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Short communication

High-performance liquid chromatographic determination of clindamycin in human plasma or serum: application to the bioequivalency study of clindamycin phosphate injections

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Abstract

This paper presents an assay of clindamycin phosphate injection in human plasma or serum. A 0.5-ml volume of plasma was used with the internal standard, propranolol. The sample was loaded onto a silica extraction column. The column was washed with deionized water and then eluted with methanol. The eluates were evaporated under nitrogen gas. The residue was reconstituted with the mobile phase and injected onto the high-performance liquid chromatographic system: a 5-μm, 25 cm×4.6 mm I.D. ODS2 column was used with acetonitrile, tetrahydrofuran and 0.05 *M* phosphate buffer as the mobile phase and with ultraviolet detection at 204 nm. A limit of quantitation of 0.05 μg/ml was found, with a coefficient of variation of 11.6% ($n=6$). The linear range is between 0.05 and 20.00 μg/ml and gives a coefficient of determination (r^2) of 0.9992. The method has been successfully applied to the bioavailability study of two commercial preparations of clindamycin phosphate injection (300 mg each) in twelve healthy adult male volunteers. © 1997 Elsevier Science B.V.

Keywords: Clindamycin

1. Introduction

Clindamycin (Fig. 1) is a semi-synthetic derivative of lincomycin. It is an antibiotic that is highly effective against Gram-positive and Gram-negative anaerobic pathogens, as well as Gram-positive aerobes [1,2]. Clindamycin phosphate is biologically inactive but is rapidly hydrolyzed in vivo to clindamycin base [3]. Clindamycin 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 infections (e.g. empyema, pneumonia, lung abscess), 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 [2,5]. Following i.m. or i.v. administration, clindamycin phosphate is rapidly hydrolyzed in plasma to active clindamycin and the peak plasma concentrations occur within 3 h in adults. The plasma half life of clindamycin is 2–3 h in adults

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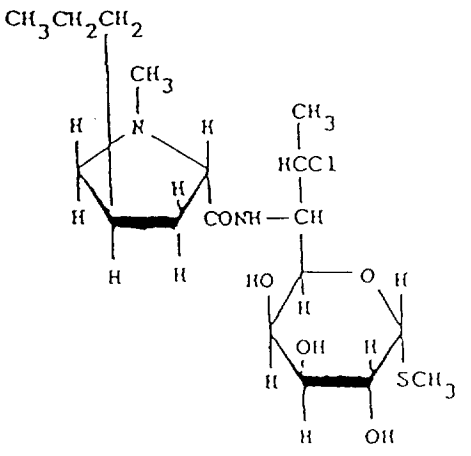
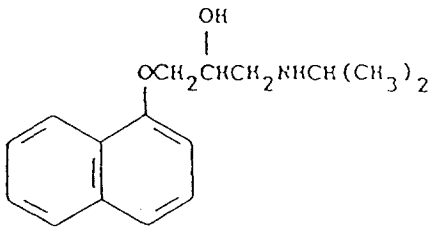


Clindamycin



Internal standard (propranolol)

Fig. 1. Structures of clindamycin and the internal standard (propranolol).

and children with normal renal function. The plasma half life is increased slightly in patients with markedly reduced renal or hepatic function [2–4,6–8].

This paper describes a high-performance liquid chromatographic (HPLC) method using UV detection. The advantages of the assay for clindamycin in plasma described in this report include greater sensitivity than that found previously [1]. The endogenous interferences in plasma samples were removed by passing plasma through silica extraction columns (Merck, 500 mg) and by washing with water. The drugs were eluted with methanol.

2. Experimental

2.1. Standards and reagents

Both clindamycin and the internal standard, propranolol (Fig. 1) were supplied by Sigma (St. Louis, MO, USA). Each silica extraction column prepared by this laboratory consisted of a plastic cartridge (total volume of 3 ml per cartridge) filled with 500 mg of silica gel 60 (Silica gel 60 was purchased from Merck, Darmstadt, Germany). Acetonitrile, methanol, tetrahydrofuran, phosphoric acid and potassium hydroxide were all of HPLC grade or reagent grade and were supplied by Fisher Scientific (Pittsburgh, PA, USA).

Stock solutions, corresponding to 1 mg/ml in methanol, were prepared for both clindamycin free base and the internal standard. Working solutions were obtained by diluting the clindamycin stock solution down to 100.00 and 10.00 μg/ml.

2.2. Instrumentation

A Waters intelligent sample processor, Model 712 (Waters Associates, Milford, MA, USA), and a Waters 510 HPLC pump (Millipore, Waters Associates) were used for this assay. The separation was achieved on a reversed-phase Keystone 5 μm ODS2 column (25 cm×4.6 mm I.D.). Elution was achieved at ambient temperature with acetonitrile, tetrahydrofuran and 0.05 *M* phosphate buffer (pH 5.00; 24:1:75, v/v/v) as the mobile phase. The flow-rate was set to 1.0 ml/min and resulted in a pressure of 1550 p.s.i. A Shimadzu SPD-10A UV–Vis detector was operated at 204 nm.

2.3. Extraction procedures

A plasma or serum sample (0.5 ml) was pipetted into a 15-ml glass test-tube, spiked with 30 μl of internal standard (6 ng/μl of propranolol) and loaded onto the silica extraction column. Each extraction column was preconditioned with one volume of methanol (about 2.5 ml) and one volume of deionized water. After loading the sample, the column was washed with 1 ml of deionized water and with 3 ml of methanol, which was added dropwise. The eluates were collected and evaporated

to dryness in a gentle stream of nitrogen. The residue was reconstituted with 200 μl of mobile phase. An aliquot (30 μl) was injected for HPLC analysis.

3. Results and discussion

The advantage of the silica extraction column used in this study was that, by passing the plasma or serum sample through the cartridge, polar impurities were removed and the clindamycin and internal standard were retained.

Two standard curves covered the range of concentration (0.05–20.00 μg/ml) for clindamycin: 0.05 to 1.00 μg/ml (low curve) for concentrations below 1.00 μg/ml and 0.05 to 20.00 μg/ml (high curve) for concentrations above 1.00 μg/ml. A linear relationship was found throughout these two ranges when the peak height ratios of clindamycin to the internal standard were plotted versus the plasma concentrations of clindamycin. Table 1 presents the accuracy, precision and linearity of six standard curves. The coefficient of variation (C.V.) was within 10%. The relative error (R.E.) of the mean of the measured concentrations ranged from −8.0 to +6.5%. The coefficient of determination (r^2) was greater than 0.9992.

Four typical chromatograms from the determination of clindamycin in human plasma are shown in Fig. 2. All samples were spiked with the internal standard at a concentration of 360 ng/ml. The clindamycin was eluted at 12 min and the internal standard at 21 min. The limit of quantification (LOQ) for clindamycin was 0.05 μg/ml. The coeffi-

Table 1
Accuracy and linearity of the HPLC method for the determination of clindamycin spiked in plasma samples

Analyte spiked (μg/ml)	Analyte found (mean±S.D., n=6) (μg/ml)	C.V. (%)	Relative error[a] (%)
0.050	0.046±0.004	8.7	−8.0
0.100	0.097±0.008	8.0	−3.0
0.200	0.213±0.009	4.5	+6.5
0.500	0.482±0.007	1.4	−3.6
1.000	1.007±0.011	1.1	+0.7
2.000	2.032±0.015	0.8	+1.6
5.000	5.122±0.033	0.7	+2.4
10.000	10.382±0.079	0.8	+3.8
20.000	19.774±0.103	0.5	−1.1

Linear regression for peak height ratios:
Low range: 0.05–1.00 μg/ml
Y=0.6547X−0.003378 (r^2=0.9992)

High range: 0.05–20.00 μg/ml
Y=0.5643X−0.005365 (r^2=0.9993)

[a] Relative error of the mean (%)=(true concentration−mean measured concentration)/true concentration×100.

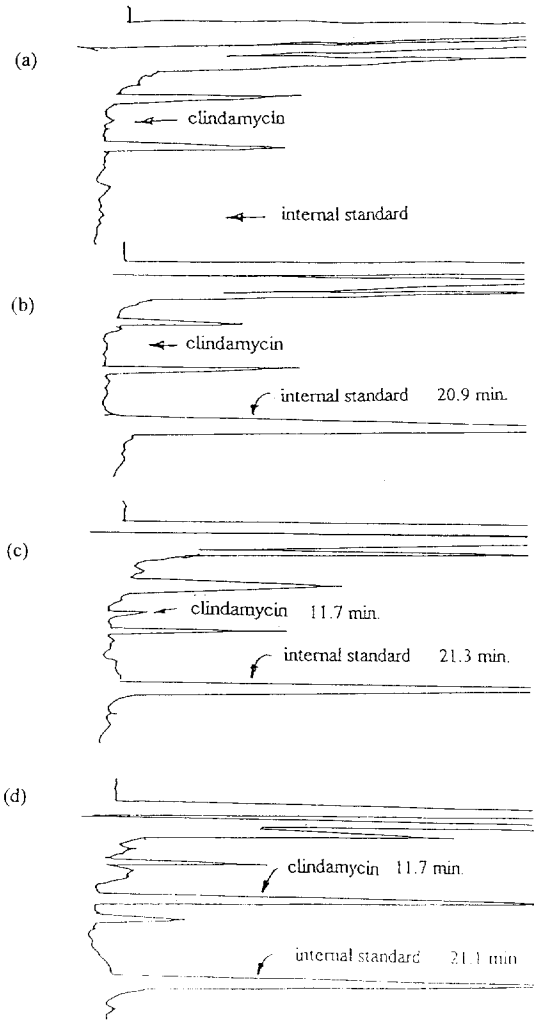


Fig. 2. Chromatograms of clindamycin in human plasma: (a) blank human plasma, (b) blank human plasma spiked with the internal standard, propranolol, (c) spiked human plasma containing 0.05 μg/ml clindamycin and (d) volunteer sample (0.83 μg/ml) 2 h after intramuscular administration of 300 mg of a clindamycin phosphate injection solution.

cient of variation of the LOQ for quality control samples was 11.6% ($n=6$).

The precision of this analysis was checked by calculating the intra-day and inter-day (six days) variation of four concentrations of quality control samples. The results are given in Table 2. The range of the C.V. was within 11%.

Extraction recovery was calculated by comparing the peak height ratios of clindamycin in plasma samples to the peak height ratios in methanol samples. The plasma samples were processed with silica extraction columns as described. Both sets of samples were spiked with equivalent amounts of internal standard before the nitrogen evaporation step. The average recovery evaluated for four drug concentrations was 97.33% for clindamycin (Table 3).

Statistical analysis of clindamycin in plasma samples was performed for a randomized, two treatments, crossover study in which twelve healthy subjects between the ages of 20 and 40 years received 300 mg of clindamycin. Fig. 3 shows a profile of the mean plasma concentrations ($n=12$) of generic clindamycin phosphate (test drug injection) and Cleocin® (reference drug injection, Upjohn)

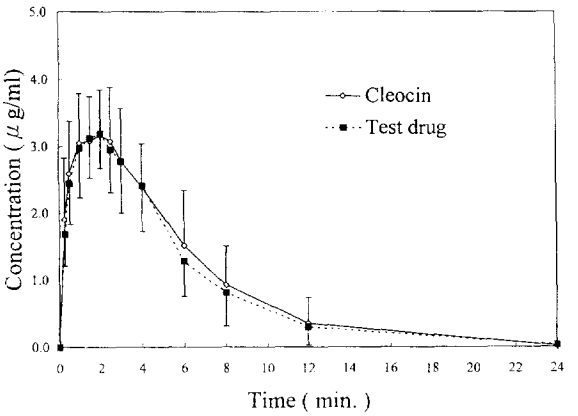


Fig. 3. Mean plasma concentration ($n=12$) versus time profile for two dosage forms.

Table 2
Reproducibility of the HPLC analysis for the determination of clindamycin in plasma samples

Spiked (μg/ml)	Found (μg/ml)	Mean±S.D.	C.V. (%)	Recovery (%)
a. Intra-day clindamycin plasma assay				
0.150	0.133, 0.127, 0.141, 0.138, 0.141, 0.138	0.136±0.005	3.68	91
0.300	0.243, 0.246, 0.273, 0.260, 0.246, 0.244	0.252±0.012	4.76	84
4.000	3.420, 3.511, 3.662, 3.445, 3.432, 3.426	3.483±0.094	2.70	87
15.000	16.501, 17.002, 16.802, 15.023, 15.602, 17.023	16.326±0.827	5.07	91
b. Inter-day (6 days) clindamycin plasma assay				
0.150	0.136, 0.138, 0.141, 0.119, 0.132, 0.151	0.136±0.011	8.09	91
0.300	0.252, 0.260, 0.276, 0.284, 0.299, 0.289	0.277±0.018	6.50	92
4.000	3.620, 3.401, 3.572, 3.895, 3.602, 3.776	3.644±0.172	4.72	91
15.000	16.301, 12.002, 12.802, 13.923, 13.502, 13.423	13.659±1.457	10.67	91

Table 3
Recovery of clindamycin in the plasma assay

Concentration (μl/ml)	Peak height ratio of four concentrations of spiked (methanol and plasma) samples				
	Methanol samples	Mean 1±S.D.	Plasma samples	Mean 2±S.D.	Recovery[a] (%)
0.150	0.061, 0.066, 0.067	0.065±0.003	0.065, 0.067, 0.064	0.065±0.002	100.00
0.300	0.114, 0.116, 0.111	0.114±0.003	0.110, 0.120, 0.109	0.113±0.006	99.12
4.000	1.472, 1.430, 1.383	1.428±0.045	1.372, 1.407, 1.441	1.407±0.035	98.53
15.000	5.817, 5.840, 5.855	5.837±0.019	5.200, 5.115, 5.518	5.278±0.212	90.42

[a] Recovery=100×(Mean 2/Mean 1).
Overall average recovery, 97.02.

versus time. Quantifiable levels of clindamycin were detected for up to 24 h after each treatment. The maximum mean plasma concentration was 3.467 μg/ml for both test and reference substances. The apparent elimination half life was about 3.5 h. No statistically significant difference was observed between the two drugs using a 90% confidence interval by two one-sided test procedures.

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