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DETERMINATION OF LINEZOLID IN HUMAN SERUM BY REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET AND DIODE ARRAY DETECTION

AGNIESZKA CIOS, KAMIL KUŚ and JOANNA SZYMURA-OLEKSIAK*

Department of Pharmacokinetics and Physical Pharmacy, Jagiellonian University, Collegium Medicum, 9 Medyczna St., 30-688 Kraków, Poland

Abstract: A high-performance liquid chromatographic (HPLC) method with UV and DAD detection for the quantitative determination of linezolid in human serum was developed in present work. Chromatography was carried out by reversed-phase technique on a RP-18 column with a mobile phase composed of 50 mM phosphate buffer and acetonitrile (76 : 26, v/v), adjusted to pH 3.5 with orthophosphoric acid. Serum samples were deproteinized with methanol centrifuged and then, the supernatant was analyzed using HPLC procedure. No interference was observed at the retention times of linezolid from blank serum or ten commonly used antibiotics. A concentration range from 0.50 to 30.0 g/mL was utilized to construct calibration curves. The lower limit of detection was determined to be 0.1 µg/mL of serum for both detectors. The lower limit of quantification of 0.25 µg/mL (CV = 2.6%) was established for determination using HPLC-UV and 0.5 µg/mL (CV = 5.42%) for HPLC-DAD. The recovery of linezolid was approximately 100%. Intra-day accuracy ranged from 0.97 to 12.63% and 0.74 to 10.85% for HPLC-UV and HPLC-DAD method, respectively. Intra-day precision was less than 4.69% for HPLC-UV and less than 5.42% for HPLC-DAD method. Tests confirmed the stability of linezolid in serum during three freeze-thaw cycles and during long-term storage of frozen serum for up to 6 weeks; in extracts it was stable in the HPLC autosampler over 24 h. Statistical analysis by Student's t-test showed no significant difference between the results obtained by these two methods. In summary, these methods will be used and adapted for infected patients in intensive care unit, to determine linezolid serum concentrations in order to know the pharmacokinetic profiles of linezolid.

Keywords: Linezolid, HPLC/UV, HPLC/DAD, validation, pharmacokinetics

Linezolid, (S)-N-{[-3-[3-fluoro-4-(4-morpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl}-acetamide (Fig. 1), is a synthetic antibiotic belonging to a new class of antimicrobials called the oxazolidinones (3).

In vitro microbiological studies showed that linezolid is effective against various antibiotic-resistant isolates of staphylococci, streptococci, enterococci, and pneumococci (4). Linezolid exhibits a unique mechanism of inhibition of bacterial protein synthesis at the initiation phase of translation (5).

The pharmacokinetics and pharmacodynamics of linezolid have been extensively investigated in healthy volunteers and patients (6-10). There are two major metabolites of linezolid (PNU-142586 and PNU-142300). Linezolid and its two main metabolites plus several minor ones are all excreted in the urine (6).

Increased knowledge of the pharmacokinetic/pharmacodynamic properties of antibiotics is useful for optimizing dosage. Linezolid is a time-dependent antimicrobial agent with persistent post-antibiotic effect. The pharmacokinetics/pharmacodynamics parameters best suitable to define its activity are time with serum concentrations higher than the minimum inhibitory concentration ($T_{C>MIC}$)

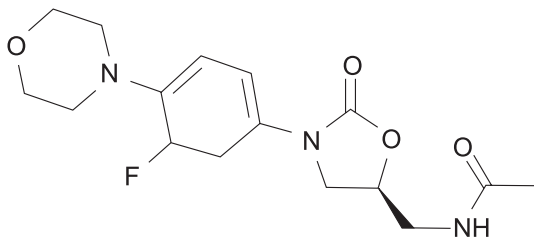

Figure 1. Chemical structure of linezolid (3)

* Corresponding author: e-mail: mfszymur@cyf-kr.edu.pl

and area under the serum concentration-time curve/minimum inhibitory concentration (AUC/MIC) ratio. Data suggest that percentage time above the MIC ($\%T_{C>MIC}$) above 85 and AUC/MIC ratio above 100 are good predictors of the development of resistance to linezolid. The pharmacokinetic parameters are highly variable between individuals so that determination of linezolid serum concentrations is necessary to ensure the treatment effectiveness. Inter-patient differences in pharmacokinetics and the frequently associated toxicity of linezolid after prolonged use makes therapeutic drug monitoring (TDM) strongly recommended. Therefore, a good analytical method for linezolid in human fluids is necessary (6, 11).

Several determinations of linezolid in human fluids using a high-performance liquid chromatographic method coupled with ultraviolet detection (HPLC/UV) have been published (12-19). Some of them described a manual solid-phase extraction (12, 13) or protein precipitation for sample clean-up (14-19). The solid-phase extraction is an expensive method which often involves multi-step purification and evaporation, and a plasma/serum volume of at least 0.5 to 1 mL is usually required. The advantages of protein precipitation over solid-phase extraction are good extraction recovery values (close to 100%) as well as simple and less-time consuming procedure.

High-performance liquid chromatographic method coupled with diode array detection (HPLC/DAD) is a commonly used method in laboratories nowadays for the analysis of many different drugs, metabolites and compounds (20-24). However, there are a few HPLC/DAD methods for measuring linezolid in human fluids (25, 26). In our paper we would like to show that HPLC/DAD method can also be used for routine determination of linezolid in human serum in clinical applications.

The objective of this study was to compare HPLC/UV method with HPLC method coupled with diode array detection. HPLC/DAD is a method less commonly used for pharmacokinetic and pharmacodynamic studies in humans and animals. The aim of the study was also to develop a simple, rapid and low-cost method for estimation of linezolid in human serum, without solid phase extraction, using HPLC method coupled with ultraviolet detection and to validate the method according to the US Food and Drug Administration (FDA) (1, 2). This method will be used and adapted for patients in intensive care unit (ICU) to determine the linezolid serum concentrations in order to analyze the pharmacokinetic profiles of linezolid in ICU infected patients.

EXPERIMENTAL

Chemicals and reagents

Linezolid was supplied by Pharmacia Corporation (Switzerland). Other antibiotics used included: amoxicillin, imipenem, meropenem, ceftazidime, cefuroxime, cefepime, cefoperazone, ceftazidime, ciprofloxacin, piperacillin (Sigma-Aldrich, Germany) and vancomycin (Merck, Germany). Acetonitrile and methanol for chromatography LiChrosolv® were obtained from Merck (Darmstadt, Germany) and analytical grade, concentrated orthophosphoric acid (85%) and potassium dihydrogen phosphate were purchased from Fluka (Germany). Drug free human serum was received from the healthy volunteer.

Apparatus and chromatographic conditions

The HPLC/UV system (Thermo Separation Products, San Jose, CA, USA) consisted of a P100 pump, a Rheodyne injector 7125 (Rheodyne, Cotati, CA, USA) with a 50 µL loop, a UV100 variable-wavelenghth UV/VIS detector, and a SP4400 (ChromJet) integrator.

The HPLC/DAD system (HITACHI LaChrom Elite) consisted of a HITACHI L-2130 pump, a HITACHI L-2350 column oven and a HITACHI L-2200 autosampler. The detector used was a HITACHI L-2455 DAD detector, which was set to scan from 190 to 800 nm. Peak areas were integrated automatically using the EZChrom Elite Client/Server version 3.2 (Scientific Software Inc. Agilent 2005-2006) workstation.

The analytical column was a LiChrospher® 100RP-18 (250 × 4 mm i.d., 5 µm particle size) from Merck (Darmstadt, Germany) coupled with a LiChroCART guard column (5 µm, 4.0 × 4.0 mm, Merck).

The mobile phase consisted of a mixture of acetonitrile – 50 mM potassium dihydrogen phosphate buffer (26 : 74, v/v). It was prepared with 6.8040 g potassium dihydrogen phosphate dissolved in 1000 mL of water. The pH was adjusted to 3.5 with concentrated orthophosphoric acid. The mobile phase was prepared daily, filtered through a 0.45 µm membrane filter (Supelco, Germany) and degassed before use. Linezolid was eluted isocratically with a flow rate of 1.0 mL/min. The peak of linezolid was detected by UV and DAD detection, the wavelength was monitored at 258 nm. The sample injection volume was 50 µL. The chromatographic run time was 9 min. The HPLC system was operated at 22 ± 1°C.

Preparation of solutions

A stock solution of linezolid (1 mg/mL) was prepared by dissolving linezolid standard in deionized water. The stock standard solution was diluted with water to obtain working solutions of 0.5; 2.5; 5.0; 10.0; 20.0 and 30.0 µg/mL for serum calibration samples.

Quality controls (QC) samples were prepared by adding an appropriate volume of the stock solutions to drug free serum to obtain a final concentration of 0.5, 1.0, 7.5 and 15.0 µg/mL (respectively LOQ, low, medium and high QC).

The stock and working standard solutions were stored in a refrigerator (4ºC) when not in use.

Sample preparation

Eppendorf tube was added of linezolid (25 µL), followed by serum (225 µL). Mixture was vortex-mixted for 10 s and the tube was added of methanol (500 µL). The solution was vortexed for 30 s and centrifuged for 10 min at 15000 rpm at room temperature. Finally, 50 µL of the clear supernatant of each sample was injected into HPLC apparatus.

Patients' blood samples were centrifuged at 1000 × g for 10 min, not later than 20 min after collection. On the day of analysis, after thawing, 250 µL of samples were vortex-mixted for 10 s. Then, serum samples were deproteinized with methanol (500 µL) and the solution was vortexed for 30 s and centrifuged for 10 min at 15 000 rpm at room temperature. Finally, 50 µL of the clear supernatant of each sample was injected into HPLC apparatus. All samples were prepared in duplicate.

VALIDATION

Validation procedures, parameters and acceptance criteria were based on FDA and recommendations in the literature (1, 2). The method was then validated regarding selectivity and specificity, linearity, accuracy and precision, limit of detection and limit of quantitation, recovery and stability.

Selectivity and specificity

The selectivity was evaluated by the analysis of samples drug-free serum applying the analytical procedure, the retention times of endogenous compounds were compared with that obtained for linezolid.

Specificity of the method was evaluated by injecting solutions containing other anti-infective drugs largely used in broad spectrum antibiotic treatment: amoxycillin, imipenem, meropenem, ceftazidime, cefuroxime, cefepime, cefoperazone, ceftazidime, ciprofloxacin, and piperacillin.

Linearity

Linearity study was performed by analysis of standards in sixplicate on 5 days between 0.5 and 30.0 µg/mL. Calibration curves of linezolid was established using peak area analyte (y) versus serum concentration (x). Slopes, intercepts and correlation coefficients (r) were obtained by linear regression analysis.

Accuracy and precision

Inter-day study was performed by assaying the six QC concentrations once-a-day on 7 separate days. Mean measured concentrations and their standard deviations (S.D.) were calculated. Precision was reported by calculating the coefficient of variation (CV) expressed as (S.D./mean) × 100 and accuracy by the bias expressed as [(measured concentration - theoretical concentration)/(theoretical concentration)] × 100. Acceptance criteria for accuracy and precision were: bias within ±15% (except ± 20% for

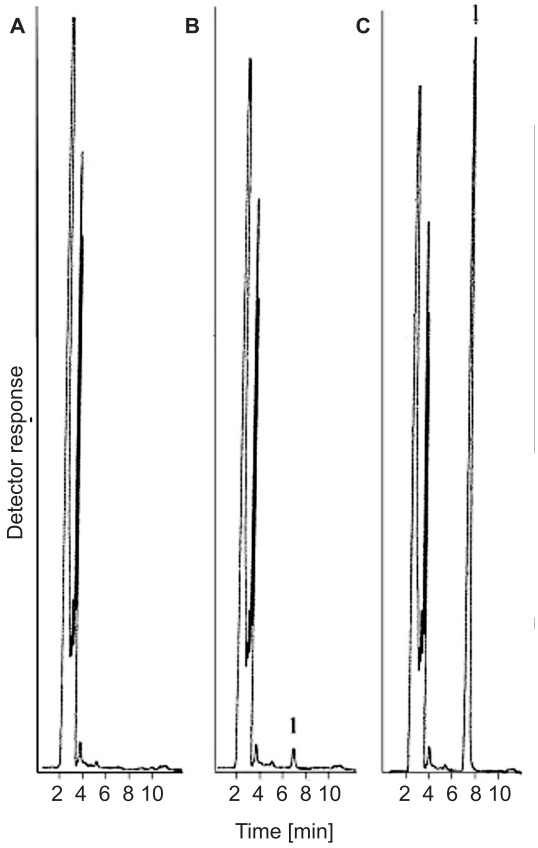

Figure 2. Chromatograms of (**A**) linezolid-free human serum, (**B**) serum spiked with 0.5 µg/mL and (**C**) 20.0 µg/mL of linezolid. (1) indicates the position of linezolid. HPLC/UV

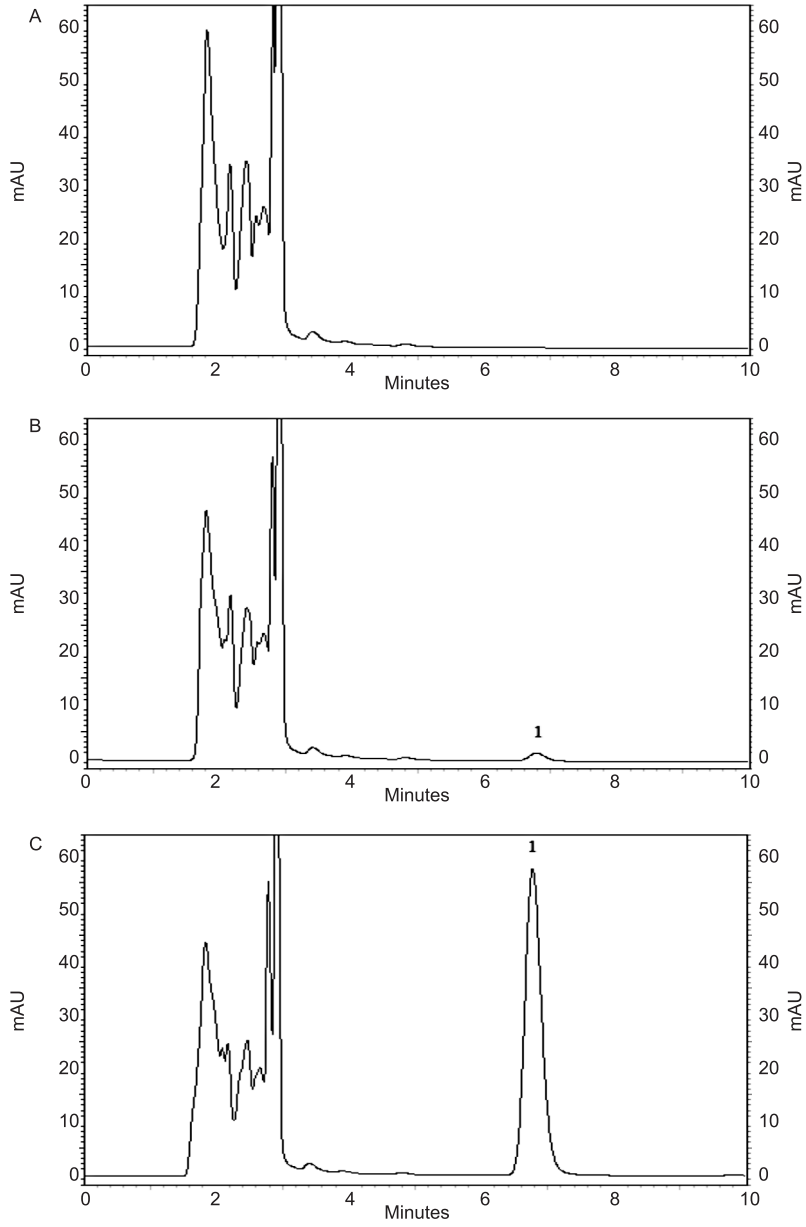

Figure 3. Chromatograms of (**A**) linezolid-free human serum, (**B**) serum spiked with 0.5 µg/mL and (**C**) 20.0 µg/mL of linezolid. (1) indicates the position of linezolid. HPLC/DAD

the LOQ QC) and CV lower than 15% (except 20% for the LOQ QC) (1, 2).

Statistical comparison of the results analysis by the two detection systems (UV and DAD) was performed with regard to accuracy and precision using Student's t-test at 95% confidence level.

Limit of detection and limit of quantitation

The limit of detection (LOD) was defined by the concentration with a signal-to-noise ratio of 3. The limits of quantification (LOQ) were experimentally determined by analyzing serum samples spiked with linezolid at 0.25, 0.5, 1.0, 2.0 and 2.5 µg/mL concentrations. The lower LOQ was chosen as the concentration which provided measurements with a precision and accuracy within the recommended ± 20% from their nominal values, in accordance with the FDA guidelines (1, 2).

Recovery

Linezolid recoveries were quantified at concentration levels corresponding to the lowest (0.5

µg/mL) medium (10.0 g/mL) and highest standards values (30.0 µg/mL) analyzed in sixplicate. Absolute recovery of linezolid from serum was estimated by the peak area integrated for linezolid in serum assayed, accordingly the procedure *versus* the peak area integrated for linezolid, after direct injection of the same concentration in purified water; expressed as percentage.

Linezolid stability

Stability of linezolid in human serum was evaluated after short-term, long-term storage, and through several freeze and thaw cycles. Low (1.0 µg/mL), medium (7.5 µg/mL) and high (15.0 µg/mL) QC concentrations were prepared in triplicate from a freshly made stock solution in human serum and were investigated under different conditions. Long-term stability was studied by assaying samples that had been stored at –30°C for 44 days. Short-term stability was assessed at different temperatures (in the HPLC autosampler at 4°C and room temperature at 22 ± 1°C). QC samples were analyzed in triplicate after different times of storage (0, 2, 5, 9 and 24 h).

All data were compared with results obtained from freshly prepared and analyzed QC samples using the formula: stability (%) = (stored QC concentration/freshly prepared QC concentration) × 100. Linezolid stability was confirmed if less than 10% difference in concentration was observed.

Linezolid stability was assayed also after one, two or three freeze-thaw cycles. This was determined by assay (n = 3) of a serum and water linezolid (15.0 µg/mL) samples thawed after storage at –30°C. The samples were returned to the freezer, frozen, thawed and assayed (n = 3) one more time. The percentage concentrations of linezolid were calculated after each cycle and compared to freshly prepared QC samples of linezolid in human serum and water. The acceptance criterion for serum and water concentrations studies was adopted as less than 15% variation.

Application of the method

One patient (K.L.) was a female, 60-year-old (weight 60 kg, height 165 cm) with respiratory tract infections hospitalized in the Department of Anesthesiology and Intensive Care, Specialistic Hospital in Kraków, Poland. Linezolid (Zivoxid®) was administered by 1 h infusion every 24 h at a dose of 600 mg. Blood samples were taken at the fourth day of treatment (steady-state) before the next dose, just after the cessation of the infusion and 4 and 7 h after infusion. Serum linezolid levels were determined by a previously reported high performance chromatography method with UV detection.

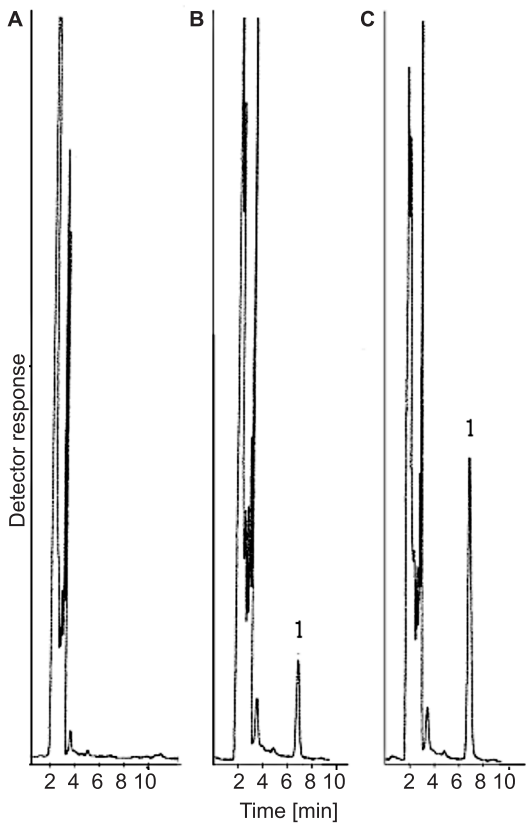

Figure 4. Chromatograms: serum control (A), serum of the patient being treated with linezolid (before infusion) (B), 7 h after the start of the infusion (C). (1) indicates the position of linezolid

Table 1. Drugs commonly prescribed with the linezolid study of the specificity of the analytical method by HPLC-UV.

Antibiotic	Retention time [min]
Linezolid	6.9
Amoxicillin	< 3.5
Imipenem	< 3.5
Meropenem	< 3.5
Vancomycin	<3.5
Ceftazidime	< 3.5
Cefuroxime	3.5
Cefepime	< 3.5
Cefoperazone	4.04
Ciprofloxacin	3.75
Piperacillin	10.0

The study protocol was approved by the Bioethics Committee of the Jagiellonian University (No. KBET/85/B/2006).

RESULTS

Chromatographic characteristics

Typical chromatograms for a blank serum, low (0.5 µg/mL) and high (20.0 µg/mL) concentration of linezolid in serum are shown in Figures 2 and 3. The retention time of linezolid was in the range 6.9 – 7.2 min and each chromatographic run required approximately 9 min. Figure 4 presents chromatograms obtained from analysis of (A) a blank serum; (B, C) a serum obtained from a patient treated with linezolid that had been administered intravenously at the standard dosage (600 mg) every 12 h.

Presented in Figure 4 chromatograms of serum samples, show, respectively, control and examined patient (K.L.) treated with linezolid, and confirmed the good separation of determined compound in the biological matrix. There was no interference of the tested antibiotic with the components of biological background, metabolites and the other drug co-administered (fraxiparine, metoclopramide, amizepine, pentoxifylline, acetylcysteine, omeprazole, fluconazole) to the patient during hospitalization.

Selectivity of the assay

Blank serum showed no interfering endogenous substances in the analysis of linezolid. Potentially co-administered drugs tested had retention times that were different from linezolid or were not extracted and detected. Retention time of linezolid was 6.9 – 7.2 min and all other drugs were eluted without interaction with the signal of linezolid (see Tab. 1).

Linearity of the assay

The linearity study was carried out with concentrations ranging from 0.5 to 30.0 µg/mL in serum. Calibration curves exhibited excellent linearity through the coefficient of correlation r = 0.9995 and 0.9997, respectively, for HPLC/UV and HPLC/DAD determination. Regression intercepts for the calibration curves were not statistically significant compared to zero. Standard curves obtained from different calibration lines performed on different days were $y = 103148 \cdot x - 47771.6$ for HPLC/UV and $y = 206185 \cdot x - 7870.8$ for HPLC/DAD determination, where y = peak area and x = concentration.

Limit of detection and lower limit of quantification

The lower limit of detection was determined to be 0.1 µg/mL of serum at a signal-to-noise ratio 3 : 1 for both detectors. The lower limit of quantification of 0.25 µg/mL (CV = 2.6%) was established for determination using HPLC/UV and 0.5 µg/mL (CV = 5.42%) for HPLC/DAD. These values are sufficient for the determination of therapeutic concentrations of studied drug. Based on the above data it can be concluded, that the UV detector has a higher sensitivity than the DAD detector.

Recovery of the analyte

Peaks area data of spiked serum samples were compared to the results of aqueous solutions at the same nominal concentrations. Recovery of spiked serum samples approximated to 100%, with average 100.5 ± 4.3% (CV = 4.27%). It shows very high extraction efficiency.

Accuracy and precision

The precision and the accuracy of the HPLC/UV and DAD developed methods are given in Tables 2 and 3. Intra-day accuracy ranged from 0.97 to 12.63% and 0.74 to 10.85% for HPLC/UV and HPLC/DAD method, respectively. However, intra-day precision was less than 4.69% for HPLC/UV and less than 5.42% for HPLC/DAD method.

From the results it can be concluded that the developed method has good precision and excellent accuracy in the range of tested concentrations.

The Student's t-test was applied between the experimental values obtained in the sample analysis by the two methods regarding the accuracy and the precision. The linezolid levels obtained for 84 blood samples by HPLC/UV (n = 42) and HPLC/DAD (n = 42). We found: HPLC/UV (µg/mL) = HPLC/DAD (µg/mL) – 0.0925 (µg/mL) with a correlation coefficient r = 0.998. Figure 5 shows the correlation and the 95% confidence interval.

The Student's t-test did not reveal significant difference between the experimental values obtained in the sample analysis by the two detectors.

Stability

The study was based on determining the concentration of linezolid in the pre-prepared samples, stored at the proper temperature for a certain time and then determining the percentage of degeneration of the drug under the influence of temperature.

The stability of linezolid was investigated at three different conditions: at 22 ± 1^{o}C (room tem-

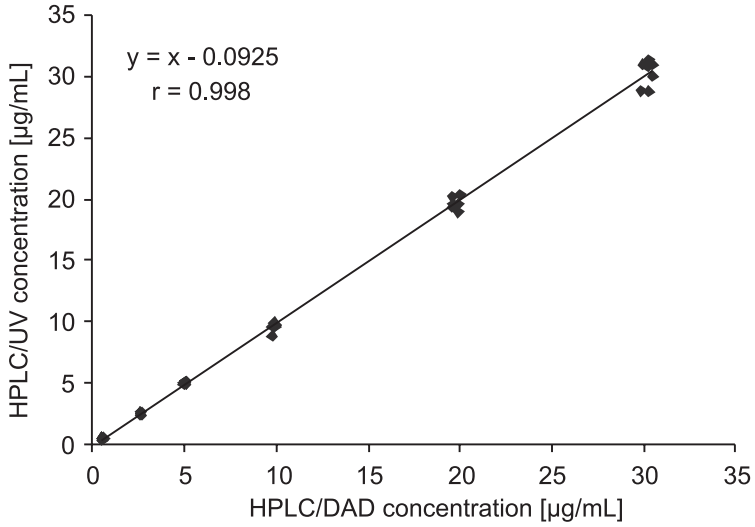

Figure 5. The HPLC/UV-HPLC/DAD correlation for 82 serum samples. The 95% confidence interval is shown

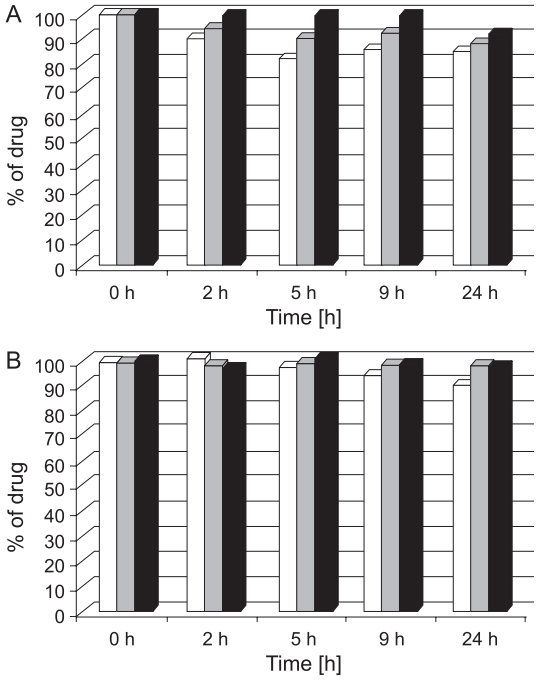

Figure 6. Short-term and long-term stability of linezolid at 1.0, 7.5 and 15.0 µg/mL in human serum samples at room temperature (**A**), at 4°C (**B**) storage for 24 h and at -30°C for 44 days (**C**)

perature), at 4°C (room in the HPLC autosampler) for 24 h and at –30°C (for 44 days). The linezolid levels in serum were measured by HPLC/UV and by HPLC/DAD. These results are shown in Figure 6.

Based on the results, we can conclude that the drug is stable and does not undergo significant disintegration in all studied temperatures. At room temperature after 24 h, a decrease of the amount of linezolid in human serum samples was 15% for lower and 7% for higher concentrations. These data indicated also that linezolid in human serum samples was stable for 24 h when stored at the temperature 4°C (room in the HPLC autosampler). Linezolid was completely stable in frozen human serum at –30°C for at least 44 days.

The stability of linezolid in serum and aqueous samples after one to three freeze-thaw cycles was evaluated. After each thawing, concentration of the drug was tested and percentage of degradation of linezolid was calculated. Results are illustrated in Figure 7.

The tests for determining the stability of linezolid in human serum and water demonstrated that the linezolid samples are stable for at least three freeze-thaw cycles. After three cycles of freeze-thaw, the concentration of linezolid in human serum was 90.7 ± 0.58% and in water was 92.8 ± 0.29% of the mean concentration of the fresh prepared samples.

Application to pharmacokinetic studies in patients

This analytical method was applied to quantify serum linezolid concentration for clinical pharmacokinetic studies. A complete steady-state patient curve after administration of 600 mg Zivoxid® *i.v.* every 12 h is shown Figure 8.

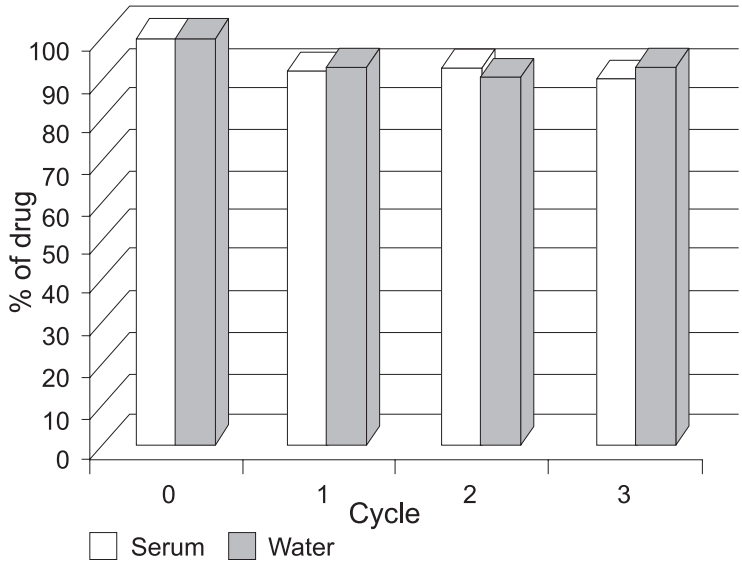

Figure 7. Tests confirmed the stability of linezolid in human serum and in water during three freeze–thaw cycles. The linezolid levels in serum were measured by HPLC/UV and by HPLC/DAD

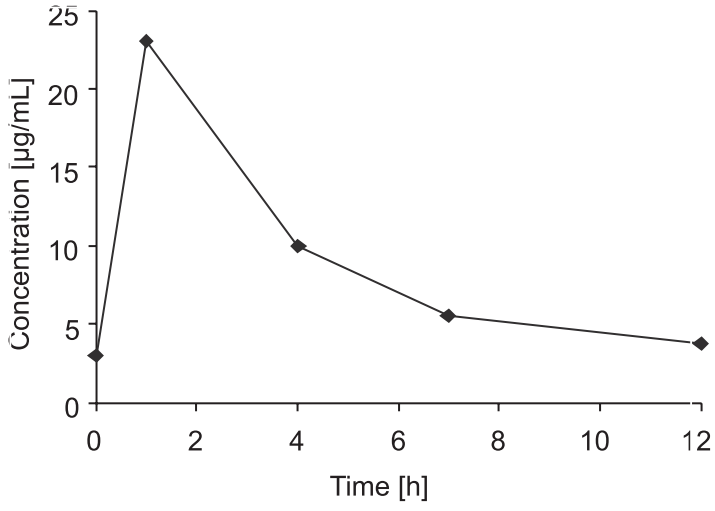

Figure 8. Serum linezolid concentration *versus* time profile at steady state in critically ill patient after its administration of a 600 mg infusion every 12 h

DISCUSSION

An analytical method for the rapid and precise determination of linezolid in human serum using protein precipitation and UV or DAD detection has been developed.

During the validation of described method, piperacillin was used as an internal standard. We gave up using this internal standard similarly as Swoboda et al. (18) because no significant differences in precision and accuracy during method validation had been found. Furthemore, longer time of analysis had been noted (15 min). We also did not encounter any problems with retention time stability or column lifetime. More than 1500 samples were successfully analyzed on the same column.

The advantage of our method over those previously published lies in the simple preparation of the samples. We evaluated the use of acetonitrile, methanol and 10% perchloric acid for protein precipitation. The best recoveries were obtained using methanol (data not shown). Compared to other procedures reported in the literature, our method is cost-effective (12, 13, 19). The relatively low price of a single column and reagents are important for pharmacokinetic and clinical laboratory investigations.

In the presented analytical method we used only 200 µL of serum samples. Small volume of sample is very important for pharmacokinetic studies, particularly in critically ill patients and children. We were able to develop a rapid HPLC assay with a total run time < 10 min. The retention times of linezolid in serum/plasma described by other authors were 10 and 20 min (10, 13, 25).

The blank serum showed no interference of endogenous substances in the analysis of linezolid. Potentially co-administered drugs had different retention times, compared to linezolid or were not extracted and detected. Calibration range was based on human serum linezolid concentrations during the pharmacokinetic steady-state when linezolid was administered at 600 mg twice daily intravenously. The calibration ranges reported in previously described linezolid assays for plasma samples extend only to 20.0 µg/mL (15, 17, 25). In contrast, the calibration range from 0.50 to 30.0 µg/mL used in this assay is more applicable for use in a clinical setting, because such high linezolid concentrations have been reported (27, 28). The complete recovery of linezolid from the serum (approximately 100%) reported for this current method provides evidence that this simple extraction procedure is satisfactory. Acceptable variations in intra-day precision and accuracy testing were observed for both method (HPLC/UV and DAD). Statistical analysis of the results obtained

Table 2. Intra-day accuracy and precision from the determination of linezolid in human serum by HPLC-UV assay (n = 7).

Linezolid concentration Cnom [µg/mL]	HPLC-UV		
	Determined concentration of linezolid [µg/mL]	CV [%]	Relative error (RE) [%]
0.5	0.56 ± 0.03	4.69	12.63
2.5	2.67 ± 0.10	3.59	6.65
5.0	5.12 ± 0.13	2.63	2.40
10.0	9.70 ± 0.35	3.56	3.04
20.0	19.66 ± 0.51	2.60	1.68
30.0	30.29 ± 1.05	3.47	0.97

Table 3. Intra-day accuracy and precision from the determination of linezolid in human serum by HPLC-DAD assay (n = 7).

Linezolid concentration Cnom [µg/mL]	HPLC-DAD		
	Determined concentration of linezolid [µg/mL]	CV [%]	Relative error (RE) [%]
0.5	0.55 ± 0.03	5.42	10.85
2.5	2.58 ± 0.04	1.74	3.18
5.0	5.06 ± 0.03	0.67	1.11
10.0	9.89 ± 0.08	0.76	1.06
20.0	19.69 ± 0.18	0.92	1.53
30.0	30.22 ± 0.29	0.97	0.74

using Student's t-test revealed no significant difference between the performance of the two methods regarding the accuracy and precision. This method showed a LOQ of 0.25 μg/mL for HPLC/UV and 0.5 μg/mL for HPLC/DAD method and required only 200 μL serum samples. Other published methods using protein precipitation showed LOQs similar to our method (0.25-1.0 μg/mL), but required much higher volume of plasma (up to 500 μL). LOQs were sufficient for clinical applications, as reported minimal concentrations were higher than 0.5 μg/mL at recommended dosages (27, 28). Moreover, according to the European Committee on Antimicrobial Susceptibility Testing, clinical breakpoints of linezolid (ranging from 0.5 to 4.0 μg/mL) are greater than our LOQ, allowing clinical applications of this method (29).

Under investigated conditions, no degradation of tested compound was observed. Linezolid concentrations remained stable after three freeze-thaw cycles of serum samples, storage on the bench top at room temperature for up to 24 h and over 6 weeks at –30°C. Extracts were also stable in the autosampler for 24 h. These results are consistent with previous reports (12, 13, 15, 17-19, 25).

The method was used for one critically ill patient for therapeutic drug monitoring, and individual dosage regimen of linezolid. Linezolid serum concentration monitored in patient was 23 μg/mL at the 1st h (15-30.0 μg/mL, reference) and through concentrations of 3.8 μg /mL at 12th h (3.0-10.0 μg/mL, reference). Obtained results indicate that HPLC/UV and DAD analyses yield reliable results for monitoring linezolid levels in blood samples. This assay shares the favorable attributes of being simple and rapid compared to two previously reported assays. Moreover, short retention time (9.0 min) is appropriate and convenient for analysis of clinical samples.

CONCLUSION

In this report, we describe an assay we developed for measuring the concentration of linezolid in low-volume serum samples. This method appears to be suitable for use in the clinical laboratory for drug monitoring and pharmacokinetic studies. Our method can be routinely performed in clinical diagnostic laboratories using a basic HPLC/UV or DAD system. The method is simple, reliable, and reproducible. The lower limits of quantification allow the measurement of linezolid concentrations in plasma and tissue down to the minimal inhibitory concentration values reported for most relevant Gram-positive pathogens, including MRSA (methicillin-resistant *Staphylococcus aureus*) and VRE (vancomycin-resistant *Enterococcus*) (29). In summary, proposed methods can be used for the determination of linezolid in human serum in laboratory as well as in clinical applications.

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