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Determination of clindamycin in human plasma by high-performance liquid chromatography using coupled columns

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Abstract

A rapid automated method has been developed for the determination of clindamycin, a lincosamide antibiotic, in human plasma. Coupled column HPLC was used after precipitation of plasma proteins with a saturated ammonium sulfate solution. As a first step, the drug and internal standard were trapped on a precolumn of LiChrospher 60RP-select B. A reversed-phase Nucleosil 100 C18 HD column then separated drug and internal standard from each other and from remaining plasma components. The assay was validated in the range 0.2–10.0 $\mu g\,ml^{-1}$ plasma. The results obtained for accuracy, intra- and inter-day precision complied very well with the generally accepted criteria for bioanalytical assays.

Keywords: Clindamycin

1. Introduction

Clindamycin (Fig. 1) is a lincosamide antibiotic with primarily bacteriostatic activity against gram-positive organisms and a wide range of anaerobic pathogens as well as some antiprotozoal efficacy. Due to side effects, it is normally used when other antibiotics are unsuitable [1]. Clindamycin can be derived from lincomycin by replacing a hydroxyl group at the 7-position of lincomycin with a chlorine group, resulting in an inversion of the configuration [2].

Various methods are described in the literature for the determination of clindamycin in biological fluids. These include microbiological [2,3], HPLC [4,5], gas

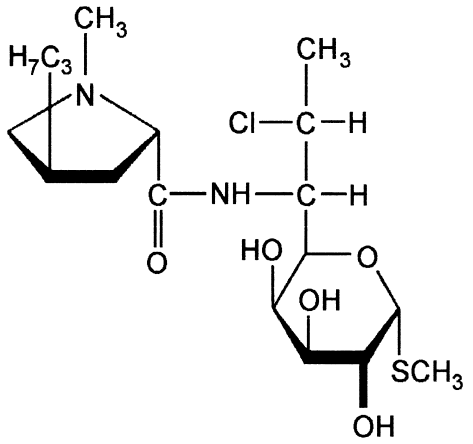

Fig. 1. Chemical structure of clindamycin.

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chromatographic [6] and radioimmuno-assays [7]. However, these methods are less specific (microbiological), more time consuming due to sample preparation [5,6] or not widely accessible. A straightforward HPLC method from the literature [4] using direct injection of plasma after a precipitation step with acetonitrile could not be reproduced in our laboratory due to interferences from plasma components. For pharmacokinetic studies, the same group later used a GC method [6] instead of the HPLC method [4,8].

The method reported here has been developed using direct injection of plasma on a coupled column system after an initial precipitation step. The present paper describes the development and validation according to international guidelines [9,10] of this rapid and robust analytical method for the determination of clindamycin in human plasma as well as its application to a pharmacokinetic study.

2. Experimental

2.1. Chemicals

Clindamycin HCl (86.6% base) Lot G-2 was obtained from USP (Rockville, USA). The internal standard, phenobarbital, was purchased from Caelo (Hilden, Germany). Monobasic potassium phosphate (p.a.), ammonium sulfate (p.a.) and acetonitrile (HPLC grade) were obtained from E. Merck (Darmstadt, Germany). Blank pool plasma originated from the Hessen Blood Bank (Frankfurt, Germany). Distilled water, prepared from demineralized water, was used throughout the study.

2.2. Stock solutions and standards

Stock solutions of clindamycin were prepared by dissolving 23.1 mg of clindamycin HCl (accurately weighed, corresponding to 20 mg clindamycin base) in methanol and making the solution up to 20.0 ml (1000 $\mu g\,ml^{-1}$). Working solutions of clindamycin were prepared by serial dilutions with monobasic potassium phosphate 0.01 *M* from the primary stock solution.

Spiked plasma samples, used as calibration standards, were prepared daily by addition of 25 μl of the working solutions to 475 μl of drug-free human plasma, resulting in calibration standards of 10.0, 5.0, 2.5, 1.2, 0.6 and 0.3 $\mu g\,ml^{-1}$. Three pools of quality control samples (QCs) for clindamycin were prepared with human plasma in the concentrations of 10.0, 3.0 and 0.3 $\mu g\,ml^{-1}$, covering the entire calibration range.

2.3. Sample preparation

After thawing at room temperature, an aliquot of each sample (500 μl) was pipetted into a conical centrifuge tube and 25 μl of internal standard solution (phenobarbital 50 $\mu g\,ml^{-1}$) were added. After vortexing briefly, 150 μl of a saturated ammonium sulfate solution were added to each sample and the samples were vortexed again for 20 s and centrifuged for 5 min at 2700 *g*. The resulting clear supernatant from each sample was transferred to an autosampler vial and a 200 μl aliquot was injected into the HPLC system.

2.4. Instrumentation and chromatographic conditions

The HPLC equipment (Fig. 2) consisted of the following components: two Kontron pumps, T414 and 420, a Kontron 460 autosampler (Kontron, Neufahrn, Germany), a UV-975 detector set at 198 nm (Jasco, Groß-Zimmern, Germany) and electronically actuated switching valves (Knauer, Berlin, Germany).

The two precolumns were filled with LiChrospher 60 RP-select B, 25 μm, (E. Merck, Darmstadt, Germany), to give a column of 5×4 mm I.D. The precolumns were dry-filled daily and packed down with methanol. The analytical column was a Nucleosil 100, C 18 HD, 5 μm, column of 250×4 mm I.D. (Macherey & Nagel, Düren, Germany) protected by a LiChrospher 60 RP-select B, 5 μm, guard column of 4×4 mm I.D. (E. Merck, Darmstadt, Germany). The analytical column was maintained at 28°C in a Knauer column oven.

Plasma samples were injected and the compounds of interest, clindamycin and the internal standard, were trapped either on precolumn 1 or 2 for 3 min with a mobile phase consisting of monobasic potassium phosphate 0.015 *M* at a flow-rate of 0.7

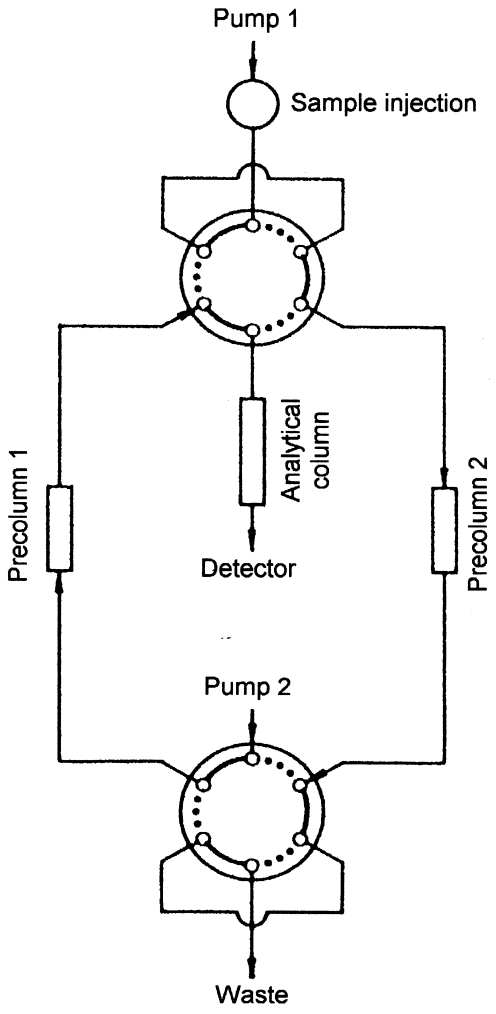

Fig. 2. Schematic diagram of the alternating coupled column HPLC system. A sample is eluted from precolumn 1 onto the analytical column while the next sample is trapped on precolumn 2.

ml min^{-1}. The compounds were then eluted from the precolumn and transferred to the analytical column with the mobile phase consisting of acetonitrile–0.028 *M* dibasic potassium phosphate (1:3, v/v) at a flow-rate of 0.7 ml min^{-1}. At the same time, a sample was concentrated on the other precolumn.

Peak recording and integration was carried out using a Kontron MT1-EMS chromatography data system. Ratios of clindamycin to the internal standard vs. concentration of clindamycin were used for quantitative computations. Calibration curves were fitted using weighted linear regression analysis ($1/x^2$).

2.5. Validation

A 3-day prestudy validation of the analytical method for clindamycin was carried out covering a concentration range of 0.3–10.0 μg ml^{-1}. A validation run included a set of calibration samples in triplicate and 6-fold QC samples at three concentration levels (10.0, 3.0 and 0.3 μg ml^{-1}). The intra- and inter-day-accuracy of the method is expressed in terms of bias and was calculated by the formula:

$$\%\ \text{Bias} = [(\text{observed concentration} - \text{nominal concentration})/\text{nominal concentration}] \times 100$$

The intra- and inter-day precision of the method was calculated by the coefficients of variation (%).

The analytical method was later validated further to extend the lower limit of quantification (LLQ) to 0.2 μg ml^{-1}. The LLQ was determined by analyzing 6-fold QCs on three separate occasions together with a set of calibration samples (0.2–10.0 μg ml^{-1}).

For the determination of the recovery of clindamycin and the internal standard phenobarbital, spiked plasma samples in one midrange concentration (3.0 μg ml^{-1}) were analyzed six times as described above. The peak areas of clindamycin and phenobarbital were compared to the peak areas (mean of six determinations) of aqueous solutions of clindamycin and phenobarbital injected directly into the system without a sample concentration process on the precolumn. Here, in order to have a meaningful comparison, peak areas instead of peak heights were assessed since direct injection results in narrower and higher peaks which nevertheless have comparable peak areas.

The specificity of the method was verified by analyzing six independent blank (drug-free) plasma samples from different volunteers. The chromatograms of these blank plasma samples were compared with chromatograms obtained after spiking the same blank plasma samples with clindamycin and internal standard in order to ascertain that endogenous substances would not interfere with the determination of the compounds of interest.

The stability of clindamycin in spiked plasma samples (10.0 and 0.3 μg ml^{-1}) was determined in three samples of each concentration after three freeze and thaw cycles. The long-term storage stability of spiked plasma samples (six samples, 3.0 μg ml^{-1}) at −80°C was determined after 6 weeks. Additionally, the stability of spiked processed plasma samples (3.0 μg ml^{-1}) during storage in the autosampler for 22 h at room temperature was determined in six samples.

2.6. Application of the method

This analytical method was applied to a pharmacokinetic study. Blood samples were taken from healthy male volunteers after the oral administration of 600 mg of clindamycin either as a tablet or capsules. Samples were obtained in heparinized tubes at 15, 30 and 45 min and 1, 1.25, 1.5, 1.75, 2, 3, 4, 6, 8, 10, 12 and 14 h after intake of the drug. Immediately after sampling, the blood was placed in an ice-water bath, then centrifuged for 8 min at 2000 *g* and the plasma was stored at −20°C within 30 min. The plasma samples were stored at −80°C within 24 h.

3. Results and discussion

The specificity of the method was demonstrated by comparing chromatograms of six independent plasma samples from different volunteers—each as a blank sample and a spiked sample. No interferences with either clindamycin or the internal standard phenobarbital were detected from blank plasma. Representative chromatograms of a blank plasma sample, a blank plasma sample spiked with clindamycin near the lower limit of quantification, and a volunteer sample are shown in Fig. 3.

The calibration curves were linear over the entire concentration range with correlation coefficients ≥0.9985. By using weighted linear regression analysis ($1/x^2$), deviations of the interpolated concentrations of all standards in the daily calibration curves of clindamycin in plasma were within an acceptable 90–110% range. The limit of quantification was determined to be 0.2 μg ml^{-1}.

The accuracy of the method, expressed in terms of bias as the percentage deviation from the nominal values, lay between −7.2% and 0.2% for the concentrations investigated (0.3, 3.0 and 10.0 μg ml^{-1}). The bias was 8.1% at the LLQ (0.2 μg ml^{-1}). The precision of the method, given by the coefficients of variation, lay between 3.3% and 4.9% during the prestudy validation and was 10.3% at the LLQ. Data are shown in Table 1.

The recovery of clindamycin was 101.7% at a concentration of 3.0 μg ml^{-1} and the recovery of the internal standard at the concentration level used in the assay (2.5 μg ml^{-1}) was 104.5%.

The stability of clindamycin in plasma was demonstrated after three freeze–thaw cycles and after 6 weeks frozen storage at −80°C. Additionally, processed samples were found to be stable on storage in the autosampler at room temperature for 22 h. No significant deterioration of the analyte was observed under any of these conditions. Data are shown in Table 2.

The method described was applied in our institute to a bioavailability study of clindamycin in human plasma samples obtained after the administration of tablets or capsules containing 600 mg clindamycin. Plasma collected from volunteers prior to drug administration did not reveal the presence of interfering endogenous peaks. A typical plasma concentration vs. time profile of clindamycin from a healthy male volunteer is shown in Fig. 4.

4. Conclusions

A rapid and convenient method for the determination of clindamycin in human plasma has been developed. The method was shown to be selective, accurate and precise. The limit of quantification was sufficient for the intended purpose. The method is appropriate for pharmacokinetic studies even if more than 1000 plasma samples are to be processed in a period of one month.

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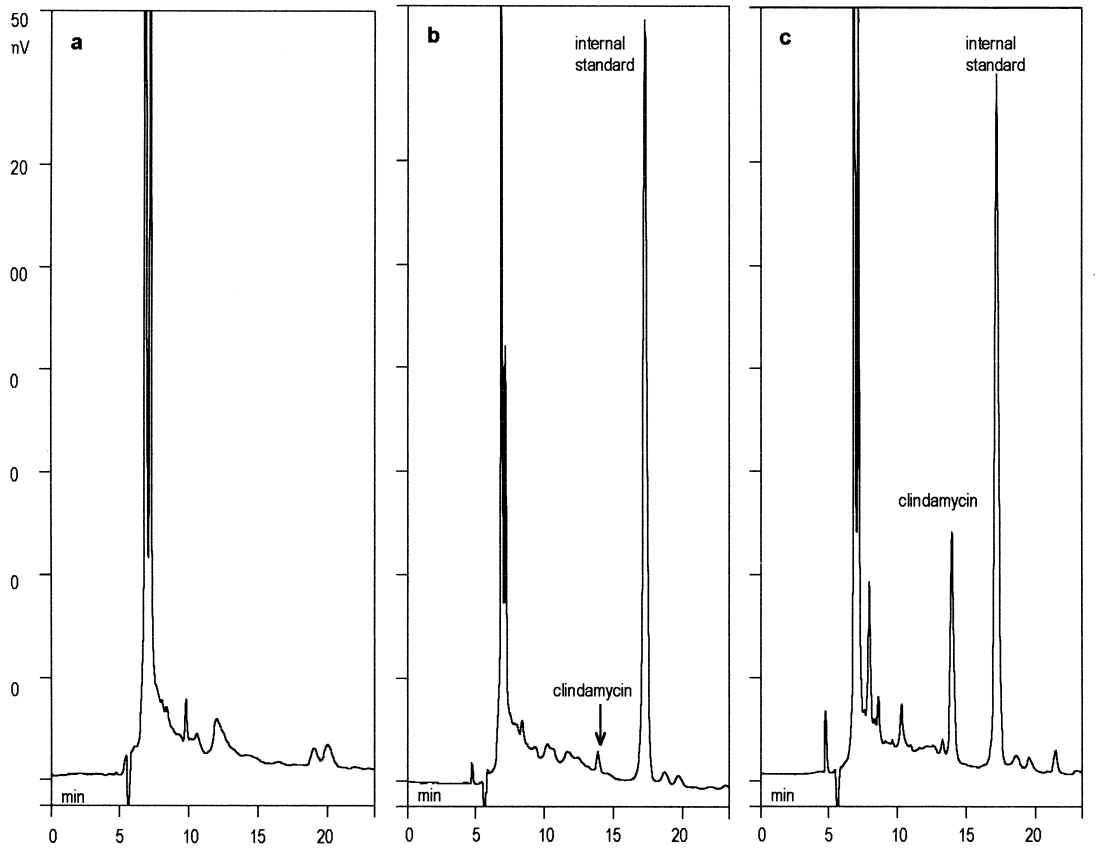

Fig. 3. Representative chromatograms of: (a) a blank plasma sample; (b) blank plasma spiked with clindamycin at the lower limit of quantitation (0.2 μg ml^{-1}) and the internal standard phenobarbital; and (c) a volunteer sample taken 1.75 h after administration of 600 mg clindamycin, corresponding to a concentration of 3.75 μg ml^{-1}.

Table 1
Intra- and inter-assay precision and accuracy for HPLC assay of clindamycin in human plasma

Nominal concentration (μg ml^{-1})	Intra-assay ($n=6$)		Inter-assay ($n=18$)	
	Accuracy (%)	C.V. (%)	Accuracy (%)	C.V. (%)
10.01	−9.4	4.7	−7.2	3.5
	−6.8	2.9		
	−5.3	0.9		
3.00	−5.8	3.7	−3.6	3.3
	−4.0	2.6		
	−1.2	1.7		
0.30	−3.9	3.4	0.2	4.9
	2.8	3.2		
	1.7	5.4		
0.20	18.4	6.7	8.1	10.3
	−2.5	9.6		
	8.3	2.4		

Table 2
Stability data for clindamycin ($n=6$ per test and each concentration)

	Nominal concentration ($\mu g\ ml^{-1}$)	Accuracy (%)	C.V. (%)
Freeze–thaw stability			
0 cycles	10.01	−9.4	4.7
3 cycles	10.01	−5.8	1.5
0 cycles	0.30	−3.9	3.4
3 cycles	0.30	−12.2	8.2
Storage stability (−80°C)			
0 weeks	3.00	−5.8	3.7
6 weeks	3.00	−1.1	3.0
Autosampler stability			
0 h	3.00	−4.0	2.6
22 h	3.00	−2.7	1.3

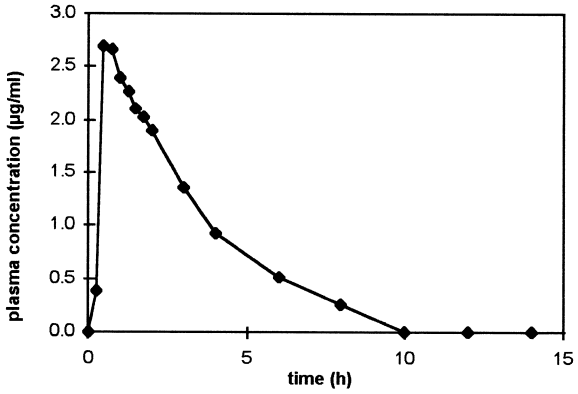

Fig. 4. Representative plasma concentration vs. time profile of clindamycin after oral administration of 600 mg of clindamycin to a healthy male volunteer.

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References

[1] J.E.F. Reynolds (Ed.), Martindale, The Extra Pharmacopoeia, 30th ed., Royal Pharmaceutical Society of Great Britain, 1993, p. 148.
[2] L.W. Brown, W.F. Beyer, Clindamycin hydrochloride, in: K. Florey (Ed.), Analytical Profiles of Drug Substances, Vol. 10, Academic Press, 1981, p. 75.
[3] C.M. Metzler, R. DeHaan, D. Schellenberg, W.D. Vandenbosch, J. Pharm. Sci. 62 (1973) 591.
[4] G. La Follette, J. Gamertoglio, J. White, J. Chromatogr. 431 (1988) 379.
[5] C. Liu, Y. Chen, T. Yang, S. Hsieh, M. Hung, E.T. Lin, J. Chromatogr. B 696 (1997) 298.
[6] G. Gatti, J. Flaherty, J. Bubp, J. White, M. Borin, J. Gambertoglio, J. Antimicrob. Agents Chemother. 37 (1993) 1137.
[7] C. Duckworth, J.F. Fisher, S.A. Carter, C.L. Newman, C. Cogburn, R.R. Nesbit, C.H. Wray, J. Antimicrob. Agents Chemother. 31 (1993) 581.
[8] J.F. Flaherty, L.C. Rodondi, B.J. Guglielmo, J.C. Fleishaker, R.J. Townsend, J.G. Gambertoglio, J. Antimicrob. Agents Chemother. 32 (1988) 1825.
[9] V.P. Shah, K.K. Midha, S. Dighe, I.J. McGilveray, J.P. Skelly, A. Yacobi, T. Layloff, C.T. Visvanathan, C.E. Cook, R.D. McDowall, K.A. Pittman, S. Spector, J. Assoc. Off. Anal. Chem. Intl. 75 (1992) 19A.
[10] CPMP Working Party on Efficacy of Medicinal Products ICH Guideline, Validation of Analytical Procedures: Methodology, Document No. CPMP/ICH/281/95, 1996.