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Compatibility and stability of vancomycin hydrochloride with PVC infusion material in various conditions using stability-indicating high-performance liquid chromatographic assay

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Abstract

The stability and compatibility of vancomycin hydrochloride injection in various diluents with polyvinyl chloride (PVC) containers were studied under different conditions of temperature and light. Drug was diluted to 5 mg/ml and 8 mg/ml in injection solutions for 1 and 24 h-simulated infusions. Vancomycin hydrochloride injection was also prepared to 5 mg/ml in PVC bags and stored at 4°C over 7 days with protection from light and at 22°C over 48 h without protection from light. Physical compatibility with PVC and chemical stability in solution of vancomycin were assessed by visual examination and by measuring the concentration of drug in duplicate with stability-indicating high-performance chromatographic assay. There were no visual change, no color change, no visible precipitation and no loss of the drug. When admixed in 0.9% sodium chloride injection or 5% dextrose injection, vancomycin hydrochloride 5 and 8 mg/ml was compatible and stable for 1 and 24 h, respectively, of simulated infusion using PVC bags through PVC administration sets without protection from light. On the other hand, in the same diluents, vancomycin hydrochloride 5 mg/ml was compatible and stable with PVC bags for at least 48 h at 22°C without protection from light and for at least 7 days at 4°C with protection from light.

Keywords: Compatibility; Stability; Vancomycin; PVC bag; Stability-indicating HPLC

Vancomycin is a tricyclic glycopeptide antibiotic discovered in 1956 and used to treat potentially life-threatening infections caused by

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susceptible gram-positive organisms, and particularly, it has become an important agent in the treatment of serious infections caused by methicillin-resistant gram-positive bacteria such as *Staphylococcus aureus* and *Clostridium difficile* induced colitis (Cheung and DiPiro, 1986). Since vancomycin is a relatively conformationally rigid glycopeptide (Harris et al., 1983), several factors (pH, temperature, solution...) can affect its chemical stability by deamidation of drug in aqueous solutions and consequently, compromise its clinical efficiency by reduction of its potential antimicrobial activity (Antipas et al., 1994).

Vancomycin is available as the injectable hydrochloride salt, and to obtain a maximum antibacterial activity, it is used commonly by administration in a 1 g slow intravenous infusion for 30 min or 24 h. For infusion, the clinical formulation is diluted with either 0.9% sodium chloride injection or 5% dextrose injection present in of glass or plastic containers. Polyvinyl chloride (PVC) bags of infusion solutions offer several advantages over conventional glass containers, such as easier storage and shipping because of their relative resistance to breakage. However, several problems are reported with their use such as the loss of substantial amounts of drug from the solution by adsorption onto the plastic bags (Kowaluk et al., 1981; D'Arcy, 1983), and the leaching of potentially harmful substances into the solution, particularly a plasticizer, di-ethylhexyl phthalate (DEHP), that is incorporated into PVC to make the bags soft and pliable (Pearson and Trissel, 1993; Faouzi et al., 1995).

The purpose of this study was to determine, in various conditions (temperature, concentrations, light, infusion solutions), the chemical stability and the compatibility of vancomycin hydrochloride 5 mg/ml with PVC bags when admixed in 0.9% sodium chloride injection and 5% dextrose injection at 22°C for up to 48 h without protection from light and at 4°C for up to 7 days with protection from light. In the same diluents, stability and compatibility of vancomycin hydrochloride (5 and 8 mg/ml) were so studied during 1 and 24 h-simulated infusions. Finally, at the same time, the extent of DEHP leaching was also determined during simulated infusion and storage.

To conduct the stability and compatibility studies, a prerequisite is to develop a stability-indicating high-performance liquid chromatographic (HPLC) method of vancomycin at various pH conditions, light conditions and temperature conditions (Trissel, 1983; Hagan, 1994). In the literature, many HPLC procedures are described to analyse vancomycin (Antipas et al., 1994; Trissel et al., 1995). The paper herein describes a simple assay using a normal-phase octyl column for stability-indicating analysis.

Vancomycin hydrochloride used was the current clinical formulation Vancomycin Qualimed* intravenous injection and was generously donated by Qualimed Laboratories (Puteaux, France) in vials of 500 mg sterile powder for injection. For simulated infusions, a volumetric infusion pump (ref. P3000) and PVC infusion sets (ref. S05, 72201) were used and obtained from Becton Dickinson Laboratories, Division Vial Medical (Saint-Etienne de Saint-Geoirs, France). Infusion bags of polyvinyl chloride (PVC, Macoflex®) were kindly provided by Macopharma Laboratories (Tourcoing, France). HPLC analyses were performed using a Hewlett-Packard 1090 M HPLC system and a Hewlett-Packard 79994 linear photodiode array U.V. detector. The method of HPLC analyses of vancomycin is described in Fig. 1. Analyses of DEHP were performed as described previously by Faouzi et al. (1995).

The HPLC method was validated as stability-indicating by accelerating the decomposition of vancomycin. Stock aqueous solutions of vancomycin and ceftazidime were prepared at 500 and 100 μg/ml, respectively, and suitable dilutions were made to prepare the standard solutions of desired concentrations. The stability-indicating assay for vancomycin was established by boiling and by adding concentrated hydrochloric acid, 1 N sodium hydroxide solution and 1% hydrogen peroxide to samples of vancomycin in injection solutions. In addition, the purity and homogeneity of the vancomycin peak in samples was confirmed by quantitating the drug at three wavelengths (230, 250 and 280 nm) using the corresponding calibration curves. During stability-indicating assay and specificity assay, vancomycin hydrochloride was chemically stable in

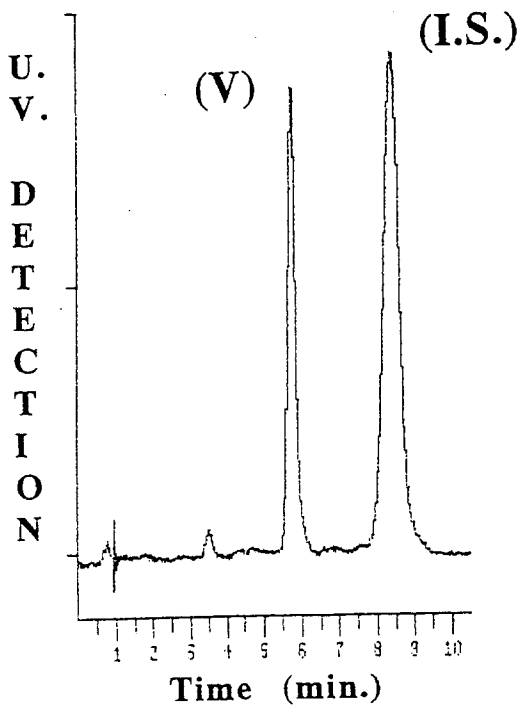

Fig. 1. HPLC analysis of vancomycin (V) and ceftazidime internal standard (IS). Operating conditions: 5 μm C18 Hypersil ODS column (150 × 4.6 mm i.d), mobile phase consisting of acetonitrile and aqueous buffer mixture (8:92, v:v). The buffer was prepared in water with 0.2% triethylamine and adjusted to pH 3 with phosphoric acid. Flow-rate, 2 ml/min, U.V. detection at 280 nm, injected volume, 10 μl.

acidic or alkaline conditions. These results were in discordance with those described by Trissel et al. (1995). They observed a reduction in the peak for intact vancomycin and the formation of new peaks after adding 1 N sodium hydroxide solution or hydrochloric acid followed by boiling for 2 h. This problem was not observed in our study, because our dilutions were not boiled for 2 h. Monitoring the solution at 280 nm yielded the best relative standard deviation and was thus selected for future studies. The three-points calibration curve of vancomycin was constructed between 50 and 100 μg/ml. The precision of the vancomycin assay was determined by using five series of five measurements at three theoretical concentrations. Table 1 summarizes the validation data of the assay procedure for the vancomycin. The results demonstrate that this analytical method had acceptable accuracy and precision in every case.

Vancomycin was resolved with a baseline separation from the internal standard under the developed conditions. Typical chromatogram of vancomycin (100 μg/ml) with internal standard ceftazidime (100 μg/ml) in solution obtained and immediately after mixing is illustrated in Fig. 1. The retention times of vancomycin and ceftazidime were 5.75 and 8.39 min, respectively. As shown by Antipas et al. (1994), the degradation of vancomycin, particularly the deamidation, was pH dependent at a high temperature. However, when dilutions were prepared at room temperature, no degradation of vancomycin was observed. So, neither ceftazidime nor the decomposition products interfered with the peak for intact vancomycin. As observed in Fig. 1, a little unidentified peak was resolved at 3.48 min. In fact, commercial vancomycin is purified by gradient elution chromatography to obtain a purity coefficient around 92%. A commercial sample of vancomycin contains approximately 8% of several

Table 1
Validation data of vancomycin HPLC assay procedure ($n = 5$)

Theoretical concentrations (μg/ml)	Average concentrations found (μg/ml) ± S.D.	CV interassay (%)	Accuracy (%)	Linear regression equation ($y = ax + b$)	Correlation coefficient
50	50.36 ± 1.42	2.84	100.72	$y = 0.015x + 0.024$	0.999
75	74.56 ± 0.72	0.94	99.41		
100	100.19 ± 0.45	0.44	100.19		

SD, standard deviation.
CV, coefficient of variation.

constituents, particularly a major component, a crystalline degradation product identified as CDP-I and formed by deamidation, consisting of a hydrolysis of the side chain amide linkage of the asparagine residue to form a free carboxylic acid (Antipas et al., 1994). In our study, the peak at 3.48 min was likely, therefore, CDP-I, but did not interfere with the peak for intact vancomycin.

Finally, an interesting previous study (Cairns and Robertson, 1987) reported that subsequent addition of vancomycin to ceftazidime (used in our study as internal standard) in the same giving sets and intravenous lines produced an instantaneous precipitate, but it was not known if either agent or both agents were precipitated. The interaction was likely to be acid-base interaction with the formation of insoluble salt or salts in an unfavourable pH environment. In our study, we have not observed precipitate when vancomycin was diluted with ceftazidime because dilutions were prepared in 5% dextrose injection or 0.9% sodium chloride injection. This procedure prevented precipitation occurring because dilutions had a pH between 4.5 and 5.5. Vancomycin solutions are acid and must remain so if precipitation is to be prevented.

Infusions of vancomycin were carried out under laboratory conditions simulating those routinely used in clinical practice to the hospital. The vancomycin injection was added to 100 ml (500 mg) or 250 ml (2 g) PVC bags to yield the initial nominal concentrations of 5 and 8 mg/ml, respectively. The bags were then attached to an administration set connected to the infusion pump that allowed the solution to flow through at a constant rate. At specified times of infusion, 1 ml of solution was withdrawn at time zero and at regular intervals from the PVC bags, and at the same time, an aliquot of effluent (1 ml) was collected from the administration set. Then, samples were kept frozen in polypropylene tubes at $-20°C$ until analysis by HPLC to assay for vancomycin and DEHP concentrations. After defrosting, one portion of sample was immediately diluted in the mobile phase and analyzed for vancomycin concentration. A second portion was diluted in acetonitrile and water and analyzed for DEHP.

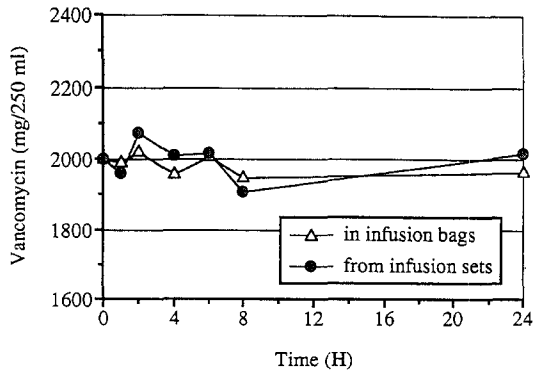

Fig. 2. Concentration kinetics of vancomycin injection during 24 h simulated infusions using PVC bags and sets ($n = 4$).

At time zero, the initial concentration of vancomycin was designated as 5 mg/ml for 1 h infusion and 8 mg/ml for 24 h infusion. All subsequently measured concentrations were expressed with respect to the initial one. Stability was defined as a concentration 90–105% of the initial one, in accordance with the Health Registration of France. Instability of drug and incompatibility with PVC were defined as a decrease of greater than 10% from the initial drug concentration.

As shown in Fig. 2, when vancomycin solutions were infused through PVC sets from PVC bags for 24 h, the variation in drug concentration in both PVC bags and effluent in no case exceeded 10%. Similar results were obtained with 1-h simulated infusions. This demonstrates that the drug was not sorbed by the plastic infusion bags and sets during infusion at ambient temperature. No additional peak corresponding to degradation products was observed on chromatograms. No significant difference was observed between 5% dextrose injection and 0.9% sodium chloride injection. So, vancomycin formulation was chemically stable for up 1 and 24 h simulated infusions. A drug is considered incompatible with the PVC containers if DEHP was detected in the drug solutions. No DEHP (< 1 μg/ml) was detected in vancomycin injections used in 1 and 24 h simulated infusions.

Vancomycin hydrochloride was reconstituted in sterile water and diluted in duplicate in 100 ml of

Table 2
Vancomycin concentrations after storage in PVC bags

Room temperature, without protection from light, concentrations (mg/100 ml)			Refrigerated temperature (4°C), with protection from light, concentrations (mg/100 ml)		
Time (h)	5% dextrose	0.9% NaCl	Time (days)	5% dextrose	0.9% NaCl
Initial	500	500	Initial	500	500
1	479	482	1	514	477
4	482	494	2	496	494
8	471	505	4	505	497
24	491	495	5	474	507
30	473	481	6	500	519
48	511	498	7	498	485

0.9% sodium chloride injection or 5% dextrose injection in PVC bags to produce a nominal vancomycin concentration of 5 mg/ml. Two of each type of container were stored at 22°C for 48 h without protection from light and two at 4°C for 7 days sheltered from light. One milliliter was removed from each bag at time zero and at regular intervals. After agitation at each time point, the samples were placed in clear glass test tubes and were visually inspected for color and clarity.

For at least 48 h of storage at 22°C without protection from light and for at least 7 days at 4°C sheltered from light, there was no substantial difference between vancomycin concentrations at time zero and at any subsequent time points (Table 2). No color variation was observed during storage, as well as no precipitation. No additional peak corresponding to degradation products was observed on chromatograms. No significant difference was observed between 5% dextrose injection and 0.9% sodium chloride injection. Finally, no DEHP (< 1 μg/ml) was detected in vancomycin solutions. So, we can conclude to a satisfactory stability and compatibility of 5 mg/ml vancomycin solutions for 48 h at room temperature and 7 days at 4°C, when reconstituted in PVC bags.

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