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# Determination of clindamycin in human plasma by liquid chromatography–electrospray tandem mass spectrometry: application to the bioequivalence study of clindamycin

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**Abstract**

A simple, sensitive and specific liquid chromatography–electrospray tandem mass spectrometry (LC–MS–MS) method for the determination of clindamycin (**I**) was developed. Both **I** and verapamil (**II**, internal standard) were analyzed using a $C_{18}$ column with a mobile phase of 80% acetonitrile–0.01% trifluoroacetic acid. Column eluents were monitored by electrospray tandem mass spectrometry. Multiple reaction monitoring (MRM) using the parent to daughter combinations of $m/z$ 425→126 and 455→165 was used to quantitate **I**. A limit of quantitation of 0.0500 μg/ml was found. The assay exhibited a linear dynamic range of 0.0500–20.0 μg/ml and gave a correlation coefficient ($r^2$) of 0.998 or better. The chromatographic run time was approximately 2 min. The intra-batch precision and accuracy of the quality controls (QCs, 0.0500, 0.150, 1.50, 15.0 and 20.0 μg/ml) were characterized by coefficients of variation (CVs) of 5.13 to 13.7% and relative errors (REs) of −4.34 to 4.58%, respectively. The inter-batch precision and accuracy of the QCs were characterized by CVs of 4.35 to 8.32% and REs of −10.8 to −4.17%, respectively. The method has successfully been applied to the analysis of samples taken up to 12 h after oral administration of 300 mg of **I** in healthy volunteers. 

*Keywords:* Clindamycin

## 1. Introduction

Clindamycin (**I**, Fig. 1), an antibiotic drug, is highly effective against Gram-positive and Gram-negative anaerobic pathogens, as well as Gram-positive aerobes [1,2]. **I** appears to inhibit protein synthesis in susceptible organisms by binding 50 S ribosomal subunits; the primary effect is inhibition of peptide bond formation [2]. It is used in the treatment of serious respiratory tract infection, serious skin and soft tissue infections, septicemia, intra-abdominal infections and in infections of the female pelvis and genital tract caused by susceptible anaerobic bacteria [2,3].

Both gas chromatography and microbiological

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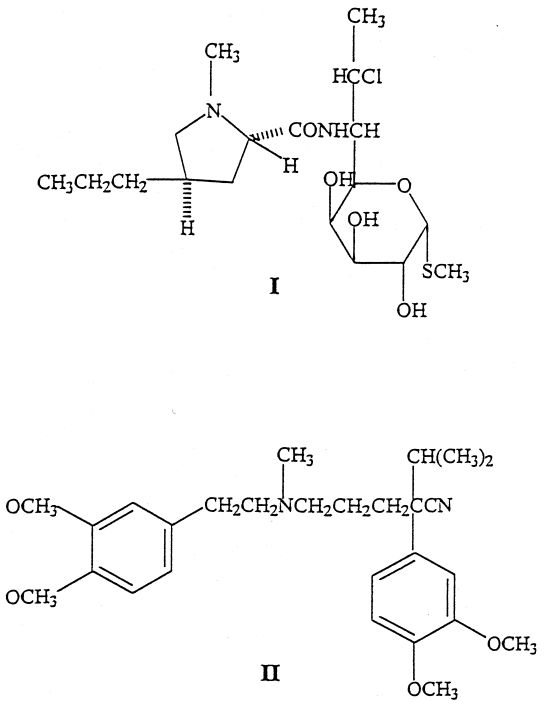


Fig. 1. Structures of clindamycin (**I**) and verapamil (**II,** I.S.).

analytical procedures exist for detecting **I** in biological fluids [4,5]. However, both are time-consuming and labor-intensive. In previous studies, we had successfully developed a more useful analytical method for **I** by high-performance liquid chromatography (HPLC) with UV detection than other reported methods [6]. Now, a simpler sample preparation and faster LC–tandem mass spectrometry (LC–MS–MS) analytical method was developed in this study.

Following oral administration of **I**, peak plasma concentrations occur within 3 h in adults. The plasma half life of **I** is 2–3 h in adults with normal renal function. The plasma half life changes slightly in patients with markedly reduced renal or hepatic function [7,8].

This paper describes a liquid chromatographic method using electrospray tandem mass spectrometry detection. The advantages of the assay for **I** in plasma described in this report include greater assay speed and a simpler sample preparation procedure than found previously [1,6].

## 2. Experimental

### *2.1. Materials and reagents*

Both **I** and internal standard, **II** (Fig. 1), were supplied by Sigma (St. Louis, MO, USA). Acetonitrile and trifluoroacetic acid were supplied by Merck (Darmstadt, Germany).

### *2.2. Instrumentation and chromatographic conditions*

LC was performed using a Waters 616 pump (Waters, Milford, MA, USA) and a Gilson 215 liquid handler autosampler (Gilson, USA). The LC was coupled to a Micromass Quattro LC electrospray tandem mass spectrometer (Micromass UK). The separation was achieved on a 5-μm Hypersil column (50×4.6 mm I.D.). Elution was achieved at ambient temperature with acetonitrile, water and 0.01% trifluoroacetic acid (80:20:0.01, v/v/v) as the mobile phase. The LC system was operated isocratically at 800 μl/min and at room temperature. The column eluent was split and approximately 80 μl/min were introduced into the electrospray ionization source. The nebulizing gas ($N_2$) flow was set at 74 l/h. The instrument was programmed for a scan dwell time of 70 ms. The responses of **I** were measured in the positive ion mode using the technique of MRM (multiple reaction monitoring). The protonated molecular ions of **I** (*m/z* 425) and **II** (*m/z* 455) were dissociated by collision in the collision cell. The mass spectrometer was set to selectively monitor parent to daughter fragments of *m/z* 425→126 for **I** and *m/z* 455→165 for **II**. The peak area ratios for calibration curves and quantitation were made using Micromass Masslynx 3.0.

### *2.3. Preparation of standard and quality control samples*

Stock solutions of **I** and **II** (1.00 mg/ml) were prepared in 50% $CH_3CN–H_2O$ and were diluted with 50% $CH_3CN–H_2O$ to obtain the desired concentrations. The stock solutions were kept refrigerated and were discarded one month after their preparation.

The nominal plasma concentrations of calibration standards were 0.0500, 0.100, 0.200, 0.500, 1.00, 2.00, 5.00, 10.0 and 20.0 μg/ml. The **II** stock solution (10.0 μl) was diluted (to 1000 ml) with 100% $CH_3CN$. Five levels of quality controls (QCs), at 0.0500, 0.150, 1.50, 15.0 and 20.0 μg/ml (LOQ, low, medium, high and maximum) were prepared.

### 2.4. Deproteination procedures

Each standard curve of plasma sample (0.100 ml) was pipetted into a 100×16 mm glass test tube, spiked with **I**, vortex-mixed for 30 s, spiked with 1.00 ml of internal standard (10.0 ng/ml of **II**) and vortex-mixed for 60 s. QC plasma samples and human samples for the determination of **I** were prepared as described above, but were not spiked with **I**. Following centrifugation at 1400 *g* for 5 min, the supernatant was transferred to a clean auto-sampler vial and 20 μl was injected into the LC–MS–MS system.

### 2.5. Method validation

Plasma calibration curves and six replicates of the QCs were analyzed. The peak areas generated by MRM of **I** and **II** were obtained. The ratios of the peak areas of **I** to the peak areas of **II** were calculated. The calibration curves were constructed by weighted ($1/y$) least-squares linear regression analysis of the peak-area ratios of **I**/**II** versus the concentrations of **I**. Calibration curve equations were used to calculate the concentrations of **I** in the samples and QCs from their peak area ratios.

The intra-batch precision and accuracy were determined by analyzing a set of QC samples ($n=6$) at each of the five levels, 0.0500, 0.150, 1.50, 15.0 and 20.0 μg/ml. The inter-batch precision and accuracy studies were also carried out by analyzing QC samples at the above five concentrations.

### 2.6. Recovery

The deproteination recoveries of **I** from human plasma were calculated by comparing the peak area ratios of the plasma samples with solvent samples. The recovery study was carried out by spiking human blank plasma samples with **I** at five concentrations, 0.0500, 0.150, 1.50, 15.0 and 20.0 μg/ml. Plasma sample data were obtained by analysis of spiked samples prepared as described except that $CH_3CN$ (1.00 ml) was added instead of internal standard and 10 μl of internal standard (10.0 ng/μl of **II**) were added after the transfer step. Solvent sample data were obtained in the same way as for plasma samples, except that the drug was added after the deproteination step.

## 3. Results and discussion

### 3.1. Method development

LC–MS–MS for the determination of **I** in human plasma was investigated. The mass spectrum (MS) of **I** showed a protonated molecular ion ($MH^+$) at $m/z$ 425 (Fig. 2a). A collisionally activated dissociation (CAD) product ion spectrum for **I** yielded a high-abundance fragment ion at $m/z$ 126 (Fig. 2b). The MS of **II** showed a protonated molecular ion ($MH^+$) at $m/z$ 455 (Fig. 2c) and the high-abundance fragment ion was observed at $m/z$ 165 (Fig. 2d). The product ions at $m/z$ 126 and 165 were used to obtain responses of **I** and **II**, respectively.

### 3.2. Separation and specificity

Four typical chromatograms from the study of **I** in human plasma are shown in Fig. 3. Short retention times of less than 2 min were achieved for both **I** and **II**. **I** eluted at 0.9 min and the internal standard at 1.1 min. Ion chromatograms for **I** and **II** from blank plasma (Fig. 3a), plasma spiked with **II** (Fig. 3b), plasma spiked with **I** (0.0500 μg/ml) and **II** (Fig. 3c) and a sample from a volunteer (3.32 μg/ml) 1 h after oral administration of 300 mg of clindamycin (Fig. 3d) are shown in Fig. 3. For both the drug and the I.S., the chromatograms were free of interfering peaks at their respective retention times.

### 3.3. Linearity, precision and accuracy

Table 1 presents the accuracy, precision and linearity of six standard curves. Calibration curves

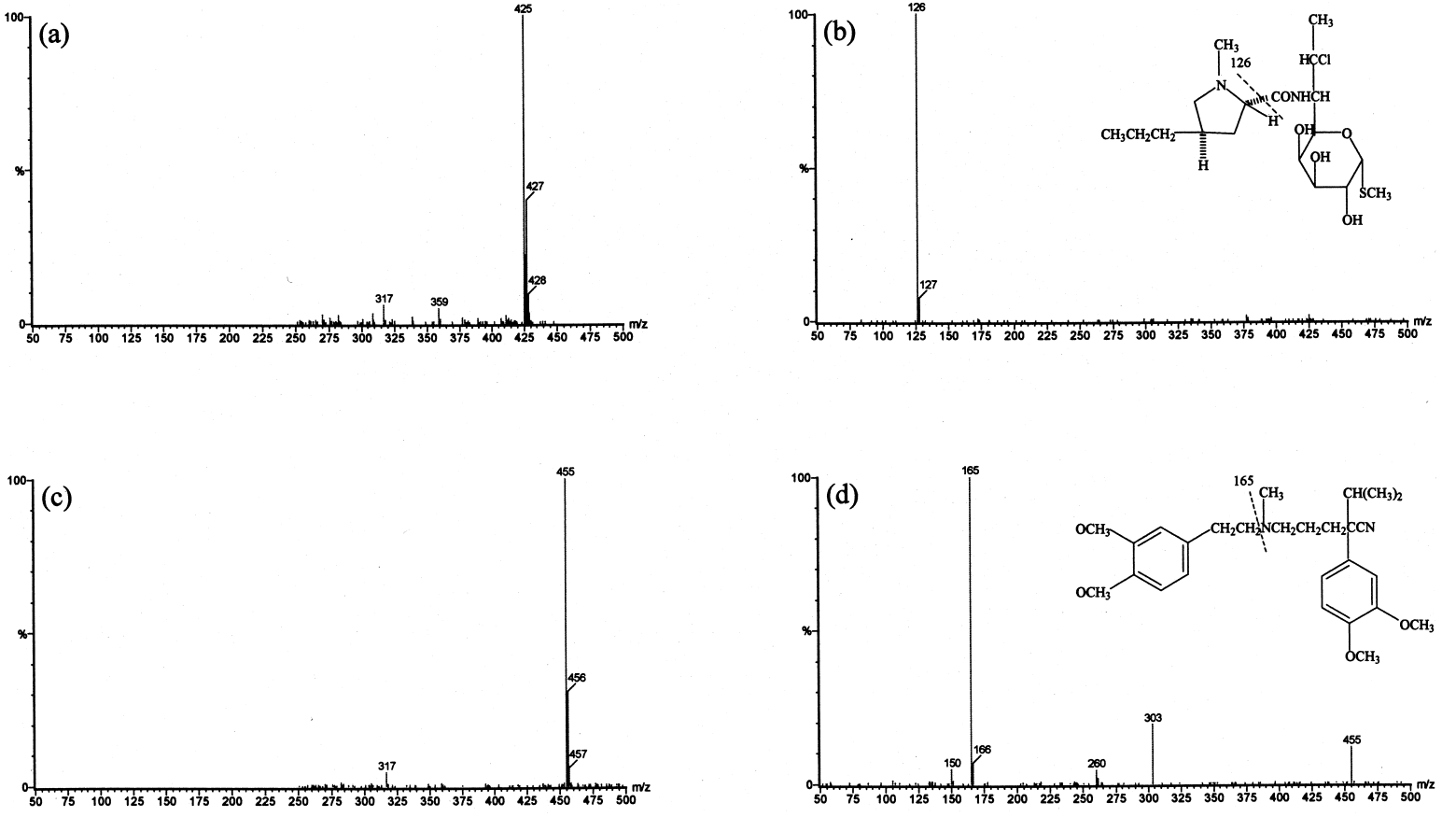


Fig. 2. Mass spectra of (a) clindamycin parent ion, (b) clindamycin daughter ion, (c) verapamil parent ion and (d) verapamil daughter ion.

were plotted as the peak area ratio (drug/I.S.) versus drug concentration. The assay was linear in the concentration range of 0.0500–20.0 μg/ml. The CVs were less than 12%. The REs of the mean of the measured concentrations ranged from −0.417 to 1.85%. The correlation coefficients ($r^2$) were greater than 0.998 for all of the curves.

The precision and accuracy of this method were checked by calculating the intra-batch and inter-batch variation at five concentrations (0.0500, 0.150, 1.50, 15.0 and 20.0 μg/ml) of QC samples in six replicates. As shown in Table 2, the intra-batch CVs and REs were less than 14% and ranged from −4.34 to 4.58%, respectively. As shown in Table 3, the inter-batch CVs and REs were less than 9% and ranged from −10.8 to −4.17%, respectively. These results indicate that the method was reliable within the analytical range, and the use of the internal standard was very effective for reproducibility by LC–MS–MS.

### 3.4. Deproteination recovery

Deproteination recovery was calculated by comparing the peak area ratios of **I** in plasma samples with the peak area ratios of solvent samples. As shown in Table 4, the recovery of **I** was determined at five different concentrations (0.0500, 0.150, 1.50, 15.0 and 20.0 μg/ml). The recoveries of **I** were

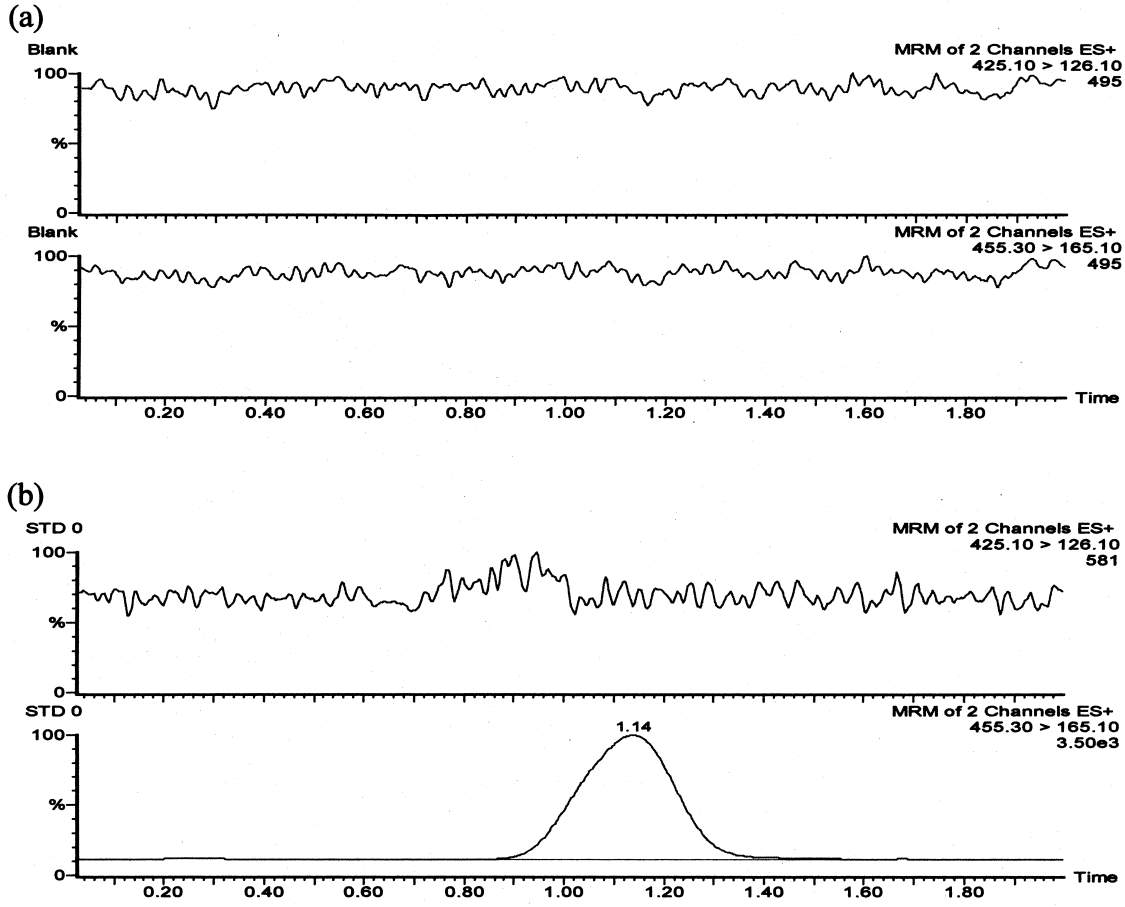


Fig. 3. Representative MRM chromatograms of clindamycin in human plasma: (a) blank human plasma, (b) blank human plasma spiked with the internal standard, verapamil, (c) spiked human plasma containing 0.0500 μg/ml clindamycin and (d) a sample from a volunteer (3.32 μg/ml) 1 h after oral administration of two capsules of clindamycin HCl.

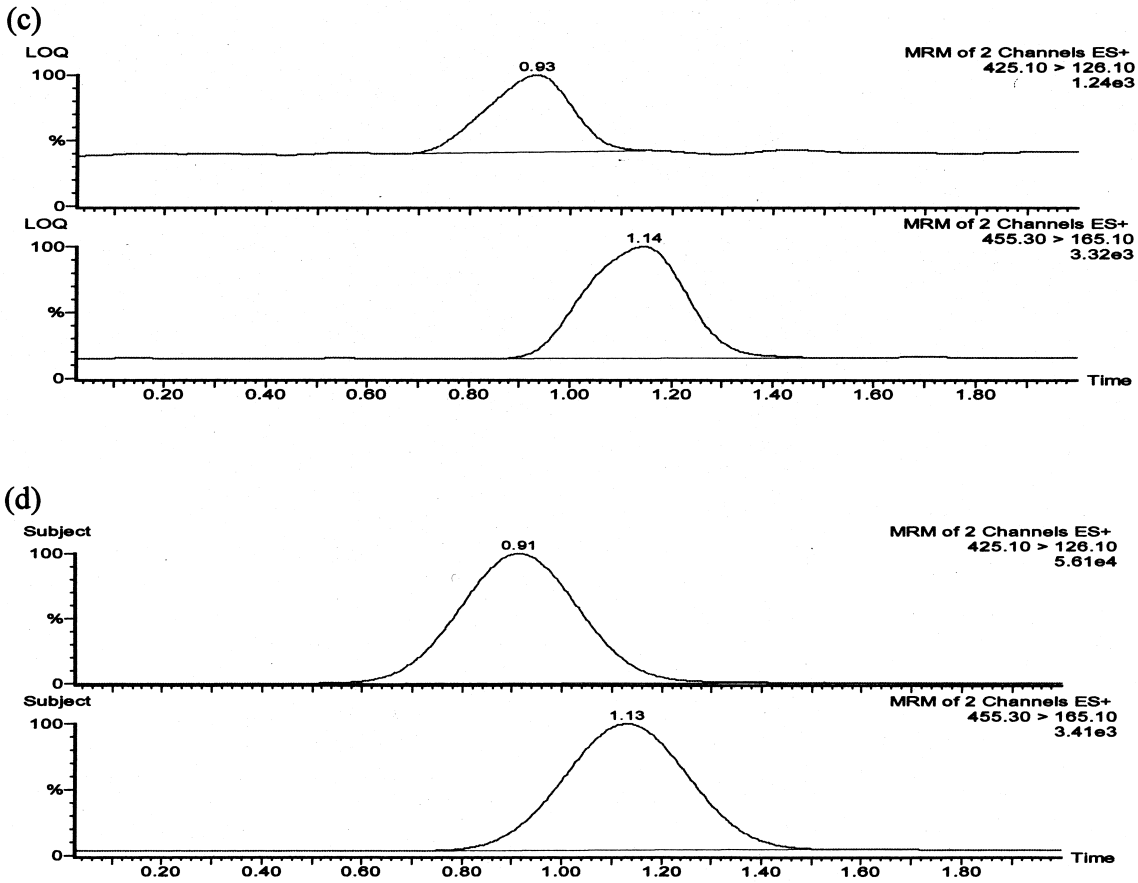


Fig. 3. (*continued*)

Table 1
Calibration curve statistics for clindamycin in plasma[a,b]

| Normal conc. (μg/ml) | Calculated conc. (μg/ml) | CV (%) | RE (%) |
|---|---|---|---|
| 0.0500 | 0.0502±0.0056 | 11.1 | 0.400 |
| 0.100 | 0.102±0.010 | 10.2 | 1.67 |
| 0.200 | 0.200±0.015 | 7.47 | −0.0833 |
| 0.500 | 0.502±0.050 | 9.87 | 0.367 |
| 1.00 | 1.01±0.07 | 6.77 | 0.467 |
| 2.00 | 1.99±0.17 | 8.56 | −0.417 |
| 5.00 | 5.01±0.29 | 5.74 | 0.133 |
| 10.0 | 10.2±0.3 | 2.98 | 1.85 |
| 20.0 | 19.9±0.5 | 2.66 | −0.667 |

[a] RE, relative error of the mean (%)=(calculated conc.−normal conc.)/normal conc.×100.
[b] $n=6$.

Table 2
Intra-batch precision and accuracy for clindamycin[a]

| Normal conc. (μg/ml) | Calculated conc. (μg/ml) | CV (%) | RE (%) |
|---|---|---|---|
| 0.0500 | 0.0491±0.0067 | 13.7 | − 1.79 |
| 0.150 | 0.154±0.010 | 6.61 | 2.60 |
| 1.50 | 1.57±0.08 | 5.13 | 4.58 |
| 15.0 | 14.3±0.8 | 5.54 | −4.34 |
| 20.0 | 19.5±1.0 | 5.33 | −2.60 |

[a] $n=6$.

Table 3
Inter-batch precision and accuracy for clindamycin[a]

| Normal conc. (μg/ml) | Calculated conc. (μg/ml) | CV (%) | RE (%) |
|---|---|---|---|
| 0.0500 | 0.0446±0.0019 | 4.35 | −10.8 |
| 0.150 | 0.144±0.012 | 8.32 | −4.17 |
| 1.50 | 1.36±0.07 | 5.39 | −9.05 |
| 15.0 | 13.6±0.6 | 4.40 | −9.49 |
| 20.0 | 18.8±1.5 | 7.80 | −5.77 |

[a] $n=6$.

84.5–99.8% for these tests, and the overall average recovery was 92.3%.

### *3.5. Application*

Statistical analysis of **I** in plasma samples was performed for a randomized, two treatment, cross-over study in which twelve healthy subjects between the ages of 20 and 40 years received 300 mg of **I**. Fig. 4 shows a profile of the mean plasma concentrations ($n=12$) of generic **I** hydrochloride (test drug) and Cleocin (reference drug, Upjohn) versus time. Quantifiable levels of **I** were detected for up to 12 h after each treatment. The maximum mean plasma concentration was 3.5–3.7 μg/ml for test and reference substances. The apparent elimination half-life was 2.4–2.6 h. No statistically significant difference was observed between the two drugs using a 90% confidence interval by two one-sided test procedures. The plasma concentrations were similar to those found earlier using an HPLC–UV method [6].

## 4. Conclusions

In conclusion, the use of LC–MS–MS allows for accurate, precise and reliable measurement of clindamycin concentrations in human plasma for up to 12 h after oral administration of 300 mg to healthy

Table 4
Recovery of clindamycin from plasma[a]

| Conc. (μg/ml) | Mean peak area | | Recovery (B/A, %) |
|---|---|---|---|
| | Solvent samples (A) | Plasma samples (B) | |
| 0.0500 | 0.366±0.020 | 0.346±0.012 | 94.6 |
| 0.150 | 1.07±0.07 | 0.908±0.052 | 84.5 |
| 1.50 | 10.6±0.3 | 9.13±0.31 | 85.9 |
| 15.0 | 82.9±3.0 | 82.7±6.5 | 99.8 |
| 20.0 | 116±3 | 112±8 | 96.6 |

[a] $n=3$.

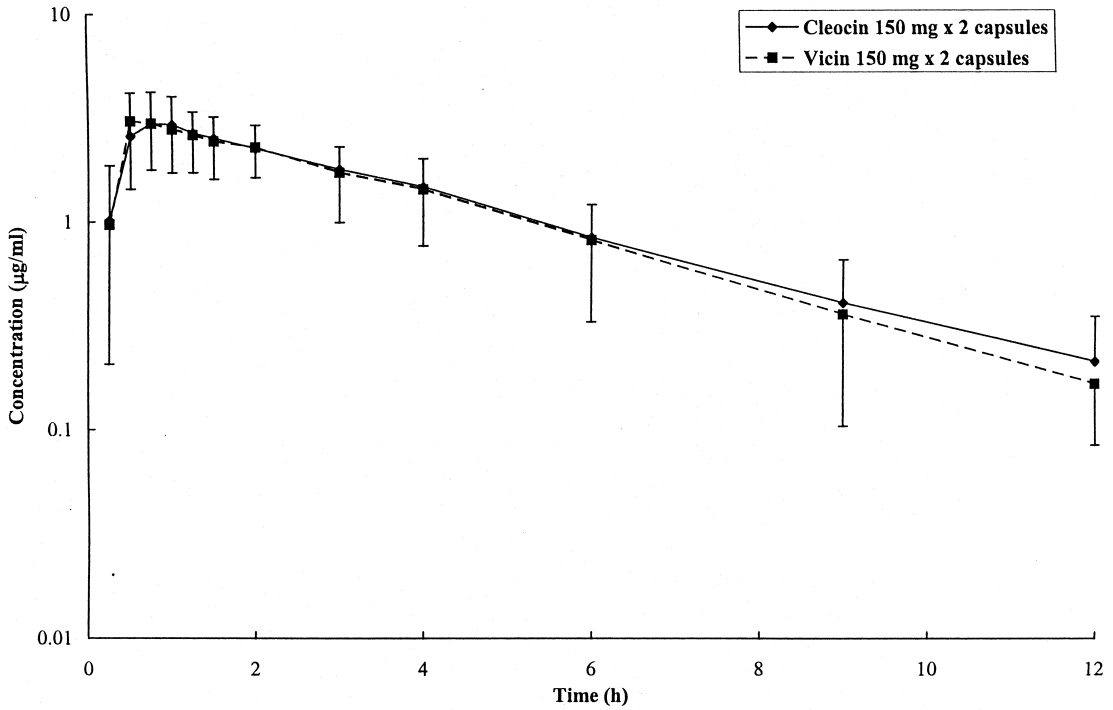


Fig. 4. Plasma concentration of clindamycin for two dosage forms.

volunteers. The assay has proven to be fast and rugged, with each sample requiring less than 2 min of analysis time. The assay method is specific due to the inherent selectivity of tandem mass spectrometry. The method described here has significant advantages over other techniques used for measuring clindamycin in biological fluids. The major advantages of this method are the simple sample preparation procedure, which is a simple protein precipitation method and the rapidity of separation.

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