ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
18 (1998) 367–372

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

A modified HPLC method for the determination of vancomycin in plasma and tissues and comparison to FPIA (TDX)

Dina Farin, Guillermo A. Piva, Igal Gozlan, Ruth Kitzes-Cohen *

Clinical Pharmacology and Infectious Diseases Unit, Carmel Medical Center, The Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, 7 Michal Street, Haifa 34362, Israel

Received 7 November 1997; received in revised form 9 February 1998; accepted 16 February 1998

Abstract

A modified high performance liquid chromatography (HPLC) method for the quantification of vancomycin levels in plasma and tissues is described. The method uses solid phase extraction (SPE) of vancomycin from the samples and reversed phase HPLC with UV detection. The method was fully validated in terms of recovery, linearity, selectivity and various stability conditions. Vancomycin was determined in plasma samples obtained from 15 patients undergoing cardiopulmonary bypass, before and repeatedly during 12 h after drug administration. The vancomycin levels in plasma were measured by HPLC and by fluorescence polarization immunoassay (FPIA) (TDX). The following correlation was found: TDX = 0.84 HPLC + 1.04. The mean vancomycin levels in skin, fat, atrium, pericardium and sternum, before and after bypass, are reported.

Keywords: Vancomycin; Reversed phase liquid chromatography; TDX; Plasma; Tissues

1. Introduction

Vancomycin is a glycopeptide antibiotic, often used against gram-positive bacteria, including methicillin-resistant staphylococci [1,2]. Monitoring vancomycin levels in the blood is considered useful in order to avoid ototoxicity and nephrotoxicity in patients under treatment and to ensure that therapeutic concentrations are achieved.

Vancomycin can be monitored in plasma by several analytical methods, such as bioassay, radioimmunoassay (RIA), fluorescence polarization immunoassay (FPIA) and high performance liquid chromatography (HPLC) [3–9].

Among these methods, HPLC is the most sensitive and specific and has the ability to detect low levels with high precision and accuracy, although not the simplest and least time consuming. HPLC was used to detect vancomycin levels in tissues and bones, where levels below the detection limit of, e.g. FPIA, are expected. Several investigators [10,11] employed solid phase extraction and reversed phase HPLC and UV detection to determine low vancomycin levels.

* Corresponding author. Tel: + 972 4 8250329; fax: + 972 4 8250684.

0731-7085/98/$ - see front matter
PII S0731-7085(98)00095-8

We report a modified HPLC method, which employs a gradient system for the determination of vancomycin in plasma, tissues and bones. The method was applied for the determination of vancomycin at different stages of a coronary artery bypass operation. We compared results obtained for plasma samples by the modified HPLC method with the FPIA results for 180 blood samples taken during cardiac surgery.

2. Materials and methods

2.1. Chemicals and reagents

Vancomycin hydrochloride (Vanco-Teva, Teva, Netanya, Israel) was used in this study. The internal standard (IS) was tinidazole (Sigma, Israel). Stock solutions in water were kept frozen (−20°C). Analytical potassium dihydrogen phosphate was obtained from Merck (Germany). Acetonitrile (HiPerSolv) and methanol (HiPerSolv) were from BDH (UK). All HPLC solvents were filtered prior to use through a 0.2 μm filter (Schleicher and Schuell). Sample clean-up solid phase extraction cartridges (SPE) were C18 Bond Elut, 3 cc 200 mg^{-1} from Varian (Harbor City, CA). Vancomycin kits for TDX were from Abbott (Cat. No. 9523-60, Abbott Laboratories, Abbott Park, IL).

2.2. Instrumentation

The HPLC system consisted of a gradient system model HP-1050 with a UV–vis detector and an autosampler (Hewlett-Packard). Chromatographic separations were performed at ambient temperature (23–25°C) on a Hypersil BDS C8 column; 10 cm × 4.6 mm, 3 μm (Shandon) with a C8 2 cm guard column (Keystone Scientific). The mobile phase consisted of 5 mM potassium dihydrogen phosphate buffer (pH 2.8)–acetonitrile, and the following gradient was applied: 3% acetonitrile for 1.5 min; 20% at 11.5 min, 20% at 14 min and 3% at 15 min; post time was 10 min. The total run time was 25 min at a flow rate of 1.5 ml min^{-1}. Detection of vancomycin and IS was at 282 nm. FPIA was performed on a TDX system (Abbott Laboratories).

2.3. Sample preparation

2.3.1. Plasma samples

To 0.5 ml spiked plasma, 100 μl IS solution (20 μg ml^{-1} in H_2O) were added. This mixture (250 μl) was applied to the Bond Elut cartridge. The cartridges were activated prior to use by successive washing with 3 ml methanol and 3 ml distilled water.

After centrifugation for 1 min at 250 rpm, the cartridge was washed with 1.5 ml H_2O. The eluate was discarded and the cartridge was washed again with a 3 ml methanol–water mixture (5:95 v/v). Vancomycin and the IS were eluted by two successive 300 μl washing with acetonitrile–50 mM KH_2PO_4 (50:50 v/v, pH = 4.0, 1 min centrifugation at 250 rpm), and the eluate was evaporated to dryness, and then reconstructed with 600 μl water. This solution (100 μl) was injected into the HPLC system.

The Bond-Elut cartridges were washed with one volume of methanol followed by one volume of water. Each cartridge was discarded after the second run.

2.3.2. Tissues and sternum

The tissue and bone samples were rinsed with saline and were pressed in sterile gauze to remove contaminating blood, and then weighed. The samples were assayed against a calibration curve of vancomycin in water. Water (500 μl) was added to the (bone or tissue) samples which were then homogenized. An additional 500 μl water were added in order to remove all sample traces from the homogenizer. The mixture was centrifuged in order to avoid loss of material on the tube walls, and then sonicated in an ice bath for 20 min. IS (200 μl) was added to this mixture. A sample of the upper layer (500 μl) was applied to the SPE cartridge. The procedure from this point was the same as for the plasma samples.

2.4. Validation of the HPLC method

Fifteen calibration curves of vancomycin, within a concentration range of 0.5–75 μg ml^{-1} in plasma and 0.25–20 μg ml^{-1} in water, were run in order to establish linearity. The calibration

curves were obtained by weighted ($1/X^2$) linear regression of the peak height of vancomycin versus vancomycin concentration. Recovery was calculated by comparing the measured values of the spiked samples with those of the standard aqueous solutions of three concentrations, namely, 2, 10 and 40 μg ml^{-1}. The extraction recovery of the IS was determined at 5 μg ml^{-1}. The stability was established with six replicates of the three above mentioned concentrations, as follows: stability on the autosampler during 24 h, long term stability up to 6 months at −36°C, and stability to two freeze-thaw cycles of the samples. Inter-day reproducibility was measured at three time points and the coefficients of variation were 7.5, 6.0 and 6.4% for 2, 10 and 40 μg ml^{-1}, respectively. The intra-day coefficients of variation, measured for eight replicates of each of the above mentioned concentrations, were 11.2, 8.5 and 8.6%, respectively .

2.5. Assay of samples

In this study 180 blood samples, and 270 various tissues and sternum samples were assayed by HPLC. The calibration curves were performed before each HPLC run. Two sets of quality controls (QCs) at three different levels (2, 10 and 40 μg ml^{-1} for blood and 1, 5 and 10 μg ml^{-1} for tissues and sternum) were tested with each HPLC run. The runs were repeated if more than two QC at different concentrations, or two QCs at the same concentration, deviated by more than 20% from their nominal range.

The blood samples were also analyzed by FPIA (TDX) on the same day. Three standard QCs, namely 12, 30 and 65 μg ml^{-1} (Biorad) were placed in each TDX monitoring cycle. The QC results were not allowed to deviate by more than 20% from their expected values.

3. Results

3.1. Validation

Fig. 1 shows typical chromatograms. No interfering peaks appear in the retention times of both vancomycin and the IS, therefore the method is proved to be selective. The extraction recoveries of vancomycin are shown in Table 1.

Linearity was established over 15 calibration curves, where $Y = 0.082X + 0.021$ (mean correlation coefficient = 0.9966) within the range 0.5–75 μg ml^{-1}. Vancomycin was found to be stable to two freeze-thaw cycles and no decomposition or deterioration was observed in the vancomycin concentration in the plasma samples kept at −36°C for 6 months.

The limit of quantification (LOQ), which is defined as the lowest concentration which was determined within less than 20% precision and accuracy, was 0.5 μg ml^{-1}.

3.2. Blood samples

The precision and accuracy of the HPLC analyses were evaluated from the quality controls included in each run. The TDX analyses were evaluated by daily testing three QC levels. The results are summarized in Table 2.

The vancomycin levels in blood were determined during various stages of cardiac surgery, for 15 patients. Altogether, 180 blood samples and 270 tissue or sternum samples were analyzed. The mean blood levels ranged between 56.4 ± 20.5 μg ml^{-1} at the end of i.v. administration and ca. 7.6 ± 2.3 μg ml^{-1} 12 h after administration. The mean plasma concentration–time curve, analyzed by HPLC, is shown in Fig. 2.

The vancomycin levels obtained for 180 blood samples by HPLC and FPIA were in good agreement. We found: μg ml^{-1} TDX = $0.84(\pm 0.02)$ μg ml^{-1} HPLC + $1.04(\pm 0.38)$ μg ml^{-1} with a correlation coefficient $r = 0.964$. Fig. 3 shows the correlation and the 95% confidence interval.

3.3. Tissues and sternum

In order to obtain reproducible results, the minimal weight of tissues was 100 mg. Vancomycin levels in tissues and sternum were calculated in μg g^{-1}, according to the following relationship:

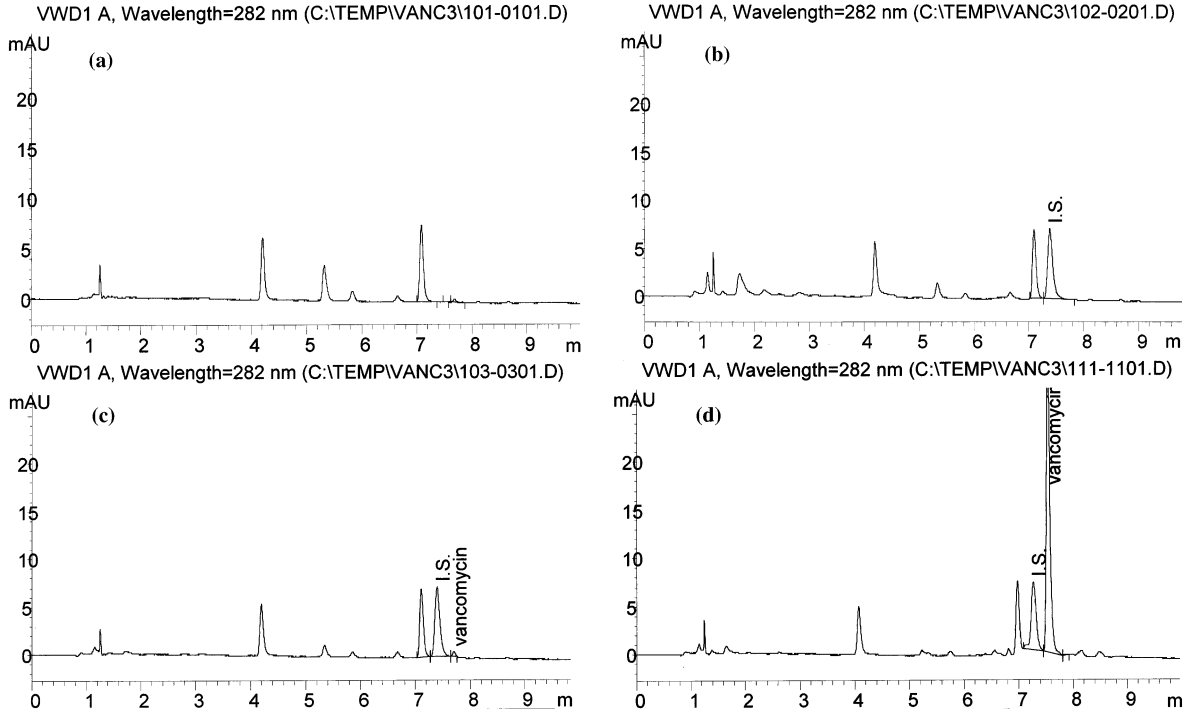

Fig. 1. Representative chromatograms of: (a) blank plasma, (b) plasma spiked with IS, (c) plasma spiked with 0.5 μg ml^{-1} vancomycin (LOQ) and (d) plasma spiked with 50 μg ml^{-1} vancomycin.

conc. (μg g^{-1}) = conc. (μg ml^{-1})

× volume of water added (ml)

/sample weight (g)

The mean vancomycin levels (μg g^{-1}) in tissues and sternum 3 h after vancomycin administration were: leg skin, 15.8 ± 14.3; leg fat, 7.7 ± 4.9; chest skin, 23.5 ± 12.4; chest fat, 6.9 ± 4.4; sternum, 10.9 ± 8.1; pericardium, 28.2 ± 12.9; and right atrium, 20.5 ± 9.3.

Table 1
Extraction recoveries of vancomycin and IS

Amount (μg ml^{-1})	Recovery (%)	CV (%)
2	96.9	9.4
10	92.1	15.7
40	97.5	15.0
IS (5)	74.0	6.2

4. Discussion

The method we used for sample preparation is a simplified modification of the methods described previously [9,10]. The washes and extraction from the SPE cartridges were carried out by placing the cartridges in a centrifuge at 250 rpm, thus achieving controlled suction through the cartridges. This also allowed the handling of up to 48 samples simultaneously and, thus, to complete the sample preparation in a short time.

Various compounds were examined in order to find internal standard which would be extracted under the same conditions as vancomycin. Ristocetin, cephalexin and cefazolin had poor extraction recovery, whereas teicoplanin, which has a similar molecular structure to vancomycin, was unsuitable due to the appearance of several wide endogenous peaks in the chromatogram. Tinidazole coeluted with vancomycin from the SPE cartridges in a good, reproducible recovery and good separation was achieved under the chromatographic conditions.

Table 2
Precision and accuracy of the HPLC analyses

Nominal concentration (μg ml^{-1})	HPLC			TDX		
	2	10	40	12	30	65
N	23	26	26	23	23	23
Observed concentration (μg ml^{-1})	1.99 ± 0.41	10.59 ± 0.84	40.61 ± 4.06	11.6 ± 1.2	28.2 ± 2.3	63.5 ± 6.6
CV (%)	20.6	8.0	10.0	10.2	8.2	10.4
Accuracy (%)*	20.6	8.2	7.9	8.8	8.6	8.4

* The accuracy, which represents the percentage deviation of the observed concentration from the nominal concentration, was calculated according to:

$$\sum|[(\text{ObservedConcentration} - \text{NominalConcentration})/\text{Nominal Concentration}]| \times (100/n)$$

.

The elution of vancomycin and the IS from the SPE cartridge was performed by applying two successive portions of 1:1 acetonitrile in buffer. Lower percentages of acetonitrile was found insufficient to elute the IS.

A gradient was applied in order to increase the signal-to-noise ratio, and hence increase the sensitivity of the method. By applying the gradient the column was washed of proteins and other impurities which tend to accumulate at the columns head at the end of each run, and thus chromatograms with no interfering peaks are obtained (Fig. 1).

Fig. 3 shows the good correlation between the results obtained by HPLC and FPIA (TDX). Similar correlations were found by other investigators, i.e. Ristuccia et al. [5] observed that μg ml^{-1} TDX = 1.0489 μg ml^{-1} HPLC − 0.737 for 100 clinical specimens, Filburn et al. [6] report μg ml^{-1} TDX = 0.8456 μg ml^{-1} HPLC + 3.3205 for 60 samples.

The results indicate that TDX analyses yield reliable results for monitoring vancomycin levels in blood samples, however, when low concentrations (μg ml^{-1}) are expected, as in the case for tissues and sternum, only the more sensitive HPLC method is appropriate.

In all tissue and bone samples the concentration of vancomycin varied among the patients with a relatively wide distribution of results. Similar distributions were also observed by other investigators [12–14]. Nevertheless, it is seen that vancomycin is present in all the tissues involved in

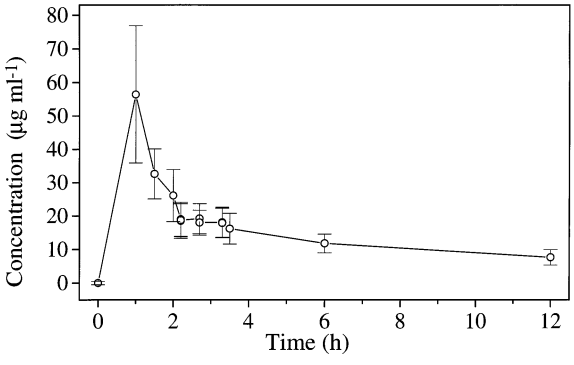

Fig. 2. The mean plasma concentration–time curve of vancomycin.

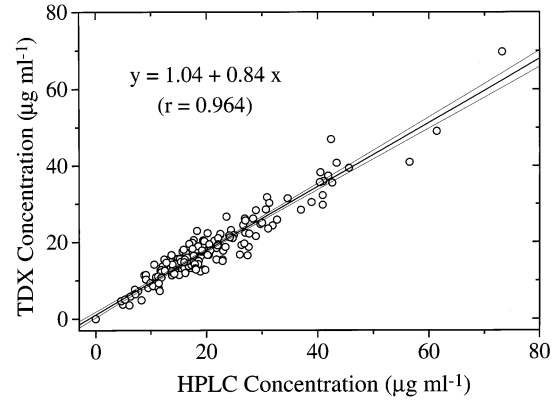

Fig. 3. The TDX–HPLC correlation for 180 blood samples. The 95% confidence interval is shown.

the surgery, and its concentration always exceeds the MIC of the common contaminating pathogens, namely higher than ca. 2 μg ml^{-1} [15]. Blood peak levels were obtained 1 h after vancomycin administration (56.4 μg ml^{-1}) and these levels remained above the MIC until the end of the operation. Further discussion of this issue, which is beyond the scope of this paper, is in preparation [16].

Acknowledgements

We gratefully thank K. Hazan and A. Ioffe for performing the analyses by TDX, and the cardiothoracic surgery staff for supplying the blood, tissue and sternum samples.

References

[1] J.E.F. Reynolds (Ed.), Martindale The Extra Pharmacopoeia, the Royal Pharmaceutical Society, London, 1996.
[2] A.G. Gilmam, T.W. Rall, A.S. Nies, P. Taylor (Eds.), Goodman and Gilman's The Pharmacological Basis of Therapeutics, Pergamon, New York, 1990.
[3] M.W. Hu, L. Anne, T. Forni, K. Gottwald, Ther. Drug. Monit. 12 (1990) 562–569.
[4] K.T. Yeo, W. Traverse, G.L. Horowitz, Clin. Chem. 35 (1989) 1504–1507.
[5] P.A. Ristuccia, A.M. Ristuccia, J.H. Bidanset, B.A. Cunha, Ther. Drug Monit. 6 (1984) 238–242.
[6] B.H. Filburn, V.H. Shull, Y.M. Tempera, J.D. Dick, Antimicrob. Agents Chemother. 24 (1983) 216–220.
[7] F. Demotes-Mainard, L. Labat, G. Vincon, B. Bannwarth, Ther. Drug Monit. 16 (1994) 293–297.
[8] F. Jehl, C. Gallion, R.C. Thierry, H. Monteil, Antimicrob. Agents Chemother. 27 (1985) 503–507.
[9] J.B.L. McClain, R. Bongiovanni, S. Brown, J. Chromatogr. 231 (1982) 463–466.
[10] J. Bauchet, E. Pussard, J.J. Garaud, J. Chromatogr. 414 (1987) 472–476.
[11] S.V. Greene, T. Abdalla, S.L. Morgan, C. Bryan, J. Chromatogr. 417 (1987) 121–128.
[12] C. Martin, M. Alaya, M.N. Mallet, et al., Antimicrob. Agents Chemother. 38 (1994) 396–399.
[13] A.L. Graziani, L.A. Lawson, G.A. Gibson, M.A. Steinberg, R.R. MacGregor, Antimicrob. Agents Chemother. 32 (1988) 1320–1322.
[14] L. Massias, C. Dubois, P. Lentdecker, O. Brodaty, M. Fischler, R. Farinotti, Antimicrob. Agents Chemother. 36 (1992) 2539–2541.
[15] V. Lorian (Ed.), Antibiotics in Laboratory Medicine, Williams and Wilkins, Baltimore, 1996.
[16] R. Kitzes-Cohen, D. Farin, G.A. Piva, A. Laor, S. Ivry, R. Sharoni, R. Amar (in preparation).