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ORIGINAL RESEARCH

Simple method for the assay of clindamycin in human plasma by reversed-phase high-performance liquid chromatography with UV detector

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ABSTRACT: A rapid and simple high-performance liquid chromatography (HPLC) method was developed and validated for the quantification of clindamycin in human plasma. After precipitation with 50% trichloroacetic acid (TCA) containing the internal standard, propranolol, the analysis of the clindamycin level in the plasma samples was carried out using a reverse-phase cyano (CN) column with ultraviolet detection (204 nm). The chromatographic separation was accomplished with an isocratic mobile phase consisting of acetonitrile–distilled water–7.6 mM tetramethylammonium chloride (TMA) (60:40:0.075, v/v/v), adjusted to pH 3.2. The proposed method was specific and sensitive with a lower limit of quantitation (LLOQ) of 0.2 μg/mL. This HPLC method was validated by examining the precision and accuracy for inter- and intraday analysis in the concentration range 0.2–20.0 μg/mL. The relative standard deviations (RSD) in the inter- and intraday validation were 6.1–14.9 and 6.0–16.1%, respectively. In the stability test, clindamycin was found to be stable in human plasma during the storage and assay procedure. The present HPLC method was applied to the analysis of samples taken up to 12 h after a single oral administration of clindamycin in healthy volunteers. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: clindamycin; HPLC; bioavailability; human plasma

INTRODUCTION

Clindamycin (Fig. 1), an antibiotic drug, is highly effective against Gram-positive and Gram-negative anaerobic pathogens, as well as Gram-positive aerobes (Sweetman, 2002). It is used in the treatment of serious respiratory tract infections, serious skin and soft tissue infections, septicemia, intra-abdominal infections and infections of the female pelvis and genital tract caused by susceptible anaerobic bacteria (Phillips, 1971). Clindamycin can be derived from lincomycin by replacing a hydroxyl group at the 7-position of this precursor with a chlorine group, resulting in an inversion of the configuration.

Several methods have been described in the literature for the determination of clindamycin in biological fluids, including assays based on microbiology (Metzler *et al.*, 1973), gas chromatography (Gatti *et al.*, 1993) and high-performance liquid chromatography (HPLC) using either an RP_{18} column (La Follette *et al.*, 1988; Fieger-Büschges *et al.*, 1999) or a coupled column (Itrud *et al.*, 1999). However, these microbial, gas chromatographic and radioimmunoassay (Duckworth *et al.*, 1993) methods are either non-specific or time-and-reagent consuming. Recently, a liquid chromatographic–tandem mass spectrometric (LC-MS/MS) method (Yu *et al.*, 1999; Cherlet *et al.*, 2002; Martens-Lobenhoffer and Banditt, 2001; Rechberger *et al.*, 2003) was developed to determine clindamycin in human plasma; however, these LC-MS/MS instruments are not yet readily available in all laboratories.

The method presented in this paper, which was validated in a human bioavailability study, was developed

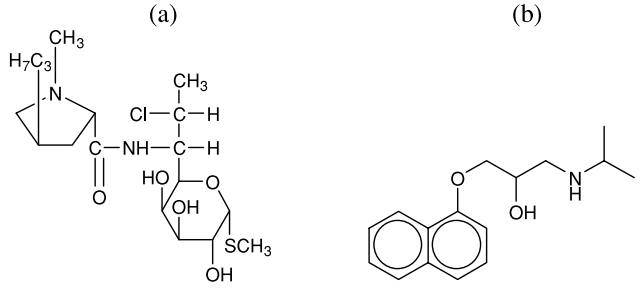

Figure 1. Structures of (a) clindamycin and (b) propranolol (internal standard).

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Abbreviations used: TCA, trichloroacetic acid; TMA, tetramethylammonium chloride.

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for the purpose of providing a simpler sample preparation procedure and more reproducible results than the HPLC methods reported previously (La Follette *et al.*, 1988; Fieger-Büschges *et al.*, 1999).

EXPERIMENTAL

Chemicals and reagents. Clindamycin, the internal standard (IS), propranolol (Fig. 1), phosphoric acid solution and trichloroacetic acid (TCA) solution were supplied by Sigma (St Louis, MO, USA). Tetramethylammonium chloride (TMA) was purchased from TCI (Tokyo Kasei, Japan). Acetonitrile and methanol were all HPLC grade and supplied by Fisher Scientific (Pittsburgh, PA, USA). Water was obtained from a Milli-Q water purification system (Millipore Corp., Bedford, MA, USA). All other chemicals and solvents used were of analytical grade.

Chromatographic conditions. The HPLC system consisted of a Waters model 515 HPLC pump, a model 717 WISP autoinjector and a model 486 tunable absorbance detector set to 204 nm (Waters Assoc., Milford, MA, USA). The chromatographic data was collected and analyzed using Millenium Chromatography Manager (Waters, version 2.15).

The separation was achieved at 35°C with a Waters Spherisorb (Waters Assoc., Milford, MA, USA) CN (250 × 4.6 mm i.d., 5 μm) reversed-phase HPLC column. The mobile phase used for analysis consisted of a mixture of acetonitrile, distilled water and 7.6 mM TMA (60:40:0.075, v/v/v). The mobile phase was adjusted to pH 3.2 with phosphoric acid, followed by filtration through a 0.45 μM filter, and was delivered at a rate of 1.0 mL/min. A constant slow bubbling of helium through the mobile phase during operation was required to keep the level of oxygen in the system at a minimum. Prior to starting the analysis, the above conditions had to be maintained for 3 h, in order for system equilibrium to be reached.

Preparation of stock solutions and spiked standards. Stock solutions of clindamycin were prepared by dissolving 10 mg of clindamycin in methanol and making the solution up to 100 mL (100.0 μg/mL); these were then stored at 4°C. Working solutions of clindamycin were prepared by serial dilutions with methanol from the primary stock solution. Spiked plasma samples, used as calibration standards, were prepared daily by the addition of 20 μL of the working solutions to 180 μL of drug-free human plasma, resulting in calibration standards with concentrations of 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 and 20.0 μg/mL.

Preparation of plasma samples. After thawing at room temperature, an aliquot of each sample (200 μL) was pipetted into a polypropylene 1.5 mL snap-cap conical-bottom centrifuge tube (Eppendorf, Hamburg, Germany) and 20 μL of internal standard solution (propranolol 20.0 μg/mL) were added. After vortexing briefly, 20 μL of 50% TCA reagent were added to each sample and the samples were vortexed again for 10 min and then centrifuged for 20 min at 15,000 rpm. The resulting clear supernatant from each sample was transferred to an autosampler vial and a 100 μL aliquot was injected into the HPLC system.

Validation of assay method. The interference of endogenous compounds was assessed by analyzing standard clindamycin, drug-free plasma, plasma spiked with clindamycin, and plasma obtained from subjects given clindamycin. The lower limit of quantitation (LLOQ) was defined as the lowest concentration yielding a precision less than 20% (relative standard deviation, RSD) and an accuracy (expressed as a percentage of the measured concentration to the theoretical concentration) of between 80 and 120% of the theoretical value. The linearity of the calibration curve for clindamycin was assessed in the range 0.2–20.0 μg/mL in the plasma samples. Standard samples were prepared by adding clindamycin to blank plasma at concentrations of 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 and 20.0 μg/mL with 20.0 μg/mL of propranolol (internal standard). The peak height ratios of each clindamycin curve were obtained from the least-squares linear regression line (no weighting factor) and were presented with their correlation coefficients. The regression line was used to calculate the respective concentrations of clindamycin in the plasma samples obtained from volunteers. The inter- and intra-assay relative standard deviation and standard errors of the mean were used to validate the precision and accuracy of the assay through the determination of standard samples of clindamycin in plasma. For the inter-day validation, five sets of control samples at seven different drug concentrations (0.2–20.0 μg/mL) were evaluated on five different days. The range of the relative standard deviations was reported. For the intra-day validation, five sets of controls at seven different drug concentrations were assayed with one standard curve on the same run. The range of the relative standard deviations was also reported. The accuracy was determined by comparing the calculated concentrations using calibration curves based on a known concentration. To assess the absolute recoveries of the clindamycin extracted from the plasma, the peak height ratios of the analytes to the internal standard, which were obtained from the extracted quality control (QC) samples, were compared with those obtained from the mobile phase having the same concentration. The mean recoveries were determined at low, medium and high concentration in three replicates. To test the short- and long-term stability of extracted clindamycin, three QC samples, containing low (0.5 μg/mL), medium (2.0 μg/mL) and high (10.0 μg/mL) concentrations, were determined after several freeze and thaw cycles. The long-term storage stability at –70°C was determined after 2 months. Moreover, the short-term stability of the extracted samples during storage for 24 h at 4°C, room temperature and –20°C was also determined.

Subjects and clinical procedure. The proposed analytical method was utilized in a bioavailability study. This study was carried out on a group of eight healthy volunteers (four male and four female), aged 21–24 years, who were taking no concurrent medication and did not consume any alcoholic beverages during the course of the study. They were an average age of 22.38 ± 1.06 years old, had an average body weight of 60.13 ± 6.47 kg and an average height of 169.5 ± 9.9 cm. All of them were informed beforehand of the content of the study and gave their written consent. On the basis of their medical

history, clinical examination and laboratory investigation, no subject had any history or evidence of renal, hepatic or gastrointestinal disease or drug allergies. The study protocol was approved by the Korean Food and Drug Administration. Subjects were advised not to take any medication for 2 weeks before study and were requested to fast for at least 10 h overnight the day before each treatment. A single dose (600 mg) consisting of four Cleocin® capsules was given to each subject in the fasting state for each treatment period. Fasting was continued for a further 4 h after drug administration. The subjects were provided a standard meal at 4 h (lunch) and 10 h (supper) after drug administration. Blood samples were taken from the healthy volunteers after the oral administration of 600 mg of clindamycin in the form of capsules. Heparinized blood samples (10 mL) were withdrawn from the forearm vein according to the time schedule, which included a blank before drug administration and then at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 6, 8 and 12 h post-dosing. Immediately after sampling, the blood was placed in an ice–water bath, then centrifuged for 10 min at 3000 rpm and the plasma was stored at –70°C until the assay was performed.

The following pharmacokinetic parameters were determined for the period of 0–12 h: the area under the plasma concentration-time curves from time zero to the last measurable clindamycin sample time ($AUC_{0-12\,h}$); the maximum plasma concentration (C_{max}); and the time to reach the C_{max} (T_{max}). The descriptive statistics for pharmacokinetic parameters were computed using WinNonlin Professional Software (version 3.1).

RESULTS AND DISCUSSION

HPLC chromatogram

Under the experimental conditions used in this study, reproducible chromatographic separations between clindamycin and propranolol were obtained in a mixture of acetonitrile, distilled water and 7.6 mM TMA adjusted to pH 3.2 with phosphoric acid (60:40:0.075, v/v/v). Representative chromatograms of a blank plasma sample, a blank plasma sample spiked with clindamycin and a volunteer sample are shown in Fig. 2. The retention times of clindamycin and propranolol (IS) were 13.5 and 18.6 min, respectively. No interferences with either clindamycin or the internal standard, propranolol, were detected in the chromatogram.

Although there have been several reports documenting HPLC assays for the quantification of clindamycin in human plasma and dog serum (Liu *et al.*, 1997; La Follette *et al.*, 1988; Fieger-Büschges *et al.*, 1999; Batzias *et al.*, 2004), these methods have some limitations. The HPLC-UV method developed by La Follette *et al.* (1988) was reported to be non-reproducible by Liu *et al.* (1997), who presented an alternative method employing a silica extraction column that had a much longer sample pre-treatment duration, due to the conditioning of the silica extraction column and the evaporation of the organic solvent, which make this method time-consuming when assaying a large number of samples. Fieger-Büschges *et al.* (1999) reported a simple automatic system for the assay of clindamycin, but unfortunately the required column switching was complex and the peak resolution achieved was ambiguous. Recently a sensitive HPLC method for the determination of clindamycin in dog plasma using a C_{18} reverse-phase column was reported (Batzias *et al.*, 2004). However, this method did not employ an internal standard to prevent possible errors which may occur during sample pre-treatment and injection.

Linearity and sensitivity

The linearity of the detector response was assessed for various extracted plasma standards over the range

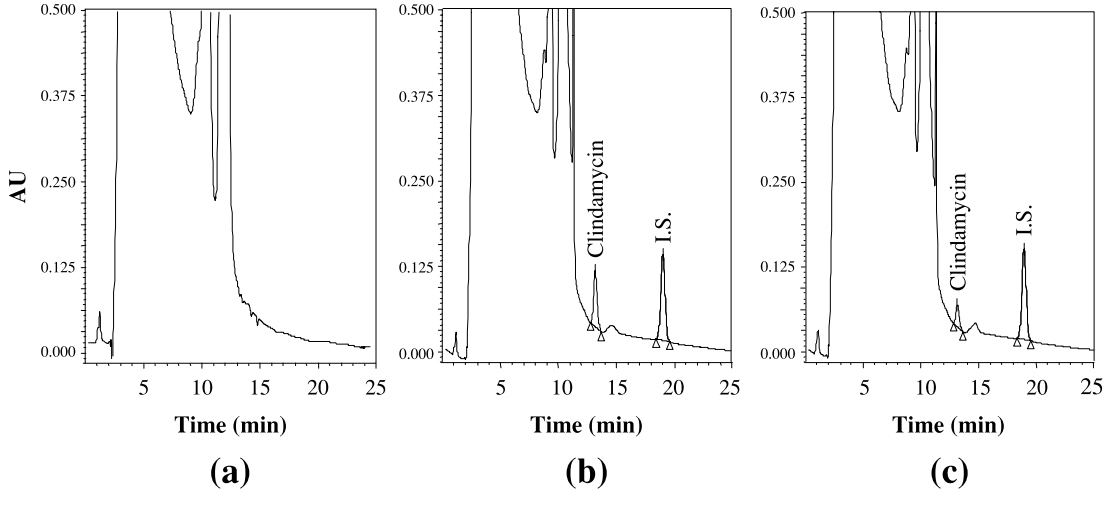

Figure 2. Representative chromatograms of: (a) a blank plasma sample; (b) blank plasma spiked with clindamycin (5.0 μg/mL) and the internal standard propranolol; and (c) a volunteer sample taken 4 h after administration of 600 mg clindamycin, corresponding to a concentration of 2.14 μg/mL.

Table 1. Intra- and inter-day assay precision and accuracy for HPLC assay of clindamycin in human plasma (n = 5 per test)

Theoretical concentration (μg/mL)	RSD (%)		Accuracy (%)	
	Intraday	Inter-day	Intraday	Inter-day
0.2	10.8	14.9	99.1	89.9
0.5	14.1	14.8	103.1	100.1
1.0	14.5	9.2	97.8	93.5
2.0	13.4	10.2	93.7	96.5
5.0	6.0	6.1	104.5	102.0
10.0	6.8	6.2	102.2	102.4
20.0	8.3	6.8	102.6	99.3

0.2–20.0 μg/mL. The calibration curve of clindamycin exhibits excellent linearity and a high correlation coefficient. The mean regression equation from five replicated calibration curves was $y = 0.1258x - 0.0099$ (y = clindamycin concentration, x = ratio of peak heights) with a correlation coefficient of 0.9995. The LLOQ was determined as the concentration of the drug giving a signal-to-noise ratio greater than 5 with an accuracy of between 80 and 120% and with a precision RSD(%) of less than 20%. The LLOQ was estimated to be 0.2 μg, as shown in Table 1.

Precision and accuracy

The inter-day precision and the accuracy were determined by analyzing plasma samples spiked at concentrations of 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 and 20.0 μg/mL. The inter-day precision was determined by analyzing five calibration curves on five different days. The intraday precision and accuracy were determined by analyzing plasma samples spiked at concentrations of 0.2, 0.5, 1.0, 2.0, 5.0, 10.0 and 20.0 μg/mL. The intraday precision was determined by analyzing five replicates on the same day. The precision of the clindamycin level calculated as a relative standard deviation (RSD) was always below 15%. The accuracy of the clindamycin level expressed as a percentage of the measured concentration to the theoretical concentration ranged from 89.9 to 104.5%. The values of the inter- and intraday precision and accuracy for clindamycin in human plasma are presented in Table 1.

Recovery and stability test

The extraction efficiency of clindamycin was evaluated in blank plasma samples and a mobile phase spiked with known amounts of clindamycin and propranolol. The plasma samples were extracted as described above and the recovery was calculated by comparing the peak height ratio of clindamycin to propranolol obtained from the extracted working standard solutions in the plasma, and those resulting from the direct injection of the working standard solutions of clindamycin prepared in the mobile phase having the same concentrations (0.5, 2.0 and 10.0 μg/mL) of clindamycin and propranolol. The mean (± SD) recovery for clindamycin (n = 3) from the plasma samples was 82.9 ± 1.9% at 0.5 μg/mL, 78.8 ± 1.0% at 2.0 μg/mL and 89.9 ± 3.3% at 10.0 μg/mL.

To evaluate clindamycin stability in human plasma, drug-free plasma samples were spiked at 0.5, 2.0 and 10.0 μg/mL. After extraction, samples were arranged in the autosampler and were analyzed. In the short-term stability study, clindamycin and propranolol were found to be stable for 24 h at 4°C, room temperature and –20°C (Table 2). In the long-term stability study, the plasma samples spiked with clindamycin and propranolol also showed no loss of analytes when they were stored for 2 months at –70°C. The final stability test was demonstrated after three freeze–thaw cycles. No significant deterioration of the analytes was observed under any of these conditions (Table 2).

Application of the analytical method to bioavailability study

The method described herein was utilized in our institute in a bioavailability study of clindamycin in human plasma samples obtained after the administration of clindamycin capsules (150 mg, four capsules). The plasma collected from the volunteers prior to the administration of the drug did not reveal the presence of any interfering endogenous peaks. A typical plasma concentration vs time profile of clindamycin from eight healthy volunteers is shown in Fig. 3. After the single oral administration of a 600 mg clindamycin capsules (150 mg, four capsules) to eight healthy volunteers, the measured AUC_{0-12}, C_{max} and T_{max} values were 20.4 μg/mL h, 6.7 μg/mL and 1.1 h, respectively (Table 3).

CONCLUSIONS

We developed a simple HPLC analytical method for the determination of clindamycin in human plasma. The simple pre-treatment procedure and the use of a

Table 2. Stability data for clindamycin (n = 3 per test and each concentration)

	Theoretical concentration (μg/mL)		
	0.5	2.0	10.0
Long-term			
2 months, –70°C (%)	93.22 ± 3.21	98.72 ± 3.13	97.67 ± 2.84
Short-term			
24 h, 4°C (%)	89.24 ± 6.63	93.10 ± 2.51	96.93 ± 5.03
24 h, room temperature (%)	90.04 ± 6.58	92.93 ± 3.84	95.54 ± 1.20
24 h, –20°C (%)	108.04 ± 2.41	104.41 ± 1.87	108.86 ± 2.51
Freeze–thaw stability (%)	89.03 ± 3.40	89.70 ± 4.62	117.20 ± 1.85

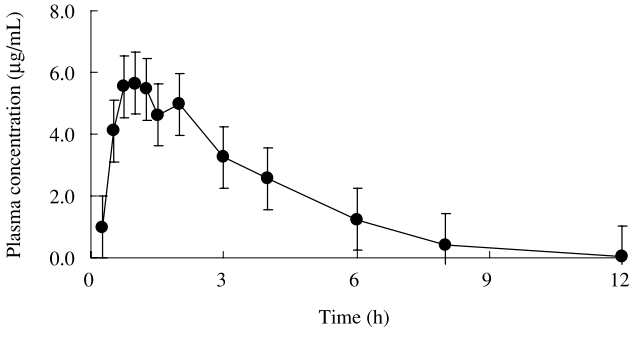

Figure 3. Mean plasma concentration-time profile of clindamycin after the oral administration of 600 mg of clindamycin to eight healthy human volunteers. Each point represents the mean ± SD.

Table 3. The pharmacokinetic parameters of clindamycin 600 mg (150 mg, four capsules) oral dose (each value presents the mean ± SD of eight volunteers)

Parameters	Mean ± SD
$AUC_{0-12\,h}$ (μg/mL h)	20.389 ± 6.117
C_{max} (μg/mL)	6.659 ± 2.144
T_{max} (h)	1.063 ± 0.347
Ke (h^{-1})	0.202 ± 0.068
$T_{1/2}$ (h)	3.767 ± 1.235

CN reverse-phase column provided a simple, rapid and practical procedure. Validation results proved that it was accurate and reproducible. The proposed method was successfully used in a bioavailability study of clindamycin in human plasma. Moreover, it is suggested that the present HPLC analysis method can be used to routinely monitor the concentration of clindamycin.

Acknowledgment

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