in the compendial methods, monodechlorovancomycin (MDCV), *N*-demethylvancomycin B (VM B − Me), aglucovancomycin (AGLUV), desvancosaminylvancomycin B (DESV), and CDP-1, other impurities with masses corresponding to an addition of a methyl group (VM B + Me), addition of chlorine (VM B + Cl), and the deamidation

succinimide intermediate (CDPi) were also measured (Table 3). Given that the enzymes involved in synthesis of nonribosomal peptides (i.e., vancomycin) do not have perfect fidelity (6) or are oxidative in nature, these compounds appear to be derivatives or degradation products of vancomycin B.

The mean antimicrobial potency values from the vancomycin products ranged from 97 to 112%, meeting the USP acceptance criteria for vancomycin HCl products (90 to 115%) as shown in Table 4. Vancomycin samples spiked with 9% CDP-1 (wt/wt), the maximum impurity limit, had mean potency values ranging from 98% to 105%. All of the products spiked with 9% CDP-1 met the USP acceptance criteria, and only one product had a statistically significant potency difference between the neat sample and the spiked sample. Vancomycin samples spiked with 25% CDP-1 (wt/wt) had mean potency values ranging from 83% to 96%. Two of the 25% CDP-1-spiked products failed to meet the USP acceptance criteria, and five of the products had significantly different average potencies in comparison of the neat samples to the CDP-1-spiked samples. CDP-1 (25 to 250 μg/ml) had a concentration-dependent increase in antimicrobial activity which was limited by solubility (Fig. 4). Nevertheless, CDP-1 at concentrations up to 10
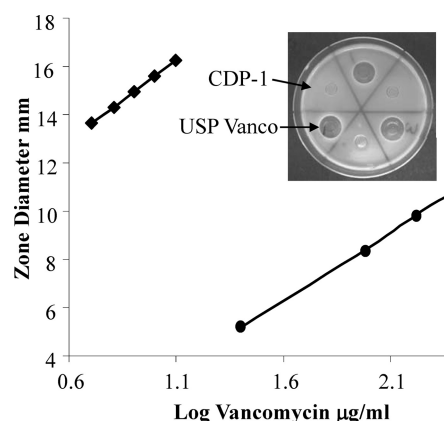

FIG 4 Antimicrobial activity comparison of USP vancomycin HCl (diamonds) and CDP-1 (circles). USP vancomycin (5 to 12.5 μg/ml) and CDP-1 (25 to 250 μg/ml) were compared using a USP cylinder plate zone-of-inhibition assay according to the USP method. After a 24-h incubation at 37°C, the kill zone diameters were measured from scanned images of each plate using Image J software. USP vancomycin and CDP-1 activities were described by the lines $y = 6.77x + 8.83$ ($R^2 = 0.99$) and $y = 5.84x − 3.25$ ($R^2 = 0.99$), respectively. The inset shows a representative plate comparing 10-μg/ml amounts of CDP-1 and USP vancomycin.

μg/ml, the median concentration of USP vancomycin, had no activity (Fig. 4, inset). At the highest concentration tested, 250 μg/ml, CDP-1 had a kill zone diameter corresponding to less than 5 μg/ml USP vancomycin, the lowest point on the standard curve. These data indicate that CDP-1 has inherent antimicrobial activity but is far less potent than vancomycin.

Conclusions. The new UPLC-UV-MS method took advantage of the higher separation efficiency afforded by a column packed with particles less than 2 μm, adopted an eluent composition compatible with mass spectrometric detection, and provided superior selectivity by using a high-mass-resolution (resolving power, >30,000), high-mass-accuracy (3-ppm) Orbitrap mass spectrometer as a detector to provide a quantitative analysis of vancomycin B products as well as to identify the majority of the components in the drug substance.

All of the market samples analyzed contained relatively small amounts of CDP-1, and in all cases, CDP-1 was not the most abundant impurity. Importantly, all the marketplace samples met USP potency criteria even when spiked with the maximum impurity limit of CDP-1 (9%), indicating that CDP-1's role as a functional antagonist is limited. When samples were spiked with 25% CDP-1, there was a decrease in potency compared with the neat samples, suggesting a possibility of weak functional antagonism by CDP-1 at very high concentrations. These data suggest that these products are well within current specifications. The impurities identified are expected from biologically derived products and are well within the limits. Moreover, naturally derived as well as exaggerated levels of CDP-1 have a marginal effect on *in vitro* potency and therefore are unlikely to change the *in vivo* efficacy of the products.

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