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Original Article

A simple and validated HPLC method for vancomycin assay in plasma samples: the necessity of TDM center development in Southern Iran

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Abstract

Background and purpose: Vancomycin is a glycopeptide antibiotic which is the drug of choice against methicillin-resistant *Staphylococcus aureus*. It has a narrow therapeutic index, and thus therapeutic drug monitoring (TDM), and clinical pharmacokinetic assessment are necessary in order to prevent adverse drug reactions such as nephrotoxicity. In this study, we aimed to develop a simple and validated HPLC method for vancomycin assay in order to establish a TDM center for patients admitted to the ICU of Nemazee Hospital in southern Iran.

Experimental approach: In this study, a brief review of different parameters and variables which could affect the sensitivity, selectivity of the validated HPLC method for vancomycin determination were considered. According to the previous studies a simple, fast, and the relatively low-cost method was established for vancomycin determination in plasma samples.

Findings/Results: The developed HPLC assay indicated a calibration curve with R-square of > 0.999, acceptable selectivity, the accuracy of 90-105%, CV% of less than 15%, the limit of quantification of 1 µg/mL, and limit of detection of 300 ng/mL. Vancomycin trough level, the area under the curve, renal clearance, the volume of distribution,, and elimination constant were measured in patients using this validated method.

Conclusion and implications: Validated method for assay of vancomycin plasma levels was used to quantify vancomycin levels of four patients who were admitted to the ICU of Nemazee Hospital. According to the results, two of these patients showed lower levels than recommended therapeutic purposes while one of them showed a toxic level. According to the results, the TDM assessment of vancomycin is strongly recommended for patients who are hospitalized in ICU.

Keywords: Acute kidney injury; AUC; HPLC; ICU; TDM; Vancomycin.

INTRODUCTION

Vancomycin is a large glycopeptide antibiotic with a molecular weight of 1450 Da. The mechanism of action is binding to the D-Ala-D-Ala dipeptide of peptidoglycan and blocking the biosynthesis of the cell wall (1). Vancomycin is the antibiotic of choice for targeted and empirical therapy of many Gram-positive microorganisms especially methicillin-resistant *Staphylococcus aureus*. It could be used in many infectious diseases such as septicemia, osteomyelitis, endocarditis, pneumonitis, meningitis, etc. (2,3). Vancomycin has very low oral absorption. Therefore, the most common route of administration is slow intravenous infusion.

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The main elimination pathway of this drug is renal and approximately 85% of the drug could be detected unchanged in urine after 24 h of a single dose administration (4). Vancomycin has a very narrow therapeutic index which is responsible for any adverse drug reactions related to vancomycin administration. The most important adverse effect is vancomycin-associated nephrotoxicity. A serum trough level of 15-20 µg/mL is recommended for vancomycin in various complicated infectious diseases (2) and in this regard, therapeutic drug monitoring (TDM) of vancomycin was suggested by several authors. The monitoring of peak and trough levels of vancomycin after steady state has been proposed in previous studies. Nevertheless according to recent studies monitoring the daily area under the curve (24 h-AUC) was suggested as a more useful pharmacokinetic parameter (5). Although in the majority of patients trough level of 15 to 20 µg/mL results in AUC/minimum inhibitory concentration (MIC) ratio of ≥ 400, but in many cases, this target value could be achieved with a trough level of less than 15 µg/mL. So, recent studies are focused on AUC/MIC ratio calculation for vancomycin TDM in order to maintain a trough level lower than 15 µg/mL to avoid vancomycin-associated nephrotoxicity which is more common with a trough level of higher than 15 µg/mL (6). Another study reported that 24 h-AUC and peak level (C_{max}) are more relevant to vancomycin-associated nephron-toxicity in comparison with the trough level. Although 24 h-AUC of 400-600 µg.h/mL could provide enough efficacy, the exact 24 h-AUC range accompanied by vancomycin-associated nephrotoxicity has not been established yet but a limit of 700 µg.h/mL was rationalized in order to avoid nephrotoxicity (7). In this study a modified and validated high-performance liquid chromatography (HPLC) method was developed in order to assess serum trough, intermediate, and peak levels of vancomycin to calculate 24h-AUC of vancomycin for TDM purposes. Although immunoassay methods because of the simplicity and fast evaluation process have been used frequently in many previous studies these methods due to the lack of enough precision and sensitivity and also due to the cross-reaction with vancomycin metabolites has its own limitations. In spite of the fact that HPLC is the most selective and sensitive method for vancomycin analysis in serum samples especially at lower concentrations (8,9), but analysis of vancomycin in biological matrices using HPLC method has been encountered many challenges that should be overcome.

Many methods have precipitated plasma proteins prior to the extraction of drugs from plasma by an organic solvent. Protein precipitation has been accomplished by using perchloric acid followed by extraction with ethyl acetate (8,10). Organic solvents such as isopropyl alcohol, acetonitrile (11), and methanol (12) have also been used for plasma protein precipitation.

Due to the effects of unexpected variables on the extraction process during the various steps of drug extraction and reducing the variations in different samples between each run, recruitment of an efficient IS in plasma samples is necessary. By the inclusion of IS, the ratio of drug to IS would be used in calculations and so the variation in extraction would be diminished to the minimum levels. The selected IS should have similar λ_{max} to vancomycin in order to obtain the possibility of simultaneous analysis. Zidovudine (9), caffeine (11), cefuroxime (13), erythromycin (14), para amino benzoic acid (15), phenacetin, and norvancomycin (16) are the examples of IS which have been considered for vancomycin assay in plasma samples.

The other parameter which could highly affect the precision and efficiency of the HPLC method for vancomycin assay is the instrument condition such as the type of HPLC column, column temperature, column length, and internal diameter, lambda max (λ_{max}) and wavelength, mobile phase, flow rate, etc. (8,9,12,17). In this study, we worked on different variables to optimize the influencing parameters to propose a simple, low-cost, and validated HPLC method for the determination of vancomycin in blood samples. Different variables including mobile phase, flow rate, wavelength, and pH were tested to achieve the optimum conditions which could precisely detect vancomycin peak among endogenous plasma peaks, IS, and other drugs in poly-pharmacy patients.

MATERIALS AND METHODS

Materials

HPLC grade acetonitrile was from Merck (Germany) and purchased from a domestic supplier. Ortho-phosphoric acid, sodium hydroxide, and perchloric acid 70% were from Merck, Germany. Pure powder of acetaminophen and vancomycin were purchased from Dena Pharmaceutical Company, Tabriz, Iran. Meropenem, ampicillin sulbactam, piperacillin tazobactam, ceftriaxone, and levofloxacin vials and amikacin ampule were prepared from the market.

Standard sample preparation

Standard stock samples were prepared in water. At first, a stock solution of 1 mg/mL was prepared and then samples with concentrations of 120, 60, 40, 20, 8, and 4 µg/mL were prepared and used to prepare plasma samples with vancomycin at 30, 15, 10, 5, 2, and 1 µg/mL. IS (acetaminophen) stock solution of 100 µg/mL was prepared by dissolving 10 mg IS in purified water, then working IS concentration was daily prepared from the stock solution at 25 µg/mL.

Drug extraction from plasma

In this regard, 0.25 mL of vancomycin standard solutions and 0.25 mL of IS solution was mixed with 0.5 mL of plasma (obtained from blood transfusion center, Shiraz, Iran.). Then 50 µL of perchloric acid 70% was added to precipitate plasma proteins. The mixture was shaken for 1 min and after that centrifuged at 12000 rpm for 15 min. The supernatant was analyzed by HPLC.

HPLC condition

Samples were analyzed by HPLC Azura (Knauer, Germany). The column was C18 (250 mm length × 4.6 mm I.D.; 5 µm pore size; Knauer, Germany). The flow rate was 0.72 mL/min and λ_{max} was adjusted on 225 nm. The mobile phase was phosphate buffer (30 mM, pH of 2.2) and acetonitrile (86:14 %v/v). The column temperature was set at 25 °C.

HPLC validation (18)

Linearity

In order to evaluate the linearity of the calibration curve with this HPLC method, samples with different concentrations (ranges between 1 to 30 µg/mL) of vancomycin and equal concentrations of IS (acetaminophen 25 µg/mL) were analyzed by HPLC. Then vancomycin concentration was plotted versus the ratio of vancomycin peak area to the acetaminophen peak area. Linearity was reported in terms of R square and adjusted R square. A regression test was done and significance F value and *P*-value of X-variable (slope) were calculated.

Selectivity

In order to evaluate the selectivity of this HPLC method for vancomycin detection, different samples with different concentrations of vancomycin and equal concentration of IS were injected and retention times of vancomycin and acetaminophen were reported.

Sensitivity

The sensitivity of this HPLC method for detection of vancomycin was assessed in terms of limit of quantification (LOQ) and limit of detection (LOD), while LOQ is based on precision and accuracy at the lowest calibration point and LOD is based on signal to noise ratio of ≥3. LOD refers to the smallest concentration or amount of analyte that could be detected and LOQ refers to the smallest concentration or amount of analyte that could be quantitatively analyzed with acceptable reliability and precision (19).

Range

This method has been validated in order to assess the vancomycin trough level for TDM purposes. Also, it could be used to assess peak level and intermediate level of vancomycin in order to calculate the area under the curve (AUC). For these purposes, a calibration curve with a vancomycin concentration range of 1 to 30 µg/mL was considered.

Precision

In order to evaluate the precision of this method, samples with a concentration range of 1 to 30 µg/mL were assessed in triplicate in one day to calculate within-day precision, and then samples were analyzed in three different days to assess between days precision (everyday

samples were prepared freshly). Within and between-day precision have been reported by the coefficient of variations (%CV). The lower the %CV values, the higher the precision of the method.

Accuracy

In order to evaluate the accuracy of this method, each sample was assessed in triplicate in one day to calculate within day accuracy, and then these samples were analyzed in three days to assess between days' accuracy. Within and between day accuracy have been reported by percent according to the following equation:

$$Accuracy = \left(\frac{Observed\ value}{True\ value}\right) \times 100 \quad (1)$$

System suitability tests

Number of theoretical plates

The number of theoretical plate (N) was calculated by the following equation:

$$N = 5.54 \left(\frac{T_R}{W_{\frac{h}{2}}}\right)^2 \quad (2)$$

while T_R is retention time and $W_{\frac{h}{2}}$ is the peak width at half of the peak height.

Tailing factor or peak symmetry

Peak symmetry (PS) was calculated by the following equation:

$$PS = \frac{W}{2f} \quad (3)$$

where, W is peak width at 0.05 of the peak height and f is front half peak width at 0.05 of the peak height.

Retention factor

Retention factor (K´) was calculated by the following equation:

$$K' = \left(\frac{T_R}{T_a}\right) - 1 \quad (4)$$

where, T_R is drug retention time and T_a is solvent retention time.

Potential interaction of vancomycin with other common drugs administered in ICU

In order to evaluate the potential chromatographic interaction of vancomycin and IS peaks with other common antibiotics which are administered simultaneously in ICU, seven drugs containing meropenem, ceftriaxone, ampicillin sulbactam, piperacillin tazobactam, levofloxacin, colistin, and amikacin were selected. In this regards, 50 µL of each of these drugs, 50 µL of vancomycin, and 50 µL of IS (acetaminophen) were mixed and added to 500 µL of plasma (obtained from blood transfusion center, Shiraz, Iran) in order to obtain therapeutic plasma concentrations. Then drug extraction was performed according to the aforementioned procedure. The supernatant of this drug cocktail was analyzed with the developed HPLC method and results were compared with a blank plasma (without any drug) and a plasma sample containing vancomycin and IS. Also, the results were compared with a plasma sample which was spiked with these seven drugs without vancomycin and IS in order to clarify all possible chromatographic interactions.

Patient blood sample analysis

After the HPLC validation process, four patients, who received vancomycin, were selected randomly from ICU of Nemazee Hospital, Shiraz, Iran (Ethics code No. 97-01-36-19208) in order to assess the performance of the developed HPLC method. A blood sample (30 min before the next dose; trough level) was collected from the first patient (a 25-year old woman, known case of CVA) who was admitted to ICU of Nemazee hospital. Her medical antibiotic regimen was colistin 3000000 IU Q8h, meropenem 2 g Q8 h, and vancomycin 1 g Q12 h through IV route of administration since 5 days before blood sampling. The blood sample was freshly centrifuged at 2500 rpm for 15 min in order to separate plasma from whole blood. Then 0.5 mL of plasma sample was mixed with 0.5 mL of acetaminophen solution (IS; concentration of 25 µg/mL). Then 50 µL of perchloric acid was added to precipitate plasma proteins. The sample was mixed for 1 min and then centrifuged at 12000 rpm for 10 min. Finally, one-half of the supernatant was directly analyzed with the developed HPLC method and the remaining supernatant was mixed with an equal volume of vancomycin solution (concentration of 50 µg/mL) in order to confirm the vancomycin peak area by spiked peak. When needed, samples were diluted prior to analysis in order to maintain the concentration range within the calibration curve concentration ranges.

The second patient (a 71-year old man, known case of status epilepticus) who was admitted to ICU of Nemazee hospital and received vancomycin for 10 days. Blood samples were collected at 1 h (peak level; just after the end of 1 h-infusion), 4 h (intermediate level), and 12 h (trough level, before the next dose administration). Patients' serum creatinine was checked at baseline and every day as routine lab data. His antibiotic regimen was vancomycin 1 g Q12 h, meropenem 1g Q8 h, ciprofloxacin 400 mg Q12 h, and ampicillin sulbactam 9 g Q8 h through IV route of administration. According to these three blood samples 12 h- and 24 h-AUC of vancomycin were calculated by the trapezoidal method. Other pharmacokinetic parameters such as clearance and elimination rate constant were also calculated from AUC according to the following equations:

$$Cl_s = \frac{X_0}{AUC} \quad (5)$$

while CLs is the systemic clearance (in L/h) which is approximately equal to renal clearance for vancomycin, X_0 is the administered dose (in mg), and AUC is 12 h-AUC (in mg.h/L).

$$K_e = \frac{Cl_r}{V_d} \quad (6)$$

where, Ke is renal excretion constant (h^{-1}) which is approximately equal to elimination constant (k) for vancomycin due to the approximately complete renal excretion of this drug, CL_r is renal clearance (in L/h), and V_d is the volume of distribution which was varied between 0.5-0.9 L/kg according to the previous literature. So, because of high inter-individual variability in vancomycin pharmacokinetics, in this study, V_d was calculated using equation 7 (20):

$$V_d = \frac{Dose}{Peak\ level - Trough\ level} \quad (7)$$

where, V_d is the volume of distribution (L), the dose is the amount of administered drug in each interval (mg), peak level, and trough level are vancomycin concentrations (mg/L) 1 h after and just before the next dose administration, respectively.

Since vancomycin half-life ($t_{1/2}$) in patients with normal renal function is about 6 h, so Ke in normal population has been assumed 0.116 h^{-1} according to Equation 8 (if a one-compartment open model is assumed).

$$K_e = \frac{0.693}{t_{1/2}} = \frac{0.693}{6} = 0.116\ h^{-1} \quad (8)$$

The third patient (a 45-year old man, known case of epilepsy) was admitted to the ICU of Nemazee Hospital and received vancomycin 3 days before blood sampling. His antibiotic regimen was vancomycin 500 mg Q24 h, azithromycin 500 mg QOD, ceftriaxone 2 g Q12 h. His baseline serum creatinine (before vancomycin administration) was 5.1 mg/dL and vancomycin dose adjustment was suggested for him according to glomerular filtration rate (GFR) value (500 mg Q24 h). Blood samples were collected at 1, 4, and 12 h after vancomycin infusion in order to assess peak, intermediate, and trough levels, respectively. AUC, V_d, K_e, and CL_r were also assessed for this patient.

The fourth patient (a 45-year old man, known case of epilepsy) who was admitted to the ICU of Nemazee Hospital received vancomycin from 2 weeks before blood sampling. His antibiotic regimen was vancomycin 1 g Q12 h and ceftriaxone 1 g Q12 h. Blood samples were collected at 1, 4, and 12 h after vancomycin infusion, and pharmacokinetic parameters were calculated and assessed.

RESULTS

HPLC validation

Calibration curve and linearity

In this study, the HPLC method has been validated and modified in order to analyze plasma samples of patients who received vancomycin in the hospital. Six concentrations of standard solutions were injected into HPLC (n = 5) and results were plotted as vancomycin concentration versus area under the peak ratio of vancomycin to IS and passed the acceptance validation criteria of calibration curve from USP guidelines which is ±15% of nominal concentrations (18). In this plot, as depicted in Fig. 1, R square was 0.9996 and linear equation was y = 0.0148 X + 0.0139. The retention time of vancomycin and acetaminophen were 5.5 and 8 min, respectively. Also, a regression test was done on these data. F-values and *P*-values were lower than 0.05.

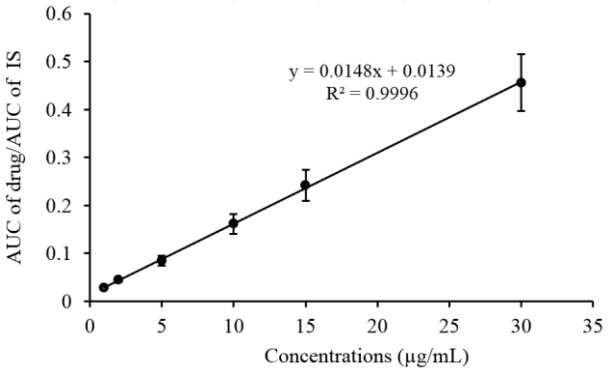

Fig. 1. Vancomycin calibration curve in biological samples.

Selectivity

Samples with different concentrations were analyzed. In all samples, vancomycin retention time was 5.5 min and IS (acetaminophen) retention time was 8 min and no interaction with plasma endogenous peaks (Fig. 2) were observed.

Sensitivity

LOD was 0.3 µg /mL and LOQ was 1 µg/mL. The analyte response at LOQ was ≥ 5-times the analyte response of the blank plasma (zero calibrator) (20).

Range

Vancomycin standard solution in different concentrations ranging between 1 and 30 µg/mL were injected into the chromatograph. According to the results, a linear correlation between the vancomycin concentrations and peak area ratios were established in this range.

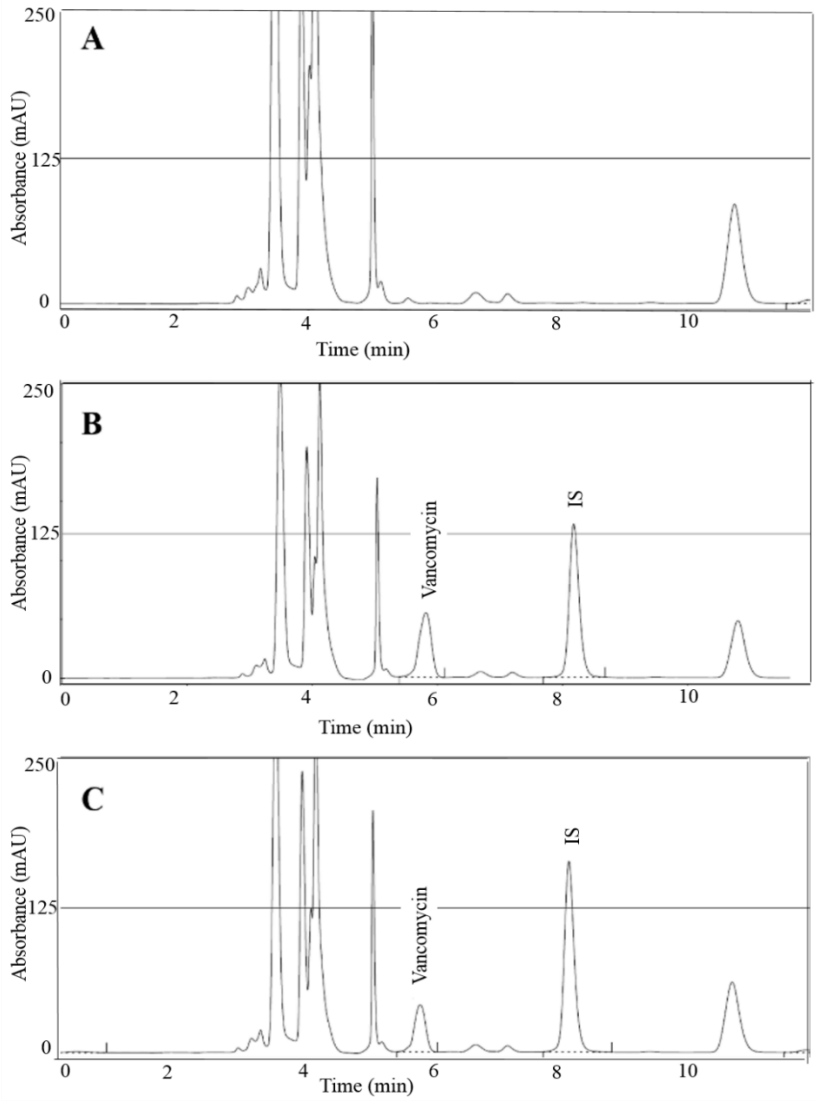

Fig. 2. Representative chromatograms of (A) blank plasma, (B) vancomycin (30 µg/mL) and IS (acetaminophen; 25 µg/mL), and (C) vancomycin (15 µg/mL) and IS (acetaminophen; 25 µg/mL). IS, internal standard.

Table 1. Within- and between-day precision and accuracy of vancomycin HPLC assay (n = 3).

Concentrations (µg/mL)	Precision		Accuracy	
	Within day (%)	Between day (%)	Within day (%)	Between day (%)
1	1.137	4.678	89.867	96.924
2	4.325	7.978	95.470	101.63
5	2.122	12.11	98.474	96.074
10	4.508	12.71	101.31	99.391
15	1.767	13.35	103.67	102.26
30	0.919	13.01	98.750	99.498

Table 2. System suitability test results (n=3)

Medications	Number of theoretical plates	Peak symmetry	Retention factor
Vancomycin	1009.665	1.250	0.657
Acetaminophen	4098.714	0.860	1.331

Precision

Between day and within-day precision results are shown in Table 1. All samples had %CV lower than 15% that was in accordance with USP guidelines (18) which suggested the precision of ± 15 %CV.

Accuracy

Between day and within-day accuracy results are shown in Table 1. All samples had accuracy ranging between 90 and 103%, which was compatible with USP validation guidelines (acceptable accuracy range of 85-115%) (18).

System suitability test

N, PS, and K´ were calculated for both vancomycin and IS, and results are shown in Table 2. N is the criterion of the column efficiency, whereas PS revealed the symmetry of the peak, and K´ shows the suitability of the method to differentiate between drug peak and solvent peak. N values should not be below 2000, acceptable PS range is 0.8-1.8, and K´ values should be at least 0.5 to pass method validation criteria.

Potential interaction of vancomycin with other common drugs administered in ICU

Chromatograms (Fig. 3) of blank plasma, a plasma sample spiked with vancomycin and IS, a plasma sample spiked with drug cocktail containing vancomycin, IS, and other seven aforementioned concomitant drugs, and a plasma sample spiked with those seven drugs without vancomycin and IS, all in therapeutic plasma concentrations, revealed that there is no interaction between vancomycin and/or IS peaks with other common drugs administered simultaneously in this developed HPLC method for vancomycin assay in plasma samples.

Patient blood sample analysis results

In order to confirm the suitability of the validated HPLC method plasma sample of the patients and spiked plasma samples with vancomycin was analyzed for a poly-pharmacy patient and chromatograms are depicted in Fig. 4. Results revealed that this method has acceptable precision and selectivity to distinguish vancomycin peak among other drugs and endogenous plasma peaks. The use of a spiked peak chromatogram confirmed the exact location of the vancomycin peak. Trough plasma level was 8.79 µg/mL which was below the recommended trough level of vancomycin (15-20 µg/mL).

Results of the second patient revealed that his vancomycin trough level was 69.365 µg/mL, intermediate (4 h) was 76.6 µg/mL, and the peak level was 122.3 µg/mL. 12 h-AUC was calculated 972.5 µg.h/mL according to Fig. 5, 24 h-AUC was 1945 µg.h/mL. Vancomycin CL_r was calculated 1.03 l/h, V_d was 18.89 L and K_e was 0.055 h^{-1}. Baseline serum creatinine for this patient was 1.2 mg/dL and serum creatinine at the day of blood sampling was 2.4 mg/dL but it was reached 3.6 mg/dL the day after the blood sampling. Finally, serum creatinine in this patient was reached 6.8 mg/dL within 10 days after blood sampling time and the patient was expired.

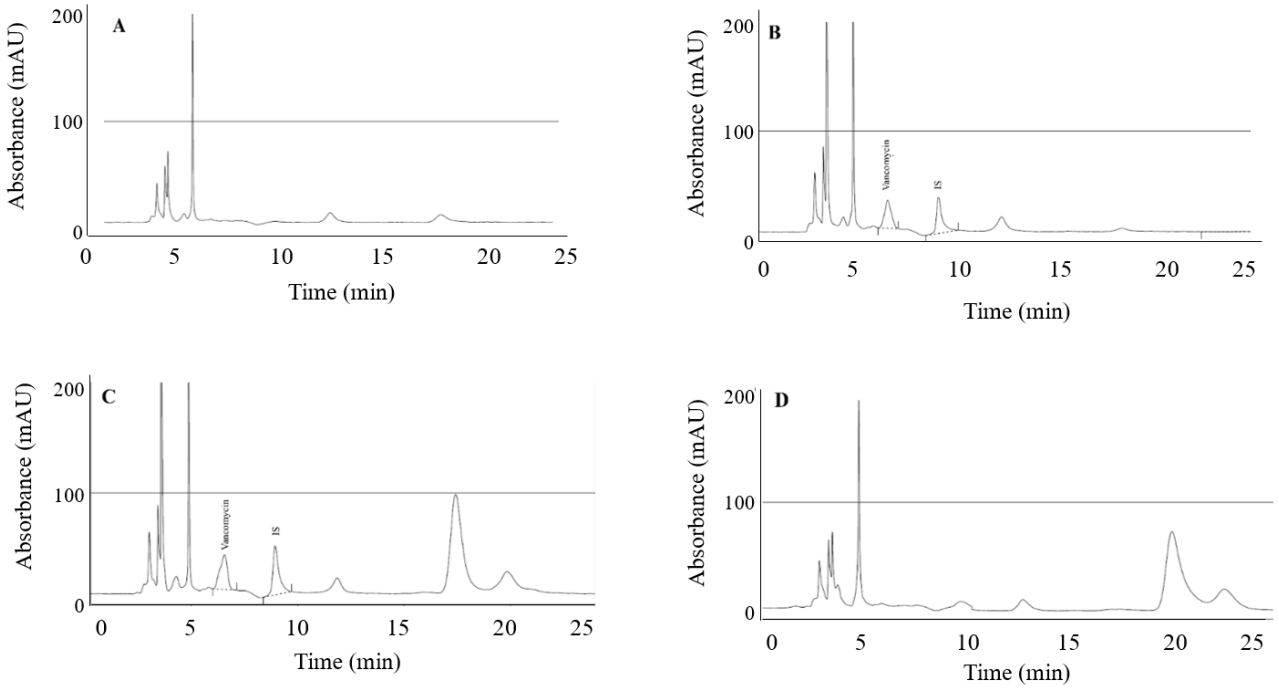

Fig. 3. Representative chromatograms of (A) blank plasma, (B) vancomycin and IS (acetaminophen), (C) drug cocktail (vancomycin, IS, and other seven drugs containing meropenem, ceftriaxone, ampicillin sulbactam, piperacillin tazobactam, levofloxacin, colistin, and amikacin), and (D) seven drugs without vancomycin and IS. IS, internal standard.

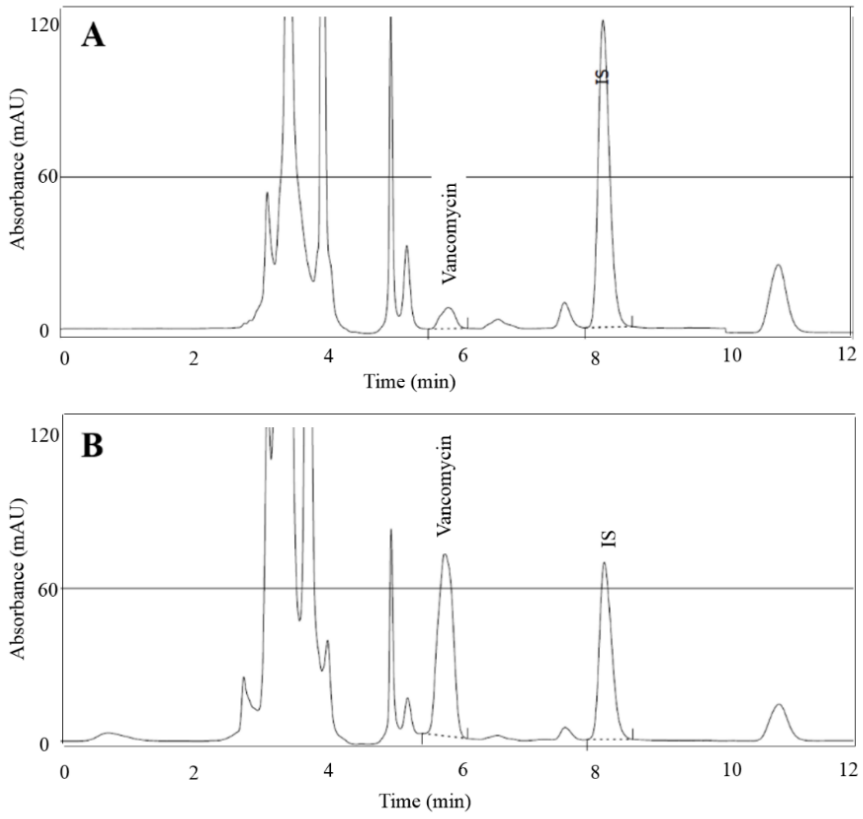

Fig. 4. Representative chromatograms of first patient (A) trough level and (B) spiked trough level. IS, internal standard.

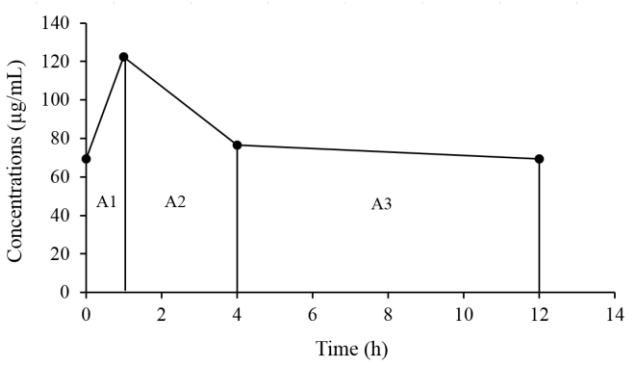

Fig. 5. 12 h-AUC which has been calculated through the trapezoidal method; AUC = A1 + A2 + A3. AUC, the area under the curve.

Results of the third patient revealed that vancomycin peak level was 24.05 µg/mL and the trough level was 0 µg/mL. 24 h-AUC was 24.3 µg.h/mL which revealed that this patient was receiving an under-dose drug and confirm that vancomycin dose adjustment solely based on GFR is not adequate and TDM is necessary.

Results of the fourth patient revealed that vancomycin peak level was 31.69 µg/mL, the intermediate level was 20.51 µg/mL, and the trough level was 13.45 µg/mL. 12 h-AUC was 245.13 µg.h/mL and 24 h-AUC was 490.26 µg.h/mL. In this patient Vd was 54.82 L, Clr was 4.08 L/h and Ke was 0.074 h-1.

DISCUSSION

In the present study, we proposed a modified and optimized extraction process which is simple and economically beneficial for plasma samples collected from critically ill patients who received vancomycin at Nemazee Hospital, Shiraz, Iran. The most important challenge in the selection of a suitable extraction method is the necessity of avoidance of peak interactions and peak overlaps of simultaneously administered drugs, internal standard (IS), and endogenous plasma peaks. In this study, a validated HPLC method has been developed to minimize these kinds of interactions. Among different IS which have been studied for vancomycin assay, acetaminophen was selected. Acetaminophen has good absorptivity and similar λ_{max} to vancomycin. Acetaminophen showed different retention time from the vancomycin and endogenous peaks of plasma samples and acceptable absorptivity at the same λ_{max} selected for vancomycin analysis. Calibration curve and linearity results showed the acceptance validation criteria of the calibration curve according to USP guidelines which are ±15% of nominal concentrations (18,21). R square of 0.9996 and adjusted R square of 0.9995 revealed that there is an acceptable linear relationship between vancomycin concentration and area of the vancomycine peak over that of IS ratios. Regression test results showed significance F values lower than 0.05 which confirmed the significant linear relationship. X-variable (slope) *P*-value was lower than 0.05 which also confirmed that the curve slope had a significant difference with zero. This method showed acceptable selectivity in the determination of vancomycin levels in plasma samples in accordance with the USP bioanalytical method validation guideline (18) which has been suggested that blank plasma should be free of interference at the retention times of the analyte and IS. The analyte response at LOQ was ≥ 5-times the analyte response of the blank plasma (zero calibrator) (18). Sensitivity results revealed that this validated method has acceptable sensitivity to analyze vancomycin trough, intermediate, and peak plasma levels. All samples had %CV lower than 15% that was in accordance with USP guidelines which suggested the precision of ± 15 %CV. Accuracy results were compatible with USP validation guidelines (acceptable accuracy range of 85-115%) (18). Therefore, according to the results, this method has acceptable accuracy and precision for vancomycin assay in plasma samples.

According to the results, in this developed HPLC method for vancomycin assay in plasma samples, there was no interference between vancomycin and/or IS peaks with other common drugs that might be administered simultaneously in ICU patients. This method showed sufficient selectivity and sensitivity for vancomycin level assay in plasma samples of critically ill patients.

Results of the first patient's samples revealed the trough level was 8.79 µg/mL which is below the recommended trough level of vancomycin (15-20 µg/mL). In this patient, after trough level measurement, vancomycin dosage was enhanced to 1 g every 8 h in order

to adjust the optimum trough level to 15 to 20 µg/mL and the patient's blood culture changed to negative after 7 days of antibiotic therapy with optimized dosage. Low vancomycin trough levels are common especially in young patients with normal renal function when vancomycin is administered with the usual dose of 1 g every 12 h (22). So, the loading and maintenance dose of vancomycin should be individualized based on patients' renal function, weight, and measured plasma levels (23). The results of this analysis confirmed the importance of TDM in patients who are receiving vancomycin.

Results of the second patient's samples revealed that 1.2 mg/dL increase in serum creatinine level within 24 h was a sign of acute kidney injury (AKI) in this patient due to the very high trough level of vancomycin (69 µg/mL) while dose adjustment between 15 and 20 µg/mL is necessary. Also, 24 h-AUC of 1945 µg.h/mL confirmed vancomycin-associated (24) nephrotoxicity which is far from a target goal of 400-600 µg.h/mL. Another pharmacokinetic parameter that revealed vancomycin-associated nephrotoxicity was K_e, in this patient K_e value of 0.055 h^{-1} was significantly lower than the vancomycin K_e value in the normal population which is 0.116 h^{-1}. Reduction in K_e value is another criterion of the occurrence of nephrotoxicity. Finally, serum creatinine in this patient was raised to 6.8 mg/dL within 10 days and the patient was expired which might be the consequence of irreversible AKI related to vancomycin nephrotoxicity. The results of this patient also revealed the necessity of TDM to avoid vancomycin-associated nephrotoxicity. According to these results consideration of optimum sampling time, which is at least 48 h after initiation of vancomycin therapy, is highly recommended in order to an early decision about dose modification and prevention of these kinds of irreversible vancomycin-induced AKIs which was seen in the second patient.

According to the results of the third patient who was in under-dose regimen, vancomycin-dose adjustment is necessary, and only based on GFR decision making is not sufficient (5) and TDM is essential to provide essential pharmacokinetic parameters. In this patient vancomycin, the dosage was modified to 1 g every 12 h and then blood culture became negative within 10 days of antibiotic therapy with modified dosage.

Results of the fourth patient emphasized the priority of AUC calculation over trough level assessment because in this patient 24 h-AUC was in acceptable range (400-600 µg.h/mL) but trough level was lower than 15 µg/mL which is the recommended clinical target level. In this patient, because of suitable vancomycin 24 h-AUC level, antibiotic therapy was continued with the previous dosage of 1 g every 12 h for 7 days until removal of clinical and laboratory presentations of infection. The results of these patients' blood sample analysis emphasized the importance of TDM of vancomycin during drug administration to avoid severe adverse drug reactions related to overdose or failure to therapy-related to under-dose drug administration in critically ill patients. The most important advantage of the HPLC method in comparison with immunoassay methods is its higher sensitivity and selectivity especially in lower concentrations, and also avoidance of cross-reactions with vancomycin metabolites which could prevent false-positive results during drug assay. Critically ill patients who are admitted to ICU are highly prone to sepsis and the occurrence of AKI, so TDM of vancomycin, as a nephrotoxic drug, is highly essential in these patients in order to prevent further complications. After pharmacists' intervention in dose adjustment by pharmacokinetic analysis, patients could gain the optimum trough level and AUC values, and clinical and laboratory presentations of infections were resolved within 10 days of antibiotic therapy with optimized dosage. According to the results of this study, vancomycin TDM is strongly recommended for patients admitted to the ICU of Nemazee Hospital, a referral center in southern Iran, which has not been performed so far.

CONCLUSION

According to the previous studies a simple, fast, and relatively low-cost method has been established and proposed for vancomycin assay. This validated HPLC method for the

assay of vancomycin plasma levels was utilized to measure vancomycin levels in four patients and pharmacokinetic parameters were calculated individually. According to the results of this study, pharmacist-guided TDM for vancomycin is strongly recommended in this center. Individualized dose adjustment of vancomycin in critically ill patients is essential in order to avoid unwanted adverse reactions especially vancomycin-induced AKI and also to achieve optimum clinical efficacy. It seems that the total costs of vancomycin TDM center establishment would be lower than the costs of nephrotoxicity associated with vancomycin overdose and its further complications. Also, applying TDM in critically ill patients would be accompanied with a shorter duration of hospitalization that can result in lower total costs for both patients and health care systems.

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CONFLICT OF INTEREST STATEMENT

The authors declared no conflict of interest in this study.

AUTHORS' CONTRIBUTION

P. Ghasemiyeh Carried out the experiment, analyzed the data, writing original draft preparation, reviewing and editing. A. Vazin supervised and carried out the methodology, writing, reviewing, and editing the manuscript. F. Zand, A. methodology, writing, reviewing, and editing the manuscript. S. Mohammadi-Samani supervised, conceptualized, methodology, writing, reviewing. and editing the manuscript.

REFERENCES

1. Eirich J, Orth R, Sieber SA. Unraveling the protein targets of vancomycin in living *S. aureus* and *E. faecalis* cells. J Am Chem Soc. 2011;133(31):12144-12153. DOI: 10.1021/ja2039979.
2. May CC, Erwin BL, Childress M, Cortopassi J, Curtis G, Kilpatrick T, *et al*. Assessment of acute kidney injury in neurologically and traumatically injured intensive care patients receiving large vancomycin doses. Int J Crit Illn Inj Sci. 2018;8(4):194-200. DOI: 10.4103/IJCIIS.IJCIIS_39_18.
3. Vazin A, Japoni A, Shahbazi S, Davarpanah MA. Vancomycin utilization evaluation at hematology-oncology ward of a teaching hospital in Iran. Iran J Pharm Res. 2012;11(1):163-170.
4. Rybak MJ. The pharmacokinetic and pharmacodynamic properties of vancomycin. Clin Infect Dis. 2006;42(Suppl 1):S35-S39. DOI: 10.1086/491712.
5. Khoei A, Soltani R, Emami J, Badri S, Taheri S. Therapeutic drug monitoring of vancomycin by AUCτ-MIC ratio in patients with chronic kidney disease. Res Pharm Sci. 2019;14(1):84-92. DOI: 10.4103/1735-5362.251856.
6. Finch NA, Zasowski EJ, Murray KP, Mynatt RP, Zhao JJ, Yost R, *et al*. A quasi-experiment to study the impact of vancomycin area under the concentration-time curve-guided dosing on vancomycin-associated nephrotoxicity. Antimicrob Agents Chemother. 2017;61(12):e01293,1-10. DOI: 10.1128/AAC.01293-17.
7. Zasowski EJ, Murray KP, Trinh TD, Finch NA, Pogue JM, Mynatt RP, *et al*. Identification of vancomycin exposure-toxicity thresholds in hospitalized patients receiving intravenous vancomycin. Antimicrob Agents Chemother. 2018;62(1):e01684-17,1-9. DOI: 10.1128/AAC.01684-17.
8. Usman M, Hempel G. Development and validation of an HPLC method for the determination of vancomycin in human plasma and its comparison with an immunoassay (PETINIA). Springerplus. 2016;5(1):124-130. DOI: 10.1186/s40064-016-1778-4.
9. Lima TdM, Seba KS, Gonçalves JCS, Cardoso FLL, Estrela RdCE. A rapid and simple HPLC method for therapeutic monitoring of vancomycin. J Chromatogr Sci. 2017;56(2):115-121. DOI: 10.1093/chromsci/bmx089.
10. Hu LQ, Yin CL, Du YH, Zeng ZP. Simultaneous and direct determination of vancomycin and cephalexin in human plasma by using HPLC-DAD coupled with second-order calibration algorithms. J Anal Methods Chem. 2012;2012:256963,1-8. DOI:10.1155/2012/256963.
11. Hagihara M, Sutherland C, Nicolau DP. Development of HPLC methods for the determination of vancomycin in human plasma, mouse serum and bronchoalveolar lavage fluid. J Chromatogr Sci. 2013;51(3):201-207. DOI: 10.1093/chromsci/bms128.
12. Favetta P, Guitton J, Bleyzac N, Dufresne C, Bureau J. New sensitive assay of vancomycin in human plasma using high-performance liquid chromatography and electrochemical detection. J Chromatogr B Biomed Sci Appl. 2001;751(2): 377-382. DOI: 10.1016/s0378-4347(00)00484-9.

13. Alasino AB, Caro YS, De Zan MM. Bioanalytical method optimization for the therapeutic drug monitoring of vancomycin. J Anal Bioanal Sep Tech. 2017;2(2):89-95.
DOI: 10.15436/2476-1869.17.1733.
14. Abu-Shandi KH. Determination of vancomycin in human plasma using high-performance liquid chromatography with fluorescence detection. Anal Bioanal Chem. 2009;395(2):527-532.
DOI: 10.1007/s00216-009-2948-9.
15. Cao Y, Yu J, Chen Y, Zhang J, Wu X, Zhang Y, *et al*. Development and validation of a new ultra-performance liquid chromatographic method for vancomycin assay in serum and its application to therapeutic drug monitoring. Ther Drug Monit. 2014;36(2):175-181.
DOI: 10.1097/FTD.0b013e3182a458bc.
16. Muppidi K, Pumerantz AS, Betageri G, Wang J. Development and validation of a rapid high-performance liquid chromatography method with UV detection for the determination of vancomycin in mouse plasma. J Chromat Separation Techniq. 2013;4(1):1-5.
DOI: 10.4172/2157-7064.1000165.
17. Plock N, Buerger C, Kloft C. Successful management of discovered pH dependence in vancomycin recovery studies: novel HPLC method for microdialysis and plasma samples. Biomed Chromatogr. 2005;19(3):237-244.
DOI: 10.1002/bmc.446.
18. Health UDo, Services H. Bioanalytical method validation, guidance for industry. 2001. Available from: http://www.fda.gov/cder/guidance/4252fnl.htm. Accessed January 02, 2019.
19. Uhrovčík J. Strategy for determination of LOD and LOQ values-some basic aspects. Talanta. 2014;119:178-180.
DOI: 10.1016/j.talanta.2013.10.061.
20. Tsang M. A Practice of anesthesia for infants and children. Can J Anesth. 2018;65(12):1392-1393.
DOI: 10.1007/s12630-018-1201-4.
21. Rezazadeh M, Emami J. A simple and sensitive HPLC method for analysis of imipramine in human plasma with UV detection and liquid-liquid extraction: Application in bioequivalence studies. Res Pharm Sci. 2016;11(2):168-176.
22. Šíma M, Hartinger J, Grus T, Slanař O. Initial dosing of intermittent vancomycin in adults: estimation of dosing interval in relation to dose and renal function. Eur J Hosp Pharm. 2019.
DOI: 10.1136/ejhpharm-2019-002013.
23. Šíma M, Hartinger J, Netíková IŠ, Slanar O. Creatinine clearance estimations for vancomycin maintenance dose adjustments. Am J Ther. 2018;25(5):e602-e604.
DOI: 10.1097/MJT.0000000000000616.
24. Soltani R, Khorvash F, Meidani M, Badri S, Alaei S, Taheri S. Vitamin E in the prevention of vancomycin-induced nephrotoxicity. Res Pharm Sci. 2020;15(2):137-143.
DOI: 10.4103/1735-5362.283813.